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## Chromatin's rising tide

By *Chris Cain, Senior Writer*

To capitalize on the full range of possible chromatin targets in and beyond oncology, industry and academia will need to delve deeper into how chromatin regulation is altered in disease, create tools that can reliably validate new targets and develop biomarkers that can improve the chances of success in clinical trials.

A wave of compounds targeting chromatin regulators entered the clinic in 2013, enabled by a decade of progress in understanding how chromatin dysfunction drives cancer.<sup>1</sup>

The first DNA methyltransferase (DNMT) and histone deacetylase (HDAC) inhibitors were approved to treat subtypes of lymphoma in 2004 and 2006, respectively. In the following decade, research linked genetic alterations in functionally diverse chromatin regulators to many additional types of cancer.

That fueled the discovery of a second generation of compounds against new targets to treat genetically defined cancers (*see Table 1*, "Select clinical-stage compounds that target epigenetic regulators").

Development of this wave of compounds was aided by technical advances that allow chromatin to be biochemically characterized in detail. Despite the progress, there remains a huge opportunity in unexplored targets and much to discover about how chromatin-dependent cellular pathways are affected in different diseases.

Against this backdrop, *SciBX* organized a panel of thought leaders to discuss the possibilities and challenges in developing chromatin-targeted compounds and outline ways to accelerate the translation of this information into disease-modifying therapies.

The panel identified three areas of chromatin drug development that are most in need of innovation.

First, new chemical tools to functionally characterize chromatin will be critical to validate targets—including those currently deemed intractable. These tools will enable a deeper understanding of how mutations in chromatin regulators alter cell signaling pathways and cell fate.

Panel member Jim Audia, CSO at **Constellation Pharmaceuticals Inc.**, said that the optimal way to generate new tool compounds would be through collaborations between industry and academia such as the **Structural Genomics Consortium** (SGC).

"We realize that, regardless of your company, the academic community from outside it is immense compared to the resources that you can muster from within," he said.

Second, identifying predictive biomarkers and developing new methods to measure target engagement *in vivo* will be needed to

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accelerate progress to the clinic. Information about how hitting chromatin targets provokes different cellular responses between individuals will help companies select appropriate patient populations.

Because little is known about how the cellular pathways involved in chromatin regulation differ cell by cell or tissue by tissue, that information will need to be integrated from multiple experimental approaches.

“The simplest readout, if you were to perform knockdowns or if you had an inhibitor, would just be to look at changes in gene expression,” said Peter Tummino, who at the time of the panel was head of **GlaxoSmithKline plc**’s Cancer Epigenetics Discovery Performance Unit (DPU). “But what we are finding is that it is entirely insufficient. You can look across a set of cell lines and there’s no similarity, there is no common gene profile. And so the next step beyond that is to think about what are the changes in histone marks—which you might expect to change. You can perform transcription factor mapping, if possible. It’s the integration of several different data sets that begins to lead toward an understanding of mechanism.”

Tummino is now VP and global head of lead discovery at the Janssen unit of **Johnson & Johnson**.

Finally, the panel said that chromatin regulators should also be targeted outside of oncology. Human genetic studies and an increased understanding of how cell fate is determined in neurology and immunology suggest that these two fields are next in line.

“We’re years behind the oncology field in terms of looking at target association in the CNS,” said Ankit Mahadevia, venture partner at **Atlas Venture** and acting CBO of **Rodin Therapeutics Inc.** “We’re not wanting for genetically implicated targets in terms of CNS applications. Really, it’s a lack of chemical probes and high-fidelity assays. The field is just so

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**Table 1. Select clinical-stage compounds that target epigenetic regulators.** The availability of new research tools and data on epigenetic regulators has driven the development of a new wave of compounds. At least nine molecules have entered clinical trials since the start of 2013, including four BET bromodomain-targeting oncology programs. **GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK) leads the pack with clinical-stage programs against three targets, while **Celgene Corp.** (NASDAQ:CELG) has rights or options to two programs.

Source: *BCIQ*; *BioCentury Archives*; *ClinicalTrials.gov*

Company	Compound	Description	Indication	Status
<b>Resverlogix Corp.</b> (TSX:RVX)	RVX-208	BET bromodomain inhibitor	Atherosclerosis	Phase II
<b>Acetylon Pharmaceuticals Inc.</b> ; Celgene	Rocilinostat (ACY-1215)	Oral selective histone deacetylase 6 (HDAC6) inhibitor	Multiple myeloma (MM)	Phase I/II
<b>Epizyme Inc.</b> (NASDAQ:EPZM); <b>Eisai Co. Ltd.</b> (Tokyo:4523)	EPZ-6438	Enhancer of zeste homolog 2 (EZH2) inhibitor	Lymphomas including those with <i>EZH2</i> mutations	Phase I/II
<b>Oryzon Genomics S.A.</b> ; <b>Roche</b> (SIX:ROG; OTCQX:RHHBY)	ORY-1001	Lysine-specific demethylase 1 (KDM1A; LSD1) inhibitor	Acute myelogenous leukemia (AML)	Phase I/IIa
<b>Constellation Pharmaceuticals Inc.</b>	CPI-0610	BET bromodomain inhibitor	Lymphoma	Phase I
Epizyme; Celgene	EPZ-5676	Histone methyltransferase DOT1L (DOT1L) inhibitor	Myeloid-lymphoid or mixed-lineage leukemia (MLL; HRX)-rearranged leukemia	Phase I
GlaxoSmithKline	GSK2879552	LSD1 inhibitor	Small cell lung cancer	Phase I
	GSK525762	BET bromodomain inhibitor	Cancers including NUT (nuclear protein in testis; C15orf55) midline carcinomas	Phase I
	GSK2816126	EZH2 inhibitor	Lymphomas including those with <i>EZH2</i> mutations	Phase I
<b>Oncoethix S.A.</b> ; <b>Mitsubishi Tanabe Pharma Corp.</b> (Tokyo:4508)	OTX015	BET bromodomain inhibitor	Hematologic malignancies	Phase I
<b>Tensha Therapeutics Inc.</b>	TEN-010	BET bromodomain inhibitor	Cancers including NUT midline carcinomas	Phase I

nascent, and there are fewer people, both in academia and in pharma, working on these associations.”

Audia, Tummino and Mahadevia participated in the panel discussion alongside Jesse Smith, executive director of biological sciences at **Epizyme Inc.**; Charles Roberts, an associate professor of pediatrics at **Harvard Medical School** and director of the research program in solid tumors at the **Dana-Farber Cancer Institute**; and Stuart Schreiber, director of the Center for the Science of Therapeutics at the **Broad Institute of MIT and Harvard**, director of chemical biology at the Broad Institute, a professor of chemistry and chemical biology at **Harvard University** and an investigator at the **Howard Hughes Medical Institute**.

The panel was part of the 2nd *SciBX* Summit on Innovation in Drug Discovery & Development and was produced with support from the following sponsors: **Acetylon Pharmaceuticals Inc.**, **Amgen Inc.**, **AstraZeneca plc**, Atlas Venture, **Biogen Idec Inc.**, **GlaxoSmithKline**, **Karus Therapeutics Ltd.**, **Merck & Co. Inc.**, **Novartis AG**, **RaNa Therapeutics Inc.**, **Sanofi** and **Zenith Epigenetics Corp.**

### Rational advancement

The first DNMT and HDAC inhibitors were developed when knowledge of chromatin regulation was sparse at best. Indeed, compounds that inhibit these targets were shown to have anticancer properties. Only later were their mechanisms of action established.

Since then, advances in whole-genome analysis and *in vitro* enzymology have ramped up knowledge about new enzymes and regulatory pathways and laid the groundwork for rational drug design.

According to the SGC, the key to exploiting this knowledge is a concerted chemical biology effort to probe the function of chromatin regulatory complexes in disease states.<sup>2</sup>

An example of the shift from phenotypic screening to rational

design is the progress made since Schreiber’s work in the mid-1990s that identified the mechanism of action of trapoxin, a natural product with anticancer activity that alters cell morphology.

Schreiber’s 1996 study published in *Science* showed that the compound inhibited a previously uncharacterized protein that became known as HDAC1.<sup>3</sup> At about the same time, independent teams identified the first mammalian histone acetyltransferase (HAT).

Now there are at least 18 known HDACs in humans, and companies including Acetylon and Karus are designing selective HDAC inhibitors.

Hundreds of additional proteins modify or alter chromatin structure. According to the SGC, there are 64 protein methyltransferases, two distinct families of protein demethylases and a slew of structurally distinct protein domains that bind acetylated or methylated histones or can physically remodel chromatin structure.

Schreiber said that the activities of chromatin-regulating protein families can be viewed as a natural extension of the signal transduction cascades that have been seen as potential drug targets for years.

“In signal transduction there are kinases that put on a phosphate mark, there are SH2 domains that bind the mark and there are phosphatases that remove the mark. And in chromatin it’s exactly the same story. I’m a little amused that we have obfuscated this connection by using new vocabulary to describe proteins that behave like kinases and phosphatases. We call kinases ‘writers’, phosphatases are now ‘erasers’ and SH2 domains are ‘readers.’”

Schreiber said that compounds that target chromatin-modifying proteins have commonly been referred to as epigenetic drugs despite the fact that there is little evidence they would meet the traditional definition of epigenetics, in which an effect is inherited and persists across multiple generations. “This is not just semantics; it is medically relevant,” he said.

For transient changes in chromatin, Schreiber added, there are clinical applications in which the effects would be seen rapidly. On the other hand, heritable changes in chromatin can involve cell fate and cellular differentiation, and drugs for those chromatin regulators could be effective in diseases of cellular deficiency.

Roberts said that genome sequencing has been central to linking chromatin-regulating enzymes to disease biology.

“I think the field really began to change when genome sequencing studies across cancers came out,” said Roberts. “I think few people would have predicted that mutations in chromatin regulators and modifiers—readers, writers and erasers—would be prevalent in cancer before those studies were done, but that’s precisely what was found.”

Roberts noted that one complex in particular has turned out to be frequently mutated in cancers—members of SWI/SNF (switch/sucrose nonfermentable) chromatin remodeling complexes.

