Nature Biotechnology's academic spinouts of 2016

Aaron Bouchie, Laura DeFrancesco, Cormac Sheridan & Sarah Webb

Our annual survey highlights several academic startups developing immunotherapies as well as ventures focusing on microbiomes, proteostasis, integrin biology, nucleic acid delivery and subcellular imaging.

Similarly to recent years, 2016 saw continued enthusiasm for investments in cutting-edge technology and early-stage products. In addition to traditional venture capital firms, corporate venture capital, non-profits and accelerators are now also contributing to seed funding. According to industry newsletter *BioCentury*, investments in life science companies by corporate venture arms continued to surge, accounting for 30% of startup financing last year.

As in previous years, our starting point for this survey was to identify ten startups originating from academic institutions. These were prioritized on the basis of the amount of early stage funding they received (a measure of commercial excitement) and then according to our editors' assessment of the novelty of disclosed research. Some firms receiving more funding than those presented here were not included because they were in 'stealth mode' or declined to be interviewed.

What follows are the stories behind the selected ventures and their technologies. We believe these represent some of the best science coming out of academia in 2016.

Forty Seven: thwarting cancer's immune evasion tactics

Pursuing the 'do not eat me' signal co-opted by tumors. Until recently, monoclonal antibodies targeting antigens unique to specific cancer cells have dominated cancer immunotherapy strategies. But researchers are increasingly trying to unmask the molecular strategies that cancer cells use to evade immune clearance. Checkpoint inhibitors, which overcome signals that suppress T cell activation, are making inroads in cancer care. One of these new drugs, Keytruda (pembrolizumab), was credited last year with putting former US President Jimmy Carter's malignant melanoma into remission.

But T cells are not the only immune cells that cancers have evolved to hide from. Cancers cells also shield themselves from macrophages by overexpressing CD47 on their surfaces. Now Forty Seven (Menlo Park, CA, USA) is leveraging a strategy of targeting CD47, and >$100 million in cumulative funding—a combination of California Institute for Regenerative Medicine (CIRM) funding and series A financing—into an ambitious clinical program to treat a range of cancers.

For more than 15 years, researchers have known that CD47 expression on the surface of cells serves as a 'don't eat me' signal to macrophages[1]. On red blood cells (RBCs), the effect is dramatic—blocking signaling between CD47 and its receptor, signal regulator protein alpha (SIRPα), leads to nearly complete clearance of these cells. Researchers speculate that waning expression of CD47 on aging RBCs prompts macrophages to 'clean house' and clear out older cells to make room for new ones.

Irving Weissman, Forty Seven founder.

Irving Weissman and Ravi Majeti at Stanford University (Stanford, CA, USA) were the first to examine the role of CD47 signaling in cancer. As abnormal cells, cancer cells express 'eat me' signals that make them vulnerable to immune clearance. But "as tumors evolve they use CD47 to avoid this process," says Craig Gibbs, Forty Seven's chief business officer (CBO). In 2009, research from the Stanford team established that CD47 overexpression allows cancer stem cells to evade phagocytosis[2]. Soon after, they demonstrated that an antibody to CD47 could eliminate acute myeloid leukemia (AML) in a mouse model[3].

These initial results highlighted the potential of CD47 as a therapeutic target for cancer. By 2008, the Stanford team had begun to discuss how they might develop a clinical antibody, says Mark Chao, Forty Seven's vice president of clinical development and a hematologist who was a postdoctoral researcher in Weissman's group at the time. One possibility was to raise venture capital funds immediately.

But though the pathway showed promise, potential pitfalls loomed large. Though more abundant on tumors, CD47 is expressed on almost all human cell types—normal and cancerous. Given the potential for off-target toxicity, the Stanford team wanted more time to develop the science in an academic setting, adds Chao. Instead of trying to spin off a company immediately, the team looked for funding that would allow them to continue their work on preclinical and early clinical antibody development in an academic setting. According to Weissman, his preference is to take research as far as phase 2 trials before licensing.

Weissman and Majeti looked to CIRM, which focuses on moving stem cell therapies into the clinic. In 2009, the team received one of the early CIRM clinical grants. Over the past eight years, CIRM funding for this effort has topped $25 million and fueled early preclinical and clinical development of their humanized anti-CD47 antibody, Hu5F9-G4. In November 2016, Forty Seven was awarded $10 million in CIRM funding to support an additional trial in colorectal cancer.

The grants, though impressive in size, were not the only way that CIRM supported the eventual launch of Forty Seven, says

Aaron Bouchie is a freelance writer based in Ithaca, New York; Cormac Sheridan is a freelance writer based in Dublin; Sarah Webb is a freelance writer based in Chattanooga, Tennessee; Laura DeFrancesco is Senior Editor, Nature Biotechnology.

Jens-Peter Volkmer, Forty Seven's vice president of research and early development. CIRM also provided mentorship and external expertise that helped to guide the clinical development of Forty Seven's research programs. "It's much more than just the funding, because you can have a lot of money and make the wrong choices, or you can have the right partners and support," Volkmer says.

To thread the needle between clinical efficacy and off-target toxicity, the Stanford team designed their antibody carefully. Instead of maximizing effector function with an IgG1 or similar antibody, they decided to graft their CD47 targeting region onto a human IgG4 antibody, a subclass with milder effector function. "I think because of that we're a lot better tolerated," Gibbs says, noting that some large pharma companies have initiated unsuccessful anti-CD47 programs with IgG1 antibodies. "I think that decision to use IgG4 was very critical."

The researchers have demonstrated that the strategy successfully targets malignancies in 23 animal models of human cancer as well as 6 types of hematological malignancy and 17 types of solid tumor, Gibbs adds. While still at Stanford, the team carried out studies to support an investigational new drug (IND) and launched a phase 1 clinical study of Hu5F9-G4 against solid tumors in August 2014. A second clinical trial using the same antibody against AML followed soon after, in December 2015.

In May 2015, Chao, Volkmer and others from Weissman's group founded Forty Seven. They soon augmented the team with industry veterans, such as CBO Gibbs, who had previously worked at Gilead Sciences (Foster City, CA, USA); and Chief Medical Officer Chris Takimoto, who came to the company from Janssen Pharmaceuticals (Spring House, PA, USA). In November 2015, Forty Seven licensed the critical Stanford patents and raised $75 million in series A financing.

Targeting CD47 as a therapeutic strategy presents a number of issues, particularly safety, notes Dennis Discher of the University of Pennsylvania (Philadelphia), who studies CD47 but is not involved with Forty Seven or competing companies. "To what extent is this targetable and still safe?" he asks. Even with overexpression of CD47 on tumor cells, safe dosage is an important question. Binding to CD47 on RBCs and other cells could sop up a lot of the therapeutic dose, he warns.

As expected from preclinical data, Hu5F9-G4 shows some off-target binding to RBCs. "When we give the antibody against CD47 we immediately see a loss of about 20% of the red blood cells," Gibbs says. On the basis of those preclinical studies, the team also designed a dosing strategy to mitigate the problem, administering an initial priming dose before the larger therapeutic doses. Within a week, RBC populations bounce back to normal levels. Dose escalation studies to assess binding site saturation showed that as the dose increases from 3 to 10 mg per kilogram of body weight (mg/kg), plasma trough levels increase by a thousand-fold, Gibbs says, which indicates that they "titrated the sink."

To address the potential for anemia, Forty Seven designed their phase 1 studies with a low priming dose (1 mg/kg) of the antibody followed by maintenance doses of up to 20 mg/kg. In clinical data reported last November at the annual meeting of the Society for Immunotherapy of Cancer in National Harbor, Maryland, and last December at the meeting of the American Society of Hematology in San Diego, they found that a subset of patients showed anemia and other blood-related side effects, but those individuals recovered with an overall younger cohort of RBCs, which express fewer 'eat me' signals on their surfaces, Gibbs notes.

The results suggest that Forty Seven's strategy might have walked the critical tightrope between sufficient potency and off-target toxicity. The quick rebound in blood cell count is "a cause for optimism" in terms of overall safety, Discher notes. Gibbs hopes that Forty Seven will have efficacy data to report later in 2017.

As with checkpoint inhibitors, questions remain about whether targeting CD47 alone will be sufficient to clear cancer in most patients. Data from the Stanford team suggest that the 'don't eat me' signals of the CD47–SIRPα pathway are paired with an intrinsic 'eat me' signal to macrophages, in the form of calreticulin[4]. Because the effect requires a sufficient suppression of the CD47–SIRPα pathway to enable the intrinsic calreticulin 'eat me' signal, "you're essentially taking your foot off the brake," Gibbs says. Though that strategy is effective for checkpoint inhibitors in a subset of tumors, it fails in others, suggesting that combination therapies might be more effective.

