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Challenges and Opportunities of Allogeneic Donor-Derived CAR T cells

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Abstract

Purpose of review—As T cells engineered with chimeric antigen receptors (CARs) are entering advanced phases of clinical trial testing with promising results, the potential implications of use in an allogeneic environment is emerging as an important consideration. This review discusses the use of allogeneic CAR therapy, the potential effects of T cell receptor (TCR) signaling on CAR T cell efficacy, and the potential for TCR elimination to generate an off-the-shelf product.

Recent findings—The majority of preclinical and clinical data regarding allogeneic T cells are focused on safety of their use given the potential for graft versus host disease (GVHD) mediated by the T cell receptor expressed with the introduced CAR. Recent clinical trials using donor derived CAR T cells are using either rigorous patient selection or T cell selection (such as enrichment for virus-specific T cells, VST). Although no GVHD has been reported, the efficacy of the allogeneic CAR treatment needs to be optimized. Several pre-clinical models limit allogeneic CAR-driven GVHD by utilizing memory T cell selection, VST, gene-editing techniques or suicide gene engineering.

Summary—In the allogeneic environment, the potential effects of TCR signaling on the efficacy of CAR could affect the clinical responses with the use of donor-derived CAR T cells. Better understanding of the functionality of donor-derived T cells for therapy is essential for the development of universal effector cells for CAR therapy.

Keywords

adoptive immunotherapy; allogeneic hematopoietic stem cell transplantation; donor derived; chimeric antigen receptors; graft-versus-host disease

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been used as an effective treatment against acute leukemia since the 1970s [1–3]. It is now well established that the efficacy of allo-HSCT is also partly dependent on the anti-leukemic effect derived from the allogeneic cells from the donor graft, also referred to as the graft-versus-leukemia (GVL). The majority of the GVL effect is mediated by donor T cells contained in the allograft[3] but is also seen following delayed infusion of donor T cells (donor lymphocyte infusion, DLI), which have the potential of inducing remission of hematopoietic malignancies that has relapsed or persists after allo-HSCT[4–8]. The desired GVL effect from donor T cells is frequently countered by the adverse effect associated with the allogeneic T cell response against normal tissue, termed graft-versus-host (GVH) response. Further, the potency of the GVL effect is often not enough for complete eradication of malignancy, particularly in the case of lymphoid malignancies[7–9]. Several methods have attempted to intensify the GVL effects of allo-HSCT while minimizing the effects of GVH in order to lower toxicity and improve the outcome of treatment with varying degrees of success[10]. Genetically engineered T cells expressing receptors to redirected specificity toward antigens expressed on malignant cells have the potential to generate a very specific GVL response.

Chimeric antigen receptors (CARs) are fusion constructs composed of the variable binding domain of a monoclonal antibody with the activation domain of the T cell receptor, with additional co-stimulatory domains of T cell signaling (i.e. CD28, 4-1BB, O×40), in second and third generation CARs [11–13]. CARs allow for the redirection and activation of effector T cells towards any cell surface molecule upon binding by the antibody derived receptor, and are independent of MHC restriction[12,13]. Arming T cells with a CAR against CD19 has been very successful in recent trials in the treatment of B cell malignancies[14–20]. In B-lineage acute lymphoblastic leukemia (B-ALL), remission rates of heavily pre-treated, relapsed/refractory patients reach as high as 70-90% after anti-CD19 CART treatment[17–19]. A significant proportion of patients receiving CAR T therapy have received an allo-HSCT, thus for CAR treatment, the T cells collected for CAR T cell production are derived from the allograft. Despite this, GVHD has not been observed in the patients following CAR infusion, perhaps since the cells have been tolerized to the transplant recipient prior to collection. Despite the lack of GVHD present in the allo-HSCT recipients, the responses of post allo-HSCT patients appears to be inferior to those in patients receiving autologous CAR T cells[18]. The discrepancy in response may be attributed to the quantitatively lower or qualitatively inferior lymphocytes at the time of collection resulting from prior chemotherapy regimens, or the qualitative defects of reconstituted allogeneic T cells in the recipient, or both[21–23]. A potential opportunity associated with the alloHSCT platform is the potential to use “healthy” T cells collected from the donor.

