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TOOLS & TECHNIQUES

THE PHENOMICS PHENOMENON

By Michael Leviten, Senior Writer

Having been upstaged by the dawning of genomics over 20 years ago, phenotypic screening is making a comeback as improvements in disease models, advances in microscopy and the rise of machine learning are making it easier to measure disease-relevant phenotypes and track down the targets responsible.

The dominant paradigm in drug discovery centers on identifying a target, often through genetic screening, and then working out how to drug it.

But many in industry believe that strategy hasn't translated well into the clinic and are opting to attack the problem from the other direction, by first screening compounds to find one that corrects a disease phenotype and then deconvoluting back to the target.

"In the past, taking the molecular approach we might have tried to pick a target of high risk and pursue it — a very hard way to go. What we now readily consider is how to begin with the phenotype, turn it into a relevant assay, and then see what sort of readouts we can establish to enable small molecule screening," said Peter Tummino, VP of lead discovery at Johnson & Johnson's Janssen Pharmaceutica N.V.

J&J is one of many companies phasing in phenotypic screening. Paul Andrews, director of operations at the U.K.'s National Phenotypic Screening Centre (NPSC), told BioCentury that pharma had "a change in mood" after several analyses published on the origins of new medicines surprisingly showed that most came from phenotypic studies rather than the target driven approaches dominating the industry.

"The hit rate in phenotypic screening is much much lower, at least 10-fold if not 100-fold lower. But what you get out usually is relevant and has an effect," said Andrews.

Traditional screening protocols involve step-by-step analyses after a target is identified, using one-dimensional biochemical assays such as receptor binding, or cell line readouts such as proliferation.

By contrast, phenotypic screens — sometimes referred to as phenomics — typically involve measuring a wide variety of cellular parameters in complex disease models that are more physiologically relevant (see "More is More").

Former Roche chemist and CEO of the non-profit Institute for Rare and Neglected Diseases Drug Discovery (iRND3) David Swinney told BioCentury the huge amount of work that has gone into increasing the efficiency of target-based drug screens hasn't paid off. "We've spent years increasing throughput and capacity, but I think sometimes we've sacrificed quality for quantity."

Swinney argued a target agnostic approach, so long as the disease model is representative and the readouts relevant, will lead to more effective therapies. "Biology is way too complex to be able to predict the exact target and mechanism beforehand."

And although phenotypic screens remain more complicated and costly than traditional ones, technology can end up being a more practical choice for some companies.

That is because developments in high throughput, high content microscopy have put a million compound phenotypic screens within reach of large pharmas, and screens of tens to hundreds of thousands of compounds in reach of many biotechs.

TOOLS & TECHNIQUES

PAINTING FOR SCIENTISTS

The laboratory of Anne Carpenter at the Broad Institute of MIT and Harvard has been one of the primary sources of innovation in tools for collecting and analyzing images for high content imaging. Carpenter is the institute's imaging platform director.

Her team described its most sophisticated imaging system to date in a *Nature Protocols* study last September. The platform, dubbed Cell Painting, lets researchers collect up to 1,500 data points from a single cell. The method uses six fluorescent dyes captured in five channels to define the features of eight cellular structures or organelles, such as their shape and dimensions. Comparison of a succession of images yields information about the dynamics of organelles over time.

To develop a robust and widely applicable assay, the researchers optimized cell lines, plates, sample layout and controls, and provided a highly detailed protocol complete with reagent preparation instructions and fixation conditions.

At the time of publication, the Broad Institute team and its collaborators had successfully used the technique on 13 different cell lines, including lab workhorses such as HeLa cells, as well as primary human cells and co-cultures of human fibroblasts and hepatocytes. The group found that adherent or flattened cells were optimal.

The system is compatible with all types of fluorescent probes, but Carpenter's team found commonly used fluorescent dyes, such as Hoechst stains to delineate nuclei, were most cost-effective for high throughput use.

According to the paper, the cell culture and imaging protocols take about two weeks, and the data analysis another one to two weeks. Although the system requires a high-quality fluorescent microscope, the assay was designed to suit standard high throughput microscopes and not be linked to any one instrument. The analysis requires special software, such as Carpenter's own Cell Profiler program, that can sift through the massive amount of data captured in the procedure.

Carpenter's team cautioned in the paper that the data analysis carries with it some challenges, including statistical issues like model overfitting and spurious correlations typical of other systems biology approaches, and the difficulty of combining data from different experiments with inherent variation in cell seeding and growth.

Both Cell Painting and Cell Profiler are open source technologies meant to be user friendly enough for non-experts yet powerful enough for a variety of applications.

Although Cell Painting was developed at the Broad Institute, the paper said it was validated at Recursion Pharmaceuticals LLC.

Recursion is a therapeutics company that is using high-content imaging to profile morphological changes in cellular models of monogenic diseases, and is looking to repurpose approved drugs. Carpenter is a scientific adviser for the company.

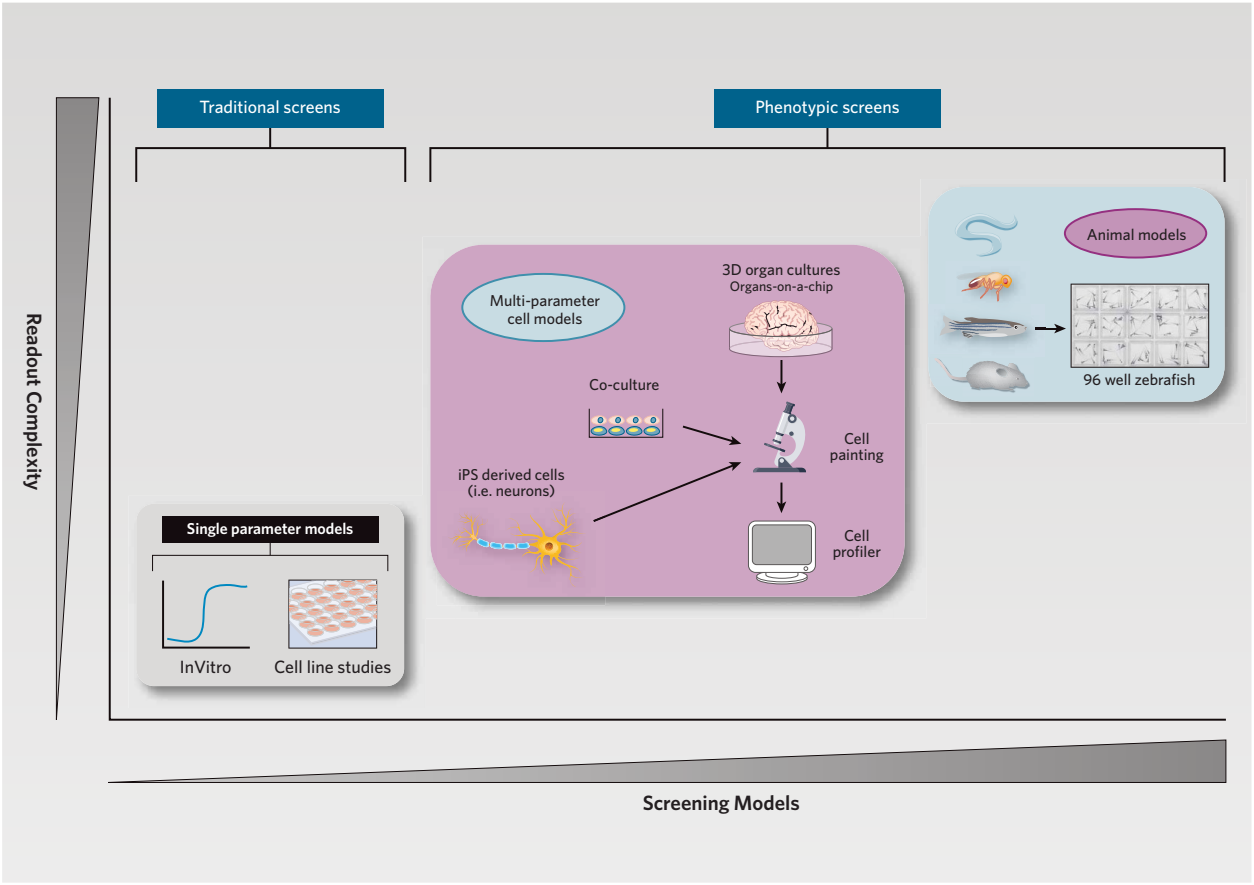
— Michael Leviten

MORE IS MORE

Traditional drug screens are typically single parameter *in vitro* or cell line assays **(gray box, left)** that measure enzyme activity, protein binding or cell properties like proliferation or migration, but screens in drug development are evolving to measure a greater number of parameters **(vertical axis)** and to incorporate more complex screening models **(horizontal axis)**.

Complex cellular models of human disease include co-cultures of two cell types, induced pluripotent stem **(iPS)** cell derived cell types, and multi-well or microfluidic organoid cultures. Readouts from the various 3-D cell models **(Pink box, center)** include protein and organelle marker expression measured using staining techniques like Cell Painting developed at the Broad Institute of MIT and Harvard and cell imaging analysis using software programs like Anne Carpenter's Cell Profiler. Current assays commonly measure more than 1,000 data points and can simultaneously analyze as many as eight different cell structures using protocols like Cell Painting. Carpenter is director of the imaging platform at Broad.

Animals such as flies, worms, and zebrafish can be used to generate genetic models of disease and provide mutant cells for phenotypic screens or potentially for organismal drug screens. Companies like Perlara PBC are generating cellular disease models from mutant flies, worms and fish and Teleos Therapeutics has screened compounds in zebrafish larvae exposed to visual and auditory stimuli for neurologic activity. Zebrafish larvae are uniquely suited to high throughput organismal screening because they can be cultured in media using multi-well plates and are permeable to small molecules that don't penetrate the cuticle of fly or worm larvae.



