



NATURE BIOTECHNOLOGY'S ACADEMIC SPINOUTS 2021

Nature Biotechnology's annual survey highlights academic startups that are, among other things, designing circular RNA therapeutics, tackling cancer with arenaviruses, creating psychedelics without the trip, editing genes and cells *in vivo*, harnessing the power of autoantibodies and editing the epigenome. **By Michael Eisenstein, Ken Garber, Esther Landhuis & Laura DeFrancesco**

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.)

Orna: circular logic
In the ascendant world of mRNA medicines, circular RNA shows promise as an alternative

way for making proteins inside human cells. The success of the Pfizer/BioNTech and Moderna COVID-19 mRNA vaccines has galvanized research on mRNA therapeutics. Rather than use mRNA to make antigens, the objective of mRNA therapeutics is to make proteins on demand inside patients' cells within a tissue of interest at a level and duration sufficient to achieve treatment benefit. Applications are many and varied: enzyme replacement therapy for rare diseases, hormone production, monoclonal antibodies or immunostimulatory proteins for cancer, cytokines or transcription factors to treat autoimmunity. Linear mRNA is the template of choice, but it has drawbacks. Synthetic mRNA

must be heavily modified to resist nuclease degradation and to avoid innate immune stimulation. Such mRNAs are inefficient to manufacture, difficult to properly incorporate into lipid nanoparticle carriers, and expensive. And modified linear mRNA is still relatively short-lived, limiting the amount of therapeutic protein produced per molecule delivered.

Circular RNA (circRNA) has emerged as an intriguing alternative, with Orna Therapeutics the most visible company advancing the technology. The circular structure can naturally arise from the 'backsplicing' of the splice site motifs flanking the 5' and 3' ends of pre-mRNA transcript exons. At first glance, circRNA is

Table 1 | The class of 2021: NBT's academic spinouts

Company	Focus area	Funding (\$ million)	Scientific founder
Orna Therapeutics	Circular RNA therapeutics	20 seed, 80 series A, 221 series B	Alex Wesselhoeft, Daniel Anderson
Abalos Therapeutics	Cancer virotherapy	12 series A, 38 series A	Karl Lang, Phillip Lang
Delix Therapeutics	Non-hallucinogenic psychedelics	70 series A	David Olson
Ensoma	In vivo gene editing	70 series A	André Lieber, Hans-Peter Kiem
Interius BioTherapeutics	In vivo cell therapy	81.5 series A	Saar Gill
Chroma Medicine	Epigenome editing	125 series A	Jonathan Weissman, David Liu, Keith Joung, Luke Gilbert, Luigi Naldini, Angelo Lombardo
Tune Therapeutics	Epigenome editing	40 series A	Fyodor Urnov, Charles Gersbach
Alchemab Therapeutics	Protective autoantibodies	82 series A	Jane Osbourn, Rachael Bashford-Rogers, Ben Larman, Uri Laserson
Adrestia Therapeutics	Synthetic rescue	Undisclosed	Steve Jackson, Gabriel Balmus, Yaron Galanty, Delphine Larrieu, Raphaël Rodriguez, Rafael Carazo Salas

an unlikely drug platform. Until 10 years ago, such RNAs had been observed rarely in nature, as idiosyncratic forms of some RNA viruses or byproducts of splicing errors in eukaryotes – basically, transcriptional ‘noise’. They were thought to be translated into proteins only rarely.



Alex Wesselhoeft, director molecular biology, Orna Therapeutics.

Attempts to make artificial circRNAs, mainly to study their in vivo properties, date back at least to the 1980s. Biologists have long observed that circRNAs are long-lived; because they lack 3' and 5' ends, where nucleases generally initiate RNA degradation, they're more stable than linear mRNAs. But only short RNAs could be

made artificially, “so the field kind of died in the nineties,” says Orna co-founder and director of molecular biology Alex Wesselhoeft.

Wesselhoeft, as a PhD student in the lab of MIT biomaterials researcher Daniel Anderson, took on the challenge. Chemically synthesizing an entire mRNA sequence in vitro and then using ligases to circularize it is inefficient. Wesselhoeft adapted an enzymatic approach that splits a cyanobacterial ribozyme sequence in two and pastes it backwards onto an mRNA sequence, with the 3' end upstream of the gene to be expressed and the 5' end downstream. The mRNA then autocatalytically

splices itself into a circle during in vitro transcription, without the need for the usual cellular protein splicing machinery. Wesselhoeft added spacer and duplex sequences that, by dampening internal splicing interference and bringing the ends together, boosted circularization efficiency for large molecules, up to 5 kilobases in length. Orna has since pushed this to over 10 kb.

Wesselhoeft confirmed that these circRNAs persist in cells longer than modified mRNA typified by the Pfizer and Moderna vaccines. The circRNA also produced larger amounts of protein than modified linear mRNA, over a longer period, in part due to the discovery of new cap-independent RNA translation sequences (IRESs, or internal ribosome entry sites), RNA elements that recruit ribosomes to internal regions of RNA. An IRES is considered less efficient than the canonical translation that begins at the 5' cap of linear mRNA, but as circRNA lacks a cap, Wesselhoeft inserted IRES sequences and discovered that some performed better than a cap.

“What we saw was actually if you pick the right IRES, you could get a lot of protein out,” he says. “That really got us thinking that maybe this was a new technology that could take the entire field of mRNA to the next step.”

On the basis of these findings, in 2019 Wesselhoeft, Anderson and economist Raffaella Squilloni, previously an entrepreneur in residence at Harvard, founded Orna, which was seeded by MPM Capital. A \$80-million series A closed in February 2021. Orna had raised a total of \$491 million by October, 2022, including a \$150 million up-front payment from

partner Merck. It employs >70 people at its Cambridge, Massachusetts facility.

CircRNA “is theoretically a really intriguing idea,” says Yale University RNA biologist Carson Thoreen. “Definitely the evidence out there so far does indicate that these are really much more stable molecules, and I think there’s potential that they’re much less immunogenic than their linear counterparts. The challenge is really engineering ways to maximize the amount of translation and protein production that can occur.”



Thomas Barnes, CEO, Orna Therapeutics.

Orna’s lead program is ‘in situ CAR’ (isCAR) therapy for cancer. CARs, or chimeric antigen receptors, express an antibody-like fusion protein linked to a T cell receptor intracellular domain, using antigen binding to activate T cells against the target.

Five autologous engineered CAR-T cell therapies are FDA approved for blood cancers, all involving ex vivo cellular engineering via lentiviral transduction before reinfusion. Pre-treatment chemotherapy-induced lymphodepletion creates a niche for CAR T cell expansion. Besides the enormous expense, “it’s hard to control how much expansion of those cells has taken place inside the patient,” says Orna CEO Thomas Barnes. Runaway expansion leads to cytokine release syndrome, a severe side effect.

Orna uses immunotropic (immune-cell specific) lipid nanoparticles, in-licensed from an unnamed academic investigator, to deliver the CAR-encoding circRNA in vivo. With this system, “biomanufacturing takes place inside the patient,” says Barnes. That makes immune cell expansion more predictable. Unlike current CAR-T therapies, “it’s not a living drug,” says Barnes. “It’s going to behave like a classic drug ... with a half-life; it’s going to have peak expression, and it’s going to taper off. And, lastly, no lymphodepletion.” The result, in theory, is a cheaper and safer treatment.

Orna has now shown that its circRNA can drive protein expression to levels that have therapeutic value in an animal model of human disease. At the May meeting of the American Society of Gene & Cell Therapy, Barnes reported that five doses of Orna’s anti-CD19 isCAR fully eradicated tumors in a mouse xenograft model of acute lymphoblastic leukemia.

