WHAT'S NEXT IN THE *Cell Therapy Revolution?*

Cell therapies have accomplished much, but what lies ahead? Experts tell us how advanced medicines can tackle solid tumors.

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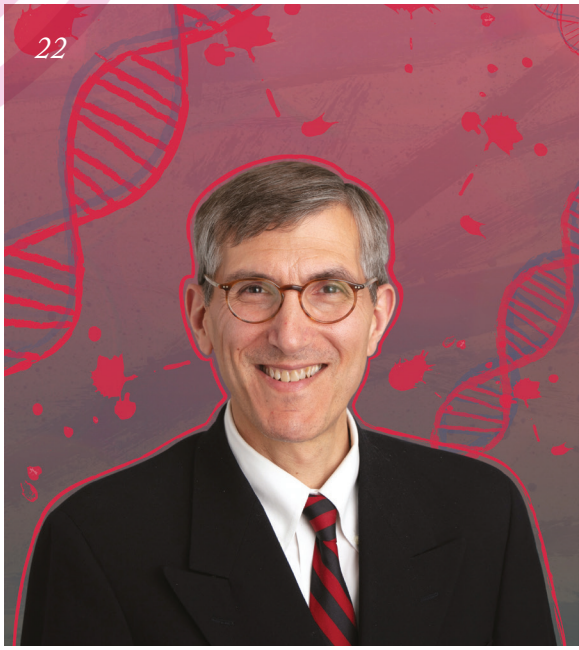

On The Cover

Searching for a way to complete the cell therapy revolution

Prepare to Be Amazed

We cannot predict what lies ahead with certainty but, given the pace of cell therapy research, I'm confident enough to feel excited

Welcome to the fourth edition of our annual supplement on the hottest area of drug development: cell and gene therapies. When I first entered the industry many years ago as a journalist, cell therapies were still within the realm of science fiction. In fact, my boss at the time tossed out my news story about cell therapy research: "These therapies will never reach the market."

But the fantastic thing about science is that it is constantly advancing – what seems impossible today may still come to pass in the years that follow. Given the trepidatious start (and the rejection of my initial excitement all those years ago), it's been fascinating to see the field of advanced medicine grow. Cell therapies have reached the market and, although treatment comes with risks, it can be life changing and curative. Record numbers of companies are now getting involved in the field, and we're seeing a record number of trials too. It's incredible given that cell therapies were seen as science fiction not that long ago.

We publish a weekly digest of news from the field via our Cell + Gene Curator newsletter – and each week the team is spoilt for choice; from early studies to trials to business deals, there is a lot going on. And greater achievements almost certainly lie ahead; for example, using cell therapies to treat solid tumors. Positive steps are being made (see our feature on page 12) – and the impact could be amazing.

If cell therapies are the science fiction of yesteryear, then "self-replicating living robots" – xenobots – tick that box today. Researchers believe such technology could eventually treat traumatic injury, birth defects, cancer, and even aging. I'll be honest: I'm skeptical. Very skeptical. And yet (remembering my old boss), I am open minded enough to accept that I may end up eating my words in the decades to come – when xenobots are busy repairing my somewhat older body…

Where will we be next year? Hopefully out of the COVID-19 pandemic – and hopefully with more cell therapy approvals. Where will we be in a few decades' time? I can't wait to see.

Sign up for The Cell + Gene Curator at: *www.texerenewsletters.com/cellandgene*

Stephanie Sutton
Editor

Reference

1. *University of Vermont, "Team Builds First Living Robots That Can Reproduce," (2021). Available at https://bit.ly/3GzAZnS*

Words From the Wise

Fancy a break from print? Check out our video interviews online...

With so much growth opportunity, it's no wonder that companies of all shapes and sizes are keen to make their mark in the cell and gene sector. At themedicinemaker.com, you'll find a variety of video interviews that give you the opportunity to hear views on trends and challenges, and why people have dedicated their careers to this space.

Tamas Masztis, Senior Director, Cell Therapy Supply Chain for Europe, Kite, a Gilead Company

Kite's cell therapy technology uses genetically modified T cells that increase the number of tumor-specific T cells and help the body to kill certain types of cancer cells.

tmm.txp.to/tamasmasztis

Sharon Brownlow, Chief Business Office at the Cell & Gene Therapy Catapult

Established as an independent center of excellence to help grow the UK's cell and gene therapy industry, the Cell and Gene Therapy Catapult works as a bridge between scientific research and manufacturing and commercialization.

tmm.txp.to/sharonbrownlow

Joe Healey, CEO and Co-Founder of NanoSyrinx

NanoSyrinx is looking to revolutionize the way medicines are administered by using naturally occurring, protein "nanosyringes" to encapsulate therapeutic payloads.

tmm.txp.to/joehealey

Edd Stone, Head of Cell and Gene at The Technology Partnership (TTP)

TTP is a technology and product development company that is active in numerous industries, including cell and gene therapy. Edd focuses on working with life sciences companies to automate complex biological workflows.

tmm.txp.to/eddstone

TIMELINE

What a Year

Our timeline chronicles key approvals and deals for cell and gene in 2021

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January

MSD and Artiva Biotherapeutics agree collaboration for CAR-NK cell therapies

February

Charles River says it will acquire Cognate BioServices

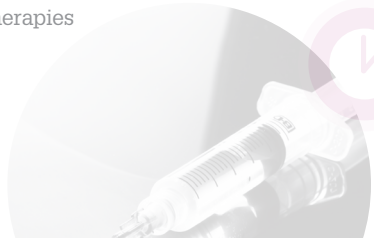
March

FDA approves cell-based gene therapy Abecma

UK's NICE cost watchdog recommends Zolgensma

May

FDA approves Breyanzi CAR T cell therapy



"A Year of Firsts and Records"

Is 2021 the best year yet for advanced medicines?

The Alliance of Regenerative Medicine has described 2021 as a "year of firsts and records" in a report that reviews the progress of the field. Here is our choice selection of key facts and figures from the report.

Key facts

- In the first half of 2021, regenerative medicine and advanced therapies raised US$14.1 billion – already 71 percent of what was raised in full-year 2020
- There are currently more than 1,300 industry-sponsored and another 1,300 plus non-industry sponsored regenerative medicine and advanced therapies trials ongoing worldwide
- Forecasts predict there will be 3,100 unique therapies in development by 2026, including 355 in phase III

Key quotes

"A scientific revolution is changing how we think about medicines. Next-generation CAR-T therapies will combine with other technologies to enhance potency and targeting. Genetic modification will allow scientists to turn therapies on or off, while gene editing platforms could help to create more potent cells. We will equip the human body to fight cancer – and the full potential of cells as medicines will bring extraordinary benefits to patients." – Bruce Levine, ARM Board Director

"In every conversation we're having with Brussels policymakers, we're advising them that the EU is at an inflection point – for its ability to compete globally and to provide continued access for European patients." – Paige Bischoff, Senior Vice President, Global Public Affairs, ARM

"Intellia's presentation of the first clinical data in history supporting precision editing of a disease-causing gene within the body following a single, systemic dose of CRISPR/Cas9 opens a new era of medicine – one that holds the potential of curing genetic disease." – John Leonard, Presidentand CEO, Intellia Therapeutics

Reference

1. *Alliance for Regenerative Medicine, "Regenerative Medicine in 2021: A Year of Firsts & Records," (2021). Available at https://bit.ly/3lRiGTv.*

Who's the Best?

