MAKING CELLS COMPUTE

By Karen Tkach Tuzman, Senior Writer

With efficiency in DNA engineering continuing to grow, and an increasing convergence between engineering and biology, synthetic biology — in its “truest” form — is coming within striking distance of translating preclinical proof of concept breakthroughs to the clinic.

And while chimeric antigen receptor (CAR) T cells could be prime beneficiaries of the advances, some basic tenets of the technology might present IP lawyers with new challenges.

The field is still carving its own definition and its boundaries, as companies across the spectrum adopt the “synthetic biology” label, blurring the line between simple engineering of individual genes and the original concept — which involves the artificial design and construction of new biological systems.

In its strictest definition, synthetic biology involves incorporation of two or more artificial components in a cell to carry out a novel combined function. That means that simply introducing a protein on an expression vector doesn’t qualify, but introducing two genes to a cell to express a protein under a set of qualified, contained circumstances does.

Broader use of the label encompasses the generation of the components to enable that process, such as synthetic biological building blocks ranging from artificial DNA bases to chimeric genes.

Although the field emerged in the early 2000s, it has been primarily tapped to engineer biosynthetic pathways in bacteria for creating industrial products such as biofuels or ingredients for cosmetics. The highest profile achievement thus far in drug development has been the engineering of yeast to generate artemisinic acid, the precursor to the anti-malarial drug artemisinin, which came out of a 2008 partnership between Amyris Biotechnologies Inc. (now Amyris Inc.), sanofi-aventis Group (now Sanofi) and Institute for OneWorld Health. Sanofi began marketing yeast-derived artemisinin in 2014.

But according to Wendell Lim, the field is now returning to applications in which “the cell itself is not just a factory, it's also a computer.”

“This field started with the computing capabilities of genetic regulatory networks, but those took a back seat as metabolic engineering became a lead application for synthetic biology. But now, I think we're really coming back to harnessing the unique capabilities of cells to be complex sensing and computing devices,” he said.
Lim is a professor of cellular and molecular pharmacology at the University of California San Francisco and scientific founder of Cell Design Labs Inc.

Cell Design Labs is one of a spate of newcos formed in the last few years that are engineering artificial sense and response circuits in cells. The company, which focuses on cellular immunotherapies, raised $34.4 million in a venture round last June led by Kleiner Perkins Caufield & Byers, Kite Pharma Inc. also invested, and signed a deal with Cell Design to use the startup’s CAR T cell on-switch technology, dubbed Throttle Switch, to make therapies for acute myelogenous leukemia (AML).

One of the leaders in the new crop is Synlogic Inc., which was founded in 2014 with backing from Atlas Venture. Following a $29.4 million Series A in 2014 and a $40 million Series B in 2016, the company disclosed on Tuesday a $42 million Series C round and announced it will become a publicly traded entity via a reverse merger with Mirna Therapeutics Inc.

CEO Jose-Carlos Gutiérrez-Ramos told BioCentury he was drawn to the idea of synthetic circuit-based therapeutics by the multi-functionality and control they afford.

"What attracted me about living biology that computes signals is that no other modality can sense and respond in this way," said Gutiérrez-Ramos. "You can have potency with conventional drugs, but they can't sense. With other living therapeutics, I think the potency piece and the control of sensing and responding is more blunted."

Atlas Venture partner and Synlogic chairman Peter Barrett said that logic prompted his firm to invest.

“What we started to realize is that there are things those cells can do that are very difficult for small molecules and antibodies to do, and that's to provide a full pathway of biological activity,” said Barrett. "Once we dug into it, the list of applications became very broad, and the challenge became, where do we feel we can best demonstrate the efficacy and safety of this approach in human disease?"

This year, Synlogic will take its lead product SYNB1020, a bacterial therapeutic that converts ammonia into arginine when it senses the anaerobic environment of the colon, into the clinic for urea cycle disorder (UCD).

Newer additions to the field include Gencirq LLC, a 2015 spinout from the University of California San Diego, and 2016 newco Senti Biosciences Inc., founded by Massachusetts Institute of Technology (MIT) professors. The companies are engineering synthetic circuits for bacterial and mammalian cell therapeutics, respectively. Neither has disclosed financing.

Although the newcos are pushing the applications of the technology in new ways, many in the field consider Intrexon Corp. a key early mover in the space. The company's RheoSwitch platform uses a pharmacologically-controlled switch to regulate therapeutic gene expression by engineered cells. Its lead product with that technology, Ad-RTS-hIL-12, is a DNA construct that enables IL-12 expression in the presence of the oral ligand veledimex and is partnered with Ziopharm Oncology Inc. The
CIRCUITING CELLS

Synthetic biology researchers are developing artificial genetic circuits that turn bacterial or mammalian cells into “computers” that respond to specific cues by executing programmed functions. Synthetic genes encoding circuit components are transduced into a bacterial or mammalian cell “chassis,” where they are expressed constitutively or conditionally depending on the logic of the circuit.

The artificial circuits include synthetic input sensor modules that detect specific cues in the cell’s environment, triggering activation of synthetic output function modules. The input and output modules are connected by signal processing machinery, which can include both synthetic and endogenous molecules.

Synthetic input sensors can take on a variety of forms, including extracellular antigen binding receptors (purple), ion channels that respond to changes in cell energetics (blue) or light-activated enzymes (green).

The signal processing machinery downstream of input sensors typically activates synthetic promoters to induce expression of synthetic genes. In clinically relevant systems, those output functions could include expression of a fluorescent diagnostic signal or a therapeutic protein such as a cytokine or mAb. More complicated circuits may generate additional sensors whose activation is needed to produce diagnostic or therapeutic proteins, thus creating “AND” gates that require multiple separate inputs to induce a clinically relevant response. They could also produce feedback machinery that boosts or dampens subsequent circuit activity.
compound is in Phase II testing for breast cancer, Phase I/II for melanoma and Phase I for brain cancer. Intrexon did not respond to requests for comment.

**IMPROVING SPECS**

The minimal requirements for the synthetic circuits include a sensing module that detects input cues, a signal processing mechanism that translates the input into new cell activity, and an output module that executes a response. Those circuits can be designed to obey logical operations, such as “AND” or “NOT” gates, that restrict the circumstances under which cells activate the output functions (see “Circuiting Cells”).

Although the cell-based “computers” sometimes tap into endogenous cell machinery, they use artificial sensors to control artificial responses via the newly created circuitry. That sets them apart from more common genetically engineered therapeutics like CAR T cells or bacterial vaccine expression systems, which typically rely on a synthetic input sensor — such as the CAR — to trigger an endogenous cellular function, or a synthetic output — such as an antigenic protein — that is constitutively produced.

According to Timothy Lu, translational applications of cell computing are growing because of technological improvements in the synthesis and editing of DNA circuit components. “It’s not necessarily a paradigm shift, but a technological acceleration of our ability to engineer life,” he said. “It’s speeding up the design-build-test cycle, and the efficiency with which you can do that dictates how quickly you converge on something that works.”

Lu is an associate professor of biological engineering, electrical engineering and computer science at MIT. He and James Collins, a professor of bioengineering at MIT, co-founded Synlogic, Senti Biosciences and the food pathogen testing company Sample6 Technologies Inc.

Collins told BioCentury the technologies have also become more reliable. “I do think the field has advanced sufficiently in making robust synthetic gene circuits and robust programmable cells that can be moved into the clinical and commercial space.”

The new momentum has also been precipitated by new interactions between orthogonal disciplines.

Martin Fussenegger, a professor of biosystems science and engineering at ETH Zurich and a Senti Biosciences co-founder, said the field had to undergo a demographic shift before it could be adapted for translation. “In the early days of synthetic biology there were no biologists involved, it was mostly electrical engineers,” he said. “But as they really moved along this learning curve, they got more and more proficient, attracted better students and specialists in the field, and are now confident enough to tackle real-world problems.”

