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THIS WEEK

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Diagnosing resistance

By Lauren Martz, Staff Writer

It might be a day late and a dollar short, but the U.S. government is finally getting around to addressing what many experts think is the big unserved need in antibiotic resistance—new diagnostics. Faster, more sensitive and versatile diagnostic technologies that can keep up with the emergence of resistant strains are needed both for improving treatment and optimizing clinical trials.

"Diagnostics are the key. It is just that we are far away from that right now and need to stimulate that," said Janet Woodcock, director of the **FDA**'s Center for Drug Evaluation and Research at a hearing last month about antibiotic resistance held by the U.S. House of Representatives' Energy and Commerce Committee's Subcommittee on Health.

The hearing was part of the committee's 21st Century Cures initiative and revolved around strategies for combating antibiotic resistance and fostering new drug development. Although the main focus was on ways to modify clinical practice and create incentives for new therapeutics, several participants highlighted the urgent need for new diagnostics.

A day earlier, the White House announced a $20 million prize for the development of a rapid point-of-care diagnostic for resistant infections, a national strategy for solving problems of antibiotic resistance and the publication of a report from the President's Council of Advisors on Science and Technology that included the importance of diagnostics in reducing the inappropriate use of antibiotics.

Experts at the hearing agreed that existing diagnostic capabilities do not adequately address the problem of antibiotic resistance.

"Louis Pasteur and Alexander Fleming would recognize the methods we use today because they invented them, so there is a lot of room at the top for improvement," Woodcock said at the hearing. "If we could bring diagnosis of infectious diseases into the 21st century, we would have made a huge advance and really accelerated the development of therapy."

The consensus of the participants was that more sensitive and faster diagnostics are needed both to help physicians pick the right antibiotic and to help companies select the right patient population for clinical trials.

"Diagnosis should be the foundation of therapy, and unfortunately in the infectious disease space often you are treating a person well before you know what the person has, and this is a fundamental problem," said Woodcock.

Using the wrong antibiotic not only fails to treat the patient properly but also contributes further to the growth of resistant strains. Woodcock noted—on the plus side—that the rapid strep test has reduced the

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misuse of antibiotics in patients with colds or other upper respiratory tract infections that resemble strep throat.

John Rex, SVP and head of infection global medicine development at **AstraZeneca plc**, told *SciBX* that in drug development, the lack of fast and specific diagnostics increases trial size and thus development costs.

"About one in four, or maybe one in three, patients that I enroll in a clinical trial are actually infected with the organism of interest. The rest of the patients don't give useful microbiotic data. All isn't lost because they do provide tolerability data, but they don't help us know if the drug is working," said Rex. "If a rapid diagnostic could simply make our guesses better, that would be fantastic. Everyone I enroll costs time, money and work."

No GAIN for diagnostics

The government launched a stimulus for new antibiotic therapies with the Generating Antibiotic Incentives Now (GAIN) Act in 2012 that provides extended exclusivity for new antibiotics and earmarks them for priority review by the FDA. That was followed last December by the introduction of the Antibiotic Development to Advance Patient Treatment (ADAPT) Act, a bill to improve the economics of antibiotic development for companies.

For therapeutics, the measures are starting to pay off. Three new antibiotics have been approved this year after being designated as Qualified Infectious Disease Products (QIDPs) under GAIN, and at least 36 more molecules in development have QIDP designation.

The approved QIDP-designated products include Dalvance dalbavancin from **Durata Therapeutics Inc.** and **Gruppo Angelini**; Sivextro tedizolid phosphate (TR-701) from **Cubist Pharmaceuticals Inc.**, **Dong-A Pharmaceutical Co. Ltd.** and **Bayer AG**; and Orbactiv

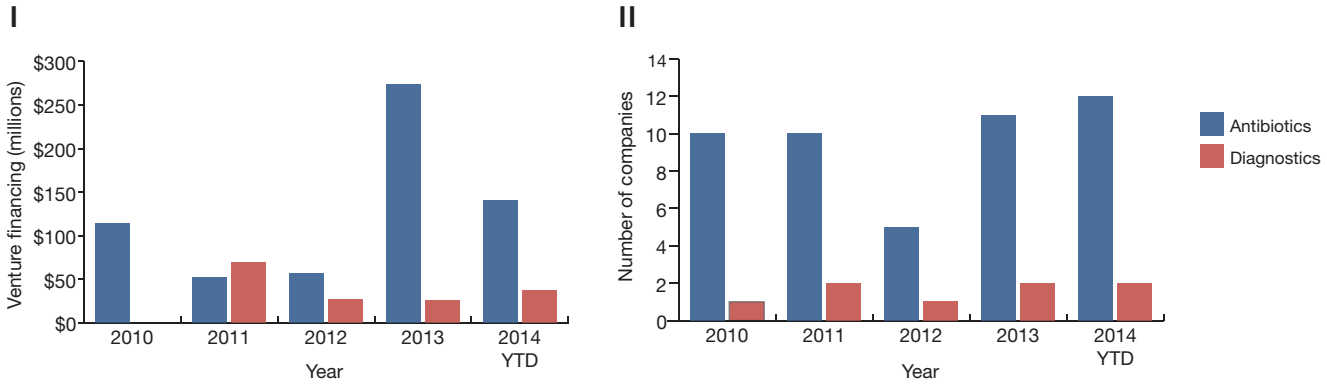

Figure 1. Venture financing for therapies and diagnostics for bacterial infections. (**I**) Total amount of venture financing for newly formed companies developing therapies or diagnostics for bacterial infections.

(**II**) Number of newly formed companies developing therapeutics or diagnostics for bacterial infections that received venture financing.

Source: BCIQ: BioCentury Online Intelligence

oritavancin from **The Medicines Co.** All three drugs are approved to treat acute bacterial skin and skin structure infections (ABSSSIs) caused by Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA).

The last two years have seen a clear uptick in venture financing for companies developing new antibacterial therapies, whereas the venture money going to new diagnostics has remained low and has barely changed in five years. Similarly, over the past 5 years, there have consistently been 5- to 10-fold more companies formed to develop therapeutics for bacterial infections than to develop diagnostics for them (*see* **Figure 1, "Venture financing for therapies and diagnostics for bacterial infections"**).

Ankit Mahadevia, entrepreneur in residence in the life sciences group at **Atlas Venture**, told *SciBX* that the new initiatives have had an impact on VC investment in new antibiotic therapeutics.

"From a VC perspective, anti-infective therapies are very attractive for investment," he said. "With the regulatory changes, the cost of development is decreasing due to decreased trial size, and GAIN makes revenues higher. We also have a clear realization from payers that we need to pay more for the effective therapeutics."