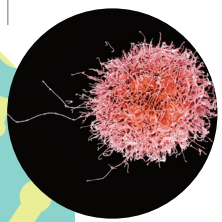
Celebrating the crème de la crème of drug development

The Medicine Maker Power List includes a category dedicated to the cell and gene therapy space. What is the Power List? It's our annual celebration of the most influential professionals that contribute to the development and manufacture of ground breaking new therapeutics – from small molecules, to biopharmaceuticals, to cell and gene therapies. And the most exciting part is that the power is in your hands to nominate the individuals that you want to see on the list — whether a mentor, colleague, hero scientist, or esteemed CEO.

You can see the latest list (published every April) and find out more about the nomination process by looking at the Power List section on our website.

July

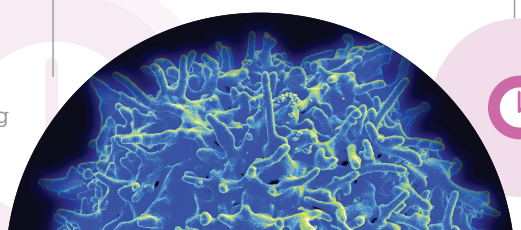
EMA approves Skysona gene therapy



August

Catalent completes acquisition of RheinCell Therapeutics

BioNTech acquires TCR R&D platform and clinical manufacturing facility from Kite



September

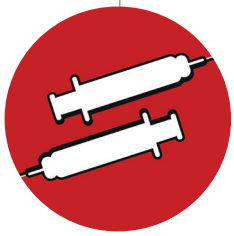
Novartis acquires Arctos Medical

October

Takeda and Poseida Therapeutics announce research collaboration

November

Sanofi invests equity in Gyroscope



December

Thermo Fisher Scientific completes acquisition of PPD

In My View

Experts from across the world share a single strongly held opinion or key idea.

Eyes on Endotoxins

Suppliers of reagents and buffers need to adhere to quality systems to ensure their products do not compromise cell and gene therapies

By Mary Medeiros, Formulation Chemist and Technical Services Manager at Teknova

Relying on the use of recombinant nucleic acids, engineered cells, and tissues, advanced therapy medicinal products (ATMPs) are attracting significant attention in the pharma industry, but their development requires extensive and complicated preclinical and clinical processes – and stringent testing. Though sterility testing of cell and gene products will rule out the presence of live microorganisms, there are other forms of contamination to consider. Here, I focus on one source of contamination in particular: endotoxins – the lipopolysaccharides from the cell wall of Gram-negative bacteria, typically released after cell death or lysis. In addition to negative effects on tissue cultures, endotoxins can cause mild-to-severe immune responses – and even death.

The raw materials that go into pharmaceutical products are not specifically regulated, but all parties involved in the development of ATMPs have an obligation to ensure their actions are documented in compliance with cGMP. Reagents and buffers are essential for pharmaceutical production, including cell and gene therapies.

The manufacturer of the final product is ultimately responsible for quality and safety. However, the significant risk posed by endotoxins means that reagent and buffer suppliers involved in production must demonstrate and document that all materials comply with specific endotoxin requirements so that the endotoxin level in the final drug product does not exceed the overall limit specified by regulators. For example, the US FDA has an algorithm to calculate the endotoxin limit for individual drug products that is based on a ratio of the threshold endotoxin dose to the maximum dose of the drug (1). The US Pharmacopeia (USP) encourages manufacturers to uphold high-quality standards through the bacterial endotoxins test (BET) described in USP Chapter 85 (USP <85>). The method of endotoxin detection used is also a crucial consideration. The Limulus amebocyte lysate (LAL) test is required for compliance with USP <85>.

Companies that develop ATMPs should carefully choose suppliers that have a sufficient quality management system in place and robust product quality control throughout the manufacturing process – from procurement of water for production to pre-filtration to final products. It is also important to consider overall supplier manufacturing standards used throughout the development process, including early-stage and research-use only (RUO) products. In my opinion, working with a company that manufactures RUO products to the higher standard – ISO 13485, rather than ISO 9001 – will confer advantages down the line. ISO 13485 represents the requirements for a quality management system for the design and manufacture of medical devices, including pharmaceuticals. It requires established processes, policies, and procedures to consistently demonstrate that products are fit for purpose.

I believe the quality of the partnership between ATMP companies and their suppliers plays an important role in laying out a smoother path to the clinic.

Reference

1. *FDA, "Bacterial Endotoxins/Pyrogens" (2014). Available at https://bit.ly/2OagSY1*

Purifying Gene Therapy

The gene therapy industry must maximize the amount of therapeutic gene payload being delivered with each vector to reduce the risk of immune reaction and, ultimately, cut costs. How? High-level purification.

By Akash Bhattacharya, Senior Application Scientist at Beckman Coulter Life Sciences

Most would agree that gene therapy holds enormous potential for treating, preventing, and even eradicating disease – and we are starting to see real results. The first gene therapy product was approved in the US by the FDA in 2017 but, since then, approvals have come rapidly.

Creating safe products that can be scaled up – and retain their safety – is key. The important element in manufacturing gene therapy products is purity, which refers to the efficiency of "packaging" for delivery into the cell. Poor packaging can lead to less effective therapy. And giving higher doses of therapy to offset poor packaging risks triggering an allergic reaction in the patient. The FDA has issued guidance for clinical trials that addresses the importance of this very issue.

As a quick recap, gene therapy typically involves inserting genetic material into cells via a harmless viral vector that "infects" the cell and delivers the genetic payload. One of the most popular vectors for packaging is the adeno-associated virus (AAV), which has two key benefits: efficiency as a gene delivery vector and low pathogenicity. The virus has been modified from the wild type to optimize its efficiency as a therapeutic gene carrier and minimize its potential to cause disease. Recombinant AAV is the leading platform for gene therapy today.

