A Phase I Study on Adoptive Immunotherapy Using Gene-Modified T Cells for Ovarian Cancer

Michael H. Kershaw,[1,3,4] Jennifer A. Westwood,[1,3] Linda L. Parker,[1] Gang Wang,[1,5] Zelig Eshhar,[6]
Sharon A. Mavroukakis,[1] Donald E. White,[1] John R. Wunderlich,[1] Silvana Canevari,[7] Linda Rogers-Freezer,[1]
Clara C. Chen,[2] James C. Yang,[1] Steven A. Rosenberg,[1] and Patrick Hwu[1,5]

Abstract **Purpose:** A phase I study was conducted to assess the safety of adoptive immunotherapy using gene-modified autologous T cells for the treatment of metastatic ovarian cancer.

Experimental Design: T cells with reactivity against the ovarian cancer–associated antigen α-folate receptor (FR) were generated by genetic modification of autologous T cells with a chimeric gene incorporating an anti-FR single-chain antibody linked to the signaling domain of the Fc receptor γ chain. Patients were assigned to one of two cohorts in the study. Eight patients in cohort 1 received a dose escalation of T cells in combination with high-dose interleukin-2, and six patients in cohort 2 received dual-specific T cells (reactive with both FR and allogeneic cells) followed by immunization with allogeneic peripheral blood mononuclear cells.

Results: Five patients in cohort 1 experienced some grade 3 to 4 treatment-related toxicity that was probably due to interleukin-2 administration, which could be managed using standard measures. Patients in cohort 2 experienced relatively mild side effects with grade 1 to 2 symptoms. No reduction in tumor burden was seen in any patient. Tracking ^{111}In-labeled adoptively transferred T cells in cohort 1 revealed a lack of specific localization of T cells to tumor except in one patient where some signal was detected in a peritoneal deposit. PCR analysis showed that gene-modified T cells were present in the circulation in large numbers for the first 2 days after transfer, but these quickly declined to be barely detectable 1 month later in most patients. An inhibitory factor developed in the serum of three of six patients tested over the period of treatment, which significantly reduced the ability of gene-modified T cells to respond against FR$^+$ tumor cells.

Conclusions: Large numbers of gene-modified tumor-reactive T cells can be safely given to patients, but these cells do not persist in large numbers long term. Future studies need to employ strategies to extend T cell persistence. This report is the first to document the use of genetically redirected T cells for the treatment of ovarian cancer.

There is increasing interest in the use of immunotherapy for the treatment of malignant disease, and some dramatic clinical responses have led to intense activity in this field. In particular, the success of adoptive immunotherapy as a treatment for melanoma has prompted us to extend this therapy to ovarian cancer. Variables important in the application of this therapy have been identified in melanoma patients, including the requirement for tumor antigen-reactive lymphocytes, high-dose interleukin-2 (IL-2; ref. 1), and more recently the benefits of prior lymphoablation (2).

However, endogenous tumor-reactive cells cannot be reproducibly found in ovarian patients. Nevertheless, several tumor-associated antigens have been identified for ovarian tumors, including Her-2 (3), tumor-associated glycoprotein 72 (4), Lewis-Y (5), and α-folate receptor (FR; ref. 6), and monoclonal antibodies exist recognizing these antigens. Recombinant genes encoding chimeric receptors incorporating antibody specificity can be used to genetically modify T cells to endow them with activity against tumor cells (7–17).

We have previously reported the generation of FR-specific T cells by modification of T cells with a gene encoding a cell surface chimeric receptor linking single-chain (scFv) anti-FR to the transmembrane and cytoplasmic domains of the Fc receptor γ chain. The scFv was derived from the MOv18 monoclonal antibody (18), and the chimeric gene is referred to as *MOv-γ*. We showed that this gene could endow *ex vivo* transduced T cells with the ability to respond against FR$^+$ tumor cells

---

Authors' Affiliations: [1]Surgery Branch, Center for Cancer Research, National Cancer Institute; [2]Department of Nuclear Medicine, Clinical Center, NIH, Bethesda, Maryland; [3]Cancer Immunology Research Program, Peter MacCallum Cancer Centre; [4]Department of Pathology, University of Melbourne, Melbourne, Australia; [5]Department of Melanoma Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; [6]Department of Immunology, Weizmann Institute of Science, Rehovot, Israel; and [7]Istituto Nazionale Tumori, Milan, Italy

Received 5/16/06; revised 6/26/06; accepted 7/18/06.