**COMPUTER BUGS**

Because the field’s roots lie with engineers who sought simple biological systems, some of the first successes came with bacteria-based products.

“We started Synlogic three years ago because we thought the bacterial technology was ready, and had the cumulative experience of 14 years,” said Lu. “People have optimized modules and methodologies to build thousands of circuits at a time, and figure out which circuits work. That effort is more mature on the bacterial side. We’ll get there with mammalian cells, but it’s more complicated.”

Much of the headway has been in diagnostics, in which microbial strains are engineered to sense disease markers and produce detectable signals in response.

Academic groups have engineered bacteria that can sense pathogens or disease-associated metabolites in patient fluids in vitro, triggering production of fluorescent reporters. Other labs have shown bacteria can be engineered to detect and report in vivo disease markers when ingested, sending colorimetric signals out through the urine or feces.

“What if you had a bacteria that you could take every day that would sense specific biomarkers of colon cancer, and when those bacteria came out of the other end, they could tell you what they saw?” said Lu.

For therapeutics, the advantages of using bacteria are the safety of commensal bacteria, and the abundance of microbial building blocks to serve as starting points for engineering.

“We developed switches using promoters and transcription factors from other commensal bacteria, which we fine-tuned,” said Gutiérrez-Ramos. “We have switches that respond to nitric
oxide, reactive oxygen species or hypoxia, and only under those conditions does the bacteria trigger a therapeutic payload.”

Synlogic is designing genetic circuits to detect and respond to disease locally. It has a collaboration with AbbVie Inc. in inflammatory bowel disease (IBD) to develop synthetic biology-based bacterial therapeutics. In addition, its pipeline includes in-house preclinical programs in metabolic diseases and cancer immunotherapy. Gutiérrez-Ramos said Synlogic’s immuno-oncology circuits are designed to turn on in response to tumor-localized cues such as hypoxia or immunosuppressive molecules, and to “deploy multiple therapeutic mechanisms at the same time.”

Gencirq also aims to exploit synthetic bacterial circuits to treat cancer. In a preclinical study of a *Salmonella enterica*-based version of the system, the bacteria homed to hypoxic tumor sites, where they expressed therapeutic proteins. The cells were engineered to lyse when they reached a high population density in the tumor, which kept bacterial populations low while enabling the organisms to release their therapeutic products.

Gutiérrez-Ramos said Synlogic is prioritizing understanding the pharmacology of its synthetic bacterial therapeutics, and is monitoring the system’s by-products to track performance.

“It’s not necessarily a paradigm shift, but a technological acceleration of our ability to engineer life.”

Timothy Lu, MIT

LOUD AND CLEAR

A major objective is to get the systems to cleanly transmit information from input to output, linking the concentration or duration of a pathogenic signal to the magnitude of response.

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Timothy Lu, MIT

SOLVING CAR PROBLEMS

One of the main testing grounds for synthetic biology will be in CAR T cells, where the technology holds the potential to solve key problems of toxicity caused by dangerous immune over-activation. At least ten companies have disclosed programs to engineer control mechanisms into their CAR T cells, mostly by using pharmacological on- or off-switches.

Cell Design Labs is developing synthetic Notch (synNotch) receptors to create artificial circuits that specify which inputs CAR T cells respond to, and which outputs they produce. The synNotch platform is based on the modular structure of Notch membrane proteins, in which ligand binding to an extracellular sensing domain triggers the release and nuclear localization of an intracellular transcription factor domain, which turns on transcription of an engineered output gene.
That enables the company to link extracellular detection of any tumor antigen to local expression of any therapeutic gene, such as IL-12 or a bispecific mAb targeting a tumor antigen. SynNotch receptors can also build logic gates that minimize CAR T cell targeting of healthy cells by requiring a synNotch to detect one tumor antigen before turning on expression of a CAR targeting a second tumor antigen.

CAR T cell newco Autolus Ltd. disclosed it is also developing “advanced cell programming” for greater potency and control of CAR T cell responses. Autolus was founded in 2015 and raised a total of $100.9 million in series A and B rounds from Syncona Partners LLP, Woodford Investment Management and Perceptive Bioscience Investments Ltd. The company did not answer requests for comment in time for publication.

Beyond immuno-oncology, synthetic biology is enabling development of systems that sense endocrine or immune dysfunction and respond by secreting therapeutic proteins. In the last 18 months, Fussenegger’s team has published four studies describing circuits that alleviate disease in mouse models of early stage diabetes, late stage diabetes, Graves’ disease and psoriasis. Respectively, those four circuits detected pathogenically high levels of insulin, glucose, thyroid hormones, or the combination of TNFα and IL-22, and in response corrected the pathogenic state by secreting adiponectin, insulin, a TSHR antagonist or the combination of IL-4 and IL-10.

The circuits were engineered into human cell lines, which were then encapsulated in alginate microbeads that sheltered them from immune attack, and implanted into mice. Although other companies are developing encapsulated cell therapies for diabetes, those products include pancreatic β cells or their precursors, rather than cells containing synthetic circuits.

SYNTHESIZING IP

The next hurdle might be carving out the right patent strategy, which could require collaboration between high tech and life sciences IP practitioners.

Lim said the modular nature of cell computing, which often involves combining pre-existing components into new architectures, is common in engineering but at odds with traditional thinking about biomedical IP.

“The way patent examiners think about this is deeply constrained by current modalities, where composition of matter is king. But there’s clearly innovation in putting together simple components into higher order structures with new function.”

Wendell Lim, UCSF

Kevin Kabler, an IP associate at Fenwick & West LLP, thinks that despite the hurdles, companies will still pursue composition of matter IP on synthetic circuits. “Composition of matter patent claim scope in synthetic biology may just end up being somewhat narrower, depending on the circumstances. This may increase the value of certain method patents that help fill that void from a scope perspective.”

But Kathleen Williams, a partner at Sunstein Kann Murphy & Timbers LLP, believes academic centers might think twice before taking on the uphill climb. “Tech transfer offices have very limited budgets, so they make these tough calls, whereas companies would not hesitate to go after it.”

Gutiérrez-Ramos believes the modifications Synlogic makes to optimize circuit components derived from commensal bacteria should be sufficient to secure IP. The company has filed
160 patent applications and obtained 14 allowances so far. “If we have done our job, the composition of matter for a potent metabolic transformation will be protected.”

“Ultimately the IP on the composition of matter of an engineered biotic to perform a specific therapeutic response will be the most valuable IP,” said Barrett.

Lu believes there are advantages to pre-competitively sharing basic components, a model he said is practiced in the software and electronics industries.

“Over time, the field benefits when you can re-use components that are compatible with each other and have standards associated them, it allows you to develop things faster,” he said. “Obviously the biotechnology industry’s not structured like that currently, but as we go forward, I would hope the field can consider alternative models of IP.”

Oslick said IP firms are increasingly seeing cases involving “fusions of engineering and life sciences.”

She said that in constructing claim language for systems biology inventions, there may be “something to be gleaned from the particular wordings and phrasings that engineers choose when drafting claims to their inventions.”

“For some of these crossovers, we’re staffing generally with two practitioners, one to cover the life sciences component and one to cover the engineering component,” she added.  

COMPANIES AND INSTITUTIONS MENTIONED
AbbVie Inc. (NYSE:ABBV), Chicago, Ill.
Amyris Inc. (NASDAQ:AMRS), Emeryville, Calif.
Autolus Ltd., London, U.K.
Intrexon Corp. (NYSE:XON), Germantown, Md.
East China Normal University, Shanghai, China
ETH Zurich, Zurich, Switzerland
Gencirr LLC, San Diego, Calif.
Institute for OneWorld Health, South San Francisco, Calif.
Kite Pharma Inc. (NASDAQ:KITE), Santa Monica, Calif.
Massachusetts Institute of Technology, Cambridge, Mass.
Mirna Therapeutics Inc. (NASDAQ:MRNA), Austin, Texas
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Senti Biosciences Inc., South San Francisco, Calif.
University of California San Diego, La Jolla, Calif.
University of California San Francisco, San Francisco, Calif.
Ziopharm Oncology Inc. (NASDAQ:ZIOP), Boston, Mass.