But for diagnostics, the outlook is less rosy. "On the diagnostic side in general, the last few years have been challenging. There are few investors, and reimbursement is cloudy. The market needs for new diagnostics are clearly there, but there is not a magic bullet to fix the health of entrepreneurial companies in diagnostics," Mahadevia told *SciBX*.

The problem is largely that without incentives such as those in the GAIN and ADAPT acts, the economics of developing diagnostics for antibiotic resistance are not attractive for companies or investors.

Oliver Schacht, CEO of diagnostics company **Curetis AG**, added, "As bad as pricing is for antibiotic drugs, it is even worse for diagnostics. To solve this problem, we need to put diagnostics in a spot where the health economic benefits are realized and the pricing and reimbursements are set accordingly."

Schacht noted that pricing for diagnostics is based on a cost-plus model, which is too low to allow companies to recover development costs. Value-based pricing could solve the problem, he said.

Stakeholders who spoke with *SciBX* agreed that the White House's $20 million prize is a step in the right direction but said it is not even remotely enough.

Joen Johansen, head of marketing at **Accelerate Diagnostics Inc.**, told *SciBX*, "It is certainly a good idea to provide financial incentives, and this prize is a good first step to help get projects off the ground, but it will take quite a bit more to get diagnostics to the market. Diagnostics don't require the same level of investment as therapeutics, but many millions more than that would be needed."

Several participants at the hearing proposed that one solution lies in increased funding for basic research at the **NIH** or universities to stimulate discoveries that can be commercialized.

"If we do not have enough basic science, the pipeline that flows to venture capital and then to the larger companies runs dry," Kevin Outterson said at the hearing. Outterson is a professor of law and of health law, bioethics and human rights at **Boston University**.

But others disagreed that increased public funding would drive commercial innovation in the absence of incentives on the commercial side.

"If you don't have somebody with the profit motive—a company, a pharmaceutical company, big or small—you can sit there doing some basic research for 100 years," but it will not get the industry where it needs to be, said Rep. Phil Gingrey, D-Ga. Gingrey was one of two congressmen who introduced the ADAPT Act in the House of Representatives.

Adrian Thomas advocated a combination of approaches to cover both academic research and commercial incentives that would promote point-of-care diagnostics, biomarkers and new diagnostic capabilities and would help advance clinical research. He said that large grants, funding and prizes would make the most sense, in addition to tax credits, to encourage broad-based academic research as well as broad-based technology development—which has shorter timelines and is managed differently than therapeutic development. Thomas is VP of global market access, commercial strategy operations and global public health at **Johnson & Johnson**'s Janssen Inc. unit.

Dennis Dixon, chief of the bacteriology and mycology branch at the NIH's **National Institute of Allergy and Infectious Diseases**, told

Table 1. Selected diagnostic tests in development for bacterial infections. Diagnostics are listed from most to least advanced stage of development.
Source: BCIQ: BioCentury Online Intelligence; company websites

Company	Product(s)	Indication(s)	Description	Development phase
Akers Biosciences Inc. (NASDAQ:AKER; LSE:AKR)	PIFA PLUSS Chlamydia Assay	Chlamydia infection	Point-of-care assay to detect chlamydia from finger-stick blood sample	Pivotal
Diaxonhit (Euronext:ALEHT)	BJI InoPlex	Articular prosthesis infections	Noninvasive, multiparameter serologic test that detects bacterial antigens	Pivotal
Accelerate Diagnostics Inc. (NASDAQ:AXDX)	Tests using the Accelerate ID/AST system	Bacterial infections	Culture-free genotypic and phenotypic microbe analysis to diagnose bacterial infections and assess antibiotic susceptibility	Pilot
Atlas Genetics Ltd.	Tests using the Atlas io system	Chlamydia; gonorrhea; methicillin-resistant *Staphylococcus aureus* (MRSA)	Point-of-care diagnostic that detects bacterial nucleic acids	Pilot
bioMerieux S.A. (Euronext:BIM)	FilmArray Meningitis/ Encephalitis Panel	Community-acquired meningitis	*In vitro* multiplex PCR platform that detects 16 bacterial, viral and fungal pathogens known to cause community-acquired meningitis and encephalitis	Pilot
Global BioDiagnostics Corp.	TB REaD	Tuberculosis	Point-of-care diagnostic to detect tuberculosis	Pilot
Great Basin Corp. (NASDAQ:GBSN)	Staph ID/R test	*Staphylococcus*	Automated molecular diagnostic combining helicase-dependent amplification of target sequence in the RNA polymerase gene with a chip-based array	Pilot
Meridian Bioscience Inc. (NASDAQ:VIVO)	Illumigene *Chlamydia trachomatis*; illumigene *Neisseria gonorrhoeae*	Chlamydia; gonorrhea	Molecular platform based on DNA amplification utilizing loop amplification technology	Pilot
Akonni Biosystems Inc.	TruArray test for MDR-TB; TruArray test for MRSA	Multidrug-resistant tuberculosis; MRSA	Point-of-care mid-multiplex assay using Akonni's TruDiagnosis platform	Preclinical

SciBX that the institute has multiple funding opportunities related to diagnostics development for antibiotic resistance, which include awards for investigator-initiated research, small business grants, special programs for diagnostic development, and clinical and preclinical services for diagnostic development.

Need for speed

Because patients usually receive treatment before their infection is properly characterized, one of the key needs in new diagnostics is faster readouts.

"The gold standard for diagnosing bacterial infections is growing the bacteria in culture for one to two days or more—depending on the type of bacteria—to identify the causal pathogen. Then, an additional 12–24 hours are required to conduct an antibiotic sensitivity screen," said Schacht. "Without a diagnosis for several days, physicians don't know which type of pathogen is responsible for the infection and treat patients with broad-spectrum antibiotics. This is the wrong treatment or inadequate in 30%–40% of cases."

"Louis Pasteur and Alexander Fleming would recognize the methods we use today because they invented them, so there is a lot of room at the top for improvement."
—Janet Woodcock, Food and Drug Administration

According to Dixon, there have been some major breakthroughs in rapid diagnostics for certain bacterial species using molecular diagnostics. These tests scan amplified genetic material or proteins for specific sequences present in the pathogen of interest.

However, AstraZeneca's Rex cautioned that although rapid readouts are valuable, molecular tests only detect resistance mutations that have been seen in the past. "This is a problem because bacteria are constantly evolving new methods of resistance," he said.

Johansen added that although molecular diagnostics can identify a known pathogen or mutation, the technique does not provide a clear indication that the organism will respond to a certain drug because other resistance mechanisms may also be at play.

Indeed, for these reasons Dixon said that molecular diagnostics are not at the point of replacing bacterial cultures.

However, there are intrinsic limitations to how much the timelines of culture-based techniques can be shortened.