Combinations could prove potent for Forty Seven as well. Hu5F9-G4 should work in combination with antibodies directed against a tumor cell–specific antigen in several indications. "We get synergy with rituximab [Rituxan], with the anti-EGFR antibodies cetuximab [Erbitux] and panitumumab [Vectibix] and others," he says. Last November, the company launched phase 1 clinical trials of combinations of Rituxan with Hu5F9-G4 for non-Hodgkin's lymphoma and Erbitux with Hu5F9-G4 for colorectal cancer. Another potential combination is with checkpoint inhibitors. Weissman's group and others have uncovered evidence that promoting phagocytosis by blocking the CD47 pathway also increases the presentation of tumor cell antigens, activating the T cell response. If an anti-CD47 strategy can activate a T cell response, a combination of anti-CD47 and checkpoint inhibitors could prove synergistic, Gibbs says. "We have a lot of interest from pharma companies in doing such combinations."

But even as clinical testing moves forward and safety data look promising, additional issues could arise. Long-term safety could prove to be an issue, Discher says, as CD47-deficient mice can succumb to autoimmune disease. In addition, a January 2017 report from the Reproducibility Project: Cancer Biology[5] highlights some of the challenges of the field.

The original paper[6] showed that CD47 was overexpressed in a variety of solid tumors, bolstering the researchers' overall conclusion that this pathway was a clinical cancer target. In the specific experiment in question, the Stanford results showed a clear reduction in the size of mouse solid tumors implanted in mice, but in the replication study, the results were heterogeneous and not statistically significant. Weissman says that a variety of factors could account for the discrepancy, including that the replication study did not attempt to reproduce the original experiments on human solid tumors and that other groups have successfully built on the original findings. "Reproducibility is so important that one must have the infrastructure funds and personnel to be able to reproduce or at least test reproducibility. The CRO selected by the Reproducibility Project, despite our efforts to help them, failed to reproduce even the most basic tumor transplants," says Weissman. The Reproducibility Project results are simply one more data point and don't necessarily invalidate the original results, says Tim Errington of the Center for Open Science (Charlottesville, VA, USA), a nonprofit organization that oversees the Reproducibility Project in partnership with Science Exchange. However, he cautions that over many replications the data might be more complicated than what was originally reported.

The Reproducibility Project data could mean that an anti-CD47 therapy alone might not be a hoped-for silver bullet in cancer treatment, Discher says. Testing an anti-CD47 antibody alone is necessary for understanding safety, but ultimately a combination strategy could be more likely to bear fruit, he says.

Anti-CD47 therapy might prove useful beyond targeting malignant cells. Weissman's group has shown that CD47 could also be important in chronic infectious diseases, atherosclerosis and fibrotic diseases. In some fat plaques in cardiovascular disease, macrophages, which can normally remove dead cells

via efferocytosis, are unable to process them because expression of 'eat me' signals, including calreticulin, is reduced. Results from Nicholas Leeper's group at Stanford, showing upregulation of CD47 in atherosclerotic human tissue that is reversible with anti-CD47 antibodies[7], were interesting to the Weissman group, leading to a recent collaboration to explore the potential connection to CD47. "We think that the implications, scientifically at least, are very broad," Volkmer says. But developing that understanding into a clinical trial in atherosclerosis or another disease is probably years away, he adds.

For now, Forty Seven is focused squarely on oncology, Gibbs stresses. The company has other molecules in the pipeline that target the CD47 pathway, including anti-CD47 fusion proteins, antibodies that target SIRPα and bi-specific molecules. These backup pipelines could eventually fuel efforts or partnerships for developing therapies in these other disease areas, he adds.

In the meantime, the Stanford startup finds itself in a field crowded by competitors. Last August, Tioma Therapeutics (St. Louis) raised an $86-million series A round to develop anti-CD47 antibodies that act selectively on leukemia cells and induce membrane potential discharge from mitochondria (the firm declined to be interviewed for this article). Alexo Therapeutics (S. San Francisco, CA, USA), another private biotech that also boasts Weissman among its founders, is developing soluble versions of SIRPα engineered to bind CD47 with greater affinity than natural SIRPα. The product is expected to enter clinical testing this year. Two public companies, Trillium Therapeutics (Mississauga, ON, Canada) and Celgene (Summit, NJ, USA), are also conducting phase 1 trials of a SIRPα CD47-binding domain–IgG Fc domain fusion and an anti-CD47 monoclonal antibody, respectively. *SW*

Magenta: innovating for stem cell transplantation

Upgrading cell conditioning, mobilization and expansion in clinical hematopoietic stem cell therapy. Hematopoietic stem cell transplants (HSCTs) have been in routine clinical use for decades as treatments for blood-based malignancies. Yet they come with considerable risks. To prepare for a transplant, traditionally recipients receive whole-body-conditioning drug regimens that are highly toxic and compromise the ability to fight infection. At the same time, if all cancerous cells are not eliminated, the risk of relapse looms. Transplant success depends on the ability of donor immune cells to eliminate any remaining tumor cells. In some cases, that process can trigger graft-versus-host disease.

Magenta Therapeutics (Cambridge, MA, USA), has proposed a new platform for reducing the risks of these procedures and expanding their application to a wider range of diseases. The company's work to create products to improve patient conditioning and stem cell mobilization and expansion is supported by $48.5 million in series A financing and a 'dream team' of internationally recognized experts David Scadden of the Harvard Stem Cell Institute (Cambridge, MA, USA), Robert Negrin of Stanford University and John DiPersio of Washington University School of Medicine in St. Louis. Magenta aims to make HSCT procedures better, says Jason Gardner, the company's CEO. "That's not just safer. It means more effective in outcomes. It means broadening the patient population that could receive a transplant, broadening the way people donate stem cells."

David Scadden, Magenta co-founder

Magenta's work—particularly in targeted conditioning—is exciting, outside experts say. However, promising preclinical results still need to translate into safety and efficacy in larger animals and eventually in humans. The company has not released any details or data about its preclinical efforts on stem cell mobilization and expansion.

Conditioning patients for HSCT typically involves some combination of chemotherapy drugs and radiation. Although these regiments are successful in younger patients, their toxicity restricts use in older patients. In recent years, researchers have developed lower-dose conditioning regimens and sought better strategies for targeted conditioning. For example, Rituxan (rituximab), a chimeric monoclonal antibody that targets CD20, a marker on B cells, is often used either before or in combination with other conditioning agents for patients receiving HSCT to treat lymphoma, says Linda Burns, a hematologist/oncologist and vice president and medical director of health services research at the National Marrow Donor Program (Minneapolis).

Magenta's conditioning strategy builds on research from Scadden's laboratory at the Harvard Stem Cell Institute. Last July, his team demonstrated that a single dose of an antibody–drug conjugate led to 90% engraftment of donor cells and eliminated sickle cell anemia in mice[8]. In their novel conditioning agent, an antibody to CD45, an antigen found only on blood cells, is linked to saporin, a ribosome-inactivating protein in the ricin family. "We were so encouraged by the limited impact on the well-being of the animal and the efficiency with which we could get new stem cell engraftment that we thought it could really change the experience for patients," says Scadden, who co-founded Magenta and chairs the scientific advisory board.

"This is a very viable and exciting approach," says Brenda Sandmaier, a hematologist/oncologist at the Fred Hutchinson Cancer Research Center (Seattle), who is not affiliated with Magenta. Magenta's team isn't the only one trying to target CD45. Sandmaier and her colleagues have their own targeted conditioning strategy, which uses antibodies to CD45 linked to beta- or alpha-emitting radionuclides that emit over a range of just 60 microns and have a short half-life. (One of antibodies is currently in phase 1 clinical trials.)

Antibody–drug conjugates have had their share of setbacks in development as immunotherapy agents. But Scadden expects the strategy to incur fewer problems in HSCT conditioning, which will probably require only limited administration of the drug. "These are potentially single-use approaches," he says. The team is also working to avoid genotoxicity to nontarget tissues, which is especially problematic when treating children.