Although the AAV itself is harmless, it is a foreign substance, so it can trigger an immune reaction in the patient. Packaging efficiency greatly affects this consideration, especially if a larger dose of the payload gene is injected in hopes of a greater therapeutic effect. For instance, if the packaging efficiency of the packaging is very poor, eight out of 10 viral packages might be empty or only partially loaded. Logically, then, to deliver the desired dose of the payload gene, you might have to dose the patient with a proportionately higher amount of total vector. This process may trigger an elevated allergic response, so gets no marks for safety (in fact, just the opposite).

To minimize risk, the obvious solution is to maximize the amount of therapeutic gene payload delivered with each vector – in other words, high-level purification. The strategies employed have been the subject of my own research over the years. Two related methods are ultracentrifugation and analytical ultracentrifugation (AUC), which can, respectively, purify and characterize a gene therapy product.

Ultracentrifugation has been around for decades, but has advanced rapidly in recent years. The machine itself is a fraction of its previous size, with many more capabilities: spinning at 100,000 rpm or greater and delivering 100,000 g. The ultracentrifuge can separate compounds of similar size, but different densities, based on a density gradient – which means it does an excellent job of isolating filled AAV vehicles from partially filled or empty vehicles. The ultracentrifuge is a method for arriving at a product of high homogeneity and purity.

Once you've isolated your product, a quality check is in order. Analytical ultracentrifugation (AUC) can be used for this purpose, because it is very good at characterizing the purified product. In short, a very small amount of the sample is run on the AUC, which employs sophisticated detectors and highly complex mathematics to determine the percentage of filled vehicles. Combining ultracentrifugation with AUC to achieve – and evaluate – a good drug product could minimize patient risk.

Gene therapy technologies are fast advancing and close interdisciplinary collaboration makes it all possible. Our virology colleagues, for instance, are working on ways to modify and scale up triple transfection, the elegant process by which the AAV itself is made. The classic method uses human embryonic kidney cells, known as HEK-293. But a newer strategy employs an insect cell line system, Sf9 , which holds a great deal of promise.

On our end, we will continue to streamline the instruments and workflow until the entire experiment and analytic process conform to current good manufacturing practices. We also hope to further reduce time and costs with the ability to analyze "dirtier" samples closer to the bioreactor and before multiple rounds of purification. Another goal is to make some of the elements fully disposable, which is important from a biohazard perspective.

Gene therapy is a powerful step in the direction of eradicating a disease – something that has only happened a few times in history. Eliminating diseases that affect a large percentage of the population or are particularly devastating – such as macular degeneration or neurodegeneration – would be an enormous advance, but one I believe will someday be achievable through simplifying workflows and increasing purity and safety. These steps will ultimately allow us to distribute gene therapy safely and affordably to much greater numbers of people.

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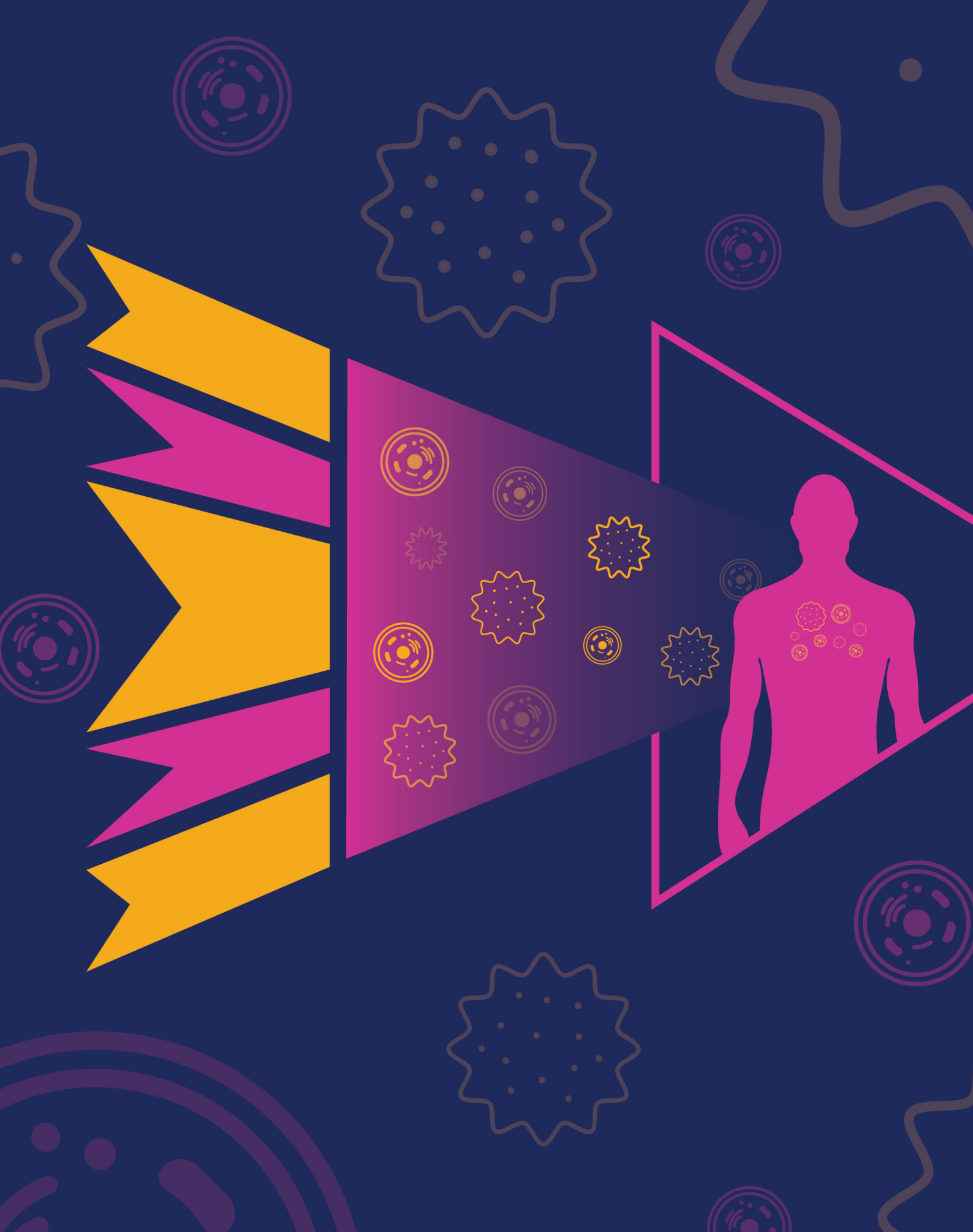


COMPLETING *The* CELL THERAPY REVOLUTION

With no specific antigens available and a highly immunosuppressive microenvironment with little blood flow, how will researchers tackle solid tumors and bring cell therapy to millions?