> "Biology is way too complex to be able to predict the exact target and mechanism beforehand."
>
> David Swinney, iRND3

However, according to Andrews, the inability to go from phenotype to mechanism has been one of the biggest hurdles to broad uptake of the phenotypic approach.

Thierry Dorval, high content screening group leader at Servier, added that pharmas are "really uncomfortable" advancing a drug candidate without knowing its target and mechanism and deconvolution remains a "roadblock" to many small companies.

But advances in proteomics, virtual target identification and chemical informatics are converging to turn target prediction into a tractable problem. According to Dorval, a number of commercial options are now available for companies that don't have the expertise or resources to build their own program in house.

NOT YOUR GRANDFATHER'S PHENOTYPING

Three key areas are fueling the renewed interest in phenotypic screening.

First is improvements in cell-based models of disease. While phenotypic screens conducted in the 1980s were typically done in a handful of cell lines, increased access to patient cells and the advent of induced pluripotent stem (iPS) cell technology are now giving rise to cellular models, including organoids and organs-on-chips, which are truer to human physiology and the diseases they are meant to represent.

According to Andrews, good cell phenotyping means moving away from freezing and thawing cells or measuring simple parameters over short periods. "I think if we ever do that I'll resign. I honestly don't think that's the space phenotypic screening should be in. Phenotyping is about trying to maintain cells as healthy and as physiologically relevant as possible."

It's also about high-content imaging, he said, which together with other aspects of microscopy has been another area of major growth.

Generally, imaging-based screens are called high content if they simultaneously image multiple data points — from a handful to hundreds. High throughput microscopes can now measure the shapes and positions of subcellular organelles, the dynamics of microtubules, the movements of proteins and a slew of other parameters.

And they can do it fast and automatically, which is critical for high throughput studies, said Andrews. "Within 10-15 minutes you can do a whole 384-well plate."

The third area driving the change is in computational tools for data analysis and target deconvolution, which Andrews says is undergoing a technological revolution.

"I think machine learning approaches will take off," he said, adding that for data analysis "the hope is that it will be able to detect differences between disease states and healthy states that the human eye can't determine, and which are not necessarily hypothesis driven."

Several experts who spoke with BioCentury pointed to Anne Carpenter's lab at the Broad Institute of MIT and Harvard as a driving force for many innovations in image analysis. In addition to developing the widely-used Cell Profiler software program, an open source tool for analyzing images of cells, Carpenter's team recently published a "cell painting" technique for ultra-high content profiling of cells. Carpenter is director of the imaging platform at Broad (see "Painting for Scientists" and "Southwestern Art").

DIFFERENT STROKES FOR DIFFERENT FOLKS

Companies are moving into phenomics at different speeds and employing the technologies in different ways.

Novartis AG has become a leader in the field. Nathan Ross, a hit discovery sciences group leader at the company's Cambridge site, said nearly all programs he directs use complex phenomics, often as a primary screen testing large chemical libraries of a million or more compounds.

Ross cited englerin A as an example of a compound found via phenomics analysis whose target was ultimately identified as an

agonist of the calcium channel TRPC4. While the compound itself is not a drug candidate, it could serve as a scaffold. More recently, he said the company performed a phenotypic screen on a cancer cell line in collaboration with the University of California San Francisco and identified a variant of the cyclic peptide ternatin that kills cancer cells.

Swinney added that Vertex Pharmaceuticals Inc. also changed course to adopt the strategy after seeing it work where standard screens had not.

"They started out as a structure-based company and then had success with the cystic fibrosis compounds discovered with phenotypic screens." Vertex was unavailable to comment in time for publication.

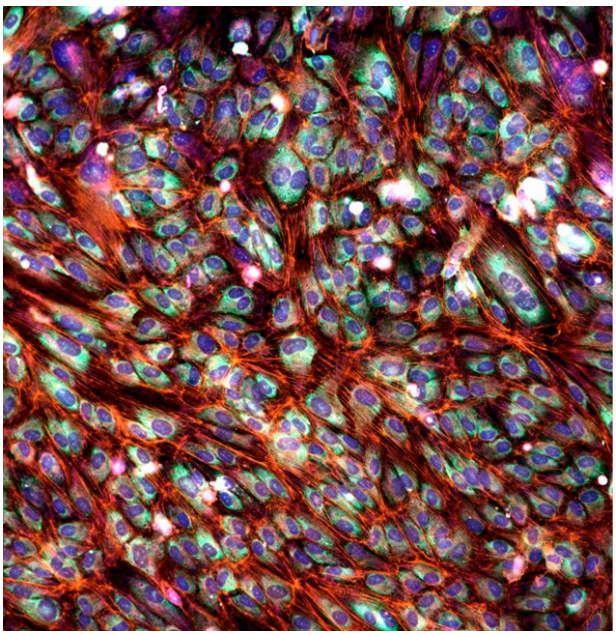
Tummino said Janssen is getting into phenotypic screening in large part through a collaboration with NPSC's Phenomics Discovery Initiative (PDi), a consortium of three academic institutions — University of Oxford, University of Dundee and University of Edinburgh. Janssen is PDi's first pharma partner.

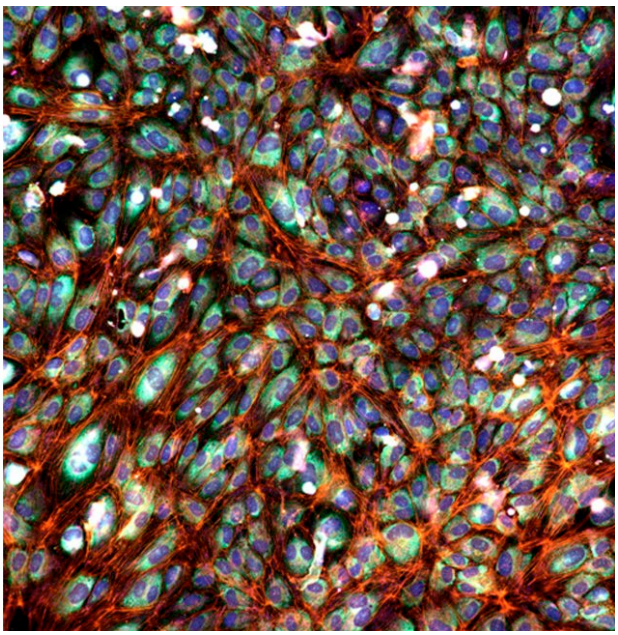
"As we thought about how to approach phenotypic screening, we thought a precompetitive consortium is a very good way to go," said Tummino.

Tummino said the consortium allows the company to tap into the biological and disease expertise of the participating academics to identify disease-relevant endpoints. Janssen's contribution is to apply its high throughput capabilities to streamline the assays.

SOUTHWESTERN ART

This pair of images shows two fields of adherent cells, used by **Recursion Pharmaceuticals LLC** in drug screens, that have been stained using the Cell Painting technique developed by Anne Carpenter's lab at the **Broad Institute of MIT and Harvard**. The technique uses five fluorescent dyes to label eight cell structures, and roughly 1,500 data points can be collected from each image. These data points include 3-D measures of organelle size, shape and location. The dyes used in the protocol stain the following structures: nuclei **(blue)**; nucleoli and cellular RNA **(green)**; F-actin cytoskeleton, Golgi and plasma membrane **(red)**; mitochondria **(deep red)**. *Source: Recursion Pharmaceuticals LLC*





"The real upside we see is that as a larger group we can develop assays that are drug discovery ready, and as we succeed we will bring some of them in-house.""

GOING *IN VIVO*

Momentum is also moving towards use of phenotypic screens on whole organisms like flies, worms and zebrafish.

Ross said Novartis has a collaboration with Perlara PBC to develop drugs for rare diseases using all three types of organisms for drug screening.

David Kokel, an assistant professor at UCSF and founder of the zebrafish-based company Teleos Therapeutics LLC, told BioCentury zebrafish screens have been a hot area in academic neuroscience for a while and have seen greater use in industry as well. "Even Novartis has a big fish tank," said Kokel.

Kokel's team was among the first to translate the utility of zebrafish for high throughput screens as the species can be cultured in multi-well plates and the skin of its larvae is permeable to small molecules. The young fish are also transparent, which allows *in vivo* fluorescence imaging.

Teleos cultures up to 10 larvae in a well and exposes them to up to six different audio and visual stimuli to measure disease-related behavioral responses reflective of underlying neural circuit defects, as well as how those behaviors change in response to drugs.

A large proportion of fly and worm genes have human counterparts, which makes them particularly useful for modeling human monogenic disorders. Multiple companies have also used them for identifying cancer-related genes, learning about disease pathways in neurological disorders like Parkinson's disease, and exploring aging mechanisms.

BEST OF BOTH WORLDS

Few in the field expect phenotypic screens to fully replace target-centered ones, but the two approaches are likely to complement one another, and some companies are attempting to combine them into a single screen.

For example, Dorval said Servier uses a hybrid strategy to capture "the best of both worlds."

His team typically designs phenotypic screens with a target in mind so that the screen provides both a readout of target engagement and metrics related to a variety of disease phenotypes. The approach not only weeds out compounds that engage the target but fail to "cure the cells," but also frequently identifies compounds that cure the cells but don't engage the target. The latter, he said, can then lead to new targets and "gives the project another chance to survive."

But while Novartis has the resources to plow through a million compounds, Dorval said Servier typically can only screen about 10,000-200,000 molecules.

Because of that, Servier is designing libraries partially tailored to the preselected target but that still cover a relatively large chemical space. In addition, the libraries comprise highly annotated compounds to make target deconvolution easier, said Dorval.