An important question, Sandmaier says, is stability of the toxin once released from the antibody conjugate and where it is eventually broken down in the body. In addition, mouse studies haven't thrown light on the potential adverse effects of a CD45-targeting drug on the liver, where up to 25% of cells are hematopoietic cells that express CD45, she adds. Furthermore, patients who have undergone multiple transfusions and might have some sensitization to minor antigens don't always respond to conditioning regimens in the same way as untreated animals in preclinical studies.

With the potential for off-target effects, Magenta researchers are looking at molecular strategies that could fine-tune their approach if needed, Scadden says. With an eye toward the clinic, they're also examining other potential payloads.

The company is not giving a target date for clinical trials, just yet. They plan to move some testing into primates relatively soon, Gardner says, with the idea of using those results as a springboard for potential clinical trials.

Conditioning is only one piece of Magenta's platform. The company is also looking at ways to improve stem cell mobilization and expansion. Mobilization involves the movement of stem cells from bone marrow to the blood stream and is critical for the donation process,

particularly in transplants done with peripheral blood. Today, stem cell donors undergo needle aspirations to remove stem cells directly from the bone marrow or, for peripheral blood donations, typically receive a 5-day series of growth factor injections with granulocyte colony-stimulating factor (G-CSF). Magenta is working on a combination approach with drugs that reproduce normal physiological mechanisms in triggering stem cells to migrate from the bone marrow to the blood, Gardner says. "[Our approach] actually takes about 15 minutes to mobilize the stem cells, in animal models." But they haven't yet revealed more details or data about this research. "This is not going to be a noncompetitive area," he says.

Treating donors with G-CSF has been the standard of care for decades, notes Rainer Storb of the Fred Hutchinson Cancer Research Center. A newer drug, the small molecule Mozobil (plerixafor), mobilizes cells more quickly and is approved by the US Food and Drug Administration (FDA) but has not become standard of care, in part because long-term studies are not yet available to verify that it doesn't trigger secondary malignancies. "We are typically able to get plenty of cells into the bloodstream for transplant. What [Magenta is] doing is trying to see if they can develop some new agents that can increase that number even more," says Burns of the National Marrow Donor Program. Increasing the number of mobilized cells is helpful—typically, the more cells, the better, she adds.

Current mobilization strategies work reasonably well in the clinic, Scadden notes. But Magenta's new technique is much less time intensive, with a different toxicity profile. In addition, emerging technologies, such as gene editing, could lead to novel therapies that rely on HCST. But incorporating gene-edited cells would require avoiding G-CSF, as this molecule can lead to maturation of cells through changes in gene expression patterns rather than simply "shaking them out," Sandmaier says.

Magenta is also working on expansion, a process that boosts the number of stem cells available for transplant outside the body. Gardner reports that Magenta has new small molecules in development. "We think that's going to be very important for stem cell transplant, and doubly important for stem cell gene therapy and gene editing," he says. This area is also competitive, and right now the company is long on enthusiasm but short on revealing specific details.

Now a mainstay treatment for hematological cancers and severe blood related disorders such as aplastic anemia and sickle cell disease, HSCT shows promise in other disorders. In Europe, HSCT is already among the treatments recommended for systemic sclerosis. Experimental studies are also examining the potential of HSCT to reset the immune system in patients with multiple sclerosis. Safer, less onerous procedures could make HSCT a more realistic choice for treating a wider range of conditions, from blood disorders to autoimmune disorders, or as a way to introduce gene modifications. That's the fundamental groundwork that Magenta is trying to build. If successful, Scadden says, "We will really change the way in which people perceive this process and the way in which it's applied."

Although many of Magenta's ideas for improving HSCT are not novel to the field, the company's leaders emphasize that the ability to bring together advances in conditioning, mobilization and expansion could energize clinical innovation. The company grew out of a unique partnership between the venture capital firms Atlas (Cambridge, MA, USA) and Third Rock (Boston). "The two firms, in the past, have not traditionally worked together to build companies," Gardner says. Gardner, a GlaxoSmithKline veteran who completed postdoctoral research with Scadden two decades ago, joined Atlas in 2015. "It was very important to have a single leading biotech company for the clinicians and the patients to understand what was coming and how we could change and innovate in this area."

At the American Society of Hematology meeting in December, Magenta solicited feedback from medical directors of 15 leading transplant centers about its initial plan and the development of a novel conditioning agent. "There was a resounding enthusiasm for the approach," Magenta's CSO Mike Cooke says. As Magenta gets closer to the clinic, he expects that there will be many opportunities to do studies in partnership with those leading centers.

Magenta also sees an opportunity to facilitate the translation of academic advances in hematopoietic stem cell biology toward the clinic, Cooke adds. With a full suite of technologies to do transplants of human and mouse cells and do mobilization studies, he expects that Magenta could quickly reproduce academic data and evaluate promising biology. "As we get more mature and able to evaluate those kinds of medicines in a rapid manner, I think that really helps push that academic science and break down that translational barrier that many of the great academic scientists really face." *SW*

Axial Biotherapeutics: drugging the gut–brain axis

Insights from the microbiome direct treatments for autism and Parkinson's. Brain disorders pose a particular challenge for drug developers. Decades of work and millions of dollars have gone into trying to move molecules

Sarkis Mazmanian, Axial Biotherapeutics founder

across the blood–brain barrier at pharmacological doses. Axial Biotherapeutics (Boston) may have found another way into the brain: through the gut. Several neurological diseases, among them autism and Parkinson's disease, are associated with a variety of gastrointestinal conditions. In the case of Parkinson's, gastrointestinal (GI) symptoms precede motor deficits by years. These observations led Sarkis Mazmanian (California Institute of Technology (Caltech), Pasadena, CA, USA) to look into the gut microbiome for clues on what could be gleaned from studying the gut microbiome that could lead to interventions.

A microbiologist by training, Mazmanian had been working on the influence of the microbiome on immune disorders such as Crohn's disease and multiple sclerosis, which spawned Symbiotix Biotherapies (Boston) in 2012. After learning from his Caltech colleague, the late Paul Patterson, that children with autism often have GI disorders, Mazmanian turned his attention there. His work in animal models of autism and later on Parkinson's disease spawned Axial Biotherapeutics last December, with a $19.2-million series A round of funding.

Previously, the groups of Mazmanian and Patterson had jointly developed the maternal immune activation (MIA) mouse model of autism, in which pups born to pregnant females exposed to polyinosinic–polycytidylic acid (polyI:polyC) exhibit the core behavioral features of autism. Mazmanian found that offspring of MIA mothers display the same intestinal changes (leaky gut) as children with autism. "It was gratifying that the mouse model reproduced not only the behavior but also the GI symptoms," he says. MIA offspring showed global changes in microbiome composition, Treating MIA mice with *Bacteroides fragilis*, a human commensal bacterium currently being tested in animal models of GI disorders, repaired the gut epithelium and corrected many behavioral abnormalities. Metabolome studies of the mice showed that 8% of metabolites detected by mass spectrometry were altered and largely returned to normal after *B. fragilis* treatment. One metabolite, 4-ethylphenylsulfate, showed a 46-fold increase[9].

These findings comprise the foundation of Axial, which Mazmanian incorporated in June 2014, to advance *B. fragilis* to the clinic as a novel therapy for the GI and behavioral symptoms of

autism spectrum disorder. Just a few months later, David Donabedian, a partner at Longwood Fund (Boston), called Mazmanian in search of early-stage programs to fund. The two had met previously, when Symbiotix was being formed and Donabedian was heading up the Ventures and Early Stage Transaction Group at AbbVie (N. Chicago, IL, USA). This time, Mazmanian had a different story to tell, and Donabedian, though skeptical initially, was sold. "There actually was real data," he says. "A lot of times as a [venture capitalist] you see a lot of interesting science projects that really are still science projects, but I felt this one is ready to make the jump from being something developed in the lab to translating it into the clinic."

Longwood partners typically take a hands-on approach and assume leadership roles in the companies they fund. Thus, Donabedian took up the reins of the spinoff Axial Biotherapeutics, bringing with him series A funding along with funding from Domain (Princeton, NJ). In the intervening years, Patterson's group repeated the experiments in two other mouse models of autism, with similar results, according to Donabedian. Particularly interesting to him were some follow-up experiments in which pups were treated with microbiomes from autistic and normal children to see if they could induce the behavior of the autistic children. "What captivated me was not only did we show that these mice started to have some of the same impairments exhibited in the human populations, but also the severity of the disease was translated as well," says Donabedian.

