C(h)AR-ting a new course in incurable lymphomas: CAR T cells for mantle cell and follicular lymphomas

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Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 has transformed the natural history of relapsed and refractory B-cell acute lymphoblastic leukemia and aggressive B-cell non-Hodgkin lymphoma. Based on these results, CD19 CAR T cells have since been tested in largely incurable lymphomas, including mantle cell lymphoma, follicular lymphoma, and marginal zone lymphoma, with promising early results that raise the question of whether this cellular immunotherapy could have curative potential and change the natural history of these diseases. This article reviews these results and this hypothesis.

Introduction

Chimeric antigen receptor (CAR) T-cell therapy has had a tremendous impact on the treatment landscape and natural history of hematologic, especially B-cell, malignancies. In diseases such as aggressive B-cell non-Hodgkin lymphoma (B-NHL) and B-cell acute lymphoblastic leukemia, a subset of patients with disease refractory to available therapies will achieve durable remissions after a single infusion of anti-CD19 CAR T cells. This is perhaps not surprising, because these diseases can be cured with upfront chemoimmunotherapy in 60% to 70% of patients, but nevertheless, this is a significant advancement in the treatment of relapsed/refractory disease. Most recently, CD19 CAR T cells were approved by the US Food and Drug Administration (FDA) for the treatment of relapsed/refractory mantle cell lymphoma (MCL) based on the results of the ZUMA-2 study of brexucabtagene autoleucel (brexu-cel) in this disease. Similarly, the interim results of the ZUMA-5 study of axicabtagene ciloleucel (axi-cel) were presented, demonstrating encouraging response rates and progression-free survival in patients with relapsed/refractory indolent B-NHL, including follicular lymphoma (FL) and marginal zone lymphoma (MZL). The expansion of CD19 CAR T cells into these traditionally incurable lymphomas poses new and provocative questions as to whether CAR T-cell therapy has the potential to cure these diseases and whether prior therapies and/or the disease itself affects the function and quality of the CAR T-cell product and thus response and toxicity outcomes, particularly in combination with Bruton tyrosine kinase (BTK) inhibitors, which have also transformed the treatment of lymphomas. In this commentary, we will review the efficacy and safety data and limitations of the currently available CAR T-cell products in lymphoma and investigate whether these products have curative potential in an incurable set of diseases. CAR T-cell therapy has an established position in the treatment paradigm of lymphoma, but how best to sequence and combine these products and how to overcome issues of resistance and toxicity remain pivotal questions in the field.

CD19 CAR T-cell therapy for relapsed/refractory B-NHL

Although a majority of patients with aggressive B-NHL will be cured with chemoimmunotherapy, 30% to 40% of patients will either have chemorefractory disease or relapse. More than half of these patients will not benefit from high-dose chemotherapy or autologous stem cell rescue, and until 2017, these patients had few effective options, with a median overall survival (OS) of only 6 months.[1] However, late 2017 and early 2018 saw the FDA approval of 2 autologous anti-CD19 CAR T-cell therapies for relapsed/refractory aggressive B-NHL in the third line and beyond: axi-cel and tisagenlecleucel (tisa-cel). These approvals were based on the results of the ZUMA-1 and JULIET studies, respectively, demonstrating