Barnes suggests that its immunotropic lipid nanoparticles can also deliver circRNAs for autoimmunity. “And we have a second delivery solution that we’ve in-licensed that is primarily hepatropic, but also myotropic to a lesser degree,” he says. At the ASGCT meeting, Barnes reported that its lipid nanoparticle, delivered intravenously in a mouse model of Duchenne muscular dystrophy, could induce limited expression in muscle of a short version of the dystrophin protein.

CircRNA has two main theoretical concerns. One is that Orna’s circularization method unavoidably leaves behind small fragments of cyanobacterial ribozyme mRNA at the ligation junction. “It’s possible that some of those remnants may, at least in some contexts, be immunogenic,” says Thoreen. “That would be bad from any kind of therapeutic standpoint because they’ll limit any kind of protein that could be produced from those RNAs.” Such an outcome is very unlikely, counters Wesselhoef. The sequence remnants “are relatively short and noncoding,” he says. “You’re not going to be creating a new synthetic peptide in the cell, through translation.”

The other theoretical concern is an immune response against the circular RNA itself, which would also limit translation. Wesselhoef and Anderson have reported that unmodified circRNA is less immunogenic than unmodified, capped linear mRNA in cells by monitoring cytokine and chemokine release into the culture medium and circRNA protein expression stability and in vivo translation. However, the process of making circRNA generates noncircular byproducts – linear

mRNA, double-stranded RNA – that do trigger an immune response. But Orna maintains that **circRNA, once rigorously purified, does not**. “The circles can be purified without this other stuff present,” says Barnes. And “the stimulation is not there.”

But whether purification is enough remains somewhat controversial. “**Other people have disputed that**,” says Thoreen. “I think part of the uncertainty really derives from uncertainty over what it is in the first place that’s triggering that response. So it could be that there are some sequences of RNA, it doesn’t really matter whether they’re in a circular context or a linear context, they’re still going to be immunogenic.”

If the circRNA story plays out as Orna expects, it could become a highly competitive platform for mRNA therapeutics. “Circles are the way to go,” says Barnes. “In every way they’re advantageous: they’re cheaper, faster, you can make them larger, you can express higher, they formulate better. It’s the gift that keeps on giving.” *KG*

Abalos Therapeutics: arenaviruses take on cancer

After >20 years of research at the intersection of virology and immunology, two brothers have launched a venture seeking to turn arenaviruses into an anticancer virotherapy.

When Karl and Philipp Lang were postdocs in Rolf Zinkernagel’s lab, they had no idea that their research on the immune system’s response to lymphocytic choriomeningitis virus (LCMV) would lead to an anticancer therapeutic, let alone a biotech company. Zinkernagel received the Nobel Prize in Physiology or Medicine in 1996 for the work showing that T cell recognition of viral antigens requires a matched major histocompatibility complex (MHC). A quarter of a century later, the brothers are tailoring knowledge of the LCMV immune response for a more prosaic purpose: in 2019, the Langs’ two German institutions – the University of Duisburg-Essen, where Karl is based, and the Heinrich Heine University Düsseldorf for Philipp – spun out Abalos to advance their arenavirus-based cancer virotherapy platform to the clinic with \$12 million in a first funding round.

The field of cancer virotherapy has not met unbridled success. To date, just a single oncolytic herpesvirus has reached the US market: Amgen’s Imlygic (talimogene laherparepvec), which was approved for melanoma in 2015 by the US Food and Drug Administration. This followed decades of research and hundreds of clinical trials employing a variety of oncolytic,



Karl Lang, co-founder; Jörg Vollmer, CSO; Marcus Kostka, CEO; Philipp Lang, co-founder; Abalos Therapeutics.

mostly adenovirus, treatments, many of which have since gone by the wayside.

But as time has progressed, appreciation has grown that a potent immune response, rather than tumor cell lysis, may be key to viral therapeutic efficacy. This is where the Langs’ decades of work looking at just such interactions promise to pay dividends. Christine Engeland, an experimental virologist at Witten/Herdecke University in Heidelberg, Germany says, “Virotherapy is fascinating because it’s the intersection of oncology, tumor biology, virology and immunology. To really understand what you are doing you need expertise in all these areas, especially now that it is seen more as an immunotherapy. It’s good to have immunologists looking into this.”

Karl Lang explains this evolution. “Initially, it was thought that you just killed the tumor with the virus, but then it was clear this will not be working as you basically would have to infect all tumor cells, which is probably impossible.” Bringing the immune system to the tumor is what the Langs believe arenaviruses can do. “We know from our work with Zinkernagel that arenaviruses are extremely strong immune activators,” Karl says. This rationale was enough to get Marcus Kostka to lead the company from Boehringer Venture Fund, which led the initial funding round. “When I started discussions in 2018, I felt that this is an interesting concept, separate from oncolytic viruses because oncolysis as the therapeutic concept we know now is not sufficient.”

The premise underlying Abalos’s formation is that viruses (oncolytic or not) have antitumor properties because they can stimulate the immune system through multiple pathways: by introducing new antigens, both viral and non-viral, that are seen as foreign; through pathogen recognition receptors that trigger the innate immune system; and by attracting T cells and cytokines to tumors. The Langs have several publications that demonstrate some of the ways arenaviruses, in particular,

accomplish these tasks. In a 2005 *Nature Medicine* paper, they showed that arenaviruses induce antigen-specific CD8⁺ T cells in peripheral niches (in the work described, beta islet cells in a study of autoimmunity) only if additional innate signals occur. LCMV, an arenavirus carried by rodents that can cause neural disease in humans, they found, was the strongest inducer of such “inflammatory signals.” In a 2017 *Nature Communications* paper, they showed that some arenavirus strains propagate preferentially in cancer cells. This site-specific virus propagation, they found, enhances the inflammatory capacity of LCMV and activates several immune components in the tumor, including interferon type 1, inflammatory macrophages, T cells and natural killer cells.

Arenaviruses have other favorable properties contributing to their efficacy. Unlike oncolytic adenoviruses and herpesviruses, which are fairly quickly cleared from the body after infection, arenaviruses induce relatively limited neutralizing antibodies that enable them to persist long enough for a full-blown CD8⁺ T cell response to unfold in infected organs. Engleland agrees in part: “One thing that is unique [to arenaviruses] is it’s difficult to raise neutralizing antibodies against the LCMV. There is a debate on whether adaptive immunity will abrogate the efficacy of oncolytic viruses,” she says.

In addition, having a RNA genome, arenaviruses can adapt to tumors by mutating and evolving, a property the Langs have exploited by ‘evolving’ viruses in the lab. In preclinical work, they employed 25 different tumor lines, infecting and passing them up to 150 times. Through this work, they identified 100 different mutations. “We learn from nature, how we can do this in a quicker time,” says Kostka.

In work not yet published, the Langs’ teams have optimized their mutated arenaviruses for increased entry and replication in tumor cells, for reduced tropism to healthy tissue, and for the ability to induce a specific cytokine profile (for example, type 1 interferon response). They screened in several murine tumor models for those mutants that reduce some of the worst side effects observed in these systems. What they were seeking was a multipronged approach, which they feel is necessary due to the complexity of the immune response. And whereas optimized arenaviruses should intrinsically activate multiple immunological pathways, the company is open to combining their viruses with other modalities, such as checkpoint inhibitors, that could have synergistic therapeutic effects. “The virus itself already

addresses this to some extent, but optimization and combining it with other modalities, like checkpoint inhibitors, will make it better,” says Kostka.

Arenaviruses have a clinical track record: they were given to people in the seventies, with some promising results and acceptable side effects, which bodes well for their entry into the clinic. Also noteworthy is Vienna-based biotech Hookipa Pharma’s efforts to use arenaviruses as delivery vehicles for tumor-associated antigens in a vaccination approach. Although the two programs employ different strategies, Hookipa’s clinical work supports the notion that arenaviruses can be used safely in the clinic.

