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Advances in T-cell therapy for ALL

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Abstract

CD19-directed chimeric antigen receptor T cells (CART19 or CTL019) have been used with success in pediatric and adult acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL) patients. While this therapy has caused toxicities, including cytokine release syndrome and macrophage activation syndrome, these conditions are reversible with IL-6 blockade using the monoclonal antibody tocilizumab. Furthermore, 90% of the very high-risk patients who underwent infusion with CTL019 achieved a complete response, despite the fact that they previously failed multiple therapies and/or transplant. With improved cell persistence, this immunotherapy may one day prove to be more than a bridge to transplant and may in fact be a transplant alternative.

Keywords

acute lymphocytic leukemia; ALL; chronic lymphocytic leukemia; CLL; cytokine release syndrome; macrophage activation syndrome; chimeric antigen receptor; IL-6

Introduction

Outcome for patients in second or later relapse of acute lymphocytic leukemia (ALL) is dismal. It is common to draw the conclusion that ALL is a solved problem in pediatric oncology because 85% or more of pediatric ALL patients do very well. However, leukemia is still the most common cause of pediatric cancer mortality, and adult patients do not achieve the cure rates that pediatric patients do. Furthermore, as outcomes have improved with initial treatment, results for those who do not respond to first-line treatment are getting worse. Patients who relapse are harder to get back into remission, harder to get to transplant, and much harder to cure. Consequently, novel therapies are absolutely still needed in ALL for adults and for those pediatric patients who relapse. In the future, as genomic characterization of ALL and identification of high-risk genetic lesions becomes and

established part of clinical practice, these patients may also be candidates for advanced therapies.

There are a variety of roadblocks to successful cellular immunotherapy for cancer (Table 1). First is the need to target the T cells to recognize and attack the cancer cell. The notion of engineering T cells to attack cancer has existed for over 20 years, with Eschar suggesting the “T body” approach of an artificial T cell receptor [1,2] that has evolved into the chimeric antigen receptor (CAR) of today [3,4]. However, it has taken time and work by many groups before these ideas could be translated into dramatic responses against CD19-positive leukemia and lymphoma.

The second problem is the ability to expand cells *ex vivo* at the appropriate number for clinical use. Engineered cells can be grown to large numbers under good manufacturing practice (GMP) conditions compatible with clinical use. However, the key is what happens after they are infused into the patient: for optimal clinical responses, engineered cells have to be able to proliferate in an antigen-driven fashion, expand significantly, and ideally persist, providing long-term immunosurveillance. This has not happened in many of the clinical trials testing gene-modified T cells. Ideally, these T cells will provide a key function of normal T cells: persist and seek antigen, which constitutes immunological memory. Excitingly, a number of groups are now getting a handle on what is required for successful cellular immunotherapy for cancer, with improvements evident in each of these key areas [5–9].

Chimeric antigen receptor (CAR) modified T cells

One strategy is to genetically modify T cells to express an antigen recognition domain of a specific antibody, such as one recognizing the B cell antigen CD19, allowing T cells to seek out a CD19-positive tumor. But CD19-positive diseases do not all respond alike. For example, chronic lymphocytic leukemia is different from ALL, which may or may not be different from some non-Hodgkin's lymphomas. The targeting portion of a CAR molecule is generally a single chain variable fragment (scFv). In principle, an scFv can be made from any monoclonal antibody with a desired specificity, and from this scFv sequence a CAR with identical specificity can be created. However CARs cannot differentiate between a normal cell that expressed the targeted antigen and a cancerous cell. In the case of CD19, the normal cell targeted is a B cell, and B cell aplasia is treatable with intravenous immunoglobulin infusions. In other diseases, depending on the antigen targeted, the risk of on-target, off-tissue toxicity can be a major concern [10], which is particularly the case for some solid tumor-associated antigens.