"The problem with accelerating culture-based tests is that you hit a biologic speed limit. Organisms will only grow so fast. We need to allow a certain number of growth cycles that each take about 60–90 minutes to get the bacteria really growing," said Rex. "What people have experimented with is trying to find ways to get the culture down to a few hours with fewer cycles, but we can't get much shorter than that."

One solution might be in bacterial cell imaging methods that monitor the response of bacteria to therapeutics on an individual-cell basis—a strategy that could enable a rapid susceptibility readout and avoid the long culture process.

Accelerate Diagnostics is developing the Accelerate ID/AST diagnostic platform using this approach and hopes to seek marketing authorization for a test in 2016.

Cleaning up

At least 11 companies have new diagnostic tests in development for bacterial infections (*see* **Table 1, "Selected diagnostic tests in development for bacterial infections"**).

Although the different technologies in development all have advantages, there are several common problems they still need to address, such as sample contamination, assay sensitivity, identifying the responsible pathogen and measuring efficacy in biofilms.

"Direct from specimen is the key enabling technology for really, really rapid diagnostics."
—John Rex, AstraZeneca plc

"Direct from specimen is the key enabling technology for really, really rapid diagnostics," said Rex.

The problem, according to Schacht, is that "native clinical samples are messy and heterogeneous, especially in tissues affected by infectious diseases such as sputum. The samples are ugly, they may have blood in them and they are all different. For a rapid bedside test, we need to develop one test that can handle very diverse clinical samples."

Certain types of tests such as next-generation sequencing also require cleaner samples than others and may be less well suited for rapid diagnostics, added Schacht.

Another challenge is developing tests with sufficient sensitivity. "Few bacteria can cause a significant response from the immune system, so we need a very sensitive test for many kinds of infections," said Johansen.

Detecting the bacteria is only part of the problem. The other part is determining whether the bacteria tested are the responsible pathogens.

For example, Dixon told *SciBX* that rapid molecular diagnostic tests for strep, chlamydia and tuberculosis have been breakthrough successes. "These tests are effective because they are each detecting bacteria that are not normally present and do so where the bacteria are present in large numbers," he said. "This is versus infections in a hospital setting that are caused by bacteria that are also part of the normal flora. These infections set a much higher bar on the threshold for what will be clinically useful tests."

Prabhavathi Fernandes, founder, president and CEO of **Cempra Inc.**, told *SciBX* that this problem is very significant in diagnosing the cause of pneumonia. "Pneumonia is caused by a number of pathogens that are all found in the saliva of healthy people. The question is, how do you distinguish the true infection from colonizing bacteria? The answer is in the numbers, which may show which is taking over."

Finally, identifying antibiotic susceptibility of chronic bacterial infections poses a separate set of challenges. Most significantly, antibiotics effective against free-floating bacteria may not be effective against biofilms.

"Minimum inhibitory concentration tests for antibiotic susceptibility test free-floating, planktonic-phase bacteria. In real life and especially in chronic infections, bacteria grow as a biofilm community and are one to two thousand times more resistant than the free-floating counterparts," said Amin Omar, technical services supervisor at **Innovotech Inc.**

However, some researchers think that diagnostic developers could learn from the success achieved in diagnosing MRSA.

"A few years ago, MRSA was the hot challenge, and companies did a fantastic job of developing a rapid test for the resistance," Schacht said. The GeneXpert system marketed by **Cepheid Inc.** can diagnose MRSA in 66 minutes or less.

Bacterial gene editing with clustered, regularly interspaced short palindromic repeats (CRISPR) may be poised for a similar breakthrough. Two new studies show that it may be possible to use CRISPR to create tailored therapies that eliminate specific resistance genes or resensitize bacteria to existing antibiotics (*see* **Programmable sensitivity, page 8**).[1,2]

"Resistance is cyclic. Any new drugs, if used long enough even appropriately, ultimately will see some level of resistance. There isn't a simple, permanent fix, and we need continuous innovation to keep up with bacterial evolution," said Rex.

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Curetis AG, Holzgerlingen, Germany
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Gruppo Angelini, Rome, Italy
Innovotech Inc. (TSX-V:IOT), Edmonton, Alberta, Canada
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
National Institute of Allergy and Infectious Diseases, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
The Medicines Co. (NASDAQ:MDCO), Parsippany, N.J.

Branching out in pancreatic cancer

By Benjamin Boettner, Senior Writer

Predicting pancreatic cancer has been a major problem in the disease, which is normally too advanced for effective treatment by the time it is diagnosed. Researchers at the **Massachusetts Institute of Technology** and the **Dana-Farber Cancer Institute** have identified a prediagnostic signature of branched-chain amino acids that could help improve risk prediction up to five years before the disease appears, but developing a viable biomarker panel will require identification of the tumor factors that trigger the change.[1]

The findings were based on four large-scale, prospective public health cohorts that each included 22,000–122,000 subjects and focused on pancreatic ductal adenocarcinoma—the major form of pancreatic neoplasms.

Currently, the most well-characterized predisposing factors are tobacco consumption and a family history of this form of pancreatic cancer, which provide a 1.8-fold increased risk of developing the disease. At diagnosis, the mean survival rate is 12 months and most patients' cancer is too advanced for surgical resection.[2,3]

"This cancer area urgently needs biomarkers that would allow more extensive screening," said Kenneth Olive. "CT and endoscopic ultrasound are used as standard diagnostic tests, but they either involve radiation or are invasive and cannot be deployed on the whole population." Olive is an assistant professor of medicine and pathology at **Columbia University** whose research focuses on pancreatic ductal adenocarcinoma.

One obstacle has been that little is known of the biology underlying early disease progression in pancreatic cancer. However, other pathologies related to altered metabolism have been linked with the disease, such as obesity, glucose intolerance and the muscle-wasting disease cancer-induced cachexia.

"Are branched-chain amino acids in particular doing something for the tumors, or is it a general supply in amino acids released by muscle tissue that promotes tumor growth? I would favor the latter possibility."

—Matthew Vander Heiden, The David H. Koch Institute for Integrative Cancer Research at MIT

Thus, Brian Wolpin and Matthew Vander Heiden thought there might be altered levels of serum metabolites that could help identify an early disease signature. Wolpin is a clinical investigator at Dana Farber and an assistant professor of medicine at **Harvard Medical School**. Vander Heiden is an associate professor of biology at **The David H. Koch Institute for Integrative Cancer Research at MIT**.

At diagnosis, most patients with pancreatic cancer have a range of comorbidities that could confound attempts to identify metabolic changes directly associated with the cancer. Thus, the researchers looked for patterns in blood samples from the four cohorts taken at least two years before the onset of disease in individuals who went on to develop pancreatic cancer.

The three branched-chain amino acids (BCAAs)—isoleucine, leucine and valine—stood out as having particularly elevated blood levels and were associated with at least a twofold increase in the risk of disease.

