Nature Biotechnology's academic spinouts of 2015

Aaron Bouchie & Laura DeFrancesco

Immuno-oncology was hotly pursued by investors in 2015, along with drug delivery platforms. In the agbiotech world, a systems biology company set up shop.

Private investors looked kindly on biotech in 2015. Twice the amount of private money flowed into biotech than in 2014, which itself had been a high point compared to the previous five years of relative stagnation.

For this year's survey *Nature Biotechnology* has chosen companies developing groundbreaking technology that were also among those that received the most funding in 2015. Firms were limited to those originating from academic institutions, prioritized on the amount of series A funding and assessed by our editors for novelty and innovation of their focus and/or platform. Companies in 'stealth mode' were excluded and some companies that had larger A rounds in 2015 were excluded because they were not deemed of interest.

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.

Gritstone Oncology and Neon Therapeutics: hunting neoantigens for cancer vaccines

Next-generation sequencing applied to immune monitoring in the search for effective cancer vaccines. The idea of invigorating a patient's immune system to fight tumors has been around for decades, but dozens of cancer vaccine programs have crashed and burned in the clinic. Two groups of researchers, with backing from eight venture capital firms between them, are betting that this is about to change. Checkpoint inhibitors—a class of drugs that unleashes an immune response against tumors by blocking immune-dampening molecules—have opened a window on the intricacies of tumor immunology and promise to reinvigorate the search for new therapies and cancer vaccines.

What set off this recent spate of startup activity is the observation that those patients who respond to checkpoint inhibitors have a larger mutational load in their tumors than those who don't. This has added proof to the notion that some of the hundreds to thousands of mutated proteins generated by the genomic instability of tumor cells—now referred to as neoantigens—must be recognized by the immune system, an idea first raised by immunologist James Allison (MD Anderson Cancer Center, Houston), who went on to develop the first checkpoint inhibitor drug against CTLA-4. Proof that such immune cells exist comes from Steven Rosenberg's work at the US National Cancer Institute (NCI) on tumor infiltrating lymphocytes (TILs). After decades of effort, in 2013 he showed that in some melanoma patients, TILs cause tumor regression owing to the presence of tumor-targeting cells in the population[1]. In 2014, Rosenberg provided the best evidence to date, and expanded the reach of this approach beyond melanoma—admittedly a cancer with a high mutational load. He showed that administering $CD4^+$ T cells that react against a single mutation ($ERBB2IP^{E805G}$) in the erbb2-interacting protein (ERBB2IP) mediated a durable regression of metastases in a patient with metastatic epithelial bile duct cancer[2].

According to Rosenberg, "This is a very exiting area, one that I am spending virtually all of my time [on] here at the NCI."

Gritstone Oncology team: (left to right) Graham Lord, Roman Yelensky, Andrew Allen, Jean-Charles Soria, Matthew Hawryluk, Tim Chan, Mark Cobbold.

But a mutated protein does not a vaccine make—that is, not until it has been shown to be immunogenic, and that is not a simple matter. As laid out by Andrew Allen, CEO of Gritstone Oncology (Emeryville, CA, USA), it is a multistep process, starting with whole genome sequencing of tumor and normal cells to compile a list of mutated proteins. The list, which can run as long as thousands of molecules, must be pared down to those that could be immunogenic (i.e., mutant peptides, produced by cellular processing machinery, that traffic and bind to human major histocompatibility complex (MHC) molecules, that are presented on the surface of tumor cells, and are capable of stimulating a productive T-cell response). Developing algorithms that identify neoantigens with the necessary predictive power remains a work in progress. Genentech's (S. San Francisco, CA, USA) Lélia Delamarre, who is doing structural modeling using mass spectrometry of potential neoantigen peptides bound to MHC proteins, points out that it is no accident that both neoantigen companies, Gritstone and Neon Therapeutics (Cambridge,

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MA, USA), are built around strong informatics expertise. Gritstone has a proprietary predictive model that is being trained on cancer patient samples, according to Allen.

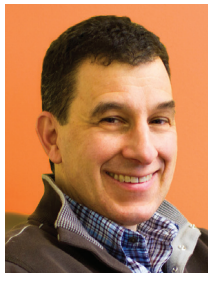
Within weeks of each other in October 2015, Neon Therapeutics and Gritstone Oncology were launched to great fanfare, with Gritstone amassing one of the largest A rounds of 2015 at $105 million. Allen, an immunologist by training with an interest in cancer, didn't see a clear future in cancer vaccinology back in 2006 when he joined Pharmion, where he was responsible for finding new therapeutics. "The insights and understanding needed for developing a tumor vaccine were lacking," he says, which certainly was borne out by the experience of cancer vaccines in the clinic. But in 2014, a paper by one of Gritstone's academic founders, Tim Chan at Memorial Sloan Kettering Cancer Center (MSKCC, New York), that reported a correlation between response to checkpoint inhibitors and mutational load in melanoma[3] made him rethink that. "That idea [that the immune response was directed against neoantigens] was an old one, but finally we had the technologies, particularly sequencing, and the T-cell analytics, to be able to put some flesh on the largely theoretical bone at this point. It was an 'Aha' moment," he says. Chan and colleagues published similar findings a few months later in non-small cell lung cancer[4].

From Chan's perspective, five or six years ago when he and his colleague at MSKCC Jedd Wolchok first embarked on a tumor sequencing project, it was a high-risk adventure that stood a good chance of failure. At the time, people thought the immune system was too complex, with too many different cell types, he says. But he scraped up $100,000 from various internal sources to start sequencing patients who responded to immunotherapies. And the signals they got were intriguing. "The more predictive neoantigens you had, the more likely you were to respond," he says.

Gritstone has negotiated an exclusive license with MSKCC for the inventions from Chan's and co-founder Nalyer Rizvi's groups. Among the intellectual property is the observation, somewhat controversial, that some of the neoantigens share certain motifs, specifically ones that resemble microbial sequences. According to Chan, as a geneticist looking at the data, he never had any doubt that the patterns he and his colleagues were seeing were nonrandom, and more recent data are "swinging their way." He points to recent work showing a connection between the microbiome and response to cancer immunotherapy. Two groups showed that the presence of particular bacterial species in the gut flora of mice correlates with response to checkpoint inhibitors, and furthermore, nonresponders could be converted to responders by a fecal transfer from a responding strain of mice (see profile on Evelo Therapeutics later in this article). To Chan, this says that there is a good chance that neoantigens are piggybacking off the immune-stimulatory properties of some intestinal microbes, and may in part be sculpted by a patient's immunologic history.

