CLUTCH CONTENT PARTNERS

Allison Bayer is an immunologist at the University of Miami, Florida, where she studies type 1 diabetes.

Pitting cell against cell

Biotechnology companies are engineering regulatory T cells to help the cells guard the body against friendly fire. **By Eric Bender**

Regulatory T cells are rare, difficult to grow in the laboratory and prone to sudden identity shifts. Furthermore, they churn out a surprising variety of molecules, the roles of which are not fully understood.

But this class of immune cell also forms the main line of defence against autoimmune disease and many inflammatory conditions in which the immune system, led by effector T (T_{eff}) cells, mistakenly turns on the body.

Clinical trials of therapies using regulatory T (T_{reg}) cells, in which a person's own T_{reg} cells are removed, expanded and re-administered, began in 2004. But the results have been less than dazzling. Dozens of small trials, to facilitate organ transplants as well to treat as autoimmune conditions, have demonstrated that although the procedure is safe, it is, in general, not that effective. Proponents, however, expect that a new class of genetically engineered T_{reg} cell transplant, which a number of companies hope to test soon in clinical trials, will prove much more effective.

The idea is that each person's T_{reg} cells will be tailored to help them better guard against rogue T_{eff} cells. To provide this defence, biotechnology companies — many of them start-ups — are leveraging two rapidly advancing technologies: chimeric antigen receptor (CAR) T-cell manipulation, which provides T cells with receptor proteins matched to specific cell targets; and CRISPR–Cas9 genome-editing tools.

"People have really recognized the power and potential of regulatory T-cell therapies," says Megan Levings, an immunologist at BC Children's Hospital Research Institute in Vancouver, Canada.

"Until now the general field of T_{reg} therapy has almost exclusively been done by academics," Levings says. "Now that companies are getting into it and putting in the resources that are needed to properly take these to the next level, there will be a lot of progress."

"This is an inflection point right now, where the academics are giving way to the industry," says immunologist Jeffrey Bluestone, co-founder of start-up Sonoma Biotherapeutics in South San Francisco, California, which launched in 2020. "Over the next five years, we'll definitely have the kind of data we need to know whether these therapies will translate into drugs."

The first trial of a CAR T_{reg} cell therapy — for people receiving kidney transplants — is expected this year. Trials in people with autoimmune diseases are not far behind. The hope is that the drugs will do more than treat symptoms. "If we select the right cells and the correct clinical design, we may aim not only to provide a therapy, but also to provide a cure," says Maria Grazia Roncarolo, an immunologist at Stanford University in California.

Honing targets

Like other T cells, T_{reg} cells originate in the bone marrow. But unlike the T_{eff} cells — killer T and helper T cells — T_{reg} cells are born to play in defence. They have more than a dozen ways to suppress immune responses that have gone wrong. One method is to soak up the signalling molecule interleukin-2 (IL-2) — a cytokine that is key to the growth and function of all T cells — reducing the amount available to T_{eff} cells.

T_{reg} cells also release other molecules to inhibit attacks or help to repair tissue. Bluestone compares the cells to pharmacies — they are capable of delivering many potential drugs.

Although there are multiple subclasses of T_{reg} cell, the ones usually proposed for clinical use express the biomarkers and cell-surface proteins CD4 and CD3, and the FOXP3 protein, which is a master regulator of T_{reg} cell function.

T_{reg} cells target specific molecules on the surface of cells that can trigger an immune response, called antigens. There are numerous antigens, and as a result many different T_{reg} cells. A blood sample of T_{reg} cells will naturally contain a mix of cells geared towards defending a variety of tissue types.

Typically, early clinical studies of T_{reg} cell therapies expanded such mixes of cells, because T_{reg} cells are difficult to isolate from each other and to expand in culture, compared with many other types of cell.

However, evidence indicates that T_{reg} cells targeted to a specific self-antigen would be more effective at treating autoimmune and inflammatory conditions than would cells that target a mixture of antigens[1]. With advances in T_{reg} cell science and cell-manipulation technologies, the field is now shifting its focus to antigen-specific therapies.

