CAR T cells Targeting Human Immunoglobulin Light Chains Eradicate Mature B-cell Malignancies While Sparing a Subset of Normal B Cells

Raghuveer Ranganathan1, Peishun Shou1, Sarah Ahn1, Chuang Sun1, John West1, Barbara Savoldo1,2, and Gianpietro Dotti1,3

ABSTRACT

**Purpose:** CD19-directed chimeric antigen receptor (CAR.CD19) T cells promote clinical responses in patients with relapsed/refractory B-cell non-Hodgkin lymphomas and chronic lymphocytic leukemia (CLL). However, patients showing sustained clinical responses after CAR.CD19-T treatment show increased infection risk due to compromised B-lymphocyte recovery. Mature B cell–derived malignancies express monoclonal immunoglobulins bearing either κ- or λ-light chains. We initially constructed CAR-T targeting the κ-light-chain (CAR.κ) and established a clinical study with it. After optimizing the CAR molecule, cells developed CAR-T targeting the λ-light chain (CAR.λ) and we explored their antitumor activity.

**Experimental Design:** Using Igλ+ lymphoma cell lines and patient-derived Igλ+ CLL cells, we evaluated the in vitro tumor cytotoxicity and cytokine profiles of CAR.λ. We also assessed the in vivo efficacy of CAR.λ in xenograft Igλ+ lymphoma models including a patient-derived xenograft (PDX) of mantle cell lymphoma, and the effects of λ- or κ-light chain–specific CAR-T on normal B lymphocytes in a humanized murine model.

**Results:** CAR.λ demonstrated antitumor effects against Igλ+ lymphoma cells and patient-derived CLL cells in vitro, and in vivo in xenograft and PDX Igλ+ lymphoma murine models. Antitumor activity of CAR.λ was superimposable to CAR.CD19. Furthermore, we demonstrated in the humanized murine model that λ- or κ-light chain–specific CAR-T cells only depleted the corresponding targeted light chain–expressing normal B cells, while sparing the reciprocal light chain carrying B cells.

**Conclusions:** Adoptive transfer of CAR.λ and CAR.κ-T cells represents a useful and alternative modality to CAR.CD19-T cells in treating mature B-cell malignancies with minimal impact on humoral immunity.

See related commentary by Jain and Locke, p. 5736

Introduction

Chimeric antigen receptors (CAR) are synthetic molecules coupling highly specific antigen-binding properties of mAbs to the T-cell signaling and costimulatory domains, leading to lytic and proliferative capacities of CAR-T cells. Infusion of CAR-T cells targeting CD19 (CAR.CD19) has shown great potential in the treatment of B cell–derived non-Hodgkin lymphomas (B-NHL) and chronic lymphocytic leukemia (CLL; refs. 1–5). Notably, a significant number of patients who received CAR.CD19-T cells exhibited durable complete responses, with subanalyses showing benefit in both older and younger patients, leading to the conclusion that CAR.CD19-T cells could be a very valuable treatment in patients with refractory and/or heavily pretreated lymphomas (3, 4).

Effective long-term disease control achieved with CAR.CD19-T cells is, however, frequently associated with B-cell depletion and resultant hypogammaglobulinemia (6). While administration of polyclonal immunoglobulins (Ig) can ameliorate the side effect of hypogammaglobulinemia, this treatment is expensive. In addition, prolonged B-cell aplasia, combined with progressive T-cell dysfunction caused by previous chemotherapies, can predispose patients to life-threatening opportunistic infections. Recent analyses of patients receiving CAR.CD19-T cells show a significant number of patients developing hypogammaglobulinemia lasting many months with an elevated risk of sinopulmonary infections requiring inpatient care in up to 20% of patients (7, 8). In addition, disease relapse due to the loss of the targeted CD19 epitope in patients receiving CAR.CD19-T cells has also been reported (9, 10), urging for the identification of additional targets for CAR-T cells in B-cell malignancies.