TARGETS
GLP-1 - Glucagon-like peptide-1
IL-4 - Interleukin-4
IL-10 - Interleukin-10
IL-12 - Interleukin-12
IL-22 - Interleukin-22
TNFa - Tumor necrosis factor α
TSHR - Thyroid stimulating hormone receptor

REFERENCES
By Michael Leviten, Senior Writer

Inflammation is one of the primary drivers of non-alcoholic steatohepatitis (NASH), but decreasing inflammation without creating susceptibility to infection remains a challenge for the field. In *Nature Medicine* this month, a Chinese team described how TMBIM1, a transmembrane regulator of vesicle trafficking, triggers breakdown of excess levels of a major inflammatory mediator of NASH when it is up-regulated during the disease, leaving normal levels of TLR4 to respond to infections.

NASH is a major complication of non-alcoholic fatty liver disease (NAFLD). As fat deposits accumulate in the liver — a process known as steatosis —, some patients progress to full-blown NASH, developing chronic inflammation that ultimately leads to fibrosis.

The excess fat primarily accumulates in hepatocytes, causing the cells to release cytokines that activate a variety of immune cells in liver and blood, leading to chronic inflammation. In turn, cytokine release from the immune cells causes liver stellate cells to become fibrotic.

The study, which was led by Hongliang Li, a professor of medicine at Wuhan University, found that TMBIM1 interacts with TLR4 and directs it to lysosomes for degradation. TLR4 is a major mediator of inflammation in NASH.

TLR4 is found on hepatocytes and liver macrophages (Kupfer cells) and is expressed at abnormally high levels in the livers of patients; preclinical studies have shown genetic or pharmacological inhibition of TLR4 can treat a variety of symptoms in mice.

There are not yet any approved therapies for NASH. And while TLR4 has been on the radar as a possible target in several inflammatory disorders, to date no TLR4 inhibitors have been approved by FDA for any indication, though at least six inhibitors are in trials for a variety of indications including sepsis, atherosclerosis, rheumatoid arthritis and skin cancer.

However, due to the central role of TLR4 in innate immunity, blocking it raises infection risk concerns. Eisai Co. Ltd’s eritoran (E5564) is in a Phase III trial for sepsis, and Takeda Pharmaceutical Co. Ltd’s TLR4 inhibitor TAK-242 failed in an earlier Phase III sepsis trial; neither company reported any infection-related adverse events, but Takeda discontinued development of its compound in 2013. VBL Therapeutics Ltd’s
dual TLR2/TLR4 antagonist VB-201 has completed Phase II in atherosclerosis and VBL has reported no adverse effects for the compound.

The Li team’s findings suggest that targeting TMBIM1 could provide a path to safe targeting of TLR4.

In mouse and monkey models of NASH, overexpressing TMBIM1 in liver decreased the levels of TLR4, without eliminating it, and ameliorated disease symptoms. Moreover, the team showed TMBIM1 induced lysosomal degradation of TLR4 only when the receptor had activated by steatosis, leaving unactivated TLR4 intact (see “Turning Over TLR4”).

CHEWING ON TLRS

Li’s team investigated whether TMBIM1 might be protective in NASH because the target is known to inhibit apoptosis and help maintain calcium homeostasis, both of which go awry in hepatocytes affected by NASH.

However, prior to this study, the role of TMBIM1 in lysosomal degradation was not known.

The team started by assessing expression of TMBIM1 in mouse models of NASH and liver tissue samples from patients. In both systems, levels of the target were lower in subjects with disease than in healthy controls. Moreover, in patients, TMBIM1 levels correlated inversely with disease severity, as patients with NASH had less TMBIM1 in their livers than patients with simple steatosis, suggesting TMBIM1 down-regulation might contribute to disease progression.

Genetic studies corroborated that idea, with TMBIM1 knockout in hepatocytes exacerbating several features of metabolic disease in mice fed a high-fat diet. Additional knockout of TLR4 in the TMBIM1-knockout mice prevented the effects on metabolic disease.

Ariel Feldstein, founder of Jecure Therapeutics Inc., told BioCentury the hepatocyte knockout data are compelling but it will be important to see the effects of activating TMBIM1 in tissues beyond hepatocytes.

Mechanistic studies showed TMBIM1 plays a role in the endosomal sorting of TLR4.

TLR4 receptors are endocytosed after activation. Once in endosomes the receptors are either sent to the lysosome for degradation or back to the plasma membrane to mediates additional signaling. Biochemical studies showed that TMBIM1 and TLR4 directly interact, and cell biology experiments confirmed the proteins co-localized in vesicles. Those data, along with TMNIM1-knockout and -overexpression studies in mouse studies confirmed that TIMB1 controlled TLR4 lysosome degradation.

Using human liver tissue samples, the team confirmed that TMBIM1 is down-regulated and demonstrated that TLR4 is also up-regulated in patients compared with controls, suggesting loss of TMBIM1 leads to chronic inflammation via a build-up of excess TLR4.

In both mice and metabolic syndrome-prone monkeys fed a high-fat diet, overexpression of TMBIM1 in the liver using an adeno-associated viral (AAV) vector decreased fasting glucose and insulin levels in blood and inflammatory cytokines and chemokines in serum compared with empty vector. In the liver, TMBIM1 overexpression decreased fat accumulation, inflammation and fibrosis.

Finally, when the team looked at the effects of hepatocyte TLR4 knockout in healthy mice versus mice with steatosis, it saw TMBIM1 only triggers degradation of TLR4 after activation by steatosis-related signals, not under basal conditions. Based on that selectivity, the authors proposed that TMBIM1 overexpression should not pose the infection risks expected for direct TLR4 antagonists.

“Our findings indicate that targeting the lysosomal degradation of TLR4 may represent a novel, and not very toxic, therapy for treating steatohepatitis and metabolic diseases,” the team wrote in its paper.
TURNING OVER TLR4

In Nature Medicine last month, scientists at Wuhan University demonstrated that adeno-associated viral (AAV)-mediated expression of TMBIM1 in liver could be a new strategy for treating inflammation and other symptoms associated with non-alcoholic steatohepatitis (NASH) by decreasing levels of the inflammatory mediator TLR4.

(1) In normal hepatocytes (pink), activation of TLR4 (blue) by their ligands (orange circles) causes internalization the receptors into endosomes. The receptors are then sorted within the multivesicular body (MVB), with some TLR4 proteins directed to the lysosome for degradation and others returned to the plasma membrane to mediate additional signaling.

The Wuhan team identified TMBIM1 as a factor in MVBs that is responsible for directing TLR4 to lysosomes.

(2) In liver tissue samples from NASH patients, as well as in mouse and monkey models of the disease, liver levels of TMBIM1 were lower and resulted in higher surface expression of TLR4 than in healthy cells.

(3) In animal models of NASH, TMBIM1 therapy via AAV-mediated overexpression of TMBIM1 increased lysosomal degradation of TLR4, which decreased the amount of the receptor on the cells’ surface to near normal levels and ameliorated inflammation.

TLR4 - Toll-like receptor 4; TMBIM1 - Transmembrane BAX inhibitor motif containing 1
NASH THERAPIES EXPLODING

According to Sanjay Bhanot, VP of clinical development & translational medicine at Ionis Pharmaceuticals Inc., drug development in NASH got a slow start for three reasons: the prevalence of the disease has only recently been appreciated; no validated regulatory path exists for getting a drug approved; and diagnosing the disease still requires a liver biopsy as there are no validated biomarkers.

