Nature Biotechnology's academic spinouts 2020

Nature Biotechnology's annual survey highlights university startups that are, among other things, rethinking how to deliver gene-editing therapy and tackling various metabolic conditions, immune disorders and cancer with microbiome treatments or immunotherapy. Michael Eisenstein, Ken Garber, Esther Landhuis, Caroline Seydel and Laura DeFrancesco report.

Michael Eisenstein, Ken Garber, Esther Landhuis, Caroline Seydel and Laura DeFrancesco

Venture capital (VC) continued to flow into the private biotech sector, as total funding outpaced that of the last five years. More than half of the new companies profiled here have had a second round of funding, in one case (Graphite Bio) not only raising a substantial B round, but also going public.

As in [previous years of our survey](#), we focus on R&D-intensive startups spun out from academic institutions (Table 1). These were first identified as having raised a series A financing in 2020. Our editors then assessed publicly available information about each firm's research to select those that appear below. (Some firms were selected but not included because they were still in 'stealth mode' or declined to be interviewed.)

IgGenix: engineering antibodies to fight food allergies

Using single-cell techniques to isolate and characterize IgE-producing B cells that can produce anti-allergy antibodies.

Derek Croote, chief technical officer of IgGenix.

University in 2013 to join Stephen Quake's lab, Croote was thrilled to apply the group's single-cell technology to a longstanding challenge in allergy research: isolating

Derek Croote has never eaten pizza, ice cream or milk chocolate; he has a lifelong dairy allergy. He's one of more than 200 million people worldwide who have food allergies. That's why, when he came to Stanford

immunoglobulin E (IgE)-producing B cells. It's been known for decades that IgE mediates allergic reactions, but neither the cells that produce these antibodies nor the individual antibodies had ever been isolated.

Allergic reactions erupt when mast cells spew out histamine and other inflammatory chemicals that cause itching, hives and potentially life-threatening anaphylaxis. Food allergy therapies aim to prevent this inflammatory release, which is triggered when IgE binds food allergens and docks onto surface receptors of histamine-containing cells.

At present, people with food allergies have limited treatment options. Beyond avoiding culprit foods and carrying epinephrine to stop emergency reactions, some get allergen immunotherapy—a regimen that uses escalating, daily doses

Table 1 | The class of 2020: NBT's academic startups

Company	Focus area	Funding (\$ millions)	Scientific founders
IgGenix	Food allergy	10, 25	S. Quake, K. Nadeau (Stanford)
SyntheKine	Engineered cytokines	82, 107.5	C. Garcia (Stanford)
Sonoma Biotherapeutics	Regulatory T cells	40, 30	J. Bluestone (UCSF), Q. Tang (UCSF), A. Rudensky (Memorial Sloan Kettering)
BeBiopharma	B cell protein factories	52	D. Rawlings, R. James (U. Washington)
Federation Bio	Microbiome	50	M. Fischbach, D. Dodd (Stanford)
Graphite Bio	Gene editing	45, 150, 274	M. Porteus, M. Roncarolo (Stanford)
Spotlight Therapeutics	In vivo gene editing delivery	30	J. Corn (ETH), A. Marson (UCSF), P. Hsu (UC Berkeley)
Bright Peak Therapeutics	Protein synthesis from scratch	35, 107	J. Bode, V. Pattabiraman (ETH)
T-knife	TCR-T cell therapy	78, 110	T. Blankenstein (Max Delbrück, Berlin)

of the food to desensitize the immune system over time. In January 2020, the US Food and Drug Administration approved standardized peanut flour capsules (Aimmune Therapeutics' Palforzia) as a therapy for children with peanut allergies; the European Medicines Agency followed with an approval several months later. A few allergists offer unregulated immunotherapy using commercial peanut powder and other food flours. However, allergic reactions are a common side effect of these repeated food exposures, making both routes infeasible for many patients—including Croote.

Kari Nadeau, co-founder of IgGenix.

Studies of the immune system's allergy arm are complicated by the rarity of IgE-producing B cells. They're outnumbered a million to one by other blood cells, and IgE represents just 0.005% of

serum proteins. Yet IgE is exceedingly potent, says physician-researcher Kari Nadeau, who directs the Sean N. Parker Center for Allergy and Asthma Research at Stanford University. For allergies, IgE "is the match that lights the fire," she says.

Quake's lab approached this by analyzing blood from patients under Nadeau's care for food allergies, to isolate the actual cells that produce high-affinity, specific IgE to food allergens. Croote used fluorescence-activated cell sorting to capture single B cells with surface IgE, then determined each cell's isotype post hoc using single-cell RNA-seq to read the immunoglobulin heavy chain sequence. Post hoc isotype assignment allowed them to sacrifice specificity and capture IgE B cells with high sensitivity without complex gating schemes, as [described](#) in a 2018

Science paper. In total, they analyzed 973 B cells from six allergy patients, of which 89 produced IgE.

On the basis of the similarity of variable gene sequences in the antibodies' antigen-binding region, the researchers clustered the cells into clonal families. Curiously, multiple individuals harbored the same clonal family with high affinity to multiple allergenic peanut epitopes—suggesting it should be possible to create a broadly applicable therapy using IgEs from a limited number of people with allergies.

For such people, B cells can be a double-edged sword. Besides producing allergy-triggering IgE antibodies, they also make inhibitory IgG antibodies that snuff out the fire lit by IgE. In fact, one person in Croote's study had IgG4 antibodies that competed for the same peanut epitope as that person's IgE. (Immunoglobulin isotypes' different Fc regions produce different functional outcomes.)

During desensitization immunotherapy, regular dosing with the culprit food triggers allergen-specific IgG levels to rise. Over time, enough IgG can be produced to bind up the allergens before they encounter the rarer IgE antibodies. But without regular exposure to the allergen, the B cells quit making IgG—so immunotherapy patients who stop dosing can lose that protection, Nadeau says.

Puzzling over these issues on a phone call with Croote and Quake in summer 2018, Nadeau recalls an 'aha' moment. "I remember Steve saying, 'Yeah, but wouldn't it be cool to clip out the [IgE] tail and put in the IgG tail,'" she says, "and boom, now you've got an inhibitory molecule."

And thus IgGenix was launched in late 2019, with Nadeau and Quake as scientific co-founders and Croote as chief technical officer. The goal is to develop therapeutic monoclonal antibodies (mAbs) that could be given as monthly shots to tame allergic

responses to specific foods. The company came out of stealth mode last August with a \$10 million series A investment led by Khosla Ventures and Parker Ventures. Jessica Grossman, a physician by training with leadership experience at several biopharmaceutical companies, is the helm. In July, Khosla co-led a second round of funding, with an additional \$25 million, with contributions from Matthias Westman of Prosperity Capital Management, Alexandria Venture Investments, ShangBay Capital and AllerFund.

Besides being passionate about empowering women—fewer than 3% of venture-funded companies are women-led—Grossman was drawn to IgGenix because food allergy treatments transform not just the lives of patients, but also their families. "And who's the chief medical officer of the family?" she says. "Well, it's Mom."

IgGenix isn't the first to develop a food-allergy biologic. Two mAbs have supplemental indication approvals for food allergy: Xolair (omalizumab), an anti-IgE humanized IgG1 mAb developed by Genentech and Novartis; and Dupixent (dupilumab), a human IgG4 mAb from Regeneron and Sanofi that binds to the interleukin-4 receptor α subunit. These were originally approved for severe asthma and eczema, respectively. IgGenix would be the first company to develop a mAb treatment designed specifically for food allergy.

The same year Croote and colleagues published their isolation of IgE-producing B cells from patients allergic to peanuts, Regeneron scientists [reported](#) developing fully human IgG4 mAbs that reduce allergy symptoms in mice and patients allergic to cats. The two approaches are conceptually similar—both harness the immune system's natural production of IgG to compete with IgE antibodies—but each goes about it differently. Whereas Regeneron generates IgG4 mAbs by immunizing transgenic

mice containing the human immune genes, IgGenix engineers antibodies based on molecules isolated directly from patients with severe allergies. “We feel that the best approach to blocking severe reactions in humans is by starting with the very molecules that are causing those reactions in the first place,” Croote says.

