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The key to unlocking CARs

Although Kymriah's approval represents a landmark for chimeric antigen receptor T-cell (CAR-T) therapy in B-cell malignancies, solid tumors remain a formidable challenge.

Six weeks ago, Novartis' Kymriah (tisagenlecleucel) became the first CAR-T therapy approved in the United States. Approval was achieved in just six months and without a phase 3 trial for one simple reason: spectacular clinical responses in patients. Within three months of Kymriah treatment, complete responses were observed in ~83% of pediatric and adult patients up to age 25 with relapsed or refractory B-cell acute lymphoblastic leukemia (ALL). Many of these remissions are sufficiently durable to suggest curative therapy. As a result, industry is taking notice. At the end of August, Kite Pharma was acquired by Gilead Sciences for $11.9 billion—a clear sign that companies are betting that Kymriah's success will extend to other CARs. But for CAR-T therapies to truly transcend their role as niche leukemia treatments, they must successfully circumvent immunosuppressive tumor microenvironments, address the heterogeneity of solid tumors and incorporate a wider range of antigens suitable for tumor targeting.

It is just five years since Novartis and the University of Pennsylvania launched their collaboration to develop CAR-T therapies for the investigational treatment of cancers. Their lead program, Kymriah, targets the B-cell marker CD19 and incorporates the 4-1BB co-stimulatory domain into the CAR to enhance cellular expansion and persistence.

Generating the therapy involves several steps: leukapheresis of a patient's blood, stimulation of the harvested T cells with mitogenic beads, transduction with a viral vector to integrate a CAR construct (comprising an scFv antibody domain coupled to intracellular co-stimulatory domains), culture and expansion of the engineered T cells and subsequent cryopreservation—a process typically taking two weeks.

Kymriah's current target ALL patient population is relatively small; Novartis estimates that ~600 patients will qualify for treatment each year. In a few months, the Swiss pharma plans to apply for supplemental approval in the larger indication of diffuse large B-cell lymphoma (DLBCL). As such, it will be in a footrace with Kite/Gilead, which awaits a November 29 decision from the US Food and Drug Administration on axicabtagene ciloleucel, its CD19 CAR-T therapy, which is also being developed in DLBCL. Both companies anticipate that the DLBCL market will be tenfold bigger than that for ALL.

Before CAR-T therapies can meet the demands of larger patient populations, however, all eyes are on Novartis' capacity to consistently deliver CAR-T batches for ALL from its centralized facility in Morris Plains, New Jersey. The Swiss drugmaker is also working to establish a network of certified treatment centers across the United States to train clinicians on the use of Kymriah and appropriate patient care.

Numerous factors can affect the potency and quality of a CAR-T therapy. These include the production process (e.g., CD4/CD8 T-cell ratios, T-cell phenotype, levels of non-transduced cells, duration of activation), transgene construct (high or low expression, insulators, etc.), vector choice (retroviral, adenoviral or transposon), CAR design (e.g., scFv affinity, stability and immunogenicity, spacer length, and signaling domain) and input donor blood cells (e.g., starting cell number and exposure to different treatments and conditioning regimens).

Some groups are also experimenting with a decentralized delivery model employing user-friendly, automated, closed manufacturing devices to grow T cells. An example is a human trial at University College London, UK, which is currently using Miltenyi Biotec's CliniMACS Prodigy bioreactor to prepare single batches of cells.

For the time being, the centralized manufacturing model is likely to be the method of choice. But if CAR-T therapies are to be expanded to address solid cancers that affect thousands of patients, industry must be convinced that a highly controlled production process can be implemented across many collection, manufacturing and treatment sites.

There is still time to work out the kinks. Thus far, clinical responses to CARs against 22 different antigens clinically tested against a variety of solid tumors have been rather underwhelming. One reason for the low effectiveness of CAR-T therapy in solid cancers is the need for cells to traffic to, and proliferate at, the tumor site. Once within the mass, T cells must also retain their killing capacity in a microenvironment comprising tumor-associated macrophages, fibroblasts, regulatory T cells, myeloid-derived suppressor cells, and all their suppressive effectors. In addition, they must contend with tumor cell heterogeneity, which means a targeting antigen might not always be expressed throughout.

Antigen tissue distribution, tumor addiction and surface density all play a role not only in treatment efficacy, but also in 'off-tumor, on-target' toxicities—a problem previously highlighted by this journal (*Nat. Biotechnol.* **31**, 365, 2013). Most antigens on solid tumors tend to be shared by normal tissues, which can give rise to toxicities; in addition, T cells can cross-react with unrelated antigens, also giving rise to toxicities. In August, the anti-IL6 receptor monoclonal antibody Actemra (tocilizumab) was approved to control T-cell-induced cytokine release syndrome; nevertheless, neurotoxicity and macrophage-activation syndrome remain serious enough to have scuppered Juno Therapeutic's lead CAR-T program earlier this year.

Ultimately, the key to unlocking CARs' potential in solid tumors will be determined by our willingness to reimagine them. We must widen our search for antigens beyond the usual suspects to new antigen targets, such as gangliosides and glycan variants. We must combat heterogeneity and tumor specificity by targeting more than one antigen with multiple CARs on the same T cell. And we must counter immunosuppression and the tumor microenvironment by combining CAR-T therapies with other drugs: checkpoint inhibitors, BET bromodomain inhibitors, STING receptor agonists, vaccines and oncolytic viruses.

In this respect, Gilead's August foray into the CAR-T space is particularly notable. $11.6 billion is a very large punt for an experimental cell therapy in a niche cancer. But what better company to potentiate CAR-T treatments than one with an established reputation for overcoming disease resistance using powerful combinations of therapies?