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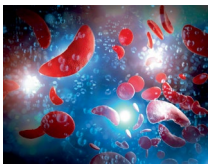

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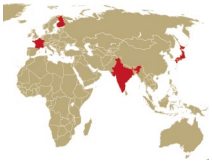

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T_{reg} engineers take aim at autoimmunity

Companies are deploying regulatory T cells, equipped with CAR and TCR constructs, to suppress immunity—locally and for the long term.

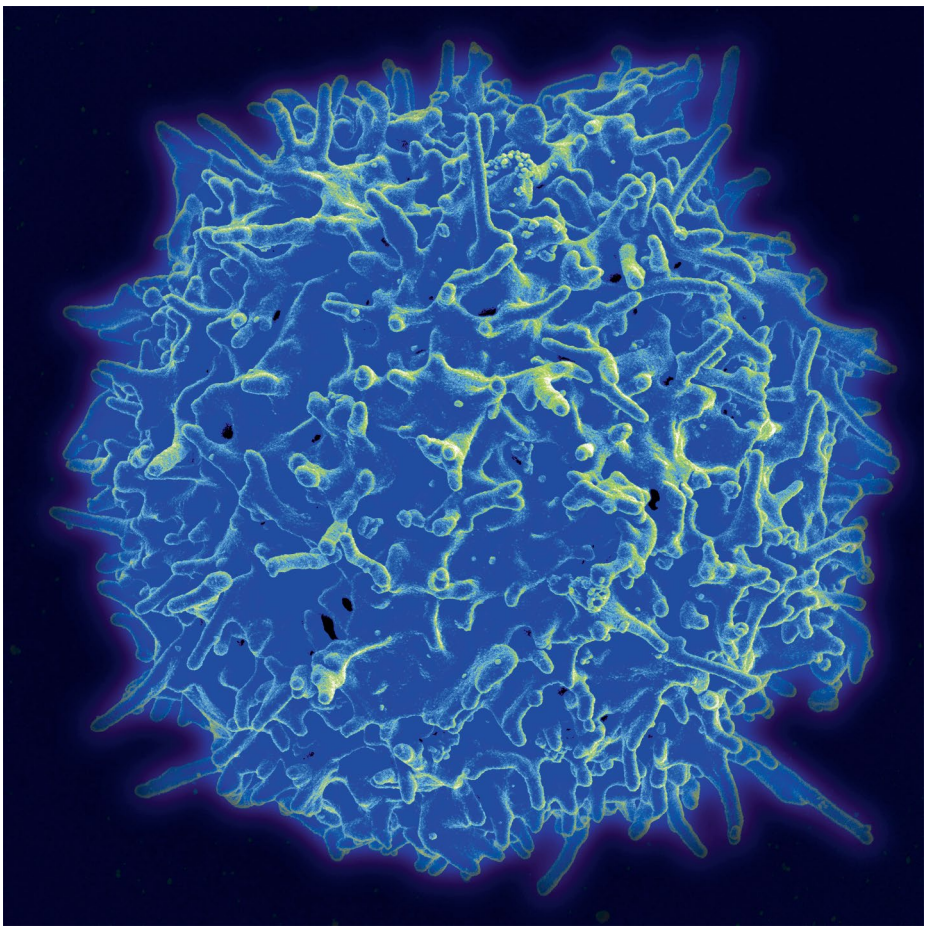
Engineered adoptive T-cell therapies have already transformed the treatment of blood cancers. Now, several startups hope to harness the power of regulatory T cells (T_{regs}) to suppress immune responses and offer tolerance to transplant recipients, or to control the inflammation driving autoimmune disorders.

Companies such as Sonoma Biotherapeutics, GentiBio and Abata Therapeutics—three leading firms that collectively raised over $500 million in recent months—are using chimeric antigen receptor (CAR) and T-cell receptor (TCR) technologies to build target specificity into T_{regs}. Other startups, including Quell Therapeutics, Kyverna Therapeutics, AZTherapies and TeraImmune, are designing new types of T_{reg} products as well.

These souped-up cell therapies, if successful, should create a type of immunosuppressive bubble at the site of disease or transplant, inducing immune-dampening effects that are targeted to hot spots of inflammation.

The first recipients of these cells will include patients undergoing solid organ transplantation or hematopoietic stem cell transfer. But companies are also pursuing applications in type 1 diabetes (T1D), rheumatoid arthritis, multiple sclerosis, hemophilia and inflammatory bowel disease. Next may be neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), and other conditions linked to chronic inflammation, including cardiovascular and metabolic diseases. Some academic groups have even begun exploring opportunities to treat allergy and to mitigate the immune responses generated after viral vector–based gene therapy as well.