This is important because IgE antibodies bind very strongly to allergens. Using patient IgE as starting material before swapping in an IgG tail, the portion of the engineered antibodies that sees the allergen remains very high affinity, says Cecilia Berin, who studies the immune basis of food allergies at Mount Sinai’s Icahn School of Medicine.

IgGenix’s antibodies, unlike immunotherapy regimens, are meant to prevent allergic reactions but would not retrain an individual’s immune response. Patients “would always be allergic and would always be treated for that allergy,” Berin says. So the company’s antibodies “would likely be an expensive therapy that may have to be given for life.”

So far, the IgGenix team has generated batches of engineered human IgG mAbs to peanut, cashew, walnut, shellfish and milk. The researchers plan to pick a lead candidate, start manufacturing it and be in phase 1 in about 18 months, according to Grossman.

And though the company is starting with food allergy, “It doesn’t end there,” she says. IgGenix’s technology can be used to isolate and re-engineer IgE mAbs as therapeutics to treat allergies to dust mite, latex and medications, as well as in a host of allergic diseases.

SyntheKine: beyond cytokines

Cytokines are problematic drugs, but Stanford structural immunologist Chris Garcia has engineered creative solutions that his company will begin testing this year in cancer.

Cytokines have a remarkable ability to potentially activate immune cells, making them attractive for cancer immunotherapy. But their lack of specificity has defeated most efforts to make them into drugs. That’s because cytokine receptors are widely distributed across many cell types, leading to side effects, often severe. Interleukin (IL)-2, for example, is a powerful survival and expansion factor for effector T cells, but it also binds to receptors on natural killer (NK) cells, regulatory T cells and vascular endothelial cells. Side effects of recombinant IL-2 (Proleukin [aldesleukin], first approved in 1992) can include massive inflammation and fatal vascular leak syndrome.

SyntheKine, founded in 2019, joins other companies seeking to harness the power

Chris Garcia, founder of SyntheKine.

of cytokines by engineering these small proteins or their receptors to generate more specific responses, reducing side effects while boosting efficacy (see Bright Peaks Therapeutics below). SyntheKine’s scientific founder, Stanford structural immunologist Chris Garcia, [who solved IL-2’s crystal structure in 2005](#), has used structural insights to create IL-2 mutants. Two licensed to SyntheKine are set to enter the clinic by the end of the year. The company closed on an \$82 million series A round in September, 2020, co-led by Canaan Partners, Samsara BioCapital and The Column Group. In June, the company pulled in a megaround of \$107.5 million from a syndicate led by Deerfield Management and Janus Henderson.

SyntheKine’s lead IL-2, STK-012, is designed to specifically bind T cells that have engaged a tumor, selectively boosting their expansion. STK-012, a partial agonist, binds only two of the three IL-2 receptor subunits— α (also known as CD25) and β , but not γ . Circulating CD8 effector T cells typically express β and γ , but upon antigen stimulation, these T cells send the α subunit to the surface. Then an IL-2- α - β trimer recruits the γ subunit, leading to intracellular signaling. By binding α and β only, STK-012 selects for the high α expressers, mainly the activated effector T cells, and avoids non- α expressers like NK cells, which—in SyntheKine’s view—cause some of IL-2’s severe side effects.

The result should be more T cells attacking the tumor, with less toxicity. With STK-012, says SyntheKine CSO Rob Kastelein, “You get a massive expansion of those specific-antigen active cells.” At the April annual meeting of the American Association for Cancer Research (AACR), SyntheKine [reported](#) that its mouse version of STK-012 boosted T cell numbers in tumors, eliminating most with few side effects as compared to wild-type IL-2.

SyntheKine’s $\alpha\beta$ approach is at odds with those of most other cytokine companies (Nektar is the most advanced), which are instead advancing IL-2 mutants with a $\beta\gamma$ bias. They seek to avoid activating CD25-expressing T regulatory (T_{reg}) cells, which would dampen the T cell antitumor response, and endothelial cells, which could lead to vascular leak syndrome.

Yet SyntheKine’s $\alpha\beta$ IL-2 is superior to the $\beta\gamma$ IL-2s, says CEO Debanjan Ray, with less

of cytokines by engineering these small proteins or their receptors to generate more specific responses, reducing side effects while boosting efficacy (see Bright Peaks Therapeutics

toxicity. Company chief development officer Martin Oft reported details for STK-012 in two colon cancer mouse models at AACR. STK-012, the company explains, enables effector T cells, with their higher receptor expression, to outcompete T_{reg} cells for activation in the tumor microenvironment. Oft’s report at AACR revealed a very high ratio of effector T cells to T_{reg} cells in mice. As for α binding causing vascular leak, “There is really very little evidence for that [in the literature],” says Kastelein. SyntheKine attributes vascular leakage from IL-2 therapy to peripheral NK cell activation, which doesn’t happen with STK-012.

Marc Ernstoff, an immuno-oncology researcher at the US National Cancer Institute (NCI), calls SyntheKine’s STK-012 “an exciting molecule.” The AACR report, he says, “provides the foundation for them to move it into a clinical trial.” But Ernstoff isn’t convinced that NK cells are responsible for the vascular leakage seen with IL-2 therapy. Before arriving at the NCI last year, he worked with a $\beta\gamma$ -biased IL-2. “We didn’t see pulmonary edema or weight gain in the animals,” Ernstoff says, despite NK cell activation.

Kastelein is confident that SyntheKine’s $\alpha\beta$ bias is the correct approach because it selectively boosts the exact T cell subpopulation that is attacking the tumor. Conversely, with $\beta\gamma$ IL-2, “if you now have an IL-2 that binds away from those cells, how can you expect to have optimal antitumor efficacy? You won’t.”

SyntheKine’s second lead program is an ‘orthogonal’ cytokine-chimeric antigen receptor (CAR)-T cell pairing for cell therapy. Garcia’s lab made a slightly modified IL-2 receptor unable to bind wild type IL-2, then screened a library of randomly mutated IL-2 variants for exclusive binding to the modified receptor. The result, [reported in Science](#) in 2018, was a mouse ligand-receptor pair that bind only each other, in lock-and-key fashion. SyntheKine is advancing a human version. SyntheKine’s IL-2 receptor, expressed by CAR-T cells, signals only when bound by the orthogonal IL-2, STK-009.

Precise, repeated dosing is the main advantage. Subcutaneous injections of STK-009, the ligand, drive CAR-T numbers higher as needed. “This private IL-2 is really what the CAR field has been waiting for. Nobody has the ability to expand CAR-T cells in vivo, in a patient,” Kastelein says. Giving a massive CAR-T dose right when the tumor burden is highest can cause cytokine release syndrome (CRS), due to activated immune cells secreting inflammatory cytokines. “Let’s come in with

a lower dose, let's build up in vivo over time, while the tumor burden is reducing," says Ray. "Hopefully that reduces the amount of CRS." In an AACR poster, SyntheKine reported complete responses in a mouse lymphoma solid tumor model at low CAR-T doses.

The NCI's Ernstoff likes the technology, but cautions that it won't solve all CAR-T therapy problems. "It's a little naïve to think that one area, one component, is going to be the solution for everything," he says. "But it may contribute," especially the ability to expand the CAR-T cells at will.

SyntheKine's Ray says its CAR-T should be effective in solid tumors, where CAR-Ts have so far failed. That's because it doesn't have to compete with tumor cells for IL-2 stimulation, which drives tumor penetration.

One limitation in solid tumors might be the inability to stimulate T cells against other antigens, says Ernstoff. If there is epitope spreading as cancer cells die and present antigen, orthogonal CAR-T cells, unlike endogenous T cells, won't adapt.

SyntheKine ultimately seeks to dispense with cytokines altogether. "Almost all [cytokines] have major problems as drugs," Garcia told *Nature Biotechnology* in 2018. Cytokine engineering has limits: "Evolution didn't anticipate using cytokines as drugs; evolution made cytokines to promote normal homeostasis of the immune system."

