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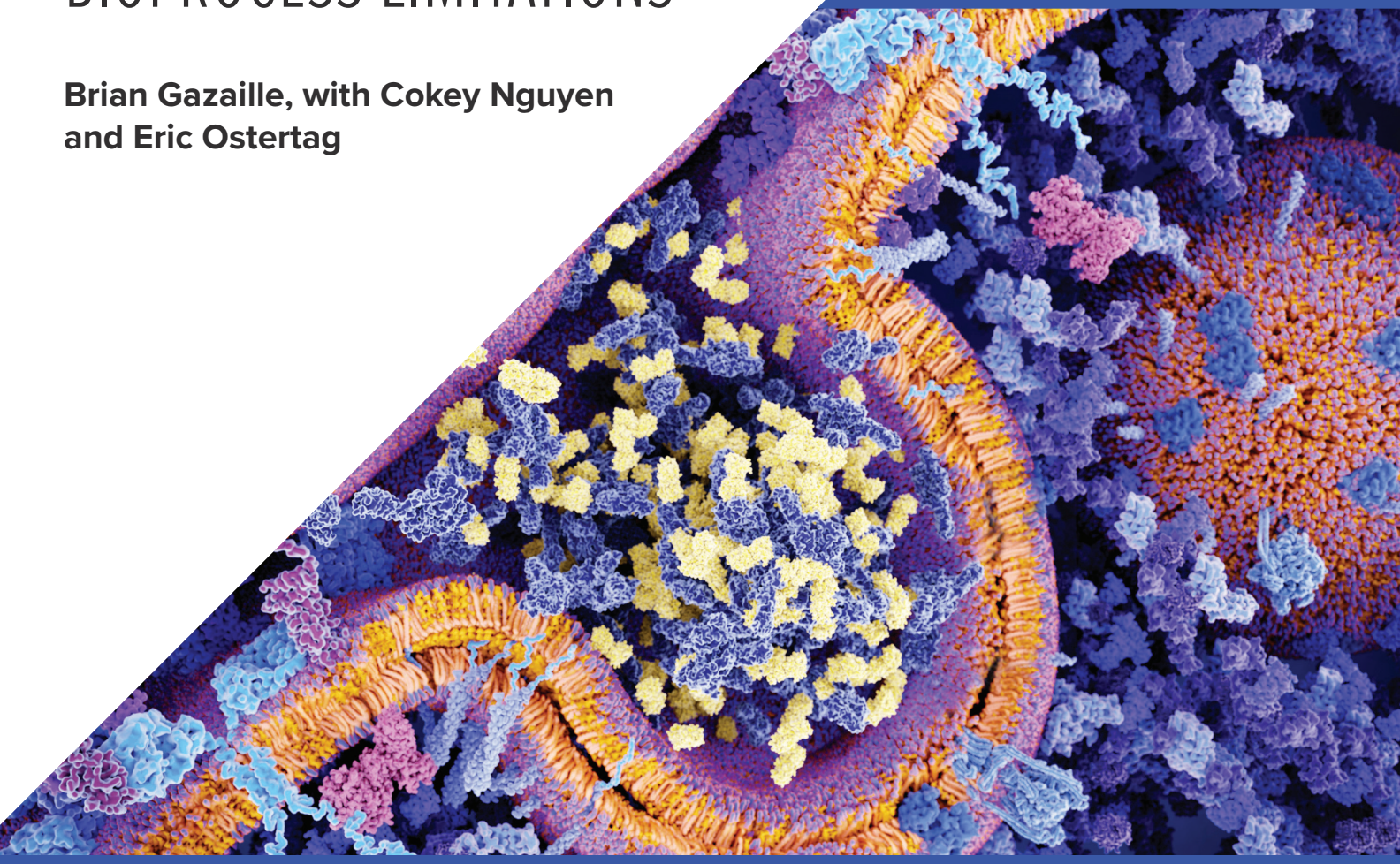
CAR-T CELL THERAPY

MITIGATING CLINICAL AND BIOPROCESS LIMITATIONS

Brian Gazaille, with Cokey Nguyen and Eric Ostertag

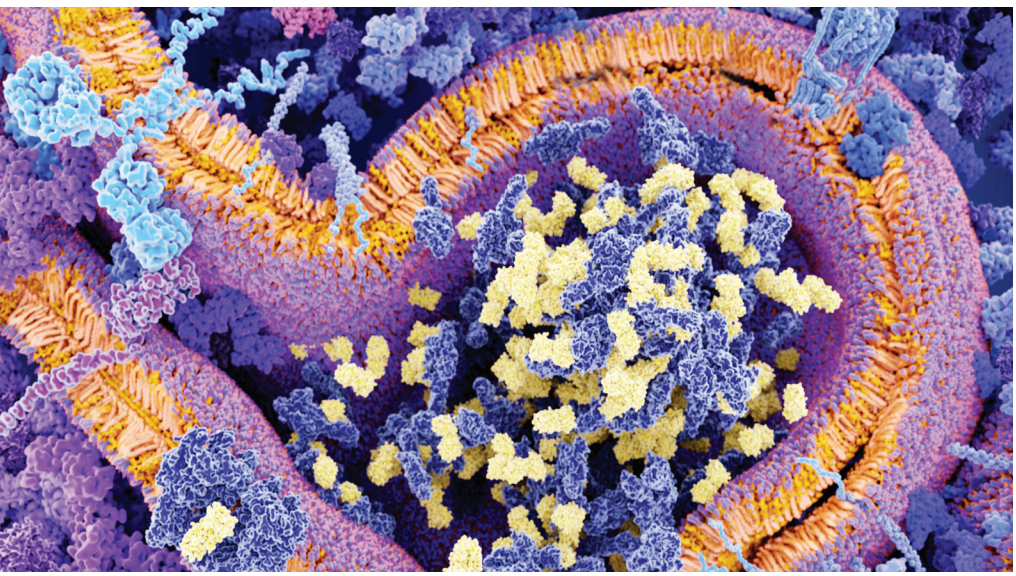


September 2021

CAR-T Cell Therapy

Mitigating Clinical and Bioprocess Limitations

by Brian Gazaille, with Cokey Nguyen and Eric Ostertag



On the cover: Chimeric antigen receptors (CARs) on the surface of a T cell bind to CD19 antigens on the surface of a leukemia cell, activating a signaling cascade that releases perforin and gramzyme molecules. CAR T-cell approaches have proven to be effective against several hematological cancers, but solid tumors remain intractable. The autologous nature of most commercially available and clinically advanced CAR-T therapies also limits how many patients can receive much-needed treatments. This eBook explores novel strategies that cell therapy companies are developing to address both of those concerns simultaneously.

