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EMERGING IMMUNOTHERAPIES FOR HEMATOLOGIC DISEASES

Defining success with cellular therapeutics: the current landscape for clinical end point and toxicity analysis

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Cellular therapies play a major and expanding role in the treatment of hematologic diseases. For each of these therapies, a narrow therapeutic window exists, where efficacy is maximized and toxicities minimized. This review focuses on one of the most established cellular therapies, hematopoietic stem cell transplant, and one of the newest cellular therapies, chimeric antigen receptor–T cells. In this review, I will discuss the current state of the field for clinical end point analysis with each of these therapeutics, including their critical toxicities, and focus on the major elements of success for each of these complex treatments for hematologic disease. (*Blood*. 2018;131(24):2630-2639)

Introduction

The scope of cellular therapeutics is broad and expanding. Although adoptive T-cell therapeutics (in particular, chimeric antigen receptor T [CAR-T] cells) have garnered the lion's share of attention in the past several years, these newly US Food and Drug Administration (FDA)-approved therapeutics still represent a small proportion of the entire scope of cellular therapies in clinical use, and those undergoing clinical investigation for patients with hematologic diseases. Indeed, the cellular therapy in widest use remains hematopoietic stem cell transplant (HCT), with many new initiatives in graft manipulation designed to increase efficacy and decrease transplant-associated complications. In addition to CAR-T therapies, there are also a number of other T-cell therapies that do not involve CARs (most prominently including antiviral T cells as well as T-cell therapeutics using tumor-specific T-cell receptors), as well other effector cells (particularly natural killer cells) designed to reduce malignant relapse. Finally, there are the regulatory cellular therapies, most prominently including $CD4^+FoxP3^+$ regulatory T cells, as well as a number of other suppressive cell-based therapies (including mesenchymal stromal cells, myeloid-derived suppressor cells, and type 1 regulatory T cells, among others). For each of these therapies, a critical therapeutic window exists, where efficacy is maximized and toxicities minimized. This review will focus on HCT and CAR-T-cell therapies, in which this therapeutic window can be small, and in which toxicities, when they occur, can be fatal. It will provide an overview of the state of the art in monitoring clinical efficacy and toxicity for each, and the surrogate markers that are most useful for interrogating their attendant risk-to-benefit ratios.

The elements of transplant success

HCT is a complex procedure with multiple stages, and at each stage, critical elements exist that define success or failure. It is useful to divide the transplant process into 4 phases: (1) transplant conditioning; (2) day of transplant; (3) preengraftment; (4) postengraftment. The gold standard of transplant success, encompassing success at all of the stages, is a patient who, a year posttransplant (and thereafter), is alive and in remission and has successfully reconstituted protective immunity without graft-versus-host disease (GVHD). The key clinical end points that encompass each of these milestones and the accompanying surrogate markers (if they exist) are discussed in the following sections.

Transplant conditioning

Conditioning for transplant can be divided into regimens that are expected to completely (or nearly completely) ablate the recipient marrow ("myeloablative") and those that would be considered nonmyeloablative and thus are not expected, in themselves, to ablate the marrow. Of the myeloablative-conditioning strategies, some are less toxic than others, with these less-toxic strategies deemed "reduced-intensity" or "reduced-toxicity" conditioning, in contrast to the standard myeloablative total body irradiation– and high-dose chemotherapy–based strategies. Although conditioning is a critical component to transplant, there are no standard surrogate markers for conditioning success, other than the eventual engraftment of donor marrow. However, a robust comorbidity index exists that helps to stratify patients for their risk of transplant-mediated toxicities and for transplant-related mortality, thereby informing decision-making about conditioning intensity.[1,2] The well-described impact of comorbidities on nonrelapse mortality (NRM) and the increasing average age of patients undergoing HCT (as well as the well-recognized increase in NRM in patients younger than 1 year of age who are often transplanted for genetic diseases) are driving research into more targeted, less globally toxic conditioning regimens. One of the most exciting strategies involves the use of targeted monoclonal antibodies, most prominently those targeting c-kit (CD117), CD47, and CD45, studies of which have recently been published by groups at the Fred Hutchinson Cancer Research Center,[3-5] Stanford University,[6] and Harvard University.[7] Although these antibodies are not specific for hematopoietic stem cells, off-target toxicity has not been a major problem to date. Although clinical

trials using these new agents are just beginning, the advent of these antibody-based approaches represents a major advance, and ushers in a new era of nongenotoxic transplantation.