Although CAR T cells are often described as having redirected specificity through the CAR receptor (i.e. toward CD19), with the presence of the endogenous T cell receptor (TCR), it is perhaps more appropriately described as additional specificity. Following alloHSCT, the precursor frequency of T cells specific for allogeneic antigen (i.e. tumor antigens, normal

antigen, viral antigens) has the potential to be relatively high, particularly if donor-derived T cells are used since these T cells will not have been tolerized in the recipient. In the presence of both TCR and CAR antigens presented to the CAR T cell, it is not known which receptor will be dominant and how signaling through the endogenous TCR affects the efficacy of the genetically engineered CAR receptor function. Further, there is the possibility of a bystander effect in which the alloreactive T cells may impact the function of T cells that do not possess specificity for alloantigens[24,25] but do express the CAR.. Thus, the use of allogeneic CAR T cells, particularly healthy donor derived CAR T cells, requires an understanding of CAR T cell function in the allogeneic setting.

Donor derived CARs in the clinic

Human CAR T cells are known to cause xenogeneic GVHD in immunocompromised mice[26,27]. However, preclinical experiments suggest that the use of donor derived allogeneic CAR T cells in immunocompetent mouse models are safe, as demonstrated by low GVHD rates[28]. Currently, there is a paucity of clinical reports in the literature addressing the risks of GVHD and the activity of donor derived CAR T cells. Kochenderfer et al had reported a small cohort of patients receiving donor derived CD19-CAR T cells for relapsed hematologic malignancies following allo-HSCT. All patients had at least 1 prior DLI, with either no GVHD, grade 1 acute GVHD, or mild chronic GVHD. There was no GVHD following donor derived CD19 CAR T cells in this cohort, however, only 3/10 patients respondeding to the CAR T cells[29]. Of note, patients on this trial did not receive lymphodepleting agents prior to CAR infusion, which is known to be significant for efficacy of adoptive T cell immunotherapy although data on the importance of lymphodepleting therapy in the context CAR T cells is lacking. Nonetheless, it is difficult to draw conclusions regarding efficacy in this small series since all autologous CAR reports incorporate lymphodepletion prior to infusion.

A second report of donor derived allogeneic CAR T therapy utilized virus specific T cells (VST) to reduce the risk of GVHD. VSTs are selected by ex-vivo antigen-driven expansion of T cells and have been used post allo-HSCT for treatment of viral induced complications[30–33] VSTs have been described as an suitable backbone for different CAR T cells (for CD30, GD2, CD19 etc), with an endogenous TCR specific to viral antigens and additional specificity through the CAR receptor against the tumor target[30,34–37]. The use of donor-derived VST-CD19 CAR was reported in a limited number of patients with no GVHD or cytokine release syndrome observed[38]. Here, expansion of CAR T cell was seen with viral infection or reactivation suggesting that TCR activation is able to enhance the expansion of infused CAR T cells. However, the increased CD19 CAR expansion did not lead to a decrease in normal or malignant B cell numbers suggesting the possibility of impaired functionality of the CAR T cells when activated through endogenous TCR.

Currently there are 6 trials on clinicaltrials.gov evaluating the use of allogeneic donor-derived CAR T cells (June 30, 2015, Table 1), with some preliminary data presented in abstract form. An ongoing trial from the MD Anderson group has treated 12 patients with pre-emptive DLI of donor-derived Sleeping-Beauty transferred CD19 CAR following allo-HSCT. Three patients, all with ALL, remained alive and in remission[39].