However, the field is now seeing a surge of activity. The scores of compounds in preclinical and clinical development can be grouped into multiple buckets targeting different mechanisms, including inflammation, lipid metabolism, reactive oxygen species (ROS) pathways, and fibrosis.

VBL is the only player in NASH pursuing TLR4 inhibition. In addition to VB-201, the company has a second dual TLR2/TLR4 inhibitor, VB-703, in preclinical testing for NASH.

In a 2016 Digestive Diseases and Sciences paper, VBL tested TLR2/TLR4 blockade in NASH using VB-201 and VB-703. While both compounds showed benefit, VB-703 reduced inflammation and liver fibrosis more effectively in a mouse model of NASH. VBL did not respond to requests for information about the safety of its compounds or how they might compare with TMBIM1 gene therapy.

Ionis is targeting hyperlipidemia rather than inflammation. Its AKCEA-ANGPTL3-LRx and DGAT2 (IONIS-DGAT2RX) antisense compounds both decrease hyperlipidemia; Ionis and partner Akcea Therapeutics Inc. have the former compound in Phase II trials for hyperlipidemia and dyslipidemia, while Ionis has the latter in preclinical testing for NASH. Bhanot believes either could modify the course of NASH because hyperlipidemia is a widely conserved, early trigger that precedes inflammation and fibrosis; but because there is no way to predict which oligo will work best in patients, Ionis wants multiple shots on goal, he said.

Jecure is taking a different tack: targeting the inflammasome to decrease hepatocyte injury, inflammation and fibrosis. Feldstein told BioCentury the company is targeting NLRP3 and expects to enter the clinic in 2019, but declined to disclose details.

Both Bhanot and Feldstein think the complicated meshwork of mechanisms that drive NASH may require combination approaches, of which TMBIM1 activation could be a component. Bhanot told BioCentury, “In the end I think it’s going to be a disease that’s likely going to get treated in a combination of drugs that act at different stages.”

Li did not respond to requests for information about his group’s plans for its gene therapy.

COMPANIES AND INSTITUTIONS MENTIONED

Akcea Therapeutics Inc., Cambridge, Mass.
Ionis Pharmaceuticals Inc. (NASDAQ:IONS), Carlsbad, Calif.
Eisai Co. Ltd. (Tokyo:4523), Tokyo, Japan
Jecure Therapeutics Inc., San Diego, Calif.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
VBL Therapeutics Ltd. (NASDAQ:VBLT), Or Yehuda, Israel
Wuhan University, Wuhan, China

TARGETS

ANGPTL3 - Angiopoietin-like 3
DGAT2 - Diacylglycerol O-acyltransferase-2
NLRP3 (NALP3; CIAS1) - NLR family pyrin domain containing 3
TLR2 - Toll-like receptor 2
TLR4 - Toll-like receptor 4
TMBIM1 - Transmembrane BAX inhibitor motif containing 1

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SPREADING CARMA

By Karen Tkach Tuzman, Senior Writer

Carma Therapeutics LLC is subverting macrophages’ tumor-promoting role by engineering in chimeric antigen receptors (CARs) to turn the phagocytic cells into agents that suppress the growth of solid tumors.

The company is a translational play from the lab of Saar Gill, and has spun out from the University of Pennsylvania with a soon-to-be-announced seed funding. Carma hopes to raise up to $50 million in a series A by year-end, and put its first CAR macrophage (CARMA), an autologous therapy, in the clinic by the end of 2018. Gill is an assistant professor of medicine at the hospital of the University of Pennsylvania.

Tumors commonly evade T cells and recruit macrophages, which take on immunosuppressive and pro-angiogenic phenotypes that promote tumor growth. Carma’s approach is to take advantage of the tumor-homing properties of macrophages, but alter the outcome when they get there.

“We’re relying on a bit of a Trojan Horse scenario,” said co-founder Michael Klichinsky, a graduate student in Gill’s UPenn group. By adding a CAR, the company is weaponizing macrophages to target and phagocytose tumor cells.

Carma’s technology uses the chimeric adenoviral vector Ad5/F35 to express CAR constructs with antigen-specific extracellular domains and CD3ζ intracellular domains, because the vector infects macrophages at a high rate, unlike standard vectors used for CAR constructs. The vector also induces CARMACs to adopt the immune-stimulating M1 phenotype, making them resistant to adopting the tumor-promoting M2 phenotype.

The CARMACs are generated from blood-derived precursor monocytes that are differentiated into macrophages ex vivo, and transduced with Ad5/F35. Because of the low proliferation rate, patients are pretreated with drugs to boost monocyte numbers and provide more starting material.

Klichinsky said harvesting a large numbers of cells instead of expanding a small population speeds up needle-to-needle time to seven days or less, compared with about two weeks for CAR T cells, because highly proliferative T cell therapies must rest for several days after ex vivo expansion.

Gill’s group presented data at this year’s American Association for Cancer Research (AACR) meeting, showing HER2-targeting CARMACs decreased tumor size over 30-fold and increased survival in xenograft mouse models of systemically disseminated ovarian cancer compared with unmodified control macrophages.

The company is now completing IND-enabling work for its lead product, an autologous anti-HER2 CARMA for undisclosed metastatic solid tumors that overexpress the target.

According to BioCentury’s BCIQ database, no companies have disclosed programs involving adoptive transfer of macrophages for any indication.

Macrophage Pharma Ltd. and GlaxoSmithKline plc have preclinical small molecules targeting macrophages for cancer

Carma plans to test CARMACs in combination with CD47 inhibitors and other immune modulators. The company is starting with autologous cell therapies, but Klichinsky said allogeneic CARMACs would be feasible therapeutic agents because macrophages don’t have TCRs that can trigger graft-versus-host disease (GvHD).

The company has filed multiple patent applications covering CARMACs and a wide range of modifications that could alter their functionality, plus methods for their use and manufacturing.

COMPANIES AND INSTITUTIONS MENTIONED

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Macrophage Pharma Ltd., Windsor, U.K.

TARGETS

CD3ζ (CD3z; CD247)
HER2 (EGFR2; ErbB2; neu) - Epidermal growth factor receptor 2
p38 MAPK (MAPK14) - p38 mitogen-activated protein kinase
TCR - T cell receptor

REFERENCES


CONVERSION THERAPY

By Lauren Martz, Senior Writer

A set of molecular guidelines devised by a University of Illinois team could provide a road map for converting Gram-positive antibiotics into broad-spectrum agents capable of targeting lesser-served Gram-negative pathogens.

Although industry has made some progress in bolstering the arsenal of antibiotics that can combat drug resistant Gram-positive bacteria, Gram-negative strains have proved a tougher challenge. That’s because, unlike Gram-positive bacteria that are enveloped by a single plasma membrane, Gram-negative bugs have a second, tougher outer membrane that few compounds can penetrate.

In its Nature study last week, the Illinois team led by Paul Hergenrother wrote that the “void in discovery is not due to a lack of effort,” citing a 2007 study in which GlaxoSmithKline plc (LSE:GSK; NYSE:GSK) screened half a million synthetic compounds for activity against the Gram-negative bacteria E. coli but identified no tractable hits. In addition, Hergenrother’s team noted that retrospective analyses by other groups have not been successful at identifying which features of compounds known to be effective against Gram-negative pathogens account for their membrane-penetrating properties. Hergenrother is chair in natural products chemistry and professor of chemistry at the University of Illinois.

Instead of brute-force screening or top-down analysis of existing compounds, Hergenrother’s team took a systematic, ground-up approach to determine the physicochemical characteristics required for a small molecule to penetrate and accumulate in Gram-negative bacteria — then used its findings to modify a Gram-positive antibiotic into a broad-spectrum agent effective against a range of Gram-negative pathogens.

The team began by screening a relatively small set of 180 small molecules for their ability to accumulate in E. coli. The results suggested a positive charge was required, as 12 of 41 positively charged molecules, but none of the negatively charged or neutral molecules, accumulated in the bacteria. Moreover, all 12 hits contained amine functional groups, and 8 of those had primary amines.

