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Form Revision Date: August 26, 2013

**Electronically Filed**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**
Carl H. June, et al.

Group Art Unit: To be assigned

**Application No.:** To be assigned

Examiner: To be assigned

**Filed:** Herewith

Attorney Docket No.
046483-6001US13 (01088)

**Title:** Compositions and Methods for Treatment of Cancer

## PRELIMINARY AMENDMENT

Prior to examination on the merits, kindly amend the above-identified application without prejudice, as follows. Please charge any applicable fees to deposit account number 50-4364.

**AMENDMENT TO THE SPECIFICATION** begins on page 2.

**AMENDMENT TO THE CLAIMS** begins on page 3.

**REMARKS** begin on page 7.

## Amendment to the Specification

On page 1, line 2, please replace the title of the invention with the following title:
--"COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER"--

On page 1, line 5 of the specification; please replace the paragraph with the following:

-- CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. Patent Application No. 13/992,622, filed June 7, 2013, which is a U.S. national phase application filed under 35 U.S.C. § 371 claiming benefit to International Patent Application No. PCT/US2011/064191, filed on December 9, 2011, which is entitled to priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 61/421,470, filed on December 9, 2010 and U.S. Provisional Patent Application No. 61/502,649, filed on June 29, 2011, each of which application is hereby incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under grant numbers K24 CA11787901, R01CA120409, 1RO1CA105216, RO1AI057838 and RO11113482 awarded by the National Institutes of Health. The Government therefore has certain rights in the invention. --

**Amendment to the Claims**

The listing of the claims will replace all prior versions, and listings, of the claims in the application.

1-89. (canceled)

90. (New) A pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD19 antigen binding domain comprising, from the amino to the carboxy terminus, a light chain variable region and a heavy chain variable region, wherein the CAR further comprises a transmembrane domain, a 4-1BB costimulatory signaling region, and a CD3 zeta signaling domain.

91. (New) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^4$ to $10^9$ cells per kg body weight of a human in need of such cells.

92. (New) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^5$ to $10^6$ cells per kg body weight of a human in need of such cells.

93. (New) The composition of claim 90, wherein said antigen binding fragment is a scFv.

94. (New) The composition of claim 93, wherein the scFv comprises the amino acid sequence of SEQ ID NO:20.

95. (New) The composition of claim 90, wherein the transmembrane domain is CD8α transmembrane domain.

96. (New) The composition of claim 95, wherein the CD8α transmembrane domain

comprises the amino acid sequence of SEQ ID NO: 22.

97. (New) The composition of claim 90, wherein the CAR further comprises a hinge domain.

98. (New) The composition of claim 97, wherein the hinge domain is a CD8α hinge domain.

99. (New) The composition of claim 98, wherein the CD8α hinge domain comprises the amino acid sequence of SEQ ID NO:21.

100. (New) The composition of claim 90, wherein the 4-1BB costimulatory signaling region comprises the amino acid sequence of SEQ ID NO:23.

101. (New) The composition of claim 90, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

102. (New) The composition of claim 90, wherein the CD19 antigen binding domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 14.

103. (New) The composition of claim 95, wherein the CD8α transmembrane domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 16.

104. (New) The composition of claim 99, wherein the CD8α hinge domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 15.

105. (New) The composition of claim 100, wherein the 4-1BB costimulatory signaling region is encoded by a nucleic acid sequence comprising SEQ ID NO: 17.

106. (New) The composition of claim 101, wherein the CD3 zeta signaling domain is



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encoded by a nucleic acid sequence comprising SEQ ID NO: 18.

107 (New) The composition of claim 90, wherein the CAR comprises the amino acid sequence of SEQ ID NO:12.

108. (New) The composition of claim 107, wherein the CAR is encoded by a nucleic acid sequence comprising SEQ ID NO:8.

109 (New) The composition of claim 90, wherein the CAR further comprises a CD28 costimulatory signaling region.

110. (New) The composition of claim 90, wherein the T cells are T cells of a human having a cancer.

111. (New) The composition of claim 110, wherein the cancer is a hematological cancer.

112. (New) The composition of claim 90, wherein the T cells comprise a vector that comprises the nucleic acid sequence.

113. (New) The composition of claim 112, wherein the vector is a lentiviral vector.

114. (New) The composition of claim 112, wherein the vector further comprises a promoter.

115. (New) The composition of claim 114, wherein the promoter is an EF-1α promoter.

116. (New) The composition of claim 90, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient.

117. (New) The composition of claim 90, wherein the pharmaceutical composition

comprises a buffer.

118. (New) The composition of claim 117, wherein the buffer is neutral buffer saline or phosphate buffered saline.

119. (New) The composition of claim 90, wherein the pharmaceutical composition further comprises a carbohydrate.

**REMARKS**

The present application is a continuation of U.S. Patent Application No. 13/992,622, filed June 7, 2013, a national phase application of PCT/US2011/064191, filed on December 9, 2011, which is entitled to priority to U.S. Provisional Patent Application No. 61/421,470, filed on December 9, 2010 and U.S. Provisional Patent Application No. 61/502,649, filed on June 29, 2011.

**Amendment to the Specification**

The specification is amended herein to properly reflect the priority information of the present application.

The specification is also amended herein to provide the statement regarding federally sponsored research or development.

No new matter is introduced by way of these amendments to the specification.

**Amendment to the Claims**

Claims 1-89 have been canceled herein and new claims 90-118 have been added. Referring to US 2013/0287748, the publication of parent U.S. Patent Application No. 13/992,622, support for new claims 90-118 is found in the application as follows:

Claim 90 - support for the recited cells comprising the recited CAR is found throughout the specification, in the Examples and in SEQ ID NO:20.

Claims 91-92 - Support for “anti-tumor effective amount” is found throughout the specification and for example, in paragraph [0224].

Claim 93 - Support for “CD19 antigen binding fragment is an scFV” is found throughout the specification and for example, in paragraph [0135].

Claims 94 and 102 – Support for “SEQ ID NO: 20” and “SEQ ID NO: 14” is found throughout the specification and for example, in paragraph [0135] and in Table 5.

Claims 95, 96 and 103 – Support for "CD8α transmembrane domain" and "SEQ ID NO: 22" and "SEQ ID NO: 16" is found throughout the specification and for example, in paragraph [0139] and in Table 5.

Claims 97, 98, 99 and 104 – Support for "hinge domain," "CD8α hinge domain," "SEQ ID NO: 21" and "SEQ ID NO: 15" is found throughout the specification and for example, in paragraph [0140] and in Table 5.

Claims 100 and 101 - Support for "SEQ ID NO: 23" and "SEQ ID NO: 24" is found throughout the specification and for example, in paragraph [0151] and in Table 5.

Claims 105 and 106 - Support for "SEQ ID NO: 17" and "SEQ ID NO: 18" is found throughout the specification and for example, in paragraph [0150] and in Table 5.

Claims 107 and 108 - Support for "SEQ ID NO: 12" and "SEQ ID NO: 8" is found throughout the specification and for example, in paragraph [0154] and in Table 5.

Claim 109 – Support for "wherein the CAR further comprises a CD28 costimulatory signaling region is found in paragraphs [0055] and [0149].

Claim 110 - Support for "wherein the T cells are T cells of a human having a cancer" is found throughout the Examples.

Claim 111 - Support for the "hematological cancer" is found throughout the specification and for example, in paragraphs [0200] and [0201].

Claims 112 and 113- Support for "wherein the T cells comprise a vector" and "the vector is a lentiviral vector" is found throughout the specification and at least in Figure 1.

Claims 114 and 115 – Support for a promoter and an EF-1α promoter is found throughout the specification and at least in Figure 1.

Claims 116, 117, 118 and 119– Support for a pharmaceutically acceptable carrier, diluent or excipient, buffer and carbohydrate is found at least in paragraph [0222].

No new matter is added by way of the addition of these claims.

**Summary**

Applicants respectfully submit that the pending claims are fully supported in the specification as filed, and that no new matter has been added by way of the present Preliminary Amendment.

Favorable examination and allowance of the claims is hereby requested.

Respectfully submitted,

**CARL H. JUNE, ET AL.**

January 15, 2016
Date

By: ______________________

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TITLE OF THE INVENTION

# USE OF CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS TO TREAT CANCER

5 ## CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 61/421,470, filed December 9, 2010, and U.S. Provisional Application No. 61/502,649, filed June 29, 2011, all of which are hereby incorporated herein by reference in their entireties.

10

## BACKGROUND OF THE INVENTION

The large majority of patients having B-cell malignancies, including chronic lymphocytic leukemia (CLL), will die from their disease. One approach to treating these patients is to genetically modify T cells to target antigens expressed on
15 tumor cells through the expression of chimeric antigen receptors (CARs). CARs are antigen receptors that are designed to recognize cell surface antigens in a human leukocyte antigen-independent manner. Attempts in using genetically modified cells expressing CARs to treat these types of patients have met with very limited success. See for example, Brentjens et al., 2010, Molecular Therapy, 18:4, 666-668; Morgan et
20 al., 2010, Molecular Therapy, published online February 23, 2010, pages 1-9; and, Till et al., 2008, Blood, 112:2261-2271.

In most cancers, tumor-specific antigens are not yet well defined, but in B cell malignancies, CD19 is an attractive tumor target. Expression of CD19 is restricted to normal and malignant B cells (Uckun, et al. *Blood*, 1988, 71:13-29), so
25 that CD19 is a widely accepted target to safely test CARs. While CARs can trigger T-cell activation in a manner similar to an endogenous T-cell receptor, a major impediment to the clinical application of this technology to date has been limited *in vivo* expansion of CAR+ T cells, rapid disappearance of the cells after infusion, and disappointing clinical activity (Jena, et al., *Blood*, 2010, 116:1035-1044; Uckun, et al.
30 *Blood*, 1988, 71:13-29).

Thus, there is an urgent need in the art for compositions and methods for treatment of cancer using CARs that can expand in vivo. The present invention addresses this need.

## SUMMARY OF THE INVENTION

The present invention provides an isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a
5 CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

In one embodiment, the nucleic acid sequence encodes a CAR comprising the amino acid sequence of SEQ ID NO: 12.

In one embodiment, the nucleic acid sequence encoding a CAR
10 comprises the nucleic acid sequence of SEQ ID NO: 8.

In one embodiment, the antigen binding domain in the CAR is an antibody or an antigen-binding fragment thereof. Preferably, the antigen-binding fragment is a Fab or a scFv.

In one embodiment, the antigen binding domain in the CAR binds to a
15 tumor antigen. In one embodiment, the tumor antigen is associated with a hematologic malignancy. In another embodiment, the tumor antigen is associated with a solid tumor. In yet another embodiment, the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any
20 combination thereof.

In one embodiment, the costimulatory signaling region in the CAR comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-
25 H3, a ligand that specifically binds with CD83, and any combination thereof.

In one embodiment, the CD3 zeta signaling domain in the CAR is encoded by the nucleic acid sequence of SEQ ID NO: 18.

The invention also provides an isolated CAR comprising an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a
30 CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

The invention also provides a cell comprising a nucleic acid sequence encoding a CAR, wherein the CAR comprises an antigen binding domain, a

transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24.

In one embodiment, the cell comprising the CAR is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

In one embodiment, the cell comprising the CAR exhibits an anti-tumor immunity when the antigen binding domain of the CAR binds to its corresponding antigen.

The invention also provides a vector comprising a nucleic acid sequence encoding a CAR, wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

The invention also provides a method for stimulating a T cell-mediated immune response to a target cell population or tissue in a mammal. In one embodiment, the method comprises administering to a mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the antigen binding domain is selected to specifically recognize the target cell population or tissue.

The invention also provides a method of providing an anti-tumor immunity in a mammal. In one embodiment, the method comprises administering to a mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, thereby providing an anti-tumor immunity in the mammal.

The invention also includes a method of treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen. In one embodiment, the method comprises administering to a mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, thereby treating the mammal.

In one embodiment, the cell is an autologous T cell.

In one embodiment, the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

5 The invention also provides a method of treating a human with chronic lymphocytic leukemia. In one embodiment, the method comprises administering to a human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24.

10 In one embodiment, the human is resistant to at least one chemotherapeutic agent

In one embodiment, the chronic lymphocytic leukemia is refractory CD19+ leukemia and lymphoma.

The invention also includes a method of generating a persisting

15 population of genetically engineered T cells in a human diagnosed with cancer. In one embodiment, the method comprises administering to a human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the persisting population of

20 genetically engineered T cells persists in the human for at least one month after administration.

In one embodiment, the persisting population of genetically engineered T cells comprises at least one cell selected from the group consisting of a T cell that was administered to the human, a progeny of a T cell that was administered to the

25 human, and a combination thereof.

In one embodiment, the persisting population of genetically engineered T cells comprises a memory T cell.

In one embodiment, the persisting population of genetically engineered T cells persists in the human for at least three months after administration. In another

30 embodiment, the persisting population of genetically engineered T cells persists in the human for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

In one embodiment, the chronic lymphocytic leukemia is treated.

The invention also provides a method of expanding a population of genetically engineered T cells in a human diagnosed with cancer. In one embodiment, the method comprises administering to a human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain,
5 a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the administered genetically engineered T cell produces a population of progeny T cells in the human.

In one embodiment, the progeny T cells in the human comprise a memory T cell.

10 In one embodiment, the T cell is an autologous T cell.

In another embodiment, the human is resistant to at least one chemotherapeutic agent.

In one embodiment, the cancer is chronic lymphocytic leukemia. In another embodiment, the chronic lymphocytic leukemia is refractory CD19+ leukemia
15 and lymphoma.

In one embodiment, the population of progeny T cells persists in the human for at least three months after administration. In another embodiment, the population of progeny T cells persist in the human for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven
20 months, twelve months, two years, or three years after administration.

In one embodiment, the cancer is treated.

## BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of preferred embodiments of the
25 invention will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

30 Figure 1, comprising Figures 1A through 1C, is a series of images of the schematic representations of the gene-transfer vector and transgene, gene modified T cell manufacturing and clinical protocol design. Figure 1A depicts the lentiviral vectors and transgene that show the major functional elements. A vesicular stomatitis virus protein G pseudotyped clinical grade lentiviral vector (designated



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pELPs 19BBz) directing expression of anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains was produced. Constitutive expression of the transgene was directed by inclusion of an EF-1α (elongation factor-1α promoter);
5 LTR, long terminal repeat; RRE, rev response element. (cPPT) and the central termination sequence (CTS). Figure is not to scale. Figure 1B depicts T cell manufacturing. Autologous cells were obtained via an apheresis, and T cells were enriched by mononuclear cell elutriation, washed and residual leukemia cells depleted by addition of anti-CD3/CD28 coated paramagnetic beads for positive selection and
10 activation of T cells. Lentiviral vector was added at the time of cell activation and was washed out on day 3 post culture initiation. Cells were expanded on a rocking platform device (WAVE Bioreactor System) for 8-12 days. On the final day of culture the beads were removed by passage over a magnetic field and the CART19 T cells harvested and cryopreserved in infusible medium. Figure 1C depicts the clinical
15 protocol design. Patients were given lymphodepleting chemotherapy as described, followed by CART19 infusion #1 by i.v. gravity flow drip over a period of 15-20 minutes. The infusion was given using a split dose approach over 3 days (10%, 30%, 60%) beginning 1 to 5 days after completion of chemotherapy. Endpoint assays were conducted on study week 4. At the conclusion of active monitoring, subjects were
20 transferred to a destination protocol for long term follow up as per FDA guidance.

Figure 2, comprising Figures 2A through 2F, is a series of images demonstrating sustained *in vivo* expansion and persistence in blood and marrow of CART19 cells. DNA isolated from whole blood as depicted in Figure 2A through 2C or marrow as depicted in Figure 2D through 2F, samples obtained from UPN 01 as
25 depicted in Figure 2A and 2D, UPN 02 as depicted in Figure 2B and 2E and UPN 03 as depicted in Figure 2C and 2F was subjected in bulk to Q-PCR analysis using a qualified assay to detect and quantify CART19 sequences. Each data point represents the average of triplicate measurements on 100-200 ng genomic DNA, with maximal % CV less than 1.56%. Pass/fail parameters for the assay included pre-established
30 ranges for slope and efficiency of amplification, and amplification of a reference sample. The lower limit of quantification for the assay established by the standard curve range was 2 copies transgene/microgram genomic DNA; sample values below that number are considered estimates and presented if at least 2/3 replicates generated a Ct value with % CV for the values 15%. CART19 cells were infused at day 0, 1, and



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2 for UPN 01 and UPN 03, and days 0, 1, 2 and 11 for UPN 02.

Figure 3, comprising Figures 3A through 3D, is a series of images demonstrating serum and bone marrow cytokines before and after CAR T cell infusion; longitudinal measurements of changes in serum cytokines, chemokines and cytokine receptors in UPN 01 as depicted in Figure 3A, UPN 02 as depicted in Figure 3B and UPN 03 as depicted in Figure 3C, on the indicated day after CART19 cell infusion and serial assessments of the same analytes in the bone marrow from UPN 03 as depicted in Figure 3D. Samples were subjected multiplex analysis using Luminex bead array technology and pre-assembled and validated multiplex kits. Analytes with a >=3 fold change are indicated, and plotted as relative change from baseline as depicted in Figure 3A through 3C or as absolute values as depicted in Figure 3D. Absolute values for each analyte at each time-point were derived from a recombinant protein-based standard curve over a 3-fold 8-point dilution series, with upper and lower limits of quantification (ULOQ, LLOQ) determined by the 80-120% observed/expected cutoff values for the standard curves. Each sample was evaluated in duplicate with average values calculated and % CV in most cases less than 10%. To accommodate consolidated data presentation in the context of the wide range for the absolute values, data are presented as fold-change over the baseline value for each analyte. In cases where baseline values were not detectable, half of the lowest standard curve value was used as the baseline value. Standard curve ranges for analytes and baseline (day 0) values (listed in parentheses sequentially for UPN01, 02 and 03), all in pg/ml: IL1-Rα: 35.5-29,318 (689, 301, 287); IL-6: 2.7-4,572 (7, 10.1, 8.7); IFN-γ: 11.2-23,972 (2.8, ND, 4.2 ); CXCL10: 2.1-5,319 (481, 115, 287 ); MIP-1β: 3.3-7,233 (99.7, 371, 174 ); MCP-1: 4.8-3,600 (403, 560, 828); CXCL9: 48.2-3,700 (1,412, 126, 177); IL2-Rα: 13.4-34,210 (4,319, 9,477, 610); IL-8: 2.4-5,278 (15.3, 14.5, 14.6); IL-10: 6.7-13,874 (8.5, 5.4, 0.7); MIP-1α: 7.1-13,778 (57.6, 57.3, 48.1).

Figure 4, comprising Figures 4A through 4D, is a series of images depicting prolonged surface CART19 expression and establishment of functional memory CARs *in vivo*. Figure 4A depicts detection of CAR-expressing CD3+ lymphocytes and absence of B cells in periphery and marrow. Freshly processed peripheral blood or marrow mononuclear cells obtained from UPN 03 at day 169 post-CART19 cell infusion were evaluated by flow-cytometry for surface expression of CAR19 (top) or presence of B cells (bottom); as a control, PBMC obtained from a



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healthy donor ND365 were stained. The gating strategy for the CD3+ and B cell populations is presented in Figure 9. To evaluate CAR19 expression in CD3+ lymphocytes, samples were co-stained with antibodies to CD14-PE-Cy7 and CD16-PE-Cy7 (dump channel) and CD3-FITC, positively gated on CD3+, and evaluated for
5 CAR19 expression in the CD8+ and CD8-lymphocyte compartments by co-staining with CD8a-PE and the anti-CAR19 idiotype antibody conjugated to Alexa-647. Data in plots are gated on the dump channel-negative/CD3-positive cell population. To evaluate the presence of B cells, samples were co-stained with antibodies to CD14-APC and CD3-FITC (dump channels) and evaluated for the presence of B cells in the
10 dump channel-negative fraction by co-staining with antibodies to CD20-PE and CD19-PE-Cy-7. In all cases, negative gate quadrants were established on no-stain controls as depicted in Figures 4B and 4C. T cell immunophenotyping of CD4+ (Figure 4B) and CD8+ (Figure 4C) T cell subsets is shown. Frozen peripheral blood samples from UPN 03 obtained by apheresis at day 56 and 169 post T cell infusion
15 were rested overnight in culture medium with no added factors, washed, and subjected to multi-parametric immunophenotyping for expression of markers of T cell memory, activation, and exhaustion. The gating strategy, as depicted in Figure 8, involved an initial gating on dump channel (CD14, CD16, Live/Dead Aqua)-negative and CD3-positive cells, followed by positive gates on CD4+ and CD8+ cells. Gates and
20 quadrants were established using FMO controls (CAR, CD45RA, PD-1, CD25, CD127, CCR7) or by gating on positive cell populations (CD3, CD4, CD8) and clearly delineated subsets (CD27, CD28, CD57); data were displayed after bi-exponential transformation for objective visualization of events. Figure 4D depicts functional competence of persisting CAR cells. Frozen peripheral blood samples
25 from UPN 03 obtained by apheresis at day 56 and 169 post T cell infusion were rested overnight in culture medium with no added factors, washed, and evaluated directly *ex-vivo* for the ability to recognize CD19-expressing target cells using CD107 degranulation assays. Following a two-hour incubation in the presence of anti-CD28, anti-CD49d, and CD107-FITC, cell mixtures were harvested, washed, and subjected
30 to multi-parametric flow cytometric analysis to evaluate the ability of CART19 cells to de-granulate in response to CD19-expressing targets. The gating strategy involved an initial gate on dump channels (CD14-PE-Cy7, CD16-PE-Cy7, Live/Dead Aqua)-negative and CD3-PE-positive cells, followed by gating on CD8-PE-Texas Red-positive cells; presented data is for the CD8+ gated population. In all cases, negative



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gate quadrants were established on no-stain controls.

Figure 5, comprising Figures 5A through 5C, is series of images depicting the results of experiments evaluating clinical responses after infusion of CART19 cells. Figure 5A depicts that UPN 02 was treated with two cycles of
5 rituximab and bendamustine with minimal response (R/B, arrow). CART19 T cells were infused beginning 4 days after bendamustine only (B, arrow). The rituximab and bendamustine-resistant leukemia was rapidly cleared from blood, as indicated by a decrease in the absolute lymphocyte count (ALC) from 60,600/μl to 200/μl within 18 days of the infusion. Corticosteroid treatment was started on day 18 post infusion
10 due to malaise and non-infectious febrile syndrome. The reference line (dotted) indicates upper limit of normal for ALC. Figure 5B depicts the results of example experiments staining sequential bone marrow biopsy or clot specimens from patient UPN 01 and 03 for CD20. Pretreatment infiltration with leukemia present in both patients was absent on post treatment specimens accompanied by normalization of
15 cellularity and trilineage hematopoiesis. UPN 01 has not had any CLL cells detected as assessed by flow cytometry, cytogenetics and fluorescence in-situ hybridization or normal B cells detected by flow cytometry in bone marrow or blood. UPN 03 had 5% residual normal CD5-negative B cells confirmed by flow cytometry on day +23, which also showed them to be polyclonal; no normal B cells were detected at day
20 +176. Figure 5C depicts the results of experiments using sequential CT imaging to assess the rapid resolution of chemotherapy-resistant generalized lymphadenopathy. Bilateral axillary masses resolved by 83 (UPN 01) and 31 (UPN 03) days post infusion, as indicated by arrows and circle.

Figure 6, comprising Figures 6A through 6C, is a series of images
25 depicting absolute lymphocyte counts and total CART19+ cells in circulation for UPN 01, 02, 03. The total number of lymphocytes (Total normal and CLL cells) vs. Total CART19+ cells in circulation is plotted for all 3 subjects using the absolute lymphocyte count from CBC values, and assuming a 5.0 L volume of blood. The total number of CART19 cells in circulation was calculated by using the tandem CBC
30 values with absolute lymphocyte counts and the Q-PCR marking values as depicted in Figure 2, converting copies/μg DNA to average % marking as described elsewhere herein. The Q-PCR % marking was found to correlate closely (<2 fold variation) with the flow cytometric characterization of the infusion products and with data from samples where concomitant flow cytometry data was available to directly enumerate

CART19 cells by staining.

Figure 7, comprising Figures 7A through 7D is a series of images depicting experiments involving the direct *ex vivo* detection of CART19-positive cells in UPN-01 PBMC 71 days post-T cell infusion. UPN-01 PBMC collected either fresh post-apheresis on day71 day post infusion, or frozen at the time of apheresis for manufacture of the T cell product(baseline) and viably thawed prior to the staining, were subjected to flow-cytometric analysis to detect the presence ofCART19 cells that express the CAR19 moiety on the surface. To evaluate the expression of CAR19 in lymphocytes, samples were co-stained with CD3-PE and the anti-CAR19 idiotype antibody conjugated to Alexa-647, or co-stained with CD3-PE alone (FMO for CAR19). Figure 7A depicts that an initial lymphocyte gate was established based on forward and side scatter (FSC vs SSC), followed by gating on CD3+ cells. Figure 7B depicts CD3+ lymphocyte gate; Figure 7C depicts CAR idiotype stain; Figure 7D depicts CAR idiotype FMO. The CAR19-positive gate was established on the CAR19 FMO samples.

Figure 8, comprising Figures 8A through 8C, is a series of images depicting the gating strategy to identify CART19 expression by using polychromatic flow cytometry in UPN 03 blood specimens. The gating strategy for Figure 8C is shown for the UPN 03 Day 56 sample and is representative of the strategy used on the UPN 03 Day 169 sample. Figure 8A depicts primary gate: Dump (CD14, CD16, LIVE/dead Aqua) negative, CD3-positive. Figure 8B depicts secondary gates: CD4-positive, CD8positive. Figure 8C depicts tertiary gates: CAR19-positive and CAR19-negative, established on CAR FMO samples (right-most panels).

Figure 9 depicts the gating strategy to directly identify CART19 expression and B cells in blood and marrow specimens. The gating strategy for Figure 4A, which shows detection of CAR-expressing CD3+ lymphocytes and absence of B cells in periphery and marrow: Leftplot: Cell gate; Upper panel: positive gate for CD3+ cells, Lower panel: negative gate (CD14-negative, CD3-negative) for B cells. NC365, peripheral blood control cells from a healthy donor

Figure 10 is an image summarizing the patient demographics and response.

Figure 11 depicts the manufacturing process of CART-19 cells

Figure 12, comprising Figures 12A through 12D, is a series of images depicting the clinical response in a patient. Figure 12A shows the lentiviral vector

used to infect T cells from the patient. A pseudotyped, clinical-grade lentiviral vector of vesicular stomatitis virus protein G (pELPs 19-BB-z) directing expression of anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and transmembrane domain, and human 4-1BB and CD3ζ signaling domains was
5 produced. Details of the CAR19 transgene, at the bottom of Figure 12A, show the major functional elements. The figure is not to scale. 3′LTR denotes 3′ long terminal repeat; 5′LTR, 5′ long terminal repeat; Amp R, ampicillin resistance gene; Bovine GH Poly A, bovine growth hormone with polyadenylation tail; cPPT/CTS, central polypurine tract with central termination sequence; EF-1α, elongation factor 1-alpha;
10 env, envelope; gag, group-specific antigen; pol, HIV gene encoding polymerase and reverse transcriptase; R, repeat; RRE, rev response element; scFv, single-chain variable fragment; TM, transmembrane; and WPRE, woodchuck hepatitis virus post-transcriptional regulatory element. Figure 12B shows serum creatinine, uric acid, and lactate dehydrogenase (LDH) levels from day 1 to day 28 after the first CART19-cell
15 infusion. The peak levels coincided with hospitalization for the tumor lysis syndrome. Figure 12C shows bone marrow–biopsy specimens obtained 3 days after chemotherapy (day −1, before CART19-cell infusion) and 23 days and 6 months after CART19-cell infusion (hematoxylin and eosin). The baseline specimen shows hypercellular bone marrow (60%) with trilineage hematopoiesis, infiltrated by
20 predominantly interstitial aggregates of small, mature lymphocytes that account for 40% of total cellularity. The specimen obtained on day 23 shows residual lymphoid aggregates (10%) that were negative for chronic lymphoid leukemia (CLL), with a mixture of T cells and CD5-negative B cells. The specimen obtained 6 months after infusion shows trilineage hematopoiesis, without lymphoid aggregates and continued
25 absence of CLL. Figure 12D shows contrast-enhanced CT scans obtained before the patient was enrolled in the study and 31 days and 104 days after the first infusion. The preinfusion CT scan reveals 1-to-3-cm bilateral masses. Regression of axillary lymphadenopathy occurred within 1 month after infusion and was sustained. Arrows highlight various enlarged lymph nodes before therapy and lymph-node responses on
30 comparable CT scans after therapy.

Figure 13, comprising Figures 13A through 13E, is a series of images depicting serum and bone marrow cytokines before and after chimeric antigen receptor T-cell infusion. Serial measurements of the cytokine interferon-γ (Figure 13A), the interferon-γ–stimulated chemokines C-X-C motif chemokine 10 (CXCL10)



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(Figure 13B) and C-X-C motif ligand 9 (CXCL9) (Figure 13C), and interleukin-6 (Figure 13D) were measured at the indicated time points. The increases in these inflammatory cytokines and chemokines coincided with the onset of the tumor lysis syndrome. Low levels of interleukin-6 were detected at baseline, whereas interferon-
5 γ, CXCL9, and CXCL10 were below the limits of detection at baseline. Standard-curve ranges for the analytes and baseline values in the patient, given in parentheses, were as follows: interferon-γ, 11.2 to 23,972 pg per milliliter (1.4 pg per milliliter); CXCL10, 2.1 to 5319 pg per milliliter (274 pg per milliliter); CXCL9, 48.2 to 3700 pg per milliliter (177 pg per milliliter); interleukin-6, 2.7 to 4572 pg per milliliter (8.3
10 pg per milliliter); tumor necrosis factor α (TNF-α), 1.9 to 4005 pg per milliliter (not detectable); and soluble interleukin-2 receptor, 13.4 to 34,210 pg per milliliter (644 pg per milliliter). Figure 13E shows the induction of the immune response in bone marrow. The cytokines TNF-α, interleukin-6, interferon-γ, chemokine CXCL9, and soluble interleukin-2 receptor were measured in supernatant fluids obtained from bone
15 marrow aspirates on the indicated days before and after CART19-cell infusion. The increases in levels of interleukin-6, interferon-γ, CXCL9, and soluble interleukin-2 receptor coincided with the tumor lysis syndrome, peak chimeric antigen receptor T-cell infiltration, and eradication of the leukemic infiltrate.

Figure 14, comprising Figures 14A through 14C, is a series of images
20 depicting expansion and persistence of chimeric antigen receptor T cells in vivo. Genomic DNA (gDNA) was isolated from samples of the patient's whole blood (Figure 14A) and bone marrow aspirates (Figure 14B) collected at serial time points before and after chimeric antigen receptor T-cell infusion and used for quantitative real-time polymerase-chain-reaction (PCR) analysis. As assessed on the basis of
25 transgenic DNA and the percentage of lymphocytes expressing CAR19, the chimeric antigen receptor T cells expanded to levels that were more than 1000 times as high as initial engraftment levels in the peripheral blood and bone marrow. Peak levels of chimeric antigen receptor T cells were temporally correlated with the tumor lysis syndrome. A blood sample obtained on day 0 and a bone marrow sample obtained on
30 day 1 had no PCR signal at baseline. Flow-cytometric analysis of bone marrow aspirates at baseline (Figure 14C) shows predominant infiltration with CD19+CD5+ cells that were clonal, as assessed by means of immunoglobulin kappa light-chain staining, with a paucity of T cells. On day 31 after infusion, CD5+ T cells were present, and no normal or malignant B cells were detected. The numbers indicate the



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relative frequency of cells in each quadrant. Both the x axis and the y axis show a log10 scale. The gating strategy involved an initial gating on CD19+ and CD5+ cells in the boxes on the left, and the subsequent identification of immunoglobulin kappa and lambda expression on the CD19+CD5+ subset (boxes on the right)

5

## DETAILED DESCRIPTION

The invention relates to compositions and methods for treating cancer including but not limited to hematologic malignancies and solid tumors. The present invention relates to a strategy of adoptive cell transfer of T cells transduced to express

10 a chimeric antigen receptor (CAR). CARs are molecules that combine antibody-based specificity for a desired antigen (e.g., tumor antigen) with a T cell receptor-activating intracellular domain to generate a chimeric protein that exhibits a specific anti-tumor cellular immune activity.

The present invention relates generally to the use of T cells genetically

15 modified to stably express a desired CAR. T cells expressing a CAR are referred to herein as CAR T cells or CAR modified T cells. Preferably, the cell can be genetically modified to stably express an antibody binding domain on its surface, conferring novel antigen specificity that is MHC independent. In some instances, the T cell is genetically modified to stably express a CAR that combines an antigen

20 recognition domain of a specific antibody with an intracellular domain of the CD3-zeta chain or FcγRI protein into a single chimeric protein.

In one embodiment, the CAR of the invention comprises an extracellular domain having an antigen recognition domain, a transmembrane domain, and a cytoplasmic domain. In one embodiment, the transmembrane domain that

25 naturally is associated with one of the domains in the CAR is used. In another embodiment, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. Preferably, the transmembrane domain is the CD8α

30 hinge domain.

With respect to the cytoplasmic domain, the CAR of the invention can be designed to comprise the CD28 and/or 4-1BB signaling domain by itself or be combined with any other desired cytoplasmic domain(s) useful in the context of the CAR of the invention. In one embodiment, the cytoplasmic domain of the CAR can



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be designed to further comprise the signaling domain of CD3-zeta. For example, the cytoplasmic domain of the CAR can include but is not limited to CD3-zeta, 4-1BB and CD28 signaling modules and combinations thereof. Accordingly, the invention provides CAR T cells and methods of their use for adoptive therapy.

5 In one embodiment, the CAR T cells of the invention can be generated by introducing a lentiviral vector comprising a desired CAR, for example a CAR comprising anti-CD19, CD8α hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains, into the cells. The CAR T cells of the invention are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained

10 tumor control.

In one embodiment the invention relates to administering a genetically modified T cell expressing a CAR for the treatment of a patient having cancer or at risk of having cancer using lymphocyte infusion. Preferably, autologous lymphocyte infusion is used in the treatment. Autologous PBMCs are collected from a patient in

15 need of treatment and T cells are activated and expanded using the methods described herein and known in the art and then infused back into the patient.

In yet another embodiment, the invention relates generally to the treatment of a patient at risk of developing CLL. The invention also includes treating a malignancy or an autoimmune disease in which chemotherapy and/or

20 immunotherapy in a patient results in significant immunosuppression in the patient, thereby increasing the risk of the patient of developing CLL.

The invention includes using T cells expressing an anti-CD19 CAR including both CD3-zeta and the 4-1BB costimulatory domain (also referred to as CART19 T cells). The CART19 T cells of the invention can undergo robust *in vivo* T

25 cell expansion and can establish CD19-specific memory cells that persist at high levels for an extended amount of time in blood and bone marrow. In some instances, the CART19 T cells of the invention infused into a patient can eliminate leukemia cells *in vivo* in patients with advanced chemotherapy-resistant CLL. However, the invention is not limited to CART19 T cells. Rather, the invention includes any

30 antigen binding moiety fused with one or more intracellular domains selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD3zeta signal domain, and any combination thereof.

Definitions



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Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the
5 present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The articles "a" and "an" are used herein to refer to one or to more
10 than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

"About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more
15 preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

"Activation", as used herein, refers to the state of a T cell that has been sufficiently stimulated to induce detectable cellular proliferation. Activation can also be associated with induced cytokine production, and detectable effector functions.
20 The term "activated T cells" refers to, among other things, T cells that are undergoing cell division.

The term "antibody," as used herein, refers to an immunoglobulin molecule which specifically binds with an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can
25 be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies in the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and $F(ab)_2$, as well as single chain antibodies and humanized antibodies (Harlow et al., 1999, In: Using Antibodies: A Laboratory Manual, Cold
30 Spring Harbor Laboratory Press, NY; Harlow et al., 1989, In: Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

The term "antibody fragment" refers to a portion of an intact antibody and refers to the antigenic determining variable regions of an intact antibody.

Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, and Fv fragments, linear antibodies, scFv antibodies, and multispecific antibodies formed from antibody fragments.

An "antibody heavy chain," as used herein, refers to the larger of the
5 two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations.

An "antibody light chain," as used herein, refers to the smaller of the two types of polypeptide chains present in all antibody molecules in their naturally occurring conformations. κ and λ light chains refer to the two major antibody light
10 chain isotypes.

By the term "synthetic antibody" as used herein, is meant an antibody which is generated using recombinant DNA technology, such as, for example, an antibody expressed by a bacteriophage as described herein. The term should also be construed to mean an antibody which has been generated by the synthesis of a DNA
15 molecule encoding the antibody and which DNA molecule expresses an antibody protein, or an amino acid sequence specifying the antibody, wherein the DNA or amino acid sequence has been obtained using synthetic DNA or amino acid sequence technology which is available and well known in the art.

The term "antigen" or "Ag" as used herein is defined as a molecule
20 that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA,
25 which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial
30 nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived

from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell or a biological fluid.

The term "anti-tumor effect" as used herein, refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the
5 number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by the ability of the peptides, polynucleotides, cells and antibodies of the invention in prevention of the occurrence of tumor in the first place.

10 The term "auto-antigen" means, in accordance with the present invention, any self-antigen which is mistakenly recognized by the immune system as being foreign. Auto-antigens comprise, but are not limited to, cellular proteins, phosphoproteins, cellular surface proteins, cellular lipids, nucleic acids, glycoproteins, including cell surface receptors.

15 The term "autoimmune disease" as used herein is defined as a disorder that results from an autoimmune response. An autoimmune disease is the result of an inappropriate and excessive response to a self-antigen. Examples of autoimmune diseases include but are not limited to, Addision's disease, alopecia greata, ankylosing spondylitis, autoimmune hepatitis, autoimmune parotitis, Crohn's disease, diabetes
20 (Type I), dystrophic epidermolysis bullosa, epididymitis, glomerulonephritis, Graves' disease, Guillain-Barr syndrome, Hashimoto's disease, hemolytic anemia, systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, pemphigus vulgaris, psoriasis, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma, Sjogren's syndrome, spondyloarthropathies, thyroiditis, vasculitis, vitiligo, myxedema,
25 pernicious anemia, ulcerative colitis, among others.

As used herein, the term "autologous" is meant to refer to any material derived from the same individual to which it is later to be re-introduced into the individual.

"Allogeneic" refers to a graft derived from a different animal of the
30 same species.

"Xenogeneic" refers to a graft derived from an animal of a different species.

The term "cancer" as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or

through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the
5 like.

"Co-stimulatory ligand," as the term is used herein, includes a molecule on an antigen presenting cell (*e.g.*, an aAPC, dendritic cell, B cell, and the like) that specifically binds a cognate co-stimulatory molecule on a T cell, thereby providing a signal which, in addition to the primary signal provided by, for instance,
10 binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A co-stimulatory ligand can include, but is not limited to, CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM), CD30L,
15 CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, HVEM, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, *inter alia*, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as, but not limited to, CD27, CD28, 4-1BB, OX40,
20 CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds with CD83.

A "co-stimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the T cell, such as, but not limited to, proliferation. Co-
25 stimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor.

A "co-stimulatory signal", as used herein, refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to T cell proliferation and/or upregulation or downregulation of key molecules.

30 A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left

untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

An "effective amount" as used herein, means an amount which provides a therapeutic or prophylactic benefit.

5 "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom.
10 Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the
15 protein or other product of that gene or cDNA.

As used herein "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

As used herein, the term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

20 The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

"Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-
25 acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

30 "Homologous" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology

between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared X 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the
5 DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

The term "immunoglobulin" or "Ig," as used herein is defined as a class of proteins, which function as antibodies. Antibodies expressed by B cells are sometimes referred to as the BCR (B cell receptor) or antigen receptor. The five
10 members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions and mucus secretions of the respiratory and genitourinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune response in most subjects. It is the most efficient
15 immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is the immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is the immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

20 As used herein, an "instructional material" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compositions and methods of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the nucleic acid, peptide, and/or composition of the
25 invention or be shipped together with a container which contains the nucleic acid, peptide, and/or composition. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compound be used cooperatively by the recipient.

"Isolated" means altered or removed from the natural state. For
30 example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

In the context of the present invention, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine.

5 Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or an RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

10 A "lentivirus" as used herein refers to a genus of the Retroviridae family. Lentiviruses are unique among the retroviruses in being able to infect non-dividing cells; they can deliver a significant amount of genetic information into the DNA of the host cell, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses. Vectors derived from

15 lentiviruses offer the means to achieve significant levels of gene transfer in vivo.

By the term "modulating," as used herein, is meant mediating a detectable increase or decrease in the level of a response in a subject compared with the level of a response in the subject in the absence of a treatment or compound, and/or compared with the level of a response in an otherwise identical but untreated

20 subject. The term encompasses perturbing and/or affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, preferably, a human.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that

25 encode proteins and RNA may include introns.

The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a

30 functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

The term "overexpressed" tumor antigen or "overexpression" of the tumor antigen is intended to indicate an abnormal level of expression of the tumor antigen in a cell from a disease area like a solid tumor within a specific tissue or organ of the patient relative to the level of expression in a normal cell from that tissue or
5 organ. Patients having solid tumors or a hematological malignancy characterized by overexpression of the tumor antigen can be determined by standard assays known in the art.

"Parenteral" administration of an immunogenic composition includes, *e.g.*, subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal
10 injection, or infusion techniques.

The terms "patient," "subject," "individual," and the like are used interchangeably herein, and refer to any animal, or cells thereof whether in vitro or in situ, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is a human.

15 The term "polynucleotide" as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric "nucleotides." The monomeric nucleotides can be
20 hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

25 As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or
30 protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments,

substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

5 The term "promoter" as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably 10 linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

15 A "constitutive" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

An "inducible" promoter is a nucleotide sequence which, when 20 operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

A "tissue-specific" promoter is a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene 25 product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

By the term "specifically binds," as used herein with respect to an antibody, is meant an antibody which recognizes a specific antigen, but does not substantially recognize or bind other molecules in a sample. For example, an antibody 30 that specifically binds to an antigen from one species may also bind to that antigen from one or more species. But, such cross-species reactivity does not itself alter the classification of an antibody as specific. In another example, an antibody that specifically binds to an antigen may also bind to different allelic forms of the antigen. However, such cross reactivity does not itself alter the classification of an antibody as

specific. In some instances, the terms "specific binding" or "specifically binding," can be used in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, to mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical
5 species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

10 By the term "stimulation," is meant a primary response induced by binding of a stimulatory molecule (*e.g.*, a TCR/CD3 complex) with its cognate ligand thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex. Stimulation can mediate altered expression of certain molecules, such as downregulation of TGF-β, and/or reorganization of
15 cytoskeletal structures, and the like.

A "stimulatory molecule," as the term is used herein, means a molecule on a T cell that specifically binds with a cognate stimulatory ligand present on an antigen presenting cell.

A "stimulatory ligand," as used herein, means a ligand that when
20 present on an antigen presenting cell (*e.g.*, an aAPC, a dendritic cell, a B-cell, and the like) can specifically bind with a cognate binding partner (referred to herein as a "stimulatory molecule") on a T cell, thereby mediating a primary response by the T cell, including, but not limited to, activation, initiation of an immune response, proliferation, and the like. Stimulatory ligands are well-known in the art and
25 encompass, *inter alia*, an MHC Class I molecule loaded with a peptide, an anti-CD3 antibody, a superagonist anti-CD28 antibody, and a superagonist anti-CD2 antibody.

The term "subject" is intended to include living organisms in which an immune response can be elicited (*e.g.*, mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof.

30 As used herein, a "substantially purified" cell is a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers



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simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some embodiments, the cells are cultured *in vitro*. In other embodiments, the cells are not cultured *in vitro*.

The term "therapeutic" as used herein means a treatment and/or
5 prophylaxis. A therapeutic effect is obtained by suppression, remission, or eradication of a disease state.

The term "therapeutically effective amount" refers to the amount of the subject compound that will elicit the biological or medical response of a tissue, system, or subject that is being sought by the researcher, veterinarian, medical doctor
10 or other clinician. The term "therapeutically effective amount" includes that amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated. The therapeutically effective amount will vary depending on the compound, the disease and its severity and the age, weight, etc., of the subject to be
15 treated.

To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

The term "transfected" or "transformed" or "transduced" as used
20 herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

The phrase "under transcriptional control" or "operatively linked" as
25 used herein means that the promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.

A "vector" is a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior
30 of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for

example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, and the like.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Description

The present invention provides compositions and methods for treating cancer among other diseases. The cancer may be a hematological malignancy, a solid tumor, a primary or a metatastizing tumor. Preferably, the cancer is a hematological malignancy, and more preferably, the cancer is Chronic Lymphocytic Leukemia (CLL). Other diseases treatable using the compositions and methods of the invention include viral, bacterial and parasitic infections as well as autoimmune diseases.

In one embodiment, the invention provides a cell (e.g., T cell) engineered to express a CAR wherein the CAR T cell exhibits an antitumor property. The CAR of the invention can be engineered to comprise an extracellular domain having an antigen binding domain fused to an intracellular signaling domain of the T cell antigen receptor complex zeta chain (e.g., CD3 zeta). The CAR of the invention when expressed in a T cell is able to redirect antigen recognition based on the antigen binding specificity. An exemplary antigen is CD19 because this antigen is expressed on malignant B cells. However, the invention is not limited to targeting CD19. Rather, the invention includes any antigen binding moiety that when bound to its cognate antigen, affects a tumor cell so that the tumor cell fails to grow, is prompted to die, or otherwise is affected so that the tumor burden in a patient is diminished or eliminated. The antigen binding moiety is preferably fused with an intracellular domain from one or more of a costimulatory molecule and a zeta chain. Preferably,

the antigen binding moiety is fused with one or more intracellular domains selected from the group of a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD3zeta signal domain, and any combination thereof.

In one embodiment, the CAR of the invention comprises a CD137 (4-1BB) signaling domain. This is because the present invention is partly based on the discovery that CAR-mediated T-cell responses can be further enhanced with the addition of costimulatory domains. For example, inclusion of the CD137 (4-1BB) signaling domain significantly increased anti-tumor activity and *in vivo* persistence of CAR T cells compared to an otherwise identical CAR T cell not engineered to express CD137 (4-1BB).

Composition

The present invention provides chimeric antigen receptor (CAR) comprising an extracellular and intracellular domain. The extracellular domain comprises a target-specific binding element otherwise referred to as an antigen binding moiety. The intracellular domain or otherwise the cytoplasmic domain comprises, a costimulatory signaling region and a zeta chain portion. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigens receptors or their ligands that are required for an efficient response of lymphocytes to antigen.

Between the extracellular domain and the transmembrane domain of the CAR, or between the cytoplasmic domain and the transmembrane domain of the CAR, there may be incorporated a spacer domain. As used herein, the term "spacer domain" generally means any oligo- or polypeptide that functions to link the transmembrane domain to, either the extracellular domain or, the cytoplasmic domain in the polypeptide chain. A spacer domain may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids.

Antigen binding moiety

In one embodiment, the CAR of the invention comprises a target-specific binding element otherwise referred to as an antigen binding moiety. The choice of moiety depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize

a ligand that acts as a cell surface marker on target cells associated with a particular disease state. Thus examples of cell surface markers that may act as ligands for the antigen moiety domain in the CAR of the invention include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

5 In one embodiment, the CAR of the invention can be engineered to target a tumor antigen of interest by way of engineering a desired antigen binding moiety that specifically binds to an antigen on a tumor cell. In the context of the present invention, "tumor antigen" or "hyperporoliferative disorder antigen" or "antigen associated with a hyperproliferative disorder," refers to antigens that are
10 common to specific hyperproliferative disorders such as cancer. The antigens discussed herein are merely included by way of example. The list is not intended to be exclusive and further examples will be readily apparent to those of skill in the art.

Tumor antigens are proteins that are produced by tumor cells that elicit an immune response, particularly T-cell mediated immune responses. The selection
15 of the antigen binding moiety of the invention will depend on the particular type of cancer to be treated. Tumor antigens are well known in the art and include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), β-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS),
20 intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-1a, p53, prostein, PSMA, Her2/neu, survivin and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor and mesothelin.

25 In one embodiment, the tumor antigen comprises one or more antigenic cancer epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for an immune attack. These molecules include but are not limited to tissue-specific antigens such as MART-1, tyrosinase and GP 100 in melanoma and prostatic acid phosphatase (PAP)
30 and prostate-specific antigen (PSA) in prostate cancer. Other target molecules belong to the group of transformation-related molecules such as the oncogene HER-2/Neu/ErbB-2. Yet another group of target antigens are onco-fetal antigens such as carcinoembryonic antigen (CEA). In B-cell lymphoma the tumor-specific idiotype immunoglobulin constitutes a truly tumor-specific immunoglobulin antigen that is

unique to the individual tumor. B-cell differentiation antigens such as CD19, CD20 and CD37 are other candidates for target antigens in B-cell lymphoma. Some of these antigens (CEA, HER-2, CD19, CD20, idiotype) have been used as targets for passive immunotherapy with monoclonal antibodies with limited success.

5 The type of tumor antigen referred to in the invention may also be a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA associated antigen is not unique to a tumor cell and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen.
10 The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development when the immune system is immature and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells but which are expressed at much higher levels
15 on tumor cells.

Non-limiting examples of TSA or TAA antigens include the following: Differentiation antigens such as MART-1/MelanA (MART-I), gp100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, p15; overexpressed embryonic antigens such
20 as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER-2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4,
25 MAGE-5, MAGE-6, RAGE, NY-ESO, p185erbB2, p180erbB-3, c-met, nm-23H1, PSA, TAG-72, CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-
30 Ag, MOV18, NB/70K, NY-CO-1, RCAS1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

In a preferred embodiment, the antigen binding moiety portion of the CAR targets an antigen that includes but is not limited to CD19, CD20, CD22, ROR1,



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Mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, MY-ESO-1 TCR, MAGE A3 TCR, and the like.

Depending on the desired antigen to be targeted, the CAR of the invention can be engineered to include the appropriate antigen bind moiety that is specific to the desired antigen target. For example, if CD19 is the desired antigen that is to be targeted, an antibody for CD19 can be used as the antigen bind moiety for incorporation into the CAR of the invention.

In one embodiment, the antigen binding moiety portion of the CAR of the invention targets CD19. Preferably, the antigen binding moiety portion in the CAR of the invention is anti-CD19 scFV, wherein the nucleic acid sequence of the anti-CD19 scFV comprises the sequence set forth in SEQ ID: 14. In one embodiment, the anti-CD19 scFV comprise the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 20. In another embodiment, the anti-CD19 scFV portion of the CAR of the invention comprises the amino acid sequence set forth in SEQ ID NO: 20.

Transmembrane domain

With respect to the transmembrane domain, the CAR can be designed to comprise a transmembrane domain that is fused to the extracellular domain of the CAR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex.

The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention may be derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. Preferably a triplet of phenylalanine, tryptophan

and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. A glycine-serine doublet provides a
5 particularly suitable linker.

Preferably, the transmembrane domain in the CAR of the invention is the CD8 transmembrane domain. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence of SEQ ID NO: 16. In one embodiment, the CD8 transmembrane domain comprises the nucleic acid sequence that encodes the
10 amino acid sequence of SEQ ID NO: 22. In another embodiment, the CD8 transmembrane domain comprises the amino acid sequence of SEQ ID NO: 22.

In some instances, the transmembrane domain of the CAR of the invention comprises the CD8α hinge domain. In one embodiment, the CD8 hinge domain comprises the nucleic acid sequence of SEQ ID NO: 15. In one embodiment,
15 the CD8 hinge domain comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 21. In another embodiment, the CD8 hinge domain comprises the amino acid sequence of SEQ ID NO: 21.

Cytoplasmic domain

20 The cytoplasmic domain or otherwise the intracellular signaling domain of the CAR of the invention is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including
25 the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such
30 truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.



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Preferred examples of intracellular signaling domains for use in the CAR of the invention include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any
5 synthetic sequence that has the same functional capability.

It is known that signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-
10 dependent primary activation through the TCR (primary cytoplasmic signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary
15 cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the invention include those derived from TCR
20 zeta, FcR gamma, FcR beta, CD3 gamma , CD3 delta , CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. It is particularly preferred that cytoplasmic signaling molecule in the CAR of the invention comprises a cytoplasmic signaling sequence derived from CD3 zeta.

In a preferred embodiment, the cytoplasmic domain of the CAR can be
25 designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the CAR of the invention. For example, the cytoplasmic domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a
30 costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, and a ligand that specifically binds

with CD83, and the like. Thus, while the invention in exemplified primarily with 4-1BB as the co-stimulatory signaling element, other costimulatory elements are within the scope of the invention.

The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR of the invention may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. A glycine-serine doublet provides a particularly suitable linker.

In one embodiment, the cytoplasmic domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment, the cytoplasmic domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28 and 4-1BB.

In one embodiment, the cytoplasmic domain in the CAR of the invention is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the nucleic acid sequence set forth in SEQ ID NO: 17 and the signaling domain of CD3-zeta comprises the nucleic acid sequence set forth in SEQ ID NO: 18.

In one embodiment, the cytoplasmic domain in the CAR of the invention is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 23 and the signaling domain of CD3-zeta comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 24.

In one embodiment, the cytoplasmic domain in the CAR of the invention is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO: 23 and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 24.

Vectors

The present invention encompasses a DNA construct comprising sequences of a CAR, wherein the sequence comprises the nucleic acid sequence of an



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antigen binding moiety operably linked to the nucleic acid sequence of an intracellular domain. An exemplary intracellular domain that can be used in the CAR of the invention includes but is not limited to the intracellular domain of CD3-zeta, CD28, 4-1BB, and the like. In some instances, the CAR can comprise any combination of
5 CD3-zeta, CD28, 4-1BB, and the like.

In one embodiment, the CAR of the invention comprises anti-CD19 scFv, human CD8 hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains. In one embodiment, the CAR of the invention comprises the nucleic acid sequence set forth in SEQ ID NO: 8. In another embodiment, the CAR
10 of the invention comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 12. In another embodiment, the CAR of the invention comprises the amino acid sequence set forth in SEQ ID NO: 12.

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by
15 screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques. Alternatively, the gene of interest can be produced synthetically, rather than cloned.

The present invention also provides vectors in which a DNA of the
20 present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as
25 hepatocytes. They also have the added advantage of low immunogenicity.

In brief summary, the expression of natural or synthetic nucleic acids encoding CARs is typically achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration
30 eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery



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protocols. Methods for gene delivery are known in the art. *See, e.g.*, U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For
5 example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of
10 a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, and lentiviruses.
15 In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

A number of viral based systems have been developed for gene
20 transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments,
25 adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown
30 to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the

promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong
5 constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor -1α (EF-1α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human
10 immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the
15 use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a
20 metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and
25 selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example,
30 antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable

property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green
5 fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents
10 for the ability to modulate promoter- driven transcription.

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical,
15 chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example,
20 Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially
25 retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell
30 include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle).

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro, ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid
5 associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a
10 suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size
15 or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

20 Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from
25 Avanti Polar Lipids, Inc. (Birmingham, AL). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as
30 having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before

the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 Glycobiology 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist
5 as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a
10 variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the
15 scope of the invention.

Sources of T cells

Prior to expansion and genetic modification of the T cells of the invention, a source of T cells is obtained from a subject. T cells can be obtained from
20 a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, may be used. In certain embodiments of the present invention, T cells can be obtained from a unit of blood
25 collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred embodiment, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the
30 cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. Again, surprisingly, initial



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activation steps in the absence of calcium lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, $Ca^{2+}$-free, $Mg^{2+}$-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$T cells, can be further isolated by positive or negative selection techniques. For example, in one embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred embodiment, the time period is 10 to 24 hours. In one preferred embodiment, the incubation time period is 24 hours. For isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or



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against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain embodiments, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process.
5 "Unselected" cells can also be subjected to further rounds of selection.

Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal
10 antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it may be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, $CD62L^{hi}$, $GITR^+$, and
15 $FoxP3^+$. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection.

For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In certain embodiments, it may be desirable to significantly decrease the
20 volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15,
25 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of
30 cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, *etc.*). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

In a related embodiment, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (*e.g.*, particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be
5 bound to the particles. For example, $CD4^+$ T cells express higher levels of CD28 and are more efficiently captured than $CD8^+$ T cells in dilute concentrations. In one embodiment, the concentration of cells used is $5 \times 10^6$/ml. In other embodiments, the concentration used can be from about $1 \times 10^5$/ml to $1 \times 10^6$/ml, and any integer value in between.

10 In other embodiments, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature.

T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the
15 cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin
20 and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used
25 as well as uncontrolled freezing immediately at -20°C or in liquid nitrogen.

In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

Also contemplated in the context of the invention is the collection of
30 blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein.

In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In
5 certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to
10 treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228,
15 and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993). In a further embodiment, the cells are isolated for a patient and frozen for
20 later use in conjunction with (*e.g.*, before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative
25 therapy such as agents that react with CD20, *e.g.*, Rituxan.

In a further embodiment of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would
30 normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic

cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is
5 favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

Activation and Expansion of T Cells

10 Whether prior to or after genetic modification of the T cells to express a desirable CAR, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Patents 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent
15 Application Publication No. 20060121005.

Generally, the T cells of the invention are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations may be stimulated as described herein,
20 such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (e.g., bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted
25 with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besançon, France) can be used as can other methods commonly known in the art (Berg et al., Transplant
30 Proc. 30(8):3975-3977, 1998; Haanen et al., J. Exp. Med. 190(9):13191328, 1999; Garland et al., J. Immunol Meth. 227(1-2):53-63, 1999).

In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell may be provided by different protocols. For example, the agents providing each signal may be in solution or coupled to a surface. When



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coupled to a surface, the agents may be coupled to the same surface (*i.e.*, in "cis" formation) or to separate surfaces (*i.e.*, in "trans" formation). Alternatively, one agent may be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal is bound to a cell surface and the agent
5 providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents may be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example, U.S. Patent Application Publication Nos.
10 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

In one embodiment, the two agents are immobilized on beads, either on the same bead, *i.e.*, "cis," or to separate beads, *i.e.*, "trans." By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an
15 antigen-binding fragment thereof and the agent providing the co-stimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for $CD4^{+}$ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28
20 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular embodiment an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one embodiment, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all
25 integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, *i.e.*, the ratio of CD3:CD28 is less than one. In certain embodiments of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular embodiment, a 1:100 CD3:CD28 ratio of antibody bound to beads is used.
30 In another embodiment, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further embodiment, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In one preferred embodiment, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:3 CD3:CD28 ratio of antibody bound to the beads



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is used. In yet another embodiment, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

Ratios of particles to cells from 1:500 to 500:1 and any integer values in between may be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells may depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain preferred values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one preferred ratio being at least 1:1 particles per T cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular embodiment, a preferred particle: cell ratio is 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one embodiment, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular embodiment, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In another embodiment, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios may be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type.

In further embodiments of the present invention, the cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to

culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

5 By way of example, cell surface proteins may be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one embodiment the cells (for example, $10^4$ to $10^9$ T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1) are combined in a buffer, preferably PBS (without divalent cations such 10 as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration may be used. For example, the target cell may be very rare in the sample and comprise only 0.01% of the sample or the entire sample (*i.e.*, 100%) may comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain embodiments, it may be 15 desirable to significantly decrease the volume in which particles and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 20 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly 25 express target antigens of interest, such as CD28-negative T cells. Such populations of cells may have therapeutic value and would be desirable to obtain in certain embodiments. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

In one embodiment of the present invention, the mixture may be 30 cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture may be cultured for 21 days. In one embodiment of the invention the beads and the T cells are cultured together for about eight days. In another embodiment, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation may also be desired such that culture time of T

cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that may contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin,
5 IFN-γ, IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFβ, and TNF-α or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, α-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer,
10 with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The
15 target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C) and atmosphere (*e.g.*, air plus 5% $CO_2$).

T cells that have been exposed to varied stimulation times may exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population ($T_H$, $CD4^+$) that is greater
20 than the cytotoxic or suppressor T cell population ($T_C$, $CD8^+$). *Ex vivo* expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of $T_H$ cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of $T_C$ cells. Accordingly, depending on the purpose of treatment, infusing a subject with a T cell
25 population comprising predominately of $T_H$ cells may be advantageous. Similarly, if an antigen-specific subset of $T_C$ cells has been isolated it may be beneficial to expand this subset to a greater degree.

Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell
30 expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

<u>Therapeutic Application</u>



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The present invention encompasses a cell (e.g., T cell) transduced with a lentiviral vector (LV). For example, the LV encodes a CAR that combines an antigen recognition domain of a specific antibody with an intracellular domain of CD3-zeta, CD28, 4-1BB, or any combinations thereof. Therefore, in some instances, 5 the transduced T cell can elicit a CAR-mediated T-cell response.

The invention provides the use of a CAR to redirect the specificity of a primary T cell to a tumor antigen. Thus, the present invention also provides a method for stimulating a T cell-mediated immune response to a target cell population or tissue in a mammal comprising the step of administering to the mammal a T cell that 10 expresses a CAR, wherein the CAR comprises a binding moiety that specifically interacts with a predetermined target, a zeta chain portion comprising for example the intracellular domain of human CD3zeta, and a costimulatory signaling region.

In one embodiment, the present invention includes a type of cellular therapy where T cells are genetically modified to express a CAR and the CAR T cell 15 is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Unlike antibody therapies, CAR T cells are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

In one embodiment, the CAR T cells of the invention can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In 20 another embodiment, the CAR T cells of the invention evolve into specific memory T cells that can be reactivated to inhibit any additional tumor formation or growth. For example, it was unexpected that the CART19 cells of the invention can undergo robust *in vivo* T cell expansion and persist at high levels for an extended amount of time in blood and bone marrow and form specific memory T cells. Without wishing 25 to be bound by any particular theory, CAR T cells may differentiate *in vivo* into a central memory-like state upon encounter and subsequent elimination of target cells expressing the surrogate antigen.

Without wishing to be bound by any particular theory, the anti-tumor immunity response elicited by the CAR-modified T cells may be an active or a 30 passive immune response. In addition, the CAR mediated immune response may be part of an adoptive immunotherapy approach in which CAR-modified T cells induce an immune response specific to the antigen binding moiety in the CAR. For example, a CART19 cells elicits an immune response specific against cells expressing CD19.



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While the data disclosed herein specifically disclose lentiviral vector comprising anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains, the invention should be construed to include any number of
5 variations for each of the components of the construct as described elsewhere herein. That is, the invention includes the use of any antigen binding moiety in the CAR to generate a CAR-mediated T-cell response specific to the antigen binding moiety. For example, the antigen binding moiety in the CAR of the invention can target a tumor antigen for the purposes of treat cancer.

10 Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the CARs of the invention include, but are not limited to, carcinoma, blastoma, and
15 sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including
20 acute leukemias (such as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's
25 lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid areas. Solid tumors can be benign or malignant. Different types of
30 solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteosarcoma, and other sarcomas, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, lymphoid malignancy,



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pancreatic cancer, breast cancer, lung cancers, ovarian cancer, prostate cancer, hepatocellular carcinoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, medullary thyroid carcinoma, papillary thyroid carcinoma, pheochromocytomas sebaceous gland carcinoma, papillary 5 carcinoma, papillary adenocarcinomas, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, Wilms' tumor, cervical cancer, testicular tumor, seminoma, bladder carcinoma, melanoma, and CNS tumors (such as a glioma (such as brainstem glioma and mixed gliomas), glioblastoma (also known as glioblastoma multiforme) astrocytoma, CNS 10 lymphoma, germinoma, medulloblastoma, Schwannoma craniopharyogioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, neuroblastoma, retinoblastoma and brain metastases).

In one embodiment, the antigen bind moiety portion of the CAR of the invention is designed to treat a particular cancer. For example, the CAR designed to 15 target CD19 can be used to treat cancers and disorders including but are not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma, salvage post allogenic bone marrow transplantation, and the like.

In another embodiment, the CAR can be designed to target CD22 to treat diffuse large B-cell lymphoma.

20 In one embodiment, cancers and disorders include but are not limited to pre-B ALL (pediatric indication), adult ALL, mantle cell lymphoma, diffuse large B-cell lymphoma, salvage post allogenic bone marrow transplantation, and the like can be treated using a combination of CARs that target CD19, CD20, CD22, and ROR1.

25 In one embodiment, the CAR can be designed to target mesothelin to treat mesothelioma, pancreatic cancer, ovarian cancer, and the like.

In one embodiment, the CAR can be designed to target CD33/IL3Ra to treat acute myelogenous leukemia and the like.

In one embodiment, the CAR can be designed to target c-Met to treat 30 triple negative breast cancer, non-small cell lung cancer, and the like.

In one embodiment, the CAR can be designed to target PSMA to treat prostate cancer and the like.

In one embodiment, the CAR can be designed to target Glycolipid F77 to treat prostate cancer and the like.



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In one embodiment, the CAR can be designed to target EGFRvIII to treat gliobastoma and the like.

In one embodiment, the CAR can be designed to target GD-2 to treat neuroblastoma, melanoma, and the like.

5 In one embodiment, the CAR can be designed to target NY-ESO-1 TCR to treat myeloma, sarcoma, melanoma, and the like.

In one embodiment, the CAR can be designed to target MAGE A3 TCR to treat myeloma, sarcoma, melanoma, and the like.

However, the invention should not be construed to be limited to solely 10 to the antigen targets and diseases disclosed herein. Rather, the invention should be construed to include any antigenic target that is associated with a disease where a CAR can be used to treat the disease.

The CAR-modified T cells of the invention may also serve as a type of vaccine for *ex vivo* immunization and/or *in vivo* therapy in a mammal. Preferably, the 15 mammal is a human.

With respect to *ex vivo* immunization, at least one of the following occurs *in vitro* prior to administering the cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a CAR to the cells, and/or iii) cryopreservation of the cells.

20 *Ex vivo* procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (preferably a human) and genetically modified (i.e., transduced or transfected in vitro) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may 25 be a human and the CAR-modified cell can be autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

The procedure for *ex vivo* expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by 30 reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of *ex vivo* expansion of the cells. Briefly, *ex vivo* culture and expansion of T cells comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding

such cells *ex vivo*. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

In addition to using a cell-based vaccine in terms of *ex vivo*
5 immunization, the present invention also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

Generally, the cells activated and expanded as described herein may be utilized in the treatment and prevention of diseases that arise in individuals who are
10 immunocompromised. In particular, the CAR-modified T cells of the invention are used in the treatment of CCL. In certain embodiments, the cells of the invention are used in the treatment of patients at risk for developing CCL. Thus, the present invention provides methods for the treatment or prevention of CCL comprising administering to a subject in need thereof, a therapeutically effective amount of the
15 CAR-modified T cells of the invention.

The CAR-modified T cells of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components such as IL-2 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present invention may
20 comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine;
25 antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The
30 quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

When "an immunologically effective amount", "an anti-tumor effective amount", "an tumor-inhibiting effective amount", or "therapeutic amount" is

indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of $10^4$ to $10^9$ cells/kg body weight, preferably $10^5$ to $10^6$ cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg et al., New Eng. J. of Med. 319:1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

In certain embodiments, it may be desired to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain embodiments, T cells can be activated from blood draws of from 10cc to 400cc. In certain embodiments, T cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc. Not to be bound by theory, using this multiple blood draw/multiple reinfusion protocol may serve to select out certain populations of T cells.

The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (*i.v.*) injection, or intraperitoneally. In one embodiment, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In another embodiment, the T cell compositions of the present invention are preferably administered by *i.v.* injection. The compositions of T cells may be injected directly into a tumor, lymph node, or site of infection.

In certain embodiments of the present invention, cells activated and expanded using the methods described herein, or other methods known in the art where T cells are expanded to therapeutic levels, are administered to a patient in



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conjunction with (*e.g.*, before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients
5 or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAM PATH, anti-CD3 antibodies or other antibody therapies, cytoxin, fludaribine,
10 cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, cytokines, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-
15 773, 1993). In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (*e.g.*, before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another
20 embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded
25 immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed
30 according to art-accepted practices. The dose for CAMPATH, for example, will generally be in the range 1 to about 100 mg for an adult patient, usually administered daily for a period between 1 and 30 days. The preferred daily dose is 1 to 10 mg per day although in some instances larger doses of up to 40 mg per day may be used (described in U.S. Patent No. 6,120,766).

## EXPERIMENTAL EXAMPLES

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of
5 illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Without further description, it is believed that one of ordinary skill in
10 the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

15

### Example 1: T cells expressing chimeric receptors establish memory and potent antitumor effects in patients with advanced leukemia

Lymphocytes engineered to express chimeric antigen receptors (CARs) have demonstrated minimal *in vivo* expansion and antitumor effects in previous
20 clinical trials. The results presented herein demonstrate that that CAR T cells containing CD137 have potent non-cross resistant clinical activity following infusion in three of three patients treated with advanced chronic lymphocytic leukemia (CLL). The engineered T cells expanded more than a thousand-fold *in vivo*, trafficked to bone marrow and continued to express functional CARs at high levels for at least 6 months.
25 On average, each infused CAR+ T cell eradicated at least 1000 CLL cells. A CD19 specific immune response was demonstrated in the blood and bone marrow, accompanied by complete remission in two of three patients. A portion of the cells persist as memory CAR+ T cells, indicating the potential of this non-MHC restricted approach for the effective treatment of B cell malignancies.

30

The materials and methods employed in these experiments are now described.

### Materials and Methods



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General laboratory statement

Research sample processing, freezing, and laboratory analyses were performed in the Translational and Correlative Studies Laboratory at the University of Pennsylvania which operates under principles of Good Laboratory Practice with established SOP and/or protocols for sample receipt, processing, freezing, and analysis. Assay performance and data reporting conforms with MIATA guidelines (Janetzki et al., 2009, Immunity 31:527-528).

Protocol Design

The clinical trial (NCT01029366) was conducted as diagramed in Figure 1. Patients with CD19 positive hematologic malignancy with persistent disease following at least two prior treatment regimens and who were not eligible for allogeneic stem cell transplantation were eligible for the trial. Following tumor restaging, peripheral blood T cells for CART19 manufacturing were collected by apheresis and the subjects given a single course of chemotherapy as specified in Figure 10 during the week before infusion. CART19 cells were administered by intravenous infusion using a 3 day split dose regimen (10%, 30% and 60%) at the dose indicated in Figure 10 and if available, a second dose was administered on day 10; only patient UPN 02 had sufficient cells for a second infusion. Subjects were assessed for toxicity and response at frequent intervals for at least 6 months. The protocol was approved by the US Food and Drug Administration, the Recombinant DNA Advisory Committee and the Institutional Review Board of the University of Pennsylvania. The first day of infusion was set as study Day 0.

Subjects: clinical summary

The clinical summaries are outlined in Figure 10 and detailed histories are provided elsewhere herein. Patient UPN 01 was first diagnosed with stage II B cell CLL at age 55. The patient was asymptomatic and observed for approximately 1-1/2 years until requiring therapy for progressive lymphocytosis, thrombocytopenia, adenopathy, and splenomegaly. Over the course of time, the patient received prior lines of therapy. The most recent therapy was 2 cycles of pentostatin, cyclophosphamide and rituximab 2 months prior to CART19 cell infusion with a minimal response. The patient then received one cycle of bendamustine as

lymphodepleting chemotherapy prior to CART-19 cell infusion.

Patient UPN 02 was first diagnosed with CLL at age 68 when the patient was presented with fatigue and leukocytosis. The patient was relatively stable for 4 years when the patient developed progressive leukocytosis (195,000/μl), anemia
5 and thrombocytopenia requiring therapy. Karyotypic analysis showed that the CLL cells had deletion of chromosome 17p. Because of progressive disease, the patient was treated with alemtuzumab with a partial response but within one and a half years the patient had progressive disease. The patient was retreated with alemtuzumab for 18 weeks with a partial response and a 1 year progression free interval. The patient
10 then received 2 cycles of bendamustine with rituximab without a significant response (Figure 5A). The patient received single agent bendamustine as lymphodepleting chemotherapy prior to CART-19 cell infusion.

