**REVIEW**

Toxicity and management in CAR T-cell therapy

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T cells can be genetically modified to target tumors through the expression of a chimeric antigen receptor (CAR). Most notably, CAR T cells have demonstrated clinical efficacy in hematologic malignancies with more modest responses when targeting solid tumors. However, CAR T cells also have the capacity to elicit expected and unexpected toxicities including: cytokine release syndrome, neurologic toxicity, “on target/off tumor” recognition, and anaphylaxis. Theoretical toxicities including clonal expansion secondary to insertional oncogenesis, graft versus host disease, and off-target antigen recognition have not been clinically evident. Abrogating toxicity has become a critical step in the successful application of this emerging technology. To this end, we review the reported and theoretical toxicities of CAR T cells and their management.

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**INTRODUCTION**

Adoptive cellular therapy (ACT) targeting malignant cells was first described decades ago in a xenogeneic murine model. Translation of this finding to human malignancies has long been a goal for cancer immunotherapy. Administration of allogeneic T cells as a component of hematopoietic stem cell transplantation (HSCT) or through donor lymphocyte infusion has been utilized to generate graft versus leukemia response secondary to human leukocyte antigen disparity in leukemia patients. Limiting this therapy is the propensity to develop graft versus host disease (GVHD) when utilizing unsel ected donor T cells. In solid tumors, the isolation and infusion of autologous tumor-infiltrating lymphocytes has shown remarkable clinical results in patients with melanoma, demonstrating the potential of ACT without the risk of GVHD. The process by which tumor-infiltrating lymphocytes are isolated and expanded is technically difficult, labor intensive, and time consuming, necessitating a method to more rapidly generate a pool of tumor-specific effectors. In response, advances in ex-vivo growth and genetic engineering of T cells have enabled rapid generation of effector cells with selectivity for tumor-associated antigens, thereby broadening applicability for cancer immunotherapy. The genetic modification of T cells to confer tumor antigen recognition is typically through transgenic expression of a high-affinity T-cell receptor or a chimeric antigen receptor (CAR).

The most common and reliable method of genetic delivery is via lentiviral and γ-retroviral-based transduction methods. These allow for stable integration with prolonged expression of the desired transgene. Alternative technologies with a goal of less durable integration and expression include electroporation as well as transposon/transposase delivery systems. T cells that are genetically modified to express a high-affinity T-cell receptor rely upon human leukocyte antigen-matched antigen presentation, which limits applicability to a diverse patient population. Additionally, given their recognition of small peptide epitopes, there exists the potential of cross-reactivity with an array of normal antigens. Alternatively, antigen recognition by CARs can be independent of human leukocyte antigen and typically occurs by engagement with larger epitopes imparting less risk of cross-reactivity. For these reasons, CAR modification of T cells may ultimately be more advantageous.

The precision with which engineered immune cells recognize targets has the potential to decrease the general toxicity traditionally seen with conventional chemotherapeutic agents. However, severe immune-mediated adverse events following CAR T-cell infusion have been appreciated. Unique to cellular therapies is the extraordinary long-term persistence of up to 10 years with ACT in human trials. This persistence extends the timeline of potential toxicities far beyond that of conventional small-molecule pharmaceuticals. Adverse events following T cell-based therapies may be immediate, delayed, mild, severe, and/or persist for the duration of the genetically modified T-cell lifespan. The focus of this review will be on the reported toxicities following CAR T-cell infusion. In an effort to provide the reader a comprehensive description of toxicities, theoretical and potential sequelae of CAR T cells will also be included (Figure 1). Preventing or managing unwanted toxicity has therefore emerged as a key component in the successful clinical application of this novel technology. Ideally, prediction of per-patient severity and onset would allow consideration of prophylactic therapy to guard against toxicity with resultant improved management.

