Published in final edited form as:

Immunotherapy. 2014 June ; 6(6): 675–678. doi:10.2217/imt.14.44.

Cancer CARtography: Charting out a new approach to cancer immunotherapy

Jaina M. Patel, Gordon A. Dale, Vincent F. Vartabedian, Paulami Dey, and Periasamy Selvaraj*

Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322. USA.

Abstract

Recently, chimeric antigen receptor (CAR) T-cell immunotherapy has entered clinical trials in patients with relapsed or refractory B-ALL. 19–28z CAR T-cells express a fusion protein comprised of an anti-CD19 mAb fused with CD28 costimulatory and CD3-zeta chain signaling domains. The current paper demonstrates that administration of 19–28z CAR T-cells in patients with relapsed or refractory B-ALL in a Phase I clinical trial has led to 88% of patients undergoing complete remission. Despite the benefits, CAR T-cell therapy is associated with cytokine release syndrome (CRS) toxicities. The authors demonstrated criteria to diagnose severe CRS (sCRS) and treated sCRS with either high dose steroids or with tocilizumab, an IL-6 receptor-specific mAb. Although both alleviated sCRS, steroid treatment negated the beneficial effects of CAR T-cell therapy, whereas tocilizumab did not. Taken together, CAR T-cell immunotherapy can be used as a safe and effective approach against tumors with known tumor-associated antigens.

Keywords

Chimeric antigen receptor; CD19; cytokine release syndrome

Adoptive immunotherapies that use a patient's own immune system to produce a response specific to the patient's cancer cells hold much promise. In particular, chimeric antigen receptor (CAR)-modified T cells have recently entered the clinical setting after years of research (1, 2, 3). The CAR is a fusion receptor composed of an extracellular fragment that is either a single chain variable fragment (scFv) or the Fab region of a monoclonal antibody fused to intracellular signaling domains of activating receptors. Upon recognition of the tumor-associated antigen (TAA) by the TAA-specific CAR extracellular region, the intracellular signaling domains initiate T cell activation and effector function against the TAA-expressing cell in a non-HLA-restricted manner (1).

*Address for Correspondence: Periasamy Selvaraj, PhD., Department of Pathology, Emory University School of Medicine, 7309 Woodruff Memorial Building, 101 Woodruff Circle, Atlanta, GA 30322, Tel: +1 404-727-5929, pselvar@emory.edu.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Writing assistance was not utilized in the production of the manuscript.

First-generation CARs consist of an extracellular antibody domain fused to an intracellular activation domain of CD3-zeta chain. These CAR T cells, however, often exhibited limited and unsustainable effector functions (1). Activation through the CD3-zeta chain alone was insufficient in activating resting T cells (4) therefore, second generation CARs arose. Second generation CARs made use of costimulatory signaling domains, such as those from CD28, 4-1BB, OX-40, and DAP10, along with CD3-zeta chain signaling, that augmented and further expanded and sustained effector T cell functions compared to first generation CARs (1, 5, 6).

Patients with B cell acute lymphoblastic leukemia (B-ALL) express the CD19 antigen on malignant and normal B cells, with limited expression on other healthy cells (7). Previously, Brentjens et al. demonstrated the therapeutic efficacy of autologous CD19-specific CAR T cells in a preliminary report of a phase I study examining a cohort of five adult patients with chemotherapy refractory B-ALL (2). Long term remission in relapsed B-ALL is dependent on achieving complete remission (CR) status after a battery of salvage chemotherapeutics. All examined patients had failed initial chemotherapeutic interventions and were subsequently started on salvage chemotherapeutics in an attempt to reach CR before undergoing the gold standard treatment of allogeneic hematopoietic stem cell transplantation (allo-SCT). In summary, all patients treated with autologous CD19-specific CAR T cells achieved CR status. However, in addition to showing therapeutic efficacy, this study also highlighted important clinical questions regarding the side effect profiles of CAR therapy - particularly that of cytokine release syndrome (CRS) and the effects of treating CRS in a patient undergoing concurrent CAR therapy. In this current follow up report by Davila *et al.* (3) these questions have largely been answered along with continued demonstration of CD19-specific CAR T cell treatment regimen efficacy against B-ALL in a cohort of 16 patients.

