CAR T-cells merge into the fast lane of cancer care

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Abstract:

Chimeric Antigen Receptors (CARs) can be introduced into T- cells redirecting them to target specific tumor antigens. CAR-modified T cells targeting CD19 have shown remarkable activity against CD19+ malignancies including B cell acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL)and non-Hodgkin lymphomas (NHL). Complete remission rates as high as 90% have been observed for patients with relapsed and refractory ALL and >50% response rates have been seen in heavily pre-treated CLL and NHL. Excitingly, some remissions have been durable without any additional therapy, a finding which correlates with in-vivo T-cell persistence and B-cell aplasia. The major treatment related toxicities include B cell aplasia, neurologic toxicities, and a potentially severe cytokine release syndrome. This review summarizes outcomes for patients treated with CD19-CAR T-cells while exploring the field's challenges and future directions.

Introduction:

The recent successes of chimeric antigen receptor (CAR) T-cell therapy highlight the remarkable ability of the human immune system to fight cancer. Genetically modified autologous T cells expressing an Anti-CD19 CAR have shown great promise in a number of different clinical trials [1-6]. Unprecedented remission rates of 67-90% have been observed in adult and pediatric patients with relapsed and refractory acute lymphoblastic lymphoma (ALL). Remissions have been sustained in many patients without subsequent therapy, a phenomenon often correlating with CAR T-cell persistence[1,3,4]. In other CD19+ malignancies including chronic lymphocytic leukemia (CLL) and B cell Non-Hodgkin Lymphomas (NHL), response rates of 50-80% have been observed even in heavily pretreated and refractory patients[2,6]. Cytokine Release syndrome (CRS), an inflammatory process correlating with the in vivo activation and expansion of CD19-CAR T-cells, is the most significant treatment related toxicity. Neurologic toxicity with encephalopathy and seizures is another important side effect[1-6]. On target, off tumor effects of CD19-CAR T-cells are fortunately limited to B-cells and B-cell aplasia occurs in patients with CAR T-cell persistence[4,6]. Given the successes of these early phase trials, larger Phase II studies are underway to assess the feasibility of expanding this treatment modality to multiple centers and to evaluate outcomes among larger numbers of patients.

CAR-modified T cells have the specificity of a targeted antibody, the ability to expand in vivo for an amplified anti-tumor response and the potential for persistence with the hope of ongoing tumor surveillance. The CAR T-cells discussed in this review target CD19, which is a pan B-cell antigen expressed on most B-cell malignancies. This ubiquitous expression on malignant B-cells and the limitation of off tumor expression to healthy B-cells, make CD19 an attractive target. Translating CAR T-cell therapies to other tumor types is underway, but limited by the choices for potential targets.

Chimeric Antigen Receptor T cells

With the use of gene transfer techniques, T-cells can be genetically modified to stably express antibodies (such as Anti-CD19) on their surface, which confers to them new antigen specificity. CARs are constructed to combine an antigen recognition domain (usually the variable regions of a specific monoclonal antibody (scFv)) with an intracellular signaling domain, allowing T-cells to exert effector functions independent of MHC[7,8]. The components of the signaling domain are critical for maximal activation, expansion and persistence of CAR T-cells and therefore a key target for manipulation. The so called, "1st generation" CARs included only a CD3ζ domain, which resulted in T-cells with limited in vivo expansion and clinical activity[9-11] (Figure 1). Their limited activity was likely due to the inability of 1st generation CARs to adequately activate T-cells especially when tumor cells lacked expression of T-cell co-stimulatory molecules[7,8,10,12]. To overcome this limitation, several groups developed "2nd generation CARs" which include an additional CD28 or CD137(4-1BB) derived co-stimulatory domain in addition to the CD3ζ domain. The strong pre-clinical suggestion that 2nd generation CARs would translate into a more potent anti-tumor product[13,14] has now been proven by dramatic clinical outcomes for patients with relapsed and refractory CD19+ malignancies (See Table 1)[1-4,6]. The optimal co-stimulatory domain for 2nd generation CARs is not known but pre-clinical studies suggest 4-1BB CARs may yield improved in vivo expansion and persistence and 4-1BB CARs have been shown to have prolonged persistence in CLL and ALL recipients, which may correlate with sustained remissions[4,6,14]. Future studies with 3rd generation CARs (which include multiple co-stimulatory domains) are in development (See Figure 1)[15].