So Garcia set out to discover 'synthekines,' new cytokine receptor ligands. The goal was to assemble [non-natural cytokine receptor heterodimers](#). These signal into immune cells in new ways—completely novel biology. Natural cytokines trigger the assembly of only a small fraction of possible receptor pairings, and SyntheKine has begun exploring the remainder. As ligands it employs nanobodies—single domain antibody fragments found in camelids (llamas, alpacas and camels)—because they're small, stable and highly specific. Kastelein says that the company, to demonstrate the principle, has created a novel IL-10 receptor/IL-2 receptor dimer that in theory will drive an antitumor T cell response without causing the anemia seen with wild-type IL-10 therapy.

Garcia considers such synthekines the future of cytokine therapy. "To really harness the power of cytokines—and they're amazingly powerful molecules—I think we've got to ... look beyond the current portfolio that our genome encodes," he said. "We have to come up with completely new activities." KG

Sonoma Biotherapeutics: rallying the regulators

Will regulatory T cells provide needed therapies against inflammatory and autoimmune disorders?

Therapeutic applications of T cells have been focused up until now on their role as immunological 'attack dogs' that can be trained to seek and destroy foreign invaders or malignant growths. This core concept underlies chimeric antigen receptor (CAR)-T cell therapies that are now delivering curative treatments for some blood cancers. But Sonoma Biotherapeutics is trying to do the opposite—to harness regulatory T (T_{reg}) cells to suppress rather than provoke an immune response, potentially offering safe and durable control for various inflammatory and autoimmune conditions.

Jeffrey Bluestone, CEO of Sonoma Biotherapeutics.

Qizhi Tang, co-founder of Sonoma Biotherapeutics.

The company is drawing on a deep bench of expertise in the fundamental biology and clinical potential of T_{reg} cells from its four co-founders: Jeffrey Bluestone, Qizhi Tang, Alexander Rudensky and Fred Ramsdell. The existence of an immunity-modulating pool of T_{reg} cells [has been recognized since at least the 1980s](#). But it wasn't until the early 2000s that researchers got a handle on their defining properties,

and Sonoma CSO [Ramsdell](#) and scientific advisor [Rudensky](#), an immunologist at the Memorial Sloan Kettering Cancer Center, helped define a gene called FOXP3 as a primary determinant of T_{reg} development and identity.

"Mice and corresponding human patients who had defects in this gene had huge defects in immune regulation," says Ramsdell. "And so the cells expressing this gene basically were really master regulators of the immune response and self tolerance." Deeper investigation of T_{reg} cells showed that they have a far-reaching capacity to selectively downregulate adaptive and innate immunity to certain targets without broadly inactivating immune defenses as a whole.

This suggested some intriguing clinical opportunities, and Sonoma CEO Bluestone and scientific advisor Tang, both at the University of California at San Francisco,

are among the pioneers in exploring the use of transplanted T_{reg} cells to modulate immunity. "We recognized that it's very hard to assimilate all the functions of the cell, and so perhaps the cells themselves can be a drug," says Tang. Animal studies from their team have offered tantalizing support for this approach in conditions like type 1 diabetes. "A few hundred thousand cells will cure the mice for life," says Tang. And since 2011, she and Bluestone have run ten phase 1 clinical trials of T_{reg} cell transplantation—five to prevent organ rejection in transplant recipients and five for autoimmune disorders. These and other studies have demonstrated the safety of this approach; the data [on efficacy in humans are relatively limited thus far](#).

Bluestone and Rudensky had been discussing the notion of a commercial venture for a few years, aware of the limitations of developing a clinical cell therapy in the academic realm. "One big impetus for getting Sonoma Bio started was the idea that if we were going to take this whole approach to the next level where we really could help patients ... it was going to require lots more resources," says Bluestone. After the founding team had assembled in late 2019, they embarked on a fundraising effort, drawing a total of \$70 million in two rounds of series A funding in February and September 2020 from more than half a dozen different backers.

The core notion of T_{reg} cell therapy is not radically different from that of CAR-T cell therapy: a patient's immune cells are harvested, purified and genetically manipulated to maximize their clinical potency before being reinfused as living drugs. But there are important distinctions between T_{reg} cells and the cytotoxic T cells used in cancer therapy, as well as some unresolved questions on how to prepare and manufacture these cells for clinical applications. " T_{reg} s are 20 years behind the rest of the engineered T cell field, and we don't necessarily have a lot of the basic science background to base decisions on," says Megan Levings, an immunologist studying clinical applications of T_{reg} cells at the University of British Columbia.

One question is how best to target these cells. Early studies used polyclonal mixtures of T_{reg} cells not preselected to recognize any particular antigen. Although simpler to prepare, these therapies tend to produce relatively weak immunomodulation. "In animal models, Tang showed 10- to 25-fold greater activity if they were specific for a particular target," says Bluestone. Sonoma will

initially focus on genetically manipulating cells to express CAR constructs in which an antigen-specific binding extracellular domain is coupled to a potent intracellular activation domain. T_{reg} cells have an important edge over cytotoxic T cells here in that they do not need to recognize a disease-related antigen per se. They need only be targeted generally to the tissue of interest—for example, an antigen expressed in the pancreas for patients with type 1 diabetes—and will then exert a ‘bystander’ effect that broadly mollifies immune cells in the area.

Evidence suggests that these cells exhibit a robust safety profile in both animals and humans. There were concerns that transplanted T_{reg} cells might lose their identity and acquire the aggressive characteristics of cytotoxic cells, but this has not been observed. “We have very good data that that’s not routinely happening in our patient,” says Bluestone. Less clear is whether the cells might prove overly suppressive of local immunity in the long term, such that infections or tumors can find safe haven. No clear evidence of this exists from the clinical work, but Levings cautions, “I think the theoretical risk is much higher with engineered T cell therapies.”

The most critical unmet challenges relate to production. “These cells are rare, so T_{reg} manufacturing will have to start with very robust methods to purify them,” says Tang. She adds that they grow slowly in culture, which could compound the problems associated with impurities—a relatively small number of contaminating cells could quickly outgrow the T_{reg} population. The most effective purification procedures are relatively low throughput—for example, using fluorescence-activated cell sorting to isolate T_{reg} cells on the basis of the presence or absence of cell-type-specific surface markers. The company is now investigating more effective and automated pipelines for isolating and cultivating the desired cells.

Addressing these questions will take hard work and substantial investment, and Levings thinks that academically rooted biotech startups like Sonoma could offer an ideal vehicle for translating this technology. “You could say they’ve got the ‘ T_{reg} dream team,’” she says. “They clearly have the academic and practical logistics background ... so they’re very well positioned to be successful.” Bluestone is encouraged rather than concerned by the mounting competition in the space. “There’s clearly been an inflection point, where people truly believe these cells can be exploited to treat a number of diseases.”

Sonoma has not yet disclosed which indications will be the initial focus of its pipeline, although its founders’ prior work in the autoimmune disease space offer some hints. But Rudensky points out that it is worth looking beyond conventional notions of what diseases might benefit from an immunological intervention. “One of the most significant conceptual advances in the last 20 or 30 years in biomedical research has been the realization that chronic inflammation underlies a broad spectrum of human disease,” he says. “So altogether, we think that the future is rich for this cell type as a therapeutic agent.” *ME*

BeBiopharma: B cells as protein factories

Plasma cells can be turned into protein factories for patients with protein deficiencies for whom one-and-done gene therapy is not an option.

As a pediatrician at the University of Washington School of Medicine, David Rawlings treats children who have hemophilia and other rare genetic diseases. “They need a therapy from day one of life,” says Rawlings, who also directs the Center for Immunity and Immunotherapies at Seattle Children’s Research Institute.

The standard of care for these disorders is protein replacement therapy, but such treatments require a lifetime of expensive infusions. Gene therapy is one alternative. For people with the bleeding disorder hemophilia B, for example, gene therapy using liver-targeting adeno-associated virus (AAV) vectors can restore long-term expression of factor IX (FIX), a clotting factor deficient in these patients. However, a major problem with AAV gene therapy is immunogenicity: subsequent infusions provoke an immune response to the virus. So “you only get one chance,” Rawlings says. That makes the approach infeasible for children, who need increasing amounts of the therapeutic protein as they grow.

Richard James, co-founder of BeBiopharma.

