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Applying high throughput to CLL

By *Chris Cain, Senior Writer*

Boston researchers have provided a detailed look at how cancer genome evolution alters clinical outcomes in chronic lymphocytic leukemia,¹ and a Michigan team has identified a new transcriptional fusion that could broadly contribute to the pathogenesis of CLL.² Both studies provide insights that could guide the development of new diagnostics and therapeutics for patients with CLL.

CLL is the second most common type of adult leukemia and has highly variable clinical progression. To better understand the origin of this variability, multiple groups have set out to conduct high throughput DNA sequencing of CLL tumor samples from patients. In the last two years, whole-exome analysis of hundreds of these tumors has shown that the disease is associated with a large number of mutations, with no one predominant mutation or pathway.³⁻⁵

“The mutational landscape of CLL that is emerging from sequencing studies is extremely challenging, in that most recurrent mutations are present at low frequencies,” said Victor Quesada, a postdoctoral fellow at the **University of Oviedo** who led one of those sequencing studies. “At this point, we need to gather information to characterize one of at least two scenarios: either there is an underlying mechanism that connects all of the driver mutations or there are many independent mechanisms that may lead to CLL. So far, the second scenario has seemed more likely.”

Now, two teams have applied new high throughput sequencing data analysis methods to gain further insight into the mechanisms underlying CLL disease progression.

A joint team from the **Dana-Farber Cancer Institute** and the **Broad Institute of MIT and Harvard** focused on tumor DNA and used whole-exome sequencing to measure mutational heterogeneity in patient samples taken before and after chemotherapy. The findings suggest that identifying driver mutations present in only small fractions of cells could help predict the clinical course of CLL.

The team expects that its computational tools could be broadly applied to measure tumor heterogeneity in a variety of cancers.

University of Michigan Medical School researchers instead focused on tumor RNA and used a recently developed approach for whole-transcriptome data analysis to identify new chimeric transcripts comprised of normally disconnected genes. The group found one such transcript in more than 95% of CLL samples and presented preliminary data showing that the truncated protein phosphatase it encodes could be oncogenic.

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PO Box 1246
San Carlos, CA 94070-1246
T: +1 650 595 5333Chadds Ford
223 Wilmington-West Chester Pike
Chadds Ford, PA 19317
T: +1 610 558 1873Chicago
20 N. Wacker Drive, Suite 1465
Chicago, IL 60606-2902
T: +1 312 755 0798Oxford
287 Banbury Road
Oxford OX4 7JA
United Kingdom
T: +44 (0)18 6551 2184Washington, DC
2008 Q Street, NW, Suite 100
Washington, DC 20009
T: +1 202 462 9582**Nature Publishing Group**New York
75 Varick Street, 9th Floor
New York, NY 10013-1917
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United Kingdom
T: +44 (0)20 7833 4000Tokyo
Chiyoda Building 6F
2-37 Ichigayatamachi
Shinjuku-ku, Tokyo 162-0843
Japan
T: +81 3 3267 8751

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However, no DNA mutations or translocations were found that could explain how the chimera is generated, and validation in additional samples will be needed before its significance becomes clear.

Evolving thinking

Recent studies have uncovered substantial mutational heterogeneity within individual tumor samples, but the reliance of those studies on single-cell methods or whole-genome deep sequencing limited the numbers of samples that could be analyzed.

For example, two of the largest tumor evolution studies in 2012 measured the mutational landscape of a total of about 30 acute myelogenous leukemia (AML) samples.^{6,7}

Because of the genetic and clinical variation in CLL, researchers at Dana-Farber wanted to scale up the analysis to ensure it could comprehensively capture diversity. They teamed up with a Broad Institute team to apply recently developed computational algorithms for DNA sequence analysis to whole-exome data, which is far less costly to produce than whole-genome sequencing data.^{8,9}

Gad Getz, director of cancer genome computational analysis at the Broad Institute and co-corresponding author on the study, told SciBX that two algorithms developed in his lab, MuTect and ABSOLUTE, were essential to handle the complexity of quantifying populations of mutations in whole-exome sequencing data derived from bulk tumors.

In heterogeneous tumor samples, some copies of a given DNA sequence may be wild-type whereas others may be mutated. This wild-type/mutant ratio is known as an allelic fraction, and analysis of the allelic fraction can predict which mutations are clonal—found in most or all cancer cells in the sample—and which are subclonal, meaning those found only in some cancer cells in the sample.

“MuTect enabled the detection of mutations that have a low allelic fraction and therefore are present in smaller subclones,” Getz said. “ABSOLUTE enables the conversion of allelic fractions to the fraction of cancer cells harboring the mutation.”

The joint team used these two approaches and additional algorithms to measure the prevalence of mutations and copy number variations within whole-exome data generated from 149 pairs of matched CLL and germline DNA samples.

The group identified 1,543 clonal mutations and 1,266 subclonal mutations in total. On average, there were about 10 clonal mutations per sample and about 5 subclonal mutations.

Catherine Wu, co-corresponding author of the study, told SciBX that the scale of the analysis provides new insights into the heterogeneity of patients with CLL.

“By studying such a large cohort we were able to trace back the genetic history of the disease,” she said. “We observed, for example, a higher proportion of clonal mutations in older individuals, probably reflecting the accumulation of passenger mutations throughout life in pretransformed cells. In addition, we were able to define groups of recurrent putative driver mutations that appear earlier and later along the disease history of CLL.

“This work shows that pretreatment clonal heterogeneity and specifically the presence of subclonal drivers can predict clinical outcomes.”

**—Catherine Wu,
Dana-Farber Cancer Institute**

The earlier mutations tended to be more B cell specific, whereas the later ones seemed to be more generic cancer drivers found across many tumor types. This suggests interesting relationships between earlier and later events that we hope to study further in model systems.”

Wu is an associate physician of medical oncology at Dana-Farber and associate professor of medicine at **Harvard Medical School**.

The team then analyzed the effect of therapeutics on the evolution of tumor mutational heterogeneity.

In 12 matched patient samples taken before and after treatment with chemotherapy or anti-CD20 antibodies, the sequencing analysis identified driver mutations that increased in prevalence after treatment and predicted poorer survival compared with what was seen in patients lacking the mutations.

Treatment was associated with an expansion of cells carrying these driver mutations, as 10 of 12 treated patients experienced clonal evolution, whereas only 1 of 5 samples from untreated patients analyzed at 2 time points underwent clonal evolution.

Results were published in *Cell*.

Wu told *SciBX* that these results could have clinical implications. “This work shows that pretreatment clonal heterogeneity and specifically the presence of subclonal drivers can predict clinical outcomes,” she said.

Quesada agreed. “Probably the most useful consequence of this work is the ability to predict the evolution of the disease before the worst symptoms arise. This is one of the most pressing issues in CLL clinical management,” he said.

“Nowadays, clinicians need to wait for the symptoms to develop before providing a treatment. The risk for overtreatment is too high if no additional information can be gathered.”

He added, “The fact that mutations that are associated with poor prognosis are already present in subclones of the CLL cells before progression begins means that clinicians may now consider treatment at early stages, which might impact on both overall and progression-free survival. This strategy has been dubbed anticipation-based chemotherapy.”

Getz said the team now plans to test these predictions in prospective clinical trials of patients with CLL. “We also plan to extend this work to other types of malignancies and evaluate to what degree the insights found in CLL are generalizable to cancer biology,” he said.