Donabedian says what sets this company apart is the focus on delivering therapies orally, to treat neurological symptoms via the gut. "We don't feel we need to have systemic circulation or get through the blood–brain barrier. I don't know how many, if any, companies are focused on the gut," he says. Their first program to go into the clinic will be a probiotic (AD-1224) in autism and is expected to start in 18–24 months.

Axial has a second program in Parkinson's disease, which Mazmanian hopes will provide more solid proof of concept. "One of the things with autism models: there's very little confidence that the models will recapitulate the disease. Thousands of labs are using them, but we have to be careful to not oversell the findings of mouse models," he says. The reason—behavioral readouts are complex. Mazmanian is hoping Parkinson's will provide clearer, verifiable endpoints.

Using a mouse model that expresses and aggregates α-synuclein in the gut, a hallmark of Parkinson's disease, Mazmanian found that the mice did not develop motor dysfunction when bred in sterile environments (lacking any gut microbes). He and his group further found that feeding sterile mice short chain fatty acids—a class of metabolites contributed by gut microbes—caused α-synuclein aggregation in regions of the brain affected by Parkinson's disease and induced motor deficits. To Donabedian, this provides a possible mechanism to pursue. Finally, transplanting fecal samples from patients with Parkinson's disease into these sterile mice causes the animals to develop parkinsonian symptoms, whereas samples from healthy controls do not[10]. "Though that paper just came out in December, ongoing work has given us a much better understanding of what we believe the mechanisms are, and we're actually moving much faster in Parkinson's, targeting that mechanism not only with bacteria but with non-bacterial interventions," says Donabedian.

The question, as always, is when are there enough data to go into humans. And Mazmanian admits that relying on mouse models requires a leap of faith. But, points out Rob Knight (University of California, San Diego (UCSD)), a collaborator on the Parkinson's work: "There's no data, which is why we need human trials. I can't see a way towards getting the data without them. It's one of these situations where every investigator—and their investors—will need to decide for themselves when they're willing to make that leap." ***LD***

ReadCoor: sequencing meets pathology

Fluorescent *in situ* sequencing, another George Church brainchild, is commercialized.

Pathologists have been working with the same set of tools for literally hundreds of years. Now, researchers at the Wyss Institute at Harvard University (Boston) and Harvard Medical School (Boston) believe they have the tool to change clinical diagnostics in a fundamental way. Fluorescent *in situ* sequencing (FISSEQ), the technology at the core of ReadCoor, enables sequencing of entire transcriptomes in immobilized cells, providing a three-dimensional display of cellular activity. This is the culmination of a vision that ReadCoor's co-founder, George Church of Harvard University (Cambridge, MA, USA), has had since the advent of DNA sequencing in the 1980s.

To move this project forward, the Wyss brought together Rich Terry, a device builder at heart, and Shawn Marcell, an economist turned pathologist. Terry, founder, president and CTO, started out in aerospace engineering before becoming enthralled with biology. co-founder, chairman and CEO Marcel, after majoring in economics, developed a passion for diagnostics and worked in that sector of the pharma industry before joining the Wyss in 2016 as an entrepreneur in residence. Explaining the allure of FISSEQ, Terry says, "It doesn't matter if your model is an animal or a tissue or an organ on a chip, what they all lack is a readout of what is going on in a complex rich environment that is a tissue or an organ or an animal." After ten years in the Church lab, culminating in a 2014 paper in *Science*[11], Terry felt a team was needed to push the commercialization, and he was ready to lead it. "It was the most exciting thing to come out of the space in diagnostics," he says. With a $23-million series A round, Terry and Marcell are moving forward.

Localizing nucleic acids on surfaces or in cells is not a new idea. Fluorescent *in situ* hybridization, the predecessor of FISSEQ, has been around for decades but in most hands can detect only one or a small number of species, limited by the color palette of fluorescent probes, low signal and an inability to distinguish individual bases. Advances in sequencing technology, particularly miniaturization, opened up new vistas, as Church had anticipated since the earliest days of DNA sequencing. In 2005, Church designed a method for sequencing samples splayed out on surfaces. With next-generation sequencing (NGS) technology, the Church group was able to take it further. "We leveraged all the existing expertise in NGS in terms of sample prep, amplification, sequencing chemistry and instrumentation and optics," says Terry.

ReadCoor team. Left to right: Allison Martin, Brian Turczyk, Steve Perrault, Conor Camplisson, Lori Johnson, Samuel Inverso, Shawn Marcell, Evan Daugharthy, Ben Pruitt and Richard Terry

Of course, sequencing is only part of the story. Various other processes needed tweaking. The molecules needed to be cross-linked in place, the sample permeabilized to allow molecules in and out, the target amplified and the amplification product kept intact and in place. A further challenge was the instrumentation for reading out the signals. Terry says, "There's not a sequencer to date outside of the ones we've produced that have been from the ground up specifically designed to produce high-resolution, high-quality, three-dimensional sequencing maps."

Once designed, the Wyss used expertise within its walls to finish the job. Arjun Raj, (University of Pennsylvania), a developer of FISH technology, thinks the Wyss is unique. "They have in-house engineering that will actually take your Scotch-tape-and-chewing-gum lab prototype and turn it into something that's actually robust," he says. And according to Marcell, the Wyss tech transfer model goes further than most academic institutions. The model 'de-risks' technologies, reduces them to practice and brings in entrepreneurs in residence (such as Marcell) to shepherd technology to market.

In their proof-of-concept paper, the Wyss and Harvard groups, along with researchers from the Allen Brain Institute (Seattle), reported results using FISSEQ in several cell systems. The process involved reverse transcribing RNA in fixed cells and amplifying the resulting cDNA by rolling-circle amplification. The resulting single-stranded nanoballs of DNA were subjected to 27–32 rounds of consecutive sequencing-by-ligation reactions. The researchers reported annotation of more than 4,000 transcripts in cultured human fibroblasts; in wounded fibroblasts, they showed that transcripts involved with wound healing were enriched, and they detected different patterns of expression in migrating versus contact-inhibited cells[11].

After the announcement of ReadCoor's spinout from the Wyss Institute, "people were literally lined up around the block from major research institutions with projects ready to sequence," says Marcell. ReadCoor has two major projects already in the works. For one, they are working on a multi-institution consortium for mapping the brain with the US Intelligence Advanced Research Projects Agency (IARPA). The aim is to develop better machine-learning algorithms based on how the brain actually works, according to Terry. "From our point of view, the brain atlas coming out of this with a number of potential targets could be used as the basis for the development of [central nervous system] CNS drugs," he says. ReadCoor also has a project with the Bill and Melinda Gates Foundation (Seattle) to colocalize different inflammatory disease agents. According to Marcell, "We'll start out with collaborations and then will expand to situations where we are supplying instruments to large institutions for core laboratories for research purposes."

In the meantime, researchers working with conventional FISH technology have made advances. With platforms like seq-FISH, developed by Long Cai (Caltech), and MER-FISH developed by Xiaowei Zhuang (Harvard), upwards of several thousand targets can be mapped. However, Raj points out that "the thing that's amazing about FISSEQ is that you don't have to build probes against particular targets. You just sequence what's in there, and see what's there."

With any *in situ* technology, there is limited physical space in which to work. "On a flow cell, you can spread out the spots and measure the spots. Inside a cell, you're limited by the physical dimensions of the cells. That's the size of your flow cell," says Raj. Wyss and collaborators are working around this with techniques such as expansion microscopy, which they are developing together with Ed Boyden (Massachusetts Institute of Technology, Cambridge, MA, USA).

Terry sees a major role for this platform along the entire continuum of drug discovery, from pathway analysis to target identification, discovery, development and validation. "We've been approached in a number of different areas. Part of our plan is to be involved in strategic areas of drug development, such as cancer, gene therapy, CNS and immunotherapy." For Church, the applications go beyond diagnostics. With DNA tagging, other types of molecules—such as proteins or small molecules—can be localized. He sees FISSEQ as the ultimate tool in personalized precision medicine in any number of diseases. Raj sums it up: "The main thing is that they dared to do it." ***LD***

Pliant Therapeutics: targeting integrins in fibrosis

Targeting tissue-specific integrins to prevent TGF-β activation in fibrotic disease. Fibrotic diseases have few good therapeutic options. Fibrosis occurs when excess connective tissue, such as insoluble collagen, forms in an organ or tissue, and it can happen nearly anywhere in the body. Historically, most biopharma companies have attacked each type of fibrosis separately. A treatment that addresses the underlying process anywhere in the body could be a magic bullet for a host of deadly diseases—and a cash cow for the company that develops it. Pliant Therapeutics (Redwood City, CA, USA) hopes to do exactly that with its focus on integrin biology and transforming growth factor-β (TGF-β). The company's lead program targets the integrin $a_vβ_6$ to treat idiopathic pulmonary fibrosis (IPF), which is currently in lead optimization and on schedule to enter the clinic in early 2019.