Mature B lymphocytes express monoclonal Igs that contain either the κ- or λ-light chains but not both simultaneously, and κ- or λ-light chain expression remains clonally restricted in malignant B cells in patients with B-NHL and CLL. Thus, CAR-T cells targeting the clonally restricted light chain expressed by the lymphoma cells should spare normal B cells expressing the reciprocal light chain, thereby lessening the negative impact on a patient’s humoral immunity. Multiple studies using flow cytometry demonstrated 92%–98% positive expression of surface Igs with light chain clonality across all subtypes of B-NHL (11–13). The most commonly associated B-NHL subtype with λ-light chain expression is mantle cell lymphoma (MCL), which has a λ:κ expression ratio of approximately 2:1 (14–16). With more than one-third of all B-NHL, and greater than two-thirds of MCL, expressing the λ-light chain monoclonally, a λ-light chain–targeting CAR-T cell approach would serve a major and critical

1Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 2Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina. 3Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://cincancersres.aacrjournals.org/).

R. Ranganathan and P. Shou contributed equally to this article.

Corresponding Authors: Raghuveer Ranganathan, Norris Comprehensive Cancer Center, University of Southern California, Jane Anne Nohl Division of Hematology and Center for the Study of Blood Diseases, Los Angeles, CA 90033. Phone: 323-442-8077; E-mail: raghuveer.ranganathan@med.usc.edu; and Gianpietro Dotti, gdotti@med.usc.edu

Clin Cancer Res 2021;27:5951-60

doi: 10.1158/1078-0432.CCR-20-2754
Translational Relevance

Adoptive transfer of chimeric antigen receptor T lymphocytes (CAR-T) directed at the CD19 antigen is a proven therapeutic option for relapsed/refractory B-cell non-Hodgkin Lymphoma (B-NHL). Targeting this antigen, however, does not distinguish between normal and malignant B cells and causes profound B-cell aplasia and agammaglobulinemia with increased infection risk since CD19.CAR-T can persist long term. Many B-NHL subtypes express surface immunoglobulin that is clonally restricted to either the kappa (Igk) or lambda (Igl) light chains. This research demonstrates targeting either Igk or Igl, individually with CAR-T can eradicate malignant B cells expressing the targeted light chain as effectively as CD19.CAR-T, but also spare B lymphocytes expressing the reciprocal light chain, which could consequently reduce the impairment of humoral immunity in CAR-T recipients and potentially improve upon the existing CAR-T approaches for B-NHL.

Materials and Methods

Cell lines and cell culture tumor cells

BV173 was obtained from the German Cell Culture Collection (DSMZ), and Daudi and Maver-1 were obtained from the ATCC. SP53 was kindly provided by Amin Hesham (M.D. Anderson Cancer Center, Houston, TX). All cells were maintained in culture with RPMI1640 medium (Gibco-BRL) containing 10% heat-inactivated FCS, 2 mmol/L glutamine, 100 IU/mL penicillin, and 100 mg/mL streptomycin (all from BioWhittaker). Cells were maintained in a humidified atmosphere containing 5% CO2 at 37°C.

Human samples of B-CLL

Samples were obtained from peripheral blood of treatment-naive patients with B-CLL through the Tissue Processing Facility at University of North Carolina at Chapel Hill (UNC, Chapel Hill, NC). T lymphocytes were isolated from these samples using CD3 microbead antibody (Miltenyi Biotec). CLL-derived T lymphocytes were then activated and genetically modified to generate autologous CAR-T cells (see “Transduction and Expansion of Human T cells”), while tumor cells were stored to be subsequently used as a target in coculture experiments (see “Coculture assays”). The protocol for collection of peripheral blood from patients with B-CLL was approved by the Institutional Review Board and ethic committee at UNC (Chapel Hill, NC).