By James Strachan

In 2017, the pharmaceutical industry erupted in celebration as the FDA approved the first two CAR T-cell therapies, Yescarta and Kymriah. Until then, the prospect of extracting a patient's cells, modifying them to express chimeric antigen receptors on the surface, and reinfusing them into the patient to latch onto specific antigens to kill tumors had seemed like science fiction to many. But FDA approvals answered the doubters: CAR T works.

However, other questions remained unanswered. Ideal drug manufacturing and logistic processes are closed and automated to eliminate the risks associated with human intervention and manual operations – but this is not the case with autologous CAR T. So how would companies handle living, breathing cells in transit? Would healthcare systems be able to cope? Pricing too was a concern. Would all stakeholders embrace evidence-based pricing?

Though these questions are yet to be fully resolved, we are seeing a conversational shift back to where it all began: scientific efficacy. We know cell therapy works in liquid tumors (leukemia and lymphoma), but what about solid tumors, which represent approximately 90 percent of adult human cancers and, therefore, a huge area of unmet need. In short, what's the hold up?

"We all thought solid tumors might be a little bit harder – but how hard could it really be?" asks Bruce Levine, Barbara and Edward Netter Professor in Cancer Gene Therapy at the University of Pennsylvania, and President of the International Society for Cell & Gene Therapy (ISCT). "Quite a lot harder, it turns out."

The central challenge is antigen specificity. The first CAR T-cell therapies were approved for beta cell-malignancies, which have easily identifiable surface markers, such as CD-19 or BCMA. An anti-CD-19 CAR T-cell therapy may wipe out most of a patient's B-cells in addition to their cancer, though this isn't a major problem. "But if you found a target that was unique to lung tissue, for example, you couldn't easily treat it with a T cell therapy because you'd run the risk of also seriously damaging the patient's lungs," says Elliot Norry, Chief Medical Officer at Adaptimmune.

Some targets, such as EGFRviii, are tumor specific – so attacking these does not risk wiping out the patient's organs. However, they're only present in about a third of glioblastomas. Finding a target that is both tumor specific and homogeneously expressed has vexed developers looking to target solid tumors. The first blood cancer cell therapies were far less challenging.

"You need to be looking at multiple targets," says Levine. "But you also need to titrate those targets." He raises the example of mesothelin, which is expressed in pancreatic adenocarcinomas, mesotheliomas, ovarian cancers, and about half of lung cancers – plus others. The catch is that mesothelin also exists at lower levels in the pleural cavity, which means any potential cell therapy targeting it could be destructive to a certain degree if not titered or controlled.

Another hurdle is the highly immunosuppressive solid tumor microenvironment, which includes the expression of checkpoint ligands, the secretion of immunosuppressive mediators like TGF-beta, the presence of regulatory T cells, and myeloid-derived suppressor cells – all of which conspire to prevent the immune system from detecting and killing the tumor. To make matters worse, solid tumors aren't well vascularized; the stroma is tightly packed and resistant to penetration by immune cells because of a matrix of cancer-associated fibroblasts.

But Levine thinks we have strategies to combat each of these problems. "It's going to take a combination of strategies and targets, including synthetic biology. The route of administration may be important too," he argues. "I'm optimistic because we do see evidence of clinical activity in both preclinical models and some early clinical trials."

"Solid tumors are the field's holy grail right now," says Tony Ting, Chief Scientific Officer at Bone Therapeutics. "This is something people have been focused on for quite some time, but we're all hopeful of strong clinical results in the near future."

SIGNS OF SCIENTIFIC EFFICACY

So how might developers go after multiple targets? Levine cites a paper by Anna Wing, Carl June, and colleagues from 2018 (1); their approach targets two antigens at once using both CAR T-cells and an oncolytic virus-driven bispecific antibody. "It's one of my favourite papers," says Levine, who worked with Carl June on developing the first CAR T-cell therapies. "Essentially, you get three for one; you have the two antigens targeted as well as the antigens released by the oncolytic vector."

With regard to synthetic biology, Levine highlights the integration of switch receptors. "This involves turning a negative signal into a positive," he explains. "You can make a switch receptor with PD-1, extracellularly, and then a signal-transducing co-stimulatory signal like CD-28. So when the tumor delivers a negative signal, the engineered T-cell sees it as a positive signal. That's really clever."

In April, the University of California San Francisco published two papers on their "SynNotch" system. In the first paper, they found that SynNotch-CAR T cells could

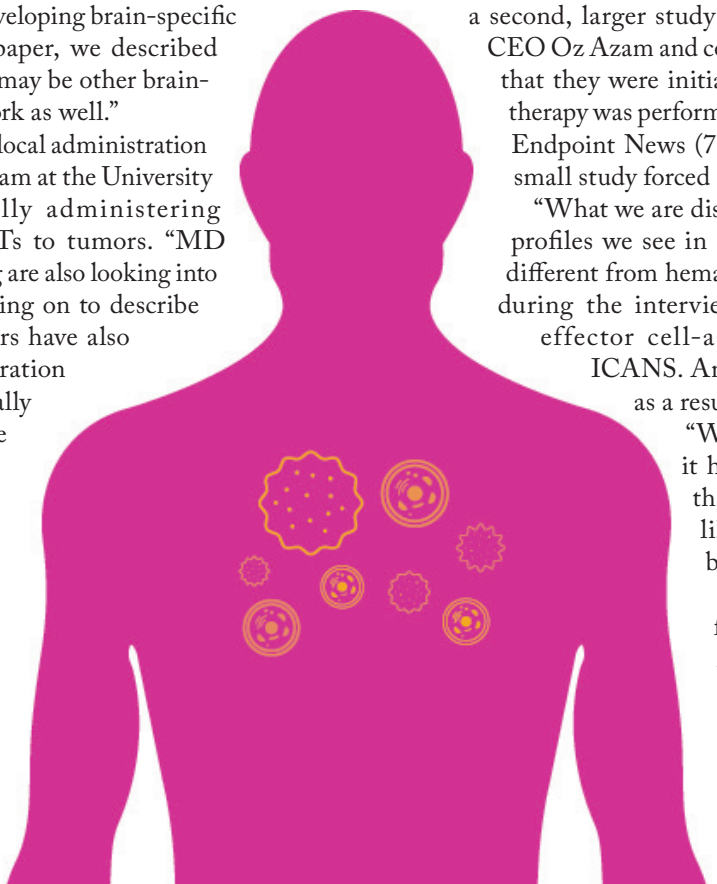
> "It's going to take a combination of strategies and targets, including synthetic biology."

completely clear human patient-derived tumors from the brains of mice – safely and without recurrence (2). In a second paper, another set of researchers showed how components of the system can be switched out to target other cancers, such as ovarian and lung (3).