Six years ago, Rawlings and Seattle Children’s colleague Richard James, associate professor in pediatrics and pharmacology at the University of Washington, came up with a possible solution: engineering B cells.

During an immune response, exposure to an antigen triggers naive B cells to proliferate and develop into plasma cells or memory cells—each with distinct advantageous

features. Plasma cells produce large quantities of disease-fighting antibodies. “They make lots and lots of proteins—comparable to industrial cell line levels,” says James. Memory B cells have longevity. After an infection subsides, memory B cells that have generated specific antibodies can reside in the bone marrow or other lymphoid organs for decades, ready to spring into action if confronted with the same antigen.

Together, Rawlings and James figured these two features make B cells ideal for treating enzyme deficiencies or other conditions in which patients require long-term protein production. In reality, though, turning B cells into medicines presented technical hurdles. For starters, delivering genes into B cells proved tricky. “It sounds like an obvious thing. Like, of course you could just put a gene in a cell. That’s really easy,” says James. “But for B cells, it wasn’t.” Plasmid delivery is toxic to B cells, and lentiviral vectors had abysmal efficiency or weren’t scalable.

Meanwhile, Rawlings and coworkers had pioneered using designer nucleases, including CRISPR–Cas9, in parallel with AAV vectors to do homology-directed gene editing of primary cells. They showed these techniques could work in [human T cells](#) and [CD34⁺ hematopoietic cells](#). Given the difficulties with other gene delivery methods in B cells, he and James decided to try this AAV technique. “It was so much better than any of the other delivery methods we had tried,” Rawlings says.

But there were more challenges. Not only did the researchers want to engineer new genes into B cells, but they also needed to get naive B cells to differentiate in vitro into protein-producing plasma cells. Rawlings’ lab had previously shown that activating the cell is important for gene editing and homology-directed repair, as terminally differentiated B cells lack endogenous repair systems. “So that was one of the first things we needed to figure out—how to activate the B cells and get them proliferating,” says James.

When the body is responding to an infection, B cells migrate to germinal centers—sites within the spleen or lymph nodes—where activated B cells proliferate and undergo somatic hypermutation, generating a variety of antibodies. From those, higher affinity antibodies are selected for further production. In addition to antigen exposure, B cell proliferation typically requires signals from helper CD4⁺ T cells and cytokines. To mimic this process in vitro, James and colleagues tested many types of reagents, eventually coming up with a cocktail that included an oligomerized form of CD40 ligand (CD40L; a protein

expressed on the surface of activated CD4⁺ T cells) as well as interleukin (IL)-2, IL-10 and IL-15.

For proof of concept, the researchers used a AAV serotype 2 (AAV-2)-mediated, homology-directed method to engineer plasma B cells to secrete FIX, as [reported](#) in *Science Translational Medicine*. They targeted FIX to the chemokine CC-motif receptor 5 (CCR5) locus—a gene that is not transcriptionally active in human B cells—and used a chromogenic assay (vitamin K-dependent FIX carboxylation) to show that the FIX produced by the engineered cells was active. To promote plasma cell production in culture, the team co-delivered CRISPR–Cas9 to disrupt a developmental gene (PAX5), which normally acts to antagonize plasma cell differentiation.

Separately, the team introduced the B-cell activating factor gene (BAFF) into the CCR5 locus and showed that BAFF-secreting plasma cells survived longer than control cells lacking the Cas9 ribonucleoprotein. Furthermore, when these BAFF-producing cells were transferred into non-obese diabetic–severe combined immunodeficient–IL-2 receptor (IL-2R)- γ knockout (NSG) mice, plasma cells engrafted in the bone marrow, produced antibodies and secreted BAFF more than a year later, as [reported](#) at the 2019 American Society for Gene and Cell Therapy meeting.

What moved these experiments beyond an academic exercise was the absence of a requirement for cumbersome co-culturing with feeder cells to differentiate the edited B cells into plasma cells. “Now that becomes something that looks a lot more like a product that can go into a patient,” says Lea Hachigian, a principal at the venture capital firm Longwood Fund. She and Longwood colleagues Aleks Radovic-Moreno and David Steinberg worked with Rawlings and James to start a biotech around the B cell engineering technology. Last October, Be Biopharma launched with a \$52 million series A financing round led by Atlas Venture and RA Capital Management, joined by Alta Partners and Takeda Ventures.

Coming up with a method to transfect primary B cells with high efficiency was “a major breakthrough,” says James Voss, an immunologist at Scripps Research Institute in San Diego. Furthermore, “it’s very visionary to imagine harnessing a B cell to express a secreted protein. It was exciting to show that these cells could live for so long, even if in an immunocompromised mouse.” Voss wonders, though, if it may be harder for engineered B cells to engraft in a patient with a fully functioning immune system.

The absence of other lymphatic cells in an immunocompromised mouse makes it “a bit easier to engraft a foreign component,” he says.

Be Biopharma researchers are doing further experiments to test for engraftment using various proteins with therapeutic potential. The company is focusing on monogenic rare diseases for now. But B cells are versatile. “We think that if you limit their differentiation and engineer B cells to have a regulatory function, you could also use those to modulate autoimmune responses,” says Rawlings, whose poster [presented](#) last March at the B Cell Renaissance Keystone Conference describes efforts to test whether antigen-specific B cells expressing IL-10 can tolerize the body to self antigens that cause autoimmune disease. On the flip side, as B cells also work as antigen-presenting cells, they could also be used to drive an anticancer response. “There are multiple ways that you could use B cells,” he says, “and their longevity gives them a lot of leverage as a cell therapeutic.”

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Federation Bio: from single strains to synthetic consortia

Transplants of engineered single-strain live biotherapeutic products and massive synthetic consortia are developed in parallel to manipulate the immune and metabolic systems in disease.

Michael Fischbach, founder of Federation Bio. Credit: Linda Cicero

The ability of the gut and skin microbiome to influence human biology represents an open frontier. Links have been found between microbiomes and conditions as diverse as eczema, cancer, metabolic disorders and

Parkinson’s disease. Federation Bio, which was co-founded by Michael Fischbach of Stanford University, is pursuing two approaches that take advantage of expanding knowledge of the gut and skin microbiome to develop live biotherapeutic products that combat secondary hyperoxaluria and cancer.

“These two platforms are running in parallel,” explains Fischbach. “The only theme that ties them together is that they both represent something unique and powerful, something that doesn’t match any current capability in the biotech arsenal, and is of very large magnitude.”

One platform involves transplants of massive synthetic microbial consortia, instead of individual species or undefined community consortia. While companies

like Vedanta Biosciences are developing synthetic consortia of 11 strains, the Federation Bio consortium contain >120 strains, likely the largest microbiota product currently under commercial development. “We wanted to be able to create a full-scale gut community from scratch, as a completely defined system,” Fischbach says.

Federation Bio’s second platform takes advantage of the skin microbiota’s ability to communicate with the immune system. *Staphylococcus epidermidis*, a commensal naturally resident on human skin, can be engineered to induce expansion of antigen-specific CD8⁺ T cells in vivo. “This is a tumor vaccine where the antigen would be something unique to the tumor, and where the adjuvant is a commensal organism,” Fischbach explains.

An assistant professor of microbiology and immunology at Stanford and director of the Stanford Microbiome Therapies Initiative, Fischbach co-founded Federation Bio with Dylan Dodd and with Racquel Bracken of Venrock, who served as the founding CEO.

Bracken and her colleagues had known Fischbach for some years before founding Federation. “Michael and I met in 2016 and did a number of brainstorming sessions around work in his lab,” she says. By 2018, she says, the work had matured to a point where it was ready to move ahead. “Michael really came at this from a confluence of different disciplines,” she says, including chemistry, microbiology and genomics.

Emily Drabant Conley, CEO of Federation Bio.

Federation launched with \$50 million in series A funding from Venrock, Altitude, Horizon Ventures and Seventure Partners/Health for Life. They also hired a new CEO, Emily Drabant

Conley, who joined the team with 10 years’ experience overseeing business development at 23andMe. During that time, she saw human genomic data grow from a niche interest into a tool for drug discovery. “It’s been a really big shift, and I got to be part of that at 23andMe. I foresee a similar shift coming in the microbiome,” she says. The chance to shepherd an emerging technology to the mainstream of health technology attracted her to Federation, she says.