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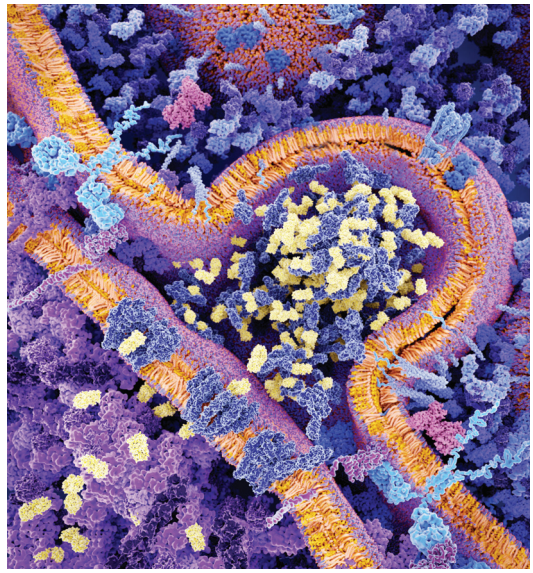
Developers of chimeric antigen receptor (CAR) T-cell therapies are working in a state of tempered optimism. Such approaches already have revolutionized the treatment of hematological malignancies. Before the advent of these products, children and young adults with relapsed/refractory B-cell acute lymphoblastic leukemia (R/R B-ALL) expected five-year survival rates of 21% and 10%, respectively. Today, 70–90% of B-ALL patients who are treated with CD19-directed CAR T cells can achieve complete remission (**1**). Much of that prognostic improvement stems from the 2017 debut of Kymriah (tisagenlecleucel, Novartis), the first CAR-T product to receive approval from the US Food and Drug Administration (FDA). Since then, four other therapies have joined the fold, all for similar indications. Coming on the heels of the Kymriah product, Kite/Gilead's Yescarta (axicabtagene ciloleucel) was approved in 2017 as a second-line treatment for diffuse large B-cell lymphoma (DLBCL). In 2020, regulators approved Kite/Gilead's Tecartus (brexucabtagene autoleucel) for mantle-cell lymphoma. And just this year, the FDA cleared Bristol-Myers Squibb's Breyanzi (lisocabtagene maraleucel) and Abecma (idecabtagene vicleucel) to treat adults with R/R LBCL and mulitple myeloma, respectively.

Now that those approved products have demonstrated the viability of CAR-based technologies, drug developers are trying to address significant limitations that have come to light with increases in available clinical data and bioprocess knowledge. One shortcoming concerns efficacy. CAR-T has shown only modest success against solid tumors, which account for the vast majority of cancers in adults (**2**). And despite remarkably positive short-term prognoses for blood-cancer patients, 30–60% of those who are treated with CAR-T products experience relapse, and roughly 20% of those patients develop CD19-negative cancers, limiting subsequent immunotherapeutic treatment options (**1**, **3**).

A second set of concerns relates to autologous processing, which remains by far the most common approach to CAR-T production — and the only strategy used for the five currently approved products. A typical process comprises

- leukapheresis of mononuclear cells from a sick patient
- shipment of donor material from a clinical site to a manufacturing facility (assuming available capacity)
- three or four weeks of processing, including steps for cellular isolation, genetic reprogramming, amplification, and purification
- shipment of a finished drug back to a clinic
- readministration to the donor.

Such a workflow raises several concerns. Highly individualized materials and process steps drive up manufacturing costs. Long production timelines can preclude treatment for patients with aggressive R/R disease, and even those who can wait might require interim chemotherapies, posing concerns for refractory malignancies (**4**). In terms of clinical outcomes, raw material gathered from truly sick patients tends to show high variability,



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An End-to-End CAR-T Cell Therapy Development Partnership

CAR-T cell therapies are continuing to gain popularity, with 5 now FDA approved and hundreds more in development.[1] While these treatments are promising, managing safety and effectiveness in patients is complex. Around the world, groups ranging from drug discovery, development, and manufacturing to clinical laboratories are using Bio-Rad's *Droplet Digital PCR (ddPCR)* technology as a reliable and scalable solution to myriad workflow challenges.

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1. Center for Biologics Evaluation and Research. Approved cellular and gene therapy products. Retrieved September 15, 2021, from https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapyproducts/approved-cellular-and-gene-therapy-products

and use of suboptimal T cells during production can diminish a final product's efficacy (**4**).

Such obstacles make allogeneic production an attractive path for developers to pursue. Basing therapies on cell banks sourced from multiple healthy donors would help to minimize variations in T-cell quality. Allogeneic production also raises opportunities for standardizing processes and increasing their scalability — the latter of which is sorely needed if cell therapy companies are to make good on their promises to treat many patients living with a broad range of highly individualized cancers (**4**–**8**).

CAR-T developers keenly understand the clinical and production-related barriers that lie ahead, yet they remain undeterred, and the clinical pipeline for such therapies continues to grow. As of September 2021, the ClinicalTrials.gov database lists 578 active trials for CAR-T candidates. The vast majority (>400) of those have reached only phase 1 studies, indicating that the field remains nascent and exploratory (**9**). However, many new candidates feature compelling new CAR designs, and developers increasingly are anticipating manufacturing requirements for their products.

This summer, I spoke with executives representing two clinical-stage cell-therapy companies to learn about different strategies for increasing CAR-T efficacy against solid tumors, transitioning from autologous to allogeneic processing, and perhaps accomplishing both goals simultaneously. Together, these interviews shed needed light on how to source sufficiently healthy T cells, improve therapeutic efficacy and longevity, and anticipate large-scale manufacturing requirements.

Leveraging Virus-Specific T Cells

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Many drug companies seeking to develop allogeneic CAR-T products begin with induced pluripotent stem cells (iPSCs), modifying those genetically to express the desired chimeric receptors, then stimulating them to differentiate into T cells (**10**, **11**). Atara Biotherapeutics leverages a novel approach, however, basing its therapeutic programs on memory T cells that recognize Epstein–Barr virus (EBV). A common pathogen in humans, EBV is attracting significant attention from researchers for its implication in a broad range of cancers and autoimmune disorders (**12**–**14**). Atara's EBV T-cell platform shows promise in treating such conditions, and it supports production of both standard and CAR-bearing T-cell products. The Atara pipeline includes a mesothelin-targeted autologous CAR-T candidate (ATA2271), an allogeneic counterpart (ATA3271), and a CD19-targeting allogeneic CAR-T therapy (ATA3219). Currently in phase 3 clinical evaluation, Atara's tab-cel (tabelecleucel) product is poised to become the first allogeneic T-cell therapy to gain regulatory approval and reach commercialization.