The researchers stratified the cases according to the interval between blood collection and diagnosis and found the strongest association between elevated BCAAs and future disease in samples taken two to five years before diagnosis.

High levels of BCAAs have also been seen in diabetes, which can precede pancreatic cancer.[4,5] However, exclusion of patients with diabetes at the time of sample collection did not change the results, which suggested that the results were not caused by a diabetes-related pathology.

Next, the team tested the connection *in vivo* using the KPC mouse model of mutant *K-ras* (*Kras*)-driven pancreatic ductal adenocarcinoma and looked for mechanisms that might link changes in BCAAs with disease pathology.

BCAAs levels were increased at subclinical stages of disease in the mice and were produced by the breakdown of long-term protein stores, most notably in the muscles. The data suggested a link to muscle wasting and indicated that muscle deterioration is initiated in pancreatic cancer long before the onset of clinical cachexia.

Olive was impressed by the *in vivo* results. "KPC mice are faithful to human disease and present an opportunity to find tumor-secreted soluble factors and to minimize less relevant factors," he said. "They are born with a histologically normal pancreas and progress through benign and pre-neoplastic to malignant stages with branched-chain amino acids rising in a time frame that is consistent with the earliest tumor stages."

The findings were published in *Nature Medicine*.

Chain links

"The discovery of a precachexic state in which the muscle breakdown process has begun well before the onset of disease is a fundamentally stunning finding," Olive told *SciBX*.

Although levels of BCAAs could lead to improved diagnostics and better understanding of the disease, the amino acids themselves are unlikely to constitute viable markers.

"The use of branched-chain amino acids alone for early diagnosis of pancreatic cancer will not be realistic," said David Whitcomb. "For a screening test, branched-chain amino acids by themselves are neither sensitive nor specific enough." Whitcomb is chief of the Division of Gastroenterology, Hepatology and Nutrition and a professor of medicine, cell biology and physiology, and human genetics at the **University of Pittsburgh**.

A first step might be to identify other markers that correlate functionally with BCAAs in patients and mice and thus serve to improve the risk prediction.

"If tumors cause cachexia early, there must be tumor-originating factors that act either directly on muscle tissue or indirectly on the CNS," Olive said. "Identifying those could be truly revolutionary for assembling a more reliable diagnostic marker panel."

Wolpin told *SciBX* that the team is looking for tumor-secreted factors and investigating biological pathways in muscle that lead to wasting. "This work can provide more opportunities for early detection of

"The discovery of a precachexic state in which the muscle breakdown process has begun well before the onset of disease is a fundamentally stunning finding."
—Kenneth Olive, Columbia University

pancreatic cancer and new therapies for cancer cachexia," he said.

Similarly, Vander Heiden said, "We now are interested in the drivers. Finding tumor-secreted factors will involve some guesswork and unbiased biochemical approaches in patient cohorts and mouse models."

Whether early cancer growth causes muscle breakdown or whether released amino acids fuel cancer growth is not yet clear. "It is an open question why muscle breakdown is really occurring," said Vander Heiden. "Are branched-chain amino acids in particular doing something for the tumors, or is it a general supply in amino acids released by muscle tissue that promotes tumor growth? I would favor the latter possibility."

Olive agreed. "There are no causal relationships yet, and this needs to be explored," he said. "But there is a possibility that branched-chain amino acids might directly feed back on tumor properties. Whether tumors are mopping up amino acids from muscle could be examined in labeling experiments."

A patent application covering the finding has been filed by Dana-Farber, and the IP is available for licensing. The team is looking for industry partners to develop or sponsor further preclinical or clinical research.

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COMPANIES AND INSTITUTIONS MENTIONED

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Harvard Medical School, Boston, Mass.
Massachusetts Institute of Technology, Cambridge, Mass.
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Programmable sensitivity

By Stephen Parmley, Senior Writer

Since the GAIN Act was passed in 2012 to stimulate new antibiotic development, there has been more interest in the field but no game changers have emerged, and antibiotic resistance is still a major problem. Two independent teams believe they could have a far-reaching solution in the gene editing technology CRISPR, which allows them to tailor therapies to specific resistance mutations.[1,2]

The most promising application of the findings is the potential to resensitize resistant bacteria to standard antibiotics, but the researchers will have to develop better delivery methods to fully capitalize on the system's possibilities.

The teams, led by Luciano Marraffini at **The Rockefeller University** and Timothy Lu at the **Massachusetts Institute of Technology** (MIT), both employed the CRISPR (clustered, regularly interspaced short palindromic repeats)-Cas9 system to produce programmable therapies that target antibiotic resistance genes directly.

Marraffini is an assistant professor in the laboratory of bacteriology at Rockefeller University. Lu is an associate professor of biological engineering and electrical engineering and computer science at MIT. He is also cofounder of **Sample6**, a company using engineered phages to develop rapid point-of-care microbial diagnostics for food safety.

"Instead of the way that antibiotics currently work—which is targeting proteins most of the time—we asked: can we actually develop a sequence-specific antimicrobial that really targets things based on genetic information?" said Lu.

Although the Generating Antibiotic Incentives Now (GAIN) Act created a new impetus for innovation in antibiotic development, most efforts on the research front have remained old school by targeting pathways or proteins unique to bacteria. Those approaches yield broad-spectrum antibiotics that carry the liability of suppressing beneficial microorganisms as well as pathogenic ones.

In addition, most compounds in development target Gram-positive bacteria, and Gram-negative infections remain severely underaddressed. In both types of organisms, genetic resistance to antibiotics can arise through mutations in the bacteria's chromosomal DNA or by acquisition of foreign episomes that contain resistance genes. Episomes are replicating extrachromosomal DNA elements that either remain as intact entities inside the bacterium or integrate into the bacterial chromosome.