Allen has assembled what he sees as all the needed expertise among his founders and management team. In addition to Chan and Rizvi, Gritstone's founders include Mark Cobbold (Massachusetts General Hospital, Boston), who brings expertise in MHC biology; Graham Lord (King's College, UK), a cancer immunologist; and Jean-Charles Soria, a lung cancer specialist at Institut Gustav Roussy (Paris). Allen also spirited Roman Yelensky, a sequencing and bioinformatics expert, away from Foundation Medicine (Cambridge, MA, USA).

On the other coast of the United States, Neon Therapeutics is taking a broader approach, in accordance with the philosophy of Third Rock, their lead investor, whence their interim CEO Cary Pfeffer comes. According to Pfeffer, Third Rock prefers to build companies that work in broad areas of biology and can provide multiple product opportunities, as opposed to a single product. And so Neon is looking at cancer vaccines, both personalized and off the shelf, as well as T-cell mediated therapies. Pfeffer was familiar with the field of immune-oncology and some of its principals, through the 2013 immune company startup Jounce Therapeutics (another Third Rock company; Cambridge, MA, USA), founded by Jim Allison and others. Eric Lander, director of the Broad Institute (Cambridge, MA, USA) introduced the work of Catherine Wu at Dana-Farber (Boston), Nir Hacohen (Broad Institute) and Ed Fritsch (Dana-Farber) to his friends at Third Rock to see if they were interested. Knowing what Jim Allison, Bob Schreiber (Washington University, St. Louis, MO, USA) and Ton Schumacher (Netherlands Cancer Institute, Amsterdam) were doing at Jounce to flesh out the potential role of neoantigens in cancer therapy, Pfeffer says, they jumped at it and brought all these experts together as founders of Neon.



Cary Pfeffer, Neon Therapeutics CEO

Notwithstanding her role as co-founder of Neon, Wu maintains an academic interest in understanding the immunological basis of curative cancer therapies. Although she originally was focused on the graft-versus-leukemia effect that is the basis of marrow transplantation-based therapy, which she addressed through T- and B-cell-based expression cloning strategies, she soon was drawn to genomics as an unbiased way to discover relevant immune targets in patient tumors. "I had looked at it from the antibody perspective, using T-cell approaches for screening, but as we learned that next-generation sequencing was coming, this became an idea that I was committed to, having done all the laborious cDNA-based approaches before," she says. Wu and colleagues at Dana-Farber embarked on large-scale whole exome sequencing of chronic lymphocytic leukemia patients' tumor cells, looking for candidate neoantigens, and then circled back to patients to see if they had T cells that could respond to the neoantigens, and they did[5].

Catherine Wu, Neon co-founder

Having coupled a neoantigen-predicting algorithm with a method of doing human leukocyte antigen (HLA) typing from exome sequencing data[6], Neon feels they are set to bring their expertise to the clinic. In fact, Dana-Farber already has two peptide antigen clinical trials in melanoma and glioblastoma ongoing, and Neon is gearing up for trials in melanoma, smoking-associated lung cancer and bladder cancer, in collaboration with Bristol-Myers Squibb (Princeton, NJ, USA), one of the pioneers of checkpoint inhibitors, such as Opdivo (nivolumab). "We're trying to see—in addition to the immune monitoring data that we are going to collect which is going to be incredibly valuable—if we can improve responses to nivolumab in certain advanced cancer patients. We feel comfortable that we can deliver the vaccine to patients in a time frame that would [be] commercially reasonable and make sense for the patient," says Pfeffer.

Yet to be solved is a way to convert checkpoint inhibitor nonresponders, which remains the majority of cancer patients, into responders. Not all highly mutated tumors, for example, respond to checkpoint inhibitors, which points to other factors, like the tumor microenvironment, that might be playing a role. In addition, Chan wonders whether some of the peptides that bind less well may turn out to be the best prospects. Rosenberg concurs, saying that it might

be the twenty-seventh peptide on the list, which, if true, makes predicting immunogenicity challenging. But Delamarre has faith. "We should not underestimate technology. Technologies are moving forward very fast because there is an incentive to develop these new capabilities. Nothing is better than doing the experiment, see what the caveats are and where we can improve," she says.

Alkahest: young blood to fight aging

Plasma from young donors could yield new treatments for diseases associated with aging, like Alzheimer's and other degenerative diseases. Traditional methods of developing treatments for numerous severe neurodegenerative diseases like Alzheimer's disease and amyotrophic lateral sclerosis (ALS) have largely been futile. Rather than continuing with the standard approach of tackling one disease at a time, Alkahest (San Carlos, California, USA) is looking at them all as a function of organismal aging. The company is developing therapies derived from blood plasma, based on recent discoveries that factors in young plasma can help reverse brain deficits in older mice. Alkahest's progress landed it a $37.5-million investment from Grifols (Barcelona) in return for a 45% equity stake in March 2015. The two companies also announced an exclusive research collaboration to develop plasma-derived products to treat cognitive decline in aging and other disorders of the central nervous system. Under the deal, Grifols paid $12.5 million upfront and will fund product development, and is on the hook for milestone payments and royalties on any sales of resulting products.

Alkahest was founded based on research done in Thomas Rando's laboratory at Stanford University (Stanford, CA, USA). In 2005, Rando's group published work led by Irina and Michael Conboy that showed that heterochronic parabiosis—an approach in which surgical connection of the circulatory systems of an old and a young mouse—leads to regeneration of muscle and liver[7].

After Conboy left, Tony Wyss-Coray and Saul Villeda continued pursuing heterochronic parabiosis' impact on brain development in Rando's laboratory. They showed that blood from old mice negatively affects neurogenesis of healthy young mice brains, and that blood from young mice increases neurogenesis of healthy old mouse brains[8]. Ecotaxin, an inflammatory chemokine, was identified as a pro-aging factor, as it increases with age and correlates with reduced neurogenesis and impaired learning and memory in both aged mice and young heterochronic parabionts. By injecting plasma from young mice into old mice and vice versa—skipping parabiosis—they were able to replicate these results. Indeed, young plasma counteracted and reversed pre-existing effects of brain aging in old mice, such as reduced plasticity and cognitive function, at the molecular, structural, functional and cognitive levels[9]. One youthful factor they discovered is cyclic AMP response element binding protein (Creb) which, when activated in the hippocampus in aging brains, partially mediates structural and cognitive improvements.