"The idea will be to take the experience from oncology and convert it to complex chronic diseases," says Tatiana Ort, immunology head at AstraZeneca, which has an active preclinical T_{reg} cell research program. "This non-conventional, innovative modality, we believe, has curative potential."



Scientists are engineering human regulatory T cells into next-generation cell therapies that can deliver local immunosuppression with exquisite specificity. Credit: Stocktrek Images, Inc. / Alamy Stock Photo

T_{regs}, in their natural form, have been evaluated in dozens of clinical trials to date. Isolated from the blood, grown in cell culture and then reintroduced into patients, these cell therapies seem to be generally safe, with hints of efficacy in some transplant settings.

The cells are thought to keep immune responses in check through a variety of mechanisms, not all of which necessarily involve the target antigen needed for activation. These include 'bystander suppression', through which T_{reg} cells act independently of their specific antigen, and 'infectious tolerance', in which cells spread

their regulatory function onto other T-cell types.

But unmodified T_{regs} are so variable in their antigen-binding repertoires that only a small proportion of these 'polyclonal' cells usually become activated and localize at target tissues to help control disease-linked inflammation. Some companies hope to address this issue through new manufacturing platforms and activation techniques designed to optimize the quality of the final product. Others are focused on advancing more conventional drugs intended to promote T_{reg} function. But a growing number of firms are embracing engineering techniques—CARs, TCRs and CRISPR-based gene editing—as the fix to the antigen-specificity problem.

"There's the potential that, with the genetic modulation of the cells, we could do better," says Everett Meyer, a clinical immunologist at the Stanford University School of Medicine in California.

For now, however, T_{reg} engineering remains in its infancy: the first clinical candidate that takes advantage of this technology, Sangamo's genetically modified autologous cell product TX200, has only just entered human testing. Most other CAR- or TCR-redirected T_{reg} therapies in development are still a year or more away from reaching patients. But a flood of such products could soon advance to the clinic. "The pace of innovation could be breathtaking," Meyer says.

TX200 originated in laboratory of Megan Levings, an immunologist at the British Columbia Children's Hospital Research Institute in Vancouver, Canada. She and her colleagues armed T_{regs} with a CAR directed against human leukocyte antigen (HLA)-A2, which is commonly mismatched in transplantation procedures. Five years ago, the researchers showed that these CAR-expressing human T_{regs} helped prevent graft rejection in a mouse model. A small biotech company called TxCell licensed the technology and began readying a clinical candidate—humanizing part of the CAR construct that was built around a mouse-derived antibody and making other modest improvements.

Sangamo then acquired TxCell in 2018. A phase 1 study of TX200, for patients undergoing HLA-A2-mismatched kidney transplants, opened for enrolment earlier this year. As no patient has ever before received engineered T_{regs}, "there's a lot to learn here," says Sangamo CSO Jason Fontenot. (As *Nature Biotechnology* went to press, study investigators were still waiting to treat their first trial participant.)

Fontenot says he hopes the therapy will prove beneficial to organ recipients: trial investigators will be looking for signs of improved graft survival and testing whether patients can safely taper off some of their immunosuppressive drugs. Yet they will also be taking tissue biopsies and blood samples from study participants in hopes of determining whether the cells are behaving as intended.

The first-in-human trial, termed STEADFAST, thus serves as "a critical test of the concept of putting a CAR on a T_{reg} and recapitulating what we've seen in our preclinical models," Fontenot notes. "Simply demonstrating that we can engineer the cells and that they do what we expect them to do is going to be a huge step forward for the whole field."

Elsewhere, with $84 million in series A financing, Quell is gearing up to launch the next human trial of a CAR–T_{reg} therapy. The company's product, QEL-001, should begin clinical testing in liver transplant recipients in early 2022. Another startup, Sonoma—flush with cash from a $265 million fundraising round announced in August—expects to follow soon behind with its lead candidate for rheumatoid arthritis, SBT-77-7101.

Neither company has disclosed the antigenic target of its CAR construct. But both Quell and Sonoma are working with thymus-derived T_{regs} isolated from patients, transduced with CARs via viral vectors and expanded clonally before being reinfused.

Although these natural T_{regs} are relatively rare, comprising only around 5% of the $CD4^+$ T cell compartment in the blood (and thus requiring precise manufacturing processes to isolate a high-purity product), Sonoma CSO Fred Ramsdell contends that they are the best cell type for therapeutics. They maintain native TCR signaling pathways and the usual epigenetic identity, he points out, which should ensure proper long-term function and consistency. "We like starting with a cell that was born to be a regulatory T cell," Ramsdell says.