Day of transplant

Transplant day (day 0) is the shortest phase of every transplant, but potentially the most important in terms of determining transplant success or failure. The prevailing graft sources (bone marrow, peripheral blood stem cells, cord blood) and the spectrum of HLA matching all make a major impact on transplant outcome, but are beyond the scope of this review. This review will focus on graft engineering, as it is this maneuver that ties HCT most closely with other cellular therapies.

One of the major strategies for graft manipulation, which has been used for several decades, is T-cell depletion. Ex vivo T-cell depletion was first applied to many graft types in the 1980s,[8-18] and gained traction with HLA-haploidentical HCT, given the significant risk of severe GVHD that occurred with haplo-HCT in the pre-posttransplant cyclophosphamide era.[19-22] This strategy was initially performed by removal of T cells from the graft,[23] and is now accomplished by positive selection of $CD34^+$ cells.[24] Although this strategy has been highly effective in controlling GVHD, other complications, including graft loss, malignant relapse, and infectious complications remain significant issues, although there are several important studies from groups in Perugia, Italy, and at the Memorial Sloan Kettering Cancer Center (MSKCC) in which relapse rates were not increased despite the control of both acute and chronic GVHD.[22,25-27]

Given the ongoing concerns with whole-scale T-cell depletion (especially without high-intensity pretransplant conditioning), recent work has concentrated on a more nuanced approach, which involves removal of the $\alpha\beta^+$ T cells[28,29] (those that are most implicated in alloreactivity) while preserving $\gamma\delta$ T cells, natural killer cells, monocytes, and dendritic cells,[28-30] thus better preserving antimicrobial protective immunity and graft-versus-leukemia.[31-34] Another approach that has been added to the $\alpha\beta-$T-cell depletion strategy has been the gene modification of T cells by the gene encoding caspase-9, iC9,[35,36] which renders them susceptible to ablation with the dimerizing agent AP1903,[36-39] and the subsequent addition of titrated amounts of these ablatable T cells posttransplant.[40] Initial results have been encouraging[41] and a number of larger trials are ongoing to fully evaluate this approach. The final strategy for graft engineering that will be discussed is naive T-cell depletion. This approach was based on substantial preclinical data supporting the critical role that naive T cells play in GVHD in murine studies.[42-44] Based on these results, several groups have begun clinical investigations using this strategy.[45-48] The results from the Fred Hutchinson Cancer Research Center have documented a striking decrease in chronic GVHD despite no substantial impact on acute GVHD when naive T-cell–depleted peripheral blood stem cells were transplanted after a high-intensity conditioning regimen.[47,48] The impact on chronic GVHD was encouraging, and larger, multicenter trials of this graft-engineering approach are currently under way.