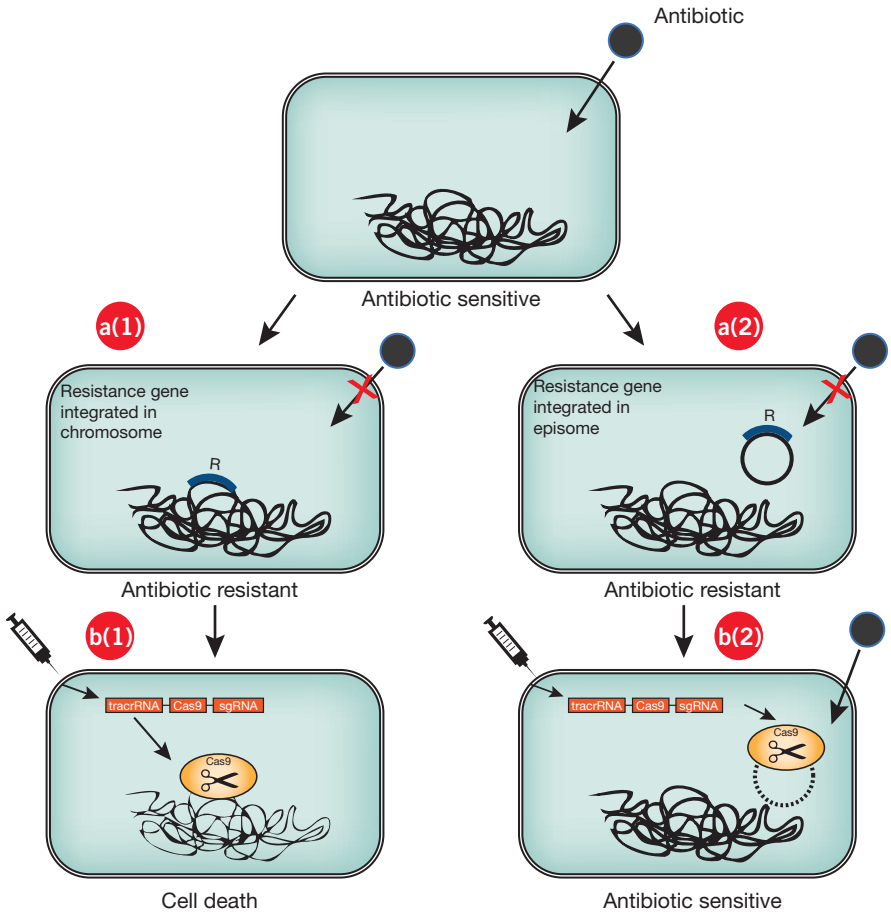

Figure 1. CRISPR's double-edged knife. Treatment of antibiotic-resistant bacteria with a clustered, regularly interspaced short palindromic repeats (CRISPR)-Cas9 agent yields different outcomes depending on whether acquired resistance genes are present on the bacterial chromosome or on an episome.

[**a(1)**] Antibiotic-sensitive bacteria can acquire resistance genes (R) from other bacteria that become chromosomally integrated and cause the bacteria to become resistant to the antibiotic.

[**b(1)**] Antibiotic-resistant bacteria can be selectively killed with a CRISPR-Cas9 agent that targets the chromosomally integrated resistance gene. Delivery of a gene cassette encoding a small *trans*-activating CRISPR RNA (tracrRNA), Cas9 and a single guide RNA (sgRNA) (orange boxes) into the cell results in expression and assembly of the CRISPR-Cas9 agent (yellow oval). This agent cleaves the targeted resistance gene, leading to chromosomal degradation and bacterial cell death.

[**a(2)**] Antibiotic-sensitive bacteria also can acquire episomes encoding resistance genes from other bacteria (R) to become antibiotic resistant.

[**b(2)**] These antibiotic-resistant bacteria can be selectively killed with a CRISPR-Cas9 agent that specifically targets a resistance gene in the episome. Delivery of the appropriate gene cassette results in expression and assembly of a CRISPR-Cas9 agent that cleaves the episomal resistance gene, leading to episomal degradation and restoring antibiotic sensitivity to the bacterium.

The CRISPR system involves using Cas9 nuclease to create double-stranded breaks in DNA and a sequence-specific guide RNA to direct Cas9 to the target sequence. By using CRIPSR to target the DNA rather than the resistance pathway, the two groups have developed techniques to attack resistance acquired through chromosomal mutations or episome acquisition that can be applied to Gram-positive or Gram-negative bacteria.

Where a resistance mutation is present on the chromosomal DNA, CRISPR-Cas9 cleaves the DNA at the site of the resistance gene, leading to chromosomal degradation and bacterial cell death. For bacteria that have acquired an episome containing a resistance gene, CRISPR-Cas9 can eliminate the episomal DNA and thus resensitize the organism to antibiotics (*see* **Figure 1, "CRISPR's double-edged knife"**).

Overcoming resistance

Both groups started by testing whether CRISPR-Cas9 could kill bacteria containing resistance genes in their chromosomes.

Lu's group created a CRISPR-Cas9 that targets a carbenicillin resistance gene of *Escherichia coli*—a Gram-negative strain. The researchers used a bacteriophage system to deliver the agent to *in vitro* cultures of *E. coli* containing a chromosomal carbenicillin resistance gene. The compound reduced bacterial viability 1,000-fold in the cultures and produced its peak bactericidal effect in 2–4 hours. Carbenicillin is a generic antibiotic in the carboxypenicillin group that is active against Gram-negative bacteria.

> **"Instead of the way that antibiotics currently work—which is targeting proteins most of the time—we asked: can we actually develop a sequence-specific antimicrobial that really targets things based on genetic information?"**
>
> ***—Timothy Lu, Massachusetts Institute of Technology***

In an *in vivo* model of systemic infection in wax moth larvae infected with a virulent strain of *E. coli*, the CRISPR-based therapy—directed against a key virulence factor gene—increased survival fourfold compared with a control bacteriophage.

Marraffini's team set out to program CRISPR to create an antibacterial that would selectively kill virulent bacteria but not affect the healthy host microbiota. The researchers designed a CRISPR-Cas9 agent to target a chromosomally integrated methicillin resistance gene in methicillin-resistant *Staphylococcus aureus* (MRSA) and used a bacteriophage delivery system to test it in a mixed culture containing equal parts MRSA and methicillin-sensitive *S. aureus*.

The percentage of methicillin-sensitive bacteria increased from 50% before treatment to 99.6% after treatment, which suggested that predominantly resistant organisms had been killed. A nonspecific control bacteriophage caused no significant change in the ratio.

In a mouse model of Gram-positive bacterial skin infection, a CRISPR-Cas9 bacteriophage targeting a kanamycin resistance gene decreased by fivefold the proportion of resistant bacteria in a mixed population of bacterial colonies on the skin.

Next, both studies used the technology to resensitize resistant bacteria to specific antibiotics.

The Rockefeller group created a Cas9-CRISPR bacteriophage that targeted an episomal tetracycline resistance gene. After treatment with bacteriophage, 99.99% of the cells became sensitive to tetracycline.

The MIT group used its carbenicillin resistance gene–targeted Cas9 bacteriophage to treat cultures of *E. coli* containing an episomal carbenicillin resistance gene. In this case, too, treatment with bacteriophage resulted in 99.9% of the cells gaining sensitivity to carbenicillin.

Results of both studies were published in *Nature Biotechnology*.

Delivery issues

Although the CRISPR approach was effective in selectively killing or resensitizing resistant bacteria, the bacteriophage-based delivery system provides both advantages and drawbacks for using the technology to develop antibacterials.