Pliant was formed by Third Rock Ventures, which creates companies from scratch after searching for technologies that could solve a particular medical problem. For fibrosis, that search led them to Dean Sheppard and Bill DeGrado at the University of California, San Francisco (UCSF). Sheppard, a professor at the UCSF School of Medicine, has spent the past 25 years studying the role of integrins in fibrosis. DeGrado, a professor in UCSF's pharmaceutical chemistry department, has expertise in integrins. According to Pliant President and CEO Bernard Coulie, combining Sheppard's and DeGrado's complementary abilities was the foundation for the company. So as to not put all of the company's eggs into one basket, Third Rock added two more UCSF professors with expertise in the epithelial–mesenchymal transition (EMT), which has an important role in fibrosis: Hal Chapman, a medicinal biologist who specializes in IPF and EMT, and Rik Derynck, a cell and tissue biologist who identified the role of TGF-β in EMT. The venture capital firm launched Pliant in February 2016 with a $45-million series A round.

Bernie Coulie, Pliant CEO

The underlying hypothesis of Pliant's approach is that TGF-β triggers EMT, activates fibroblasts and leads to the remodeling of the extracellular matrix (ECM). Together, these actions result in fibrotic disease. Other attempts at treating IPF have directly targeted TGF-β; one of them is Esbriet (pirfenidone) from Genentech (S. San Francisco, CA, USA), which was approved in 2014. However, Esbriet slows the progression of IPF and can cause intolerable side effects in large numbers of patients. Pliant's primary approach is to move upstream and target integrins, which activate TGF-β. By inhibiting the cytokine, the company hopes to stop disease progression completely.

Coulie says it is important that integrins show tissue specificity, which allows targeting of fibrosis in specific areas of the body, thus avoiding systemic side effects. Of the 24 known integrins, Coulie says six or seven are implicated in

fibrosis. The best characterized of them is $a_v\beta_6$, which is lung specific and expressed at relatively high levels in biopsies from IPF patients compared to healthy lung tissue. In 1999, Sheppard's group was the first to show that $a_v\beta_6$ activates TGF-β[12]. More recently, he showed that inhibiting $a_v\beta_6$ prevents IPF in mice[13]. Steven Nathan, Medical Director at Inova Fairfax Hospital's Advanced Lung Disease and Transplant Program (Falls Church, VA, USA), believes that Pliant's approach to targeting integrins could be a good strategy for treating IPF and fibrosis in general. However, Nathan cautions, many other agents that made biological sense haven't panned out as useful therapies for IPF.

In anticipation of its lead compound entering human trials in early 2019, Pliant has begun building a prospective 125-patient registry in hopes of discovering a biomarker for IPF progression. Patients also will undergo regular high-resolution computed tomography (CT) lung scans for a year, and the company will collect blood and urine for genomic, proteomic and metabolomic analyses. Coulie hopes the company can discover a biomarker that will allow it to shorten the typical clinical trial length of 6–12 months that is common in IPF.

As patients with IPF have a life expectancy of only 2.5 years, Pliant's goal with its lead compound is to halt disease progression. It's unknown whether IPF is reversible, as no one knows whether the insoluble collagen laid down during fibrosis can be turned back into soluble collagen. Coulie notes that other companies and researchers are attempting to do that (e.g., by targeting lysyl oxidase homolog 2, which can cross-link collagen type IV), but there have been no commercial successes thus far. Coulie expects that Pliant's integrin inhibitors could ultimately be used in combination with other drugs that could help reverse the disease. In the case of liver fibrosis, the tissue itself is regenerative, so stopping disease progression could potentially allow the liver to heal itself, thereby reversing disease.

Pliant is initially focusing on IPF not only because of the scientific founders' expertise in the area and the well-characterized role of $a_v\beta_6$, but also because it has a regulatory precedent in Esbriet and Ofev (nintedanib) from Boehringer Ingelheim (Ingelheim am Rhein, Germany). Pliant plans to also develop compounds for fibrotic diseases that have no regulatory precedents, such as fibrosis of the liver, kidney, gastrointestinal tract, skin and heart. For now, Coulie says the company is building functional *in vitro* assays and translational animal models that can better predict human outcomes than current assays and models can.

Coulie would not disclose how much cash Pliant has on hand, but he notes that it would be difficult for a company like Pliant to fully develop and commercialize compounds on its own. He thus aims to focus on building a fibrotic platform, bringing compounds to the clinic and then partnering with a company that has the scale and expertise to develop product candidates in multiple indications and geographies. Pliant also is developing compounds against EMT-related targets. The company's second compound, which is in discovery, is part of this program. ***AB***

C4 Therapeutics: tagging disease-causing proteins for the trash

Using conjugates of phthalimide and small-molecule inhibitors to selectively target proteins for degradation by the ubiquitin–proteasome system. Drug developers have focused largely on inhibiting unwanted or errant proteins to restore normal function in disease states. But what if you could just get rid of a bad actor? This is the approach taken by a cadre of biotechs that are exploiting the cellular protein degradation pathway to rid cells of disease-causing proteins. The newest company to take this approach, C4 Therapeutics (Cambridge, MA, USA), spun out of Dana Farber Cancer Institute (Boston) is repurposing thalidomide to recruit unwanted proteins to the proteasome, the cellular trash bin.

Andy Phillips, C4 Therapeutics CSO

The name C4 has a double meaning. The first thing (some) people might think of is composition C4, an explosive, points out CSO Andy Phillips. "We refer to what we're doing as find and destroy," he says. C4 also refers to a new tool in the ubiquitin–proteasome pathway of protein degradation, in which a fourth, all-chemical step is added to E1, E2 and E3, the pathway's three well-characterized steps. That is where C4 Therapeutics is placing its focus.

C4's approach derives from work from the lab of James (Jay) Bradner at Dana Farber, after some detective work on a decades-old mystery by colleagues at his institute and elsewhere. (In 2015, Bradner took up the mantle of president of the Novartis Institutes of Biomedical Research (Cambridge, MA, USA).) Although thalidomide's teratogenic properties were revealed more than 50 years ago, when it was taken off the market after being found to induce congenital limb malformations in children born to mothers exposed to the drug during pregnancy, the mechanism by which thalidomide does this was discovered relatively recently. Once versions of the drug were found to benefit certain indications (e.g., multiple myeloma and leprosy) and became a successful commercial franchise for Celgene, several groups set out to work out its mechanism of action. In 2010, Hiroshi Honda (Integrated Research Institute, Tokyo) solved the three-dimensional structure of thalidomide, revealing that it binds cereblon, an E3 ligase that catalyzes the transfer of ubiquitin to substrate proteins, the third step in the proteasome pathway[14]. Following that, Benjamin Ebert and colleagues at Dana Farber showed that thalidomide recruits for destruction two transcription factors, IKZF1 and IKZF3, that are vital for the survival of multiple myeloma cells[15]. According to Phillips, Bradner did something "really quite magical" and saw what others didn't see. "At last, we understand how thalidomide works. But Bradner said, 'Great, not only do I see that, but I see how I can make use of this'," Phillips says.

Bradner's idea was to make bivalent derivatives of thalidomide that would bind other proteins and use them to hijack the protein degradation pathway. The final piece of the puzzle came in work from his own lab describing JQ1, a cell-permeable selective binder of the transcription factor BRD4, a bromodomain-containing protein involved in transcriptional regulation of oncogenes in certain cancers. In a 2015 *Science* paper[16], the foundational work of C4, Bradner and his group put it all together. They created bifunctional molecules bearing an imide arm derived from a phthalimide molecule that binds cereblon (and ubiquitinates proteins, hence recruiting them for degradation) and a selective small-molecule protein binder arm that grabs onto target proteins. They provided two examples—cereblon-dependent bromo- and extra-terminal domain binder 1 (dBET-1), which binds BRD4, and cereblon-dependent FKBP12 binder 1, which bears ligands for the cytoplasmic signaling protein dFKBP1. Both molecules, which they dubbed degronimids, knocked down their target proteins, and dBET-1 slowed progression of leukemia in a mouse xenograft model.