NPSC's Andrews said companies designing purely phenotypic screens are also looking for ways to create smaller, "smarter" libraries that "although lower throughput, have a higher value proposition."

"I'm sure quite a lot of pharma companies also don't see the need to automatically go to a full deck screen," he said.

COMPANIES AND INSTITUTIONS MENTIONED

Broad Institute of MIT and Harvard, Cambridge, Mass.
Institute for Rare and Neglected Diseases Drug Discovery (iRND3), Mountain View, Calif.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
National Phenotypic Screening Centre, Dundee, U.K.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Perlara PBC, San Francisco, Calif.
Phenomics Discovery Initiative, Dundee, U.K.
Recursion Pharmaceuticals LLC, Salt Lake City, Utah
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Servier, Neuilly-sur-Seine, France
Teleos Therapeutics LLC, Medford, Mass.
University of California San Francisco, San Francisco, Calif.
University of Dundee, Dundee, U.K.
University of Edinburgh, Edinburgh, U.K.
University of Oxford, Oxford, U.K.
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Boston, Mass.

TARGETS

TRPC4 - Transient receptor potential cation channel subfamily C member 4

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Source: Thinkstock

STRATEGY

CHANGE FROM WITHIN

By Mark Zipkin, Staff Writer

The proposed $5.8 billion cut in the NIH budget in the president's FY18 spending proposal sent shock waves through the scientific community for its size and its message. But while few worry the cuts will ever pass, a caucus of prominent researchers has been building a case for making changes at NIH that advocates a restructuring and takes aim at what some consider the gravy train of administrative costs enjoyed by universities.

In a report titled *A Vision and Pathway for NIH*, an *ad hoc* group composed of two former directors of NIH, two former directors of individual NIH institutes, and 16 other scientific leaders present a vision for how to increase the efficiency of funding and ensure more is channeled into actual research and less into indirect costs.

The report was delivered to the Trump transition team last November and the researchers are continuing to push its case. To date, the Administration has provided no response.

The group was chaired by Keith Yamamoto, vice chancellor for science policy and strategy at the University of California San Francisco, and included Harold Varmus, NIH director from 1993-99; Elias Zerhouni, NIH director from 2002-08; and industry veteran Mark Fishman, professor of stem cell and regenerative biology at Harvard University who was previously president of Novartis AG's Novartis Institutes for BioMedical Research (NIBR).

Varmus is now professor of medicine at Weill Cornell Medical College and Zerhouni is president of global R&D at Sanofi.

Unlike the standard drumbeat of asking for more funding, the pitch centers on policies designed to break down silos, simplify the process of applying for funding, and shift additional responsibility for researcher pay to academic institutions.

The report reflects a sizable voice from inside the research community for re-examining the status quo at NIH.

"Everything should be in play. We should be looking at all the ways NIH spends its dollars and not leave or single out anything," Yamamoto told BioCentury.

DEATH BY 6 BILLION CUTS

According to Yamamoto, the group drafted the report last year before the election's outcome was known. He has since been "shopping it around" on the Hill, where he said people across the aisle are willing to engage in discussion.

However, the huge cut in the president's budget blueprint will likely change the conversation.

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>
> Keith Yamamoto, UCSF

The proposed $25.9 billion for NIH is an 18% drop from the anticipated FY17 authorizations, which have not been finalized, and is the largest year-on-year reduction proposed in the agency's 87-year history.

Since 2001, Congress has not appropriated NIH funds more than 3% higher than the amount requested by the president (see "Ask, But Shall Ye Receive?").

NIH has benefited from bipartisan support recently. For FY16, the agency received a $2 billion increase in budget, its largest gains since 2003, and in December, Congress passed the 21st Century Cures Act authorizing an additional $4.8 billion in NIH funding over the next 10 years.

The consensus in the biopharma industry is that this support will hold up. Rachel King, CEO of GlycoMimetics Inc. and former chair of BIO, told BioCentury, "It's hard to think it won't have an effect on research, but bipartisan support has been so consistent, it's unlikely to pass."

Still, Yamamoto believes that's no reason to ignore the cuts and says it sends a clear message, which might make selling his group's proposal harder. "I share the view of many it's never going to happen. But what it does do is put a mark on Trump's view of NIH research. You have to make it at least a marker of what he believes."

PAVING A NEW PATH

Yamamoto said that although the report doesn't advocate a drop or a rise in the NIH budget, it acknowledges the need to use the funding better.

"NIH could spend its money more wisely, where 'wisely' is defined as finding ways to put more funding into experimental research itself – as distinct from paying for research buildings or salaries of researchers," he said.

The report contains dozens of recommendations, including a push to keep basic research funding above 55% of all grant spending; a task force from across the sector to strengthen research and innovation; an increase in multidisciplinary research by expanding the Common Fund budget to 5% of all NIH research; and a move to a rolling five-year professional judgment budget to increase predictability.

However, Zerhouni told BioCentury that last week's budget proposal goes directly against the recommendations made in the report, and wouldn't achieve the Administration's stated aim of improving efficiency and prioritizing innovation. Since NIH grants typically last five years, a 20% single-year cut would effectively lead to an abrupt stop in grant renewals and new research for FY18 which would have a "dramatic if not catastrophic" effect, he said.

He added: "Science cannot be done year-on-year; it cannot be turned on or off like the production of widgets."

Within NIH, the largest change in the researchers' report is to merge or consolidate some of the institutes, which Zerhouni thinks is a healthy exercise for any organization.

That's an area of some overlap with the budget blueprint, which also includes "a major reorganization of NIH's Institutes and Centers to help focus resources on the highest priority research and training activities," but achieves that by eliminating the Fogarty International Center and consolidating the Agency for Healthcare Research and Quality (AHRQ) within NIH.

The idea of restructuring the agency isn't new and dates back at least to 2001, when former director Varmus advocated for reorganizing NIH's then-24 institutes into five or six larger ones.

Five years later, Zerhouni helped craft and push the NIH Reform Act of 2006 through Congress. Among other institutional changes, the law established an independent Strategic Management Review Board to make recommendations at least every seven years on modifying NIH's structure, and established the Common Fund, designed to break down silos.

The Board's recommendations are not always followed. In 2010, current director Francis Collins announced plans backed by the

ASK, BUT SHALL YE RECEIVE?

National Institutes of Health (NIH) appropriations have increased by nearly 250% since FY97, due to steep increases through FY03, and a shallower rate of rise since then. Year-on-year NIH funding has decreased five times in the last 20 years (in FY06, FY11, FY12, FY13 and FY15), and the requested amount, which reflects the amount given in the president's budget request to Congress, has been met or exceeded in authorized spending 11 times. Numbers reflect the actual requests and spending levels and have not been adjusted for inflation. Funding authorized for a fiscal year was likely approved during the previous calendar year.

The FY18 amount reflects the cut proposed in the president's budget blueprint, not a formal budget request. Spending for the full FY17 and FY18 have not yet been authorized, and FY16 authorized spending is the most recent estimate available, shown in the FY17 budget request. FY09 data does not include additional funding authorized for NIH through the American Recovery and Reinvestment Act of 2009.

Years when the White House **(bar at top)**, House of Representatives **(above dashed line)** or Senate **(below dashed line)** were controlled by Republicans are shaded **pink/red**; years when the White House, House or Senate were controlled by Democrats are shaded **blue**. *Source: Government Publishing Office*

Board to merge the National Institute on Drug Abuse (NIDA) and the National Institute on Alcohol Abuse and Alcoholism (NIAAA). But that plan was scuppered by an outcry from researchers and fierce political headwinds, which made the practicalities of achieving change almost impossible. Many see the benefits as minimal.

Yamamoto concedes that it's "politically very difficult," to restructure the agency, but said "that doesn't mean it's not important and shouldn't be tried." And he disputes the idea that consolidation wouldn't make a difference.

"Research would be better if there were more cooperation between institutes," he said. "For example, institutes have data systems that don't talk to each other. They're not linked. But we're in an era of precision medicine that depends on being able to analyze large amounts of data. So for these to be kept separate inhibits the ability to do good work."

INDIRECT QUESTION

Another point high on Yamamoto's list of changes is a demand that institutes take on more responsibility for paying researchers salaries. Often, he said, business plans build in salaries that come out of the NIH budget. All or at least a significant fraction of that should come from the institutes themselves, leaving more funding available for the research.

Those changes would need to be negotiated thoughtfully and phased in over time, said Yamamoto, because many operating plans are running based on current policies. "But even a gradual change in this profile would have an immediate impact on the dollars available for research," he said.

The amount taken by universities, including administrative costs more broadly, have long been a bone of contention in the research community. Most "indirect costs," also known as facilities and administrative costs, fall into the 80% of the NIH budget spend on extramural costs, and are negotiated between academic institutions and the U.S. Department of Health and Human Services (HHS) or the U.S. Department of Defense.

The rate for administrative indirect costs is capped at 26% of the modified total direct cost of a grant — which accounts for a proportion of institutional personnel costs and graduate student services — but there is no cap for indirect costs related to facilities, which can include operations and maintenance of facilities, as well as depreciation on the buildings and interest on debt for capital improvements. Facilities costs can vary widely based on region.

The report does not make direct recommendations about changes to direct or indirect costs but calls for changes to the funding model to allow NIH to respond better to research needs and elevating fixed costs.

Zerhouni said that changes in cost structures aren't necessarily the best solution.

"This issue of limiting indirect costs has always been there," he said, adding that because of "the stagnation of the budget" over the past few years NIH has lost much of its purchasing power. "So that also affects indirect costs, and what I found when I was director was that it would be destabilizing to the system if you abruptly change the direct cost formula."