By showing that they could rejuvenate synaptic plasticity and improve cognitive function without permanently connecting circulatory systems, the researchers opened up the potential for therapeutics. This advance led Wyss-Coray and biotech industry veteran Karoly Nikolich to form Alkahest in 2014 with $3.5 million in seed financing. They began a small clinical study to see if plasma from young donors (aged 30 or younger) could treat patients with mild-to-moderate Alzheimer's disease. This Plasma for Alzheimer Symptom Amelioration (PLASMA) study is currently recruiting 18 patients who will receive four weekly infusions and be followed for nine weeks. The primary endpoints are safety and tolerability as measured by adverse events, and feasibility as measured by number of subjects who comply with the research protocol. Secondary endpoints include various measures of efficacy, such as a change in the score on the 13-point Alzheimer's disease assessment scale for cognition. The company will also assess the composition of plasma to identify any components that might be associated with aging or Alzheimer's. Chairman and CEO Nikolich says he expects the study will be completed by year's end.

Karoly Nikolich, Alkahest CEO

Alkahest is in the process of setting up another, expanded version of the PLASMA trial. Whereas PLASMA uses donated plasma from the Stanford blood center, future trials will utilize plasma from Grifols, which has more than 150 collection centers in the United States and processes >12 million liters of plasma every year. This steady source of human plasma was a major attraction to the Grifols deal, Nikolich adds, as well as the Spanish company's excitement about the science. Indeed, Grifols is already conducting the phase 2/3 AMBAR (Alzheimer Management by Albumin Replacement) study to evaluate plasmapheresis with an infusion of human albumin and intravenous immunoglobulin in Alzheimer's patients. Alkahest also is designing more clinical studies in other neurodegenerative diseases. Nikolich says they haven't yet decided which specific diseases, but they are looking at Huntington's disease, amyotrophic lateral sclerosis, multiple sclerosis, diabetic neuropathy and post-surgery neuropathy.

Now at the University of California, Berkeley, Irina Conboy questions whether just delivering plasma or plasma fractions will be enough for therapeutic efficacy in humans, especially for diseases of the brain. She says that studies show pro-aging factors are dominant over youthful factors. She believes that a big reason for this pattern is that young mice also have a young set of kidneys, liver and other organs that can clear out the old factors, and this benefit of parabiosis goes away when you are delivering just young blood. Furthermore, many biologically active molecules won't cross the blood-brain barrier. She thus believes the answer is to inhibit old factors while also administering youthful factors. Conboy is currently pursuing a TGF-beta inhibitor, which she has shown rejuvenates hippocampal neurogenesis and muscle regeneration in old mice[10]. She plans to combine this knowledge with her earlier discovery that oxytocin is a youthful factor that rapidly improves muscle regeneration by enhancing aged stem cell activation and proliferation[11].

Nikolich agrees that this approach may be valid because aging leads to both a loss of good factors in plasma and an increase in detrimental components. He points to the discoveries by Wyss-Coray's group of both pro-aging and youthful factors as further evidence that both types of factors might be potential therapeutics. Nikolich says Alkahest is currently exploring some opportunities brought by potential pharma partners to develop therapeutic proteins, antibodies and small molecules that antagonize pro-aging factors or mimic mechanisms of youthful factors.

Wave Life Sciences: stereopure oligos

Pure stereoisomers of nucleic acid-based therapies could be safer and more efficacious than stereo mixtures. Nucleic acid-based therapies have had many well-documented struggles with delivery and immunogenicity. Decades of chemistry work has helped overcome many of these issues to the point where there are now dozens of candidates in late-stage development. Wave Life Sciences (Cambridge, Massachusetts, USA) believes it can improve on them all with its platform technology to design and manufacture stereopure oligos.

Wave is developing compounds that work through antisense, RNA interference (RNAi) and exon-skipping mechanisms. The company caught the eyes of numerous investors in 2015, during which Wave raised $18 million in a Series A round in February, $66 million in a series B round in August, and then $102 million in an initial public offering in November. As of April 13, 2016, the company had a market cap of $271 million.

According to president and CEO Paul Bolno, Wave's foundations began with a hypothesis from company co-founder Gregory Verdine of Harvard University (Cambridge, MA, USA), who wondered if chirality plays a part in the function of nucleic acid-based therapeutics. (A chiral molecule has two enantiomeric forms that are not superimposable.) Many small-molecule therapeutics have had their safety or efficacy improved by delivering a pure stereoisomer; Verdine wondered if that would also be the case for oligos because phosphorothioate linkages, commonly built into nucleic acid therapeutics to protect RNA from degradation, also create a chiral backbone. Consider that a 20-mer antisense compound like the US Food and Drug Administration (FDA)-approved Kynamro (mipomersen; Genzyme, Cambridge, MA, USA) for homozygous familial hypercholesterolemia, has 2^{19}—or 524,288—possible stereoisomers. Because nucleic acid therapies interact with enzymes—antisense therapies with RNase H to mediate gene knockdown and RNAi therapeutics with Ago2—it seemed plausible that stereo mixtures could hinder these interactions as well as enhance immunogenic interactions.

Paul Bolno, Wave Life Sciences CEO

To elucidate the role of stereoisomers in the safety and efficacy of oligo therapeutics, Shin Nippon Biomedical Laboratories, a contract research organization in Tokyo, formed two companies in 2009. Ontorii was formed in Boston around Verdine's design work, and Chiralgen in Japan to focus on scalable manufacturing methods developed by Wave's other scientific co-founder, Takeshi Wada. After the two companies had made progress and built some intellectual property, it made sense to combine them into a single entity in 2013, which is when Wave was formed and Bolno joined from GlaxoSmithKline (Brentford, UK).

Wave's platform technology can precisely control stereochemistry while building the oligos one nucleic acid at a time. The technology utilizes a proprietary chirally controlled process and novel monomers, as well as a design element that is part of the compound synthesizers. Making different stereopure forms of an oligo helps the company discover the rules that govern the compound's pharmacology, such as stability, specificity, activity and immunogenicity. Wave then couples this information with structure-activity relationship rational design to optimize its oligo.