“We’re at the beginning part of the curve. There’s better tools, there’s more understanding, and we have an inkling that the microbiome is very important for human health,” Conley says, “I foresee

that it will become a powerful therapeutic modality in the next ten years.”

Federation’s lead program aims to treat secondary hyperoxaluria, which causes kidney stones. In these patients, the gastrointestinal tract absorbs too much oxalate from food. Transplanting gut microbes that break down the oxalate can reduce kidney stone formation, and these microbes stand a better chance of establishing themselves if they are transplanted as part of a thriving microbial ecosystem.

“You have to create the ecosystem for your organism to live and also satisfy all its metabolic requirements,” says Bryan Coburn of the University of Toronto. The gut microbiome is an interconnected ecology, just like any macroecology. Although many in the field are trying to tease out the right combination of microbes to create a scalable and efficacious therapy, it’s a daunting task. “A lot of the work has been observational and has not clearly disentangled the roles of specific microbes from the role of a healthy microbial community,” Coburn says.

Synthetically designed consortia attempt to improve on fecal microbiota transplants (FMTs), in which an entire microbial community is extracted from a healthy donor and introduced into patients. FMTs have successfully treated chronic infection with *Clostridioides difficile*, a pathogenic bacterium that can take over the gut after antibiotic use. The transplant approach avoids the difficulty of identifying individual species, while benefiting from having a healthy, interconnected microbial community. But, although donors are carefully screened, the FMT approach is not without risks. The FDA has documented six patients who became infected with pathogenic strains of *Escherichia coli* following FMT, with two deaths possibly resulting.

“What if we could progress the field from FMT, which is more like a natural product, to synthetically designed consortia?” says Bracken. To assemble a stable consortium, Federation Bio cultivates bacterial strains individually to characterize the chemical phenotype contributed by each species. That allows them to optimize the community under controlled conditions, by adding or dropping out one species at a time. “We’re able to apply and develop design rules around what microbes need to be in the consortia to change the phenotype,” Bracken says.

“We’ve had to make a major investment in learning to isolate not just a lot of strains but the right strains from a sample,” Fischbach adds. He can’t give too many specifics about as-yet-unpublished work,

but he says where Federation Bio stands out is the number of species in their consortia. “For a variety of reasons, I think that people have been overestimating the complexity of real native communities,” he says.

Coburn points out that it’s impossible at this stage to know whether replacing a person’s native microbiome with a synthetic consortium could have unanticipated negative effects. “One of the long-range concerns is that we have short-term therapeutic goals, which we may achieve, but that may have long-term ecological consequences for that individual that we might not see until years later,” he says. Still, he adds, “every area of drug development deals with this. This is what phases of clinical research are for.”

Federation Bio’s platform of immune-system-stimulating skin microbes Fischbach sees as a simple immunotherapy. Chimeric antigen receptor (CAR)-T cell therapy has had some spectacular successes, particularly in blood cancers, but it’s invasive, time consuming and expensive. “The whole reason that people make CAR-T cells is so they can endow the T cell with the right antigen, so that it goes after the right target cell,” Fischbach explains. But what if there were a less invasive way to endow T cells with the cancer antigen?

Turns out, microbes on the skin can direct the T cells. “One of the most striking things the microbiome does to the host is modulate the immune function very potently,” says Fischbach. In a recent bioRxiv preprint, Fischbach reports that, when applied to the skin, an *S. epidermidis* strain engineered to express ovalbumin stimulates both a CD4⁺ and a CD8⁺ T cell response against an ovalbumin-expressing B16 melanoma subcutaneously implanted in a mouse.

Developing an efficient method for genetically engineering *S. epidermidis* was an initial hurdle, he says. “It was one of these problems that was solved not by one magical ‘aha’ moment, but by carefully tuning the parameters around the source of the DNA and the conditions to electroporate the strain,” Fischbach says. Once that was solved, the bacteria performed admirably. “The effect is striking. You take a Q-tip and swab it on the head of the mouse, and their tumors grow much smaller,” Fischbach says. The method could be adapted for use in other skin commensals and with other tumor antigens.

These programs may represent the tip of the iceberg as researchers discover more ways to tap into the microbiome’s “superpowers.” “I’m delighted for everybody’s success,” Fischbach says.

“I’m hoping we see a broad infiltration of biotech by the microbiome.”

CS

Graphite Bio: gene editing blood stem cells for sickle cell disease

Ex vivo gene editing of hematopoietic stem cells using CRISPR-Cas9 and adeno-associated virus serotype 6 is ready for trials in people with sickle-cell disease.

Sickle cell disease has been a neglected area of drug development, with limited treatment options to prevent the associated debilitating pain and early mortality. With the maturation of cell and gene therapy, however, several cutting-edge treatments that have entered clinical testing aim to undo the damage caused by a mutation in the β -globin gene. Most existing gene therapy approaches supplement faulty production of β -globin with a compensating foreign gene—in the case of bluebird bio, for example, patients are given a lentiviral vector containing a T87Q-engineered form of the human β -globin gene that inserts at distant points in the genome and confers potent anti-sickling activity.

Matthew Porteus,
co-founder of
Graphite Bio.

Maria Grazia Roncarolo,
co-founder of
Graphite Bio.

Stanford University spinout Graphite Bio has a different goal in mind. Leveraging the expertise of co-founders Matthew Porteus and Maria Grazia Roncarolo in genome manipulation and hematopoietic stem cell therapy, Graphite is pursuing a strategy of repairing the mutations behind sickle cell and other genetic diseases at the source. “When people say genome editing, the implication is that you’re fixing typos,” says Porteus.

“That’s actually what we’re doing—almost all the other approaches are workarounds” in which aberrant function is counteracted by supplementing a foreign gene product.

Trained as a pediatric hematologist, Porteus has been working in gene editing for nearly 20 years; his lab is currently focused on pushing the performance of CRISPR-Cas9 at fixing rather than supplementing disease mutations. Using electroporation, his team was able to deliver complexes of the Cas9 nuclease with its guide RNA,

which targets enzyme to a desired site in the genome. This triggers a selective genomic cutting and repair process that can inactivate targeted genes. To replace them, a 'donor DNA' sequence is required; it undergoes targeted recombination at the cleavage site. But foreign DNA can trigger cellular alarms as a sign of a viral infection. Porteus's team [overcame this in 2016](#) by using an adeno-associated virus serotype 6 (AAV-6) gene therapy vector to shepherd donor DNA into cells, achieving unprecedented efficiencies in this 'homology-directed recombination' (HDR) process. "All of a sudden we're going from 1% targeted integration to 30–50% targeted integration," says Porteus.

Around this same time, Roncarolo was recruited to Stanford. As director of the San Raffaele Telethon Institute for Gene Therapy in Milan, she had experience with the clinical application of genetically manipulated hematopoietic stem cells (HSCs), including testing and developing Orchard Therapeutics' Strimvelis, the first European Medicines Agency-approved gene therapy. Much of this work involved lentiviruses, which can shoehorn foreign DNA into host genomes, but with little control over its destination. This can be problematic if the foreign DNA ends up disrupting essential genes or regulatory elements.

Roncarolo, tasked with setting up an academic facility for the development, manufacture and clinical testing of cell and gene therapy candidates, immediately recognized the promise of CRISPR-based HDR. "It was clear to me that Matt's gene-editing technology was really a priority for the center," she says. Together, they developed a robust protocol for extracting HSCs from patients with sickle cell disease, correcting them via HDR, and transplanting them back into patients. Support from the California Institute for Regenerative Medicine helped bring them to the brink of clinical testing.

At this point Roncarolo, Porteus and his former postdoc Daniel Dever decided they had gone as far as they could without the resources of a commercial venture, and so Graphite Bio was born. The company drew \$45 million in series A funding from Versant Ventures and Samsara BioCapital in September 2020. Its founders attribute their fundraising success to their work in preparing for clinical testing, including preliminary toxicology studies, which in turn led to an approval by the US Food and Drug Administration for an investigational new drug (IND) application. "We had an incredible jumpstart because I don't know how many companies start with an IND

approved by the FDA—the very first IND approved for [ex vivo] gene correction," says Roncarolo. This funding helped Graphite to build out its team, including CEO Joshua Lehrer, who oversaw the development of the sickle cell disease-modifying therapy Oxbraya (voxelotor) at Global Blood Therapeutics.