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>
> Elias Zerhouni, Sanofi

He said that although it's an easy target, limiting indirect costs has a knock-on effect. "There are things — services and supports and buildings and rent — that no one pays but are paid through indirect costs." If you take the "indirect cost strategy, it's just going to shift expenses from one bucket to another."

"I'm not sure that the indirect costs are right for every institution, but I really believe you need indirect costs to sustain the system," said Zerhouni.

A 2013 U.S. Government Accountability Office study reported that indirect costs accounted for a fifth of NIH's total budget, or $6.2 billion, in FY12.

By comparison, charities like the Bill & Melinda Gates Foundation cap U.S. university indirect costs at 10% of the direct costs.

> "I share the view of many it's never going to happen. But what it does do is put a mark on Trump's view of NIH research."
>
> Keith Yamamoto, UCSF

Other co-authors of the report include: Steven Hyman, director of the Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard and former director of NIH's National Institute of Mental Health (NIMH); Story Landis, former director of NIH's National Institute of Neurological Disorders and Stroke (NINDS); Bonnie L. Bassler, Howard Hughes Medical Institute (HHMI) investigator and professor of molecular biology at Princeton University; Tom Cech, professor of chemistry and biochemistry at University of Colorado Boulder and former president of the HHMI; R. Alta Charo, professor of law and bioethics at University of Wisconsin Law School; H. Robert Horvitz, professor of biology at Massachusetts Institute of Technology (MIT); Philippa Marrack, faculty member in the department of immunology at National Jewish Health and professor of biochemistry and molecular biology, immunology, and medicine at University of Colorado Anschutz Medical Campus; and Shirley Tilghman, president emerita and professor of molecular biology at Princeton.

COMPANIES AND INSTITUTIONS MENTIONED

Agency for Healthcare Research and Quality (AHRQ), Rockville, Md.
Bill & Melinda Gates Foundation, Seattle, Wash.
Biotechnology Innovation Organization (BIO), Washington, D.C.
Broad Institute of MIT and Harvard, Cambridge, Mass.
GlycoMimetics Inc. (NASDAQ:GLYC), Rockville, Md.
Harvard University, Cambridge, Mass.
Howard Hughes Medical Institute (HHMI), Chevy Chase, Md.
Massachusetts Institute of Technology (MIT), Cambridge, Mass.
National Institute on Alcohol Abuse and Alcoholism (NIAAA), Bethesda, Md.
National Institute on Drug Abuse (NIDA), Bethesda, Md.
National Institutes of Health (NIH), Bethesda, Md.
National Institute of Mental Health (NIMH), Bethesda, Md.
National Institute of Neurological Disorders and Stroke (NINDS), Bethesda, Md.
National Jewish Health, Denver, Colo.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
Princeton University, Princeton, N.J.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
University of California San Francisco, San Francisco, Calif.
University of Colorado Anschutz Medical Campus, Aurora, Colo.
University of Colorado Boulder, Boulder, Colo.
University of Wisconsin Law School, Madison, Wisc.
U.S. Department of Defense, Washington, D.C.
U.S. Department of Health and Human Services (HHS), Washington, D.C.
Weill Cornell Medical College, New York, N.Y.

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"The problem is that public and private funding is too focused on a narrow set of hypotheses for dementia that so far haven't panned out."

Tetsu Maruyama, Dementia Discovery Fund

FINANCE

BIOLOGY ON THE MIND

By Lauren Martz, Senior Writer

Less than a year and a half after its launch, the Dementia Discovery Fund has spawned four new companies and invested almost 10% of the roughly $100 million it raised in 2015. With a goal of pushing forward new therapeutics that go beyond the amyloid hypothesis, the firm believes it can make enough small bets in enough new areas to kick-start discovery in Alzheimer's disease.

On March 15, DDF announced its latest seed investment with a £400,000 ($496,760) commitment to Autifony Therapeutics Ltd., a hearing loss company whose pathology could have overlapping biology with neurodegenerative diseases such as Alzheimer's disease.

On February 28, DDF made its first infrastructure investment with the purchase of a 513,000 compound, CNS-focused small molecule library from Aptuit LLC, and the formation of a subsidiary, DDF ChemCo Ltd., to manage the library. The deal includes a strategic partnership, allowing DDF companies, partners and collaborators to run high throughput screens for molecules with the right properties for CNS drug discovery.

The idea is to provide researchers with a quick, easy way to get relevant molecules to screen that they wouldn't get in other ways, said DDF CSO Tetsu Maruyama. "That is good for our deal flow, because early stage companies always wonder how to get access to good molecules."

DDF is managed by SV Life Sciences, and was launched in 2015 with £82 million ($102 million) of its target £230 million, from six strategic pharma investors, Alzheimer's Research UK and the U.K. government. The firm plans to raise the balance of the funds this year.

The U.K. goverment's £15 million contribution was part of a 2012 pledge to spend £300 million on dementia research, when the government issued a challenge to find a disease-modifying therapeutic for dementia by 2025.

The companies, which include the Astex Pharmaceuticals subsidiary of Otsuka Pharmaceutical Co. Ltd., Biogen Inc., Johnson & Johnson, Eli Lilly and Co., Pfizer Inc. and Takeda Pharmaceutical Co. Ltd., all have late stage AD programs in the clinic, most of which act via traditional targets such as β-amyloid, BACE1 and tau.

The fund offers the companies an avenue into new biology for AD and other forms of dementia, and is structured to emphasize four emerging areas of disease biology: microglial biology and inflammation, mitochondrial dynamics, synaptic physiology and function, and trafficking and membrane biology.

According to Kate Bingham, managing partner of SV Life Sciences and DDF, the fund was launched because the current model for dementia research and funding isn't working.

Despite the large amount of money pharma has thrown at the problem, "the reality is we still have no disease-modifying drug," said Bingham.

According to Maruyama, that is largely because funding is being directed at the wrong projects.

"The problem is that public and private funding is too focused on a narrow set of hypotheses for dementia that so far haven't panned out," he said.

TWO WAYS IN TO NEUROINFLAMMATION

DDF has invested in two programs in its microglial biology and inflammation focus, one via Alector LLC and the other in planned newco Tiaki Therapeutics, both of which are aiming to stoke the immune system to treat neurodegeneration by restoring the activity of dysfunctional microglial cells, which act as the brain's macrophages.

The central hypothesis of both companies' strategies is that degenerative brain disorders are caused in part by a dysfunctional immune system, including chronically activated microglial cells that damage synapses and fail to clear β-amyloid and other toxic proteins from the brain.

Alector, DDF's first investment, is developing antibodies to help boost microglial-mediated clearance of disease-associated proteins from the brain.

In 2015, DDF led a $29.5 million series D round, bringing the total raised by Alector to $61.5 million. Alector has four projects ready to move to the clinic, and plans to take one of them forward in 2018. The company has seven assets — six of which are in neurology — about 15-18 months from IND.

Tiaki hasn't disclosed the modality of its microglial-targeted therapeutics but it intends them to complement other approaches, such as reduction or clearance of β-amyloid. DDF has invested $1 million in Tiaki to establish methods for interrogating microglial biology and validating new targets that are genetically linked to dementia.

Other companies targeting microglia to treat neurodegeneration include Cardeus Pharmaceuticals Inc., which is developing the COX-1 inhibitor ASP6537 to treat cognitive dysfunction. The compound, which was licensed from Astellas Pharma Inc., blocks microglial activation and was previously tested by Astellas to treat pain, but Cardeus is exploring other indications including neurodegeneration.

DEMENTIA DRUG DISCOVERY

The Dementia Discovery Fund (DDF) has invested about $10 million of its over $100 million in funding in research projects and companies pursuing novel biology to treat dementia. The fund is focusing investments in four areas of dementia biology — microglial biology/inflammation, synaptic physiology and function, mitochondrial dynamics, and trafficking and membrane biology — but has also made investments outside those areas. To date, the fund has made eight investments, six of which have been disclosed. *Source: Dementia Discovery Fund*

AREA	COMPANY/PROJECT	DATE OF INVESTMENT	INVESTMENT	DESCRIPTION
Microglial biology / inflammation	**Alector LLC**	December 2015	$5 million	Develop immunomodulatory antibodies to treat neurodegenerative disorders including Alzheimer's disease, frontotemporal dementia (FTD) and Parkinson's disease
	Tiaki Therapeutics (planned new company; not yet operational)	Undisclosed	$1 million	Restore microglial function by exploiting genetically validated targets
Synaptic physiology and function	**Autifony Therapeutics Ltd.**	March 2017	£400,000 ($496,760); **Innovate UK** also provided £895,000 ($1.1 million)	Leverage hearing loss platform to validate hypothesis that modulating potassium channel Kv3.4 (KCNC4) channel can restore healthy synapses and neuronal activity
Mitochondrial dynamics	Rheo Stat Therapeutics (planned new company; not yet operational)	Undisclosed	$1 million	Restore and increase mitophagy to treat PD using a genetics-based autophagy screen
Other	DDF ChemCo subsidiary of **DDF**	February 2017	£1 million ($1.24 million)	DDF subsidiary to provide DDF portfolio companies and projects with access to a CNS-focused small molecule library to test discovery ideas
	Gen2 Neuroscience Ltd.	May 2016	£300,000 ($372,570) invested of a £600,000 ($745,140) commitment	Develop mAbs to differentiate between extracellular neurotoxic microtubule-associated protein τ (tau, MAPT; FTDP-17) species and non-pathogenic forms of tau

EIP Pharma LLC and Vertex Pharmaceuticals Inc. have the p38 MAPK inhibitor neflamapimod in Phase II for AD. p38 MAPK is expressed in brain where regulates inflammation in microglia and synaptic plasticity in neurons.