While the scFv provides antigen specificity, CAR-modified T cells must then be activated with an activation domain. CD3 zeta has been used to provide the T-cell activation signal (signal 1). A recent innovation that has greatly increased the success of this approach is the addition of a costimulatory signal (signal 2) to the CAR design. A number of groups have focused on the CD28 [5,6,9] costimulatory domain, and our group at the University of Pennsylvania focused on 4-1BB (CD137) [7,8,11,12]. The use of a CD3 zeta domain only has been referred to as a first generation CAR, and the addition of a single (second

generation) or multiple costimulatory domains (third generation) is seen in almost all current CAR designs [13]. CARs in clinical use in which high-level proliferation and high percentages of clinical responses have been reported are all currently second generation.

To activate and expand the genetically modified T cells, some combination of these signals must also be provided during the culture process. Many prior trials utilized an approach of OKT3 (which binds CD3) and IL-2 to activate and expand the T cells [14,15]. More recently, several groups have utilized a bead-based approach pioneered by Bruce Levine and Carl June. In this ex vivo expansion protocol, the T cells are surrounded by beads conjugated to antibodies that bind to and activate CD3 and CD28 [16,17]. Thus, both signal 1 and signal 2 are induced by a bead that essentially acts as an artificial antigen presenting cell. This process produces a large number of T cells and may also preserve beneficial T cell functional phenotypes after infusion into the patient, such as long telomeres [18], central memory T cells [19], and fewer markers of T-cell exhaustion [20].

One of the most important responses that relates to clinical effectiveness of these cells is expansion. A number of the studies prior to 2010 were able to see small numbers of T cells by polymerase chain reaction [18,22–24]. This can be demonstrated with data from ongoing clinical trials at the University of Pennsylvania and Children's Hospital of Philadelphia, using a CD19-targeted, second-generation CAR that uses 4-1BB as the costimulatory domain. This CD19/4-1BB/CD3 zeta CAR has been referred to as CART19 or CTL019, and was recently given the generic name of tisagenlecleucel-T. To examine peripheral expansion of CTL019 cells after adoptive transfer (Fig. 1), flow cytometry can be used. This technique is not nearly as sensitive as PCR, but has rapid turnaround, is well suited to a circumstance where large numbers of engineered T cells can be detected, and also only detects gene-modified cells in which the transgene is expressed. One day after infusion, no CD3-positive T cells are found in the peripheral blood compartment, even in patients who will eventually respond. The cells are either absent or have migrated to tissues, despite a large dose of cells infused. The fact that the cells have not failed to “engraft” after adoptive transfer is demonstrated at 2 weeks after infusion, where (in the case depicted in Fig. 1) around 70% of the circulating T cells are genetically engineered. In some of the cases we have treated, half of circulating white cells are active, CAR+ T cells. Given that these percentages exceed the percentage of CAR-modified cells in the product (11%–40%), this is strongly indicative of antigen-driven cell proliferation, and not merely homeostatic proliferation in a lymphodepleted host. In patients who achieve complete response to treatment, CTL019 can persist up to 24 months, while patients who do not reach complete response have minimal proliferation (at least as detected by flow) and persistence of about 28 days. The probability of persistence of CTL019 cells at 6 months was 68% in our recently reported cohort of 30 children and adults [8], although some patients experienced loss of CTL019 cells and B cell aplasia earlier, with one patient losing cells after initial robust proliferation after 15 days in what was apparently a rejection event.

Cytokine release syndrome (CRS)

Toxicity remains a problem, with one significant toxicity being cytokine release syndrome. Our initial patient on the pediatric ALL CTL019 study experienced a life-threatening

cytokine release syndrome. She started treatment with very low counts due to high-dose chemotherapy received 6 weeks prior to infusion, and so did not require or receive further lymphodepleting chemotherapy treatment. The cells were infused as divided doses over 3 days (Fig. 2), and after a few days, the patient started to have high fever, was admitted to the ICU, and required intensive support for hypotension and respiratory failure, including 3 vasopressors and 100% oxygen on an oscillating ventilator. The patient received steroids per protocol but only experienced a decrease in her hectic fever curve, without improvement in her cardio-respiratory status. She received etanercept, based on data suggesting that it is helpful in patients with cytokine-induced lung injury [25,26], but this also did not improve her status.