Grant support: The National Health and Medical Research Council (M. Kershaw), National Breast Cancer Foundation of Australia (M. Kershaw), Bob Parker Memorial Fund (J. Westwood), and Peter MacCallum Cancer Centre Foundation (J. Westwood).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Patrick Hwu, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 430, Houston, TX 77030. Phone: 713-792-2921; Fax: 713-745-1046; E-mail: phwu@mdanderson.org.

doi:10.1158/1078-0432.CCR-06-1183

in vitro (19). In addition, adoptive transfer of anti-FR mouse T cells could inhibit tumor growth in lung metastases and i.p. models of disease in mice (20).

More recently, we have generated FR-reactive cells from populations of T cells with endogenous specificity for allogeneic antigen. We showed that these T cells could respond to both tumor and allogeneic antigen and referred to these T cells as dual specific (21). The rationale behind the generation of dual-specific T cells was to provide a population of FR-reactive T cells that could expand *in vivo* in response to allogeneic immunization, which is not possible in response to FR alone. Indeed, adoptive transfer of mouse dual-specific T cells into mice followed by allogeneic immunization resulted in expansion of transferred cells and enhanced inhibition of s.c. tumor growth without the need for administration of IL-2 (21).

Based on these encouraging results in mice, we initiated a two-cohort phase I clinical study in ovarian cancer patients. Cohort 1 patients were treated with adoptive transfer of bulk peripheral blood–derived T cells gene-modified with the anti-FR chimeric receptor in combination with high-dose IL-2. Cohort 2 involved the generation of dual-specific T cells from autologous peripheral blood mononuclear cells (PBMC) and their transfer into patients followed by s.c. immunization with allogeneic PBMCs.

Materials and Methods

Treatment regimen. Patients received adoptive transfer of autologous T cells gene-modified to express a chimeric receptor specific for the tumor-associated antigen FR. The study was divided into two cohorts, with cohort 1 receiving T cells and high-dose IL-2 and cohort 2 receiving dual-specific T cells and s.c. immunization with allogeneic PBMCs but no IL-2.

Eight patients were enrolled in cohort 1, each receiving up to three cycles of treatment, with each cycle consisting of administration of gene-modified T cells and IL-2 (720,000 IU/kg body weight). Approximately 4 weeks elapsed between the start of each cycle. Following activation, transduction, and G418 selection, T cells were expanded in culture, harvested, washed, and resuspended in 100 mL of saline and given to patients by i.v. drip over 20 to 30 minutes. The first five patients received a dose escalation regimen beginning at 3×10^9 transduced T cells. If no grade 3 or 4 toxicity was observed, not easily rectified within 24 hours, the patient was eligible to proceed to the next dose level of 1×10^{10} T cells at the start of the next cycle and subsequently to the highest test dose level of 3×10^{10} to 5×10^{10} cells at the start of the third cycle. IL-2 was given i.v. on the day of T cell transfer and every 12 hours for up to six doses if tolerated.

Six patients were enrolled in cohort 2, each receiving up to two cycles of treatment, with each cycle consisting of adoptive transfer of gene-modified dual-specific T cells followed by immunization with allogeneic PBMCs. Eight to 12 weeks elapsed between the start of each cycle. Following two *in vitro* allogeneic stimulations and expansion in culture, T cells were given to patients as in cohort 1. Allogeneic immunization consisted of s.c. injection of ~2.0×10^9 to 4.0×10^9 allogeneic PBMCs (viable and nonirradiated) from the same donor used to stimulate T cells during their generation *in vitro*. Each dose of allogeneic PBMCs was split into four equal parts and injected s.c. into separate sites on the lower extremities in 1 mL saline per site. Immunization was done 1 day after T cell transfer and again 1 week later because multiple allogeneic immunizations had shown better effect in mouse studies (21).