To date, DDF's only investment in the traditional AD targets is in Gen2 Neuroscience Ltd., a company developing mAbs that differentiate between extracellular neurotoxic tau species and non-pathogenic forms of the protein to block neuronal transfer of only the pathogenic species. Last May, DDF provided the first half of the committed £600,000 ($745,140) convertible loan (see "Dementia Drug Discovery").

Although tau biology is already being explored in later-stage trials, Maruyama told BioCentury the technology was interesting enough that the fund wanted to explore the new biology.

SMALL AND PLENTIFUL

According to Maruyama, while the ChemCo deal is the first of its kind for DDF, it's not an outlier — the fund plans to make similar deals in the future.

"Although we do not have any specific near-term plans, our thought is that it might be useful to consider other things that would enable many of our companies to reach their goals more quickly," said Maruyama. A patient-derived iPS cell repository is one example, he said.

And while it's rare for VC funds to make infrastructure investments, it fits with the fund's different approach from most others, said Bingham. "I don't know of another venture fund who has bought a chemical library," she added.

Maruyama pointed to three key differences between DDF and most VCs.

First, it has its own CSO. "Our belief is that if you're going to invest in really novel science and have one focus area, it is useful to have your own scientific team," said Maruyama.

Second, it is embedded within a traditional life science VC firm, bringing together scientific and investment experts to evaluate potential investments and advance the chosen programs.

Third, all investors in the fund have strategic as well as financial motives, which has advantages for DDF. "We can start more projects, take more time and take more risks than the standard venture firm would do," said Maruyama.

He told BioCentury the fund is considering bringing on one more strategic investor this year, plus other investors from a variety of backgrounds that share the same dementia-focused mission.

Over its 15-year term, the goal is to make about 40 investments through the fund, and to bring three to five of those into the clinic.

Bingham told BioCentury that the collaborative structure with its pharma LPs allows DDF to make a collection of smaller, earlier-stage investments than its standard VC fund counterparts, with an understanding that the attrition rate will be higher than the 25% seen for SV Life Science's other funds.

"Having third party input to pressure test new biology or approaches with teams from within pharma is generally helpful, provided it isn't competitive with what they're doing," added Bingham.

The networks in place through SV Life Sciences provide overlap with other areas of biology that could be co-opted for neurodegeneration. For example, Autifony is developing

> "One way we're diversifying discovery in dementia is by starting in immunology and other related fields, and helping people understand how to apply their research to dementia."
>
> Tetsu Maruyama, Dementia Discovery Fund

potassium channel inhibitors for hearing loss, and with the DDF funding will explore the role of KCNC4, a related channel, in AD.

"One way we're diversifying discovery in dementia is by starting in immunology and other related fields, and helping people understand how to apply their research to dementia," said Maruyama. "This allows experienced people to move laterally into the dementia area, and start from a more advanced position."

Maruyama and Bingham both think the value of the model extends beyond dementia.

> "We can start more projects, take more time and take more risks than the standard venture firm would do."
>
> Tetsu Maruyama, Dementia Discovery Fund

"Our responsibility is not only to find treatments for dementia. Part of our responsibility is just to show that the model can be effective in general and applied to other problems," said Maruyama.

He noted that the funding mechanism, or variants of it, could be applied to any disease area "where you need to build a new infrastructure for science to solve a problem with a big unmet need."

An obvious example, he said, is antimicrobial resistance. Like dementia, it could benefit from the structure because the field hasn't yielded results and requires more scientific investigation.

However, while the market is ready for a dementia drug, it's a bigger challenge for antibiotics, he said.

Other potential indications include different CNS disorders that lack effective treatment and funding, like treatment-resistant depression and schizophrenia, he added.

Bingham told BioCentury that stakeholders in other disease areas have already expressed interest in forming similar types of constructs.

COMPANIES AND INSTITUTIONS MENTIONED

Alector LLC, San Francisco, Calif.
Alzheimer's Research UK, Cambridge, U.K.
Aptuit LLC, Greenwich, Conn.
Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
Autifony Therapeutics Ltd. London, U.K.
Biogen Inc. (NASDAQ:BIIB), Cambridge, Mass.
Cardeus Pharmaceuticals Inc., Menlo Park, Calif.
EIP Pharma LLC , Cambridge, Mass.
Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.
Gen2 Neuroscience Ltd., Cambridge, U.K.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Vertex Pharmaceuticals Inc. (NASDAQ:VRTX), Boston, Mass.

TARGETS

BACE1 - β-site APP-cleaving enzyme 1
COX-1 - Cyclooxygenase 1
KCNC4 - Potassium channel Kv3.4
p38 MAPK (MAPK14) - p38 mitogen-activated protein kinase
tau (MAPT; FTDP-17) - Microtubule-associated protein τ

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Hansen, S. "Exploratory cash for dementia." *BioCentury* **(2015)**

TRANSLATION IN BRIEF

PRECISION-ENGINEERED CARS

By Karen Tkach, Senior Writer

A *Nature* study suggests inserting chimeric antigen receptor (CAR) DNA within the locus encoding T cell receptor α (TCRA) could produce less exhausted and more effective antitumor agents than the common strategy of scattering CARs throughout the genome. The more consistent CAR expression and improved performance of the precision-engineered CAR T cells could mean lower doses are needed for treatment, reducing manufacturing burden for the autologous cell therapy.

> "Inserting the CAR at the TRAC locus diminished tonic signaling and delayed the development of exhaustion, resulting in superior tumor rejection with lower T cell doses."
>
> Michel Sadelain, MSKCC

Study leader Michel Sadelain told BioCentury his team started the project looking for a way to make CAR T cell products more uniform than retrovirus-, lentivirus- or transposon-based engineering methods allow.

"One of the features inherent to these viral vectors is that they randomly integrate in the genome, so you don't have two T cells with the same integration sites," he said. "We know that the same gene, flanked by the same promoter or enhancer, won't function equally at different integration sites. We asked whether we could do away with such variability by precisely targeting the CAR cDNA to defined locations in the T cells."

Sadelain is a physician-scientist at Memorial Sloan Kettering Cancer Center and director of its Center for Cell Engineering & Gene Transfer.

In the study, his team took T cells from healthy volunteers and used CRISPR-based gene editing to knock in a CD19-targeting CAR at a locus encoding the TCRA constant region, known as TRAC. The team first showed the knock-in was successful in over 40% of donor T cells, with very little variability in levels of CAR expression across the edited cells.

"It was a huge pleasure to see that, because for so many years, we and others had used these randomly integrating viral vectors that are efficient for transduction but result in a broad spectrum of expression spanning several logs of CAR molecules per T cell," said Sadelain.

The team then compared the CRISPR-engineered CAR T cells to conventional retrovirus-engineered cells in a xenograft mouse model of pre-B cell acute lymphoblastic leukemia (ALL), and showed they had less baseline CAR signaling in the absence of antigen, and more potent antitumor activity. "Inserting the CAR at the TRAC locus diminished tonic signaling and delayed the development of exhaustion, resulting in superior tumor rejection with lower T cell doses," said Sadelain (see Distillery, Cancer: Acute Lymphoblastic Leukemia).

To figure out how the genomic localization improved therapeutic performance, the group performed a series of *in vitro* and *in vivo* experiments comparing T cells with a TRAC-localized CAR construct, T cells with the TRAC construct controlled by a different promoter, and T cells with the CAR construct inserted at the β-2 microglobulin (B2M) locus.

They found the TRAC-localized CAR induced more potent antitumor immunity than the other constructs because of its internalization and re-expression dynamics at T

cell surfaces. "That taught us that the level of CAR expression ought to be much more tightly regulated than anyone knew before," Sadelain said.

He noted that knocking CARs into the TRAC locus disrupts endogenous TCR expression, which reduces the risk of off-target CAR T cell activity. Cellectis S.A. (Euronext:ALCLS; NASDAQ:CLLS) has also knocked out TCR genes in CAR T cells to prevent its lentivirally engineered allogeneic UCART cells from inducing graft-versus-host disease (GvHD).

Sadelain's team is now conducting safety studies with the goal of taking its CRISPR-engineered CAR T cells into the clinic.

"One of the important safety questions is whether your nuclease is cutting other sites in the genome, and whether donor DNA is randomly integrating," he said. "We show in this study that there are no hotspots for off-target integration of donor DNA. We're now examining this in greater depth to gear up for clinical applications."

He said that if the mouse results hold up in human studies, CAR T cell therapies could become much cheaper to produce. "By making better cells, you need far fewer of them, and that means the scale, duration and cost of the cultures should all go down."

MSKCC has filed a patent application on the CAR T cell engineering method, and is seeking partners to develop the technology. *Eyquem, J., et al. "Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection." Nature (2017)*

SALMONELLA-1, TUMORS-0

By Mary Romeo, Staff Writer

A group led by researchers at Chonnam National University has shown that a non-virulent strain of *Salmonella typhimurium* expressing an immune-stimulating protein from another bacterial species shrank tumors, providing a bacteria-based immunotherapy that could potentially treat cancer in a single dose.

Salmonella and other anaerobic bacteria are attractive agents for delivering cancer therapies because the microbes target the hypoxic tumor microenvironment. In 2002, now-defunct Vion Pharmaceuticals Inc. demonstrated limited efficacy of VNP20009, an intratumorally injected, attenuated *S. typhimurium* strain, in Phase I cancer trials. In preclinical studies, attenuated *Salmonella* strains have been used to deliver cytokines to tumors, and last year, a University of California San Diego group used attenuated *Salmonella* to deliver pulses of therapeutic proteins to tumor tissues (see "Bacteria with a Pulse." *BioCentury Innovations* (Sept. 1, 2016)).

