Brief report

Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19

James N. Kochenderfer,1 Wyndham H. Wilson,2 John E. Janik,2 Mark E. Dudley,1 Maryalice Stetler-Stevenson,3 Steven A. Feldman,1 Irina Maric,4 Mark Raffeld,3 Debbie-Ann N. Nathan,1 Brock J. Lanier,1 Richard A. Morgan,1 and Steven A. Rosenberg1

Adoptive transfer of genetically modified T cells is an attractive approach for generating antitumor immune responses. We treated a patient with advanced follicular lymphoma by administering a preparative chemotherapy regimen followed by autologous T cells genetically engineered to express a chimeric antigen receptor (CAR) that recognized the B-cell antigen CD19. The patient's lymphoma underwent a dramatic regression, and B-cell precursors were selectively eliminated from the patient's bone marrow after infusion of anti–CD19-CAR-transduced T cells. Blood B cells were absent for at least 39 weeks after anti–CD19-CAR-transduced T-cell infusion despite prompt recovery of other blood cell counts. Consistent with eradication of B-lineage cells, serum immunoglobulins decreased to very low levels after treatment. The prolonged and selective elimination of B-lineage cells could not be attributed to the chemotherapy that the patient received and indicated antigen-specific eradication of B-lineage cells. Adoptive transfer of anti–CD19-CAR-expressing T cells is a promising new approach for treating B-cell malignancies. This study is registered at www.clinicaltrials.gov as #NCT00924326. (Blood. 2010;116(20):4099-4102)

Methods

This clinical trial was approved by the National Cancer Institute Institutional Review Board. Design and construction of the mouse stem cell virus-based splice-gag retroviral vector MSGV-FMC63-28Z encoding the anti-CD19 CAR used in our clinical trial have been described (GenBank HM852952).1 The anti-CD19 CAR contains an antigen-recognition moiety consisting of the variable regions of the FMC63 monoclonal antibody joined to part of the CD28 molecule and the signaling domains of the CD3ζ molecule.

Peripheral blood mononuclear cells were transduced with retroviruses encoding the anti-CD19 CAR and cultured in an almost identical manner as previously described.20 As measured by flow cytometry, the CAR was expressed on 64% of the infused cells, which were 98% CD3+ T cells (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). The T cells were 66% CD8+ and 34% CD4+. The anti-CD19-CAR-transduced T cells specifically recognized CD19+ target cells (supplemental Table 1). Methods of T-cell preparation, flow cytometry, polymerase chain reaction, and immunohistochemistry are in the supplemental data. For the immunohistochemistry images in Figures 1 and 2, images were obtained via digital microscopy using an Olympus BX51 microscope (Olympus America) equipped with a UPlanFL 10×/0.3 numeric aperture and UPlanFL 40×/0.75 numeric aperture objectives. Images were captured using an Olympus DP70 digital camera system. Imaging software was Adobe Photoshop CS3 (Adobe Systems).

Results and discussion

The patient was diagnosed with grade 1, stage IVB follicular lymphoma in 2002. Before enrollment on our protocol, he had received the following treatments for his lymphoma: PACE (prednisone, doxorubicin, cyclophosphamide, and etoposide), an idiotype vaccine, the anti–CTLA-4 monoclonal antibody ipilimumab, and EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab). The last cycle of EPOCH-R was administered in January 2008. The EPOCH-R caused a partial remission; however, progressive disease was noted in July 2008. The patient received no further treatment before he was evaluated for enrollment on our trial of anti–CD19-CAR-transduced T cells.

When we evaluated the patient in May 2009, he had progressive lymphoma that involved all major lymph node areas (Figure 1A). He had bilateral pleural effusions, night sweats, and a recent weight loss of 10 pounds. Flow cytometry of a fine needle aspirate from an
enlarged cervical lymph node demonstrated a monoclonal B-cell process consistent with follicular lymphoma that uniformly expressed CD19, CD20, CD22, CD10, and IgM-kappa. Flow cytometry showed that 14.5% of the blood lymphoid cells had a phenotype that was consistent with the lymphoma and 0.7% of the blood lymphoid cells were normal polyclonal B cells (data not shown). Before treatment, 35% of bone marrow lymphoid cells expressed CD19 (Figure 1B). A total of 55% of these CD19+ cells were monoclonal \( \kappa \)-positive and \( \lambda \)-negative lymphoma cells; 45% of the bone marrow CD19+ cells were normal surface-immunoglobulin (Ig)-negative immature B-cell precursors (Figure 1C). The immature B-cell precursors demonstrated a pattern of antigen expression consistent with normal maturation, namely, CD22+ B cells with decreasing CD10 expression correlating with increasing CD20 expression (Figure 1D-E).\(^{21,22}\) Large numbers of bone marrow CD19+ cells and CD79a+ cells were detected by immunohistochemistry before treatment (Figures 1G, 2A).