Patient treatment and monitoring procedures were reviewed by the Institutional Review Board of the Center for Cancer Research, National Cancer Institute, and informed consent was obtained from all patients before treatment.

Patient eligibility. Patients had biopsy-proven recurrent, resected recurrent, or residual epithelial FR$^+$ ovarian cancer that failed standard effective therapy, including cisplatin/carboplatin– or paclitaxel-containing regimens. Patients ranged in age from 33 to 60 years and had clinical Eastern Cooperative Oncology Group performance status of 0 or 1. Eligibility criteria required serum creatinine levels ≤1.6 mg/dL and bilirubin <2.0 mg/dL. Blood eligibility criteria included hemoglobin >9.0 g/dL, WBC >3,000/mm^3, and platelets >100,000/mm^3 and an intact immune system as evidenced by a positive reaction to *Candida albicans* skin test, mumps skin test, or tetanus toxoid skin test on a standard anergy panel.

Response assessment. Patients received radiologic evaluation by magnetic resonance imaging, computed tomography, or sonography immediately before treatment and at completion of therapy. Disease response was determined by comparison of pretreatment and post-treatment images. In addition, serum CA-125 levels were determined following treatment and compared with pretreatment CA-125 levels.

T cell generation. A detailed description of the generation and characterization of T cells used in cohort 1 of the study has been published previously (22). Briefly, patient PBMCs derived from leukapheresis were stimulated with anti-CD3 (OKT3, Ortho Biotech, Raritan, NJ) and human recombinant IL-2 (600 IU/mL; Chiron, Emeryville, CA). After 3 days of culture, ~5×10^7 to 1×10^8 lymphocytes were taken and transduced with retroviral vector supernatant (Cell Genesys, San Francisco, CA) encoding the chimeric *MOv-γ* gene and subsequently selected for gene integration by culture in G418.

For the generation of dual-specific T cells used in cohort 2, stimulation of T cells was achieved by coculture of patient PBMCs with irradiated (5,000 cGy) allogeneic donor PBMCs from cryopreserved apheresis product (mixed lymphocyte reaction). The MHC haplotype of allogeneic donors was determined before use, and donors that differed in at least four MHC class I alleles from the patient were used. Culture medium consisted of AimV medium (Invitrogen, Carlsbad, CA) supplemented with 5% human AB$^-$ serum (Valley Biomedical, Winchester, VA), penicillin (50 units/mL), streptomycin (50 mg/mL; Bio Whittaker, Walkersville, MD), amphotericin B (Fungizone, 1.25 mg/mL; Biofluids, Rockville, MD), L-glutamine (2 mmol/L; Mediatech, Herndon, VA), and human recombinant IL-2 (Proleukin, 300 IU/mL; Chiron). Mixed lymphocyte reaction consisted of 2×10^6 patient PBMCs and 1×10^7 allogeneic stimulator PBMCs in 2 mL AimV per well in 24-well plates. Between 24 and 48 wells were cultured per patient for 3 days, at which time transduction was done by aspirating 1.5 mL of medium and replacing with 2.0 mL retroviral supernatant containing 300 IU/mL IL-2, 10 mmol/L HEPES, and 8 μg/mL polybrene (Sigma, St. Louis, MO) followed by covering with plastic wrap and centrifugation at 1,000 × *g* for 1 hour at room temperature. After overnight culture at 37°C/5% CO_2, transduction was repeated on the following day, and then medium was replaced after another 24 hours. Cells were then resuspended at 1×10^6/mL in fresh medium containing 0.5 mg/mL G418 (Invitrogen) in 175-cm^2 flasks for 5 days before resuspension in media lacking G418.