“Eight different subunits of this complex are recurrently mutated in cancer. The latest data suggest that 20% of all human cancers have a mutation of one or another SWI/SNF subunit. Furthermore, the genes encoding these subunits are being validated as bona fide tumor suppressors using mouse models. Now, an important question is what can we do about it therapeutically?” asked Roberts.

SWI/SNF is also an example of how hard it can be to convert genetic findings to new drug candidates when so little is known about their precise mechanism.

Multiple independent groups including Roberts’ have shown that components of the SWI/SNF complex can be targeted in cancers harboring mutations in BRG1 (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily a member 4; SMARCA4) or ARID1A (AT rich interactive domain 1A), two of the complex’s subunits.

But—as Smith pointed out—determining the best approach for targeting SWI/SNF complex members as drug targets is a difficult question because they can have multiple functions within the cell, including depositing chromatin marks, binding histones and facilitating protein-protein scaffolding interactions.

In addition, the SWI/SNF complexes are large, multiprotein complexes with a diverse set of configurations based upon the various proteins that can comprise these assemblies. This phenomenon poses a significant challenge to biochemical and biophysical assay development for these targets.

For example, knockdown of BRM (SMARCA2) kills *BRG1*-mutant cancer cells, but it is not yet known whether small molecules that specifically target the protein’s bromodomain region will reproduce the effect.<sup>4</sup>

### Chemicals matter

There was wide agreement among panel members that the field needs more and better chemical probes to understand how dysfunctional chromatin regulators contribute to disease.

“I think the power of the probes has been demonstrated on many occasions, and I think there’s clearly room for additional probes,” said Audia. “If you look at the SGC, or you look across the industry, we’re

getting a fair number of probes targeting bromodomains and a few targeting select SET domain-containing proteins. We’re starting to get a few examples for the demethylases. But there are vast classes of these chromatin regulators and modifiers that do not have adequate probes for even doing rudimentary experiments.”

SET is the catalytic domain found in many lysine methyltransferases.

Thus far, the SGC has made available 18 probes targeting chromatin regulators. One of the most recent, the chemical probe PFI-3, inhibits the bromodomains of BRM, BRG1 and polybromo 1 (PBRM1; PB1). Although the chemical properties of the compound are available on SGC’s website, no studies detailing functional data for PFI-3 have been published.

The ability of a probe to shape a therapeutic space was best demonstrated by

the rapid pace of development of BET bromodomain inhibitors since the first probe was made available in 2011. BET bromodomain inhibitors have been critical for target validation in NUT (nuclear protein in testis; C15orf55) midline carcinoma and additional cancers.<sup>5,6</sup>

At least five BET bromodomain inhibitors are now in the clinic (see Table 1). These include TEN-010, a compound being developed by **Tensha Therapeutics Inc.** that was synthesized by James Bradner’s lab.

His lab also synthesized the widely used research tool BET inhibitor JQ1 in collaboration with the SGC.<sup>5</sup>

Bradner is an attending physician in the Department of Medical Oncology at the Dana-Farber Cancer Institute, an assistant professor in the Department of Medicine at Harvard Medical School and associate director of the Center for the Science of Therapeutics at the Broad Institute.

All panelists agreed that more probes are needed but noted that the field needs to focus on generating high-quality probes that work across multiple assays and both *in vitro* and *in vivo*.

“If you get poor probes, you generate an awful lot of data that tends to confound the conclusions that are reached. I think that’s one of the things that we spend a great deal of time trying to do—to thoroughly characterize all of the probes that we utilize internally in order to determine whether they selectively inhibit the appropriate target in a cellular context,” said Smith.

He added, “There are plenty of examples of published inhibitors that have good biochemical potency and selectivity, but for various reasons they lack specific cellular target engagement, at least as measured by global or local methyl mark changes. If you treat cells with high enough concentrations of these inhibitors, you often will observe phenotypic effects, which are most likely due to off-target activity. So I think it is important for investigators in our field to be very clear about how their probes should and should not be used experimentally: can they be used in cell-based assays? Can they be used in animal studies? What are appropriate concentrations to use?”

Multiple panelists said that compounds with defined *in vitro* effects may behave differently inside cells. “We need compounds to do what we think they’re doing, and we need to be certain of it,” said Schreiber. “If we look to the kinase world, we’ve learned that, over and over, compounds work on their targets in cells in ways that can be context dependent. That’s not seen biochemically *in vitro*.”

**“There are vast classes of these chromatin regulators and modifiers that do not have adequate probes for even doing rudimentary experiments.”**

—Jim Audia,  
*Constellation Pharmaceuticals Inc.*

Schreiber told *SciBX* that investigating new target classes may require creating new chemical compounds to screen against. “Screening collections are limited in what they can do, so a lot of this starts from getting the right chemistry,” he said.

Schreiber is a cofounder of **Forma Therapeutics Holdings LLC**, which is developing diversity-oriented synthesis technology that the Broad Institute uses.

Mahadevia said that new chemical matter will be especially important in indications beyond oncology in which toxicity is less tolerable and the bar is higher for achieving an acceptable therapeutic index.

For example, he said, “one of our programs at Rodin is an isoform-specific HDAC inhibitor being developed to enhance cognition. It’s a case where the biology has been under discussion for about half a decade but no one has been able to identify that exquisite isoform specificity that correlates with the better safety profile.”

Karus CEO Simon Kerry added that in the case of HDAC6, it is clear that the enzyme acts on different substrates inside and outside of the nucleus, which highlights how it can be challenging to understand the cellular biology underlying the therapeutic effect of HDAC6 inhibitors even when they are highly selective.

Karus is developing selective HDAC6 inhibitors to treat cancer and inflammation, and Acetylon is developing selective HDAC inhibitors in both oncology and non-oncology indications.

### Signature effects

Another major challenge highlighted by the panelists is the lack of predictive biomarkers that could help explain the cellular effects of inhibiting particular chromatin targets. That lack of biomarkers has made it difficult to expand into cancers with no known genomic alterations.

Schreiber noted BET inhibitors as an example. “I still have not seen any predictor of response to BET inhibition other than the hematopoietic lineage or if BET itself is genomically altered. That’s it, basically. The exception might be this very intriguing notion of these super-enhancers,” he said.

Super-enhancers are a class of regulatory elements that control the expression of genes—including oncogenes—involved in determining cellular identity. They were first described last year in work published by Bradner and researchers from Richard Young’s laboratory.<sup>7,8</sup>

In that study, the expression of some genes associated with super-enhancers, most notably *c-Myc* (*MYC*), was dramatically reduced by treatment with the BET inhibitor JQ1. In addition, super-enhancers were bound by less bromodomain containing 4 (*BRD4*) upon JQ1 treatment than regular enhancers.

Young is a member of the **Whitehead Institute for Biomedical Research** and a professor of biology at the **Massachusetts Institute of Technology**. He and Bradner have cofounded **Syros Pharmaceuticals Inc.** to develop compounds that disrupt super-enhancers or the genes they regulate in cancer.<sup>9</sup>

Late last year, the group also published details of a methodology called Chem-seq, which enables the mapping of interactions between small molecules and proteins bound to chromatin.<sup>10</sup>

Audia said that the super-enhancer theory is intriguing but is not sufficient to explain differential sensitivity to BET inhibitors.

“We spent a great deal of time and energy trying to find the molecular characteristics that infer sensitivity to BET inhibitors,” he said. “And I don’t think, except for very isolated cases, we’ve got a real clear answer. I do think this super-enhancer story is interesting, but we’re not finding that it’s really a simplifying assumption. We’re finding we can reach similar levels of predictive power, both with and without super-enhancers. And so, therefore, it doesn’t seem either necessary or sufficient to account for the sensitivity that we’re seeing, at least *in vitro*.”

Epizyme’s Smith said that enhancer of zeste homolog 2 (*EZH2*) inhibitors are an example of an area in which some progress is being made. *EZH2* is a methyltransferase in the polycomb repressive complex 2 (*PRC2*).

He noted that the company is optimistic about its ability to identify additional indications for *EZH2* inhibitors, with one example being recent work showing the therapeutic potential of *EZH2* inhibitors in malignant rhabdoid tumors with *SNF5* (*SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily b member 1; SMARCB1*) mutations. This was enabled by in-house screening efforts and the research of Charlie Roberts and others who have studied the relationship between *SWI/SNF* and *EZH2* in soft tissue sarcomas.<sup>11–13</sup>

Tummino agreed that in addition to identification of biomarkers, a deep understanding of the biological effect of inhibiting chromatin-regulator targets can also guide drug development.

He pointed to inhibitors that GSK and other companies have developed against lysine-specific demethylase 1 (*LSD1*; *KDM1A*) as an example of a program against a chromatin-modifying target that was not guided by the discovery of genomic alterations but instead by studies of the function of the protein in normal cells.

“In really nice work, *LSD1* was shown to have a direct role in pluripotency and differentiation in hematopoietic cells. It was not a big leap then to at least hypothesize you’d have a similar role in acute myelogenous leukemia (*AML*). And it gives you greater confidence if you understand the role of the protein in its normal context,” he said. “First Oryzon showed it with Tim Somerville, and we’ve seen the same with our *LSD1* inhibitor.”

“If you’re affecting the biology of a tumor type, and if you understand the function of a chromatin protein, there’s a path forward,” he said.

GSK’s *LSD1* inhibitor, GSK2879552, is in Phase I trials to treat small cell lung cancer.

**Oryzon Genomics S.A.**’s *LSD1* inhibitor, ORY-1001, is in Phase I/IIa testing to treat *AML*. In 2012, Somerville, group leader of the Leukaemia Biology Laboratory at the **Cancer Research UK Manchester Institute**, published work in *Cancer Cell* using an *LSD1* inhibitor designed by Oryzon.<sup>14</sup>

In April, Oryzon granted **Roche** exclusive worldwide rights to its *LSD1* inhibitor program.

Tummino also noted that understanding the mechanism can complicate the picture—for example, the mechanism of cell death is not uniform across all cell types.

“We see some cells where there is an apoptotic response, where we see other outcomes in other cell types. We see activity that’s broad, but a variety of different downstream mechanisms appear to be in play. And I think this is not just true for the *BET* inhibitors; it seems to apply to other chromatin-based targets as well.”

**“If you understand the function of a chromatin protein, there’s a path forward.”**

—Peter Tummino,  
**GlaxoSmithKline plc**

Although that provides an opportunity to understand what is going on mechanistically, he said, “it makes it challenging because you’re not looking for a singular mechanism; you’re looking for the mechanism in a particular context.”