Abalos is planning safety studies for early to mid-2023, with clinical studies to follow in 2024, with an additional \$37 million coming in a second A round. According to Kostka, once they had achieved their goal of optimizing virus, getting more investors on board was relatively easy. “We were quite quick, by convincing other investors, within 2 months we had investors together,” he says. Choosing indications and combinations is “the million dollar question,” he says. Translating from animal models is limited. “An animal model is only good to address specific questions – like, is this pathway involved? It cannot give you more confidence as to taking this alone,” he says.

The work is still very much a collaboration among the two universities, with six or seven students, and with work being done at the Abalos facility in Dusseldorf. Philipp Lang has nothing but praise for the support that the universities provided. “It was really great. Everybody was excited about the project. They all worked together to make it happen,” he says. As basic scientists, he says they were a bit naive about bringing a therapy to the clinic: “Having this development and seeing it develop in the clinic is like a dream come true.” LD

Delix Therapeutics: psychedelics without the trip

The field of psychedelic medicines – therapeutics that trigger non-ordinary states of consciousness – has spawned dozens of companies, including several that are publicly traded. Investors have recently been pouring money into them on the back of several trials indicating that [these drugs could work in neuropsychiatric disorders](#) like depression, addiction and [post-traumatic stress disorder \(PTSD\)](#). In 2019, the US Food and Drug Administration (FDA) gave marketing approval to Janssen’s Spravato (esketamine), the S-enantiomer of ketamine, for depression that has failed

to respond to two or more antidepressants. MDMA (methylenedioxymethamphetamine, or ‘ecstasy’) may be headed for FDA approval after a positive phase 3 trial [for PTSD](#), with a second phase 3 trial nearing completion. Delix Therapeutics is taking a different approach: it’s developing analogs of psychedelic drugs called psychoplastogens that reproduce the changes in neuronal activity without the often extreme subjective psychological effects.



David Olson, co-founder and chief innovation officer, Delix Therapeutics.

Many people suffering from mental illness can’t handle a hallucinogenic experience. For those who can, they need psychological advance preparation for a psychedelic as well as trained therapists to accompany them during treatment and in later integration of the insights gained. This “is just not a very scalable model,” says Delix sci-

entific co-founder David Olson. “So we coined the term psychoplastogen to really help us differentiate between these hallucinogenic neuroplasticity-promoting compounds and these non-hallucinogenic neuroplasticity-promoting compounds.”

Seven years ago Olson, an organic chemist starting his faculty career at the University of California, Davis, noted ketamine’s remarkable ability to very quickly overcome treatment-resistant depression. [The drug induced rapid synaptic plasticity](#) in the prefrontal cortex (PFC) of rodents. In depression and other neuropsychiatric illnesses, “neurons in the PFC tend to atrophy; they shrivel up, synapses are lost, branches actually physically retract,” Olson says. “Ketamine, very excitingly, rapidly regrows these neurons within 24 hours, and it’s also one of the fastest-acting antidepressants that we have discovered to date.”

Could potentially longer-lasting serotonergic psychedelics, like LSD (lysergic acid diethylamide) and psilocybin, and empathogens, like MDMA, be acting the same way? Although they don’t share ketamine’s molecular target, Olson decided to find out. In 2018, his group reported that LSD, psilocin (from magic mushrooms), DMT (*N,N*-dimethyltryptamine) and [MDMA increase dendritic spine density](#), synapse formation and intrinsic excitability in cultured rodent cortical neurons, with many

of the same effects visible in *Drosophila* larvae after drug exposure. Their effects and ketamine's seem to converge on a common downstream signaling pathway. Using basic structure-activity relationships and medicinal chemistry to create variants, and cell-based assays to screen for effects, Olson's group, [working with others](#), then discovered drug variants that did not seem to be hallucinatory in rodents, but strongly promoted neural plasticity while showing antidepressant-like effects.



Mark Rus, CEO,
Delix Therapeutics.

Such compounds had obvious and commercial potential, leading to Delix's founding in 2019. Compared with the classic serotonergic psychedelics, "these non-hallucinogenic neuroplasticity promoting compounds ... we believe could be more scalable alternatives," Olson says. "More patients would be able to benefit from them, for a variety of reasons." The company completed a \$70 million series A funding round in September 2021 and has since raised another \$30 million in convertible notes. Delix's derivative of the psychedelic ibogaine and the company's MDMA analog are both progressing through Investigational New Drug (IND)-enabling studies. "We hope to move phase 1's for one or both of those assets through the end of the calendar year this year and into 2023," says Delix CEO Mark Rus.

Non-hallucinogenic psychedelics, if they work, could have the kind of market impact that selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs) enjoy. "If you block the subjective effects and still get therapeutic benefit, that changes the whole landscape of how these drugs can be used, how they need to be regulated, the numbers of patients with various mental illnesses who can now gain access to them," says Stanford neuroscientist Rob Malenka. He hopes Delix succeeds. But, Malenka adds, finding such psychedelic analogs that work in mental illness is anything but guaranteed. "There's a large segment of the clinical research community – that is the people who have been studying these drugs for a while – who are of the opinion that that is probably not possible."

These skeptics argue that a powerful conscious experience is necessary for the drugs to work. "I think the subjective experience, and

some form of trip ... is going to be required for therapeutic benefit," Malenka says. "That's where I stand just based on intuition." There is also correlative evidence. In clinical trial patient surveys, [emotional breakthrough during treatment is highly correlated with ultimate well-being](#), "People have an altered sense of self and world view. This may also be accompanied by psychological insights," said Johns Hopkins University psychedelics researcher Roland Griffiths at a March workshop organized by the US National Academies of Sciences, Engineering and Medicine. "There's something about these experiences that's integral to the construction of meaning-making." Griffiths has pointed out that, in human psilocybin trials, [mystical-type experiences predicted therapeutic benefit](#) in depression, even when controlling for the overall psilocybin effect.

Correlation, of course, does not prove causation. Molecular interactions – for example, LSD or psilocybin engagement of the 5-hydroxytryptamine (serotonin) 2A (5-HT_{2A}) receptor and subsequent downstream signaling, inducing synaptic plasticity in the prefrontal cortex – may determine most patient benefit, independent of subjective effects. "A mystical-type experience could be simply a biomarker for activation of the 5-HT_{2A} receptor," says Olson. His [preclinical work in animals](#) suggests that such target engagement, along with therapeutic benefit, can be achieved without altered consciousness, although these models are crude surrogates for human experience.

Olson stresses that subjective versus molecular effects is not an either-or proposition. And he agrees that subjective experiences probably do matter. But he's betting his company's future on the expectation that molecular effects alone will make enough of an impact to see Delix's drugs through to market. "Only clinical data will tell," he says. Delix isn't the only company pursuing psychoplastogen drugs. Gilgamesh Pharma, for example, has a non-hallucinogenic 5-HT_{2A} receptor-targeted compound in preclinical development. "It's a competitive landscape out there," says Malenka.

Malenka is cautiously optimistic about the prospects of psychedelics companies (he advises two of them), but regrets the hype that the field attracts. "We have many years if not a decade or two of work before we really understand how to use [psychedelics and MDMA], and who should be receiving them, and how frequently they should be given, all those kinds of issues," he says. "If you actually count of the number of patients who have actually

received these drugs in well-controlled clinical trials, it's very small numbers."

The Delix founders see themselves "as a neuroplasticity company, not a psychedelics company," says Olson. Atrophied cortical neurons and synapse loss also underpin neurodegenerative diseases like Alzheimer's, and drugs that safely restore such neurons to functionality could have an enormous impact, beyond mental illness, if they work as Olson hopes.