The computational tools used in this study have been patented and are available for licensing from the Broad Institute. Getz said his lab has made the tools freely available to academic and not-for-profit organizations.

Chimeric RNA riddle

Although DNA sequencing efforts have characterized the genetic landscape of CLL, the Michigan team turned to RNA sequencing to identify new transcripts that could drive disease progression but would not be detected solely through genome analysis.

In the last several years, RNA sequencing analyses by the lab of Arul Chinnaiyan, professor of pathology at the University of Michigan Medical School, have led to the identification of many new cancer-associated chimeric transcripts—RNA species carrying sequences normally found in separate genes—with diagnostic and therapeutic potential in prostate cancer.¹⁰

The most obvious cause of a chimeric transcript would be a chromosomal translocation event, such as the well-known fusion of the *BCR* and *ABL* genes. However, at least one recently identified chimera is not associated with DNA translocation and is likely to be produced by splicing, suggesting that traditional DNA sequencing could miss fusion transcripts or proteins with oncogenic potential.¹¹

To look for such fusions in CLL, Kojo Elenitoba-Johnson, director of translational pathology at the University of Michigan Medical School, teamed up with Chinnaiyan to sequence the transcriptome of seven CLL patient samples. This led to the identification of RNA fusions of two transcripts from different chromosomes, *yippee-like 5* (*YPEL5*) and *protein phosphatase 1 catalytic subunit β -isozyme* (*PPP1CB*), expressed as either *YPEL5-PPP1CB* or *PPP1CB-YPEL5*.

The *PPP1CB-YPEL5* transcript was predicted to produce wild-type *YPEL5*, whereas the *YPEL5-PPP1CB* transcript was predicted to produce a truncated form of *PPP1CB* lacking its first 28 amino acids.

One or both transcripts were detected in 97 of 103 CLL samples (95%). Neither were detected in noncancerous B cells or in a variety of other leukemia or lymphoma samples.

The team tried numerous techniques to detect a DNA fusion between *YPEL5* and *PPP1CB* but found nothing. Elenitoba-Johnson told *SciBX* that result made the team’s finding even more intriguing. “We worked hard at establishing whether there was a genomic origin of the chimera—which we notably did not show—and part of the interest in this study is that it provides further evidence that you can have an RNA chimera that is not driven by a genomic event that may participate in the pathogenesis of some forms of cancer,” he said.

To test the functional significance of *YPEL5-PPP1CB*, the team expressed the transcript in cell culture and confirmed that it produced truncated *PPP1CB* protein. *In vitro*, this purified protein had less phosphatase activity than purified, full-length *PPP1CB*. In cultured leukemia cell lines,

small hairpin RNA against *PPP1CB* increased proliferation compared with control shRNA, suggesting that reduced *PPP1CB* function could have an oncogenic effect.

Results were published in the *Proceedings of the National Academy of Sciences*.

Quesada said the results are promising but emphasized that they must be validated by independent labs. “In this study, we have the first genomic hint that there is indeed a common biochemical mechanism at the origin of CLL,” he said. “In my opinion the first order of business is to independently confirm these results. Even though the authors provide ample and satisfying evidence for their case, confirmation from other laboratories is a must for an important discovery like this.”

Elenitoba-Johnson agreed that the result must be independently investigated and said he expects it to be quickly picked up by other labs.

“We suspect that due to the attention this result is getting because of its frequency, other labs will rapidly try to reproduce this finding in additional populations. After that, the next step will be to look and see how early this transcript occurs in the evolutionary process of the disease,” he said.

“In this study, we have the first genomic hint that there is indeed a common biochemical mechanism at the origin of CLL.”

**—Victor Quesada,
University of Oviedo**

He noted that although the functional evidence is still early, one future direction could be to screen for compounds that activate PPP1CB and test their effects in CLL.

Patent and licensing details were undisclosed.

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Contact: Catherine J. Wu, Dana-Farber Cancer Institute, Boston, Mass.
e-mail: cwu@partners.org
Contact: Gad Getz, Broad Institute of MIT and Harvard, Cambridge, Mass.
e-mail: gadgetz@broadinstitute.org
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Contact: Kojo S.J. Elenitoba-Johnson, University of Michigan Medical School, Ann Arbor, Mich.

e-mail: kojoelen@med.umich.edu

Contact: Arul M. Chinnaiyan, same affiliation as above
e-mail: arul@umich.edu

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COMPANIES AND INSTITUTIONS MENTIONED

Broad Institute of MIT and Harvard, Cambridge, Mass.

Dana-Farber Cancer Institute, Boston, Mass.

Harvard Medical School, Boston, Mass.

University of Michigan Medical School, Ann Arbor, Mich.

University of Oviedo, Oviedo, Spain

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Trapping influenza neuraminidase

By Lev Osherovich, Senior Writer

Researchers at **The University of British Columbia** have used a mechanism-based approach to design irreversible inhibitors of influenza virus neuraminidase that could have activity against flu strains resistant to marketed inhibitors of the target.¹ The new molecules are being developed by **CDRD Ventures Inc.**, the venture arm of **The Centre for Drug Research and Development**, a public-private partnership that commercializes discoveries from Canadian researchers.

Influenza virus uses neuraminidase to cleave sugars from host glycoproteins at the cell surface, allowing newly formed virions to escape from the infected cells and spread to others.

Two reversible inhibitors of influenza A virus neuraminidase—**Roche** and **Gilead Sciences Inc.**'s Tamiflu oseltamivir and Relenza zanamivir from **GlaxoSmithKline plc** and **Biota Pharmaceuticals Inc.**—are marketed in the U.S. to treat and prevent influenza A. Two other reversible neuraminidase inhibitors are marketed outside the U.S.—PeramiFlu peramivir from **BioCryst Pharmaceuticals Inc.**, **Green Cross Corp.** and **Shionogi & Co. Ltd.**, and Inavir laninamivir from **Daiichi Sankyo Co. Ltd.** and Biota.

Although these inhibitors bind tightly to the active site of the enzyme, mutations in nearby residues of the viral protein can reduce drug affinity and even lead to resistance.

Over time, widespread use of marketed neuraminidase inhibitors could favor the spread of resistant strains, similar to what occurs with antibiotics. Indeed, Tamiflu-resistant influenza strains, including variants of the highly virulent H5N1 bird flu strain, have emerged in Asia in recent years.

Stephen Withers, professor of chemistry and biochemistry at the University of British Columbia, thus set out to design neuraminidase inhibitors that target the enzyme differently from the approved flu drugs.

Using a mechanism-based approach, his team designed a class of molecules based on 2,3-difluorosialic acid (DFSA), a compound that closely resembles sialic acid, the natural target of neuraminidase. DFSA, which is highly reactive, becomes covalently trapped in the enzyme's active site and eliminates the enzyme's activity.

The compounds were effective against existing resistant strains. They also could lower the odds of new resistant strains emerging, Withers said, because the new compounds' close mimicry of the natural substrate makes it difficult for the virus to evolve resistance-associated mutations without compromising the enzyme's normal function.

"If you minimize differences between the structure of the inhibitor and the natural substrate, it will be harder to develop resistance," said Withers. "This is also a covalent modification, which is essentially irreversible. It should thus be slower for resistance to develop."