Cells were expanded to 2×10^9 and then restimulated with allogeneic PBMCs from the same donor to enrich for T cells specific for the donor allogeneic haplotype. Restimulation was done by incubating patient T cells (1×10^6/mL) and stimulator PBMCs (2×10^6/mL) in 3-liter Fenwall culture bags in AimV + additives and IL-2 (no G418). Cell numbers were adjusted to 1×10^6/mL, and IL-2 was added every 2 days, until sufficient numbers for treatment were achieved.

Cell lines, flow cytometry, and IFN-γ secretion assay. Tumor cell lines used in assays of T cell function were the FR$^+$ human ovarian cancer cell line IGROV-1 (23) and FR$^-$ melanoma cell lines Mel 526, Mel 624, Mel 888, and Mel 1866 (Surgery Branch, National Cancer Institute, Bethesda, MD). Tumor cells were maintained in RPMI supplemented with 10% FCS (Invitrogen), penicillin (50 units/mL), streptomycin (50 mg/mL), amphotericin B (1.25 mg/mL), and L-glutamine (2 mmol/L). A melanoma-specific T cell line used in some experiments was derived from tumor-infiltrating lymphocytes of a patient at National Cancer Institute and maintained in T cell culture medium described above.

Expression of chimeric MOv-γ receptor by transduced T cells was determined using flow cytometry following staining with phycoerythrin-conjugated Id18.1, a monoclonal antibody specific for the MOv-18 idiotype (24). T cells were stained with phycoerythrin-conjugated mouse IgG1 as a control for nonspecific binding.

Transduced T cells were assessed for their ability to respond against the FR antigen by coculture with IGROV-1 ovarian cancer cells. T cells (1×10^5) were incubated with IGROV-1 cells (1×10^5; or FR-negative control tumor cells) in triplicate wells of 96-well plates. After overnight culture, supernatant was taken and assayed for IFN-γ using ELISA kits according to manufacturer's instructions (Endogen, Woburn, MA). T cells in cohort 2 of the study were also assessed for their ability to secrete IFN-γ in response to allogeneic stimulator PBMCs freshly thawed from cryopreserved stocks. Anti-human CD3 (OKT3) was also used to stimulate T cells to gauge their maximal capacity to respond to TCR-CD3 engagement. OKT3 was immobilized on 96-well plastic plates at 0.5 μg/well in 100 μL PBS overnight at 4°C.

In some experiments, a 25% proportion of patient serum was included in T cell cocultures to determine possible effects of patient serum on T cell function. In some assays, protein G (Amersham Biosciences, Piscataway, NJ; 20 μL/mL) was added to serum before coculture and incubated for 1 hour at 4°C with gentle rocking to deplete patient serum of immunoglobulin.

Serum FR titer assay. A double-determinant assay was done essentially as described (25) using MOv19, a non–cross-reacting antibody directed against FR (18), as catcher. Briefly, 96-well flat-bottomed maxisorp plates (Nunc, Roskilde, Denmark) were coated with 200 μL of MOv19, at 1 μg/mL in PBS, and incubated overnight at 4°C. Plates were washed and blocked for 1 hour with 200 μL/well of 0.5% bovine serum albumin in PBS; 100 μL of sample was added to wells. A positive control consisted of tissue culture supernatant from IGROV-1 cells. Plates were incubated 2 hours and washed, and 100 μL of biotinylated MOv18 (0.25 μg/mL) were added followed by incubation at room temperature for 2 hours. Plates were washed, and streptavidin-horseradish peroxidase in PBS/0.5% bovine serum albumin was added 100 μL/well and incubated for 0.5 hour. Plates were washed, and 100 μL/well trimethylbenzidine was added and incubated for 5 to 10 minutes, and reaction was stopped with 1 mol/L H_2SO_4. Plates were read on spectrophotometer 450 nm within 0.5 hour of stopping reaction. Concentrations of FR in patient sera were expressed as dilution until absorbance reached background levels of media alone.