Patient UPN 03 presented at age 50 with asymptomatic stage I CLL and was followed with observation for years. The patient had progressive
15 leukocytosis (white blood count 92,000/μl) and progressive adenopathy requiring therapy. The patient received 2 cycles of rituximab with fludarabine that resulted in normalization of blood counts and significant improvement though not complete resolution in adenopathy. The patient had an approximately 3 year progression free interval. Karyotypic testing showed cells to contain deletion of chromosome 17p with
20 FISH demonstrating a *TP53* deletion in 170 of 200 cells. Over the next years the patient required 3 different lines of therapy (Figure 10) for progressive leukocytosis and adenopathy, last receiving alemtuzumab with a partial response 6 months prior CART19 cell infusion. The patient received pentostatin and cyclophosphamide as lymphodepleting chemotherapy prior to CART-19 cell infusion.

25

Vector Production

The CD19-BB-z transgene (GeMCRIS 0607-793) was designed and constructed as described (Milone et al., 2009, Mol Ther. 17:1453-1464). Lentiviral vector was produced according to current good manufacturing practices using a three-
30 plasmid production approach at Lentigen Corporation as described (Zufferey et al., 1997, Nature biotechnol 15:871-875).

Preparation of CART19 cell product

Methods of T cell preparation using paramagnetic polystyrene beads

coated with anti-CD3 and anti-CD28 monoclonal antibodies have been described (Laport et al., 2003, Blood 102: 2004-2013). Lentiviral transduction was performed as described (Levine et al., 2006, Proc Natl Acad Sci U S A 103:17372-17377).

5 Methods for tumor burden calculation

CLL burden at baseline was estimated as shown in Figure 10. The amount of CLL cells were calculated in bone marrow, blood, and secondary lymphoid tissues as described below.

*Bone marrow:* In healthy adults, the bone marrow represents
10 approximately 5% of total body weight (Woodard et al., 1960, Phys Med Biol, 5:57-59; Bigler et al., 1976, Health Phys 31:213-218). The bone marrow in iliac crest samples has an increasing percentage of inactive (fatty) marrow with age, rising from 20% of the total marrow at age 5 to about 50% by age 35, when it remains stable until age 65, and then rises to about 67% inactive marrow by age 75 (Hartsock et al., 1965,
15 Am J Clin Path 43:326-331). The international reference value for the total skeletal weight of active (red) and inactive (fatty) marrow for males at age 35 is currently set at 1170g and 2480g, respectively (Basic anatomical and physiological data for use in radiological protection: The Skeleton in Annals of the ICRP, Vol. 25 (ed. Smith, H.) 58-68 (A report of a Task Group of Committee 2 of the International Commission on
20 Radiological Protection, Oxford, 1995)). Adult males between ages 35 to 65 have marrow that represents 5.0% total of body weight, comprised of 1.6% as active (red) marrow and 3.4% as inactive (fatty) marrow (Basic anatomical and physiological data for use in radiological protection: The Skeleton in Annals of the ICRP, Vol. 25 (ed. Smith, H.) 58-68 (A report of a Task Group of Committee 2 of the International
25 Commission on Radiological Protection, Oxford, 1995)). Based on the bone marrow biopsy and aspirate specimens, the weight of CLL cells for the three patients at baseline was calculated as shown in the Table 1. These estimates of total CLL marrow mass were then converted to total CLL cell number in the marrow using 1Kg $= 10^{12}$ cells, and the resulting numbers are shown in Figure 10. These calculations are
30 based on the assumption that the CLL has a uniform distribution in the bone marrow. For patient UPN 01, calculations are shown for a marrow biopsy that was obtained before bendamustine chemotherapy, and for an aspirate obtained after bendamustine and pre-CART19 infusion. The numbers are less precise for the day-1 aspirate compared to the day -14 biopsy specimen due to technical limitations of the day-1



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aspirate. Patient UPN 02 had a single pre-treatment biopsy specimen showing complete replacement of marrow by CLL. This patient had an unchanged specimen on day 30 post CART19. The marrow burden for patient UPN 03 was calculated based on a post-chemotherapy and pre-CART19 biopsy.

5 Table 1: Marrow Mass

| | Wt of Active Marrow (kg) | Wt of Inactive Marrow (kg) | Total marrow (kg) |
|---|---|---|---|
| Normal males (ICRP reference standard) | 1.17 | 2.48 | 3.65 |
| UPN 01 day -14 (95% cellular) | 3.47 | 0.18 | 3.65 |
| UPN 02 day -47 (95% cellular) | 3.47 | 0.18 | 3.65 |
| UPN 03 day -1 (60% cellular) | 2.19 | 1.46 | 3.65 |
| | **Wt of CLL (kg)** | | |
| UPN 01 day -14 (70% CLL) | 2.43 | | |
| UPN 01 day -1 (50% CLL by clot) | 1.73 | | |
| UPN 02 day -47 (>95% CLL) | 3.29 | | |
| UPN 03 day -1 (40% CLL) | 0.88 | | |

*Blood:* Only patient UPN 02 had substantial CLL tumor burden in the blood pre-CART19 infusion. Flow cytometry showed that the cells had a typical phenotype as a clonal population with a dim surface kappa-restricted CD5+ CD10-
10 CD19+ CD20(dim)+ CD23(variable)+ IgM-B cell population. Approximately 35% of the CLL cells coexpressed CD38. The CLL burden did not clear with 3 cycles of bendamustine chemotherapy and was present at the time of CART19 infusions. At the time of CART19 infusion, the CLL count in blood was 55,000 cells/μL. Assuming a blood volume of 5.0 L, patient UPN 02 had $2.75 \times 10^{11}$ CLL cells in blood
15 on day 0. Given the normal overall WBC in patients UPN 01 and 03, the circulating disease burden in these patients was not calculated, which would lead to a slight underestimate of total body burden.

*Secondary lymphoid tissues*: The volume of lymphadenopathy and splenomegaly was quantified on axial CT scans using FDA-approved software. The
20 volumes are for chest, abdomen and pelvis only. Masses from the T1 vertebral body to the level of the bifurcation of the common femoral artery were measured in all patients, and in some, the nodes in the inguinal area were also included. Nodes in the head/neck and extremities were excluded from analysis and excluded from the



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baseline CLL target cell number, which would also lead to a slight underestimate of total body burden. Patients UPN 01 and 03 have had sustained complete remissions beyond 6 months, and thus the formula (baseline volume -month 3 volume) was used to determine the reduction in tumor burden from baseline; patient UPN 02 had stable
5 disease in adenopathy, and thus the baseline tumor mass is estimated by subtracting the reference splenic volume from age matched healthy males (Harris et al., 2010, Eur J Radiol 75:e97-e101). Baseline tumor mass was converted to CLL cells using a density approach (1 Kg/L density, and 1 Kg = 1012cells) cells or a volume approach (CLL cells are 10 μM diameter or 600 fL, assuming spherical shape), and both values
10 presented in Figure 10. The tumor volumes in secondary lymphoid tissues in the three patients are shown below in Table 2 as calculated from the available CT scans.

*Table 2: Tumor Volumes*

| Patient | Study Day | LN volume (mm3) | Spleen volume (mm3) | Total volume (mm3) |
|---|---|---|---|---|
| UPN 01 | -37 | 239655 | 1619180 | 1858835 |
| | 1 month | 105005 | 1258575 | 1363580 |
| | 3 month | 65060 | 1176625 | 1241685 |
| UPN 02 | -24 | 115990 | 1166800 | 1282790 |
| | 1 month | 111755 | 940960 | 1052715 |
| UPN 03 | -10 | 239160 | 435825 | 674985 |
| | 1 month | 111525 | 371200 | 482725 |
| | 3 month | 47245 | 299860 | 347105 |

The baseline CT scan for patient UPN 01 was performed 8 days after 2
15 cycles of pentostatin/ cyclophosphamide/ rituximab, and showed no response to this chemotherapy regimen compared to the previous CT scan. The patient had one cycle of bendamustine before CART19, and thus, the change in tumor volume from Day -37 to Day +31 for UPN 01 cannot exclude the potential contribution of the bendamustine as well as CART19. Similarly, the change in tumor volume for UPN
20 03 reflects the combined effect of 1 cycle of pentastatin/ cyclophosphamide and CART19.

<u>Method for estimating effective in vivo E:T ratio in patients</u>

The E:T ratio of infused CAR T cells to the number of tumor cells killed was calculated using the number of tumor cells present at the time of CAR T cell injection and the number of CAR T cells injected (Carpenito et al., 2009, Proc Natl Acad Sci U S A 106:3360-3365). For the present invention, the number of

5 CART19+ T cells injected as shown on Figure 10 was used because it is not possible to determine the absolute number of CART19+ T cells present *in vivo* with sufficient accuracy or precision. The available data on CART19 expansion in blood and marrow is robust as depicted in Figure 2 and Figure 6. However it was not possible to determine the trafficking of CART19 to other sites such as secondary lymphoid

10 tissues, creating substantial uncertainty on the total number of CART19 cells achieved *in vivo* at the time of maximal tumor reduction. The calculated values from Table 3 were used to derive the effective E:T ratios.

*Table 3: Calculated CART19 E:T ratios achieved in vivo*

| Patient | Tumor Burden (Baseline and Delta) | | | | CART19+ cells Infused | In Vivo E:T |
|---|---|---|---|---|---|---|
| | Bone marrow Baseline | Blood Baseline | Nodes/Spleen[1] Baseline | Total Change in CLL Burden | | |
| UPN 01 | 1.70E+12 | N/A | 8.1E+11 | 2.51E+12 | 1.13E+09 | 1:2200 |
| UPN 02 | 3.20E+12 | 2.75E+11 | 1.6E+12 | 2.74E+11[2] | 5.80E+08 | 1:1000 |
| UPN 03 | 8.80E+11 | N/A | 4.4E+11 | 1.32E+12 | 1.42E+07 | 1:93,000 |
| | | | | | Range | 1000 - 93,000 |

15

1 = average of density and volume method
2 = Patient UPN02 did not respond in bone marrow and had a partial reduction in adenopathy (3.1E+11 cells) in the tumor masses measured by CT in spleen and lymph nodes. See Figure 5A for response in blood.

20

Sample processing and freezing

Samples (peripheral blood, marrow) were collected in lavender top (K2EDTA,) or red top (no additive) vacutainer tubes (Becton Dickinson) and delivered to the TCSL within 2 hours of draw. Samples were processed within 30

25 minutes of receipt according to established laboratory SOP. Peripheral blood and marrow mononuclear cells were purified via Ficoll density gradient centrifugation using Ficoll-Paque (GE Health care, 17-1440-03) and frozen in RPMI (Gibco 11875-135) supplemented with 4% human serum albumin (Gemini Bio-Products, 800-120), 2% Hetastarch (Novaplus, NDC0409-7248-49), and 10% DMSO (Sigma, D2650)



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using 5100 Cryo 1° freezing containers; after 24-72 hours at -80° C, cells were transferred to liquid Nitrogen for long-term storage. Apheresis samples were obtained through the Hospital of the University of Pennsylvania Blood Bank and processed in the CVPF by Ficoll gradient purification and frozen as above. Viability immediately
5 post-thaw was greater than 85% when assessed. For serum isolation, samples were allowed to coagulate for 1.5-2 hours at room temperature; serum isolated by centrifugation, and single use 100 µl aliquots frozen at -80° C.

Cell lines

K562 (CML, CD19-negative) was obtained from ATCC (CCL-243).
10 K562/CD19, a generous gift of Carmine Carpenito, and is K562 lentivirally transduced at 100% frequency to express the CD19 molecule. NALM-6, a CD19-positive non-T, non-B ALL precursor B cell line (Hurwitz et al., 1979, Int J Cancer 23:174-180), and confirmed to express the CD19 antigen was a generous gift of Laurence Cooper. The above cell lines were maintained in R10 medium (RPMI 1640
15 (Gibco, 11875) supplemented with 10% fetal bovine serum (Hyclone), and 1% Pen-Strep (Gibco, 15140-122). Peripheral mononuclear cells (ND365) from a healthy donor were obtained by apheresis from the Human Immunology Core at the University of Pennsylvania, processed, and frozen as above.

20 DNA isolation and Q-PCR analysis

Whole-blood or marrow samples were collected in lavender top (K3EDTA) BD vacutainer tubes (Becton Dickinson). Genomic DNA was isolated directly from whole-blood using QIAamp DNA blood midi kits (Qiagen) and established laboratory SOP, quantified by spectrophotometer, and stored at -80°C. Q-
25 PCR analysis on genomic DNA samples was performed in bulk using 123-200 ng genomic DNA/time-point, ABI Taqman technology and a validated assay to detect the integrated CD19 CAR transgene sequence. Pass/fail parameter ranges, including standard curve slope and $r^2$ values, ability to accurately quantify a reference sample (1000 copies/plasmid spike) and no amplification in healthy donor DNA sample were
30 calculated from the qualification studies and pre-established acceptance ranges. Primer/probes for the CD19 CAR transgene were as described (Milone et al., 2009, Mol Ther 17:1453-1464). To determine copy number/unit DNA an 8-point standard curve was generated consisting of $10^6$-5 copies lentivirus plasmid spiked into 100 ng non-transduced control genomic DNA. Each data-point (samples, standard curve,

reference samples) was evaluated in triplicate with average values reported. For patient UPN 01, all reported values were derived from a positive Ct value in 3/3 replicates with % CV less than 0.46%. For patient UPN 02, with the exception of the day +177 sample (2/3 replicates positive, high % CV), all reported values were
5 derived from a positive Ct value in 3/3 replicates with % CV less than 0.72%. For patient UPN 03, with the exception of the day +1 sample (2/3 replicates positive, 0.8% CV) and the day +3 sample (2/3 replicates positive, 0.67% CV), all reported values were derived from a positive Ct value in 3/3 replicates with % CV less than 1.56%. The lower limit of quantification (LLOQ) for the assay was determined from
10 the standard curve at 2 copies/microgram DNA (10 copies/200 ng input DNA); average values below LLOQ (i.e. reportable not quantifiable) are considered approximate. A parallel amplification reaction to control for the quality of interrogated DNA was performed using 12-20 ng input genomic DNA, a primer/probe combination specific for non-transcribed genomic sequence upstream of the
15 CDKN1A gene (GENEBANK: Z85996 ) (sense primer: GAAAGCTGACTGCCCCTATTTG; SEQ ID NO. 25, antisense primer: GAGAGGAAGTGCTGGGAACAAT; SEQ ID NO. 26, probe: VIC- CTC CCC AGT CTC TTT; SEQ ID NO. 27), and an 8 point standard curve created by dilution of control genomic DNA; these amplification reactions produced a correction factor
20 (CF) (ng detected/ng input). Copies transgene /microgram DNA were calculated according to the formula: copies calculated from CD19 standard curve/input DNA (ng) x CF x 1000 ng. Accuracy of this assay was determined by the ability to quantify marking of the infused cell product by Q-PCR according to the formula: Average marking = detected copies/input DNA x 6.3 pg DNA/male somatic cell x CF versus
25 transgene positivity by flow cytometry using CAR-specific detection reagents. These blinded determinations generated 22.68% marking for the UPN 01infusion product (22.6% by flow cytometry), 32.33% marking for UPN 02 infusion product (23% by flow cytometry), and 4.3% marking for the UPN 03 infusion product (4.7% marking by flow cytometry).

30

Cytokine analyses

Quantification of soluble cytokine factors was performed using Luminex bead array technology and kits purchased from Life technologies (Invitrogen). Assays were performed as per the manufacturer protocol with an 8 point



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standard curve generated using a 3-fold dilution series. Each standard point and sample was evaluated in duplicate at 1:3 dilution; calculated % CV for the duplicate measures were less than 15%. Data were acquired on a Bioplex 200 and analyzed with Bioplex Manager version 5.0 software using 5-parameter logistic regression
5 analysis. Standard curve quantification ranges were determined by the 80-120% (observed/expected value) range. Individual analyte quantification ranges are reported in the Figure legends.

Cellular assay to detect CAR function

10 Cells were evaluated for functionality after thaw and overnight rest in TCM by measuring CD107 degranulation in response to target cells. Degranulation assays were performed using 1 x $10^6$PBMC and 0.25 x $10^6$ target cells in a final volume of 500 μl in 48-well plates for 2 hours at 37°C in the presence of CD49d (Becton Dickinson), anti-CD28, monensin (e-Bioscience) and CD107a-FITC antibody
15 (eBiosciences) essentially as described (Betts et al., 2003, J Immunol Methods 281:6578).

Antibody reagents

The following antibodies were used for these studies: MDA-CAR, a murine anti CD19 CAR antibody conjugated to Alexa647 was a generous gift of Drs.
20 Bipulendu Jena and Laurence Cooper (MD Anderson Cancer Center). For multi-parametric immunophenotyping and functional assays: anti-CD3-A700, anti-CD8-PE-Cy7, anti-PD-1-FITC anti-CD25-AF488, anti-CD28-PercP-Cy5.5, anti-CD57-eF450, anti-CD27-APC-eF780, anti-CD17-APC-eF780, anti-CD45RA-eF605NC, CD107a-FITC (all from e-Bioscience), anti-CD4-PE-Texas Red and Live/Dead Aqua (from
25 Life Technologies) and anti-CD14-V500, anti-CD16-V500 (from Becton Dickinson). For general immunophenotyping: CD3-PE, CD14-APC, CD14-PE-Cy7, CD16-FITC, CD16PE-Cy7, CD19-PE-Cy7, CD20-PE, all from Becton Dickinson.

Multi-parameter flow cytometry

30 Cells were evaluated by flow cytometry either fresh after Ficoll-Paque processing or, if frozen, after overnight rest at a density of 2 x $10^6$cells/ml in T cell medium (TCM) (X-vivo 15 (Lonza, 04-418Q) supplemented with 5% human AB serum (GemCall, 100-512), 1% Hepes (Gibco, 15630-080), 1% Pen-Strep (Gibco,

15140-122), 1% Glutamax (Gibco, 35050-061), and 0.2% N-Acetyl Cysteine (American Regent, NDC0517-7610-03). Multi-parametric immunophenotyping was performed on 4 x $10^6$ total cells/condition, using FMO stains as described in the text. Cells were stained at a density of 1 x $10^6$ cells/100 μl PBS for 30 minutes on ice using
5 antibody and reagent concentrations recommended by the manufacturer, washed, re-suspended in 0.5% paraformaldehyde and acquired using a modified LSRII (BD Immunocytometry systems) equipped with Blue (488 nm) Violet (405 nm), Green (532), and Red (633 nm) lasers and appropriate filter sets for the detection and separation of the above antibody combinations. A minimum of 100,000 CD3+ cells
10 were acquired) for each stain. For functional assays, cells were washed, stained for surface markers, re-suspended in 0.5% paraformaldehyde and acquired as above; a minimum of 50,000 CD3+ events were collected for each staining condition. Compensation values were established using single antibody stains and BD compensation beads (Becton Dickinson) and were calculated and applied
15 automatically by the instrument. Data were analyzed using FlowJo software (Version 8.8.4, Treestar). For general immunophenotyping cells were acquired using an Accuri C6 cytometer equipped with a Blue (488) and Red (633 nm) laser. Compensation values were established using single antibody stains and BD compensation beads (Becton Dickinson) and were calculated manually. Data were analyzed using C-Flow
20 software analysis package (version 1.0.264.9, Accuri cytometers).

Patient past medical histories and response to therapy

The clinical treatment summaries are outlined in Figure 10. Patient UPN 01 was first diagnosed with stage II B cell CLL at age 55. The patient was
25 asymptomatic and observed for approximately 1-1/2 years until requiring therapy for progressive lymphocytosis, thrombocytopenia, adenopathy, and splenomegaly. After 4 cycles of fludarabine the patient had complete normalization of blood counts and a complete response by CT scans. Progression was noted within 5 months with asymptomatic lymphocytosis, thrombocytopenia, and increasing adenopathy. The
30 patient was observed without symptoms for approximately 3 years, and later required treatment with Rituximab and fludarabine for progressive leukocytosis, anemia, and thrombocytopenia. The patient was treated with 4 cycles of rituximab with fludarabine with partial improvement in blood counts. The patient again had progression within one year requiring therapy manifested by leukocytosis (WBC



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150,000/µl) and thrombocytopenia (platelets 30,000/µl) and was treated with alemtuzumab with normalization of blood counts. Progression was noted within 13 months. The patient then received single agent rituximab without a significant response and followed by rituximab, cyclophosphamide, vincristine, and prednisone
5 (R-CVP) for 2 cycles with minimal response and followed by lenalidomide. Lenalidomide was discontinued because of toxicity. The patient received 2 cycles of pentostatin, cyclophosphamide and rituximab with a minimal response.

Later, the patient received bendamustine as lymphodepleting chemotherapy 4 days prior to CART19 cell infusion. Prior to therapy, WBC was
10 14,200/µl, hemoglobin 11.4 gm/dl, platelet count 78,000/µl and ALC was 8000/µl. The CT scan showed diffuse adenopathy and bone marrow was extensively infiltrated with CLL (67% of cells). The patient received 1.6 x $10^7$ CART-19 cells/kg (1.13 x $10^9$ total CART19 cells as assessed by FACS). There were no infusional toxicities. The patient became neutropenic approximately 10 days after bendamustine and 6 days
15 after CART19 cell infusions, and beginning 10 days after the first CART19 infusion, the patient developed fevers, rigors and transient hypotension. At the same time, a chest X-ray and CT scan demonstrated a left upper lobe pneumonia treated with antibiotics. The fevers persisted for approximately 2 weeks and resolved when there was neutrophil recovery. The patient has had no further infectious or constitutional
20 symptoms.

The patient achieved a rapid and complete response as depicted in Figure 5. Between 1 and 6 months after infusion no circulating CLL cells have been detected in the blood by flow cytometry. Bone marrow at 1, 3 and 6 months after CART-19 cell infusions shows sustained absence of the lymphocytic infiltrate by
25 morphology and flow cytometry testing. The CT scans at 1 and 3 months after infusion show complete resolution of abnormal adenopathy. The patient has had a persistent leukopenia (WBC 1000-3900/ul) and thrombocytopenia (platelets ~100,000/ul), and mild hypogammaglobulinia (IgG 525 mg/dL, normal 650-2000 mg/dL) but no infectious complications.

30 Patient UPN 02 was treated with CART19 cells at age 77. The patient had a relevant history of coronary artery disease and was first diagnosed with CLL in 2000 at age 68 when the patient presented with fatigue and leukocytosis. The patient was relatively stable for 4 years when the patient developed progressive leukocytosis (195,000/µl), anemia and thrombocytopenia requiring therapy. Genetic testing at that



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time showed that the CLL cells had deletion of chromosome 17p. Because of progressive disease, the patient was treated with a 12 week course of alemtuzumab with a partial response and improvement in blood counts. Within one and a half years the patient had progressive leukocytosis, anemia, thrombocytopenia, and splenomegaly. Karyotypic analysis confirmed deletion of chromosome 17p now with a deletion of chromosome 13q. The patient was retreated with alemtuzumab for 18 weeks with improvement of leukocytosis and stabilization of anemia and splenomegaly. The patient had evidence of progressive leukocytosis, anemia, and thrombocytopenia within one year. Treatment included 2 cycles of bendamustine with rituximab resulting in stable disease but no significant improvement as shown in Figure 5A.

The patient received bendamustine alone as lymphodepleting chemotherapy prior to CART-19 cell infusion. The patient received 4.3 x $10^6$ CART19 cells/kg (4.1 x $10^8$ total cells) in 3 split infusions complicated by transient fevers as high as 102° degrees for 24 hours. On day 11 after the first infusion, the patient received a boost of 4.1x$10^8$ (4.3x$10^6$/kg) CART19 cells and this infusion was complicated by fevers, rigors and shortness of breath without hypoxia requiring a 24 hour hospitalization. There was no evidence for cardiac ischemia, and the symptoms resolved. On day 15 after the first CART-19 infusion and day 4 after the boost CART19 cell infusion the patient was admitted to the hospital with high fevers (up to 104° F), chills and rigors. Extensive testing with blood and urine cultures and CXR failed to identify a source of infection. The patient complained of shortness of breath but had no hypoxia. An echocardiogram showed severe hypokinesis. Ejection fraction was 20%. The patient received prednisone 1 mg per kilogram for one day and 0.3 mg per kilogram for approximately one week. This resulted in rapid resolution of fevers and cardiac dysfunction.

Coincident with the onset of high fevers, the patient had a rapid drop in lymphocytes from peripheral blood as depicted in Figure 5A. Although the patient had normalization of white blood count, the patient had persistent circulating CLL, stable moderate anemia and thrombocytopenia. Bone marrow showed persistent extensive infiltration of CLL one month after therapy despite dramatic peripheral blood cytoreduction, and CT scans showed a partial reduction of adenopathy and splenomegaly. Five months after CART19 cell infusions the patient developed progressive lymphocytosis. Nine months after infusions the

patient has lymphocytosis (16,500/μl) with stable modest anemia and thrombocytopenia with stable adenopathy. The patient remains asymptomatic and has not had further therapy.

Patient UPN 03 was diagnosed with asymptomatic stage I CLL at
5 age 50 and was followed with observation for 6 years. Later, the patient had progressive leukocytosis (white blood count 92,000/μl) and progressive adenopathy requiring therapy. The patient received 2 cycles of rituximab with fludarabine that resulted in normalization of blood counts and significant improvement though not complete resolution in adenopathy. The patient had
10 approximately a 3 year progression free interval followed over the next 6 months by rapidly progressive leukocytosis (WBC 165,000/μl) and progressive adenopathy requiring therapy. The patient received one cycle of fludarabine and 3 cycles of rituximab with fludarabine with normalization of blood counts and resolution of palpable adenopathy. The patient had an approximate 20 month
15 progression free interval until the patient again developed rapidly progressing leukocytosis and adenopathy. At this time, bone marrow was extensively infiltrated with CLL and karyotypic analysis showed cells to contain deletion of chromosome 17p with FISH demonstrating a *TP53* deletion in 170/200 cells. The patient received one cycle of rituximab with bendamustine followed by 4 cycles of
20 bendamustine only (due to a severe allergic reaction to rituximab). The patient had initial normalization of blood counts but shortly after discontinuation of therapy had progressive leukocytosis and adenopathy.

Autologous T cells were collected by apheresis and cryopreserved from Patient UPN3. The patient was then treated with alemtuzumab for 11 weeks
25 through with an excellent hematologic response. There was improvement though not complete resolution in adenopathy. The patient had active but stable disease over the next 6 months. Later, the patient received pentostatin and cyclophosphamide as lymphodepleting chemotherapy prior to CART19 cell infusion.

30 Three days after chemotherapy but prior to cell infusion, the bone marrow was hypercellular (60%) with approximately 40% involvement by CLL. Because of manufacturing limitations inherent in apheresis collections from CLL patients as depicted in Table 3 and (Bonyhadi et al., 2005, J Immunol 174:2366-2375), the patient was infused with a total of 1.46 x $10^5$CART19+ cells per kg

($1.42 \times 10^7$ total CART19+ cells) over 3 days. There were no infusional toxicities. Fourteen days after the first infusion, the patient began having chills, fevers as high as 102° F, rigors, nausea and diarrhea treated symptomatically. The patient had no respiratory or cardiac symptoms. By day 22 after infusion, a tumor lysis syndrome
5 was diagnosed manifested by an elevated LDH, uric acid, and complicated by renal insufficiency. The patient was hospitalized and treated with fluid resuscitation and rasburicase with rapid normalization of uric acid and renal function. A detailed clinical evaluation with a CXR, blood, urine, and stool cultures were performed and were all negative or normal.

10 Within 1 month of CART-19 infusions, the patient had clearance of circulating CLL from the blood and bone marrow by morphology, flow cytometry, cytogenetic, and FISH analysis and CT scans showed resolution of abnormal adenopathy (Figure 5C). The patient's remission has been sustained beyond 8 months from the initial CART19 cell infusion.

15 The results of the experiments are now described.

Clinical protocol

Three patients with advanced, chemotherapy-resistant CLL were enrolled on a pilot clinical trial as depicted in Figure 1. All patients were extensively
20 pretreated with various chemotherapy and biologic regimens as shown in Figure 10. Two of the patients had p53 deficient CLL, a deletion that portends poor response to conventional therapy and rapid progression (Dohner et al., 1995, Blood, 851580-1589). Each of the patients had large tumor burdens following the preparative chemotherapy, including extensive marrow infiltration (40 to 95%) and
25 lymphadenopathy; patient UPN 02 also had significant peripheral lymphocytosis. The CART19 T cells were prepared as depicted in Figure 1B and details of the cell manufacturing and product characterization for each patient are shown in Table 4. All patients were pretreated 1-4 days before CART19 T cell infusions with lymphodepleting chemotherapy. A split dose cell infusion schedule was used because
30 the trial testing a CAR incorporating a 4-1BB costimulatory signaling domain as depicted in Figure 1A.

*Table 4: Apheresis products and CART19 product release criteria*



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| | Assay | Specification | UPN 01 | UPN 02 | UPN 03 |
|---|---|---|---|---|---|
| **Apheresis Product** | | | | | |
| | Flow Cytometry For CD3+ of CD45+ | N/A | 4.46% | 2.29% | 2.67% |
| **CART19 Product** | | | | | |
| | Total Cell Number Infused | ~2-5 x $10^9$ | 5 x $10^8$ | 1.275 x $10^9$<br>1.275 x $10^9$<br>[2.55 x $10^9$ total] | 3 x $10^8$ |
| | Cell Viability | >= 70% | 96.2% | 95.3 (90.5)[1] | 90.3 |
| | % CD3+ Cells | >= 80% | 88.9% | 98.8 | 98.9 |
| | Residual Bead # | <= 100 beads / 3 x $10^6$ Cells | 3.95 | 1 | 4 |
| | Endotoxin | <= 3.5 EU/mL | <0.5 EU/mL | <0.5 EU/mL | <0.5 EU/mL |
| | Mycoplasma | Negative | Negative | Negative | Negative |
| | Sterility (Bactec) | No Growth | No Growth | No Growth | No Growth |
| | Fungal Culture | No Growth | No Growth | No Growth | No Growth |
| | BSA ELISA | <= 1 ug/mL | <0.5 ng/mL | <0.5 ng/mL | <0.5 ng/mL |
| | Replication Competent Lentivirus (RCL) | RCL Not Detectable | Not Detectable | Inconclusive[2] | Inconclusive[2] |
| | Transduction Efficiency (scFv Expression) | >= 20% | 22.6% | 23% | 4.74%[4] |
| | Vector DNA Sequence (CART19 PCR) | 0.2 - 3 copies/cell | 0.15[3] | 0.275 | 0.101 |

1 = Dose #2.
2 = Assay value at Day 12 below LOQ and had been decreasing from earlier in expansion consistent with carryover of plasmid DNA from vector generation.
5 Submitted to the FDA as an informational amendment.
3 = Product release based on surface staining by FACS.
4 = Treatment exception granted for release criteria by external DSMC and IRB.

*In vivo* expansion and persistence of CART19 and trafficking to bone marrow

10 CAR+ T cells expanded using CD3/CD28 beads and expressing a 4-1BB signaling domain is believed to be in improvement to CARs lacking 4-1BB. A Q-PCR assay was developed to enable quantitative tracking of CART19 cells in blood and bone marrow. All patients had expansion and persistence of the CART19-cells in blood for at least 6 months as depicted in Figures 2A and 2C. Notably, patients UPN
15 01 and UPN 03 had a 1,000 to 10,000 fold expansion of CAR+ T cells in blood during the first month post infusion. The peak expansion levels coincided with onset of the post-infusion clinical symptoms in patient UPN 01 (day 15) and patient UPN 03 (day 23). Furthermore, following an initial decay that can be modeled with first order kinetics, the CART19 T cell levels stabilized in all 3 patients from day 90 to 180 post

infusion. Significantly, the CART19 T cells also trafficked to bone marrow in all patients, albeit at 5-to 10-fold lower levels than observed in blood as depicted in Figures 2D through 2F. Patients UPN 01 and 03 had a log linear decay in the marrow, with a disappearance T½ of ~35 days.

5 Induction of specific immune responses in the blood and bone marrow compartments following CART19 infusion

Serum samples from all patients were collected and batch analyzed to quantitatively determine cytokine levels, assessing a panel of cytokines, chemokines, and other soluble factors to assess potential toxicities and to provide

10 evidence of CART19 cell function as depicted in Figure 3. Of thirty analytes tested, eleven had a 3-fold or more change from baseline, including 4 cytokines (IL-6, INF-γ, IL-8 and IL-10), 5 chemokines (MIP-1α, MIP-1β, MCP-1, CXCL9, CXCL10) and soluble receptors for IL-1Rα and IL-2Rα. Of these, interferon-γ had the largest relative change from baseline. Interestingly, the peak time of cytokine elevation in

15 UPN 01 and UPN 03 correlated temporally with the previously described clinical symptoms and the peak levels of CART19 cells in the blood in each patient. Only modest changes were noted in patient UPN 02, perhaps as a result of corticosteroid treatment given to this patient. Elevation of soluble IL-2 was not detected in the serum of the patients, even though one of the pre-clinical rationales for developing

20 CAR+ T cells with 4-1BB signaling domains was the reduced propensity to trigger IL-2 secretion compared to CD28 signaling domains (Milone et al., 2009, Mol Ther. 17:1453-1464). This may be relevant to sustained clinical activity as previous studies have shown that CAR+ T cells are potentially suppressed by regulatory T cells (Lee et al., 2011, Cancer Res 71:2871-2881), cells that could be elicited by

25 CARs that secrete substantial amounts of IL-2 or by the provision of exogenous IL-2 post-infusion. Finally, a robust induction of cytokine secretion in the supernatants from bone marrow aspirates of UPN 03 was observed as depicted in Figure 3D that also coincided with the development of tumor lysis syndrome and complete remission.

30

Prolonged expression and establishment of a population of memory CART19 cells in blood

A central question in CAR-mediated cancer immunotherapy is

whether optimized cell manufacturing and costimulation domains enhance the persistence of genetically modified T cells and permit the establishment of CAR+ memory T cells in patients. Previous studies have not demonstrated robust expansion, prolonged persistence and/or expression of CARs on T cells after
5 infusion (Kershaw et al., 2006, Clin Cancer Res 12:6106-6115; Lamers et al., 2006, J Clin Oncol 24:e20-e22; Till et al., 2008, Blood, 112, 2261-2271; Savoldo et al., 2011, J Clin Invest doi:10.1172/JCI46110). Flow-cytometric analysis of samples from both blood and marrow at 169 days post infusion revealed the presence of CAR19 expressing cells in UPN 03 (Figures 4A and 4B), and an absence of B cells
10 as depicted in Figure 4A. Notably, by Q-PCR assay, all three patients have persisting CAR+ cells at 4 months and beyond as depicted in Figures 2 and Figures 6. The *in vivo* frequency of CAR+ cells by flow cytometry closely matched the values obtained from the PCR assay for the CART19 transgene. Importantly, in patient UPN 03, only CD3+ cells expressed the CAR19, as CAR19+ cells were not
15 detectable in CD16-or CD14-positive subsets as depicted in Figure 4A. CAR expression was also detected on the surface of 4.2% of T cells in the blood of patient UPN 01 on day 71 post infusion as depicted in Figure 7.

Next, polychromatic flow cytometry was used to perform detailed studies to further characterize the expression, phenotype, and function of CART19
20 cells in UPN 03 using an anti-CAR idiotype antibody (MDA-647) and a gating strategy shown in Figure 8. Notable differences in the expression of memory and activation markers in both CD8+ and CD4+ cells based on CAR19 expression was observed. At day 56, CART19 CD8+ cells displayed primarily an effector memory phenotype (CCR7-CD27-CD28-) consistent with prolonged and robust exposure to
25 antigen as depicted in Figure 4C. In contrast, CAR-negative CD8+ cells consisted of mixtures of effector and central memory cells, with CCR7 expression in a subset of cells, and substantial numbers in the CD27+/CD28-and CD27+/CD28+ fractions. While both CART19 and CAR-negative cell populations substantially expressed CD57, this molecule was uniformly co-expressed with PD-1 in the CART19 cells, a
30 possible reflection of the extensive replicative history of these cells. In contrast to the CAR-negative cell population, the entirety of the CART19 CD8+ population lacked expression of both CD25 and CD127. By day 169, while the phenotype of the CAR-negative cell population remained similar to the day 56 sample, the CART19 population had evolved to contain a minority population with features of

central memory cells, notably expression of CCR7, higher levels of CD27 and CD28, as well as CAR+ cells that were PD-1-negative, CD57-negative and CD127-positive.

5 In the CD4+ compartment, at day 56 CART19 cells were characterized by uniform lack of CCR7 and a predominance of CD27+/CD28+/PD-1+ cells distributed within both CD57+ and -compartments, and an essential absence of CD25 and CD127 expression as depicted in Figure 4B. In contrast, CAR-negative cells at this time-point were heterogeneous in CCR7, CD27 and PD-1 expression, expressed CD127 and also contained a substantial CD25+/CD127-

10 (potential regulatory T cell) population. By day 169, while CD28 expression remained uniformly positive in all CAR+CD4+ cells, a fraction of the CART19 CD4+ cells had evolved toward a central memory phenotype with expression of CCR7, a higher percentage of CD27-cells, the appearance of a PD-1-negative subset, and acquisition of CD127 expression. CAR-negative cells remained

15 reasonably consistent with their day 56 counterparts, with the exception of a reduction in CD27 expression a decrease in the percentage of CD25+/CD127-cells.

CART19 cells can retain effector function after 6 months in blood

In addition to short persistence and inadequate in vivo proliferation, a

20 limitation of previous trials with CAR+ T cells has been the rapid loss of functional activity of the infused T cells *in vivo*. The high level CART19 cell persistence and surface expression of the CAR19 molecule in patient UPN 01 and 03 provided the opportunity to directly test anti-CD19-specific effector functions in cells recovered from cryopreserved peripheral blood samples. PBMC from patient UPN 03 were

25 cultured with target cells that were either positive or negative for CD19 expression (Figure 4d). Robust CD19-specific effector function of CART19 T cells was demonstrated by specific degranulation against CD19-positive but not CD19-negative target cells, as assessed by surface CD107a expression. Notably, exposure of the CART19 population to CD19-positive targets induced a rapid internalization

30 of surface CAR-19 as depicted in Figure 8 for surface expression of CAR19 in the same effector cells in standard flow-cytometric staining. The presence of costimulatory molecules on target cells was not required for triggering CART19 cell degranulation because the NALM-6 line does not express CD80 or CD86 (Brentjens et al., 2007, Clin Cancer Res 13:5426-5435). Effector function was evident at day

56 post infusion and was retained at the day 169 time-point. Robust effector function of CAR+ and CAR-T cells could also be demonstrated by pharmacologic stimulation.

5 Clinical activity of CART19 cells

There were no significant toxicities observed during the four days following the infusion in any patient, other than transient febrile reactions. However, all patients subsequently developed significant clinical and laboratory toxicities between day 7 and 21 following the first infusion. These toxicities were
10 short-term and reversible. Of the three patients treated to date, there are 2 CRs and 1 PR at >6 months post CART19 infusion according to standard criteria (Hallek et al., 2008, Blood 111:5446). Details of past medical history and response to therapy for each patient are depicted in Figure 10.

In brief, patient UPN 01 developed a febrile syndrome, with rigors
15 and transient hypotension beginning 10 days after infusion. The fevers persisted for approximately 2 weeks and resolved; the patient has had no further constitutional symptoms. The patient achieved a rapid and complete response as depicted in Figure 5. Between 1 and 6 months after infusion, no circulating CLL cells have been detected in the blood by flow cytometry. Bone marrow at 1, 3, and 6 months
20 after CART19 cell infusions shows sustained absence of the lymphocytic infiltrate by morphology and flow cytometric analysis as depicted in Figure 5B. CT scans at 1 and 3 months after infusion show resolution of adenopathy as depicted in Figure 5C. Complete remission was sustained for 10+ months at the time of this report.

Patient UPN 02 was treated with 2 cycles of bendamustine with
25 rituximab resulting in stable disease as depicted in Figure 5A. The patient received a third dose of bendamustine as lymphodepleting chemotherapy prior to CART19 T cell infusion. The patient developed fevers to 40°C, rigors and dyspnea requiring a 24 hour hospitalization on day 11 after the first infusion and on the day of the second CART19 cell boost. Fevers and constitutional symptoms persisted and on
30 day 15, the patient had transient cardiac dysfunction; all symptoms resolved after corticosteroid therapy was initiated on day 18. Following CART19 infusion, and coincident with the onset of high fevers, the patient had rapid clearance of the p53-deficient CLL cells from peripheral blood as depicted in Figure 5A and a partial reduction of adenopathy, bone marrow showed persistent extensive infiltration of

CLL one month after therapy despite dramatic peripheral blood cytoreduction. The patient remains asymptomatic.

Patient UPN 03 received pentostatin and cyclophosphamide as lymphodepleting chemotherapy prior to CART19 cell infusion. Three days after chemotherapy but prior to cell infusion, bone marrow was hypercellular (60%) with approximately 50% involvement by CLL. The patient received a low dose of CART19 cells ($1.5x10^5$ CAR+ T cells/kg divided over 3 days). Again, there were no acute infusional toxicities. However, 14 days after the first infusion, the patient began having rigors, fevers, nausea and diarrhea. By day 22 after infusion, tumor lysis syndrome was diagnosed requiring hospitalization. The patient had resolution of constitutional symptoms, and within 1 month of CART19 infusions, the patient had clearance of circulating CLL from the blood and bone marrow by morphology, flow cytometry, cytogenetic, and FISH analysis. CT scans showed resolution of abnormal adenopathy as depicted in Figures 5B and 5C. Complete remission was sustained beyond 8 months from the initial CART19 cell infusion.

Considerations of *in vivo* CART19 effector to CLL target cell ratio

Pre-clinical studies showed that large tumors could be ablated, and that the infusion of $2.2x10^7$CARs could eradicate tumors comprised of $1x10^9$ cells, for an *in vivo* E:T ratio of 1:42 in humanized mice (Carpenito et al., 2009, Proc Natl Acad Sci U S A 106:3360-3365), although these calculations did not take into account the expansion of T cells after injection. Estimation of CLL tumor burden over time permitted the calculation of tumor reduction and the estimated CART19 E:T ratios achieved *in vivo* in the three subjects based on number of CAR+ T cells infused. Tumor burdens were calculated by measuring CLL load in bone marrow, blood and secondary lymphoid tissues. The baseline tumor burdens as shown in Figure 10 indicate that each patient had on the order of $10^{12}$ CLL cells (i.e. 1 kilogram tumor load) before CART19 infusion. Patient UPN 03 had an estimated baseline tumor burden of $8.8x10^{11}$ CLL cells in the bone marrow on day -1 (i.e. post chemotherapy and pre-CART19 infusion), and a measured tumor mass in secondary lymphoid tissues of 3.3 -$5.5x10^{11}$CLL cells, depending on the method of volumetric CT scan analysis. Given that UPN 03 was infused with only $1.4x10^7$CART19 cells, using the estimate of initial total tumor burden ($1.3x10^{12}$ CLL cells), and that no CLL cells are detectable post treatment, a striking 1:93,000 E:T ratio was achieved.

By similar calculations, an effective E:T ratio *in vivo* of 1:2200 and 1:1000 was calculated for UPN 01 and UPN 02 as shown in Table 3). In the end, a contribution of serial killing by CART19 T cells, combined with *in vivo* CART19 expansion of >1,000-fold likely contributed to the powerful anti-leukemic effects mediated by
5 CART19 cells.

T cells expressing chimeric receptors establish memory and potent antitumor effects in patients with advanced leukemia

Limited *in vivo* expression and effector function of CARs has been a
10 central limitation in the trials testing first generation CARs (Kershaw et al., 2006, Clin Cancer Res 12:6106-6115; Lamers et al., 2006, J Clin Oncol 24:e20-e22; Till et al., 2008, Blood, 112, 2261-2271; Park et al., 2007, Mol Ther 15:825833; Pule et al., 2008, Nat Med 14:1264-1270). Based on pre-clinical modeling demonstrating enhanced persistence of CARs containing a 4-1BB signaling module (Milone et al.,
15 2009, Mol Ther. 17:1453-1464; Carpenito et al., 2009, Proc Natl Acad Sci U S A 106:3360-3365), experiments were designed to develop a second generation of CARs engineered with lentiviral vector technology. This second generation of CARs was found to be safe in the setting of chronic HIV infection (Levine et al., 2006, Proc Natl Acad Sci U S A 103:17372-17377). The present results show that when this second
20 generation CAR was expressed in T cells and cultured under conditions designed to promote engraftment of central memory T cells (Rapoport et al., 2005, Nat Med 11:1230-1237; Bondanza et al., 2006, Blood 107:1828-1836), improved expansion of CAR T cells after infusion was observed compared to previous reports. CART19 cells established CD19-specific cellular memory, and killed tumor cells at E:T ratios
25 *in vivo* not previously achieved.

CART19 is the first CAR trial to incorporate a 4-1BB signaling domain and the first to use lentiviral vector technology. The present results demonstrate efficient tracking of CARs to sites of tumor, with the *de facto* establishment of "tumor infiltrating lymphocytes" that exhibited CD19 specificity.
30 The pronounced *in vivo* expansion permitted the first demonstration that CARs directly recovered from patients can retain effector function *in vivo* for months. A previous study had suggested that introduction of a first generation CAR into virus specific T cells is preferable to primary T cells (Pule et al., 2008, Nat Med 14:1264-1270), however the results with second generation CARs introduced into optimally



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costimulated primary T cells calls this notion into question. Without wishing to be bound by any particular theory, a cautionary note is raised that the clinical effects were profound and unprecedented with the lysis of kilogram sized tumor burdens in all three patients accompanied with the delayed release of potentially dangerously
5 high levels of cytokines in two of the patients. Classical cytokine storm effects were not observed. However, the present study was designed to mitigate this possibility by deliberate infusion of CART19 over a period of three days.

It was found that very low doses of CARs can elicit potent clinical responses. This was a pilot study that demonstrated safety of the CART19 vector
10 design. The observation that doses of CART19 cells several orders of magnitude below those tested in previous trials can have clinical benefit may have important implications for future implementation of CAR therapy on a wider scale, and for the design of trials testing CARs directed against targets other than CD19.

The present studies further indicate that CART19 is expressed in
15 both central memory and effector T cells, and this likely contributes to their long term survival compared to previous reports. Without wishing to be bound by any particular theory, CAR T cells may differentiate *in vivo* into a central memory-like state upon encounter and subsequent elimination of target cells (e.g. CLL tumor cells or normal B cells) expressing the surrogate antigen. Indeed signaling of 4-1BB
20 has been reported to promote the development of memory in the context of TCR signaling (Sabbagh et al., 2007, Trends Immunol 28:333-339).

The extended proliferation and survival of CART19 has revealed aspects of the pharmacokinetics of CAR T cells that have not previously been reported. It was observed that the kinetics of cytokine release in serum and marrow
25 correlated with peak CART19 levels, so that it is possible that the decay is initiated when cellular targets expressing CD19 become limiting. The mechanism of the extended survival of CART19 may relate to the aforementioned incorporation of the 4-1BB domain or to signaling through the natural TCR and/or CAR. An intriguing possibility is that the extended survival is related to the population of CART19 that
30 has been identified in marrow specimens, raising the hypothesis that CD19 CARs could be maintained by encounter with B cell progenitors in the bone marrow. Related to this question is what drives the initial expansion of CART19 cells *in vivo*? With rare exceptions (Savoldo et al., 2011, J Clin Invest doi:10.1172/JCI46110; Pule et al., 2008, Nat Med 14:1264-1270), the present study

is the only trial to have omitted IL-2 infusions, so that the CART19 cells likely either expanded in response to homeostatic cytokines or more likely, to CD19 expressed on leukemic targets and/or normal B cells. In the latter case, this could be an attractive feature for CARs directed against targets on normal APCs such as
5 CD19 and CD20, as it is possible that self renewal of CART19 occurs on the normal cells, providing a mechanism for CAR memory by means of "self vaccination/boosting" and therefore, long term tumor immunosurveillance. The mechanisms of CART19 homeostasis may require further study to elucidate cell intrinsic and extrinsic mechanisms of persistence. Previous to these results, most
10 investigators have viewed CAR therapy as a transient form of immunotherapy, however CARs with optimized signaling domains may have a role in both remission induction and consolidation as well as for long term immunosurveillance.

Potent anti-leukemic effects have been observed in all three patients, including two patients with p53 deficient leukemia. Previous studies with CARs
15 have had difficulty separating antitumor effects from lymphodepleting chemotherapy. However, the delayed cytokine release combined with the kinetics of tumor lysis in fludarabine-refractory patients that was coincident, and possibly dependent on *in vivo* CAR expansion in the present study, indicate that CART19 mediates potent antitumor effects. The present results do not exclude a role for
20 chemotherapy in potentiating the effects of CARs.

A thorough comparison of the vector, transgene and cell manufacturing procedures with results from ongoing studies at other centers may be required to gain a full understanding of the key features required to obtain sustained function of CAR T cells *in vivo*. Unlike antibody therapies, CAR-modified T cells
25 have the potential to replicate *in vivo*, and long-term persistence could lead to sustained tumor control. The availability of an off the shelf therapy comprised of non-cross resistant killer T cells has the potential to improve the outcome of patients with B cell malignancies. A limitation of antibody therapy, as for example, with agents such as rituximab and bevicizumab, is that the therapy requires repeated
30 antibody infusions, that is inconvenient and costly. The delivery of prolonged antibody therapy (in this case for at least 6 months in 3 of 3 patients treated to date) with anti-CD19 scFv expressed on T cells following a single infusion of CART19 cells has a number of practical advantages, including conveniences and cost savings.



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Example 2: Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia

A lentiviral vector expressing a chimeric antigen receptor with
5 specificity for the B-cell antigen CD19, coupled with CD137 (a costimulatory receptor in T cells [4-1BB]) and CD3-zeta (a signal-transduction component of the T-cell antigen receptor) signaling domains, was designed. It was observed that a low dose (approximately $1.5\times10^5$ cells per kilogram of body weight) of autologous chimeric antigen receptor–modified T cells reinfused into a patient with refractory
10 chronic lymphocytic leukemia (CLL) expanded to a level that was more than 1000 times as high as the initial engraftment level *in vivo*. It was also observed that the patient exhibited delayed development of the tumor lysis syndrome and with complete remission.

Apart from the tumor lysis syndrome, the only other grade 3/4 toxic
15 effect related to chimeric antigen receptor T cells was lymphopenia. Engineered cells persisted at high levels for at least 6 months in the blood and bone marrow and continued to express the chimeric antigen receptor. A specific immune response was detected in the bone marrow, accompanied by loss of normal B cells and leukemia cells that express CD19. Remission was ongoing 10 months after treatment.
20 Hypogammaglobulinemia was an expected chronic toxic effect.

The materials and methods employed in these experiments are now described.

25 Materials and Methods

Study Procedures

A self-inactivating lentiviral vector (GeMCRIS 0607-793) was designed, which was subjected to preclinical safety testing, as reported previously (Milone et al., 2009, Mol Ther, 17: 1453-64). Methods of T-cell preparation have also
30 been described previously (Porter et al, 2006, Blood, 107:1325-31). Quantitative polymerase-chain-reaction (PCR) analysis was performed to detect chimeric antigen receptor T cells in blood and bone marrow. The lower limit of quantification was determined from the standard curve; average values below the lower limit of quantification (i.e., reportable but not quantifiable) are considered approximate. The

lower limit of quantification of the assay was 25 copies per microgram of genomic DNA.

Soluble-factor analysis was performed with the use of serum from whole blood and bone marrow that was separated into aliquots for single use and
5 stored at −80°C. Quantification of soluble cytokine factors was performed with the use of Luminex bead-array technology and reagents (Life Technologies).

Apheresis #1

A 12-15 liter apheresis procedure is carried out at the apheresis center.
10 Peripheral blood mononuclear cells (PBMC) are obtained for CART-19 T cell generation during this procedure. From a single leukapheresis, at least $50 \times 10^9$ white blood cells are harvested to manufacture CART-19 T cells. Baseline blood leukocytes are also obtained and cryopreserved.

15 Cytroreductive Chemotherapy

Chemotherapy is started approximately 5-10 days before infusion so that CART-19 cells may be given 1-2 days after completion of the chemotherapy. The timing of chemotherapy initiation therefore depends on the length of the regimen. The purpose of the chemotherapy is to induce lymphopenia in order to facilitate
20 engraftment and homeostatic expansion of CART-19 cells. The chemotherapy may be chosen also to reduce disease tumor burden. The cytoreductive chemotherapy is chosen and administered by community oncologists. The choice of chemotherapy depends on the patients underlying disease and prior therapies. Fludarabine (30 mg/m2/day x 3 days) and cyclophosphamide (300 mg/m2/day x 3 days) are the agents
25 of choice, as there is the most experience with the use of these agents in facilitating adoptive immunotherapy. Several other acceptable regimens using FDA-approved drugs are appropriate, including CHOP, HyperCVAD, EPOCH, DHAP, ICE or other regimens.

30 Restaging assessment

A limited restaging is performed at the completion of chemotherapy in order to provide baseline tumor burden measurements. This includes imaging, physical examination, and minimal residual disease (MRD) assessments. Subjects undergo the following for pre-infusing testing: physical exam, documentation of

adverse events and blood draws for hematology, chemistry and pregnancy testing (if applicable).

Preparation of CART-19 T cells

5 Autologous T cells are engineered to express an extracellular single chain antibody (scFv) with specificity for CD19. The extracellular scFv can redirect specificity of the transduced T cells for cells that express CD19, a molecule that is restricted in expression on the surface of the malignant cells and on normal B cells. In addition to CD19 scFv, the cells are transduced to express an intracellular signaling

10 molecule comprised of either the TCRζ chain or a tandem signaling domain comprised of 4-1BB and TCRζ signaling modules. The scFv is derived from a mouse monoclonal antibody, and thus contains mouse sequences, and the signaling domains are entirely of the native human sequences. CART-19 T cells are manufactured by isolating the T cells by apheresis, and using lentiviral vector technology (Dropulic et

15 al., 2006, Human Gene Therapy, 17: 577-88; Naldini et al., 1996, Science, 272: 263-7; Dull et al., 1998, J Virol, 72: 8463-71) to introduce the scFv:TCRζ:4-1BB into CD4 and CD8 T cells. In some patients, a control scFv:TCRζ: is introduced into a portion of the cells for a competitive repopulation experiment. These receptors are "universal" in that they bind antigen in an MHC-independent fashion, thus, one

20 receptor construct can be used to treat a population of patients with CD19 antigen-positive tumors.

The CAR constructs were developed at the University of Pennsylvania, and the clinical grade vector was manufactured at Lentigen Corporation. The CART-19 cells are manufactured in the Clinical Cell and Vaccine Production Facility at the

25 University of Pennsylvania according to the process shown in Figure 11. At the end of cell cultures, the cells are cryopreserved in infusible cryomedia. A single dose of CART-19 transduced T cells comprising of the infusion of $2.5 \times 10^9$ to $5 \times 10^9$ total cells, are administered in either 1 or 2 bags. Each bag contains an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents

30 (% v/v): 31.25 plasmalyte-A, 31.25 dextrose (5%), 0.45 NaCl, up to 7.50 DMSO, 1.00 dextran 40, 5.00 human serum albumin with approximately $2.5\text{-}5 \times 10^9$ autologous T cells per bag. For increased safety, the first dose is given as a split dose on days 0,1 and 2, with ~10% of the cells on day 0, 30% on day 1, and 60% on day 2.



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Storage

Bags (10 to 100 ml capacity) containing CART-19-transduced T cells are stored in blood bank conditions in a monitored -135°C freezer. Infusion bags are stored in the freezer until needed.

5

Cell thawing

After logging the cells in the investigational pharmacy, frozen cells are transported in dry ice to the subject's bedside. The cells are thawed at the bedside one bag at a time using a water bath maintained at 36°C to 38°C. The bag is gently

10 massaged until the cells have just thawed. There should be no frozen clumps left in the container. If the CART-19 cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused.

Premedication

15 Side effects following T cell infusions may include transient fever, chills, and/or nausea. It is recommended that the subject be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or IV, prior to the infusion of CART-19 cells. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory

20 medication may be prescribed if the patient continues to have fever not relieved by acetaminophen. It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells. If corticosteroids are required for an acute

25 infusional reaction, an initial dose of hydrocortisone 100 mg is recommended.

Administration/Infusion

Infusions begin 1 to 2 days after completion of chemotherapy. The day of the first infusions, patients have a CBC with differential, and assessment of CD3,

30 CD4 and CD8 counts since chemotherapy is given in part to induce lymphopenia. Without wishing to be bound by any particular theory, it is believed that an initial i.v. dose of $2.5\text{-}5 \times 10^9$ CART-19 cells is optimal for this protocol. Because there are about $1 \times 10^{12}$ T cells in a healthy adult, the proposed total dose is equivalent to about 0.5% of the total body mass of T cells (Roederer, 1995, Nat Med, 1: 621-7; Macallan et al.,

2003, Eur J Immunol, 33: 2316-26). The first dose is administered using a split dose on days 0 (10%), 1 (30%) and 2 (60%). Subjects receive infusion in an isolated room. The cells are thawed at the patient's bedside as described elsewhere herein. The thawed cells are given at an infusion rate as quickly as tolerated so that the duration of the infusion is approximately 10-15 minutes. The transduced T cells are administered by rapid intravenous infusion at a flow rate of approximately 10mL to 20mL per minute through an 18-gauge latex free Y-type blood set with a 3-way stopcock. The duration of the infusion is approximately 15 minutes. One or two bags of CART-19 modified cells are delivered on ice, and the cells are administered to the subject while cold. In subjects receiving mixtures of CART-19 cells, in order to facilitate mixing, the cells are administered simultaneously using a Y-adapter. Subjects are infused and premedicated as described elsewhere herein. Subjects' vital signs are assessed and pulse oximetry is done prior to dosing, at the end of the infusion and every 15 minutes thereafter for 1 hour and until these are stable and satisfactory. A blood sample for determination of baseline CART-19 level is obtained before infusion and 20 minutes post infusion. Patients experiencing toxicities from their preceding cytoreductive chemotherapy have their infusion schedule delayed until these toxicities have resolved. The specific toxicities warranting delay of T cell infusions include: 1) Pulmonary: Requirement for supplemental oxygen to keep saturation greater than 95% or presence of radiographic abnormalities on chest x-ray that are progressive; 2) Cardiac: New cardiac arrhythmia not controlled with medical management. 3) Hypotension requiring pressor support. 4) Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48-hours of T cell infusion. A serum sample for potassium and uric acid is collected before the first infusion as well as two hours after each subsequent infusion.

Post infusion laboratories to assess graftment and persistence

Subjects return at day 4 and 10 after the initial CART-19 cell infusion to have blood drawn for serum cytokine levels, and CART-19 PCR in order to evaluate the presence of CART-19 cells. Subjects return once a week for three weeks to undergo the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs.



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Second infusion

Without wishing to be bound by any particular theory, it is believed that a second dose of CART-19 cells can be given on day 11 to patients, provided that they exhibit adequate tolerance to the first dose and sufficient CART-19 cells were
5 manufactured. The dose is 2-5 x $10^9$ total cells. A serum sample for potassium and uric acid can be collected two hours after the infusion.

Second apheresis

A 2 liter apheresis procedure is carried out at the apheresis center.
10 PBMC are obtained for research and cryopreserved. Subjects undergo the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. In addition restaging is done in order to provide tumor burden measurements. Restaging testing is determined by disease type and includes imaging, MRD assessments, bone
15 marrow aspirate and biopsy and/or lymph node biopsy.

Monthly evaluations 2 to 6 months post infusion

Subjects return on a monthly basis during months 2 to 6 post CART-19 cell infusion. At these study visits, subjects undergo the following: concomitant
20 medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. The HIV DNA assay is performed at months 2-6 post CART-19 cell infusion to exclude the presence of detectable RCL.

25 Quarterly evaluations up to 2 years post infusion

Subjects are evaluated on a quarterly basis until 2 years post infusion. At these study visits, subjects undergo the following: concomitant medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CART-19 cells and research labs. The HIV
30 DNA assay is performed at months 3 and 6 post CART-19 cell infusion to exclude the presence of detectable RCL.

The results of the experiments are now described



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Patient history

The patient received a diagnosis of stage I CLL in 1996. He first required treatment after 6 years of observation for progressive leukocytosis and adenopathy. In 2002, he was treated with two cycles of rituximab plus fludarabine;
5 this treatment resulted in normalization of blood counts and partial resolution of adenopathy. In 2006, he received four cycles of rituximab and fludarabine for disease progression, again with normalization of blood counts and partial regression of adenopathy. This response was followed by a 20-month progression-free interval and a 2-year treatment-free interval. In February 2009, he had rapidly progressive
10 leukocytosis and recurrent adenopathy. His bone marrow was extensively infiltrated with CLL. Cytogenetic analysis showed that 3 of 15 cells contained a deletion of chromosome 17p, and fluorescence in situ hybridization (FISH) testing showed that 170 of 200 cells had a deletion involving *TP53* on chromosome 17p. He received rituximab with bendamustine for one cycle and three additional cycles of
15 bendamustine without rituximab (because of a severe allergic reaction). This treatment resulted in only transient improvement in lymphocytosis. Progressive adenopathy was documented by means of computed tomography (CT) after therapy.

Autologous T cells were collected by means of leukapheresis and cryopreserved. The patient then received alemtuzumab (an anti-CD52, mature-
20 lymphocyte, cell-surface antigen) for 11 weeks, with improved hematopoiesis and a partial resolution of adenopathy. Over the next 6 months, he had stable disease with persistent, extensive marrow involvement and diffuse adenopathy with multiple 1- to 3-cm lymph nodes. In July 2010, the patient was enrolled in a phase 1 clinical trial of chimeric antigen receptor–modified T cells.
25

Cell Infusions

Autologous T cells from the patient were thawed and transduced with lentivirus to express the CD19-specific chimeric antigen receptor (Figure 12A); sequence identifiers for the lentiviral vector and relevant sequences are depicted in
30 Table 5. Four days before cell infusion, the patient received chemotherapy designed for depletion of lymphocytes (pentostatin at a dose of 4 mg per square meter of body-surface area and cyclophosphamide at a dose of 600 mg per square meter) without rituximab (Lamanna et al., 2006, J Clin Oncol, 24: 1575-81). Three days after chemotherapy but before cell infusion, the bone marrow was hypercellular with



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approximately 40% involvement by CLL. Leukemia cells expressed kappa light chain and CD5, CD19, CD20, and CD23. Cytogenetic analysis showed two separate clones, both resulting in loss of chromosome 17p and the *TP53* locus (46,XY,del(17)(p12)[5]/46,XY,der(17)t(17;21)(q10;q10)[5]/46,XY[14]). Four days

5 after chemotherapy, the patient received a total of $3 \times 10^8$ T cells, of which 5% were transduced, for a total of $1.42 \times 10^7$ transduced cells ($1.46 \times 10^5$ cells per kilogram) split into three consecutive daily intravenous infusions (10% on day 1, 30% on day 2, and 60% on day 3). No postinfusion cytokines were administered. No toxic effects of infusions were noted.

10

Table 5: Sequence identifiers for pELPS-CD19-BBz transfer vector

| SEQ ID NO: # | IDENTITY |
|---|---|
| SEQ ID NO: 1 | pELPS-CD19-BBZ transfer vector (nucleic acid sequence) |
| SEQ ID NO: 2 | RSV's U3 (nucleic acid sequence) |
| SEQ ID NO: 3 | HIV R repeat (nucleic acid sequence) |
| SEQ ID NO: 4 | HIV U5 Repeat (nucleic acid sequence) |
| SEQ ID NO: 5 | Partial Gag/Pol (nucleic acid sequence) |
| SEQ ID NO: 6 | cPPT (nucleic acid sequence) |
| SEQ ID NO: 7 | EF1 alpha Promoter (nucleic acid sequence) |
| SEQ ID NO: 8 | CD19-BBzeta CAR (nucleic acid sequence) |
| SEQ ID NO: 9 | Hu Woodchuck PRE (nucleic acid sequence) |
| SEQ ID NO: 10 | R Repeat (nucleic acid sequence)t |
| SEQ ID NO: 11 | U5 Repeat (nucleic acid sequence) |
| SEQ ID NO: 12 | CD19-BBzeta CAR (amino acid sequence) |
| SEQ ID NO: 13 | CD8 Leader (nucleic acid sequence) |
| SEQ ID NO: 14 | Anti-CD19scFv (nucleic acid sequence) |
| SEQ ID NO: 15 | CD8 Hinge (nucleic acid sequence) |
| SEQ ID NO: 16 | CD8 Transmembrane (nucleic acid sequence) |
| SEQ ID NO: 17 | 4-1BB (nucleic acid sequence) |
| SEQ ID NO: 18 | CD3zeta (nucleic acid sequence) |
| SEQ ID NO: 19 | CD8 Leader (amino acid sequence) |
| SEQ ID NO: 20 | Anti-CD19scFv (amino acid sequence) |
| SEQ ID NO: 21 | CD8 Hinge (amino acid sequence) |



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| | |
|---|---|
| SEQ ID NO: 22 | CD8 Transmembrane (amino acid sequence) |
| SEQ ID NO: 23 | 4-1BB (amino acid sequence) |
| SEQ ID NO: 24 | CD3zeta (amino acid sequence) |

Clinical Response and Evaluations

Fourteen days after the first infusion, the patient began having chills and low-grade fevers associated with grade 2 fatigue. Over the next 5 days, the chills intensified, and his temperature escalated to 39.2°C (102.5°F), associated with rigors, diaphoresis, anorexia, nausea, and diarrhea. He had no respiratory or cardiac symptoms. Because of the fevers, chest radiography and blood, urine, and stool cultures were performed, and were all negative or normal. The tumor lysis syndrome was diagnosed on day 22 after infusion (Figure 12B). The uric acid level was 10.6 mg per deciliter (630.5 μmol per liter), the phosphorus level was 4.7 mg per deciliter (1.5 mmol per liter) (normal range, 2.4 to 4.7 mg per deciliter [0.8 to 1.5 mmol per liter]), and the lactate dehydrogenase level was 1130 U per liter (normal range, 98 to 192). There was evidence of acute kidney injury, with a creatinine level of 2.60 mg per deciliter (229.8 μmol per liter) (baseline level, <1.0 mg per deciliter [<88.4 μmol per liter]). The patient was hospitalized and treated with fluid resuscitation and rasburicase. The uric acid level returned to the normal range within 24 hours, and the creatinine level within 3 days; he was discharged on hospital day 4. The lactate dehydrogenase level decreased gradually, becoming normal over the following month.

By day 28 after CART19-cell infusion, adenopathy was no longer palpable, and on day 23, there was no evidence of CLL in the bone marrow (Figure 12C). The karyotype was now normal in 15 of 15 cells (46,XY), and FISH testing was negative for deletion *TP53* in 198 of 200 cells examined; this is considered to be within normal limits in negative controls. Flow-cytometric analysis showed no residual CLL, and B cells were not detectable (<1% of cells within the CD5+CD10–CD19+CD23+ lymphocyte gate). CT scanning performed on day 31 after infusion showed resolution of adenopathy (Figure 12D).

Three and 6 months after CART19-cell infusion, the physical examination remained unremarkable, with no palpable adenopathy, and CT scanning performed 3 months after CART19-cell infusion showed sustained remission (Figure

12D). Bone marrow studies at 3 and 6 months also showed no evidence of CLL by means of morphologic analysis, karyotype analysis (46,XY), or flow-cytometric analysis, with a continued lack of normal B cells as well. Remission had been sustained for at least 10 months.

5

Toxicity of CART19 Cells

The cell infusions had no acute toxic effects. The only serious (grade 3 or 4) adverse event noted was the grade 3 tumor lysis syndrome described above. The patient had grade 1 lymphopenia at baseline and grade 2 or 3 lymphopenia beginning
10 on day 1 and continuing through at least 10 months after therapy. Grade 4 lymphopenia, with an absolute lymphocyte count of 140 cells per cubic millimeter, was recorded on day 19, but from day 22 through at least 10 months, the absolute lymphocyte count ranged between 390 and 780 cells per cubic millimeter (grade 2 or 3 lymphopenia). The patient had transient grade 1 thrombocytopenia (platelet count,
15 98,000 to 131,000 per cubic millimeter) from day 19 through day 26 and grade 1 or 2 neutropenia (absolute neutrophil count, 1090 to 1630 per cubic millimeter) from day 17 through day 33. Other signs and symptoms that were probably related to the study treatment included grade 1 and 2 elevations in aminotransferase and alkaline phosphatase levels, which developed 17 days after the first infusion and resolved by
20 day 33. Grade 1 and 2 constitutional symptoms consisted of fevers, chills, diaphoresis, myalgias, headache, and fatigue. Grade 2 hypogammaglobulinemia was corrected with infusions of intravenous immune globulin.

Analysis of Serum and Bone Marrow Cytokines

25 The patient's clinical response was accompanied by a delayed increase in levels of inflammatory cytokines (Figure 13A through Figure 13D), with levels of interferon-γ, the interferon-γ–responsive chemokines CXCL9 and CXCL10, and interleukin-6 that were 160 times as high as baseline levels. The temporal rise in cytokine levels paralleled the clinical symptoms, peaking 17 to 23 days after the first
30 CART19-cell infusion.

The supernatants from serial bone marrow aspirates were measured for cytokines and showed evidence of immune activation (Figure 13E). Significant increases in interferon-γ, CXCL9, interleukin-6, and soluble interleukin-2 receptor were noted, as compared with the baseline levels on the day before T-cell infusion;

the values peaked on day 23 after the first CART19-cell infusion. The increase in bone marrow cytokines coincided with the elimination of leukemia cells from the marrow. Serum and marrow tumor necrosis factor α remained unchanged.

5 Expansion and Persistence of Chimeric Antigen Receptor T Cells

Real-time PCR detected DNA encoding anti-CD19 chimeric antigen receptor (CAR19) beginning on day 1 after the first infusion (Figure 14A). More than a 3-log expansion of the cells *in vivo* was noted by day 21 after infusion. At peak levels, CART19 cells in blood accounted for more than 20% of circulating
10 lymphocytes; these peak levels coincided with the occurrence of constitutional symptoms, the tumor lysis syndrome (Figure 12B), and elevations in serum cytokine levels (Figure 13A through Figure 13D). CART19 cells remained detectable at high levels 6 months after the infusions, though the values decreased by a factor of 10 from peak levels. The doubling time of chimeric antigen receptor T cells in blood was
15 approximately 1.2 days, with an elimination half-life of 31 days.

Chimeric Antigen Receptor T Cells in Bone Marrow

CART19 cells were identified in bone marrow specimens beginning 23 days after the first infusion (Figure 14B) and persisted for at least 6 months, with a
20 decay half-life of 34 days. The highest levels of CART19 cells in the bone marrow were identified at the first assessment 23 days after the first infusion and coincided with induction of an immune response, as indicated by cytokine-secretion profiles (Figure 13E). Flow-cytometric analysis of bone marrow aspirates indicated a clonal expansion of CD5+CD19+ cells at baseline that was absent 1 month after infusion and
25 in a sample obtained 3 months after infusion (data not shown). Normal B cells were not detected after treatment (Figure 14C).

Treatment with autologous genetically modified CART19 cells

Described herein is the delayed development of the tumor lysis
30 syndrome and a complete response 3 weeks after treatment with autologous T cells genetically modified to target CD19 through transduction with a lentivirus vector expressing anti-CD19 linked to CD3-zeta and CD137 (4-1BB) signaling domains. Genetically modified cells were present at high levels in bone marrow for at least 6 months after infusion. The generation of a CD19-specific immune response in bone

marrow was demonstrated by temporal release of cytokines and ablation of leukemia cells that coincided with peak infiltration of chimeric antigen receptor T cells. Development of the tumor lysis syndrome after cellular immunotherapy has not been reported previously (Baeksgaard et al., 2003, Cancer Chemother Pharacol, 51: 187-
5 92).

Genetic manipulation of autologous T cells to target specific tumor antigens is an attractive strategy for cancer therapy (Sadelain et al., 2009, Curr Opin Immunol, 21: 215-23; Jena et al., 2010, Blood, 116: 1035-44). An important feature of the approach described herein is that chimeric antigen receptor T cells can
10 recognize tumor targets in an HLA-unrestricted manner, so that "off-the-shelf" chimeric antigen receptors can be constructed for tumors with a wide variety of histologic features. HIV-derived lentiviral vectors were used for cancer therapy, an approach that may have some advantages over the use of retroviral vectors (June et al., 2009, Nat Rev Immunol, 9: 704-16).

15 In previous trials of chimeric antigen receptor T cells, objective tumor responses have been modest, and *in vivo* proliferation of modified cells has not been sustained (Kershaw et al., 2006, Clin Cancer Res, 12: 6106-15; Till et al., 2008, Blood, 112: 2261-71; Pule et al., 2008, Nat Med, 14: 1264-70). Brentjens and colleagues reported preliminary results of a clinical trial of CD19-targeted chimeric
20 antigen receptors linked to a CD28 signaling domain and found transient tumor responses in two of three patients with advanced CLL (Brentjens et al., 2010, Mol Ther, 18: 666-8); however, the chimeric antigen receptors rapidly disappeared from the circulation.