Non-hallucinogenic versions of drugs like LSD, psilocybin and MDMA could unlock their potential in mental illness. KG

Ensoma and Interius BioTherapeutics: in vivo veritas

Two startups are developing tools to do cell and gene engineering directly in the human body. The cell and gene therapy field is experiencing a renaissance of sorts, with about a dozen [products approved](#) in the past few years. However, most of these therapies require physicians to collect and purify cells from patients, manipulate them and deliver them back into the patient. Such ex vivo protocols subject patients to long hospital stays and expensive procedures that can be performed only at specialized medical centers. Two companies are developing tools to perform cell and gene engineering in vivo in hopes that these new technologies could make gene therapy widely available as a one-time injection to a much larger number of patients.



Andre Lieber,
co-founder, Ensoma.

The first of these, Ensoma, is developing an adenoviral delivery platform that brings therapeutic genes and gene editing machinery into stem and immune cells to target the root cause of genetic diseases. The technology builds on decades of research on gene therapy and adenoviral vectors by Seattle-based co-founders Hans-Peter Kiem of the Fred Hutchinson Cancer Research Center and André Lieber of the University of Washington. During a May 18 keynote symposium at the annual conference of the American Society of Gene & Cell Therapy (ASGCT), senior fellow Chang Li from the Lieber lab reported the team's latest achievement: correcting the sickle cell mutation in hematopoietic stem cells (HSCs) of mice by in vivo prime editing – a genome editing technology that makes precise DNA modifications

without causing double-stranded breaks as does CRISPR–Cas9.

Sickle cell disease afflicts millions of people worldwide, producing ~300,000 new cases each year, most commonly in sub-Saharan Africa, the Middle East and India. In people with the condition, normally disc-shaped red blood cells deform to resemble crescent-shaped sickles due to a single mutation in the hemoglobin subunit- β gene (*HBB*). The misshapen cells cause severe anemia by stiffening and clumping together in vessels, slowing blood flow, and by breaking down faster than usual, depleting the body's supply of red blood cells. Owing to its straightforward genetic cause, sickle cell disease has attracted attention from gene therapy researchers and companies, several of which have products under clinical development. However, so far, human trials have only investigated ex vivo approaches, which entail sophisticated equipment and trained personnel that are only available at a few clinical centers – facilities that are not available in rural areas and developing nations.

Enter Ensoma. “I believe we are the only company that can in vivo engineer the hematopoietic stem cell today,” says Emile Nuwaysir, who joined Ensoma last October as president and CEO. Being pluripotent, HSCs are “an incredibly powerful nexus,” he says. In principle, Ensoma's technology should be able to not only program all cell types derived from HSCs using a strong, constitutive promoter, but also to turn on genes in a single cell type (for example, red blood cells in the case of sickle cell disease) using lineage-specific regulatory sequences, Nuwaysir says.

Being able to engineer HSCs in vivo could make gene therapy more widely accessible – a goal that has captivated Kiem and Kush Parmar, managing partner at 5AM Ventures, for more than five years. “Kush would always say, let me know when you have the right platform,” Kiem says.

For nearly 30 years, Lieber has worked on adenoviruses – non-enveloped viruses that serve as gene delivery vectors, most commonly for vaccine applications. Adenoviruses transduce a wide range of cells and can be purified easily. Recent versions of adenoviral vectors are “gutless,” or devoid of all viral genes, so they have a large packaging capacity (~35 kilobases).

Lieber and colleagues have engineered adenovirus to target HSCs by combining the backbone of the well-characterized Ad5 serotype with the receptor-interacting domain (called ‘fiber’) of a different serotype

(Ad35) that targets the membrane protein CD46, which is highly expressed on primitive HSCs. The team further optimized the system by screening a library of Ad35 fibers for CD46-binding properties. That effort helped them construct Ad5/35⁺ vectors that have **60-fold higher affinity for CD46** than Ad35, according to Lieber. These Ad5/35⁺ vectors could presumably transduce HSCs more efficiently and at lower doses.

But there was another hurdle: HSCs are hard to reach and ultra-rare. As the emergency regenerator for the blood and immune system, hematopoietic stem cells don't circulate in the periphery. They're sequestered deep in the bone marrow, and within this physically secluded space they make up less than 1 in 10,000 cells. “We knew that as long as [HSCs] remain in the bone marrow, we cannot reach them with our vectors,” says Lieber.

The solution, as it turned out, emerged through discussions Lieber had with Thalia Papayannopoulou, a colleague down the hall whose lab has expertise luring stem cells out of the bone marrow and into the bloodstream by mobilizing them using procedures developed for bone marrow transplantation. Mobilization involves giving an injection of a growth factor to boost blood cell production and stimulate HSC release into peripheral blood. What if – instead of harvesting the cells and manipulating them in the lab – “we leave them inside the body and try to transduce them?” Lieber says.

While straightforward in principle, that twist on a decades-old protocol – engineering the mobilized cells instead of extracting them – was initially presumed too good to be true. “It was generally thought that HSCs would just disappear somewhere or die off,” Lieber says. Papayannopoulou, for one, did not believe that mobilized, transduced HSCs would return to the bone marrow and repopulate all blood lineages. “In her direct way, she called this BS,” Lieber quips. “This is now a running joke between us.”

A joke, because it actually works. In immunodeficient mice with engrafted human CD34⁺ cells, HSCs that were mobilized and transduced in the periphery could in fact **return to the bone marrow**, where some of them stably expressed a green fluorescent protein transgene and formed multilineage progenitor colonies. But the process was inefficient. Twenty weeks after transduction, fewer than 1% of peripheral blood cells expressed the transgene. That doesn't bode well for treating inherited blood disorders, which would require

long-term expression of the modified gene in differentiated cells.

To address this drawback, the group needed some way to give transduced HSCs a proliferative advantage. Kiem and his Fred Hutchinson coworkers had developed a system for in vivo HSC selection based on a mutant of the *O*⁶-methylguanine-DNA methyltransferase (*Mgmt*^{P140K}) gene that confers resistance to the combination of *O*⁶-benzylguanine and bis-chloroethylnitrosourea (BCNU), a chemotherapy regimen used in some cancer patients. This selection system helped gene-modified stem cells survive in mice, dogs, non-human primates and even people with glioblastoma. Given these successes, the team incorporated the *Mgmt*^{P140K} gene into their adenoviral green fluorescent protein (GFP) vector and showed that low doses of *O*⁶-benzylguanine/BCNU – 50-fold lower than what is used in chemotherapy – led to **stable transgene expression in HSC progeny cells**. “Eighty percent of peripheral cells would have our gene and express it,” Lieber says. “This is when Kush and Hans-Peter got interested in the technology.”

After several years incubating at 5AM Ventures, Ensoma launched publicly in February 2021 with \$70 million in series A financing led by the San Francisco venture capital firm, with participation from more than half a dozen other funds. In addition, the company signed a deal with Takeda to work on several rare disease targets with \$100 million in up-front and preclinical research payments as part of a strategic collaboration worth up to \$1.25 billion.

At the May ASGCT presentation, Li showed as yet unpublished results from experiments using the company's adenoviral vector to directly repair the sickle cell mutation in a mouse model, the Townes/CD46 mouse. In this work, the researchers mobilized HSCs from the bone marrow into the peripheral blood, then gave an intravenous injection of adenoviral vector HDAd5/35⁺ carrying a 17.5-kilobase payload that contained the *Mgmt*^{P140K} drug resistance gene and the prime editor and pegRNA for prime editing. With a single injection of adenoviral vector and several rounds of selection with *O*⁶-benzylguanine/BCNU, they achieved an editing level of 60%, enough to replace 50% of sickling hemoglobin S with normal hemoglobin A.

“I thought their data was exciting,” says Miriam Kim, a physician-scientist at Washington University School of Medicine in St. Louis who studies T cell immunotherapies for myeloid malignancies. She had assumed in vivo gene editing in HSCs was “a little bit

more unattainable,” but “they made it happen,” she says. “It was earlier than I expected.”