Tummino added that understanding the mechanism of some targets may involve looking at effects outside of chromatin regulation. “There are going to be nonhistone substrates for a lot of these enzymes. And some have already been identified. A lot of that work could be quite interesting, and it might be that not all the effects we’re seeing in targeting these enzymes or readers are coming at the level of the chromatin. It may be in the cytosol or in the nucleus outside of chromatin,” he said.

### Mapping potential

Panelists agreed that expanding chromatin-targeted drug development beyond genomically altered cancers will require increased commitment to chromatin profiling and follow-up functional studies to test new hypotheses.

Chromatin profiling involves mapping the locations and levels of histone modifications throughout the genome.

“The technology we have with ChIP-seq today, and even newer versions of this, are enabling a kind of analysis that’s never been seen before,” said Schreiber.

Many labs have taken advantage of the fact that genome-sequencing technologies are more widely available and have seen significant reductions in cost in the last decade.

For example, Bradley Bernstein, who is heading up the global chromatin mapping effort at the Broad Institute, has published protocols for performing nano-ChIP-seq on samples with small numbers of cells.<sup>15</sup> Bernstein is a senior associate member of the Broad Institute and co-directs its epigenomics program. He also is a professor in the Department of Pathology at **Massachusetts General Hospital** and Harvard Medical School and an early career scientist at the Howard Hughes Medical Institute.

But Schreiber said that the technology still has a lot of room for improvement. “We need a big leap in the technology to lower the cost and improve the throughput to be able to do single-cell analysis. Right now, it’s slow and costly, and so you have to pick and choose,” he said.

Cliff Meyer, a research scientist at Dana-Farber, asked the panelists their thoughts on the quality of data in genomewide chromatin profiling studies.

Smith said that it has been a mixed bag. “We’ve got experience in both directions, where we’ve had good success merging our datasets with publicly available datasets and getting consistent answers. We’ve also seen real inconsistencies where we struggle to interpret our data in the context of the publicly available data,” he said, which is “likely due to differences in methodologies and experimental conditions.”

“My sense is that we probably have a little more confidence in public data than we did two or three years ago,” added Tummino. “I remember the discussion then around the nonselectivity of antibodies that were

being used for ChIP. I feel like some of the most basic issues like that are being dealt with. We’re not there yet, but one could take a little bit of optimism from the progress in the last two or three years that we’re getting there.”

One effort to improve antibody quality was a 2012 partnership between the SGC and Life Technologies Corp. to make available a master set of recombinant antibodies against epigenetic targets. **Thermo Fisher Scientific Inc.** acquired Life Technologies earlier this year.

Tummino said that he was impressed by a recent collaboration between the Broad Institute and the **Novartis Institutes for BioMedical Research**, which used mass spectrometry to map histone methylations in the Cancer Cell Line Encyclopedia.<sup>16</sup> The method led the team to discover activating mutations in *nuclear SET domain-containing protein 2 (MMSET; WHSC1; NSD2)*.

“This is clearly an example of a high-quality, large-scale endeavor that impacts the cancer epigenetics field,” said Smith. “And it is also a good example of a productive collaboration between a large pharma and an academic entity.”

Roberts was less certain about the discovery potential of the mass spectrometry approach. “I’m torn on how useful this method is going to be. On one hand, we’re picking up new things. On the other hand, if it’s just picking up a proxy—that is, another marker—driven by a DNA mutation, you could argue we should just sequence more,” he said. “But I think it could have the potential to identify real changes in chromatin state that may have a shot at being therapeutically modulated.”

### Expanding the scope

Although chromatin-targeted compounds are starting to produce results in cancer, applications in non-oncology indications are still far behind. For existing targets such as HDACs and BET bromodomains, developing selective compounds with appropriate safety profiles for chronic use remains a prime challenge.

Mahadevia told *SciBX* that most people in the field are focused on oncology. “There are fewer folks working outside of oncology, so the pace of identification and validation of new targets is slower. In this ‘second act’ of epigenetics, you really can’t own a space such as CNS or inflammation. You really have to look on a target-by-target basis.”

He added, “HDACs are a good example of a target where multiple labs have identified corroborating findings, looking at the impact on things like cognition and the regulation of genes in the hippocampus. There are targets like HATs that are emerging and are getting to that similar level of validation. It’s happening in pockets, with a small cadre of investigators in the neurosciences, but it’s not happening at the scale that it should.”

Other examples include work published by GSK’s EpiNova DPU on the role of BET bromodomain proteins in inflammation, and a recent immunology study from Constellation showing that the protein family also regulates T helper type 17 (Th17) cell function.

Tummino said that there are other scattered cases of intriguing results outside of oncology, including the use of compounds to treat

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—Stuart Schreiber,  
Broad Institute of MIT and Harvard

latent viral infections, and work from Harvard pediatric oncology professor Stuart Orkin on reactivating fetal hemoglobin expression to treat sickle-cell anemia.

The challenge will be showing that these targets can be inhibited with a benign safety profile. Donald McCaffrey said that for BET bromodomain inhibitors, improved safety may be possible by increasing selectivity of compounds for specific bromodomains.

McCaffrey is cofounder, president and CEO of **Resverlogix Corp.** and spinout company Zenith Epigenetics, which is developing BET inhibitors for oncology and autoimmune indications.

Mahadevia said that the same was true for the use of HDACs in particular in chronic neurological diseases. “I think we have to have a very early and intensive focus on improving safety,” he said. “Especially in the case of HDACs, selectivity matters both in probing the biology and in ultimately developing a drug to use in populations—such as patients with mild to moderate cognitive impairment—where people have some of their activity of daily living intact under acetylcholinesterase inhibitor therapy.”

Karus’ Kerry added, “Tool compounds like Tubastatin have been helpful because they are quite HDAC6 selective. It gives you a fairly reasonable idea of what the effects of an inhibitor might be. But designing a selective drug-like compound is challenging as there are no crystal structures of HDAC6.”

He also added that it may not always be better to be more isoform selective because you may need activity against other HDACs. He said that it is important to test compounds with a range of potencies and selectivities in preclinical models.

Schreiber said that he would turn to human genetic studies to guide target selection. “I would first turn to germline heritable genetics. I would look at genetic variations associated with any non-oncology disease and see ones that are enriched in chromatin regulators.” In those diseases, he added, “I would do deep sequencing to look, for example, for rare protective variants in chromatin regulators associated with some chronic heritable disorder.”

Roberts agreed but said that the need for more chromatin profiling is particularly pronounced in diseases outside of oncology. Even though mutations may still be found in genomewide association studies, they cannot be counted on to play the same driving role they have in cancer.

“I think the developmental biology of chromatin state is going to be absolutely key because we’re back into correlative biology,” he said. “With DNA mutations, we have an absolute result—mutation or no mutation. With chromatin biology, what do you compare it to? We know what our disease state looks like, but we have an extremely poor ability to identify what normal is, and therefore we can’t really identify what the aberrancy is.”

### Chromatin clinic

The panel and audience members returned frequently to a discussion of clinical development challenges for compounds targeting chromatin regulators.

Richard Gregory, head of Sanofi’s Genzyme R&D center, asked if any tumors have developed resistance to new compounds such as the BET bromodomain inhibitors.

Tummino expects resistance to emerge but said that it is too early

to predict resistance mutations. “Our expectation is that there will be resistance that emerges from targeting epigenetic regulators. I think that is a relatively safe assumption. However, we don’t have enough clinical experience to have observations of resistance at this point. We are able to generate resistant clones for some of these targets *in vitro*.”

Henry Long, associate director for the Center for Functional Cancer Epigenetics at Dana-Farber, asked whether new compounds might be combined with and synergize with existing drugs.

Schreiber highlighted work from Jeff Settleman on combining HDAC inhibitors with Tarceva erlotinib, an epidermal growth factor receptor 1 (EGFR1; HER1; ErbB1) inhibitor, to treat lung cancer by eliminating persisting cells that may cause Tarceva resistance.

“Personally, I think that there’s going to be a lot of new and different ideas about combinations,” said Schreiber.

Settleman is senior director of discovery oncology at the **Genentech Inc.** unit of Roche. **Astellas Pharma Inc.**, **Chugai Pharmaceutical Co. Ltd.** and Roche market Tarceva for pancreatic cancer and non-small cell lung cancer (NSCLC).

Indeed, Audia said that it will be critical to see how new chromatin-modulating therapies work in conjunction with standard of care. “One of the things that we do is to look at the compatibility of our drug with the existing standard of care. We look to see if there’s cumulative efficacy or synergistic toxicities to find out if it can be added to standard of care,” he said. “We’ve certainly seen that with EZH2 inhibitors combined with CHOP [cyclophosphamide, hydroxydaunorubicin, oncovin and prednisone]—a very nice tumor regression without any evident preclinical exacerbation of the toxicities.”

He added that rationally designing combination strategies will require better understanding of the signaling pathways downstream of these new compounds.

Panelists agreed that an important factor for advancing new compounds into the clinic will be the ability to collaborate with clinicians. This is not a problem specific to chromatin regulation but is more pronounced because there are so few genetically altered cancers in which compounds are being tested.

Tummino said that if they have an agent with new activity, they would work with clinical scientists to get primary samples and get researchers’ input on other models because most preclinical data does not translate well to the clinic.

“We are not just thinking about tumor types but [also] subtypes to understand the relevance of the findings. That is essential; we shouldn’t be progressing without that insight.”

Roberts agreed. “A lot of the indications we are seeing sensitivity in aren’t going to be in a standard panel of cell lines. It really does require expertise from those who do translational work and have expertise in those particular cancer types,” he said.

### The next frontier

Finally, panelists discussed the prospects for developing compounds that act by causing cells to differentiate into other cell types rather than by killing cells.

“There are far fewer cell differentiation-type therapies than there are cytotoxic or proapoptotic therapies,” Audia said. “ATRA [all-*trans* retinoic acid] is certainly one of the better examples of a differentiation therapy,” he added. But he said that it would be complicated to unravel

the biology—in particular in oncology or other indications—in which cell death is commonly the desired outcome.

“I think it will be a challenge to interpret data going forward, if things like histone methyltransferase and histone demethylase inhibitors have more of a differentiation effect. How do we deal with that data, in hematological malignancies in particular, where some of the criteria for response are really based on classic human therapies and wiping out bone marrow?”

“People want to see tumors melt,” said Tummino. “Everybody would like that because we’ve been testing cell-cycle inhibitors and kinase inhibitors, and they’re looking for that profile. So part of it is changing that mindset.”

He added, “From a mechanistic standpoint, I don’t think we have all the tools we need to understand the therapeutic potential of differentiation therapy. We’d like to go in that direction. But then the second part of it is to have a bit of a cultural change within drug discovery. And it’s been reasonably positive so far, but there’s a lot to do.”

“I couldn’t agree more,” said Schreiber. “I love this, and it’s harder because we know less, and it has less precedence. But we have to tackle this.”

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- Acetylon Pharmaceuticals Inc.**, Boston, Mass.  
**Amgen Inc.** (NASDAQ:AMGN), Thousand Oaks, Calif.  
**Astellas Pharma Inc.** (Tokyo:4503), Tokyo, Japan  
**AstraZeneca plc** (LSE:AZN; NYSE:AZN), London, U.K.  
**Atlas Venture**, Cambridge, Mass.  
**Biogen Idec Inc.** (NASDAQ:BIIB), Weston, Mass.  
**Broad Institute of MIT and Harvard**, Cambridge, Mass.  
**Cancer Research UK Manchester Institute**, Manchester, U.K.  
**Chugai Pharmaceutical Co. Ltd.** (Tokyo:4519), Tokyo, Japan  
**Constellation Pharmaceuticals Inc.**, Cambridge, Mass.  
**Dana-Farber Cancer Institute**, Boston, Mass.  
**Epizyme Inc.** (NASDAQ:EPZM), Cambridge, Mass.  
**Forma Therapeutics Holdings LLC**, Watertown, Mass.  
**Genentech Inc.**, South San Francisco, Calif.  
**GlaxoSmithKline plc** (LSE:GSK; NYSE:GSK), London, U.K.  
**Harvard Medical School**, Boston, Mass.  
**Harvard University**, Cambridge, Mass.  
**Howard Hughes Medical Institute**, Chevy Chase, Md.  
**Johnson & Johnson** (NYSE:JNJ), New Brunswick, N.J.  
**Karus Therapeutics Ltd.**, Chilworth, U.K.  
**Massachusetts General Hospital**, Boston, Mass.  
**Massachusetts Institute of Technology**, Cambridge, Mass.  
**Merck & Co. Inc.** (NYSE:MRK), Whitehouse Station, N.J.  
**Novartis AG** (NYSE:NVS; SIX:NOVN), Basel, Switzerland  
**Novartis Institutes for BioMedical Research**, Cambridge, Mass.  
**Oryzon Genomics S.A.**, Barcelona, Spain  
**RaNa Therapeutics Inc.**, Cambridge, Mass.  
**Resverlogix Corp.** (TSX:RVX), Calgary, Alberta, Canada  
**Roche** (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland  
**Rodin Therapeutics Inc.**, Cambridge, Mass.  
**Sanofi** (Euronext:SAN; NYSE:SNY), Paris, France  
**Structural Genomics Consortium**, Oxford, U.K.  
**Syros Pharmaceuticals Inc.**, Watertown, Mass.  
**Tensha Therapeutics Inc.**, Cambridge, Mass.  
**Thermo Fisher Scientific Inc.** (NYSE:TMO), Waltham, Mass.  
**Whitehead Institute for Biomedical Research**, Cambridge, Mass.  
**Zenith Epigenetics Corp.**, Calgary, Alberta, Canada

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# The DR is out

By Lev Osherovich, Senior Writer

Five years after a **Genentech Inc.** team proposed that the receptor DR6 might be involved in Alzheimer's disease, the company and **The Rockefeller University** have concluded that the proapoptotic pathway is unlikely to affect the most overt feature of the disease—amyloid plaque formation.

The **Roche** unit said that the new findings<sup>1,2</sup> mark the stopping point for its discovery efforts to target DR6 (tumor necrosis factor receptor superfamily member 21; TNFRSF21) in AD. Although the company is backing off DR6, the academics involved in the work think further studies with better disease models are warranted.

DR6 is a member of a family of cell surface receptors that activates caspases—intracellular proapoptotic proteases—when neurons are deprived of trophic factors such as nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF).

In 2009, Genentech discovered an interaction between DR6 and amyloid precursor protein (APP),<sup>3</sup> a neuronal surface protein that undergoes a series of proteolytic cleavages to yield the  $\beta$ -amyloid ( $A\beta$ ) fragment.  $A\beta$  aggregates form the amyloid plaques that are the hallmarks of AD and are thought to be a main upstream trigger for neurodegeneration.

The group, led by then-CSO Marc Tessier-Lavigne, found that DR6 was activated by a fragment of APP left behind after the proteolytic processing formed  $A\beta$ .<sup>4</sup>

Tessier-Lavigne is now a professor and president at The Rockefeller University.

Because activation of DR6 by the stray APP fragment led to neuronal apoptosis, the next question was whether DR6 activation played a role in  $A\beta$  toxicity.

“We set out to test a specific hypothesis about the potential role of DR6 in an APP-driven model of AD,” said team leader and Genentech senior scientist Robby Weimer. “The conclusion from our results is that we didn't find a role for DR6 in amyloid formation, synapse loss or behavior consequence. Our take home is that DR6 doesn't represent a good target for APP-driven AD pathology.”

## Death receptor dead

The hope was that DR6 inhibition might enhance neuronal branching and thus counteract the neurotoxic effects of  $A\beta$ . However, the new findings suggest that will not be the case.

In two mouse models of AD in which overproduction of  $A\beta$  leads to disease, DR6 deletion increased branching of mature neurons in some brain regions compared with no alteration. However, DR6 deletion did not reduce plaque formation or the behavioral and cognitive consequences of  $A\beta$  toxicity.

Results were reported in *The Journal of Neuroscience*.

Weimer and Tessier-Lavigne said that the findings indicate that one of APP's normal functions outside of AD may be to interact with DR6 to control neuronal branching in the adult nervous system. However, this process likely occurs independently of the abnormal proteolytic processing of APP that leads to AD.

“It seems that APP has multiple functions,” said Weimer. “Its normal physiological function could be through DR6. We did find evidence for DR6 and APP acting together in the mature nervous system to regulate synapse sensitivity.”

Tessier-Lavigne said that the findings do not absolutely shut the door on DR6's role in AD. He noted that the mouse models did not display some key features of AD such as neurodegeneration.

Thus, he said that other models of neurodegeneration featuring abnormal expression and phosphorylation of microtubule-associated protein- $\tau$  (MAPT; tau; FTDP-17) are an alternative place to look for effects of DR6 deletion. Some of those models display clear patterns of axonal degeneration, a terminal stage of AD pathology.

“We need to understand the extent to which DR6 participates in axon degeneration in other preclinical models with frank axonal pathology,” said Tessier-Lavigne. “For example, it would make sense to study the DR6 knockout in the context of some tauopathy models.”

Last year, Tessier-Lavigne's team and collaborators reported that in an assay of neuronal growth, adult DR6 knockout mice had less axonal degeneration than wild-type controls.<sup>5</sup>

Tessier-Lavigne said that his team now is investigating how DR6 affects axonal degeneration using induced pluripotent stem (iPS) cells from patients with AD and another common neurodegenerative disorder, frontotemporal dementia.

Weimer said that Genentech has discontinued its DR6 studies until a more solid connection to disease emerges. “At this point we're not planning any additional experiments” concerning DR6, said Weimer.

Genentech's most advanced AD candidate is crenezumab, a mAb that targets soluble or oligomeric  $A\beta$ . It was developed in partnership with **AC Immune S.A.** and is in Phase II testing to treat mild to moderate AD. Roche's gantenerumab, developed with **MorphoSys AG**, also targets  $A\beta$  plaques and is in Phase III testing for AD.

Osherovich, L. *SciBX* 7(19); doi:10.1038/scibx.2014.546  
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## COMPANIES AND INSTITUTIONS MENTIONED

**AC Immune S.A.**, Lausanne, Switzerland  
**Genentech Inc.**, South San Francisco, Calif.  
**MorphoSys AG** (Xetra:MOR; Pink:MPSYF), Martinsried, Germany  
**Roche** (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland  
**The Rockefeller University**, New York, N.Y.

# Blood test for asthma

By Lauren Martz, Staff Writer

Asthma is notoriously difficult to accurately diagnose because its symptoms can be transient and common tests are susceptible to user error. A **University of Wisconsin–Madison** team believes that it can improve the process by using a microfluidic device that analyzes the mobility of inflammatory cells contained in a single drop of blood.<sup>1</sup>

**Salus Discovery LLC** has exclusively licensed the technology and is seeking development partners.

Asthma is diagnosed using a combination of qualitative and quantitative assessments. Qualitative measures typically include surveys of patient symptoms and medical history, whereas quantitative measures include spirometry, which can measure lung function and airway constriction, and the nitric oxide test, which relies on elevated nitric oxide in the breath as a marker of airway inflammation.

Because quantitative tests require the presence of symptoms at the time of the test, they can sometimes report false-negative results. In addition, compliance for airway tests can be difficult in young children.

Shawn Aaron, a professor in the Department of Medicine at the **University of Ottawa**, told *SciBX* that current asthma diagnostic methods cause both under- and overdiagnosis. About 30% of treated patients do not actually have asthma, and the diagnostic criteria also miss a significant proportion of actual patients.

There are other well-known indicators of asthma, but they are difficult to measure noninvasively. These include an increase in numbers of immune cells, such as eosinophils and neutrophils, in bronchoalveolar lavage samples.<sup>2,3</sup>

Researchers have also shown that inflammatory cells from patients with asthma can behave differently than cells isolated from healthy individuals.

David Beebe and colleagues at the University of Wisconsin–Madison hypothesized that in addition to increases in the levels of inflammatory cells in the lungs of patients with asthma, the ability of these cells to migrate may also be affected. Thus, the team sought to adapt a microfluidic assay that it had previously developed to study neutrophil function for use with samples from patients with asthma.<sup>4</sup>

Beebe is associate chair of research and faculty development and a professor in the Department of Biomedical Engineering at the University of Wisconsin–Madison.

The kit-on-a-lid-assays (KOALA) platform consists of self-contained microfluidic chambers containing prepared reagents and microchannels that can be used to measure cell functions such as migration and chemotaxis.<sup>5</sup>

To adapt the device, they designed chambers with P selectin (SELP; CD62P)-coated polystyrene, which can bind neutrophils and allows

the cells to be purified from whole-blood samples with a simple wash step. Once the neutrophils are purified, a hydrogel chemoattractant lid is applied to the chamber, and chemotaxis of the cells toward the lid is measured with tracking software.

The researchers first tested whether the device can help discriminate between asthma and nonasthmatic allergic rhinitis, two conditions with similar clinical presentation. In blood samples from 23 mildly asthmatic patients, neutrophil movement velocity toward chemoattractants including IL-8 (CXCL8) and formyl-methionyl-leucine-phenylalanine was slower than that in samples from 11 nonasthmatics with allergic rhinitis. Importantly, most of the patients with asthma were not symptomatic at the time of the test, suggesting that the test may help overcome the issue of false negatives caused by transient symptoms.

Using a cutoff velocity of 1.545  $\mu\text{m}/\text{min}$ , the microfluidic device accurately identified 22 of 23 asthmatic subjects and 8 of 11 nonasthmatic controls, for a sensitivity of 96% and specificity of 73%.

The team also compared the accuracy of its test to the published accuracy of existing diagnostics, including the nitric oxide test. The researchers found that the new method had the best sensitivity of any test but was usually less specific.

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

## Diagnostic applications

Although the University of Wisconsin–Madison team found that the device can discriminate between patients with asthma and allergic rhinitis, a key next step for the team is to determine whether the device can also differentiate between asthma and other inflammatory diseases, such as chronic obstructive pulmonary disease (COPD).

“The next steps are to test broader patient populations, including some with inflammatory disorders that may affect neutrophil function, and see how the results hold up,” Beebe told *SciBX*.

He added that compared with existing diagnostic tests, the microfluidic test has the potential to be more accurate in these populations because it directly measures the function of inflammatory cells involved in the pathology. Other indicators of asthma measure indirect effects of the inflammation.

The team is in discussions with potential industry and academic collaborators to gain access to the clinical samples required for the testing and expand into indications beyond asthma.

Prajak Barde, associate director of clinical research at **Rhizen Pharmaceuticals S.A.**, said that testing the device in patients with COPD would be particularly valuable in determining its diagnostic utility.

Aaron agreed. “Clinically, it is very important to be able to differentiate between

asthma and COPD because the diseases present similarly but are treated very differently,” he said. “Asthma is currently discriminated from COPD based on an evaluation of the clinical history of the patient, because a long history of smoking is often associated with COPD, and based on how they respond to bronchodilators. Problems with lung function are often reversible with bronchodilators and/or inhaled steroids in asthma

**“Recent studies indicate that many of the diagnoses of asthma in the primary care setting rely solely on clinical evaluation or response to treatment and may lead to misdiagnosis.”**

**—Prajak Barde,  
Rhizen Pharmaceuticals S.A.**

patients, while patients with COPD continue to exhibit chronic airflow obstruction that does not resolve despite intensive treatments.”

Rhizen has asthma therapeutics in preclinical testing including the dual phosphoinositide 3-kinase- $\delta$  (PI3K $\delta$ ) and PI3K $\gamma$  inhibitor RP6503 and the calcium release-activated calcium channel (CRAC) inhibitor RP3128.

Barde noted that the lack of easy and reliable confirmatory quantitative tests can lead to overdiagnosis of asthma. “Recent studies indicate that many of the diagnoses of asthma in the primary care setting rely solely on clinical evaluation or response to treatment and may lead to misdiagnosis,” he said. “Inevitably, any misdiagnosed cases lead to overtreatment or inappropriate treatments and increased risk of side effects in the absence of any pharmacological benefit.”

He added that a potential disadvantage of the test is its low specificity but said it will be important to determine whether the specificity can be improved in studies in larger populations.

Aaron also wanted to see the test used in more patients. He added that a specificity of 73% would lead to a lot of false positives and therefore would not resolve the issue of unnecessary treatments for nonasthmatics, but he was optimistic the specificity could be improved.

“This test was only used in a limited number of patients, and the researchers seem to have chosen a relatively arbitrary cutoff point based on the velocity that gave the best sensitivity and specificity in the data that they have. The researchers need to conduct a larger validation study, which could lead to a new cutoff point that provides improved specificity.”

### Salus discovery

Although the researchers are moving forward with the asthma diagnostic indication, Salus’ initial plans for commercial development are in the R&D tool space, in which products can be brought to market quickly.

Beebe, who is also the founder of Salus, said that the company has a suite of technologies for next-generation sample preparation and cell-based

assays. “These technologies simplify bioassays by making them smaller, cheaper and better.”

He added that Salus’ initial focus is on developing products for sample preparation for researchers based on a separate technology platform, and the first product is scheduled for launch by the end of this year.

“The initial products and markets for the KOALA technology, upon which the asthma assay was built, will likely be in the R&D market, not the diagnostic, where KOALA can greatly simplify cell-based assays. We are actively seeking commercial partners for this application of KOALA now,” Beebe said. He added that validating the asthma diagnostic will require more patient studies as a first step.

The **Wisconsin Alumni Research Foundation**, which handles IP for the University of Wisconsin–Madison, has filed several patent applications covering the technology. The IP is exclusively licensed to Salus, and the company is seeking partnerships to develop bioassays including the asthma diagnostic assay.

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**Rhizen Pharmaceuticals S.A.**, La Chaux-de-Fonds, Switzerland

**Salus Discovery LLC**, Madison, Wis.

**University of Ottawa**, Ottawa, Ontario, Canada

**University of Wisconsin–Madison**, Madison, Wis.

**Wisconsin Alumni Research Foundation**, Madison, Wis.

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## 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
<b>Autoimmune disease</b>				
Autoimmune disease	Long noncoding RNA (lncRNA)	Cell-based studies suggest stimulating expression of a lncRNA found in dendritic cells (DCs) could help treat autoimmune diseases. In differentiating human DCs, shRNA targeting the DC-specific lncRNA encoded by the <i>WDM1</i> -like pseudogene decreased antigen uptake and allogeneic CD4 <sup>+</sup> T cell proliferation compared with nontargeting shRNA. Biochemical analysis showed that the lncRNA specifically associated with and promoted the activity of signal transducer and activator of transcription 3 (STAT3), a key regulator of DC function. Next steps include determining whether the lncRNA levels are related to the outcome of immunological disorders.	Patent application filed; licensing status unavailable	Wang, P. <i>et al. Science</i> ; published online April 18, 2014; doi:10.1126/science.1251456 <b>Contact:</b> Xuetao Cao, Second Military Medical University, Shanghai, China e-mail: <a href="mailto:caoxt@immunol.org">caoxt@immunol.org</a>
<b>SciBX 7(19); doi:10.1038/scibx.2014.548 Published online May 15, 2014</b>				
Autoimmune disease	RAR-related orphan receptor C thymus-specific isoform (ROR $\gamma$ 2; ROR $\gamma$ T)	<i>In vitro</i> and mouse studies suggest ROR $\gamma$ T inhibitors could suppress T helper type 17 (Th17) cells to help treat autoimmune diseases. In cultured human Th17 cells, three ROR $\gamma$ T inhibitors identified from a small molecule library screen each suppressed production of the proinflammatory cytokine IL-17. In a mouse model of experimental autoimmune encephalomyelitis (EAE), oral GSK805, which was found to inhibit ROR $\gamma$ T, or subcutaneous injection of one of the ROR $\gamma$ T inhibitors from the screen delayed disease onset and decreased disease severity compared with vehicle. Next steps could include testing the inhibitors in additional autoimmune disease models. Innovimmune Biotherapeutics Inc. has the ROR $\gamma$ T inverse agonist INV-17 in preclinical development for various autoimmune and inflammatory conditions. GSK805 is an investigational compound to inhibit the HCV NS5A protein that the Janssen Pharmaceutical Inc. unit of Johnson & Johnson acquired from GlaxoSmithKline plc.	Patent and licensing status unavailable	Xiao, S. <i>et al. Immunity</i> ; published online April 17, 2014; doi:10.1016/j.immuni.2014.04.004 <b>Contact:</b> Vijay K. Kuchroo, Brigham and Women's Hospital and Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:vkuchroo@rics.bwh.harvard.edu">vkuchroo@rics.bwh.harvard.edu</a> <b>Contact:</b> Alexander Marson, University of California, San Francisco, Calif. e-mail: <a href="mailto:alexander.marson@ucsf.edu">alexander.marson@ucsf.edu</a>
<b>SciBX 7(19); doi:10.1038/scibx.2014.549 Published online May 15, 2014</b>				