a 30% to 50% complete response (CR) rate to and a 30% to 40% long-term disease-free survival rate after a single infusion of these engineered cell therapy products.[2,3] A third product, lisocabtagene maraleucel (liso-cel), is expected to be approved by the end of 2020 based on the similarly promising results of the TRANSCEND NHL-001 study.[4,5] As second-generation CAR T cells, these products differ with respect to their second costimulatory domain, which has been hypothesized to result in different CAR T-cell pharmacokinetics and possibly contributes to different rates of toxicities like cytokine release syndrome (CRS) and immune effector cell therapy–associated neurotoxicity syndrome (ICANS). For example, the CD28 costimulatory domain used in axi-cel may lead to more rapid CAR T-cell activation and expansion when compared with the 4-1BB CAR T cells tisa-cel and liso-cel, and this may result in increased rates of high-grade CRS and ICANS.[2-4] Although axi-cel, tisa-cel, and liso-cel have each revolutionized the therapeutic landscape in aggressive B-NHL, their toxicity profile limits their utility, and their benefit is seen in fewer than half of treated patients. Ongoing preclinical and clinical studies are exploring the development of potentially safer and more effective CAR T cells: CAR T cells requiring more complex and/or physiologic interactions at the immune synapse, including Boolean-gated CARs or T-cell antigen coupler or T-cell receptor fusion constructs)[6-8]; CAR T cells that target >1 tumor antigen[9,10]; and CAR T cells that do more to recruit native antitumor immune cells (including armored CAR T cells).[11] Studies are also investigating the use of new toxicity mitigation strategies using competitors to the interleukin-1 (IL-1) receptor (anakinra; registered at www.clinicaltrials.gov as #NCT04150913), antibodies to IL-6 (siltuximab) or granulocyte-macrophage colony-stimulating factor (lenzilumab; registered at www.clinicaltrials.gov as #NCT04314843), and endothelial stabilizing agents (defibrotide; registered at www.clinicaltrials.gov as #NCT03954106) or CAR T cells derived from T cells of healthy donors (allogeneic CAR T and natural killer cells; registered at www.clinicaltrials.gov as #NCT03939026 and #NCT03666000)[12] to improve accessibility for a greater number of patients.

CD19 CAR T-cell therapy for relapsed/refractory MCL

The most recent FDA approval in the space, the use of brexu-cel for the treatment of relapsed/refractory MCL, marks another milestone in the field. Brexu-cel is a relative of axi-cel and as such is also a CD19-directed autologous CAR T-cell product with a CD28 costimulatory domain. Its manufacturing process includes an additional step before T-cell activation and engineering, which is to select out any CD19$^+$ tumor cells from the pheresis product. It is the first engineered cell therapy to be approved for MCL, an intermediate-grade B-cell lymphoma that is incurable, with a median OS of ~10 years. The ZUMA-2 study enrolled 74 patients with relapsed/refractory MCL after at least chemoimmunotherapy and BTK inhibitor therapy.[13] Of the 68 patients treated, the median number of prior therapies was 3, 82% had Ki67 >30%, 31% had blastoid or pleomorphic variant, and 17% had a *TP53* mutation. By definition, all patients had received a BTK inhibitor; 85% of patients had received ibrutinib, and the remaining patients had received acalabrutinib. In 1 retrospective series, the expected median OS in this population was 5.8 months.[14] Among these high-risk patients, the objective response rate (ORR) was 93% and the CR rate was 67%; with a median follow-up of 12.3 months, 57% of patients

remained disease free, with estimated 12-month progression-free survival and OS rates of 61% and 83%, respectively. Toxicity was similar to that seen with axi-cel in aggressive B-NHL, with rates of any-grade and high-grade CRS of 91% and 15%, respectively, and rates of any-grade and high-grade neurologic toxicity of 63% and 31%, respectively. There were no grade 5 events resulting from CRS or neurotoxicity, although there was 1 case of grade 4 reversible cerebral edema. Based on these results, the FDA approved brexu-cel for a broader indication than had been included in the ZUMA-2 clinical trial, namely any relapsed/refractory MCL regardless of line of therapy, perhaps with an eye toward a dynamic frontline treatment landscape for this disease, with ongoing investigations of combination chemoimmunotherapy and BTK inhibition as well as chemotherapy-free strategies in the frontline setting.