"If you minimize differences between the structure of the inhibitor and the natural substrate, it will be harder to develop resistance."

—**Stephen Withers,**
The University of British Columbia

Tight fit

The group developed a series of DFSAs with modifications designed to fit tightly into the neuraminidase active site and tested the compounds against a panel of eight clinically relevant neuraminidase variants. *In vitro*, the best compound had an IC₅₀ value of less than 50 nM for 7 of 8 neuraminidase variants, including enzymes that were resistant to Relenza and/or Tamiflu.

On the flip side, the DFSAs tested had higher *in vitro* IC₅₀ values than Relenza and Tamiflu against most nonresistant strains, suggesting the new compounds could benefit from further optimization.

In a cell culture plaque-formation assay, the best new compound and Relenza showed similar prevention of viral growth. In mice infected with a nonresistant H3N3 strain, the DFSAs showed efficacy comparable to that of Relenza. The team has not yet tested the compounds in animals with resistant strains.

The compounds from Withers' team were more effective *in vitro* against influenza B virus neuraminidase than Relenza. Influenza B infects only humans and seals and is considered less of a public health threat than influenza A, which infects a wide range of domestic animals as well as humans.

Findings were reported in *Science*.

Withers said the next step is to use a ferret model of influenza infection to test the compounds' efficacy against resistant and nonresistant strains. His team also is collaborating with the Centre for Drug Research and Development to synthesize orally available prodrug forms of the compounds.

Michael Parr, director of commercial project development at CDRD Ventures, said prior attempts by other researchers to design new inhibitors of influenza neuraminidase based on traditional models of the active site met with failure, so the approach had "fallen out of favor."

The new compounds work better than previous covalent inhibitors because of new information about the mechanism of the enzyme.

Parr said that Withers "understood the true nature of the enzyme reaction. We have capitalized on a unique mechanism of action."

Jonathon Jafari, director of business development at CDRD Ventures, said that the initial market for drugs based on Withers' compounds could be government stockpiles to combat a large-scale outbreak of virulent, drug-resistant strains.

CDRD Ventures expects that the compounds could be used as a potential second

line of defense if approved drugs eventually lose their effectiveness. The company is investing an undisclosed amount of money in the preclinical development of the compounds.

"There's a potential for this product to be best in class, with the potential for oral availability," said Jafari.

Jafari and Parr said preclinical proof of concept should be completed this year, at which time CDRD Ventures will seek to partner or out-license the compounds. They said another option is to raise additional venture capital or nondilutive grant funding to bring the compounds into clinical testing.

Covalent inhibitors based on Withers' DFSAs might be more potent than marketed drugs, but delivering the compounds to patients early

during infection remains a challenge. In the U.S., most patients wait until the infection has nearly run its course before seeking treatment. Jafari noted that in Japan, for example, patients typically consult doctors earlier during the illness and could potentially benefit from early intervention.

Withers thinks that his compounds could be used to stop the spread of flu, not just to treat already infected patients.

“I would anticipate that this would be a prophylactic taken ahead of time if your family gets the flu or there’s a pandemic,” he said.

Other flu compounds in the clinic include VX-787 from **Vertex Pharmaceuticals Inc.** In March, Vertex reported positive results for the compound in a Phase IIa trial to treat H3N2 influenza A virus. Vertex did not disclose the target of VX-787.

The findings have been patented by the University of British Columbia, which has granted CDRD Ventures an option to license the technology.

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Contact: Stephen G. Withers, The University of British Columbia, Vancouver, British Columbia, Canada
e-mail: withers@chem.ubc.ca

COMPANIES AND INSTITUTIONS MENTIONED

BioCryst Pharmaceuticals Inc. (NASDAQ:BCRX), Durham, N.C.
Biota Pharmaceuticals Inc. (NASDAQ:BOTA), Rockville, Md.
The Centre for Drug Research and Development, Vancouver, British Columbia, Canada
CDRD Ventures Inc., Vancouver, British Columbia, Canada
Daiichi Sankyo Co. Ltd. (Tokyo:4568; Osaka:4568), Tokyo, Japan
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Green Cross Corp., Yongin-Si, South Korea
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
Shionogi & Co. Ltd. (Tokyo:4507; Osaka:4507), Osaka, Japan
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Understanding fibrosis

By Tracey Baas, Senior Editor

Researchers at **The University of Alabama at Birmingham** have identified idiopathic pulmonary fibrosis as a repurposing opportunity for fasudil, a Rho kinase inhibitor that **Asahi Kasei Pharma Corp.** markets as Eril to treat aneurysm. The key observation was that the drug acts on a pathway that converts biochemical or biomechanical stimuli into fibrogenic signals that sustain myofibroblast activation and survival.¹

The team now needs to develop a formulation of fasudil or other Rho kinase inhibitors that works only in the lung and thus avoids systemic side effects.

In normal wound healing, fibroblasts that reside in tissues are activated and transform into myofibroblasts, which are characterized by high contractile activity that results from actin $\alpha 2$ smooth aorta muscle (ACTA2; α -SMA) accumulation in stress fibers. The myofibroblasts help remodel and repair tissue by secreting extracellular matrix components, such as collagen and fibronectin.

Once the wound is re-epithelialized, the myofibroblasts undergo apoptosis.²

In fibrotic disease, apoptotic-resistant myofibroblasts persist in injured tissues and can lead to excessive accumulation of fibrous connective tissue and tissue stiffening. This accumulation can result in permanent scarring, organ malfunction and possibly death, as seen in end-stage idiopathic pulmonary fibrosis (IPF).

Fibrogenic cytokines and growth factors, such as transforming growth factor- $\beta 1$ (TGFB1), are known promoters of fibroblast-to-myofibroblast differentiation, but the Alabama team has been searching for other contributors.

In 2012, the group showed that biomechanical properties of the extracellular matrix contribute to differentiation. The researchers

Figure 1. Myofibroblast dynamics. Lung fibroblasts undergo actin cytoskeleton and actomyosin contractile system remodeling in response to biomechanical and biochemical stimuli. This remodeling leads to enhanced fibroblast contraction, which results in the translocation of myocardin-related transcription factor A (MKL1; MAL; MRTF-A) from the cytoplasm to the nucleus. The result is activation of fibrotic genes that induce myofibroblast differentiation, such as *actin $\alpha 2$ smooth aorta muscle* (ACTA2; α -SMA) and *collagen type I $\alpha 2$* (COL1A2).

The Alabama team showed that the Rho kinase inhibitor fasudil disrupts the actin cytoskeleton required for myofibroblast contractility in pre-existing myofibroblasts. This deactivates a constitutively activated MKL1 nuclear signal in myofibroblasts, resulting in blockade of fibroblast-to-myofibroblast differentiation and downregulation of the antiapoptotic protein B cell lymphoma 2 (BCL-2; BCL2) to activate apoptosis.

Rho kinase inhibitors could provide additive or synergistic anti-fibrotic effects with inhibitors of transforming growth factor- $\beta 1$ (TGFB1) such as Esbriet pirfenidone. (Figure based on Figure 7 in ref. 1.)

reported that mouse lung fibroblasts cultured on a stiff matrix showed greater actin cytoskeletal reorganization and myofibroblast differentiation than those cultured on soft matrix.³

However, inhibiting Rho kinase activity prevented those changes. Rho kinases—including Rho-associated coiled-coil containing protein kinase 1 (ROCK1) and ROCK2—are involved in regulating cell contractility, actin cytoskeletal organization, stress fiber formation and focal adhesion assembly.