Cloning of the single-chain antibody and generation of CARs

We cloned VH and VL variable regions of the antibody targeting the λ-light chain of human immunoglobulins from the murine hybridoma HP6054 (ATCC) and generated a scFv. The scFv sequence was cloned in frame with the human CD86 hinge and transmembrane domain and with the CD28 costimulatory endodomain and the intracytoplasmic CD3ζ chain of the TCR/CD3 complex in the SFG retroviral backbone, as established previously (19). An additional retroviral vector was constructed to label tumor cells, which encoded the firefly luciferase gene (FFLuc). This vector was used for stable transduction of tumor cell lines (17). After transduction, cells were selected in puromycin (Sigma), grown in culture, and used to inoculate SCID mice for in vivo experiments. Retroviral supernatants were prepared as described previously (17, 20). Briefly, 293T cells were transfected with three plasmids (retroviral transfer vector, Peg-Pam encoding gag-pol, and RFP encoding the RDR114 envelope), using Genejuice transfection reagent (Novagen). Supernatants were collected at 48 and 72 hours for transduction of T cells.

Transduction and expansion of human T cells

Buffy coats from healthy donors were obtained through the Gulf Coast Regional Blood Center, Houston, TX. Peripheral blood mononuclear cells (PBMC) were isolated with Lymphoprep density separation (Fresenius Kabi Norge) andactivated with 1 μg/mL α-CD3 (Miltenyi Biotec) and 1 μg/mL α-CD28 (BD Biosciences) mAb–coated plates. Forty-eight hours later, T lymphocytes were transduced with retroviral supernatants using retroenectin-coated plates (Takara Bio), and expanded in complete medium [45% RPMI1640 and 45% Click’s medium (Irvin Scientific), 10% FBS (Hyclone), 2 mmol/L Glutamax, 100 IU/mL of penicillin and 100 μg/mL of streptomycin] with IL7 (10 ng/mL; PeproTech) and IL15 (5 ng/mL; PeproTech). Seven to 12 days later, cells were collected for in vitro or in vivo experiments, as described previously (18, 20).

Immunophenotyping

Phycoerythrin-conjugated, FITC-conjugated, allophycocyanin (APC)-conjugated, brilliant violet 510- and 650-conjugated, allophycocyanin-cyanine7 tandem (APC-Cy7)-conjugated, and peridinin chlorophyll protein (PerCP)-conjugated CD3, CD4, CD8, and CD45 mAbs were used to stain T lymphocytes, whereas CD19, CD20, anti-Igk, and anti-Igl antibodies were used to stain tumor cells. All antibodies were from Becton Dickinson. To detect the expression of CARs, T lymphocytes were stained with an mAb conjugated with the Alexa Fluor 647 fluorophore provided by Jackson ImmunoResearch recognizing the antigen-binding fragment component of mouse IgG that is part of the CAR construct. Cells were analyzed by FACS FACScan (Becton Dickinson). A flow cytometry assay was also used to measure the proliferative capacity of CAR-T cells against various tumor cell lines. Briefly, 2 × 107 T lymphocytes were incubated for 10 minutes at room temperature with 1.5 μmol/L carboxyfluorescin diacetate succinimidyl ester (CFSE; Molecular Probes Europe). These cells were cocultured either alone or with tumor cell lines (Daudi or SP53) at a 10:1 effector-to-target (E:T) ratio for 4 to 5 days at 37°C in 5% CO2. After incubation, CFSE-labeled cells were counted by FACScan gating on viable T cells.

Coculture assays

CD19+ BV173, CD19+ Igc+ Daudi, and either CD19+ Igl+ SP53 or Maver-1 tumor cells were seeded into 24-well plates at 5 × 105 cells/well and nontransduced (NTD) or CAR-T cells were added at different E:T ratios (E:T = 1:5 or 1:10). After 24 hours, supernatants were collected for IFNγ and IL2 measurement. Five days later, cells were collected and stained with CD3 and CD19 antibodies for flow cytometry analysis (FACS Canto; Becton Dickinson). For assessing CAR-T
cell cytotoxicity against primary human tumors, human B-CLL samples were obtained. NTD or irrelevant CAR-T cells serving as negative control and CAR.A+T cells derived from the patients’ PBMCs were plated and cultured with the B-CLL cells in an autologous manner at an effector to tumor ratio of 1:10. Supernatants were collected at 24 hours for cytokine measurement. After 48 hours, all cells were collected, stained with CD3 and CD19 antibodies, and then analyzed by flow cytometry (FACsCanto; Becton Dickinson).