Luminex analysis of serum from the patient showed very significant elevations in a number of inflammatory cytokines, such as IFN- γ and IL-2R, but IL-6 was also markedly elevated [27,28]. Because tocilizumab, a drug commonly used in rheumatoid arthritis, targets IL-6 by blocking its receptor and has both a pediatric indication and known pediatric dose, the patient was given tocilizumab and began rapid improvement within hours. She became afebrile and no longer needed vasopressors or ventilator support. In subsequent analysis, we have shown that the level of IL-6 correlates with severity of cytokine release syndrome, with peak IL-6 being 2 orders of magnitude higher in patients with severe CRS compared to those with mild or moderate CRS [8]. Patients who have these high levels of IL-6 after treatment typically receive 1 (or occasionally 2) doses of tocilizumab and then have rapid responses. Tocilizumab does have rare side effects of transaminitis and neutropenia. Blinatumomab, a bispecific CD3/CD19-binding antibody also causes significant cytokine release syndrome. This can be associated with high IL-6 concentrations, and may also improve with tocilizumab [29]. This suggests that increases in IL-6 are characteristic of therapies that result in powerful, nonphysiologic T-cell activation, and not just our specific CAR technology.

CD19 escape

Testing bone marrow cells for minimal residual disease (MRD) reveals that >85% of the ALL patients we have treated enter an MRD-negative complete remission. In addition, there is complete absence of the CD19 compartment in responding patients, due to the action of CTL019 cells against both normal and malignant B cells. However, we have observed 3 patients who have recurred with CD19-negative disease [8]. In two cases, the patients had previously been treated with CD19-directed blinatumomab, which may have increased the risk of CD19 escape. In one of these cases, a tiny peak in CD19-negative disease was observed retrospectively, that later caused the patients' recurrence after all CD19+ cells were destroyed [7]. The CD19(-) and CD19(+) cells from the pretreatment sample show the same phenotype after engraftment and proliferation in immunodeficient mice, and the CD19-negative cells are genetically related to the bulk clone with the same antigen receptor gene arrangement, but are not targeted by the CAR cells. Work to understand the mechanism of CD19 loss in these leukemias is underway.

Trafficking of cells to cerebral spinal fluid (CSF)

CSF is an important sanctuary site for ALL. As a result, therapies for ALL must be effective in the CSF as well as other sites of disease. The vast majority (17/19 tested patients) who have received CTL019 and entered a complete remission show the presence of the CAR cells in CSF as well as peripheral blood and bone marrow. CSF white counts range from 1 to 25 cells/uL, with most or all of these cells being engineered T cells. While those with ALL with overt central nervous system involvement (CNS3) are not currently eligible for CTL019 ALL trials, we have treated two patients with CNS2 disease, and both of these patients experienced BM and CSF remissions. No CNS relapses have been seen in our ALL cohort to date. Treatment of CNS3 ALL is currently under consideration to better test the efficacy of these cells against central nervous system disease.

Results of CTL019 treatment

Across the CTL019 program, well over 70 patients with both CLL and ALL have been treated with these CAR cells. In a recently reported cohort of 30 patients, 27 (90%) achieved complete response [8]. Three of the patients had previously failed blinatumomab therapy, and two of these responded. There have been 6 relapses, including 2 CD19-negative relapses. Responses in adults and children, and in patients who had never been treated with allogeneic bone marrow transplant (BMT) or had relapsed after a BMT were similar. Overall survival after CTL019 infusion is shown in Fig. 3. Most patients had refractory, often significant disease burden at the time of CTL019 infusion, and 60% were treated after relapsing after transplant. The majority had also proved refractory to multiple prior therapies. T cells collected from patients who had undergone prior transplant were mostly of donor origin, with median donor chimerism of 100%. No patient showed evidence of graft-vs-host disease after CTL019 infusion.