The CD19-CAR T-cells used across the clinical trial programs summarized in Table 1 differ not only in CAR design but also in other aspects of their manufacturing processes including choice of gene transfer technique and cell culture environment. The basic steps involved in the manufacture of CD19-CAR T–cells in our clinical trial programs are summarized in Figure 2. In our and other trials, patients typically undergo leukapheresis for collection of autologous T-cells. One limitation to this therapy is that adequate functional T-cells may not be present particularly in heavily or recently treated patients. Once collected, T-cells are genetically modified and expanded ex-vivo for clinical use. While lenti- and retroviral vectors are the methods of gene transfer in the represented studies, other methods such as electroporation or RNA-based methods can be employed[11,16]. Different cell culture manufacturing systems have been developed which may impact the final phenotypic composition of the CAR T-cell product[17-19]. As an example, our manufacturing system uses an anti-CD3/CD28 antibody-coated magnetic bead system which results in a final product capable of memory function and persistence[18]. Prior to CD19-CAR T-cell infusion, patients typically receive chemotherapy in an effort to induce further lymphodepletion and enhance CAR T-cell expansion and persistence in vivo[20]. In addition to variations in the investigational product, other differences across protocols include type and intensity of lymphodepletion, timing and dose of CD19-CAR T-cell infusion and of course underlying disease.

Clinical Outcomes of CD19-CAR T-Cell Therapy

ALL

Adult patients with relapsed or refractory ALL have a poor prognosis and almost all patients will die from their disease[21-28]. While children are initially more responsive to traditional salvage chemotherapy approaches than adults, these remissions are often not sustained and relapsed ALL remains a leading cause of cancer deaths in children[29,30]. It is this dismal prognosis and lack of conventional treatment options, which underscores the impact of the dramatic responses observed with CD19-CAR T-cell therapy in patients with relapsed and refractory ALL[1,3,4,31,32].

Outcomes from single center studies of relapsed and refractory ALL patients treated with 2nd generation CARs have been remarkable with complete remission (CR) rates of 67-90% (See Table 1)[1,3,4]. In our program, we reported a 90% CR rate in 30 pediatric and adult patients treated with CD19-CAR T-cells incorporating the 4-1BB co-stimulatory domain, referred to as CTL019 cells[4]. Assessment for minimal residual disease (MRD) by multiparametric flow cytometry was negative in 22 patients, positive in 3 patients (0.1%, 0.09% and 0.22%) and not performed in 2 patients who achieved a CR. Of interest, responders included 2 patients who were refractory to blinatumomab, another CD19-targeting approach that uses a bispecific antibody to redirect cytotoxic T-cells to B-cells with its anti-CD3 and anti-CD19 arms[33]. Eighteen of the 30 patients treated had relapsed after a prior allogeneic SCT, with T-cells successfully collected and manufactured from the recipient and no graft versus host disease was observed[4]. Similarly high response rates have also been observed employing a CD28 co-stimulatory domain by both the Memorial Sloane Kettering Cancer Center(MSKCC) (88% CR rate for 16 adult patients) and the National Cancer Institute(NCI) (CR rate of 67% in an intent to treat analysis of 20 children and young adults)[1,3].

Of importance, remissions were sustained from 2-24+ months in 19 patients from our initial cohort, with 15 of these patients never receiving further treatment. The durable remissions observed in patients not bridged to allogeneic SCT correlated with CAR T-cell persistence and the biological correlate of ongoing CAR T-cell activity, B-cell aplasia[4].