The new approach has two steps. The first step uses SynNotch to grant CAR Ts the ability to "judge" whether they are in a tumor. The second step uses a different set of SynNotch sensors to ensure a strong tumor-killing response. "Our approach allows us to prime the expression of the CAR against broad tumor antigens only in conditions where the T cells see tumor-specific or brain-specific signals," says Hideho Okada, co-author of the first paper. "As such, the SynNotch-T cells are safer and more effective."

Okada and his team are actively working on moving into the clinic. "We're also developing brain-specific priming," he says. "In the paper, we described priming by MOG, but there may be other brain-specific antigens that may work as well."

Levine is also excited about local administration of CAR T-cell therapy. His team at the University of Pennsylvania are locally administering mesothelin-targeted CAR Ts to tumors. "MD Anderson and Sloan Kettering are also looking into this approach," he says, moving on to describe how City of Hope researchers have also incorporated local administration into the CNS. "That's technically challenging, but they did see some evidence of clinical efficacy." They've also used lentiviral transfer of CAR, targeting mesothelin. "We saw clinical activity in one-out-of-five pancreatic cancer patients using that approach," says Levine. UPenn and City of Hope have also targeted EGFRviii in glioblastoma. In the University of Pennsylvania clinical trial, investigators saw tumor necrosis and downregulation of the target in patient tumor tissue.



Another promising area is macrophage-based cell therapy. In 2020, University of Pennsylvania researchers genetically engineered macrophages to kill solid tumors in both mouse models and human samples (4). Then, in March 2021, Carisma Therapeutics – a company founded by researchers at the University of Pennsylvania – announced that it had dosed its first human participant in a phase I clinical study assessing the safety of CAR macrophages (5).

"Engineered macrophages may be particularly suited to the very challenging microenvironment of solid tumors," says Levine. In a review of recent developments in CAR-macrophage-based treatments for solid tumors from Anhui Medical University, China, researchers cited "great potential" when it came to migration to tumor and recruitment of immune effector cells (6).

However, a central challenge with engineered cell therapy is the potential for toxicity and cytokine release syndrome. Tmunity recently suffered a serious setback after the company was forced to shut down and modify their lead program for prostate cancer after two patients died following CAR T-cell therapy. The researchers had taken PSMA-specific and TGFβ-resistant CAR-modified autologous T cells into an 18-subject phase I prostate cancer trial in 2017. Tmunity then began a second, larger study late in 2019. President and CEO Oz Azam and co-founder Carl June explained that they were initially shocked at how well the therapy was performing in a recent interview with Endpoint News (7). But the two deaths in the small study forced a rethink.

"What we are discovering is that the cytokine profiles we see in solid tumors are completely different from hematologic cancers," said Azam, during the interview. "We observed immune effector cell-associated neurotoxicity – ICANS. And we had two patient deaths as a result of that."

"We didn't see this coming until it happened," said June. "But I think we'll engineer around just like we did with tocilizumab back in 2012."

"We've been lulled into a false sense of security by the rapid progress with blood cancers," says Levine. "But with solid tumors, while

Bruce Levine

we're making progress – we have more centers working on the problem, as well as new tools and technologies – we're going to need long attention spans."

THE CAR ALTERNATIVES

Another set of promising non-CAR-based approaches to the development of solid tumor therapy involves T-cell receptors (TCRs). CAR technology uses an artificial receptor introduced into the immune effector cells to recognize tumor cell surface proteins (such as CD-19 or EGFRviii). In contrast, TCR-engineered effector cells use naturally occurring (or minimally modified) TCRs that have been selected for their ability to recognize tumor-specific epitopes presented by the major histocompatibility complex (MHC) molecules on the tumor cell surface.

"Here, you're targeting peptide fragments from intracellular targets expressed on the cell surface in the context of HLA, which only TCRs can address," says Norry, who has been actively researching this area alongside his colleagues at Adaptimmune. "This increases the number of potential targets and allows for greater specificity – you can more readily differentiate between cancer and healthy tissue."

Our T-cell receptors may be recognizing malignant cells all the time and destroying them without us ever realizing. Some malignant cells avoid this protective mechanism and become tumors. By enhancing the affinity of these receptors, researchers can give TCRs the ability to recognize a tumor as foreign – and then attack it.

In addition to enhancing the affinity of the T-cell receptor, researchers are also focused on improving the potency of T cells as a whole. "We and other groups are focusing on increasing the ability of T cells to overcome the inhibitory features of the tumor microenvironment," says Norry. "We've also shown, in a laboratory setting, that we can enhance their ability to recruit the rest of the immune system once activated."

The ability to recognize intracellular antigen fragments presented by MHC molecules increases the number of targets available to TCR therapies; however, it also makes the therapy "MHC restricted," which means their activity depends on presentation by MHC molecules to recognize targets and activate T cell functions. "This is a potential limitation because we all have our own MHC (or HLA) types – some are more or less common," says Norry. "This means that a given TCR may only work in a certain sub-population."

Norry and his team are developing TCRs that work across various HLA types. "We're also developing something called an HLA-independent TCR, which would expand the applicability of the therapy to a broader population."

Researchers from the MD Anderson Cancer Center recently reviewed the current technology and early clinical development of TCR-based therapy in patients with solid tumors, concluding that, while still early stage, TCR therapies may prove to be a "more effective option for solid tumors where intracellular antigens presented in MHC." The researchers also thought it "plausible" that TCR therapies could be cheaper, given the "substantially lower costs" associated with the manufacturing processes. However, Levine is skeptical of the costs being substantially lower. "I'm not aware of how this would be true for TCRs and not for CARs," he says.

"We're very optimistic about TCRs," says Norry. "We have a first-generation TCR in the clinic for patients with sarcoma, which we believe will become the first registered TCR-based

> "But treating hundreds of thousands – or even millions – of patients using this relatively complicated, somewhat manual manufacturing process seems unlikely."

therapy for solid tumors. We also have a next-generation TCR therapy in the clinic that incorporates a CD8-alpha cofactor, which enhances the killing capability of the product (giving it enhanced killer T-cell properties)."

In addition to TCR therapy, researchers are also interested in tumor-infiltrating lymphocyte (TIL) therapy, which involves harvesting infiltrated lymphocytes from tumors, then culturing and amplifying them in vitro, and finally infusing them back to treat patients.

"I remember listening to a talk by Steve Rosenberg about TILs in 1986," says Ting, who also recounted how Rosenberg isolated TILs from multiple mouse tumor models in 1982 – the first time in history. In fact, the earliest attempt at TIL therapy in the clinic goes back to 1988, in which a 60 percent objective response rate in metastatic melanoma was achieved. "Now they're being used to treat solid tumors in clinical trials."

Because TILs are composed of T cells with multiple TCR clones capable of recognizing an array of tumor antigens, a TIL-based approach may allow researchers to tackle tumor heterogeneity more easily than in CAR T and TCR T-cell therapy.