Preengraftment, hematologic engraftment, and immune reconstitution

In the preengraftment period, there are a number of significant risks, associated both with the ongoing toxicities of conditioning and with the inherent risks of pancytopenia. In this phase of transplant, the surrogate markers are well established, and include the day of neutrophil engraftment, the day of platelet engraftment, and the degree of donor chimerism (with day 30 assessments standardly performed). Although the concept of successful engraftment has historically been associated with the rise in neutrophil count (and its attendant decrease in the risk of serious bacterial and fungal infection), there has been less certainty about what constitutes fully functional hematologic and immunologic reconstitution. In particular, surrogate markers for successful immunologic reconstitution, as it relates to lymphocyte engraftment, antiviral immunity, and the impact on late-onset immune dysregulation have not yet been established. Although historically a CD4 count of $>200 \times 10^3$ cells per μL had been implicated as at least partially protective against some viral infections,[49] our understanding of the pace and character of effective immune reconstitution is becoming more comprehensive, as has been our understanding of the impact that latent viruses can make on this reconstitution. Thus, recent work from my laboratory and others in both unrelated donor and haploidentical HCT has documented the significant quantitative and qualitative effect that cytomegalovirus (CMV) reactivation can make on posttransplant T-cell expansion.[50,51] Of note, this work has underscored the fact that, although CMV is the major driver of the quantitative reconstitution of effector CD8 cells, this does not necessarily equate to functional competence. Indeed, our calculations of the number of deficits in the T-cell repertoire in patients who reactivated CMV compared with those who did not suggest that CMV-reactivating patients may have significant defects in T-cell–mediated protective immunity.[50] This observation is consistent with a recent study from MSKCC, which focused on T-cell–depleted HCT, and demonstrated that functional competence of T cells after mitogen stimulation is more predictive of survival advantage after HCT than quantitative lymphocyte expansion.[52] This functional competence, and the diversity of the T-cell repertoire, can also be linked to thymic health after transplant.[53-59] The surrogate markers for this competence are also becoming more sophisticated and include T-cell receptor excision circle assays, naive T-cell regeneration, and T-cell receptor repertoire diversity in both naive and antigen-experienced CD4 and CD8 T-cell populations.[54-59]

Postneutrophil engraftment

The period after neutrophil engraftment and through the first year posttransplant is dominated by 2 major toxicities, each of which there has been the subject of major initiatives to identify surrogate markers to help guide preemptive and treatment strategies. The first toxicity, GVHD, represents the major cause of NRM for the vast majority of transplant paradigms. Although clinical staging and grading of both acute and chronic GVHD are well established, our ability to predict and develop accurate prognoses for patients with GVHD is still in its infancy. Given the importance of preventing and treating GVHD, over the past decade, there has been a major emphasis on developing biomarkers for this disease. For acute GVHD, much progress has been made in identifying a panel of serum biomarkers that can risk-stratify patients who develop acute GVHD.[60-73] This work has now matured to the point where clinical trials can incorporate biomarkers as well as sophisticated clinical staging[74] to risk-stratify patients for treatment studies, representing a major step forward for the field. The field of chronic GVHD is also making strides in the areas of soluble biomarkers,[75-85] although

these are not yet as robust as those for acute GVHD, likely due to the inherent complexity of chronic GVHD, in terms of its timing, clinical presentation, and underlying immunopathologic drivers. There is also a growing focus in the field in identifying cellular biomarkers of this disease. This work has been driven by detailed flow cytometric evaluation of the T- and B-cell subpopulations driving both acute and chronic disease,[86-102] as well as increasing use of systems-based transcriptomic approaches to identifying the pathways associated with GVHD, both in preclinical models and clinical samples.[50,103-107] This work is identifying a new cohort of targetable pathways for GVHD control, many of which are amenable for clinical translation.

Although GVHD is the most significant cause of NRM after HCT, the primary cause of death after transplant for patients with leukemia or lymphoma remains relapse of their primary disease. The issue of disease relapse, and the development of strategies to prevent and/or treat relapse has undergone an explosion of activity in the 5 five years, based on the landmark success of cellular therapies designed to eliminate malignancies. The most striking successes have been with the CAR-T-cell therapies targeting the CD19 antigen. However, as with other cellular therapies, CAR-T cells are associated with a complex risk-to-benefit profile.

CAR-T cells: new efficacy, new toxicities

For patients with relapsed leukemia, and for those with primary refractory disease, the outlook with conventional chemotherapy remains dismal. In the past 5 years, a subset of these patients, predominantly including those with CD19-expressing acute lymphoblastic leukemia (ALL), have been successfully treated with CD19-redirected CAR-T cells, which has represented a watershed moment for cellular therapeutics. This success has been built on decades of research and the development of both immune-engineering and gene transfer capabilities, with the first successful trials published in pediatric B-cell ALL in 2013,[108] with multiple follow-up studies now completed. The striking efficacy of this therapeutic strategy has led to the rapid approval of 2 CAR-T therapies: tisagenlecleucel and axicabtagene ciloleucel, with more approvals expected. Although the vast majority of CAR-T-cell trials for hematologic diseases have focused on CD19-redirected T cells, there are a number of new trials targeting other B-cell antigens (including CD20, CD22, CD30, and B-cell maturation antigen[109-112]), T-cell antigens, as well as early trials targeting antigens associated with acute myeloid leukemia.[113-124]