Stanford's IND has been transferred to Graphite, and the company is launching its first phase 1/2 clinical trial. "We're open for business," says Lehrer, "and the next step will be identifying and screening and enrolling that first patient." The company has good cause for optimism; other ongoing HSC-based transplantation and gene therapy trials have shown the feasibility of achieving disease control after transplantation. For example, CRISPR Therapeutics and Vertex Pharmaceuticals are now conducting a trial in which CRISPR–Cas9 is being used to switch on the production of fetal hemoglobin, a fully functional form of hemoglobin that is normally absent in adult cells. A [recent publication](#) has described robust and durable improvement in symptoms for one patient treated in this trial.

But it's unclear what level of gene correction is necessary in the modified HSC pool to provide clinical benefit. Akshay Sharma, a bone marrow transplantation specialist at St. Jude Children's Research Hospital—and one of the investigators on the CRISPR Therapeutics/Vertex trial—notes that data suggest that a pool comprising 25–30% modified cells might do the trick, but adds that little information is available from genome-editing trials.

"It is safe to say right now that in the gene therapy setting, more is better," he says, "although we don't know quite how much more." Porteus says they can now routinely achieve 50% editing efficiency in HSCs at clinical scale, and is optimistic that this should be sufficient. However, it remains unclear how well these edited cells can engraft in the marrow. "That's one of those questions that keeps me up at night," says Porteus, although [recent work](#) from his group in mice suggests that durable engraftment is achievable.

There are also some safety concerns associated with the chemotherapy 'conditioning' regimen used to kill existing HSCs before transplantation, a standard part of HSC gene therapy—including Graphite's. The potential consequences can include genetic damage, sterility, and endocrine problems. In severe cases of sickle cell disease, this risk may be worth it. "Sickle cell is a very debilitating disorder ... and there is no doubt it can reduce lifespan," says Sharma. But he also notes that existing

disease-modifying treatments may be a better fit for patients with milder disease.

Safer conditioning has been targeted by startups such as Magenta Therapeutics; it is also a priority at Graphite. Jasper Therapeutics, another Stanford spinout, has developed a monoclonal antibody that effectively kills marrow-resident HSCs without inflicting broader damage. The two companies are partnering on a clinical program to treat X-linked severe combined immune deficiency, in which this antibody-based conditioning will replace chemotherapy. If successful, this could become a general component of Graphite's treatment process.

Lehrer sees this as a potential game-changer not just for Graphite, but the field as a whole. "This could be an outpatient procedure," he says. "If you're talking about one antibody infusion followed by a cell infusion and they're cured for life, then you can see how this would really expand the potential to transform a lot of lives."

The Graphite team is planning next steps, with half a dozen indications in the pipeline, such as the rare metabolic disorder Gaucher syndrome. The investor community is also enthusiastic—in mid-March, a round of series B funding netted the company another \$150 million. And the money keeps rolling in. A June, 2021, initial public offering brought in a whopping \$274 million.

The work is progressing quickly, but Porteus is mindful of how many incremental successes were necessary to get to this point, and he believes that stepwise progress will be a key component of the company's future growth and development. "To use a baseball analogy, we're going to get there by singles and doubles and walks and stealing bases, not just trying to hit the home run," he says. ME

Spotlight Therapeutics: making CRISPR deliver

Moving beyond viral vectors and lipid nanoparticles, Spotlight is conjugating Cas proteins to agents that will home the endonuclease and their guide RNA to targets in vivo.

Less than a decade after being harnessed as a programmable gene-editing tool, CRISPR has marched into the clinic. Advances continue to be made in refining the editing reagents themselves—finessing both the guide RNAs (gRNAs) and Cas endonucleases to improve cutting precision and minimize genotoxic effects—but, for clinical applications, systemic delivery remains a work in progress, relying on the viral vector and lipid nanoparticle

technologies (LNPs) used for other nucleic acid therapeutics.

Jacob Corn, co-founder of Spotlight Therapeutics.

“Those are fine technologies, but they have some serious flaws,” says Jacob Corn of ETH Zürich, one of Spotlight’s co-founders.

“When people are trying to make them better, they’re iterating on the

core idea. They’re trying to make new AAV capsids or they’re trying to make different types of lipids for the LNPs. They’re not thinking different[ly] about how you could do this.”

Alex Marson, co-founder of Spotlight Therapeutics. © Anastasiia Sapon for UCSF.

Spotlight Therapeutics was born of a vision to re-imagine CRISPR delivery. The company’s three co-founders—Corn; Alex Marson of the University of California, San Francisco; and Patrick Hsu of the University of California,

Berkeley—zeroed in on delivery as an area in the CRISPR field that required new thinking.

“We thought there was an opportunity, based on some preliminary work that was happening in our labs and some things we were seeing in the field, to take advantage of the fact that Cas9 is a protein, and that we could use the power of medicinal biochemistry to start addressing that protein to particular cell types,” explains Marson.

The central idea is to target Cas endonuclease and its gRNA to specific cell types in vivo using a “library of parts” consisting of cell-penetrating peptides (CPPs), antibodies, and ligands. These parts can be recombined into a selection of targeted active gene editors (TAGEs). TAGEs combine a cell-targeting antibody with CPPs linked to Cas endonuclease preloaded with a gRNA of interest. They seek out the intended cells, traverse the cell membrane and penetrate the nucleus, where they can edit the locus of interest.

“We have a really rich toolbox of editors available to us, and one of the remaining hurdles is the ability to deliver these tools carefully to different cell types in vivo,” says Ben Kleinstiver of the Center for Genomic Medicine at Massachusetts General Hospital. “They’re addressing a

really critical need in the field, and if they can make progress, it will be very impactful.”

To identify targeting molecules that can be used in TAGEs, Spotlight uses a combination of rational design and high-throughput screening. The company says that their discovery pipeline allows them to identify targeting molecules that work in practice, not just in theory. Their screening platform ensures that a given molecule effectively delivers the editors to the nucleus. “If you just make an antibody, sure, it’s going to bind to the cell surface receptor, but how do you know it gets in?” says Corn. “If it hits in our system, it has actually made it into the nucleus.”

So far, most gene editing in humans has been done using cells engineered outside the body, expanded and then reinfused. Chimeric antigen receptor (CAR)-T cells in cancer are one example; another is the engineering of hematopoietic stem cells to treat blood disorders like sickle cell disease or β -thalassemia. These ex vivo therapies allow direct delivery of the gene editing reagents to the desired cells. But because they’re usually autologous, N-of-1 approaches, they’re also expensive, complicated and labor intensive. In some cases, the patient must endure a toxic conditioning regimen to clear the way for the edited cells.

“That’s where Spotlight has an enormous opportunity,” says Marson. “The strongest motivation to move to in vivo editing is that this will democratize the benefits of CRISPR. Editing will go from an extremely complicated process with, in some cases, toxicities to something that could be done directly inside the body, hopefully with a one-time injection that would have a curative effect.”

One benefit of ex vivo therapies, of course, is the chance to perform quality control on the edited cells. Injecting gene-editing reagents directly into the body takes that control out of researchers’ hands, leaving no room for error. Even as editing reagents become better characterized and controllable, questions remain about off-target or other unintended genomic changes. In vivo delivery is also heavily biased towards certain tissues, such as liver sinusoidal endothelial cells, and hyperabundant asialoglycoprotein receptors on hepatocytes have thus far been the main target for successful conjugate-mediated delivery in the clinic.

Although Spotlight’s focus is on optimizing delivery technology and not the editors themselves, any in vivo gene editing therapy will have to contend with the possibility of the “unknown unknowns”

of genome editing that takes place in the body rather than outside the body. “Systematic, unbiased profiling of the genomic consequences of genome editing in in vivo models has been a focus in the field,” says Hsu. “But it will need to be tackled individually for each new therapeutic development candidate.”