**TOXICITIES OF CAR T-CELL THERAPY**

Cytokine release syndrome (CRS)

To date, the most prevalent adverse effect following infusion of CAR T cells is the onset of immune activation, known as CRS. CRS has

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also been seen following the infusion of therapeutic monoclonal antibodies (mAbs), systemic interleukin-2 (IL-2), and the bispecific CD19-CD3 T-cell engaging antibody blinatumomab. In the setting of early CAR T-cell trials utilizing “first-generation” constructs (without costimulatory signaling elements), insufficient T-cell proliferation/cytokine production and lack of antitumor response were noted. The addition of costimulatory signaling in second-generation CAR design (CD28 or 41BB) translated to improved T-cell activation/expansion, cytokine production, and most notably dramatic antitumor responses in patients with hematologic malignancies. However, the “double-edged sword” of CAR T cells is demonstrated in the similarly impressive and potentially life-threatening CRS following CAR T-cell administration.

The hallmark of CRS is immune activation resulting in elevated inflammatory cytokines. Clinical and laboratory measures range from mild CRS (constitutional symptoms and/or grade-2 organ toxicity) to severe CRS (sCRS; grade ≥3 organ toxicity, aggressive clinical intervention, and/or potentially life threatening). Clinical features include: high fever, malaise, fatigue, myalgia, nausea, anorexia, tachycardia/hypotension, capillary leak, cardiac dysfunction, renal impairment, hepatic failure, and disseminated intravascular coagulation. Dramatic elevations of cytokines including interferon-gamma, granulocyte macrophage colony-stimulating factor, IL-10, and IL-6 have been shown following CAR T-cell infusion. The cost and technical difficulties inherent to “real-time” monitoring of serum cytokines have precluded the clinical application of this methodology to identify evolving CRS. Currently, under investigation is the use of C-reactive protein, which is made by hepatocytes in response to IL-6, as a laboratory marker of CRS onset and severity. The presence of CRS generally correlates with expansion and progressive immune activation of adoptively transferred cells. It has been demonstrated that the degree of CRS severity is dictated by disease burden at the time of infusion as patients with high tumor burden experience a more sCRS. In reports of patients treated with CD19-specific CAR T cells for relapsed/refractory B-cell acute lymphoblastic leukemia, the incidence of sCRS has ranged from 19% to 43%, with variability likely due to differences in clinical identification of the syndrome, chimeric receptor designs, and infused cellular phenotypes. Clinical outcome is not predicated on the development of sCRS as patients may exhibit an antitumor response in the absence of this toxicity. However, in the context of hematologic malignancies the majority of patients who respond exhibit at least mild CRS (fever) following CAR T-cell infusion.

Following diagnosis of CRS, a challenge has been choosing appropriate therapy to mitigate the physiological symptoms of uncontrolled inflammation without dampening the antitumor efficacy of the engineered cells. Systemic corticosteroid has been shown to rapidly reverse symptoms of sCRS without compromising initial antitumor response. However, prolonged use (e.g., >14 days) of high-dose corticosteroids has also resulted in ablation of the adoptively transferred CAR T-cell population potentially limiting their long-term antileukemia effect. As an effective alternative IL-6 receptor (IL-6R) blockade with the Food and Drug Administration-approved mAb, tocilizumab has demonstrated near-immediate reversal of CRS. Investigators are now determining the effect of IL-6R blockade on CAR T-cell proliferation, persistence, and most importantly, antitumor effect. Despite this unknown, the use of IL-6R blockade has generally been accepted as front-line treatment for sCRS following CAR T-cell infusion. It is also unclear whether blocking other cytokine/receptor partners would effectively treat CRS and maintain antitumor efficacy. Future studies are warranted and ongoing.
Neurological toxicity
The development of neurologic toxicities including confusion, delirium, expressive aphasia, obtundation, myoclonus, and seizure has been reported in patients receiving CD19-specific CAR T cells. The causative pathophysiology of these neurologic side effects is unknown, though given similar events reported with blinatumomab administration, it is plausible that elevated cytokine levels are partly responsible for the neurologic sequelae. Conversely, direct CAR T-cell toxicity on the central nervous system is possible but has not been demonstrated. Patient correlates have not been informative as reports have conflicted on the correlation between the number of engineered or nonengineered T cells in spinal fluid and status of central nervous system leukemia with neurological complications. Similarly, EEG traces have not reliably identified seizure activity despite clinical symptoms demonstrating such activity. To date, the neurologic toxicity has been reversible in a majority of cases and it is unclear if this toxicity is restricted to CD19-specific CAR T cells or will be exhibited by the targeting of other tumor-associated antigens.