Salmonella and other anaerobic bacteria are attractive agents for delivering cancer therapies because the microbes target the hypoxic tumor microenvironment.

However, *Salmonella* used to deliver human proteins must be dosed repeatedly for full therapeutic effect because the bacteria's pathogenicity limits the therapeutic window.

In its *Science Translational Medicine* study, the Chonnam team got around this problem by building on two earlier findings: its own work developing an attenuated strain of *S. typhimurium* that elicited antibody responses in mice with a 100,000- to 1,000,000-fold increase in median lethal dose over the wild-type bacteria, and work by another group showing *S. typhimurium* flagellin suppressed tumor growth in a mouse model of breast cancer. However, compared with endogenous *S. typhimurium* flagellin, *Vibrio vulnificus* flagellin flaB induces more potent toll-like receptor 5 (TLR5) signaling in

mice. The team therefore chose to engineer its strain of *S. typhimurium* with a plasmid encoding flaB, and induced its expression by co-administering L-arabinose.

In mouse models of colon cancer and melanoma, a single IV injection of the flaB-expressing bacteria plus L-arabinose decreased tumor growth and increased survival compared with flaB alone or the *S. typhimurium* strain engineered with an empty plasmid. The flaB-expressing bacteria also decreased tumor growth and metastasis in a mouse model of metastatic colon cancer (see Distillery, Cancer: Cancer, Colorectal Cancer, Melanoma).

Next, the team confirmed the engineered bacteria thrived only in the tumor microenvironment. In a xenograft mouse model of colorectal cancer, L-arabinose-stimulated flaB-expressing bacteria proliferated 10,000-fold more in tumors than in the liver, spleen and lungs. In addition, flaB RNA and protein were detected only in tumors treated with the bacteria, and various serum markers indicated the therapy caused no inflammation, sepsis, renal dysfunction or liver dysfunction, suggesting the bacteria had minimal effects outside tumor tissues.

Finally, the Chonnam team showed the mechanism involved the toll-like receptors TLR4 and TLR5. Colonization by flaB-expressing *Salmonella* stimulated TLR4 in the host to induce infiltration of neutrophils, macrophages and other immune cells into the tumor, where TLR5 signaling triggered by secretion of flaB further activated the immune cells. In the colorectal xenograft model, the engineered bacteria had minimal effects on tumor growth in mice with TLR5 knocked out, and no effect in TLR4-knockout mice. The TLR4-knockouts also had no tumor infiltration of macrophages and neutrophils.

In its study, the team wrote that the safety profile of the flaB-expressing bacteria makes the strain a promising therapeutic for multiple types of cancer. The authors did not respond to requests for comment. *Cai, Z., et al. "Activation of toll-like receptor 5 on breast cancer cells by flagellin suppresses cell proliferation and tumor growth." Cancer Research (2011); Zheng, J., et al. "Two-step enhanced cancer immunotherapy with engineered Salmonella typhimurium secreting heterologous flagellin." Science Translational Medicine (2017)*

HIGH-ALTITUDE HYPOXIA

By Mary Romeo, Staff Writer

Researchers have uncovered a pathway in human blood that helps cells cope with low oxygen levels at high altitudes, and have shown in mice that a target in pathway — solute carrier family 29 nucleoside transporter member 1 (SLC29A1; ENT1) — may be useful for treating hypoxia.

The group, led by researchers at the University of Texas Health Science Center, started with data from the Human AltitudeOmics Study — in which 21 healthy volunteers hiked up and down Bolivia's Mount Chacaltaya multiple times to help characterize the biology behind successful acclimatization and why the process happens faster after a second climb.

Previous findings from the AltitudeOmics study had shown that while increased production of red blood cells and arterial oxygenation contribute to acclimatization,

In a series of mouse studies, the team uncovered a signaling pathway by which hypoxia increases circulating levels of NT5E, inducing production of extracellular adenosine.

neither can explain the faster re-acclimatization, suggesting additional factors remained to be discovered.

The Texas team hypothesized that a build up of adenosine in blood might play a role, as increased adenosine production is a well-known adaptive response to hypoxia that reduces vascular leakage and inflammation and dilates blood vessels to protect against tissue damage.

In blood samples from the volunteers, plasma adenosine levels increased after the initial ascent to 5,260 meters from 1,525 meters, and rose even higher and faster upon re-ascending either one or three weeks later. Statistical analyses showed elevated levels of adenosine and ecto-5'-nucleotidase (NT5E; NT; CD73), which converts adenosine monophosphate to adenosine, correlated with extent and speed of acclimatization.

Adenosine uptake assays in erythrocytes from healthy mice and humans identified SLC29A1 as the cells' primary adenosine transporter.

In a series of mouse studies, the team uncovered a signaling pathway by which hypoxia increases circulating levels of NT5E, inducing production of extracellular adenosine. Adenosine then signals via the adenosine A2B receptor (ADORA2B) to induce protein kinase A (PKA)-mediated phosphorylation, ubiquitination, and proteosomal degradation of membrane-bound SCL29A1 on erythrocytes. The result of lowering SCL29A1 levels is that erythrocytes take up less adenosine, which then accumulates in extracellularly in blood.

The team confirmed the results in erythrocytes isolated from subjects in the AltitudeOmics study, showing that high-altitude hypoxia leads to PKA-mediated degradation of erythrocyte SCL29A1 that is retained and further decreased upon re-ascent.

In a mouse model of hypoxia, knockout of SLC29A1 increased plasma adenosine levels; decreased tissue hypoxia in the heart, kidneys and lungs; and decreased vascular leakage and inflammation in the lungs compared with normal SLC29A1 expression (see Distillery: Other, Inflammation: Hypoxia, Inflammation).

The team hypothesized that erythrocytes maintain a hypoxic adenosine "memory" that facilitates re-acclimatization to high altitude until the cells are replaced by newly created ones expressing higher levels of SCL29A1. The authors suggest targeting SCL29A1, NT5E or ADORA2B could enhance the endogenous adenosine response to help treat or prevent hypoxia. The authors did not respond to requests for comment. *Song, A., et al. "Erythrocytes retain hypoxic adenosine response for faster acclimatization upon re-ascent." Nature Communications (2017); Subudhi, A., et al. "AltitudeOmics: The integrative physiology of human acclimatization to hypobaric hypoxia and Its retention upon reascent." PLoS One (2014)*

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: Ulcerative colitis; colitis

Patient samples and mouse studies suggest CD126 inhibitors or the *Lachnospiraceae* family of bacteria could help treat colitis. In gut mucosal samples from ulcerative colitis patients, levels of NLRP12, a negative regulator of inflammation, were lower than in samples from healthy volunteers. In a mouse model of chemical-induced colitis, knockout of NLRP12 increased disease progression and decreased colonic populations of *Lachnospiraceae* bacteria compared with normal NLRP12 expression. In the same model, an antibody against CD126 decreased weight loss and clinical disease scores and increased survival compared with vehicle. Also in the model, gut colonization by oral gavage with a mixture of 23 genera and species of *Lachnospiraceae* decreased weight loss, colon pathology, clinical disease scores and shortening of the colon. Next steps include testing *Lachnospiraceae* strains in models of ulcerative colitis and inflammatory bowel disease (IBD).

TARGET/MARKER/PATHWAY: Interleukin-6 receptor (CD126); NLR family pyrin domain containing 12 (NLRP12; NALP12)

LICENSING STATUS: Patent status undisclosed; available for licensing or partnering

PUBLICATION DETAILS: Chen, L. et al. *Nat. Immunol.*; published online March 13, 2017

doi:10.1038/ni.3690

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CANCER

INDICATION: Acute lymphoblastic leukemia (ALL)

Mouse studies suggest chimeric antigen receptor (CAR) T cells targeting a TCRA locus could help treat ALL. The T cells were engineered from healthy donor T cells using CRISPR-based gene editing to knock in a construct encoding a CD19-targeting CAR at a locus within the TCRA gene. In a xenograft mouse model of ALL, the CRISPR-engineered CAR T cells increased survival, decreased tumor growth and exhibited decreased expression of exhaustion markers compared with CAR T cells engineered using a retrovirus-encoded CAR. Next steps include IND-enabling safety studies of the CRISPR-engineered CAR T cells (see "Precision-Engineered CARs").

TARGET/MARKER/PATHWAY: T cell receptor α chain (TCRA); CD19

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

PUBLICATION DETAILS: Eyquem, J. et al. *Nature*; published online Feb. 22, 2017

doi:10.1038/nature21405

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THERAPEUTICS

CANCER

INDICATION: Adenocarcinoma

Patient sample and cell culture studies suggest inhibiting VAV2 could help treat adrenocortical carcinoma (ACC). In patients, high tumor expression of VAV2 was associated with poor overall survival and disease-free survival. In a chicken chorioallantoic membrane-based invasiveness assay, siRNA targeting VAV2 in a human ACC cell line decreased the percentage of membrane-invading cells compared with non-specific siRNA. Next steps include identifying VAV2 inhibitors.

TARGET/MARKER/PATHWAY: Vav guanine nucleotide exchange factor 2 (VAV2)
LICENSING STATUS: Patent and licensing status unavailable
PUBLICATION DETAILS: Ruggiero, C. et al. *Sci. Signal.*; published online March 7, 2017
doi:10.1126/scisignal.aal2464
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INDICATION: Brain cancer

Patient sample and mouse studies suggest inhibiting MCP-1 could help treat glioblastoma multiforme (GBM). In patients, high tumor levels of MCP-1 were associated with poor survival. In a mouse model of GBM, knockout of MCP-1 increased survival compared with normal MCP-1 expression. Next steps could include identifying and testing MCP-1 inhibitors in GBM models.