The elements of success with CAR-T therapies

As with HCT, CAR-T-cell therapies are composed of multiple stages, and at each stage, critical elements exist that contribute to success or failure. It is useful to divide CAR-T cellular therapies into 4 distinct stages: (1) patient selection and cell manufacturing; (2) preinfusion chemotherapy; (3) remission-induction and CAR-T–associated toxicities; and (4) postremission therapeutic strategies. Given the early stage of the field of CAR-T cellular therapeutics, there is still considerable debate about what constitutes success in this field. Thus far, the definition of success has focused on remission induction, with many patients receiving CAR-T cells going on to have further consolidative therapy (most commonly including HCT). Indeed, the reimbursement strategy for the first FDA-approved CD19-CAR-T product, tisagenlecleucel, includes charging for this therapy only if successful remission (but not long-term cure) is achieved. However, as CAR-Ts are more widely used, and used earlier in the treatment pipeline, these definitions of success will need to evolve, and critical questions about the acceptability of CAR-T–associated toxicities, and the need for additional treatment after CAR-T therapy (and how to select patients that do or do not need further treatment) will need further refinement.

Cell manufacturing and patient selection

Although there is growing interest in the development of universal, "off-the-shelf" CAR-T products,[125,126] the prevailing paradigm for CAR-T-cell manufacture currently relies on the creation of a patient-specific product. To manufacture this product, T cells need to be obtained from the patient (usually by apheresis) and then expanded ex vivo to reach the desired infusion dose. This process can be challenging, especially in the heavily pretreated patient populations currently being treated with CAR-Ts. Although the first studies of CAR-Ts included a relatively high rate of failure to successfully manufacture a CAR-T product (and included a preexpansion feasibility assessment, which eliminated significant numbers of prospective patients),[127] newer studies have documented high rates of success (>95% for ALL and >89% for neuroblastoma in the largest study of manufacturing efficacy to date).[128]

The composition of CAR-T-cell products has also evolved, although there is not yet a recognized gold standard. Two key elements of CAR-T composition need to be considered: (1) the structure of the CAR construct itself, and (2) the cellular composition of the infused product. (1) The structure of the CAR transgene has evolved from a "first-generation" structure, which expressed the CD3-ζ signaling domain, to "second-generation" CARs, in which one of many possible costimulatory signals (the 2 most common being CD28 and 41BB signals) were added to CD3-ζ, to "third-generation" CARs, which contain 2 costimulatory signals in tandem (CAR construct structure reviewed in June et al[129]). Although the identity of the costimulatory domains have gained the most attention of late, there are multiple other considerations in engineering an optimal CAR construct, and a comprehensive discussion of these is beyond the scope of this review. These engineering considerations have been recently reviewed by Srivastava and Riddell, Gomes-Silva and Ramos, and Lim and June.[130-132] (2) The cellular composition of the CAR-T-cell infusion is similarly complex and is also rapidly evolving. The original CAR-T infusions contained unselected products from expansion cultures; thus, patient-specific variability in terms of CD4:CD8 ratio, naive/central/effector memory composition, and the proportion of CAR-T–transduced cells ensued. Given the enhanced ability of central memory T cells to expand and persist after adoptive transfer,[133-136] several studies have enriched CAR-T products for this subpopulation. Although current CAR-T infusion strategies often do not include the purification of specific memory subpopulations, CAR-T culture conditions optimized separately for CD4 and CD8 expansion have been developed that are also geared toward producing a cell product with a defined CD4:CD8 ratio, and with optimized expansion and persistence characteristics.[137,138] Although many strategies for defining and optimizing CAR-T products exist, a gold standard for this aspect of CAR-T preparation has not been

established, with wide variation in the culture conditions and final composition of CAR-T-cell products. It is also important to consider the fact that in any CAR-T production process, not all T cells are successfully transduced with the CAR-T construct. Therefore, most manufacturing processes now include enrichment and/or selection for the transduced cells, such that the infused product does not contain a surfeit of non-CAR-Ts. Importantly, although boutique manufacturing processes have, in the past, been able to handle the demand for CAR-Ts, as the number of products needed grows, more industrial, "untouched" manufacture processes will be required, and creating a system for CAR-T manufacturing that can more closely resemble a blood bank than a research laboratory (without compromising efficacy) will be a critical area of future development.