Cynthia Dunbar of the US National Health, Lung, and Blood Institute says it’s “a very nice system” but raised concern about the need for multiple rounds of alkylating agents to select for transduced cells. “The point of doing in vivo gene therapy is to avoid having to give cytotoxic chemotherapy conditioning.” Kim agrees that with the current protocol, “the chemo would be a hard sell.”

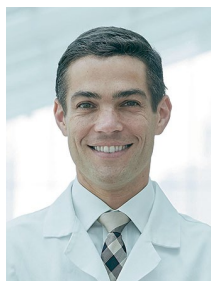
Lieber notes that the *O*⁶-benzylguanine/BCNU dosing for in vivo selection are 50-fold lower than the doses typically used for chemotherapy. His team is also studying alternative approaches – such as [knocking out the *Cd33* gene in HSCs](#) to give transduced cells a survival edge after treatment with CD33-directed immune therapy.

On the whole, Ensoma’s efforts center around delivery. “That’s what is limiting the field,” Nuwaysir says. “It’s not the ability to modify DNA. It’s the ability to deliver the right stuff to the right number of cells.” The Ensoma vector could be the answer – if it can sidestep the toxicity concerns that have plagued adenoviral vectors since the tragic death in 1999 of Jesse Gelsinger, who, after receiving a adenovirus-vectored gene therapy for a rare metabolic disorder (ornithine transcarbamylase deficiency) suffered an intense inflammatory response and blood-clotting disorder.

The toxicity concern applies broadly to in vivo approaches, Lieber says. “Anything you inject in vivo is taken up by macrophages, and they release pro-inflammatory cytokines. You can get really bad side effects, down to multi-organ failure.” Thus, he acknowledges, “you have to do something against these innate toxicities.”

That he and others have deeply studied their biology for decades gives adenoviral platforms an advantage, Lieber says. “We knew where the pathways were that could trigger these toxicities and we could counteract them pharmacologically.” Kim also points out that there are other ways to prevent these problems, such as administering steroids to “pre-empt anything that might be toxic.” But, she points out, it will be on them to convince the FDA that it is safe.

A second company, Interius BioTherapeutics, is also focused on in vivo delivery – in its case delivering chimeric antigen receptors (CARs) into T cells. A decade ago, “nobody even thought this CAR-T cell thing was going to be a thing. And now it absolutely is,” says co-founder Saar Gill, a physician-scientist in the Center for Cellular Immunotherapies



Saar Gill,
co-founder, Interius
BioTherapeutics.

at the University of Pennsylvania School of Medicine.

Since 2017, the US Food and Drug Administration has approved six CAR-T cell therapies for treating refractory or relapsed lymphomas and leukemias and, recently, multiple myeloma. Thousands of patients have been treated

with CAR-T cells. “The platform is clearly validated,” Gill says. But CAR-T cells are highly personalized therapies with six-figure price tags. Current protocols require extracting and purifying blood cells from the patient, manipulating them in a laboratory and infusing the modified cells back into the patient.

Early strategies for expanding access to CAR-T cells focused on producing allogeneic ‘universal donor’ T cells or developing procedures for ex vivo cell manipulation closer to bedside. These efforts are ongoing, but concerns linger over the numerous edits introduced (for example, into TCR loci) to ensure allogeneic cells do not cause graft-versus-host disease associated with a risk of off-target events and abnormalities that might then expand due to selective pressure for mitogenic cells. As a result, these days research interest – and investor dollars – are turning to the in vivo frontier.

Seattle-based Sana Biotechnology, [featured among the journal’s 2019 academic spinouts](#), is pursuing in vivo CAR-T cell production using fusogens – specialized viral proteins that not only promote virus–cell membrane fusion, but also undergo structural rearrangements that unleash the energy to get the virus into the cell once binding occurs. Interius is also pursuing a similar strategy. The company has yet to publish or publicly present any of its findings, but that hasn’t put a damper on its venture financing. Last May, Interius announced its \$76 million series A round, co-led by Cormorant Asset Management and Fairmount Funds and joined by lead founding investor Tellus BioVentures and others.

To generate CAR-T cells in vivo, Interius, and Sana before them, are using lentiviral vectors, which integrate into the host genome. As T cells are highly proliferative, “you need a vector that is going to remain there for the life of the T cell and its daughter cells,” Gill

says. “The other advantage is that it’s largely non-immunogenic.”

Lentiviral vectors are typically pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G) machinery – a highly promiscuous set of surface molecules – to efficiently transduce many types of cells. To specifically target T cells, scientists at Interius did two things to re-engineer the vector. First, they removed VSV-G – essentially “blinding the viral-like particle such that it cannot see or transduce any targets,” Gill says. Next, they studied the virus membrane with proprietary molecules to specifically transduce T cells.

“Achieving specificity has been relatively easy,” Gill says. However, “when you go from the optimized VSV-G system to anything fancier and more specific, you tend to take a hit on efficiency. So that’s been the key challenge – how to retain exquisite specificity while improving the efficiency of the transduction.”



Dora Mitchell,
senior VP of
operations and chief
of staff, Interius
BioTherapeutics.

So far, the Interius vector shows target cell specificity >99.9% in cultured human peripheral blood mononuclear cells, says Dora Mitchell, senior vice president of operations and chief of staff at Interius. In humanized mouse models, the vector “efficiently creates CAR-T cells that eliminate normal and malignant B cells.”

Although Interius has not yet reported data, at the May ASGCT conference, Sana presented a proof-of-concept experiment using fusogens directed against T cell receptors CD8, CD4 and CD3. Viral vectors pseudotyped with CD4- or CD8-targeted fusogens and carrying a chimeric antigen receptor directed against CD19 were able to reduce CD19⁺ tumor burden in preclinical mouse models.

A critical issue for any in vivo gene therapy approaches is giving the modified cells a survival advantage. “You will never hit all your therapy-relevant cells. You will only hit a very tiny fraction,” says Christian Buchholz, head of the molecular biotechnology and gene therapy group at the Paul Ehrlich Institute in Langen, Germany. “You have to make sure that this tiny fraction has a selective advantage.”

In addition to showing tumor-killing efficacy in humanized mouse models, in vivo technologies should also be compared directly with CAR-T cells generated with ex vivo protocols. “Make them in vivo, take them out and show they’re equivalent on a cell by cell basis with traditionally made CAR-T cells,” Kim says.

“I really do think the in vivo CAR-T stuff is going to go forward,” says Dunbar, adding “it could go forward reasonably quickly.” *EL*

Chroma Medicine and Tune Therapeutics: epigenome editing Defanged CRISPR systems hooked up to enzymatic domains may bring epigenome therapies to precision medicine.

Since the 1990s, attempts to manipulate the epigenome to fight diseases like cancer have relied on DNA-modifying drugs such as azacitidine. These drugs act more like therapeutic sledgehammers than precision medicines. Now, with decades of research on DNA-binding domains and a burgeoning number of enzymatic effectors that can write or erase epigenetic marks, precision epigenome editing is coming into view. These advances have not been lost on investors, who in 2021 poured \$165 million into two epigenome-editing companies: Tune Therapeutics and Chroma Medicine. Earlier this year, a third firm, Epic Bio, spun out of Stanford University, with a \$55 million A round.



Fyodor Urnov,
co-founder, Tune
Therapeutics.

It doesn’t hurt that the founders of Tune and Chroma are part of a veritable ‘who’s who’ of gene regulation, many of whom pioneered the design and refinement of molecular tools for targeting, binding and modifying DNA and the histone proteins that control access to it. Tune’s co-founders, Fyodor

Urnov and Charles Gersbach, together have many decades of experience in the field, with the latter’s 2015 paper showing transcriptional activation of four endogenous genes after targeted histone acetylation of the promoters.