T cell tracking. A fraction of transduced T cell cultures (17-50%, 1.5×10^9 to 7.5×10^9 cells) were radiolabeled with ^{111}In-oxine as described previously (26). Briefly, this involved incubation with 750 μCi ^{111}In-oxine per 10^{10} cells in 30 to 50 mL PBS for 15 minutes with gentle rocking. Labeled cells were then washed and resuspended in 100 mL of saline containing 5% human serum albumin and 75,000 IU IL-2 for i.v. infusion into patients over a period of 10 to 20 minutes. Gamma camera images were obtained at intervals for up to 5 days where practicable.

T cell persistence: PCR-ELISA. Patient peripheral blood was analyzed for persistence of transduced T cells by detection of the neomycin phosphotransferase (neo) gene using a PCR-ELISA DIG Detection kit (Roche, Basel, Switzerland), as per the manufacturer's directions using 50 pmol forward neo primer (ATTGAACAAGATGGATTGCACGCAG), 50 pmol reverse neo primer (TCAGAAGAACTCGTCAAGAAGGCG), 0.25 unit Taq DNA polymerase (Promega, Madison, WI) and 50% by volume of patient PBMC lysate. A series of lysed samples of Jurkat-22 neo cells (containing 1 copy of neo per cell) was prepared as standards, consisting of 1% of cells and decreasing in multiples of 2 down to

Table 1. Sites of disease and treatment history

Patient	Prior treatment	Metastatic disease status on enrollment in study
1	Hysterectomy, BSO, debulked, Taxol, Carboplatin, Cisplatin, bone marrow transplant, etoposide	Lower abdominal s.c. mass, two inguinal nodules
2	THA/BSO, omentectomy, appendectomy, nodectomy, Taxol, Cisplatin, Topotecan, Hexamethylmelamine	Retroperitoneal and left cervical lymph nodes
3	Radical hysterectomy, debulked, Carboplatin, Cytoxan, Adriamycin, Mitoxantrane, Tamoxifen, etoposide, radiation	Liver and vaginal cuff
4	TAH/BSO Taxol, Carboplatin, Doxil, Topotecan, Gemzar	Perihepatic lesion, midabdominal s.c. nodule
5	TAH/BSO, Carboplatin, Cytoxan, Taxol, Topotecan	Perihepatic, ascites, sigmoid mass, omental disease
6	Debulked, Carboplatin, Taxol, Doxil	Pelvic mass, para-aortic adenopathy
7	TAH/BSO, debulked, Carboplatin, Taxol	Multiple sites periaortic retroperitoneal adenopathy
8	TAH/BSO, debulked, Cisplatin, Cytoxan, Carboplatin, Taxol, Topotecan, Doxel	Liver, pericolonic, pelvic lymph node
9	TAH/BSO, debulked, Carboplatin, Taxol, Cytoxan	Liver and rectal muscle mets
10	TAH/BSO, debulked, Carboplatin, Taxol, Cisplatin, Taxol, monoclonal vaccine, Tamoxifen	Omentum, peritoneal implants, diaphragm, right supraclavicular nodes, pelvis
11	TAH/BSO, omentectomy, appendectomy, pancreatic reduction, splenectomy, Cytoxan, Cisplatin, vincristine, Hexalen, etoposide, Taxol, Carboplatin, Adriamycin, Topotecan, Gemzar	Peritoneal implants, left pleural effusion, liver, retroperitoneal nodes
12	TAH/BSO, omentectomy, Taxol, Carboplatin, bone marrow transplant, Taxane, Doxil, Herceptin, Gemcitabine, Topotecan	Pelvic and mediastinal mets
13	Ovarian cystectomy, hysterectomy, pelvic lymphadenectomy, Taxotere, Carboplatin, Topotecan, Doxyl, Gemzar	Omentum, mediastinum
14	TAH/BSO, omentectomy, pelvic and para-aortic lymphadenectomy, Taxol, Cisplatin, external beam radiation, Topotecan, Thalidomide, etoposide, Hexalen	Epigastric intra-abdominal mass, right pelvic mass

NOTE: Patients had advanced ovarian cancer with metastases to various sites. Before enrolling in the current study, total abdominal hysterectomy and bilateral salpingo-oophorectomy were done, and most patients had undergone debulking surgery (patients 1-8 enrolled in cohort 1 and patients 9-14 in cohort 2).
Abbreviations: TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy.