Now, the team has probed the underpinnings of the pathway that converts biomechanical stimuli into biochemical fibrogenic signals.

For biochemical stimuli the group used healthy human lung fibroblasts treated with TGFB1, and for biomechanical stimuli the group cultured the fibroblasts on a stiff matrix. In both cases, fasudil blocked the fibroblast-to-myofibroblast differentiation and myofibroblast contractility that was seen in untreated cells.

The team concluded that the drug targets both biochemical and biomechanical signals that mediate myofibroblast differentiation.

The Alabama team also showed that fasudil led to apoptosis of α -SMA⁺ lung myofibroblasts but not normal fibroblasts.

Notably, fasudil showed antifibrotic effects even when given to mice with established fibrosis.

Further work with fasudil-treated IPF myofibroblasts provided a clear mechanistic picture of what was occurring. Specifically, the drug impeded differentiation and induced apoptosis through blocking nuclear

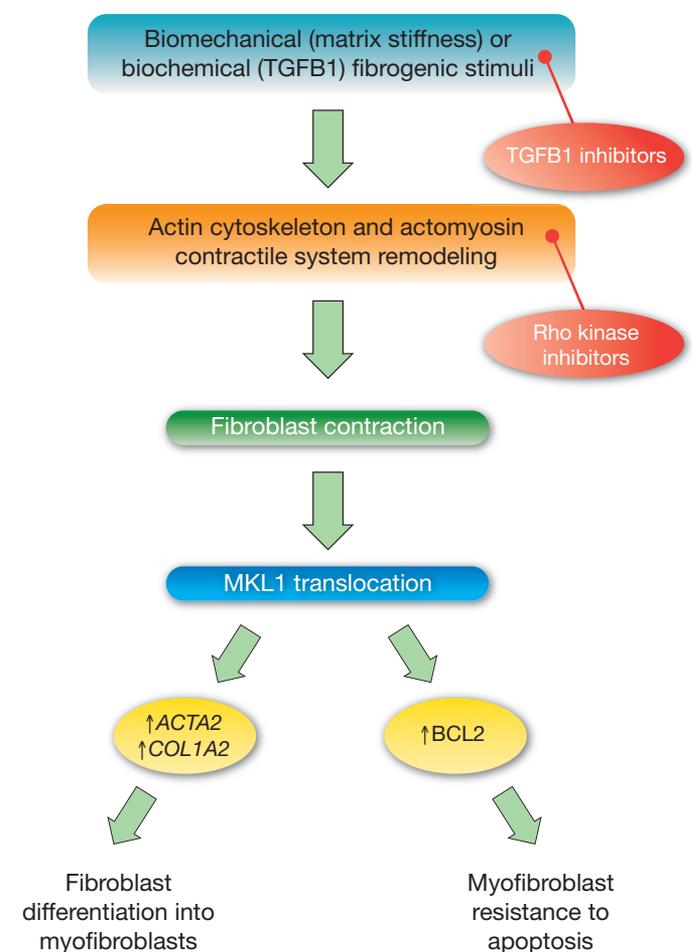


Table 1. TGFB1 and Rho kinase inhibitors.

Transforming growth factor- β 1 (TGFB1) inhibitors				
Company	Product	Disease category	Indication	Development
InterMune Inc. (NASDAQ:ITMN)	Esbriet pirfenidone	Pulmonary disease	Pulmonary fibrosis	Marketed in Europe and Canada
Acceleron Pharma Inc./Celgene Corp. (NASDAQ:CELG)	ACE-536	Hematology	Anemia and thalassemia	Phase II
Digna Biotech S.L.	Disitertide (P144)	Autoimmune disease	Scleroderma	Phase II
Digna Biotech	Disitertide (P144)	Dermatology	Dermatology	Phase II
Eli Lilly and Co. (NYSE:LLY)	LY2382770	Renal disease	Diabetic nephropathy and renal disease	Phase II
Renovo Group plc (LSE:RNVO)	Juvidex	Dermatology	Scars and wrinkles	Phase II
Yuhan Corp.	YH14618	Musculoskeletal disease	Cartilage repair	Phase I/II
Digna Biotech	Disitertide (P144)	Dermatology	Actinic keratosis	Phase I
Rho kinase inhibitors				
Asahi Kasei Pharma Corp.	Eril fasudil	Cardiovascular disease	Aneurysm	Marketed
D. Western Therapeutics Institute Inc. (JASDAQ:4576)	K-115	Ophthalmic disease	Glaucoma and ocular hypertension	Phase III
Aerie Pharmaceuticals Inc.	AR-13324	Ophthalmic disease	Glaucoma	Phase II
Amakem N.V.	AMA0076	Ophthalmic disease	Glaucoma and ocular hypertension	Phase II
Asahi Kasei Pharma	ATS907	Ophthalmic disease	Glaucoma	Phase II
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508; Osaka:4508)	Y-39983	Ophthalmic disease	Glaucoma	Phase II
Aerie Pharmaceuticals	AR-12286	Ophthalmic disease	Glaucoma	Phase II/III

localization of myocardin-related transcription factor A (MKL1; MAL; MRTF-A) and downregulating B cell lymphoma 2 (BCL-2; BCL2) and α -SMA (see Figure 1, “Myofibroblast dynamics” and Table 1, “TGFB1 and Rho kinase inhibitors”).

“This new work very elegantly shows the mechanistic details to unravel how biochemical and biomechanical stimulus leads to remodeling and especially differentiated apoptosis of myofibroblasts versus resident fibroblasts,” said Dirk Leysen, CSO and founder of Amakem N.V. “Many people are working on the various triggers and processes that lead to fibrosis, including persistent inflammation. This work focuses on the postinflammatory fibrotic phase, which is much more useful for late-stage fibrosis such as that seen in IPF. What they find suggests that not only could Rho kinase inhibition stop fibrosis from progressing but might also be able to reverse fibrosis.”

The new work shows that “cellular sensing of and response to tissue stiffness almost certainly plays a significant role in the progression of fibrosis,” said Scott Seiwert, SVP of research and technical development at InterMune Inc.

InterMune markets Esbriet pirfenidone to treat IPF in Europe and Canada. Esbriet is a small molecule inhibitor of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and IL-1 β as well as profibrotic cytokines, including platelet derived growth factor (PDGF) and TGFB (TGF β).

Making it work

Seiwert cautioned that “fasudil is a rather nonspecific kinase inhibitor, and so its ability to probe the role of Rho kinases is limited.”

Regardless, Martin Kolb, research director of the Firestone Institute for Respiratory Health and associate professor of medicine, pathology

and molecular medicine at McMaster University, ultimately wanted to see studies of pirfenidone plus fasudil.

Kolb is collaborating with Genoa Pharmaceuticals Inc. to develop an aerosol formulation of pirfenidone for IPF to avoid the drug’s nausea side effect. With aerosol delivery, the hope is the drug would be concentrated in the lungs and avoid high concentrations systemically, which can lead to nausea.