However, screening of 68 additional molecules — all containing primary amines — identified only 36 that accumulated in the bacteria, suggesting factors beyond amine functionality were also involved. By analyzing 297 different molecular descriptors for each, the group determined that the molecule’s shape and flexibility were also key factors. Specifically, molecules with low globularity and a small number of rotatable bonds were most likely to cross E. coli’s outer membrane and accumulate inside.

The group proposed that these molecular criteria could be used in two ways to identify new Gram-negative antibiotics. First, libraries of molecules matching the criteria could be used for high throughput screens to identify new antimicrobial agents; second, modifications could be made to existing Gram-positive antibiotics to convert them into broad-spectrum therapies.

“Our rules provide actionable guidance when developing compounds — what charge, shape and flexibility to build into candidate antibiotics.”

Paul Hergenrother, University of Illinois
Hergenrother told BioCentury that both strategies are likely to lead to new antibiotics. “Our rules provide actionable guidance when developing compounds — what charge, shape and flexibility to build into candidate antibiotics.”

As proof-of-concept for the conversion approach, his team selected a molecule with activity against Gram-positive bacteria — deoxynybomycin — that had the preferred shape and flexibility for Gram-negative bacterial accumulation but lacked a primary amine.

When a primary amine was added to deoxynybomycin, the new compound, 6DNM-NH₃, not only accumulated in *E. coli* but inhibited growth of a panel of 22 Gram-negative and Gram-positive pathogens.

One Gram-negative bacteria the compound did not inhibit was *P. aeruginosa*. In the paper, the team hypothesized that was because "*P. aeruginosa* is known to be even less sensitive to antibiotics than most other Gram-negative bacteria,” and wrote that it might be necessary to repeat the experiments in *P. aeruginosa* to identify a specific set of molecular criteria required for compound accumulation in that species.

“We would like to extend this approach to other problematic microorganisms, and to use what we have learned to convert high-value, Gram-positive-only drugs into broad-spectrum antibiotics,” said Hergenrother, adding that his team also plans to continue development of 6DNM-NH₃.


**BEETTER OFF-TARGET PROFILING**

By Lauren Martz, Senior Writer

As CRISPR-Cas9 gene editing agents are being translated from research tools to therapeutic candidates, safety concerns over off-target activity have come to the fore. Caribou Biosciences Inc. and DuPont Pioneer have developed a new technology for mapping a fuller set of off-target cut sites than other profiling techniques capture.

In *Nature Methods* this month, a team from Caribou and the Dupont Pioneer subsidiary of E. I. du Pont de Nemours and Co. (NYSE:DD) described the technique, dubbed SITE-Seq (Selective Enrichment and Identification of Tagged Genomic DNA Ends by Sequencing), which Caribou is already putting to use.

The two-stage system first pinpoints all sites cleaved by CRISPR-associated protein 9 (Cas9) in genomic DNA *in vitro*, then sequences those sites in cells of interest to identify which off-target edits actually occur.

The benefit of the two-part system is fewer false negatives, said Caribou’s CEO Rachel Haurwitz. “Many other methods attempt to identify the breadth of all potential off-target sites and the depth of editing at each of those sites in a single experimental step. This is very challenging and often results in false negatives.”

In addition, most other genome-wide, off-target profiling systems rely on libraries of synthetic variants — homologous sequences with random, varying degrees of
mismatch to the sgRNA — to predict off-target sites, which biases the results towards those sequences.

By contrast, the first step of SITE-Seq is agnostic: cell-free genomic DNA is combined with a single-guide ribonucleoprotein (sgRNP) complex, consisting of Cas9 and a single-guide RNA (sgRNA) to direct Cas9 to a target site. After Cas9-mediated cleavage, all cut sites are tagged, and the tagged sites are amplified and sequenced. In DNA from HEK cells, the technique identified 771 potential cut sites.

Because Cas9’s access to a DNA site in vitro may differ from its access within a cell, SITE-Seq’s second step involves deep sequencing, within a cell type of interest, of the cut sites identified in the first step. Of the 68 cut sites in HEK cell-free DNA selected for additional analysis in the live cell, 29 were confirmed off-target sites in cells with mutation frequencies between 0.1 and 66.6%.

Haurwitz noted that SITE-Seq differentiates between natural breaks in the DNA and sites broken by Cas9 using a unique signature in the sequencing data created when the enzyme makes cuts. “There may also be some sites in the genome that are ‘hot spots’ for double-stranded breaks in cells,” she said. “Inclusion of negative control samples that are not digested with Cas9 allows us to pinpoint and filter out those sites” as false positives.

In addition, sites identified at low sgRNP concentrations in vitro were more likely to be confirmed in cells, indicating those sites were easily cleaved by Cas9. But when sgRNPs were delivered at higher concentrations or expressed for longer periods of time, SITE-Seq found other sites less readily cleaved by Cas9 that other methods might miss — but that nonetheless could have major functional consequences to the cell.

When compared with three other off-target identification platforms that produced non-overlapping lists of off-target sites, SITE-Seq identified all the sites found by all three platforms, as well as 11 new ones.

“This is critical and the main strength of our method,” said Haurwitz. “Simply put: the high sensitivity of SITE-Seq enables us to identify more cellular off-targets than with other competing approaches.”

She told BioCentury that Caribou is already using the platform, but will continue to “improve it and evaluate its use for other gene editing technologies.”

But while identifying each potential off-target site is important to the development of CRISPR-based therapeutics, it is only the first step, and must be paired with analysis of the functional consequences of that off-target event, she said. “It’s possible that the off-target editing has no likely functional consequence or risk and may be acceptable as a result.”

Patent applications covering the SITE-Seq platform have been filed and assigned to DuPont Pioneer. Caribou has a license to the IP for use in certain undisclosed fields. Cameron, P., et al. “Mapping the genomic landscape of CRISPR-Cas9 cleavage.” Nature Methods (2017)
DISTILLERY

THE DISTILLERY brings you this week’s most essential scientific findings in therapeutics, distilled by BioCentury Innovations editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable. This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

THERAPEUTICS

AUTOIMMUNE DISEASE; GASTROINTESTINAL

INDICATION: Arthritis; psoriasis; colitis
Patient sample and mouse studies suggest inhibiting MAP3K8 could help treat arthritis, psoriasis and colitis. In inflamed colon tissue or skin lesion samples from Crohn’s disease or psoriasis patients, respectively, levels of MAP3K8 mRNA levels were higher than in uninflamed colon tissue or non-lesional skin samples. In a mouse model of both psoriasis and psoriatic arthritis, MAP3K8 knockout or a MAP3K8 inhibitor tool compound decreased paw swelling and skin lesion scores compared with normal expression or vehicle. In a rat model of arthritis, the MAP3K8 inhibitor decreased joint inflammation and increased cortical bone volume compared with vehicle. In a mouse model of chemical-induced colitis, the inhibitor increased colon length and decreased crypt epithelial cell loss. Next steps by the Genentech Inc. unit of Roche could include studies of chronic MAP3K8 inhibition to examine the protein’s tumor suppressor functions.

TARGET/MARKER/PATHWAY: Mitogen-activated protein kinase kinase kinase 8 (MAP3K8; COT; TPL2)
LICENSING STATUS: Patent and licensing status undisclosed
PUBLICATION DETAILS: Senger, K. et al. Sci. Signal.; published online April 18, 2017
doi:10.1126/scisignal.aah4273
email: zarrin.ali@gene.com

AUTOIMMUNE DISEASE; INFECTIOUS DISEASE

INDICATION: Lupus; sepsis; influenza virus
Patient sample, cell culture and mouse studies suggest agonizing CR3 could help treat lupus, sepsis and influenza. Lupus patients harboring loss-of-function SNPs on CR3 had higher serum levels of type I interferons than patients with wild-type CR3. In a mouse model of lupus, a CR3 agonist tool compound decreased glomerular damage, renal immune complex deposition, serum levels of inflammatory cytokine levels, and other lupus markers compared with vehicle. In a mouse model of sepsis, the CR3 agonist resulted in survival of 50% of treated animals at day 240, compared with 10% for vehicle-treated mice. In a mouse model of influenza, the CR3 agonist decreased weight loss compared with vehicle. Next steps include developing an optimized small molecule CR3 agonist for clinical studies.