Preinfusion chemotherapy

To optimize CAR-T-cell expansion after infusion, a state of lymphodepletion is usually induced prior to cell delivery. This approach capitalizes on the well-documented phenomenon of homeostatic T-cell proliferation, which leads to T-cell expansion, activation, and memory differentiation.[139-141] Initial studies often used cyclophosphamide alone for lymphodepletion, but enhanced expansion and persistence has been documented when cyclophosphamide is combined with other agents, most commonly including fludarabine. Although there has been some concern that fludarabine may have increased the risk for neurotoxicity (given its known association with neurologic events at very high doses), which is observed after CAR-T therapy (discussed in "CRES"), the predominance of the data argues against this association being causative,[142,143] and cyclophosphamide/fludarabine chemotherapeutic regimens are widely used with both CD19-directed and other CAR-T therapies. The goal of preinfusion chemotherapy is twofold: (1) to deplete endogenous T cells that might increase the risk of T-mediated rejection of CAR-T cells,[144] and (2) to enhance CAR-T expansion in the lymphodepleted host.[139-141]

Remission induction and associated toxicities

One of the most remarkable results observed with CAR-T-cell therapies against B-cell malignancies has been the high rates of complete remission (CR) that have been observed (over 90% in some studies),[108,136-138,144-155] which are particularly striking given the high-risk patient populations that have been treated. However, despite the successful remission induction with CD19 CAR-Ts, these cells have also been associated with significant toxicities. These include both a cytokine release syndrome (CRS) and a neurotoxicity syndrome (newly termed CAR-T–related encephalopathy syndrome [CRES]). CRS was the first major toxicity measured in patients treated with CD19-CAR-T cells, and remains the most commonly observed toxicity.[108] This entity encompasses a large number of signs and symptoms, which range from low-grade fever and constitutional symptoms to life-threatening multiorgan dysfunction, high fever, and hypotension. Neurotoxicity, or CRES, is the more rare, but most deadly complication of CAR-T cells, and is characterized by confusion, delirium, language disturbance, seizures, and cerebral edema. Given the potential severity of both of these complications, there have been significant efforts made in recent years in defining these toxicities and testing potential prevention or treatment modalities, including the formation of toxicity working groups[156] to define cross-institution standards for diagnostic criteria and treatment algorithms. Although significant advances have been made, universal consensus has not been reached,[157] and remains a critical unmet need in the field, especially in the setting of FDA approval, as these therapies move from specialized centers to more broad implementation.

CRS CRS begins with the activation of T cells, when the CAR engages its cognate antigen on both malignant and nonmalignant cells. The active mechanisms causing CRS include the release of cytokines and chemokines by the CAR-Ts themselves (prominently including interleukin-6 [IL-6], IL-2, soluble CD25, interferon γ) along with activated "bystander" immune cells (including monocytes and macrophages) that secrete multiple inflammatory mediators.[137,152,158-161] Although all of the risk factors for severe CRS have not been determined, patients at higher risk have been found to have higher CD19 antigen load (either from disease or normal B cells), and to develop CRS earlier after cell infusion (usually <3 days after infusion[138,152,161,162]). Surrogate markers for severe CRS that are both sensitive and specific are still being elucidated, with these studies garnering increasing statistical power and predicative capability as increasing numbers of patients are treated with CAR-T cells. Two robust correlative markers of severe CRS have been (1) the degree of expansion (and the peak levels) of CAR-T cells measured in the peripheral blood and (2) the presence of highly elevated serum IL-6 levels.[137,138,142,152,156,159] Moreover, the functional association of IL-6 levels with clinical disease has been demonstrated by the ability of the anti-IL6R antibody tocilizumab (FDA approved for CAR-T-cell therapy in 2017) and the anti-IL-6 monoclonal antibody siltuximab to effectively treat symptoms of CRS.[138,152,162-165] In addition to IL-6 blockade, corticosteroids have also been used to treat CRS,[163,165,166] but their use has been viewed with caution, given concerns that this treatment might blunt the antileukemia efficacy of CAR-T-cell therapy. Despite the understandable concerns, several studies have observed that use of steroids did not appear to affect CR rates nor durability of CAR-T cells, although extensive follow-up has not yet been completed.[138,156,159,160]