This approach provides multiple benefits, according to Samie Jaffrey (Weill Cornell Medical College, New York). For one, degronimids can be used to inhibit proteins when conventional small-molecule inhibitors that bind active sites don't exist, since the binder can attach anywhere on the molecule. In addition, because they lead to the degradation of their targets, they can be more effective than reversible small-molecule inhibitors, which

often require multiple or large doses for effective inhibition. And finally, even degronimids with low binding affinities could be effective as, once the target protein is degraded, the binder is released and can bind again, acting catalytically. "This is one of the magical things about this drug, you can use sub-stoichiometric amounts of the drug—really a tiny amount can bind to its target, kill it and then come off and bind to another target and kill that," says Jaffrey.

Potentially, anything that binds proteins could be hooked up to an imide. Phillips says, "If there's a binder out there we can turn it into a degrader and very quickly start to understand the pharmacology. It gives us an opportunity to shorten the discovery path that leads to drugs." To find binders, Phillips says that they are searching the literature of known chemical space, as well screening for binders and designing some from scratch.

C4 was put together by Marc Cohen, the executive chairman, who, as a trustee at Dana Farber, has worked along with the institute's technology transfer group to find support for promising lines of work. "I'm particularly interested in accelerating the translation of discoveries, from the bench to the clinic, not leaving things hanging around on the shelf," says Cohen. In January 2016, the company launched with $73 million in a series A round from Cobro Ventures (Fairfax, VA, USA), a discovery venture firm founded by Cohen, and some super-angels. At its founding, C4 announced a partnership with Roche for undisclosed oncology targets potentially worth $750 million (upfront money was not disclosed).

Much remains to be worked out, according to Jaffrey. Right now, the strategy has been shown to work with only a few E3 ligases, those to which the imide binds. One question is whether degronimids can be developed that use other ligases, potentially some that are tissue specific. Also, the phthalimides could have some toxicities (class effects) that would show up when applied systemically. Expanding the chemistry to other ligases might help here, if they provide tissue specificity. Finally, the rate at which target proteins are degraded will have to exceed the target's rate of synthesis, particularly in the case of weak binders. These parameters will have to be established for each target, says Jaffrey.

Phillips, a native New Zealander who came to the United States 20 years ago as a postdoc, finds Cambridge a fabulous place to do science. And when he heard about Bradner's work, his first thought was: "Will it be a company?" The second: "How do I get a ticket on this train?" He says, "It's something that, to me, was honestly the best opportunity I've ever seen."

Now 50 people strong, the company is deeply invested in oncology with its Roche partnership, but it is also looking to targets in infectious diseases and other indications. At the moment, Phillips feels that there's tremendous excitement in the space, but so far, they have only a handful of tools in the toolkit. "To really make the difference on the scale we'd like to impact, we need additional tools. We need to know which E3 ligase is the next cereblon," he says. ***LD***

Gadeta: extending the reach of CAR T cells

By targeting the γδ T cell receptor, Gadeta combines immunotherapy with metabolomics. Chimeric antigen receptor T cell (CAR-T) therapies are electrifying the cancer field but have so far been aimed only at CD19-bearing malignancies. Progress in other cancer types, particularly in solid tumors, has been stymied by a lack of suitable targets as well as insufficient homing of CAR-T cells to tumor sites and poor persistence of the transplanted cells[17]. Dutch biotech firm Gadeta (Utrecht), a spinout from the University Medical Center Utrecht (UMC Utrecht), is putting a new twist on adoptive T cell therapy, by grafting T cell receptors (TCRs) derived from γδ T cells (γδ TCRs) onto conventional T cells, which ordinarily express α and β TCR chains. This approach, originally developed in the lab of Jürgen Kuball, Gadeta's scientific founder and CSO, could bring a new set of tumor-associated antigens into play, at the same time building on the manufacturing and clinical development know-how the CAR-T cell therapy field has generated. This theory will soon be put to the test in a clinical trial of aβ T cells engineered to express a γδ TCR in leukemia or multiple myeloma set to get under way this year.

Jürgen Kuball, Gadeta CEO

First discovered in the 1980s, γδ T cells combine characteristics of both the innate and adaptive immune systems. Unlike aβ TCRs, γδ TCRs do not require major histocompatibility complex (MHC) molecules to recognize their target antigen. Along with innate immune effector cells, γδT-cells respond rapidly to cellular stress or infection, before an aβ T cell response emerges. Although they constitute a minority of circulating T cells in the blood, γδT-cells are highly abundant in epithelial tissues[18]. They appear to have a key role in cancer immune surveillance by recognizing newly transformed cells during the initial steps of tumorigenesis. "Each time a cell turns out funny, these cells kick in," says Kuball, who also chairs the adult hematology department at UMC Utrecht. An unexpected finding from a recent gene expression analysis of 18,000 human tumors has further galvanized interest in their therapeutic potential. The study, led by Ash Alizadeh at Stanford University, found that the presence of γδ T cells in patients' tumors—inferred by computational analysis of bulk tumor transcriptomes—was associated with a better clinical outcome than the presence of 21 other leukocyte populations, across 25 different cancer types[19].

Early efforts to develop cancer therapies that employ γδ T cells foundered owing to poor persistence of the transferred cells[20]. "It's very hard to get them to proliferate," says Kuball. This is particularly difficult in patients with advanced cancer, even though they can readily mount aβ T cell responses to an infectious agent. His group's effort to use aβ T cells as carriers for γδ TCRs showed preliminary *in vivo* and *in vitro* proof of concept several years ahead of Alizadeh's findings[21]. "It was the first circumstantial evidence that we were able to target leukemic stem cells," he says. Since then, the company has developed a screening platform, combinatorial T cell receptor exchange (CTE), to identify optimized, high-affinity combinations of γ and δ TCR chains that respond to metabolic changes in cancer cells. "We're able to target not only hematological malignancies. The pathway we're targeting is a metabolic pathway that is active in many cancer cells," Kuball says. So far, the company has identified more than five candidate gδ TCRs that are at various stages of development.

The overall approach is not obvious, particularly as it is not clear precisely how gδ TCRs interact with their targets. "Intuitively, it doesn't make a lot of sense. If it works, it's cool," says Immo Prinz at the Institute of Immunology, Hanover Medical School (Hanover, Germany). "It's possible that the threshold to activate these chimeric aβ T cells is lower," he says. "That could be the big advantage." Long-term persistence of the transplanted cells appears not to be a problem in challenge experiments, Kuball says, although clinical trials will have to determine whether this holds true in humans.

Kuball was joined as co-founder and CEO by Marc DeBoer, venture partner at London-based Medicxi Ventures (previously the life sciences arm of Index Ventures), who had to overcome his initial skepticism. "My first

perception was those cells were difficult. What do they recognize?" he recalls. He became increasingly intrigued—particularly, he says, as he learned more about how γδ TCRs interact with their targets. "γδ T cell receptors seem to recognize their targets more like an antibody in a protein–protein interaction," he says. As a self-described "antibody guy," he found this an attractive proposition. However, the financing strategy for building out Gadeta's cell therapy approach is radically different from what an antibody development program requires. Medicxi—and Index before it—is closely associated with an asset-centric financing model, which typically involves the creation of a virtual company around a single program. DeBoer is conscious of the need to build out the company's technology platform, which requires an in-house scientific team, as well as Kuball's research group at UMC Utrecht.

The company is currently expanding its staff and has enough cash, including an innovation loan from the Dutch government, to take it through a first clinical readout. Its strategic plan, which covers the next three years, calls for a significant uptick in investment. "We need about $40 million–50 million to really get solid proof of concept on the technology in solid tumors," DeBoer says. "We are comfortable we will raise the money in one or two steps, starting this year."

First out of the gate is an investigator-initiated study to treat patients who have leukemia or refractory multiple myeloma with TEG-001, an αβ T cell engineered to express a γδ TCR that is activated by butyrophilin-3A1, a conformation of CD277 that results from cancer-induced changes in cholesterol metabolism[22]. A company-sponsored trial of TEG-002, which also targets CD277, in solid tumors will follow in late 2018 or early 2019. *CS*

Lodo Therapeutics: natural products from soil metagenomics

Heterologous expression of biosynthetic gene clusters identified via soil metagenomics opens the path to new chemical space. Microbes have been the starting point for drug discovery efforts for decades, at least since Alexander Fleming isolated penicillin from *Penicillium rubens* in 1929. More than 60% of all FDA-approved anti-infective and anticancer drugs have been derived from environmental microbes. But all have come from microbes that can be cultured, leaving a huge untapped potential from the roughly 99% of soil microbes that cannot be cultured. Lodo Therapeutics (New York) is applying its large-scale metagenomic profiling technologies to uncover bioactive compounds from soil microbes that so far have been overlooked.

