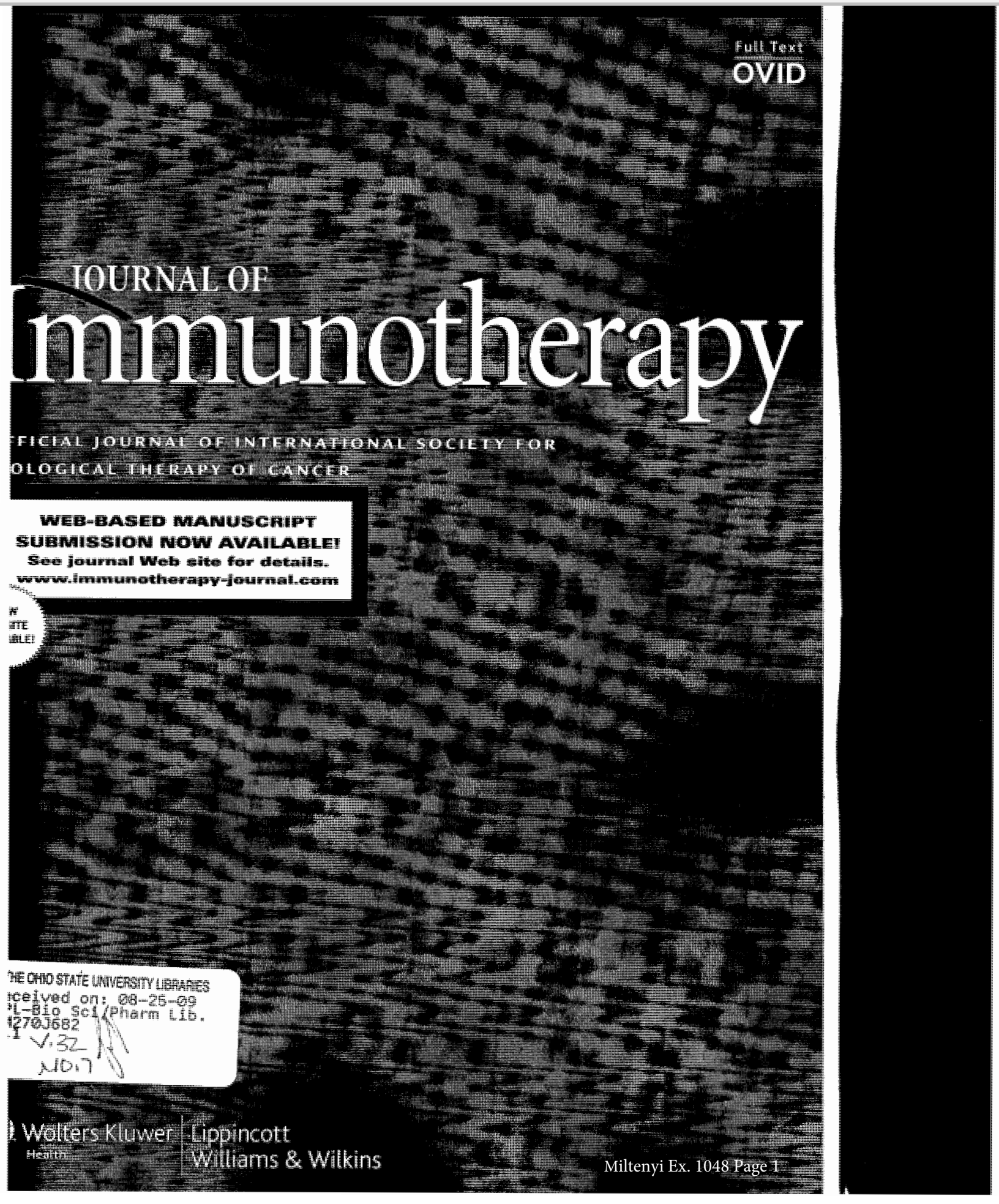

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Construction and Preclinical Evaluation of an Anti-CD19 Chimeric Antigen Receptor

James N. Kochenderfer, Steven A. Feldman,* Yangbing Zhao,* Hui Xu,* Mary A. Black,* Richard A. Morgan,* Wyndham H. Wilson,† and Steven A. Rosenberg**