Chroma Medicine also was founded by a group of scientific pioneers: Jonathan Weissman, David Liu, Luke Gilbert, Keith Joung, Luigi Naldini and Angelo Lombardo. In 2016, Naldini and Lombardo published groundbreaking work in *Cell* showing they could heritably and specifically silence genes by DNA methylation.



Charles Gersbach,
co-founder, Tune
Therapeutics.

An analysis of 150,000 human genomes in the UK Biobank by Kári Stefánsson, published earlier this year in *Nature*, shows conclusively what had been gleaned a decade earlier during the ENCODE (Encyclopedia of DNA Elements) project: that the vast majority (89%) of disease-causing variants occur in non-coding regulatory elements of the genome. Says Urnov, “Mother Nature wants to protect them because they matter. It means that when we get sick, these are diseases of gene control, not what the genes themselves say.”

To create therapeutics that can act at specific genetic loci, both companies couple DNA-binding proteins – a ‘dead’ Cas9 (dCas9) protein with RuvC and HNH endonuclease domain mutations or a zinc finger – to different enzymatic effectors, including transcriptional activators (for example, VP16), transcriptional repressors (for example, KRAB), epigenetic ‘writer’ enzymes (for example, DNA methyltransferases or histone acetyltransferase) or epigenetic ‘eraser’ enzymes (for example, DNA demethylase, histone demethylase or histone deacetylase).

Tune co-founder Gersbach has been creating large numbers of CRISPR–dCas9-based editors to screen for editable sites in the genome. “Because it’s so easy to design thousands, hundreds of thousands, or millions of guide RNAs and to get them synthesized quickly, we can use that to target epigenome editors to over a million sites in the genome in a single sample and then measure the outcome on the cells for every epigenetic perturbation at every one of those thousands or hundreds of thousands of sites,” says Gersbach.

In recent years, the Gersbach group has been building epigenome editing screens at an ever-increasing scale. A 2016 *Nature Biotechnology* paper used dCas9^{KRAB} repressor and dCas9^{p300} activator constructs to screen >10,000 gRNAs for activity against a several-megabase region around two genes

Both companies are working toward the same end. As Gersbach explains, “Our goal is fundamentally to have complete control of gene expression – to turn things on or off, for as long as we want, for as long as we want, in the cell types that we want.” Urnov also points to a large body of foundational work.

(*HBB*, encoding β -globin, and *HER2*) in human cells to find genomic control regions. In a subsequent paper, the same team used dCas9^{VP64} and a library of >8,000 gRNAs targeting ~1,500 genes encoding human transcription factors to screen for loci that drive cell differentiation and maturation. In 2020, they showed that the same epigenetic effector targeted to the gene encoding transcription factor PAX7, which controls skeletal muscle cell differentiation, could drive human induced pluripotent stem cells (iPSCs) to mature into myogenic precursors. Gene activation lasted longer than when PAX7 was overexpressed directly as a transgene, corresponding with histone marks characteristic of expression including H3K4me3 and H3K27ac, which were absent in PAX7-overexpressing cells. In a recent preprint, they describe a screen using the dCas9^{KRAB} transcriptional repressor and >1.1 million gRNAs to screen >100,000 regions of open chromatin in K562 human lymphoblast cells to identify regulatory elements involved in gene regulation and cell fitness.

Tune’s CEO Matt Kane has been in the gene-editing field for some time, having founded and run Precision BioSciences. He took the post at Tune because he believes epigenome editing is now sufficiently mature to make commercial therapeutic development feasible. “We understand diseases better, and how to deliver the actual editors better than we would have, even just a couple of years ago,” he says. “We know where all the obstacles are so we can see where the near-term opportunities might be, as well as laying some of the groundwork now for some of the longer term ambitions.” Urnov also believes decades of work in the gene-editing field will stand epigenome-editing in good stead. “The vision of human genetic engineering as disease therapy is 50 years old exactly this year. The only reason, for example, that the recent gene-editing approaches for sickle cell anemia have gone so well [is] because they are standing on the shoulders of 20 years of gene therapy; and gene therapy in turn is standing on 30 years of bone marrow transplantation,” he says.

Tune’s headquarters in Durham, North Carolina, has been able to recruit many employees from the Gersbach lab at Duke University. The company also has a second site in Seattle. Gersbach explains, “We got paired up with some pioneers in [the] cell and gene therapy field out there. There were introductions made to us from some of our investors. It’s one of our strengths that we are in an area

that is less crowded with cell and gene therapy companies than other parts of the country.”

“We have an initial portfolio of programs, and the good news is that it is working really well. The bad news is that it starts getting more expensive,” Kane adds. Gersbach says, “We want to prioritize areas where some of the other technical risks are minimized.”

Chroma’s founders have created their own set of epigenomic editors, which they call ‘CRISPRoff’ and ‘CRISPRon’ editors. The technology was showcased in a [2021 Cell paper](#) from the labs of co-founders Luke Gilbert at the University of California, San Francisco (UCSF) and Jonathan Weissman at the Whitehead Institute. According to Weissman, “We first saw the Lombardo paper as the starting point. Once it showed it was possible at least for a handful of genes, [we asked] could we make a robust system that we could program to any gene or least a majority of genes; then what would be the rules for silencing and how strict would it be?” The 2016 Cell paper by Lombardo and Naldini used a cocktail of CRISPR–dCas9 tethered to KRAB and/or the DNA methyltransferases DNMT3A or DNMT3L to silence several highly expressed genes in K562 cells.



Jonathan Weissman, co-founder, Chroma Medicine.

Building on this system, Gilbert and Weissman’s groups designed epigenome editors for >20,000 human genes, tested in several cell types (iPSCs, HeLa cells, U2OS human epithelial cells or K562 cells). These studies have shown that, in some cell lines, silencing is heritable over >450 cell generations and reversible with CRISPRoff (which substitutes a demethylase for a methylase). “They took a lot of the foundational work done by Luigi [Naldini] and Angelo [Lombardo] and patched it into a single construct,” says Vic Myer, Chroma’s president and CSO.

Last year, Naldini and Lombardo joined the Chroma team when their company, Milan-based Epsilon Bio, merged with the US company. According to Chroma CEO Catherine Stehman-Breen, the buyout let Chroma leverage Epsilon’s experience – they had a year or so head start compared with the US startup. “Also, they had experiments already set up and running, so it gave us a running start. They also brought with them some early [intellectual



Catherine Stehman-Breen, CEO, Chroma Medicine.

Society for Gene and Cell Therapy meeting held in May in Washington, DC. With a single administration of engineered transcriptional repressors programmed to target the PCSK9 locus, they were able to lower circulating levels of PCSK9 by 50% as compared with controls (mock edited) for over 200 days after administration, with a significant reduction in low-density lipoproteins as measured at day 30.

David Segal, a longtime gene-editing researcher at University of California, Davis, says that one constraint on epigenome editing (and indeed for gene therapy and gene editing) is the poor efficiency of in vivo delivery: it simply has been hard to fit epigenome effector proteins into company delivery vectors like adeno-associated virus (AAV). “This has been a major drawback and has led to a lot of innovation in the field,” he says. Lipid nanoparticles (LNPs), which don’t have packaging constraints, are being used already in clinical trials, but they are only efficient in targeting the liver. “So if you’re going after liver disease, it’s great. But if you want to go after anything else, it’s hard to do that with LNPs,” says Segal.

Notably, the in vivo experiment by Lombardo used a commercially available LNP to target the liver, according to Myer.

Another issue, Segal says, “is any consensus on what kind of epigenetic editing would be required for a persistent, durable editing to increase gene expression. We know more about how to silence genes and keep them durably silent.” Whether a cell will tend to reverse the pattern being forced on it remains a nagging question. Weissman acknowledges that there have been fewer demonstrations of activating genes, but he says a priori, there’s no reason why it shouldn’t work. Besides, “at the moment we have plenty of targets that are interesting that we know we can get silencing, though there might be a subset that is recalcitrant to silencing,” he says.

property], which we also felt was important for the company.”