“We know that CRISPR can trigger some chromosomal rearrangements,” says Philippe Duchateau, CSO of Collectis. He cautions that careful dosing will be critical to avoiding off-target effects. “You want to use just enough to have the effect that you want, but not too much,” he says. “If you put too much nuclease into the cell, you start having these off-site effects. This technology is going to be difficult to fine tune the dose that is needed.” He adds, “The gene editing field is still in infancy. In vivo application is the future, but for today, we are not there yet.”

Ready or not, however, in vivo gene editing has begun. In March 2020, Editas Medicine (with Allergan) announced treatment of its first patient with an injected CRISPR therapy to correct a mutation in centrosomal protein 290 (CEP290) that causes blindness. The treatment delivers Cas9/gRNAs specific to CEP290 via an adeno-associated virus serotype 5 (AAV-5) vector injected directly into the retina. Last month, Intellia Therapeutics also [published results](#) of durable knockout of transthyretin (TTR) in TTR amyloidosis via a LNP-delivered Cas9 endonuclease infused intravenously.

Manufacturing viruses is complicated and costly, however. “At the time that we got started with Spotlight, the noise that was ringing in our ear was that Editas had just failed a clinical manufacturing batch of AAV,” recalls Corn. “Some of these delivery technologies are very difficult to engineer, they’re not modular, and they’re hard to produce.”

“The idea for TAGEs is that you have all the scalability in terms of manufacturing, quality control, of a biotherapeutic, but you do it with a gene-editing reagent,” Corn explains.

Spotlight has their sights on three applications: ocular indications, where the TAGEs would be delivered directly to the subretina; immuno-oncology, where TAGEs would be injected directly into the tumor microenvironment to potentiate T cells and macrophages; and blood disorders, where TAGEs would be injected into the bone marrow to access hematopoietic stem cells, avoiding cumbersome extraction and reinfusion protocols. Because in many of these cases ex vivo CRISPR gene editing has already been validated by other groups,

these provide a solid base on which to build TAGE approaches off the ground.

A key feature for selecting indications, Marson says, is that editing only a subset of cells is enough to deliver a therapeutic benefit. “They’re diseases where you don’t have to correct every cell in the body, where getting a targeted gene modification into even a small number of cells would totally transform the way that the body responds and have a major effect on treating that disease,” he says.

So far, the company hasn’t published many data demonstrating their platform’s efficacy. “The data that’s been driving some of this is with transgenic reporter mice,” says Corn. “When you get editing, you get a reporter signal in the mice. In all three of the programs, there’s very clear evidence of in vivo activity following a single administration.” For the first TAGEs, the company has selected CD34 and c-Kit antibodies to target hematopoietic stem cells, and these are being tested in humanized mice.

Marson acknowledges that Spotlight is launching at a very early stage. “It was not something that was fully de-risked in the academic setting,” he says. “It’s been wonderful to have GV [formerly Google Ventures] as a partner that’s been committed to this, recognizing the importance and showing a willingness to be bold.”

Spotlight closed their series A funding round with \$30 million from GV and a few other, unnamed, funders. Mary Haak-Frendscho, former president of Takeda San Francisco, joins the team as president and CEO.

Bright Peak Therapeutics: better biologics through chemistry

Chemical synthesis matures as an alternative to cell culture for made-to-order small to medium-sized proteins.

Recombinant proteins are a mainstay in today’s pharmacopeia, but manufacturing them at scale remains technically and logistically challenging. Cell-based systems—whether they be bacterial, plant or mammalian—are inherently cumbersome to manipulate and maintain. Present-day capacity can’t always meet the demand, as the early days of the pandemic demonstrated when supplies of monoclonal antibodies for COVID-19 were limited to the rich and famous. And the ability to modify proteins at specific sites is not always straightforward, taking months or sometimes years to accomplish.

Now Jeff Bode’s group at ETH in Zurich has come up with a way to construct proteins from scratch using a solid-phase peptide synthesis platform that allows

fine-tuning of “biological activity by making small, but important, changes in amino acid side chains,” says Bode. Putting unnatural amino acids into a recombinant protein produced in cells is possible but complicated (requiring expanding the genetic code) and expensive. With a totally synthetic process, Bode says that many non-canonical amino acids can be added into a peptide chain, wherever they want.

In addition, with their particular ligation process, which pairs an α -ketoacid with a hydroxylamine—a so-called KAHA reaction, which Bode’s group first described in 2006—they can make proteins of several hundred amino acids by making 30- to 40-mer peptides and then stitching them together. Technologies for ligating small peptides together to make larger peptides or small proteins have been around for decades, but serial purification steps can reduce yields, making this process unfit for protein manufacturing at the scale needed for biologic production. However, after a decade of trying different combinations of N- and C-terminal end groups, Bode’s group found that this KAHA pairing, unlike most organic reactions, works well in the presence of other organic functional groups found on amino acid side chains. “We sometimes call it the ‘teenager in love reaction,’ as the ketoacid and hydroxylamine just stick to each other and completely ignore everything else around them,” jokes Bode.

Jeff Bode and Vijay Pattabiraman, co-founders of Bright Peak Therapeutics. Credit: Stefan Weiss, ETH Zürich

some small globular proteins. Key was the choice of hydroxylamine. What they found, quite by accident, was that when oxaproline is used as the hydroxylamine of the pair, ester (rather than amide) bonds are created, which are stable to all standard reactions, reagents and purification schemes. Then, under conditions for protein folding, the ester bond spontaneously converts to an amide. “We got lucky in that oxaproline behaves in this unexpected way, which proved crucial for making some really difficult proteins,” Bode remarks.

Vijaya Pattabiraman (known as Vijay), Bright Peak’s senior vice president and head of technology, notes that the hydroxylamine functional group opened doors for them, allowing them to move beyond 100 amino acids to chain lengths that are at least the size of

With this combination, the group set its sights first on a class of proteins that is both potent and relevant in therapeutic settings: cytokines. Alex Mayweg, who sits on Bright Peak’s board and whose venture capital firm Versant Ventures put in the whole \$35 million series A round in September 2020, says, “Cytokines are a wonderful playground; they are very potent molecules. We know the efficacy is there, but the field needs to learn how to tame them to make really good drugs.” In June Versant and a group of new investors, among them RA Capital, funded Bright Peak’s B round of \$107 million.

To use interleukin (IL)-2 as a cancer therapeutic, for example, requires eliminating its effects on immune modulating cells by blocking the interaction with the α -subunit of the IL-2 receptor. Most of the other groups trying to create an IL-2 cancer therapy (and [there are many](#)) achieve this by attaching polyethylene glycol (PEG) groups at the α -subunit receptor binding site. Bright Peak’s approach, instead, is to create a permanent ‘dead alpha’ (PEG molecules notoriously fall off the molecule over time, which can lead to unwanted effects.) Bright Peak still employs PEG, which enhances stability in vivo, but its use is independent of the blocking of α -receptor binding.

In addition to introducing non-canonical amino acids, Bright Peak can add chemical conjugation handles anywhere in the protein. “That’s where things really get interesting because we can then enhance the therapeutic potential of the cytokine depending on what we attach to the handle,” says Vijay. The modular feature also provides advantages, as they can modify one part of the protein without reengineering an entire construct. “We can just swap out one or two segments, and then you actually have a new construct,” he says. By doing so, they can address multiple aspects of the same protein, he points out.

Anne Conibear, a protein chemist at the University of Queensland in Brisbane Australia, points out that a totally synthetic platform provides greater flexibility than engineering proteins in a living system. “You can vary the conditions a lot more than you could with a bacterial system. If [a protein] doesn’t fold in a bacterial system, there’s only so much you can do without killing that bacteria,” she notes.

Moving beyond single cytokines, Bright Peak is gearing up to create immunocytokines. “This is where we think we have a truly differentiated product,” says Vijay. A newly announced deal with Tokyo-based Ajinomoto aims to use the latter’s Ajicap [bioconjugation](#)

technology—which uses a cyclic peptide to site-specifically modify residues in antibodies, allowing them to be chemoselectively conjugated afterwards—to join an antibody anywhere onto Bright Peak's synthetic proteins. "You can literally walk to the pharmacy and buy the available monoclonal. You can then modify it site selectively using Ajinomoto Ajicap technology, to introduce a conjugation handle on the monoclonal." This Vijay believes will be the next generation of immuno-oncology drugs.