Summary: T cells can be engineered to express the genes of chimeric antigen receptors (CARs) that recognize tumor-associated antigens. We constructed and compared 2 CARs that contained a single chain variable region moiety that recognized CD19. One CAR contained the signaling moiety of the 4-1BB molecule and the other did not. We selected the CAR that did not contain the 4-1BB moiety for further preclinical development. We demonstrated that gammaretroviruses encoding this receptor could transduce human T cells. Anti-CD19-CAR-transduced $CD8^+$ and $CD4^+$ T cells produced interferon-γ and interleukin-2 specifically in response to $CD19^+$ target cells. The transduced T cells specifically killed primary chronic lymphocytic leukemia (CLL) cells. We transduced T cells from CLL patients that had been previously treated with chemotherapy. We induced these T cells to proliferate sufficiently to provide enough cells for clinical adoptive T cell transfer with a protocol consisting of an initial stimulation with an anti-CD3 monoclonal antibody (OKT3) before transduction followed by a second OKT3 stimulation 7 days after transduction. This protocol was successfully adapted for use in CLL patients with high peripheral blood leukemia cell counts by depleting $CD19^+$ cells before the initial OKT3 stimulation. In preparation for a clinical trial that will enroll patients with advanced B cell malignancies, we generated a producer cell clone that produces retroviruses encoding the anti-CD19 CAR, and we produced sufficient retroviral supernatant for the proposed clinical trial under good manufacturing practice conditions.

Key Words: chimeric antigen receptor, gene therapy, CD19, T cell, gammaretrovirus, adoptive T cell therapy

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Approximately 22,000 people die because of B cell malignancies each year in the United States.[1] Patients with some B cell malignancies including mantle cell lymphoma and chronic lympocytic leukemia (CLL) cannot be cured by therapies such as conventional chemotherapy and monoclonal antibodies,[2,3] but some patients with these diseases can achieve prolonged disease-free survival after allogeneic stem cell transplantation.[4–6] Unfortunately, allogeneic stem cell transplantation is limited by significant transplant-related mortality and a shortage of suitable donors.[2,6,7] In patients with B cell malignancies that relapse after allogeneic stem cell transplantation, infusion of allogeneic donor lymphocytes can induce remissions.[8–10] The effectiveness of these lymphocyte infusions provides a rationale for attempts to develop other cellular immunotherapies for B cell malignancies.

Adoptive transfer of autologous T cells that are cultured from tumor infiltrating lympohocytes can cause regressions of advanced melanoma in humans.[11,12] Because tumor-reactive T cells cannot be reliably cultured from most of human tumors, methods have been developed to engineer T cells to express genes encoding tumor antigen-specific T cell receptors.[13,14] Adoptive transfer of these genetically modified T cells is a promising approach to cancer immunotherapy.[15] Another approach to adoptive T cell therapy is to engineer T cells to express chimeric antigen receptors (CARs).[16,17] CARs are made up of an antigen-recognizing receptor coupled to signaling molecules that can activate T cells expressing the CAR.[18–20] The antigen-receptors most commonly incorporated into CARs are single chain variable region moieties (scFv) that consist of the light chain and heavy chain variable regions of a monoclonal antibody joined by a peptide linker. Murine models have shown that T cells transduced with retroviruses encoding CARs can protect mice from tumor challenges in vivo.[21,22]

Our group has completed a phase I clinical trial in which patients with ovarian carcinoma were treated with T cells that were transduced with a CAR that was specific for the ovarian carcinoma-associated antigen α-folate receptor.[23] No objective tumor regressions were seen.[23] The CAR used in this clinical trial incorporated the Fc receptor-γ cytoplasmic signaling chain without any costimulatory molecules such as CD28 or 4-1BB. More recent work in mice has demonstrated that CARs containing the T cell receptor (TCR)-ζ cytoplasmic signaling chain had superior in vitro function and in vivo antitumor efficacy than CARs containing the Fc receptor-γ cytoplasmic signaling chain.[24] In addition, in vitro studies with human cells and murine in vivo studies have shown that incorporating the signaling domain of CD28 into CARs enhances function and in vivo antitumor efficacy.[22,25–27] Signaling of the 4-1BB costimulatory molecule has been shown to enhance T cell proliferation and persistence,[28,29] and 4-1BB signaling enhanced the function of CARs in vitro.[30,31] Thus, significant advances in CAR design have occurred since our last clinical trial using CAR-transduced T cells.

CD19 is a promising target for antigen-specific T cell

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