Summary of methods & results

Davila *et al.* conducted a Phase I study to determine the toxicity effects of administering 19-28z CAR T cells to 16 patients with relapsed or refractory B-ALL. These CAR T cells were developed by retroviral transduction of leukapheresis-obtained T cells with a gene encoding an extracellular anti-CD19 variable region antibody domain fused to intracellular domains of CD28 and CD3-zeta chain. After individually generating these 19-28z CAR T cells from patients' peripheral blood lymphocytes, the CAR T cells were administered back to each of the patients, whom by that time had also undergone salvage chemotherapy. Out of the 16 patients, 88% (14 patients) demonstrated an overall complete response rate, a response rate higher than patients who received only salvage chemotherapy, and 75% of patients reached CR with no evidence of minimal residual disease (MRD). Furthermore, after CAR T cell immunotherapy, 10 patients were eligible for curative allo-SCT treatment.

CAR T cell immunotherapy has been associated with toxicities in some patients that are characterized by fevers, hypoxia, hypotension and also an increase in many serum cytokine levels that are associated with neurologic disturbances. These symptoms are addressed as cytokine release syndrome (CRS). There are different levels of CRS: severe CRS (sCRS), which requires immediate intervention and immunosuppression or nCRS in which patients

do not need intervention and are able to continue immunotherapy. sCRS patients exhibited a higher fever with higher duration than patients with nCRS. In order to differentiate between sCRS and nCRS, Davila *et al.* established criteria to diagnose sCRS. The diagnosis included fevers that persists for three or more consecutive days, a 75-fold elevation in cytokine levels of two of the main seven CRS-associated cytokines (IFN- γ , IL-6, Flt-3L, Fractalkine, IL-5, IL-10, and GM-CSF) or a 250-fold elevation of one of the cytokines, a clinical toxicity (hypotension), hypoxia of a PO₂ of less than 90%, and neurologic changes within the patient. Furthermore, patients with sCRS consistently showed increased levels of C reactive protein (CRP) of 20 mg/dl or more in the serum compared to patients with nCRS, supporting the use of CRP levels as a biomarker for sCRS.

Davila *et al.* additionally explored treatment modalities for CAR T cell therapy induced CRS. The authors showed that patients with sCRS could be treated with either high dose lymphotoxic steroids or with tocilizumab, a monoclonal antibody that blocks the IL-6 receptor. Although both treatments were effective in treating sCRS and reducing fevers in the patients after 1–3 days, the steroid treatment decreased the efficacy of the CAR T cell immunotherapy. Steroid administration reduced the number of CAR T cells that were found in the bone marrow and thus resulted in the patients relapsing. On the other hand, tocilizumab similarly treated sCRS as marked by reduced fevers and decreased CRP levels, but without affecting CAR T cells. Therefore, tocilizumab was suggested as a treatment for sCRS associated with CAR T cell immunotherapy.

After patients relapse from B-ALL, the standard of care is to undergo allo-SCT, the only therapy currently known to potentially cure B-ALL. Out of the 16 patients enrolled, only 3 patients were ineligible for allo-SCT because they did not exhibit CR after CAR T cell therapy. Three more patients were ineligible due to medical conditions. Out of the 10 eligible patients that responded well to CAR T cell immunotherapy, 7 received an allo-SCT and have remained CR without relapsing.

Discussion & significance

Currently, B-ALL patients are treated with salvage chemotherapy until the disease relapses, after which the standard of care is to receive an allo-SCT; however, the frequency of patients that receive allo-SCT after only receiving salvage chemotherapy is merely 5% (8). In order to bridge the gap between patients that receive salvage chemotherapy and then receive an allo-SCT, CAR T cell immunotherapy has been introduced in clinical trials. Davila *et al.* demonstrated that the introduction of 19–28z CAR T cells to B-ALL relapsed or refractory patients that underwent salvage chemotherapy led to 44% of the patients transitioning to receive an allo-SCT. However, CAR T cell immunotherapies are associated with CRS due to activation of the T cells upon receptor targeting. Davila *et al.* demonstrated that CRP levels correlated to sCRS and therefore could be monitored in patients to determine which patients would need further treatment against CRS. They also demonstrated that tocilizumab treats sCRS as effectively as high-dose steroids but without diminishing the effects of 19–28z CAR T cell immunotherapy. Therefore, these results enhance the efficacy of CAR T cell immunotherapy to successfully transition relapsed or refractory B-ALL patients to receive allo-SCTs.