CRES Less prevalent than CRS, but clinically more concerning, is CRES, the toxic encephalopathic syndrome that can accompany CD19 CAR-T therapy.[137,142,144,155,167,168] Although often self-limiting, the syndrome can also be severe, and result in seizures, obtundation, increased intracranial pressure, and cerebral edema, which has, in a small proportion of patients, led to death.[137,142,144,155,161,167-169] Given the severity and potential morbidity/mortality associated with CRES, there has been a significant effort made to understand its pathophysiology. Several hypotheses have been put forth. The first focuses on the inflammatory cytokine milieu that accompanies CD19 CAR-T therapy, and the accumulation of these cytokines in the brain in the setting of high serum levels.[151,156,159,160,165] The second focuses on direct T-cell infiltration into the cerebral spinal fluid (CSF) and brain.[108,151,152,165,170] Compelling new data also implicate breakdown of the blood-brain barrier (BBB) in CRES, supported by both histopathologic evidence and by the measurement of systemic biomarkers for endothelial disruption during CRES.[142,168] A recent study by Gust et al has proposed a pathophysiologic model of the interplay between CAR-Ts, cytokines, the BBB, and CRES.[142] In this model, the inflammation associated with CRS leads to activation of endothelial cells in the central nervous system (CNS), which drives release of 2 key mediators of the ensuing BBB disruption. These are ANG2 and von Willebrand factor, which together drive endothelial cell

activation and BBB disruption as well as the coagulopathy that is often observed during CRES. The BBB disruption results in a "feed forward" loop in which more cells and cytokines can cross into the CNS, leading to further activation of endothelial cells and thus further CNS inflammation. This has important clinical implications, as it suggests that therapeutic strategies that restore normal ANG2 or von Willebrand factor levels may be able to prevent or treat CRES. Given the concern that corticosteroids may impair long-term CAR efficacy, finding more targeted agents, such as those that target ANG-2, would be a major advance for the field.

Although the clinical studies are now starting to yield important clues to the pathophysiology of CRES, one of the major barriers to understanding the molecular pathobiology of CAR-T–mediated neurotoxicity has been the lack of animal models for this disorder. To address this, my research group, in collaboration with Michael Jensen's laboratory, has recently developed the first nonhuman primate (NHP) model of CRS and neurologic toxicity, using CD20 CAR-T cells in rhesus macaques[171] and Bruce Blazar's group has recently developed a mouse model of CAR-T toxicity, in which human CD19-specific mouse CAR-T cells were adoptively transferred into mice whose normal B cells express a hCD19 transgene at hemizygous levels.[172] Using the NHP model, we demonstrated CAR-T-cell expansion and B-cell aplasia, as well as CRS and neurotoxicity that closely mirrors what has been observed clinically.[171] Thus, this model induces elevations in the serum of multiple cytokines, and has documented disproportionately high concentrations of several cytokines in the CSF. Importantly, it has also been able to recapitulate clinical and histopathologic neurotoxicity. Coincident with the clinical neurotoxicity, we identified significant encephalitis, which was characterized not only by the accumulation of CAR-T cells, as expected, but also by the accumulation of non–CAR-T cells that infiltrated both the CSF and brain parenchyma. The results of the NHP model and of the new clinical studies suggest that neurotoxicity is associated with a complex program of immune activation, which encompasses multiple cellular and soluble mediators, which include (1) an increase in multiple cytokines in the CSF compared with the serum and (2) the development of encephalitis, in which both CAR and non–CAR-T cells accumulate in both the CSF and the brain. The NHP model thus also suggests that the breakdown in the integrity of the BBB is key to clinical neurotoxicity and that strategies designed to protect this barrier may be key to protecting patients from this major complication of CAR-T-cell therapy.