Seiwert was also interested in seeing the effects of that combination. “Pirfenidone reduces TGFB1 levels in dozens of animal models of fibrosis across several organ systems,” he said. “Since pirfenidone lowers TGFB1 levels and fasudil disrupts TGFB1-induced myofibroblast differentiation, the two compounds could potentially display additive or synergistic antifibrotic effects *in vivo*.”

Prior to any combination studies, there was consensus that fasudil needs to be reformulated for IPF.

“Fasudil could not be used to treat pulmonary fibrosis as is,” said Leysen. “Rho kinase activity is needed for vascular smooth muscle homeostasis, so systemic exposure to Rho kinase inhibitors will lead to dangerous side effects such as extremely low blood pressure. One way around this would be to apply a very specific lung delivery technology.”

An alternative, he said, would be using Amakem’s approach of chemically modifying Rho kinase inhibitors “so that they are only active in the tissue they intend to treat and degraded once they reach systemic circulation.”

Amakem’s AMA0076 is in Phase II trials for glaucoma and ocular hypertension.

Although Asahi’s Eril is the only Rho kinase inhibitor on the market and is used to treat aneurysm, multiple companies are pursuing the target for glaucoma, neurodegeneration, pain and solid tumors.

“What they find suggests that not only could Rho kinase inhibition stop fibrosis from progressing but might also be able to reverse fibrosis.”

—Dirk Leysen, Amakem N.V.

Asahi declined requests for interviews.

The Alabama team has filed an IP disclosure with the **UAB Research Foundation**, but the work is currently unpatented. The team is looking to find a partner that is currently working on formulating Rho kinase inhibitors for cancer or other clinical indications to start Phase II studies of Rho kinase inhibitors in patients with IPF.

Baas, T. *SciBX* **6**(11); doi:10.1038/scibx.2013.255
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Contact: Victor J. Thannickal, The University of Alabama at Birmingham, Birmingham, Ala.
e-mail: vjthan@uab.edu

Contact: Yong Zhou, same affiliation as above
e-mail: yzhou@uab.edu

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COMPANIES AND INSTITUTIONS MENTIONED

Amakem N.V., Diepenbeek, Belgium
Asahi Kasei Pharma Corp., Tokyo, Japan
Firestone Institute for Respiratory Health, Hamilton, Ontario, Canada
Genoa Pharmaceuticals Inc., San Diego, Calif.
InterMune Inc. (NASDAQ:ITMN), Brisbane, Calif.
McMaster University, Hamilton, Ontario, Canada
UAB Research Foundation, Birmingham, Ala.
The University of Alabama at Birmingham, Birmingham, Ala.



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Nanoparticles for lupus

By Lauren Martz, Staff Writer

A Yale University group has designed a nanoparticle that selectively delivered an immunosuppressant to immune cells and improved survival in mouse models of lupus.¹ The nanoparticle delivery platform has been exclusively licensed to Immunova LLC, which is selecting an anti-inflammatory compound to take into the clinic.

Lupus is an autoimmune disease characterized by excessive production of proinflammatory cytokines and autoantibodies that cause damage to multiple tissues, including the kidneys, CNS, joints and skin.

Marketed drugs for the disease have side effects including GI toxicities and increased risk of infection from immunosuppression. Thus, a continuing challenge is figuring out how to target and suppress only the immune cells that trigger autoimmunity.

The Yale group, led by Tarek Fahmy, hypothesized that a nanoparticle drug delivery system could selectively deliver immunosuppressants to autoreactive immune cells. Prior work by a University of Virginia team showed that nanoparticles traveled to the sites of renal tissue inflammation in a lupus mouse model.²

“We are proposing that the current challenge in autoimmune diseases is not exclusively limited to drug discovery but in essence is a problem of directed drug delivery to pathogenic immune cells,” Fahmy told *SciBX*.

Fahmy, who is associate professor of biomedical and chemical engineering and of immunobiology at Yale, decided to use a nanoparticle delivery platform to administer the small molecule mycophenolic acid (MPA). Roche markets the MPA prodrug CellCept mycophenolate mofetil to prevent organ transplant rejection. CellCept is in Phase III testing for lupus and is used off label for the indication.

The nanoparticle delivery platform consisted of liposomes and biodegradable polymers that were stable and could encapsulate a range of small molecules.

In a mouse model for systemic lupus erythematosus (SLE), the MPA-loaded nanoparticles extended survival to 50–51 weeks compared with 38 weeks for saline control, whereas free MPA did not extend survival at the equivalent dose or at a 16-fold higher dose. Treatment also delayed onset of proteinuria and decreased glomerular damage.

Importantly, the nanoparticles did not reduce body weight or cause hepatic or renal toxicities.

Finally, Fahmy and colleagues showed that dendritic cells took up the nanoparticles and decreased both production of proinflammatory cytokines and T cell activation. Those findings suggested that the nanoparticles suppress the antigen-presenting and proinflammatory functions of dendritic cells.

The findings were published in *The Journal of Clinical Investigation*.

“Our next steps are focused on the choice of drug candidates to be encapsulated for lupus clinical translation and then initiating safety trials in humans,” said Fahmy, while also continuing development of the MPA-loaded nanoparticles.

Fahmy’s team also is considering other autoimmune indications for the nanoparticle technology and is working with the Human and Translational

Immunology Program at Yale to explore using the platform to treat type 1 diabetes.

Getting optimal

Meanwhile, Immunova has exclusively licensed the platform. The company is optimizing the nanoparticle content and methods of production, while Fahmy and his team are further characterizing the nanoparticle, potential drug candidates and indications under a sponsored research agreement with the company.

Immunova declined to provide a timeline for when nanoparticle-based therapeutics might reach the clinic.

“The next step to consider is the safety of the nanoparticle for use in humans,” said Keith Elkon, professor of medicine and head of the Division of Rheumatology at the University of Washington. “The current concern is about medium- and long-term effects of their administration.”

Thus, Elkon wanted to see long-term safety studies in animals. He said nanoparticles have been highly reactive with a number of human cell types, including neurons, depending on the type and composition of the nanoparticles. Resulting problems could include carcinogenesis and CNS toxicity due to the nanoparticle crossing the blood brain barrier.

Michael Look, a postdoctoral fellow in Fahmy’s lab at Yale, pointed out that although some types of nanomaterials do cause problems that make them unsuitable for use in humans, the team formulated its nanogels “with this concern in mind and chose materials that are biocompatible and have a robust track record of being safe.”

The nanoparticle also may carry the risk of immunosuppression. “Dendritic cells are needed for normal immune function, and dampening their antigen-presenting function long term would require consideration of how it affects patients’ abilities to fight infection,” added Elkon.

According to Look, the team has two reasons to suspect that risk of infection should not necessarily increase with the nanoparticles.

First, they found that total white blood cell numbers remained normal following very high-dose treatment, which is in contrast with conventional methods of immunosuppression, for which depletion of white blood counts is often associated with opportunistic infection. Second, the mice were still able to generate functional immune responses after immunization. “These observations lead us to believe that a patient could still mount an immune response to an infection even with therapy. Though, of course, we’d still have to check to see if this is true in human trials,” said Look.

Fahmy said Yale has filed a patent application covering nanoparticle composition and methods of manufacture and use.

Martz, L. *SciBX* 6(11); doi:10.1038/scibx.2013.256
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e-mail: tarek.fahmy@yale.edu
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COMPANIES AND INSTITUTIONS MENTIONED

Immunova LLC, New Haven, Conn.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University of Virginia, Charlottesville, Va.
University of Washington, Seattle, Wash.
Yale University, New Haven, Conn.