Cytokine measurements
Cytokines in culture supernatants and plasma were measured using ELISA following manufacturer’s instructions (R&D Systems). Data were collected and analyzed using the Lumina-200 System and the BioPlex Manager 6.1 software (Bio-Rad).

In vivo studies in a xenograft NSG murine model

CAR.A+T cells were tested in NSG (NOD-scid IL2Rgnull) mice injected intravenously with 2 × 10⁶ CD19+Igκ+ Daudi tumor cell line labeled with the Firefly luciferase gene (Daudi-FFLuc). Four days later, mice received control T cells or CAR.CD19 or CAR.A+T cells. CAR.A+T cells were tested in NSG mice injected intravenously with 2 × 10⁶ CD19+Igκ+ Mäver-1 tumor cell line labeled with the Firefly luciferase gene (Mäver-FFLuc). Four days later, mice received control T cells or CAR.CD19, CAR.A+T cells. Tumor growth was monitored weekly by injecting mice intraperitoneally with α-luciferin (150 mg/kg, Xenolight, PerkinElmer). Photon emission was then analyzed within 20 minutes of α-luciferin injection, using the Xenogen-IVIS Imaging System as validated previously (17, 19).

Patient-derived xenograft murine model
A patient-derived, Igκ+ mantle cell lymphoma (MCL) cell line was purchased from a public repository (Dana-Farber Cancer Institute, Boston, MA) to create a patient-derived xenograft (PDX) murine model. NSG mice were injected in the flank with 3 × 10⁶ tumor cells mixed with 100 μL of Matrigel matrix (Corning). After tumor engraftment defined as 250 mm² on size measurement with calipers, mice were treated with either NTD, CAR.CD19, or CAR.A+T cells intravenously. Tumor sizes were measured weekly for up to 60 days, or until the length of tumor reached 15 mm at which time mice were euthanized.

In vivo studies in a humanized murine model

To assess the effect of CAR-T cells on normal human B lymphocytes in vivo, NSG mice were first irradiated at 2.8 Gy to ablate their native bone marrow (19). Human umbilical cord blood-derived CD3⁴ hematoPoietic stem cells were injected intravenously into the mice 2–3 hours after irradiation. Human cell engraftment, and specifically B-lymphocyte reconstitution, was then measured through serial blood measurements over 8–10 weeks. Once cell engraftment was achieved, B and T lymphocytes were quantified by flow cytometry. Mice with T-cell engraftment or no B-cell engraftment was sacrificed. The remaining mice were infused intravenously with either NTD T cells or CAR.CD19, CAR.A+T cells. Mice were bled 7 days after CAR-T cell infusion to measure CD19+Igκ+ and CD19+Igκ+ B cells through flow cytometry.

Statistical analysis
Data were presented with their means unless indicated otherwise. Statistical analyses were performed using GraphPad Prism software. Two-tailed unpaired t test, one-way ANOVA, two-way ANOVA, Kruskal–Wallis multiple comparisons test, and log-rank Mantel–Cox tests were used. Bonferroni’s correction for multiple comparisons was used to calculate adjusted P values when appropriate. The exact P values were shown in the figures and/or their legends; ns, not significant. The specific statistical test used for each figure was described in the corresponding figure legend.