It was unexpected that the very low dose of chimeric antigen receptor
25 T cells that were infused would result in a clinically evident antitumor response. Indeed, the infused dose of $1.5\times10^5$ chimeric antigen receptor T cells per kilogram was several orders of magnitude below doses used in previous studies of T cells modified to express chimeric antigen receptors or transgenic T-cell receptors (Kershaw et al., 2006, Clin Cancer Res, 12: 6106-15; Brentjens et al., 2010, Mol Ther,
30 18: 666-8; Morgan et al., 2010, Mol Ther, 18: 843-51; Johnson et al., 2009, Blood, 114: 535-46). Without being held to any particular theory, it is speculated that the chemotherapy may potentiate the effects of chimeric antigen receptor.

The prolonged persistence of CART19 cells in the blood and bone marrow of the patient results from inclusion of the 4-1BB signaling domain. It is



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likely that the CART19-cell–mediated elimination of normal B cells facilitated the induction of immunologic tolerance to the chimeric antigen receptor, since the CART19 cells that express the single-chain Fv antibody fragment containing murine sequences were not rejected. Given the absence of detectable CD19-positive leukemia
5 cells in this patient, and without being held to any particular theory, it is possible that homeostasis of the chimeric antigen receptor T cells was achieved at least in part from stimulation delivered by early B-cell progenitors as they began to emerge in the bone marrow. The invention relates to the discovery that a new mechanism may exist to maintain "memory" chimeric antigen receptor T cells.

10 Although CD19 is an attractive tumor target, with expression limited to normal and malignant B cells, there is concern that persistence of the chimeric antigen receptor T cells may mediate long-term B-cell deficiency. In fact, in the patient, B cells were absent from the blood and bone marrow for at least 6 months after infusion. This patient did not have recurrent infections. Targeting B cells through CD20 with
15 rituximab is an effective and relatively safe strategy for patients with B-cell neoplasms, and long-term B-cell lymphopenia is manageable (Molina, 2008, Ann Rev Med, 59: 237-50). Patients treated with rituximab have been reported to have a return of B cells within months after discontinuation of therapy. It is not yet clear whether such recovery occurs in patients whose anti–B-cell T cells persist in vivo.

20 Patients who have CLL with *TP53* deletions have short remissions after standard therapies (Dohner et al., 1995, Blood, 85: 1580-9). Allogeneic bone marrow transplantation has been the only approach that has induced long-term remissions in patients with advanced CLL (Gribben et al., 2011, Biol Blood Marrow Transplant, 17: Suppl:S63-S70). However, the resulting potent graft-versus-tumor
25 effect is associated with considerable morbidity because of the high frequency of chronic graft-versus-host disease, which is often especially severe in older patients — those who are typically affected by CLL (Gribben et al., 2011, Biol Blood Marrow Transplant, 17: Suppl:S63-S70; Sorror et al., 2008, Blood, 111: 446-52). The data presented herein suggests that genetically modified autologous T cells may
30 circumvent this limitation.

The delayed onset of the tumor lysis syndrome and cytokine secretion, combined with vigorous in vivo chimeric antigen receptor T-cell expansion and prominent antileukemia activity, points to substantial and sustained effector functions of the CART19 cells. Experiments described herein highlights the potency of this

therapy and provides support for the detailed study of autologous T cells genetically modified to target CD19 (and other targets) through transduction of a chimeric antigen receptor linked to potent signaling domains. Unlike antibody-mediated therapy, chimeric antigen receptor–modified T cells have the potential to replicate *in*
5 *vivo*, and long-term persistence could lead to sustained tumor control. Two other patients with advanced CLL have also received CART19 infusions according to this protocol, and all three have had tumor responses. These findings warrant continued study of CD19-redirected T cells for B-cell neoplasms.

10 The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the
15 invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.



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CLAIMS

What is claimed is:

1. An isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

2. The isolated nucleic acid sequence of claim 1, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 12.

3. The isolated nucleic acid sequence of claim 1 comprising the nucleic acid sequence of SEQ ID NO: 8.

4. The isolated nucleic acid sequence of claim 1, wherein the antigen binding domain is an antibody or an antigen-binding fragment thereof.

5. The isolated nucleic acid sequence of claim 4, wherein the antigen-binding fragment is a Fab or a scFv.

6. The isolated nucleic acid sequence of claim 1, wherein the antigen binding domain binds to a tumor antigen.

7. The isolated nucleic acid sequence of claim 6, wherein the tumor antigen is associated with a hematologic malignancy.

8. The isolated nucleic acid sequence of claim 6, wherein the tumor antigen is associated with a solid tumor.

9. The isolated nucleic acid sequence of claim 6, wherein the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

10. The isolated nucleic acid sequence of claim 1, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

11. The isolated nucleic acid sequence of claim 1, wherein the CD3 zeta signaling domain is encoded by the nucleic acid sequence of SEQ ID NO: 18.

12. An isolated chimeric antigen receptor (CAR) comprising an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

13. The isolated CAR of claim 12, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 12.

14. The isolated CAR of claim 12, wherein the antigen binding domain is an antibody or an antigen-binding fragment thereof.

15. The isolated CAR of claim 14, wherein the antigen binding fragment is a Fab or a scFv.

16. The isolated CAR claim 12, wherein the antigen binding domain binds to a tumor antigen.

17. The isolated CAR of claim 16, wherein the tumor antigen is associated with a hematologic malignancy.

18. The isolated CAR of claim 16, wherein the tumor antigen is associated with a solid tumor.

19. The isolated CAR of claim 16, wherein the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin,

CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

20. The isolated CAR of claim 12, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

21. A cell comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), the CAR comprising an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24.

22. The cell of claim 21, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 12.

23. The cell of claim 21, wherein the nucleic acid comprises the nucleic acid sequence of SEQ ID NO: 8.

24. The cell of claim 21, wherein the antigen binding domain is an antibody or an antigen-binding fragment thereof.

25. The cell of claim 24, wherein the antigen-binding fragment is a Fab or a scFv.

26. The cell of claim 21, wherein the antigen binding domain binds to a tumor antigen.

27. The cell of claim 26, wherein the tumor antigen is associated with a hematologic malignancy.

28. The cell of claim 26, wherein the tumor antigen is associated with a solid tumor.

29. The cell of claim 26, wherein the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

30. The cell of claim 21, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

31. The cell of claim 21, wherein the CD3 zeta signaling domain is encoded by the nucleic acid sequence of SEQ ID NO: 18.

32. The cell of claim 21, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

33. The cell of claim 21, wherein the cell exhibits an anti-tumor immunity when the antigen binding domain binds to its corresponding antigen.

34. A vector comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

35. The vector of claim 34, wherein the CAR comprises the amino acid sequence of SEQ ID NO: 12.

36. The vector of claim 34, wherein the isolated nucleic acid sequence encoding a CAR comprises the nucleic acid sequence of SEQ ID NO: 8.

37. The vector of claim 34, wherein the antigen binding domain is an antibody or an antigen-binding fragment thereof.

38. The vector of claim 37, wherein the antigen-binding fragment is a Fab or a scFv.

39. The vector of claim 34, wherein the antigen binding domain binds to a tumor antigen.

40. The vector of claim 39, wherein the tumor antigen is associated with a hematologic malignancy.

41. The vector of claim 39, wherein the tumor antigen is associated with a solid tumor.

42. The vector of claim 39, wherein the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

43. The vector of claim 34, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

44. The vector of claim 34, wherein the CD3 zeta signaling domain is encoded by the nucleic acid sequence of SEQ ID NO: 18.

45. A method for stimulating a T cell-mediated immune response to a target cell population or tissue in a mammal, the method comprising administering to a mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the antigen binding domain is selected to specifically recognize the target cell population or tissue.



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46. A method of providing an anti-tumor immunity in a mammal, the method comprising administering to the mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, thereby providing an anti-tumor immunity in the mammal.

47. A method of treating a mammal having a disease, disorder or condition associated with an elevated expression of a tumor antigen, the method comprising administering to the mammal an effective amount of a cell genetically modified to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, thereby treating the mammal.

48. The method of claim 47, wherein the cell is an autologous T cell.

49. The method of claim 47, wherein the tumor antigen is selected from the group consisting of CD19, CD20, CD22, ROR1, mesothelin, CD33/IL3Ra, c-Met, PSMA, Glycolipid F77, EGFRvIII, GD-2, NY-ESO-1 TCR, MAGE A3 TCR, and any combination thereof.

50. A method of treating a human with chronic lymphocytic leukemia, the method comprising administering to the human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24.

51. The method of claim 50, wherein the human is resistant to at least one chemotherapeutic agent.

52. The method of claim 50, wherein the chronic lymphocytic leukemia is refractory CD19+ leukemia and lymphoma.

53. A method of generating a persisting population of genetically engineered T cells in a human diagnosed with cancer, the method comprising administering to the human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the persisting population of genetically engineered T cells persists in the human for at least one month after administration.

54. The method of claim 53, wherein the persisting population of genetically engineered T cells comprises at least one cell selected from the group consisting of a T cell that was administered to the human, a progeny of a T cell that was administered to the human, and a combination thereof.

55. The method of claim 53, wherein the persisting population of genetically engineered T cells comprises a memory T cell.

56. The method of claim 53, wherein the cancer is chronic lymphocytic leukemia.

57. The method of claim 56, wherein the chronic lymphocytic leukemia is refractory CD19+ leukemia and lymphoma.

58. The method of claim 53, wherein the persisting population of genetically engineered T cells persists in the human for at least three months after administration.

59. The method of claim 53, wherein the persisting population of genetically engineered T cells persists in the human for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

60. The method of claim 56, wherein the chronic lymphocytic leukemia is treated.

61. A method of expanding a population of genetically engineered T cells in a human diagnosed with cancer, the method comprising administering to the human a T cell genetically engineered to express a CAR wherein the CAR comprises an antigen binding domain, a costimulatory signaling region, and a CD3 zeta signaling domain comprising the amino acid sequence of SEQ ID NO: 24, wherein the administered genetically engineered T cell produces a population of progeny T cells in the human.

62. The method of claim 61, wherein the progeny T cells in the human comprise a memory T cell.

63. The method of claim 61, wherein the T cell is an autologous T cell.

64. The method of claim 61, wherein the human is resistant to at least one chemotherapeutic agent.

65. The method of claim 61, wherein the cancer is chronic lymphocytic leukemia.

66. The method of claim 65, wherein the chronic lymphocytic leukemia is refractory CD19+ leukemia and lymphoma.

67. The method of claim 61, wherein the population of progeny T cells persists in the human for at least three months after administration.

68. The method of claim 61, wherein the population of progeny T cells persist in the human for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, two years, or three years after administration.

69. The method of claim 61, wherein the cancer is treated.

## ABSTRACT

The present invention provides compositions and methods for treating cancer in a human. The invention includes relates to administering a genetically modified T cell to express a CAR wherein the CAR comprises an antigen binding
5 domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain.




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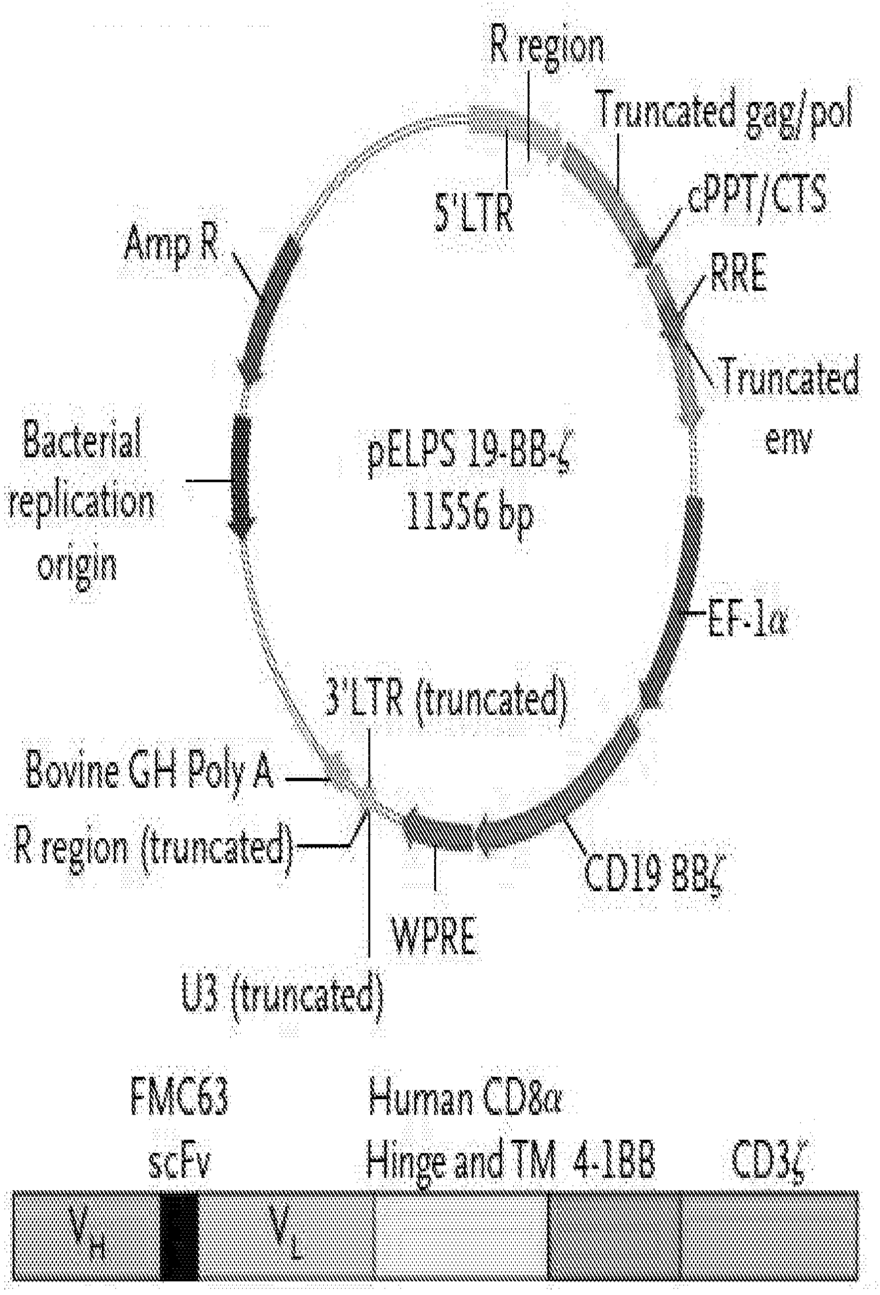


Figure 1A

Day 0 | Day 0-1 | Day 3 | Day 5 | Harvest Day (10 ± 2)

Leukocyte Apheresis → CD3/28 positive selection of T-cells with anti-CD3/anti-CD28 mAb coated magnetic beads → Seed in gas permeable bags. Transduction w/ αCD19-41BBζ vector → Vector wash out. Culture in gas permeable bags → Culture in Wave Bioreactor → Remove Beads

↓

Harvest, Wash, Concentrate

↓

Cryopreserve Final Product in Infusible Cryomedia

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Figure 1B

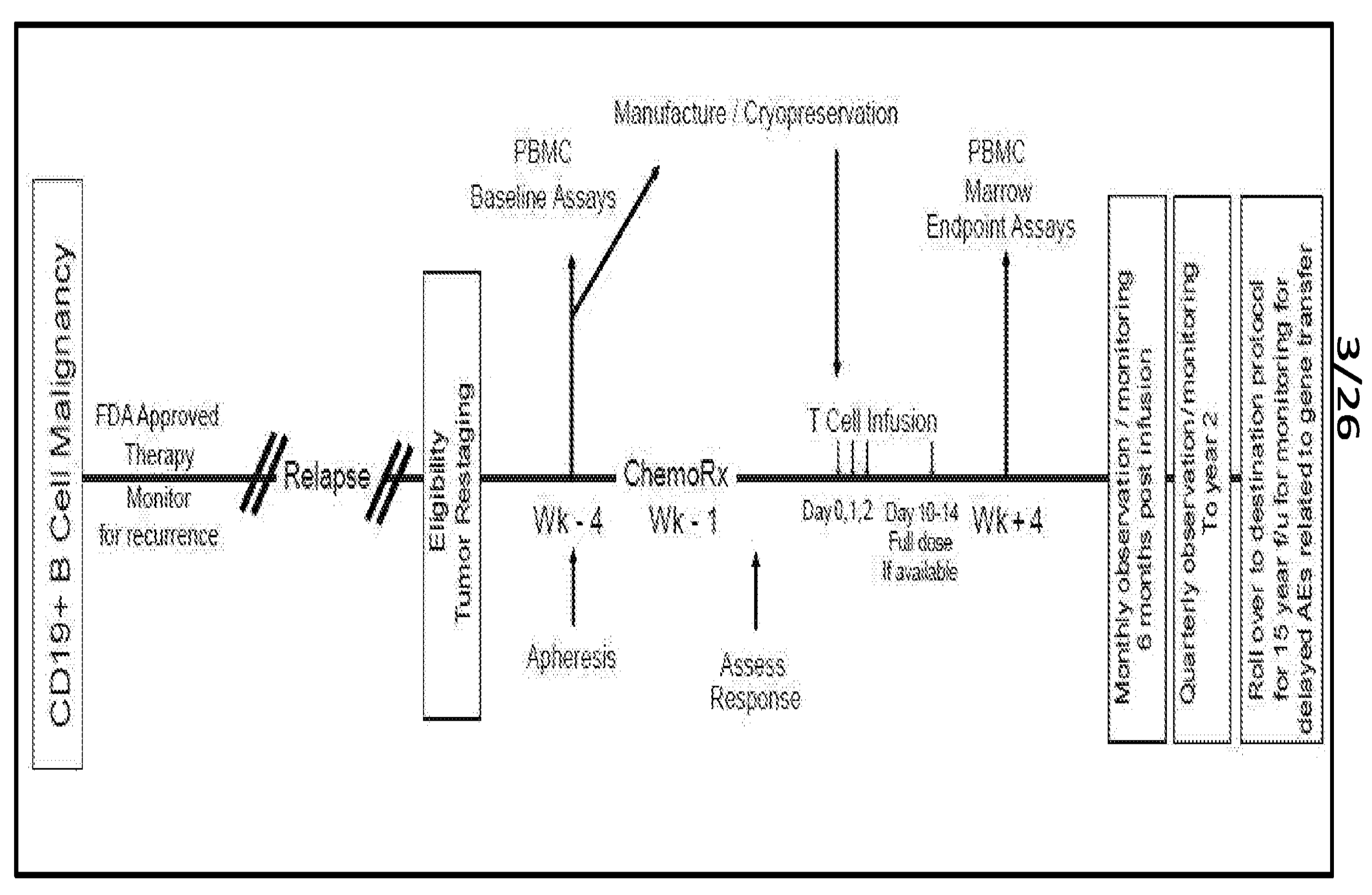


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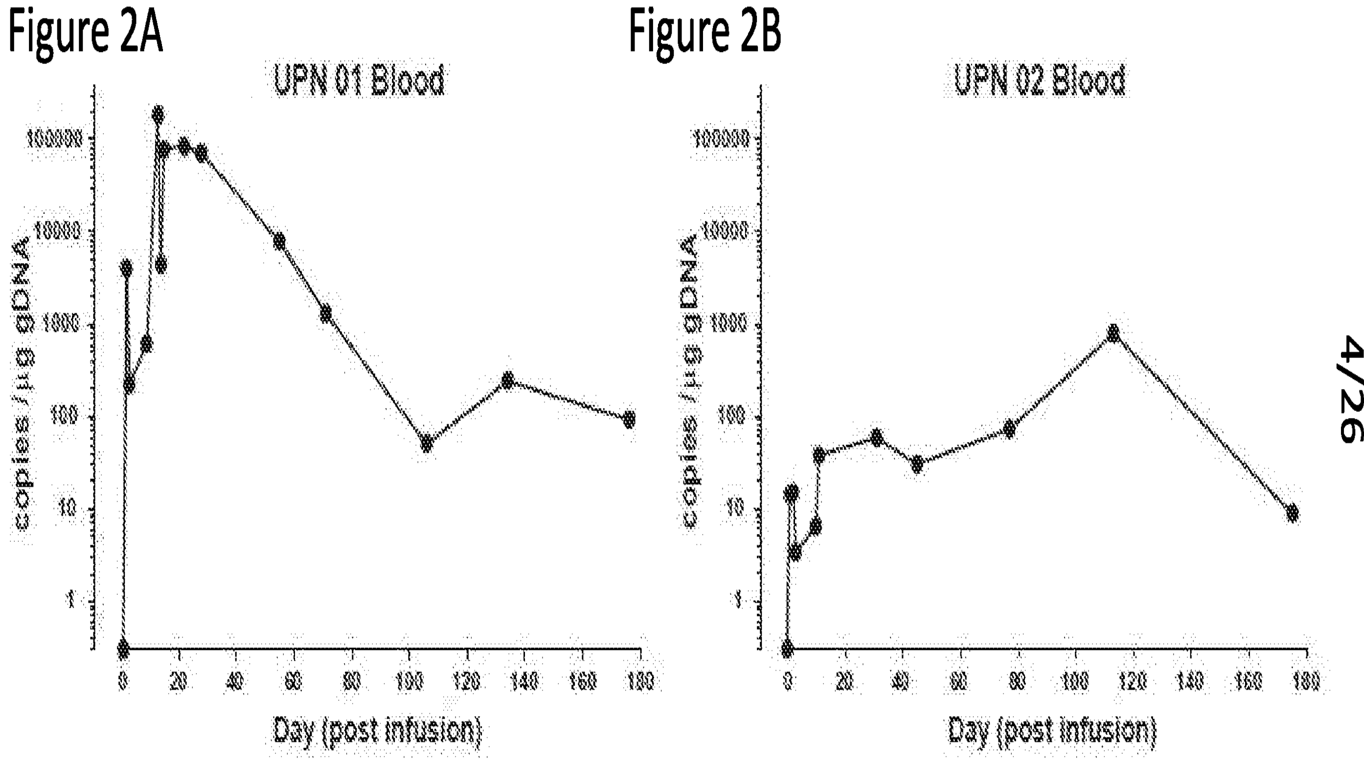
Figure 1C

Figure 2A

Figure 2B

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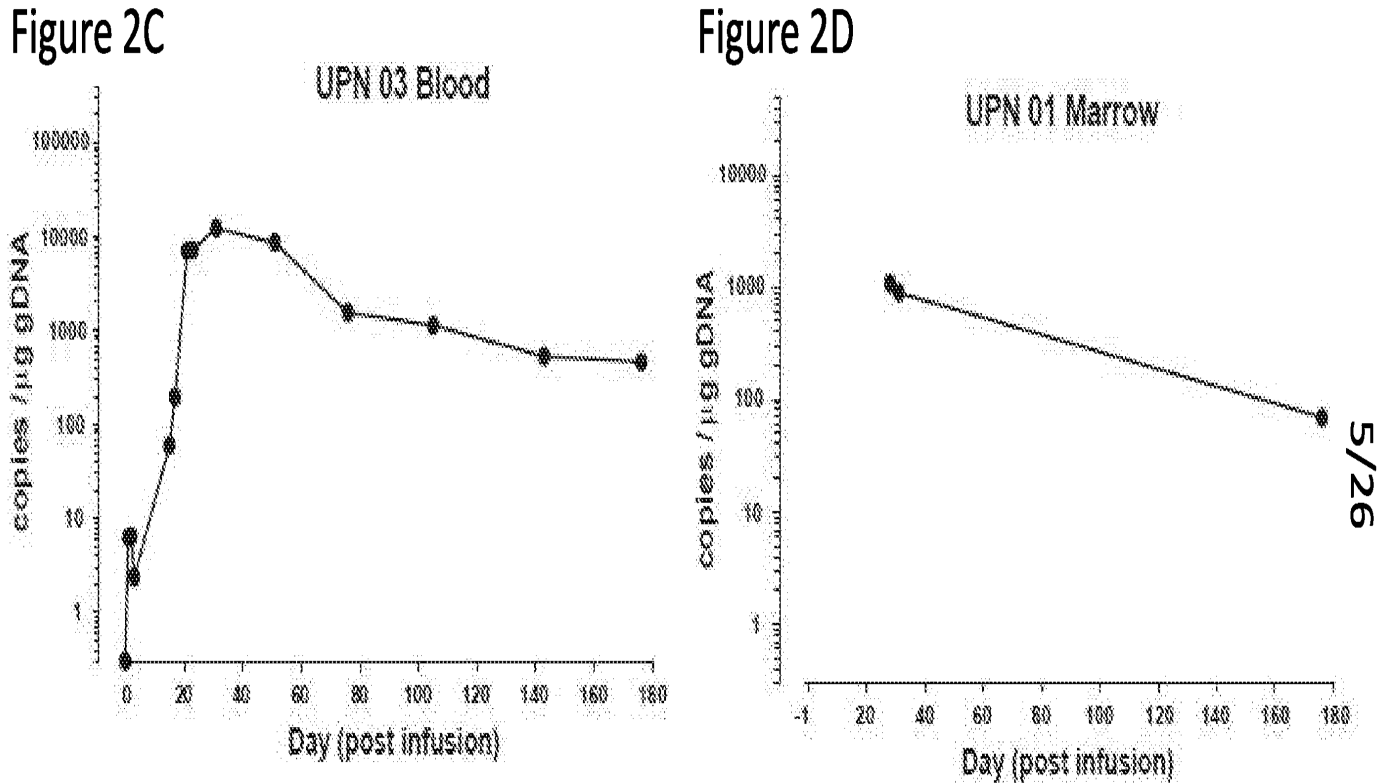
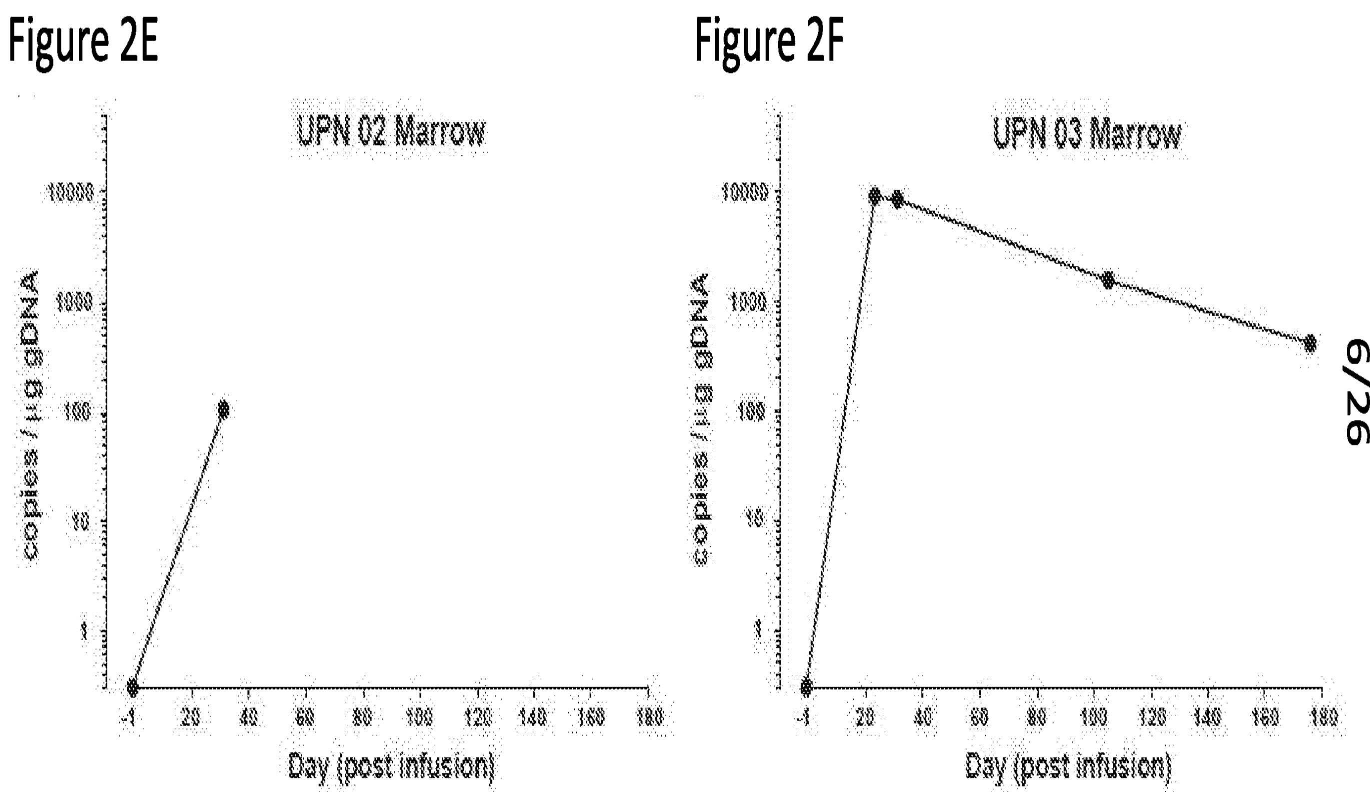


Figure 2C
UPN 03 Blood
100000
10000
1000
100
10
1
copies / µg gDNA
0
20
40
60
80
100
120
140
160
180
Day (post infusion)
Figure 2D
UPN 01 Marrow
10000
1000
100
10
1
copies / µg gDNA
-1
20
40
60
80
100
120
140
160
180
Day (post infusion)
5/26

Figure 2E

Figure 2F

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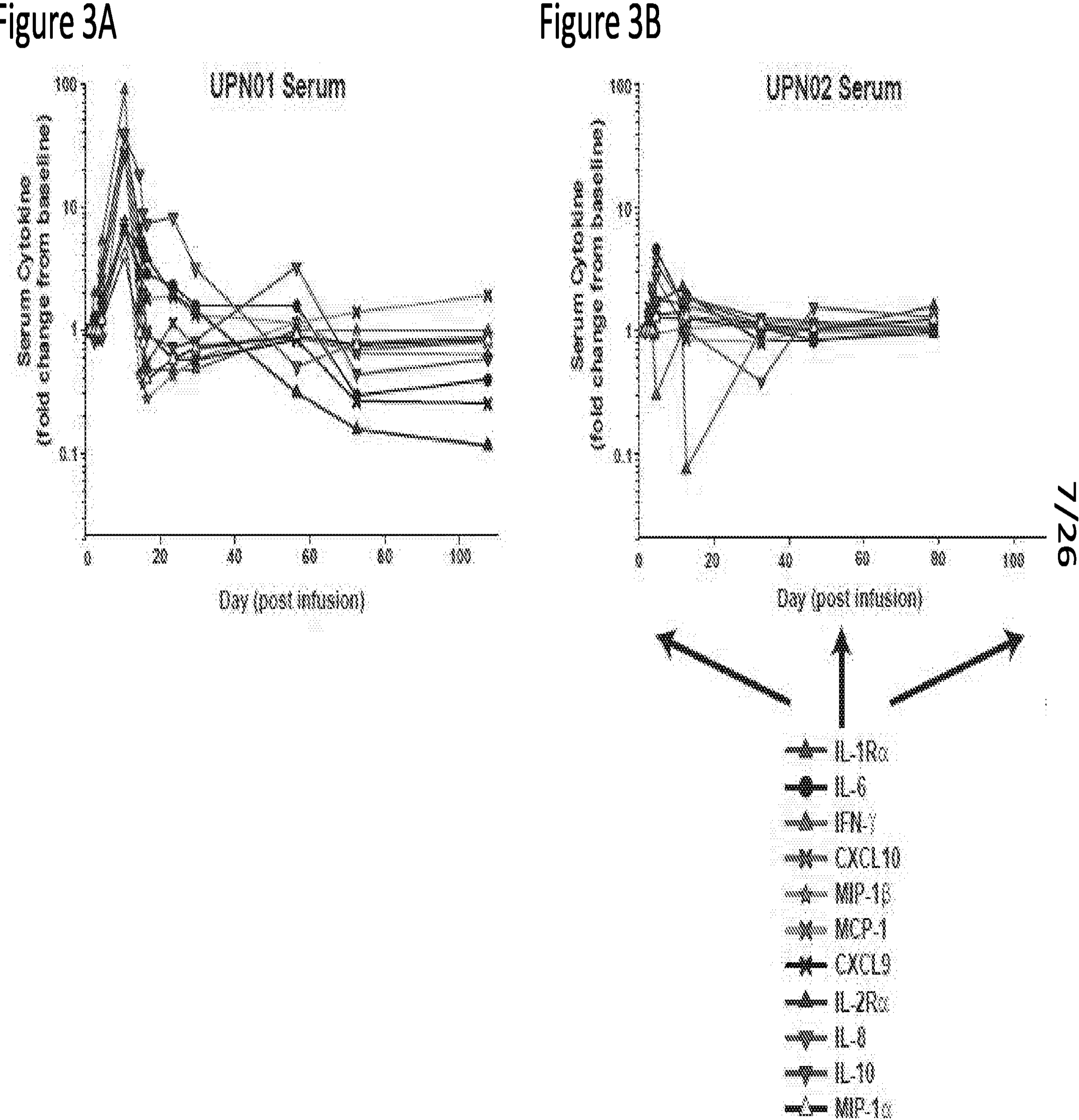


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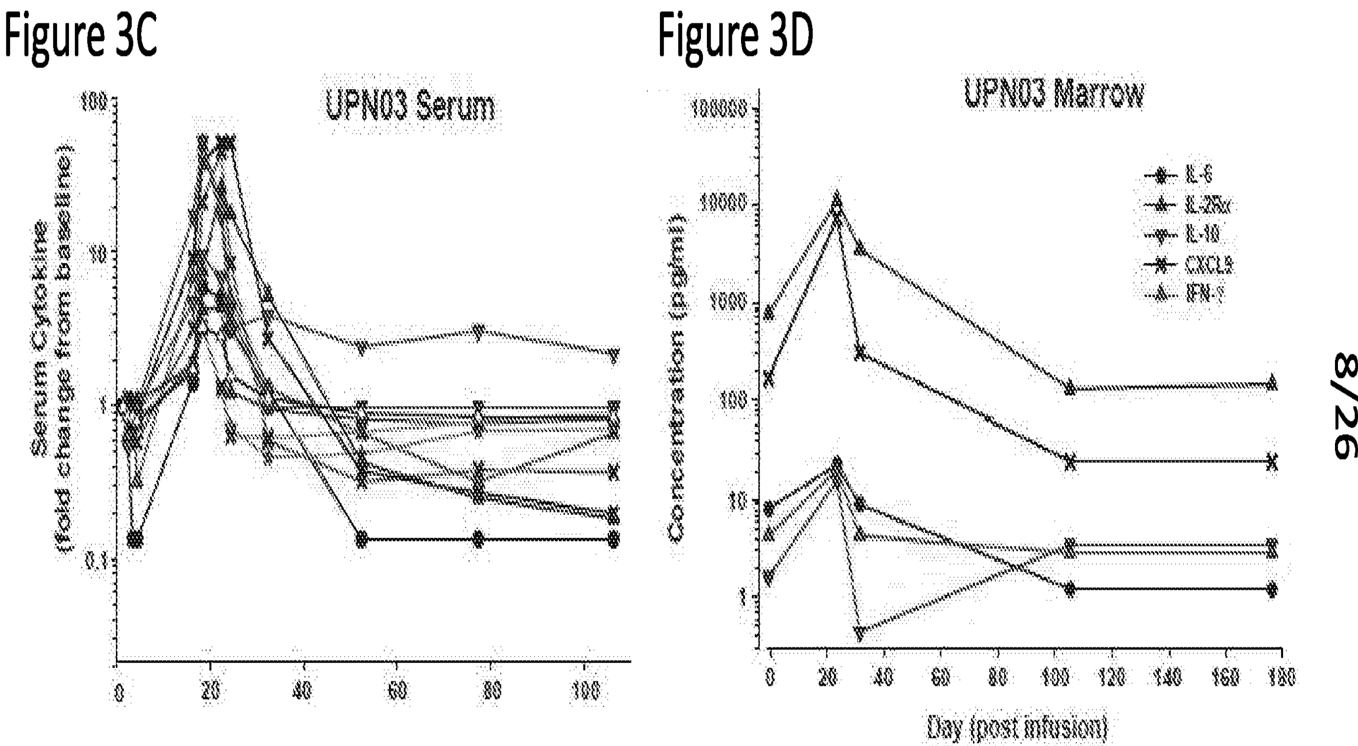


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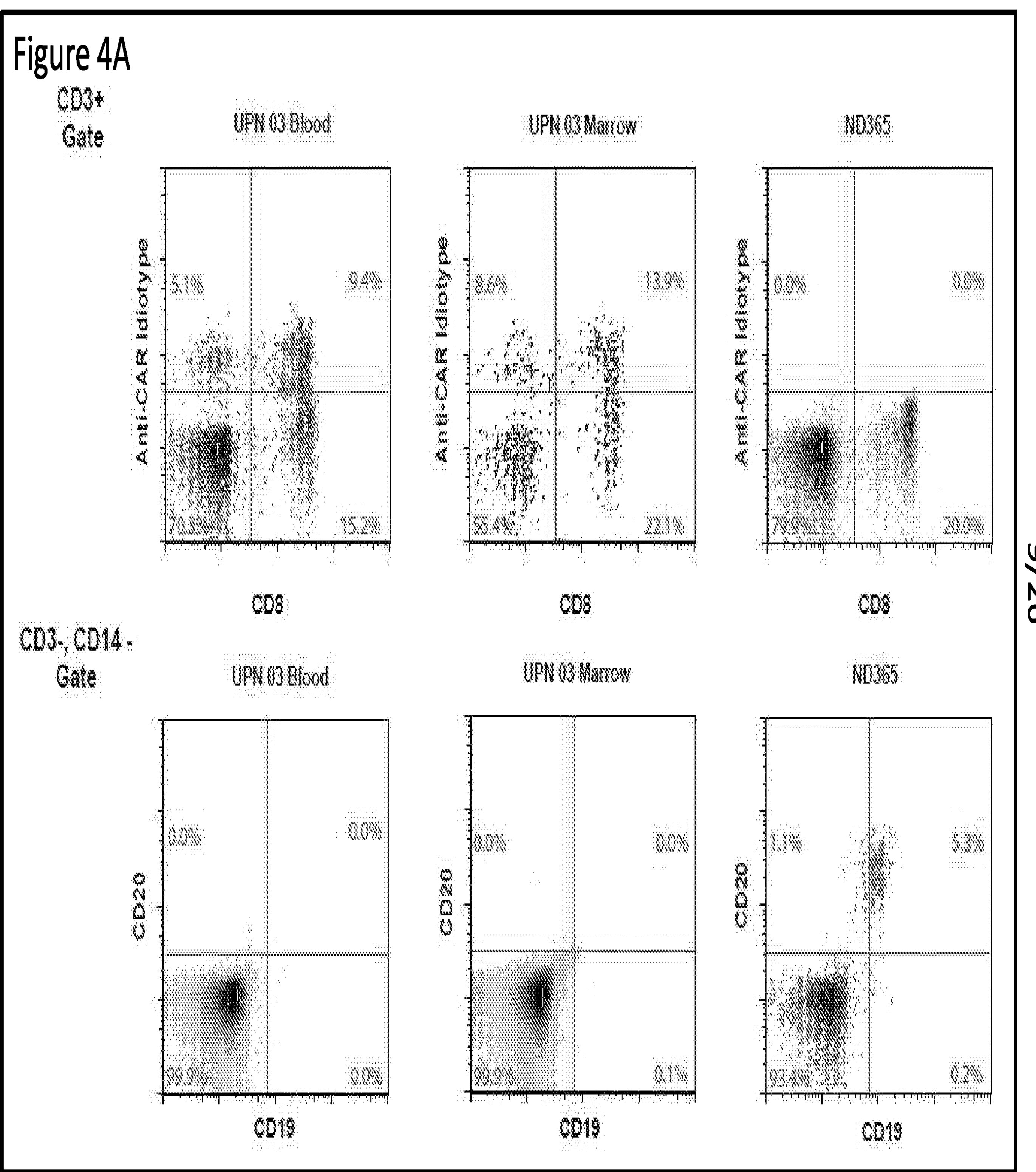


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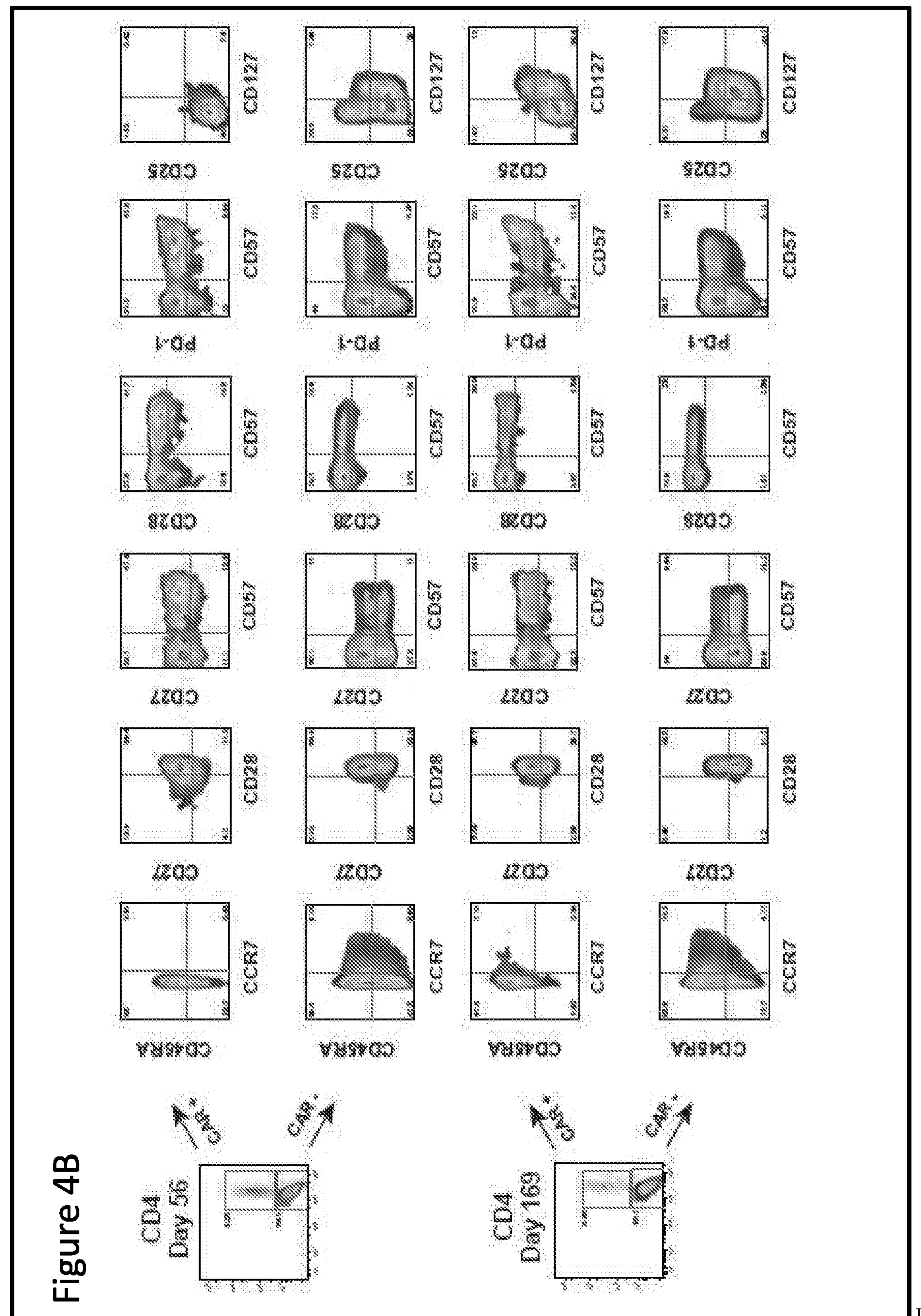
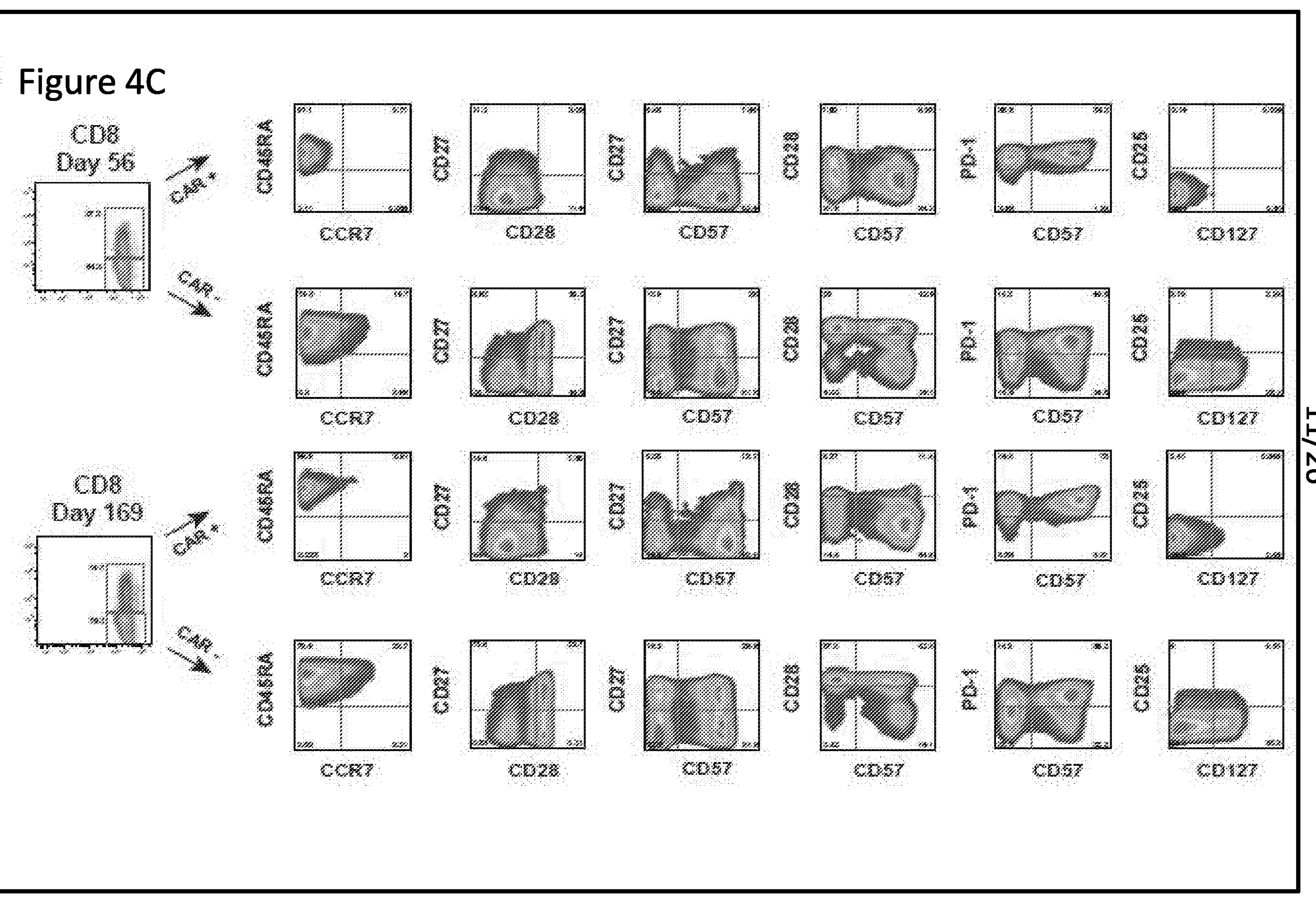



Figure 4B

Figure 4C



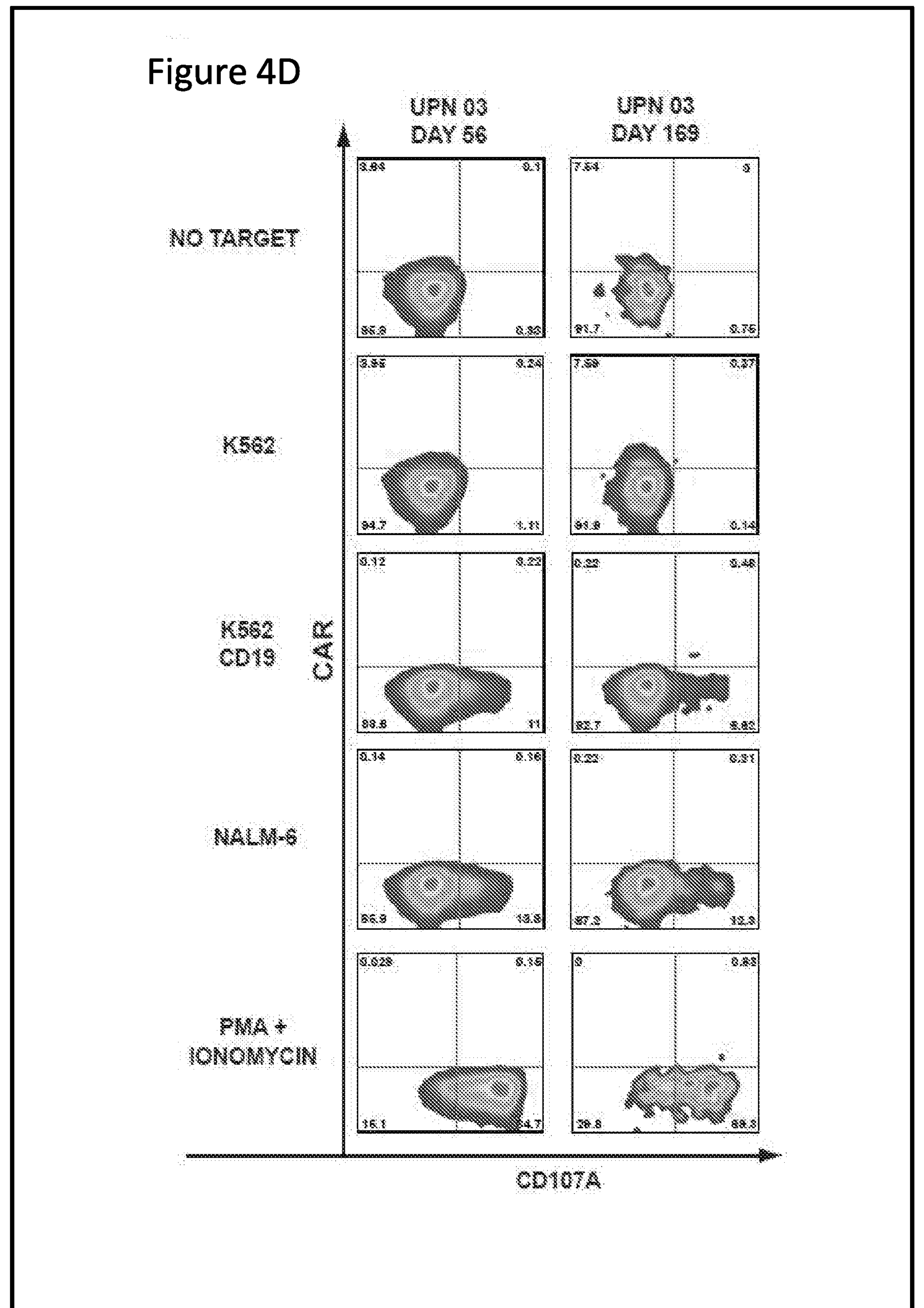


Figure 4D
UPN 03
DAY 56
UPN 03
DAY 169
NO TARGET
K562
K562
CD19
NALM-6
PMA +
IONOMYCIN
CAR
CD107A

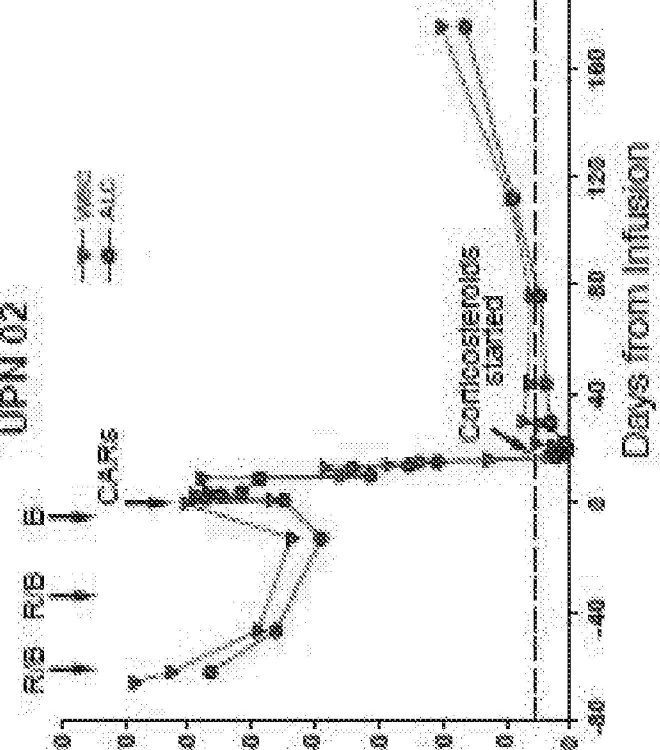



Figure 5A

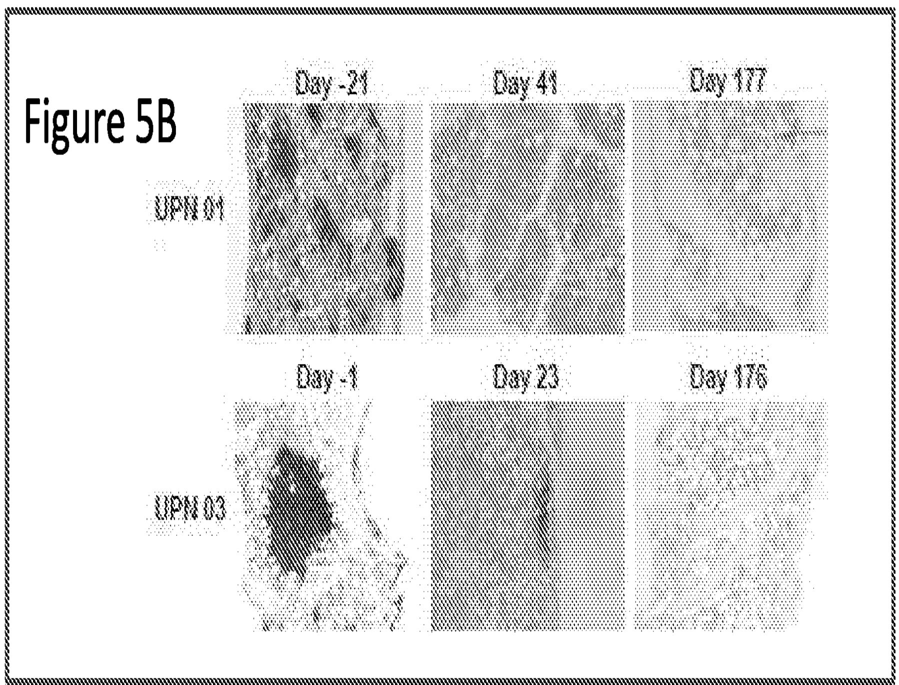


Figure 5B
Day -21
Day 41
Day 177
UPN 01
Day -1
Day 23
Day 176
UPN 03

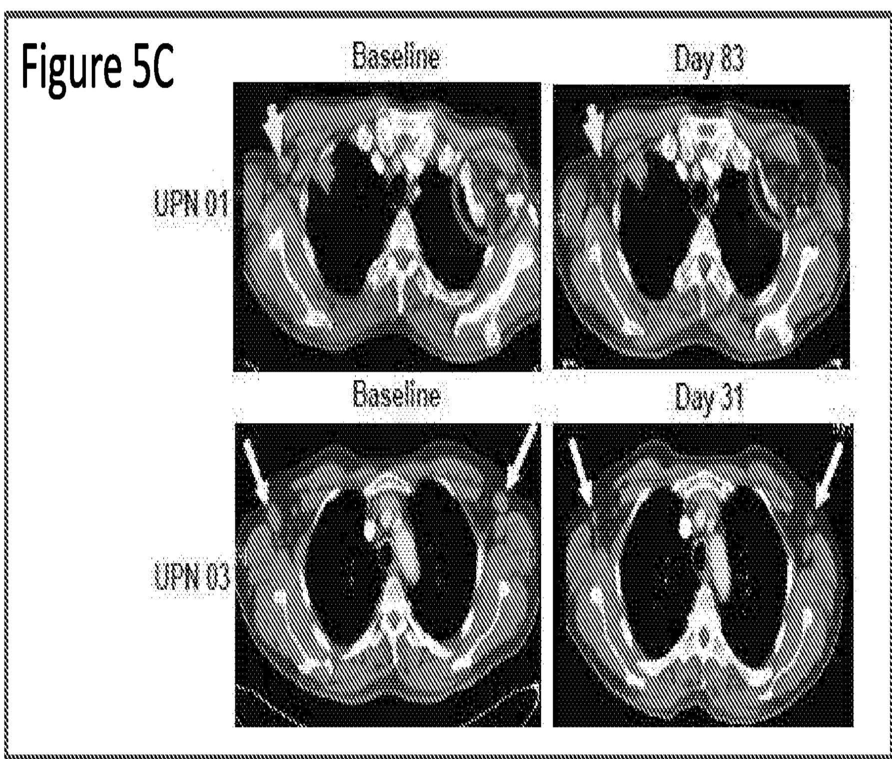
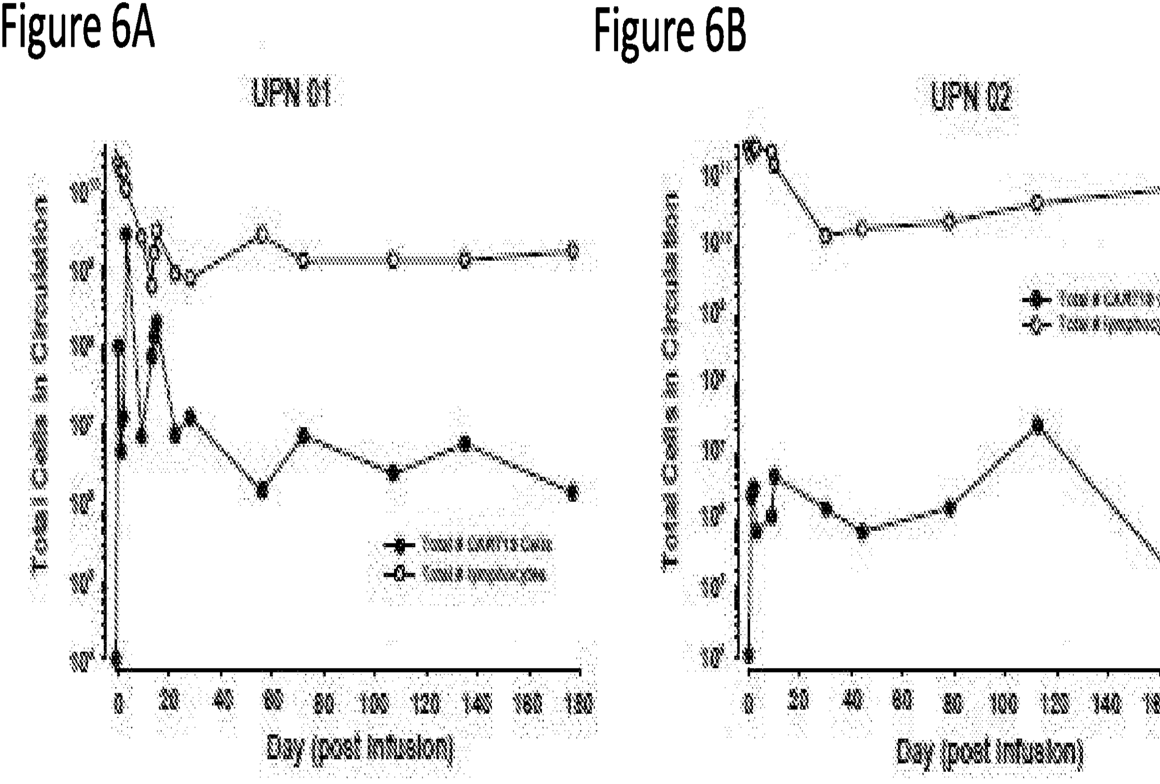
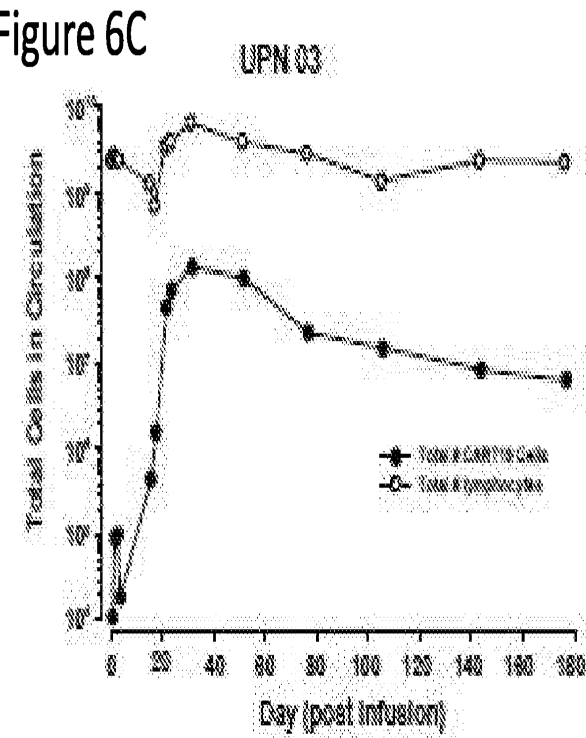


Figure 5C
Baseline
Day 83
UPN 01
Baseline
Day 31
UPN 03

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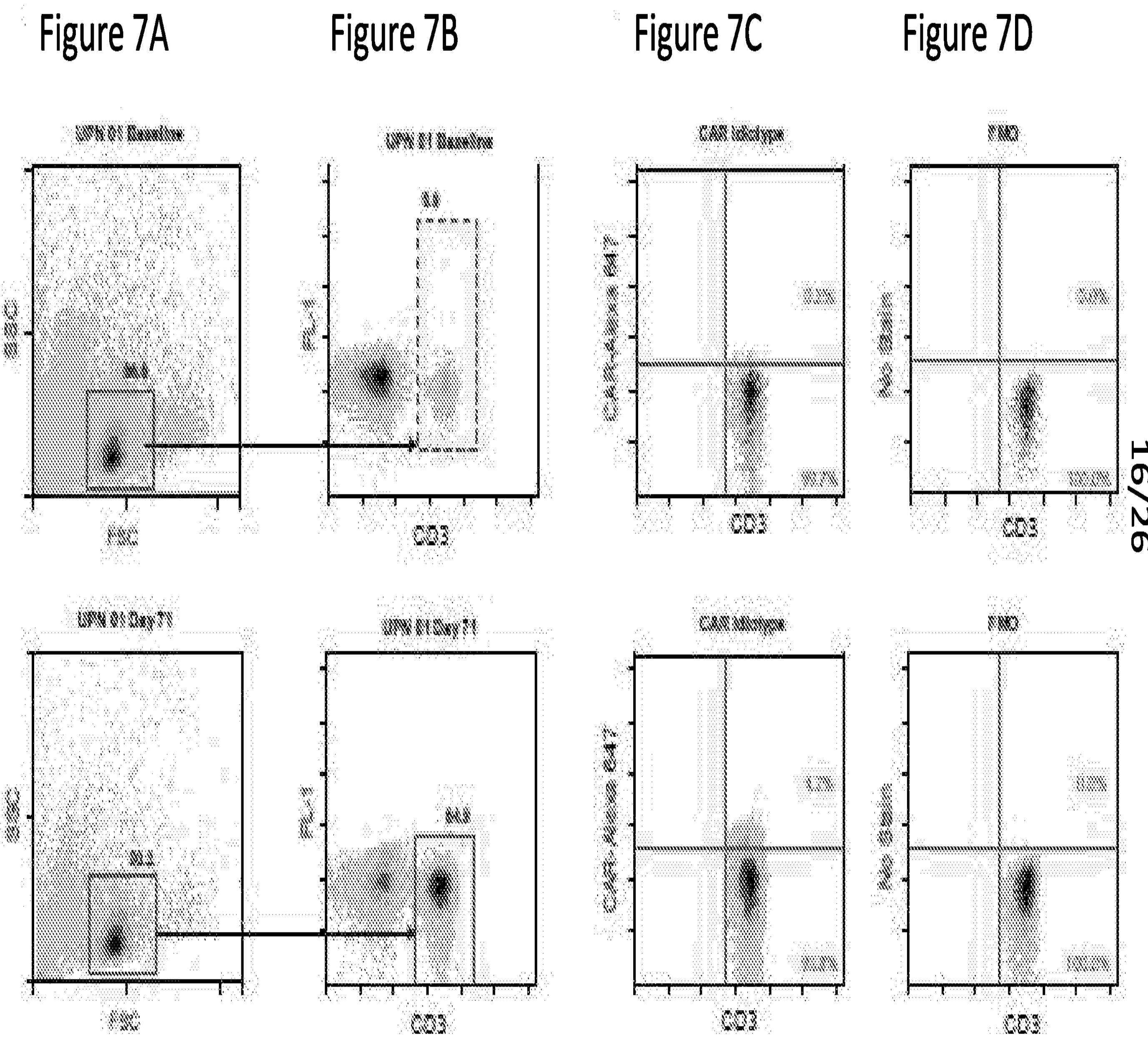
Figure 7A
Figure 7B
Figure 7C
Figure 7D
SSC
FSC
FL-1
CD3
CD3
CD3
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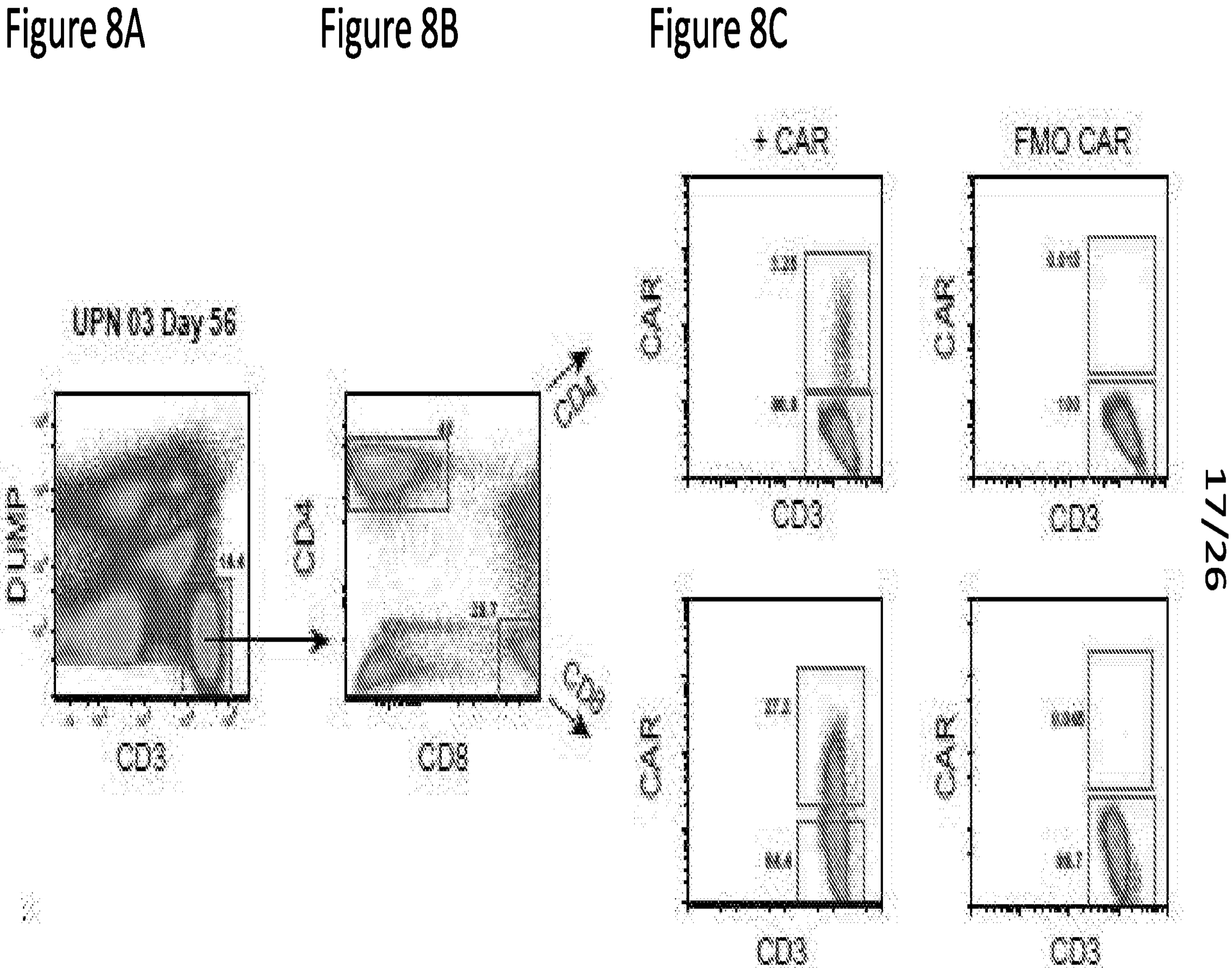
Figure 8A Figure 8B Figure 8C



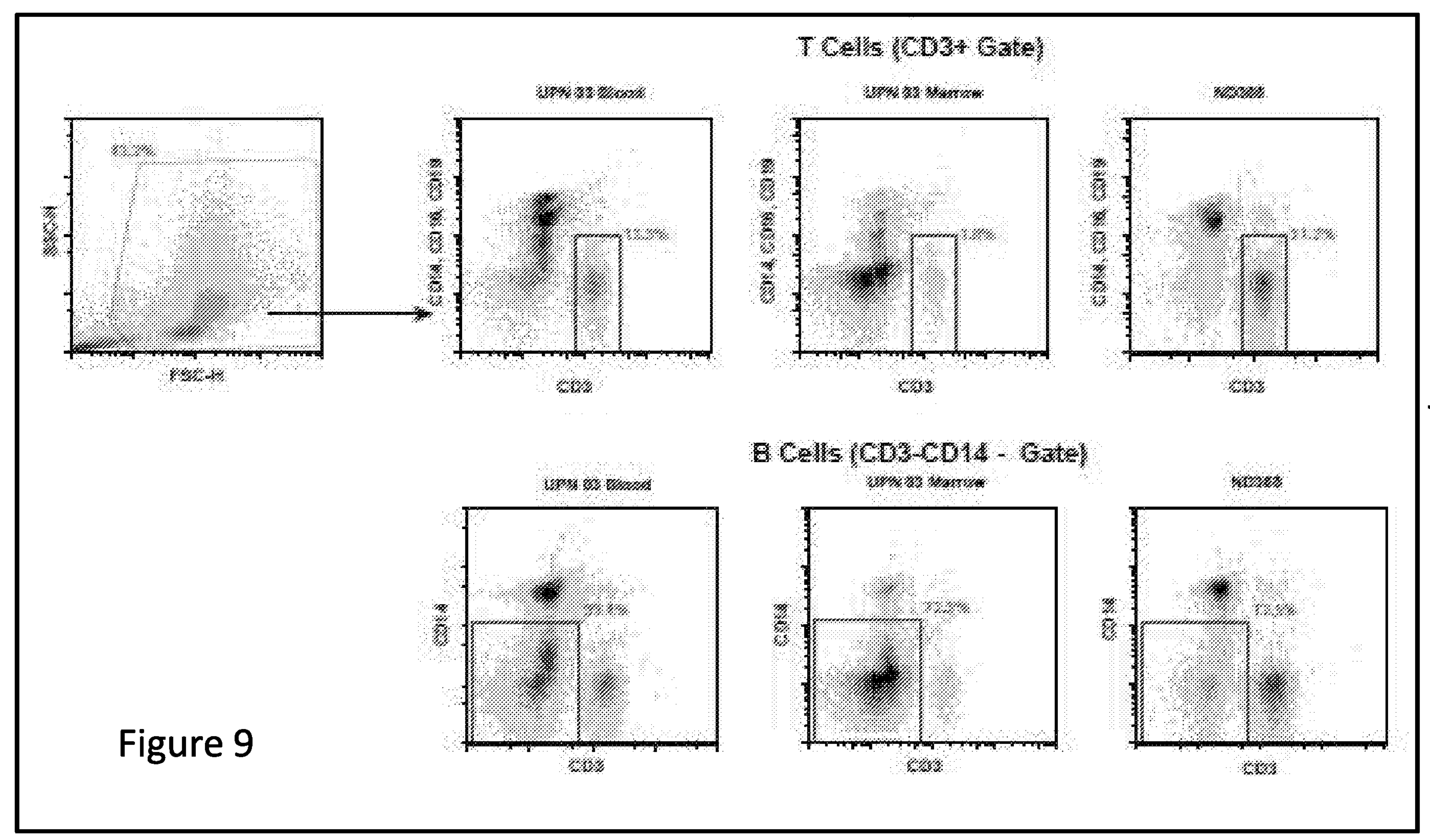



Figure 9

| Subject UPN | Age/sex Karyotype | Previous therapies | CLL Tumor Burden at Baseline | | | Total Dose of CART19 (cells/kg) | Response D +30 (Duration) |
|---|---|---|---|---|---|---|---|
| | | | Bone marrow[3] (Study Day) | Blood[3] (Study Day) | Nodes/spleen[3] (Study Day) | | |
| 01 | 65/M normal | Fludarabine x 4 cycles (2002)<br>Rituximab/fludarabine x 4 cycles (2005)<br>Alemtuzumab x 12 wks (2006)<br>Rituximab (2 courses 2006-2009)<br>R-CVP x 2 cycles (2009)<br>Lenalidomide (2009)<br>PCR x 2 cycles (5/18-6/18/2010)<br>Bendamustine x 1 cycle (7/31-8/1/10) pre-CART19 | Hypercellular<br>70% CLL<br>$2.4x10^{12}$ CLL cells<br>(Day -14)<br>$1.7x10^{12}$ CLL cells<br>(Day -1) | N/A | $8.2x10^{11}$ - $1.0x10^{12}$<br>CLL cells<br>(Day -37) | $1.1x10^{9}$<br>($1.6x10^{7}$/kg) | CR<br>(8+months) |
| 02 | 77/M<br>del(17)(p13)[1] | Alemtuzumab x 16 wks (6/2007)<br>Alemtuzumab x 18 wks (3/2009)<br>Bendamustine/ Rituximab:<br>7/1/2010 (cycle 1)<br>7/28/2010 (cycle 2)<br>8/26/2010 (cycle 3) pre-CART19 | Hypercellular<br>>95% CLL<br>$3.2x10^{12}$ CLL cells<br>(Day -47) | $2.75 x 10^{11}$<br>CLL Cells<br>(Day -1) | $1.2x10^{12}$ -$2.0x10^{12}$<br>CLL cells<br>(Day -24) | $5.8x10^{8}$<br>($1.0x10^{7}$/kg) | PR<br>(5 months) |
| 03 | 64/M<br>del(17)(p13)[2] | R- Fludarabine x 2 cycles (2002)<br>R-Fludarabine x 4 cycles (10/06-1/07)<br>R- Bendamustine x1 cycle (2/09)<br>Bendamustine x 3 cycles (3-6/09)<br>Alemtuzumab x 11 wks (12/09-3/10)<br>Pentostatin/cyclophosphamide (9/10/10) pre-CART19 | Hypercellular<br>40% CLL<br>$8.8x10^{11}$ CLL cells<br>(Day -1) | N/A | $3.3x10^{11}$ - $5.5x10^{11}$<br>CLL cells<br>(Day -10) | $1.4x10^{7}$<br>($1.46x10^{5}$/kg) | CR<br>(7+ months) |