As chief scientific officer at Atara, Cokey Nguyen leads the company's efforts to develop next-generation allogeneic cell therapies for cancer and autoimmune diseases. To that task he

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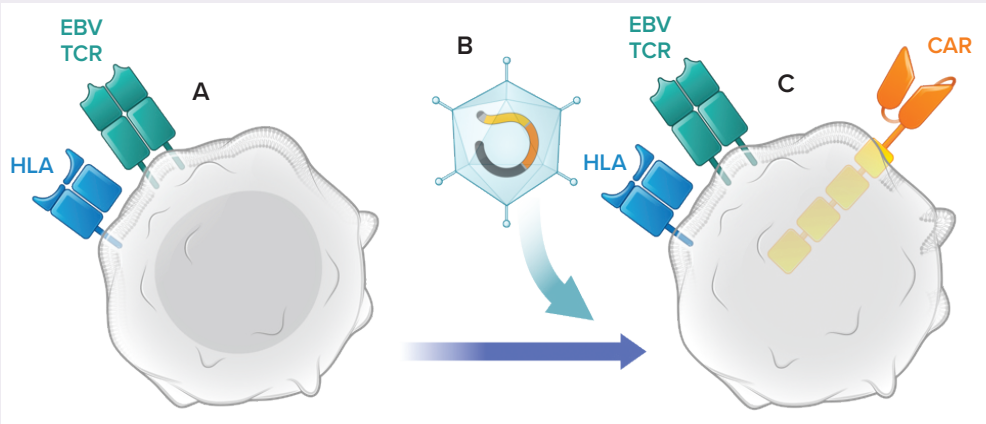
brings considerable experience, including work as a vice president of research and development (R&D) at Fate Therapeutics and as a leader of Pfizer's targeted-immunotherapy group. At Janssen, he helped to evaluate Legend Biotech's B-cell maturation antigen (BCMA)-directed CAR-T program. I spoke with Nguyen in August 2021 about the value of EBV T-cell platforms, especially their potential for enabling allogeneic production of CAR-T therapies.

Figure 1: Epstein–Barr virus (EBV)-specific T cells can support development of several types of cell therapies, including (A) allogeneic EBV T-cell platforms. Combined with (B) next-generation chimeric antigen receptor (CAR) technologies, such platforms could facilitate development of (C) allogeneic CAR-T approaches for a broad range of indications. (HLA = human leukocyte antigen, TCR = T-cell receptor, IMAGE COURTESY OF ATARA BIOTHERAPEUTICS, HTTPS://WWW.ATARABIO.COM)



Why does Atara focus on developing EBV T-cell therapies? EBV T cells could address high unmet medical need. EBV is associated with several illnesses, including posttransplant lymphoproliferative disease (PTLD), which arises after patients undergo solid-organ or hematopoietic-cell transplantation. Patients who develop that condition often die within two to three months after failure of initial treatment. That is why our investigational tab-cel EBV T-cell immunotherapy could be transformative. Patients with those conditions usually have limited recourse, but the tab-cel product could offer them off-the-shelf treatment.

EBV also has been implicated in multiple sclerosis (MS). Although treatments exist for relapsing-remitting MS, few options are available for the progressive form of the disease, and antibodies don't cross into the central nervous system (CNS) efficiently. We believed that a T-cell product would be a smart play comparatively, and our published data support that conviction. We plan to report phase 1 clinical-trial data for our investigational ATA188 allogeneic treatment for progressive MS in October 2021.

A second reason for focusing on EBV T cells is that they have compelling biological features. These cells have been selected through evolution to persist in the human body. They have a central memory (T_{CM}) phenotype, meaning that they are highly specific for EBV and circulate constantly to detect latent virus that can arise when an infected person is stressed or immunosuppressed. And like other memory T cells, EBV T cells can expand, persist, and travel to sites of disease. They also show enhanced cytotoxicity compared with naive T cells.

Such qualities make EBV T cells ideal platforms for treating EBV-driven diseases. Furthermore, current literature indicates that >90% of adults have experienced EBV infection. Except in cases of immunosuppression, people's immune systems handle the virus well. Those factors enable developers to isolate EBV T cells from a

Emerging CAR-T Technologies

Multitargeted chimeric antigen receptors (CARs) enable dual targeting with "and/or" gating to prevent on-target–off-tumor activity.

Novel costimulatory domains could reduce T-cell exhaustion, leading to longer functional persistence.

Programmed cell death protein 1 dominant-negative receptors (PD-1 DNRs) could provide intrinsic checkpoint inhibition to help unlock solid-tumor microenvironments.

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wide range of healthy donors, making possible allogeneic production approaches that could be game-changing compared with complex autologous workflows. Even a state-of-the-art autologous T-cell process can require 20–27 days after leukapheresis. That process is rigorous, but manufacturing failures can occur, and sometimes patients can't wait. By harnessing EBV-specific T cells from multiple healthy donors, we can identify a patient, select for appropriate human leukocyte antigen (HLA) profiles, and begin treatment within three days.

EBV T cells also raise advantages for cellular expansion steps. T cells proliferate in response to physiological signals. During an autologous CAR-T process, activation typically is performed using anti-CD3/28 beads — a kind of "atomic hammer." I don't know how you feel when you've had soda and sugar cookies all day, but that's akin to the stimulatory effects of the beads, and it is not likely to yield healthy T cells. EBV T cells can be expanded using EBV peptides. Thus, we believe that EBV-based approaches will produce high-quality T cells "out of the gate."

We have the advantage of knowing that our EBV T-cell platform has the potential to treat a wide range of EBV-driven diseases and other serious diseases through incorporation of engineered CARs or T-cell receptors (TCRs).

Why do so many patients with solid-tumor cancers have shown lackluster responses to CAR-T products? When the biopharmaceutical industry started working with CAR T cells, it focused on hematologic malignancies. Developers wanted highly potent T cells — and such cells continue to dominate the pipeline. In that first generation of CAR-T therapies, companies also concentrated on optimizing T-cell expansion. That all still suits the hematological niche: Memory and even naive T cells travel easily to lymph nodes and bone marrow, where most such tumors develop. But when we apply the same thinking to solid tumors, treatment falls flat. Common T-cell activation strategies can result in premature "exhaustion."