0.016%. PCR cycling consisted of 96°C for 6 minutes followed by 35 cycles of 95°C for 1 minute, 57°C for 1 minute, and 72°C for 2 minutes. The ELISA was done using 7.5 μL of the PCR product and 15 pmol/mL probe (Biotin-AGCAAGGTGAGATGACAGGAGAT), with hybridization done at 48°C.

Results

Patient characteristics. All patients had been diagnosed with metastatic ovarian cancer. Sites of metastases varied between patients but involved peritoneal disease with lymph node involvement (Table 1). Previous treatments received by patients before enrollment in the study varied but included surgical removal of primary lesion, debulking, and chemotherapy (Table 1). Previous therapies ceased at least 2 weeks before receiving gene-engineered T cells.

Characterization of gene-modified T cells used in cohort 1. A complete characterization of T cells used in cohort 1 has already been described previously (22), but briefly, T cell cultures were stimulated with anti-CD3 antibody and were shown to expand from 11,000- to 3,000,000-fold. The mean time of culture of T cells from patients in cohort 1 was 47 days (range, 25-56 days). The T cells secreted IFN-γ specifically in response to FR and could lyse FR^+ tumor cells. Phenotypically, the bulk lymphocyte population was composed of both $CD4^+$ and $CD8^+$ T cells and was shown to consist of a diverse range of clones able to secrete a variety of cytokines, including IFN-γ, IL-10, granulocyte macrophage colony-stimulating factor, and IL-2, in response to FR. Percentages of $CD4^+CD8^-$ T cells varied between 1% and 39%, and $CD4^-CD8^+$ T cell percentages ranged from 47% to 94%.

Expansion of T cells used in cohort 2. In this cohort of the study, T cells received an initial stimulation with allogeneic stimulator cells and transduction with retroviral vector for a culture period of between 21 and 38 days. During this stimulation period, T cell expansion from patients varied from 12- to 325-fold. To further expand T cell numbers and enrich for allo-specific T cells, a second stimulation with PBMCs from the original PBMC donor was done. This restimulation resulted in a further expansion in T cells of ~50-fold. A representative growth curve for T cells over two stimulations is presented in Fig. 1A. The mean time of T cell culture for cohort 2 was 40.5 days (range, 37-48 days).

To promote high levels of allo stimulation, stimulator PBMCs were HLA typed at MHC class I loci to check for allelic differences to patient HLA type. Patient HLA type was determined to be largely dissimilar to stimulator HLA, with differences in at least four of six alleles.

Phenotype of T cells in cohort 2. T cells are characterized into two major phenotypic subsets, either $CD4^+$ or $CD8^+$, which have different fundamental abilities of helper function or cytotoxic function, respectively. Because this could affect on the overall function of bulk T cell populations and interpretation of clinical results, the relative proportions of $CD4^+$ and $CD8^+$ T cells were determined for each culture. Following two stimulations with allogeneic PBMCs, expression of CD4 and CD8 T cell markers was determined using specific monoclonal antibodies and flow cytometry. Percentages of $CD4^+CD8^-$ T cells varied between 2% and 82%, and $CD4^-CD8^+$ T cell percentages ranged from 13% to 85%. $CD4^-CD8^-$ cells were present in all cultures but only as a minor population (2-15%; Fig. 1B).