One difference between using unmanipulated polyclonal donor derived T cells and VST-CAR T cells is that a substantially smaller percentage of the polyclonal population would be predicted to encounter TCR antigen in an allo-recipient based on the precursor frequency reported for allo-specific TCRs. With the use of VST-CAR T cells, every T cell infused could potentially encounter a TCR and CAR antigen simultaneously in the presence of a viral infection or reactivation. If there were an advantage of one receptor over another, the prediction would be a much more pronounced effect with the use of VST-CAR T cells. VST-CAR T cells seem to benefit from enhanced long term expansion due to stimulation through TCR by viral antigens as compared stimulation solely through the CAR [30,34,35], but this expansion does not necessarily correlate with CAR efficacy, suggesting a distraction through activation of the TCR. To further understand the explanation for the suboptimal results with the use of allo-CAR T cells as well as to improve such approaches in the future, there is a need to better understand basic immunobiology of T cells in the allogeneic environment, and the interaction of the CAR and the TCR.

CAR versus TCR: Immunobiology in the allogeneic recipient

The density of CAR receptors on the T cells, as well as target antigen density, are important to the functionality of CAR T cell therapy[40]. However, CAR density differs between different CAR constructs and varies heavily depending on the method used to genetically modify the T cell to express the CAR, and is not typically measured systematically in most CAR reports. The density of TCR on the T cell surface has been extensively researched, and found to be about 40,000 molecules on a single normal CD4+ T cell[41]. Investigators also found that CD8+ T cells are able to produce cytokines with as little as 500-1000 TCRs on the surface, but low receptor levels affect the potency and lytic activity of the T cell[42]. The efficacy of a CAR T cells has been reported to be co-dependent on the expression level of the CAR receptor and expression level of the target antigen[40]. In the case of a second-generation CD20-CAR T cell it was found that direct binding of hundreds of target antigens was required to activate lytic capacity and thousands of antigens interactions required before the cytokine production occurred[43]. It is possible that there are more TCRs than CARs on a surface of a given T cell, potentially resulting in a TCR advantage. TCR and CAR interactivity and dynamics are of special importance in the context of allogeneic or VST-CARs, where the presence of both antigens for a substantial proportion of T cells is likely.

The binding specificity of a CAR is typically derived from a monoclonal antibody and significant discrepancies in affinity and avidity between the CAR or the TCR and a target cell may exist thus affecting T cell efficacy mediated by either receptor. The inherently low affinity of most endogenous TCRs that survive thymic selection maintains high potency by allowing for a sufficient “on/off rate”[44–46]. There is very little known about the importance of affinity for CAR function. High affinity binding by the antibody portion may not allow for frequent engagement and disengagement thus affecting signaling through the CD3zeta chain. Thus, T cells might prefer signaling through native TCR and confer higher response against TCR antigen than CAR antigen. Indeed, investigators have studied activation thresholds of T cells transduced such that there is similar surface expression of TCR and CAR receptors demonstrating that TCRs were more active in the presence low antigen levels than cells transduced with CAR. In fact in the presence of low antigen

density, stimulation through TCR resulted in maximum INFγ secretion compared to ~50% less secretion by the CAR in the presence of low-density CAR antigen [47]. Again, these differences would be accentuated in the allogeneic environment due to the presence of both TCR and CAR antigens. Dominance of TCR signal could potentially lead to increased GVHD and lowered CAR-mediated GVL, although this has not been observed in the very limited number of patients treated with donor-derived CAR T cells as discussed above. Nonetheless, the interaction between the CAR and the TCR has not been thoroughly studied and will be critical for the optimization of CAR T cells in the clinic.

Improving donor derived CARs through T cell selection

Today, many major pharmaceutical companies are investing in the research and development of CAR T cell design and manufacturing. Ideally, a universal, off-the-shelf, product would offset the costs of individual cell preparation required in trials today[48]. These products would likely be analogous to the donor-derived CAR T cells currently being given after allogeneic HSCT in limited numbers of ongoing trials. A goal of generating off-the-shelf products would be to potentially administer these without the requirement for a prior HSCT from the same donor. To achieve this, methods to minimize the potential for donor cell rejection by the recipient and to reduce the alloreactive potential of the product are needed. The safety and efficacy of such off-the-shelf products remains to be systematically tested. Several approaches have been proposed to increase the efficacy of donor-derived allogeneic CAR T cells with the intention of developing general produced cell products available to patients not qualified for autologous infusions due to challenges such as low lymphocyte numbers or poor lymphocyte quality (and expansion *in vitro*).