To treat solid tumors, T cells need to persist in a challenging microenvironment. Lymph nodes — where most B-cell lymphomas reside — are not hostile to T cells, whereas solid tumors do everything that they can to suppress T cells. Such cancers require a multidimensional approach — potentially a different kind of T cell.

How might Atara's candidates address those limitations? One such program, ATA3219, is an allogeneic, CD19-directed immunotherapy based on our EBV T-cell platform. Rather than using the kind of chimeric receptor that is engineered into many first-generation products, ATA3219 is designed to express a 1928ζ mutant (1XX). The 1XX CAR design enables attenuated T-cell signaling — like a "Goldilocks zone" for T-cell signaling that helps the cells to persist longer. We are on schedule to submit an investigational new drug (IND) application for that candidate early in 2022.

We also are working with Bayer to advance an "armored," next-generation allogeneic T-cell immunotherapy (ATA3271) and an

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autologous version (ATA2271), both of which target mesothelin, a membrane-bound surface glycoprotein that is highly expressed in many solid tumors. Like our other CAR-T program, ATA3271 uses a next-generation design, but in addition to 1XX, it bears a programmed cell death protein 1 dominant-negative receptor (PD-1 DNR). Many drug developers are considering ways to combine CAR T cells with PD-1 inhibitors or to generate complicated chimeric receptors based on PD-1. Using a PD-1 DNR offers an elegant solution to protect T cells from hostile environments that exert tremendous selectional pressure to suppress and exhaust them. We plan to file an IND for that candidate late in 2022.

We are excited about both CAR-T programs, and manufacturing knowledge that we are gaining from our tab-cel and ATA188 candidates will help to ensure that those programs run smoothly. That knowledge also will enable us to build up our pipeline early.

What is your company learning about manufacturing and scale-up as its lead candidates progress through clinical stages? We've learned to build our manufacturing process around the "three Ps" of healthcare economics: patients, physicians, and payers. We've needed to ask critical questions about how we can get our drug into patients, how we can simplify its administration requirements for physicians and nurses, and how we can decrease its costs. Scalability can address all those concerns, and that's the beauty of an allogeneic approach. We have invested in scalable technologies, so we're transitioning from static, gas-permeable vessels to stirred-tank perfusion bioreactors and to closed systems with automation capabilities. Scaling up in that way will help us to produce enough drug to address unmet medical needs. Leveraging an allogeneic approach also enables us to cryopreserve a large inventory of healthy EBV T cells so that as soon as we recruit a clinical site and a patient is identified, we can send a drug product.

We have given serious consideration to comparability assessment. Such studies are critically important when you begin working with regulatory agencies, so we're continually building out assay suites and quality teams. Internally, we can analyze a drug's strength, purity, potency, and specificity. We also can qualify all of our reagents. Identified quality attributes and process parameters need to meet standards for good manufacturing practice (GMP) in Europe and the United States. All that analytical work requires significant effort and resources, but it's been worthwhile to invest in such capabilities.

You've already noted some advantages to allogeneic production processes. What challenges come with that approach? Variability in donor material is bound to happen, but that can be mitigated with sheer numbers of donors. Atara has assembled a large network of healthy donors to ensure a large enough pool of raw material to minimize the impacts of variability. And once you find a healthy donor, you want to be able to recruit that person again. We have resources for doing so, too. But establishing such infrastructure requires significant investment.

Using a PD-1 DNR offers an elegant solution to **PROTECT** T cells from hostile environments that exert tremendous selectional pressure to suppress and exhaust them.

—C. Nguyen

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Background

Cell-based chimeric antigen receptor (CAR) T cell therapies have rapidly advanced from preclinical research—with a variety of targets in clinical research and several FDA-approved products currently on the market [1]. This success has driven an influx of companies to further develop CAR T cell constructs to make them more effective, safe, and persistent. On the manufacturing side, however, errors, lot-to-lot variation, and contamination can be associated with open processing and manual handling of CAR T products. Overcoming the bioprocessing bottleneck remains a critical challenge in CAR T cell therapy scalability, which can potentially hinder both product development and patient access. It has been reported that about 7–9% of patients have been unable to receive one of the FDA-approved CAR T cell therapies because of manufacturing failures [2].

Autologous CAR T cell therapies are donor-specific, where a donor's own immune cells are used to create therapeutic CAR T (Figure 1A). During the manufacturing process, a Leukopak™ bag from the donor is received by a GMP facility, where the T cells are isolated from peripheral blood mononuclear cells (PBMCs), activated, and genetically engineered by viral transduction to express a CAR. The activated T cells are expanded in a T cell–specific cell culture medium, typically for 7–10 days to reach a therapeutically relevant number, and then they are cryopreserved. The cryopreserved CAR T cell product is then characterized and analyzed before being shipped to the treatment center, where it will be thawed and administered to the donor via infusion. This complicated, labor-intensive process usually involves many open manipulations and manual procedures, potentially

We also think a lot about preventing immunogenicity. Other companies have different solutions for that concern depending on their products' features and requirements, but our T-cell products retain their TCRs, building in specificity for their particular targets. We also select products based on appropriate patient HLA profiles. To date, we have dosed >300 patients with our tab-cel product, with no reports of graft rejection, graft loss, or cytokine storms attributed to it.

What are the biggest challenges facing developers of CAR-T products? The science needs to catch up to address unmet medical need for solid tumors. To do that, CAR-T developers need to implement next-generation approaches, focusing especially on T-cell quality. Cells must be safe and specific for their targets, and they must be produced to meet high standards. T-cell products also need to serve their purpose. Your cells must be capable of traveling to target regions — and still be cytolytic effector cells when they get there. Data from recent CD19 CAR-T trials also remind us that T cells must show strong expansion capability and persistence. When cells have not proliferated or persisted, the therapies either have lacked efficacy or not produced durable responses. You need to ensure that your product truly provides clinical benefits for patients.

My company takes such considerations seriously. It might seem that we are moving at a deliberate pace, but we take pains to ensure that we've addressed safety and quality. The benefit of that strategy is coming through now. You don't get to close to submitting a biologics license application (BLA) by accident. It takes a lot of infrastructure to get there.

The cell therapy industry also needs to bring manufacturing to the next level. Compared with the high titers that are generated in the antibody industry, cell therapy developers are a few generations behind. But because our products are living drugs in culture, we need to think about production differently, especially when using closed systems. Not only do we need to determine optimal growth rates, nutrient levels over time, and maximum cell densities, but we also must determine T-cell proliferation and whether that correlates with a therapy's function. It's all a moving target, and that's just at small scales. Scale-up introduces all kinds of complexity, so a process that works in a 300-mL bioreactor is not guaranteed to work at 50-L scales.