TARGET/MARKER/PATHWAY: Complement receptor 3 (CR3; CD11b; ITGAM)
LICENSING STATUS: Patent application filed; licensed to Adhaere Pharmaceuticals Inc.
doi:10.1172/JCI88442
CONTACT: Vineet Gupta, Rush University Medical Center, Chicago, Ill.
email: vineet_gupta@rush.edu
**EMERGING COMPANY PROFILE**

**TRANSLATION IN BRIEF**

**PRODUCT R&D**

**TARGETS & MECHANISMS**

**EMERGING COMPANY PROFILE**

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**THERAPEUTICS**

**AUTOIMMUNE DISEASE; NEUROLOGY**

**INDICATION: Arthritis; pain**

Cell culture and mouse studies suggest a pyrazolopyridine-based GPR4 inhibitor could help treat arthritic or pain. Chemical synthesis of pyrazolopyridine analogs and testing in a human cell line yielded an oxadiazole-containing compound that inhibited GPR4 activity with an IC₅₀ of 70 nM. In a rat model of antigen-induced arthritis, the compound decreased knee swelling compared with the generic steroid dexamethasone. In a rat model of adjuvant-induced pain, the compound decreased mechanical hyperalgesia with comparable potency to the generic NSAID diclofenac. Next steps could include testing the compound in other animal models of arthritis and pain.

**TARGET / MARKER / PATHWAY:** G protein-coupled receptor 4 (GPR4)

**LICENSING STATUS:** Patent and licensing status unavailable

**PUBLICATION DETAILS:** Velcicky, J. et al. *J. Med. Chem.*; published online April 26, 2017
doi: 10.1021/acs.jmedchem.6b01703

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**CANCER**

**INDICATION: Breast cancer**

Cell culture and mouse studies suggest inhibiting GPNMB or its regulator MAFK could help treat breast cancer. Human triple-negative breast cancer (TNBC) cell lines expressed higher levels of MAFK than luminal- or basal A-type human breast cancer cell lines. In four TNBC human breast cancer cell lines, knockdown of GPNMB or MAFK decreased number of spheres formed and cell migration compared with normal MAFK and GPNMB expression. In a mouse allograft model of advanced breast cancer, MAFK knockdown in tumor cells decreased tumor growth and the number of metastases compared with normal MAFK expression. Next steps could include testing inhibition of MAFK or GPNMB in animal models of other breast cancer subtypes.

**TARGET / MARKER / PATHWAY:** MAF bZIP transcription factor K (MAFK); glycoprotein NMB (GPNMB)

**LICENSING STATUS:** Patent and licensing status unavailable

**PUBLICATION DETAILS:** Okita, Y. et al. *Sci. Signal.*; published online April 11, 2017
doi: 10.1126/scisignal.aak9397

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**INDICATION: Breast cancer**

Cell culture studies suggest that artificial vesicles loaded with Zybrestat fosbretabulin and doxorubicin could help treat doxorubicin-resistant breast cancer. The artificial vesicles consisted of amphiphilic block co-polymers loaded with Zybrestat fosbretabulin and doxorubicin. In a doxorubicin-resistant human breast cancer cell line, the vesicles decreased growth, spheroid growth, and activity of the drug resistance markers PRKCA and ABCB1, and increased apoptosis compared with free doxorubicin, free Zybrestat or artificial vesicles loaded with Zybrestat or doxorubicin alone. Next steps could include testing the drug-loaded artificial vesicles in animal models of doxorubicin-resistant breast cancer.

**Mateon Therapeutics Inc.** has Zybrestat (Combretastatin A4), an A4 prodrug phosphatase-activated tumor vascular target agent, in Phase II/III testing to treat ovarian and thyroid cancers, Phase II testing to treat neuroendocrine tumors and non-small cell lung cancer (NSCLC), and Phase I testing to treat solid tumors.

**TARGET / MARKER / PATHWAY:** ATP-binding cassette sub-family B member 1 (ABCB1; MDR1; PGP; CD243); protein kinase C α (PRKCA)

**LICENSING STATUS:** Patent and licensing status unavailable

**PUBLICATION DETAILS:** Zhu, J. et al. *J. Pharm. Pharmacol.*; published online April 20, 2017
doi: 10.1111/jphp.12725

**CONTACT:** Liyan Qiu, Zhejiang University, Hangzhou, China
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THERAPEUTICS

CANCER

INDICATION: Cancer
Cell culture studies suggest simultaneously inhibiting PARP-3 and stabilizing G quadruplex (G4) DNA could help treat cancers containing high levels of G4 DNA structures. In a human lung adenocarcinoma cell line treated with a tool compound that stabilizes G4 DNA, PARP-3 knockout decreased cell survival compared with normal PARP-3 expression. Also in the cell line, the G4 DNA stabilizer plus a PARP-3 inhibitor tool compound decreased survival compared with vehicle. Next steps include investigating the mechanism by which PARP-3 interacts with G4 DNA.

TARGET/MARKER/PATHWAY: Poly(ADP-ribose) polymerase-3 (PARP-3)

LICENSING STATUS: Unpatented; available for partnering


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CONTACT: David M. Weinstock, same affiliation as above
email: dweinstock@partners.org

INDICATION: Colorectal cancer
In vitro and cell culture studies identified a quinolone-quinazoline conjugate-based DNMT3A inhibitor that could help treat colorectal cancer. Chemical synthesis and testing of quinolone-quinazoline conjugate analogs in vitro yielded a compound that inhibited the activity of the catalytic domain of DNMT3A with an EC50 of 1.1 μM. In a human colorectal cancer cell line, the compound decreased CDKN2A promoter methylation — a marker of colon cancer — and increased expression of CDKN2A compared with vehicle. Next steps by Pierre Fabre Group could include testing the compound in xenograft models of colorectal cancer.

TARGET/MARKER/PATHWAY: DNA (cytosine-5-) methyltransferase 3 α (DNMT3A); cyclin dependent kinase inhibitor 2A (CDKN2A; INK4a; ARF; p16INK4a)

LICENSING STATUS: Patent and licensing status unavailable


CONTACT: Paola B. Arimondo, CNRS-Pierre Fabre, Toulouse, France
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INDICATION: Multiple myeloma (MM)
Cell culture and mouse studies identified a rocaglate-based inhibitor of protein translation that could help treat MM. Screening of a small molecule library in eight human MM cell lines identified a rocaglate analog that inhibited proliferation with IC50 values below 10 nM. In four of the cell lines and one additional cell line, the compound decreased cellular concentrations of ribosome components and increased concentrations of transcriptional activators compared with vehicle. In two of the cell lines, the compound increased apoptosis. In one xenograft mouse model of MM, the compound decreased tumor burden and increased survival and in another, it decreased cell proliferation in tumors. Next steps include testing the compound in additional animal models of MM.

TARGET/MARKER/PATHWAY: An undetermined target

LICENSING STATUS: Patent application filed; unavailable for licensing; available for partnering


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email: irene.ghobrial@dfci.harvard.edu
CONTACT: Salomon Manier, same affiliation as above
email: salomon_manier@dfci.harvard.edu
## THERAPEUTICS

### CANCER

**INDICATION: Pancreatic cancer**

Mouse studies suggest inhibiting ZEB1 could help treat pancreatic ductal adenocarcinoma (PDAC). In a genetic mouse model of PDAC, tumor-specific ZEB1 knockout decreased tumor grade, tumor invasiveness, the number of metastases, the number of acinar-to-ductal metaplastic lesions, and the size and grading of pancreatic intraepithelial neoplasm (PanIN) lesions, which are precursors to PDAC, compared with normal ZEB1 expression. Next steps could include identifying and testing ZEB1 inhibitors in PDAC models.

**TARGET/MARKER/PATHWAY:** Zinc finger E-box binding homeobox 1 (ZEB1)

**LICENSING STATUS:** Patent and licensing status unavailable

**PUBLICATION DETAILS:** Krebs, A. et al. Nat. Cell Biol.; published online April 17, 2017 doi:10.1038/ncb3513

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**CONTACT:** Thomas Brabletz, same affiliation as above email: thomas.brabletz@fau.de

### INDICATION: Skin cancer

Mouse and cell culture studies suggest inhibiting TCF3 and TCF4 could help treat squamous cell carcinoma (SCC) of the skin. In two mouse models of SCC of the skin, tumor levels of TCF3 were higher than in skin samples from normal mice. In a human skin SCC cell line, dual knockdown of TCF3 and TCF4 decreased proliferation compared with normal expression. In a xenograft mouse model of SCC of the skin, TCF3/TCF4 knockdown decreased tumor size and SCC tumor cell proliferation. Next steps could include identifying and testing dual TCF3/TCF4 inhibitors.

**TARGET/MARKER/PATHWAY:** Transcription factor 3 (TCF3; E2A); TCF4

**LICENSING STATUS:** Patent and licensing status details unavailable

**PUBLICATION DETAILS:** Ku, A. et al. eLife; published online May 3, 2017 doi:10.7554/elife.23242

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### DERMATOLOGY

**INDICATION: Dermatosis**

Mouse studies suggest inhibiting SYK could help treat neutrophilic dermatosis. In a mouse model of the disease, myeloid-specific knockout of SYK delayed disease onset and lesion severity in the footpad compared with normal SYK expression. Next steps could include testing SYK inhibitors in models of neutrophilic dermatosis. Rigel Pharmaceuticals Inc. has Tavalisse fostamatinib disodium, an oral SYK inhibitor, in registration to treat idiopathic thrombocytopenic purpura (ITP) and Phase II testing to treat chronic autoimmune hemolytic anemia and IgA nephropathy. Gilead Sciences Inc. has the two SYK inhibitors: GS-9876 in Phase II testing to treat rheumatoid arthritis and Sjogren’s syndrome; and GS-9973 in Phase II testing to treat relapsed or refractory hematologic malignancies and in Phase I testing to treat RA.

**TARGET/MARKER/PATHWAY:** Spleen tyrosine kinase (SYK)

**LICENSING STATUS:** Patent and licensing status unavailable

**PUBLICATION DETAILS:** Gurung, P. et al. Immunity; published online April 11, 2017 doi:10.1016/j.immuni.2017.03.014

**CONTACT:** Thirumala-Devi Kanneganti, St. Jude Children’s Research Hospital, Memphis, Tenn. email: thirumala-devi.kanneganti@stjude.org

**CONTACT:** Takeda Pharmaceutical Co. Ltd. has TAK-659, a SYK inhibitor, in Phase I testing to treat hematologic malignancies and solid tumors.
**THERAPEUTICS**

**ENDOCRINE / METABOLIC**

**INDICATION: Diabetes**

Mouse studies suggest a smartphone-controlled hydrogel implant containing a far-red light (FRL) source and cells engineered to secrete insulin or GLP-1 when exposed to FRL could help treat Type I or Type II diabetes, respectively. The hydrogel implant contains wirelessly powered, FRL light-emitting diodes (LEDs); engineered HEK cells optogenetically expressing an FRL-responsive bacterial enzyme that generates diguanylate monophosphosphate (c-di-GMP); a c-di-GMP-activated synthetic transcription factor complex; and a genetic construct encoding insulin or a short variant of GLP-1 whose expression is induced by the transcription factor complex. The smartphone-based part of the system regulated the implant's secretion of insulin and GLP-1 by converting input from a blood glucose meter into signals that controlled the LED emissions. In mouse models of Type I and Type II diabetes, insulin- and glucose-secreting versions of the system decreased blood glucose compared with version with the FRL emitters turned off. Next steps include developing an autologous version of the system using engineered patient-derived cells.

**TARGET/MARKER/PATHWAY:** Insulin; glucagon-like peptide-1 (GLP-1)

**LICENSING STATUS:** Patent application filed; available for licensing or partnering

**PUBLICATION DETAILS:** Shao, J. et al. Sci. Transl. Med.; published online April 26, 2017
doi:10.1126/scitranslmed.aal2298

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**NEUROLOGY**

**INDICATION: Alzheimer’s disease (AD)**

Cell culture and mouse studies suggest that arylbenzofuran-based inhibitors of neuronal cell death could help treat AD. Chemical synthesis and testing of arylbenzofuran analogs in cell-based assays yielded two compounds that inhibited glutamate-mediated neurotoxicity with EC$_{50}$ values of 33 and 46 μM, respectively. In primary mouse cortical neurons pretreated with β amyloid, the compounds increased viability compared with vehicle. In a mouse model of AD, the two compounds decreased abnormal nest-building behaviors, a marker of AD. Next steps include PK and metabolic studies of the compounds.

**TARGET/MARKER/PATHWAY:** An undetermined target

**LICENSING STATUS:** Patent application filed; unavailable for licensing; available for partnering

**PUBLICATION DETAILS:** Chen, P.-C. et al. J. Med. Chem.; published online May 1, 2017
doi:10.1021/acs.jmedchem.7b00376

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**CONTACT:** Wen-Tai Li, same affiliation as above
email: wtl@nricm.edu.tw
<table>
<thead>
<tr>
<th>INDICATION: Huntington's disease (HD)</th>
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<tbody>
<tr>
<td>Cell culture studies identified a benzonorbornene diphosphate-based inhibitor of mutant HTT protein aggregation, a previously reported benzonorbornene diphosphate compound that bound a 17-residue domain of the N-terminal domain of mutant HTT ($K_d = 20 \mu M$) increased time to HTT aggregation compared with no treatment. In a rat neuronal cell line expressing mutant HTT, the compound increased neurite length. Next steps could include testing the compound in animal models of HD.</td>
</tr>
<tr>
<td><strong>IONIS-HTTRx</strong> by Ionis Pharmaceuticals Inc. and Roche have an antisense molecule targeting HTT, in Phase I/II testing for HD.</td>
</tr>
<tr>
<td><strong>VY-HTT01</strong> by Voyager Therapeutics Inc. and CHDI Foundation Inc. have an adeno-associated virus (AAV)-based gene therapy that knocks down HTT, in preclinical testing for HD.</td>
</tr>
<tr>
<td><strong>PRO289</strong> by BioMarin Pharmaceutical Inc. has a RNA-based antisense oligonucleotide targeting HTT, in preclinical testing for HD.</td>
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<th>INDICATION: Pain</th>
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<tr>
<td>Cell culture and mouse studies identified adenosine-based dual-acting ADORA1 agonists/ADORA3 antagonists that could help treat pain. Chemical synthesis and testing of adenosine analogs in cell-based receptor binding and activity assays yielded three compounds that bound human ADORA1 and ADORA3 with $K_i$ values of 0.45-3.56 nM and 0.31-0.48 nM, respectively. In adenylyl cyclase activity assays, the compounds agonized human ADORA1 and antagonized ADORA3 with IC$_{50}$ values of 7.21-97.2 nM and 2.2-14.4 nM, respectively. In a mouse model of chemical-induced pain, pretreatment with two of the compounds decreased licking, biting and other pain-related behaviors compared with vehicle. Next steps include testing the compounds in additional animal models of pain.</td>
</tr>
<tr>
<td><strong>Adenosine A1 receptor (ADORA1); ADORA3</strong> by Petrelli, R. et al. have unpatented; licensing status undisclosed</td>
</tr>
<tr>
<td><strong>VY-HTT01</strong> by Voyager Therapeutics Inc. and CHDI Foundation Inc. have an adeno-associated virus (AAV)-based gene therapy that knocks down HTT, in preclinical testing for HD.</td>
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<td><strong>PRO289</strong> by BioMarin Pharmaceutical Inc. has an RNA-based antisense oligonucleotide targeting HTT, in preclinical testing for HD.</td>
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<tr>
<th>INDICATION: Spinal muscular atrophy (SMA)</th>
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<tbody>
<tr>
<td>Cell culture and mouse studies identified benzamide-based SMN2 splicing modifiers that could help treat SMA. Chemical synthesis, testing and optimizing of benzamide analogs in cell-based splicing assays yielded two compounds that increased expression of full-length SMN2 mRNA 1.5-fold at concentrations of 29 and 12 nM and SMN2 protein levels at concentrations of 158 and 246 nM, respectively. In two mouse models of SMA, the compounds increased SMN2 protein levels in brain and quadriceps muscle compared with vehicle. Next steps could include testing the compounds in additional animal models of SMA.</td>
</tr>
<tr>
<td><strong>Survival of motor neuron 2 centromeric (SMN2)</strong> by Pinard, E. et al. have patent and licensing status details unavailable</td>
</tr>
<tr>
<td><strong>VY-HTT01</strong> by Voyager Therapeutics Inc. and CHDI Foundation Inc. have an adeno-associated virus (AAV)-based gene therapy that knocks down HTT, in preclinical testing for HD.</td>
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<tr>
<td><strong>PRO289</strong> by BioMarin Pharmaceutical Inc. has an RNA-based antisense oligonucleotide targeting HTT, in preclinical testing for HD.</td>
</tr>
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TECHNIQUES