On-target/off-tumor recognition
The ideal target antigen is restricted to the tumor cell and provides a critical survival signal for the malignant clone. Unfortunately, most targets of CAR T cells have shared expression on normal tissues and some degree of “on-target/off-tumor” toxicity occurs through engagement of target antigen on nonpathogenic tissues. The severity of reported events has ranged from manageable lineage depletion (B-cell aplasia) to severe toxicity (death). “On-target/off-tumor” recognition is predictably seen in a variety of organ systems, including gastrointestinal, hematologic, and pulmonary. One of the earliest trials utilizing a carboxyhydrate-IX-specific CAR T cell for renal cell carcinoma resulted in the development of cholestasis due to expression of carboxyhydrate-IX on bile duct epithelium. Targeting of carcinoembryonic antigen by CAR T cells in patients with colon cancer resulted in severe, albeit transient, colitis due to antigen recognition of normal colonic tissue. In the setting of CD19-specific CAR T cells, the targeting of normal B cells results in B-cell aplasia which may require intermittent infusion of pooled immunoglobulin as prophylaxis from infectious complications. CAR T cells or will be exhibited by the targeting of other tumor-associated antigens.

Insertional oncogenesis
The risk of insertional oncogenesis in human cells has been established in the context of gene therapy of hematopoietic stem cells for X-linked severe combined immunodeficiency and chronic granulomatous disease. In the majority of cases retroviral vector insertion near the LMO-2 oncogene has been implicated. Insertion of a transgene into differentiated T cells also carries the risk of induced malignant transformation. However, to date, no cases of transformation have been reported following infusion of genetically modified T cells. Notably, the LMO-2 oncogene is silent in T cells making this site an unlikely locus of retroviral integration. In practice, the use of genetically modified T cells has a decade-long safety profile without evidence of vector-induced immortalization, clonal expansion, or enrichment for integration sites near genes implicated in growth control or transformation. Taken together, the risk of insertional oncogenesis following gene transfer into T cells is seemingly low; however, investigators must remain vigilant and adhere to strict monitoring set forth in current clinical trial design.

Graft versus host disease
ACT with autologous/patient-derived tumor-specific CAR T cells has demonstrated clinical benefit for patients with cancer. In the context of CD19-specific CAR T cells, a number of patients have been treated following allo-HSCT with confirmation that infused CAR T cells were donor in origin. Despite the risk of alloreactivity, CD19-specific CAR T cells collected post allo-HSCT have not demonstrated the propensity to induce GVHD. Even more impressive is the lack of reported GVHD in a study utilizing CD19-specific CAR T cells generated from allogeneic donors in adult patients with relapsed/refractory B-cell malignancies following allo-HSCT. In this study, patients were required to tolerate donor lymphocyte infusion without evidence of GVHD prior to donor-derived CAR T-cell infusion. It is unclear, if this selection resulted in a population of patients/donors with mitigated risk for alloreactivity. Despite this proven safety, the generation of a “personalized” product (patient or allo-HSCT donor derived) on a patient-by-patient basis is time consuming and expensive. Establishment of an “off-the-shelf” or “third-party” cell bank is an attractive solution with the possibility of reducing time to treatment and cost. Two methodologies are currently at the forefront of these efforts: CAR-transduced viral-specific cells and endogenous T-cell receptor silencing. Studies utilizing these technologies are in process and may ultimately broaden the applicability of engineered cell technology.