A primary goal of using virus-specific T cells for CAR production is the selection of a T cell product that does not contain an alloreactive TCR. An additional advantage of CAR products with a virus-specific TCR is the ability to utilize enhanced expansion of the T cells by stimulation through the native TCR using a vaccine approach (such as the varicella-zoster virus vaccine) following adoptive CAR T cell transfer [49]. A clinical trial addressing this method is currently open for autologous anti-GD2 CAR T cells (NCT01953900).

Another method to enrich for non-allogeneic T cells is by using antigen-experienced memory T cells for CAR transduction[27,36]. It would be predicted that the vast majority of the T cell population with a memory phenotype is likely to have encountered antigens other than allogeneic antigens, and T cells carrying TCRs specific to allogeneic antigens would maintain their naive properties. Thus, selection for memory phenotype cells should enrich for a non-alloreactive repertoire. Indeed, memory T cells have been shown to have less potential to generate GVHD[50–52] in murine models, in part due to non-alloreactive TCR enrichment with evidence that memory cells are less likely to traffic to GVHD target tissues, such as the GI tract. One marker that can distinguish memory from naïve T cells is CD45RA. Several pre-clinical models have selected for CD45RA-CD62L+CD8+ central memory T cells[36] or CD45RA- T cells [27] to use for CAR production. Both techniques demonstrated good CAR transduction, in-vitro functionality and in-vivo effect. In addition, the memory CAR T cells maintained anti-leukemic ability without causing xenogeneic GVHD, a major survival limitation with the use of immunodeficient mice treated with

human T cells[27]. There are currently several clinical trials using central memory T cells for anti-CD19 CAR based immunotherapy in the autologous setting against non-Hodgkin lymphoma or ALL (NCT02051257, NCT01815749, NCT02146924, NCT2153580, NCT01318317), and another using anti-IL-13Ra2 for glioma (NCT02208362). One trial is evaluating central-memory donor-derived CD19 CAR T cells following allogeneic transplant (NCT01475058, table 1).

Another source of T cells that could be used for CAR immunotherapy is the γδ T cell subset. These T cells recognize antigens that are distinct from the protein-derived peptides that comprise allogeneic antigens recognized by the αβ TCR. Thus, γδ T cells have less allogeneic potential but have been shown to mediate anti-tumor responses. Although they consist a minority of peripheral T cells, a single subtype (vδ2) can be expanded in vivo prior to T cell collection after stimulation with zolendronic acid[53]. The MD Anderson group used a different expansion approach, with polyclonal γδ T cell transduction with CD19 CAR followed by ex-vivo expansion on an antigen-presenting layer expressing the CD19 target. This resulted in expanded polyclonal γδ T cells that demonstrated *in vitro* and *in vivo* effects in murine models, although the efficacy was not as dramatic as αβ CAR T cells in immunodeficient mice[54]. Interestingly, T cells derived from induced pluripotent stem cells (originally induced from T cells) transduced with CAR have very similar gene expression to γδ T cells, providing general as well as CAR-specific antitumor response[55]. Despite initial enthusiasm, recent discoveries demonstrate potential tumor-promoting effects of IL17-producing γδ T cells[56]. Nonetheless, should methods for efficient selection for the IFNγ producing γδ T cells be elucidated, this T cell subset will be a potential candidate for CAR T cell production.

In addition to T cells, NK cells can be used for the generation of CAR products for immunotherapy. NK cells have been shown to contribute significantly to the GVL effect, especially in haplo-identical MHC mismatched/KIR-mismatched settings[57–59]. Generally, NK Cells are considered to have lower allogenicity compared to T cells; nevertheless, NK-mediated

GVHD has been reported[60]. NK-CAR cells has shown preclinical efficacy against a variety of tumor-associated antigens [61–65]. Major challenges with the use of NK-CAR cells include low persistence inherent to NK cell immunotherapy, and complex intracellular signaling machinery potentially not compatible or optimal when using of conventional T cell activation domains. The use of specific NK cell activation domains such as DAP12[63,66] is currently being evaluated in preclinical studies.