Both labs showed that their CRISPR-based antimicrobials killed pathogenic bacteria *in vitro* and *in vivo*, but the reductions were modest and were most likely limited by the number of cells successfully infected with the Cas9 bacteriophage.

The two studies showed at least a three-log reduction in pathogenic bacteria *in vitro*. However, the Rockefeller group saw only about one-log reduction *in vivo*, and the MIT group saw only a twofold protective effect *in vivo*. Typically, antibiotics reduce the counts of sensitive bacteria *in vivo* by greater than four logs and fully protect infected animals.

According to Marraffini, a modest effect might be enough. "We only need to help the immune system when it is overloaded, and studies have shown that in some cases reducing the bacterial pathogen count by only one log will be sufficient and the immune system clears the rest," he said.

Marraffini added that although the number of phage-susceptible bacterial strains amenable to this delivery system is relatively small, the CRISPR-based approach provides a selectivity advantage over other bacteriophage delivery systems because the latter kill pathogenic and beneficial bacteria indiscriminately.

Lu told *SciBX* that his team is developing a better delivery system by using rational engineering and high throughput screening to modify the bacteriophage and by exploring alternative delivery methods as well.

The use of the phage system to deliver CRISPR-Cas9 might also face difficulties in the clinic because clinicians are reluctant to give bacteriophage-based therapies orally or parenterally owing to the potential safety risk from contaminating endotoxins.

James Collins—an early developer of bacteriophage systems that sensitize cells to antibiotics—said that one option might be to start with other routes of administration. "You could use the phage in a topical application in conjunction with existing antibiotics," he said. "Or you could envision using the phage in an aerosolized fashion to go after lung infections." Collins is a professor of biomedical engineering at **Boston University**.

He added that although the two studies provide powerful proof of concept for the approach, key questions still have to be addressed, such as what level of penetrance into virulent bacterial populations can be achieved and how fast resistance could arise.

"Bacteria evolve quickly against any strategy we appear to be coming up with, so the issue is how quickly?" Collins said. "Bugs are quite good at evolving against bacteriophage and evolving ways to pump out antibiotics. What would be the signs to pick up that resistance is developing and needs to be controlled?"

Indeed, a key point raised at a hearing last month about antibiotic resistance held by the U.S. House of Representatives' Energy and Commerce Committee's Subcommittee on Health was the need for diagnostic technologies that can keep pace with the emergence of antibiotic-resistant bacteria (*see* **Diagnosing resistance, page 1**).

Rockefeller University and MIT have both filed patent applications covering their respective *Nature Biotechnology* studies, but neither Marraffini nor Lu disclosed the licensing status.

Parmley, S. *SciBX* 7(41); doi:10.1038/scibx.2014.1198
Published online Oct. 23, 2014

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   Contact: Timothy K. Lu, Massachusetts Institute of Technology, Cambridge, Mass.
   e-mail: timlu@mit.edu
2. Bikard, D. *et al. Nat. Biotechnol.*; published online Oct. 5, 2014; doi:10.1038/nbt.3043
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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
Autoimmune disease				
Osteoarthritis	Matrix metalloproteinase 13 (MMP13)	*In vitro* and rat studies identified selective MMP13 inhibitors that could be useful for treating osteoarthritis. An *in vitro* high throughput screen and SAR studies identified a carboxylic acid compound that selectively inhibited the osteoarthritis-associated enzyme MMP13 at an IC_{50} value of 3.9 picomolar. In a rat model of osteoarthritis, an orally available monosodium salt of the lead inhibitor decreased markers of cartilage damage compared with vehicle without causing overt toxicity. Next steps could include evaluating the salt of the lead inhibitor in additional preclinical osteoarthritis models. ***SciBX* 7(41); doi:10.1038/scibx.2014.1199** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Nara, H. *et al. J. Med. Chem.*; published online Sept. 29, 2014; doi:10.1021/jm500981k **Contact:** Hiroshi Nara, Takeda Pharmaceutical Co. Ltd., Kanagawa, Japan e-mail: hiroshi.nara@takeda.com
Cancer				
Brain cancer	Spermidine/spermine-N1-acetyltransferase 1 (SAT1; SSAT)	Studies in patients, mice and cell culture suggest inhibiting SAT1 could help overcome resistance to radiotherapy in glioblastoma multiforme (GBM). In patients with various brain tumors including GBM, elevated SAT1 expression was associated with decreased survival. In human GBM cell lines, shRNA knockdown of *SAT1* increased GBM cell sensitivity to ionizing radiation exposure compared with knockdown of a control gene. In mice injected with primary GBM cell lines, *SAT1* knockdown led to increased survival compared with knockdown of a control gene. Next steps include identifying therapeutic agents that can inhibit SAT1. ***SciBX* 7(41); doi:10.1038/scibx.2014.1200** **Published online Oct. 23, 2014**	Unpatented; unlicensed	Brett-Morris, A. *et al. Cancer Res.*; published online Oct. 2, 2014; doi:10.1158/0008-5472.CAN-14-1249 **Contact:** Scott M. Welford, Case Western Reserve University, Cleveland, Ohio e-mail: scott.welford@case.edu
Cancer	AT rich interactive domain 1A (ARID1A); ARID1B; breast cancer 1 early onset (BRCA1); BRCA2; K-Ras (KRAS); phosphoinositide 3-kinase (PI3K)	Genetic studies identified mutations in chromatin-remodeling genes that could be corrected to help treat carcinosarcomas. Whole-exome sequencing of 22 gynecological carcinosarcomal tumors and matching normal tissues from patients identified previously unknown mutations in *ARID1A*, *ARID1B* and other chromatin-remodeling genes. Sequencing also identified mutations in multiple genes—including *BRCA1*, *BRCA2*, *KRAS* and *PI3K*—encoding targets associated with other cancers but not previously considered targets in carcinosarcomas. Next steps could include investigating the gene mutations and their downstream effects to identify therapeutic targets for carcinosarcomas. ***SciBX* 7(41); doi:10.1038/scibx.2014.1201** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Jones, S. *et al. Nat. Commun.*; published online Sept. 19, 2014; doi:10.1038/ncomms6006 **Contact:** Victor E. Velculescu, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: velculescu@jhmi.edu **Contact:** Christoph Lengauer, Blueprint Medicines, Cambridge, Mass. e-mail: clengauer@blueprintmedicines.com