CLL and NHL

Despite a wide array of available treatment options, CLL remains incurable without an allogeneic SCT and prognosis is quite poor for patients with multiply relapsed disease and short progression free intervals between treatments[34]. CD19-CAR T-cells have been used by several programs to treat patients with relapsed and high risk CLL[2,6,31]. We recently reported long term follow up of the first 14 patients with CLL treated at PENN using CTL019 cells[6]. The overall response rate in this heavily pretreated cohort was 57% with 4 CRs and 4 partial responses (PR). Similar to our ALL experience, durable remissions have been observed correlating with CD19-CAR T-cell expansion and persistence. Remarkably, 2 of these patients with CLL are now over 4 years out from CAR T-cell infusion, in molecular remissions with detectable functional CD19-CAR T-cells[6]. While responses in CLL are impressive we note that they do not approach the dramatic 70-90% CR rates observed in ALL. Reasons for this may be due to functional defects in CLL patients' T-cells with resulting differences in the functionality and composition of the manufactured T-cell product or differences in engagement with cancer cells due to the CLL tumor microenvironment[15,35,36]. To date, in CLL we have not been able to identify baseline patient or disease

defining characteristics that differentiate responders from non-responders[6]. Patients are currently being enrolled in an expanded Phase II clinical trial open at PENN.

The NCI reported their experience using CD19-CAR T-cells with CD28 co-stimulatory domain for patients with CLL and other NHL. Of the 4 reported patients with CLL, all had a response with 3 having CRs that have been maintained for over a year[2]. Nine patients with diffuse large B-cell lymphoma or primary mediastinal B-cell lymphoma were treated with 4 achieving a CR, 2 a PR, 1 stable disease and 2 not evaluable for response[2]. MSKCC's initial experience using CD19-CAR T cells with CD28 domain were more disappointing with 0 of 7 evaluable CLL patients responding. Reasons for this differential response compared with the NCI and PENN experience are not clear but may be due to differences in the protocol design (a Phase I design with 3 of the 7 subject not receiving lymphodepletion) or patient selection[31].

Toxicity

These dramatic successes with CAR T-cells fuel the commitment to larger phase clinical trials and the scientific pursuit with next generation signaling domains and new antigen specificity. Investigations to better understand, predict and manage CD19-CAR T-cell side effects are a key part of ongoing clinical trials.

CRS, an inflammatory process marked by dramatic elevations in cytokine levels, has emerged as the most significant toxic effect from CART19 therapy. Neurologic toxicity has also been observed with encephalopathy and seizures occurring in patients with different diseases and treated with different constructs. Finally, as anticipated, B-cell aplasia is noted in essentially all patients throughout the duration of CD19-CAR T-cell persistence.

Cytokine Release Syndrome

CRS is a systemic inflammatory response which correlates with the in vivo activation and proliferation of CAR T-cells. The clinical features of the syndrome are associated with high levels of inflammatory markers and cytokines including C reactive protein (CRP), ferritin, interferon-ɣ (INFɣ) and interleukin-6 (IL-6). The first clinical sign of CRS is fever which often starts low but escalates to levels as high as 104-105°F. In the vast majority of patients, CRS occurs within 1-14 days of CD19-CAR T-cell infusion. Unfortunately, this syndrome can progress beyond fevers and malaise to life threatening vasodilatory shock. Depending on its severity, CRS can either be self-limited (requiring only supportive care with antipyretics and intravenous fluids) or it may require intervention with anti-cytokine directed therapy. The duration of CRS is variable and dependent on intervention, with resolution typically by 2-3 weeks after CD19-CAR T-cell infusion.