Results

CAR.A+T cells selectively eliminate human Igκ+ lymphoma tumor cell lines and Igκ+ primary CLL cells

We have previously described a κ-light chain–targeting CAR construct (CAR.κ) in which we used a long spacer region (CH2–CH3 region and hinge of IgG1) and the transmembrane and signaling domains of CD28 along with the CD3ζ chain (17, 18). On the basis of emerging data demonstrating the impact of the length and type of hinge and transmembrane domains on CAR functionality (21, 22), we investigated whether alternative CAR formats may affect the antitumor activity of the CAR.κ+T cells. In particular, we modified the spacer and transmembrane domains by using the stalk (hinge and transmembrane domain) of the human CD8z (Supplementary Fig. S1A), which is used in the CAR.CD19 construct encoding the 4-1BB endodomain recently approved by the FDA for the treatment of B-cell malignancies, and fused it with the endodomains containing the CD28 signaling domains and the CD3ζ chain (19, 23, 24). The new design of the CAR.κ displayed improved in vivo antitumor activity, which was superimposable to that of CAR.CD19+T cells (Supplementary Fig. S1B–S1F). On the basis of this new evidence, we constructed the CARs targeting the human immunoglobulin κ-light chain (CAR.κ) maintaining the same vector design. T lymphocytes obtained from five healthy donors were transduced using retroviral vectors expressing the CAR.CD19, CAR.κ or CAR.λ, while NTD T cells were used as a negative control. As shown in Fig. 1A and B, all CARs were equally expressed in T cells. CAR-T cells and NTD cells were then cocultured with CD19+ B cell–derived tumor lines expressing either κ- or λ-light chains or no light chain expression (Fig. 1C). Both CAR.λ and CAR.κ+T cells specifically recognized their targeted tumor cell lines Mäver-1/SP53 and Daudi, respectively, while sparing the reciprocal light chain–expressing tumor cells. NTD cells did not display any antitumor activity, while CAR.CD19+T cells eliminated all three cell lines indiscriminately (Fig. 1D and E). IFNY and IL2 detected in the supernatants collected from the coculture assays correlated with the observed antitumor effects (Fig. 1F). These results were reproducible at E:T ratios of 1:5 and 1:10. We also analyzed CAR-T cell proliferative capacity in response to their specific targets using a CFSE staining assay. CAR.λ+T cells showed significant proliferation when plated with Mäver-1 cells, while conversely CAR.κ+T cells exhibited much less proliferation when exposed to the same tumor target, further demonstrating the selective tumor recognition of the CAR constructs (Fig. 1G).

To assess the antitumor effects of CAR.λ+T cells against primary human tumor samples in an autologous setting, we used samples from treatment-naive patients with B-CLL expressing the λ-light chain. CD3⁴ T lymphocytes were isolated from peripheral blood samples and engineered to express CAR.CD19, CAR.λ, or a CAR targeting an unrelated antigen (CAR.GD2; Fig. 2A; ref. 25). Upon in vitro expansion, CAR-T cells were plated with their respective autologous B-CLL cells expressing different levels of Igκ (Fig. 2B) at an E:T of 1:10 and cocultured for 48 hours. While control T cells did not significantly affect B-CLL cells, both CAR.CD19 and CAR.λ+T cells displayed comparable ability to eliminate leukemic cells in culture (Fig. 2C and D). Supernatants collected at 24 hours from the coculture assays
In collected at day 5 of culture and tumor cells and T cells quantified Daudi, BV173, Maver-1, and SP53. T lymphocytes obtained from healthy donors. NTD indicates nontransduced control T cells. Not significant; one-way ANOVA. CAR.

Illustrated in dilution assay of CAR.

Antitumor activity of CAR.

Exert similar antitumor activities compared with CAR.CD19. CD19.

Demonstrated elevated IFNγ and IL2 cytokine production for both CAR.CD19 and CAR.λ-T cells, while control T cells did not display cytokine production (Fig. 2E). Overall, these data show that CAR.λ-T cells specifically target Igλ-light chain–expressing human tumors and exhibit similar antitumor activities compared with CAR.CD19.

Antitumor activity of CAR.λ-T cells in vivo is equal to CAR.CD19-T cells

We established a xenograft lymphoma model in NSG (NOD-Scid IL2Rnull) mice and used a bioluminescence system to track tumor growth in vivo. T cells expressing CAR.CD19, CAR.κ, or CAR.λ were inoculated intravenously in CD19−Igλ− lymphoma-bearing mice (Fig. 3A). In mice treated with CAR.κ-T cells, the lymphoma grew unchecked and mice needed to be sacrificed after 3 weeks. In contrast, CAR.λ-T cells demonstrated substantial tumor control and showed equivalent efficacy to CAR.CD19-T cells (Fig. 3B and D).