Individual autoimmune diseases are likely to need their own targeting strategy. For some diseases, including rheumatoid arthritis and type 1 diabetes, researchers think that they have a good handle on appropriate antigen targets, says Jane Buckner, a rheumatologist at Benaroya Research Institute in Seattle, Washington. Buckner is also co-founder of start-up Gentibio in Boston, Massachusetts, which was set up in August 2020 to focus on T_{reg} cell therapeutics. For autoimmune conditions such as lupus that affect multiple tissues, it might be more difficult to identify targets and measure therapeutic effectiveness.

Special effects

Fortunately, getting close to the target is often good enough for T_{reg} cells. Through a process called bystander suppression, T_{reg} cells that are activated by one antigen can also guard against an immune response to another. "If you've got the cells in the right place and activated, they don't necessarily need to be specific for the antigens that are driving the actual autoimmune disease," says Levings.

That ability might prove useful for cases in which the antigen isn't known or wouldn't make a good CAR target, or when many antigens are under attack. In type 1 diabetes, T_{eff} cells target at least five antigens among the pancreatic islet cells. If bystander suppression is activated, "I don't have to make a T_{reg} for every potential antigen", Buckner says. "I just need to make a T_{reg} that will see an antigen in the location where I need the activity."

The ability of therapies to induce bystander suppression is particularly relevant because it is unclear how long infused T_{reg} cells will stay healthy doing their job. Research in mice and people has shown that the numbers of transplanted cells can drop fairly quickly, meaning therapies might need to be repeated[2].

"People have really recognized the power and potential of regulatory T-cell therapies."

"Cell stability is definitely a concern," says Levings. "A lot of it has to do with the purity of your starting product, because one of the defining characteristics of T_{reg} cells is their slow rate of proliferation. So even if you have a few contaminating effector T cells, they can take over your culture quickly, because they have this growth advantage."

In addition, under sufficiently inflammatory conditions, T_{reg} cells sometimes lose their suppressive abilities or even switch sides and express inflammatory cytokines. This is a significant concern, but can be addressed by several cell-manipulation techniques, says immunologist Marc Martinez-Llordella, co-founder of start-up Quell Therapeutics in London, which was launched in 2019 to develop T_{reg} cell therapies.

On the flip side, however, T_{reg} cells can also trigger a phenomenon known as infectious tolerance, in which one cell population spreads immune-suppressive abilities to another cell population. This capability has been demonstrated in mice genetically modified to have autoimmune pathologies[3]. If the pathology is suppressed by transferred T_{reg} cells and those T_{reg} cells are then wiped out, the pathology doesn't re-emerge. Presumably, this is because the cells that originally caused the pathology have become dormant. But this kind of resetting of the immune system awaits clinical proof.

CAR T cell lessons

Designing and manufacturing CAR T_{reg} cells is no easy task, but the field is drawing on the lessons learnt from cancer-killing CAR T cells and the rapid advances in cell engineering and manufacturing technologies that have followed. "I'm not worrying about manufacturing," says Nathalie Belmonte, who leads research and translation at Quell Therapeutics.

CAR T-cell therapies remain dauntingly expensive — they often cost more than US$1 million per person, much too high to treat the millions of people with chronic autoimmune diseases. But companies such as Quell think that they can manufacture T_{reg} cell products for much less, Belmonte says.

Still, CAR T_{reg} cells are new. "So far, the field has basically just copied what the oncology people have done," says Levings. "Fortunately that mostly seems to work, but the design rules are not necessarily the same. What's the dose, what's the time course, what's the manufacturing? For CAR T_{reg} cells, it's one big question mark after another."

Some CAR T_{reg} cell therapies also bring their own unique puzzles to solve — for instance, Roncarolo leads development of treatments based on type 1 T_{reg} cells, a subclass that has performed well in early trials for graft-versus-host disease, a condition that can occur after a transplant. These cells produce a highly suppressive cytokine called IL-10 and not only suppress T_{eff} cells, but also form allies among immune cells called dendritic cells and other types of T cell by infectious tolerance. "They gather the army," as Roncarolo puts it. She is refining a second generation of these T_{reg} cells and looking to start a spin-off company based on her research.