Improving donor derived CARs through gene editing

To develop donor derived T cells without the effects of TCR activation, CAR T cells with CAR-only specificity have been generated by selectively deleting the endogenous TCR. With current gene editing technologies, the endogenous TCR could be excised through the use of nucleases such as zinc-finger nucleases (ZFNs)[67], transcription activator-like effector nucleases (TALEN)[68,69], and the CRISPR/Cas9 system[70]. The absence of an endogenous TCR eliminates the possibility of GVHD or the potential distraction of TCR receptor signaling. Using the same techniques, MHC class I could be deleted on donor-

derived, off-the-shelf T cells to avoid rejection of transferred cells[71]. Even though novel gene editing tools may prevent GVHD and rejection, significant potential issues remain to be fully tested. Absence of MHC may elicit an NK response against allogeneic T cells. Also, the functionality of a CAR containing a CD3ζ domain has been shown to be dependent on its ability to dimerize with the endogenous TCR in order to activate downstream pathways through the interactions with the TCR/CD3 complex[72,73]. The results of these studies suggest that the efficacy of CAR T cell activity and persistence could be diminished by eliminating or mutating the endogenous TCR or MHC with the use of nucleases.

Lastly, adding a suicide gene to the CAR construct may be beneficial in minimizing the risk of GVHD after allogeneic CAR T cell infusion. Inducible caspase 9 (iC9) is an intrinsic activator of apoptosis that can be transduced into allogeneic T cells that are administered following allo-HSCT and can effectively abrogate GVHD[74,75]. Preclinical models have shown that activation of the suicide iC9 in CAR therapy rescued mice from xenogeneic GVHD in a CD44v6-CAR model[76]. Ongoing clinical trials have incorporated the iC9 construct into CAR T cell products to provide a method to eliminate autologous CAR T cells in the event of potential off tumor toxicity (NCT02107963, NCT01822652, NCT02439788).

Conclusions

CAR T cells are one of the exciting achievements of current adoptive immunotherapy, with noteworthy clinical successes in treating ALL. Currently, many patients may experience lower efficacy due to general T cell defects from prior chemotherapy or allo-HSCT. The use of donor-derived cells, especially on route to off-the-shelf T cells needs to be evaluated. Many current studies address safety questions, with multiple techniques being employed to avoid GVHD. Little is known about TCR/CAR interactions and the effects TCR signaling has on therapy outcome. Better understanding of the immunobiology of CAR and TCR signaling and functionality is required before we determine the benefits or pitfalls of including or deleting the endogenous TCR for CAR therapy. Altogether, solving challenges in MHC-matched allogeneic CAR T cells will pave the way for off the shelf universal CAR T cells.

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Key Points

■ No reported GVHD with the use of donor-derived CAR T cells in patients following allo-HSCT.

■ TCR stimulation confer enhanced expansion of CAR T based on viral-specific T cells, in-vitro and in clinical trials.

■ TCR activation could be eliminated with ZFN, TALEN, or CRISPR/CAS9 technology, with potential consequences on CAR functionality.

Table 1

Current clinical trials using donor-derived CAR modified T cells

Study Center	Targeted Diseases	CAR construct	T cell origin/type	phase	NCT#
National Cancer Institute	NHL / B-ALL	2nd gen. CD19 CAR	Polyclonal T cells	Phase I	NCT01087294
Fred Hutchinson Cancer Research Center	B-ALL / NHL / CLL	2nd gen. CD19 CAR	Central memory - CMV-VST-CD8	PhaseI/II	NCT01475058
Memorial Sloan Kettering Cancer Center	B-ALL	2nd gen. CD19 CAR	EBV-VST	Phase I	NCT01430390
Baylor College of Medicine	NHL / B-ALL / CLL	2nd gen. CD19 CAR	Polyclonal T cells	Phase I	NCT02050347
University College of London	B-ALL	1st gen. CD19 CAR	EBV-VST	Phase I	NCT01195480
M.D. Anderson Cancer Center	NHL / ALL / B-ALL	CD19 CAR (unknown gen.)	Umbilical cord derived T cells	Phase I	NCT01362452