BIOMARKERS

TECHNOLOGY: Gene profiling

Tumor levels of eight proteins could help predict survival in melanoma. In patients, low tumor levels of eight tumor suppressors — tumor necrosis factor α-induced protein 3 (TNFAIP3; A20), PR domain containing 1 with ZNF domain (PRDM1; BLIMP1), B cell CLL lymphoma 10 (BCL10), NK3 homeobox 1 (NKX3-1), ArfGAP with GTase domain ankyrin repeat and PH domain 2 (AGAP2), potassium channel tetramerization domain containing 11 (KCTD11), RAP1A member of RAS oncogene family (RAPIA) and FES proto-oncogene tyrosine kinase (FES) — were independently associated with poor survival. In 176 metastatic melanoma patients, low tumor levels of FES were associated with poor survival. Next steps could include validating all eight markers in a larger cohort of melanoma patients.

DESCRIPTION: Tumor levels of eight tumor suppressor proteins to predict survival in melanoma

LICENSED STATUS: Patent and licensing status unavailable


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CHEMISTRY

TECHNOLOGY: Synthetic chemistry

A stereoselective method for synthesizing phosphoramidate-containing nucleosides could enable efficient synthesis of pronucleotide (ProTide) nucleoside prodrugs. The ProTide synthesis method involves adding a phosphoramidate functional group to the 5' position of a nucleoside, but lacks selectivity for the biologically relevant (R)-enantiomer at the phosphorus stereocenter, yielding unwanted analogs. The stereoselective method utilizes a lutidine analog to catalyze the reaction between phosphoramidate chloride and uridine to produce the prodrug MK-3682 in a single step with a combined yield of 92% for the phosphorus (R)- and (S)-enantiomers of the 5' analog and 99:1 selectivity for the (R)-enantiomer over the (S)-enantiomer. When applied to a range of other nucleoside analogs, the method produced the corresponding phosphoramidate-containing compound with combined yields of up to 96% for the 5' analogs and up to 98:2 selectivity for the phosphorus (R)-enantiomers. Next steps by Merck & Co. Inc. could include applying the method to other nucleoside-based therapies in its pipeline.

Merck has MK-3682, a uridine nucleotide analog that inhibits HCV NS5B polymerase, in Phase I/II testing for HCV infection.

DESCRIPTION: Synthesis method for selective, high-yield production of (R)-enantiomers of 5'-phosphoramidate nucleoside prodrugs

LICENSED STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: DiRocco, D. et al. Science; published online April 27, 2017 doi:10.1126/science.aam7936

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TECHNIQUES

DISEASE MODELS

TECHNOLOGY: Cell models

Macrophages derived from human iPS cells could be used to model chlamydia and identify therapeutic targets. The model consisted of infecting human iPS cell-derived macrophages with a *Chlamydia trachomatis* strain expressing GFP to allow monitoring of infection by fluorescent microscopy. Over a 48-hour period, the model recapitulated the full infectious cycle observed in patients, including macrophage phagocytosis of bacteria, distortion of macrophage morphology, development of intracellular inclusions containing elementary bodies, and release of the elementary bodies. Transcriptomic analysis of infected iPS cell-derived macrophages and infected blood-derived primary human macrophages identified 1,194 up-regulated and 835 down-regulated genes common to both types of infected macrophages. Next steps include using the model to identify therapeutic targets for the disease.

DESCRIPTION: Human induced pluripotent stem (iPS) cell-derived macrophage model of chlamydia

LICENSED STATUS: Unpatented; unavailable for licensing


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email: bob@hancocklab.com

DISEASE MODELS; DRUG PLATFORMS

TECHNOLOGY: Animal models; cell therapy

An *in vitro* method for generating hPSCs with extended developmental potency could be used to generate cell-based regenerative therapies and chimeric animal models of disease. The method involved treating hPSCs with a cocktail of leukemia inhibitory factor (LIF), a glycogen dependent kinase 3 (GSK3) inhibitor tool compound, dimethindene maleate, and the generic antibiotic minocycline to generate cells with embryonic and extraembryonic developmental potency, whereas untreated hPSCs lacked extraembryonic potency. Extended potency cells derived from three hPSC types — a pluripotent stem cell line, primary embryonic stem cells and induced pluripotent stem (iPS) cells — were capable of differentiating into endoderm, mesoderm and ectoderm tissues. In mouse dams, implantation of embryos injected with extended cells derived from any one of the three hPSC types produced 24 of 54 viable conceptuses (embryos plus extraembryonic tissues) that contained human cells in the embryo and 6 of 54 viable conceptuses that contained human cells in both the embryo and the placenta, whereas implantation of embryos injected with extended cells from any one of the three hPSC types produced 54 conceptuses that contained no human cells. Next steps include using the extended pluripotent stem cells to generate human cell types for cell therapy and developing chimeric tissue, organ and mouse models of disease.

DESCRIPTION: In vitro generation of human pluripotent stem cells (hPSCs) with extended developmental potency for use in chimeric disease models and cell-based regenerative therapies

LICENSED STATUS: Patent application filed; available for licensing or partnering

PUBLICATION DETAILS: Yang, Y. et al. Cell; published online April 6, 2017; doi:10.1016/j.cell.2017.02.005

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The 4th BioCentury China Healthcare Summit will gather top thinkers from industry, academia and finance to identify who will lead China’s biopharma innovation ecosystem, how innovation will be funded, and the business strategies required to transform both domestic and multinational biopharmas and medtech companies as China advances its innovation agenda.

**HIGHLIGHTS**

- A special half-day showcase focused on science, innovation and entrepreneurship in China.
- Pharma GM plenary panel focused on growing your China business in the wake of major regulatory and reimbursement reforms.
- The 8th annual BayHelix China Healthcare Awards Ceremony to celebrate R&D and commercial achievements in China.
- Attendance is limited to senior executives and investors to ensure intimate dialogue and networking among peers.

Our agenda is being developed by an organizing committee that includes China industry KOLs and BioCentury’s editorial team. Simultaneous translation will be provided in all sessions.

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