1. UPN 02 Karyotype (ISCN Nomenclature): 45,XY,del(1)(q25),+del(1)(p13),t(2;20)(p13;q11.2),t(3;5)(p13,q35),add(9)(p22),?del(13)(q14q34),-14,del(17)(p13)[cp24]

2. UPN 03 Karyotype (ISCN Nomenclature): 46,XY,del(17)(p12)[18]/44~46,idem,der(17)t(17;21)(p11.2;q11.2)[cp4]/40~45,XY,-17[cp3]

3. See Supplementary Materials for methods of tumor burden determination.

Figure 10

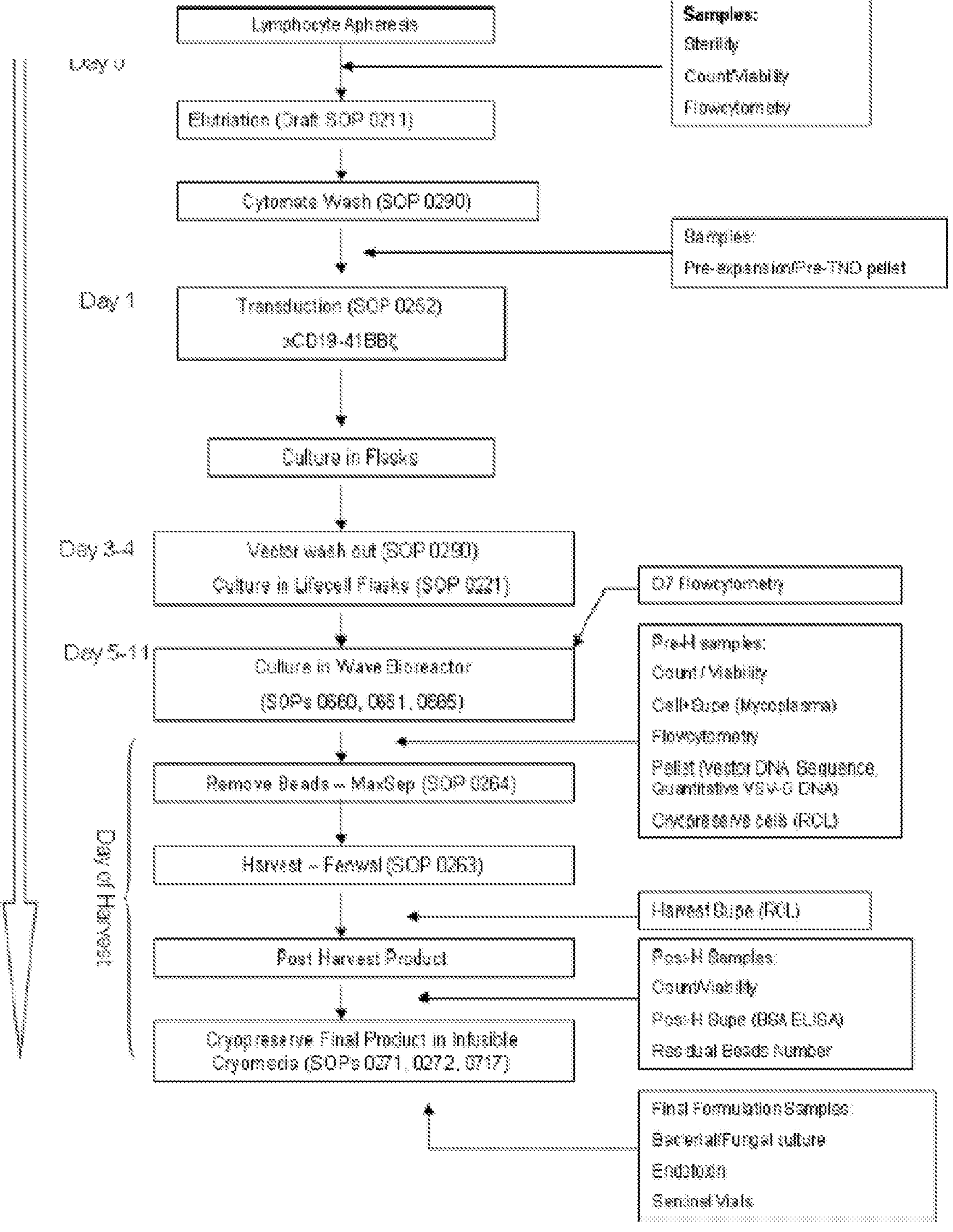



Figure 11

Figure 12A

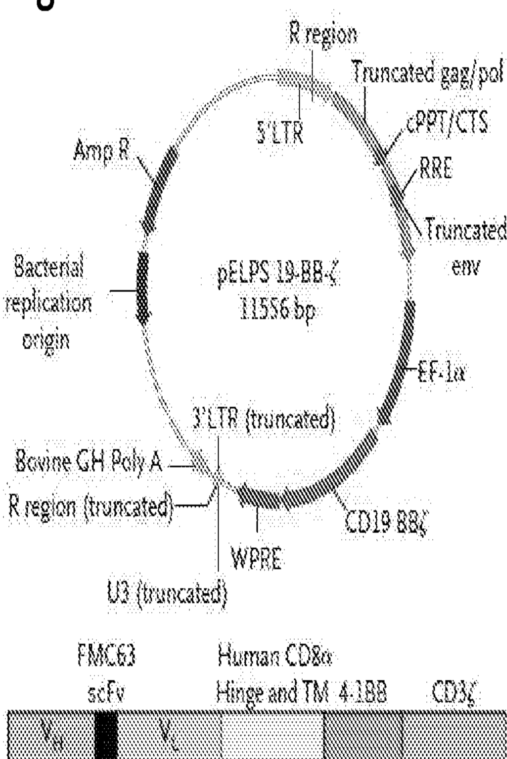
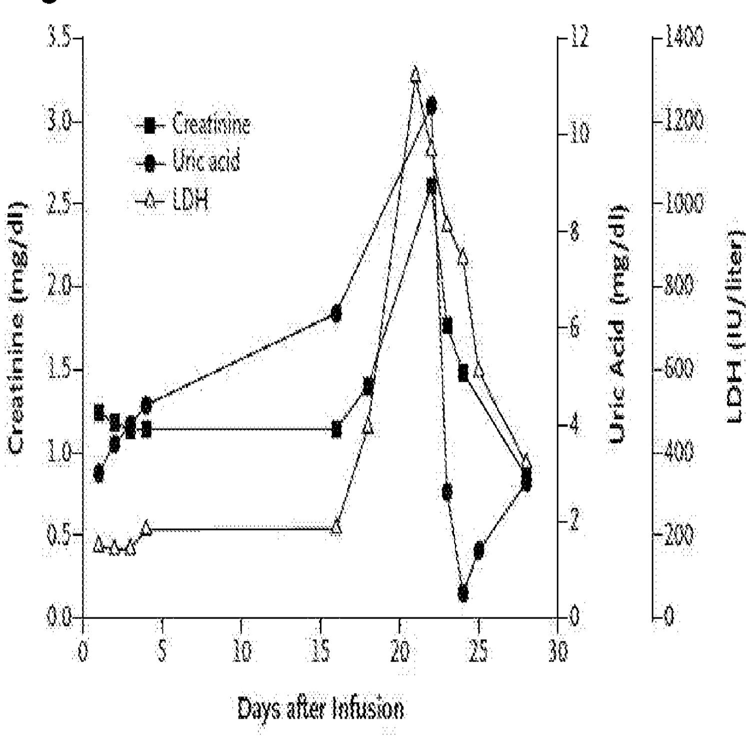
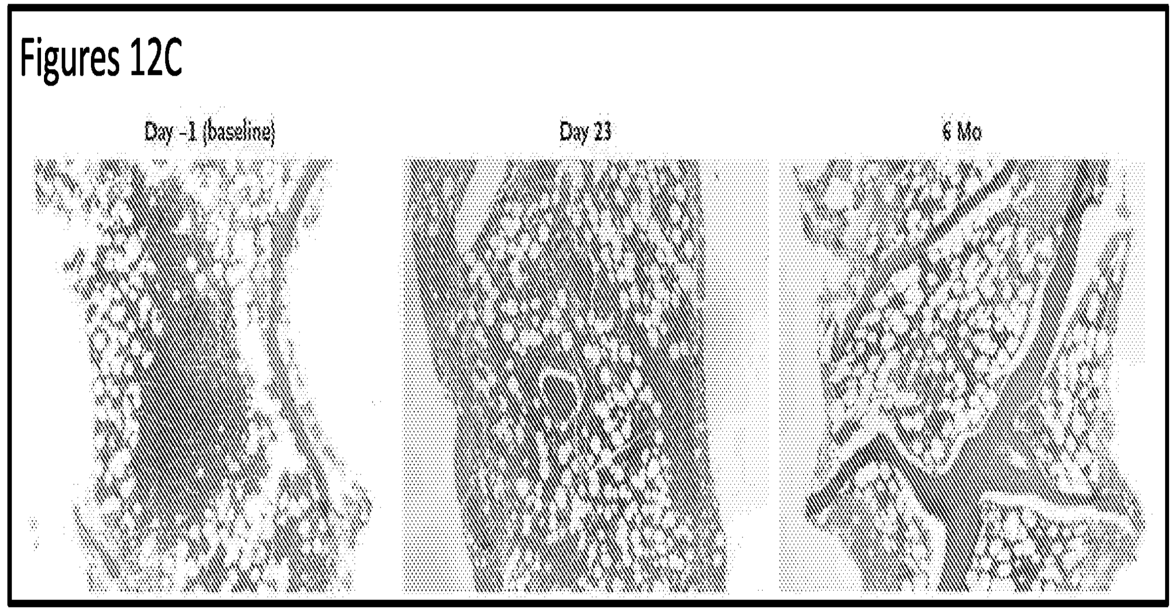
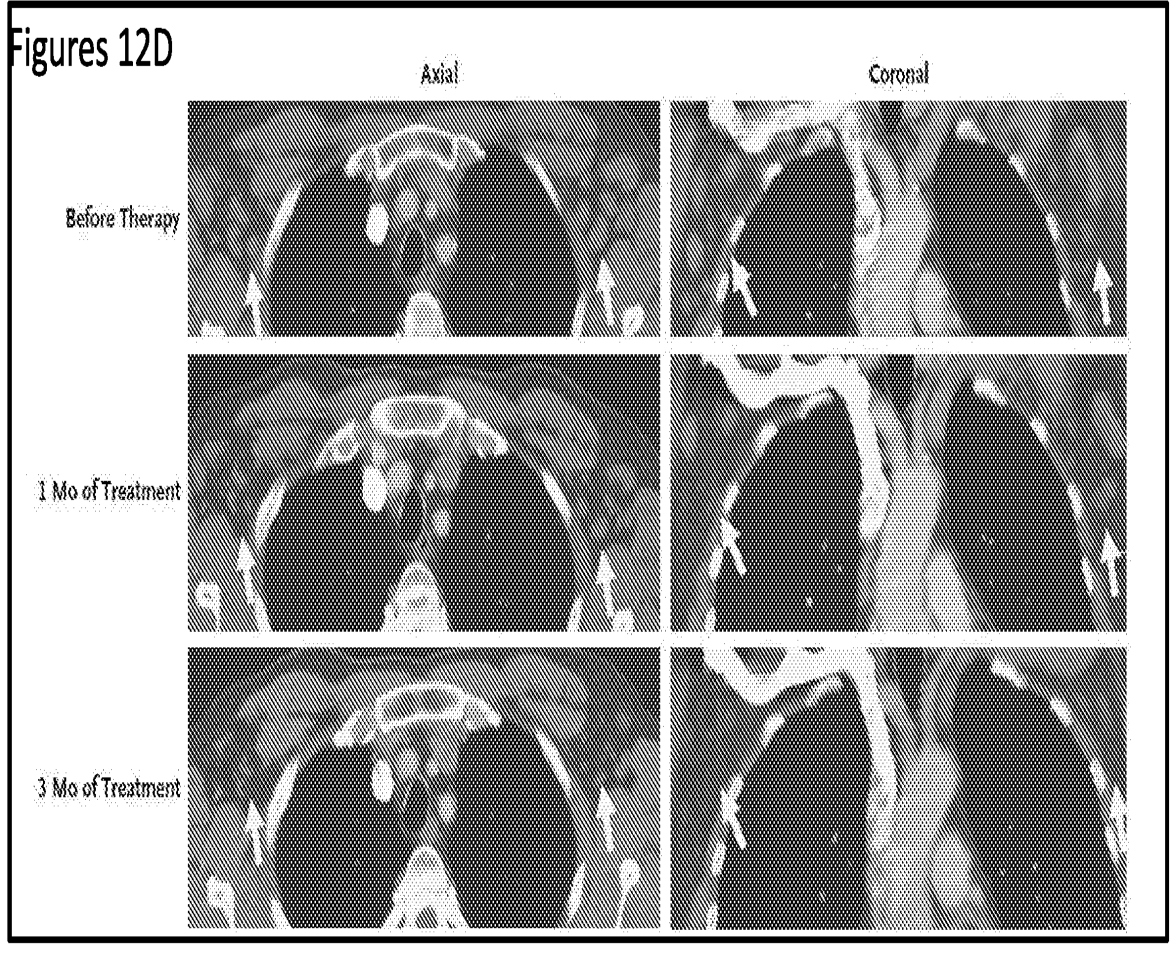

R region
Truncated gag/pol
5'LTR
cPPT/CTS
Amp R
RRE
Truncated env
Bacterial replication origin
pELPS 19-BB-ζ
11556 bp
EF-1α
3'LTR (truncated)
Bovine GH Poly A
R region (truncated)
CD19 BBζ
WPRE
U3 (truncated)
FMC63 scFv
Human CD8α Hinge and TM
4-1BB
CD3ζ
$V_H$
$V_L$

Figure 12B

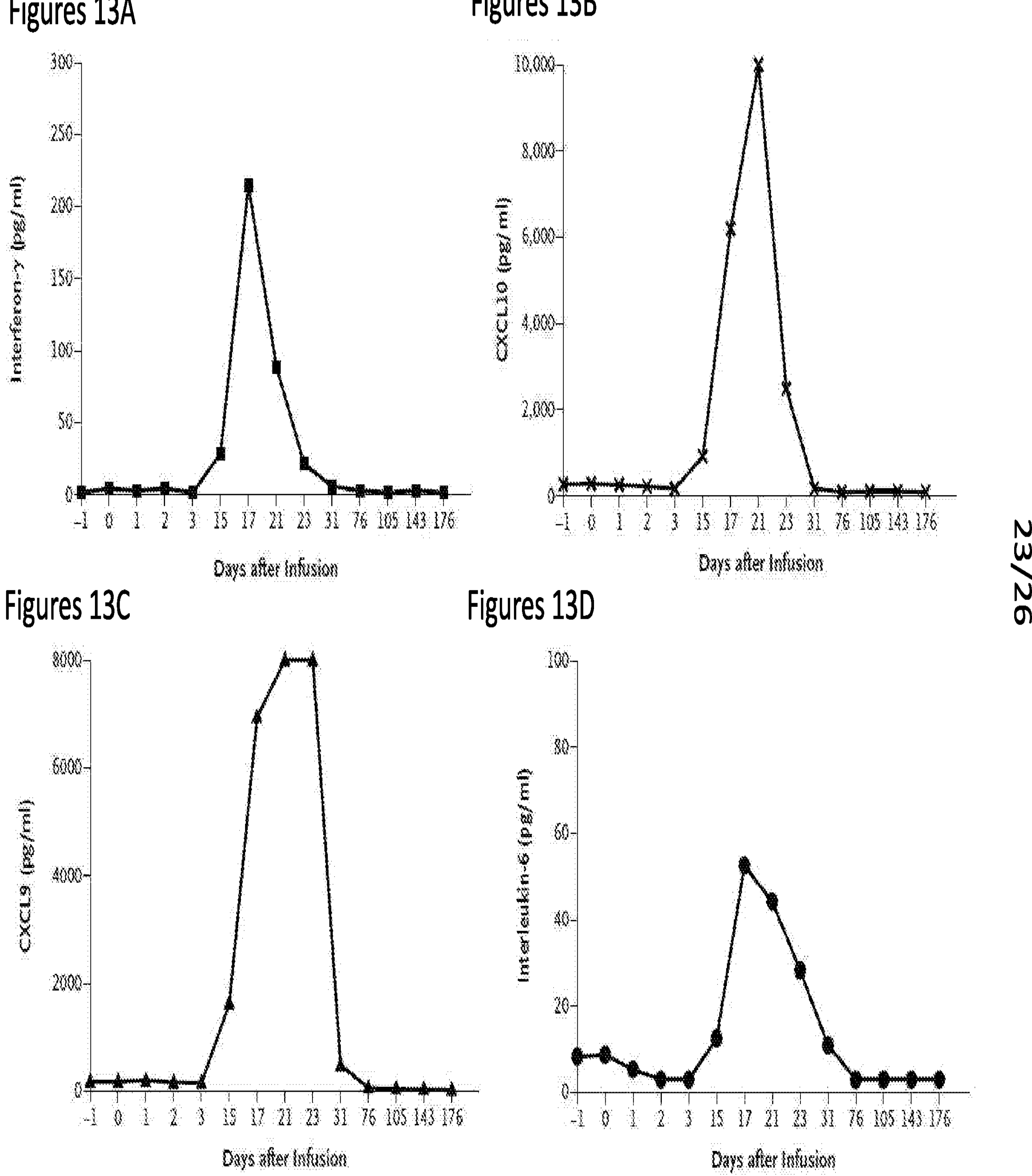

Creatinine
Uric acid
LDH
Creatinine (mg/dl)
Uric Acid (mg/dl)
LDH (IU/liter)
Days after Infusion
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0
2
4
6
8
10
12
200
400
600
800
1000
1200
1400
5
10
15
20
25
30



Figures 12C
Day -1 (baseline)
Day 23
6 Mo

Figures 12D
Axial
Coronal
Before Therapy
1 Mo of Treatment
3 Mo of Treatment




23/26

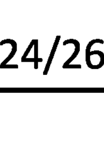


Figure 13E
1 Day before infusion
23 Days after infusion
31 Days after infusion
105 Days after infusion
176 Days after infusion
Concentration (pg/ml)
0
10
20
30
40
50
TNF-α
Interleukin-6
Interferon-γ
Concentration (pg/ml)
0
2,000
4,000
6,000
8,000
10,000
12,000
CXCL9
Interleukin-2 receptor

Figure 14A

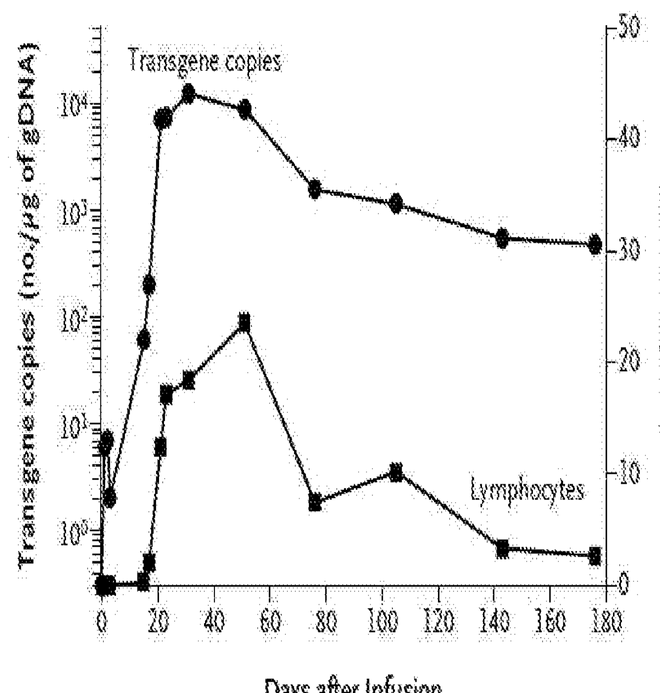
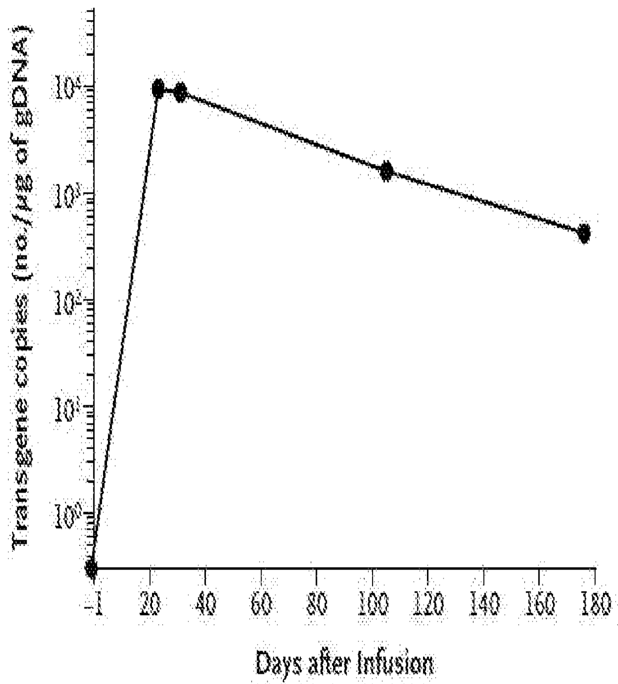


Figure 14B

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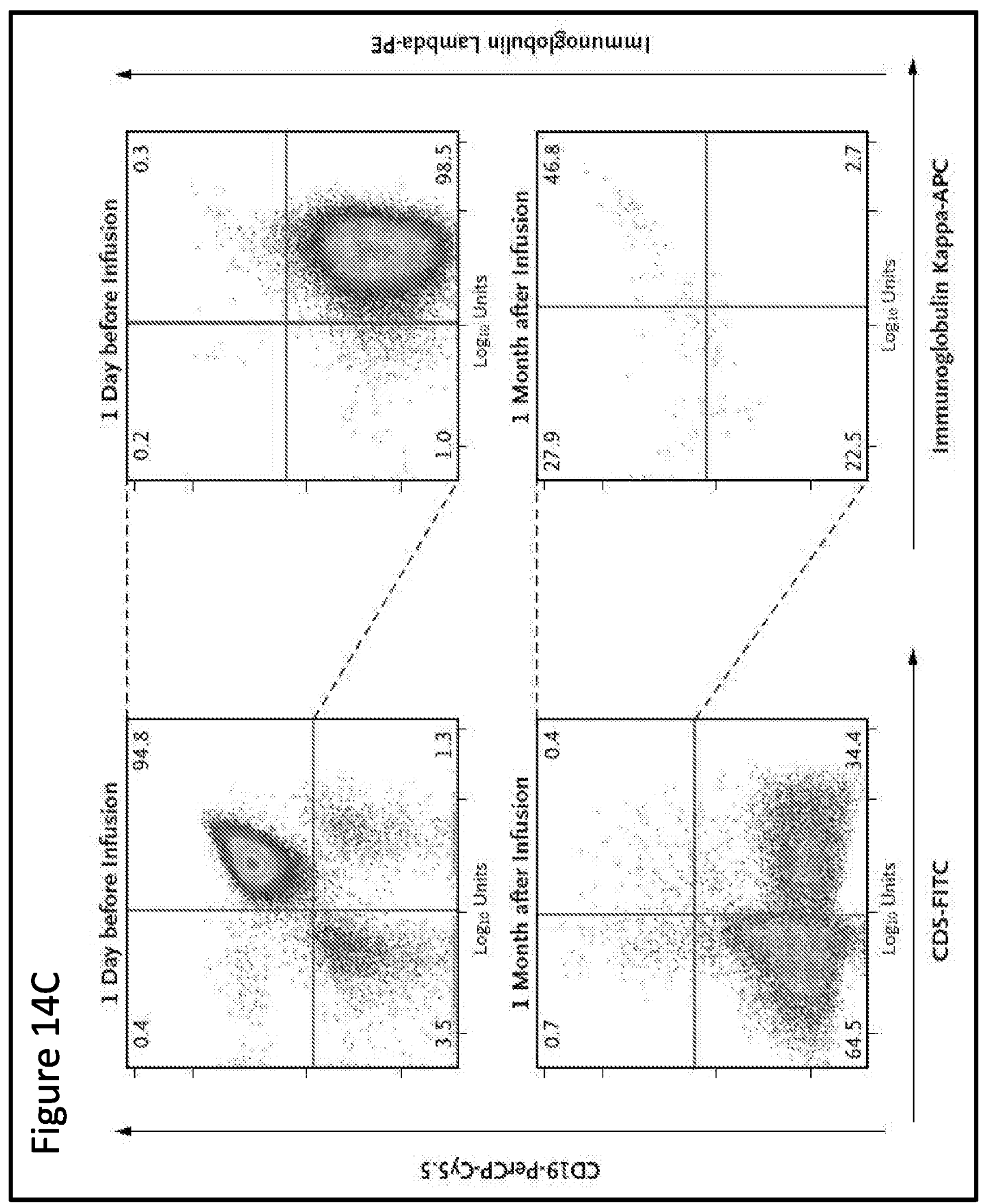


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Page 139

PATENT

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>Not Yet Assigned |
| Serial No.: | Not Yet Assigned | : | Examiner:<br>Not Yet Assigned |
| Filed: | Herewith | : | |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(001088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**INFORMATION DISCLOSURE UNDER 37 CFR 1.97(b)**

The attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached Form PTO-1449. Copies of the cited documents may be found in parent application, Application No. 13/992,622, filed June 7, 2013.

No fee or certification is required in connection with this Information Disclosure, since it is being submitted prior to the last of 1) issuance of a first Office Action on the merits, or 2) expiration of the three-month period following filing of the above-identified application.

It is respectfully requested that the information be considered by the Examiner and that a copy of the attached Form PTO-1449 be returned indicating that such information has been considered.

The references listed in this Information Disclosure Statement comprise the most pertinent prior art known to Applicants and their attorneys as of the date hereof. This Information Disclosure Statement should not be construed as a representation that the cited references are material or that no better art exists.

In the event any fees are required in connection with this paper, please charge Deposit Account No. 50-4364

Applicants' undersigned attorney may be reached by telephone at (215) 972-7734. All correspondence should be directed to the below-listed address.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: January 15, 2016

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| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>To Be Assigned |
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| | **APPLICANT:**<br>Carl H. June et al. | |
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PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**In re Application of:**
Carl H. June, et al.

Group Art Unit: Not Yet Assigned

**Application No.:** To Be Assigned

Examiner: Not Yet Assigned

**Filed:** Herewith

Attorney Docket No.
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**Title:** Compositions and Methods for Treatment of Cancer

Commissioner for Patents
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**STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 CFR §§ 1.821 THROUGH 1.825**

I hereby state, in accordance with the requirements of 37 C.F.R. §1.821(f), that the contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR §1.821(c) and (e), respectively are the same.

I further state that the submission filed in accordance with 37 CFR §1.821(g) does not include new matter.

Respectfully submitted,

CARL H. JUNE, ET AL.

Date: January 15, 2016

By: ______________________________

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SEQUENCE LISTING

<110>  The Trustees of the University of Pennsylvania
       June, Carl H
       Porter, David L
       Kalos, Michael
       Levine, Bruce L
       Milone, Michael C.

<120>  COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

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ggatgtgaac tgagagtgaa gttcagcagg agcgcagacg cccccgcgta caagcagggc      7560
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ccagcggacc ttccttcccg cggcctgctg ccggctctgc ggcctcttcc gcgtcttcgc      8400
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cggtaccttt aagaccaatg acttacaagg cagctgtaga tcttagccac tttttaaaag 8520

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gtactgggtc tctctggtta gaccagatct gagcctggga gctctctggc taactaggga 8640

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tatttatgca gaggccgagg ccgcctcggc ctctgagcta ttccagaagt agtgaggagg 9120

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<210> 2
<211> 228
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthesized

<400> 2
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gccttacaag gagagaaaaa gcaccgtgca tgccgattgg tggaagtaag gtggtacgat 120

cgtgccttat taggaaggca acagacgggt ctgacatgga ttggacgaac cactgaattg 180

ccgcattgca gagatattgt atttaagtgc ctagctcgat acataaac 228

<210> 3
<211> 98
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthesized

<400> 3
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ctgcttaagc ctcaataaag cttgccttga gtgcttcca 98

<210> 4
<211> 85
<212> DNA
<213> Artificial Sequence

<220>

<223>  Chemically Synthesized

<400>  4
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gtcagtgtgg aaaatctcta gcagt                                            85


<210>  5
<211>  1377
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  5
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ttgctgaagc gcgcacggca agaggcgagg ggcggcgact ggtgagtacg ccaaaaattt     120

tgactagcgg aggctagaag gagagagatg ggtgcgagag cgtcagtatt aagcggggga     180

gaattagatc gcgatgggaa aaaattcggt taaggccagg gggaaagaaa aaatataaat     240

taaaacatat agtatgggca agcagggagc tagaacgatt cgcagttaat cctggcctgt     300

tagaaacatc agaaggctgt agacaaatac tgggacagct acaaccatcc cttcagacag     360

gatcagaaga acttagatca ttatataata cagtagcaac cctctattgt gtgcatcaaa     420

ggatagagat aaaagacacc aaggaagctt tagacaagat agaggaagag caaaacaaaa     480

gtaagaccac cgcacagcaa gcggccgctg atcttcagac ctggaggagg agatatgagg     540

gacaattgga gaagtgaatt atataaatat aaagtagtaa aaattgaacc attaggagta     600

gcacccacca aggcaaagag aagagtggtg cagagagaaa aaagagcagt gggaatagga     660

gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc gtcaatgacg     720

ctgacggtac aggccagaca attattgtct ggtatagtgc agcagcagaa caatttgctg     780

agggctattg aggcgcaaca gcatctgttg caactcacag tctggggcat caagcagctc     840

caggcaagaa tcctggctgt ggaaagatac ctaaaggatc aacagctcct ggggatttgg     900

ggttgctctg gaaaactcat ttgcaccact gctgtgcctt ggaatgctag ttggagtaat     960

aaatctctgg aacagatttg gaatcacacg acctggatgg agtgggacag agaaattaac    1020

aattacacaa gcttaataca ctccttaatt gaagaatcgc aaaaccagca agaaaagaat    1080

gaacaagaat tattggaatt agataaatgg gcaagtttgt ggaattggtt taacataaca    1140

aattggctgt ggtatataaa attattcata atgatagtag gaggcttggt aggtttaaga    1200

atagtttttg ctgtactttc tatagtgaat agagttaggc agggatattc accattatcg    1260

tttcagaccc acctcccaac cccgagggga cccgacaggc ccgaaggaat agaagaagaa    1320

ggtggagaga gagacagaga cagatccatt cgattagtga acggatctcg acggtat       1377

<210>  6
<211>  547
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  6
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ggcaagaaac agcatacttc ctcttaaaat tagcaggaag atggccagta aaaacagtac     180

atacagacaa tggcagcaat ttcaccagta ctacagttaa ggccgcctgt tggtgggcgg     240

ggatcaagca ggaatttggc attccctaca atccccaaag tcaaggagta atagaatcta     300

tgaataaaga attaaagaaa attataggac aggtaagaga tcaggctgaa catcttaaga     360

cagcagtaca aatggcagta ttcatccaca attttaaaag aaaagggggg attggggggt     420

acagtgcagg ggaaagaata gtagacataa tagcaacaga catacaaact aaagaattac     480

aaaaacaaat tacaaaaatt caaaattttc gggtttatta cagggacagc agagatccag     540

tttggct                                                               547

<210>  7
<211>  1178
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  7
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gaggggtcgg caattgaacc ggtgcctaga gaaggtggcg cggggtaaac tgggaaagtg     120

atgtcgtgta ctggctccgc ctttttcccg agggtggggg agaaccgtat ataagtgcag     180

tagtcgccgt gaacgttctt tttcgcaacg ggtttgccgc cagaacacag gtaagtgccg     240

tgtgtggttc ccgcgggcct ggcctcttta cgggttatgg cccttgcgtg ccttgaatta     300

cttccacctg gctgcagtac gtgattcttg atcccgagct tcgggttgga agtgggtggg     360

agagttcgag gccttgcgct taaggagccc cttcgcctcg tgcttgagtt gaggcctggc     420

ctgggcgctg gggccgccgc gtgcgaatct ggtggcacct tcgcgcctgt ctcgctgctt     480

tcgataagtc tctagccatt taaaattttt gatgacctgc tgcgacgctt tttttctggc     540

aagatagtct tgtaaatgcg ggccaagatc tgcacactgg tatttcggtt tttggggccg     600

cgggcggcga cggggcccgt gcgtcccagc gcacatgttc ggcgaggcgg ggcctgcgag     660

cgcggccacc gagaatcgga cgggggtagt ctcaagctgg ccggcctgct ctggtgcctg     720

gcctcgcgcc gccgtgtatc gccccgccct gggcggcaag gctggcccgg tcggcaccag     780

ttgcgtgagc ggaaagatgg ccgcttcccg gccctgctgc agggagctca aaatggagga 840

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cctcagccgt cgcttcatgt gactccactg agtaccgggc gccgtccagg cacctcgatt 960

agttctcgag cttttggagt acgtcgtctt taggttgggg ggaggggttt tatgcgatgg 1020

agtttcccca cactgagtgg gtggagactg aagttaggcc agcttggcac ttgatgtaat 1080

tctccttgga atttgccctt tttgagtttg gatcttggtt cattctcaag cctcagacag 1140

tggttcaaag tttttttctt ccatttcagg tgtcgtga 1178

<210> 8
<211> 1459
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthesized

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ccggacatcc agatgacaca gactacatcc tccctgtctg cctctctggg agacagagtc 120

accatcagtt gcagggcaag tcaggacatt agtaaatatt taaattggta tcagcagaaa 180

ccagatggaa ctgttaaact cctgatctac catacatcaa gattacactc aggagtccca 240

tcaaggttca gtggcagtgg gtctggaaca gattattctc tcaccattag caacctggag 300

caagaagata ttgccactta cttttgccaa cagggtaata cgcttccgta cacgttcgga 360

ggggggacca agctggagat cacaggtggc ggtggctcgg gcggtggtgg gtcgggtggc 420

ggcggatctg aggtgaaact gcaggagtca ggacctggcc tggtggcgcc ctcacagagc 480

ctgtccgtca catgcactgt ctcaggggtc tcattacccg actatggtgt aagctggatt 540

cgccagcctc cacgaaaggg tctggagtgg ctgggagtaa tatggggtag tgaaaccaca 600

tactataatt cagctctcaa atccagactg accatcatca aggacaactc caagagccaa 660

gttttcttaa aaatgaacag tctgcaaact gatgacacag ccatttacta ctgtgccaaa 720

cattattact acggtggtag ctatgctatg gactactggg gccaaggaac ctcagtcacc 780

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tatatattca aacaaccatt tatgagacca gtacaaacta ctcaagagga agatggctgt 1080

agctgccgat ttccagaaga agaagaagga ggatgtgaac tgagagtgaa gttcagcagg 1140

agcgcagacg cccccgcgta caagcagggc cagaaccagc tctataacga gctcaatcta 1200

ggacgaagag aggagtacga tgttttggac aagagacgtg gccgggaccc tgagatgggg 1260

ggaaagccga gaaggaagaa ccctcaggaa ggcctgtaca atgaactgca gaaagataag 1320

atggcggagg cctacagtga gattgggatg aaaggcgagc gccggagggg caaggggcac 1380

gatggccttt accagggtct cagtacagcc accaaggaca cctacgacgc ccttcacatg 1440

caggccctgc cccctcgct 1459

<210> 9
<211> 591
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthesized

<400> 9
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cttttacgct atgtggatac gctgctttaa tgcctttgta tcatgctatt gcttcccgta 120

tggctttcat tttctcctcc ttgtataaat cctggttgct gtctctttat gaggagttgt 180

ggcccgttgt caggcaacgt ggcgtggtgt gcactgtgtt tgctgacgca acccccactg 240

gttggggcat tgccaccacc tgtcagctcc tttccgggac tttcgctttc cccctcccta 300

ttgccacggc ggaactcatc gccgcctgcc ttgcccgctg ctggacaggg gctcggctgt 360

tgggcactga caattccgtg gtgttgtcgg ggaagctgac gtcctttcca tggctgctcg 420

cctgtgttgc cacctggatt ctgcgcggga cgtccttctg ctacgtccct tcggccctca 480

atccagcgga ccttccttcc cgcggcctgc tgccggctct gcggcctctt ccgcgtcttc 540

gccttcgccc tcagacgagt cggatctccc tttgggccgc ctccccgcct g 591

<210> 10
<211> 98
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthezied

<400> 10
gggtctctct ggttagacca gatctgagcc tgggagctct ctggctaact agggaaccca 60

ctgcttaagc ctcaataaag cttgccttga gtgcttca 98

<210> 11
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> Chemically Synthesized

<400>  11
agtagtgtgt gcccgtctgt tgtgtgactc tggtaactag agatccctca gacccttttta      60

gtcagtgtgg aaaatctcta gcag                                              84

<210>  12
<211>  486
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  12

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu
            20                  25                  30

Ser Ala Ser Leu Gly Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln
        35                  40                  45

Asp Ile Ser Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr
    50                  55                  60

Val Lys Leu Leu Ile Tyr His Thr Ser Arg Leu His Ser Gly Val Pro
65                  70                  75                  80

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile
                85                  90                  95

Ser Asn Leu Glu Gln Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly
            100                 105                 110

Asn Thr Leu Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr
        115                 120                 125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
    130                 135                 140

Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser
145                 150                 155                 160

Leu Ser Val Thr Cys Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly
                165                 170                 175

Val Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly
            180                 185                 190
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```
Val Ile Trp Gly Ser Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser
        195                 200                 205

Arg Leu Thr Ile Ile Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys
    210                 215                 220

Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys
225                 230                 235                 240

His Tyr Tyr Tyr Gly Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly
                245                 250                 255

Thr Ser Val Thr Val Ser Ser Thr Thr Thr Pro Ala Pro Arg Pro Pro
            260                 265                 270

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
        275                 280                 285

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
    290                 295                 300

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
305                 310                 315                 320

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
                325                 330                 335

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
            340                 345                 350

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
        355                 360                 365

Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
    370                 375                 380

Pro Ala Tyr Lys Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
385                 390                 395                 400

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
                405                 410                 415

Pro Glu Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu
            420                 425                 430

Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile
        435                 440                 445
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```
Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr
    450                 455                 460

Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met
465                 470                 475                 480

Gln Ala Leu Pro Pro Arg
                485


<210>  13
<211>  63
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  13
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ccg                                                                    63


<210>  14
<211>  726
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  14
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atcagttgca gggcaagtca ggacattagt aaatatttaa attggtatca gcagaaacca     120

gatggaactg ttaaactcct gatctaccat acatcaagat tacactcagg agtcccatca     180

aggttcagtg gcagtgggtc tggaacagat tattctctca ccattagcaa cctggagcaa     240

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tccgtcacat gcactgtctc aggggtctca ttacccgact atggtgtaag ctggattcgc     480

cagcctccac gaaagggtct ggagtggctg ggagtaatat ggggtagtga aaccacatac     540

tataattcag ctctcaaatc cagactgacc atcatcaagg acaactccaa gagccaagtt     600

ttcttaaaaa tgaacagtct gcaaactgat gacacagcca tttactactg tgccaaacat     660

tattactacg gtggtagcta tgctatggac tactggggcc aaggaacctc agtcaccgtc     720

tcctca                                                                726


<210>  15
<211>  135
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<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  15
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tccctgcgcc cagaggcgtg ccggccagcg gcggggggcg cagtgcacac gagggggctg      120

gacttcgcct gtgat                                                       135


<210>  16
<211>  72
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  16
atctacatct gggcgccctt ggccgggact tgtggggtcc ttctcctgtc actggttatc       60

accctttact gc                                                           72


<210>  17
<211>  126
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  17
aaacggggca gaaagaaact cctgtatata ttcaaacaac catttatgag accagtacaa       60

actactcaag aggaagatgg ctgtagctgc cgatttccag aagaagaaga aggaggatgt      120

gaactg                                                                 126


<210>  18
<211>  336
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  18
agagtgaagt tcagcaggag cgcagacgcc cccgcgtaca agcagggcca gaaccagctc       60

tataacgagc tcaatctagg acgaagagag gagtacgatg ttttggacaa gagacgtggc      120

cgggaccctg agatgggggg aaagccgaga aggaagaacc ctcaggaagg cctgtacaat      180

gaactgcaga aagataagat ggcggaggcc tacagtgaga ttgggatgaa aggcgagcgc      240

cggaggggca aggggcacga tggcctttac cagggtctca gtacagccac caaggacacc      300

tacgacgccc ttcacatgca ggccctgccc cctcgc                                336

<210>  19
<211>  21
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  19

```
Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15


His Ala Ala Arg Pro
            20
```

<210>  20
<211>  242
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  20

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Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1               5                   10                  15


Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Lys Tyr
            20                  25                  30


Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35                  40                  45


Tyr His Thr Ser Arg Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
65                  70                  75                  80


Glu Asp Ile Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Tyr
                85                  90                  95


Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Thr Gly Gly Gly Gly Ser
            100                 105                 110


Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Lys Leu Gln Glu
        115                 120                 125


Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Val Thr Cys
    130                 135                 140
```

```
Thr Val Ser Gly Val Ser Leu Pro Asp Tyr Gly Val Ser Trp Ile Arg
145                 150                 155                 160

Gln Pro Pro Arg Lys Gly Leu Glu Trp Leu Gly Val Ile Trp Gly Ser
                165                 170                 175

Glu Thr Thr Tyr Tyr Asn Ser Ala Leu Lys Ser Arg Leu Thr Ile Ile
            180                 185                 190

Lys Asp Asn Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln
        195                 200                 205

Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Lys His Tyr Tyr Tyr Gly
    210                 215                 220

Gly Ser Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val
225                 230                 235                 240

Ser Ser


<210>  21
<211>  47
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Chemically Synthesized

<400>  21

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1               5                   10                  15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
            20                  25                  30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp Ile Tyr
        35                  40                  45


<210>  22
<211>  22
<212>  PRT
<213>  Artificial Sequence

<220>
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<400>  22

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PTO/SB/424 (12-11)

## CERTIFICATION AND REQUEST FOR PRIORITIZED EXAMINATION UNDER 37 CFR 1.102(e) (Page 1 of 1)

| First Named Inventor: | Carl H. June, et al. | Nonprovisional Application Number (if known): | |
|---|---|---|---|
| Title of Invention: | Compositions and Methods for Treatment of Cancer | | |

**APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS PRIORITIZED EXAMINATION FOR THE ABOVE-IDENTIFIED APPLICATION.**

1. The processing fee set forth in 37 CFR 1.17(i), the prioritized examination fee set forth in 37 CFR 1.17(c), and if not already paid, the publication fee set forth in 37 CFR 1.18(d) have been filed with the request. The basic filing fee, search fee, examination fee, and any required excess claims and application size fees are filed with the request or have been already been paid.

2. The application contains or is amended to contain no more than four independent claims and no more than thirty total claims, and no multiple dependent claims.

3. The applicable box is checked below:

I. ☑ **Original Application (Track One) - Prioritized Examination under § 1.102(e)(1)**

i. (a) The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a). This certification and request is being filed with the utility application via EFS-Web.
---OR---
(b) The application is an original nonprovisional plant application filed under 35 U.S.C. 111(a). This certification and request is being filed with the plant application in paper.

ii. An executed oath or declaration under 37 CFR 1.63 is filed with the application.

II. ☐ **Request for Continued Examination - Prioritized Examination under § 1.102(e)(2)**

i. A request for continued examination has been filed with, or prior to, this form.
ii. If the application is a utility application, this certification and request is being filed via EFS-Web.
iii. The application is an original nonprovisional utility application filed under 35 U.S.C. 111(a), or is a national stage entry under 35 U.S.C. 371.
iv. This certification and request is being filed prior to the mailing of a first Office action responsive to the request for continued examination.
v. No prior request for continued examination has been granted prioritized examination status under 37 CFR 1.102(e)(2).

| Signature /Kathryn Doyle/ | Date January 15, 2016 |
|---|---|
| Name (Print/Typed) Kathryn Doyle, Ph.D., J.D. | Practitioner Registration Number 36,317 |

***Note:*** *Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required in accordance with 37 CFR 1.33 and 11.18. Please see 37 CFR 1.4(d) for the form of the signature. If necessary, submit multiple forms for more than one signature, see below*.*

☐ *Total of ________ forms are submitted.

# Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

# DECLARATION FOR PATENT APPLICATION

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

ATTORNEY DOCKET NO.: 046483-6001-00-US.602564

As a below named inventor, I hereby declare that:

## Use of Chimeric Antigen Receptor-Modified T-Cells to Treat Cancer

The specification of which:

is attached hereto; or

was filed as United States application Serial No. 13/992,622 on June 7, 2013 ; and
was filed as PCT international application Number PCT/US2011/064191 on December 9, 2011.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate or Section 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign applications(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

PRIOR FOREIGN APPLICATION(S):

| COUNTRY (If PCT, indicate PCT) | APPLICATION NUMBER | DATE OF FILING (day, month, year) | PRIORITY CLAIMED |
|---|---|---|---|
| | | | ☐ Yes ☐ No |
| | | | ☐ Yes ☐ No |
| | | | ☐ Yes ☐ No |
| | | | ☐ Yes ☐ No |

I hereby claim the benefits under Title 35, United States Code Section 119(e) of any United States provisional application(s) listed below.

| U.S. PROVISIONAL APPLICATIONS | |
|---|---|
| U.S. PROVISIONAL APPLICATION NO. | U.S. FILING DATE: |
| 61/421,470 | December 9, 2010 |
| 61/502,649 | June 29, 2011 |
| | |

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT:

| U.S. OR PCT INTERNATIONAL APPLICATIONS | | STATUS (Check One) | | |
|---|---|---|---|---|
| APPLICATION NO. | FILING DATE | PATENTED | PENDING | ABANDONED |
| | | ☐ | ☐ | ☐ |
| | | ☐ | ☐ | ☐ |
| | | ☐ | ☐ | ☐ |
| | | ☐ | ☐ | ☐ |

Direct Telephone Calls To:

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317 at
215-268-3888



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Miltenyi v. UPenn
IPR2022-00855
Page 174

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

| | | | |
|---|---|---|---|
| FULL NAME OF SOLE OR FIRST INVENTOR | Carl H. JUNE | | |
| RESIDENCE & CITIZENSHIP | Merion Station, Pennsylvania | COUNTRY OF CITIZENSHIP US | |
| POST OFFICE ADDRESS | 409 Baird Road<br>Merion Station, PA 19066<br>United States of America | | |
| FIRST OR SOLE INVENTOR'S SIGNATURE | | DATE 7/1/2013 | |
| FULL NAME OF SECOND INVENTOR | Bruce L. LEVINE | | |
| RESIDENCE & CITIZENSHIP | Cherry Hill, New Jersey | COUNTRY OF CITIZENSHIP US | |
| POST OFFICE ADDRESS | 1258 Liberty Bell Drive<br>Cherry Hill, NJ 08003<br>United States of America | | |
| SECOND INVENTOR'S SIGNATURE | | DATE 7/1/2013 | |
| FULL NAME OF THIRD INVENTOR | David L. PORTER | | |
| RESIDENCE & CITIZENSHIP | Springfield, Pennsylvania | COUNTRY OF CITIZENSHIP US | |
| POST OFFICE ADDRESS | 821 Crum Creek Road<br>Springfield, PA 19064<br>United States of America | | |
| THIRD INVENTOR'S SIGNATURE | | DATE 7-1-13 | |

Listing of Inventors Continued on attached page(s): ☒ Yes ☐ No



UPenn Ex. 2047
Miltenyi v. UPenn
IPR2022-00855
Page 175

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

| FULL NAME OF FOURTH INVENTOR | Michael D. KALOS | |
|---|---|---|
| RESIDENCE & CITIZENSHIP | Philadelphia, Pennsylvania | COUNTRY OF CITIZENSHIP US |
| POST OFFICE ADDRESS | 716 Carpenter Lane<br>Philadelphia, PA 19119<br>United States of America | |
| FOURTH INVENTOR'S SIGNATURE | | DATE June 21, 2013 |
| FULL NAME OF FIFTH INVENTOR | Michael C. MILONE | |
| RESIDENCE & CITIZENSHIP | Cherry Hill, New Jersey | COUNTRY OF CITIZENSHIP US |
| POST OFFICE ADDRESS | 314 Surrey Road<br>Cherry Hill, NJ 08002<br>United States of America | |
| FIFTH INVENTOR'S SIGNATURE | | DATE Jun 30, 2013 |
| FULL NAME OF SIXTH INVENTOR | | |
| RESIDENCE & CITIZENSHIP | | COUNTRY OF CITIZENSHIP |
| POST OFFICE ADDRESS | | |
| SIXTH INVENTOR'S SIGNATURE | | DATE |

Listing of Inventors Continued on attached page(s): ☐ Yes ☒ No



UPenn Ex. 2047
Miltenyi v. UPenn
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Page 176

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | |
| **Filing Date:** | |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Attorney Docket Number:** | 046483-6001US13(01088) |

Filed as Large Entity

**Filing Fees for** **Track I Prioritized Examination - Nonprovisional Application under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| Utility application filing | 1011 | 1 | 280 | 280 |
| Utility Search Fee | 1111 | 1 | 600 | 600 |
| Utility Examination Fee | 1311 | 1 | 720 | 720 |
| Request for Prioritized Examination | 1817 | 1 | 4000 | 4000 |
| **Pages:** | | | | |
| **Claims:** | | | | |
| Claims in Excess of 20 | 1202 | 10 | 80 | 800 |
| **Miscellaneous-Filing:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **6400** |

# Electronic Acknowledgement Receipt

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|---|---|
| **EFS ID:** | 24641752 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 15-JAN-2016 |
| **Filing Date:** | |
| **Time Stamp:** | 17:27:52 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $6400 |
| RAM confirmation Number | 4593 |
| Deposit Account | 504364 |
| Authorized User | DOYLE, KATHRYN |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Application Data Sheet | 046483-6001US13_Application_Data_Sheet.pdf | 1823607<br>6a6f9a8e440ca832a0cc90b9bde4604cfcdd1547 | no | 10 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Preliminary Amendment | 046483-6001US13_Preliminary_Amendment.pdf | 188228<br>2af6a3ff59227cf24766345b373bfe5c33d0c834 | no | 9 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Specification | 046483-6010US13_specification.pdf | 1526646<br>7784f0813ea7c71f7ea34aed8d7b11a7550d56dc | no | 102 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Drawings-other than black and white line drawings | 046483_6001US13_drawings.pdf | 1454540<br>a83af818a9afff2493378aaf70e82f89863e00bb | no | 26 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Information Disclosure Statement (IDS) Form (SB08) | 046483-6001US13_IDS_Support_Statement.pdf | 134031<br>309f3acad03e31f270d606534143e8c6b479cdbb | no | 2 |
| **Warnings:** | | | | | |
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| This is not an USPTO supplied IDS fillable form | | | | | |
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| 7 | CRF Statement Paper and CRF are the same | 046483-6001US13_Sequence_support_statement.pdf | 130365<br>7c59b47b3587491bc9597957e9ad81df4be39e4c | no | 1 |
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| 9 | Power of Attorney | 046483_6001US13_Power_of_Attorney.pdf | 939415<br>354d8a54a2e8092f03f4e066084a50c038f01dc1 | no | 1 |
| **Warnings:** | | | | | |
| The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing | | | | | |
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| 10 | Miscellaneous Incoming Letter | 046483-6001US13_Statement_under_37_CFR_3_73b.pdf | 74916<br>df453791812283d14d1ba861852ea124d5d0cba2 | no | 2 |
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| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 7135707 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |

| Title of Invention | Compositions and Methods for Treatment of Cancer |
|---|---|

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

## Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

## Inventor Information:

**Inventor 1** Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Carl | H. | June | |

**Residence Information (Select One)** ● US Residency ○ Non US Residency ○ Active US Military Service

| City | Merion Station | State/Province | PA | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| Address 1 | 409 Baird Road | | |
|---|---|---|---|
| Address 2 | | | |
| City | Merion Station | State/Province | PA |
| Postal Code | 19066 | Country | US |

**Inventor 2** Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Bruce | L. | Levine | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | Cherry Hill | State/Province | NJ | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| Address 1 | 1258 Liberty Bell Drive | | |
|---|---|---|---|
| Address 2 | | | |
| City | Cherry Hill | State/Province | NJ |
| Postal Code | 08003 | Country | US |

**Inventor 3** Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | David | L. | Porter | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service



UPenn Ex. 2047
Miltenyi v. UPenn
IPR2022-00855
Page 183

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

| City | Springfield | State/Province | PA | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| Address 1 | 321 Crum Creek Road | | |
|---|---|---|---|
| Address 2 | | | |
| City | Springfield | State/Province | PA |
| Postal Code | 19064 | Country | US |

**Inventor** 4 Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Michael | D. | Kalos | |

**Residence Information (Select One)** ◉ US Residency  Non US Residency  Active US Military Service

| City | Philadelphila | State/Province | PA | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| Address 1 | 716 Carpenter Lane | | |
|---|---|---|---|
| Address 2 | | | |
| City | Philadelphia | State/Province | PA |
| Postal Code | 19119 | Country | US |

**Inventor** 5 Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Michael | C. | Milone | |

**Residence Information (Select One)** ◉ US Residency  Non US Residency  Active US Military Service

| City | Cherry Hill | State/Province | NJ | Country of Residence | US |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| Address 1 | 314 Surrey Road | | |
|---|---|---|---|
| Address 2 | | | |
| City | Cherry Hill | State/Province | NJ |
| Postal Code | 08002 | Country | US |

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

## Correspondence Information:

**Enter either Customer Number or complete the Correspondence Information section below.**
**For further information see 37 CFR 1.33(a).**

☐ **An Address is being provided for the correspondence Information of this application.**



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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
| --- | --- | --- |
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

| | | | |
| --- | --- | --- | --- |
| **Customer Number** | 78905 | | |
| **Email Address** | IPGroupMailbox@saul.com | Add Email | Remove Email |

## Application Information:

| | | | |
| --- | --- | --- | --- |
| **Title of the Invention** | Compositions and Methods for Treatment of Cancer | | |
| **Attorney Docket Number** | 046483-6001US13(01088) | **Small Entity Status Claimed** | ☐ |
| **Application Type** | Nonprovisional | | |
| **Subject Matter** | Utility | | |
| **Total Number of Drawing Sheets (if any)** | 26 | **Suggested Figure for Publication (if any)** | |

## Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

| Application number of the previously filed application | Filing date (YYYY-MM-DD) | Intellectual Property Authority or Country |
| --- | --- | --- |
| | | |

## Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

## Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

| | | | |
| --- | --- | --- | --- |
| Please Select One: | ● Customer Number | US Patent Practitioner | ○ Limited Recognition (37 CFR 11.9) |
| Customer Number | 78905 | | |

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

## Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the “Application Number” field blank.

| Prior Application Status | Pending | | Remove |
|---|---|---|---|
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| | Continuation of | 13992622 | 2013-07-09 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| 13992622 | a 371 of international | PCT/US2011/064191 | 2011-12-09 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| PCT/US2011/064191 | Claims benefit of provisional | 61421470 | 2010-12-09 |
| Prior Application Status | Expired | | Remove |
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| PCT/US2011/064191 | Claims benefit of provisional | 61502649 | 2011-06-29 |

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

## Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

| Application Number | Country[i] | Filing Date (YYYY-MM-DD) | Access Code[i] (if applicable) |
|---|---|---|---|
| | | | |

Remove

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

## Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |

| Title of Invention | Compositions and Methods for Treatment of Cancer |
|---|---|

## Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

**NOTE**: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

**1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)**

**A. Priority Document Exchange (PDX)** - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

**B. Search Results from U.S. Application to EPO** - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

**2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)**

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

**NOTE:** Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

## Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

**Applicant** 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section. Clear

| ● Assignee | Legal Representative under 35 U.S.C. 117 | Joint Inventor |
|---|---|---|
| Person to whom the inventor is obligated to assign. | Person who shows sufficient proprietary interest | |

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

| Name of the Deceased or Legally Incapacitated Inventor: | | | |
|---|---|---|---|
| If the Applicant is an Organization check here. | ☒ | | |
| Organization Name | The Trustees of the University of Pennsylvania | | |
| **Mailing Address Information For Applicant:** | | | |
| **Address 1** | Center for Technology Transfer | | |
| Address 2 | 3160 Chestnut Street, Suite 200 | | |
| **City** | Philadelphia | **State/Province** | PA |
| **Country** | US | Postal Code | 19104 |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Applicant Data may be generated within this form by selecting the Add button. Add

## Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (11-15)
Approved for use through 04/30/2017. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

**Assignee 1**

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

| Prefix | **Given Name** | Middle Name | **Family Name** | Suffix |
|---|---|---|---|---|
| | | | | |

**Mailing Address Information For Assignee including Non-Applicant Assignee:**

| **Address 1** | | | |
|---|---|---|---|
| Address 2 | | | |
| **City** | | **State/Province** | |
| **Country** i | | Postal Code | |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

## Signature:

Remove

**NOTE:** This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

| Signature | /Kathryn Doyle/ | | | Date (YYYY-MM-DD) | 2016-01-15 |
|---|---|---|---|---|---|
| First Name | Kathryn | Last Name | Doyle | Registration Number | 36,317 |

Additional Signature may be generated within this form by selecting the Add button. Add

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 046483-6001US13(01088) |
|---|---|---|
| | Application Number | |
| Title of Invention | Compositions and Methods for Treatment of Cancer | |

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

# Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

PTO/SB/80 (11-08)
Approved for use through 11/30/2011. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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# POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

☑ Practitioners associated with the Customer Number: 78905

*OR*

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

| Name | Registration Number | Name | Registration Number |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

☑ The address associated with Customer Number: 78905

OR

| ☐ Firm or Individual Name | Saul Ewing, LLP | | | | |
|---|---|---|---|---|---|
| Address | 1500 Market Street | | | | |
| City | Philadelphia | State | PA | Zip | 19102-2186 |
| Country | US | | | | |
| Telephone | 215-972-7734 | Email | ipgroupmailbox@saul.com | | |

Assignee Name and Address:

The Trustees of the University of Pennsylvania
3160 Chestnut Street, Suite 200
Philadelphia, PA 19104

**A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.**

**SIGNATURE of Assignee of Record**

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

| Signature | [signature] | Date | 11-20-13 |
|---|---|---|---|
| Name | Jennifer Langenberger | Telephone | 215-573-4508 |
| Title | Director of Intellectual Property, The Trustees of the University of Pennsylvania | | |

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: The Trustees of the University of Pennsylvania

Application No./Patent No.: TBD Filed/Issue Date: Herewith

Titled: Compositions and Methods for Treatment of Cancer

The Trustees of the University of Pennsylvania , a university
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. [X] the assignee of the entire right, title, and interest in;

2. [ ] an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is ________ %); or

3. [ ] the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel ________, Frame ________, or for which a copy therefore is attached.

OR

B. [X] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Inventors June, Levine, Porter and Kalos To: The Trustees of the University of Pennsylvania
The document was recorded in the United States Patent and Trademark Office at
Reel 028482 , Frame 0357 , or for which a copy thereof is attached.

2. From: Inventor Milone To: The Trustees of the University of Pennsylvania
The document was recorded in the United States Patent and Trademark Office at
Reel 030530 , Frame 0519 , or for which a copy thereof is attached.

3. From: ________ To: ________
The document was recorded in the United States Patent and Trademark Office at
Reel ________, Frame ________, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet(s).

[X] As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Kathryn Doyle/ January 15, 2016
Signature Date

Kathryn Doyle, Ph.D., J.D., Reg No. 36317 Patent Attorney
Printed or Typed Name Title

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

# Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 02/19/2016

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01 FC : 1830 140.00 DA

============================================================

Sequence Listing was accepted.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).

Reviewer: Saleem, Syed (ASRC)

Timestamp: [year=2016; month=2; day=3; hr=8; min=47; sec=39; ms=277; ]

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Application No: 14997136 Version No: 1.0

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | 1653 | 2400 | 046483-6001US13(01088) | 30 | 1 |

**CONFIRMATION NO. 4164**

**FILING RECEIPT**

OC000000080817586

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Date Mailed: 02/23/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Inventor(s)**

Carl H. June, Merion Station, PA;
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Michael D. Kalos, Philadelphila, PA;
Michael C. Milone, Cherry Hill, NJ;

**Applicant(s)**

The Trustees of the University of Pennsylvania, Philadelphia, PA;

**Power of Attorney:** The patent practitioners associated with Customer Number 78905

**Domestic Priority data as claimed by applicant**

This application is a CON of 13/992,622 07/09/2013
which is a 371 of PCT/US2011/064191 12/09/2011
which claims benefit of 61/421,470 12/09/2010
and claims benefit of 61/502,649 06/29/2011

**Foreign Applications** for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
*Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.*

**Permission to Access Application via Priority Document Exchange:** Yes

**Permission to Access Search Results:** Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

**If Required, Foreign Filing License Granted:** 02/04/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/997,136**

**Projected Publication Date:** To Be Determined - pending completion of Corrected Papers

**Non-Publication Request:** No

**Early Publication Request:** No
**Title**

Compositions and Methods for Treatment of Cancer

**Preliminary Class**

435

**Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:** No

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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## Title 35, United States Code, Section 184

## Title 37, Code of Federal Regulations, 5.11 & 5.15

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technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875 | Application or Docket Number<br>14/997,136 |
|---|---|

## APPLICATION AS FILED - PART I

| FOR | NUMBER FILED (Column 1) | | NUMBER EXTRA (Column 2) | SMALL ENTITY RATE($) | SMALL ENTITY FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY FEE($) |
|---|---|---|---|---|---|---|---|---|
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | | N/A | N/A | | | N/A | 280 |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | | N/A | N/A | | | N/A | 600 |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | | N/A | N/A | | | N/A | 720 |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 30 | minus 20 = | * 10 | | | OR | x 80 = | 800 |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 1 | minus 3 = | * | | | | x 420 = | 0.00 |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | | 0.00 |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | | 0.00 |
| | | | | TOTAL | | | TOTAL | 2400 |

* If the difference in column 1 is less than zero, enter "0" in column 2.

## APPLICATION AS AMENDED - PART II

### AMENDMENT A

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

### AMENDMENT B

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE($) | ADDITIONAL FEE($) | OR | RATE($) | ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) |

**CONFIRMATION NO. 4164**

**FORMALITIES LETTER**

*OC000000080817587*

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Date Mailed: 02/23/2016

# NOTICE TO FILE CORRECTED APPLICATION PAPERS

***Filing Date Granted***

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - Numbers, letters, and reference characters on the drawings must measure at least 0.32 cm (1/8 inch) in height. See Figure(s) 4A-4D, 5A, 6A-6C, 7A-7D, 8A-8C, 9, 10, 11.
  - The drawings submitted to the Office are not electronically reproducible because portions of figures 4A-4D, 5A, 6A-6C, 7A-7D, 8A-8C, 9, 10, 11 are missing and/or blurry.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ygizaw/

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**Electronically Filed**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1653 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Not Yet Assigned |
| Filed: | January 15, 2016 | : | Confirmation No. 4164 |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(01088) |

**SECOND PRELIMINARY AMENDMENT**

Prior to examination on the merits, kindly amend the above-identified application without prejudice, as follows. Please charge any applicable fees to deposit account number 50-4364.

**AMENDMENT TO THE CLAIMS** begins on page 2.

**REMARKS** begin on page 6.

**Amendment to the Claims**

The listing of the claims will replace all prior versions, and listings, of the claims in the application.

1-89. (canceled)

90. (Currently amended) A pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD19 antigen binding domain comprising, from the amino to the carboxy terminus, a light chain variable region and a heavy chain variable region of SEQ ID NO:20, wherein the CAR further comprises a transmembrane domain, a 4-1BB costimulatory signaling region, and a CD3 zeta signaling domain.

91. (Previously presented) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^4$ to $10^9$ cells per kg body weight of a human in need of such cells.

92. (Previously presented) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^5$ to $10^6$ cells per kg body weight of a human in need of such cells.

93. (Previously presented) The composition of claim 90, wherein said antigen binding fragment is a scFv.

94. (Previously presented) The composition of claim 90, wherein the scFv comprises the amino acid sequence of SEQ ID NO:20.

95. (Previously presented) The composition of claim 90, wherein the transmembrane domain is CD8α transmembrane domain.

96. (Previously presented) The composition of claim 95, wherein the CD8α transmembrane domain comprises the amino acid sequence of SEQ ID NO: 22.

97. (Previously presented) The composition of claim 90, wherein the CAR further comprises a hinge domain.

98. (Previously presented) The composition of claim 97, wherein the hinge domain is a CD8α hinge domain.

99. (Previously presented) The composition of claim 98, wherein the CD8α hinge domain comprises the amino acid sequence of SEQ ID NO:21.

100. (Previously presented) The composition of claim 90, wherein the 4-1BB costimulatory signaling region comprises the amino acid sequence of SEQ ID NO:23.

101. (Previously presented) The composition of claim 90, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

102. (Previously presented) The composition of claim 90, wherein the CD19 antigen binding domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 14.

103. (Previously presented) The composition of claim 95, wherein the CD8α transmembrane domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 16.

104. (Previously presented) The composition of claim 99, wherein the CD8α hinge domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 15.

105. (Previously presented) The composition of claim 100, wherein the 4-1BB costimulatory signaling region is encoded by a nucleic acid sequence comprising SEQ ID NO: 17.

106. (Previously presented) The composition of claim 101, wherein the CD3 zeta signaling domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 18.

107 (Previously presented) The composition of claim 90, wherein the CAR comprises the amino acid sequence of SEQ ID NO:12.

108. (Previously presented) The composition of claim 107, wherein the CAR is encoded by a nucleic acid sequence comprising SEQ ID NO:8.

109 (Previously presented) The composition of claim 90, wherein the CAR further comprises a CD28 costimulatory signaling region.

110. (Previously presented) The composition of claim 90, wherein the T cells are T cells of a human having a cancer.

111. (Previously presented) The composition of claim 110, wherein the cancer is a hematological cancer.

112. (Previously presented) The composition of claim 90, wherein the T cells comprise a vector that comprises the nucleic acid sequence.

113. (Previously presented) The composition of claim 112, wherein the vector is a lentiviral vector.

114. (Previously presented) The composition of claim 112, wherein the vector further



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Miltenyi v. UPenn
IPR2022-00855
Page 220

comprises a promoter.

115. (Previously presented) The composition of claim 114, wherein the promoter is an EF-1α promoter.

116. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient.

117. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition comprises a buffer.

118. (Previously presented) The composition of claim 117, wherein the buffer is neutral buffer saline or phosphate buffered saline.

119. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition further comprises a carbohydrate.

## REMARKS

Claim 90 has been amended herein to include recitation of SEQ ID NO:20. Referring to US 2013/0287748, the publication of parent U.S. Patent Application No. 13/992,622, support for this amendment is found throughout the specification and for example, in paragraph [0135] and in Table 5.

No new matter is added by way of this amendment to claim 90.

**Summary**

Applicants respectfully submit that the pending claims are fully supported in the specification as filed, and that no new matter has been added by way of the present Preliminary Amendment.

Favorable examination and allowance of the claims is hereby requested.

Respectfully submitted,

**CARL H. JUNE, ET AL.**

February 29, 2016 By: [signature]
Date

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*Attorney for Applicant*

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1653 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Not Yet Assigned |
| | | : | Confirmation No. 4164 |
| Filed: | January 15, 2016 | : | |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(01088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**SUPPLEMENTAL INFORMATION DISCLOSURE UNDER 37 CFR 1.97(b)**

The attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached Form PTO-1449. One copy of each of these documents is attached, if required.

No fee or certification is required in connection with this Information Disclosure, since it is being submitted prior to the issuance of a first Office Action on the merits, or expiration of the three-month period following filing of the above-identified application.

It is respectfully requested that the information be considered by the Examiner and that a copy of the attached Form PTO-1449 be returned indicating that such information has been considered.

The references listed in this Information Disclosure Statement comprise the most pertinent prior art known to Applicants and their attorneys as of the date hereof. This Information Disclosure Statement should not be construed as a representation that the cited references are material or that no better art exists.

In the event any fees are required in connection with this paper, please charge Deposit Account No. 50-4364

Applicants' undersigned attorney may be reached by telephone at (215) 972-7734. All correspondence should be directed to the below-listed address.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: February 29, 2016

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| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Supplemental**<br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>14/997,136 |
|---|---|---|
| | **APPLICANT:**<br>Carl H. June, et al. | |
| | **FILING DATE:**<br>January 15, 2016 | **GROUP:**<br>1653 |

**U.S. PATENT DOCUMENTS**

| Examiner Initial | | Document Number | Date | Name | Class | Subclass | Filing Date if appropriate |
|---|---|---|---|---|---|---|---|
| | | 7,402,431 | 07-22-2008 | Michael Har-Noy | | | |
| | | | | | | | |

**FOREIGN PATENT DOCUMENTS**

| | | Document Number | Date | Country | Class | Subclass | TranslationYes/No /Abstract |
|---|---|---|---|---|---|---|---|
| | | WO 2005/044996 | 19 May 2005 | PCT | | | |
| | | | | | | | |
| | | | | | | | |

**OTHER DOCUMENT(S)** (*Including Author, Title, Date, Pertinent Pages, etc.)*

| | | |
|---|---|---|
| | | Colombian Patent Application No. 15-80428 – Office Action issued December 23, 2015. |
| | | Colombian Patent Application No. - No. 13-137536 – Office Action issued November 23, 2015 |
| | | Eurasian Patent Application No. 201390847 – Office Action issued February 14, 2016 |
| | | U.S. Patent Application No. 13/992,622 – Final Office Action issued January 5, 2016 |
| | | U.S. Patent Application No. 14/996,953 – non-final Office Action issued February 22, 2016 |
| | | “Genetically Engineered Lymphocyte Therapy in Treating Patients with B-Cell Leukemia or Lymphoma That is Resistant or Refractory to Chemotherapy” ClinicalTrials.gov Identifier NCT01029366; Retrieved from the internet on January 29, 2016. Found at https://www.clinicaltrials.gov/ct2/show/NCT01029366?term=NCT01029366&rank=1 |
| | | “Pilot Study for Patients with Chemotherapy Resistant or Refractory CD19 Leukemia and Lymphoma (CART-19)” ClinicalTrials.gov Identifier: NCT00891215; Retrieved from the internet on September 2, 2015. Found at http://web.archive.org/web/20090903002304/http://clinicaltrials.gov/ct2/show/NCT00891215 |
| | | AppliChem product sheet for RPMI-1640, 2 pages, downloaded 12/28/2015 |
| | | Milone, M. et al., Supplementary Materials and Methods, Mol Ther, Vol. 17, 2009, 7 pages |
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**(54) Title:** CHIMERIC RECEPTORS WITH 4-1BB STIMULATORY SIGNALING DOMAIN


**(57) Abstract:** The present invention relates to a chimeric receptor capable of signaling both a primary and a co-stimulatory pathway, thus allowing activation of the co-stimulatory pathway without binding to the natural ligand. The cytoplasmic domain of the receptor contains a portion of the 4-1BB signaling domain. Embodiments of the invention relate to polynucleotides that encode the receptor, vectors and host cells encoding a chimeric receptor, particularly including T cells and natural killer (NK) cells and methods of use. Also included is a method for obtaining an enriched population of NK cells from a mixed population of NK cells and T cells.