Mayweg is excited about this possibility. "There is a potential to create a portfolio of portfolios by combining antibodies with cytokine payloads. We can optimize their effect on the immune system and disease and also discover new biology depending on how and where we target them. That matrix you can create here is incredibly fascinating."

There remain limitations. Many proteins are too large to be made using this technology. At the moment, Bright Peak can make proteins 200 amino acids long, and Bode thinks they may be able to stretch it to 300. Even if they cannot go any higher, the Bright Peak team thinks there are enough important proteins—including protein hormones and cytokines—that fall in the <300 amino acid range. In addition, there are some of what he refers to as crazy structures—plant proteins, some with lots of cysteines—that are very difficult to make via recombinant expression.

Bright Peak, which has sites in San Diego and in Basel, Switzerland, will be taking its first molecule, an IL-2, into the clinic in the first half of next year and has programs to develop IL-7 and IL-18, according to Vijay. "The molecules are quite homogeneous and clean. You do not really see any kind of typical frame shifts that you see with recombinant proteins." Conibear sums it up: "I think it is a big step and bridging that gap between the small molecule, medicinal chemistry and the biologics. Making biologics chemically I think is the best use of these technologies." Mayweg finds the possibilities almost limitless. "Now it's down to our imagination of what we would like to do."

LD

T-knife: unleashing T cell receptors on cancer

Human TCR-based adoptive T cell cancer therapy is entering clinical testing. Can it succeed in cancers where CAR-T cell therapy has failed?

After 17 years of painstaking work, this year Thomas Blankenstein, founder of the Berlin-based start-up T-knife, will finally see how his T cell receptor (TCR) cancer immunotherapy works in real life.

Thomas Blankenstein, co-founder of T-knife Therapeutics.

The idea was conceptually simple and not entirely original: replace the TCR genes of a mouse with those from a human, as had previously been done with antibody genes. Transgenic mice with the entire complement of human immunoglobulin genes have been the starting point for several commercialized fully human monoclonal antibody therapies. But conceptually simple does not mean it was simple to do. It required inserting genes to encompass both the human TCR repertoire and human major histocompatibility complex (MHC) molecules and knocking out the cognate mouse genes, generating mouse lines bearing human genes, and then subjecting them to a succession of crosses to get all the genes together in a single mouse—the HuTCR mouse. "It was very difficult and laborious to create a transgenic mouse that allows the discovery of humanized TCRs so efficiently, and I believe others have tried and failed," says Alex Mayweg, a partner at Versant Ventures and board member of T-knife.

Why would anyone do such a thing? One reason is any other way of creating high-affinity human TCRs against tumor targets has proven elusive. During thymic T cell development, the human immune system is tolerized to antigens containing shared epitopes with normal human proteins—the case for most tumor antigens. The advantage of using a mouse is that its immune system does not remove such T cell clones, so injecting human proteins into a mouse generates a robust cellular immune response. And when you do this with a mouse that has only human TCR genes, the TCRs are fully human. In fact, as demonstrated in a 2015 [paper](#) by Blankenstein's group, the TCRs derived from his transgenic mice against human MAGE-1A antigen had higher affinity than TCRs from human subjects. Straight out of the mouse, the affinities are such that no further optimization is needed. "This is a fundamental part of their platform, allowing the company to rapidly generate diverse human TCRs with optimal affinity and high specificity," says Mayweg. "It's really quite beautiful. It's [analogous] to how we successfully created fully human antibody drugs, and still do today."

T cell with engineered TCRs have some compelling advantages over CARs.

The idea was conceptually simple and not entirely original: replace the TCR genes of a mouse with those from a human, as had previously been done with antibody genes. Transgenic mice with the entire complement

Engineered TCRs integrate seamlessly into the signal transduction pathways of T cells; they have more subunits in their receptor structure (ten subunits in a TCR versus one subunit in a CAR); they have more ITAM (immunoreceptor tyrosine-based activation motif) motifs (ten, versus three in a CAR); and they are associated with more co-stimulatory receptors (CD3, CD4, CD28 and others). As a result, TCR-T cells are capable of more extended signaling and killing than CAR-T cells. Of perhaps greater importance, unlike CARs—which bind only to those antigens present on cell surfaces—TCRs bind epitopes presented in the context of MHC molecules and thus can target intracellular tumor antigens as well as extracellular. This leads to another advantage: whereas CAR-T cells remain localized mostly to periphery of a tumor to access surface antigens, TCR-T cells have a greater likelihood of penetrating tumors, a property that ultimately may prove useful for addressing solid tumors.

In this context, 17 years of work may seem like a long time, but Blankenstein never wavered. "It was already clear to me that the main purpose of these mice is using these T cell receptors for clinical trials. I had before worked on cancer vaccines, which was notoriously frustrating. And I saw what a qualitative difference adoptive T cells can achieve." He acknowledges that there were setbacks along the way. "Where we lost at least one year was introducing megabase large TCR gene loci into mouse embryonic stem cells by fusing embryonic stem cells with yeast cells containing yeast artificial chromosomes."

Elisa Kieback, Chief Technology Officer of T-knife Therapeutics.

Elisa Kieback, a member of the Blankenstein group since 2015 and T-knife's founding CEO, who now serves as CTO, says there were two triggers—a 2010 *Nature Medicine* [article](#) describing the creation

of HuTCR mice and the 2015 *Nature Biotechnology* paper analyzing a mouse that produced HuTCRs against MAGE-1A—that led them to start thinking about forming a company. "We had already seen for a couple of years how many receptors we could generate. It seemed to work well. But to leverage the full potential of the mouse platform activity, there were also more resources needed." In 2018, they closed their first financing, a seed round of \$9.6 million from several European investors, followed

two years later by \$78.2 million from Versant along with RA Capital, Andrea Partners and the Boehringer Ingelheim Venture Fund—one of the largest rounds in Europe in the recent past.

Kieback sees the ability of TCR-T cells to bind intracellular targets as impetus for adoptive cell therapy moving away from CARs and towards TCRs. “What this allows us to do with TCRs is target a single point mutation, as many tumors accumulate point mutations. We’re able to target those very specifically, and then again have that specificity to only affect tumor cells and not healthy tissue,” says Kieback.

Immuno-oncologist Jeffrey Weber of NYU Langone Health finds the concept sound but sees a couple of potential problem areas. One is that even if TCR-T cells can better infiltrate tumors than CAR-T cells, will they be able to overcome the hostile tumor environment? So far, Blankenstein has shown solid tumor regression in mice, but it remains to be demonstrated in people. Another potential pitfall is cross reaction

with shared epitopes expressed on normal tissues. This is not entirely theoretical; a clinical trial in cancer of a MAGE-A3 TCR was abruptly stopped after causing two deaths in patients who had a cross reaction with an unrelated muscle peptide. To check for this possibility when targeting MAGE-A1, Blankenstein’s group mutated each amino acid in the T cell recognition region and tested the resulting variants against the TCR to identify the peptide being recognized by the TCR. They then did a search in the human proteome for identical peptides. According to Kieback, this preclinical routine safety screening is performed for all their TCRs, but it still may not be enough. “It is very rare that TCRs with relevant cross-reactivity are detected,” she says.

In March of this year, the first patient received therapy for multiple myeloma using TCR cell therapy produced using HuTCR mice. Eleven more patients are expected to be dosed in this phase 1 trial, which is being conducted by Max Delbrück

Center for Molecular Medicine and Charité’s Institute of Medical Immunology. The T-knife team is gearing up to enter the clinic later this year, which will doubtless be aided by a new funding round—a \$110 million series B—announced in early August. Kieback says they have created close to 100 TCRs with the HuTCR mice, but declined to disclose their next clinical targets. Kieback calls Blankenstein, who is staying put in academia, a visionary. Twenty years ago “nobody was thinking about going into the clinic and being able to treat patients with tumors with T cell receptors,” she says. *LD*

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