A recent review of TIL therapy for solid tumors found that there have been 79 trials of TIL therapy, including 22 kinds of TIL products between 2011 and 2020 – and factoring in two successful phase II trials by Iovance in 2018 (8). The researchers highlighted "impressive clinical benefits" in metastatic melanoma and advanced cervical cancer, even in patients treated with checkpoint inhibitors, while emphasizing that "the laborious, expensive, and time-consuming tissue collection and production process" means TILs are only currently being developed at a few leading research institutions and companies in a handful of countries.

"It has become increasingly apparent that TIL therapies will have a role to play in selected indications," says Norry. "This is why we are working with the CCIT in Denmark to develop a next-generation TIL product. We believe the ability to modify TILs with our next-gen scientific capabilities to potentially enhance efficacy has great promise."

ALLO VERSUS AUTO

So far, the CAR T-cell therapies that have made it to the market have been autologous (the patient's own cells are taken out of their body, modified so they target cancer cells, and then reinjected). But treating hundreds of thousands – or even millions – of solid tumor cancer patients using this relatively complicated, somewhat manual manufacturing process seems unlikely. One alternative is allogeneic cell therapy – an off-the-shelf alternative in which donor cells (rather than the patient's own cells) are modified, which can reduce production time, cost, manufacturing delays, and dependence on the functional fitness of patient T cells.

The major downside of allogeneic cell therapy is the potential for graft-versus-host disease, and host allorejection. There are, however, several approaches to overcome or at least ameliorate this difficulty, such as the generation of TCR-deficient T cells using genome editing tools such as CRISPR/Cas9. Researchers are also evaluating repeated rounds of administration, using chemotherapy-resistant CAR T-cell or genetically eliminating key molecules governing CAR T-cell immunogenicity (9).

Besides T cells, other cells are also being explored to generate allogeneic cell therapies. Most commonly this applies to NK cells because of their potent cytotoxic anti-tumor activity and favorable safety profile. NK cells tend to possess a smaller risk of inducing GVHD because (as opposed to T cells) NK cells kill independently of MHC expression – though one of the ways by which NK cells kill is by sensing the absence of self MHC. In 2020, Fate Therapeutics announced encouraging preliminary phase I data for their iPSC-derived allogeneic NK-cell therapy in advanced solid tumors – the first study in the US to evaluate an iPSC-derived cell product. Among 15 heavily pre-treated patients (nine of whom were refractory to prior therapy), 11 had a best overall response of Stable Disease (10).

SO, IS ALLOGENEIC THE ANSWER?

"There are some great qualities to allogeneic therapies," says Levine. "They can be made in advance, stored in the freezer, and ready to go within days. And there are certainly patients from whom we cannot collect or generate enough quality CAR

T or even CAR-NK cells for autologous cell therapy.

"But I think it's going to be both – I just can't see allo-therapies ever reaching the potency of autologous therapies. For me, it's more a question of how these therapies will evolve together – because they aren't being developed independently of one another."

But Norry believes that allogeneic approaches are particularly exciting: "The product can be more consistent from patient to patient, and you have the ability to gene edit rather than using a viral vector to introduce a piece, or multiple pieces of genetic material into the cell.

"Really, all of the various iterations of TCR therapy can be made using an allogeneic platform, and we – alongside several other companies – are making good progress in the allogeneic space. Ultimately, it's about making a real difference to the patient and I think both allogeneic and autologous approaches can do that for solid tumors."

In the end, the successful approach may be something totally out of the box. "There's got to be a revolution," says Levine. "When we're thinking about autologous therapy: integrating automation for sure, but maybe even going beyond that and generating CAR T-cells in vivo. There are several companies – probably a dozen now – using viral vectors or nanoparticles to create CAR T-cells in the patients without having to extract, modify, and readminister."

Recently, researchers from Nanjing University generated CAR T-cells in vivo using AAV vectors carrying the CAR gene. This "AAV delivering CAR gene therapy" (ACG) resulted in tumor regression in a mouse model of human T-cell leukemia (11).

"Just look at the disruption we've seen in the vaccine field with the development of mRNA lipid nanoparticles," says Levine. "I think the in vivo approach has the potential for massive disruption, and we'll soon see clinical data from some of these therapies.

"When one looks at solid tumors, treating hundreds of thousands of patients with the current autologous manufacturing methods wouldn't be sustainable. I don't know how it's going to shake out, but I think we'll find out by the latter end of this decade."

References

1. *A Wing et al., "Improving CART-Cell Therapy of Solid Tumors with Oncolytic Virus–Driven Production of a Bispecific T-cell Engager," Can Imm Res, 6, 5 (2018). DOI: 10.1158/2326-6066.CIR-17-0314*
2. *JH Choe et al., "SynNotch-CAR T cells overcome challenges of specificity, heterogeneity, and persistence in treating glioblastoma," Sci Trans Med, 13, 591 (2021). DOI: 10.1126/scitranslmed.abe7378*
3. *A Hyrenius-Wittsten et al., "SynNotch CAR circuits enhance solid tumor recognition and promote persistent antitumor activity in mouse models," Sci Trans Med, 13, 591 (2021). DOI: 10.1126/scitranslmed.abd8836*
4. *M Klichinsky et al., "Human chimeric antigen receptor macrophages for cancer immunotherapy," Nat Biot, 38, 947-953 (2020). DOI: 10.1038/s41587-020-0462-y*
5. *PR Newswire, "CARISMA Therapeutics to Present Data at The American Association for Cancer Research Annual Meeting" (2021). Available at: https://prn.to/3bTkh68*
6. *Y Chen et al., "CAR-macrophage: A new immunotherapy candidate against solid tumors" (2021). Available at: https://bit.ly/2RRto0r*
7. *Endpoints (2021). Available at: https://bit.ly/3vWdHTI*
8. *S Wang et al., "Perspectives of tumor-infiltrating lymphocyte treatment in solid tumors," BMC Med, 19, 140 (2021). DOI: 10.1186/s12916-021-02006-4*
9. *DM Beoya, V Dutoit and Migliorini, "Allogeneic CAR T Cells: An Alternative to Overcome Challenges of CAR T Cell Therapy in Glioblastoma," Front Immunol (2021). DOI: 10.3389/fimmu.2021.640082*
10. *Fate Therapeutics (2020). Available at: https://bit.ly/3BsHibp*
11. *W Nawaz et al, "AAV-mediated in vivo CAR gene therapy for targeting human T-cell leukemia," Blood Can J, 11, 119 (2021). DOI: 10.1038/s41408-021-00508-1*

AUTOMATING CELL THERAPY MANUFACTURING

Fabian Gerlinghaus, Co-Founder and Chief Executive Officer at Cellares, believes he can make autologous cell therapy a realistic proposition for solid tumors by closing and automating manufacturing

How did you become interested in cell and gene therapies?