BTK inhibitors and synergy with CAR T-cell therapy

Unlike the trials for relapsed/refractory aggressive B-NHL with axi-cel and tisa-cel, treatment with BTK inhibition was required for eligibility in the ZUMA-2 study of brexu-cel in MCL.[13] It remains unclear whether the higher response rate and slightly higher toxicity rate observed in ZUMA-2 compared with ZUMA-1 were results of differences in the underlying disease, slight differences in the manufacturing process of the CAR T-cell products, or the quality of the T cells collected during pheresis. The fact that all the MCL patients had received prior BTK inhibition also brings into question the potential role that ibrutinib or acalabrutinib played in modifying either the quality of the T cells or the underlying disease. Ibrutinib is an irreversible inhibitor of not just BTK but also inducible T-cell kinase (ITK), which results in enhanced Th1-type cellular immunity functions in both infection[15] and solid tumors treated with programmed death-1 (PD-1) blockade.[16] One of us also demonstrated that prolonged treatment with ibrutinib restored T-cell functions and proliferative capacity both ex vivo and in vivo, in both bulk T-cell populations and CAR-transduced T cells from patients with relapsed/refractory chronic lymphocytic leukemia (CLL).[17] Ibrutinib treatment resulted in higher T-cell quality, as determined by both phenotype and function, and BTK-resistant tumor xenograft models demonstrated that ibrutinib had direct effects in the T-cell compartment.[17] Ibrutinib treatment in mice bearing MCL xenografts also resulted in improved responses to 4-1BB CAR T cells[18] and, unlike what was observed in ZUMA-2, reduced CRS.[19] This finding was consistent with the hypothesis that ibrutinib also reduced tumor burden through its effect on BTK inhibition in B cells. Interestingly, a follow-up study in patients with CLL who were treated with either ibrutinib or acalabrutinib demonstrated that ibrutinib increased both the function and quantity of CD4 and CD8 T cells, particularly in the effector and effector/memory subsets, whereas these effects were not observed with acalabrutinib, a more selective BTK inhibitor that is not expected to inhibit ITK in T cells. Both ibrutinib and acalabrutinib reduced expression of the inhibitory markers PD-1 and CTLA-4 in the T-cell compartment, consistent with reduced immunosuppression by tumor, and this reduction in expression was correlated with reduced expression of CD200 and BTLA and IL-10 production by the CLL cells.[20]

The optimal timing of ibrutinib treatment with CAR T-cell therapy remains to be determined. Although not a registration trial, Turtle et al[21] also found that 4-1BB CAR T cells were highly effective in

a study of 24 CLL patients who had experienced treatment failure with ibrutinib. The same group then demonstrated that concurrent ibrutinib therapy with CAR T cells was well tolerated, with lower CRS and higher response rates, in a trial of 19 patients with CLL who started ibrutinib therapy before pheresis and continued the drug for at least 3 months after CAR T-cell infusion (which was administered as planned and without dose reduction in 68% of patients). The 4-week ORR was 83%, which was higher but not statistically different than the 56% ORR in a similar cohort that had not received ibrutinib, and the rate of CRS was lower despite equivalent CAR T-cell expansion compared with CLL patients who had not received ibrutinib. Brentjens et al[22] also observed improved efficacy and CAR T-cell phenotypes in patients with CLL who were receiving ibrutinib (for a median of 4.8 months) at the time of leukapheresis in their study of CAR T cells bearing the CD28 signaling domain. In the ZUMA-2 study, a majority (88%) of patients enrolled had disease that was refractory to or had relapsed after BTK therapy, and a majority (85%) had received ibrutinib, with only 24% having prior exposure to acalabrutinib. The breakdown of responses and toxicities with respect to type of BTK inhibitor has not yet been described, but it would be interesting to further clarify the role of pure BTK inhibition in the B-cell compartment relative to the role of combined BTK and ITK inhibitors in both the B- and T-cell compartments. The synergy between BTK (and/or ITK) inhibition and CAR T-cell therapy for MCL and CLL leaves open the tantalizing possibility of chemotherapy-free curative treatment (aside from the low-dose cyclophosphamide and fludarabine conditioning regimen for CAR T cells) for these previously incurable malignancies.