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	Dermcidin (DCD)	Cell culture studies suggest the *Serinicoccus*-derived natural compound seriniquinone could help treat cancer. A chemical screen of marine bacteria metabolites identified seriniquinone as an anticancer candidate that inhibited the growth of cancer cells at nanomolar concentrations. In melanoma cell lines, seriniquinone and its analogs upregulated DCD expression and increased both autophagy and apoptosis compared with no treatment. Next steps could include elucidating the mechanism of seriniquinone's anticancer activity and testing the molecule in mouse models of cancer. ***SciBX* 7(41); doi:10.1038/scibx.2014.1202** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Trzoss, L. *et al. Proc. Natl. Acad. Sci. USA*; published online Sept. 30, 2014; doi:10.1073/pnas.1410932111 **Contact:** William Fenical, University of California, San Diego, La Jolla, Calif. e-mail: wfenical@ucsd.edu **Contact:** James J. La Clair, same affiliation as above e-mail: jlaclair@ucsd.edu **Contact:** Letícia V. Costa-Lotufo, same affiliation as above e-mail: costalotufo@gmail.com
Cancer	Protein disulfide isomerase (PDI)	Cell culture studies suggest PDI inhibitors could sensitize cancers to etoposide chemotherapy. In a panel of cancer cell lines, micromolar concentrations of a PDI inhibitor plus subtoxic nanomolar concentrations of etoposide increased apoptosis compared with either agent alone. In cultured pancreatic tumors, the synergistic combination decreased tumor growth compared with either agent alone. Next steps could include testing PDI inhibitors with other chemotherapy agents and evaluating combination approaches in animal cancer models. ***SciBX* 7(41); doi:10.1038/scibx.2014.1203** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Eirich, J. *et al. Angew. Chem. Int. Ed.*; published online Sept. 26, 2014; doi:10.1002/anie.201406577 **Contact:** Stephan A. Sieber, Technical University Munich, Garching, Germany e-mail: stephan.sieber@tum.de **Contact:** Angelika M. Vollmar, Ludwig Maximilian University of Munich, Munich, Germany e-mail: angelika.vollmar@cup.uni-muenchen.de
Chronic lymphocytic leukemia (CLL)	Lymphotoxin-α (LTα); lymphotoxin-β receptor (LTBR); chemokine CXC motif ligand 13 (CXCL13); CXC chemokine receptor 5 (CXCR5)	Mouse studies suggesting inhibiting LTα-LTBR signaling or CXCL13-CXCR5 signaling could help treat CLL. In a mouse model of CLL, Ltα secreted by leukemia cells interacted with Ltbr in the stroma to induce stromal cell production of Cxcl13, which then bound to Cxcr5 on the leukemia cells. In the mouse model, knocking out *Cxcr5* or *Ltα* or treatment with an LTBR decoy protein led to decreased tumor burden or slowed disease progression compared with no genetic alteration or treatment with an inactive control protein. Next steps could include testing inhibitors of CXCL13, CXCR5 or LTBR in the CLL models. ***SciBX* 7(41); doi:10.1038/scibx.2014.1204** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Heinig, K. *et al. Cancer Discov.*; published online Sept. 24, 2014; doi:10.1158/2159-8290.CD-14-0096 **Contact:** Uta E. Höpken, Max Delbrueck Center for Molecular Medicine, Berlin, Germany e-mail: uhoepken@mdc-berlin.de
Kidney cancer	L-2-hydroxyglutarate dehydrogenase (L2HGDH)	*In vitro* studies suggest agonizing L2HGDH could help treat renal cell carcinoma (RCC). In primary RCC tumor samples, levels of L2HGDH were lower and levels of its substrate, L-2-hydroxyglutarate (L-2-HG), were higher than in normal kidney tissue. In RCC cell lines, lentiviral-mediated expression of *L2HGDH* decreased L-2-HG levels, cancer cell proliferation and colony formation compared with no *L2HGDH* expression. Next steps could include identifying strategies to augment L2HGDH levels or activity in patients with cancer. ***SciBX* 7(41); doi:10.1038/scibx.2014.1205** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Shim, E.-H. *et al. Cancer Discov.*; published online Sept. 2, 2014; doi:10.1158/2159-8290.CD-13-0696 **Contact:** Sunil Sudarshan, The University of Alabama at Birmingham, Birmingham, Ala. e-mail: sudarshan@uab.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Myelodysplastic syndrome (MDS)	Casein kinase 1 (CSNK1; CKI)	*In vitro* and mouse studies suggest inhibiting CSNK1 could help treat MDS. Deletion of the long arm of chromosome 5q, which contains *CSNK1*, is associated with MDS. In lethally irradiated mice, transplantation of *Csnk1*-deficient hematopoietic stem cells (HSCs) led to higher numbers of circulating HSCs and a greater self-renewing capacity in those HSCs than transplantation of wild-type HSCs. In mice receiving transplants of *Csnk1*-deficient HSCs, pretreatment of the HSCs with a CSNK1 inhibitor led to lower levels of *Csnk1*-deficient HSCs and myeloid progenitor cells in bone marrow and circulation compared with vehicle pretreatment. Next steps could include identifying a selective CSNK1 inhibitor to treat patients with MDS with 5q deletions. ***SciBX* 7(41); doi:10.1038/scibx.2014.1206** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Schneider, R.K. *et al. Cancer Cell*; published online Sept. 18, 2014; doi:10.1016/j.ccr.2014.08.001 **Contact:** Benjamin L. Ebert, Harvard Medical School, Boston, Mass. e-mail: bebert@partners.org
Endocrine/metabolic disease				
Diabetes	Not applicable	Mouse and *in vitro* studies suggest an ethanolamine salt of the antihelminthic drug niclosamide could be useful for treating type 2 diabetes. In mitochondria isolated from mouse livers, niclosamide ethanolamine induced oxygen consumption, a hallmark of mitochondrial uncoupling. In wild-type mice fed a high-fat diet, dietary supplementation with niclosamide ethanolamine increased energy expenditure compared with no supplementation. In a mouse model of diet-induced diabetes, dietary supplementation with niclosamide ethanolamine decreased fasting blood glucose levels and increased glucose tolerance and insulin sensitivity compared with no supplementation. Mito BioPharm LLC, a startup cofounded by the senior investigator on the study, is planning IND-enabling studies of niclosamide ethanolamine. ***SciBX* 7(41); doi:10.1038/scibx.2014.1207** **Published online Oct. 23, 2014**	Patent pending; licensed to Mito BioPharm	Tao, H. *et al. Nat. Med.*; published online Oct. 5, 2014; doi:10.1038/nm.3699 **Contact:** Shengkan Jin, Rutgers Robert Wood Johnson Medical School, Piscataway, N.J. e-mail: victor.jin@rutgers.edu
Diabetes; obesity	G protein–coupled receptor 119 (GPR119)	*In vitro* and mouse studies suggest gordonoside F could help treat obesity and diabetes. In a human cell line expressing human GPR119, gordonoside F, a steroid glycoside derived from the *Hoodia gordonii* herb, activated the receptor. In normal rat islet cells, but not in *Gpr119*-deficient islets, gordonoside F increased glucose-stimulated insulin secretion compared with vehicle. In wild-type mice, but not in *Grp119*-deficient mice, gordonoside F increased glucose tolerance and decreased food intake. Next steps include making structural modifications to gordonoside F to improve potency. CymaBay Therapeutics Inc. has the GPR119 agonist MBX-2982 in Phase II testing to treat diabetes. Daiichi Sankyo Co. Ltd. has the GPR119 agonist DS-8500 in Phase II testing to treat diabetes. At least three other companies have GPR119 agonists in Phase I or earlier testing. ***SciBX* 7(41); doi:10.1038/scibx.2014.1208** **Published online Oct. 23, 2014**	Patent application filed; available for licensing	Zhang, S. *et al. Proc. Natl. Acad. Sci. USA*; published online Sept. 22, 2014; doi:10.1073/pnas.1324130111 **Contact:** Xin Xie, Shanghai Institute of Materia Medica, Shanghai, China e-mail: xxie@simm.ac.cn