Verdine has conducted multiple proof-of-concept studies establishing that Wave's technology can create stereopure oligos that are superior to parent mixtures. The first compound the company looked at was mipomersen, developed by Ionis Pharmaceuticals (Carlsbad, California, USA) and Genzyme. When Wave prepared hundreds of stereopure sequences and compared them to the FDA-approved mixture, they claim to have found some isomers superior to the mixture in the approved drug in terms of stability and catalytic activity. The company also has evidence that isomers with increased stability and catalytic activity were superior in efficacy and duration of response in the ApoB transgenic mouse model, with some compounds' activity lasting for weeks longer than the mixture. Wave also has studied its isomers in a complement activation assay and established that some designs have potential to reduce immunogenicity. The company is currently preparing a manuscript describing these studies for publication.

Wave currently has a pipeline of 23 candidates, with a goal of having six programs in the clinic by the end of 2018. The company's lead stereopure oligos are in preclinical development for Huntington's disease, which is caused by an expansion of CAG repeating units in the Huntingtin (*HTT*) gene. Because CAG repeats are present in both the mutated and normal gene, they can't be targeted. However, single-nucleotide polymorphisms (SNP), flanking the CAG repeats, have been identified that are specific for either normal or mutated *HTT* genes.

A stereo mixture of an antisense molecule can't discriminate the healthy *HTT* from the mutants, but Wave's stereopure isomers can silence mutant alleles while leaving the healthy allele functional. Bolno aims to take two of these chirally pure compounds into the clinic by the end of this year or early next year. Each of the two oligos targets a different *HTT* SNP, which together account for about 75% of those found in Huntington's disease patients.

The program next furthest along in Wave's pipeline is Duchenne muscular dystrophy (DMD), a fatal X-linked genetic neuromuscular disorder in which dystrophin is prematurely truncated and thus nonfunctional. Of the 3,500 boys born with DMD in the United States each year, 13% of them have mutations in exon 51 of the dystrophin gene. Wave is thus developing an oligo that utilizes an exon-skipping antisense approach to enable production of an internally deleted but partially functioning dystrophin. Although other companies are also developing exon-skipping antisense molecules to accomplish the same feat, Wave is counting on its stereopure oligo being best in class.

Bolno says the company plans to develop its central nervous system- and muscle-targeted products in-house, including the Huntington's and DMD candidates. Wave also is developing products in liver disease, skin diseases, eye diseases and gastrointestinal diseases. Bolno is looking for external resources to take those forward, either through partnerships or by spinning off assets into a separate company.

Codiak Biosciences: moving exosomes into the clinic

As exosome biology matures, diagnostic and therapeutic uses are emerging. In the past ten years, exosome biology has been steadily advancing, and these tiny, free-floating vesicles have gone from a curiosity to a potential tool as both a diagnostic and a delivery vehicle. Formed from the fusion of endosomes with the plasma membrane, exosomes contain a sampling of cell material, including DNA, RNA and protein. Once thought to be a dumping ground for cellular detritus, they are now known to traffic around the body—they are found in all bodily fluids—and pass material from cell to cell. Several companies have formed around the idea of using exosomes as an alternative to a biopsy, a so-called liquid biopsy; already an exosome-based diagnostic for lung cancer has made it to market[12].

Josh Levine Photography

Douglas Williams, Codiak Biosciences co-founder

Codiak Biosciences (Cambridge, MA, USA), which set up shop in November 2015 has a bolder idea, and the backing of $92 million in funding to take it forward. CEO Douglas Williams, a biotech veteran, most recently the head of R&D at Biogen (Cambridge, MA, USA,), thinks that exosome biology has reached a tipping point. "I became intrigued by the story and the possibilities of thinking of this as a mechanism for delivering macromolecules

into cells in a broad sense. It just struck me as an emerging area of biology with a lot of possibilities," he says.

Williams was ready to return to a small company environment, and took the advice of his longtime colleague Steve Gillis, a partner at Arch Ventures (Seattle), who suggested he take a look at exosomes. That brought Raghu Kalluri's work at MD Anderson Cancer Center (Houston) to his attention. After seeing a 2015 paper in which Kalluri described a sensitive method for detecting pancreatic cancer using exosomes[13], Williams was hooked. Arch became the lead investor and Gillis a director of Codiak. In that paper, Kalluri and colleagues characterized a population of exosomes with a unique surface protein (glypican-1) isolated from the blood of pancreatic cancer patients, the presence of which tracked with the severity of the disease. In mice, glypican-positive exosomes could be isolated from blood before lesions were detected by MRI, suggesting that they could be used for early diagnosis.

Raghu Kalluri, Codiak Biosciences co-founder

From his side, Kalluri and the MD Anderson team, which owned the intellectual property from Kalluri's discoveries, had initiated discussions with their contacts in the industry, realizing the broad potential of exosomes. In addition, he sought out Broad Institute's (Cambridge, MA, USA) Eric Lander, as Kalluri was looking to enlist a genomics expert, having first shown that whole genome sequencing can be done on exosomal DNA[14]. "This gives you a window on what is going on in the parenchymal cells in the body, including cancer cells," he says.

Providentially, Williams learned that Flagship Ventures (Cambridge, MA, USA) had an on going project on exosomes in their venture laboratory, and had been working with one of the leaders in exosome biology, the Swedish cell biologist Jan Lötvall, at University of Gothenburg, Sweden. Lötvall showed in 2007 that exosomes transfer information between cells[15], the finding largely credited with raising the level of excitement in the field. The two venture groups decided to join the intellectual property into one company and Codiak was formed with Flagship, along with Arch and MD Anderson as investors, Kalluri and Lander as co-founders, with Lötvall staying on as an advisor.

Codiak has plans to exploit exosomes as diagnostics as well as therapeutics. According to Williams, unpublished preclinical results on animal models from Kalluri's laboratory are hastening them into the clinic with an exosome therapy for pancreatic cancer, a notoriously difficult cancer to treat. Codiak is also developing an exosome to deliver inhibitory RNA (to an undisclosed target) and the plan is for MD Anderson to take the molecule into clinical trials by early next year. "We're being quiet about the specifics of that study other than to say that it's sufficiently compelling to want to move ahead into the clinic very rapidly, essentially to recapitulate in man what's been seen in the mouse models in pancreatic cancer," he hints. There are already ongoing investigator-initiated clinical trials with native exosomes, which Williams says give them some assurance that exosomes are safe to administer to people.