CD19 CAR T cells for relapsed/refractory indolent B-NHL

Like MCL, indolent B-NHL, which includes FL and MZL, is chemoimmunotherapy sensitive but not curable with these therapies. Anti-CD19 CAR T cells have been tested in these diseases and have led to high rates of complete remissions, which in some cases have been encouragingly durable.[23-26] The largest series presented to date, the ZUMA-5 study of axi-cel in relapsed/refractory FL and MZL, evaluated 96 patients for efficacy (n = 80 and 16 for FL and MZL, respectively) and 140 patients for safety (n = 124 and 16 for FL and MZL, respectively).[23] In all patients, the ORR was 93%, and 80% of these responses were CRs; in FL, the ORR was 95%, with a CR rate of 81%. Sixty-eight percent of patients with FL had maintained their response at the median follow-up of 15.3 months. Although toxicity in MZL was similar to that seen in aggressive B-NHL, there were fewer instances of any-grade and high-grade CRS and ICANS in the FL cohort, and the onset of CRS was delayed compared with that seen in aggressive lymphomas. Other smaller series of FL patients after CD19 CAR T-cell therapies have had longer median follow-up and have similarly demonstrated ongoing responses at ≥2 years.[25,26] The phase 2 study of tisa-cel in relapsed/refractory FL has completed accrual and did meet its primary end point (CR rate); results are expected to be presented soon (registered at www.clinicaltrials.gov as #NCT03568461).

Making the incurable curable?

The field is largely accepting that CD19 CAR T cells offer a potentially curative option for ∼40% of patients with relapsed/refractory aggressive B-NHL. Clearly, longer follow-up is needed for

studies of CD19 CAR T cells in MCL and FL, but against comparable third-line treatment strategies for these diseases, CD19 CAR T-cell therapy has already surpassed expectations, and indeed, a handful of patients remain in remission ≥5 years after their treatment. This inspires cautious optimism and raises the question of how CAR T cells might transform diseases from incurable to curable.

Aggressive B-NHL, including diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma, is potentially curable with upfront chemoimmunotherapeutic strategies. This is in contrast to diseases like MCL and indolent B-NHL, including FL and MZL, as well as CLL, which are highly chemoimmunotherapy responsive but, given enough time, will invariably recur. The precise reason behind the differential curability of these lymphomas is unknown, but a proposed explanation postulates the existence of a lymphoma stem cell or lymphoma-initiating cell.[27] Although diseases like DLBCL and Burkitt lymphoma arise from mature B cells that are rapidly dividing and thus sensitive to chemotherapy, diseases like MCL, FL, and MZL may arise from a quiescent lymphoma-initiating cell that, after acquiring additional mutations, presents itself as the clinically active malignancy. After chemoimmunotherapy, it is this latter population of cells that is killed, but the lymphoma-initiating cell, in its quiescent state, is left behind to mutate once again, leading to clinical relapse.

Clonogenic MCL-initiating cells have been identified in human and murine samples.[28,29] These cells, which were CD45$^+$ but CD19$^-$, had self-renewal and tumorigenic properties in xenograft models but displayed a quiescent status. In FL, a malignant population of interfollicular B lymphocytes, distinct from lymphoma cells within the follicles, has been identified.[30] Similar to MCL-initiating cells, these cells displayed a quiescent phenotype with downregulation of CD19.

If these lymphoma-initiating cells exist, understanding their biology is of great therapeutic importance, because they may be sensitive to immunologic or targeted treatments, and the eradication of these cells may render these once incurable diseases curable. Allogeneic stem cell transplantation, perhaps the only curative modality for these diseases, provides indirect support for this hypothesis. However, the allogeneic graft resides within the patient in perpetuity, thereby providing continuous immune surveillance for microscopic relapse, and thus, cure may not rely on the eradication of a lymphoma stem cell population. However, if allogeneic transplantation does work via immune attack on a lymphoma stem cell, engineered cell therapies, like CAR T cells, could prove curative by a similar mechanism, assuming the tumor antigen of interest is present on the lymphoma-initiating cell. Given that the lymphoma-initiating cells identified to date in FL and MCL either downregulate or do not express CD19, how a CD19-directed CAR T-cell therapy might transform their natural history into curable diseases is unclear. There is evidence that CAR T cells may not be responsible for all of the antitumor cell killing after their infusion, because a majority of activated T cells found in the tumor microenvironment do not express the CAR itself.[31] Therefore, it is possible that it is the recruitment of these T cells, either already in the microenvironment or through epitope spreading, that could eliminate the lymphoma stem cells.