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Scleroderma	Toll-like receptor 4 (TLR4); fibronectin extra domain A (FnEDA)	<p><i>In vitro</i> and mouse studies suggest inhibiting TLR4 signaling in skin lesions could help treat scleroderma. In samples from skin lesions and sera from patients with scleroderma and in mouse models of cutaneous fibrosis, compared with healthy controls, the TLR4 ligand FnEDA was upregulated. In a mouse model of drug-induced cutaneous fibrosis, knockout of <i>Fned</i> or subcutaneous treatment with a small molecule TLR4 inhibitor prevented fibrosis and dermal thickening. In mice with established disease, the inhibitor reversed cutaneous fibrosis. Next steps include identifying new TLR4 inhibitors for scleroderma.</p> <p>At least three companies have TLR4 antagonists in Phase II or earlier testing for autoimmune and other indications.</p> <p>In addition, Eisai Co. Ltd.'s Eritoran (E5564), a synthetic lipid A analog that blocks TLR4 activation, failed to meet the primary endpoint of reduced mortality in Phase III testing for sepsis, and development is on hold.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.550</b> Published online May 15, 2014</p>	Patent application pending covering the use of new TLR4 inhibitors in scleroderma; BioLineRx Ltd. has an exclusive licensing option	<p>Bhattacharyya, S. <i>et al. Sci. Transl. Med.</i>; published online April 16, 2014; doi:10.1126/scitranslmed.3008264</p> <p><b>Contact:</b> Swati Bhattacharyya, Northwestern University Feinberg School of Medicine, Chicago, Ill. e-mail: <a href="mailto:s-bhattacharyya@northwestern.edu">s-bhattacharyya@northwestern.edu</a></p> <p><b>Contact:</b> John Varga, same affiliation as above e-mail: <a href="mailto:j-varga@northwestern.edu">j-varga@northwestern.edu</a></p>
<b>Cancer</b>				
Brain cancer	Activin receptor-like kinase 2 (ALK2; ACVR1); SMAD family member 1 (MADH1; SMAD1); inhibitor of DNA binding 1 (ID1)	<p>Three studies in primary tumors and cell culture suggest inhibiting ALK2 could help treat a subset of pediatric brain tumors. Whole-genome sequencing of diffuse intrinsic pontine gliomas and midline high-grade astrocytomas identified <i>ALK2</i> mutations in 19 of 101 tumors. In human astrocyte cell lines, the mutant <i>ALK2</i> increased cell growth, levels of activated SMAD1 and expression of <i>ID1</i> compared with wild-type <i>ALK2</i>. In cultures of primary diffuse intrinsic pontine glioma cells, a research compound that inhibited ALK2 blocked proliferation at low micromolar to high nanomolar <math>GI_{50}</math> values. Ongoing work includes additional <i>in vitro</i> and <i>in vivo</i> studies of mutant <i>ALK2</i> function and optimization of the ALK2 inhibitor to treat diffuse intrinsic pontine glioma.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.551</b> Published online May 15, 2014</p>	<p>For findings from first study, ALK2 inhibitor patented by Harvard University; unlicensed</p> <p>Patent and licensing status unavailable for findings from second study</p> <p>Findings from third study unpatented; unlicensed</p>	<p>Taylor, K.R. <i>et al. Nat. Genet.</i>; published online April 6, 2014; doi:10.1038/ng.2925</p> <p><b>Contact:</b> Chris Jones, The Institute of Cancer Research, Sutton, U.K. e-mail: <a href="mailto:chris.jones@icr.ac.uk">chris.jones@icr.ac.uk</a></p> <p>Buczkwicz, P. <i>et al. Nat. Genet.</i>; published online April 6, 2014; doi:10.1038/ng.2936</p> <p><b>Contact:</b> Cynthia Hawkins, The Hospital for Sick Children, Toronto, Ontario, Canada e-mail: <a href="mailto:cynthia.hawkins@sickkids.ca">cynthia.hawkins@sickkids.ca</a></p> <p>Fontebasso, A.M. <i>et al. Nat. Genet.</i>; published online April 6, 2014; doi:10.1038/ng.2950</p> <p><b>Contact:</b> Nada Jabado, McGill University, Montreal, Quebec, Canada e-mail: <a href="mailto:nada.jabado@mcgill.ca">nada.jabado@mcgill.ca</a></p>
Brain cancer	Hyaluronan-mediated motility receptor (RHAMM; CD168)	<p>Human samples and mouse studies suggest inhibiting RHAMM could help treat glioblastoma. Analysis of human glioma samples revealed higher <i>RHAMM</i> expression than that in normal brain tissue. In mice bearing human glioblastoma cells, shRNA knockdown of <i>RHAMM</i> decreased tumor-forming potential compared with no alteration, whereas overexpression of <i>RHAMM</i> increased expression of stem cell markers and tumor growth. Next steps include testing the effects of chemical compounds that inhibit <i>RHAMM</i> expression in glioma stem cells.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.552</b> Published online May 15, 2014</p>	Patent and licensing status undisclosed	<p>Tilghman, J. <i>et al. Cancer Res.</i>; published online April 7, 2014; doi:10.1158/0008-5472.CAN-13-2103</p> <p><b>Contact:</b> Mingyao Ying, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: <a href="mailto:ying@kenedykrieger.org">ying@kenedykrieger.org</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Breast cancer	MicroRNA-105 (miR-105)	<p>Mouse studies suggest inhibiting miR-105 could help prevent breast cancer metastasis. In a mouse xenograft model of human breast cancer, overexpression of <i>miR-105</i> increased metastasis to the lung and brain compared with normal expression. In xenograft mice bearing a breast cancer cell line with high <i>miR-105</i> expression, an anti-miR-105 compound restored the integrity of the vascular barrier and decreased metastasis compared with a control compound. Next steps could include developing a therapeutic strategy based on the selective blockade of miR-105 signaling.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.553</b> <b>Published online May 15, 2014</b></p>	Patent and licensing status unavailable	<p>Zhou, W. <i>et al. Cancer Cell</i>; published online April 14, 2014; doi:10.1016/j.ccr.2014.03.007 <b>Contact:</b> Shizhen Emily Wang, Beckman Research Institute at City of Hope, Duarte, Calif. e-mail: <a href="mailto:ewang@coh.org">ewang@coh.org</a></p>
Cancer	MEK	<p>Cell culture and mouse studies suggest the MEK inhibitor Mekinist trametinib could be useful for treating <i>K-Ras</i> (<i>KRAS</i>)-mutant cancers. In a mouse model of <i>Kras</i>-mutant pancreatic cancer, Craf (Raf1)-targeting shRNAs promoted MEK inhibitor-induced growth suppression, suggesting resistance to MEK inhibitors in such cancers could be driven by RAF1 signaling. In <i>KRAS</i>-mutant human cancer cells, trametinib, which disrupted the interaction between RAF1 and MEK, decreased proliferation compared with a MEK inhibitor, which did not disrupt the interaction. Next steps could include designing clinical trials to test trametinib and other newer MEK inhibitors in patients with <i>KRAS</i>-mutant cancers. Japan Tobacco Inc. markets Mekinist to treat melanoma. The drug is also in Phase II testing to treat non-small cell lung cancer (NSCLC) and pancreatic cancer.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.554</b> <b>Published online May 15, 2014</b></p>	Patent and licensing status unavailable	<p>Lito, P. <i>et al. Cancer Cell</i>; published online April 15, 2014; doi:10.1016/j.ccr.2014.03.011 <b>Contact:</b> Neal Rosen, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: <a href="mailto:rosenn@mskcc.org">rosenn@mskcc.org</a> <b>Contact:</b> Scott Lowe, same affiliation as above e-mail: <a href="mailto:lowes@mskcc.org">lowes@mskcc.org</a></p>
Colorectal cancer	MicroRNA-135b (miR-135b)	<p>Studies in human samples and mice suggest inhibiting miR-135b could help treat colon cancer. In two mouse models of colon cancer, genomewide miRNA profiling showed that miR-135b is upregulated in tumor tissue versus normal tissue. The miRNA also was upregulated in human cancer tissues and correlated with poor prognoses. In two mouse models of colon cancer, intraperitoneal injection of an anti-miR-135b oligonucleotide decreased tumor growth and size compared with injection of a scrambled oligonucleotide. Next steps could include testing miR-135b inhibition in additional cancer models.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.555</b> <b>Published online May 15, 2014</b></p>	Patent and licensing status unavailable	<p>Valeri, N. <i>et al. Cancer Cell</i>; published online April 14, 2014; doi:10.1016/j.ccr.2014.03.006 <b>Contact:</b> Carlo M. Croce, The Ohio State University Comprehensive Cancer Center, Columbus, Ohio e-mail: <a href="mailto:carlo.croce@osumc.edu">carlo.croce@osumc.edu</a> <b>Contact:</b> Nicola Valeri, University of Glasgow, Glasgow, U.K. e-mail: <a href="mailto:nicola.valeri@icr.ac.uk">nicola.valeri@icr.ac.uk</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Liver cancer	Fibroblast growth factor 19 (FGF19); fibroblast growth factor receptor 4 (FGFR4; CD334)	<p>Mouse studies suggest an FGF19 variant that activates a subset of pathways downstream of FGFR4 could help treat liver cancer. In mice, adeno-associated viral (AAV) vector-induced expression of FGF19 variants showed that the M70 variant bound Fgfr4 but did not induce tumor formation or activation of a tumor-associated signaling pathway in liver cells. In mice, coexpression of FGF19 and the M70 variant resulted in decreased tumor formation compared with expression of FGF19 alone, indicating that M70 can counter the known tumor-promoting activity of wild-type FGF19. Next steps include evaluating M70 for clinical use in primary biliary cirrhosis, hepatocellular carcinoma and nonalcoholic steatohepatitis.</p> <p>Isis Pharmaceuticals Inc. has ISIS-FGF4Rx, an antisense oligonucleotide targeting <i>FGFR4</i> mRNA, in Phase I testing to treat obesity.</p> <p>Blueprint Medicines has BLU9931, a small molecule inhibitor of FGFR4, in preclinical development to treat liver cancer.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.556</b> Published online May 15, 2014</p>	Patent applications filed; available for licensing from NGM Biopharmaceuticals Inc.	<p>Zhou, M. <i>et al. Cancer Res.</i>; published online April 11, 2014; doi:10.1158/0008-5472.CAN-14-0208</p> <p><b>Contact:</b> Lei Ling, NGM Biopharmaceuticals Inc., South San Francisco, Calif. e-mail: <a href="mailto:lling@ngmbio.com">lling@ngmbio.com</a></p>
Lung cancer	EPH receptor A2 (EPHA2)	<p>Cell culture and mouse studies suggest inhibiting EPHA2 could help treat lung cancer. In a <i>K-Ras</i> (<i>KRAS</i>)-driven mouse model of lung cancer, <i>Epha2</i> knockout decreased tumor burden and increased tumor cell apoptosis compared with no alteration. In cultured human non-small cell lung cancer (NSCLC) cells, an inhibitor of EPHA2 increased cell death compared with an inactive analog. In a mouse xenograft model of NSCLC, the inhibitor decreased tumor growth and increased tumor cell apoptosis compared with the analog or vehicle. Next steps could include developing inhibitors with higher specificity for EPHA2 or with increased bioavailability.</p> <p>Daiichi Sankyo Co. Ltd. has the anti-EPHA2 antibody DS-8859a in Phase I testing to treat solid tumors.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.557</b> Published online May 15, 2014</p>	Patent and licensing status unavailable	<p>Amato, K.R. <i>et al. J. Clin. Invest.</i>; published online April 8, 2014; doi:10.1172/JCI72522</p> <p><b>Contact:</b> Jin Chen, Vanderbilt University School of Medicine, Nashville, Tenn. e-mail: <a href="mailto:jin.chen@vanderbilt.edu">jin.chen@vanderbilt.edu</a></p>
<b>Cardiovascular disease</b>				
Ischemia/reperfusion injury	Neuron-derived neurotrophic factor (NDNF); endothelial cell nitric oxide synthase 3 (NOS3; eNOS); protein kinase B (PKB; PKBA; AKT; AKT1)	<p>Mouse studies suggest NDNF could be used to stimulate revascularization in ischemic vascular disease. In a mouse model of hindlimb ischemia, <i>Ndnf</i> expression was increased. siRNA against <i>Ndnf</i> impaired blood flow recovery and decreased eNos and Akt activation compared with control siRNA. In the same model, pretreatment with an intramuscular injection of an adenoviral vector expressing <i>Ndnf</i> led to eNos and Akt activation and increased blood flow recovery compared with pretreatment using an adenoviral vector expressing a control protein. Next steps include examining the effect of <i>Ndnf</i> on ischemic peripheral artery disease in diabetic mice.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.558</b> Published online May 15, 2014</p>	Unpatented; licensing status not applicable	<p>Ohashi, K. <i>et al. J. Biol. Chem.</i>; published online April 6, 2014; doi:10.1074/jbc.M114.555789</p> <p><b>Contact:</b> Noriyuki Ouchi, Nagoya University Graduate School of Medicine, Nagoya, Japan e-mail: <a href="mailto:nouchi@med.nagoya-u.ac.jp">nouchi@med.nagoya-u.ac.jp</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
<b>Endocrine/metabolic disease</b>				
Contraception; infertility	Folate receptor 4 $\delta$ (FOLR4)	Cell culture and mouse studies suggest FOLR4-targeting compounds could be useful as fertility treatments or contraceptives. A screen using epithelial cells that express mouse oocyte genes identified Folr4 as a surface receptor for izumo sperm-egg fusion (Izumo1), a protein shown to maintain fertility in male mice. In <i>in vitro</i> fertilization assays, anti-Folr4 antibodies blocked fertilization. Folr4-deficient female mice were infertile despite normal growth and natural mating behavior. Next steps include exploring the role of IZUMO1 and FOLR4 in human fertilization and looking for a commercial partner.	Patent application filed; available for licensing from the Wellcome Trust Sanger Institute <b>Contact:</b> Ross Rounsevell, Wellcome Trust Sanger Institute, Hinxton, U.K. e-mail: <a href="mailto:rr10@sanger.ac.uk">rr10@sanger.ac.uk</a>	Bianchi, E. <i>et al. Nature</i> ; published online April 16, 2014; doi:10.1038/nature13203 <b>Contact:</b> Gavin J. Wright, Wellcome Trust Sanger Institute, Hinxton, U.K. e-mail: <a href="mailto:gw2@sanger.ac.uk">gw2@sanger.ac.uk</a>
<b>SciBX 7(19); doi:10.1038/scibx.2014.559</b> Published online May 15, 2014				
<b>Infectious disease</b>				
Bacterial infection	FabI enoyl-(acyl carrier protein) reductase	Mouse and <i>in vitro</i> studies suggest a 4-pyridone fabI inhibitor could help treat Gram-positive and Gram-negative bacterial infections. Crystal structures of a narrow-spectrum, pyridone-based fabI inhibitor in complex with fabI from <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> were used to rationally design a 4-pyridone fabI inhibitor. In a panel of bacterial cell cultures, the 4-pyridone fabI inhibitor showed an extended spectrum of bactericidal activity compared with the narrow-spectrum inhibitor. In a mouse model of MRSA infection, the 4-pyridone fabI inhibitor decreased infection compared with the generic, methicillin-based antibiotic oxacillin. Next steps include developing fabI inhibitors with further improvements in potency and spectrum of activity.	Compounds covered by filed and pending patents; available for licensing from the Research Foundation for SUNY	Schiebel, J. <i>et al. J. Biol. Chem.</i> ; published online April 16, 2014; doi:10.1074/jbc.M113.532804 <b>Contact:</b> Caroline Kisker, University of Wuerzburg, Wuerzburg, Germany e-mail: <a href="mailto:caroline.kisker@virchow.uni-wuerzburg.de">caroline.kisker@virchow.uni-wuerzburg.de</a> <b>Contact:</b> Peter J. Tonge, Stony Brook University, Stony Brook, N.Y. e-mail: <a href="mailto:peter.tonge@stonybrook.edu">peter.tonge@stonybrook.edu</a>
<b>SciBX 7(19); doi:10.1038/scibx.2014.560</b> Published online May 15, 2014				
Measles	Viral RNA- dependent RNA polymerase	Cell culture and ferret studies suggest an orally available inhibitor of <i>Morbillivirus</i> RNA-dependent RNA polymerase could be used to treat measles. In human primary cells and cell lines infected with measles virus clinical isolates, the inhibitor showed nanomolar antiviral potency with limited cellular cytotoxicity. In ferrets intranasally infected with the <i>Morbillivirus</i> canine distemper virus (CDV), treatment with the oral inhibitor one day prior to infection and for the two weeks following resulted in increased survival and decreased viral load compared with vehicle treatment. In the ferret model, treatment with the oral inhibitor three days postinfection and for the following two weeks resulted in 100% survival and a 99% decrease in viral load compared with what was seen in vehicle-treated controls. Next steps include testing the compound in a nonhuman primate model of measles virus infection.	Patent applications pending; available for licensing from Emory University	Krumm, S.A. <i>et al. Sci. Transl. Med.</i> ; published online April 16, 2014; doi:10.1126/scitranslmed.3008517 <b>Contact:</b> Richard K. Plemper, Georgia State University, Atlanta, Ga. e-mail: <a href="mailto:rplemper@gsu.edu">rplemper@gsu.edu</a>
<b>SciBX 7(19); doi:10.1038/scibx.2014.561</b> Published online May 15, 2014				