Co-founder Lombardo, continues to do groundbreaking work at the San Raffaele Telethon Institute for Gene Therapy in Milan, presenting results of in vivo epigenome editing in mice at this year’s American

Like Tune’s CEO Kane, Chroma’s Myer was previously involved with genome editing, being part of Editas Medicine’s team. “I thought I would never go back to genome editing,” he says. “Then this technology came along – and this is really game changing.” What differentiates epigenome editing from gene editing is that epigenomic editors don’t break DNA, and don’t mutate irreversibly, “when you want to turn things on to the right level or turn things down, potentially silencing completely. With gene activation, it is possible to modulate in a way that genes are still responsive to environmental signals – those are things that are uniquely possible with epigenome editing. This is essentially the normal way cells regulate gene expression,” says Myer. Stehman-Breen agrees: “What I really loved was that ... we are taking a highly evolved, highly conserved endogenous mechanism and leveraging it,” she says. Myer is confident that the company can surmount any remaining hurdles. “There have been no ‘Oh no’ moments yet,” he says. LD

Alchemab Therapeutics: cracking the secrets of protective autoimmunity *Searching for protective antibodies in ‘resilient’ people may provide a new approach for discovering drug targets.*

The presence of autoantibodies is usually a red flag, indicating a damaging B cell response against healthy tissues. But London, UK-based Alchemab Therapeutics is turning this paradigm on its head, looking instead for instances in which autoantibodies are actually fighting disease. “Our hypothesis is that, at least in some cases, people have protective autoantibodies that are providing them with some disease resilience,” explains Jane Osbourn, the company’s CSO and co-founder.



Jane Osbourn, founder and CSO, Alchemab Therapeutics.

to uncover new drug targets and drugs for challenging conditions such as pancreatic cancer and Alzheimer’s disease.

This unconventional strategy has elicited occasional skepticism – including, initially, from Alchemab’s recently appointed CEO, Young Kwon. “I spent the past part of my career focusing on pathogenic autoantibodies,” says Kwon. “When the idea was broached that there was a company focusing on protective ones, I didn’t believe it.” But research demonstrating the existence of such autoantibodies won him over, including a handful of published studies reporting the discovery of naturally occurring, disease-mitigating antibodies. For example, a [2020 report](#) from a team led by Almudena Ramiro of the Centro Nacional de Investigaciones Cardiovasculares in Madrid determined that mice that spontaneously produce antibodies against an enzyme called ALDH4A1 experience slower progression of atherosclerosis than animals that do not. Alchemab is now seeking other such antibodies and has already built a pipeline with several promising drug candidates that could be ready for clinical testing within the next few years.

Many groups in both academia and industry are leveraging high-throughput sequencing technology to perform immune repertoire profiling. The goal is to selectively sequence the genes that encode the highly variable antigen-recognition domains expressed by cells in the adaptive immune system. By analyzing large numbers of T cell or B cell receptor sequences – the latter of which encode antibodies – one can learn how the immune response has been ‘tuned’ in response to infection, vaccination, disease pathology or other physiological triggers. “It enables diagnosis of immune status or health, and also potentially prognosis and to decide on different treatment options based on the repertoire,” explains Victor Greiff, who studies adaptive immunity at the University of Oslo.

Alchemab’s approach focuses on so-called ‘resilient’ cohorts – people who are healthy despite being at high risk of developing diseases such as early-onset Alzheimer’s or who experience unusual longevity following a cancer diagnosis. By comparing immune repertoires of B cells from resilient individuals to those from healthy controls, as well as from people who ultimately develop disease, Alchemab aims to uncover distinctive antibodies that contribute to the protective response. These can then be characterized with proteomic arrays and other assays to identify their cognate antigen, thereby revealing targets that may be druggable to achieve similar protective benefits in other patients.

The concept came from conversations between Osbourn and co-founder Houman



Olivia Cavlan,
chief corporate
development
and strategy
officer, Alchemab
Therapeutics.

Ashrafian, a managing partner at SV Health Investors, which led them to consider exploring B cell repertoires to uncover immunological correlates associated with wellness. “Houman came up with this ‘convergence’ concept,” says Olivia Cavlan, Alchemab’s chief corporate development and strategy officer. “If you’ve got a common antibody between different individuals, it must be because the body’s selecting it.” The founders also include a number of academic experts, including University of Oxford immune repertoire profiling specialist Rachael Bashford-Rogers.

Osbourn notes that at the time of its inception, in 2019, Alchemab was built entirely on a conceptual framework, with no experimental hardware in place. It received critical help from sequencing giant Illumina, becoming one of the first European companies to receive funding, equipment and technical support through the Illumina Accelerator in early 2020.

Around this time, Alchemab partnered with clinicians in the UK National Health Service to test its platform as a means for comparing antibody repertoires in patients who developed serious COVID-19 symptoms versus those who did not. This research allowed the company to optimize its analytical pipeline and also yielded some potentially useful findings, including a set of [naturally occurring antibodies capable of neutralizing SARS-CoV-2](#).

Over the course of 2020 and early 2021, the company found other collaborators with potentially valuable clinical samples to share, including the European Prevention of Alzheimer’s Dementia initiative and the Cure Huntington’s Disease Initiative Foundation. And in April 2021, Alchemab announced that it had raised US\$82 million in series A funding.

But finding clinically interesting signals in vast immune repertoire datasets is no simple task. For one thing, the sequence space of the immune repertoire is vast. Osbourn notes that any given B cell could theoretically encode roughly 10^{13} different antibody sequences, although the number likely expressed in any one person is closer to 10^9 . “We try and sample at least 1% of that 10^9 ,” she says.

This represents a tremendous number of sequences, but perhaps still not enough to find the antibodies they are seeking. Greiff notes that although antigen-specific antibody signals may surge immediately after vaccination or infection, those associated with chronic disease are often subtler and harder to track. “For autoimmune diseases, the signal is very low,” he says. Blood-based sampling can also create challenges; although such specimens are relatively easy to collect and process, the highest density of B cells is found in the spleen and bone marrow. “So you are basically constantly undersampling,” says Greiff. Peripheral blood is still the primary sample type used by Alchemab, although Osbourn says that the company is also working on samples from more disease-relevant sites such as tumor infiltrates and cerebrospinal fluid.

Clever algorithms can help stack the odds in favor of a successful search. Alchemab has developed a specialized machine learning-based framework known as [AntiBERTa](#), which they are using to find meaningful patterns in B cell repertoire data. Beyond identifying shared repertoire features that may distinguish disease-resilient individuals, Osbourn says that AntiBERTa can identify key structural features in the antibody’s antigen-binding domain that might help uncover the cognate protein target. The algorithm can even help distinguish antibodies derived from mature and immunologically active B cells – which are likely to be responding to an ongoing disease state – versus those from naive, immunologically inactive B cells.

Greiff, who also uses machine learning in his research, is enthusiastic about the company’s algorithmic approach. “Machine learning and adaptive immune repertoire analysis is a perfect match,” he says. “I think the company is really at the cutting edge of immune repertoire science.” Over the past summer, Alchemab has further optimized and battle-tested their algorithm through a partnership with the technology company Nvidia, which brought six weeks of access to the Cambridge-1 supercomputer.

The sequences flagged as promising by the machine learning algorithm are synthesized and subjected to rigorous experimental testing and validation. “We’re going from millions and millions of sequences to choosing maybe 50 that we then put into our biology assays,” says Osbourn. However, she adds that this isn’t strictly a computational decision; oversight by antibody experts is also critical here. The sequences that make the cut are assessed to identify their biological target, and these data then provide the foundation for the drug

development and optimization process. Kwon believes that starting with data collected from actual patients will give the company a considerable edge relative to more traditional strategies that begin with cell- or animal-based screening. “We’re essentially identifying physiologically validated molecules,” he says.