Paul Burke of Burke Bioventures (Cambridge, MA, USA), who previously was executive director, RNA Therapeutics at Merck, is impressed by the pace of progress on exosomes. "The understanding in that area of biology is changing almost day by day," he says. However, the biology is still not well understood, he feels. "The flip side is that the path to commercialization is in no way close to being clear. Cell-derived exosomes will be very difficult to commercialize," he says. From his days at Merck, he knows that it takes a lot of capital to turn a great new biology concept into a pipeline of therapeutics. "Alnylam burned a billion dollars, and Merck spent probably half that [on short interfering RNA (siRNA) delivery] but never reached the clinic," he says.

An alternative, according to Burke, are synthetic exosomes, admittedly "futuristic." But he says, "Thirty years from now we're going to look back at lipid nanoparticles…as the model-T."

Matthew Wood, of the University of Oxford, agrees that some engineering of exosomes is going to be needed. Wood reported in 2011 on work his group did to modify exosomes, loaded with siRNA, to express a tag that targets neurons[16]. In his hands, loading the payload was the tough part. "On the plus side, exosomes are natural and they have all sorts of valuable biological properties we don't fully understand, one of which seems to be very good ability to deliver RNA effectively into cells," he says. (Wood has also founded a company based on exosome technology himself, EvOx Therapeutics, Oxford, UK, which launched in early April.)

Kalluri would be the first to admit that there's a lot of exosome biology to be learned. His laboratory is trying to get a handle on how exosomes travel around the body, and what gives them a survival advantage over other kinds of nanoparticles. He plans to exploit their ability to deliver molecules intracellularly to attack previously undruggable targets. His laboratory has already shown that they can inhibit Dicer by delivering an antibody against it by means of exosomes.

Rubius: harnessing red cells to make deliveries

Work at the Whitehead Institute for Biomedical Research and DARPA to create a source of red cell prophylactics has morphed into a commercial entity. The Massachusetts Institute of Technology and Whitehead Institute's (Cambridge, MA, USA) Harvey Lodish has worked for much of his career on understanding the basic biology and developmental pathway of red blood cells with an eye toward medical applications. Six years ago, he received a call from a biotech company that had a contract with The Defense Advanced Research Projects Agency (DARPA, Arlington, VA, USA) to produce red cells in culture from bone marrow or cord blood stem cells, but they couldn't get it to work. Their erythrocyte precursors wouldn't mature into red blood cells that eject their nuclei. Could he help? For Lodish, there was an easy solution—the culture medium was short on iron-loaded transferrin, without which red cells can't make hemoglobin.

Harvey Lodish, Rubius co-founder

However, things didn't stop there. DARPA was after a ready supply of safe O-negative blood in the event of a national security threat. And although they now could produce red cells *in vitro*, the cost of culturing the cells was not competitive in the real world. So DARPA looked at the platform from a different angle. Daniel Wattendorf, then at DARPA's Biological Technologies Office, felt that there was potential value with a cultured blood product. "Instead of competing with the human donor blood supply, we could have a product with novel capability," he says. With that in mind, he and Lodish set out to find "something useful" for their platform, building a relationship between DARPA and the Whitehead.

In 2014, Lodish with his faculty colleague Hidde Ploegh published findings that were the first step toward this goal; he demonstrated

that red blood cells could be decorated with various molecules using the bacterial enzyme sortase to covalently attach molecules to the red cell surface[17]. To do this, he engineered mouse erythroid precursors to express sortase-modifiable proteins by attaching a five-amino-acid tag that the enzyme recognizes to two red cell membrane proteins, Kell and Glycophorin A. He showed that such engineered red blood cells could be produced *in vitro*, decorated with a small molecule (biotin) as well as a protein (single-domain antibody) and persist in the circulation of mice for 28 days.

In a Boston area pow-wow on synthetic biology, Noubar Afeyan, CEO of Flagship Ventures and a longtime friend of Lodish's, heard him talk about this work, and said to him afterward, "I think we have a company." In typical Flagship fashion, there ensued a deep dive into the technology, and they discovered that whereas others had attempted to harness red cells for various purposes, Lodish was just flat out better at manufacturing differentiated red cells. "We saw publications where people were claiming that they made red cells, and just by looking at the images you could tell they were not even close to red cells," according to Avak Kahvejian, a Flagship partner at the time, and now CEO at Rubius. "We realized that there were quite a few different dots we could connect to make this quite an impactful technology," says Kahvejian, who took the helm at the company at its launch in late 2015 with $25 million in Flagship money with Lodish and Ploegh as co-founders.

Kahvejian puts the potential for the technology into four buckets: enzyme replacement therapy, using the red cells as a biotherapeutic with enzymatic capability. The first program that Rubius is talking about does just that, in harnessing the cells to express a phenylalanine-degrading enzyme that would replace the enzyme missing in people with phenylketonuria (PKU). A second use would be to coat red cells with antibodies to neutralize or capture pathogens or otherwise unwanted proteins in circulation; to support this, Rubius has licensed some unpublished work by the Lodish laboratory in collaboration with Charles Shoemaker of The Tufts Veterinary School (Grafton, MA, USA). They have put a single-chain antibody (a camelid nanobody) against a particular toxin on red cells and have shown that mice are protected from multiple lethal doses of the toxin with only a half percent of their red cells expressing the nanobody. Providing capture antibodies in this fashion provides a longer window (the life of an erythrocyte is about four months), compared to the half-life of an antibody, which is measured in days. A third program makes multimodal red cells that can target and agonize or antagonize surface receptors on tissues. Kahvejian says they have figured out how to put multiple molecules on the surface, which he thinks will be useful, particularly in oncology. A final, more speculative, idea is to tether antigens to red cells to tamp down specific immune reactions. According to Kahvejian, evidence is mounting that red cell surface proteins induce anergy, which could find a place in autoimmune disease therapies.

Johannes Winkler, of the University of Vienna's Department of Pharmaceutical Chemistry, says, Rubius's approach "stands apart from other approaches, as it is especially attractive for having long circulation time in the blood and keeping it in the blood, not having it in tissue." But he points out, like all new technologies, it's too soon to say whether it will have commercial uptake. "The whole success of the approach depends on good selection of the diseases and targets. It's necessary to get into the clinic, and a lot of surprising things can happen there," he says. Rubius thinks they have made a good selection with PKU. For many PKU patients, the only remedy is diet, which is restrictive and hard to maintain throughout life, which leaves them in pain. For DARPA, the ability to scale up production is attractive, when thinking about mounting a rapid response to new or emerging pathogens. Initial proof-of-concept experiments show that only one milliliter of blood has a prophylactic effect in animals. "Imagine the possibilities if you only need one drop to protect a person from a toxin or pathogen, and we can scale up production of these kind of blood products. You could protect entire populations."