Lodo was founded on the basis of work done by Sean Brady, head of The Rockefeller University's Laboratory of Genetically Encoded Small Molecules. Brady has spent eight years devising the most efficient way to access natural molecules from unculturable soil bacteria. He starts with DNA isolated directly from soil, which can contain upwards of 10,000 different microbial species. Then, using a mix of degenerate primers of similar, but not identical, sequences, Brady conducts PCR on the soil DNA samples to find motifs that are conserved across biosynthetic pathways of interest. These motifs are sequenced to generate what he calls natural product sequence tags (NPSTs) and compared to other bacterial genomic sequences using computational searches of curated genetic reference databases. Based on these *in silico* searches, Brady can prioritize which soil samples have the highest chances of containing novel biosynthetic gene clusters of interest, including those from cryptic DNA. He then creates large-insert (40 kb) cosmid libraries for discovery studies. The cloned gene clusters can be engineered into model microbes and heterologously expressed, and the resulting biosynthetic compounds isolated and characterized. "We've essentially figured out a way to clone and sequence whole environments, and then pull out biosynthetic gene clusters of interest to discover new compounds," Brady says.

Sean Brady, Lodo founder

Using this platform, his team has identified several novel compounds as potential starting points for new therapeutics. For example, they searched for epoxyketone proteasome inhibitors from 185 soil samples from around the world and found 99 unique epoxyketone NPSTs. They were then able to recover nine complete gene clusters associated with the NPSTs. Heterologous expression in five *Streptomyces* host strains yielded seven potent epoxyketone proteasome inhibitors, including some with novel warhead structures and a naturally occurring halohydrin prodrug[1].

Although these various technologies are not proprietary, Brady has been able to scale them up to analyze hundreds of soil samples in a month. "About three years ago, I decided that what we'd developed in the lab had to move out of the lab in order to scale it up and translate into real drug discovery," Brady says. Around that time, he serendipitously caught the eye of the Bill and Melinda Gates Foundation (Seattle), which provided funding for his lab to discover compounds for treating tuberculosis—a global health problem due in part to the emergence of multidrug-resistant strains. Rockefeller University's technology transfer office reached out to Accelerator Corporation (Seattle), which is a syndicate of venture capital investors including ARCH Venture Partners (Chicago) and state funds such as the Innovate NY Fund and others. In January 2016, Accelerator launched Lodo with a $17-million series A round.

Lodo is maintaining its initial focus on tuberculosis. The company's lead compound is undergoing optimization, which has increased its activity against drug-resistant strains. Lodo is also looking for compounds against Gram-negative bacteria, an area with great unmet medical need. CSO and co-founder David Pompliano, a partner at Apple Tree Partners (New York), points out that there are evolutionary reasons to focus on leads derived from antibacterial natural products. "Molecules developed by nature are under selection pressure. One pressure is an arms race for microbes to develop antibiotics to kill other microbes, then those microbes develop resistance to those antibiotics, and new antibiotics evolve, and then more resistance and on and on. We have found some compounds that look like they will overcome resistance," Pompliano says.

The New York–based startup plans to expand its efforts into indications such as oncology and immune-related diseases, for which many treatments have been derived from soil microbes. Pompliano notes that the interplay between humans and microbes has existed since humans first evolved, and bacteria have evolved ways to suppress inflammation and the immune response. UCSD's Rob Knight agrees that Lodo's initial disease areas "are good places to start." He added that he is aware of other companies attempting to mine the soil metagenome using an approach similar to that of Lodo, "including major agribusinesses."

One company with a head start on Lodo is Warp Drive Therapeutics (Cambridge, MA, USA), which launched, to great fanfare, in 2012. To date, Warp Drive has focused its efforts on discovering novel natural products against drug-resistant infectious agents. It has amassed a database of 135,000 actinomycete genomic sequences, containing 148 complete genome sequences and 148 biosynthetic gene clusters that encode the enzymes to make ~3.5 million natural products. Last November, the company achieved its first milestone, providing a set of novel aminoglycoside antibiotics to Sanofi (Paris).

At the moment, Lodo has ~$20 million on hand, which is expected to last several years, according to David Schubert, COO of Accelerator. Lodo is in active discussions with potential partners with an eye for a company that can provide complementary expertise. "Our core expertise is to discover molecules. Once they are generated, then we will figure out what to do with them: develop them ourselves or find a partner," Brady says. ***AB***

Exicure: nucleic acids come full circle

Enhanced cellular uptake of spherical nucleic acids aims to unlock therapeutic applications beyond the liver. Nucleic acid–based therapies have long wrestled with the challenges of efficient cellular delivery and low immunogenicity. Advances in chemical modifications in oligonucleotides over the past few decades have minimized these issues, but Exicure (Skokie, IL, USA) thinks it has found an approach to trump them all. The startup is developing its spherical nucleic acid (SNA) platform, which consists of densely packed oligonucleotides arranged radially on a liposomal (e.g., 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPC) nanoparticle. The company is using the technology to attack diseases via a variety of mechanisms, including antisense oligonucleotides, small-interfering RNAs (siRNAs), microRNAs (miRNAs), aptamers and CpG Toll-like receptor 9 (TLR) agonists.

John Giljohann, Exicure CEO

SNAs were first developed by Chad Mirkin (Northwestern University, Evanston, IL, USA) in 1996. The multimeric SNA constructs were found to bind complementary extracellular nucleic acids rapidly, and Nanosphere, was founded to commercialize diagnostic applications of the technology. While Exicure CEO David Giljohann was obtaining his PhD at Northwestern with Mirkin, he worked on methods for delivering nucleic acids into cells. When he tested SNAs, he tried traditional delivery technologies, such as encapsulation with lipids and polymers, to enhance uptake. "These delivery technologies worked okay," he remembers. But when he looked at SNAs without any transfection vehicle, he found, to his amazement, that the control worked best. "It turns out the SNAs go into cells all on their own," Giljohann says. Furthermore, SNAs enjoy high cellular uptake in many tissue types.

On the basis of this discovery, in 2011 the group formed AuraSense, Exicure's predecessor, to develop SNA-based therapeutics. The company's underlying premise was that the three-dimensional orientation of SNAs would allow their use in treating diseases throughout the body, not just in the liver—the latter being the predominant focus of most other nucleic acid therapies because it takes up non-nanoparticle oligonucleotides more readily.

In a 2013 publication, AuraSense described the mechanism by which SNAs enter cells: they are actively taken up by class A scavenger receptors located on the cell surface[23]. The company has thus far found the scavenger receptor on more than 60 different cell types, including those that have proved difficult to target with oligos, such as the brain, eye, skin, lungs and gastrointestinal tract. "With the exception of mature red blood cells, we have yet to find a cell type that does not take up the SNAs," Giljohann says.

Giljohann is unsure why the scavenger receptor takes up SNAs so readily, but, he says, "We think there is some multivalency recognition." He says that if there are ten nucleic acids on the SNA, the receptor will ignore it, but if there are 50 or more, then it will take them up. Once the SNAs enter the cells, they are protected from nucleases by virtue of their density, which impedes nucleases from latching onto any single oligo strand. In addition, the negatively charged cloud around the outside of the SNA structure attracts a positive-charged 'salt cloud', which inhibits nucleases.

Exicure has published work showing that SNAs can perform any mechanism performed by simple RNA oligos. "Cellular uptake of SNAs is agnostic as to what type of RNA is on there, but once inside the cell you can leverage all different pathways," Giljohann says. The company is focusing its drug discovery and development efforts on two mechanisms: immunomodulatory and antisense. The company's lead molecule, AST-005, uses antisense synthesized using phosphoramidite to target tumor necrosis factor (TNF), a proinflammatory cytokine. A topical gel formulation of AST-005 recently completed a phase 1 trial in 15 patients with chronic plaque psoriasis, meeting safety and tolerability endpoints.

In December 2016, Exicure partnered with Purdue Pharma (Stamford, CT, USA) to develop AST-005 for mild-to-moderate psoriasis. The pharma obtained full worldwide development and commercial rights to the compound, as well as an option to three additional collaboration targets. Financial details of the deal were undisclosed but included an upfront payment and equity investment, which, together with potential milestone payments, could total up to $790 million plus royalties.