Survival curves also exhibited equivalency between CAR.λ and CAR.CD19-T cells (Fig. 3E; Supplementary Figs. S2 and S3). Mice treated with CAR.λ and CAR.CD19-T cells were bled 30 days after CAR-T cell injection to examine the persistence of the CAR-T cells. Both groups showed presence of CAR-T cells in the peripheral blood (Fig. 3F). When mice treated with CAR.λ-T cells were sacrificed to analyze for the presence of lymphoma at day 80, no tumor cells were detected by flow cytometry analysis in blood, bone marrow, liver, and spleen in mice without bioluminescence signal, while CD19+ Igλ− tumor cells were detected in mice showing bioluminescence signal (Fig. 3C; Supplementary Fig. S4).

To further demonstrate the clinical relevance of the proposed CAR-T cells, we implemented a PDX mouse model of Igλ− MCL to test CAR.λ-T cells in vivo. NSG mice were inoculated in the flank with a CD19−Igλ− MCL.PDX, and tumor engrafted for 2–3 weeks (Fig. 4A and B). After tumor engraftment, mice were injected intravenously...
with either NTD, CAR.CD19, or CAR.λ-T cells and then had their tumors measured prospectively. CAR.CD19 and CAR.λ-T cells demonstrated similar control of tumor growth (Fig. 4C). Survival curves also demonstrated equivalency between the two treatment groups (Fig. 4D). To assess CAR-T cell persistence, mice were bled at day 30 after CAR-T cell injection to check for persistence as well as for presence of tumor. Despite the location of the tumor in the flank, flow cytometry analysis showed the presence of circulating tumor cells in mice treated with NTD cells (prior to euthanization), while mice treated with CAR.CD19 and CAR.λ-T cells showed very low or no detectable circulating tumor cells, with T cells were also equally detectable in the peripheral blood (Fig. 4E). Two mice that had tumor recurrence in the CAR.λ-T cell treatment group, were sacrificed and had their tumors harvested. Flow cytometry analysis demonstrated continued Igλ expression by tumor cells indicating that tumor growth in these mice may not be due to antigen loss (Fig. 4F). These results were reproducible when this PDX murine model was repeated with CAR-T cells from another healthy donor (Supplementary Fig. S5). Overall, these data show that both CAR.λ and CAR.CD19-T cells control MCL PDX in vivo.

**Immunoglobulin light chain–specific CAR-T cells spare normal B lymphocytes expressing the reciprocal light chain in a humanized murine model**

To further characterize and evaluate the selectivity of our CAR constructs, and also to approximate conditions which are closer to the human immune system, we generated a humanized murine model (Fig. 5A). These mice showed engraftment of human CD19⁺ B
lymphocytes that expressed either the Igκ or Igλ, recapitulating the repertoire of human B cells (Fig. 5B). Engrafted mice were then injected intravenously with either NTD, CAR.CD19, CAR.k, or CAR.λ-T cells. The levels of the CD19+ Igκ+ and CD19+ Igλ+ B cells were measured in the peripheral blood 7 days after CAR-T cell infusion (Fig. 5C). Mice infused with control NTD cells did not show any modification in B-cell subset composition, while mice treated with CAR.CD19-T cells showed full depletion of B cells. Mice treated with either CAR.k-T cells or CAR.λ-T cells demonstrated a significant reduction only of the appropriately targeted CD19+ Igκ+ or CD19+ Igλ+ B cells, respectively, while sparing CD19+ Igλ+ or CD19+ Igκ+ B cells, respectively. Overall, these data reinforce the target selectivity on normal B lymphocytes of the proposed immunoglobulin light chain–specific CAR-T cells.