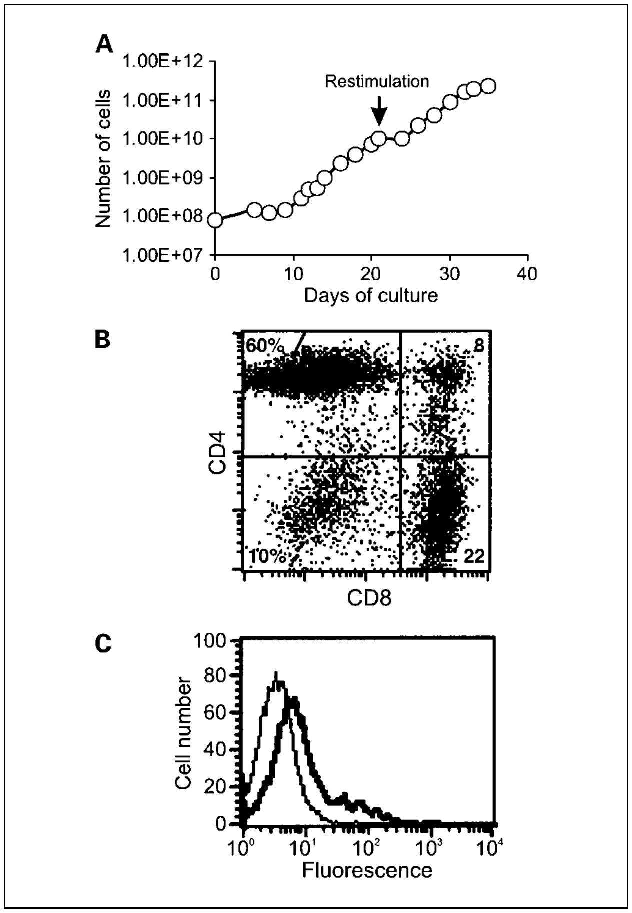

Fig. 1. Growth and phenotype of gene-modified T cells. *A,* following allogeneic stimulation, 8 × 10^7 T cells were transduced with retroviral vector encoding the MOv-γ receptor and maintained at 1 to 2 × 10^6/mL in media containing IL-2. Transduced T cells were restimulated with allogeneic PBMCs on day 21, which resulted in further expansion of T cells. Using this method, large numbers of dual-specific T cells could be generated. T cell expansion depicted is for patient 9. Representative of all six patients in cohort 2. The phenotype of transduced T cells from cohort 2 of the study was determined with respect to T cell subset markers and chimeric receptor expression using specific antibodies and flow cytometry. *B,* although the relative proportions of $CD4^+$ and $CD8^+$ T cells varied between patients, the culture was made up predominantly of $CD4^+$ and $CD8^+$ cells as seen in the representative plot. *C,* expression of the chimeric MOv-γ receptor was evident following staining with anti-idiotype antibody (*thick line*) compared with isotype control antibody (*thin line*). Representative of all patients.

All T cells were transduced, as indicated by G418 resistance, and expressed the chimeric MOv-γ receptor, as determined, in flow cytometry, by an increase in fluorescence staining in presence of Id 18.1, an anti-MOv18 idiotype monoclonal antibody compared with staining in presence of isotype control antibody (Fig. 1C). Although expressed at low level, the chimeric receptor endowed T cells with the ability to respond specifically against FR^+ target cells (see next section).

IFN-γ secretion by T cells in response to tumor cells and allogeneic stimulator PBMC. T cell cultures from patients in cohort 1 were shown to secrete IFN-γ specifically in response to FR, and this has been previously reported (22). IFN-γ levels in response to FR^+ IGROV-1 cells varied from 1,749 to 28,560 pg/mL. With respect to patients in cohort 2, an important requirement of dual-specific T cells was their ability to respond

Table 2. Anti-FR and anti-allo responses of transduced T cells from patients in cohort 1 and 2 of the study

	Cohort 2							Cohort 1, median
	Pt. 9	**Pt. 10**	**Pt. 11**	**Pt. 12**	**Pt. 13**	**Pt. 14**	**Median**	
Media alone	36	60	34	42	0	19	35	153
Melanoma (FR$^-$)	35	54	28	26	14	27	28	139
IGROV-1 (FR$^+$)	1,375	2,960	8,010	1295	1,340	9,050	2,168	6,501
Allogeneic stimulator PBMCs	2,270	1,555	2,995	5625	>4,320	603	2,633	
Autologous PBMCs	210	456	36	140	39	298	175	
OKT3	5,784	13,317	9,620	2890	>3,760	>59,000	7,702	7,457