Exicure has three other topical antisense SNAs in preclinical development in the dermatology space: XCUR17, targeting interleukin 17 receptor A (IL-17RA) to treat mild-to-moderate psoriasis; an SNA targeting interleukin 4 receptor A (IL-4RA) to treat mild-to-moderate ectopic dermatitis; and an SNA targeting interleukin 1β (IL-1β) to treat epidermolysis bullosa simplex. In the first half of 2017, AST-005 is expected to begin a phase 1b trial, and XCUR17 to begin a phase 1 trial, with data expected from both by year's end.

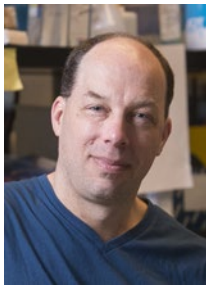
The company also is developing SNAs for cancer that agonize TLR9, which activates the immune system. Exicure has published data showing that compared to monovalent oligos of the same sequence, its SNA constructs have 80-fold greater potency *in vitro*, 700-fold higher antibody titers in mice and 400-fold higher cellular responses to model antigen in mice, resulting in improved treatment in a mouse model of lymphoma[24]. The company believes that this stronger innate immune response will lead to a greater therapeutic efficacy than competing oligo technologies. The lead in this program, AST-008, is expected to enter a phase 1 trial in combination with a checkpoint inhibitor to treat solid tumors in the first half of 2017.

When AuraSense morphed into Exicure, the company switched from a gold nanoparticle to a DOPC-liposomes. Apart from AST-005 and AST-008, the rest of the company's pipeline is in early stage and is employing other local delivery technologies. Giljohann says the company has done preliminary work on a simple saline formulation for delivery to the eye for ophthalmic indications, a nebulized formulation for delivery to the lungs and an oral formulation for delivery to the gastrointestinal tract. He notes that such local delivery has benefits over systemic delivery, namely a reduction in systemic side effects, lower costs and increased efficacy, "because you're putting all of your drug where you want it to go and not fighting any distribution problems."

Exicure last reported raising funds in February 2016, with $33.6 M in funding bringing its total to $42.6 M, including earlier funding from Bill Gates and other high net worth individuals. Giljohann says the company currently has enough cash to last a year and is expecting to receive some milestone payments from Purdue in 2017. Exicure also is currently working with a number of additional pharmas on undisclosed targets and may be announcing additional partnerships. ***AB***

Agenovir: editing antivirals

Transforming CRISPR–Cas9 into a human antiviral treatment for treating latent infections. Viruses have a nasty habit of hiding out in reservoirs in various places around the body, only to reappear and cause further mayhem at some later date. Stephen Quake of Stanford University had what he calls a moment of inspiration when he envisioned a role for gene-editing tools—particularly CRISPR–Cas9, the most recently discovered one—in ridding people of latent viruses. "It seemed like we ought to use them like God intended them, as antiviral agents," he says, referencing the fact that CRISPR is a bacterial system for destroying invasive phage. From this insight now comes Agenovir, spun out from Stanford, moved to an incubator run by Johnson and Johnson incubator (JLABS; S. San Francisco, USA, CA) in November 2015, and raising $10.6 million in a series A round to develop therapies for attacking viruses as they lie dormant in cellular reservoirs.



Steve Quake, Agenovir founder

In 2014, Quake's group provided a proof of principle in a model system for latent virus, the Raj cell line, which, cultured from patients infected with Epstein–Barr (EB) virus, retains on average 45 copies of the viral genome per cell. They designed a set of guide RNAs to target EBV genes required for viral replication, for membrane proteins and structural (repeat) regions scattered around the genome. The idea was that these guide RNAs would fragment the genome into pieces, eliminating any possibility of recovery or repair of the damage by the viral machinery. Transfecting Raj cells with combinations of these guide RNAs resulted in impaired cell replication; in addition, the number of viruses per cell was reduced between 65–85%, compared to untreated control cells. In a fraction of cells, the entire reservoir was apparently eliminated—no viral sequences were detected[25].

When applying gene-editing tools in human therapeutic settings, off-target effects are a major concern. However, Quake points out that in this case, no human sequences are targeted, as they are taking aim solely at viral genes. "It's very far from the human genome, so you have a much easier problem to solve," says Quake. Furthermore, unintended effects that might compromise viability, which would be undesirable when editing a human gene, would instead be desirable, in the case of virally infected cells.

Nonetheless, the fledgling company has expended considerable effort in making sure their guides are as specific as possible, according to Agenovir CEO Dirk Thye. "Off-target activity is an important safety concern for the first products in this new field and therefore important to us," he says. Using *in silico* approaches, the researchers first identify the most conserved regions of the viral genomes to capture as many serotypes as possible while choosing regions that affect genes that are critical to the life cycle. They use various algorithms to calculate the probability of having incidental homology to human genes. Using these filters, Thye reports that they are reaching the limits of specificity (99.9%) reported in the literature by different methods. "The *in silico* methods give us a good starting point for estimating specificity of the target, but then we empirically test the off-target activity using the GUIDE-seq assay. This is important because the software algorithms are far from perfect, and used primarily as a screening tool," says Thye.

The company has judiciously chosen human papilloma virus (HPV) for their first target. It is confined to a easily accessible sites—the anus and the cervix—where it has reservoirs in the mucosal epithelium that can cause high-grade squamous interepithelial lesions. In the cervix in particular, these latent infections can cause cancer; cervical cancer kills 250,000 women worldwide annually. This means that local application is possible, simplifying delivery, which is a major issue yet to be solved with systemic *in vivo* gene-editing-based therapies. Furthermore, taking a topical approach, which would mean that systemic exposure of the drug is expected to be negligible, will further mitigate any concern for off target activity.

Agenovir's initial product will contain a single guide RNA targeting a gene encoding a key (undisclosed) viral enzyme and packaged with the mRNA for Cas9 in undisclosed cationic lipid nanoparticles. Thye thinks this is the most efficient pathway from proof of concept to an approved drug, but he concedes that in the future the company might employ multigene strategies, which have proven efficient in model systems.

Dirk Thye

However, whether multigene strategies are required—that is, whether total annihilation of the viral genomes is necessary for a good clinical outcome—is unknown. Joel Palefsky, a clinician at UCSF and a consultant with Agenovir, says this is totally unchartered territory. Palefsky works with patients who have AIDS, where the incidence of high-grade lesions from HPV is 50%. (With uninfected men who have sex with men, the incidence is 25%; in the general population, the incidence of lesions is lower.) "We really don't know what the natural history is of a really small focused latent infection," he says. Even if not every cell harboring virus is destroyed, the reservoir may be knocked down to the point that it behaves as it would in a person with a normal immune response, which can control a small focus. Quake concurs: "Most of the viral experts we talked to believe that once you knock it down, the immune system will take over and do the rest for you," he says. "When all is said and done, it's like everything else; I think we're going to assess that in the human model," says Thye.

Robert Jan Lebbink, at the University Medical Center in Utrecht, who works on CRISPR–Cas9 gene editing of herpes viruses, feels Agenovir has mapped out a good strategy for proof of principle, which may well extend to other viruses, such as several herpes viruses that reside in specific neurons at defined locations in the body. Upon reactivation, these viruses can cause diseases such as recurrent eye infection and shingles. However, he points out that local administration of CRISPR–Cas9 will work only for a subset of viruses. Many viruses have latent reservoirs scattered throughout the body or in hard-to-reach places. "Such viruses will be more challenging to combat, as antiviral CRISPR–Cas9 would need to be administered systemically, or (if applicable) used to cripple or eliminate the viruses *ex vivo* when used for transplantation purposes," he says.

In addition, the potency of antiviral CRISPR targeting is unknown. "At this point, there is a need for more detailed studies on the *in vivo* potency of antiviral CRISPRs targeting virus at defined sites by using local administrations," says Lebbink. Aside from the risk of establishing virus escape mutants due to CRISPR–Cas9 editing at the target site, he says, there could be immunological responses, for example, to the Cas9 protein, that might limit efficacy. However, Thye believes that a small amount of inflammation could be beneficial, as it could stimulate the immune system in the area and help clear out the residual cells. Furthermore, in places such as the liver, where all the cells might be infected, eliminating them all might create other problems.

Thye says that a lot of progress in HPV has been made since the company launched, and he is poised to reveal their lead product candidate

in Q2 of this year. The initial indication will be anal high-grade squamous intraepithelial lesions in men, where the prospect of reproductive toxicity is less than in women. *LD*

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