Discussion

Despite clinical efficacy of CAR.CD19-T cells in patients with B-NHL and B-CLL, increased infection risk due to B-cell aplasia and hypogammaglobulinemia remains a concern. Taking advantage of the clonally restricted expression of κ- and λ-light chains of human immunoglobulins on normal and malignant B cells, our proposed approach to target selectively the immunoglobulin light chains will spare a compartment of normal B cells while targeting tumor cells. This approach was initially translated into a phase I clinical study that demonstrated feasibility, safety, and antitumor activity in patients with B-cell malignancies infused with autologous CAR.k-T cells (18). In this clinical trial, patients were infused with CAR.k-T cells without prior lymphodepleting chemotherapy, and clinical activity was observed without interference of soluble immunoglobulins as

Figure 3.
CAR.λ and CAR.CD19-T cells show equivalent antitumor activity against Igλ+ lymphoma in vivo. A, Schema of the lymphoma xenograft model. NSG mice bearing firefly luciferase (FFLuc) labeled Igλ+ Maver-1 tumor cells were treated with CAR.k (negative control), CAR.CD19 (positive control) or CAR.λ-T cells. B, Representative IVIS bioluminescence imaging (BLI) showing tumor growth of different groups. C, Mice treated with CAR.λ-T cells were sacrificed at day 75 and analyzed for presence of tumor. In mice with no detectable BLI signal, flow cytometry confirmed the lack of tumor cells in bone marrow, spleen, and liver. In contrast, in mice with BLI signal, flow cytometry confirmed the presence of CD19+ tumor cells that retained Igλ expression. Flow plots shown here are of bone marrow samples. D, Graph summary of tumor BLI kinetics of all treated mice described in B (n = 5). E, Kaplan–Meier survival curves of treated mice described in B. *** P < 0.001 (log-rank Mantel–Cox test) when comparing CAR.λ with CAR.k-T cells (n = 5). F, Representative flow cytometry plots (left) and quantification (right) of CAR-T cells detected in the peripheral blood of the mice treated as described in B at day 30.
CARλ and CAR.CD19-T cells demonstrate equivalent antitumor effects in a PDX MCL model. A, Schema of PDX model in which MCL cells derived from a patient were injected in the flank of NSG mice. After tumor engraftment (tumor size of 250 mm³), mice were injected intravenously with either NTD, CAR.CD19, or CARλ-T cells. B, Flow cytometry plots showing CD19 and Igλ expression in PDX cells. C, Kinetics of tumor growth in all treated mice described in A. D, Kaplan-Meier survival curves of mice treated as described in A. E, Log-rank Mantel-Cox test (p = 0.001). F, Representative flow cytometry plots showing the expression of CD19 and Igλ in PDX cells that recurred in two mice treated with CARλ-T cells showing no loss of the Igλ.

The anticipated the proposed strategy by generating CARλ-T cells. On the basis of our previous preclinical and clinical experience (17, 18), we first refined the structure of the backbone of the CAR cassette by modifications of the hinge and transmembrane regions and by adding these modifications we achieved increased antitumor activity in vivo. This novel design of the CARκ is currently under clinical investigation in B-cell lymphoid malignancies in which CARκ-T cells are infused in lymphodepleted patients (ClinicalTrials.gov Identifier: NCT04223765). Using the same CAR design, CARλ-T cells demonstrated effective in vitro cytotoxicity toward Igλ⁺ lymphoma/leukemia cells, while sparing the Igλ⁻ expressing cells. In our xenograft murine model, CARλ-T cells displayed equivalent antitumor activity as CAR.CD19-T cells without showing escape of Igλ⁺ tumor cells, though this finding does not preclude the possibility of Igλ antigen loss from ever occurring. This equivalency in tumor eradication compared with CAR.CD19-T cells was also seen in vitro with patient-derived CLL cells and in vivo with a PDX model of MCL. In addition, we implemented a humanized murine model showing reconstituted normal Igκ- and Igκ-restricted normal B cells, and demonstrated the selective B-cell depletion of CARλ and CARκ-T cells, while CAR.CD19-T cells depleted all normal B cells.