The patient underwent apheresis, and peripheral blood mononuclear cells were used to prepare anti–CD19-CAR-transduced T cells. The patient received a lymphocyte-depleting regimen consisting of 60 mg/kg cyclophosphamide daily for 2 days followed by 5 daily doses of 25 mg/m\(^2\) fludarabine. The day after the last fludarabine dose, the patient received \( 1 \times 10^8 \) anti–CD19-CAR-transduced T cells intravenously. The next day, he received \( 3 \times 10^8 \) anti–CD19-CAR-transduced T cells intravenously. After the second anti–CD19-CAR-transduced T-cell infusion, the patient received 720,000 IU/kg interleukin-2 (IL-2) intravenously every 8 hours. Eight doses of IL-2 were administered. The only acute toxicities that the patient experienced were cytopenias that were attributable to chemotherapy and a fever that lasted 2 days (maximum temperature, 38.5°C). The patient was discharged 11 days after his second anti–CD19-CAR-transduced T-cell infusion, and he resumed full-time employment.

After therapy, computed tomography scans revealed an impressive partial remission of the lymphoma that lasted 32 weeks (Figure 1A); 32 weeks after treatment, progressive CD19+ lymphoma was noted in right cervical and retroperitoneal lymph nodes.

Blood B cells were absent from 9 weeks after anti–CD19-CAR-transduced T-cell infusion until at least 39 weeks after anti–CD19-CAR-transduced T-cell infusion (Figure 2C; supplemental Figure 2). This prolonged B-cell depletion cannot be attributed to the chemotherapy that the patient received. Neither the New York esophageal squamous cell carcinoma antigen-1 (NY-ESO) nor the melanoma antigen gp100 is expressed by B cells.\(^{23,24}\) In prior clinical trials, patients treated with the
same chemotherapy and IL-2 regimen as the patient described in this report along with T cells retrovirally transduced with receptors that recognized either NY-ESO or gp100 did not experience prolonged B-cell depletion (Figure 2D).

Except for B cells and a mild thrombocytopenia, all blood cell counts, including neutrophils, erythrocytes, T cells, and NK cells, of the patient treated with anti–CD19-CAR-transduced T cells recovered to normal levels by 9 weeks after treatment (Figure 2E-F).

Thirty-six weeks after anti–CD19-CAR-transduced T cells were infused, CD19+ cells were absent from the bone marrow as measured by flow cytometry (Figure 1F) and immunohistochemistry (Figure 1H-I). CD79a+ cells were undetectable in the bone marrow by immunohistochemistry 14 weeks after treatment (data not shown). CD79a+ cells were detected at greatly below normal frequency 36 weeks after anti–CD19-CAR-transduced T-cell infusion (Figure 2B). CD79a is expressed earlier in B-cell development than CD19, so the presence of a small number of CD79a+ cells while CD19+ cells were absent suggests early recovery of B-lineage cells.

A decrease in serum IgG levels occurred after treatment (Figure 2G). Serum IgM was undetectable from 9 to at least 39 weeks after treatment. Serum IgA was 66.8 mg/dL before treatment. Serum IgA decreased to below the detectable limit of 10 mg/dL after treatment (supplemental Figure 3). Five months after treatment, the patient developed pneumonia of unknown etiology that required hospitalization. After a course of antibiotics, the patient recovered completely. The patient has subsequently received intravenous Ig replacement, and he has not had further infections.

The anti-CD19 CAR transgene was detected in peripheral blood mononuclear cells from one to 27 weeks after anti-CD19-CAR-transduced T-cell infusion with a quantitative real-time polymerase chain reaction assay (Figure 2H).

This is the first patient treated on our trial and the only patient with long enough follow-up to evaluate B-cell depletion. The prolonged elimination of CD19+ cells in this patient indicates in vivo antigen-specific activity of anti–CD19-CAR-expressing T cells. Our findings should encourage continued study of anti–CD19-CAR-transduced T cells.

Acknowledgments

The authors thank Margaret Brown for flow cytometry, Manuel Van Deventer for Ig assays, Laura Devillier for T-cell preparation, and Hui Xu, Mary Black, and Zhili Zheng for technical assistance.
This work was supported by the Center for Cancer Research, National Cancer Institute, National Institutes of Health (intramural funding).

Authorship

Contribution: J.N.K. designed the protocol, provided patient care, conducted experiments, analyzed data, and wrote the paper; W.H.W., J.E.J., D.-A.N.N., and B.J.L. provided patient care, assisted protocol design, and edited the paper; S.A.F. and R.A.M. provided reagents and interpreted data; M.E.D. conducted experiments and edited the paper; M.S.-S., I.M., and M.R. conducted experiments, interpreted data, and edited the paper; and S.A.R. designed the protocol, interpreted data, and edited the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: James N. Kochenderfer, National Institutes of Health, 10 Center Dr, CRC Rm 3-3888, Bethesda, MD 20892; e-mail: kochendj@mail.nih.gov.

References

Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19


Updated information and services can be found at:
http://www.bloodjournal.org/content/116/20/4099.full.html

Articles on similar topics can be found in the following Blood collections
- Brief Reports (1949 articles)
- Clinical Trials and Observations (4609 articles)
- Gene Therapy (585 articles)
- Immunobiology and Immunotherapy (5507 articles)
- Lymphoid Neoplasia (2618 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml