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Driving CAR T cell translation forward

Liora Schultz[1] and Crystal Mackall[1,2,3]*

Successes in CAR T cell translation have propelled their commercial launch, but expanding the impact of cancer immunotherapies remains challenging.

Adoptive cell therapy for cancer has been revolutionized by the development of chimeric antigen receptor (CAR) T cells. CARs are synthetic T cell receptors that fuse tumor-specific binding domains to T cell activation signals to confer tumor-specific cytotoxicity. Patient-derived T cells can be collected and transduced ex vivo to express CARs and then returned to the patient to mediate tumor eradication. Following decades of preclinical development, striking clinical responses have been achieved using CAR T cells targeting CD19, a B cell antigen with conserved expression by the majority of B cell malignancies. The success of CD19-CAR T cells has propelled commercial launch of this class of therapeutics, but realizing the full potential of CAR T cells will require parallel scientific progress to overcome primary and secondary resistance and addressing practical challenges relating to affordability and scalability (Fig. 1).

DISCOVERING CAR T CELL POTENCY

In addition to a successful trial in indolent lymphoma, an early report of CAR T cell efficacy in patients with chronic lymphocytic leukemia (CLL) by Kalos *et al.* (*1*) was published in *Science Translational Medicine*. These studies demonstrated that CD19-CAR T cells could induce lasting antitumor responses and establish CAR^+ memory T cells. Subsequent results in pediatric and adult acute lymphoblastic leukemia (ALL) revealed exquisite sensitivity to CD19-CAR T cell therapy, driving global collaborative efforts to centralize manufacturing and confirm results at scale. A phase 2 multi-institutional trial testing CD19-specific CAR T cells in pediatric patients and young adults reported a remission rate of 81% within 3 months of infusion in the subset of patients for whom ex vivo T cell engineering was successful and whose clinical status was such that they could wait for manufacture of the T cell product (*2*). This led the U.S. Food and Drug Administration (FDA) in 2017 to approve tisagenlecleucel, a CD19-specific 4-1BB-ζ CAR construct for treating relapsed or refractory $CD19^+$ B cell ALL in children and young adults. Shortly thereafter came FDA approval of axicabtagene ciloleucel, a CD19-specific CD28-ζ CAR construct, for treating refractory large B cell lymphoma after a phase 2 multi-institutional study in adults, which demonstrated complete responses in 54% of patients (*3*). Thus, in the 8 years since the Kalos *et al.* (*1*) study was published, the field has progressed rapidly, resulting in FDA-approved CAR T cell therapies for treating hematological malignancies. Commercialization of individualized and engineered cell products was a major hurdle but now provides post-marketing clinical data to enhance understanding of the correlates of success or failure for these therapeutics.

CAR T CELL EXPANSION AND PERSISTENCE

Dissecting drivers of durable clinical responses remains a challenge. Studies have variably reported on the relationship between CAR T cell expansion, persistence, and clinical responses. The experience in CLL demonstrated that in vivo expansion correlated with CAR T cell persistence and clinical response, and studies in large B cell lymphoma also showed that expansion correlated with clinical response (*3*). The phase 2 study of tisagenlecleucel in pediatric ALL did not reveal differential CAR T cell expansion between responders and nonresponders, but persistence did correlate with sustained clinical responses (*2*).

Lymphodepletion of the recipient is vital for permitting homeostatic CAR T cell expansion after infusion. Comparison of CAR T cell expansion and clinical outcomes in patients with non-Hodgkin's lymphoma who received cyclophosphamide-based lymphodepletion demonstrated superior survival outcomes with the addition of fludarabine (8% clinical response compared to 50% clinical response) (*4*). Although inter-institutional conditioning regimens were quite variable during early stages of CAR T cell translation, cyclophosphamide- and fludarabine-based regimens are now standard for lymphodepletion.

CAR design and intrinsic properties of resident T cells influence CAR T cell expansion and persistence. Second-generation CARs, incorporating a CD3-ζ signal domain and a costimulatory domain (typically CD28 or 4-1BB), remain the most common constructs in clinical use but demonstrate distinct kinetics. CD28 achieves a more rapid expansion of CAR T cells and potentially faster tumor elimination, as demonstrated using a preclinical model where CAR T cells are titrated down to identify differences in efficacy (*5*). However, costimulation also impacts CAR T cell persistence and exhaustion. A model of chronic CAR signaling demonstrated that T cell exhaustion could be ameliorated by 4-1BB costimulation (*6*), providing a biologic explanation to why 4-1BB–bearing CAR T cells are more persistent than CD28-bearing CAR T cells. Third-generation CARs, including those with two costimulatory domains, have not demonstrated clinical superiority over second-generation CARs.

Regarding CAR T cell persistence, the type of disease influences clinical outcomes. Persistent 4-1BB–containing CAR T cells are associated with sustained clinical remission in B-ALL. CD28-bearing CAR T cells unable to achieve long-term persistence are effective in lymphoma but not ALL. We anticipate that decreased persistence of CAR T cells may be effective and even desirable for treating acute myeloid leukemia (AML). Many AML targets of CAR T cells are primed for translation but have conserved expression on hematopoietic stem cells and progenitor cells, making long-term persistence

[1]Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305, USA. [2]Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA. [3]Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA 94305, USA.
*Corresponding author. Email: cmackall@stanford.edu

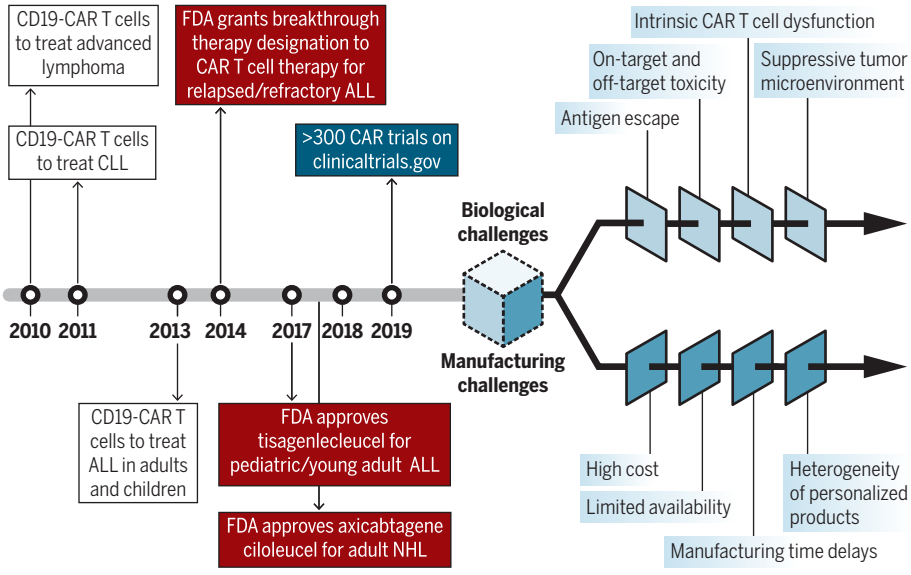

Fig. 1. Progress in CAR T cell therapy. Success in early CAR T cell clinical trials propelled rapid evolution of the field from small early-phase trials to FDA approval of commercialized cell products within a decade. Continued progress will require solutions to biological challenges, including barriers that have limited the efficacy of CAR T cells in non–B cell malignancies, as well as addressing the manufacturing challenges currently limiting accessibility of these therapies for patients.

possibly undesirable. It seems that desirable CAR T cell properties vary among diseases, and thus it is unlikely that one configuration will emerge as the optimal therapeutic for all malignancies.

NAVIGATING CAR T CELL TOXICITY

Cumulative experience has facilitated standardized clinical guidelines to enhance the safety of CAR T cells. Cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome (ICANS), which can range from mild to life-threatening, have emerged as dominant CAR T cell–mediated toxicities, with the toxicity risk paralleling disease burden (*7*). Cytokine release syndrome is now treated with the IL-6 receptor–targeting antibody tocilizumab, although other cytokines are involved as well. Early in clinical translation, it was unclear if aborting cytokine release syndrome would in parallel disrupt CAR T cell efficacy, and so this intervention was often delayed until later stages of toxicity. Studies have since shown that tocilizumab or steroids are not independent covariates influencing clinical response rates (*3*) and the use of these agents to manage CAR T cell–related toxicities has been liberalized. Clinical management algorithms for both types of toxicity are now established, allowing more centers to safely offer this treatment (*7*).

CD19 ANTIGEN LOSS IS A MAJOR DRIVER OF RELAPSE

Although the initial clinical remission rates after CAR T cell therapy in ALL patients are as high as 90%, survival rates with extended follow-up are substantially lower. In the previously mentioned phase 2 pediatric ALL study, despite an overall remission rate of 81% at 3 months postinfusion, event-free survival at 12 months decreased to 50%. In 15 of 16 evaluable patients who relapsed in this study, the cause was the emergence of leukemia that lacked CD19 and thus escaped recognition by the CD19–CAR T cells (*2*). Mechanistic studies of relapsed tumors negative for CD19 describe alternatively spliced isoforms lacking exons critical for CAR binding, including loss of epitopes recognized by CAR or proteins involved in surface expression. Patients have additionally experienced relapse associated with myeloid transformation and CD19 loss. Future work focused on more precise immune profiling of disease to quantify antigen density and identify minor subclones with subthreshold CD19 expression, or variant exon mutations could identify predictive biomarkers that confer increased risk of immune escape.

WHAT IS BEYOND CD19?

Hematological malignancies

The phenomenon of CD19-negative B cell leukemia relapse has prompted targeting of alternative B cell antigens. A major advance has been the successful treatment of patients with B-ALL who had, in some cases, been previously treated with CD19-CAR T cell therapy using CD22-specific CAR T cells. Patients achieved clinical remission rates of 73% using CD22-CAR T cells at biologically active dosing (*8*). Clinical remission rates were comparable to rates seen with CD19-CAR T cells, but antigen remodeling and CD22 downregulation were also observed. Preclinical studies developing CAR T cells with dual targeting of CD19/CD22 or CD19/CD20 have demonstrated promise, and trials studying bispecific targeting to circumvent antigen down-regulation are ongoing. Efforts targeting alternative antigens, including CD30 in refractory Hodgkin's lymphoma; CD33, CD123, and FLT3 in AML; and BCMA in multiple myeloma are under way. These promising agents are still in the early stages of clinical translation.

Solid tumors

Studies are ongoing to extend CAR T cell applications to solid tumors, yet effects comparable to CD19-CAR T cell therapy for hematological cancers have not yet been achieved. Constrained clinical responses seen with solid tumors can be explained by limited CAR T cell trafficking, intrinsic T cell dysfunction in the recipient due to T cell exhaustion, extrinsic T cell suppression mediated by a hostile tumor microenvironment, and antigenic heterogeneity. Identification of appropriate antigens with high on-tumor expression and absent or subthreshold expression on normal tissues has been challenging. Enhancing CAR T cell efficacy using next-generation CAR design and intratumoral injections for solid tumors is under way. Notably, IL-13Rα2-specific CAR T cells delivered intracranially achieved a 7.5-month regression in patients with glioblastoma (*9*). This experience emphasizes the possible need for alternative dosing and delivery strategies in solid tumors and invigorates promise for CAR T cells for treating solid tumors.

DESIGNING NEXT-GENERATION CAR T CELL PRODUCTS

The basic CAR structure is modular, allowing targeted modification of single chain variable fragments (scFvs), flexible linkers within scFvs, activation domains, spacers, and transmembrane domains to improve therapeutic effects. Further engineering strategies are also under way to permit CAR T cell–mediated

cytokine delivery, secretion of checkpoint-blocking moieties, modulation of T cell exhaustion, and regulatable "on/off" switches. Precise gene editing techniques are being leveraged to develop off-the-shelf CAR T cell products, including CRISPR-mediated elimination of endogenous T cell receptors on donor CAR T cells to prevent graft-versus-host disease and to generate CAR T cells that are less likely to be rejected by host allogeneic immune responses. The majority of clinical studies to date use mixed populations of T cells within the CAR T cell product, which vary across individuals. The consequences of variable T cell subset composition are currently being investigated to determine whether defined and potent subsets can be identified (*4*).

ONGOING CHALLENGES

Although scientific challenges relating to CAR T cell therapy optimization are manyfold, the need to render these therapies more affordable and available is equally pressing. The field, which sprung from individual academic centers, has evolved into a centralized model of commercial distribution. The prices for these products are high, at $373,000 per product for axicabtagene ciloleucel and $475,000 for tisagenlecleucel. Although CAR T cells in ALL can at times replace stem cell transplantation, CAR T cell therapy is often used as a bridge to transplant, incurring the costs of both therapies (*10*). It is anticipated that improvements in the manufacturing process and reductions in the cost of goods may ultimately result in lower prices and support scalability. The generation of off-the-shelf CAR T cells, currently in early-phase clinical trials, is an alternative strategy to address the complexities of manufacturing and high costs of individualized CAR T cell products.

The rapid translation of CAR T cell therapies from studies in academic centers to commercialized global manufacturing within the span of a decade is a remarkable success story. Future scientific and clinical progress will extend the reach of these therapeutics to even more patients. Unleashing the full curative potential of this potent therapy hinges on advancing our understanding of the basis for primary and secondary resistance in hematological cancers and solid tumors, development of next-generation products that leverage improvements in CAR engineering, as well as overcoming major barriers related to cost and scalability.

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