What do you find most encouraging about Atara's work and about the state of CAR-T more broadly? Having joined Atara recently, I am amazed that we are working to get drugs to patients who have limited options. For instance, patients with EBV-associated PTLD often die shortly after initial treatment failure. But when other options have not worked, such patients might be able to receive our drug in the future. It also amazes me that we can take the same EBV T-cell technology and apply it to progressive MS. I am excited to know that we have a clinical-stage platform with potential applications for multiple diseases, and having that enables me to focus on early discovery phases.

T-cell products need to serve their **PURPOSE**. Your cells must be capable of traveling to target regions — and still be **CYTOLYTIC** effector cells when they get there.

—C. Nguyen

When I consider today's CAR-T field, I think about how the landscape has changed. Patients with B-cell lymphomas and other such cancers did not have a lot of hope 20 or even 10 years ago. Chemotherapies would fail; antibodies would fail. Now, we are talking about off-the-shelf CAR-T therapies, and clinical trials are starting to send early positive signals for treating solid tumors. Given more time, I believe that CAR-T will transform medicine.

Moving forward, I believe that cell therapy developers need to ask how much thought they can put into their CAR-T designs — that is, how much they need to worry about applying next-generation biology and how much of that they realistically can incorporate into one cell.

My company learns something new about T-cell therapy development every day. As we continue to understand the mechanisms behind our tab-cel and ATA188 products, we're repeatedly surprised by the biology of EBV. From outside the company, our interest in EBV might seem to be a weird investment, but now clinical data are emerging to show just how much EBV influences our biology.

Preserving Proliferative Capacity

Improving CAR-T outcomes will require drug developers not only to devise GMP-grade allogeneic production strategies, but also to find ways of boosting their cells' abilities to expand and persist in a patient's body. Such considerations will be especially important for advancing the treatment of solid tumors. In September 2021, I learned from Eric Ostertag (chief executive officer of Poseida Therapeutics) that several options exist for mitigating T-cell exhaustion. The most popular approach, he explained, is the "armoring" strategy, which involves engineering of T cells to express and possibly secrete additional proteins (e.g., PD-1 DNRs) to reduce the immunosuppressive effects of solid-tumor microenvironments (**15**–**16**). Armored designs could help ensure that CAR T cells proliferate widely enough — and for long enough — to exact their antitumor effects. Osertag observed, however, that "cell type matters" and that even some early stage T-cell phenotypes might be too differentiated to serve as effective therapeutic platforms.

Poseida's CAR-T therapies rely on *T stem-cell memory* (T_{SCM}) cells: multipotent, self-renewing cells that have long lifespans and can

Cell Type Matters: Memory T Cells

Memory T cells are antigen-specific T lymphocytes that augment adaptive immune responses. All such cells live long and expand quickly into large numbers of effector cells upon recognition of their cognate antigens. But several subtypes exist, each with distinctive properties — and thus different advantages and disadvantages for production of cell therapies.

T central memory (T_{CM}) cells share several features with stem cells, including capacity for self-renewal. That property derives from the cells' high level of phosphorylation on key transcription factor STAT5. T_{CM} cells confer more powerful immunity against viruses, bacteria, and cancer cells than do effector cells.

T effector memory (T_{EM}) cells serve primarily as CD8 variants, inciting cytotoxic action against pathogens. They lack lymph-node–homing receptors and thus are found in peripheral circulation and tissues.

T tissue resident-memory (T_{RM}) cells occupy barrier tissues (e.g., epithelial tissues) for long periods and do not recirculate. They initiate quick responses to pathogens and are known to secrete granzyme B.

T stem-cell memory (T_{SCM}) cells are multipotent, self-renewing cells with long lifespans. They can reconstitute the full spectrum of T-cell subsets. Much remains to be learned about these cells.

T virtual memory (T_{VM}) cells circulate in the periphery in low numbers. Currently, their only known function is cytokine production.

Reference: Memory T Cell. *Wikipedia* 9 September 2021; https://en.wikipedia.org/wiki/Memory_T_cell.

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reconstitute the full spectrum of T-cell subsets. The company's CAR-T pipeline features several candidates based on such cells, including autologous and allogeneic products for multiple myeloma (P-BCMA-101 and P-BCMA-ALLO1, respectively), autologous and allogeneic therapies for prostate cancer (P-PSMA-101 and P-PSMA-ALLO1), and an allogeneic treatment for multiple solid-tumor indications (P-MUC1C-ALLO1). I spoke with Ostertag about how T_{SCM} cells could facilitate development of off-the-shelf therapies and about what technologies could improve processing and scalability.

How would you characterize the different generations of CAR-T approaches, and how do Poseida's products fit into the CAR-T landscape? Early CAR-T products focused on what was happening inside T cells. First- and second-generation approaches explored what stimulatory domains to incorporate and how to increase cells' proliferative capacity and persistence. Since then, the industry overwhelmingly has switched to a third-generation CAR design, which incorporates multiple costimulatory domains. The newest wave of treatments, however, seeks to armor CAR T cells against solid-tumor microenvironments.

Poseida's CAR-T products use a third-generation signaling domain. We, too, have developed armoring platforms but haven't needed them yet because we have focused on maximizing the percentage f the best type of T cells in our final products. A T cell's type significantly influences its metabolic activity and proliferative capacity, and we've figured out how to create a CAR-T product with extremely high percentages of desirable T_{SCM} cells. That strategy is not an armoring approach as such; our T cells are just protected naturally against the effects of exhaustion. So our products seem to be in a class of their own, not fitting cleanly into any of the prior generations of CAR-T therapy development.

Why do cancer patients with solid tumors show lackluster responses to CAR-T products? The reasons behind the poor outcomes are multifactorial, but a problem that seems to be inherent to most CAR-T products is exhaustion. Typically, target cells are grown, then modified and manipulated outside of the body. Such an approach doesn't do anything to select for or maintain the best cell type (T_{SCM}), but rather it yields an exhausted product that comprises more differentiated cells, such as effector (T_{EFF}), effector memory (T_{EM}), and T_{CM} cells.