I originally trained as an aerospace engineer in Germany. I considered careers in aerospace or robotics, but when I went to the US I became fascinated with life-sciences and genetics. I ended up co-inventing an RNA synthesizer technology, which I then helped commercialize at Synthego, a leading genome editing company. We started with five employees in a garage and grew to more than 230 employees over the course of my five-year tenure. Later in my career I was attending a lot of conferences, and speakers would often talk about the challenges of commercial scale cell therapy manufacturing. In particular, I kept hearing that the industry needed closed and fully automated manufacturing technologies. We thought we could make a difference with our experience in inventing, developing and commercializing new bioprocessing technologies, so we set out to build the most advanced cell therapy manufacturing technology to accelerate access to life-saving cell therapies. This was the birth of Cellares.

How does your technology work?

We are fully automating and closing the entire cell therapy manufacturing process, to enable commercial scale manufacturing in a way that is cost-efficient, robust and scalable. Current cell therapy processes involve a plethora of different benchtop instruments, each with their own respective consumables. Everything is disjointed and made by different vendors. We're bringing it all together in an all-in-one single-use cartridge, which supports all of the unit operations end-to-end. The entire manufacturing process takes place in one closed tubing set, which itself, is contained within a secondary hard-shell that was designed with automation in mind. By closing and automating the process in this way – without compromising on process flexibility – we are radically reducing the risk of process failure due to contamination or operator error, while also pushing down costs.

Could closed and automated manufacturing tech benefit therapies for solid tumors?

We are looking at the prospect of treating hundreds of thousands of patients per year, per drug. Those kinds of numbers simply aren't possible with current manual processes. Our modular manufacturing platform, the Cell Shuttle, is essentially a factory in a box. It contains all of the required bioprocessing instruments inside a robotic workcell that maintains an ISO 7 cleanroom internally. Inside the Cell Shuttle, the robot moves single-use cartridges from one instrument to the next in accordance with the process you previously designed in software. Importantly, you can load 10 single-use cartridges and execute up to 10 autologous or allogeneic processes simultaneously. This is an order of magnitude improvement in throughput! By combining end-to-end automation with an order of magnitude improvement in instrument throughput, Cellares enables cell therapy manufacturers to meet commercial scale patient demand and overcome this manufacturing bottleneck. We're seeing that closed and automated technology can bring down manufacturing costs by up to 70 percent for autologous cell therapy manufacturing workflows. I genuinely believe our technology will benefit therapies for solid tumors by enabling cost-efficient manufacturing of hundreds of thousands of doses per year, per drug.

Where do you stand on the "allo versus auto" debate, particularly with regard to solid tumors?

I've discussed this question with our advisor, Carl June, and I think we're on the same page: there's going to be a place for both autologous and allogeneic cell therapies. Of course, one of the main drivers for allogeneic therapies is the autologous scalability issue. We think that closed and automated technology can shift the balance here by making autologous more scalable and cost-efficient.

Which therapy approaches do you think are most promising?

I will leave it to the clinical experts to comment on therapeutic approaches, however we are obviously impressed by those of our partners PACT Pharma and Poseida Therapeutics. PACT Pharma is working on neoTCR-T cell therapies, which I think have tremendous potential, and Poseida Therapeutics has a very strong pipeline with both autologous and allogeneic CAR T-cell therapies.

NextGen

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Mind the Cell and Gene Gap

What can we do about the shortage of people and skills in the advanced medicine space?

By Anshul Mangal, President, Project Farma and Tony Khoury, Executive Vice President, Project Farma

A 2018 study by Deloitte suggested that more than 2.4 million manufacturing jobs in the US would remain unfilled in 2028 because of the widening – and often generational – skills gap. This approaching pain point may sting particularly badly in the emerging cell and gene therapy sector, where there is already a shortage of skilled people. Put bluntly, without further training and a boost in headcount, we will see a bottleneck in the rollout of advanced medicines that could ultimately cost the lives of patients.

Proposing solutions is easy enough, but doing something is harder. When considering the cell and gene skills gap at home and abroad, we have a favorite online adage at Project Farma that we return to again and again: "Teach me and I may remember, involve me and I'll learn."

Most posts online attribute the words to either Benjamin Franklin or Confucius, but the true author is Xunzi, a Confucian scholar writing in the 3rd century BCE. Translating and distilling classical Chinese is a tricky business, but fortunately the US Department of Health handled this task for us back in 1953, when in their Public Health Reports periodical they boiled Xunzi's esoteric words down to:

When I hear it, I forget it
When I see it, I remember it
When I do it, I know it

Anshul Mangal

Tony Khoury

We all likely sat through at least a few "hear it and forget it" conferences (you might know the type: hand-waving and sales pitches). And that's exactly what we don't do at Project Farma; before and during the pandemic, in conference halls and in cyberspace, we have presented in dialogue and in panels, using real case studies as the basis for our discussions. We believe this sharing of experience is one form of reinvesting because it helps disseminate knowledge that will help us close the skills gap. The key topic we hit on at these conferences is the journey of developing a therapeutic and getting it to market. The listeners we are hoping our words will benefit? The companies and professionals a few rungs below us on the ladder.

Saving lives with talent

Moving aside from the ancients, let's look at the stakes today. Advanced therapies are often a patient's only or last hope, so the time to market for these drugs is critical. If we make wrong moves, we create bottlenecks or even put drug approval at risk. And it's not only the health of our patients at stake – it's also their hope.

Though we don't have exact data on the talent gap, we can measure the need by the amount of financial investment going into the space and a number of products that will be commercialized over the next decade. To date, 19 therapies have been approved for about a half a dozen diseases. The FDA expects to approve 10–20 new therapies a year

starting in 2025. There are over 1200 clinical trials worldwide, with at least half of these trials in the US. In terms of investment, over $20 billion went into the cell and gene space alone in 2020; up $10 billion from 2019. With that amount of investment pouring into the space, the workforce will need to expand.

Even while most of the world was grappling with a pandemic, we saw a record year for investment in cell and gene therapy. Do you need any clearer signal of the confidence that the investment community has in the sector?

But which roles have fallen into this skills gap. Well, manufacturing for one. We are now seeing demand for manufacturing capacity significantly outstripping supply. One of those constraints is talent – manufacturing talent specifically. But really the demand for talent in cell and gene therapy runs across every functional area, including regulation and commercialization. After all, there are relatively few commercially proven products on the market today, so naturally that means few people have experience with commercially launching cell and gene therapy products.

The FDA has commented that there's a general problem at the moment getting sufficiently trained staff to even review gene therapy applications – not only in the review of manufacturing, but in all aspects of the FDA review process.