In our first 30 adult and pediatric ALL patients, all experienced some degree of CRS. This was defined as severe in 8 patients who required ICU level care for vasopressor support and supplemental oxygen[4]. Other programs, as summarized in Table 1, have noticed a similar incidence, duration and severity of CRS. Given the life threatening nature of CRS, it is helpful to try and identify disease, patient or therapy related factors which may predict for the severity of CRS. Disease type (ALL) and disease burden (in ALL) are strongly correlative with the severity of CRS[1,4,16]. Unlike more traditional agents, there is not an

obvious dose: toxicity relationship with CD19-CAR T-cell therapy where the infusion dose grossly underestimates the final expanded active dose, and dose is only one of many factors which may correlate with peak in vivo expansion. However, there is suggestion that the infusion dose of CD19-CAR T-cells may impact the severity of CRS. In the Phase I portion of their ALL study, the NCI found an increase in the severity of CRS with escalating dose levels[3].

Clinically available laboratory markers of inflammation including CRP and ferritin are universally elevated in patients with CRS from CAR T-cells[4,6]. We and other groups have also noted investigational cytokine activation profiles which correlate with clinical syndrome of CRS[1-4,6,32]. Effector cytokines such as INFγ and soluble interleukin-2 receptor α (sIL2Ra) are elevated but so are cytokines traditionally associated with macrophage activation such as IL-6 and interleukin-10. Indeed, many of the clinical manifestations of CRS overlap with those of macrophage activation syndrome/hemophagocytic lymphohistiocytosis[37]. An area of ongoing investigation is whether cytokine profiles can be used to predict severity of CRS and be used to guide pre-emptive anti-cytokine directed treatment[1,16].

Given the very high levels of IL6 observed in patients with CRS and the initial observation that tocilizumab, an antibody against the IL-6 receptor, rapidly reversed fevers, hypotension and life-threatening hypoxia in a child with CRS after CTL019[32], the use of tocilizumab to manage CRS has become standard. Tocilizumab remains an attractive choice as it continues to be effective for most patients and has limited inherent toxicity. In addition, it may have a less negative impact on the anti-tumor effect of CD19-CAR T-cells when compared to steroids or other potential agents against other cytokine targets. MSKCC has shown differential survival of CD19-CAR T cells in the bone marrow at Day 28 based on whether patients' severe CRS was managed with steroid or tocilizumab based approaches[1]. It remains to be seen whether the administration of tocilizumab earlier in the clinical course of CRS will have a negative impact on response rates.

Neurologic

Neurologic adverse events have been observed in some patients receiving CD19-CAR T-Cell therapy (see Table 1). In our 30 patient ALL study, 13 patients had neurologic side effects, all with resolution to baseline within 3-4 weeks of treatment. Central nervous system (CNS) manifestations in our cohort ranged from delirium to global encephalopathy with findings that included aphasia, confusion, hallucinations and seizure[4]. These events occurred within the first few weeks after CD19-CAR T-cell infusion but were not directly linked with clinical signs and symptoms of systemic CRS. In our small numbers, administration of tocilizumab did not seem to impact neurologic symptoms. Neurologic events as summarized in Table 1, have been observed in varying degrees across different tumor types and different CD19-CAR T-cell products. The mechanisms involved in mediating CD19-CAR T-Cell CNS events are unclear. Possibilities include direct T-cell mediated toxicity or cytokine mediated events. We know that CD19-CAR T-cells are able to cross the blood brain barrier (which may actually be beneficial and result in successful surveillance of the CNS for disease). Of the 19 patients in our ALL study with evaluable CSF, 17 had detectable CD19-CAR T-cells. However, only a fraction of this subset had CNS toxicity[4]. Further studies are needed to determine the mechanism of action, risk factors and optimal management of neurologic toxicity.

B-cell Aplasia:

As predicted, off tumor on target toxicity from anti-CD19 CAR T cells has been limited to healthy B-cells. In our patients with CD19-CAR T-cell persistence, B-cell aplasia and associated hypogammaglobulinemia has been observed. Indeed, B cell aplasia may be a biological marker for ongoing CAR T-cell activity, or a measure of "functional persistence". To date, with follow up limited to 4 years, this has been successfully managed with intravenous immunoglobulin replacement therapy[4,6].