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Viral infection	IL-6	<p>Cell culture and mouse studies suggest inhibiting IL-6 could help treat Ross River virus (RRV)-induced bone loss. In cultured human osteoblasts, RRV infection increased osteoclastogenesis and the production of proinflammatory factors, including IL-6, compared with saline. In mice, RRV infection led to viral replication within the bone and bone loss, but an IL-6-neutralizing antibody decreased both bone loss and viral titers in joints compared with a control antibody. Next steps include testing anti-IL-6 drugs in the model.</p> <p>Johnson &amp; Johnson's anti-IL-6 antibody Sylvant siltuximab is approved to treat multicentric Castleman's disease (MCD).</p> <p>At least 11 other companies have IL-6 antibodies in Phase II or earlier testing for indications including cancer and autoimmune diseases.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.562</b> Published online May 15, 2014</p>	Patent status not applicable; unavailable for licensing	<p>Chen, W. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online April 14, 2014; doi:10.1073/pnas.1318859111</p> <p><b>Contact:</b> Suresh Mahalingam, Griffith University, Southport, Queensland, Australia e-mail: <a href="mailto:s.mahalingam@griffith.edu.au">s.mahalingam@griffith.edu.au</a></p>
<b>Musculoskeletal disease</b>				
Muscular dystrophy	Dystrophia myotonica protein kinase (DMPK)	<p><i>In vitro</i> and mouse studies identified a DMPK CUG repeat-binding inhibitor that could help treat myotonic dystrophy type 1 (DM1). DM1 is caused by CUG repeats in the DMPK mRNA that bind and sequester the splicing regulator muscleblind (MBNL1). In HeLa cells expressing CUG repeat-containing DMPK, an inhibitor that bound the repeats and blocked binding to MBNL1 decreased formation of ribonuclear foci and partially corrected splicing of two genes compared with no treatment. In a transgenic <i>Drosophila</i> model of DM1, the inhibitor suppressed disease phenotypes. Next steps could include testing the inhibitor in additional DM1 models and improving its potency.</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.563</b> Published online May 15, 2014</p>	Patent and licensing status unavailable	<p>Wong, C.-H. <i>et al. J. Am. Chem. Soc.</i>; published online April 4, 2014; doi:10.1021/ja5012146</p> <p><b>Contact:</b> Steven C. Zimmerman, University of Illinois at Urbana-Champaign, Urbana Ill. e-mail: <a href="mailto:sczimmer@illinois.edu">sczimmer@illinois.edu</a></p> <p><b>Contact:</b> Paul J. Hergenrother, same affiliation as above e-mail: <a href="mailto:hergenro@illinois.edu">hergenro@illinois.edu</a></p>
<b>Neurology</b>				
Depression	DNA-damage-inducible transcript 4 (DDIT4; RTP801; REDD1)	<p>Rodent and human studies suggest inhibiting REDD1 could help treat major depressive disorder (MDD). In postmortem human prefrontal cortex samples from patients with MDD, mRNA expression of the mammalian target of rapamycin complex 1 (mTORC1) inhibitor REDD1 was increased compared with that in samples from individuals without MDD. <i>Redd1</i> knockout mice showed resistance to chronic stress-induced behaviors and decreases in mTORC1 signaling and the number and function of spine synapses compared with wild-type mice. In rats, injection of a <i>REDD1</i>-expressing viral vector into the prefrontal cortex induced behaviors and neuronal atrophy similar to those caused by chronic stress. Next steps include screening for compounds that disrupt the effect of REDD1.</p> <p>Kunshan RiboQuark Pharmaceutical Technology Co. Ltd. and Pfizer Inc. have PF-655, an siRNA targeting REDD1, in Phase II testing to treat diabetic macular edema (DME) and wet age-related macular degeneration (AMD).</p> <p><b>SciBX 7(19); doi:10.1038/scibx.2014.564</b> Published online May 15, 2014</p>	Unpatented; unavailable for licensing	<p>Ota, K.T. <i>et al. Nat. Med.</i>; published online April 13, 2014; doi:10.1038/nm.3513</p> <p><b>Contact:</b> Ronald S. Duman, Yale School of Medicine, New Haven, Conn. e-mail: <a href="mailto:ronald.duman@yale.edu">ronald.duman@yale.edu</a></p>