One limitation is that red blood cells can act only on places where circulation reaches. On the plus side, many of the regulatory concerns that typically come with cell-based therapies don't apply here. *In vitro*-differentiated red cells have two strong safety features in therapeutic settings: because the cells are enucleated, they pose no risk of transformation, and radiation can remove any nucleated stragglers before administering them to patients. "So you have a stem cell-like product with your protein of interest designed into it, but it no longer has the chassis of the gene therapy," Wattendorf says. Nonetheless, Lodish says he spends considerable time planning ways of getting the cells back, should some disaster scenario play out (although he couldn't think of one). The foreign protein itself could serve as a tag for removing the engineered cells, particularly if it's on the surface, if the need arose. "Our inclination is to go slowly, do PKU and one or two others, make sure that's working and then go to more interesting ones," he says.

Benson Hill team: Tom Brutnell, Matt Crisp, Todd Mockler

Benson Hill Biosystems: better photosynthesis through systems approaches

Benson Hill Biosystems aims to improve crop yields by applying systems biology to crop engineering. Improving crop yield through manipulating photosynthesis is not a new idea. But researchers at Donald Danforth Plant Science Center (St. Louis, MO, USA) think their systems biology approach to the problem will produce greater yields. Two Danforth researchers, Todd Mockler and Tom Brutnell, together with CEO Matthew Crisp have been putting this idea to the test since 2012 when they started Benson Hill Biosystems. After raising more than $2 million in seed funding, new series A investors got on board with an $8-million A round led by Middleland Capital, an agriculture-focused venture fund, with participation from Mercury Fund, Prelude Ventures, Prolog Ventures and others. Crisp describes the present state of agbiotech as somewhat archaic. "Agriculture already enjoys the highest rate of biotech penetration of any sector in the economy, and to put it quite bluntly, we're still using first-generation tools and technologies," he says. Benson Hill was set up to change that, he says, and "establish the core capabilities and the platform that would enable us and our partners to innovate and to do so more rapidly than was previously possible."

That's where Mockler and Brutnell come in. At Danforth, their groups had been developing bioinformatics tools and platforms for mapping out the gene networks that regulate photosynthesis, not just in the amount of CO_2 fixed, but also in time and space. "We use all the genomic knowledge, transcriptomic knowledge, understanding the predictive function of genes and so on to not only pick the candidates, but we predict the optimal expression level, so the promoters that express those genes put them at the right level in the right cell type at the right time of day under the right conditions," says Mockler, the lead bioinformatics expert in the group.

Over the past several decades, Brutnell and collaborators from Europe and China have been

developing methods to compare the transcriptional networks from groups of C4 plants—maize, sorghum and green foxtail—which are efficient at photosynthesis, and they have compared them to those from the less efficient C3 plants, such as rice. (C3 and C4 refer to the number of carbons in the first molecule produced by CO_2 fixation, 3-phosphoglycerate versus malate.) By looking for genes whose expression patterns matched those of classic C4 genes (a gradient of expression that increases as you move through the leaf from the base to the tip), they identified 478 candidate C4-specific genes, and from among them they could pick out 128 rate-limiting ones, by comparing one C4 plant against another and C4 plants to the C3 plant[18]. For example, some of the classic C4 genes (carbonic anhydrase and phosphoenol pyruvate carboxylase kinase) they find not to be rate-limiting, as their expression patterns in C3 and C4 plants are similar. Through comparisons of gene expression patterns of photosynthetic genes in C3 and C4 plants, they have also defined candidate lists of transcription factors that likely drive C4 photosynthesis[19]. From data such as these, they created a list of 38 novel candidate genes that might be useful to engineer better photosynthesis in C3 plants.

Mockler contrasts their approach with the more typical approach, where researchers typically choose one gene (albeit a rationale one), overexpress it and "then hope for the best." Instead, combining the information Brutnell and colleagues and others have acquired on spatial expression as well as what is known about circadian control, they can predict how a particular gene needs to be expressed and where in the leaf to improve photosynthesis. "Those predictions are made off the gene network modules, so we express something very selectively and carefully and that doesn't mean expressing it massively, like 100-fold. Sometimes it can be as modest as 1.5 times (50%) more than what the gene would normally be expressed." This sentiment is echoed by Crisp, "If you stick *35S* or a ubiquitin promoter in front of everything, you overexpress it, we, like many others, have learned, it's the wrong approach when you are trying to perturb metabolism," he says. "[It's like] using a sledge hammer instead of a scalpel."

Brutnell says he had been looking for a tractable model system to dissect C4 photosynthesis his whole career, and with *Setaria*, he thinks he has found it. By manipulating photosynthesis in *Setaria viridis*—a small transformable C4 plant with a tractable genome and a short life cycle so that thousands of lines can be grown rapidly in growth chambers—they have substantial percentage gains in yield both in seed and biomass, although in field trials with corn, Mockler says they are seeing more modest gains. Still, "Speak with someone in the industry" he suggests. "When they talk about step function gains they want to see a minimum of 8–10% gain." Martha Ludwig of the University of Western Australia (Perth), says that progress is always likely to be slow due to pleiotropic effects of the engineered pathways in particular crops—sometimes adding a new pathway can create havoc in endogenous pathways. "We can't just fast-track evolution in these plants," she says. "It's just going to be try and see—it's exactly what evolution has done—and see what works."

Crisp came to Benson Hill from synthetic biology company Intrexon, where he was working on the investment side before he moved over to run their agbiotech division. He says, "I really fell in love with this intersection of agriculture and synthetic biology." But what he learned at Intrexon is, "if you want to design and build the biological tools to do this, you need a strong underpinning of computational and systems biology expertise." Without having invested in computational and systems biology, which Benson Hill calls CropOS, he feels they would never have been able to raise institutional funds and succeed in the way they have succeeded thus far.