This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* 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.

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer				
Basal cell carcinoma (BCC)	Protein kinase C α (PRKCI; aPKC-1 λ); sonic hedgehog homolog (SHH)	Studies <i>in vitro</i> , in mice and in human tissue suggest inhibiting PRKCI could help treat SHH-driven BCC. A proteomic screen identified PRKCI as a new component of the SHH signaling pathway. In primary human BCC tumors, levels of total and activated PRKCI were higher than those in healthy human skin samples. In a mouse model for BCC, topical treatment with a peptide inhibitor of PRKCI decreased SHH signaling and tumor growth compared with vehicle treatment. Next steps include screening for more potent and selective peptide or small molecule inhibitors of PRKCI and exploring the effects of PRKCI inhibition in other SHH-driven cancers. SciBX 6(11); doi:10.1038/scibx.2013.257 Published online March 21, 2013	Patent application filed; available for licensing	Atwood, S.X. <i>et al. Nature</i> ; published online Feb. 27, 2013; doi:10.1038/nature11889 Contact: Anthony E. Oro, Stanford University School of Medicine, Stanford, Calif. e-mail: oro@stanford.edu Contact: Scott X. Atwood, same affiliation as above e-mail: satwood@stanford.edu
Cancer	IL-11	Mouse and cell culture studies suggest inhibiting IL-11 signaling could help treat hypoxic solid tumors. Human colorectal cancer cells cultured under hypoxic conditions had greater IL-11 mRNA levels and anchorage-independent growth than cells cultured under normal oxygen conditions. In a mouse xenograft model for human prostate cancer, small hairpin RNA against IL-11 decreased tumor growth compared with scrambled shRNA. Next steps could include evaluating the combination of IL-11 signaling inhibitors with other cancer therapeutics. SciBX 6(11); doi:10.1038/scibx.2013.258 Published online March 21, 2013	Unpatented; licensing not applicable	Onnis, B. <i>et al. J. Clin. Invest.</i> ; published online March 15, 2013; doi:10.1172/JCI59623 Contact: Giovanni Melillo, Bristol-Myers Squibb, Princeton, N.J. e-mail: giovanni.melillo@bms.com Contact: Annamaria Rapisarda, SAIC-Frederick Inc., Frederick, Md. e-mail: rapisardaa@mail.nih.gov
Cancer	Nicotinamide N-methyltransferase (NNMT)	Cell culture studies suggest inhibiting NNMT could help treat cancer. Aggressive cancer cell lines showed higher NNMT levels and NNMT activity than nonaggressive cancer cell lines. In aggressive cancer cell lines, knockdown of NNMT decreased expression of multiple cancer-associated genes and invasiveness compared with no knockdown. Next steps include developing NNMT inhibitors and testing them in preclinical cancer models. SciBX 6(11); doi:10.1038/scibx.2013.259 Published online March 21, 2013	Unpatented; licensing status not applicable	Ulanovskaya, O.A. <i>et al. Nat. Chem. Biol.</i> ; published online March 3, 2013; doi:10.1038/nchembio.1204 Contact: Benjamin F. Cravatt, The Scripps Research Institute, La Jolla, Calif. e-mail: cravatt@scripps.edu
Cancer	Tubulin	<i>In vitro</i> and mouse studies identified tubulin polymerization inhibitors that could help treat cancer. In seven cancer cell lines, inhibitors from the series showed more potent inhibition of proliferation than the parent compound. The most potent compounds inhibited tubulin polymerization and induced mitochondrial dysfunction and apoptosis in cancer cells but not in normal human lymphocytes. In mice with hepatocellular carcinoma (HCC) tumors, the most potent compound decreased tumor growth compared with vehicle control. Next steps could include optimizing the lead compound and testing it in additional animal cancer models. SciBX 6(11); doi:10.1038/scibx.2013.260 Published online March 21, 2013	Patent and licensing status unavailable	Romagnoli, R. <i>et al. J. Med. Chem.</i> ; published online Feb. 28, 2013; doi:10.1021/jm400043d Contact: Giampietro Viola, University of Padua, Padua, Italy e-mail: giampietro.viola1@unipd.it Contact: Pier Giovanni Baraldi, University of Ferrara, Ferrara, Italy e-mail: pgb@unife.it Contact: Romeo Romagnoli, same affiliation as above e-mail: rmr@unife.it

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	Prion protein (PRNP; PrP; CD230)	<p>Patient sample and mouse studies suggest inhibiting PRNP could help treat metastatic colorectal cancer (CRC). In primary tumor samples from patients with CRC, increased PRNP expression in tumors was associated with increased risk of metastasis and decreased survival. In two mouse xenograft models for patient-derived metastatic CRC, animals implanted with PRNP⁺ CRC stem cells showed higher rates of liver metastasis than mice that received PRNP⁻ CRC stem cells. In one of the mouse models, PRNP-targeting antibodies decreased metastasis and tumor weight compared with control IgG. Next steps include testing the antibodies in mouse models for other cancer types.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.261 Published online March 21, 2013</p>	Patent and licensing status undisclosed	<p>Du, L. <i>et al. Cancer Res.</i>; published online Feb. 15, 2013; doi:10.1158/0008-5472.CAN-12-3759 Contact: Quan Chen, Chinese Academy of Sciences, Beijing, China e-mail: chenq@ioz.ac.cn</p>
Liver cancer	Glypican 3 (GPC3)	<p><i>In vitro</i> and mouse studies identified an anti-GPC3 antibody that could help treat hepatocellular carcinoma (HCC). In human HCC cell lines, an antibody against a core functional portion of GPC3 inhibited growth of GPC3⁺ HCC cells but not GPC3⁻ or GPC3 knockout cells. In a mouse xenograft model for HCC, the antibody decreased tumor growth compared with control antibodies. Next steps include completing preclinical testing.</p> <p>Chugai Pharmaceutical Co. Ltd. and Roche have the anti-GPC3 antibody GC33 in Phase II testing to treat liver cancer.</p> <p>Bristol-Myers Squibb Co. has the anti-GPC3 antibody MDX-1414 in preclinical development for the same indication.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.262 Published online March 21, 2013</p>	Patent application filed; available for licensing	<p>Feng, M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 5, 2013; doi:10.1073/pnas.1217868110 Contact: Mitchell Ho, National Cancer Institute, Bethesda, Md. e-mail: homi@mail.nih.gov</p>
Neuroendocrine tumors	CD56	<p>Patient sample and mouse studies suggest CD56⁺ NK cells could help treat neuroblastoma. <i>In vitro</i>, peripheral blood mononuclear cells from healthy donors were selected for cell surface CD56 expression, mixed 1:1 with CD56⁻ cells and then activated using a combination of IL-12, IL-15 and a CD3 antibody. In xenograft mice with neuroblastoma cell lines, the activated mixture decreased tumor growth compared with nonactivated NK cells or no treatment. Next steps include a clinical trial of the cells in patients with neuroblastoma.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.263 Published online March 21, 2013</p>	Patent and licensing status undisclosed	<p>Rujkijyanont, P. <i>et al. Cancer Res.</i>; published online Feb. 25, 2013; doi:10.1158/0008-5472.CAN-12-3322 Contact: Wing Leung, St. Jude Children's Research Hospital, Memphis Tenn. e-mail: wing.leung@stjude.org</p>
Pancreatic cancer	K-Ras; 3-phosphoinositide-dependent protein kinase-1 (PDPK1)	<p>Mouse studies suggest inhibiting PDPK1 could help prevent mutant <i>K-Ras</i>-driven pancreatic ductal adenocarcinomas (PDACs). In the epithelial cells of <i>K-ras</i> mutant mice, <i>Pdpk1</i> inactivation prevented acinar-to-ductal development of premalignant pancreatic intraepithelial neoplasias and PDACs. The mice also had higher median survival than <i>K-ras</i> mutant mice expressing functional <i>Pdpk1</i>. Next steps could include testing clinical-stage PDPK1 inhibitors in mouse models for PDAC.</p> <p>Arno Therapeutics Inc.'s AR-12, a small molecule PDPK1 inhibitor that blocks the phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB; PKBA; AKT; AKT1) pathway, is in Phase I testing to treat solid tumors and lymphoma.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.264 Published online March 21, 2013</p>	Patent and licensing status unavailable	<p>Eser, S. <i>et al. Cancer Cell</i>; published online Feb. 28, 2013; doi:10.1016/j.ccr.2013.01.023 Contact: Dieter Saur, Technical University Munich, Munich, Germany e-mail: dieter.saur@lrz.tum.de</p>