Off-target antigen recognition
The majority of CAR T cells recognize antigen through single-chain variable fragments derived from mAbs. For some, the corresponding mesothelin-specific CAR T cells. In this report, one of four patients treated with multiple infusions of mesothelin-specific CAR T cells developed cardiorespiratory failure at the conclusion of the third infusion. The design of this study employed multiple infusions of T cells expressing a transient CAR (mRNA vector) in an effort to reduce “on-target/off tumor” toxicity. Detailed investigation following the cardiorespiratory event confirmed the presence of human antimouse antibodies and elevated trypsin in the patient’s serum supporting the finding of an IgE-mediated anaphylactic event. Diligent surveillance, prompt recognition, and immediate treatment of this life-threatening side effect are critical for patients receiving genetically modified T cells.
mAb has established a proven safety record in clinical use. For others, the toxicity profile is uncertain. Confounding this is a discrepancy when comparing mAb to CAR T-cell binding, as illustrated in the case of trastuzumab (anti-HER2/neu). The toxicity profile of the mAb (cardiotoxicity) is not mirrored by the toxicity profile of HER-2/neu-specific CAR T cells. In addition to target-antigen binding, there is a distinct possibility of off-target antigen recognition by genetically modified tumor-specific T cells. In cases where “first-in-human” antigen recognition motifs are used, prediction of off-target antigen recognition and subsequent toxicities is exquisitely difficult. To date, off-target recognition of cross-reactive antigens has not been evident in CAR T-cell trials. However, fatal cardiac toxicity due to off-target reactivity has been seen in 2/2 patients infused with autologous T cells engineered to express an enhanced affinity T-cell receptor directed against the cancer testis antigen MAGE-A3. Cross reactivity occurred against titin, a protein for which expression is only evident during contraction and expansion of cardiac tissue. This finding illustrates the difficulty in predicting off-target antigen toxicity especially in the case of complex antigen presentation at different stages of cellular differentiation and activity. To this end, diligent surveillance and prompt recognition of any potential toxicity is a requisite when CART cells target novel tumor-associated antigens.

TOXICITY MANAGEMENT
In hematologic malignancies, significant clinical benefit in targeting of CD19 antigen on malignant B cells has been demonstrated. However, the intense immune activation has resulted in unwanted side effects despite optimism that direct targeting of tumor cells would avoid systemic toxicity. Management of these toxicities has become an integral step in the successful clinical application of CAR T cells. Several methods have been proposed to ameliorate toxicity, including nonspecific immune suppression or selective depletion of modified cells through “suicide” or “elimination” genes (Figure 2). An obvious caveat to any suppression or elimination of the tumor-specific cell population would be the concurrent abrogation of any persistent antitumor surveillance.

Pharmacological Immunosuppression
As noted, the most common toxicity following CD19-specific CAR T-cell infusion has been uncontrolled immune activation in the form of CRS. The use of tocilizumab to provide IL-6R blockade has demonstrated near-immediate reversal of CRS symptomatology including fever and hypotension. The impact of IL-6R blockade on the neurologic sequelae following CD19-specific CART cells is unknown. Immunosuppression with systemic corticosteroid can also improve the symptoms of CRS, with dexamethasone as a logical first choice agent due to its superior central nervous system penetration. Not surprisingly, the use of prolonged systemic corticosteroids has been shown to diminish the persistence and potentially, the efficacy of CAR T cells. Alternative immunosuppression with cell-specific mAbs or lymphodepleting chemotherapy (cyclophosphamide) is theoretically possible but as of yet, untested or reported.

Figure 2  Management of chimeric antigen receptor (CAR) T-cell toxicity. (a) CAR T cells can be further engineered to express “suicide genes” or “elimination genes” such as herpes simplex virus thymidine kinase (HSV-tk) which converts the prodig drug ganciclovir (GCV) into GCV-triphosphate resulting in cell death by incorporation into replicating DNA; inducible caspase 9 (iCasp9) a chimeric protein that binds the small molecule AP1903, leading to caspase 9 dimerization and ultimate apoptosis; truncated endothelial growth factor receptor (tEGFR) which is a targetable antigen which allows for elimination of modified cells following infusion of associated anti-EGFR MAb (cetuximab). (b) Pharmacological immunosuppression will ameliorate toxicity from CART including blockade of IL-6R and/or treatment with systemic corticosteroid. ADCC, antibody-dependent cellular cytotoxicity.
and successfully reported in preclinical or clinical testing. The first suicide gene evaluated in human trials was the herpes simplex virus thymidine kinase whose expression renders modified cells susceptible to treatment with the acyclic nucleoside analog ganciclovir. Once expressed, herpes simplex virus thymidine kinase catalyzes phosphorylation of ganciclovir resulting in competitive inhibition of guanosine incorporation with subsequent disruption of DNA polymerization and synthesis. While effective, this approach is limited by the immunogenicity of herpes simplex virus thymidine kinase expression with resultant rejection of modified cells. Secondly, reliance on inhibition of DNA replication as a method of cell death may delay clinical benefit. Furthermore, the widely used antiviral ganciclovir is precluded from therapeutic use as administration would result in toxicity to modified cells. Another approach utilizes the transgenic introduction of a mutated thymidylate kinase responsible for phosphorylation of the HIV produg 3′-azido-3′-deoxythymidine with subsequent DNA chain termination and cell death in modified cells. However, this system was proven to have suboptimal efficacy when compared with herpes simplex virus thymidine kinase.