Some companies say that their products are based on "early memory" CAR T cells. But such products include T_{CM} cells, and those are not the earliest type of memory T cell. We have found that using T_{CM} cells doesn't cut it. They do not have the same properties as T_{SCM} cells or more differentiated T_{EM} and effector-cell products. T_{CM} cells are already exhausted. They don't persist as long as T_{SCM} cells do. Products composed of more differentiated T cells are plagued by toxicity problems that, many researchers believe, are caused by the cytokines that such cells secrete. Products based on more differentiated cells can immediately initiate cytokine release cascades that can harm patients.

Typically, target cells are grown, then modified and manipulated outside of the body. But such an approach doesn't do anything about the **CELL TYPE**, so what it yields is an **EXHAUSTED** product that acts more like an effector memory cell than as a central memory stem-cell memory T cell.
—E. Ostertag

Although CAR-T limitations are multifactorial, we believe that many of the problems derive from competitor products' use of inappropriate cell types. That said, some issues are indication or target specific. Even though CAR-T approaches initially showed incredible success in treating B-cell leukemia and lymphoma, some patients still have relapsed. Sometimes that stems from antigen escape; sometimes other issues are to blame. Redosing can be difficult. Immune responses to drug products can generate cytotoxic events, and antidrug antibodies can be stimulated.

We are trying to solve such problems by focusing on cell type, specifically on producing high-T_{SCM} products. Other companies and research institutions already have correlated our approach with positive clinical responses. It can provide unprecedented efficacy. Not all patients benefit from such an approach, as is true with all candidate therapeutics. But some patients in our clinical trials are showing two-year stringent complete responses (sCRs), with detectable cells in the periphery. Such an outcome would be impossible using previous, more effector-like products because such cells just don't live for more than weeks to months.

We think of our products as acting like prodrugs that go into patients, engraft, and set off multiple waves of effector cells. Why is a multiple-wave approach important? In liquid tumors especially, you can achieve long-lived responses without needing to readminister your product, even using more differentiated cells. We've observed that result in both mouse models and human subjects. However, we believe that a multiple-wave approach will be key to treating solid tumors. Essentially, a single administration of a T_{SCM} "prodrug" could produce the equivalent of multiple doses of a more differentiated product, enabling cells to work away at tumors such that they "melt" over time. Our P-PSMA-101 candidate is showing the possibility of such outcomes. During the August 2021 virtual CAR-TCR Summit, we released what could be the best clinical results yet recorded for a CAR-T product in a solid-tumor indication, specifically metastatic, castrate-resistant prostate cancer (mCRPC).

We are finding that treatments based on T_{SCM} cells take a little longer to expand than effector-like products do. Our candidates reach much the same peak concentration (C_{max}) on a pharmacokinetics curve but a bit longer after administration. That result, we think, contributes greatly to our products' safety profiles. Our candidates show low rates of cytokine release syndrome (CRS). To date, clinical trials for our P-BCMA-101 autologous therapy have enrolled over 100 patients. None of those subjects has recorded grade three (or higher) CRS or required entry to an intensive care unit (ICU). None have experienced fatal toxicity events, and neurotoxicity levels have been quite low. Given the safety advantage of our product, many of our patients have received fully outpatient dosing: They receive their CAR T cells, go home, and often never need to return to a hospital except for laboratory testing.

Again, we believe such benefits derive from our products' cell type. Is that the only key to treating liquid and/or solid tumors?

Perhaps it is, but we and other companies have developed sophisticated armoring platforms as another recourse.

How might armoring platforms increase CAR-T efficacy? Five or six years ago, our company thought like everyone else in the field that we absolutely would need to armor our CARs to survive solid-tumor microenvironments. Limited access to all parts of a tumor, hypoxia in a tumor, and expression of immune checkpoint inhibitors are all factors that can present challenges to T cells. But mechanistically speaking, although some of those factors can hinder a CAR T cell's ability to kill a solid tumor, they shouldn't eliminate that capability altogether. The reason is that you can put any kind of binder or ligand on a CAR T cell, and it will serve as a mechanical receptor. Its binding interaction will create torsion, which then releases killing enzymes such as perforin and granzyme. So long as a T cell creates that interaction and maintains an appropriate distance from its target, those killing enzymes will be released.

CAR T cells can interact with their solid-tumor targets — maybe just not as efficiently as they might with a B-cell tumor, which would be right there in circulation. Thus, we have produced armoring platforms that induce cytokine secretion or deliver a checkpoint inhibitor drug only at the interaction site and only when a CAR-T engages its target.

Why has your company held off on armoring its CAR T cells? Rather than applying our armoring platforms at the onset of drug development, we began by focusing on evaluating different binders. At that time, we were working with difficult-to-treat solid-tumor models, including lymph-node carcinoma of the prostate (LNCaP) cells. To evaluate and distinguish between different binders, you don't want to dump an overwhelming number of CAR T cells into your model because doing that would produce a response no matter what; it would be artificial. Thus, we titrated down to what we call a "stress-test dose." We thought that such a low level of drug would be barely efficacious, but even without any armoring, that dose resulted in 100% tumor elimination in every animal model, every time. We wondered, how did that work?

When we investigated our animal models' blood, bone marrow, and lymph nodes, we found (as I mentioned) that our T_{SCM} cells acted as a prodrug. They homed to bone marrow and lymph nodes, then started making wave after wave of effector cells that chipped away at the tumors until they were gone. Those shorter-lived, more differentiated cells eventually died off, as predicted. But what remained were engrafted cells that served essentially as a secondary vaccination, and if a model relapsed, those cells

Figure 2: A scientist pipettes bioprocess material at Poseida's pilot facility in San Diego, CA (PHOTO COURTESY OF POSEIDA THERAPEUTICS)

produced a secondary response without the need for redosing. During clinical trials for our P-BCMA-101 candidate, we have observed the same effect in humans. Such outcomes would be impossible to achieve using more effector-like products.

How does your company approach CAR-T production and processing? As does every company that develops an autologous CAR-T product, we observed considerable variability in donor material early in process development. Sometimes we would find high transposition frequency on the front end of the process; other times, that value would be low. Thus, we sought out methods to select for cells that have been transposed.

On their own, regular T cells can't do anything for patients, but they certainly can release cytokines. To correct for donor-cell variability and minimize the number of nontransposed cells that appear in a drug product, we decided to perform positive selection. We explored different methods of accomplishing that, including the addition of a surface marker to transposed cells followed by selection of cells showing that marker. Ultimately, we chose to use a dihydrofolate reductase (*dhfr*) gene. It is a fully human gene that gives cells a slight resistance to methotrexate, enabling us to add very low doses of that nongenotoxic drug during our manufacturing process to ensure 100% transposition of CAR-positive cells at the end of the manufacturing process. Being able to achieve such high rates of CAR positivity also contributes to our candidates' strong safety profile.