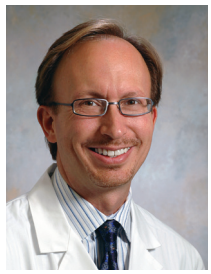
And by succeeding thus far, Crisp refers to the staff of 40 that he has assembled in sites in St. Louis and Durham, North Carolina, and the build-out of CropOS, which has generated candidate traits but also provides decision support for breeding and genome editing. "This process so far has been more successful than we would have anticipated," says Mockler. "Our hit rate, even for somebody who does this for a living and believes in this process, I've been shocked by our hit rate in terms of picking gene candidates that in our model systems and crops improve photosynthesis."

Evelo: microbiome meets immuno-oncology

Mining the microbiome for clues to counter immunosuppressive tumor microenvironments. The immuno-oncology field has been reignited since checkpoint inhibitors first arrived on the market in 2011. Despite impressive clinical responses in some patients, clinicians have observed response rates in many cancers of only 20–50%. This is spurring a search for ways of expanding the number of patients who can respond to these treatments by manipulating tumor microenvironments. Evelo Therapeutics (Cambridge, MA, USA) is hoping that the microbiome can provide clues for how to circumvent immune suppression.

The startup is the latest microbiome-based company spun out of Flagship VentureLabs, the Flagship Partners' unit that proactively scours academia for innovative research that can be combined to form a biotech company. Evelo CEO Simba Gill says the original transformative idea of using the microbiome to disrupt tumors had been incubating at Flagship VentureLabs for about three years while the partners formed a series of academic collaborations. One of those collaborations, with Thomas Gajewski of the University of Chicago (Chicago), resulted in a discovery that helped trigger the formation of Evelo in November 2015 with a $35-million series A investment.

Gajewski's group compared the growth of subcutaneous B16.SIY melanoma cells in C57BL/6 mice from Jackson Laboratory (Bar Harbor, ME, USA) to mice from Taconic Biosciences (Hudson, NY, USA). They found that tumors in the Jackson Labs' mice grew less aggressively because they had significantly higher tumor-specific T-cell responses and intratumoral $CD8^+$ T cell accumulation compared with Taconic's mice. The researchers found that co-housing the mice before implantation eliminated the differences, and they theorized that the microbiome was involved because mice tend to eat each other's feces. Indeed, feeding Taconic mice fecal material from Jackson Labs' mice before implantation delayed tumor growth and enhanced induction and infiltration of tumor-specific $CD8^+$ T cells.



Tom Gajewski, Evelo collaborator

The researchers further showed that ingestion of fecal material from Jackson Labs' mice was as effective against tumor growth as a checkpoint inhibitor. A combination of the fecal material with the checkpoint inhibitor was the most efficacious. By comparing fecal bacterial content over time with activated antigen-specific T cells within the tumor microenvironment, the researchers determined that *Bifidobacterium* was the beneficial bug. Indeed, treatment with oral *Bifidobacterium* worked as well as the anti-PD-L1 monoclonal antibody in the mouse model of melanoma, and together, the combination nearly eliminated tumor outgrowth. They further found that the bacteria modulated the activation of dendritic cells, which in turn improved the effector function of tumor-specific $CD8^+$ T cells[20]. Diane Mathis of Harvard Medical School (Cambridge, MA, USA) says the results of the study are striking, particularly considering that another group showed that anti-tumor effects of anti-CTLA-4 antibodies in mice relied on the presence in

the gut of certain species of *Bacteroides* in the same issue of *Science*[21]. She noted the key to this therapeutic approach will be to show that treatment with bacteria can contribute to reducing the size of established tumors in humans, rather than just slowing their growth.

Gajewski's results showed a clear role for specific bacteria in activating and priming the immune system to treat cancer, which Evelo calls bacterial immune activators. Gill says the microbiome also can be used to disrupt the tumor microenvironment by interfering with tumor metabolism and by modifying tumor interactions with stromal cells. These discoveries came from the observation that some bacteria are specific to particular cancers, bacteria they call cancer-associated bacteria or CABs. Gill says that although it has been known for over 100 years that some bacteria are correlated with certain cancers, the discovery of tumor-specific microbiomes is new. Many bacterial species associated with tumor-specific microbiomes can live anaerobically, which helps them survive in hypoxic environments.

In collaboration with several academic groups, Evelo is systematically building a database of CABs according to the tumor types they associate with, as well as which drugs, such as chemotherapies, CABs modify or are modified by. Gill claims the company has unpublished results that show that small genetic differences lead to very different biological activity. Evelo will use the resulting information to determine how they might home the bacteria of interest to tumors, as well as develop small molecules and antibodies that can disrupt the tumor–bacteria microenvironment.

He hopes to enter clinical trials in 2017 with oral bacteria products from Gajewski's discoveries, as well as some from Evelo's internal work on CABs. Although the company believes the approach will work for many types of cancer, they are focusing on tumor types in which checkpoint inhibitors have shown activity, such as non-small cell lung cancer, melanoma, renal cancer and bladder cancer. The company is looking at developing its products both as monotherapies and in combination with other cancer drugs, especially checkpoint inhibitors.

Rigontec: pathogen detectors engaged for cancer therapies

Oligos that mimic viral double-stranded RNA could prove effective in killing tumors. Cells detect the presence of invading organisms using pattern recognition receptors (PRRs), of which the most widely studied are toll-like receptors (TLRs). Recent work on an intracellular PRR, RIG-I, encoded by the helicase retinoic acid-inducible gene-I, suggests

Christian Schetter, Rigontec CEO

it could be exploited for battling tumors. Researchers at the University of Bonn (Germany) showed that targeting RIG-I has potent anti-tumor activity in mice. To capitalize on these advances, Rigontec (Bonn, Germany) was founded in 2014 to explore the use of synthetic RIG-I agonists developed by scientific co-founders Gunther Hartmann and Veit Hornung.

Hartmann and Hornung were the first to describe RIG-I's ligand, the 5′ triphosphate end of viral RNAs[22], and have since identified three different mechanisms by which RIG-I agonists could kill tumors. First, they found that human melanoma cells were more susceptible to apoptosis when treated with synthetic double-stranded RNA (dsRNA) RIG-I agonists *in vitro* and *in vivo*. Specifically, they found their synthetic dsRNA oligos reduced human tumor metastases in immunodeficient mice[23]. The researchers also found that interferon production induced by their agonists within tumor cells lead them to secrete chemokine-containing exosomes. The released chemokines activate natural killer (NK) cells, thus triggering the innate immune system within the tumor microenvironment. Finally, an adaptive immune response is triggered because the tumor cells destroyed by NK cells release antigens, as do the exosomes themselves, thus stimulating an antigen-specific T-cell response.