Noxxon Pharma N.V. has Emapticap pegol, an L-aptamer MCP-1 inhibitor developed using Spiegelmer technology, in Phase II testing to treat diabetic nephropathy and Phase I testing to treat diabetes-related complications.

TARGET/MARKER/PATHWAY: Monocyte chemoattractant protein-1 (MCP-1; CCL2)
LICENSING STATUS: Patent and licensing status unavailable
PUBLICATION DETAILS: Chen, Z. et al. *Cancer Res.*; published online Feb. 24, 2017
doi:10.1158/0008-5472.CAN-16-2310
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INDICATION: Cancer; colorectal cancer; melanoma

Mouse studies suggest an engineered *Salmonella* strain that agonizes TLR4 and TLR5 could help treat melanoma, colorectal and other cancers. An attenuated strain of *S. typhimurium* was engineered to express a plasmid encoding *Vibrio vulnificus* flaB. In mouse models of colon cancer and melanoma, the flaB-expressing bacteria decreased tumor size and increased survival compared with flaB alone or bacteria engineered to express empty plasmid. In a mouse model of metastatic colon cancer, the flaB-expressing bacteria decreased tumor size and metastasis compared with vehicle or bacteria engineered to express empty plasmid. In the colon cancer model, the flaB-expressing bacteria decreased tumor growth to a lesser extent in mice with TLR4 or TLR5 knockout than in mice with normal TLR4 and TLR5 expression, suggesting the bacteria acted by agonizing the two receptors. Next steps could include testing the flaB-expressing *S. typhimurium* in models of other cancers (see "Salmonella-1, tumors-0").

TARGET/MARKER/PATHWAY: Toll-like receptor 4 (TLR4); TLR5; V. *vulnificus* flagellin flaB (flaB)
LICENSING STATUS: Patent and licensing status unavailable
PUBLICATION DETAILS: Zheng, J. et al. *Sci. Transl. Med.*; published online Feb. 8, 2017
doi:10.1126/scitranslmed.aak9537
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THERAPEUTICS

CANCER

INDICATION: Cancer; renal cancer

Cell culture and mouse studies suggest the NME2 inhibitor stauprimide could help treat MYC-driven cancer. Stauprimide is known to inhibit NME2-mediated MYC transcription in normal cells. In multiple MYC-driven human cancer cell lines — including melanoma, renal cell carcinoma (RCC), leukemia, breast, colorectal, lung and pancreatic cancers — stauprimide inhibited NME2-mediated MYC transcription with EC_{50} values of 30 nM-8 µM. In one of the renal cancer cell lines, stauprimide inhibited proliferation with an IC_{50} of 780 nM. In two xenograft mouse models of MYC-driven renal cancer, stauprimide decreased tumor growth compared with vehicle. Next steps could include testing stauprimide and its analogs in models of other MYC-driven cancers.

TARGET/MARKER/PATHWAY: NME/NM23 nucleoside diphosphate kinase 2 (NME2); v-myc myelocytomatosis viral oncogene homolog (MYC; c-Myc)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Bouvard, C. et al. *Proc. Natl. Acad. Sci. USA*; published online March 14, 2017

doi:10.1073/pnas.1702663114

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INDICATION: Lung cancer; melanoma

Mouse studies suggest inhibiting SMAD3 could help treat lung cancer and melanoma. In mouse models of lung cancer and melanoma, systemic knockout of SMAD3 decreased tumor growth and metastasis to the lung and increased survival compared with normal SMAD3 expression. Also in the models, a SMAD3 inhibitor tool compound decreased tumor growth and increased NK cell numbers in tumors, tumor and blood levels of markers of NK cell anticancer cytotoxicity, and survival compared with vehicle. In the melanoma model, systemic SMAD3 knockout also increased the numbers of NK cells in the bone marrow, spleen, lung and blood compared with normal SMAD3 expression, and the SMAD3 inhibitor also decreased angiogenesis compared with vehicle. Next steps include developing a SMAD3-knockout NK cell-based immunotherapy in models of lung cancer and melanoma.

TARGET/MARKER/PATHWAY: SMAD family member 3 (SMAD3; MADH3)

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

PUBLICATION DETAILS: Tang, P. et al. *Nat. Commun.*; published online March 6, 2017

doi:10.1038/ncomms14677

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THERAPEUTICS

CANCER

INDICATION: Multiple myeloma (MM)

Patient sample, cell culture and mouse studies suggest bispecific antibodies targeting CD3 and either BCMA or FCRL5 could help treat multiple myeloma (MM). In MM cells from patients and human MM cell lines, an anti-CD3/BCMA (EM801) or an anti-CD3/FCRL5 bispecific antibody increased apoptosis compared with a control bispecific antibody or vehicle. In a xenograft mouse model of MM, the antibodies decreased tumor growth. Next steps by the Genentech Inc. unit of Roche include additional, undisclosed preclinical testing of the anti-CD3/FCRL5 bispecific antibody.

Celgene Corp. has EM801 in preclinical testing for MM.

TARGET/MARKER/PATHWAY: Fc receptor-like 5 (FCRL5; FCRH5); tumor necrosis factor receptor superfamily member 17 (BCMA; TNFRSF17; CD269)

LICENSING STATUS: Patent application filed; licensing status unavailable

PUBLICATION DETAILS: Li, J. et al. *Cancer Cell*; published online March 2, 2017

doi:10.1016/j.ccell.2017.02.001

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LICENSING STATUS: Patent status unavailable; licensing status unavailable.

PUBLICATION DETAILS: Seckinger, A. et al. *Cancer Cell*; published online March 2, 2017

doi:10.1016/j.ccell.2017.02.002

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CARDIOVASCULAR

INDICATION: Ischemia / reperfusion injury

Patient sample, cell culture and mouse studies suggest promoting SNRK expression could help treat ischemia/reperfusion injury. In heart tissue samples from cardiomyopathy patients, levels of SNRK were higher than in samples from organ donors with no history of cardiac disease. In a mouse cardiomyocyte cell line cultured under hypoxic conditions, SNRK overexpression increased the efficiency of mitochondrial ATP production and decreased cell death compared with normal SNRK expression. In a mouse model of acute ischemia/reperfusion, transgenic cardiomyocyte-specific expression of human SNRK decreased infarct size. Next steps could include identifying and testing a compound that promotes SNRK expression in models of ischemia/reperfusion injury.

TARGET/MARKER/PATHWAY: SNF related kinase (SNRK)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Rines, A. et al. *Nat. Commun.*; published online Jan. 24, 2017

doi:10.1038/ncomms14095

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THERAPEUTICS

DERMATOLOGY

INDICATION: Ichthyosis

Cell culture studies suggest acylceramide or agents that promote its biosynthesis could help treat ichthyosis caused by PNPLA1 mutations. In a HEK cell-based activity assay, expression of disease-associated PNPLA1 mutants decreased PNPLA1 activity and production of acylceramide — a lipid involved in epidermal barrier function — compared with expression of wild-type PNPLA1. In keratinocytes from PNPLA1-knockout mice, acylceramide increased epidermal barrier development, as measured by a marker of terminal differentiation, compared with no treatment. Next steps could include identifying compounds that promote acylceramide biosynthesis.

TARGET/MARKER/PATHWAY: Patatin like phospholipase domain containing 1 (PNPLA1)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Hirabayashi, T. et al. *Nat. Commun.*; published online March 1, 2017

doi:10.1038/ncomms14609

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LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Ohno, Y. et al. *Nat. Commun.*; published online March 1, 2017

doi:10.1038/ncomms14610

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ENDOCRINE / METABOLIC

INDICATION: Hyperuricemia / gout

Rat and cell culture studies suggest β-hydroxybutyrate or promoters of its biosynthesis could help treat gout flares. In primary mouse bone marrow-derived macrophages stimulated with monosodium urate, β-hydroxybutyrate decreased IL-1β levels and NLRP3 inflammasome activation — both markers of gout flares — compared with vehicle. In a rat model of the flares, a ketogenic diet that promoted β-hydroxybutyrate biosynthesis decreased knee swelling, serum levels of IL-1β and inflammation and necrosis in the knee compared with a normal diet. Next steps could include identifying compounds that promote β-hydroxybutyrate biosynthesis.

TARGET/MARKER/PATHWAY: NLR family pyrin domain containing 3 (NLRP3; NALP3; CIAS1)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Goldberg, E. et al. *Cell Rep.*; published online Feb. 28, 2017

doi:10.1016/j.celrep.2017.02.004

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THERAPEUTICS

GASTROINTESTINAL

INDICATION: Colitis

In vitro and mouse studies identified a chlorobenzamide-based inhibitor of bromodomain 1 of BET bromodomain proteins that could help treat colitis. Structure-based design, chemical synthesis and *in vitro* competitive bromodomain binding assays on hits yielded a chlorobenzamide-based compound that inhibited bromodomain 1 of BRD2, BRD3 and BRD4 with K_i values of 77-110 nM and two- to 10-fold selectivity over bromodomain 2 of the three proteins. In an adoptive T cell transfer mouse model of colitis, the compound decreased maturation of T helper type 17 (Th17) cells, shortening and inflammation of the colon, clinical disease scores and weight loss compared with vehicle. Next steps could optimizing and testing the compound in other models of colitis.