It has been observed that about two-thirds of patients receiving CAR.CD19-T cells develop hypogammaglobulinemia. In one long-term study with a median follow-up of 28 months, 86 patients showed persistent hypogammaglobulinemia (IgG < 400 mg/dL) and infectious events. Starting 90 days after CAR.CD19-T cell infusion, 20% and 5% of patients needed inpatient or intensive care for infections, respectively, with the majority of the infections being sinopulmonary (8). A second study showed nearly half of patients receiving CAR.CD19-T cells exhibiting hypogammaglobulinemia...
Alternative targets to CD19 in B-cell malignancies are actively being investigated. The receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed at high levels in MCL and CLL, with analyses implicating higher ROR1 levels with accelerated disease progression and negative survival prognosis in CLL (31, 32). ROR1-targeting CAR-T cells displayed promising antitumor effects in preclinical studies against CLL and MCL (33). However normal B-cell precursors in the bone marrow also express ROR1, as do large areas of the gastrointestinal tract, so ROR1-specific CAR-T cells may not only negatively impact the B-cell compartment, but also could have significant on-target but off-tumor toxicity (34). CD79b is a normal component of the multimeric B-cell receptor complex, and is reliably expressed in many B-cell malignancies (35). Targeting CD79b with antibody-drug conjugates like polatuzumab vedotin is already part of the treatment repertoire in B-NHL, and CAR-T cells against CD79b showed effective tumor control in preclinical models (36). CD20 is also a well-known target in B-NHL and CD20-specific CAR-T cells have been clinically tested (37). However, both CD79b and CD20 share the same issue as targeting CD19 in being uniformly expressed in normal B cells. Our approach targeting the immunoglobulin light chains could lend an advantage in this respect, because our data show significant sparing of nonmalignant B cells expressing the nontargeted light chain. Despite our demonstration of efficacy with our CAR, there are factors which could potentially impact the translational efficacy of any CAR-T approach.
in patients with B-NHL and which cannot be recapitulated fully within preclinical models. In particular, the xenograft tumor models do not recapitulate the complexity of the human diseases. However, our proposed experimental models are similar to those used to validate the antitumor activity of CAR.CD19-T cells which proved to be effective in clinical studies stimulating the clinical translation of preclinical findings.

In summary, our data suggest that a strategy targeting the ι-light chain as well as the κ-light chain of human immunoglobulins with CAR-T cells could represent a valuable and important modality in treating all patients with B-NHL and B-CLL. Because the humoral immune response against antigens is typically polyclonal, the sparing of a subset of reciprocal ι-light chain expressing, nonmalignant B cells could lessen deleterious effects on humoral immune function.

Authors' Disclosures

P. Shou reports a patent for U.S. Patent 63/108,047: “Dual Targeting Chimeric Antigen Receptors” pending and a patent for U.S. Patent 62/928,675: “Methods and Compositions for Chimeric Antigen Receptor Targeting Cancer Cells” pending. B. Savolod reports grants from NCi during the conduct of the study; grants and other from Tessa Therapeutic, grants from Bluebird bio; grants and personal fees from Bellicum Pharmaceuticals; personal fees from Catamaran, Tessa Therapeutic outside the submitted work. No disclosures were reported by the other authors.

Authors’ Contributions

R. Ranganathan: Conceptualization, resources, data curation, software, formal analysis, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. F. Shou: Conceptualization, data curation, formal analysis, visualization, methodology, writing–review and editing. S. Ahn: Resources, data curation, formal analysis, methodology, writing–review and editing. C. Sun: Data curation, methodology. J. West: Methodology. B. Savolod: Conceptualization, resources, supervision, writing–review and editing. G. Dotti: Conceptualization, resources, supervision, writing–review and editing.

Acknowledgments

This work was supported by R01 CA193140 (to G. Dotti), T32 CA211058 (to R. Ranganathan), and Lymphoma Research Foundation Clinical Investigator Career Development Grant 610516 (to R. Ranganathan). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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.

Received July 30, 2020; revised February 8, 2021; accepted April 12, 2021; published first April 15, 2021.

References