The company has already identified interesting targets for cancer and neurodegenerative disease. Osbourn says that the company is now pursuing a cancer therapeutic candidate that could be ready for Investigational New Drug-enabling studies in 2023, and estimates that their first neurodegenerative disease candidate could reach that stage by the following year. Alchemab has also forged several external collaborations, including a prostate cancer program with AstraZeneca and a Huntington’s disease initiative with the Medicines Discovery Catapult.

The Alchemab team is excited about the insights that they expect to emerge as the company continues to accrue and analyze ever-larger sets of repertoire data. “We have around 250 million B cell receptor sequences already, and that number’s growing,” says Osbourn. With large enough cohorts, it should become possible to begin identifying shared mechanisms and pathways that connect different conditions, and thereby identify targets that might prove more broadly effective for drug development for various disease families.

And, of course, practice makes perfect. “Over the past year, we’re now deconvoluting more targets, and getting better at triaging our antibodies and choosing the most convergent and most evolved,” says Osbourn, “and so we’re now in a position where we’ve got actually a lot more choice in terms of programs.”

Adrestia Therapeutics: gene networks to the rescue

Identifying synthetic rescue mutations in healthy people may provide new avenues for fighting disease. Decades of research on genetic mutations have had one overriding goal: to understand how specific mutations interfere with physiological processes and promote disease. Adrestia Therapeutics, a spinout of the University of Cambridge in the UK, is taking a new tack. Rather than looking at how mutations cause illness, they are scouring the genome for mutations that keep people healthy despite a genetic predisposition to disease. The idea is that mutations that put an individual at high risk of developing a disease can be overridden by mutations elsewhere in

the genome. This phenomenon, called ‘synthetic rescue’, holds out the tantalizing possibility of providing an entirely new strategy for discovering therapeutic targets and drugs.

Genes do not act in isolation, but form nodes in a vast network. Some can regulate the activity of other genes that act downstream in a particular pathway whereas others might serve redundant functions and thus act as a buffer against the damaging effects of mutations in other genes.



Steve Jackson,
co-founder, Adrestia
Therapeutics.

to cells only when combined with other – in his case, cancer-causing – mutations. A previous startup that Jackson launched, KuDOS Pharmaceuticals, applied this approach to develop a drug called olaparib, which sabotages a DNA repair mechanism that cancer cells with particular driver mutations rely on for survival. AstraZeneca acquired KuDOS in 2006 and has subsequently shepherded olaparib into the clinic as a treatment for multiple cancer indications.

The synthetic rescue interactions sought by Adrestia represent the flip side of the coin: genes that, when disabled, buffer the detrimental effects of other gene mutations. Jackson’s academic team has described at least one example of this kind of interaction, in which mutations in the *USP48* gene mitigate the pathological effects of gene mutations that cause the blood disease Fanconi’s anemia, which commonly gives rise to leukemia. He notes that studies by others in large patient cohorts have identified individuals that are surprisingly ‘resilient’ against the otherwise-detrimental effects of known disease mutations. “These patients probably have variants in their genetic background in modifier genes,” says Jackson.

The possibility of discovering and drug-ging those modifier genes led Jackson to launch Adrestia in 2017, in partnership with Cambridge colleagues Gabriel Balmus and Yaron Galanty, Delphine Larrieu of the Cambridge Institute for Medical Research, Raphaël

Adrestia co-founder Steve Jackson, a cancer biologist at the University of Cambridge, is interested in finding ways to exploit these network effects for therapeutic benefit. Previously he focused on discovering ‘synthetic lethal’ interactions. These are secondary gene mutations that are deadly

Rodriguez at the Institut Curie in Paris, and Rafael Carazo Sachs, now at the University of Bristol. Three years later, the company would draw series A funding from Ahren Innovation Capital and GlaxoSmithKline (GSK), the latter of which is collaborating with Adrestia on up to 5 programs, with the promise of \$230 million in milestone payments per successful program. GSK is also working in close collaboration with Adrestia on multiple drug development programs. “They are a super partner,” says CEO Robert Johnson. “And I think GSK, like many companies in the industry, are struggling to find high-quality validated targets.”



Robert Johnson,
CEO, Adrestia
Therapeutics.

the disease; in some cases, the company might use patient-derived induced pluripotent stem cells, whereas other projects might use CRISPR-based genome manipulation to inactivate a gene or introduce selected point mutations. But the key question is always the same, says Jackson: “Which of the 20,000 human genes will allow you to modulate that phenotype in the right direction?” He adds that the methods themselves are not the key ingredient here, but rather the experience and knowhow of a research team with many years of gene network research.

Jolanda van Leeuwen, a functional genomics researcher at the University of Lausanne in Switzerland, sees promise in this approach based on her own large-scale analyses of gene-gene interactions that enable synthetic rescue. For example, her group has repeatedly shown that the loss of many so-called essential genes in yeast and human cells can be countered by secondary mutations in ‘suppressor’ genes. “It is still so surprising to me how common this is,” says van Leeuwen. The work to date in this field leads her to suspect that “for almost every disease allele in humans, there must be a way to ameliorate the disease.”

Adrestia is also planning to bolster their screening results with complementary data from large patient cohorts and resources like

the UK Biobank, which contains clinical and genomic data from half a million individuals. Showing that people possessing rescue mutations exist in these populations could provide evidence for the safety of targeting those genes therapeutically.

These cohort data could even offer direct, real-world confirmation that these apparent rescue mutations mitigate the effects of other disease-causing mutations. However, van Leeuwen cautions that the complexity of the genome can confound the interpretation of these results. “You can identify a suppressor in one patient population or cell line,” she says, “but it may not actually work as a suppressor in different genetic backgrounds.” Thus, extensive validation in a variety of animal models and other experimental systems will also be essential.

The company is also building out its computational capabilities and recently brought on board John Perry – Jackson’s colleague and long-time collaborator at Cambridge – as VP of human genetics. Among Perry’s tasks will be building out the analytical capabilities needed to sift through tremendous amounts of experimental and human data. “We’re delving into datasets in a more systematic way now,” says Jackson.

The company has yet to announce its clinical pipeline, but has disclosed exploratory research in areas including cardiac disease and Huntington’s disease. For the latter indication, Adrestia is working closely with Cambridge and University College London researchers to develop strategies for inducing protection against the inevitable neurodegenerative decline experienced by patients with Huntington’s disease. The idea would be to find genetic modifiers to counter the effect of the loss of DNA repair mechanisms in Huntington’s disease that leads to the expansion of triplet repeats.

Johnson believes their approach should be suitable for identifying targets in many disorders and tissue types. “We’ve done nearly 30 screens, and all of them have identified at least one target that could be druggable with a small molecule,” he says. “And furthermore, none of the targets that have been identified by the screens have yet failed in validation.” His ultimate vision is for Adrestia to grow into a fully integrated drug company, covering the entire spectrum of pharmaceutical development from target discovery to drug manufacturing. In the nearer term, partnerships are likely to be critical to make the most

of the company’s approach, and talks are currently underway with a number of potential industry partners in addition to GSK.

But Jackson is excited about the discovery opportunities, including the opportunity to find hidden threads that contribute to the pathology of multiple disease states. This information could in turn dramatically accelerate the discovery of more broadly useful drugs, as well as the repurposing of existing agents that might offer previously unrecognized value for other conditions. “Each disease is distinct, but we’re already seeing crossover between the hits that we’re getting,” he says. “I think an ultimate direction of travel is generating a ‘synthetic rescue atlas’, which would be a network model that would allow one to discover these connections in a more systematic way.” *ME*

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