TARGET/MARKER/PATHWAY: BET bromodomain proteins; bromodomain containing 4 (BRD4); BRD2; BRD3

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Cheung, K. et al. *Proc. Natl. Acad. Sci. USA;* published online March 6, 2017

doi:10.1073/pnas.1615601114

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NEUROLOGY

INDICATION: Neuroinflammation

Patient sample, mouse and cell culture studies suggest ORM2 could help treat neuroinflammation. Levels of ORM2 were higher in blood from patients with cognitive impairment and Alzheimer's disease and in the hippocampus of a mouse model of lipopolysaccharide (LPS)-induced neuroinflammation than in healthy volunteers and normal mice, respectively. In a mouse microglia cell line pretreated with LPS, ORM2 decreased levels of inflammatory cytokines compared with vehicle. In two mouse models of LPS-induced neuroinflammation, intracerebroventricular injection of ORM2 decreased numbers of activated microglia cells — a marker of neuroinflammation — in the hippocampus and cortex compared with vehicle. In one of the models, intracerebroventricular injection of ORM2 decreased cognitive impairment and anhedonic behavior, a marker of neuroinflammation. Next steps include testing ORM2 in additional models of neuroinflammation and neurodegeneration.

TARGET/MARKER/PATHWAY: Orosomucoid 2 (ORM2; AGP2)

LICENSING STATUS: Patent application filed in South Korea; available for licensing or partnering

PUBLICATION DETAILS: Jo, M. et al. *J. Neurosci.;* published online Mar. 15, 2017

doi:10.1523/JNEUROSCI.2534-16.2017

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THERAPEUTICS

OTHER; INFLAMMATION

INDICATION: Hypoxia; inflammation

Mouse studies suggest inhibiting SLC29A1 could help treat hypoxia and hypoxia-induced inflammation. In a mouse model of hypoxia, knockout of SLC29A1 increased plasma adenosine levels — a marker of an antihypoxic vasodilative response — decreased tissue hypoxia in the heart, kidneys, and lungs, and decreased vascular leakage and inflammation in the lungs compared with normal expression. Next steps could include identifying and testing SLC29A1 inhibitors in additional models of hypoxia and hypoxia-induced tissue damage (see "High-Altitude Hypoxia").

TARGET/MARKER/PATHWAY: Solute carrier family 29 nucleoside transporter member 1 (SLC29A1; ENT1)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Song, A. et al. *Nat. Commun.;* published online Feb. 7, 2017

doi:10.1038/ncomms14108

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PULMONARY

INDICATION: Chronic obstructive pulmonary disease (COPD)

In vitro and cell culture studies identified a dual-acting PDE-4 inhibitor/ADRB2 agonist that could help treat COPD. In *in vitro* activity assays, a quinolone analog conjugated to a PDE-4 inhibitor tool compound inhibited human PDE-4B activity with an IC_{50} of 1.38 nM. In human lung endothelial cell-based activity assays, the compound agonized ADRB2 with an EC_{50} of 39 pM. In a human lung endothelial cell line, the compound increased expression of anti-inflammatory genes compared with vehicle, and produced a comparable gene expression profile to that produced by combination of another PDE-4 inhibitor tool compound and the ADRB2 agonist Arcapta indacaterol. Next steps could include testing the dual-acting compound in animal models of COPD.

Novartis AG markets Arcapta Neohaler to treat COPD.

AstraZeneca plc, Allergan plc, Merck & Co. Inc. and Mitsubishi Tanabe Pharma Corp. market Daliresp roflumilast, a PDE-4 inhibitor, to treat COPD.

Chiesi Farmaceutici S.p.A. has CHF 6001, an inhaled PDE-4 inhibitor, in Phase II testing for COPD.

TARGET/MARKER/PATHWAY: Phosphodiesterase-4 (PDE-4); PDE-4B; adrenergic receptor β2 (ADRB2)

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Joshi, T. et al. *J. Pharmacol. Exp. Ther.;* published online Dec. 7, 2016

doi:10.1124/jpet.116.237743

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TECHNIQUES

ASSAYS AND SCREENS; DRUG PLATFORMS

TECHNOLOGY: Cellular assays; peptides

A genomic and proteomic screening method could identify neoantigens as personalized immunotherapy targets for cancer. The method involved collecting tumor and non-tumor tissue samples from a patient; immunoprecipitating human leukocyte antigen class I A (HLA-A) and other class I alleles and major histocompatibility complex class II DR (HLA-DR) from the tumor samples; then separating tumor peptides bound to those HLAs and analyzing their sequences by mass spectrometry. Next, sequences of the HLA-binding tumor peptides were compared with whole-exome sequencing and direct immunoglobulin gene sequencing data from the patient's samples and data in public peptide databases to identify mutant tumor peptides (neoantigens) presented on the HLA class I molecules or HLA-DR. In 17 mantle cell lymphoma (MCL) patients, the method identified a total of 66 neoantigens that were fragments of immunoglobulin heavy chain variable region (Ighv) or immunoglobulin light chain (IgLC) and all presented on HLA-DR, which stimulates $CD4^+$ T cells. In $CD4^+$ T cells from one of the patients, a pool of that patient's Ighv and IgLC neoantigens induced activation of neoantigen-specific T cells. In co-cultures, the activated T cells killed the patient's MCL cells, whereas the activated T cells had no effect on the patient's normal B cells, which lacked the neoantigens, that had been transformed with Epstein-Barr virus (EBV). Next steps include using the method to identify neoantigens in patients with other cancers.

DESCRIPTION: Genomic- and proteomic-based method to identify cancer neoantigens for personalized immunotherapy

LICENSING STATUS: Unpatented; available for licensing and partnering

PUBLICATION DETAILS: Khodadoust, M. et al. *Nature*; published online March 22, 2017

doi:10.1038/nature21433

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BIOMARKERS

TECHNOLOGY: Gene profiling

Tumor aneuploidy could help predict tumor responses to checkpoint inhibitors. In 5,255 paired tumor samples from patients and normal tissue samples from healthy volunteers representing 12 cancer types, high tumor aneuploidy was associated with high expression of genes promoting tumor cell proliferation and low expression of genes promoting cytotoxic immune responses. In two independent sets of melanoma patients treated with the anti-CTLA4 (CD152) mAb Yervoy ipilimumab, high tumor aneuploidy was associated with poor long-term survival. Next steps could include testing the association between tumor aneuploidy in patient samples and resistance to other checkpoint inhibitors in patients.

Bristol-Myers Squibb Co. and Ono Pharmaceutical Co. Ltd. market Yervoy for melanoma and have the compound in Phase I through Phase III testing for various cancers.

DESCRIPTION: Tumor aneuploidy for predicting response to checkpoint inhibitors

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Davoli, T. et al. *Science*; published online Jan. 20, 2017

doi:10.1126/science.aaf8399

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TECHNIQUES

BIOMARKERS

TECHNOLOGY: Plasma markers

ICOS-positive and CD38-positive Tfh cells in the blood could help monitor immune responses to prophylactic influenza vaccines. The numbers of ICOS-positive and CD38-positive follicular helper T cells were higher in blood from healthy volunteers vaccinated with Fluarix than in unvaccinated volunteers. In the vaccinated volunteers, 11% of the Tfh cells were specific for influenza antigens, whereas only 0.03% of memory T cells were antigen-specific. Next steps include validating the Tfh cell response to influenza vaccines in elderly, HIV-infected and pediatric cohorts that typically have poor vaccine responses.

GlaxoSmithKline plc markets the inactivated influenza vaccine Fluarix to prevent seasonal influenza virus.

DESCRIPTION: Blood levels of inducible T cell co-stimulator (ICOS)-positive and CD38-positive follicular helper T (Tfh) cells for monitoring immune responses to influenza vaccines

LICENSING STATUS: Unpatented; available for licensing and partnering

PUBLICATION DETAILS: Herati, R. et al. *Sci. Immunol.*; published Feb. 17, 2017

doi:10.1126/sciimmunol.aag2152

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TECHNOLOGY: Tissue markers

Tumor levels of integrin β4 could help predict outcomes in lung, ovarian, gastric and breast cancers. In tumor samples from 461 lung, 143 serous ovarian and 641 gastric cancer patients, high levels of integrin β_4 correlated with poor progression-free survival. In tumor samples from 173 triple-negative breast cancer (TNBC) patients and 399 basal-like breast cancer patients receiving chemotherapy, high levels of integrin β_4 correlated with poor relapse-free survival. Next steps could include validating the findings in larger cohorts of cancer patients.

DESCRIPTION: Integrin β4 as a prognostic marker for lung, ovarian, gastric and breast cancers

LICENSING STATUS: Patent and licensing status unavailable

PUBLICATION DETAILS: Bierie, B. et al. *Proc. Natl. Acad. Sci. USA*; published online March 7, 2017

doi:10.1073/pnas.1618298114

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DRUG PLATFORMS

TECHNOLOGY: Cell therapy

An *in vitro* protocol could differentiate human MSCs into stable cartilage tissue for use in regenerative therapies. The protocol involves using a culture system of two compartments separated by a collagen-coated membrane to generate hyaline cartilage disks. First, human bone marrow-derived MSCs are cultured on the membrane with chondrogenic medium containing transforming growth factor β3 (TGFB3) in both compartments to generate the disks. Next, the chondrogenic medium in the basal compartment is replaced with hypertrophic medium containing the thyroid hormone receptor agonist thyroxine to form a basal mineralization layer in the disks. In mice, subcutaneous transplants of hyaline cartilage disks produced with the protocol had less of the endochondral ossification and collagen type II (COL2) loss associated with transplant instability than cartilage produced with the protocol by not replacing the basal compartment medium in the second step or by replacing the medium in both compartments with hypertrophic medium in the second step. Next steps include testing cartilage produced by the protocol in animal models of cartilage damage.

DESCRIPTION: In vitro protocol for differentiating human mesenchymal stem cells (MSCs) into stable cartilage tissue for regenerative therapies

LICENSING STATUS: Unpatented; unavailable for licensing; available for partnering

PUBLICATION DETAILS: Ng, J. et al. *Proc. Natl. Acad. Sci. USA*; published online Feb. 22, 2017

doi:10.1073/pnas.1611771114

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