This week in therapeutics

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Prostate cancer	Fibroblast growth factor 19 (FGF19)	Cell culture and mouse studies suggest inhibiting FGF19 could help treat prostate cancer. In human prostate cancer cell lines, FGF19 induced proliferation and anchorage-independent cell growth and increased invasiveness compared with control vectors. In these cell lines, small hairpin RNA against FGF19 decreased proliferation and invasiveness compared with control vectors. In mice implanted with prostate cancer cells, shRNA against FGF19 decreased tumor growth compared with control vectors. Next steps could include testing anti-FGF19 antibodies in prostate cancer models. SciBX 6(11); doi:10.1038/scibx.2013.265 Published online March 21, 2013	Patent and licensing status unavailable	Feng, S. <i>et al. Cancer Res.</i> ; published online Feb. 25, 2013; doi:10.1158/0008-5472.CAN-12-4108 Contact: Michael M. Ittmann, Baylor College of Medicine, Houston, Texas e-mail: mittmann@bcm.edu
Hepatic disease				
Liver disease	Farnesoid X receptor (FXR; NR1H4); G protein-coupled bile acid receptor 1 (GPBAR1; TGR5)	Mouse studies suggest the FXR and TGR5 dual agonist INT-767 could help treat nonalcoholic fatty liver disease (NAFLD). In a mouse model for obesity-induced NAFLD, INT-767 decreased hepatic steatosis and inflammation compared with no agonist. Next steps could include evaluating INT-767 in additional mouse models of NAFLD. Intercept Pharmaceuticals Inc. has INT-767 in preclinical development to treat renal diseases. SciBX 6(11); doi:10.1038/scibx.2013.266 Published online March 21, 2013	Multiple patents covering FXR agonists filed by Intercept Pharmaceuticals; licensing status unavailable	McMahan, R. <i>et al. J. Biol. Chem.</i> ; published online March 4, 2013; doi:10.1074/jbc.M112.446575 Contact: Hugo R. Rosen, University of Colorado Denver, Aurora, Colo. e-mail: hugo.rosen@ucdenver.edu
Infectious disease				
Bacterial infection; herpes simplex virus (HSV)	Interferon-ε (IFNE; IFNε)	Mouse studies suggest increasing IFNE might help prevent sexually transmitted infections. In mouse models for HSV infection, IFNE-deficient mice had severe genital lesions and greater virus levels in vaginal tissues, spinal cord and brain stem than wild-type mice. In mouse models for <i>Chlamydia muridarum</i> infection, IFNE-deficient mice had higher vaginal and uterine bacteria levels than wild-type mice. In the <i>Chlamydia</i> infection mouse model, intravaginal pretreatment with IFNE six hours before challenge protected against bacterial infection. Next steps could include testing the protective effects of IFNE treatment in additional models for sexually transmitted infections. SciBX 6(11); doi:10.1038/scibx.2013.267 Published online March 21, 2013	Patent and licensing status unavailable	Fung, K.Y. <i>et al. Science</i> ; published online March 1, 2013; doi:10.1126/science.1233321 Contact: Paul J. Hertzog, Monash University, Clayton, Victoria, Australia e-mail: paul.hertzog@monash.edu
Influenza	Protectin D1 (PD1)	<i>In vitro</i> and mouse studies suggest PD1 could help treat influenza. In normal lung epithelial cells infected with influenza A virus, a lipid screen identified the lipid mediator PD1 as a suppressor of influenza replication. In mice, i.v. treatment with PD1 48 hours after infection increased survival compared with vehicle treatment. In the mouse model, PD1 plus the neuraminidase (NEU1; SIAL1) inhibitor peramivir led to 100% survival. Next steps could include testing PD1 in different animal models of influenza virus infection. BioCryst Pharmaceuticals Inc. discontinued a Phase III trial of peramivir in November 2012 for futility following an interim analysis. SciBX 6(11); doi:10.1038/scibx.2013.268 Published online March 21, 2013	Patent and licensing status unavailable	Morita, M. <i>et al. Cell</i> ; published online March 7, 2013; doi:10.1016/j.cell.2013.02.027 Contact: Yumiko Imai, Akita University, Akita, Japan e-mail: imai@med.akita-u.ac.jp

This week in therapeutics

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Pneumonia	IL-1 β ; IL-18; NLR family CARD domain containing 4 (NLRC4); caspase-1 (CASP1)	<p>Mouse studies suggest inhibiting inflammasome activation could help treat <i>Pseudomonas aeruginosa</i> pneumonia. In mice infected with <i>P. aeruginosa</i>, knockout of inflammasome activation and signaling components, including Nlr4, Casp1, Il-1β or Il-18, led to increased bacterial clearance from the airways and decreased tissue damage compared with no knockout. Next steps include testing available inhibitors of inflammasome signaling in patients with pneumonia.</p> <p>At least seven companies have inhibitors or antibodies targeting IL-1β in development stages ranging from preclinical to marketed for various indications. GlaxoSmithKline plc's 1070806, an mAb against IL-18, is in Phase I testing for inflammatory bowel disease (IBD) and diabetes.</p> <p>Vertex Pharmaceuticals Inc.'s VX-765, a CASP1 inhibitor, is in Phase II testing to treat epilepsy.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.269 Published online March 21, 2013</p>	Patent status not applicable; unavailable for licensing	<p>Cohen, T.S. & Prince, A.S. <i>J. Clin. Invest.</i>; published online March 8, 2013; doi:10.1172/JCI66142</p> <p>Contact: Alice Prince, Columbia University, New York, N.Y. e-mail: asp7@columbia.edu</p>
Various				
Cardiovascular disease; neurology	AMP-activated protein kinase (AMPK); toll-like receptor 9 (TLR9)	<p>Cell culture studies suggest activating TLR9 could help protect cardiomyocytes and neurons from stress-induced damage. In cultured cardiomyocytes, a synthetic TLR9 ligand increased survival under hypoxia-induced stress compared with vehicle. In cultured neurons, the TLR9 ligand increased AMPK activation and decreased H₂O₂-induced cell death compared with vehicle. Next steps include testing the protective effects of activating TLR9 in <i>in vivo</i> models.</p> <p>SciBX 6(11); doi:10.1038/scibx.2013.270 Published online March 21, 2013</p>	Unpatented; licensing status not applicable	<p>Shintani, Y. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online March 11, 2013; doi:10.1073/pnas.1219243110</p> <p>Contact: Ken Suzuki, Queen Mary, University of London, London, U.K. e-mail: ken.suzuki@qmul.ac.uk</p> <p>Contact: Yasunori Shintani, same affiliation as above e-mail: y.shintani@qmul.ac.uk</p>