Others are taking a different tack. Seeking to maximize the number of cells generated—and citing fears that endogenous T_{regs} might spontaneously convert to effector T cells that could propagate, rather than limit, disease pathologies—some companies are collecting bulk $CD4^+$ T cell populations and then deploying gene-editing techniques to force the expression of FOXP3 (forkhead box P3) or some other protein critical to the function of T_{regs}. (AstraZeneca scientists recently completed a genome-wide screen looking for additional factors that can help ensure a consistent T_{reg} phenotype; according to Ort, validation experiments are planned.)

Using these synthetic biology tactics, companies aim to grow huge batches of cells with a stable T_{reg}-like identity. "We put a lot of emphasis up front in being able to have a process where we can reprogram the cells—and make enough of the cells—to generate efficacious doses," says Kyverna cofounder and CSO Jeffrey Greve. Greve and his colleagues at the company have tied FOXP3 expression to their CAR construct, so that the activation of the two is linked. Kyverna is focused on using its T_{reg}-like cells to treat Crohn's disease and ulcerative colitis, among other autoimmune conditions.

GentiBio, meanwhile, is using a strong gene promoter to stably express FOXP3 in its cells. Company cofounder David Rawlings and his colleagues at the Seattle Children's Research Institute described the method last year. As a further guarantee of cellular fidelity, GentiBio scientists then add another engineering trick: a special type of small-molecule-switchable interleukin-2 receptor introduced into their T_{reg}-like cells.

Interleukin-2 is an essential growth factor normally used in the manufacturing of T_{regs} to promote their expansion in culture conditions. Take the cytokine away and the cells typically won't grow. With GentiBio's drug-inducible system, however, any cells with the desired modifications will trigger their own downstream interleukin-2 signaling, even in the absence of the cytokine. "So, we're able to selectively expand only those cells that have all the edits we want," says Andy Walker, chief technology officer and cofounder of the company. What's more, the drug involved, rapamycin, can be given to patients at subtherapeutic doses to further expand and activate the T_{reg}-like cells for added benefit. The company's lead candidate is an autologous TCR–T_{reg} cell product directed at an islet cell antigen associated with T1D.

Abata is also taking a TCR-based approach, endowing natural T_{regs} with receptors directed against myelin basic protein, a key target of the autoreactive immune response in multiple sclerosis. According to CSO Andrea Van Elsas, those TCRs, once activated, should direct the cells to take up residency at sites of inflammation around the brain. There, the T_{regs} change their characteristics, he explains: "They start to produce factors that help the brain, and they help local tissues to regenerate." If enough damage can be repaired, he adds, "maybe we can dream of a one-and-done therapy that will have an effect for years."

Most startups in this space, whether working with TCRs or CARs, natural or induced T_{regs}, are continuing to tinker with their engineering strategies—because as Levings, an advisor to AZTherapies, points out: "The design rules for T_{regs} are not just

the same as for conventional effector T cells." She and her colleagues showed as much last year when they took second-generation CARs encoding either a CD28 or 4-1BB co-receptor signaling domain. Both designs are found in commercial CAR T-cell therapies for cancer, but only CD28-containing CARs worked well in a T_{reg} context for preventing graft-versus-host disease in mice.

The bottom line, says Sonoma cofounder and scientific adviser Qizhi Tang, a translational immunologist at the University of California, San Francisco: much remains to be learned about the optimal design principles for these cells. "Can you make them better?" she asks. "At this stage, everything is worth trying."

To that end, various companies are planning to incorporate additional genetic enhancements into their future engineered cell products. Sangamo, for example, intends to use its zinc-finger nuclease genome-editing technologies to create off-the-shelf allogeneic therapies or to augment the cells' functionalities in disease-specific ways; AstraZeneca is exploring whether the introduction of integrins or chemokines can strengthen the cells' tissue-homing potential; and Sonoma—with an eye to its preclinical programs for irritable bowel disease and T1D—is considering ways in which the cells might locally produce tissue-restorative factors. "I would expect pretty much every generation beyond the first one to have additional attributes," says Ramsdell, "and those attributes will be tuned to the biology we're trying to address."

But those next-generation products will take time to reach the clinic. And several companies think that, by refining manufacturing protocols for unmodified, polyclonal T_{regs}, they can forge a quicker path to market.