If instead transplantation works through perpetual immune surveillance, attacking microscopic relapse before it can cause a clinical relapse, CAR T cells will only provide long-sought-after cures if they

too persist and provide prolonged immune surveillance. In this case, CAR T cells with different pharmacokinetics and persistence may prove equally efficacious in diseases that can be cured with chemoimmunotherapy, like DLBCL, but may differ with respect to long-term disease control in diseases historically destined to relapse, like MCL and FL. Increasing evidence suggests that the costimulatory domain does matter in this regard. As mentioned previously, CD28 CAR T cells are activated and expand more rapidly, and although they can be detected genetically months after infusion, it is not clear that these cells are immunologically active. 4-1BB CAR T cells, in contrast, expand more slowly and persist longer in both xenograft models and reported human cases.[32-34] Several recent reports have identified transcriptional and signaling pathway differences between CAR T cells with CD28 and 4-1BB costimulatory domains, which offers insight into their differential persistence.[35-37] Specifically, 4-1BB CAR T cells have increased expression of HLA class II genes and IL-21 axis genes and decreased expression of PD-1 compared with CD28 CAR T cells and are enriched in a central memory phenotype.[36] These cells also activated noncanonical nuclear factor κB in an ex vivo system, whereas CD28 CAR T-cells did not, and abrogation of non-canonical nuclear factor κB led to a reduction in expansion and survival of 4-1BB CAR T cells.[35] It seems that a single amino acid residue change in CD28 can improve CD28 CAR T-cell persistence, thus opening the door for additional CAR construct modifications to be tested in future studies.[37] Understanding the downstream effects of the costimulatory domain may then improve outcomes and expand indications of this powerful new therapy.

Future directions for CAR T cells in lymphoma

It is clear now that CAR T-cell therapy for lymphoma is here to stay, given that substantial numbers of patients are achieving durable complete remissions; it remains to be seen whether a particular CAR design or cell type will become the clear winner in terms of efficacy and less toxicity, and there are multiple new therapeutic cell products entering the field. These include CAR T cells bearing different costimulatory domains or additional functionalities, such as transgenic cytokine secretion, CAR-modified natural killer cells, gene-edited allogeneic CAR T cells, and T cells derived from induced pluripotent stem cells. Questions remain about what product attributes drive the mechanisms of durability and long-term remission, including whether tumor cell death is mediated only by the gene-modified T cells or if bystander T cells also lyse tumor or even if the activated macrophages that drive CRS are also responsible for tumor eradication. A third major question for the future, as with any novel therapeutic modality, will be what mechanisms of resistance will emerge, and how physician investigators will circumvent these; antigen escape variants justify the rapid development of dual-targeting CAR T cells, drugs such as BTK and/or ITK inhibitors may be used routinely specifically to enhance T-cell quality and potentiate durable remissions, and novel therapeutics targeting macrophages or innate immunity may also modify the tumor microenvironment, which may (or may not) be a driver of resistance. Finally, acute toxicity of CAR T cells, although transient in a vast majority of patients, is still a major factor limiting widespread use of this therapy in the community. New products or prophylactic interventions to mitigate toxicity are certainly desirable and represent active areas of investigation, but even now, given the potential for cure and the limited long-term toxicities observed to date, the risk/benefit ratio of CAR T cells is highly favorable for patients with lymphoma.

Authorship

Contribution: C.A.J. and M.V.M. contributed equally to the writing, review, and editing of this manuscript.

Conflict-of-interest disclosure: C.A.J. reports consulting for Kite, Novartis, Bristol-Myers Squibb, Celgene, Precision Biosciences, Nkarta, Lonza, and AbbVie and research funding from Pfizer. M.V.M. reports consulting for Novartis, Kite, BluebirdBio, Bristol-Myers Squibb, and Allogene, has sponsored research from KitePharma and Novartis, and reports equity interests in TCR2 and Century Therapeutics.

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