Future Directions:

The successes of CD19-CAR T-cell therapy for patients with relapsed and refractory chemotherapy resistant CD19+ malignancies have been dramatic, particularly for patients with ALL. Larger studies are ongoing to describe outcomes across larger numbers of patients and to assess the feasibility of expanding this therapy beyond just a few highly specialized centers. Trials are in progress to both optimize outcomes (through manipulations of CD19-CAR T-cell manufacturing techniques and cell dose) and better understand and manage toxicities. Efforts to identify modifiable predictors for response in CLL and NHL are underway.

The success of second generation CD19-CARs also inspires the exploration of next generation CARs and CARs to target new antigens and other tumor types. Other approaches that may be tested in the near future include administration of combinations of CAR T-cells with different targets, and combining CAR-T cells with other therapies such as check point inhibitors or other immune modifying therapies. The pursuit to manufacture universal CAR-modified T-cells as an "off the shelf" product is particularly interesting, potentially allowing for more rapid treatment and circumventing the disadvantage inherent in using autologous T cells and finding an appropriate window between treatments to collect T-cells, which for some patients are either low in number or dysfunctional. No doubt, these CARs are now merging into the mainstream of cancer immunotherapy, and are continuing to pick up speed.

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Table 1: Clinical Trials with 2nd Generation CD19-CAR T-cells

Reference	Signaling Domain	Lymphodepletion	Population	Response	CRS	Neurologic Toxicity
Acute Lymphoblastic Leukemia						
4	CD3z-4-1BB	Varied	N=30 (ALL) Pediatric & Adults	CR=90%	100% CRS 27% Severe	43% Total Encephalopathy Aphasia Seizure (1)
1	CD3z-CD28	Cyclophosphamide	N=16 (ALL) Adults	CR=88%	43% Severe	25% Grade 3-4 Encephalopathy Seizure
3	CD3z-CD28	Fludarabine/ Cyclophosphamide	N=21 (ALL) Pediatric & Young Adults	CR=67%	76% CRS 28% Severe	29% Total hallucinations Dysphasia encephalopathy
Chronic Lymphocytic Leukemia & Non-Hodgkin Lymphoma						
2	CD3z-CD28	Fludarabine/ Cyclophosphamide	N=15 (NHL/CLL)	CR=53% PR=27%	27% Severe	40% Total Encephalopathy Aphasia Myoclonus Ataxia
6	CD3z-4-1BB	Varied	N=14(CLL)	CR=29% PR=29%	9/14 Total 5/14 Severe	43% Total 1/14 Grade 4
31	CD3z-CD28	3/8: None 5/8: Cyclophosphamide	N=8 (CLL)	No PR/CR	8/8 fever 1/8 Gr5	NR
10	CD3z-CD28	None	N=8 (NHL)	No PR/CR	NR	NR

Abbreviations: CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ALL, acute lymphoblastic leukemia; CLL. chronic lymphocytic leukemia; NHL, Non-Hodgkin Lymphoma; CR, complete remission; PR, partial response; NR, not reported

Figure 1: Chimeric Antigen Receptors. Next generation CARs have additional modifications to their intracellular stimulatory domains.

Figure 2: Manufacturing Overview of CTL019 (a CD19-CAR T–cell product)