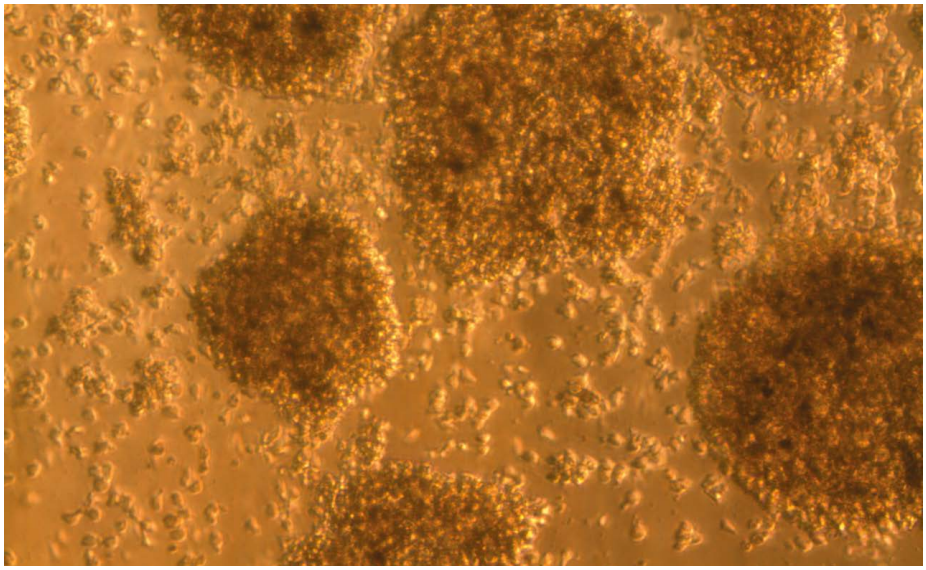
Combine and conquer

As in cancer care, it's unreasonable to think that one treatment will necessarily furnish a cure, says Bluestone. "In autoimmunity, we're trying to rebalance an immune system that has effectively outwitted all of the components of control that the immune system has put in place," he says. "We have to be prepared to think about combination therapies that will set the system up to regain that balance." There are a host of potential candidates to bolster T_{reg} cells or weaken T_{eff} cell attacks.

People with autoimmune conditions still need healthy immune systems to handle threats such as, say, a viral pandemic. "We just want to fix the problem of the autoimmune response," says Allison Bayer, an immunologist at the University of Miami, Florida. "We want to leave the rest of the immune system intact."

In mouse models of type 1 diabetes, Bayer's lab has shown[4] the benefits of depleting existing T-cell populations with other drugs before infusing antigen-specific T_{reg} cells. "When you make this space, you limit the competition," she says. "You don't need tons and tons of these cells."

Targeting the CD3 cell-surface protein, a biomarker for all types of T cell, has proved particularly effective in Bayer's work. One CD3 inhibitor is close to US Food and Drug Administration (FDA) approval as the first drug for people at high risk of developing type 1 diabetes.



JEFFREY BLUESTONE

Regulatory T cells grown at Jeffrey Bluestone's laboratory in San Francisco, California.

Among other candidates for combination treatments, IL-2 is a leading contender. Early clinical trials that use low-dose IL-2 or synthesized versions of the molecule that hook up with target T_{reg} cells have shown promise against a number of autoimmune conditions[5].

Cells on trial

Sangamo Therapeutics in Brisbane, California, expects to launch the first CAR T_{reg} cell clinical trial, for kidney transplants, this year, says Jason Fontenot, an immunologist at the biotechnology company.

T_{reg} cell trials for organ transplants often precede those for autoimmune diseases. One reason for this is that it's relatively easy to pick an antigen target — such as the human leukocyte antigen (HLA) cell-surface proteins on donor kidneys that are not shared by the recipient. Researchers also know exactly when the immune attack begins.

It's also straightforward to assess whether a drug is successful at reducing inflammation associated with a transplant — whereas making this judgement is trickier for autoimmune drug candidates. In fact, the difficulty in measuring clinical outcomes for these drugs has been a major stumbling block for academic labs trying to move therapies past phase I trials.