# Chimeric Receptors with 4-1BB Stimulatory Signaling Domain

## Government Interest

This invention was made in part with U.S. Government support under National
5 Institutes of Health grant no. CA 58297. The U.S. Government may have certain rights in this invention.

## Field of the Invention

This invention relates to chimeric cell membrane receptors, particularly chimeric T-
10 cell receptors.

## Background

Regulation of cell activities is frequently achieved by the binding of a ligand to a surface membrane receptor comprising an extracellular and a cytoplasmic domain. The
15 formation of the complex between the ligand and the extracellular portion of the receptor results in a conformational change in the cytoplasmic portion of the receptor which results in a signal transduced within the cell. In some instances, the change in the cytoplasmic portion results in binding to other proteins, where other proteins are activated and may carry out various functions. In some situations, the cytoplasmic portion is autophosphorylated or
20 phosphorylated, resulting in a change in its activity. These events are frequently coupled with secondary messengers, such as calcium, cyclic adenosine monophosphate, inositol phosphate, diacylglycerol, and the like. The binding of the ligand to the surface membrane receptor results in a particular signal being transduced.

For T-cells, engagement of the T-cell receptor (TCR) alone is not sufficient to induce
25 persistent activation of resting naive or memory T cells. Full, productive T cell activation requires a second co-stimulatory signal from a competent antigen-presenting cell (APC). Co-stimulation is achieved naturally by the interaction of the co-stimulatory cell surface receptor on the T cell with the appropriate counter-receptor on the surface of the APC. An APC is normally a cell of host origin which displays a moiety which will cause the stimulation of an
30 immune response. APCs include monocyte/macrophages, dendritic cells, B cells, and any number of virally-infected or tumor cells which express a protein on their surface recognized by T cells. To be immunogenic APCs must also express on their surface a co-stimulatory molecule. Such APCs are capable of stimulating T cell proliferation, inducing cytokine production, and acting as targets for cytolytic T cells upon direct interaction with the T cell.



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See Linsley and Ledbetter, Ann. Rev. Immunol. 4:191-212 (1993); Johnson and Jenkins, Life Sciences 55:1767-1780 (1994); June et al., Immunol. Today 15:321-331 (1994); and Mondino and Jenkins, J. Leuk. Biol. 55:805-815 (1994).

Engagement of the co-stimulatory molecule together with the TCR is necessary for optimal levels of IL-2 production, proliferation and clonal expansion, and generation of effector functions such as the production of immunoregulatory cytokines, induction of antibody responses from B cells, and induction of cytolytic activity. More importantly, engagement of the TCR in the absence of the co-stimulatory signal results in a state of non-responsiveness, called anergy. Anergic cells fail to become activated upon subsequent stimulation through the TCR, even in the presence of co-stimulation, and in some cases may be induced to die by a programmed self-destruct mechanism.

In certain situations, for example where APCs lack the counter-receptor molecules necessary for co-stimulation, it would be beneficial to have the co-stimulatory signal induced by virtue of employing a ligand other than the natural ligand for the co-stimulatory receptor. This might be, for example, the same ligand as that recognized by the TCR (i.e., the same moiety, such that if one signal is received, both signals will be received), or another cell surface molecule known to be present on the target cells (APCs).

Several receptors that have been reported to provide co-stimulation for T-cell activation, including CD28, OX40, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), and 4-1BB. The signaling pathways utilized by these co-stimulatory molecules share the common property of acting in synergy with the primary T cell receptor activation signal.

Previously the signaling domain of CD28 has been combined with the T-cell receptor to form a co-stimulatory chimeric receptor. See U.S. Patent No. 5,686,281; Geiger, T.L. *et al., Blood 98*: 2364-2371 (2001); Hombach, A. *et al., J Immunol 167*: 6123-6131 (2001); Maher, J. *et al. Nat Biotechnol 20*: 70-75 (2002); Haynes, N.M. *et al., J Immunol 169:* 5780-5786 (2002); Haynes, N.M. *et al., Blood 100*: 3155-3163 (2002). These co-stimulatory receptors provide a signal that is synergistic with the primary effector activation signal, i.e. the TCR signal or the chimeric effector function receptor signal, and can complete the requirements for activation under conditions where stimulation of the TCR or chimeric effector function receptor is suboptimal and might otherwise be detrimental to the function of the cell. These receptors can support immune responses, particularly of T cells, by permitting the use of ligands other than the natural ligand to provide the required co-stimulatory signal.

Chimeric receptors that contain a CD19 specific single chain immunoglobulin extracellular domain have been shown to lyse CD19+ target cells and eradicate CD19+ B cell



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lymphomas engrafted in mice [Cooper LJ, et al., Blood 101:1637-1644 (2003) and Brentjens RJ, et al., Nature Medicine 9:279-286 (2003)]. Cooper et al. reported that T-cell clones transduced with chimeric receptors comprising anti-CD19 scFv and CD3ζ produced approximately 80% specific lysis of B-cell leukemia and lymphoma cell lines at a 1:1 effector to target ratio in a 4-hour Cr release assay; at this ratio, percent specific lysis of one primary B-lineage ALL sample tested was approximately 30%. Brentjens et al. reported that T-cells bearing anti-CD19 scFv and CD3ζ chimeric receptors could be greatly expanded in the presence of exogenous IL-15 and artificial antigen-presenting cells transduced with CD19 and CD80. The authors showed that these T cells significantly improved the survival of immunodeficient mice engrafted with the Raji B-cell lymphoma cell line. Their results also confirmed the importance of co-stimulation in maximizing T-cell-mediated anti-leukemic activity. Only cells expressing the B7 ligands of CD28 elicited effective T-cell responses. This could be a major obstacle in the case of B-lineage ALL because leukemic lymphoblasts typically do not express B7 molecules.

In addition to T cell immune responses, natural killer (NK) cell responses appear to be clinically relevant. While T cells recognize tumor associated peptide antigen expressed on surface HLA class I or class II molecules, antigen nonspecific immune responses are mediated by NK cells that are activated by the failure to recognize cognate "self" HLA class I molecules. The graft-versus-tumor effect of transplants using HLA matched donors is mediated by antigen specific T cells, while transplantation using HLA mismatched donors can also lead to donor NK cells with potent antitumor activity. HLA mismatched haplo-identical transplants can exert a powerful anti-leukemia effect based on expansion of antigen nonspecific donor NK cells.

Immunotherapy with NK cells has been limited by the inability to obtain sufficient numbers of pure NK cells suitable for manipulation and expansion. The established methods for cell expansion favor T cell expansion and even after T cells are depleted, residual T cells typically become prominent after stimulation. Thus there is a need for better methods to expand NK cells from a population without expanding T cells.

## Summary of the Invention

The present invention provides a chimeric receptor containing a co-stimulatory signal by incorporation of the signaling domain of the 4-1BB receptor. The chimeric receptor comprises an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic domain wherein the cytoplasmic domain comprises the signaling domain of 4-

1BB. In one embodiment of the invention the signaling domain of 4-1BB used in the chimeric receptor is of human origin. In a preferred embodiment, human 4-1BB consists of SEQ ID NO:2. In another embodiment the signaling domain comprises amino acids 214 – 255 of SEQ ID NO:2.

5 In another embodiment of the invention the cytoplasmic domain of the chimeric receptor comprises the signaling domain of CD3ζ in addition to the signaling domain of 4-1BB. In another embodiment the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody. In another embodiment the transmembrane domain comprises the hinge and transmembrane domains of CD8α. In a most preferred
10 embodiment of the invention the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody, the transmembrane domain comprises the hinge and transmembrane domain of CD8α, and the cytoplasmic domain comprises the signaling domain of CD3ζ and the signaling domain of 4-1BB.

Other aspects of the invention include polynucleotide sequences, vectors and host
15 cells encoding a chimeric receptor that comprises the signaling domain of 4-1BB. Yet other aspects include methods of enhancing T lymphocyte or natural killer (NK) cell activity in an individual and treating an individual suffering from cancer by introducing into the individual a T lymphocyte or NK cell comprising a chimeric receptor that comprises the signaling domain of 4-1BB. These aspects particularly include the treatment of lung cancer,
20 melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. Preferred cancer targets for use with the present invention are cancers of B cell origin, particularly including acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphoma.

25 A different but related aspect of the present invention provides a method for obtaining an enriched NK cell population suitable for transduction with a chimeric receptor that comprises the signaling domain of 4-1BB. This method comprises the expansion of NK cells within a mixed population of NK cells and T cells by co-culturing the mixed population of cells with a cell line that activates NK cells and not T lymphocytes. This NK activating
30 cell line is composed of cells that activate NK cells, but not T lymphocytes, and which express membrane bound interleukin-15 and a co-stimulatory factor ligand. In a particular embodiment the NK activating cell line is the K562 myeloid leukemia cell line or the Wilms tumor cell line HFWT. In another embodiment of the invention the co-stimulatory factor ligand is CD137L.



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Description of the Sequence Listing

SEQ ID No. 1 is the nucleotide sequence for human 4-1BB mRNA. The coding sequence for the human 4-1BB protein begins at position 129 and ends at position 893.

5 SEQ ID No. 2 is the amino acid sequence of human 4-1BB. The signaling domain begins at position 214 and ends at position 255.

SEQ. ID. No. 3 is the nucleotide sequence for murine 4-1BB mRNA. The coding sequence for the murine 4-1BB protein begins at position 146 and ends at position 916.

SEQ ID. No. 4 is the amino acid sequence of murine 4-1BB. The signaling domain

10 begins at position 209 and ends at position 256.

Description of the Figures

Figure 1 is a schematic representation of the CD19-truncated, CD19-ζ, CD19-28-ζ and CD19-BB-ζ receptor constructs.

15 Figure 2 shows the percent of CD19-positive leukemia cell recovery in four different cell lines (380, 697, KOPN-57bi and OP-1) after 24 hours of culture with NK cells with or without a chimeric receptor at a 1:1 ratio relative to cultures with no NK cells. The bars represent each of the 4 cell lines that are co-cultured with NK cells containing either "vector" which is MSCV-IRES GFP only; "trunc." which is vector containing truncated anti-CD19;

20 "ζ" which is vector containing anti-CD19- CD3ζ; "28 ζ" which is vector containing anti-CD19-CD28α- CD3ζ; or "BB- ζ" which is vector containing anti-CD19- 4-1BB intracellular domain- CD3ζ. This figure shows that chimeric receptors confer anti-ALL activity to NK cells which is improved by the addition of the co-stimulatory molecules CD28 or 4-1BB.

25 Detailed Description of the Invention

Definitions

4-1BB: The term "4-1BB" refers to a membrane receptor protein also termed CD137, which is a member of the tumor necrosis factor receptor (TNFR) superfamily expressed on the surface of activated T-cells as a type of accessory molecule [Kwon et al., Proc. Natl.

30 Acad. Sci. USA 86:1963 (1989); Pollok et al., J. Immunol. 151:771 (1993)]. 4-1BB has a molecular weight of 55 kDa, and is found as a homodimer. It has been suggested that 4-1BB mediates a signal transduction pathway from outside of the cell to inside [Kim et al., J. Immunol. 151:1255 (1993)].



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A human 4-1BB gene (SEQ ID NO:1) was isolated from a cDNA library made from activated human peripheral T-cell mRNA [Goodwin et al., Eur. J. Immunol. 23:2631 (1993);]. The amino acid sequence of human 4-1BB (SEQ ID NO: 2) shows 60% homology to mouse 4-1BB (SEQ ID NO:4)[Kwon et al., Proc. Natl. Acad. Sci. USA 86:1963 (1989);
5 Gen Bank No: NM_011 612] which indicates that the sequences are highly conserved. As mentioned supra, 4-1BB belongs to the TNFR superfamily, along with CD40, CD27, TNFR-I, TNFR-II, Fas, and CD30 [Alderson et al., Eur. J. Immunol. 24:2219 (1994)]. When a monoclonal antibody is bound to 4-1BB expressed on the surface of mouse T-cells, anti-CD3 T-cell activation is increased many fold [Pollok et al., J. Immunol. 150:771 (1993)].

10 4-1BB binds to a high affinity ligand (4-1BBL, also termed CD137L) expressed on several antigen-presenting cells such as macrophages and activated B cells [Pollok et al., J. Immunol. 150:771 (1993) Schwarz et al., Blood 85:1043 (1995)]. 4-1BBL is claimed and described in US Patent 5,674,704. The interaction of 4-1BB and its ligand provides a co-stimulatory signal leading to T cell activation and growth [Goodwin et al., Eur. J. Immunol.
15 23:2631 (1993); Alderson et al., Eur. J. Immunol. 24:2219 (1994); Hurtado et al., J. Immunol. 155:3360 (1995); Pollock et al., Eur. J. Immunol. 25:488 (1995); DeBenedette et al., J. Exp. Med. 181:985 (1995)]. These observations suggest an important role for 4-1BB in the regulation of T cell-mediated immune responses [Ignacio et al., Nature Med. 3:682 (1997)].

The term IL-15 (interleukin 15) refers to a cytokine that stimulates NK cells [Fehniger
20 TA, Caligiuri MA. Blood 97(1):14-32 (2001)]. It has become apparent that IL-15 presented through cell to cell contact has a higher NK stimulating activity than soluble IL-15 [Dubois S, et al., Immunity 17(5):537-547 (2002); Kobayashi H, et al., Blood (2004) PMID: 15367431; Koka R, et al., J Immunol 173(6):3594-3598 (2004); Burkett PR, et al., J Exp Med 200(7):825-834 (2004)]. To express membrane-bound IL-15 a construct consisting of human
25 IL-15 mature peptide (NM172174) was fused to the signal peptide and transmembrane domain of human CD8$\alpha$.

To specifically expand NK cells means to culture a mixed population of cells that contains a small number of NK cells so that the NK cells proliferate to numbers greater than other cell types in the population.

30 To activate T cells and NK cells means to induce a change in their biologic state by which the cells express activation markers, produce cytokines, proliferate and/or become cytotoxic to target cells. All these changes can be produced by primary stimulatory signals. Co-stimulatory signals amplify the magnitude of the primary signals and suppress cell death



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following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity.

The terms T-cell and T lymphocyte are interchangeable and used synonymously herein.

5 The term "chimeric receptor" as used herein is defined as a cell-surface receptor comprising an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic co-stimulatory signaling domain in a combination that is not naturally found together on a single protein. This particularly includes receptors wherein the extracellular domain and the cytoplasmic domain are not naturally found together on a single receptor 10 protein. The chimeric receptors of the present invention are intended primarily for use with T cells and natural killer (NK) cells.

The term "host cell" means any cell of any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or 15 an enzyme. Host cells of the present invention include T cells and NK cells that contain the DNA or RNA sequences encoding the chimeric receptor and express the chimeric receptor on the cell surface. Host cells may be used for enhancing T lymphocyte activity, NK cell activity, treatment of cancer, and treatment of autoimmune diseases.

The terms "express" and "expression" mean allowing or causing the information in a 20 gene or DNA sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an "expression product" such as a protein. The expression product itself, e.g. the resulting protein, may also be said to be "expressed" by the cell. An expression product can be characterized as intracellular, 25 extracellular or transmembrane. The term "intracellular" means something that is inside a cell. The term "extracellular" means something that is outside a cell. The term transmembrane means something that has an extracellular domain outside the cell, a portion embedded in the cell membrane and an intracellular domain inside the cell.

The term "transfection" means the introduction of a foreign nucleic acid into a cell 30 using recombinant DNA technology. The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include



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regulatory or control sequences, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "transformed" and is a "transformant" or a
5 "clone." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species.

The term "transduction" means the introduction of a foreign nucleic acid into a cell using a viral vector.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by
10 which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence. Vectors include plasmids, phages, viruses, etc.

Description of the Invention

In accordance with the present invention there may be employed conventional
15 molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology"
20 Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984); CURRENT PROTOCOLS IN
25 IMMUNOLOGY (J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); ANNUAL REVIEW OF IMMUNOLOGY; as well as monographs in journals such as ADVANCES IN IMMUNOLOGY. All patents, patent applications, and publications mentioned herein are hereby incorporated herein by reference.

Primary T cells expressing chimeric receptors specific for tumor or viral antigens
30 have considerable therapeutic potential as immunotherapy reagents. Unfortunately, their clinical value is limited by their rapid loss of function and failure to expand in vivo, presumably due to the lack of co-stimulator molecules on tumor cells and the inherent limitations of signaling exclusively through the chimeric receptor.



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The chimeric receptors of the present invention overcome this limitation wherein they have the capacity to provide both the primary effector activity and the co-stimulatory activity upon binding of the receptor to a single ligand. For instance, binding of the anti-CD19-BB-ζ receptor to the CD19 ligand provides not only the primary effector function, but also a
5 proliferative and cytolytic effect.

T cells transduced with anti-CD19 chimeric receptors of the present invention which contain co-stimulatory molecules have remarkable anti-ALL capacity. However, the use of allogenic receptor-modified T cells after hematopoietic cell transplantation might carry the risk of severe graft-versus–host disease (GvHD). In this setting, the use of CD3-negative NK
10 cells is attractive because they are not expected tocause GvHD.

Studies suggest an anti-tumor effect of NK cells and Zeis et al., Br J Haematol 96: 757-61 (1997) have shown in mice that NK cells contribute to a graft-versus-leukemia effect, without inducing GvHD.

Obtaining an enriched population of NK cells for use in therapy has been difficult to
15 achieve. Specific NK cell expansion has been problematic to achieve with established methods, where CD3+ T cells preferentially expand. Even after T cell depletion, residual T cells typically become prominent after stimulation. However, in accordance with the teachings of the present invention NK cells may be expanded by exposure to cells that lack or poorly express major histocompatibility complex I and/or II molecules and which have been
20 genetically modified to express membrane bound IL-15 and 4-1BB ligand (CD137L). Such cell lines include, but are not necessarily limited to, K562 [ATCC, CCL 243; Lozzio et al., Blood 45(3): 321-334 (1975); Klein et al., Int. J. Cancer 18: 421-431 (1976)], and the Wilms tumor cell line HFWT. [Fehniger TA, Caligiuri MA. Int Rev Immunol 20(3-4):503-534 (2001); Harada H, et al., Exp Hematol 32(7):614-621 (2004)], the uterine endometrium tumor
25 cell line HHUA, the melanoma cell line HMV-II, the hepatoblastoma cell line HuH-6, the lung small cell carcinoma cell lines Lu-130 and Lu-134-A, the neutoblastoma cell lines NB19 and NB69, the embryonal carcinoma cell line from testis NEC14, the cervix carcinoma cell line TCO-2, and the bone marrow-metastated neuroblastoma cell line TNB1 [Harada H., et al., Jpn. J. Cancer Res 93: 313-319 (2002)]. Preferably the cell line used lacks or poorly
30 expresses both MHC I and II molecules, such as K562 and the HFWT cell lines.

Expanding NK cells which can then be transfected with chimeric receptors according to this method represents another aspect of the present invention.



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The chimeric receptors of the present invention comprise an extracellular domain, a transmembrane domain and a cytoplasmic domain. The extracellular domain and transmembrane domain can be derived from any desired source for such domains.

As described in U.S. Patents Nos. 5,359,046, 5,686,281 and 6,103,521, the
5 extracellular domain may be obtained from any of the wide variety of extracellular domains or secreted proteins associated with ligand binding and/or signal transduction. The extracellular domain may be part of a protein which is monomeric, homodimeric, heterodimeric, or associated with a larger number of proteins in a non-covalent complex. In particular, the extracellular domain may consist of an Ig heavy chain which may in turn be
10 covalently associated with Ig light chain by virtue of the presence of CH1 and hinge regions, or may become covalently associated with other Ig heavy/light chain complexes by virtue of the presence of hinge, CH2 and CH3 domains. In the latter case, the heavy/light chain complex that becomes joined to the chimeric construct may constitute an antibody with a specificity distinct from the antibody specificity of the chimeric construct. Depending on the
15 function of the antibody, the desired structure and the signal transduction, the entire chain may be used or a truncated chain may be used, where all or a part of the CH1, CH2, or CH3 domains may be removed or all or part of the hinge region may be removed.

Wherein an antitumor chimeric receptor is utilized, the tumor may be of any kind as long as it has a cell surface antigen which may be recognized by the chimeric receptor. In a
20 specific embodiment, the chimeric receptor may be for any cancer for which a specific monoclonal antibody exists or is capable of being generated. In particular, cancers such as neuroblastoma, small cell lung cancer, melanoma, ovarian cancer, renal cell carcinoma, colon cancer, Hodgkin's lymphoma, and childhood acute lymphoblastic leukemia have antigens specific for the chimeric receptors.

25 The transmembrane domain may be contributed by the protein contributing the multispecific extracellular inducer clustering domain, the protein contributing the effector function signaling domain, the protein contributing the proliferation signaling portion, or by a totally different protein. For the most part it will be convenient to have the transmembrane domain naturally associated with one of the domains. In some cases it will be desirable to
30 employ the transmembrane domain of the .zeta., .eta. or Fc.epsilon.R1.gamma. chains which contain a cysteine residue capable of disulfide bonding, so that the resulting chimeric protein will be able to form disulfide linked dimers with itself, or with unmodified versions of the .zeta., .eta. or Fc.epsilon.R1.gamma. chains or related proteins. In some instances, the transmembrane domain will be selected or modified by amino acid substitution to avoid

binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In other cases it will be desirable to employ the transmembrane domain of .zeta., .eta., Fc.epsilon.R1-.gamma. and -.beta., MB1 (Ig.alpha.), B29 or CD3-.gamma., .zeta., or
5 .epsilon., in order to retain physical association with other members of the receptor complex.

The cytoplasmic domain of the chimeric receptors of the invention will comprise the 4-1BB signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of this chimeric receptor type. In a most preferred embodiment of the invention the extracellular domain comprises a single chain variable domain of an anti-CD19
10 monoclonal antibody, the transmembrane domain comprises the hinge and transmembrane domain of CD8α, and the cytoplasmic domain comprises the signaling domain of CD3ζ and the signaling domain of 4-1BB. The extracellular domain of the preferred embodiment contains the anti-CD19 monoclonal antibody which is described in Nicholson IC, et al., Mol Immunol 34:1157-1165 (1997) plus the 21 amino acid signal peptide of CD8α (translated
15 from 63 nucleotides at positions 26-88 of GenBank Accession No. NM_001768). The CD8α hinge and transmembrane domain consists of 69 amino acids translated from the 207 nucleotides at positions 815-1021 of GenBank Accession No. NM_001768. The CD3ζ signaling domain of the preferred embodiment contains 112 amino acids translated from 339 nucleotides at positions 1022-1360 of GenBank Accession No. NM_000734.

20 Antigen-specific cells can be expanded in vitro for use in adoptive cellular immunotherapy in which infusions of such cells have been shown to have anti-tumor reactivity in a tumor-bearing host. The compositions and methods of this invention can be used to generate a population of T lymphocyte or NK cells that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of cancer, in particular the
25 treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector
30 cells include cytotoxic T cells and NK cells. The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth.

In adoptive immunotherapy, the patient's circulating lymphocytes, or tumor infiltrated lymphocytes, are isolated in vitro, activated by lymphokines such as IL-2 or transduced with



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genes for tumor necrosis, and readministered [Rosenberg et al., N. Engl. J. Med. 319:1767 (1988)]. To achieve this, one would administer to an animal, or human patient, an immunologically effective amount of activated lymphocytes genetically modified to express a tumor-specific chimeric receptor gene as described herein. The activated lymphocytes will most preferably be the patient's own cells that were earlier isolated from a blood or tumor sample and activated and expanded in vitro. In aspects of the present invention T lymphocytes or NK cells from a patient having a cancer of B cell origin such as lymphoblastic leukemia, B-cell chronic lymphocytic leukemia or B-cell non-Hodgkin's lymphoma would be isolated and tranduced with the CD19-BB-ζ polynucleotide so that a chimeric receptor containing 4-1BB in the cytoplasmic domain is express on the cell surface of the T cell or NK cell. The modified cells would then be readministered into the patient to target and kill the tumor cells.

As shown in one Example infra, primary T-cells were transduced with the anti-CD19-BB-ζ receptor of the present invention. One week after transduction the T-cells had expanded 3-4 fold in contrast to cells that were transduced with a chimeric receptor that lacked 4-1BB. After 3 weeks in culture the T-cells had expanded by more than 16 fold.

T-cells that were transduced with the anti-CD19-BB-ζ receptor and cultured in 200 IU/mL of IL-2 for two weeks, then removed from IL-2 and exposed to irradiated OP-1 cells underwent apoptosis. However, cells cultured in 10 IU/mL of IL-2 and exposed to irradiated OP-1 cells for two weeks after transduction remained viable. T-cells that were transduced with CD19 chimeric receptors that lacked 4-1BB underwent apoptosis under these same conditions. These results show that 4-1BB co-stimulation confers a survival advantage on lymphocytes, which overcomes a major obstacle with current chimeric receptors used in immunotherapy.

To determine if T-cells transduced with the anti-CD19-BB-ζ receptor exhibited cytotoxic activity under conditions necessary for immunotherapy, their cytotoxic activity at low effector:target (E:T) ratios were measured. As described in the Example infra, T-cells transduced with the anti-CD19-BB-ζ receptor and control vectors were expanded in vitro for two weeks and mixed with OP-1 cells at various E:T ratios (1:1, 0.1:1, and 0.01:1). Viable leukemic cells were counted after one week of culture. T-cells expressing the anti-CD19-BB-ζ receptor exhibited cytotoxic activity at the 1:1 and 0.1:1 ratios against all $CD19^{+}$ cell lines tested. The anti-CD19-BB-ζ receptor was not effective at the 0.01:1 ratio. The CD19 chimeric receptor that lacked 4-1BB showed cytotoxic activity at the 1:1 ratio, but at the 0.1:1 ratio the results were inferior to the anti-CD19-BB-ζ receptor.



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A surprising result obtained with the anti-CD19-BB-ζ receptor was that the T-cells transduced with the receptor exhibited cytotoxic activity toward $CD19^+$ leukemic cells at a ratio of 0.01:1 when the leukemic cells were co-cultured with bone marrow-derived mesenchymal cells. This result shows that T-cells transduced with the anti-CD19-BB-ζ
5 receptor exhibit cytotoxic activity in an environment critical for B-lineage leukemic cell growth. Another unexpected result was that expression of the anti-CD19-BB-ζ receptor caused higher levels of TRAIL stimulation.

Furthermore, IL-2, which causes $CD8^+$ cells to expand more vigorously, levels in cells expressing the anti-CD19-BB-ζ receptor were higher than in cells expressing the other
10 receptors tested. These results further support the use of the anti-CD19-BB-ζ receptor for immunotherapy.

Construction of the anti-CD19-BB-ζ receptor

The present invention provides a chimeric receptor construct which contains the signaling domain of 4-1BB and fragments thereof. In a preferred embodiment of the
15 invention, the genetic fragments used in the chimeric receptor were generated using splicing by overlapping extension by PCR (SOE-PCR), a technique useful for generating hybrid proteins of immunological interest. [Warrens AN, et al. Gene 20;186: 29-35 (1997)]. Other procedures used to generate the polynucleotides and vector constructs of the present invention are well known in the art.
20 Transduction of T-cells

As shown in the Examples, infra, a polynucleotide expressing a chimeric receptor capable of providing both primary effector and co-stimulatory activities was introduced into T-cells and NK cells via retroviral transduction. References describing retroviral transduction of genes are Anderson et al., U.S. Pat. No. 5,399,346; Mann et al., Cell
25 33:153 (1983); Temin et al., U.S. Pat. No. 4,650,764; Temin et al., U.S. Pat. No. 4,980,289; Markowitz et al., J. Virol. 62:1120 (1988); Temin et al., U.S. Pat. No. 5,124,263; International Patent Publication No. WO 95/07358, published Mar. 16, 1995, by Dougherty et al.; and Kuo et al., Blood 82:845 (1993). International Patent Publication No. WO 95/07358 describes high efficiency transduction of primary B lymphocytes.
30 Expansion of NK cells

The present invention shows that human primary NK cells may be expanded in the presence of a myeloid cell line that has been genetically modified to express membrane bound IL-15 and 4-1BB ligand (CD137L). A cell line modified in this way which does not



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have MHC class I and II molecules is highly susceptible to NK cell lysis and activates NK cells.

For example, K562 myeloid cells can be transduced with a chimeric protein construct consisting of human IL-15 mature peptide fused to the signal peptide and transmembrane
5 domain of human CD8α and GFP. Transduced cells can then be single-cell cloned by limiting dilution and a clone with the highest GFP expression and surface IL-15 selected. This clone can then be transduced with human CD137L, creating a K562-mb15-137L cell line.

Peripheral blood mononuclear cell cultures containing NK cells are cultured with a K562-mb15-137L cell line in the presence of 10IU/mL of IL-2 for a period of time sufficient
10 to activate and enrich for a population of NK cells. This period can range from 2 to 20 days, preferably about 5 days. Expanded NK cells may then be transduced with the anti-CD19-BB-ζ chimeric receptor.

Administration of Activated T Cells and NK Cells

Methods of re-introducing cellular components are known in the art and include
15 procedures such as those exemplified in US Patent Nos. 4,844,893 and 4,690,915. The amount of activated T cells or NK cells used can vary between in vitro and in vivo uses, as well as with the amount and type of the target cells. The amount administered will also vary depending on the condition of the patient and should be determined by considering all appropriate factors by the practitioner.

20

**EXAMPLES**

**Example 1**

INTRODUCTION

In approximately 20% of children and 65% of adults with acute lymphoblastic
25 leukemia (ALL), drug-resistant leukemic cells survive intensive chemotherapy and cause disease recurrence. [Pui CH et al, Childhood acute lymphoblastic leukemia - Current status and future perspectives. Lancet Oncology2:597-607 (2001); Verma A, Stock W. Management of adult acute lymphoblastic leukemia: moving toward a risk-adapted approach. Curr Opin Oncol 13:14-20T (2001)] lymphocyte-based cell therapy should bypass cellular
30 mechanisms of drug resistance. Its potential clinical value for leukemia is demonstrated by the association between T-cell-mediated graft-versus-host disease (GvHD) and delay or suppression of leukemia recurrence after allogeneic stem cell transplantation. [Champlin R. T-cell depletion to prevent graft-versus-host disease after bone marrow transplantation.



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Hematol Oncol Clin North Am 4:687-698 (1990); Porter DL, Antin JH. The graft-versus-leukemia effects of allogeneic cell therapy. Annu Rev Med 50:369-86.:369-386 (1999); Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. Nature 411:385-389 (2001)] Manipulation of GvHD by infusion of donor lymphocytes can produce a measurable
5 anti-leukemic effect. [Porter DL, et al. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med 330:100-106 (1994); Kolb HJ, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 6:2041-2050 (1995); Slavin S, et al. Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse
10 after allogeneic bone marrow transplantation . Blood 87:2195-2204 (1996); Collins RH, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 15:433-444 (1997)] However, in patients with ALL this effect is often limited, [Kolb HJ, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 86:2041-2050 (1995); Verdonck LF, et al.
15 Donor leukocyte infusions for recurrent hematologic malignancies after allogeneic bone marrow transplantation: impact of infused and residual donor T cells. Bone Marrow Transplant 22:1057-1063 (1998); Collins RH, Jr., et al. Donor leukocyte infusions in acute lymphocytic leukemia. Bone Marrow Transplant 26:511-516 (2000)] possibly reflecting inadequate T-cell stimulation by leukemic lymphoblasts.

20 T lymphocyte specificity can be redirected through expression of chimeric immune receptors consisting of an extracellular antibody-derived single-chain variable domain (scFv) and an intracellular signal transduction molecule (e.g., the signaling domain of CD3ζ or FcγRIII). [Geiger TL, Jyothi MD. Development and application of receptor-modified T lymphocytes for adoptive immunotherapy. Transfus Med Rev 15:21-34 (2001); Schumacher
25 TN. T-cell-receptor gene therapy. Nat Rev Immunol. 2:512-519 (2002); Sadelain M, et al. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3:35-45 (2003)] Such T lymphocytes can be activated by cell surface epitopes targeted by the scFv and can kill the epitope-presenting cells. The first requirement to redirect T cells against ALL cells is the identification of target molecules that are selectively expressed by leukemic cells.
30 In B-lineage ALL, CD19 is an attractive target, because it is expressed on virtually all leukemic lymphoblasts in almost all cases. [Campana D, Behm FG. Immunophenotyping of leukemia. J Immunol Methods 243:59-75 (2000)] It is not expressed by normal non-hematopoietic tissues, and among hematopoietic cells, it is expressed only by B-lineage lymphoid cells. [Campana D, Behm FG. Immunophenotyping of leukemia. J Immunol



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Methods 243:59-75 (2000); Nadler LM, et al. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J Immunol 131:244-250 (1983)] Recent studies have shown that T-cells expressing anti-CD19 scFv and CD3ζ signaling domain can proliferate when mixed with $CD19^+$ cells and can lyse $CD19^+$ target
5 cells. [Cooper LJ, et al. T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. Blood 101:1637-1644 (2003); Brentjens RJ, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003)]

A prerequisite for the success of T-cell therapy is the capacity of the engineered T
10 lymphocytes to expand and produce a vigorous and durable anti-leukemic response in vivo. The engagement of the TCR, although necessary, is not sufficient to fully activate T cells; a second signal, or co-stimulus, is also required. [Liebowitz DN, et al. Costimulatory approaches to adoptive immunotherapy. Curr Opin Oncol 10:533-541 (1998); Allison JP, Lanier LL. Structure, function, and serology of the T-cell antigen receptor complex. Annu
15 Rev Immunol 5:503-540 (1987); Salomon B, Bluestone JA. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol 19:225-52.:225-252 (2001)] This could be a major obstacle for chimeric receptor-based therapy of B-lineage ALL, because B-lineage leukemic lymphoblasts generally lack B7 molecules that bind to CD28 on T-lymphocytes and trigger the CD28-mediated co-
20 stimulatory pathway. [Cardoso AA, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. Blood 88:41-48 (1996)] This limitation might be overcome by incorporating the signal transduction domain of CD28 into chimeric receptors. [Eshhar Z, et al. Functional expression of chimeric receptor genes in human T cells. J Immunol Methods 2001;248:67-76 (2001); Hombach A, et al. Tumor-specific T cell
25 activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. J Immunol 167:6123-6131 (2001); Geiger TL, et al. Integrated src kinase and costimulatory activity enhances signal transduction through single-chain chimeric receptors in T lymphocytes. Blood 98:2364-2371 (2001); Maher J, et
30 al. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta /CD28 receptor. Nat Biotechnol 20:70-75 (2002)] Murine T cells bearing such receptors have shown a greater capacity to inhibit cancer cell growth and metastasis in mice than those with chimeric receptors lacking this domain. [Haynes NM, et al. Rejection of syngeneic colon carcinoma by CTLs expressing single-chain antibody receptors codelivering



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CD28 costimulation. J Immunol 169:5780-5786 (2002); Haynes NM, et al. Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. Blood 100:3155-3163 (2002)]

A second co-stimulatory pathway in T cells, independent of CD28 signaling, is mediated by 4-1BB (CD137), a member of the tumor necrosis factor (TNF) receptor family. [Sica G, Chen L. Modulation of the immune response through 4-1BB. In: Habib N, ed. Cancer gene therapy: past achievements and future challenges. New York: Kluwer Academic/Plenum Publishers; 355-362 (2000)] 4-1BB stimulation significantly enhances survival and clonal expansion of $CD8^+$ T-lymphocytes, and $CD8^+$ T-cell responses in a variety of settings, including viral infection, allograft rejection, and tumor immunity. [Shuford WW, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp Med 186:47-55 (1997); Melero I, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 3:682-685 (1997); Melero I, et al. Amplification of tumor immunity by gene transfer of the co-stimulatory 4-1BB ligand: synergy with the CD28 co-stimulatory pathway. Eur J Immunol 28:1116-1121 (1998); Takahashi C, et al. Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. J Immunol 162:5037-5040 (1999); Martinet O, et al. T cell activation with systemic agonistic antibody versus local 4-1BB ligand gene delivery combined with interleukin-12 eradicate liver metastases of breast cancer. Gene Ther 9:786-792 (2002); May KF, Jr., et al. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific CD8+ T cells. Cancer Res 62:3459-3465 (2002)] However, the natural ligand of 4-1BB is weakly and heterogeneously expressed in B-lineage ALL cells (C. Imai, D. Campana, unpublished observations). Therefore, it is likely that this important co-stimulatory signal, like CD28, can become operational only if 4-1BB is added to chimeric receptors. However, it is not known whether such receptors would help deliver effective T-cell responses to cancer cells and, if so, whether these would be equivalent to those elicited by receptors containing CD28.

We constructed a chimeric T-cell receptor specific for CD19 that contains a 4-1BB signaling domain. We determined whether T cells transduced with these receptors could effectively destroy B-lineage ALL cell lines and primary leukemic cells under culture conditions that approximate the in vivo microenvironment where leukemic cells grow. We compared the properties of T-cells expressing the 4-1BB-containing receptor to those of T-cells expressing an equivalent receptor lacking 4-1BB or containing CD28 instead.



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## MATERIALS AND METHODS

***Cells***

Available in our laboratory were the human B-lineage ALL cell line OP-1, developed from the primary leukemic cells of a patient with newly diagnosed B-lineage ALL with the t(9;22)(q34;q11) karyotype and the *BCR-ABL* gene fusion; [Manabe A, et al. Interleukin-4 induces programmed cell death (apoptosis) in cases of high-risk acute lymphoblastic leukemia. Blood 83:1731-1737 (1994)] the B-lineage ALL cell lines RS4;11, [Stong RC, et al. Human acute leukemia cell line with the t(4;11) chromosomal rearrangement exhibits B lineage and monocytic characteristics. Blood 1985;65:21-31 (1985)] and REH [Rosenfeld C, et al. Phenotypic characterisation of a unique non-T, non-B acute lymphoblastic leukaemia cell line. Nature 267:841-843 (1977)]; the T-cell lines Jurkat [Schneider U, et al. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int J Cancer 1977;19:621-626 (1977)] and CEM-C7 [Harmon JM, et al. Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. J Cell Physiol 98:267-278 (1979)]; and the myeloid cell lines K562 [Koeffler HP, Golde DW. Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. Science 200:1153-1154 (1978)] and U-937. [Sundstrom C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 1976;17:565-577 (1976)] Cells were maintained in RPMI-1640 (Gibco, Grand Island, NY) with 10% fetal calf serum (FCS; BioWhittaker, Walkersville, MD) and antibiotics. Human adenocarcinoma HeLa cells and embryonic kidney fibroblast 293T cells, maintained in DMEM (MediaTech, Herndon, VA) supplemented with 10% FCS and antibiotics, were also used.

We used primary leukemia cells obtained from 5 patients with newly diagnosed B-lineage ALL with the approval of the St. Jude Children's Research Hospital Institutional Review Board and with appropriate informed consent. The diagnosis of B-lineage ALL was unequivocal by morphologic, cytochemical, and immunophenotypic criteria; in each case, more than 95% of leukemic cells were positive for CD19. Peripheral blood samples were obtained from 7 healthy adult donors. Mononuclear cells were collected from the samples by centrifugation on a Lymphoprep density step (Nycomed, Oslo, Norway) and were washed two times in phosphate-buffered saline (PBS) and once in AIM-V medium (Gibco).

**Plasmids**

The plasmid encoding anti-CD19 scFv was obtained from Dr. I. Nicholson (Child Health Research Institute, Adelaide, Australia). [Nicholson IC, et al. Construction and



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characterisation of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukaemia and lymphoma. Mol Immunol 34:1157-1165 (1997)] The pMSCV-IRES-GFP, pEQPAM3(-E), and pRDF were obtained from Dr. E. Vanin at our institution. Signal peptide, hinge and transmembrane domain of CD8α, and intracellular domains of 4-1BB, CD28, CD3ζ and CD19 were subcloned by polymerase chain reaction (PCR) using a human spleen cDNA library (from Dr. G. Neale, St. Jude Children's Research Hospital) as a template. Figure 1 shows a schematic representation of the anti-CD19-ζ, anti-CD19-BB-ζ, anti-CD19-28-ζ and anti-CD19-truncated (control) constructs. We used splicing by overlapping extension by PCR (SOE-PCR) to assemble several genetic fragments. [Warrens AN, et al. Splicing by overlap extension by PCR using asymmetric amplification: an improved technique for the generation of hybrid proteins of immunological interest. Gene 20;186:29-35 (1997)] The sequence of each genetic fragment was confirmed by direct sequencing. The resulting expression cassettes were subcloned into *Eco*RI and *Xho*I sites of MSCV-IRES-GFP.

To transduce CD19-negative K562 cells with CD19, we constructed a MSCV-IRES-DsRed vector. The IRES and DsRed sequences were subcloned from MSCV-IRES-GFP and pDsRedN1 (Clontech, Palo Alto, CA), respectively, and assembled by SOE-PCR. The IRES-DsRed cassette was digested and ligated into *Xho*I and *Not*I sites of MSCV-IRES-GFP. The expression cassette for CD19 was subsequently ligated into *Eco*RI and *Xho*I sites of MSCV-IRES-DsRed vector.

**Virus production and gene transduction**

To generate RD114-pseudotyped retrovirus, we used calcium phosphate DNA precipitation to transfect 3 x $10^6$ 293T cells, maintained in 10-cm tissue culture dishes (Falcon, Becton Dickinson, Franklin Lakes, NJ) for 24 hours, with 8 μg of one of the vectors anti-CD19-ζ, anti-CD19-BB-ζ, anti-CD19-28-ζ or anti-CD19-truncated, 8 μg of pEQ-PAM3(-E) and 4 μg of pRDF. After 24 hours, medium was replaced with RPMI-1640 with 10% FCS and antibiotics. Conditioned medium containing retrovirus was harvested 48 hours and 72 hours after transfection, immediately frozen in dry ice, and stored at –80 °C until use. HeLa cells were used to titrate virus concentration.

Peripheral blood mononuclear cells were incubated in a tissue culture dish for 2 hours to remove adherent cells. Non-adherent cells were collected and prestimulated for 48 hours with 7 μg/mL PHA-M (Sigma, St. Louis, MO) and 200 IU/mL human IL-2 (National Cancer Institute BRB Preclinical Repository, Rockville, MD) in RPMI-1640 and 10% FCS. Cells were then transduced as follows. A 14-mL polypropylene centrifuge tube (Falcon) was



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coated with 0.5 mL of human fibronectin (Sigma) diluted to100 μg/mL for 2 hours at room temperature and then incubated with 2% bovine serum albumin (Sigma) for 30 minutes. Prestimulated cells (2 x $10^5$) were resuspended in the fibronectin-coated tube in 2-3 mL of virus-conditioned medium with polybrene (4 μg/mL; Sigma) and centrifuged at 2400 x *g* for
5 2 hours. The multiplicity of infection (4 to 8) was identical in each experiment comparing the activity of different chimeric receptors. After centrifugation, cells were left undisturbed for 24 hours in a humidified incubator at 37 °C, 5% $CO_2$. The transduction procedure was repeated on two successive days. Cells were then washed twice with RPMI-1640 and maintained in RPMI-1640, 10% FCS, and 200 IU/mL of IL-2 until use.

10 A similar procedure was used to express chimeric receptors in Jurkat cells, except that cells were not prestimulated. K562 cells expressing CD19 were created by resuspending 2 x $10^5$ K562 cells in 3 mL of MSCV-CD19-IRES-DsRed virus medium with 4 μg/mL polybrene in a fibronectin-coated tube; the tube was centrifuged at 2400 x *g* for 2 hours and left undisturbed in an incubator for 24 hours. Control cells were transduced with the vector
15 only. These procedures were repeated on 3 successive days. After confirming CD19 and DsRed expression, cells were subjected to single-cell sorting with a fluorescence-activated cell sorter (MoFlo, Cytomation, Fort Collins, CO). The clones that showed the highest expression of DsRed and CD19 and of DsRed alone were selected for further experiments.

**Detection of chimeric receptor expression**

20 Transduced Jurkat and peripheral blood cells were stained with goat anti-mouse $(Fab)_2$ polyclonal antibody conjugated with biotin (Jackson Immunoresearch, West Grove, PA) followed by streptavidin conjugated to peridinin chlorophyll protein (PerCP; Becton Dickinson, San Jose, CA). Patterns of CD4, CD8, and CD28 expression were also analyzed by using anti-CD4 and anti-CD28 conjugated to PE and anti-CD8 conjugated to PerCP
25 (antibodies from Becton Dickinson, and Pharmingen, San Diego, CA). Antibody staining was detected with a FACScan flow cytometer (Becton Dickinson).

For Western blotting, 2 x $10^7$ cells were lysed in 1 mL RIPA buffer (PBS, 1% Triton-X100, 0.5% sodium deoxycholate, 0.1% SDS) containing 3 μg/mL of pepstatin, 3 μg/mL of leupeptin, 1 mM of PMSF, 2mM of EDTA, and 5 μg/mL of aprotinin. Centrifuged lysate
30 supernatants were boiled with an equal volume of loading buffer with or without 0.1 M DTT, then were separated by SDS-PAGE on a precast 12% acrylamide gel (BioRad, Hercules, CA). The proteins were transferred to a PVDF membrane, which was incubated with primary mouse anti-human CD3ζ monoclonal antibody (clone 8D3; Pharmingen), 1μg/mL for 12 hours at 4 °C. Membranes were then washed, incubated with a 1:500 dilution of goat anti-



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mouse IgG horseradish peroxidase-conjugated second antibody for 1 hour, and developed by using the ECP kit (Pharmacia, Piscataway, NJ).

**Changes in gene expression and cytokine production after receptor ligation**

Jurkat cells transduced with the chimeric receptors were cocultured with OP-1 leukemic cells fixed with 0.5% paraformaldehyde at an effector : target (E : T) ratio of 1 : 1. RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA). Gene expression of Jurkat cells was analyzed using HG-U133A GeneChip microarrays (Affymetrix, Santa Clara, CA) as previously described. [Yeoh EJ, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002;1:133-143 (2002); Ross ME, et al. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. May 2003; 10.1182/blood-2003-01-0338 (2003)] Arrays were scanned using a laser confocal scanner (Agilent, Palo Alto, CA) and analyzed with Affymetrix Microarray suite 5.0. We used an arbitrary factor of 2 or higher to define gene overexpression. IL-2, TNF-related apoptosis-inducing ligand (TRAIL), OX40, IL-3 and β-actin transcripts were detected by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using Jurkat cells stimulated as above; primers were designed using the Primer3 software developed by the Whitehead Institute for Biomedical Research.

For cytokine production, Jurkat cells and primary lymphocytes ($2 \times 10^5$ in 200 μl) expressing chimeric receptors were stimulated with OP-1 cells at a 1:1 E:T ratio for 24 hours. Levels of IL-2 and IFNγ in culture supernatants were determined with a Bio-Plex assay (BioRad). Lymphocytes before and after stimulation were also labeled with anti-TRAIL-PE (Becton Dickinson).

Expansion and purification of receptor-transduced primary T cells

Receptor-transduced lymphocytes ($3 \times 10^5$) were co-cultured with $1.5 \times 10^5$ irradiated OP-1 cells in RPMI-1640 with 10% FCS with or without exogenous IL-2. Cells were pulsed weekly with irradiated target cells at an E : T ratio of 2 : 1. Cells were counted by Trypan-blue dye exclusion and by flow cytometry to confirm the presence of GFP-positive cells and the absence of CD19-positive cells. To prepare pure populations of $CD8^+$ cells expressing chimeric receptors, we labeled cells with a PE-conjugated anti-CD8 antibody (Becton Dickinson) that had been previously dialyzed to remove preservatives and then sterile-filtered. $CD8^+$ $GFP^+$ cells were isolated using a fluorescence-activated cell sorter (MoFlo).



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**Cytotoxicity assays**

The cytolytic activity of transductants was measured by assays of lactate dehydrogenase (LDH) release using the Cytotoxicity Detection Kit (Roche, Indianapolis, IN) according to the manufacturer's instructions. Briefly, 2 x $10^4$ target cells were placed in 96-well V-bottom tissue culture plates (Costar, Cambridge, MA) and cocultured in triplicate in RPMI-1640 supplemented with 1% FCS, with primary lymphocytes transduced with chimeric receptors. After 5 hours, cell-free supernatant was harvested and immediately analyzed for LDH activity. Percent specific cytolysis was calculated by using the formula: (Test – effector control – low control / high control – low control) x 100, in which "high control" is the value obtained from supernatant of target cells exposed to 1% Triton-X-100, "effector control" is the spontaneous LDH release value of lymphocytes alone, "low control" is the spontaneous LDH release value of target cells alone; background control (the value obtained from medium alone) was subtracted from each value before the calculation.

The anti-leukemic activity of receptor-transduced lymphocytes was also assessed in 7-day cultures using lower E : T ratios. For this purpose, we used bone marrow–derived mesenchymal cells to support the viability of leukemic cells. [Nishigaki H, et al. Prevalence and growth characteristics of malignant stem cells in B-lineage acute lymphoblastic leukemia. Blood 89:3735-3744 (1997); Mihara K, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 120:846-849 (2003)] Briefly, 2 x $10^4$ human mesenchymal cells immortalized by enforced expression of telomerase reverse transcriptase were plated on a 96-well tissue culture plate precoated with 1% gelatin. After 5 days, 1 x $10^4$ $CD19^+$ target cells (in case of cell lines) or 2 x $10^5$ $CD19^+$ target cells (in case of primary ALL cells) were plated on the wells and allowed to rest for 2 hours. After extensive washing to remove residual IL-2-containing medium, receptor-transduced primary T cells were added to the wells at the proportion indicated in Results. Cultures were performed in the absence of exogenous IL-2. Plates were incubated at 37°C in 5% $CO_2$ for 5-7 days. Cells were harvested, passed through a 19-gauge needle to disrupt residual mesenchymal-cell aggregates, stained with anti-CD19-PE antibody, and assayed by flow cytometry as previously described. [Ito C, et al. Hyperdiploid acute lymphoblastic leukemia with 51 to 65 chromosomes: A distinct biological entity with a marked propensity to undergo apoptosis. Blood 93:315-320 (1999); Srivannaboon K, et al. Interleukin-4 variant (BAY 36-1677) selectively induces apoptosis in acute lymphoblastic leukemia cells. Blood 97:752-758



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(2001)] Expression of DsRed served as a marker of residual K562 cells. Experiments were done in triplicate.

**RESULTS**

**Transduction of primary human T lymphocytes with anti-CD19-BB-ζ chimeric receptors**

In preliminary experiments, transduction of lymphocytes stimulated with PHA (7 μg/mL) and IL-2 (200 IU/mL) for 48 hours, followed by centrifugation (at 2400 x *g*) of the activated lymphocytes with retroviral supernatant in tubes coated with fibronectin, consistently yielded a high percentage of chimeric receptor and GFP expression; this method was used in all subsequent experiments. In 75 transduction experiments, 31% to 86% (median, 64%) of mononuclear cells expressed GFP. In experiments with cells obtained from 6 donors, we tested the immunophenotype of the cells transduced with anti-CD19-BB-ζ receptors. Fourteen days after transduction a mean (± SD) of 89.6% ± 2.3% (n = 6) of $GFP^+$ cells also expressed CD3; 66.2% ± 17.9% of $CD3^+$ T lymphocytes were transduced. Among $GFP^+$ cells, 21.1% ± 8.8% (n = 6) were $CD4^+$, 68.1% ± 8.1% (n = 6) were $CD8^+$, 38.1% ± 16.1% (n = 3) were $CD28^+$ and 24.2% ± 11.6% (n = 3) were $CD8^+CD28^+$. These proportions were similar to those obtained with the anti-CD19-ζ receptors lacking 4-1BB. In this case, 85.4% ± 11.0% (n = 6) of $GFP^+$ cells expressed CD3; 60.8% ± 10.1% of $CD3^+$ cells were transduced. Among $GFP^+$ cells, 18.0% ± 8.7% (n = 6) were $CD4^+$, 66.1% ± 11.7% (n = 6) were $CD8^+$, 41.2% ± 12.2% (n = 3) were $CD28^+$ and 20.6% ± 11.3% (n = 3) were $CD8^+CD28^+$. In these experiments, median transduction efficiency was 65% (range, 31% to 86%) for anti-CD19-BB-ζ receptors, and 65% (range, 37% to 83%) for anti-CD19-ζ receptors.

The surface expression of the chimeric receptors on $GFP^+$ cells was confirmed by staining with a goat anti-mouse antibody that reacted with the scFv portion of anti-CD19. Expression was detectable on most $GFP^+$ cells and was not detectable on $GFP^-$ cells and vector-transduced cells. The level of surface expression of anti-CD19-BB-ζ was identical to that of the receptor lacking 4-1BB. Expression was confirmed by Western blot analysis; under non-reducing conditions, peripheral blood mononuclear cells transduced with the chimeric receptors expressed them mostly as monomers, although dimers could be detected.

**Signaling function of anti-CD19-BB-ζ chimeric receptors**

To test the functionality of the anti-CD19-BB-ζ chimeric receptor, we used the T-cell line Jurkat and the $CD19^+$ ALL cell line OP-1. After transduction, >95% Jurkat cells were



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GFP+. Exposure of irradiated OP-1 cells to Jurkat cells transduced with anti-CD19-BB-ζ triggered transcription of IL-2. Notably, in parallel experiments with Jurkat cells transduced with the anti-CD19-ζ receptor lacking 4-1BB, the level of IL-2 transcription was much lower. No IL-2 transcription was detected in Jurkat cells transduced with the anti-CD19-truncated
5 control receptor lacking CD3ζ.

To identify further changes in molecules associated with T-cell activation, survival or cytotoxicity induced by anti-CD19-BB-ζ receptors, Jurkat cells were either transduced with these receptors or with anti-CD19-ζ receptors and then stimulated with paraformadehyde-fixed OP-1 cells. After 12 hours of stimulation, we screened the cells' gene expression using
10 Affymetrix HG-U133A chips. Genes that were overexpressed by a factor of 2 or higher in cells with anti-CD19-BB-ζ. included the member of the TNF family TRAIL, the TNF-receptor member OX40, and IL-3. Overexpression of these molecules after stimulation was validated using RT-PCR. In cells bearing the anti-CD19-ζ receptor, there were no overexpressed genes with a known function associated with T-cells. Therefore, anti-CD19-
15 BB-ζ receptors elicit transcriptional responses that are distinct from those triggered by receptors lacking 4-1BB.

**Expansion of T cells expressing anti-CD19-BB-ζ receptors in the presence of CD19+ cells**

To measure the ability of anti-CD19-BB-ζ transduced lymphocytes to survive and expand in vitro, we first analyzed primary T cells (obtained from 2 donors), 7 days after
20 transduction. Transduction efficiency with the 3 receptors was similar: 72% and 67% for anti-CD19-BB-ζ, 63% and 66% for anti-CD19-ζ and 67% and 68% for the truncated anti-CD19 receptor. When cocultured with irradiated OP-1 ALL cells in the absence of exogenous IL-2, cells transduced with anti-CD19-BB-ζ expanded: after only 1 week of culture, GFP+ cells recovered were 320% and 413% of input cells. T cells that expressed the anti-CD19-ζ
25 receptor but lacked 4-1BB signaling capacity remained viable but showed little expansion (cell recovery: 111% and 160% of input cells, respectively), whereas those that expressed the truncated anti-CD19 receptor underwent apoptosis (<10% of input cells were viable after 1 week). Lymphocytes transduced with anti-CD19-BB-ζ continued to expand in the presence of irradiated OP-1 cells. After 3 weeks of culture, they had expanded by more than 16-fold, with
30 98% of the cells at this point being GFP+. By contrast, cells transduced with only anti-CD19-ζ survived for less than 2 weeks of culture.

We performed the next set of experiments with T cells (obtained from 3 donors) 14 days after transduction with anti-CD19-BB-ζ, anti-CD19-ζ or anti-CD19-truncated, and expanded with high-dose IL-2 (200 IU/mL). Recovery of lymphocytes of each donor with



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anti-CD19-BB-ζ receptors was significantly higher than that of lymphocytes with anti-CD19-ζ receptors in all 3 comparisons (P<0.005). When IL-2 was removed, exposure of the transduced cells to irradiated OP-1 cells induced apoptosis, irrespective of the chimeric receptor expressed. This was in contrast to results with cells 7 days post-transduction, and in
5 accord with the loss of T cell functionality after prolonged culture in IL-2 observed by others. [Brentjens RJ, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003); Rossig C. et al. Targeting of G(D2)-positive tumor cells by human T lymphocytes engineered to express chimeric T-cell receptor genes. Int J Cancer 94:228-236 (2001)] However, low-dose
10 IL-2 (10 IU/mL) was sufficient to maintain most lymphocytes transduced with anti-CD19-BB-ζ viable after 2 weeks of culture with irradiated OP-1 cells, but did not prevent apoptosis of cells transduced with the other receptors. Taken together, these data indicate that 4-1BB-mediated costimulation confers a survival advantage on lymphocytes.

**Cytotoxicity triggered by anti-CD19-BB-ζ chimeric receptors**

15 Lymphocytes obtained from two donors and transduced with anti-CD19-BB-ζ and anti-CD19-ζ exerted dose-dependent cytotoxicity, as shown by a 5-hour LDH release assay using the OP-1 B-lineage ALL cell line as a target. Transduction efficiencies were 41% and 73% for empty vector, 40% and 67% for anti-CD19-truncated, 43% and 63% for anti-CD19-ζ, and 46% and 72% for anti-CD19-BB-ζ. No differences in cytotoxicities mediated by the
20 two receptors were detectable with this assay. Although no lysis of target cells was apparent at a 1:1 ratio in the 5-hour LDH assay, most leukemic cells were specifically killed by lymphocytes expressing signaling chimeric receptors when the cultures were examined at 16 hours by flow cytometry and inverted microscopy.

To better mimic the application of T-cell therapy, we determined whether T cells
25 expressing the chimeric receptor would exert significant anti-leukemic activity when present at low E : T ratios in prolonged culture. Lymphocytes from various donors were expanded in vitro for 14 days after transduction and were mixed at different ratios with OP-1, RS4;11, or REH B-lineage ALL cells, or with K562 (a CD19-negative myeloid cell line that lacks HLA antigens) transduced with CD19 or with vector alone. Co-cultures were maintained for 7
30 days, and viable leukemic cells were counted by flow cytometry. As observed in short term cultures, at a 1:1 ratio, T cells expressing signaling chimeric receptors eliminated virtually all leukemic cells from the cultures. At a 0.1 : 1 ratio, however, T cells transduced with anti-CD19-BB-ζ receptors were markedly more effective than those lacking 4-1BB signaling. Chimeric receptor-transduced T cells had no effect on cells lacking CD19. The presence of 4-



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1BB in the chimeric receptor did not increase background, non-CD19-mediated cytotoxicity, in experiments using CEM-C7, U-937 and K-562. As in other experiments, transduction efficiencies with the two chimeric receptors were equivalent, and range from 62% to 73% for anti-CD19-ζ and from 60% to 70% for anti-CD19-BB-ζ.

5 Cells present in the bone marrow microenvironment may decrease T-cell proliferation in a mixed lymphocyte reaction. [Bartholomew A, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30:42-48 (2002); Krampera M, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101:3722-3729 10 (2003); Le Blanc K, et al. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 57:11-20 (2003)] To test whether these cells would also affect T-cell-mediated antileukemic activity, we repeated the experiments with OP-1 in the presence of bone marrow-derived mesenchymal cell layers. [Mihara K, et al. Development and functional 15 characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 2003;120:846-849 (2003)] T-cell cytotoxicity under these conditions was even greater than that observed in cultures without mesenchymal cells. Remarkably, T cells transduced with anti-CD19-BB-ζ were markedly cytotoxic even at a ratio of 0.01 : 1 in this assay, whereas those transduced with anti-CD19-ζ were not.

20 **Effect of receptor-transduced T cells on primary leukemic cells**

We co-cultured primary B-lineage ALL cells with bone marrow-derived mesenchymal cells, which are essential to preserve their viability in vitro. [Nishigaki H, et al. Prevalence and growth characteristics of malignant stem cells in B-lineage acute lymphoblastic leukemia. Blood 1997;89:3735-3744 (1997); Mihara K, et al. Development 25 and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 120:846-849 (2003)] We tested the effect of T cells expressing anti-CD19-BB-ζ on primary leukemic cells obtained from 5 patients at the time of diagnosis; these patients included 3 who had B-lineage ALL with 11q23 abnormalities, a karyotype associated with drug resistance. [Pui CH, et al. Childhood acute 30 lymphoblastic leukemia - Current status and future perspectives. Lancet Oncology 2:597-607 (2001)] Mesenchymal cells supported ALL cell survival in vitro: in cultures not exposed to exogenous T cells, recovery of leukemic cells from the 5 patients after 5 days of culture ranged from 100.1% to 180.7% of the input cell number. Leukemic cells incubated at a 0.1 : 1 ratio with lymphocytes expressing anti-CD19-BB-ζ were virtually eliminated in all 5



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cultures. Remarkable cytotoxicity was also seen at a 0.01 : 1 ratio. Importantly, at this ratio, lymphocytes expressing anti-CD19-BB-ζ were consistently more cytotoxic than those expressing the anti-CD19-ζ receptor alone (P<0.01 by *t* test for all comparisons).

**Comparisons between chimeric receptors containing signaling domains of 4-1BB and of CD28**

We compared responses induced by anti-CD19-BB-ζ to those of an equivalent receptor in which 4-1BB signaling domains were replaced by CD28 signaling domains (Figure. 1). Expression of the latter was similar to that of anti-CD19-BB-ζ and anti-CD19-ζ receptors: >95% Jurkat cells were consistently $GFP^+$ after transduction with anti-CD19-28-ζ and most of these cells had detectable receptors on the cell surface. In 6 experiments with primary lymphocytes, transduced cells ranged from 42% to 84% (median, 72%).

We tested production of IL-2 in Jurkat cells transduced with the three receptors and stimulated with the $CD19^+$ ALL cell line OP-1. Production of IL-2 was the highest in cells expressing anti-CD19-BB-ζ (P<0.05). Production of IL-2 was also tested in primary lymphocytes, which were transduced with the chimeric receptors and then expanded for 5 weeks with pulses of OP-1. The pattern of IL-2 production was similar to that observed in Jurkat cells. Cells expressing anti-CD19-BB-ζ produced higher levels of IL-2 (P <0.01). Chimeric receptors containing the co-stimulatory molecules induced a higher IFN-γ production in primary lymphocytes. IFN-γ levels were the highest with the anti-CD19-28-ζ receptor (P <0.05). Finally, we tested surface expression of TRAIL protein in primary lymphocytes by staining with a specific antibody. Levels of TRAIL were the highest in cells transduced with the anti-CD19-BB-ζ receptor. These results indicate that anti-CD19-BB-ζ receptors are functionally distinct from those lacking co-stimulatory molecules or containing CD28 instead of 4-1BB.

Next, we compared the cytotoxicity exerted by primary T cells transduced with anti-CD19-BB-ζ receptors to those exerted by T cells bearing receptors lacking 4-1BB. For these experiments, we transduced primary lymphocytes from 2 donors with anti-CD19-BB-ζ anti-CD19-28-ζ, anti-CD19-ζ and anti-CD19-truncated, we expanded them for 2-3 weeks with IL-2, and then purified $CD8^+$, $GFP^+$ cells by fluorescence activated cell sorting. Confirming our previous results with unsorted cells, $CD8^+$ cells expressing anti-CD19-BB-ζ receptors were significantly more effective than those with anti-CD19-ζ receptors, and were as effective as those with anti-CD19-BB-ζ Finally, we determined the capacity of the purified CD8 cells transduced with the various receptors to expand in the presence of low dose (10 U/mL) IL-2.



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Cells transduced with anti-CD19-BB-ζ receptor had a significantly higher cell growth under these conditions than those bearing the other receptors (P<0.001).

**DISCUSSION**

Results of this study indicate that anti-CD19-BB-ζ receptors could help achieve
5 effective T-cell immunotherapy of B-lineage ALL. Lymphocytes expressing anti-CD19-BB-ζ survived and expanded better than those with equivalent receptors lacking 4-1BB. These lymphocytes also had higher anti-leukemic activity and could kill B-lineage ALL cells from patients at E : T ratios as low as 0.01 : 1, suggesting that the infusion of relatively low numbers of transduced T cells could have a measurable anti-leukemic effect in patients.
10 Finally, lymphocytes transduced with anti-CD19-BB-ζ were particularly effective in the presence of bone marrow-derived mesenchymal cells which form the microenvironment critical for B-lineage ALL cell growth, further supporting their potential for immunotherapy.

Two recently reported studies used anti-CD19 scFv as a component of a chimeric receptor for T-cell therapy of B-cell malignancies. Cooper et al. Blood 101:1637-1644 (2003)
15 reported that T-cell clones transduced with chimeric receptors comprising anti-CD19 scFv and CD3ζ produced approximately 80% specific lysis of B-cell leukemia and lymphoma cell lines at a 1:1 E : T ratio in a 4-hour $^{51}$Cr release assay; at this ratio, percent specific lysis of one primary B-lineage ALL sample tested was approximately 30%. Brentjens et al. Nat Med 279-286 (2003) reported that T-cells bearing anti-CD19 scFv and CD3ζ chimeric receptors
20 could be greatly expanded in the presence of exogenous IL-15 and artificial antigen-presenting cells transduced with CD19 and CD80. The authors showed that these T cells significantly improved the survival of immunodeficient mice engrafted with the Raji B-cell lymphoma cell line. Their results demonstrated the requirement for co-stimulation in maximizing T-cell-mediated anti-leukemic activity: only cells expressing the B7 ligands of
25 CD28 elicited effective T-cell responses. However, B-lineage ALL cells typically do not express B7-1(CD80) and only a subset expresses B7-2 (CD86) molecules. [Cardoso AA, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. Blood 88:41-48 (1996)]

4-1BB, a tumor necrosis factor-receptor family member, is a co-stimulatory receptor
30 that can act independently from CD28 to prevent activation-induced death of activated T cells. [Kim YJ, et al. Human 4-1BB regulates CD28 co-stimulation to promote Th1 cell responses. Eur J Immunol 28:881-890 (1998); Hurtado JC, et al. Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J Immunol 158:2600-2609 (1997); DeBenedette MA, et al. Costimulation of CD28- T



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lymphocytes by 4-1BB ligand. J Immunol 1997;158:551-559 (1997); Bukczynski J, et al. Costimulation of human CD28- T cells by 4-1BB ligand. Eur J Immunol 33:446-454 (2003)] In our study, we found that chimeric receptors containing 4-1BB can elicit vigorous signals in the absence of CD28-mediated co-stimulation. Cytotoxicity against $CD19^+$ cells mediated by
5 these receptors was as good as that mediated by CD28-containing receptors and was clearly superior to that induced by receptors lacking co-stimulatory molecules. It is known that, in contrast to CD28, 4-1BB stimulation results in a much larger proliferation of $CD8^+$ cells than $CD4^+$ cells. [Shuford WW, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp
10 Med 1997;186:47-55 (1997)] We found that T cells expressing the anti-CD19-BB-ζ receptor produced more IL-2 upon stimulation, and that $CD8^+$ cells expanded in the presence of low-dose IL-2 more vigorously than those expressing receptors lacking 4-1BB domains, including those containing CD28. Therefore, the presence of 4-1BB in the chimeric receptors may support more durable T cell responses than those induced by other receptors.

15 Experimental evidence indicates that harnessing 4-1BB signaling could have useful application in antitumor therapy. Melero et al. Nat Med 3:682-685 (1997) found that antibodies to 4-1BB significantly improved long-lasting remission and survival rates in mice inoculated with the immunogenic P815 mastocytoma cell line. Moreover, immunogenic murine tumor cells made to express 4-1BB ligand were readily rejected and induced long
20 term immunity. [Melero I, et al. Chen L. Amplification of tumor immunity by gene transfer of the co-stimulatory 4-1BB ligand: synergy with the CD28 co-stimulatory pathway. Eur J Immunol 28:1116-1121 (1998)] Dramatic results were also observed in vaccination experiments using other tumor cell lines expressing 4-1BB ligands. [Ye Z, et al. Gene therapy for cancer using single-chain Fv fragments specific for 4-1BB. Nat Med 8:343-348 (2002);
25 Mogi S, et al. Tumour rejection by gene transfer of 4-1BB ligand into a CD80(+) murine squamous cell carcinoma and the requirements of co-stimulatory molecules on tumour and host cells. Immunology 101:541-547 (2000); Yoshida H, et al. A novel adenovirus expressing human 4-1BB ligand enhances antitumor immunity. Cancer Immunol Immunother 52:97-106 (2003)] Of note, experiments with the poorly immunogenic Ag104A fibrosarcoma cell line
30 provided some evidence that 4-1BB could be superior to CD28 in eliciting anti-tumor responses: 80% of mice showed tumor regression with 4-1BB stimulation and 50% of mice with widespread metastasis were cured, [Melero I, Shuford WW, Newby SA, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 3:682-685 (1997)] whereas CD28 costimulation was not effective alone and



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required simultaneous CD2 stimulation. [Li Y, et al. Costimulation by CD48 and B7-1 induces immunity against poorly immunogenic tumors. J Exp Med 1996;183:639-644 (1996)] These data, together with our results, indicate that the addition of 4-1BB to the chimeric receptor should significantly increase the probability that transduced T-cells will survive and continue to proliferate when the receptor is engaged in vivo. We think it noteworthy that T cells with chimeric receptors containing 4-1BB expressed the highest levels of TRAIL upon stimulation, given the known tumoricidal activity of this molecule. [Schmaltz C, et al. T cells require TRAIL for optimal graft-versus-tumor activity. Nat Med 8:1433-1437 (2002)]

Clinical precedents, such as administration of T-cell clones that target CMV epitopes [Walter EA, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 333:1038-1044 (1995)] or EBV-specific antigens, [Rooney CM, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345:9-13 (1995)] attest to the clinical feasibility of adoptive T-cell therapy. Transfer of chimeric receptor-modified T cells has the added advantage of permitting immediate generation of tumor-specific T-cell immunity. Subsequently, therapeutic quantities of antigen-specific T cells can be generated quite rapidly by exposure to target cells and/or artificial antigen-presenting cells, in the presence of ligands of co-stimulatory molecules and/or exogenous cytokines such as IL-2, IL-7, and IL-15. [Geiger TL, Jyothi MD. Development and application of receptor-modified T lymphocytes for adoptive immunotherapy. Transfus Med Rev 15:21-34 (2001); Schumacher TN. T-cell-receptor gene therapy. Nat Rev Immunol. 2:512-519 (2002); Sadelain M, et al. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3:35-45 (2003); Brentjens RJ, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003)] A specific risk of the strategy proposed here relates to the transforming potential of the retrovirus used to transduce chimeric receptors. [Baum C, Dullmann J, Li Z, et al. Side effects of retroviral gene transfer into hematopoietic stem cells. Blood 101:2099-2114 (2003)] We therefore envisage the coexpression of suicide genes as a safety measure for clinical studies. [Marktel S, et al. Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation. Blood 101:1290-1298 (2003)] This approach would also ensure that the elimination of normal CD19$^+$ B-lineage cells is temporary and should therefore have limited clinical consequences.