Selective depletion of genetically modified cells can also occur through the dimerizable death molecules inducible Fas or caspase 9 (ICasp9). These dimerizable chimeric elements are engineered to contain components of an FK506-binding protein with cross-linking triggered by exposure to an otherwise inert bivalent small molecule. In the case of both inducible Fas and ICasp9, dimerization leads to activation of downstream caspases with induction of the apoptotic pathway. As a proof-of-principal, ICasp9-modified donor T cells were evaluated in haploidentical HSCT recipients. A single dose of a small-molecule dimerizing agent (AP1903) eliminated more than 90% of ICasp9-modified T cells within 30 minutes of administration. This rapid onset of action without adverse event resulted in reversal of GVHD without recurrence. Despite these dramatic results, when ICasp9 is introduced into other engineered T-cell platforms, the minority cell population unaffected by suicide gene activation may perpetuate toxicity. Conversely, there is a possibility of tonic ICasp9 dimerization in the absence of the small-molecule dimerizing agent, seen in transduced cell lines in vitro. The resultant increase in basal apoptosis potentially limits widespread utility.

Elimination genes

Another method to induce selective depletion of genetically modified cells is the expression of a targetable moiety. Modified cells can be programmed to express a known cell-surface antigen, such as CD20 or EGFR, with subsequent cell death triggered via infusion of the associated mAb (rituximab for CD20 and cetuximab for EGFR). This is an attractive strategy given the familiarity of clinicians with the use and safety profile of several Food and Drug Administration-approved mAbs. Expression of known antigens on the surface of CAR T cells also enables for the selection (ex vivo) and tracking (in vivo) of genetically modified cell populations. Factors limiting this methodology are any on-target side effects inherent to mAb binding to normal tissue as well as dependence on antibody-dependent cellular cytotoxicity for clearance of the cell population. Heavily pretreated cancer patients with dysfunctional immune systems may also have limited ability to remove unwanted populations of genetically modified cells, especially in the setting of toxicity. In a robust preclinical comparison of various safety switch technologies, the IC9 system and the combination of CD20 and rituximab were the methods most likely to have translatable utility based on both rapid onset of action and superior efficacy.

Targeted activation

Functional control of the intensity or toxicity of T-cell activation is possible through the inclusion of an “on-switch” in CAR design. T-cell response can be controlled through combinatorial antigen targeting with separation of T-cell activation signals. This requires the identification of two target antigens with coexpression on malignant tissue for T-cell activation. Dual-antigen binding is then necessary for complete T-cell activation. In contrast, normal tissue (expressing one target antigen) provides incomplete activation thereby limiting “on-target/off tumor” toxicity. Alternatively, if presentation of dual antigens is exclusive to normal tissue, inclusion of inhibitory signaling in CAR design allows for selective targeting of malignant/pathogenic tissue (expressing one antigen) while normal tissue is spared. Finally, separation of the antigen recognition moiety of a CAR from the intracellular signaling domain is a strategy that promises exquisite control by incorporating heterodimerizable elements responsive to small-molecule binding. This approach has the potential to control T cell activation and toxicity through titration of the small-molecule “on-switch.”

CONCLUSION

The utility of CAR T cells as ACT for the treatment of malignancy will depend both on the ability to easily manufacture the cellular product as well as the feasibility of safe administration. Given the dramatic responses seen in hematological malignancies, we should attempt to diminish the barriers to widespread access and perfect the response seen in solid tumors. Toxicity management should necessarily become a focus of implementation to allow for administration beyond specialized centers. Improved understanding of the immunological response following CAR T-cell infusion will improve clinical management and enhance our investigation into activation or elimination of CAR T cells thereby reducing hazards following infusion. It is our hope that toxicities will be anticipated and manageable, allowing for improved quality and universal benefit.

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