Postremission therapeutic strategies

One of the key unanswered questions in CAR-T-cell therapeutics, even for the most efficacious CD19-CAR-T products, is how best to manage patients after successful remission induction. The first wave of studies enrolled patients who were at very high risk of imminent death from their primary disease: these patients had been refractory to standard treatment approaches or had relapsed (often multiple times). They had very few, if any, alternatives among the more conventional therapeutic approaches. In these patients, the achievement of a CR was a major achievement. However, although the successful induction of CR is critical, this is not, in itself, a sufficient end point to determine the ultimate success or failure of CAR-Ts. Longer follow-up, focused on the stability of CR for years after infusion, is beginning to be reported and provides both reason for celebration and also a mandate for further optimization of these therapeutics.[138,144,152,173] Thus, recent work from multiple centers in the United States and China have documented high CR rates as well as sustained remissions in patients treated with CD19-CAR-T cells, both with and without additional consolidation,[108,111,136-138,144-155,173] striking results given the high-risk patient populations that have been treated. However, it is now clear that for most patients, the CR is not followed by long-term remission, with more than half of patients ultimately relapsing following CAR-T therapy. Thus, several recent studies continue to document high (~70% to 90%) remission rates in pediatric and adult patients with B-cell ALL,[144,145,151,152,162,174-176] but with significant relapse rates, even in short-term follow-up, and with a recent long-term analysis by the MSKCC group documenting event-free survival of 6.1 months and overall survival of 12.9 months in patients treated with CAR-T therapy.[173] Markers that distinguish a high risk of relapse vs long-term relapse-free survival after CAR-T therapy are actively being sought. Although greater persistence of CAR-T cells has been correlated with long-term survival in some studies (especially in studies of 4-1BB–containing CARs),[137,138,144,155] this is not a universal finding, and, especially with CD28-containing CARs, long-term survival was most strongly correlated with the development of "deep" minimal residual disease–negative remission after CAR-T therapy and a lower disease burden at the start of treatment.[173] Given the early stage of most of the CAR-T-cell studies, additional long-term studies and the discovery of surrogate markers of a sustained response to CAR-Ts are increasingly critical, as they will help inform decision-making about risk stratification post-CAR-T therapy. This risk stratification is of central importance in making decisions about what other therapies (if any) to offer patients who have been treated with CAR-Ts. Perhaps the most common of these postremission strategies is HCT. And, of note, although the MSKCC long-term data described similar outcomes for patients treated and not treated with HCT after CAR-T-cell therapy,[173] this was a retrospective, not randomized-controlled analysis. However, these data and those from other trials suggest that carefully designed randomized trials of post–CAR-T-cell therapies (including determining whether post-CAR-T-cell HCT improves outcomes) are now warranted. These trials, as well as moving CAR-Ts earlier in the treatment paradigm for ALL represent the critical next phase of CD19-CAR-T clinical investigation. When discussing postremission therapeutic strategies for CAR-Ts, it is also critical to carefully consider the types of relapse that occur with the current generation of CAR-T products. In B-cell ALL, these relapses are both of $CD19^+$ leukemia (indicating lack of long-term efficacy of the CARs) and of $CD19^-$ clones, indicating mutational escape as a pivotal event in disease relapse.[111,112,137,138,144,152,154,155,173,175-177] The next generation of CARs is tackling both of these modes of treatment failure, and if successful, could further reduce the need for postremission therapies.

Summary

In this review, one of the oldest (HCT) and one of the newest (CAR-T) cellular therapies have been discussed, each of which plays a critical role in the treatment of patients with hematologic diseases. The reality of cellular therapeutics is that they have a narrow therapeutic window, with significant toxicities that often accompany their efficacy. Understanding the mechanisms driving both of these processes, and identifying surrogates to help optimize the risk-to-benefit ratio of these and other cellular

therapies, will be critical to their successful implementation in patients with hematologic diseases.

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Footnote

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