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In view of the limited effectiveness and the high risk of the currently available treatment options for chemotherapy-refractory B-lineage ALL and other B cell malignancies, the results of our study provide compelling justification for clinical trials using T cells expressing anti-CD19-BB-ζ receptors. Donor-derived T cells endowed with chimeric receptors could replace infusion of non-specific lymphocytes post-transplant. To reduce the risk of GvHD mediated by endogenous T-cell receptors, it may be beneficial to use T cells with restricted endogenous specificity, for example, Epstein-Barr-virus-specific cytotoxic T-lymphocyte lines. [Rossig C, et al. Epstein-Barr virus-specific human T lymphocytes expressing antitumor chimeric T-cell receptors: potential for improved immunotherapy. Blood. 99:2009-2016 (2002)] Therefore, it would be important to test the effects of adding 4-1BB to chimeric receptors transduced in these lines. The reinfusion of autologous T cells collected during clinical remission could also be considered in patients with persistent minimal residual disease. In our experiments, T cells expressing anti-CD19-BB-ζ receptors completely eliminated ALL cells at E:T ratios higher than 1:1, and autologous B lymphocytes became undetectable shortly after transduction of anti-CD19-BB-ζ, suggesting that the potential leukemic cell contamination in the infused products should be greatly reduced or abrogated by the procedure.

**Example 2**

T lymphocytes transduced with anti-CD19 chimeric receptors have remarkable anti-ALL capacity in vitro and in vivo, suggesting the clinical testing of receptor-modified autologous T cells in patients with persistent minimal residual disease. However, the use of allogeneic receptor-modified T lymphocytes after hematopoietic cell transplantation (HCT) might carry the risk of severe graft-versus-host disease (GvHD). In this setting, the use of CD3-negative natural killer (NK) cells is attractive because they should not cause GvHD.

Spontaneous cytotoxicity of NK cells against ALL is weak, if measurable at all. To test whether anti-CD19 chimeric receptors could enhance it, we developed methods to specifically expand human primary NK cells and induce high levels of receptor expression. Specific NK cell expansion has been problematic to achieve with established methods which favor CD3+ T cell expansion. Even after T-cell depletion, residual T cells typically become prominent after stimulation.

We overcame this obstacle by generating a genetically-modified K562 myeloid leukemia cell line that expresses membrane-bound interleukin-15 (IL-15) and 4-1BB ligand (CD137L) (K562-mb15-137L). The K562-mb15-137 cell line was generated by retrovirally



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transducing K562 cells with a chimeric protein construct consisting of human IL-15 mature peptide fused to the signal peptide and transmembrane domain of human CD8alpha, as well as GFP. Transduced cells were single cell-cloned by limiting dilution and a clone with the highest expression of GFP and membrane-bound (surface) IL-15 was selected. Then, the
5 clone was transduced with human CD137L.

Peripheral blood mononuclear cells from 8 donors were cultured with K562-mb15-137L in the presence of 10 IU/mL IL-2. After 1 week of culture with K562-mb15-137L, NK cells expanded by 16.3 ± 5.9 fold, whereas T cells did not expand. The stimulatory effect of K562-mb15-137L was much higher than that of K562 cells transduced with control vectors,
10 K562 expressing membrane-bound IL-15 or CD137L alone, or K562 expressing wild-type IL-15 instead of membrane-bound IL-15.

NK cells expanded with K562-mb15-137L were transduced with a retroviral vector and the anti-CD19-BB-ζ chimeric receptor. In 27 experiments, mean transduction efficiency (± SD) after 7-14 days was 67.5% ± 16.7%. Seven to fourteen days after transduction, 92.3%
15 (range 84.7%-99.4%) of cells were CD3- CD56+ NK cells; expression of receptors on the cell surface was high. NK cells expressing anti-CD19-BB-ζ had powerful cytotoxicity against NK-resistant B-lineage ALL cells. NK cells transduced with anti-CD19-BB-ζ had consistently higher cytotoxicity than those transduced with receptors lacking 4-1BB.

**Transduction of NK cells with chimeric receptors**

20 Peripheral blood mononuclear cells were stimulated with the K562-mb15-137L cells prior to their exposure to retroviral vectors containing anti-CD19 receptor constructs and GFP. In 10 experiments, median percent of NK cells was 98.4% (93.7-99.4%) 7-11 days after transduction; 77.4% (55.2-90.0%) of these cells were $GFP^+$. We observed high levels of surface expression of the anti-CD19 chimeric receptors.

25 NK activity against the CD19-negative cells K562 and U937 was not affected by the expression of anti-CD19 receptors. The receptors, however, markedly increased NK activity against $CD19^+$ ALL cells. The following summarizes results obtained with NK cells from 2 donors. At an E : T ratio of 1:1, NK cells from donor 1 lacked cytotoxicity against $CD19^+$ RS4;11 cells and exerted ~50% cytoxicity against $CD19^+$ 697 cells after 24 hours. NK cells
30 from donor 2 had no cytotoxicity against RS4;11 or 697 cells. Expression of the anti-CD19-CD3ζ receptor overcame NK resistance. NK cells from donor 1 became cytotoxic to RS4;11 cells and those from donor 2 become cytotoxic to both RS;11 and 697 cells. Moreover, when

control cells had some cytotoxicity, this was significantly augmented by expression of signaling anti-CD19 receptor.

Subsequently, we found that addition of the co-stimulatory CD28 or 4-1BB to the anti-CD19 receptor markedly enhanced NK cytotoxicity against NK-resistant ALL cells
5 (Figure 2). For example, after 24 hours of culture at 1 : 1 E : T ratio, the cytotoxicity mediated by the anti-CD19-BB-ζ receptor against the NK-resistant $CD19^{+}$ ALL cell lines 380, 697, KOPN57bi and OP1 ranged from 86.5% to 99.1%. Therefore, the inclusion of co-stimulatory molecules enhances not only the cytoxicity of T lymphocytes but also that of NK cells.



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We claim:

1. A chimeric receptor comprising an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic domain, wherein said cytoplasmic domain comprises the signaling domain of 4-1BB.
2. The chimeric receptor of claim 1 wherein said 4-1BB is human 4-1BB.
3. The chimeric receptor of claim 2 wherein said human 4-1BB has the amino acid sequence set forth in SEQ ID NO:2.
4. The chimeric receptor of claim 3 wherein said signaling domain comprises amino acids 214 to 255 of SEQ ID NO:2.
5. The chimeric receptor of claim 1 wherein said cytoplasmic domain further comprises the signaling domain of CD3ζ.
6. The chimeric receptor of claim 1 wherein said extracellular ligand binding domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody.
7. The chimeric receptor of claim 1 wherein said transmembrane domain comprises the transmembrane domain of CD8α.
8. A polynucleotide encoding the chimeric receptor of claim 1.
9. A vector for recombinant expression of a chimeric receptor, said vector comprising the polynucleotide of claim 8, operatively linked to at least one regulatory element in the appropriate orientation for expression.
10. A host cell expressing the chimeric receptor of claim 1.
11. The host cell of claim 10 selected from the group consisting of T lymphocytes and natural killer (NK) cells.
12. A chimeric receptor having a cytoplasmic domain comprising the signaling domain of 4-1BB.
13. The chimeric receptor of claim 12 wherein said 4-1BB is human 4-1BB.
14. The chimeric receptor of claim 13 wherein said human 4-1BB has the amino acid sequence set forth in SEQ ID NO:2.
15. The chimeric receptor of claim 14 wherein said signaling domain comprises amino acids 214 to 255 of SEQ ID NO:2.

16. A method of enhancing T lymphocyte or natural killer cell activity of an individual by introducing into said individual a T lymphocyte or natural killer cell comprising a chimeric receptor having a cytoplasmic domain comprising the signaling domain of 4-1BB.
17. A method for treating an individual suffering from cancer by introducing into said individual a T lymphocyte or natural killer cell comprising a chimeric receptor wherein said chimeric receptor comprises an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic domain, wherein said cytoplasmic domain comprises the signaling domain of 4-1BB.
18. The method of claim 17 wherein the cancer is selected from the group consisting of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma.
19. The method of claim 17 wherein the extracellular ligand binding domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody.
20. The method of claim 19 wherein the cancer is of B cell origin.
21. The method of claim 20 wherein the cancer is selected from the group consisting of B-lineage acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphoma.
22. A cell line comprising cells that activate natural killer (NK) cells, lack or poorly express major histocompatibility complex I molecules and do not activate T lymphocytes wherein such NK activating cells express membrane bound interleukin-15 and a co-stimulatory factor ligand..
23. The cell line of claim 22 wherein said cell line also lack or poorly expresses major histocampatibility complex II molecules.
24. The cell line of claim 23 wherein the NK activating cells are selected from the group consisting of K562 myeloid leukemia cells and WFWT Wilms tumor cells.
25. The cell line of claim 22 wherein the co-stimulatory factor ligand is CD137L.
26. A method of expanding natural killer (NK) cells which comprises culturing a population of cells comprising NK cells with a cell line that activates NK cells,



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wherein the cell line that activates NK cells expresses membrane bound interleukin-15 and a co-stimulatory factor ligand.

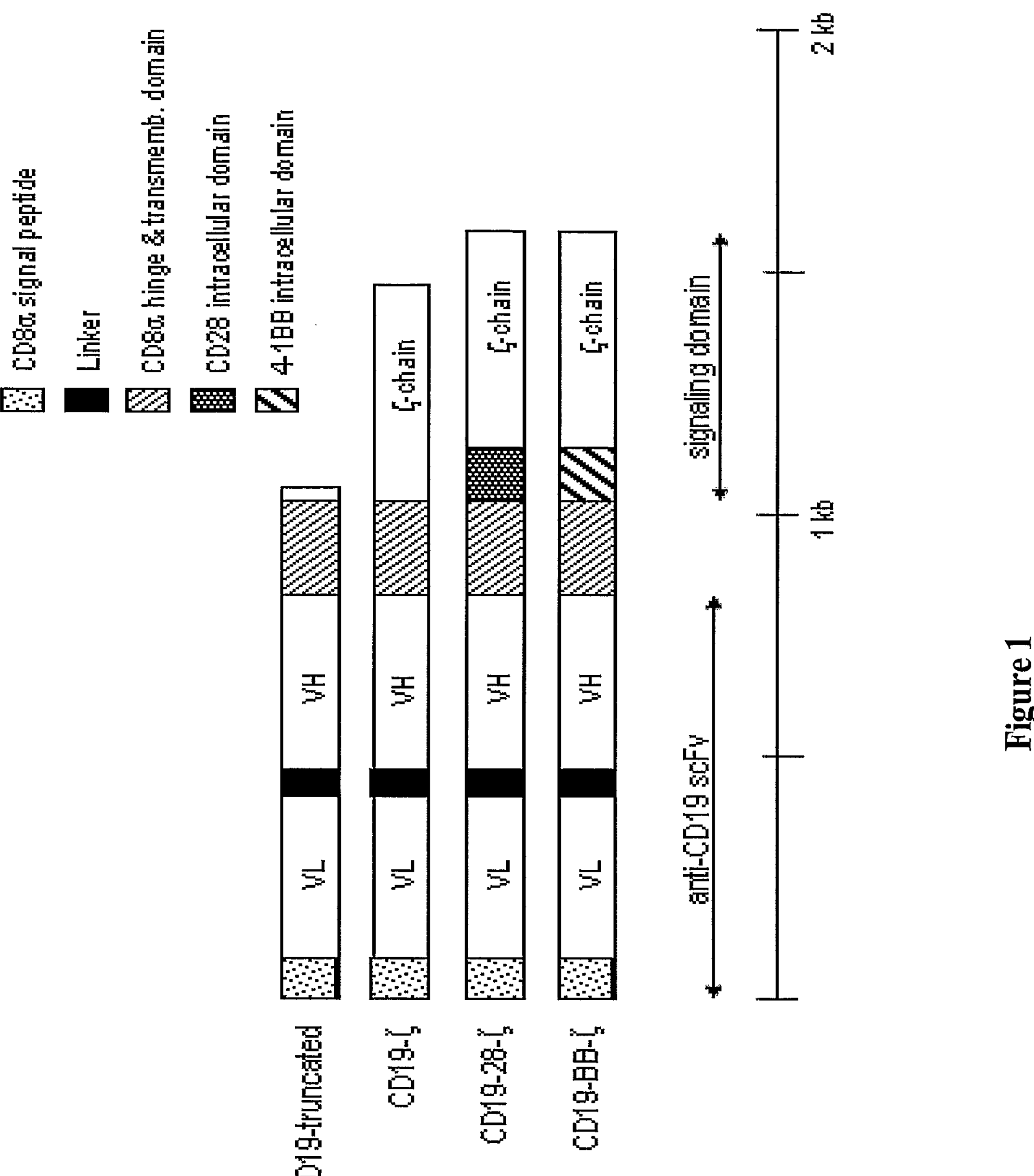


**Figure 1**

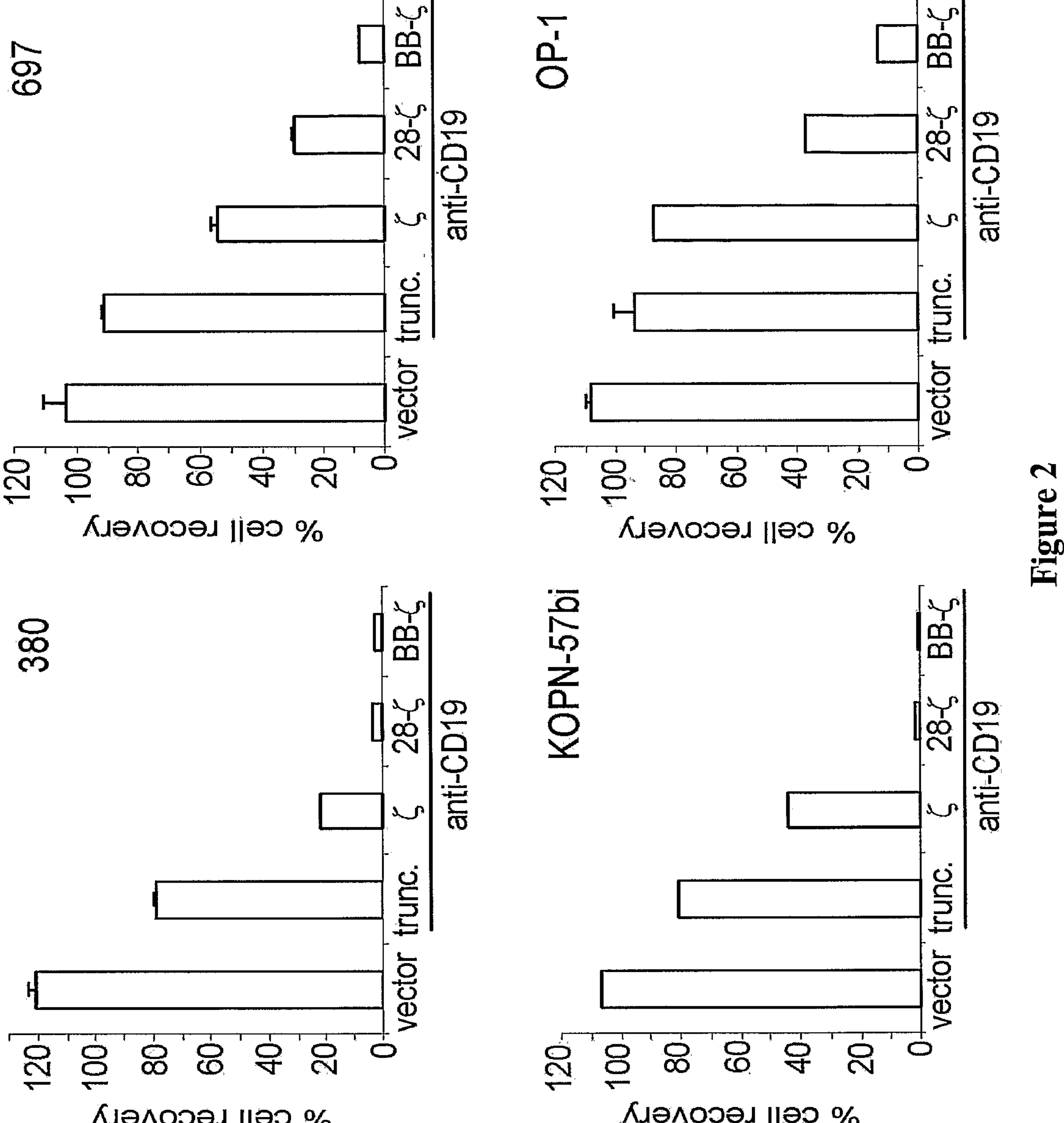


Figure 2

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# Electronic Acknowledgement Receipt

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| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 29-FEB-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 11:56:07 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

| **PATENT APPLICATION FEE DETERMINATION RECORD** Substitute for Form PTO-875 | Application or Docket Number 14/997,136 | Filing Date 01/15/2016 | ☐ To be Mailed |
|---|---|---|---|

**ENTITY:** ☒ LARGE ☐ SMALL ☐ MICRO

## APPLICATION AS FILED – PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | RATE ($) | FEE ($) |
|---|---|---|---|---|
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X $ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X $ = | |
| ☐ APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| ☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

## APPLICATION AS AMENDED – PART II

| AMENDMENT 01/15/2016 | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * 30 | Minus | ** 30 | = 0 | x $80 = | 0 |
| Independent (37 CFR 1.16(h)) | * 1 | Minus | ***3 | = 0 | x $420 = | 0 |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | **0** |

| AMENDMENT 02/29/2016 | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * 30 | Minus | ** 30 | = 0 | x $80 = | 0 |
| Independent (37 CFR 1.16(h)) | * 1 | Minus | *** 3 | = 0 | x $420 = | 0 |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | **0** |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
VERONICA DAY EVERETT

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

PATENT

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1653 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Not Yet Assigned |
| Filed: | January 15, 2016 | : | Confirmation No. 4164 |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(01088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**RESPONSE TO NOTICE TO**
**FILE CORRECTED APPLICATION PAPERS**

In response to the Notice to File Corrected Application Papers dated February 23, 2016, Applicant submits herewith replacement drawings for all figures consisting of Twenty (26) sheets. No new matter has been added.

No fee is believed due for the filing of this Response. Please charge any fee that may be due, and credit any overpayment, to deposit account no. 50-4364.

Favorable examination and allowance of the claims is hereby requested.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: March 22, 2016

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

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Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102
Telephone: (215) 972-7734
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Email: kdoyle@saul.com
*Attorney for Applicant*

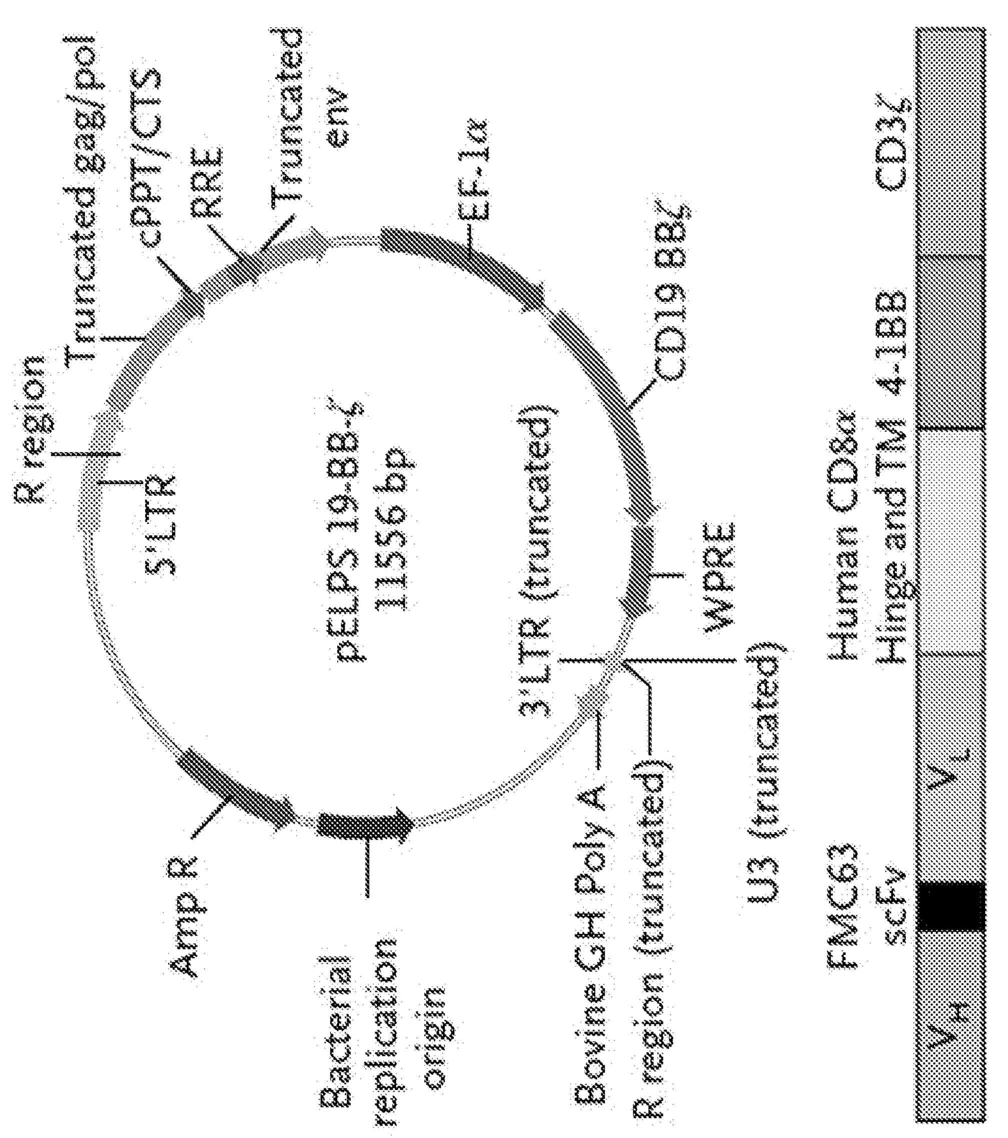



Figure 1A

Replacement Sheet

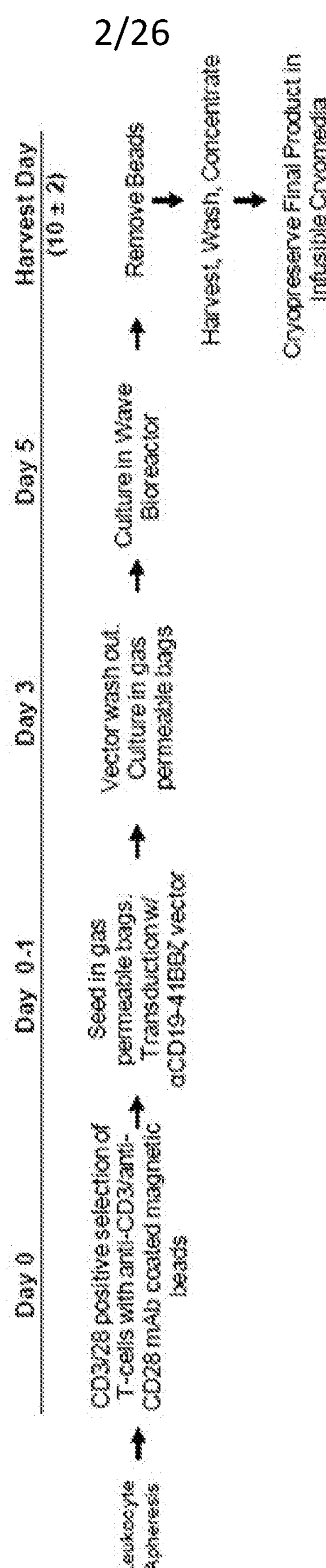
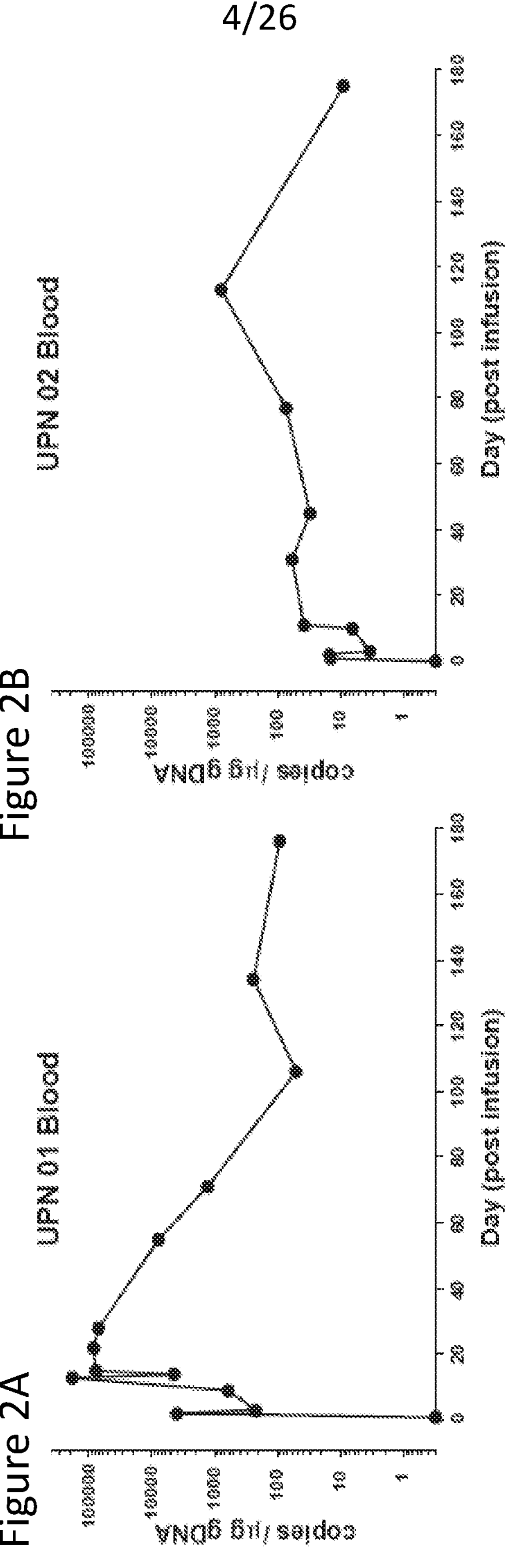



Figure 1B

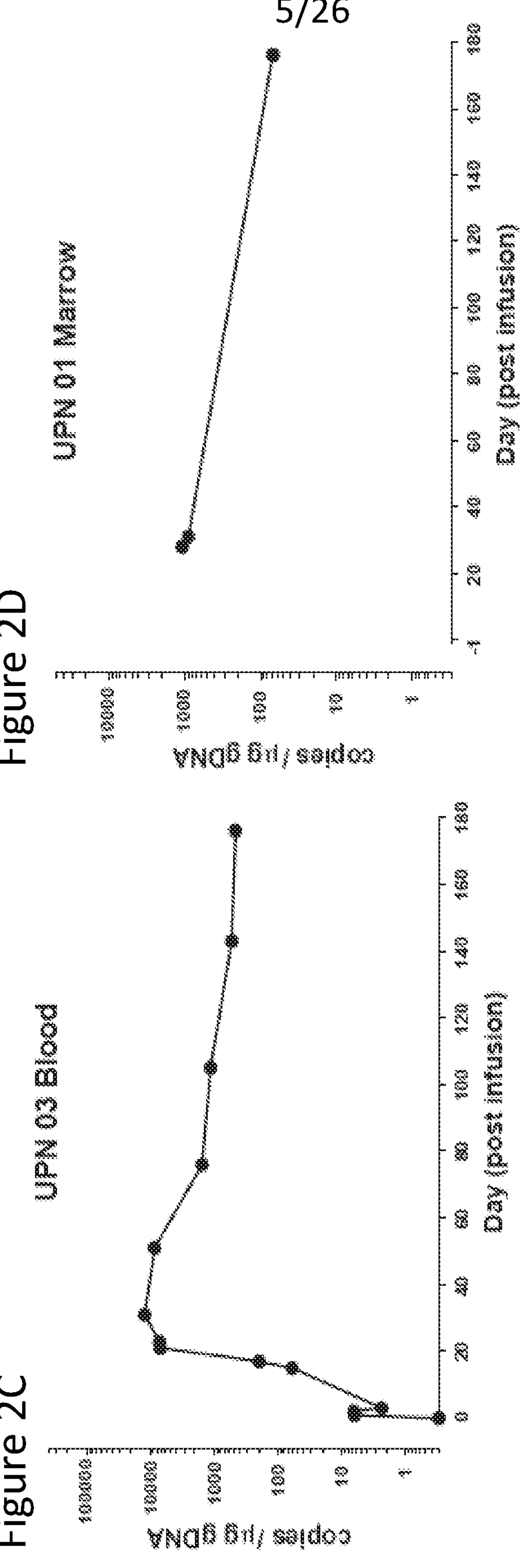
Replacement Sheet

Figure 1C

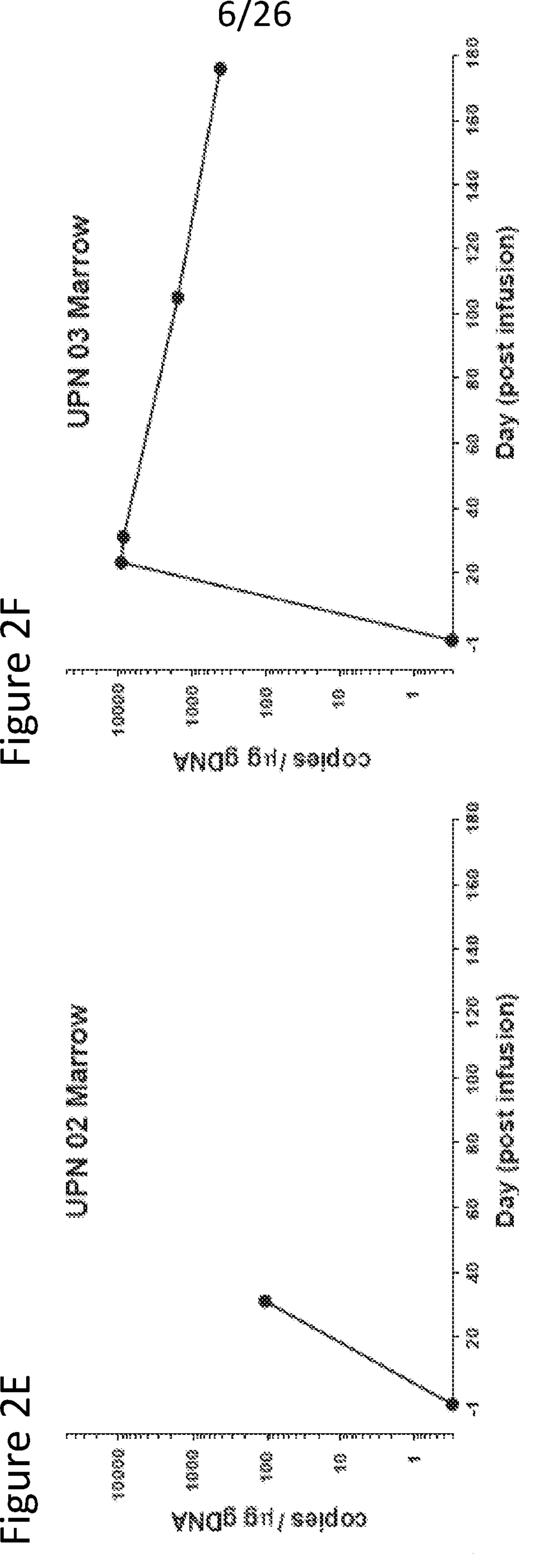
Replacement Sheet



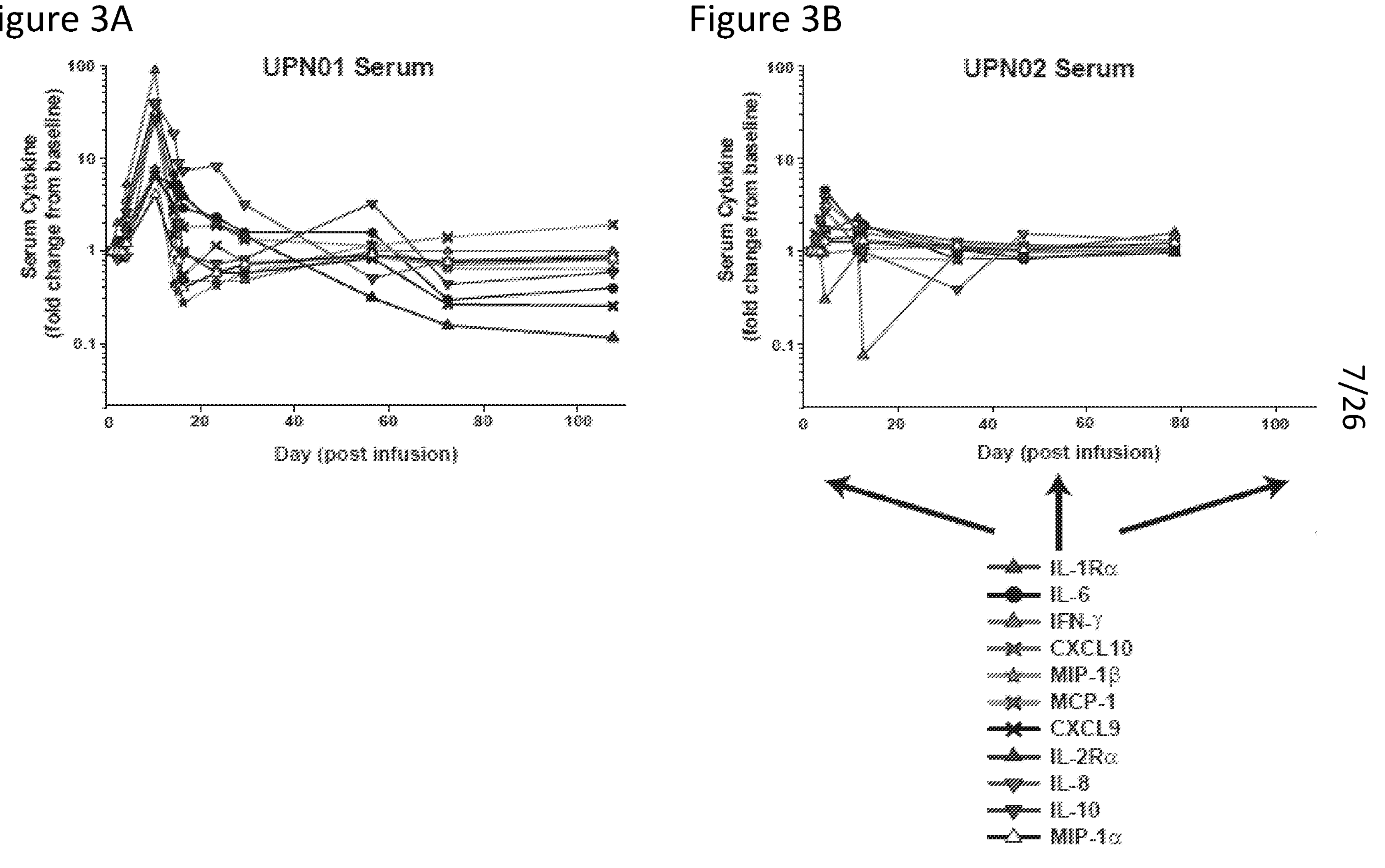

Replacement Sheet




Replacement Sheet




Replacement Sheet

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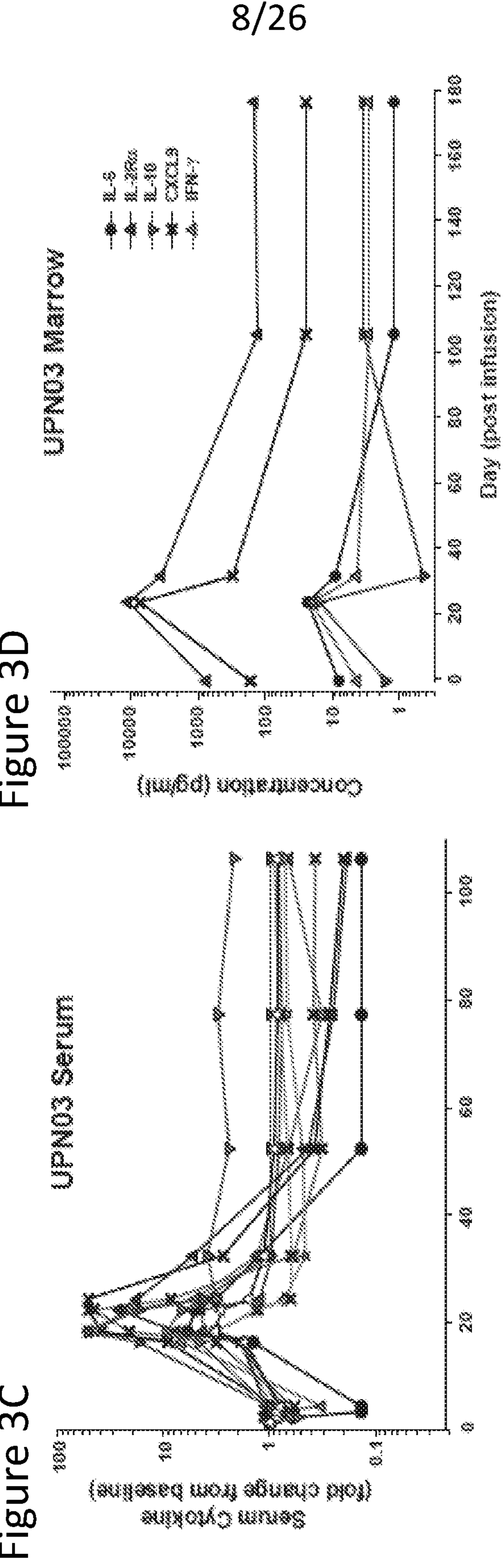
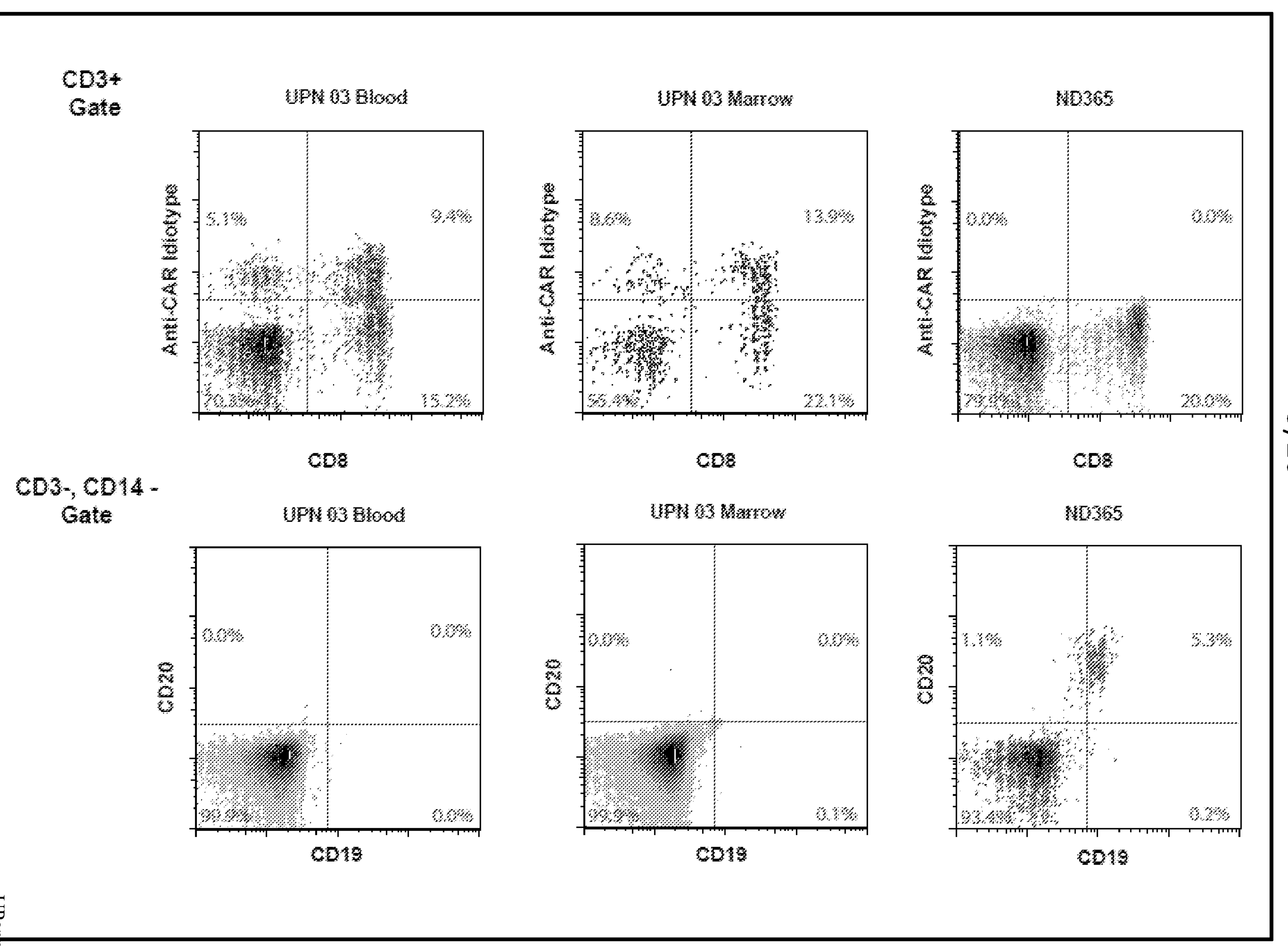



Replacement Sheet

Figure 4A




Replacement Sheet

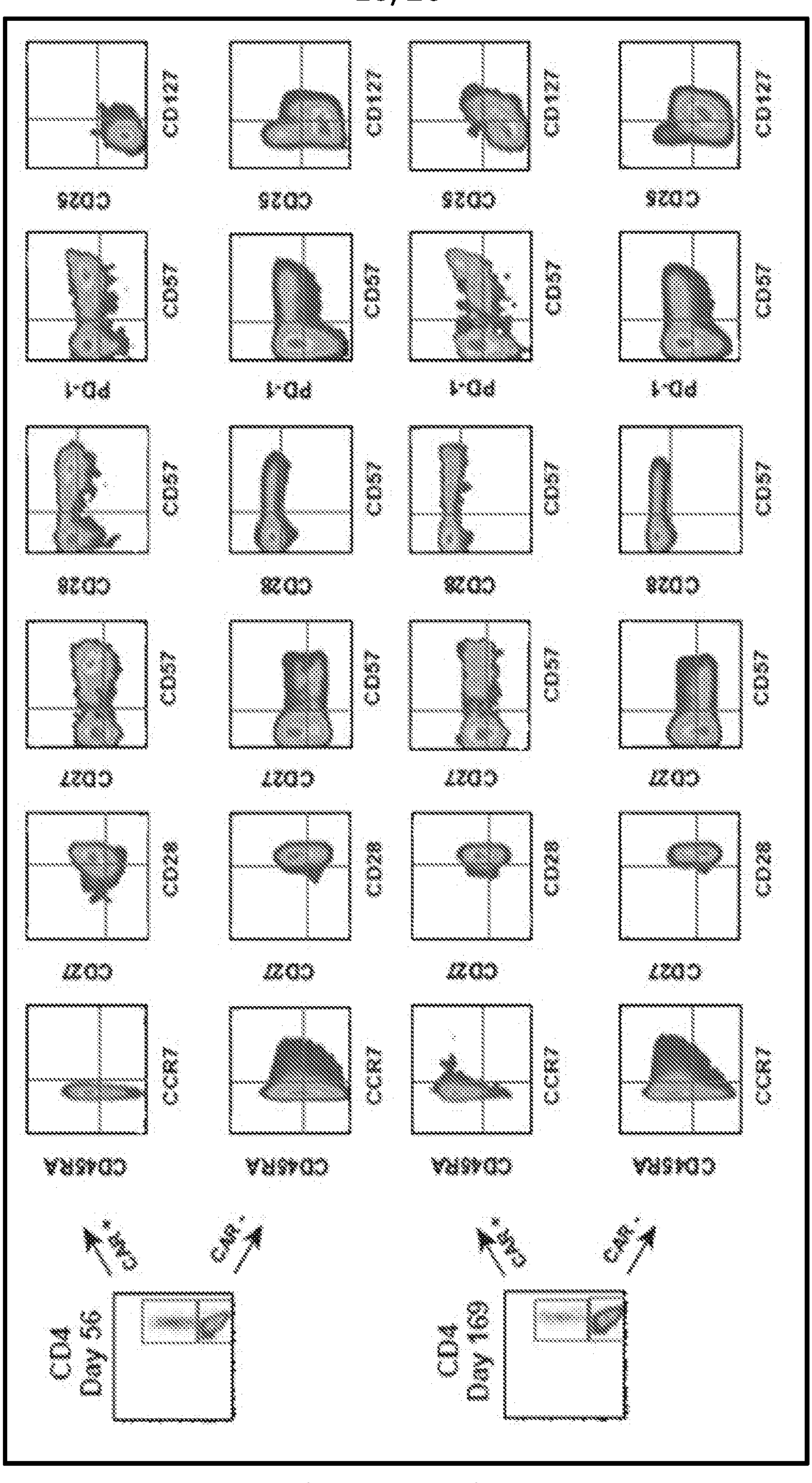



Figure 4B

Replacement Sheet

Figure 4C

CD8 Day 56

CD8 Day 169

CAR+

CAR-

CD45RA CCR7

CD27 CD28

CD27 CD57

CD28 CD57

PD-1 CD57

CD25 CD127

Replacement Sheet

Figure 4D

| | UPN 03 DAY 56 | | | | UPN 03 DAY 169 | | | |
|---|---|---|---|---|---|---|---|---|
| | Upper left | Upper right | Lower left | Lower right | Upper left | Upper right | Lower left | Lower right |
| NO TARGET | 3.64 | 0.1 | 95.9 | 0.33 | 7.64 | 0 | 91.7 | 0.79 |
| K562 | 3.95 | 0.24 | 94.7 | 1.11 | 7.69 | 0.37 | 91.8 | 0.14 |
| K562 CD19 | 0.13 | 0.22 | 88.6 | 11 | 0.22 | 0.48 | 92.7 | 6.62 |
| NALM-6 | 0.14 | 0.16 | 85.9 | 13.8 | 0.22 | 0.31 | 87.2 | 12.3 |
| PMA + IONOMYCIN | 0.029 | 0.16 | 15.1 | 84.7 | 0 | 0.93 | 29.8 | 69.3 |

CAR

CD107A

Replacement Sheet

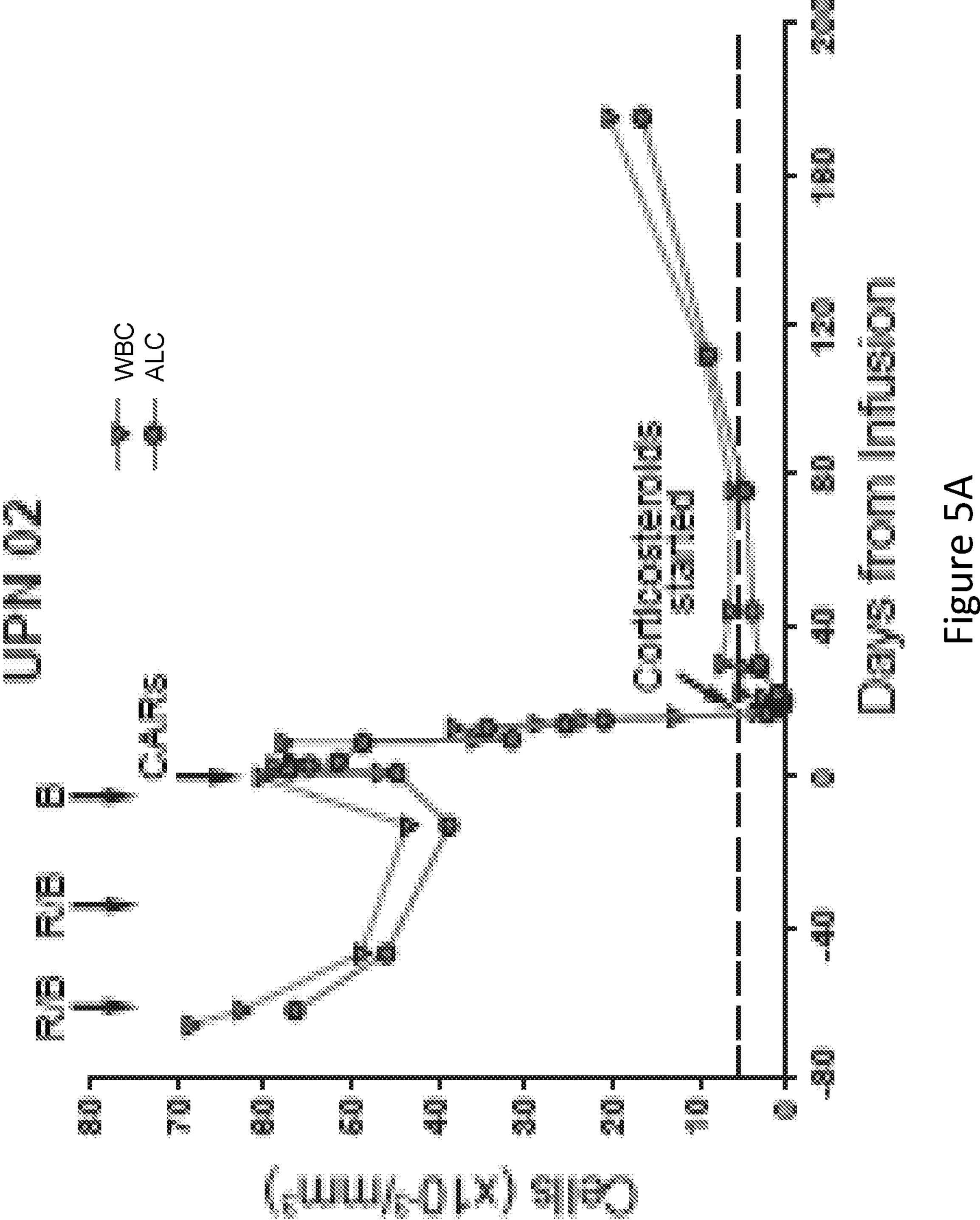
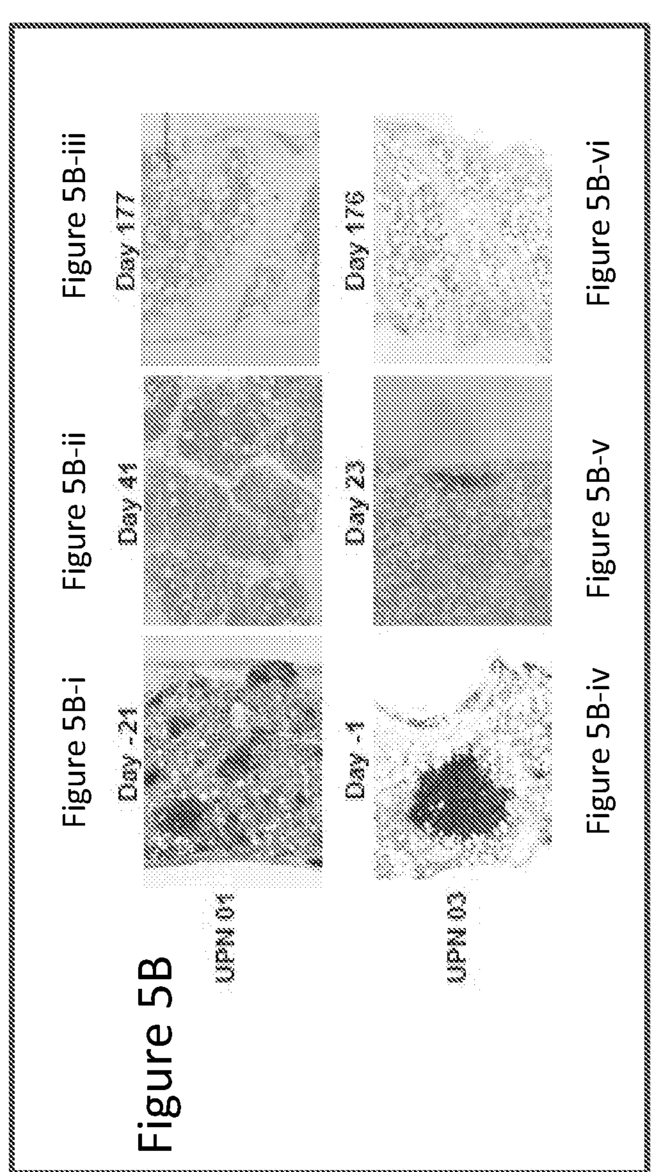
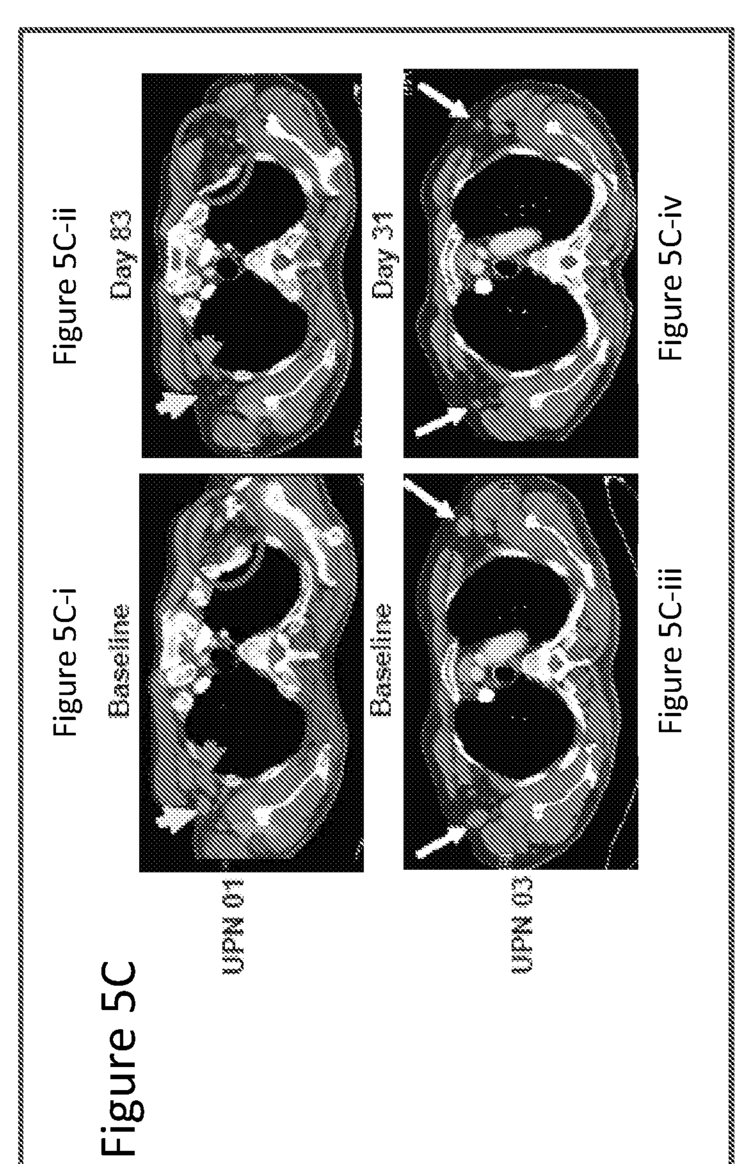



Figure 5A

Replacement Sheet



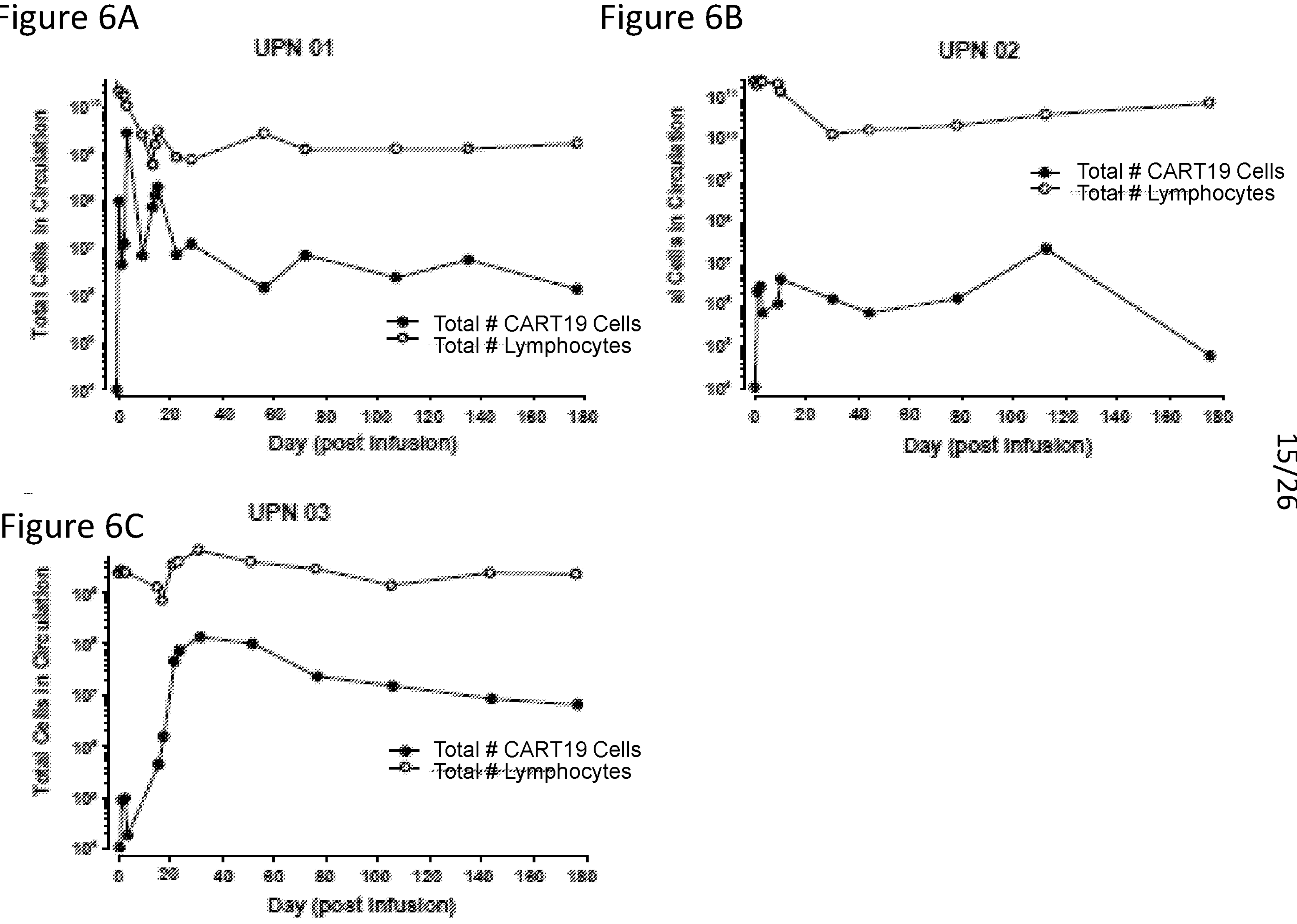



UPenn Ex. 2047
Miltenyi v. UPenn
IPR2022-00855
Page 292

Figure 6A

Figure 6B

Figure 6C

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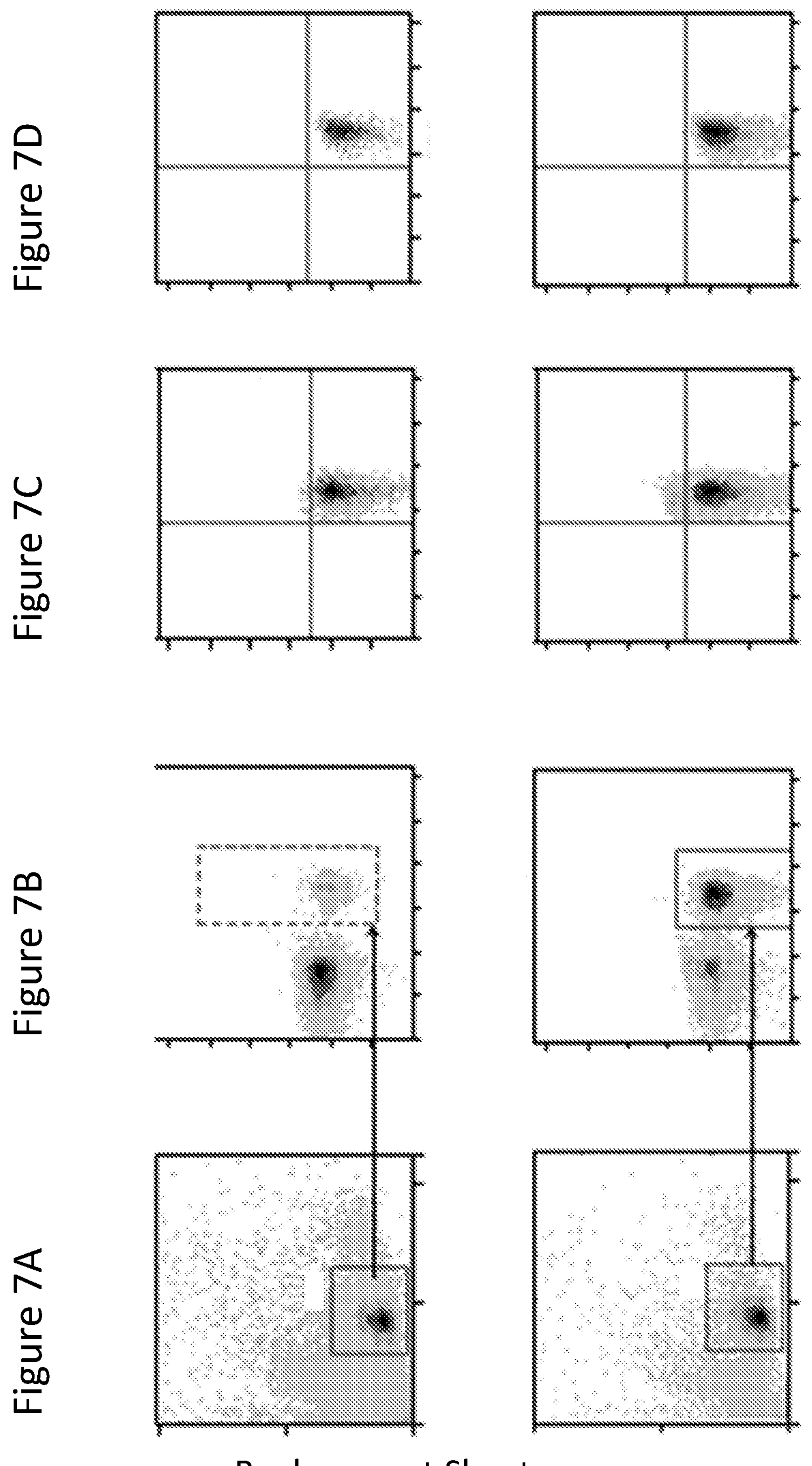


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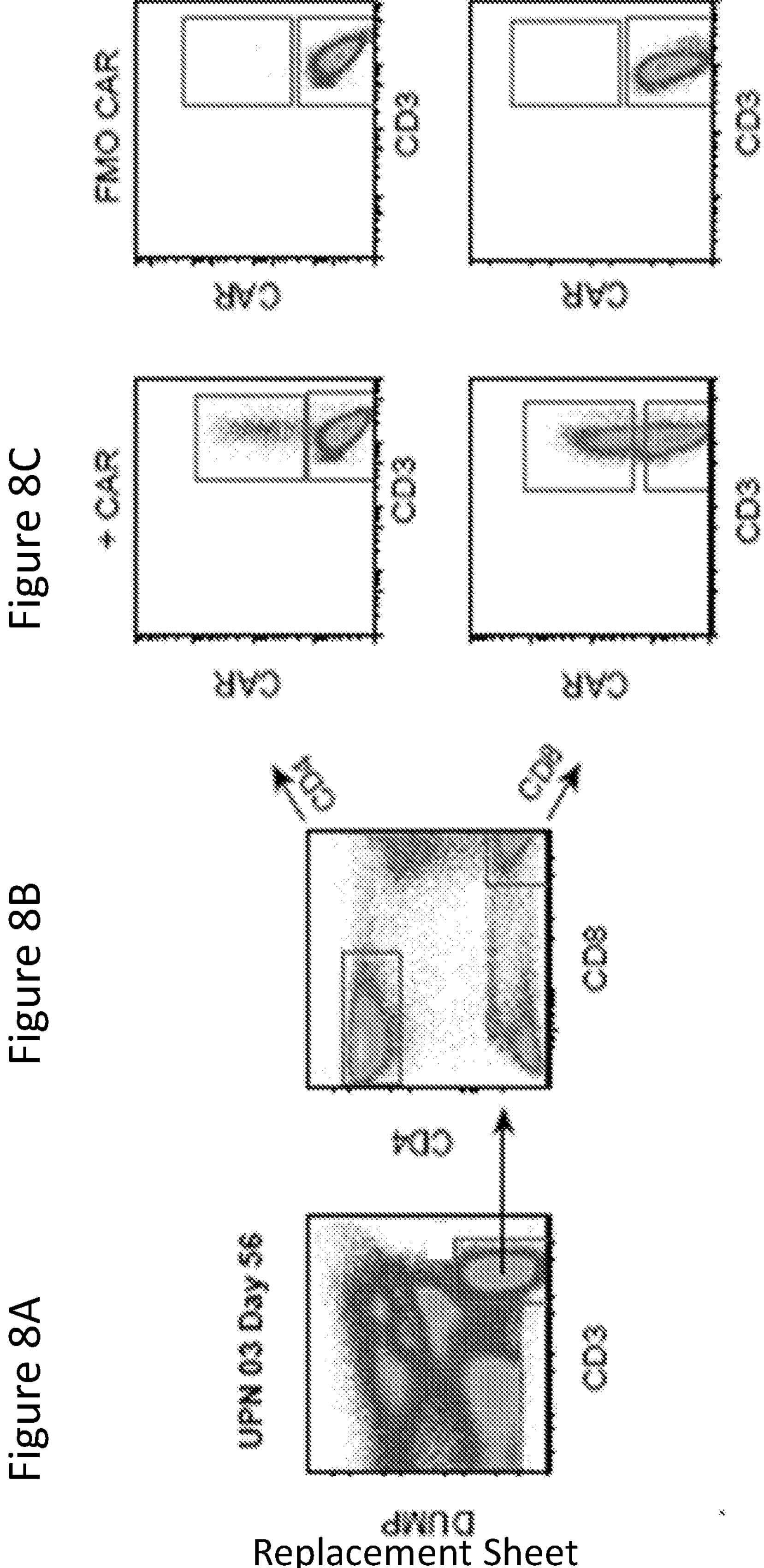


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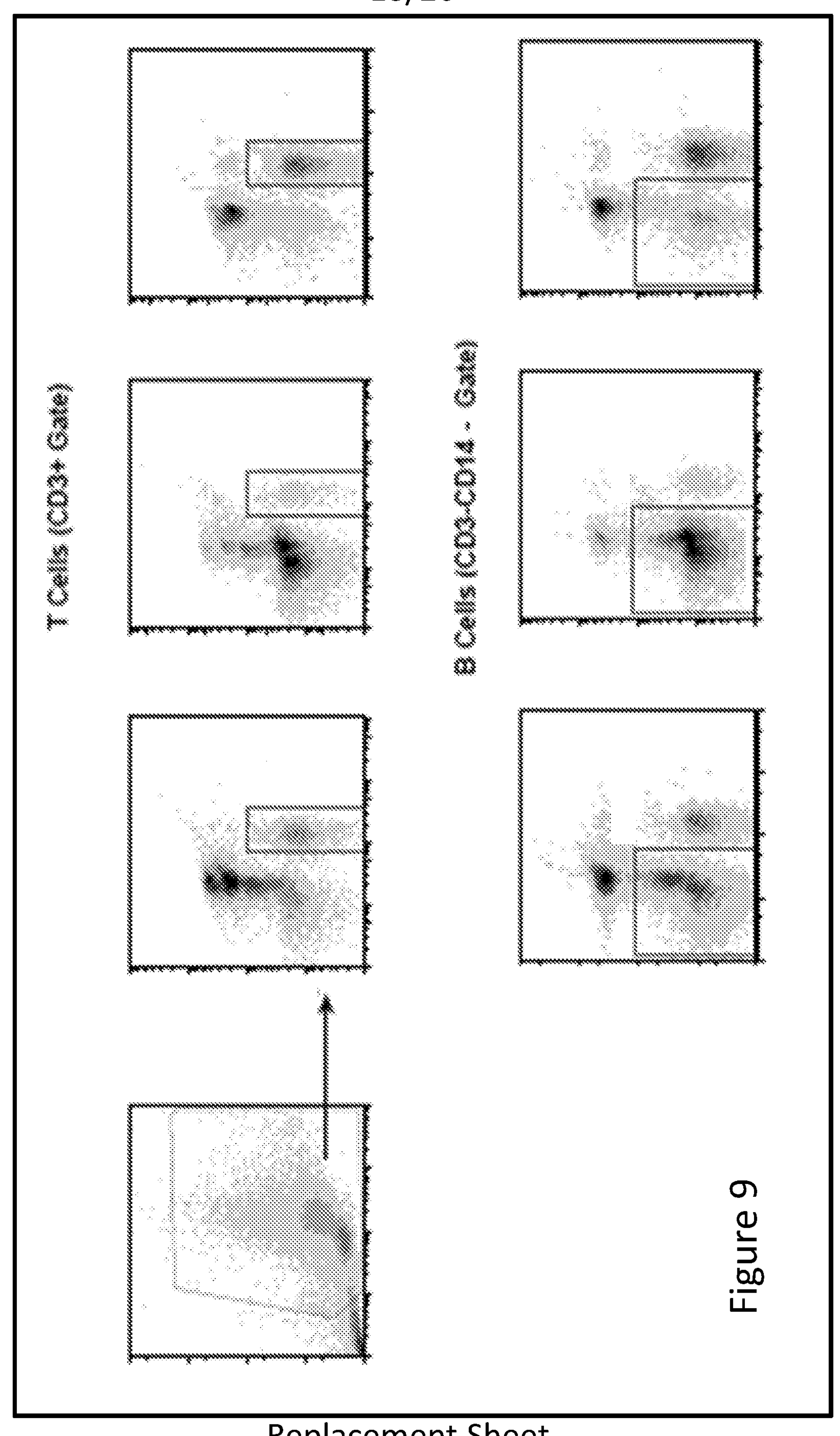



Figure 9

Replacement Sheet

| Subject UPN | Age/sex Karyotype | Previous therapies | CLL Tumor Burden at Baseline | | | Total Dose of CART19 (cells/kg) | Response D +30 (Duration) |
|---|---|---|---|---|---|---|---|
| | | | Bone marrow[3] (Study Day) | Blood[3] (Study Day) | Nodes/spleen[3] (Study Day) | | |
| 01 | 65/M normal | Fludarabine x 4 cycles (2002)<br>Rituximab/fludarabine x 4 cycles (2006)<br>Alemtuzumab x 12 wks (2006)<br>Rituximab (2 courses 2008-2009)<br>R-CVP x 2 cycles (2009)<br>Lenalidomide (2009)<br>PCR x 2 cycles (5/18-6/18/2010)<br>Bendamustine x 1 cycle (7/31-8/1/10) pre-CART19 | Hypercellular<br>70% CLL<br>$2.4x10^{12}$ CLL cells<br>(Day -14)<br>$1.7x10^{12}$ CLL cells<br>(Day -1) | N/A | $6.2x10^{11}$ - $1.0x10^{12}$<br>CLL cells<br>(Day -37) | $1.1x10^{9}$<br>($1.6x10^{7}$/kg) | CR<br>(8+months) |
| 02 | 77/M del(17)(p13)[1] | Alemtuzumab x 16 wks (6/2007)<br>Alemtuzumab x 18 wks (3/2009)<br>Bendamustine/ Rituximab:<br>7/1/2010 (cycle 1)<br>7/28/2010 (cycle 2)<br>8/26/2010 (cycle 3) pre-CART19 | Hypercellular<br>>95% CLL<br>$3.2x10^{12}$ CLL cells<br>(Day -47) | $2.75 x 10^{11}$<br>CLL Cells<br>(Day -1) | $1.2x10^{12}$ -$2.0x10^{12}$<br>CLL cells<br>(Day -24) | $5.8x10^{8}$<br>($1.0x10^{7}$/kg) | PR<br>(5 months) |
| 03 | 64/M del(17)(p13)[2] | R- Fludarabine x 2 cycles (2002)<br>R-Fludarabine x 4 cycles (10/06-1/07)<br>R- Bendamustine x1 cycle (2/09)<br>Bendamustine x 3 cycles (3-5/09)<br>Alemtuzumab x 11 wks (12/09-3/10)<br>Pentostatin/cyclophosphamide (9/10/10) pre-CART19 | Hypercellular<br>40% CLL<br>$8.8x10^{11}$ CLL cells<br>(Day -1) | N/A | $3.3x10^{11}$ - $5.5x10^{11}$<br>CLL cells<br>(Day -10) | $1.4x10^{7}$<br>($1.46x10^{5}$/kg) | CR<br>(7+ months) |