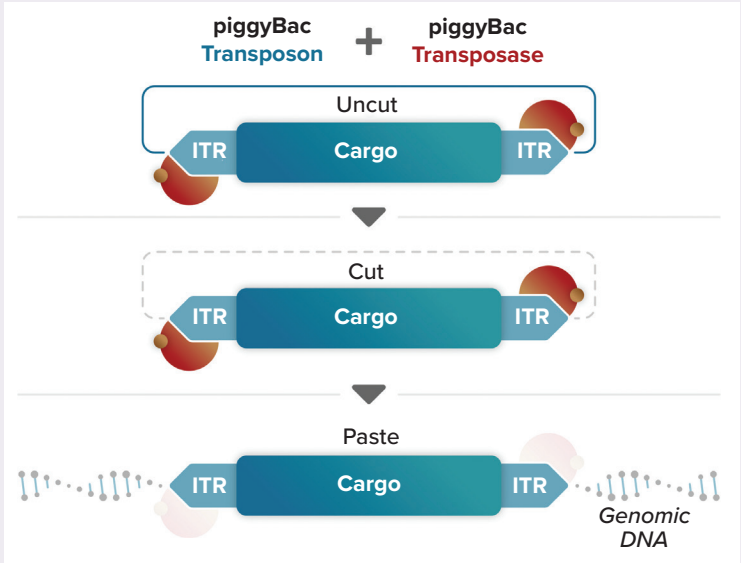
What factors limit CAR-T_{SCM} expansion, and what technologies does your company leverage to negotiate such hurdles? Some patients simply do not produce abundant T_{SCM} cell populations. As we age, our numbers of T_{SCM} cells diminish. That also happens after you've undergone multiple prior lines of therapy, as is usually the case for patients with multiple myeloma or mCRPC. Thus, patients' bodies tend to produce low percentages of what otherwise would be the most desirable cell type for treating their conditions. Thus, such cells cannot be extracted by conventional methods. They are rare, so you can't just pass them over a cell-isolation column or perform flow sorting.

Compounding that problem is the fact that virus-based technologies are almost completely unable to infect and transduce T_{SCM} cells. Drug developers often use lentivirus to transduce CAR T cells, but that step requires virions to bind T-cell surface receptors, and those generally are expressed only after cells have been activated. However, once you have activated your cells, they are well on their way toward differentiation and exhaustion. Even if they bear a couple surface markers that call them out as T_{SCM} cells, further investigation using more sensitive methods will show that they are not T_{SCM} cells, metabolically speaking.

Given such factors, we selected our proprietary Super piggyBac (PB) gene-delivery system to transpose T_{SCM} cells (Figure 3, next page). The nonviral technology uses a transposon and transposase to deliver nucleic acids into the genome. We were pleased to find

that although a PB system performs consistently well across multiple T-cell subsets, it preferentially transposes T_{SCM} cells. So even if such cells are rare, a PB method will reach them and deliver a therapeutic transgene. Combined with a positive-selection step to ensure 100% CAR positivity in our transposed cells, our process increases the percentage of T_{SCM} cells during manufacturing. Regardless of how many such cells a patient had been producing at the time of leukapheresis, we can shift the T-cell population heavily toward the T_{SCM} subtype. We further enhance the final product by favoring T_{SCM} self-renewal and blocking differentiation during manufacturing.

Figure 3: The Super *piggy*Bac DNA modification system (ITR = inverted terminal repeat sequence, PHOTO COURTESY OF POSEIDA THERAPEUTICS, HTTPS://WWW.POSEIDA.COM)



That advantage can be pushed further when moving from autologous to allogeneic processing. You've probably heard the term *allo tax*, which usually connotes that bad things happen when genetically modifying donor cells ex vivo. They can become exhausted. Moreover, TCRs must be removed from most products to prevent graft-versus-host disease, and doing that limits T-cell expansion capability. We have found, however, that both the PB-based systems for transgene delivery and our proprietary Cas-Clover platform for multiplexed gene editing work well in resting T cells. Thus, all of our gene delivery and editing can be completed before we activate our T cells, and we can maintain a high percentage of T_{SCM} cells even after gene editing. We achieve even higher percentages of T_{SCM} cells in an allogeneic process than in our autologous workflow because we will start with material from younger, healthier subjects and have fewer worries about donor variability. It's as if using those technologies gives us an "allo credit" rather than imposing a "tax."

The merits of allogeneic production are widely known, but what obstacles accompany such processes? Removal of endogenous TCRs diminishes cells' expansion potential. Part of the advantage of an off-the-shelf, allogeneic approach is supposed to be that many doses can be produced, which in turn would decrease production costs and enable manufacturing of off-the-shelf products. But no CAR-T company that I am aware of truly has figured out how to accomplish that. Typically, developers of allogeneic CAR-T therapies produce roughly a dozen doses from a single manufacturing run. That is an improvement over what can be realized from an autologous process — but not by much, especially if the cells that they are ending with are in worse shape than those that would be in the autologous version of the product.

To mitigate that concern, we have developed a "booster molecule" that can substitute functionally for TCRs that are

removed during production steps. That enables us to expand cells using the same reagents as we would apply during autologous manufacturing. The resulting process not only maintains a high percentage of "stemness" among our cells, but also provides excellent productivity, with hundreds of doses per run. If a dose equals ~50 million cells — and our candidates have shown efficacy against both liquid and solid tumors at lower doses than that — then some of our donors are providing us with enough material in a single leukopak to treat between 500 and 1,000 patients. That level of productivity can drop the cost of a cell therapy to that of a monoclonal antibody (MAb), and the resulting product would have a high percentage of T_{SCM} cells. Currently, our allogeneic products contain 60–80% T_{SCM} cells. The highest range that I have found reported among other companies is 6–8%. We believe that having such a substantial amount of T_{SCM} cells in the product will enable us to offer fully outpatient dosing — a significant advantage to add to a potentially best-in-class safety profile, strong efficacy, and great persistence of product cells in a patient's body.

What lessons have you learned from developing your autologous candidates, and how might they inform your work on allogeneic approaches? Sometimes I think that autologous programs are the "price of admission" for successful allogeneic production. The latter require significant understanding of T-cell biology, including knowledge about T-cell subsets and metabolism. We learned all of that through our autologous manufacturing processes. Because a CAR-T product is a "living drug," even more so than a conventional biologic is, its expansion potential is much more important than the number of cells in it. For that reason, patients sometimes exhibit better responses from low T-cell doses than they do from products with high numbers of cells. We learned that lesson as we started to manufacture our autologous candidates.