This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* 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 techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
An automated microfluidic tissue processor (MTP) for immunohistochemistry	An automated MTP could allow immunohistochemistry to detect cancer biomarkers. The microfluidic device delivered fluorescently labeled antibodies and other bioreagents to 5 µm-thick tissue samples and subsequently generated immunohistochemistry (IHC) images of the tissues. In a set of 76 human invasive ductal breast carcinomas, MTP-IHC resulted in 73 accurate HER2 (EGFR2; ErbB2; neu) diagnoses and 3 ambiguous scores, whereas traditional IHC resulted in 49 accurate HER2 diagnoses and 27 ambiguous scores. Next steps include optimizing the MTP to automatically quantify HER2 expression and comparing the results with current clinical scoring. SciBX 6(11); doi:10.1038/scibx.2013.271 Published online March 21, 2013	Patent application filed; unlicensed	Ciftlik, A.T. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 11, 2013; doi:10.1073/pnas.1211273110 Contact: Martin A.M. Gijs, Swiss Federal Institute of Technology Lausanne, Lausanne, Switzerland e-mail: martin.gijs@epfl.ch
Drug delivery			
Formulation of T cell vaccines for cancer	Mouse studies suggest saline formulations of T cell-targeted cancer vaccines could provide greater antitumor activity than conventional adjuvant carriers. In a mouse model for melanoma, subcutaneous injection with a tumor antigen vaccine formulated in incomplete Freund's adjuvant (IFA), a widely used oil-based carrier, caused accumulation of antigen-specific T cells at the injection site and elicited lower antitumor activity than injection of a saline-based control formulation of the same antigen. Mice receiving the saline-based formulation with an adjuvant cocktail of IL-2, a toll-like receptor 7 (TLR7) agonist and an antibody against CD40 had higher levels of tumor-infiltrating T cells than mice receiving an IFA-based vaccine. Next steps include optimizing the vaccine formulation to maximize dendritic cell uptake of the antigen and testing the formulation in an investigator-led trial for melanoma. SciBX 6(11); doi:10.1038/scibx.2013.272 Published online March 21, 2013	Unpatented; licensing status not applicable	Hailemichael, Y. <i>et al. Nat. Med.</i> ; published online March 3, 2013; doi:10.1038/nm.3105 Contact: Willem W. Overwijk, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: woverwijk@mdanderson.org
Nanogel-mediated delivery of anti-inflammatory therapeutics	Nanogel-mediated delivery of anti-inflammatory therapeutics could help treat autoimmune diseases such as lupus. Nanogels were composed of a gel-like core containing the anti-inflammatory mycophenolic acid (MPA) inside a lipid bilayer. In a mouse model for lupus, prophylactic or therapeutic delivery of the nanogel decreased renal damage and increased survival compared with delivery of the same dose of free MPA. Next steps include exploring potential anti-inflammatory drugs to deliver in the nanogel particles for clinical translation in lupus and investigating the potential of the nanogel to treat type 1 diabetes (<i>see Nanoparticles for lupus</i> , page 10). SciBX 6(11); doi:10.1038/scibx.2013.273 Published online March 21, 2013	Patent application filed covering nanogel compositions and methods of manufacture and use; licensed by Immunova LLC	Look, M. <i>et al. J. Clin. Invest.</i> ; published online March 1, 2013; doi:10.1172/JCI65907 Contact: Tarek M. Fahmy, Yale University, New Haven, Conn. e-mail: tarek.fahmy@yale.edu
Drug platforms			
Bacterial synthesis of small interfering RNA	siRNA synthesized in bacteria could have better potency than chemically synthesized siRNA. In <i>Escherichia coli</i> , expression of p19 plus a target gene hairpin enabled the isolation of 21-nucleotide siRNA fragments from the target sequence. p19 is a protein that binds and stabilizes 21-nucleotide siRNA fragments in bacteria. Bacteria-produced siRNA knocked down their targets with potency that was comparable to or greater than that of chemically synthesized siRNAs. Next steps include optimizing the technology to improve the yield of the bacteria-synthesized siRNA. SciBX 6(11); doi:10.1038/scibx.2013.274 Published online March 21, 2013	Patent application filed; available for licensing	Huang, L. <i>et al. Nat. Biotechnol.</i> ; published online March 10, 2013; doi:10.1038/nbt.2537 Contact: Judy Lieberman, Boston Children's Hospital, Boston, Mass. e-mail: judy.lieberman@childrens.harvard.edu

This week in techniques

Approach	Summary	Licensing status	Publication and contact information
Controlled Fab-arm exchange (cFAE) approach to develop bispecific antibodies for therapeutic use	<i>In vitro</i> and mouse studies identified a method to develop bispecific antibodies as therapeutics. The cFAE method involved expressing matched mutant methyl domains in two IgG1 antibodies and combining the antibodies with a weak reducing agent to preserve the heavy-chain and light-chain pairings. Bispecific antibodies were designed including those that target both CD20 and HER2 (EGFR2; ErbB2; neu), as well as those that target HER2 and CD3. In cancer cells and in mice xenograft models for gastric carcinoma, the HER2- and CD3-targeting antibody decreased cancer cell survival compared with a control antibody. Next steps include developing bispecific antibody therapeutic candidates.	Patent applications filed; licensed to Novartis AG, Janssen Pharmaceuticals N.V., Kyowa Hakko Kirin Co. Ltd. and an undisclosed company for specific programs; available for licensing	Labrijn, A.F. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online March 11, 2013; doi:10.1073/pnas.1220145110 Contact: Paul W.H.I. Parren, Genmab A/S, Brooklyn Park, Minn. e-mail: p.parren@genmab.com
	SciBX 6(11); doi:10.1038/scibx.2013.275 Published online March 21, 2013		

Markers

Carbamylated serum albumin as a risk factor for mortality in end-stage renal disease (ESRD)	Carbamylated serum albumin could be a useful marker of increased mortality risk in patients with ESRD. In two cohorts totaling 1,348 patients with ESRD, a higher percentage of carbamylated serum albumin was associated with a higher risk of death. Next steps could include determining if reducing the levels of carbamylated serum albumin helps lower mortality in animal models for ESRD.	Patent and licensing status unavailable	Berg, A.H. <i>et al. Sci. Transl. Med.</i> ; published online March 6, 2013; doi:10.1126/scitranslmed.3005218 Contact: Anders H. Berg, Beth Israel Deaconess Medical Center, Boston, Mass. e-mail: ahberg@bidmc.harvard.edu
	SciBX 6(11); doi:10.1038/scibx.2013.276 Published online March 21, 2013		

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