1. UPN 02 Karyotype (ISCN Nomenclature): 45,XY,del(1)(q25),+del(1)(p13),t(2;20)(p13;q11.2),t(3;5)(p13;q35),add(9)(p22),?del(13)(q14q34),-14,del(17)(p13)[cp24]

2. UPN 03 Karyotype (ISCN Nomenclature): 46,XY,del(17)(p12)[16]/44~46,idem,der(17)t(17;21)(p11.2;q11.2)[cp4]/40~45,XY,-17[cp3]

3. See Supplementary Materials for methods of tumor burden determination.

Figure 10

Replacement Sheet

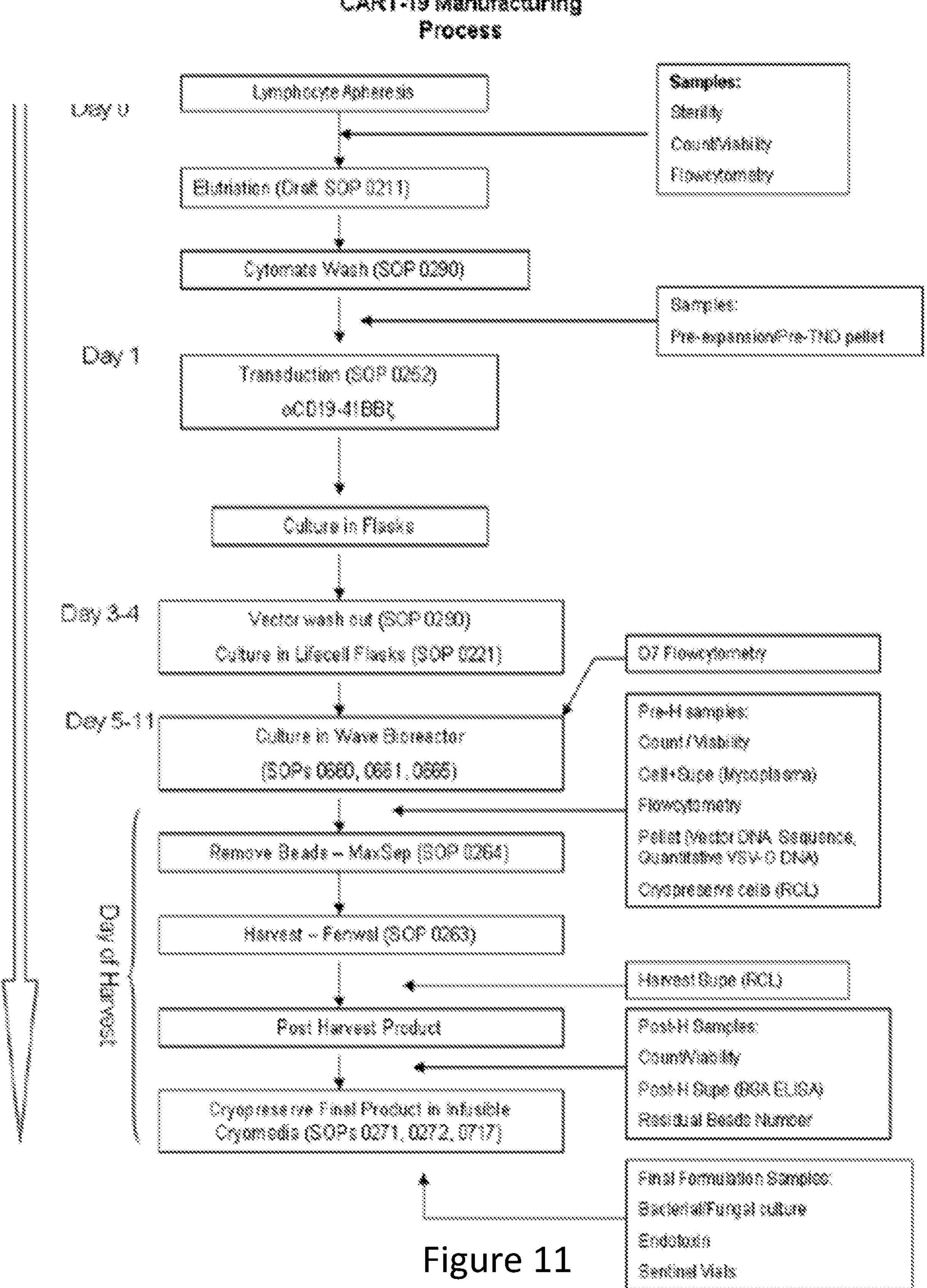



Figure 11

Replacement Sheet

Figure 12B

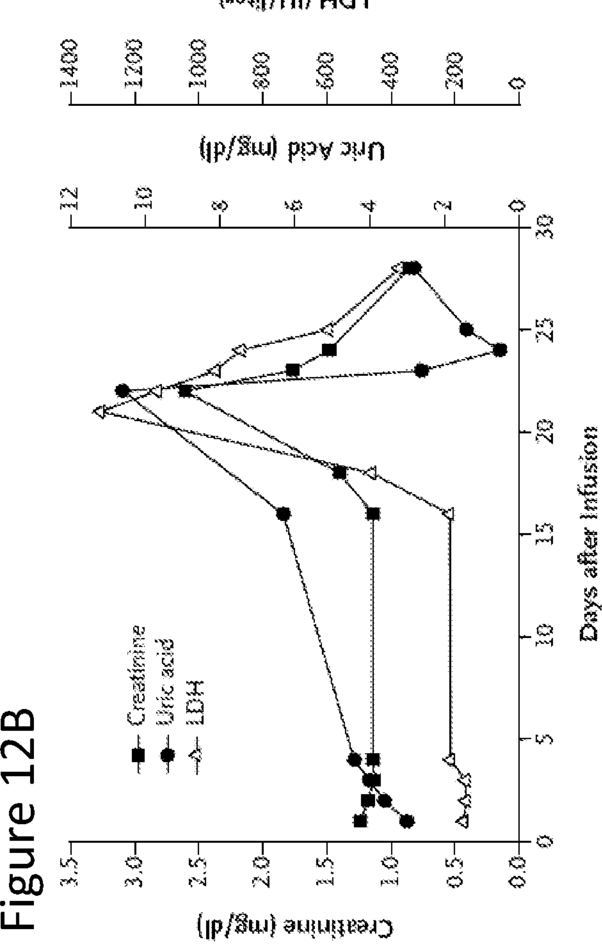
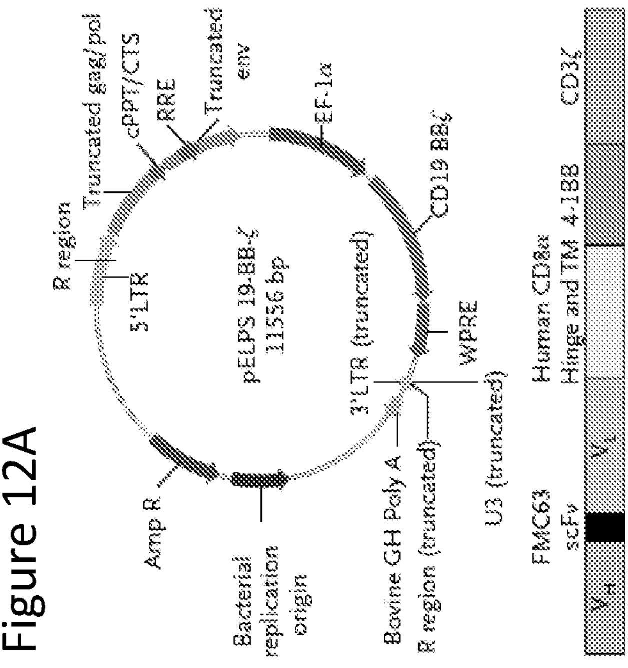



Figure 12A

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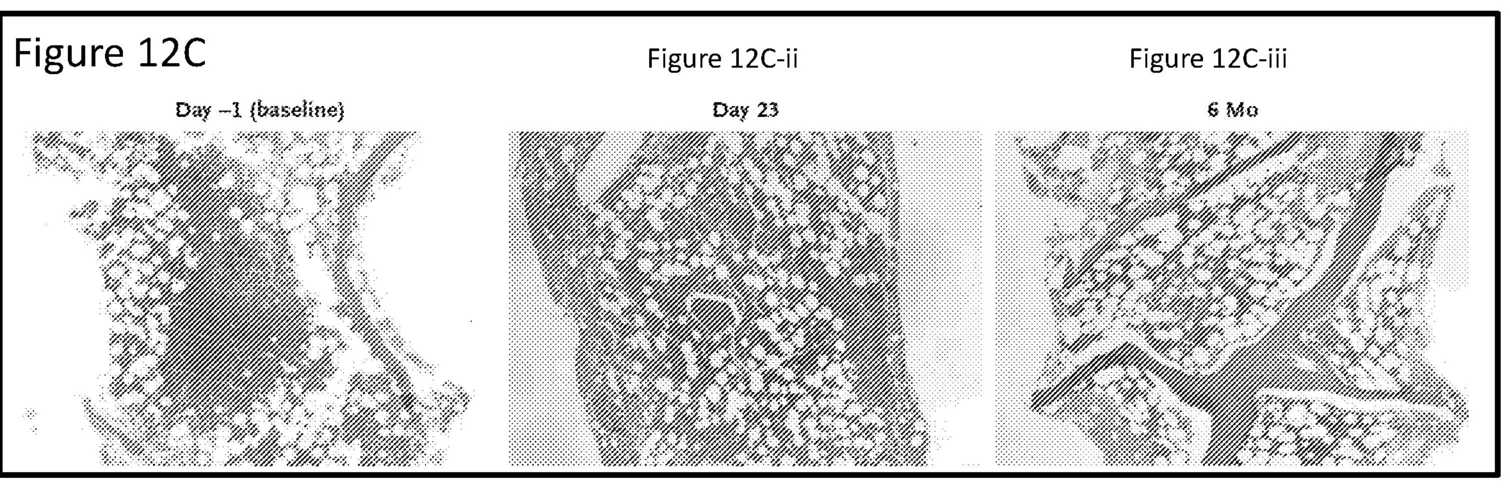


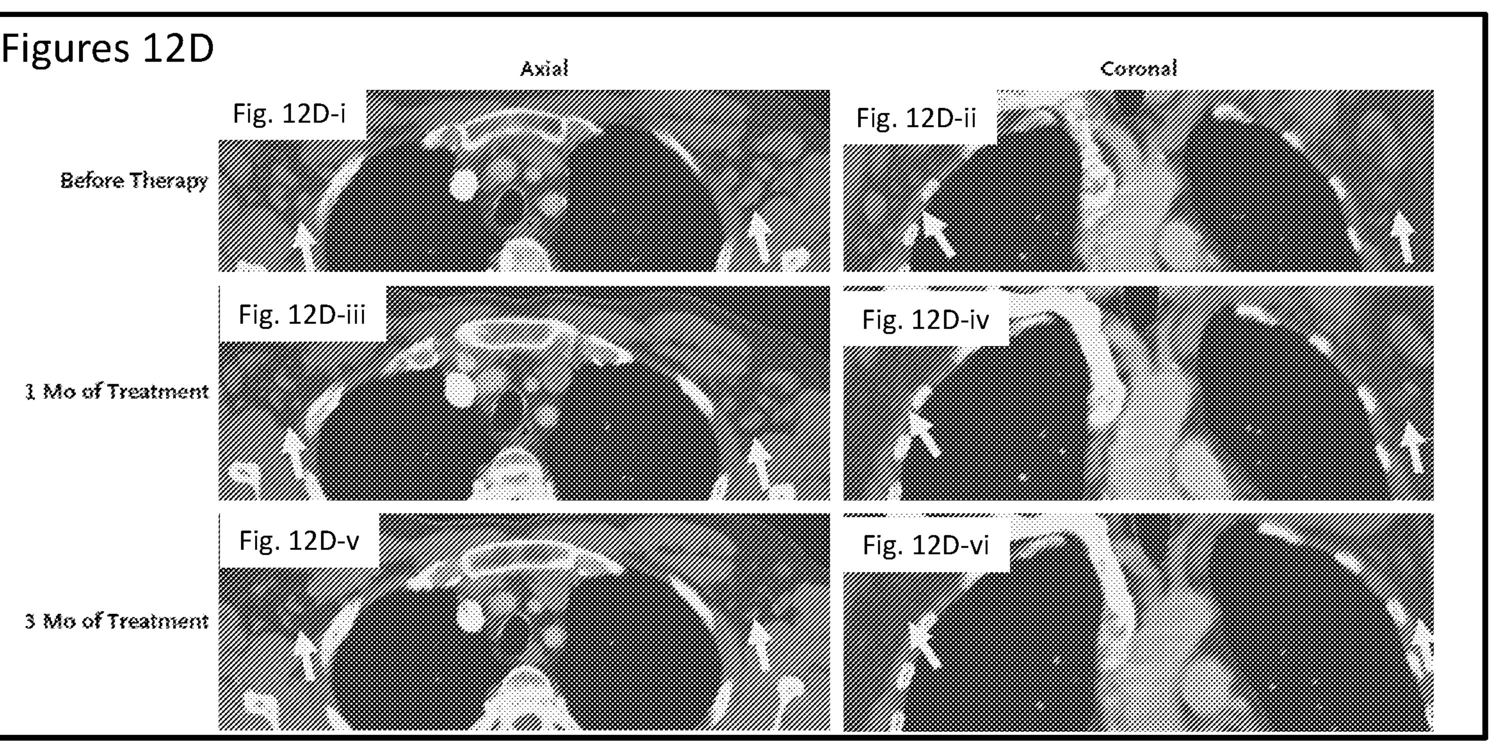


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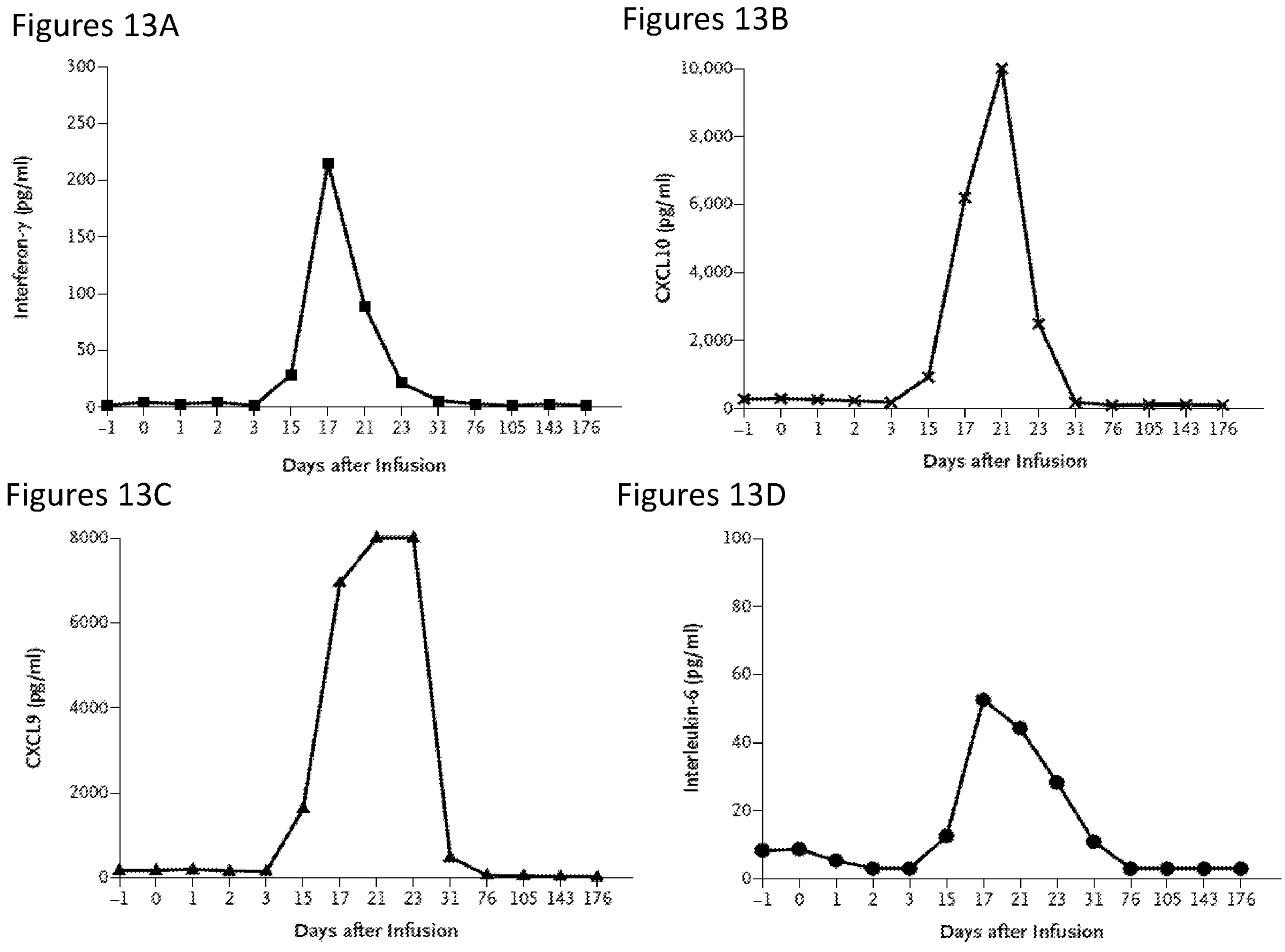


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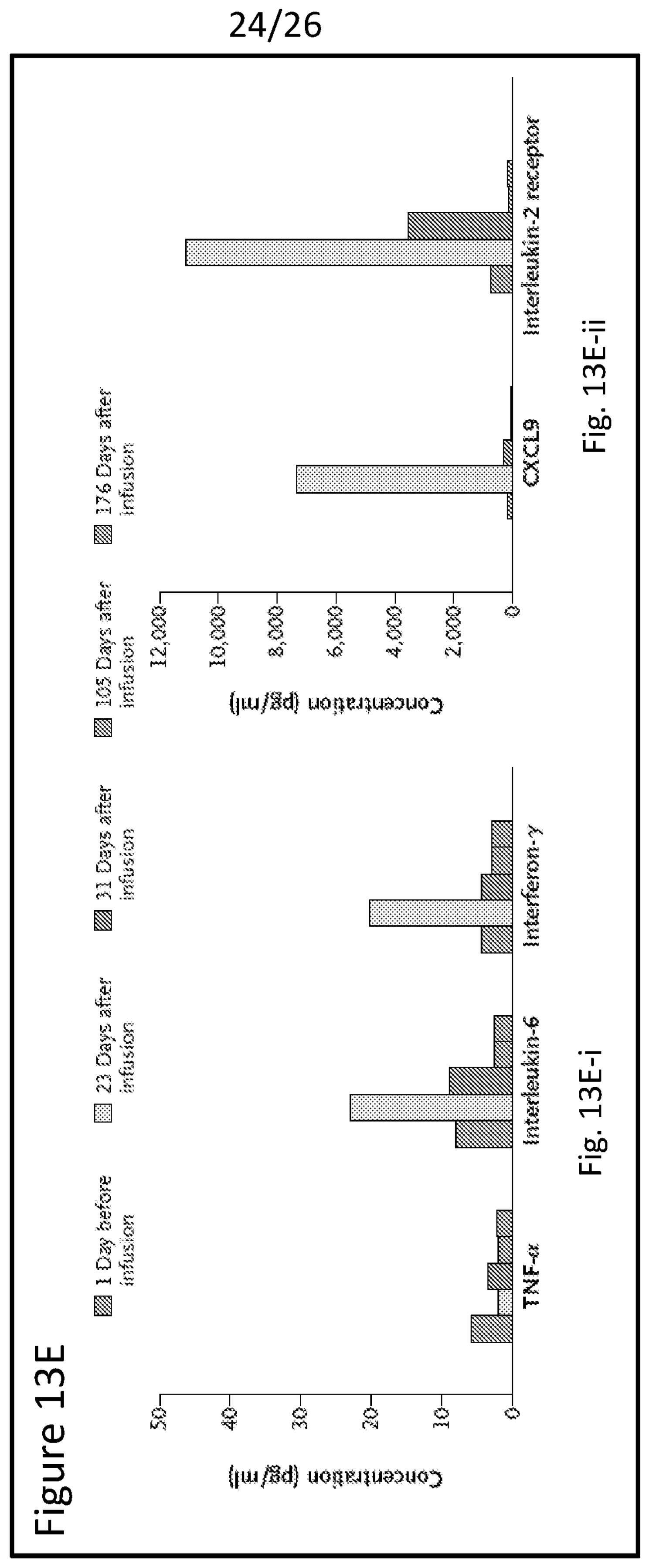



Figure 13E

Fig. 13E-i

Fig. 13E-ii

Replacement Sheet

Figure 14A

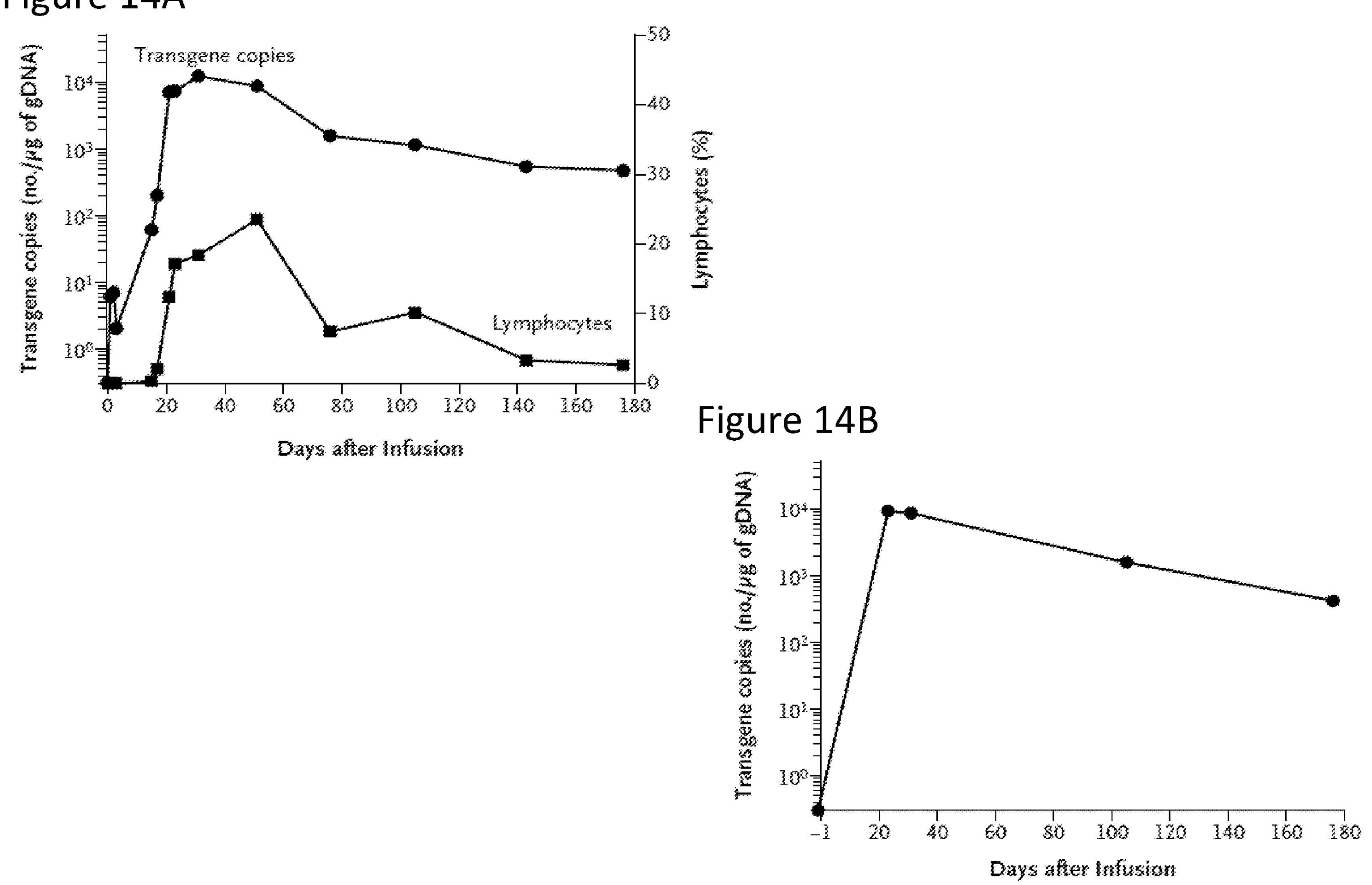
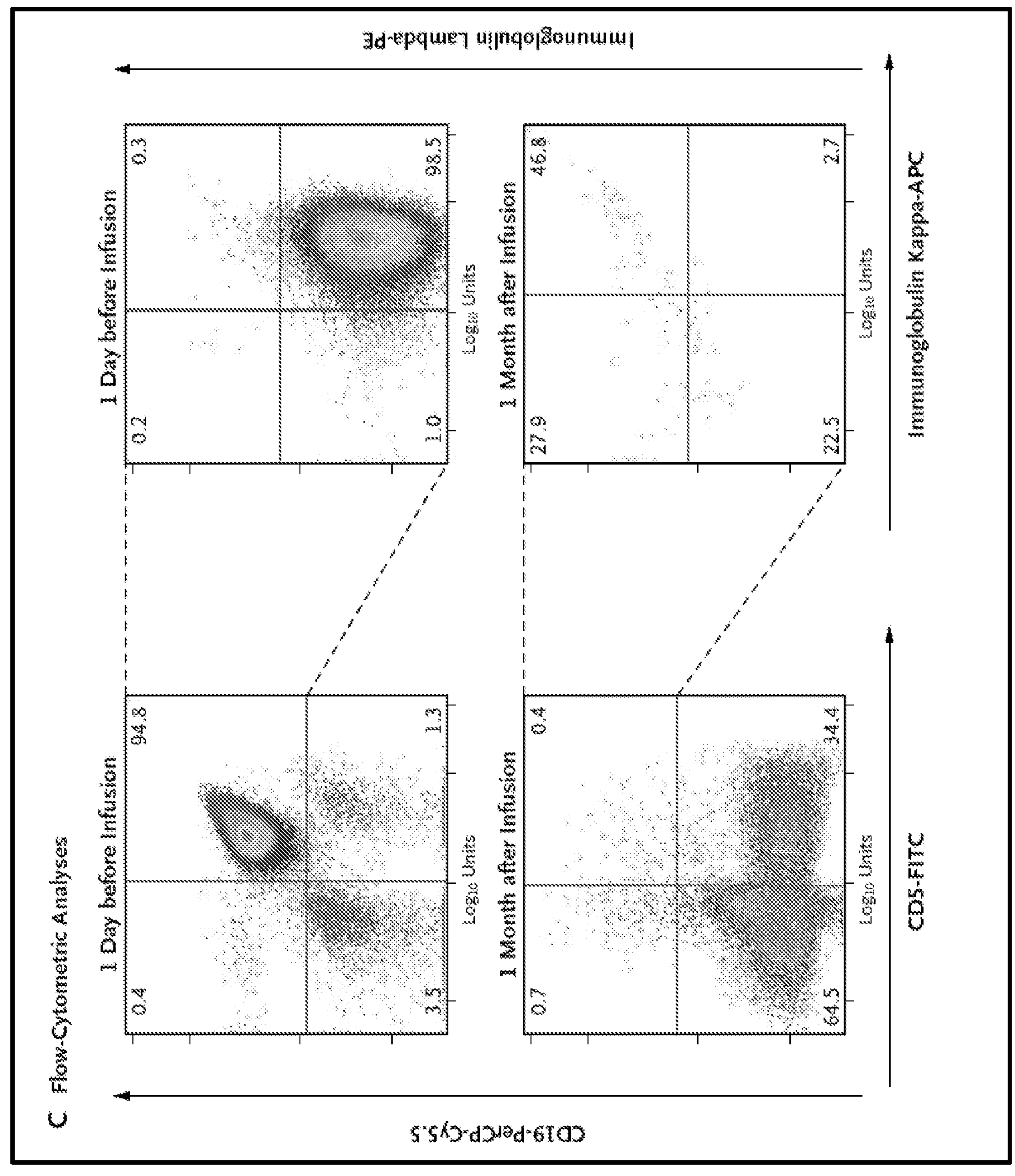



Figure 14B

Replacement Sheet

Figure 14C




Replacement Sheet

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 25272139 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 22-MAR-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 16:19:44 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Applicant Response to Pre-Exam Formalities Notice | 046483-6001US13_Response_to_Notice_to_File_Corrected_Application_Papers_of_2-23-16.pdf | 135112<br>86b9516a21e2812f19e713d686659044c5649c44 | no | 2 |

**Warnings:**

**Information:**

| 2 | Drawings-only black and white line drawings | 046483-6001US13_Replacement_figures.pdf | 6362306<br>d3506bf3a6d5eed6c733a1bf307fecf05d985867 | no | 26 |
|---|---|---|---|---|---|

**Warnings:**

**Information:**

| **Total Files Size (in bytes):** | 6497418 |
|---|---|

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**<u>New Applications Under 35 U.S.C. 111</u>**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**<u>National Stage of an International Application under 35 U.S.C. 371</u>**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**<u>New International Application Filed with the USPTO as a Receiving Office</u>**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

# SCORE Placeholder Sheet for IFW Content

Application Number: 14997136 Document Date: 03/22/2016

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions below.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

## PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
14/997,136

### APPLICATION AS FILED - PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | SMALL ENTITY RATE($) | SMALL ENTITY FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY FEE($) |
|---|---|---|---|---|---|---|---|
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | 280 |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | 600 |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | 720 |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 30 minus 20 = | * 10 | | | OR | x 80 = | 800 |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 1 minus 3 = | * | | | | x 420 = | 0.00 |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | 0.00 |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | 0.00 |
| | | | TOTAL | | | TOTAL | 2400 |

* If the difference in column 1 is less than zero, enter "0" in column 2.

### APPLICATION AS AMENDED - PART II

**AMENDMENT A**

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

**AMENDMENT B**

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
| --- | --- | --- | --- |
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) |

**CONFIRMATION NO. 4164**

**FORMALITIES LETTER**

*OC000000081863518*

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Date Mailed: 04/01/2016

## NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)

*Filing Date Granted*

The U.S. Patent and Trademark Office has received your reply on 03/22/2016 to the Notice to File Missing Parts (Notice) mailed 02/23/2016 and it has been entered into the nonprovisional application. The reply, however, does not include the following items required in the Notice. A complete reply must be timely filed to prevent ABANDONMENT of the above-identified application. Replies should be mailed to: Mail Stop Missing Parts, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450.

Applicant is given **TWO MONTHS** from the date of the Notice to File Missing Parts (Notice) mailed 02/23/2016 within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

**Items Required to Avoid Abandonment:**

The required items noted below SHOULD be filed along with any items required above. The filing date of this nonprovisional application will be the date of receipt of the items required above.

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The drawings submitted to the Office are not electronically reproducible because portions of figures 5A,6A-6C are missing and/or blurry.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/dgela/

---

UNITED STATES PATENT AND TRADEMARK OFFICE

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United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
| --- | --- | --- | --- |
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) |

**CONFIRMATION NO. 4164**

**FORMALITIES LETTER**

*OC000000081863518*

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Date Mailed: 04/01/2016

# NOTICE OF INCOMPLETE REPLY (NONPROVISIONAL)

*Filing Date Granted*

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Applicant is given **TWO MONTHS** from the date of the Notice to File Missing Parts (Notice) mailed 02/23/2016 within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

**Items Required to Avoid Abandonment:**

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The application is informal since it does not comply with the regulations for the reason(s) indicated below.

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Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/dgela/

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# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 25438325 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 08-APR-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 13:49:48 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Applicant Response to Pre-Exam Formalities Notice | 046483-6001US13_Response_to_Notice_of_Incomplete_Reply_dated_4_1_16.pdf | 135990<br>102be52d90515d037551061b44641d9dd41a2529 | no | 2 |

**Warnings:**

**Information:**

| | | | | | |
|---|---|---|---|---|---|
| 2 | Drawings-only black and white line drawings | 046483-6001US13_Replacement_figures_5A-6C.PDF | 215440<br>7c019ce55b57ccdbb6f11cc0cf7fb59353abfe53 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Applicant Response to Pre-Exam Formalities Notice | 046483-6001US13-notice.PDF | 74187<br>d201fd81775f586f817ef2956435114ab3fd21fd | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 425617 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PATENT

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1653 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Not Yet Assigned |
| Filed: | January 15, 2016 | : | Confirmation No. 4164 |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(01088) |

Commissioner for Patents
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Alexandria, VA 22313-1450

**RESPONSE TO NOTICE OF INCOMPLETE REPLY**

This Response is being filed in answer to the Notice of Incomplete Reply dated April 1, 2016 ("Notice"). The Response is timely filed in view of the deadline being two months from the Notice to File Corrected Application Papers dated February 23, 2016.

Applicant hereby submits replacement drawing sheets for Figures 5A and 6A-C.

No fee is believed due for the filing of this Response. Please charge any fee that may be due, and credit any overpayment, to deposit account no. 50-4364.

Favorable examination and allowance of the claims is hereby requested.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: April 8, 2016

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

Saul Ewing LLP
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102
Telephone: (215) 972-7734
Fax: (215) 972-1818
Email: *kdoyle@saul.com*
*Attorney for Applicant*

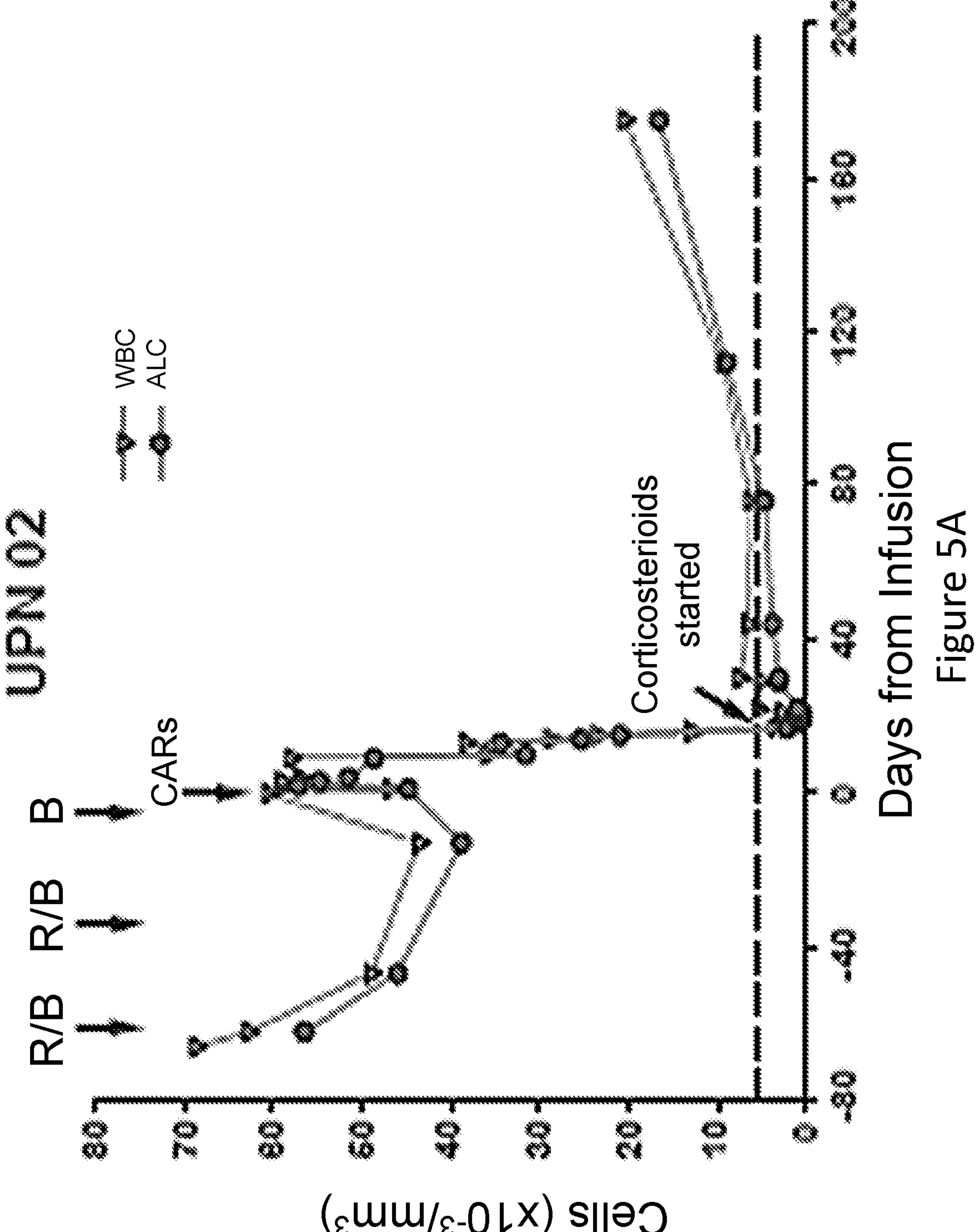


Figure 5A

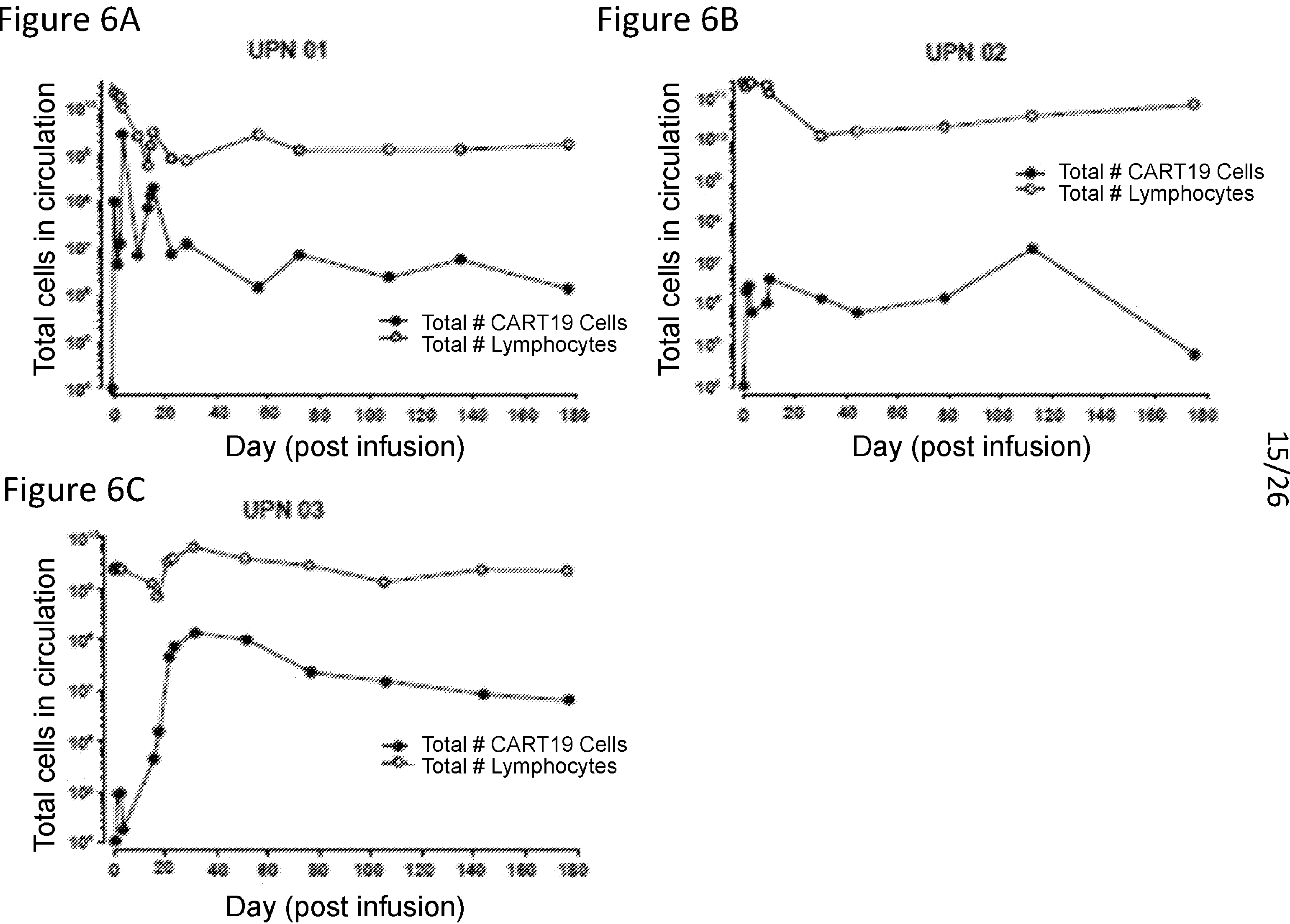
Figure 6A
UPN 01
Total cells in circulation
Total # CART19 Cells
Total # Lymphocytes
0 20 40 60 80 100 120 140 160 180
Day (post infusion)
Figure 6B
UPN 02
Total cells in circulation
Total # CART19 Cells
Total # Lymphocytes
0 20 40 60 80 100 120 140 160 180
Day (post infusion)
Figure 6C
UPN 03
Total cells in circulation
Total # CART19 Cells
Total # Lymphocytes
0 20 40 60 80 100 120 140 160 180
Day (post infusion)

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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | 1653 | 2400 | 046483-6001US13(01088) | 30 | 1 |

**CONFIRMATION NO. 4164**

**UPDATED FILING RECEIPT**

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Date Mailed: 04/15/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Inventor(s)**

Carl H. June, Merion Station, PA;
Bruce L. Levine, Cherry Hill, NJ;
David L. Porter, Springfield, PA;
Michael D. Kalos, Philadelphila, PA;
Michael C. Milone, Cherry Hill, NJ;

**Applicant(s)**

The Trustees of the University of Pennsylvania, Philadelphia, PA;

**Power of Attorney:** The patent practitioners associated with Customer Number 78905

**Domestic Priority data as claimed by applicant**

This application is a CON of 13/992,622 07/09/2013
which is a 371 of PCT/US2011/064191 12/09/2011
which claims benefit of 61/421,470 12/09/2010
and claims benefit of 61/502,649 06/29/2011

**Foreign Applications** for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
*Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.*

**Permission to Access Application via Priority Document Exchange:** Yes

**Permission to Access Search Results:** Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

**If Required, Foreign Filing License Granted:** 02/04/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 14/997,136**

**Projected Publication Date:** 07/21/2016

**Non-Publication Request:** No

**Early Publication Request:** No
**Title**

Compositions and Methods for Treatment of Cancer

**Preliminary Class**

435

**Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:** No

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific

countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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## Title 35, United States Code, Section 184

## Title 37, Code of Federal Regulations, 5.11 & 5.15

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

**NOT GRANTED**

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technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

## PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number
14/997,136

### APPLICATION AS FILED - PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | SMALL ENTITY RATE($) | SMALL ENTITY FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY FEE($) |
|---|---|---|---|---|---|---|---|
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | 280 |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | 600 |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | 720 |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 30 minus 20= | * 10 | | | OR | x 80 = | 800 |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 1 minus 3 = | * | | | | x 420 = | 0.00 |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | 0.00 |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | 0.00 |
| | | | TOTAL | | | TOTAL | 2400 |

* If the difference in column 1 is less than zero, enter "0" in column 2.

### APPLICATION AS AMENDED - PART II

**AMENDMENT A**

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

**AMENDMENT B**

| | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|
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| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
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* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
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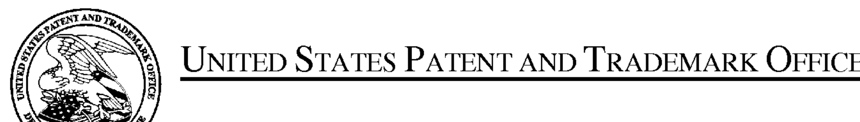
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) | 4164 |

78905 7590 04/21/2016

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
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Philadelphia, PA 19102-2186

| EXAMINER | |
|---|---|
| BURKHART, MICHAEL D | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1633 | |

| NOTIFICATION DATE | DELIVERY MODE |
|---|---|
| 04/21/2016 | ELECTRONIC |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@saul.com

| Office Action Summary | Application No. 14/997,136 | Applicant(s) JUNE ET AL. | |
|---|---|---|---|
| | Examiner Michael Burkhart | Art Unit 1633 | AIA (First Inventor to File) Status No |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --***

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☐ Responsive to communication(s) filed on _____.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.
3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims***

5)☒ Claim(s) *90-119* is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.
6)☐ Claim(s) _____ is/are allowed.
7)☒ Claim(s) *90-119* is/are rejected.
8)☐ Claim(s) _____ is/are objected to.
9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

**Application Papers**

10)☐ The specification is objected to by the Examiner.
11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
**Certified copies:**
a)☐ All b)☐ Some** c)☐ None of the:
1.☐ Certified copies of the priority documents have been received.
2.☐ Certified copies of the priority documents have been received in Application No. _____.
3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1)☐ Notice of References Cited (PTO-892)
2)☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *1/15/16;2/29/16*.
3)☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
4)☐ Other: _____.



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## DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

### *Priority*

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of 35 U.S.C. 112(a) or the first paragraph of pre-AIA 35 U.S.C. 112, except for the best mode requirement. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994)

The disclosure of the prior-filed application, Application Nos. 61/502,649 and 61/421,470, fail to provide adequate support or enablement in the manner provided by 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph for one or more claims of this application. The '649 and '470 applications do not disclose any of the SEQ ID NOs recited in the claims. The first disclosure of such SEQ ID NOs was in PCT/US11/64191, thus, the benefit of priority for the claims is given to the filing date of the application, 12/9/2011.

## *Claim Rejections - 35 USC § 112*

The following is a quotation of 35 U.S.C. 112(b):
(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:
The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 94 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

Claim 94 recites the limitation "the scFv" in line 1. There is insufficient antecedent basis for this limitation in the claim.

## *Double Patenting*

Claims 90, 93, 95-115 of this application are patentably indistinct from claims 90-92, 94-113 of Application No. 14/997,042. Pursuant to 37 CFR 1.78(f) or pre-AIA 37 CFR 1.78(b), when two or more applications filed by the same applicant contain patentably indistinct claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicant is required to either cancel the patentably indistinct claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822.

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and

useful process... may obtain a patent therefor..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the claims that are directed to the same invention so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claims 90, 93, 95-115 are provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 90-92, 94-113 of copending Application No. 14/997,042 (reference application). This is a provisional statutory double patenting rejection since the claims directed to the same invention have not in fact been patented. The cells claimed in both applications have the same structural limitations, and the cells claimed in the '042 application are clearly directed to a "pharmaceutical composition." See, e.g. claims 114-116 of the '042 application, and the specification.

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably

distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/forms/. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-21 of U.S. Patent No. 8,911,993. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the '933 patent, or are disclosed as preferred embodiments.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-30 of U.S. Patent No. 8,906,682. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those used in the methods claimed in the '682 patent, or are disclosed as preferred embodiments.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-30 of U.S. Patent No. 9,102,760. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the '760 patent, or are disclosed as preferred embodiments.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-30 of U.S. Patent No. 9,101,584. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those used in the methods claimed in the ‘584 patent, or are disclosed as preferred embodiments.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-30 of U.S. Patent No. 9,102,761. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the ‘761 patent, or are disclosed as preferred embodiments.

Claims 90-119 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-15 of U.S. Patent No. 9,328,156. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the ‘156 patent, or are disclosed as preferred embodiments.

Claims 90-119 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 21-25, 31-36, 44-48, 50-69, 93-103 of copending Application

No. 13/992,622. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the ‘622 application, or are disclosed as preferred embodiments.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 90-119 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 90-119 of copending Application No. 14/996,953. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta signaling domain) as those claimed in the ‘953 application, or are disclosed as preferred embodiments.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Claims 90-119 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 90-119 of copending Application No. 14/984,371. Although the claims at issue are not identical, they are not patentably distinct from each other because the instant cells comprise the same elements (e.g. a CAR comprising a CD19 binding domain encoded by SEQ ID NO: 20, TM and hinge domains, 41BB costimulatory domain, a CD3 zeta

signaling domain) as those claimed in the ‘371 application, or are disclosed as preferred embodiments.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

***Conclusion***

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Burkhart whose telephone number is (571)272-2915. The examiner can normally be reached on M-F 8AM-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner’s supervisor, Christopher Babic can be reached on (571) 272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Michael Burkhart/
Primary Examiner, Art Unit 1633

| *Search Notes* | Application/Control No. 14997136 | Applicant(s)/Patent Under Reexamination JUNE ET AL. |
|---|---|---|
| | Examiner MICHAEL BURKHART | Art Unit 1633 |

## CPC- SEARCHED

| Symbol | Date | Examiner |
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## CPC COMBINATION SETS - SEARCHED

| Symbol | Date | Examiner |
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## US CLASSIFICATION SEARCHED

| Class | Subclass | Date | Examiner |
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## SEARCH NOTES

| Search Notes | Date | Examiner |
|---|---|---|
| Inventor name search (Medline, EAST), USPat, USPgPub, EPO, JPO, Derwent keyword search (EAST) | 4/18/2016 | MB |
| Parent cases reviewed | 4/18/2016 | MB |
| STIC search SEQ ID Nos | 4/18/2016 | MB |

## INTERFERENCE SEARCH

| US Class/ CPC Symbol | US Subclass / CPC Group | Date | Examiner |
|---|---|---|---|
| | | | |

| | /M.B./<br>Primary Examiner.Art Unit 1633 |
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| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>To Be Assigned |
|---|---|---|
| | **APPLICANT:**<br>Carl H. June et al. | |
| | **FILING DATE:**<br>Herewith | **GROUP:**<br>Not Yet Assigned |

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| **Examiner Signature:** /Michael Burkhart/ | **Date Considered:** 04/18/2016 |
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

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| **Examiner Signature:** /Michael Burkhart/ | **Date Considered:** 04/18/2016 |
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| **Examiner Signature:** /Michael Burkhart/ | **Date Considered:** 04/18/2016 |
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

| Sheet 4 of 10 | | |
|---|---|---|
| Form PTO-1449<br>**U.S. Department of Commerce**<br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>To Be Assigned |
| | **APPLICANT:**<br>Carl H. June et al. | |
| | **FILING DATE:**<br>Herewith | **GROUP:**<br>Not Yet Assigned |

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| | **FILING DATE:**<br>January 15, 2016 | **GROUP:**<br>1653 |

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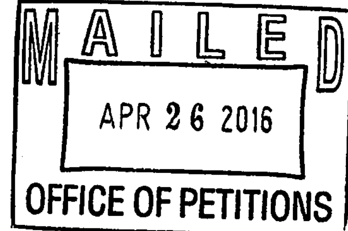


Doc Code: TRACK1.GRANT

| ***Decision Granting Request for Prioritized Examination (Track I or After RCE)*** | Application No.: 14/997,136 |
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1. THE REQUEST FILED 1/15/16 IS **GRANTED.**

The above-identified application has met the requirements for prioritized examination

A. ☒ for an original nonprovisional application (Track I).
B. ☐ for an application undergoing continued examination (RCE).

2. **The above-identified application will undergo prioritized examination.** The application will be accorded special status throughout its entire course of prosecution until one of the following occurs:

A. filing a **petition for extension of time** to extend the time period for filing a reply;
B. filing an **amendment to amend the application to contain more than four independent claims, more than thirty total claims**, or a multiple dependent claim;
C. filing a **request for continued examination**;
D. filing a notice of appeal;
E. filing a request for suspension of action;
F. mailing of a notice of allowance;
G. mailing of a final Office action;
H. completion of examination as defined in 37 CFR 41.102; or
I. abandonment of the application.

Telephone inquiries with regard to this decision should be directed to Cheryl Gibson-Baylor at (571)272-3213, Office of Petitions. In his/her absence, calls may be directed to Brian W. Brown, (571)272-5338.

Cheryl Gibson-Baylor
/Cheryl Gibson-Baylor/
[*Signature*]

Petitions Paralegal Specialist
(Title)

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| | | Australian Application No. 2011338200 – Patent Examination Report No. 1, dated April 11, 2016 |
| | | Chinese Patent Application No. 201180067173X – Third Office Action dated March 28, 2016 |
| | | U.S. Patent Application No. 14/984,371 – non-final Office Action dated March 11, 2016 |
| | | U.S. Patent Application No. 14/997,042 – non-final Office Action dated April 13, 2016 |
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| **Examiner Signature:** | **Date Considered:** |
|---|---|
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 25756615 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 12-MAY-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 10:55:29 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Transmittal Letter | 046483-6001US13_Supplemental_IDS_Statement.pdf | 135033<br>791b2147e106d73621924813922aa055f515fe3c | no | 2 |

**Warnings:**

**Information:**

| 2 | Information Disclosure Statement (IDS) Form (SB08) | 046483-6001US13_IDS_form_1449_for_Supplemental_IDS.pdf | 157084<br>ea651a1c38bcd0cc160160ea5188bb548d335654 | no | 1 |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| 3 | Other Reference-Patent/App/Search documents | AU-Appl-20160411-ExaminationReportNo1-April112016.pdf | 170149<br>2eeaa703402a6ac172a47ac103fb35db8e2e9a3c | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Other Reference-Patent/App/Search documents | CN-Third_Office_Action_March282016.pdf | 10275862<br>69fe65f0874d73582b8955272500aa891a6d9f3d | no | 7 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 5 | Other Reference-Patent/App/Search documents | 14984371-non-final-OA-March112016.pdf | 355320<br>af790bcfca1868851f166805f3036817d84e99e0 | no | 9 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Other Reference-Patent/App/Search documents | 14997042-non-final-OA-April132016.pdf | 353421<br>a2590bcbc379c17331a03e096d6945b54de999dd | no | 9 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 11446869 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PATENT

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | :<br>:<br>: | Group Art Unit:<br>1633 |
| Serial No.: | 14/997,136 | :<br>:<br>: | Examiner:<br>Burkhart, Michael D. |
| | | : | Confirmation No. 4164 |
| Filed: | January 15, 2016 | : | |
| | | : | Attorney Docket No.: |
| For: | Compositions and Methods for Treatment of Cancer | :<br>: | 046483-6001US13(01088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**SUPPLEMENTAL INFORMATION DISCLOSURE UNDER 37 CFR 1.97(e)**

In compliance with the duty of disclosure under 37 CFR § 1.56 and in accordance with the practice under 37 CFR § 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached Form PTO-1449. A copy of each of the cited documents is attached, if required.

**CERTIFICATION**

It is hereby certified that the references contained in this Information Disclosure were cited in a communication from a foreign Patent Office in a counterpart foreign application no more than three months prior to the filing date of this Information Disclosure.

**CONCLUSION**

It is respectfully requested that the information be considered by the Examiner and that a copy of the attached Form PTO-1449 be returned indicating that such information has been considered.

In the event any fees are required in connection with this paper, please charge Deposit Account No. 50-4364.

Applicant's undersigned attorney may be reached by telephone at (215)972-7734. All correspondence should be directed to the below-listed address.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: May 12, 2016

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

Saul Ewing LLP
Centre Square West
1500 Market Street, 38th Floor
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*Attorney for Applicant*

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
| --- | --- | --- | --- |
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) |

**CONFIRMATION NO. 4164**

**PUBLICATION NOTICE**

*OC000000084526707*

78905
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

**Title:**Compositions and Methods for Treatment of Cancer

**Publication No.**US-2016-0208012-A1
**Publication Date:**07/21/2016

## NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

| **Doc Code: DIST.E.FILE**<br>**Document Description: Electronic Terminal Disclaimer - Filed** | PTO/SB/25<br>PTO/SB/26<br>U.S. Patent and Trademark Office<br>Department of Commerce |
|---|---|

| Electronic Petition Request | **TERMINAL DISCLAIMER TO OBVIATE A PROVISIONAL DOUBLE PATENTING REJECTION OVER A PENDING "REFERENCE" APPLICATION AND TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT** |
|---|---|
| Application Number | 14997136 |
| Filing Date | 15-Jan-2016 |
| First Named Inventor | Carl June |
| Attorney Docket Number | 046483-6001US13(01088) |
| Title of Invention | Compositions and Methods for Treatment of Cancer |

☒ Filing of terminal disclaimer does not obviate requirement for response under 37 CFR 1.111 to outstanding Office Action

☒ This electronic Terminal Disclaimer is not being used for a Joint Research Agreement.

| Owner | Percent Interest |
|---|---|
| The Trustees of the University of Pennsylvania | 100 % |

The owner(s) of percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number(s)

14997042 filed on 01/15/2016
14984371 filed on 12/30/2015
14996953 filed on 01/15/2016
13992622 filed on 07/09/2013

as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that any such patent granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

The owner(s) with percent interest listed above in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent number(s)

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8906682

as the term of said prior patent is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later:

- expires for failure to pay a maintenance fee;
- is held unenforceable;
- is found invalid by a court of competent jurisdiction;
- is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
- has all claims canceled by a reexamination certificate;
- is reissued; or
- is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

◉ Terminal disclaimer fee under 37 CFR 1.20(d) is included with Electronic Terminal Disclaimer request.

○ I certify, in accordance with 37 CFR 1.4(d)(4), that the terminal disclaimer fee under 37 CFR 1.20(d) required for this terminal disclaimer has already been paid in the above-identified application.

Applicants claims the following fee status:

○ Small Entity

○ Micro Entity

◉ Regular Undiscounted

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

- [x] An attorney or agent registered to practice before the Patent and Trademark Office who is of record in this application

  Registration Number 36317

- [ ] A sole inventor
- [ ] A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application
- [ ] A joint inventor; all of whom are signing this request

| Signature | /Kathryn Doyle/ |
|---|---|
| Name | Kathryn Doyle |

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 14997136 |
| **Filing Date:** | 15-Jan-2016 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Attorney Docket Number:** | 046483-6001US13(01088) |

Filed as Large Entity

**Filing Fees for** **Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| Statutory or Terminal Disclaimer | 1814 | 1 | 160 | 160 |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
| --- | --- | --- | --- | --- |
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | **Total in USD ($)** | | | **160** |

Doc Code: DISQ.E.FILE
Document Description: Electronic Terminal Disclaimer – Approved

Application No.: 14997136

Filing Date: 15-Jan-2016

Applicant/Patent under Reexamination: June et al.

Electronic Terminal Disclaimer filed on July 21, 2016

☒ APPROVED

**This patent is subject to a terminal disclaimer**

☐ DISAPPROVED

Approved/Disapproved by: Electronic Terminal Disclaimer automatically approved by EFS-Web

U.S. Patent and Trademark Office

# Electronic Acknowledgement Receipt

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|---|---|
| **EFS ID:** | 26416505 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 21-JUL-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 16:59:18 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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|---|---|
| Submitted with Payment | yes |
| Payment Type | Deposit Account |
| Payment was successfully received in RAM | $160 |
| RAM confirmation Number | 6810 |
| Deposit Account | 504364 |
| Authorized User | DOYLE, KATHRYN |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 CFR 1.16 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 CFR 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 CFR 1.19 (Document supply fees)

Charge any Additional Fees required under 37 CFR 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 CFR 1.21 (Miscellaneous fees and charges)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Electronic Terminal Disclaimer-Filed | eTerminal-Disclaimer.pdf | 42077<br>95c5164282c0ace57d5cec5ecfdcc199f9e3ab18 | no | 3 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Fee Worksheet (SB06) | fee-info.pdf | 30329<br>6c9c8d6010144cc83c483cb68c75c0c8fda9ff2d | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 72406 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1633 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Burkhart, Michael D |
| Filed: | January 15, 2016 | : | Confirmation No. 4164 |
| For: | Compositions and Methods for Treatment of Cancer | : | Attorney Docket No.:<br>046483-6001US13(01088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

# AMENDMENT

This Amendment responds to the non-final Office Action dated April 21, 2016, sent in connection with the above-identified application.

A Terminal Disclaimer and accompanying fee are being filed simultaneously herewith.

A Supplemental Information Disclosure Statement is also being filed simultaneously herewith.

Please charge any underpayment of fee, or credit any overpayment, to Deposit Account No. 50-4364.

**AMENDMENT TO THE CLAIMS** begins on page 2 of this paper.

**REMARKS** begin on page 6 of this paper.

**Amendment to the Claims**

The listing of the claims will replace all prior versions, and listings, of the claims in the application.

1-89. (canceled)

90. (Currently amended) A pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells, wherein the T cells comprise a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a CD19 antigen binding domain comprising, from the amino to the carboxy terminus, a light chain variable region and a heavy chain variable region of SEQ ID NO:20, wherein the CAR further comprises a transmembrane domain, a 4-1BB costimulatory signaling region, and a CD3 zeta signaling domain, wherein the T cells are from a human having cancer.

91. (Previously presented) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^4$ to $10^9$ cells per kg body weight of a human in need of such cells.

92. (Previously presented) The composition of claim 90, wherein the anti-tumor effective amount of T cells is $10^5$ to $10^6$ cells per kg body weight of a human in need of such cells.

93. (Previously presented) The composition of claim 90, wherein said antigen binding fragment is a scFv.

94. (Currently amended) The composition of claim ~~90~~ 93 , wherein the scFv comprises the amino acid sequence of SEQ ID NO:20.

95. (Previously presented) The composition of claim 90, wherein the transmembrane domain is CD8α transmembrane domain.

96. (Previously presented) The composition of claim 95, wherein the CD8α transmembrane domain comprises the amino acid sequence of SEQ ID NO: 22.

97. (Previously presented) The composition of claim 90, wherein the CAR further comprises a hinge domain.

98. (Previously presented) The composition of claim 97, wherein the hinge domain is a CD8α hinge domain.

99. (Previously presented) The composition of claim 98, wherein the CD8α hinge domain comprises the amino acid sequence of SEQ ID NO:21.

100. (Previously presented) The composition of claim 90, wherein the 4-1BB costimulatory signaling region comprises the amino acid sequence of SEQ ID NO:23.

101. (Previously presented) The composition of claim 90, wherein the CD3 zeta signaling domain comprises the amino acid sequence of SEQ ID NO: 24.

102. (Previously presented) The composition of claim 90, wherein the CD19 antigen binding domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 14.

103. (Previously presented) The composition of claim 95, wherein the CD8α transmembrane domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 16.

104. (Previously presented) The composition of claim 99, wherein the CD8α hinge domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 15.

105. (Previously presented) The composition of claim 100, wherein the 4-1BB costimulatory signaling region is encoded by a nucleic acid sequence comprising SEQ ID NO: 17.

106. (Previously presented) The composition of claim 101, wherein the CD3 zeta signaling domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 18.

107 (Previously presented) The composition of claim 90, wherein the CAR comprises the amino acid sequence of SEQ ID NO:12.

108. (Previously presented) The composition of claim 107, wherein the CAR is encoded by a nucleic acid sequence comprising SEQ ID NO:8.

109 (Previously presented) The composition of claim 90, wherein the CAR further comprises a CD28 costimulatory signaling region.

110. (Previously presented) The composition of claim 90, wherein the T cells are T cells of a human having a cancer.

111. (Previously presented) The composition of claim 110, wherein the cancer is a hematological cancer.

112. (Previously presented) The composition of claim 90, wherein the T cells comprise a vector that comprises the nucleic acid sequence.

113. (Previously presented) The composition of claim 112, wherein the vector is a lentiviral vector.

114. (Previously presented) The composition of claim 112, wherein the vector further comprises a promoter.

115. (Previously presented) The composition of claim 114, wherein the promoter is an EF-1α promoter.

116. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient.

117. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition comprises a buffer.

118. (Previously presented) The composition of claim 117, wherein the buffer is neutral buffer saline or phosphate buffered saline.

119. (Previously presented) The composition of claim 90, wherein the pharmaceutical composition further comprises a carbohydrate.

## REMARKS

Claims 90-119 are pending in the application.

Claim 90 is amended herein to recite *wherein the T cells are from a human having cancer*. Support for this amendment is found throughout the Examples where the T cells used are from a human having cancer.

Claim 94 is amended herein to depend from claim 93.

No new matter is added by way of these amendments to claims 90 and 93.

**Rejection of Claim 94 under pre-AIA 35 U.S.C. § 112**

Claim 94 has been rejected under 35 U.S.C. § 112(b) or 35 U.S.C. § 112 (pre-AIA), second paragraph, as being indefinite for lacking antecedent basis.

Claim 94 has been amended herein to depend from claim 93, thereby correcting antecedent basis for this claim.

Applicant respectfully requests that the rejection of this claim be withdrawn.

**Statutory Double Patenting Rejection of Claims 90, 93 and 95-115**

Claims 90, 93, 95-115 have been provisionally rejected as being patentably indistinct from claims 90-92, 94-113 of co-pending Application No. 14/997,042. According to the Examiner, the cells claimed in both applications have the same structural limitations, and the cells claimed in co-pending Application No. 14/997,042 are clearly directed to a "pharmaceutical composition," citing claims 114-116 of co-pending Application No. 14/997,042. The Examiner states that Applicant is required to either cancel the patentably indistinct claims from all but one application or maintain a clear line of demarcation between the applications. See MPEP § 822.

Applicant respectfully traverses this rejection for the reasons now given.

The present claims recite "*a pharmaceutical composition comprising an anti-tumor effective amount of a population of human T cells*, wherein the T cells comprise… ." while the co-pending Application No. 14/997,042 recites "*a human T cell comprising*... ." As noted, the present claims recite "*an antitumor effective amount*," whereas the claims of co-

pending Application No. 14/997,042 do not recite this feature. The scope of the claims in the two applications is therefore different. As such, present claims 90, 93, 95-115 are patentably distinct over claims 90-92 and 94-113 in co-pending Application No. 14/997,042 and Applicant respectfully requests that the rejection be withdrawn.

While not conceding that the present claims are obvious over the recited claims in co-pending Application No. 14/997,042, in an effort to avoid an anticipated final rejection of the present claims over claims 90-92 and 94-113 in co-pending Application No. 14/997,042 on the grounds of non-statutory obviousness-type double patenting, Applicant is filing simultaneously herewith a Terminal Disclaimer to obviate any such rejection.

**Double Patenting Rejection**

The Examiner has rejected claims 90-119 on the grounds of non-statutory double patenting as being obvious over various claims in various other members of this patent family.

While Applicant does not agree with the Examiner regarding the merits of these rejections, Applicant is filing herewith the appropriate Terminal Disclaimers which serve to obviate these rejections. Terminal Disclaimers are being filed concurrently herewith that address the following rejections:

Rejection of claims 90-119 over claims 1-21 of U.S. Patent No. 8,911,993;

Rejection of claims 90-119 over claims 1-30 of U.S. Patent No. 8,906,682;

Rejection of claims 90-119 over claims 1-30 of U.S. Patent No. 9,102,760;

Rejection of claims 90-119 over claims 1-30 of U.S. Patent No. 9,101,584;

Rejection of claims 90-119 over claims 1-30 of U.S. Patent No. 9,102,761;

Rejection of claims 90-119 over claims 1-15 of U.S. Patent No. 9,328,156;

Provisional rejection of claims 90-119 over claims 21-25, 31-36, 44-48, 50-69, 93-103 of copending Application No. 13/992,622 (now allowed);

Provisional rejection of claims 90-119 over claims 90-119 of copending Application No. 14/996,953 (now allowed);

Provisional rejection of claims 90-119 over claims 90-119 of copending Application No. 14/984,371 (now allowed);

And as noted elsewhere herein, anticipated provisional rejection of claims 90-119 over claims 90-92 and 94-113 in co-pending Application No. 14/997,042.

In view of the filing of these Terminal Disclaimers, Applicant submits that the rejections have been overcome and respectfully requests withdrawal of same.

**Summary**

Applicants respectfully submit that the pending claims are in full condition for allowance and such action is respectfully requested at the earliest possible time.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: July 21, 2016

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

Saul Ewing LLP
Centre Square West
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*Attorney for Applicant*

PATENT

**ELECTRONICALLY FILED**
**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| In re: | Patent Application Of<br>Carl H. June, et al. | : | Group Art Unit:<br>1633 |
| Serial No.: | 14/997,136 | : | Examiner:<br>Burkhart, Michael D. |
| | | : | Confirmation No. 4164 |
| Filed: | January 15, 2016 | : | |
| | | : | Attorney Docket No.: |
| For: | Compositions and Methods for Treatment of Cancer | : | 046483-6001US13(01088) |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

## SUPPLEMENTAL INFORMATION DISCLOSURE UNDER 37 CFR 1.97(e)

In compliance with the duty of disclosure under 37 CFR § 1.56 and in accordance with the practice under 37 CFR § 1.98, the attention of the Patent and Trademark Office is hereby directed to the documents listed on the attached Form PTO-1449. A copy of each of the cited documents is attached, if required.

## CERTIFICATION

It is hereby certified that the references contained in this Information Disclosure were cited in a communication from a foreign Patent Office in a counterpart foreign application no more than three months prior to the filing date of this Information Disclosure.

## CONCLUSION

It is respectfully requested that the information be considered by the Examiner and that a copy of the attached Form PTO-1449 be returned indicating that such information has been considered.

In the event any fees are required in connection with this paper, please charge Deposit Account No. 50-4364.

Applicant's undersigned attorney may be reached by telephone at (215)972-7734. All correspondence should be directed to the below-listed address.

Respectfully submitted,
*CARL H. JUNE, ET AL.*

Dated: July 21, 2016

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

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1500 Market Street, 38th Floor
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Email: *kdoyle@saul.com*
*Attorney for Applicant*

| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Supplemental**<br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>14/997,136 |
|---|---|---|
| | **APPLICANT:**<br>Carl H. June, et al. | |
| | **FILING DATE:**<br>January 15, 2016 | **GROUP:**<br>1633 |

**U.S. PATENT DOCUMENTS**

| Examiner Initial | | Document Number | Date | Name | Class | Subclass | Filing Date if appropriate |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |

**FOREIGN PATENT DOCUMENTS**

| | | Document Number | Date | Country | Class | Subclass | TranslationYes/No /Abstract |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**OTHER DOCUMENT(S)** (*Including Author, Title, Date, Pertinent Pages, etc.*)

| | | |
|---|---|---|
| | | Mexican Patent Application No. MX/a/2013/006570 – Office Action dated May 27, 2016. |

| **Examiner Signature:** | **Date Considered:** |
|---|---|
| | |

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 26421117 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 21-JUL-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 17:04:10 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Amendment Copy Claims/Response to Suggested Claims | 046483-6001US13_RESPONSE_to_non-final_OA_of_April_21__2016-.pdf | 204766<br>c87841200fe24e61423af467a003c7e6cdf33821 | no | 9 |

**Warnings:**

| Information: | | | | | |
|---|---|---|---|---|---|
| 2 | Transmittal Letter | 046483-6001US13_Supplemental_IDS_Support_statement_-_July_2016.pdf | 134885<br>808edb81f99fb7664637982190f85b630108db7f | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Information Disclosure Statement (IDS) Form (SB08) | 046483-6001US13_IDS_form_1449_for_Supplemental_IDS_-_July_2016.pdf | 154212<br>289cb7efe468f16e2126109670f0ad77a5ff3356 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| This is not an USPTO supplied IDS fillable form | | | | | |
| | | | **Total Files Size (in bytes):** | 493863 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 26421245 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | Compositions and Methods for Treatment of Cancer |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle |
| **Filer Authorized By:** | |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 21-JUL-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 17:10:56 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Other Reference-Patent/App/Search documents | MX_a_2013_006570_Office_Action_dated_5-27-16-.pdf | 746072<br>62ffa0c36014cf698c9b8a8f17fd3120ee6986ee | no | 4 |

**Warnings:**

| Information: | |
|---|---|
| Total Files Size (in bytes): | 746072 |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| **PATENT APPLICATION FEE DETERMINATION RECORD** Substitute for Form PTO-875 | Application or Docket Number 14/997,136 | Filing Date 01/15/2016 | ☐ To be Mailed |
|---|---|---|---|

**ENTITY:** ☒ LARGE ☐ SMALL ☐ MICRO

## APPLICATION AS FILED – PART I

| FOR | NUMBER FILED (Column 1) | NUMBER EXTRA (Column 2) | RATE ($) | FEE ($) |
|---|---|---|---|---|
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X $ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X $ = | |
| ☐ APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| ☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

## APPLICATION AS AMENDED – PART II

| AMENDMENT 07/21/2016 | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * 30 | Minus | ** 30 | = 0 | x $80 = | 0 |
| Independent (37 CFR 1.16(h)) | * 1 | Minus | ***3 | = 0 | x $420 = | 0 |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | **0** |

| AMENDMENT | CLAIMS REMAINING AFTER AMENDMENT (Column 1) | | HIGHEST NUMBER PREVIOUSLY PAID FOR (Column 2) | PRESENT EXTRA (Column 3) | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | X $ = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | X $ = | |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/Theresa Dawkins/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

# NOTICE OF ALLOWANCE AND FEE(S) DUE

78905 7590 08/31/2016

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

| EXAMINER | |
|---|---|
| BURKHART, MICHAEL D | |
| ART UNIT | PAPER NUMBER |
| 1633 | |

DATE MAILED: 08/31/2016

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) | 4164 |

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | UNDISCOUNTED | $960 | $0 | $0 | $960 | 11/30/2016 |

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.**

**THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.**

**HOW TO REPLY TO THIS NOTICE:**

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.**

# PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail** **Mail Stop ISSUE FEE**
**Commissioner for Patents**
**P.O. Box 1450**
**Alexandria, Virginia 22313-1450**
**or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

78905 7590 08/31/2016

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

**Certificate of Mailing or Transmission**

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)

(Signature)

(Date)

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) | 4164 |

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | UNDISCOUNTED | $960 | $0 | $0 | $960 | 11/30/2016 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| BURKHART, MICHAEL D | 1633 | 435-372300 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 ____________________

2 ____________________

3 ____________________

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:

❑ Issue Fee

❑ Publication Fee (No small entity discount permitted)

❑ Advance Order - # of Copies ____________________

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**

❑ A check is enclosed.