Future perspective

The current paper by Davila *et al.* demonstrated that 19–28z CAR T cells can be used to successfully treat patients with relapsed or refractory B-ALL permitting the patients to undergo the standard of care allo-SCT therapy. The authors were able to discriminate between severe and non-severe toxicities associated with CAR T cell administration by identifying a biomarker for sCRS and were able to treat sCRS without affecting the efficacy of the CAR T cell immunotherapy. The results obtained from this Phase I clinical trial warrant the use of 19–28z CAR T cells in an expanded Phase II clinical trial. Although Phase I clinical trials are used to test the safety and maximal tolerated dose (MTD), the MTD may not be as applicable (1). These second generation CAR T cells were found to expand and contract in the patient by correlating to the presence of CD19+ cells. Also, if a patient relapses after CAR T cell immunotherapy, the same CAR T cell immunotherapy can in theory be readministered without harming the patient.

Emerging research also suggests that modified immunosuppressive molecules, such as PD-1, can be coupled with CAR T cell therapy - leading to enhanced anti-tumor efficacy of CAR T cell immunotherapy (9) while limiting off target effects. Many of the tumor-associated antigens (TAAs), such as CD19 in B-ALL, are also found on normal healthy cells although they may be upregulated on tumor cells. In an attempt to control this off-target autoimmunity, inhibitory CAR (iCAR) T cells have been generated (10). Fedorov et al. have created an antigen-specific receptor coupled with an inhibitory signaling domain from CTLA-4 or PD-1 that can suppress the signaling of an activated CAR. Thus, upon activation of the iCAR, the T cell remains unresponsive against the target cell even if a CAR on the same T cell is stimulated. Therefore, T cells transfected with both iCARs and CARs are able to preferentially target malignant cells while greatly reducing harm to off-target normal cells that share similar antigens as the malignant cells.

Though the Phase I study examined by Davila et al. is extremely promising, CAR T cell therapy still needs to pursue and emerge from subsequent phase II and III clinical trials. Should the results obtained by Brentjens et al. (2) and Davila *et al.* be replicated in each of these, CAR T cell therapy will likely emerge as a new lifesaving treatment option among B-ALL patients.

Acknowledgments

This work was supported by NIH grants R01 CA138993 (PS), and F31 CA165632 (JMP).

References

1. Davila ML, Brentjens R, Wang X, Riviere I, Sadelain M. How do CARs work?: Early insights from recent clinical studies targeting CD19. *Oncoimmunology*. 2012; 1(9):1577–1583. [PubMed: 23264903]
2. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Science translational medicine*. 2013; 5(177):177ra138.

3. Davila ML, Riviere I, Wang X, et al. Efficacy and Toxicity Management of 19–28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. *Science translational medicine*. 2014; 6(224): 224ra225.
4. Brocker T, Karjalainen K. Signals through T cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes. *J. Exp. Med.* 1995; 181(5):1653–1659. [PubMed: 7722445]
5. Gong MC, Latouche JB, Krause A, Heston WD, Bander NH, Sadelain M. Cancer patient T cells genetically targeted to prostate-specific membrane antigen specifically lyse prostate cancer cells and release cytokines in response to prostate-specific membrane antigen. *Neoplasia*. 1999; 1(2):123–127. [PubMed: 10933046]
6. Savoldo B, Ramos CA, Liu E, et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *The Journal of clinical investigation*. 2011; 121(5):1822–1826. [PubMed: 21540550]
7. Kochenderfer JN, Feldman SA, Zhao Y, et al. Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. *J. Immunother.* 2009; 32(7):689–702. [PubMed: 19561539]
8. Thomas DA, Kantarjian H, Smith TL, et al. Primary refractory and relapsed adult acute lymphoblastic leukemia: characteristics, treatment results, and prognosis with salvage therapy. *Cancer*. 1999; 86(7):1216–1230. [PubMed: 10506707]
9. John LB, Kershaw MH, Darcy PK. Blockade of PD-1 immunosuppression boosts CAR T-cell therapy. *Oncoimmunology*. 2013; 2(10):e26286. [PubMed: 24353912]
10. Fedorov VD, Themeli M, Sadelain M. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. *Sci. translational medicine*. 2013; 5(215):215ra172.

Executive summary

- Davila *et al.* report the promising 88% complete response rate of 19–28z CAR T cell immunotherapy in B-ALL patients as well as characterizing cytokine release syndrome (CRS), the therapy's main side effect.
- CRP-monitoring was suggested as a method of determining severe CRS from normal CRS, and anti-IL-6 receptor monoclonal antibody, tocilizumab, was suggested to treat severe CRS, as it alleviates symptoms without compromising efficacy.
- These results suggest that CAR T cell therapy represents an autologous, targeted immunotherapy with manageable side effects.