Coya Therapeutics is one such company. "We've really worked out the formula for expansion," says cofounder and CEO Howard Berman. As he explains, "We've introduced bioreactors that automate the process and allow much faster expansion and better quality control" than existing cell isolation methods. Coya's T_{reg} product, which is based on a therapy initially developed and clinically evaluated by Stanley Appel from the Houston Methodist Neurological Institute, will soon begin testing in a placebo-controlled trial involving patients with ALS.

ActiTrexx is similarly leaving the genetics of its T_{regs} untouched. But instead of growing millions or billions of cells in the lab, as most others have done, the company simply adds an activating protein to its cell culture broth—no expansion needed. That activating agent is a CD4-binding protein derived from HIV, gp120, which stimulates nearly all the T_{regs} collected from a person's blood. "This is our unique selling point," says ActiTrexx CEO Andrea Tüttenberg. "We can isolate regulatory T cells and, within 24 hours, make them ready to go into the patient." A first trial involving patients with leukemia undergoing hematopoietic stem cell transplants is planned for next year.

Meanwhile, Cellenkos, a company founded by hematologist Simrit Parmar from The University of Texas MD Anderson Cancer Center in Houston, is using allogeneic T_{reg} cells derived from umbilical cord blood that, because of their immunologically naive nature, allow off-the-shelf treatment of inflammatory conditions. In small trials involving patients with bone marrow failure disorders or COVID-19-associated lung damage, the cell therapy has proven safe, despite only partial or no HLA matching across diseases, Parmar notes. Early trial data hint at signs of clinical benefit as well.

All of these cell therapy approaches come with manufacturing challenges and costs, though. So, many companies continue to search for more conventional drugs capable of boosting the immunosuppressive function of T_{regs}. Several large pharmaceutical firms now have altered forms of interleukin-2 in clinical trials designed to trigger potent and selective expansion of T_{regs}—and earlier this year, Merck paid $1.85 billion for Pandion Therapeutics, the maker of two interleukin-2-based molecules, so it could enter this development race.

But rather than follow the pharma crowd, TRexBio has opted to go back to the drawing board for T_{reg}-related targets. The company's scientists isolated T_{regs} from the gut, lung and skin of people with or without autoimmune diseases and performed systematic functional profiling of the cells in each tissue. This analysis yielded more than 20 novel tissue-focused targets that the company aims to modulate with monoclonal antibody therapeutics—and it raised $59 million earlier this year in support of those efforts. As CEO Johnston Erwin explains, instead of harnessing T_{regs} themselves, "we are trying to use the cells to teach us their biology so we can create the therapeutics." ❐

Elie Dolgin
Somerville, MA, USA

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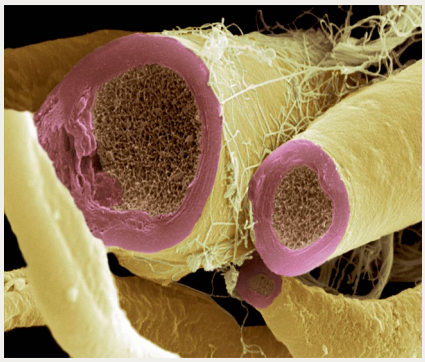
First gene therapy for adrenoleukodystrophy



Myelinated nerve cells. Credit: Science Photo Library / Alamy Stock Photo

Bluebird Bio has earned a marketing authorization from the European Commission for its single-dose gene therapy Skysona (elivaldogene-autotemcel; Lenti-D) to treat a severe form of adrenoleukodystrophy. The go-ahead is for patients younger than 18 years with cerebral adrenoleukodystrophy (CALD) for whom a matched hematopoietic stem cell donor is not available. CALD is a rare X-linked neurodegenerative disease caused by a mutation in the *ABCD1* gene; it affects one in 21,000 male newborns. The mutation affects the production of the adrenoleukodystrophy protein (ALDP), which normally allows long-chain fatty acids to enter the peroxisome for their degradation. Without ALPD, these fats build up in the adrenal glands and myelin sheaths of the nervous system, resulting in rapid and irreversible neurodegeneration and death. Skysona uses the patient's $CD34^+$ stem cells modified ex vivo using a lentiviral vector encoding functional copies of the human *ABCD1* cDNA under the control of a modified enhancer/promoter of myeloproliferative sarcoma virus. The European approval was based on phase 2/3 data from a pivotal study of the compound, ALD-102, in 32 patients, 90% of whom maintained neurological function after two years, meeting the primary efficacy endpoint. Should ALDP expression be maintained, as the company expects, the effect of Skysona will be life-long. In August, the US Food and Drug Administration placed the therapy ALD-104 on clinical hold following a report of a suspected serious adverse reaction.

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