In addition to the cytokine release syndrome, patients experienced macrophage activation syndrome (MAS; also referred to as hemophagocytic lymphohistiocytosis or HLH), which is indicated by very high ferritin levels (16,000 to 415,000 ng/mL) and coagulopathy with elevated D-dimer (in all patients) and low fibrinogen (in several patients). Our data suggest that there may be a positive feedback loop between the macrophage system and the T cells that produces the high IL-6 levels and MAS. Two patients with grade 4 cytokine release syndrome also had a potentially predisposing hypomorphic perforin mutation that has been associated with secondary HLH. MAS/HLH appears to also be entirely reversible with cytokine blockade.

B-cell aplasia has been observed in all responding patients to date. B-cell aplasia in peripheral blood continues for a number of months beyond the ability to detect the CTL019 cells in peripheral blood by flow cytometry, suggesting that there are low levels of CTL019 cells that are sufficient to control normal B cell formation. This is confirmed by quantitative PCR testing. B-cell aplasia has continued for up to 2 years post-infusion and requires intravenous immunoglobulin replacement.

In addition to the B-cell aplasia, CTL019 has been associated with other toxicities. Tumor lysis syndrome (TLS) has not been a large problem in the pediatric population but may be

delayed for 20–50 days post infusion in chronic lymphocytic leukemia, where the tumor burden may be much larger [11,12]. The severity of cytokine release syndrome, which has been shown to be reversible with tocilizumab, is highly correlated with pre-infusion tumor burden. Currently, CAR T-cell therapy is best pursued by centers with transplant and cell therapy experience. However, in the future, treating high-risk patients who still have somewhat responsive disease in an MRD status, where the risk of severe cytokine release syndrome [3] may be far lower, may allow the use of this therapy in smaller centers. CTL019 also causes some neurotoxicity, manifesting as significant confusion and aphasia. This has occurred in a small number of patients, generally after the cytokine release syndrome, lasts for several days, and has fully resolved in our patients [8,28,30].

Conclusion

What is the potential for CAR cell immunotherapy? It may be an option for consolidation for patients with persistent MRD and can reinduce as many as 90% of relapsed patients in to remission. This may also be helpful to achieve an MRD-negative state prior to allotransplant and can be used as a bridge to stem cell transplant. Most of the patients who have achieved complete response with CTL019 in our pediatric trial have chosen not to undergo transplantation, despite the fact that transplant after first complete response is standard of care. This suggests for the first time that this treatment, with demonstration of adequate persistence, might in the future be considered as an alternative to transplant. B-cell aplasia continues while CLT019 cells persist, which can be for many months or even years, and requires intravenous immunoglobulin replacement to avoid serious infectious risks.

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Persistence out to 6-18 months in responding patients

	CR	No CR	
Persistence of CTL019 cells	≥ 145 days* Range 15-512 *many ongoing	28 days Range 11-45	$P < .05$

Fig. 1.

Peripheral expansion of CTL019 cells as detected by flow cytometry. CR, complete response; D, day.

Fig. 2.

Lymphocyte and neutrophil reconstitution in a refractory ALL patient after CTL019. The 3 downward-facing arrows represent 3 daily CTL019 infusions at 10%, 30% and 60% of the eventual total dose.

Fig. 3.

Overall survival in 30 children and adults treated with CTL019 therapy. Of this group, 5 patients who entered a CR went on to further therapy, 3 of which received an allogeneic BMT. The rest have not received further therapy to consolidate their remissions. Figure adapted from The New England Journal of Medicine; Maude SL, Frey N, Shaw PA et al., Chimeric antigen receptor T cells for sustained remissions in leukemia, Volume No 371, Page No 1510, ©2014 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

Table 1

Roadblocks to successful cellular immunotherapy for cancer.

Problem	Solution
Targeting	CAR or TCR
Expansion ex vivo	GMP cell culture
Expansion in the host	?Young T cells
Persistence	?Memory T cells

CAR, chimeric antigen receptor; GMP, good manufacturing practice; TCR, T-cell receptor.