❑ Payment by credit card. Form PTO-2038 is attached.

❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number ____________ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

❑ Applicant asserting small entity status. See 37 CFR 1.27

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

❑ Applicant changing to regular undiscounted fee status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature ____________________ Date ____________________

Typed or printed name ____________________ Registration No. ____________________

UNITED STATES PATENT AND TRADEMARK OFFICE

**UNITED STATES DEPARTMENT OF COMMERCE**
**United States Patent and Trademark Office**
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) | 4164 |

78905 7590 08/31/2016

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

| EXAMINER | |
|---|---|
| BURKHART, MICHAEL D | |
| **ART UNIT** | **PAPER NUMBER** |
| 1633 | |

DATE MAILED: 08/31/2016

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

## OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

## Privacy Act Statement

**The Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

| Notice of Allowability | Application No. 14/997,136 | Applicant(s) JUNE ET AL. | |
|---|---|---|---|
| | Examiner Michael Burkhart | Art Unit 1633 | AIA (First Inventor to File) Status No |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--***

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the response dated 7/21/2016*.
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on______.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *90-119*. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   **Certified copies:**

   a) ☐ All b) ☐ Some *c) ☐ None of the:
   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. ______ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE**.

5. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

1. ☒ Notice of References Cited (PTO-892)
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 5/12;7/21
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
5. ☒ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/Michael Burkhart/
Primary Examiner, Art Unit 1633



UPenn Ex. 2047
Miltenyi v. UPenn
IPR2022-00855
Page 382

## EXAMINER'S COMMENT

### *Terminal Disclaimer*

The terminal disclaimer filed on 7/21/2016 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of 8,911,993; 8,906,682; 9,102,760; 9,101,584; 9,102,761; 9,328,156; 13/992,622; 14/107,302; 14/996,953; 14/984,371; and 14/997,042 has been reviewed and is accepted. The terminal disclaimer has been recorded.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Burkhart whose telephone number is (571)272-2915. The examiner can normally be reached on M-F 8AM-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Babic can be reached on (571) 272-8507. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Michael Burkhart/
Primary Examiner, Art Unit 1633

| Notice of References Cited | Application/Control No. 14/997,136 | Applicant(s)/Patent Under Reexamination JUNE ET AL. | |
|---|---|---|---|
| | Examiner Michael Burkhart | Art Unit 1633 | Page 1 of 1 |

**U.S. PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Name | CPC Classification | US Classification |
|---|---|---|---|---|---|---|
| * | A | US-2016/0208012 A1 | 07-2016 | June; Carl H. | A61K35/17 | 1/1 |
| | B | US- | | | | |
| | C | US- | | | | |
| | D | US- | | | | |
| | E | US- | | | | |
| | F | US- | | | | |
| | G | US- | | | | |
| | H | US- | | | | |
| | I | US- | | | | |
| | J | US- | | | | |
| | K | US- | | | | |
| | L | US- | | | | |
| | M | US- | | | | |

**FOREIGN PATENT DOCUMENTS**

| * | | Document Number Country Code-Number-Kind Code | Date MM-YYYY | Country | Name | CPC Classification |
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**NON-PATENT DOCUMENTS**

| * | | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |
|---|---|---|
| | U | |
| | V | |
| | W | |
| | X | |

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Supplemental**<br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>14/997,136 |
|---|---|---|
| | **APPLICANT:**<br>Carl H. June, et al. | |
| | **FILING DATE:**<br>January 15, 2016 | **GROUP:**<br>1633 |

**U.S. PATENT DOCUMENTS**

| Examiner Initial | | Document Number | Date | Name | Class | Subclass | Filing Date if appropriate |
|---|---|---|---|---|---|---|---|
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**FOREIGN PATENT DOCUMENTS**

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**OTHER DOCUMENT(S)** (*Including Author, Title, Date, Pertinent Pages, etc.)*

| | | |
|---|---|---|
| | | Australian Application No. 2011338200 – Patent Examination Report No. 1, dated April 11, 2016 |
| | | Chinese Patent Application No. 201180067173X – Third Office Action dated March 28, 2016 |
| | | U.S. Patent Application No. 14/984,371 – non-final Office Action dated March 11, 2016 |
| | | U.S. Patent Application No. 14/997,042 – non-final Office Action dated April 13, 2016 |
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| **Examiner Signature:** | **Date Considered:** |
|---|---|
| /Michael Burkhart/ | 08/22/2016 |

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

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# BIB DATA SHEET

**CONFIRMATION NO. 4164**

| SERIAL NUMBER | FILING or 371(c) DATE | CLASS | GROUP ART UNIT | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016<br>RULE | 424 | 1633 | 046483-6001US13(01083) |

**APPLICANTS**
The Trustees of the University of Pennsylvania, Philadelphia, PA;

**INVENTORS**
Carl H. June, Merion Station, PA;
Bruce L. Levine, Cherry Hill, NJ;
David L. Porter, Springfield, PA;
Michael D. Kalos, Philadelphila, PA;
Michael C. Milone, Cherry Hill, NJ;

** **CONTINUING DATA** *************************
This application is a CON of 13/992,622 07/09/2013
which is a 371 of PCT/US2011/064191 12/09/2011
which claims benefit of 61/421,470 12/09/2010
and claims benefit of 61/502,649 06/29/2011

** **FOREIGN APPLICATIONS** *************************

** **IF REQUIRED, FOREIGN FILING LICENSE GRANTED** **
02/04/2016

| Foreign Priority claimed ❑ Yes ☑ No<br>35 USC 119(a-d) conditions met ❑ Yes ❑ No<br>Verified and Acknowledged /MICHAEL D BURKHART/ Examiner's Signature | ❑ Met after Allowance<br>/MB/ Initials | STATE OR COUNTRY | SHEETS DRAWINGS | TOTAL CLAIMS | INDEPENDENT CLAIMS |
|---|---|---|---|---|---|
| | | PA | 26 | 30 | 1 |

**ADDRESS**

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186
UNITED STATES

**TITLE**

Compositions and Methods for Treatment of Cancer

| FILING FEE RECEIVED | | |
|---|---|---|
| 2400 | FEES: Authority has been given in Paper No.____________ to charge/credit DEPOSIT ACCOUNT No.____________ for following: | ❑ All Fees<br>❑ 1.16 Fees (Filing)<br>❑ 1.17 Fees (Processing Ext. of time)<br>❑ 1.18 Fees (Issue)<br>❑ Other ________________<br>❑ Credit |

BIB (Rev. 05/07).

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /MB/



| Form PTO-1449<br>**U.S. Department of Commerce**<br><br>**Supplemental**<br>**Information Disclosure Statement** | **DOCKET NO.**<br>046483-6001US13(01088) | **APPLN. NO.**<br>14/997,136 |
|---|---|---|
| | **APPLICANT:**<br>Carl H. June, et al. | |
| | **FILING DATE:**<br>January 15, 2016 | **GROUP:**<br>1633 |

**U.S. PATENT DOCUMENTS**

| Examiner Initial | | Document Number | Date | Name | Class | Subclass | Filing Date if appropriate |
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**FOREIGN PATENT DOCUMENTS**

| | | Document Number | Date | Country | Class | Subclass | TranslationYes/No /Abstract |
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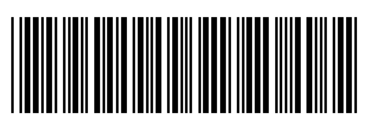
**OTHER DOCUMENT(S)** (*Including Author, Title, Date, Pertinent Pages, etc.*)

| | | |
|---|---|---|
| | | Mexican Patent Application No. MX/a/2013/006570 – Office Action dated May 27, 2016. |

| **Examiner Signature:** /Michael Burkhart/ | **Date Considered:** 08/22/2016 |
|---|---|

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

| *Issue Classification*  | Application/Control No. 14997136 | Applicant(s)/Patent Under Reexamination JUNE ET AL. |
|---|---|---|
| | Examiner MICHAEL BURKHART | Art Unit 1633 |

| CPC | | | | |
|---|---|---|---|---|
| **Symbol** | | | **Type** | **Version** |
| C07K | 16 | 2896 | F | 2013-01-01 |
| A61K | 35 | 17 | I | 2013-01-01 |
| A61K | 39 | 0011 | I | 2013-01-01 |
| A61K | 39 | 39558 | I | 2013-01-01 |
| C12N | 5 | 0636 | I | 2013-01-01 |
| A61K | 2039 | 5156 | A | 2013-01-01 |
| A61K | 2039 | 5158 | A | 2013-01-01 |
| C12N | 2501 | 515 | A | 2013-01-01 |
| C12N | 2510 | 00 | A | 2013-01-01 |
| A61K | 2039 | 505 | A | 2013-01-01 |
| C07K | 2317 | 80 | A | 2013-01-01 |
| C12N | 15 | 85 | I | 2013-01-01 |
| C07K | 14 | 70517 | I | 2013-01-01 |
| C07K | 14 | 70578 | I | 2013-01-01 |
| C07K | 14 | 70596 | I | 2013-01-01 |
| A61K | 48 | 005 | I | 2013-01-01 |
| A61K | 2039 | 585 | A | 2013-01-01 |
| C07K | 14 | 525 | I | 2013-01-01 |
| C07K | 14 | 7051 | I | 2013-01-01 |
| C07K | 16 | 3061 | I | 2013-01-01 |
| C07K | 2317 | 622 | A | 2013-01-01 |
| C07K | 2319 | 00 | A | 2013-01-01 |
| C07K | 2319 | 02 | A | 2013-01-01 |
| C07K | 2319 | 03 | A | 2013-01-01 |
| C12N | 7 | 00 | I | 2013-01-01 |
| C12N | 2740 | 15034 | A | 2013-01-01 |
| C12N | 2740 | 15043 | A | 2013-01-01 |
| A61K | 38 | 177 | I | 2013-01-01 |
| A61K | 38 | 1774 | I | 2013-01-01 |
| A61K | 45 | 06 | I | 2013-01-01 |
| C07K | 16 | 2803 | I | 2013-01-01 |

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|---|---|---|---|
| NONE<br><br>(Assistant Examiner) | (Date) | **Total Claims Allowed:**<br>30 | |
| /MICHAEL BURKHART/<br>Primary Examiner.Art Unit 1633<br><br>(Primary Examiner) | 8/22/2016<br><br>(Date) | O.G. Print Claim(s)<br>1 | O.G. Print Figure<br>none |

| *Issue Classification* | Application/Control No.<br>14997136 | Applicant(s)/Patent Under Reexamination<br>JUNE ET AL. |
|---|---|---|
| | **Examiner**<br>MICHAEL BURKHART | **Art Unit**<br>1633 |

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|---|---|---|---|---|---|
| C07K | 16 | / | 30 | I | 2013-01-01 |
| C07K | 2317 | / | 76 | A | 2013-01-01 |
| C07K | 2319 | / | 33 | A | 2013-01-01 |
| C07K | 2319 | / | 74 | A | 2013-01-01 |
| C07K | 2319 | / | 30 | A | 2013-01-01 |
| A61K | 2039 | / | 5256 | A | 2013-01-01 |
| C07K | 14 | / | 70521 | I | 2013-01-01 |
| C07K | 2317 | / | 53 | A | 2013-01-01 |
| C12N | 2740 | / | 15071 | A | 2013-01-01 |

**CPC Combination Sets**

| Symbol | | | | Type | Set | Ranking | Version |
|---|---|---|---|---|---|---|---|
| A61K | 39 | / | 39558 | I | 1 | 1 | 2013-01-01 |
| A61K | 2300 | / | 00 | A | 1 | 2 | 2013-01-01 |

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|---|---|---|---|
| NONE<br><br>(Assistant Examiner) | (Date) | **Total Claims Allowed:**<br>30 | |
| /MICHAEL BURKHART/<br>Primary Examiner.Art Unit 1633<br><br>(Primary Examiner) | 8/22/2016<br><br>(Date) | O.G. Print Claim(s)<br>1 | O.G. Print Figure<br>none |

| *Issue Classification* | Application/Control No. | Applicant(s)/Patent Under Reexamination |
|---|---|---|
| | 14997136 | JUNE ET AL. |
| | **Examiner** | **Art Unit** |
| | MICHAEL BURKHART | 1633 |

| US ORIGINAL CLASSIFICATION | |
|---|---|
| **CLASS** | **SUBCLASS** |
| 435 | 372.3 |

**CROSS REFERENCE(S)**

| CLASS | SUBCLASS (ONE SUBCLASS PER BLOCK) | | | | |
|---|---|---|---|---|---|
| 435 | 328 | 330 | | | |

**INTERNATIONAL CLASSIFICATION**

| CLAIMED | | | | | NON-CLAIMED |
|---|---|---|---|---|---|
| C | 1 | 2 | N | 5 / 071 (2010.01.01) | |
| C | 1 | 2 | N | 5 / 0783 (2010.01.01) | |
| C | 1 | 2 | N | 5 / 22 (2006.01.01) | |

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| NONE<br>(Assistant Examiner) (Date) | Total Claims Allowed:<br>30 | |
| /MICHAEL BURKHART/<br>Primary Examiner.Art Unit 1633 8/22/2016<br>(Primary Examiner) (Date) | O.G. Print Claim(s)<br>1 | O.G. Print Figure<br>none |

| *Issue Classification* | Application/Control No.<br>14997136 | Applicant(s)/Patent Under Reexamination<br>JUNE ET AL. |
|---|---|---|
| | Examiner<br>MICHAEL BURKHART | Art Unit<br>1633 |

☒ **Claims renumbered in the same order as presented by applicant** ☐ **CPA** ☒ **T.D.** ☐ **R.1.47**

| Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original | Final | Original |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
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| NONE<br><br>(Assistant Examiner) (Date) | Total Claims Allowed:<br>30 | |
|---|---|---|
| /MICHAEL BURKHART/<br>Primary Examiner.Art Unit 1633 8/22/2016<br><br>(Primary Examiner) (Date) | O.G. Print Claim(s)<br>1 | O.G. Print Figure<br>none |



UPenn Ex. 2047
Miltenyi v. UPenn
IPR2022-00855

| | L # | Hits | Search Text | DBs | Time Stamp |
|---|---|---|---|---|---|
| **1** | L1 | 2 | "20160208012" | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:04 |
| **2** | L2 | 246 | michael near2 kalos.in. | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **3** | L3 | 64 | L2 and car | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **4** | L4 | 28 | L3 and cd19 | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **5** | L5 | 410 | carl near june.in. | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **6** | L6 | 131 | L5 and car | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **7** | L8 | 160 | bruce near2 levine.in. | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **8** | L9 | 65 | L8 and car | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **9** | L10 | 31 | L9 and cd19 | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **10** | L7 | 50 | L6 and cd19 | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **11** | L11 | 1885 | david near2 porter.in. | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **12** | L12 | 48 | L11 and car | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **13** | L13 | 23 | L12 and cd19 | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |

| **14** | L14 | 67 | michael near2 milone.in. | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
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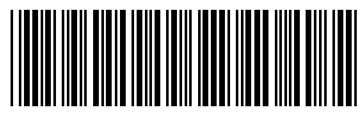
| | L # | Hits | Search Text | DBs | Time Stamp |
|---|---|---|---|---|---|
| **15** | L15 | 36 | L14 and car | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |
| **16** | L16 | 27 | L15 and cd19 | US-PGPUB; USPAT; FPRS; EPO; DERWENT | 2016/08/22 13:05 |

| *Search Notes*  | Application/Control No. 14997136 | Applicant(s)/Patent Under Reexamination JUNE ET AL. |
|---|---|---|
| | **Examiner** MICHAEL BURKHART | **Art Unit** 1633 |

## CPC- SEARCHED

| Symbol | Date | Examiner |
|---|---|---|
| | | |

## CPC COMBINATION SETS - SEARCHED

| Symbol | Date | Examiner |
|---|---|---|
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## US CLASSIFICATION SEARCHED

| Class | Subclass | Date | Examiner |
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## SEARCH NOTES

| Search Notes | Date | Examiner |
|---|---|---|
| Inventor name search (Medline, EAST), USPat, USPgPub, EPO, JPO, Derwent keyword search (EAST) | 4/18/2016 | MB |
| Parent cases reviewed | 4/18/2016 | MB |
| STIC search SEQ ID Nos | 4/18/2016 | MB |
| Updated | 8/22/2016 | MB |

## INTERFERENCE SEARCH

| US Class/ CPC Symbol | US Subclass / CPC Group | Date | Examiner |
|---|---|---|---|
| none | none | 8/22/2016 | MB |

| | /M.B./<br>Primary Examiner.Art Unit 1633 |
|---|---|

## PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail** **Mail Stop ISSUE FEE**
**Commissioner for Patents**
**P.O. Box 1450**
**Alexandria, Virginia 22313-1450**
**or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

78905 7590 08/31/2016
Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

**Certificate of Mailing or Transmission**
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/15/2016 | Carl H. June | 046483-6001US13(01088) | 4164 |

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

| APPLN. TYPE | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE |
|---|---|---|---|---|---|---|
| nonprovisional | UNDISCOUNTED | $960 | $0 | $0 | $960 | 11/30/2016 |

| EXAMINER | ART UNIT | CLASS-SUBCLASS |
|---|---|---|
| BURKHART, MICHAEL D | 1633 | 435-372300 |

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
   - ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
   - ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
   (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
   (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
   1 Saul Ewing LLP
   2 Kathryn Doyle
   3 ______

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: The Trustees of the University of Pennsylvania
(B) RESIDENCE: (CITY and STATE OR COUNTRY): Philadelphia, Pennsylvania (US)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies ______

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ☐ A check is enclosed.
- ☒ Payment by credit card. Form PTO-2038 is attached.
- ☒ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number 50-4364 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
  NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
  NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ☐ Applicant changing to regular undiscounted fee status.
  NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Kathryn Doyle/  Date November 28, 2016

Typed or printed name Kathryn Doyle, Ph.D., J.D.  Registration No. 36,317

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 14997136 |
| **Filing Date:** | 15-Jan-2016 |
| **Title of Invention:** | COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Attorney Docket Number:** | 046483-6001US13(01088) |

Filed as Large Entity

**Filing Fees for** **Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| UTILITY APPL ISSUE FEE | 1501 | 1 | 960 | 960 |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **960** |

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 27620796 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Lisa Sapovits |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 28-NOV-2016 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 14:26:29 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | CARD |
| Payment was successfully received in RAM | $960 |
| RAM confirmation Number | 112916INTEFSW14273100 |
| Deposit Account | 504364 |
| Authorized User | Kathryn Doyle |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

37 CFR 1.20 (Post Issuance fees)

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Issue Fee Payment (PTO-85B) | 046483-6001U13-issue-fee-transmittal.pdf | 62156<br>8bb2db89ab13f228adf595d7776759c5cd3ed1a7 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Fee Worksheet (SB06) | fee-info.pdf | 30598<br>c5abf2c8b44d02e77d4870758069ac647a0375d2 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | | **Total Files Size (in bytes):** | 92754 | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | ISSUE DATE | PATENT NO. | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 14/997,136 | 01/10/2017 | 9540445 | 046483-6001US13(01088) | 4164 |

78905 7590 12/21/2016

Saul Ewing LLP (Philadelphia)
Attn: Patent Docket Clerk
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102-2186

# ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

**Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)**
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

The Trustees of the University of Pennsylvania, Philadelphia, PA;
Carl H. June, Merion Station, PA;
Bruce L. Levine, Cherry Hill, NJ;
David L. Porter, Springfield, PA;
Michael D. Kalos, Philadelphila, PA;
Michael C. Milone, Cherry Hill, NJ;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

PTO/SB/44 (09-07)
Approved for use through 01/31/2020. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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(Also Form PTO-1050)

# UNITED STATES PATENT AND TRADEMARK OFFICE
## CERTIFICATE OF CORRECTION



PATENT NO. : 9,540,445 B2

APPLICATION NO.: 14/997,136

ISSUE DATE : January 10, 2017

INVENTOR(S) : Carl H. June, Bruce L. Levine, David L. Porter, Michael D. Kalos and Michael C. Milone

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On column 1, lines 21-25, of the specification, after the "STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT", please replace the existing paragraph with the following paragraph:
-- This invention was made with government support under grant number K24 CA117879, R01 CA120409, R01 CA105216 and R01 AI057838 awarded by the National Institutes of Health. The government has certain rights in the invention. --

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):

Kathryn Doyle, Ph.D., J.D., Saul Ewing Arnstein & Lehr LLP
Centre Square West, 1500 Market Street, 38th Floor
Philadelphia, PA 19102

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

# Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

# Electronic Patent Application Fee Transmittal

| Application Number: | 14997136 |
|---|---|
| Filing Date: | 15-Jan-2016 |
| Title of Invention: | COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER |
| First Named Inventor/Applicant Name: | Carl H. June |
| Filer: | Kathryn R. Doyle/Katie Wray |
| Attorney Docket Number: | 046483-6001US13(01088) |

Filed as Large Entity

**Filing Fees for** **Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |
| CERTIFICATE OF CORRECTION | 1811 | 1 | 150 | 150 |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **150** |

# Electronic Acknowledgement Receipt

| | |
|---|---|
| **EFS ID:** | 33451549 |
| **Application Number:** | 14997136 |
| **International Application Number:** | |
| **Confirmation Number:** | 4164 |
| **Title of Invention:** | COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER |
| **First Named Inventor/Applicant Name:** | Carl H. June |
| **Customer Number:** | 78905 |
| **Filer:** | Kathryn R. Doyle/Katie Wray |
| **Filer Authorized By:** | Kathryn R. Doyle |
| **Attorney Docket Number:** | 046483-6001US13(01088) |
| **Receipt Date:** | 13-AUG-2018 |
| **Filing Date:** | 15-JAN-2016 |
| **Time Stamp:** | 16:06:40 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | yes |
| Payment Type | CARD |
| Payment was successfully received in RAM | $150 |
| RAM confirmation Number | 081418INTEFSW16065700 |
| Deposit Account | |
| Authorized User | |

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Transmittal Letter | 046483-6001US13-Request-Certificate-Correction.pdf | 108568<br>191c06cefe62e3b57c7e2ed20b30a08dbf2956b3 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Request for Certificate of Correction | 046483-6001US13-Certificate-of-Correction.pdf | 154235<br>394c78263dcea9d515ec056ce21d624e80d397ff | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | Fee Worksheet (SB06) | fee-info.pdf | 30259<br>87466b2705107c8eef031c0bdda5628e1566b81b | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 293062 | | |

**This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.**

**<u>New Applications Under 35 U.S.C. 111</u>**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**
**<u>National Stage of an International Application under 35 U.S.C. 371</u>**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**
**<u>New International Application Filed with the USPTO as a Receiving Office</u>**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Patent Number: 9,540,445 B2

Issued: January 10, 2017

Name of Patentee: Carl H. June et al.

Title of Invention: Compositions and Methods for Treatment of Cancer

Atty. Docket No. 046483-6001US13(01088)

# FILED ELECTRONICALLY

**COMMISSIONER FOR PATENTS**
**P.O. Box 1450**
**Alexandria, VA 22313-1450**

**Attention: OFFICE OF PETITIONS**

**REQUEST UNDER 37 CFR §1.323 FOR CERTIFICATE OF CORRECTION OF PATENT FOR APPLICANT'S MISTAKE**

Applicant hereby requests, pursuant to 37 CFR 1.323, that a Certificate of Correction be issued to correct Applicant's mistake with regard to correction improper grant numbers in the Statement Regarding Federally Sponsored Research or Development.

The error occurred in good faith. Correction thereof does not involve such changes in the patent as would constitute new matter or would require re-examination. A certificate of correction is requested.

Submitted also herewith is Form PTO-1050, suitable for printing. Please send the Certificate to the undersigned attorney at the address that appears below.

The required fee of $150.00 pursuant to 37 CFR 1.20(a), is hereby authorized to be charged to Deposit Account No. 50-4364.

Respectfully submitted,

Dated: August 13, 2018

Kathryn Doyle, Ph.D., J.D.
Registration No. 36,317

Saul Ewing Arnstein & Lehr LLP
Centre Square West
1500 Market Street, 38th Floor
Philadelphia, PA 19102
Telephone: (215) 972-7734
Fax: (215) 972-1818
Email: *kathryn.doyle@saul.com*
*Attorney for Applicant*

UNITED STATES PATENT AND TRADEMARK OFFICE

# CERTIFICATE OF CORRECTION



PATENT NO. : 9,540,445 B2
APPLICATION NO. : 14/997136
DATED : January 10, 2017
INVENTOR(S) : Carl H. June et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

On Column 1, Lines 21-25, after the "STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT", please replace the existing paragraph with the following paragraph:
-- This invention was made with government support under grant number K24 CA117879, R01 CA120409, R01 CA105216 and R01 AI057838 awarded by the National Institutes of Health. The government has certain rights in the invention. --

Signed and Sealed this
Eleventh Day of September, 2018

Andrei Iancu
*Director of the United States Patent and Trademark Office*

Trials@uspto.gov
571-272-7822

Paper 10
Entered: October 11, 2022

UNITED STATES PATENT AND TRADEMARK OFFICE

---

BEFORE THE PATENT TRIAL AND APPEAL BOARD

---

MILTENYI BIOMEDICINE GmbH and MILTENYI BIOTEC INC.,
Petitioner
v.

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,
Patent Owner

---

IPR2022-00855
Patent 9,540,445 B2

---

Before ULRIKE W. JENKS, SUSAN L. C. MITCHELL, and
ROBERT A. POLLOCK, *Administrative Patent Judges.*

JENKS, *Administrative Patent Judge*.

# DECISION
## Granting Institution of *Inter Partes* Review
*35 U.S.C. § 314*

# I. INTRODUCTION

## *A. Background*

Miltenyi Biomedicine GmbH and Miltenyi Biotec Inc. (collectively, "Petitioner") filed a Petition for an *inter partes* review of claims 1–19 and 21–30 of U.S. Patent No. 9,540,445 B2 ("the '445 Patent," Ex. 1001). Paper 1 ("Pet."). Trustees of the University of Pennsylvania ("Patent Owner") timely filed a Preliminary Response. Paper 7. ("Prelim. Resp."). Petitioner further filed an authorized Reply to the Preliminary Response (Paper 8, "Reply"); Patent Owner filed a responsive Sur-Reply (Paper 9, "Sur-Reply").

We have authority, acting on the designation of the Director, to determine whether to institute an *inter partes* review under 35 U.S.C. § 314 and 37 C.F.R. § 42.4(a)(2020). *Inter partes* review may not be instituted unless "the information presented in the petition filed under section 311 and any response filed under section 313 shows that there is a reasonable likelihood that the petitioner would prevail with respect to at least 1 of the claims challenged in the petition." 35 U.S.C. § 314(a). The Supreme Court held that a decision to institute under 35 U.S.C. § 314 may not institute on fewer than all claims challenged in the petition. *SAS Inst., Inc. v. Iancu*, 138 S. Ct. 1348, 1359–60 (2018).

For the reasons set forth below, upon considering the Petition, Preliminary Response, and supporting evidence of record, we determine that Petitioner has sufficiently shown for the purpose of institution that the information presented in the Petition establishes a reasonable likelihood that Petitioner will prevail with respect to at least one of the challenged claims.

Accordingly, we institute *inter partes* review on all of the challenged claims based on all of the grounds identified in the Petition.

Our findings of fact, conclusions of law, and reasoning discussed below are based on the evidentiary record developed thus far, and made for the sole purpose of determining whether the Petition meets the threshold for initiating review. This decision to institute trial is not a final decision as to the patentability of any challenged claim or the construction of any claim limitation. Any final decision will be based on the full record developed during trial.

*B. Real Parties-in-Interest*

Petitioner identifies itself, Miltenyi Biomedicine GmbH and Miltenyi Biotec Inc. as the real parties-in-interest. Pet. 11. Patent Owner, identifies itself, The Trustees of the University of Pennsylvania and its licensee, Novartis Pharma AG, as real parties-in-interest. Paper 5, 2.

*C. Related Matters and Chain of Priority*

The ’445 patent issued from application No. 14/997,136 (“the ’136 application”) which is a continuation of application No. 13/992,622 (“the ’622 application”), filed as application No. PCT/US2011/064191 (“the PCT application”) on December 9, 2011. The ’445 patent further claims benefit of priority to provisional application No. 61/421,470. filed on December 9, 2010, and provisional application No. 61/502,649. filed on June 29, 2011.

Petitioner reasonably contends that the challenged claims of the ’445 patent are not entitled to benefit of the provisional applications. Pet. 13, 71 (citing, e.g., Ex. 1021, 402). Patent Owner does not presently contest this assertion. *See* Prelim. Resp. 41. On the present record, we consider

December 9, 2011, filing date of the PCT application, to be the earliest possible priority date for the challenged claims.

Petitioner concurrently challenges claims of related U.S. Patent Nos. 9,518,123 B2 (“the ’123 patent) and 9,464,140 B2 (“the ’140 patent”) in IPR2022-00852 and IPR2022-00853, respectively. The ’123 and ’140 patents similarly issued from continuation applications of the ’622 parent application and, thus, share the substantially the same specification. The ’622 parent application issued as U.S. Patent No. 9,499,629 B2 (“the ’629 patent.). The ’123, ’140, ’445, and ’629 patents were Examined by the same Examiner.

*D. Asserted Grounds of Unpatentability*

Petitioner asserts the following grounds of unpatentability (Pet. 5):

| Ground | Claims Challenged | 35 U.S.C §[1] | Reference(s)/Basis |
|---|---|---|---|
| 1 | 1–4, 6, 8, 9, 11, 16, 21, 22, 27–30 | § 103 | Campana,[2] Nicholson,[3] Honsik,[4] CART-19 ClinicalTrials.gov[5] |
| 2 | 1–6, 8, 9, 11, 13, 16, 21, 22, 27–30 | § 103 | Campana, Jensen,[6] Honsik, CART-19 ClinicalTrials.gov |
| 3 | 1–30 | § 103 | Campana, Milone,[7] CART-19 ClinicalTrials.gov, Nicholson, Jensen, Littman,[8] Sadelain,[9] Honsik, Riddell[10] |
| 4 | 1–30 | § 103 | Campana, Porter,[11] Nicholson, Jensen, Littman, Sadelain, Honsik, Riddell |

[1] The Leahy-Smith America Invents Act ("AIA") included revisions to 35 U.S.C. § 103 that became effective on March 16, 2013. Because the '140 patent issued from an application that is a continuation of an application filed before March 16, 2013, we apply the pre-AIA version of the statutory basis for unpatentability.

[2] US 2005/0113564, publ. May 26, 2005. Ex. 1003 ("Campana").

[3] Nicholson et al., "*Construction and Characterisation of a Functional CD19 Specific Single Chain Fv Fragment for Immunotherapy of B Lineage Leukaemia and Lymphoma*," 34 MOL. IMMUNOL. 1157 (1997). Ex. 1004 ("Nicholson").

[4] US 4,844,893, issued July 4, 1989. Ex. 1005 ("Honisk").

[5] "*Pilot Study for Patients with Chemotherapy Resistant or Refractory CD19 Leukemia and Lymphoma (CART-19)*," https:/clinicaltrials.gov/ct2/show/NCT00891215. Ex. 1006 (includes Declaration of Duncan Hall).

[6] US 2004/0126363, published July 1, 2004. *Ex.* 1007 ("Jensen") .

[7] Milone et al., "*Chimeric Receptor Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy In Vivo*," 17 MOL. THERAPY 1453 (2009). Ex. 1008 ("Milone").

Petitioner further relies on, inter alia, the Declaration of Richard P. Junghans, M.D., Ph.D. Ex. 1002. Patent Owner did not choose to submit testimony of a technical expert at this stage of the proceeding.

*E. The '445 Patent*

*1) Background and Specification*

The '445 patent, titled "Compositions and Methods for Treatment of Cancer," issued on January 10, 2017, naming Carl H. June, Bruce L. Levine, David L. Porter, Michael D. Kalos, and Michael C. Milone as inventors. Ex. 1001, code (45), (72). The '445 patent discloses administering immune cells—including T cells—modified to express a chimeric antigen receptor or CAR construct. *See generally, id.* at code (57), 1:29–42, 2:40–43.[12] The disclosed CARs generally

> comprise an extracellular domain having an antigen binding domain fused to an intracellular signaling domain of the T cell antigen receptor complex zeta chain (e.g., CD3 zeta). The CAR of the invention when expressed in a T cell is able to redirect antigen recognition based on the antigen binding specificity.

*Id.* at 19:6–12.

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[8] Littman et al., "*The Isolation and Sequence of the Gene Encoding T8: A Molecule Defining Functional Classes of T Lymphocytes*," 40 CELL 237 (1985). Ex. 1009 ("Litmann").
[9] US 2004/0043401, published March 4, 2004. Ex. 1010 ("Sadelain").
[10] US 2008/0131415 A1, published June 5, 2008. Ex. 1011 ("Ridell").
[11] Porter et al., "Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia," 365 N. ENGL J. MED. 725 (2011), Ex. 1012 ("Porter").
[12] The '445 patent variously refers to T cells transduced with CAR constructs as CAR modified T cells, CART, CAR T, or CAR-T cells. *Id.* at 3:59–62, 5:23–30, 52:46–48.

The ’445 patent variously refers to T cells transduced with CAR constructs as CAR modified T cells, CART, CAR T, or CAR-T cells. *Id.* at 3:59–62, 5:23–30, 52:46–48. The CAR modified cells may be autologous T cells from a patient in need of treatment. *See e.g., id.* at 3:5–15, 9:49–58. Dr. Junghans explains: “When a patient’s own T cells are transduced and infused into the same patient, the T cells are identified as ‘autologous.’ This is in contrast to a cancer patient receiving transduced T cells from a healthy donor, wherein the T cells would be considered ‘allogeneic.’” Ex. 1002 ¶ 42. The ’445 patent discloses that in some embodiments the treatment is directed against a B-cell malignancy, such as Chronic Lymphocytic Leukemia (CLL). Ex. 1001, 18:29–31. 65–67.

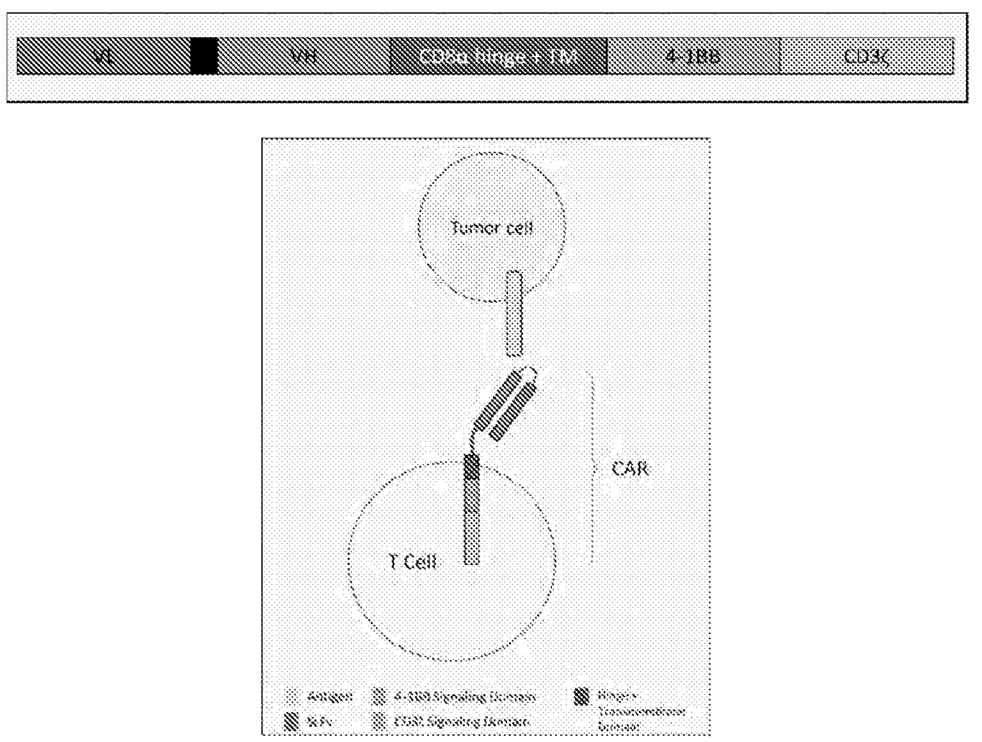
According to the ’445 patent, “[a]ttempts in using genetically modified cells expressing CARs to treat [patients having B-cell malignancies] have met with very limited success.” *Id.* at 1:29–42 (citations omitted). “[A] major impediment to the clinical application of this technology to date has been limited in vivo expansion of CAR+ T cells, rapid disappearance of the cells after infusion, and disappointing clinical activity.” *Id.* at 1:48–54. (citations omitted).

The ’445 patent discloses T cells modified to express a “CAR compris[ing] an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain.” Ex. 1001, code (57). In a preferred embodiment the CAR comprises an “anti-CD19 scFv[13] derived from FMC63 murine monoclonal antibody, human CD8α

[13]A “scFV” or “single chain variable fragment” is a recombinant antibody fragment in which Vl and Vh antigen recognition elements are fused with a short peptide linker to form a single polypeptide chain having an antigen recognition moiety derived from the parent antibody. *See Ex.* 1002 ¶ 33.

hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains." *Id.* at 4:37–40 (referencing Figs. 1A–1C). "The CD 19-BB-z transgene (GeMCRIS 0607-793) was designed and constructed as described (Milone et al., 2009, Mol Ther. 17:1453-1464) [Ex. 1008]." *Id.* at 41:5–7.

In the figure below, Dr. Junghans provides "A schematic of the major functional elements of a preferred embodiment of the CAR and an illustration how the CAR could appear when inserted into the T cell surface." Ex. 1002 ¶ 51.




The above figure illustrates a CAR protein anchored in the T cell membrane via a CD8α hinge and transmembrane domain with 4-1BB co-stimulatory and CD3ζ signaling domains in the interior of the T cell, and extracellular VL and VH ScFv domains interacting with CD19 antigen on the surface of a tumor cell. *See id.*

*2) Challenged Claims*

Petitioner challenges claims 1–30 of the '445 patent, of which only claim 1 is independent. Claim 1 recites:

> ([a]) A pharmaceutical composition comprising
>
> ([b]) an anti-tumor effective amount of a population of human T cells,
>
> ([c]) wherein the T cells comprise a nucleic acid sequence encoding a chimeric antigen receptor (CAR),
>
> ([d]) wherein the CAR comprises a CD19 antigen binding domain comprising, from the ammo to the carboxy terminus, a light chain variable region and a heavy chain variable region of SEQ ID NO:20,
>
> ([e]) wherein the CAR further comprises a transmembrane domain, a 4-1BB co stimulatory signaling region, and a CD3 zeta signaling domain,
>
> ([f]) wherein the T cells are from a human having cancer.

Ex. 1001, claim 1 (paragraphing and reference letters [a]–[f] as added by Petitioner (*see* Pet. 28–29)).

The challenged dependent claims recite limitations directed to the amount or structure of the T cells (claims 2–4); the identity or nucleic acid sequence of the CAR or its components (claims 6, 8, 9, 11, 16); the source of the T cells (claims 21, 22); and components of a pharmaceutical composition comprising the CAR construct (claims 27–30).

## II. ANALYSIS

### *A. Legal Standards*

"In an IPR, the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable." *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C.

§ 312(a)(3) (requiring *inter partes* review petitions to identify "with particularity . . . the evidence that supports the grounds for the challenge to each claim")). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

Petitioner challenges claims 1–30 as obvious under 35 U.S.C. § 103. The Supreme Court in *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398 (2007), reaffirmed the framework for determining obviousness set forth in *Graham v. John Deere Co*., 383 U.S. 1 (1966). The *KSR* Court summarized the four factual inquiries set forth in *Graham* (383 U.S. at 17–18) that are applied in determining whether a claim is unpatentable as obvious under 35 U.S.C. § 103 as follows: (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and the claims at issue; (3) resolving the level of ordinary skill in the art; and (4) considering objective evidence indicating obviousness or non-obviousness, if present. *KSR*, 550 U.S. at 406.

"[W]hen a patent 'simply arranges old elements with each performing the same function it had been known to perform' and yields no more than one would expect from such an arrangement, the combination is obvious." *Id.* at 417 (quoting *Sakraida v. Ag Pro, Inc.*, 425 U.S. 273, 282 (1976)). But in analyzing the obviousness of a combination of prior art elements, it can also be important to identify a reason that would have prompted one of skill in the art "to combine . . . known elements in the fashion claimed by the patent at issue." *Id.* at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, "any need or problem known in the field of endeavor at the time of

invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so. *In re Magnum Oil Tools International, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (quotations and citations omitted). Under the proper inquiry, "obviousness cannot be avoided simply by a showing of some degree of unpredictability in the art so long as there was a reasonable probability of success." *Pfizer, Inc. v. Apotex, Inc.*, 480 F.3d 1348, 1364 (Fed. Cir. 2007).

*B. Level of Ordinary Skill in the Art*

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *See Custom Accessories, Inc. v. Jeffrey-Allan Industries, Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *see also Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

In addressing the level of ordinary skill in the art, Petitioner contents that:

> A POSA is a person skilled in the art of administering CAR T-cell therapies. The person would possess a relatively high level of skill and have at least an MD, together with several years of experience in administering CAR T-cell therapies. The person would also have experience designing CARs. The POSA would have knowledge of the scientific literature pertaining to

> immunology, including CARs and methods for utilizing CARs before the priority date. A POSA would also be knowledgeable about laboratory techniques related to engineering and testing the function of CAR T cells. A POSA would also be knowledgeable about designing clinical trials, including selecting dose ranges, that evaluate CAR T-cell therapies.

Pet. 18. Patent Owner does not presently contest this definition.

Although Petitioner's definition generally comports with our understanding of the high level of skill in the art, we are not persuaded all of the asserted qualifications must be embodied in a single person. For example, it seems unduly limiting to require that the person of ordinary skill in the art have "at least an MD" degree, and "be knowledgeable about laboratory techniques related to engineering and testing the function of CAR T cells," as Petitioner suggests. Accordingly, we provisionally adopt Petitioner's definition with the caveat that the asserted qualifications may be shared among multiple individuals working as part of a multidisciplinary team.

*C. Claim Construction*

We interpret a claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. 282(b)." 37 C.F.R. § 42.100(b) (2020). Under this standard, we construe the claim "in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.* Moreover, "the specification 'is always highly relevant to the claim construction analysis. Usually it is dispositive; it is the single best guide to the meaning of a disputed term.'" *In re Abbott Diabetes Care Inc.*, 696 F.3d 1142, 1149 (Fed. Cir. 2012) (quoting *Phillips v. AWH Corp.*, 415 F.3d 1303, 1315 (Fed. Cir. 2005) (en banc)).

*1) "anti-tumor effective amount"*

Claim 1 recites "an anti-tumor effective amount of a population of human T cells." Claim 2 limits the anti-tumor effective amount of T cells recited in claim 1 to "$10^4$ to $10^9$ cells per kg body weight of a human in need of such cells."[14] Relying on the testimony of Dr. Junghans, Petitioner proposes that the term "anti-tumor effective amount" should be understood to encompass at least "$10^4$ to $10^9$ cells/kg body weight," and any other amount of CAR T cells that would have at least one of the biological effect specifically described in the specification, including "a decrease in the number of tumor cells." Pet. 19–21 (citing Ex. 1001, 37:60–64, 12:28–37; Ex. 1002 ¶¶ 56–61); Reply 3–5.

In support of this position, Petitioner argues that "[w]hile claim 1 does not expressly state a numerical range for an "anti-tumor effective amount," claim 1 necessarily encompasses at least the numerical range stated in dependent claim 2." Pet. 19–20. Petitioner further argues that one of ordinary skill in the art would understand that a dose outside the range of $10^4$ to $10^9$ cells per kg can also satisfy the claim limitation of "anti-tumor effective amount" if it provides at least one "anti-tumor effect" as defined in the Specification. *Id.* at 20. In this respect, Petitioner points to the '445 patent's express definition of "anti-tumor effect" as

> a biological effect which can be manifested by [(i)] a decrease in tumor volume, [(ii)] ***a decrease in the number of tumor cells***, [(iii)] a decrease in the number of metastases, [(iv)] an increase in life expectancy, ***or*** [(v)] amelioration of various physiological symptoms associated with the cancerous condition. An "anti-tumor effect" can also be manifested by [(vi)] the ability of the peptides, polynucleotides, cells and

[14] Claim 3 further limits this range to "$10^5$ to $10^6$ cells per kg."

> antibodies of the invention in prevention of the occurrence of tumor in the first place.

*Id.* (quoting Ex. 1001, 12:28–37 (emphasis by Petitioner)).

As we understand it, Patent Owner's response does not appear to contest that "anti-tumor effect" can comprise a decrease in the number of tumor cells, but argues that the claim term requires "a palpable therapeutic effect," which is more than "just the death of a single cancer cell or handful of cells." Prelim. Resp. 17–21; Sur-reply 3–5. In support, Patent Owner points to the '445 patent's express definition of "[a]n 'effective amount'" as meaning "an amount which provides a therapeutic or prophylactic benefit." Prelim. Resp. 18 (citing Ex. 1001, 13:53–54). Relatedly, we note that the Specification also teaches that a "'therapeutically effective amount' includes that amount of a compound that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the signs or symptoms of the disorder or disease being treated." Ex. 1001, 18:12–15.

Patent Owner further contends that

> [t]he specification teaches that "an antitumor effective amount" is an amount to be administered that is "determined by a physician" as part of determining "[t]he optimal dosage and treatment regime for a particular patient . . . by monitoring the patient for signs of disease and adjusting the treatment accordingly."

Prelim. Resp. 20 (citing Ex. 1001, 37:53–38:5).

Considering the argument and evidence of record at this stage of the proceeding, we agree with Patent Owner that one of ordinary skill in the art would understand an "anti-tumor effective amount" to require more than the killing of a single tumor cell. Accordingly, and consistent with our determination in IPR2022-00853, we provisionally construe "anti-tumor effective amount" as meaning any amount of CAR T cells when

administered to a patient in need of cancer treatment that reduces the frequency or severity of at least one clinically relevant sign or symptom of the disease.

We note that the "anti-tumor effective amount" as recited in the independent claim could reasonably be construed as a functional limitation.[15] The patentability of a composition claim, however, "depends on the claimed structure, not on the use or purpose of that structure." *Catalina Mktg. Int'l, Inc. v. Coolsavings.com, Inc.*, 289 F.3d 801, 809 (Fed. Cir. 2002). Although statements of intended use often appear in the preamble, a statement of intended use or purpose can appear elsewhere in a claim. *In re Stencel*, 828 F.2d 751, 754 (Fed. Cir. 1987). Here, the pharmaceutical composition of claim 1 is defined by elements (c)–(f) which describes the structure of the CAR T cells, while the "anti-tumor effective amount" limitation (element (b)) represents an intended use that is embedded in a composition claim. "An intended use or purpose usually will not limit the scope of the claim because such statements usually do no more than define a context in which the invention operates." *See Boehringer Ingelheim Vetmedica, Inc. v. Schering-Plough Corp.*, 320 F.3d 1339, 1345 (Fed. Cir. 2003). As discussed above, we have provisionally construed the "anti-tumor effective amount" limitation as meaning the administration of an amount[16]

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[15] As discussed in the preceding paragraph, we interpret the "anti-tumor effective amount" as requiring an embedded method step that requires administering CAR T cells to a subject so one can then select only those compositions that show relief "of at least one clinically relevant sign or symptom of the disease." *See MasterMine Software, Inc. v. Microsoft Corp.*, 874 F.3d 1307, 1312–16 (Fed. Cir. 2017) (explaining impermissibility of mixing products with processes in claims); *see also* MPEP 2173.05(p).

[16] The recitation of $10^4$ to $10^9$ CAR T cells/kg body weight as set forth in claim 2 could be construed as a structural limitation as urged by Petitioner.

of CAR T cells to a patient in order to reduce the frequency or severity of at least one clinically relevant sign or symptom of the disease. As such, this limitation merely describes the context in which the CAR T cells operate – in a cancer patient – but does not further define the structure of the composition.

The parties are invited to brief this issue further at trial, if deemed necessary.

*2) Remaining Claim Terms*

At this stage of the proceeding, no other term requires construction. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd. v. Matal*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy.'" (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))).

*D. Over view of Asserted References*

*1) Campana (Exhibit 1003)*

Campana is a US Patent Application Publication, published May 26, 2005, and listing Dario Campana and Chilhaya Imai as inventors. Ex. 1003, codes (43), (75). Campana discloses the results of in vitro studies using an anti-CD19 CAR having the same overall structure as the CAR described in claim 1 of the '445 patent.

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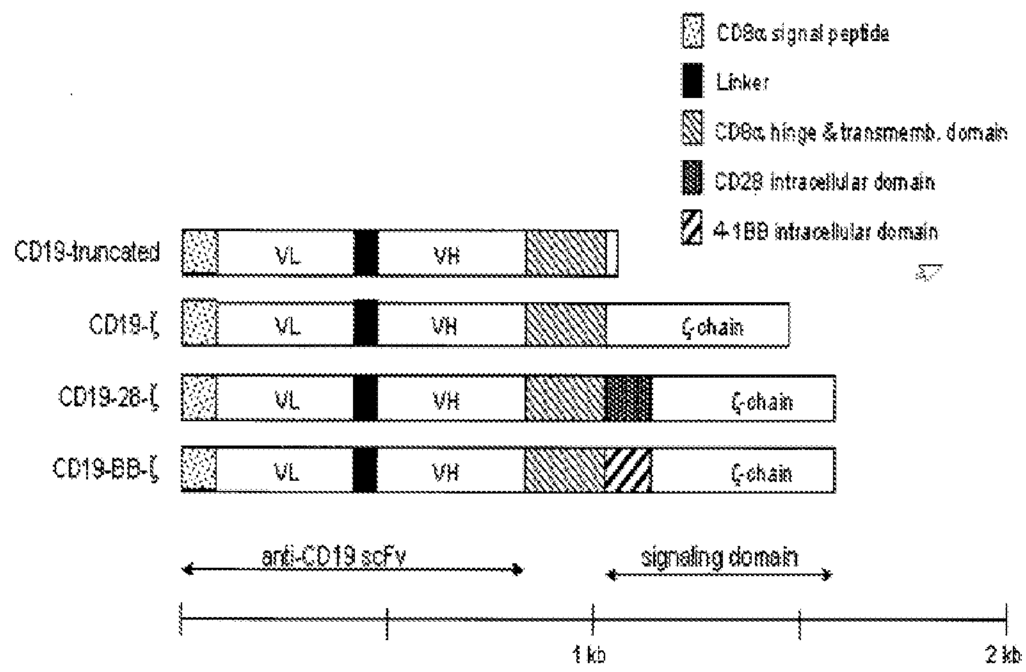
*See* Pet. 38–39. As such, a product with the recited structure in the recited amount would reasonably meet the functional limitation of being "anti-tumor effective" because it is an inherent characteristic of the product. Pet. 41 ("a POSA would have been motivated to use the CART-19 ClinicalTrials.gov dose (which necessarily satisfies the limitation of 'anti-tumor effective amount')"); *see* MPEP 2114.

Campana discloses the use of CARs containing a co-stimulatory 4-1BB signaling domain in T cells and natural killer (NK) cells. *Id.* at, code (57). With respect to T cells, Campana states:

> We constructed a chimeric T-cell receptor specific for CD19 that contains a 4-1BB signaling domain. We determined whether T cells transduced with these receptors could effectively destroy B-lineage ALL cell lines and primary leukemic cells under culture conditions that approximate the in vivo microenvironment where leukemic cells grow. We compared the properties of T-cells expressing the 4-1BB-containing receptor to those of T-cells expressing an equivalent receptor lacking 4-1BB or containing CD28 instead.

*Id.* ¶ 69. Figure 1 of Campana, reproduced below, is a schematic representation of constructs used in this work.




*Id.* at Fig. 1, ¶ 20.

Figure 1 discloses representative CAR CD19-BB-ζ comprising a CD8α signal peptide, an anti-CD19 scFv binding region, a CD8α hinge and transmembrane domain, a 4-1BB costimulatory domain, and a CD3-ζ

signaling domain. *Id.* at Fig. 1, ¶ 13; Ex. 1002, ¶ 63. Reflecting its antigen specificity, Campana elsewhere refers to this same construct as "anti-CD19-BB-ζ." *See, e.g., id.* ¶¶ 21, 38, 53.

Campana discloses that primary T-cells were transduced with anti-CD19-BB-ζ, expanded in culture, and tested for activity in vitro. *Id.* ¶¶ 50–51. According to Campana, "[t]hese results show that 4-1BB co-stimulation confers a survival advantage on lymphocytes, which overcomes a major obstacle with current chimeric receptors used in immunotherapy." *Id.* ¶ 51. Subsequent experiments showed that "T-cells expressing the anti-CD19 BB-ζ receptor exhibited cytotoxic activity at the 1:1 and 0.1:1 ratios against all CD19+ cell lines tested." *Id.* ¶ 52. Campana reports that other experiments "show[] that T-cells transduced with the anti-CD19-BB-ζ receptor exhibit cytotoxic activity in an environment critical for B-lineage leukemic cell growth," and that anti-CD19-BB-ζ expression "caused higher levels of TRAIL stimulation" and increased IL-2-mediated T-cell expansion. *Id.* ¶¶ 53, 115, 116 (noting that T cells require TRAIL for optimal graft-versus-tumor activity).

According to Campana, "[r]esults of this study indicate that anti-CD19-BB-ζ receptors could help achieve effective T-cell immunotherapy of B-lineage ALL." *Id.* ¶ 113; *see also id.* ¶ 54 ("These results further support the use of anti-CD19-BB-ζ receptor for immunotherapy."). Campana concludes that, "[i]n view of the limited effectiveness and the high risk of the currently available treatment options for chemo therapy-refractory B-lineage ALL and other B cell malignancies, the results of our study provide compelling justification for clinical trials using T cells expressing anti CD19-BB- ζ receptors." *Id.* ¶ 118. For example, "[d]onor-derived T cells

endowed with chimeric receptors could replace infusion of non-specific lymphocytes post-transplant." *Id.* In addition, "[t]he reinfusion of autologous T cells collected during clinical remission could also be considered in patients with persistent minimal residual disease." *Id.* Consistent with these assertions, Campana claims

> 17. A method for treating an individual suffering from cancer by introducing into said individual a T lymphocyte or natural killer cell comprising a chimeric receptor wherein said chimeric receptor comprises an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic domain, wherein said cytoplasmic domain comprises the Signaling domain of 4-1BB.

*Id.* at claim 17.

*2) CART-19 ClinicalTrials.gov (Exhibit 1006)*

CART-19 ClinicalTrials.gov is a printout of a government website disclosing "Pilot Study for Patients with Chemotherapy Resistant or Refractory CD19 Leukemia and Lymphoma (CART-19)." Ex. 1006, 5; *see also id.* at 1–2 (Declaration of Duncan Hall).

In setting forth the purpose of the study, CART-19 ClinicalTrials.gov explains that "[t]he subject's T cells will be modified in one or two different ways that will allow the cells to identify and kill the tumor cells (B cells)." *Id.* at 5. In particular,

> The two types of CART-19 T cells will be given back to subject's through an infusion. In addition to determining the safety of this approach, the purpose of the study is to determine which way of modifying the T cells works better in turning them "on" to fight cancer. This is done by monitoring levels of both types of modified cells in the subject's blood stream, and if possible, in the bone marrow and tumor tissue for four weeks after the infusion.

*Id.*

The reference further discloses that the study, designated NCT00891215, would be conducted at the University of Pennsylvania, Philadelphia, and identifies Dr. Carl H. June and David Porter, MD, as the "Responsible Party" and "Principle Investigator," respectively. Ex. 1006, 6. The reference describes the NCT00891215 as "an open label, single center, pilot study to evaluate the safety and tolerability, and differential persistence and engraftment of autologous T cells engineered to express a chimeric antigen receptor targeting CD19 which is linked either to the CD3 or CD3:4-1 BB signaling chains in a competitive repopulation setting." *Id.* at 5.

NCT00891215 was expected to enroll 10 patients, each of which would receive three infusions of CART-19 cells for a total dose of ~2xE9 - 5xE10 cells. The reference also discloses an evaluation and monitoring schedule and notes that "[a]nnual follow-up for lentiviral vector safety will be carried out for 15 years in accordance with FDA guidelines for retroviral vectors." *Id.*

*3) Nicholson (Exhibit 1004)*

Nicholson discloses "a single chain Fv (scFv) fragment from the mouse hybridoma cell line FMC63 which produces monoclonal antibody specific for CD19." Ex. 1004, 1157, 1160 (DNA and amino acid sequence information).

*4) Honsik (Exhibit 1005)*

Honsik is a United States Patent issued July 4, 1989. Ex. 1005, code (45). Honsik discloses pharmaceuticals compositions for infusing activated leukocytes (including T cells) using "a physiologically tolerable diluent aqueous medium." *Id.* at 4:45–5:9, 13:58–64; see Ex. 1002 ¶ 116. According to Honsik, "[e]xempary aqueous media include water, normal

saline, PBS, Ringer's solution, lactated Ringer's solution, and the like." Ex. 1005, 13:62–64.

*E. Obviousness Based on Campana, Nicholson, Honsik, and CART-19 ClinicalTrials.gov (Ground 1)*

For Ground 1 Petitioner contends claims 1–4, 6, 8, 9, 11, 16, 21, 22, and 27–30 are obvious in view of Campana, Nicholson, Honsik, and CART-19 ClinicalTrials.gov. Pet. 13, 28–48. Patent Owner argues that the combination of Campana and CART-19 ClinicalTrials.gov does not provide a reasonable expectation of success. Prelim. Resp. 21–39.

Following the approach set forth in the Petition, we address first the claim elements directed to the CAR T cell structure (elements [c]–[f]), and then claim elements directed to a pharmaceutical composition that comprises the claimed CAR T cells (elements [a]–[b]). *See* Pet. 29.

*1) Petitioner's Contentions*

*a) CAR T Structure*

Petitioner relies on Campana as disclosing a CAR T cell within the scope of elements [c]–[f]. Pet. 28–37.

> ([c]) wherein the T cells comprise a nucleic acid sequence encoding a chimeric antigen receptor (CAR)
>
> ([d]) wherein the CAR comprises a CD19 antigen binding domain comprising, from the ammo to the carboxy terminus, a light chain variable region and a heavy chain variable region of SEQ ID NO:20
>
> ([e]) wherein the CAR further comprises a transmembrane domain, a 4-1BB co stimulatory signaling region, and a CD3 zeta signaling domain
>
> ([f]) wherein the T cells are from a human having cancer.

Petitioner relies on Campana as teaching element (c) with the transduction of a viral vector encoding anti-CD19-BB-ζ CAR into T cells,

and the subsequent expression of the encoded CAR. Pet. 30–31 (citing Ex. 1003 ¶¶ 75–83; Ex. 1002 ¶¶ 36–37, 130–133); *see generally* Section II.D.1, above. Petitioner reasonably contends that the anti-CD19-BB-ζ CAR construct—illustrated in Campana's Figure 1—comprises, from its amino to carboxy terminus: (1) a CD8α signal peptide, (2) an anti-CD19scFv comprising a light chain variable region and a heavy chain variable region separated by a linker, (3) a CD8α hinge and transmembrane domain, (4) a 4-1BB costimulatory signaling region, and (5) a CD3 signaling domain. Pet. 32 (citations omitted); Ex. 1002 ¶¶ 60–62; 135–136; Section II.D.1, above.

With respect to the identity of the CD19 antigen binding domain of element (d), the '445 patent defines SEQ ID NO: 20 as the amino acid sequence of an anti-CD19scFv. Ex. 1001, 61:21. As noted by Petitioner, although "Campana itself does not recite sequences for a VL region and a VH region in an anti-CD19 scFv, it discloses that these can be obtained from Nicholson." Pet. 32 (citing Ex. 1003 ¶ 47; Ex. 1004, 1160). According to Dr. Junghans, SEQ ID NO: 20 corresponds to VH and VL regions of the CD19 antigen binding antibody FMC63 disclosed in Nicholson. *Id.* 33–34; Ex. 1002 ¶¶ 68–71, 138–139, 140–141 (noting that, as compared to SEQ ID NO: 20, Nicholson discloses an additional 16 amino acids at the end of the VL region).

Petitioner relies on Campana as teaching element (e) "wherein the CAR further comprises a transmembrane domain, a 4-1BB costimulatory signaling region, and a CD3 zeta signaling domain." Petitioner contends that Campana's CAR contains a "transmembrane domain[] of CD8α," a "signaling domain of CD3ζ[zeta]," and a costimulatory "signaling domain of 4-1BB." Pet. 35 (citing Ex. 1003 ¶ 13, Figure 1; Ex. 1002 ¶¶ 144–145).

Petitioner relies on Campana as teaching element (f) "wherein the T cells are from a human having cancer." Petitioner contends that Campana "proposes autologous CAR T-cell therapy." Pet. 37 (citing Ex. 1003 ¶ 119; Ex. 1002 ¶¶ 146–147).

Patent Owner does not presently contest that the prior art teaches or suggests element [c]–[f]. On the record before us, we find that Petitioner has shown sufficiently that the prior art teaches or suggests these elements as set forth above.

*b) Pharmaceutical Composition*

As briefly addressed above, Petitioner relies on Campana as disclosing the structural limitations of the CAR T and relies on the CART-19 ClinicalTrials.gov teachings to arrive at the pharmaceutical composition of elements [a]–[b]. Pet. 37–45.

> ([a]) A pharmaceutical composition comprising
> ([b]) an anti-tumor effective amount of a population of human T cells

Petitioner relies on Campana as teaching element (a) a "pharmaceutical composition." According to Petitioner, Campana discloses that methods of CAR T-cell "administration" to patients generally, and relies on Honisk for providing "exemplar pharmaceutical compositions for administering leucocytes, including T leucocytes (i.e., T cells)." Pet. 38 (citing Ex. 1003 ¶ 64; Ex. 1005, 13:58–64; Ex. 1002 ¶ 114).

Petitioner relies on CART-19 ClinicalTrials.gov as teaching element (b) reciting an "anti-tumor effective amount" that encompasses a range of cells from $10^4$ to $10^9$ per kg body weight. Pet. 38 (citing Ex. 1002 ¶ 154). Specifically, Petitioner contends that CART-19 ClinicalTrials.gov "disclosed dose of $2\times10^9$ to $5\times10^{10}$ total cells infused is equivalent to

$2.5 \times 10^7$ to $6.1 \times 10^8$ cells per kg body weight, based on an average adult body mass of 81.5 kg." Pet. 39–40 (citing Ex. 1002 ¶¶ 101-103, 161).

*2) Patent Owner's Contention*

Patent Owner argues, inter alia, that Petitioner cannot demonstrate a reasonable expectation of success in light of the unpredictability of cancer therapy and the "uninspiring" results and failures of earlier CAR-T clinical trials. Prelim. Resp. 23–28 (*citing e.g.*, Ex. 2037, 1036; Ex. 2011, 2269–2270; Ex. 2032, 1250–1251; Ex. 2038, 6106, 6115; Ex. 2039, 20; Ex. 2035, 956). In support, Patent Owner points to *OSI Pharms.* as "directly on point." *Id.* at 22 (citing *OSI Pharms., LLC v. Apotex Inc.*, 939 F.3d 1375 (Fed. Cir. 2019)).

*3) Motivation and Reasonable Expectation of Success*

According to Petitioner, "because the CART-19 ClinicalTrials.gov teaches a dose falling within an express range of 'anti-tumor effective amount,' this claim limitation does not separately require that a POSA would have had a reasonable expectation of an actual anti-tumor effect in a patient." Pet. 41. Here, CART-19 ClinicalTrials.gov suggests using a dose of CART-19 T cells falling within the range set forth in claim 2. Ex. 1005, 5.

Petitioner contends that the skilled artisan "would have reasonably expected, based on Campana and the CART-19 ClinicalTrials.gov, that administering T cells expressing the anti-CD19-BB-ζ CAR to cancer patients would at least result in a decrease in the number of tumor cells." Pet. 42 (citing Ex. 1002 ¶¶ 175, 184). Petitioner points to Campana's disclosure that T-cells expressing the anti-CD19-BB-ζ CAR exhibited cytotoxic activity against CD19+ leukemic cells, its conclusions that this result "provide[s] compelling justification for clinical trials using T cells expressing anti-CD19-BB-ζ," and its claims to a method of treating cancer



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using a CAR of this general structure. Pet. 42 (emphasis omitted) (citing Ex. 1003 ¶¶ 50–54, 118; Ex. 1002 ¶¶ 166-173).[17] Petitioner contends that Campana's in vitro results and proposed use of anti-CD19-BB-ζ in patients, would have led the skilled artisan to reasonably expect that "administering T cells transduced with the anti-CD19-BB-ζ CAR to patients with cancer would result in at least a decrease in the number of tumor cells," as required for "an anti-tumor effective amount." *Id.* at 43 (citing Ex. 1002 ¶ 175).

With respect to ClinicalTrials.gov's disclosure of the NCT00891215 study, Petitioner contends that just the "initiation of a clinical trial using anti-CD19-BB-ζ is indicative of a reasonable expectation of success." *Id.* at 44 (citing Ex. 1002 ¶¶ 176-177; M.P.E.P. § 2107.03(IV); 45 C.F.R. § 46.111(a)(2)). Petitioner further argues that the ordinarily skilled artisan reading the CART-19 ClinicalTrials.gov would have understood that "several other anti-CD19 CAR T cells in the art had been used successfully in cancer patients" (*id.* at 43–44 (citing Ex. 1002 ¶¶ 178–184)); that the number of anti-CD19-BB-ζ T-cells administered in NCT00891215 "used a dose that was within the range of 'therapeutically effective doses' for anti-CD19 CAR T cells taught by the art" (*id.* (citing Ex. 1002 ¶¶ 165, 176–177); and that "the study investigators believed this dose to be safe and likely effective in the treatment of CD19+ cancers such as '[a]cute lymphoblastic leukemia, follicular lymphoma, chronic lymphocytic leukemia, mantle cell

[17] Petitioner further contends that "[t]he Campana inventors also published their results in Imai, a peer-reviewed journal article that also advocated for clinical trials using anti-CD19-BB-ζ." Pet. 43 (citing Ex. 1022, 683 ("[T]he results of our study justify clinical trials using T cells expressing anti-CD19-BB-ζ receptors."); Ex. 1002 ¶¶ 60–65).

lymphoma, and diffuse large cell lymphoma'" (*id.* at 43 (citing Ex. 1002 ¶¶ 176–177; Ex. 1006, 5–7)).

In light of the above, Petitioner contends that a person of ordinary skill in the art "would have reasonably expected that the disclosed dose of anti-CD19-BB-ζ CAR T cells at least would reduce the number of tumor cells in a human patient with cancer, thereby rendering obvious 'an anti-tumor effective amount' of the claimed CAR T cells." Pet. 45 (emphasis omitted) (citing Ex. 1002 ¶¶ 165–184).

Ground 1 requires us to find a reasonable expectation of success in creating a pharmaceutical composition comprising CAR-T cells using a "light chain variable region and a heavy chain variable region of SEQ ID NO:20." Ex. 1001, 91:16–16 (claim 1). Campana teaches the use of CAR CD19-BB-ζ comprising a CD8α signal peptide, an anti-CD19 scFv binding region, a CD8α hinge and transmembrane domain, a 4-1BB costimulatory domain, and a CD3-ζ signaling domain. Ex. 1003, Fig. 1, ¶¶ 13, 65. Petitioner acknowledges that Campana does not teach the sequence set out in SEQ ID NO:20 but relies on the teachings of Nicholson for this limitation. *See* Pet. 32. Nicholson teaches the light chain variable and heavy chain variable regions as set out in SEQ ID NO:20. Ex. 1004, 1157, 1160 (DNA and amino acid sequence information). Petitioner's reliance on ClinicalTrials.gov's disclosure of the NCT00891215 showing the "initiation of a clinical trial using anti-CD19-BB-ζ is indicative of a reasonable expectation of success" evidences, based on the preliminary record, that the production of a pharmaceutical composition as recited in the claim would have been obvious. *See* Pet. 44 (citing Ex. 1002 ¶¶ 176-177; M.P.E.P. § 2107.03(IV); 45 C.F.R. §46.111(a)(2)). The success in making the

pharmaceutical composition for administration to patients regardless of the clinical outcome supports a finding of obviousness. Petitioner, therefore, has directed us to sufficient teachings in Campana, Nicholson, Honsik, and CART-19 ClinicalTrials.gov from which to conclude on the record before us that the CAR T structure as recited in claim 1 would have been obvious to one of ordinary skill in the art at the time the invention was made. *See* Pet. 28–48.

We are not persuaded by Patent Owner's contention that failures of earlier clinical trials using CAR-T cells supports the conclusion that there is lack of reasonable expectation of success for products that are useful for treating cancer. Prelim. Resp. 22. The claims at issue in *OSI Pharmaceutical* were method claims directed to treating cancer, while the present claims are composition claims directed to CAR T cells having the structure of elements (c)–(f) as recited in claim 1 of the '445 patent. For the reasons discussed above, a functional limitation in a product claim does not necessarily limit the scope of the claim because it only describes the context in which the CAR T cell product operates, but does not otherwise limit the structure of the product. *See supra* § II.C.1. Here, there is a sufficient reason in the record to have selected the number of CAR-T cells for use in a patient from the ClinicalTrials.gov's disclosure to meet the product claim limitation of an "amount" as recited in element 1(b). A person of ordinary skill in the art would have reasonably expected the product to work on some level based on the disclosure of ClinicalTrials.gov even if it does not reach the level of clinical effectiveness in treating cancer.

Based on the evidence and arguments presented by the parties at this stage in the proceeding, Petitioner shows that there is a reasonable likelihood

that claim 1 of the ’445 patent would have been obvious over Campana, Nicholson, Honsik, and CART-19 ClinicalTrials.gov under Ground 1.

### *F. Other arguments*

Patent Owner argues that Porter is not prior art. *See* Prelim Resp. 39–40. This argument is essentially identical to the argument presented in IPR2022-00853, and for the same reason discussed in our co-pending decision we agree with Patent Owner that Petitioner has not shown sufficiently Porter is prior art. *See* IPR2022-00853, Paper 11 at 30–33 (“[W]e are not persuaded that Petitioner can establish that Porter qualifies as prior art under §102(a).”).

Patent Owner additionally argues that we should deny institution pursuant to 35 U.S.C. § 325(d), because “the same or substantially the same prior art or arguments previously were presented to the Office.” *See* Prelim Resp. 42–52. This argument is essentially identical to the argument presented in IPR2022-00853. For the same reason discussed in our co-pending decision we agree with Petitioner that Campana was cited on an IDS but not substantively discussed during prosecution. *See* IPR2022-00853, Paper 7 at 42–48. Because the Petition includes references not substantively discussed by the Office during prosecution we are not persuaded by Patent Owner’s argument that we should deny institution.

## III. CONCLUSION

On the preliminary record before us, Petitioner demonstrates a reasonable likelihood of prevailing at trial in showing that at least one claim of the ’445 patent would have been obvious over the cited prior art. In accordance with the Court’s decision in *SAS Institute, Inc.*, 138 S. Ct. at

1359–60, and Office Guidance, we institute an *inter partes* review of all challenged claims (1–19 and 21–30) of the '445 patent on all grounds (Grounds 1–4) asserted by Petitioner.

At this stage of the proceeding, the Board has not made a final determination as to the patentability of any challenged claim or the construction of any claim term.

## IV. ORDER

It is hereby:

ORDERED that pursuant to 35 U.S.C. § 314(a), an *inter partes* review of claims 1–19 and 21–30 of the '445 patent is instituted with respect to all grounds set forth in the Petition; and

FURTHER ORDERED that, pursuant to 35 U.S.C. § 314(c) and 37 C.F.R. § 42.4(b), *inter partes* review of the '445 patent shall commence on the entry date of this Order, and notice is hereby given of the institution of a trial.

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