In advanced blood cancers, for which CAR T-cell therapies are approved, outcomes are cut and dried. "You measure survival, and you're either alive or you're dead at the end of six months," says Fontenot. But in a disease such as rheumatoid arthritis, for which outcomes are based on a person's reported pain, measuring change owing to a treatment is much more difficult. For many autoimmune diseases, it is "extraordinarily challenging" to quantify success, Fontenot says.

In CAR T_{reg} cell autoimmune trials, the bar for safety will be set very high. Unlike the advanced cancers targeted by approved CAR T-cell therapies, autoimmune diseases are generally chronic and not immediately life-threatening. Moreover, "many autoimmune diseases are most prevalent in women, often young women in their childbearing years", Fontenot notes (see page S51).

> **"I tell my students that with CRISPR, basically your imagination is the limit."**

T_{reg} cells fit the bill. "The T_{reg} by itself is a nice cell," Martinez-Llordella says. "We know that it's super safe. It's not a cell whose innate function is to kill."

Although there are concerns that the cell-manufacturing process could introduce impurities, researchers say that rigorous enforcement of proper T_{reg} cell manufacturing methods will prevent this. Moreover, although any genetically engineered cell might have the potential to turn cancerous, genetic switches that would cause a cell to kill itself if this happened can be incorporated.

Going corporate

The use of T_{reg} cells in autoimmunity and transplantation will eventually open the door for their use in other roles, too. This might include tissue regeneration, Martinez-Llordella predicts. Powerful cell-modification tools such as CRISPR gene editing could allow T_{reg} cells and other T cells to deliver molecular payloads for many conditions, says David Hafler, an immunologist at Yale University in New Haven, Connecticut. He points out that T cells are not only proficient at slipping into tissues — they can, for instance, cross the barrier between the brain and circulating blood — but might also function in normal health. For example, T_{reg} cells might help to maintain healthy salt balance in cells.

If and when T_{reg} cells emerge as a platform for delivering treatments, they might fit into a broad range of therapeutic roles, says Levings. "The best therapies are the ones where you're harnessing a natural biological process," she says. "I tell my students that with CRISPR, basically your imagination is the limit. Just imagine what you want your T cell to do for you, and make it happen."

That broad potential is highly appealing to biotech venture capitalists, and has helped to fuel the recent wave of start-ups specializing in T_{reg} cells. Researchers working in the field think that this fresh investment in T_{reg} cells is exactly what it needs to progress. Successful cell therapies won't follow the well-trodden path for developing small-molecule drugs. "This is not a simple chemistry problem," Bluestone says. "This requires learning about how to do manufacturing, how to do cell selection, how to dose it, how to think about it. In an academic setting, it's been a challenge because there are only limited resources."

Levings agrees that commercial investment is crucial to moving forward. "These cell therapies are just so expensive and logistically complicated that it's really hard for academics to get to the next step," she says.

The continued involvement of the researchers who have been working on the problem for decades is also valuable, however. Bluestone says that he left his academic career to start Sonoma not just because of the great promise of the approach and the availability of the cell-engineering tools to make it a reality, but also because he had a sense of responsibility to drive the approach towards a therapy. The CD3 inhibitor he led research into as an academic decades ago is only now approaching FDA approval, and he is not willing to wait that long again. "If we're going to do this, we really need to give it 100% and not hope other people will make it fly," he says.

Eric Bender is a science writer in Newton, Massachusetts.

1. Ferreira, L. M. R., Muller, Y. D., Bluestone, J. A. & Tang, Q. *Nature Rev. Drug Discov.* **18**, 749–769 (2019).
2. Tran, L. M. & Thomson, A. W. *Front. Immunol.* **11**, 614578 (2020).
3. Kendal, A. R. *et al. J. Exp. Med.* **208**, 2043–2053 (2011).
4. Cabello-Kindelan, C. *et al. Diabetes* **69**, 215–227 (2020).
5. Graßhoff, H. *et al. Front. Immunol.* **12**, 648408 (2021).