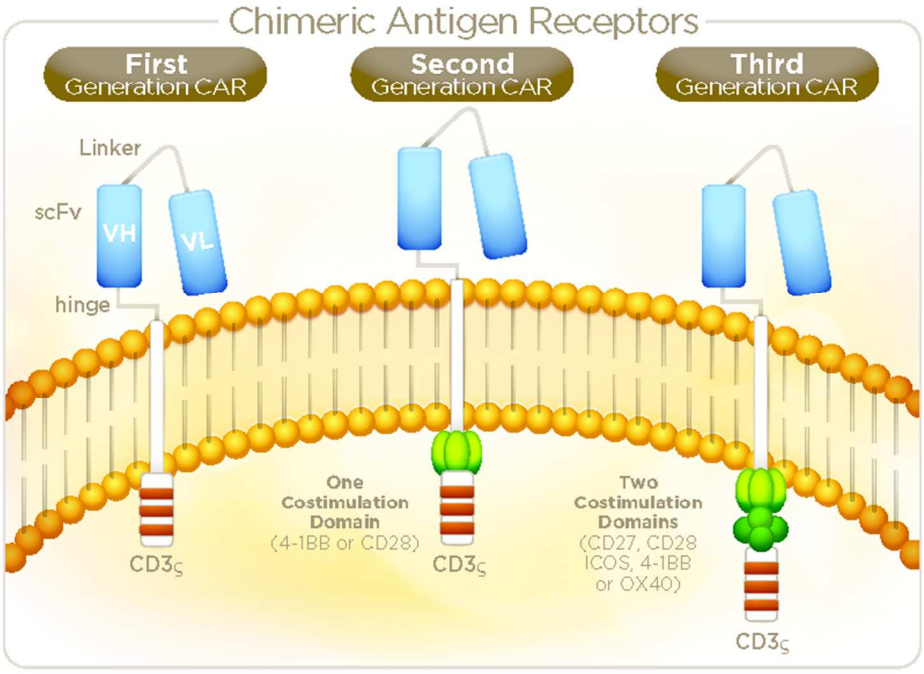

Figure 1: Chimeric Antigen Receptors. Next generation CARs have additional modifications to their intracellular stimulatory domains.
228x165mm (96 x 97 DPI)

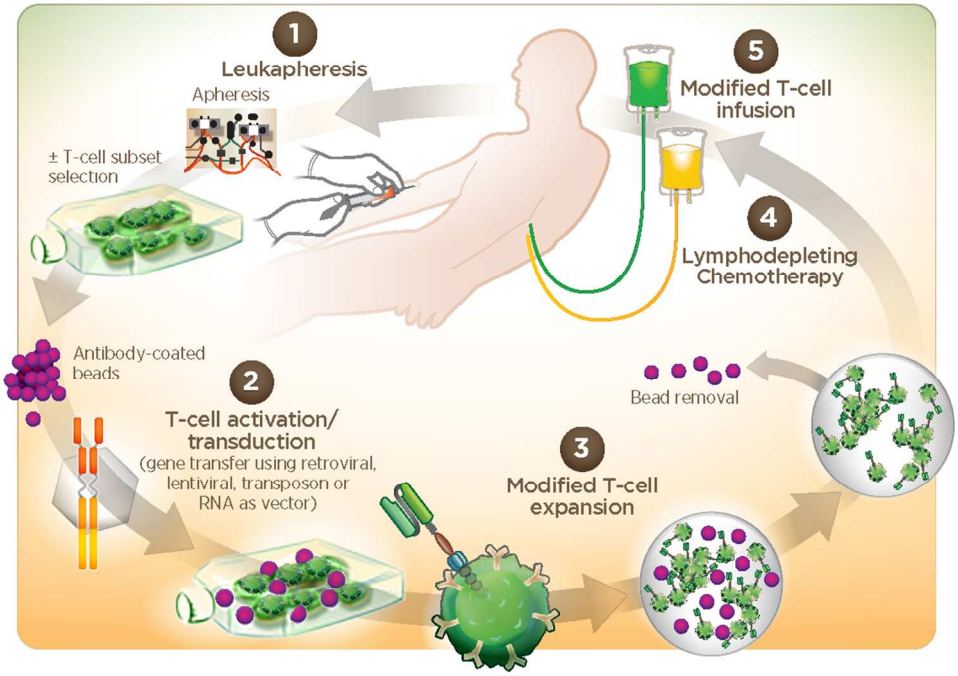

Figure 2: Manufacturing Overview of CTL019 (a CD19-CAR T–cell product)
228x165mm (132 x 133 DPI)