But we also need to think about the corporate level too; this is about catching the folks that have the experience, know-how, and credibility to start and run companies as they step out of academia. Many of today's new medicines are coming out of both academia and private equity, so we need strong talent and experience in dealing with supply chains, technical operations, business strategy, regulatory affairs… And the list goes on.

At Project Farma, we've sought to address the skills gap not only through recruitment but also training. We have set up a free, internal "university" for our engineers. The aim is to provide them with a baseline of knowledge for their own benefit and to accelerate the development of the cell gene therapies we work on.

Looking to role models

Let's think back to those words from Xunzi. To learn, students need to do. Take a look at NC State University's BTech; it's a bio manufacturing, training, and education center, and it's home to a manufacturing suite that the students have regular access to. When people first start their careers in engineering, they are often terrified of interfering with equipment – mainly through the fear of losing the company half a billion dollars in batch costs! But in environments like BTech, professionals are freed-up to learn by doing because they have an education centre that mimics the environment of a pharma facility, without the expensive batches.

Finally, we should not underestimate the importance of partnerships. In fact, we believe nothing will help fill the skills gap quicker than partnerships between private and public organizations. For example, Ohio State University announced a new collaboration between Nationwide Children's Hospital and Jobs Ohio on a massive US$1.1 billion cell and gene project in Columbus. Ohio State is committing to producing over 20,000 graduates over the next 15 years in the STEM field, including health science, vital materials, computer science, and other fields as part of this investment.

In the US, we are fortunate to have many success stories like this, and they are something to be proud of. But we need more of them. To take these stories to heart, people need to see them happen. To build the industry, people need to take part in its construction.

Anshul Mangal and Tony Khoury are President and Executive Vice President of Project Farma, respectively

The other gap…

The gear in advanced therapy is simply not as sophisticated or mature as that of other therapeutic areas, nor has it gone through as much rigorous testing and development over the years. In 2015, our company went through quite a serious struggle, because we were attempting to work with equipment such as bioreactors and modular cleanrooms that had never been used to manufacture advanced therapies. We could not have done it alone. In this industry, the word "collaboration" cannot be stressed enough. Collaboration. Collaboration. Collaboration!

Companies need to evaluate the available technology, find ways to work with their clients, and effectively manage all the materials tied into the supply chain: critical starting materials, raw materials, plasmids, vectors, disposables – every detail becomes a weed. To succeed, you need to strategize.

At Project Farma our way of dealing with this is to run "make versus buy" or facility-build analyses, and form manufacturing strategies with our clients. It is not enough to simply evaluate internal versus outsourced production. Companies need to analyze every step of every process on the pipeline. Foresight and preparation are essential for success.

The Watcher

Sitting Down With… Peter Marks,
Director of the Center for Biologics Evaluation and Research (CBER) at the FDA, USA

How did you start your career?

I went into college thinking I was probably going to become a PhD biochemist. At the time, cell and molecular biology were becoming more popular and they caught my interest. After I got a part-time job at a hospital taking blood, I started to think about going to medical school and working in medical research. Ultimately, I chose to become a physician-scientist but, over the years I've occupied several different roles in academia and industry.

How did you get involved with the FDA?

My first industry role was with Genzyme, involving interacting with the Center for Biologics. The Center had both applied scientific research and regulatory components, as well as a nifty set of products. It was so interesting to me that, in 2011, I applied for a job there. At the time, gene and cell therapies were becoming exciting – and, as a hematologist-oncologist, blood products interested me. The opportunity to have an impact on the development and availability of important medical products was attractive. It meant I could make use of different skills in one job. What I do now combines science, medicine, administration, and even a little teaching.

What skills are important for a regulator?

You need to understand science and medicine really well to do a good job as a regulator. This includes the fundamental science, product manufacturing, and the technologies involved. Without that knowledge, you can't make necessary decisions about cutting-edge products. You also have to know how to manage people. The Center for Biologics has around 1,300 full-time equivalents. They are mostly knowledge workers… and managing knowledge workers can be challenging. You need to know when to zoom in to the data, and when to zoom out and let others deal with the weeds while you make the high-level decisions. That, to me, is an important balance to have.

What is the biggest challenge you face?

The biggest challenge is uncertainty. For example, on one hand, a gene therapy may help to cure or treat a disease long-term. On the other hand, there may be side effects. Not knowing exactly what will happen ahead of time is what makes the job challenging. Sometimes, it takes a long time to know whether a decision was a good idea or a bad one. The challenge is to negotiate the uncertainty as skilfully as possible.

What work are you doing in terms of harmonizing gene therapies?

This is one of my favorite topics and an area we are actively working on. We're developing a white paper on global harmonization of cell and gene therapy regulatory approaches. If we have different regulatory frameworks in different countries, then patients in different countries likely will be deprived of these therapies simply because of the cost of market entry. If studies are performed in one location and are then required in a different location, that will present a barrier. We're going to need a lot of work to move toward harmonization and we'll need to start small. Right now, if someone in the US develops a therapy for mucopolysaccharidosis type I and someone in the EU develops a therapy for mucopolysaccharidosis type III, the regulatory requirements may be different and the therapies may never cross the Atlantic. This means patients would have to travel to get access. Harmonization could help therapies enter other countries.

How far are we from being able to manufacture gene therapies at scale?

We're not that far away, but there are challenges. With mAbs, people came together to help develop technologies that could produce and purify large protein quantities. With gene therapies, there is still a lot of proprietary work that can limit information-sharing. One of my goals is to help the field share information and grow. I think we can make better cell lines and purification methods and develop continuous methodologies for producing these gene therapies. But that will require a type of collaboration and cooperation that we haven't yet fully achieved.

What advances in gene therapy do you think could be transformative?

In vivo genome editing has tremendous potential because it can help overcome some of the problems we have with current gene therapy vectors, including longevity of expression. You need expression of an editing construct for a period of time – ideally in the dividing cells – but after your correction occurs, you're done. Too much persistence is undesirable because it can lead to off-target effects. Genome editing could treat many diseases, but there is a whole regulatory paradigm that we have yet to fully create for genome editing. It's very exciting – and it's advances like this that keep me coming to work every day.

What advice do you have for developers of therapies?

Engage with the FDA – or whatever regulatory authority you are applying to – frequently. Do not be afraid to ask the hard questions. And do not be afraid to question the regulator's responses if they don't make sense. Such dialogue between developer and regulator is really important. I recommend closing the loop: you ask a question, the regulator responds, and then you respond back with, "From your response, we think we need to do X." Often, regulator comments can be open to interpretation but, if you close the loop and give feedback to the regulator on what you have heard, it becomes unambiguous. For example: "We hear you. We need to have a potency assay before we proceed to phase III. Is that what you mean?" The regulator can then confirm, and you'll know you're on the right track.

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