Another important learning was that developers can change their production processes in small but advantageous ways during clinical trials. Clearly, companies seek to lock down their processes as early as possible. Last year, however, we were able to adjust our process to incorporate Nanoplasmid expression vectors (Nature Technology Corporation). Those plasmids have been excellent additions because they are smaller, less toxic, and easier to produce than standard plasmids. They also eliminate concerns about bacterial selection and antibiotic residuals. Most important for our purposes, however, is that Nanoplasmid technology increases our transposition frequencies on the front end of the process. That enables us to start with more cells and thus reduces requirements for ex vivo expansion, which ultimately saves our T_{SCM} cells' highly desirable in vivo proliferation capacity for doing their job in a patient's body.

When we dosed clinical subjects with drug products made using the Nanoplasmid system during manufacturing, we confirmed that despite the therapeutic transgene remaining unchanged, our candidate's efficacy had improved with the use of Nanoplasmid technology. That was a compelling result and a significant

Because a CAR-T product is a "living drug," even more so than a conventional biologic is, its **EXPANSION POTENTIAL** is much more important than the number of cells in it.
—E. Ostertag

learning. We have now incorporated Nanoplasmid technology in all of our autologous and allogeneic product candidates.

How difficult is it to base a production process on a nonviral gene-delivery system, and how might you improve your process moving forward? Conceptually speaking, nonviral gene delivery is simple. You're just performing electroporation, enabling DNA and RNA to pass transiently into cells. Everything else in that process is natural; the PB system does what it's supposed to do. Then we perform our methotrexate selection step. We also apply our gene-editing reagents at the front end of the process, and like other parts of the production process, they just do what they're designed to do. Our allogeneic candidate also requires a purification step to remove residual TCR-positive cells. Then, we freeze the drug product and ship it to clinical sites to administer to patients.

Despite that simplicity, we are working continually toward developing a fully closed manufacturing process. We also want to ensure that our process will be highly scalable. Our booster molecule will play an important role in that endeavor. It enables allogeneic production of 500–1,000 doses from a single manufacturing run — enough material to supply an entire phase 1 or phase 2 clinical trial. Of course, we don't want to produce all of our drug product based on material from one donor; much can be learned from trying material from different donors and observing how that works in humans. But high scalability potential is vitally important. In the United States alone, multiple myeloma tallies nearly 35,000 new cases per year. The ability to make potentially 1,000 doses from a single manufacturing run would be a significant advantage in treating those emerging cases.

What do you find most encouraging about the state of CAR-T production, and what goals should the cell therapy industry adopt as research and process knowledge advance? One of the most significant lessons of the past five or six years is that a T cell's subtype will shape its therapeutic potential. I don't think that the early CAR-T industry gave much thought to those subtypes. "A T cell is a T cell, right?" No, it's not. Many subtypes exist. At Poseida, we believe that the T_{SCM} phenotype is the best cell type to use for development of CAR-T products; however, some researchers are beginning to focus on γδ and other T-cell types.

CAR-T products need clean targets to diminish risks for on-target–off-tumor toxicity. The industry also has learned that binders matter. To that end, drug developers now are considering what binders to select, how strong they must be, and how close or far they must be from target cells. We and others are working towards approaches that use multiple binders to the same target and/or binders to multiple different targets. We believe the best multibinder approach is to put each binder on a separate CAR molecule, which can rapidly increase the size of the therapeutic transgene. Our nonviral piggyBac method would greatly facilitate this multi-CAR approach because its cargo capacity is more than 20× greater than that of viral-based methods.

Two relatively recent advances will be vital as the cell therapy industry moves forward. One is that allogeneic approaches can work. Such approaches vary right now. Some companies already have adopted a "semiallogeneic" process, although we prefer a fully allogeneic approach. But at least we, as a field, now know from clinical experience that donor T cells can be modified in an allogeneic context to treat patients safely. The second advance is what Poseida described at the CAR-TCR Summit in August: that CAR-T can work against solid tumors. The CAR-T treatment modality is no longer restricted to liquid tumors, and in fact, it can treat some patients with solid tumors quite effectively.

Together, those advances indicate that CAR-T has a bright future. Now we can realistically conceive of an allogeneic CAR-T product for solid tumors. We have just received a "safe to proceed" notification from the US Food and Drug Administration for our first fully allogeneic CAR-T product, P-BCMA-ALLO1, and we plan to submit an IND application for another allogeneic CAR-T product candidate, P-MUC1C-ALLO1, later this year. That candidate has a compelling target: transmembrane glycoprotein mucin 1 C-terminal domain (MUC1-C), which is expressed in high abundance in epithelial-cell solid tumors such as breast, ovarian, colorectal, nonsmall-cell lung, and head and neck cancers. So now we are discussing the exciting possibility of an off-the-shelf, "pan–solid-tumor" therapeutic.

Still on the Brink of a Breakthrough

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Cell-therapy industry insiders generally agree that allogeneic CAR-T products and effective treatments for solid tumors are unlikely to reach patients as approved commercial products for at least five years (**17**). However, drug developers are close to realizing significant advances that will facilitate subsequent CAR-T development and production. New understanding of T-cell biology is improving selection and bolstering amplification of cells and important reagents used in autologous and allogeneic processing. Enhanced CAR designs and novel armoring technologies will help cells survive the immunosuppressive effects of difficult tumor microenvironments. And increasing availability of closed and automated processing systems can enable developers to standardize their processes while ensuring drug safety. Obstacles certainly remain, but with good reason, cautious optimism abounds in the CAR-T community.

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About the Author and Interviewees

Brian Gazaille *is associate editor for* BioProcess International, *part of Informa Connect; 1-212-600-3594; brian.gazaille@informa.com.*
Cokey Nguyen, *PhD, is chief scientific officer at Atara Biotherapeutics, 611 Gateway Boulevard, Suite 900, South San*

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Francisco, CA 94080; 1-650-278-8930; office@atarabio.com. And ***Eric Ostertag,*** *MD, PhD, is founder and chief executive officer at Poseida Therapeutics as well as founder of Transposagen, Hera Therapeutics, and Demeetra AgBio. Please direct inquires about Poseida's activities to 9390 Towne Centre Drive #200, San Diego, CA 92121; 1-858-779-3100; info@poseida.com.*