Rigontec will further develop the RIG-I agonists into immuno-oncology treatments. The company closed a EUR14.25 million series A in March 2015 co-led by investors Wellington Partners (Munich) and Boehringer Ingelheim Venture Fund (Ingelheim am Rhein, Germany). The company's lead compound, RGT100, is 20–24 nucleotides long with 5′-triphosphate and additional structural components required for optimal RIG-I activation. The compound's backbone also includes some conventional chemistry to increase stability and additional modifications to eliminate crosstalk to TLRs, which can lead to an inflammatory response. RGT100 is formulated with an undisclosed cationic polymer. The resulting complex is taken up by cells where the oligos activate RIG-I in the cytoplasm, and according to Hartmann, the compound actually works better as a complex, and the company doesn't thus need to worry about releasing the oligo from the complex.

Hartmann says that they have targeted several cancers in mouse models, with data still in preparation. He says they have shown that RGT100 works in mouse models of melanoma, ovarian cancer, lung metastasis melanoma, prostate carcinoma, colon carcinoma, mammary carcinoma, ovarian cancer hepatocellular carcinoma and fibrosarcoma, adding they have seen the product work with both intratumor and intravenous injections, including responses in distant tumors and metastases.

The company aims to get RGT100 into the clinic by 1Q17, according to CEO Christian Schetter. He anticipates the first phase 1/2 trial will begin in patients with lesional tumors, such as melanoma and other cutaneous tumors, which will allow intratumoral injections. This trial will also have a second arm focused on liver metastases. The company will look at delivering RGT100 systemically in an all-comers study with solid tumors, after which it could expand into indications that show the best outcomes or the best commercial opportunity. Schetter adds that Rigontec could combine its compound with other therapeutic modalities in these expanded indications. He believes that RIG-I activation will be safe and efficacious enough to work as a monotherapy, but he suspects that it would also work well in combination with drugs like checkpoint inhibitors.

Anna Marie Pyle, of Yale University (New Haven, CT, USA), who led a team that solved the structure of RIG-I in 2011 (ref. 24), says one concern about Rigontec's approach is the possibility of side effects due to the compound's activity in healthy cells. According to Pyle, RIG-I hyperactivity is linked to autoimmune disorders like Singleton–Merten syndrome and Sjögren's syndrome. However, so far, Hartmann has not seen any autoimmune responses in any of their mouse studies. He notes that introducing Rigontec's dsRNA constructs are similar to introducing a virus; doing so creates an alarm signal that there is a virus around, but there is no signal to kill a specific cell. The compound's anti-cancer properties are due to activity inside the cancer cells, not its direct action on immune cells. Another safety concern, that the compounds could trigger apoptosis in healthy cells, is kept in check by the upregulation of Bcl-XL in those cells in reaction to the dsRNA.

Gunter Hartmann, Rigontec co-founder

Another important aspect of RGT100 is that it is a short dsRNA, only 20–24 nucleotides long. Longer dsRNA molecules trigger another

helicase, MDA5, as well as TLR3 and protein kinase R (PKR), which trigger inflammation. Hartmann claims RGT100 was developed to not trigger any TLRs, and the company has not seen any inflammatory response as of yet in its mouse models. However, he does expect some mild flu-like symptoms due to the local type I interferon response. As these are locally induced rather than systemic, he hopes these reactions are less severe than symptoms caused by injections of recombinant interferon.

According to Pyle, small molecules could potentially be an effective and less costly way to stimulate RIG-I, and she notes some companies are developing compounds that target the helicase. But Schetter says Hartmann's group has structural data suggesting that small molecules will not specifically and effectively activate RIG-I, similar to what has been observed with attempts to target TLR9 with small molecules. They concluded that a complex molecule like RNA or an RNA mimic is needed to activate RIG-I. Schetter suspects that some of the reports of small molecules activating RIG-I may be off-target effects that resemble RIG-activation.

Rigontec is also using its platform technology to develop a pipeline of compounds, which will be pursued once the RTG100 clinical program is on track. Schetter says the company is developing compounds that combine RIG-I activation with gene silencing in a single molecule. Given RIG-I's natural function as part of the viral detection system, antiviral indications are logical indications for the technology. Hepatitis B virus and influenza are highest on Schetter's list, but the company would need partners with established know-how and disease models.

Schetter estimates Rigontec has enough cash to complete its phase 1 trial, but he plans on concluding a series B finance round later this year, which would fund the company's clinical program into the middle of 2019.

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Corrected after print 27 July 2016.

Erratum: Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells

Daesik Kim, Jungeun Kim, Junho K Hur, Kyung Wook Been, Sun-heui Yoon & Jin-Soo Kim
***Nat. Biotechnol.* doi:10.1038/nbt.3609; corrected online 18 July 2016**

In the version of this article initially published, the year in the received date on the first page was given as "2015," but should be "2016." The error has been corrected for the print, PDF and HTML versions of this article.

Erratum: No longer going to waste

Ken Garber
***Nat. Biotechnol.* 34, 458–461 (2016); published online 6 May 2016; corrected after print 27 July 2016.**

In the version of this article initially published, on p.460, columns 1 and 2, the number following "ACE-" was given as "2454" instead of "2494." The errors have been corrected in the HTML and PDF versions of the article.

Erratum: *Nature Biotechnology*'s academic spinouts of 2015

Aaron Bouchie & Laura DeFrancesco
***Nat. Biotechnol.* 34, 484–492 (2016); published online 6 May 2016; corrected after print 27 July 2016.**

In the version of this article initially published, on p.491, the subtitle read "double-stranded DNA" instead of "double-stranded RNA". The error has been corrected in the HTML and PDF versions of the article.

Erratum: Near-optimal probabilistic RNA-seq quantification

Nicolas L Bray, Harold Pimentel, Páll Melsted & Lior Pachter
***Nat. Biotechnol.* 34, 525–527 (2016); published online 4 April 2016; corrected after print 27 July 2016**

In the version of this article initially published, in the HTML version only, the equation "$\alpha_t N > 0.01$" was written as "$\alpha_{tN} > 0.01$." In addition, in the Figure 1 legend, the formatting of the nodes was incorrect (v_1, etc., rather than v_1). The errors have been corrected in the HTML and PDF versions of the article.