NOTE: T cell reactivity towards the FR tumor antigen and allogeneic stimulator PBMCs was determined by assaying IFN-γ secretion (pg/mL) using ELISA following overnight incubation of T cells with the targets listed. Plastic-coated anti-CD3 (OKT3) was used as an indicator of maximal T cell response. Transduced T cells from all patients were reactive with FR, and T cells from patients in cohort 2 were reactive with allogeneic PBMCs. Nontransduced T cells did not respond against IGROV-1, except for patient 14 in whom 548 pg/mL IFN-γ was secreted (data not shown).

to both FR and allogeneic stimulator PBMCs that were to be used as immunogen following T cell transfer. IFN-γ secretion following coculture of T cells with tumor or allogeneic PBMC was used as an indicator of T cell response. Although there was some variation between patients in IFN-γ levels in response to FR$^+$ IGROV-1 cells (1,295-9,050 pg/mL; Table 2), secretion was always greater than that in response to FR$^-$ melanoma cells (14-54 pg/mL), thereby showing that transduced T cells could respond specifically against FR. The specificity of the response was also supported by the observed lack of IFN-γ secretion

Table 3. Summary of treatment regimen and toxicity for patients receiving gene-modified T cells

Patient	Cycle no.	No. T cells × 10^{-9}	No. IL-2 injections	Grades of adverse events	Grade 1 and 2 toxicity events	Grade 3 and 4 toxicity events
1	1	3.0	6	1, 2, 3, 4	HB, FAT, NAU, PU, PE	LEU, HYP, PCD
	2	3.0*	5	2, 3	ED	RIG
	3	10.0	3	2, 3	ED, LEU, PCD, PU, PE	HB, HYP
2	1	3.0[†]	5	1, 2	PCD, FAT, NAU, BIL, PU	
	2	9.0	4			
	3	47.0[†]	4			
3	1	3.0	5	3		DIA, FAT
	2	10.0	3	2	LUO	
	3	17.5	1	3		HYP, STC
4	1	3.0	5			
	2	11.4[†]	1	3		DYS
	3	21.9[†]	0			
5	1	3.0	3	2	ASC	
6	1	28.57[†]	6	2	FAT, DIA	
	2	11.0	6	2	FAT	
7	1	22.0[†]	0	2, 4	RIG, VOM	DYS[‡]
8	1	44.0[†]	2	3		HYP
	2	43.5	1	2	DYS	

	Cycle no.	No. T cells × 10^{-9}	No. allo immunizations[§]	No. allo cells × 10^{-9}	Grades of adverse events	Grade 1 and 2 toxicity events
9	1	46.5	2	4.55	1, 2	ISR, URT, DYS
	2	169.0	2	6.5	None	
10	1	13.17[†]	2	4.5	1, 2	RIG, ISR, NAU
	2	50.0	2	7.0	None	
11	1	4.0	2	7.8	2	ISR
12	1	11.7	2	7.32	1	ISR
13	1	36.7	2	6.72	None	
14	1	9.0	2	7.45	1, 2	HYP, RIG, FAT, ISR, NAU, VOM

Abbreviations: ASC, ascites; HB, hemoglobin; PE, pleural effusion; BIL, bilirubin increased; HYP, hypotension; PU, pulmonary; DIA, diarrhea; LEU, leukopenia; RIG, rigors; DYS, dyspnea; LUO, low urine output; STC, sinus tachycardia; ED, Edema; NAU, nausea; URT, urticaria; FAT, fatigue; PCD, platelet count decreased; VOM, vomiting; ISR, injection site reaction (allogeneic immunization).

*No progression to higher dose in this cycle due to grade 4 toxicity in previous cycle.

[†] Some of these cells were labeled with ^{111}In for trafficking.

[‡] Off protocol after one cycle due to dyspnea concerns.

[§] Dose divided into two to four injections given on day 1 and 8 following MOv-γ T cells.

DOCKET
ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

WHAT WILL YOU BUILD? | **sales@docketalarm.com** | **1-866-77-FASTCASE**

fastcase®
Smarter legal research.