## This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Stroke; nerve damage	Casein kinase 1 (CSNK1; CKI); RE1-silencing transcription factor (REST; NRSF)	<i>In vitro</i> and rat studies suggest activating CKI could help protect against stroke-associated neuronal death. In rats, global ischemia decreased <i>Cki</i> expression, increased <i>Rest</i> expression and increased death of CA1 hippocampal neurons compared with no ischemia. In rats, intracerebroventricular delivery of the CKI activator pyrvinium decreased ischemia-induced <i>Rest</i> expression and death of CA1 neurons compared with no treatment. Next steps could include developing selective CKI activators and evaluating them in stroke models.	Patent and licensing status unavailable	Kaneko, N. <i>et al. J. Neurosci.</i> ; published online April 23, 2014; doi:10.1523/JNEUROSCI.4045-13.2014 Contact: R. Suzanne Zukin, Albert Einstein College of Medicine of Yeshiva University, New York, N.Y. e-mail: <a href="mailto:suzanne.zukin@einstein.yu.edu">suzanne.zukin@einstein.yu.edu</a>
		<i>SciBX</i> 7(19); doi:10.1038/scibx.2014.565 Published online May 15, 2014		



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## 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
<b>Assays &amp; screens</b>			
Cancer personalized profiling by deep sequencing (CAPP-seq) to characterize cancer using blood samples	Detection, quantification and analysis of circulating tumor DNA using CAPP-seq could help diagnose cancer and monitor response to therapy. CAPP-seq quantifies and identifies known mutations in circulating tumor DNA. In tumor samples from 13 patients with non-small cell lung cancer (NSCLC) and samples from 5 healthy controls, CAPP-seq detected cancer with 96% specificity, and quantification of plasma circulating tumor DNA levels correlated with tumor volume. In repeated sampling of three patients throughout treatment, circulating tumor DNA quantity correlated with tumor volume and better predicted residual disease and recurrence than imaging. Next steps could include validating the method in additional patients.  <b>SciBX 7(19); doi:10.1038/scibx.2014.566</b> <b>Published online May 15, 2014</b>	Patent and licensing status unavailable	Newman, A.M. <i>et al. Nat. Med.</i> ; published online April 6, 2014; doi:10.1038/nm.3519 <b>Contact:</b> Maximilian Diehn, Stanford University, Stanford, Calif. e-mail: <a href="mailto:diehn@stanford.edu">diehn@stanford.edu</a>
Lentivirus-based clustered, regularly interspaced short palindromic repeats (CRISPR) library for high throughput screening	A high throughput screening system using lentivirus-based CRISPR libraries could be used to identify therapeutic targets. A CRISPR library was constructed using lentiviral DNA to target 291 human genes with 3 different kinds of single guide RNAs (sgRNAs). In a functional screening study designed to identify genes associated with bacterial toxin resistance, known toxin targets as well as previously unknown genes were identified. In HeLa cells, knockdown of the newly identified genes caused toxin resistance. Next steps include expanding the size of the library and the number of sgRNAs targeting each gene.  <b>SciBX 7(19); doi:10.1038/scibx.2014.567</b> <b>Published online May 15, 2014</b>	Patent application filed; unavailable for licensing	Zhou, Y. <i>et al. Nature</i> ; published online April 9, 2014; doi:10.1038/nature13166 <b>Contact:</b> Wensheng Wei, Peking University, Beijing, China e-mail: <a href="mailto:wswwei@pku.edu.cn">wswwei@pku.edu.cn</a>
Signature of 24 genes to predict prostate cancer recurrence	Sequencing studies identified a 24-gene panel that could help predict disease recurrence in patients with prostate cancer. In 106 prostate tumor samples, RNA sequencing led to the identification of a 24-gene signature that differentiated patients at high and low risk of disease recurrence when combined with assessment of clinical variables. In a validation dataset from 140 patients with prostate cancer, assessing clinical variables together with the 24-gene panel resulted in increased predictive capability of disease recurrence compared with assessing clinical variables alone or in combination with a gene signature from a panel of 31 cell-cycle progression genes. Next steps include validating the biomarkers in a larger patient cohort.  <b>SciBX 7(19); doi:10.1038/scibx.2014.568</b> <b>Published online May 15, 2014</b>	Patent application filed; available for licensing	Long, Q. <i>et al. Cancer Res.</i> ; published online April 8, 2014; doi:10.1158/0008-5472.CAN-13-2699 <b>Contact:</b> Carlos S. Moreno, Emory University, Atlanta, Ga. e-mail: <a href="mailto:cmoreno@emory.edu">cmoreno@emory.edu</a>
<b>Chemistry</b>			
Bacterial polysialyltransferase engineered to generate uniform polysaccharides for therapeutic applications	<i>In vitro</i> studies suggest a bacterial polysialyltransferase could be used to improve drug pharmacology. Pharmacological properties of therapeutic proteins can be enhanced by attached polysialic acid, but polysialyltransferases usually generate polysialic chains of varying lengths because the enzyme retains its substrate for more than one catalytic cycle. Mutational analysis of polysialyltransferase from <i>Neisseria meningitidis</i> identified a point mutation that resulted in the enzyme releasing the substrate after every catalytic cycle, allowing for control over chain length. Next steps include investigating whether uniform polysialylation can be obtained on clinically relevant proteins including interferon- $\alpha$ (IFNA; IFN $\alpha$ ).  <b>SciBX 7(19); doi:10.1038/scibx.2014.569</b> <b>Published online May 15, 2014</b>	Unpatented; licensing status not applicable	Keys, T.G. <i>et al. Nat. Chem. Biol.</i> ; published online April 13, 2014; doi:10.1038/nchembio.1501 <b>Contact:</b> Rita Gerardy-Schahn, Hannover Medical School, Hannover, Germany e-mail: <a href="mailto:gerardy-schahn.rita@mh-hannover.de">gerardy-schahn.rita@mh-hannover.de</a>

## This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
<b>Drug platforms</b>			
Direct <i>in vivo</i> reprogramming of pancreatic acinar cells into $\delta$ -like and $\alpha$ -like pancreatic cells	Direct <i>in vivo</i> reprogramming of pancreatic acinar cells into $\delta$ - and $\alpha$ -like pancreatic islet cells could be used to generate pancreatic islets to treat diseases such as type 1 diabetes. In mice, intrapancreatic injection of an adenoviral vector encoding <i>neurogenin 3</i> ( <i>Ngn3</i> ) converted acinar cells into $\delta$ -like pancreatic islet cells. In mice, intrapancreatic injection of adenoviral vectors encoding <i>Ngn3</i> and <i>v-maf musculoaponeurotic fibrosarcoma oncogene homolog A</i> ( <i>Mafa</i> ; <i>Ripe3b1</i> ) converted acinar cells into $\alpha$ -like pancreatic islet cells. Next steps include creating islets that contain the full repertoire of acinar tissue-derived endocrine cells.  <b>SciBX 7(19); doi:10.1038/scibx.2014.570</b> <b>Published online May 15, 2014</b>	Patented; available for licensing	Li, W. <i>et al. eLife</i> ; published online April 8, 2014; doi:10.7554/eLife.01846 <b>Contact:</b> Qiao Zhou, Harvard University, Cambridge, Mass. e-mail: <a href="mailto:qiao_zhou@harvard.edu">qiao_zhou@harvard.edu</a>
<b>Markers</b>			
Forssman disaccharide antibodies predict positive response to ProstVac-VF prostate cancer vaccine	Patient sample studies suggest serum antibodies against Forssman disaccharide could help predict a positive response to ProstVac-VF, a poxvirus-based cancer vaccine that induces responses to prostate-specific antigen (KLK3; PSA) as well as a poxvirus-specific carbohydrate called Forssman disaccharide. In two independent cohorts of over 100 patients, ProstVac-VF-treated patients who had high Forssman disaccharide serum antibodies showed increased overall survival compared with those who had low serum antibody levels and those who received a control vector. In an additional cohort of 13 patients receiving both ProstVac-VF and the radiopharmaceutical Quadramet, seven patients had high levels of Forssman disaccharide serum antibodies and increased overall survival compared with those who had low serum levels of the antibodies. Next steps could include validating the marker in additional patient cohorts. Bavarian Nordic A/S has ProstVac-VF rilimogene galvacirepvec in Phase III testing to treat prostate cancer. Jazz Pharmaceuticals plc markets Quadramet samarium 153 lexidronam to treat bone pain associated with cancer.  <b>SciBX 7(19); doi:10.1038/scibx.2014.571</b> <b>Published online May 15, 2014</b>	Patent and licensing status unavailable	Campbell, C.T. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online April 14, 2014; doi:10.1073/pnas.1314722111 <b>Contact:</b> Jeffrey C. Gildersleeve, National Cancer Institute, Bethesda, Md. e-mail: <a href="mailto:gildersj@mail.nih.gov">gildersj@mail.nih.gov</a>
<i>Hydroxyprostaglandin dehydrogenase 15 NAD</i> (HPGD; <i>15-PGDH</i> ) expression as a biomarker of aspirin-associated decrease in colorectal cancer risk	Measuring HPGD expression could help predict whether regular aspirin use will help decrease colorectal cancer risk. In a study with 127,865 participants, regular aspirin use was associated with decreased incidence of colorectal cancer in subjects who had high HPGD expression in the colonic mucosa but not in subjects who had low HPGD expression. Next steps include carrying out a prospective clinical trial to determine whether levels of HPGD in patients are a significant predictor of whether aspirin therapy can decrease recurrent colorectal polyps.  <b>SciBX 7(19); doi:10.1038/scibx.2014.572</b> <b>Published online May 15, 2014</b>	Use of HPGD to select individuals for treatment with aspirin patented; available for licensing	Fink, S.P. <i>et al. Sci. Transl. Med.</i> ; published online April 23, 2014; doi:10.1126/scitranslmed.3008481 <b>Contact:</b> Andrew T. Chan, Harvard Medical School, Boston, Mass. e-mail: <a href="mailto:achan@mgh.harvard.edu">achan@mgh.harvard.edu</a> <b>Contact:</b> Reiko Nishihara, Dana-Farber Cancer Institute, Boston, Mass. e-mail: <a href="mailto:rnishiha@hsph.harvard.edu">rnishiha@hsph.harvard.edu</a> <b>Contact:</b> Sanford D. Markowitz, Case Western Reserve University, Cleveland, Ohio e-mail: <a href="mailto:sxm10@cwru.edu">sxm10@cwru.edu</a>