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Infertility	Neurokinin 3 receptor (TACR3; NK3R); gonadotropin-releasing hormone (GnRH)	*In vitro* and goat studies identified peptidomimetic NK3R agonists that could help treat infertility. Senktide, a peptide agonist of NK3R, upregulates NK3R-GnRH signaling to treat infertility in animal models but is prone to enzymatic degradation. Chemical synthesis and *in vitro* testing of senktide peptidomimetics identified a lead compound that exhibited greater resistance to proteolytic degradation than senktide while retaining comparable agonistic activity toward NK3R. In ovariectomized goats, injection of the lead compound induced GnRH production with a longer duration of effect than senktide injection. Next steps could include studies to assess how the lead compound affects pregnancy rates in animals. ***SciBX* 7(41); doi:10.1038/scibx.2014.1209** **Published online Oct. 23, 2014**	Patent and licensing status unavailable	Misu, R. *et al. J. Med. Chem.*; published online Sept. 23, 2014; doi:10.1021/jm500771w **Contact:** Nobutaka Fujii, Kyoto University, Kyoto, Japan e-mail: nfujii@pharm.kyoto-u.ac.jp **Contact:** Satoshi Ohkura, same affiliation as above e-mail: soishi@pharm.kyoto-u.ac.jp
Infectious disease				
Bacterial infection	Interleukin-1β (IL-1β)	Mouse studies suggest altering the composition of the intestinal microbiome could help prevent osteomyelitis (bone infection). In a mouse model of hind limb osteomyelitis, a high-fat diet decreased signs of inflammatory bone disease, hind paw levels of proinflammatory Il-1β and intestinal levels of *Prevotella*—a genus of bacteria that causes some cases of osteomyelitis—compared with a low-fat diet. In models fed the low-fat diet, oral gavage with fecal microbiota from mice fed the high-fat diet decreased *Prevotella* levels and signs of osteomyelitis compared with oral gavage using vehicle. Next steps include identifying the specific bacterial communities and microbial-encoded metabolites that are responsible for promoting protection against inflammatory bone disease. ***SciBX* 7(41); doi:10.1038/scibx.2014.1210** **Published online Oct. 23, 2014**	Unpatented; licensing status not applicable	Lukens, J.R. *et al. Nature*; published online Sept. 28, 2014; doi:10.1038/nature13788 **Contact:** Thirumala-Devi Kanneganti, St. Jude Children's Research Hospital, Memphis, Tenn. e-mail: thirumala-devi.kanneganti@stjude.org
Inflammation				
Inflammation	Transient receptor potential vanilloid 1 (TRPV1; VR1)	*In vitro* and mouse studies suggest antagonizing TRPV1 could decrease inflammation in colitis and inflammatory bowel disease (IBD). In mouse or human primary $CD4^+$ T cells, *TRPV1* knockout or treatment with a TRPV1 antagonist decreased inflammatory cytokine production after T cell receptor activation compared with wild-type *TRPV1* expression or vehicle treatment. In a mouse model of $Cd4^+$ T cell–mediated colitis, *Trpv1* knockout or treatment with a TRPV1 antagonist decreased colonic inflammation and production of T cell–derived inflammatory cytokines compared with wild-type *Trpv1* expression or vehicle treatment. Next steps include additional preclinical studies to evaluate the potential of TRPV1 inhibition in autoimmune and inflammatory diseases. At least five companies have TRPV1 inhibitors in Phase II or earlier testing to treat various conditions including autoimmune and inflammatory diseases. ***SciBX* 7(41); doi:10.1038/scibx.2014.1211** **Published online Oct. 23, 2014**	Patent application filed; available for licensing	Bertin, S. *et al. Nat. Immunol.*; published online Oct. 5, 2014; doi:10.1038/ni.3009 **Contact:** Eyal Raz, University of California, San Diego, La Jolla, Calif. e-mail: eraz@ucsd.edu **Contact:** Wilfred A. Jefferies, The University of British Columbia, British Columbia, Vancouver, Canada e-mail: wilf@msl.ubc.ca

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			
Label-free electrochemical detection of methyltransferases	A label-free electrochemical system for detecting methyltransferases could help distinguish tumor tissue from normal tissue without the use of radioactive or fluorescent probes. The multiplexed detection system used a substrate plate containing a 15-electrode array and a complementary patterning and detection plate containing another 15-electrode array for high-sensitivity, selective measurement of DNA (cytosine-5-)-methyltransferase 1 (DNMT1) activity. In cell culture lysates prepared from biopsies from patients with colorectal carcinoma, the system detected higher levels of DNMT1 activity in carcinoma tissue than in the surrounding normal tissue. Next steps include testing whether incorporation of microfluidics technology into the system could decrease the sample sizes needed for detection. ***SciBX*** **7(41); doi:10.1038/scibx.2014.1212** **Published online Oct. 23, 2014**	Patent pending; available for licensing	Furst, A.L. *et al. Proc. Natl. Acad. Sci. USA*; published online Oct. 6, 2014; doi:10.1073/pnas.1417351111 **Contact:** Jacqueline K. Barton, California Institute of Technology, Pasadena, Calif. e-mail: jkbarton@caltech.edu
Disease models			
Genetically defined triple-knockout mouse model of high-grade superficial bladder cancer	A triple-knockout mouse model of bladder cancer could be useful for studying disease biology and evaluating new candidate therapies for high-grade superficial bladder cancer. Adult mice with conditional deletion of *retinoblastoma 1* (*Rb1*), *retinoblastoma-like 2* (*Rbl2*; *p130*) and *Rbl1* (*p107*) developed lesions in the bladder lumen with histological features that recapitulated high-grade, nonmuscle-invasive carcinoma. Genomic analysis of tumor samples from the models and from patients identified an RB-E2F-enhancer of zeste homolog 2 (EZH2) signaling axis that drove cancer development. Next steps include evaluating inhibitors of EZH2 signaling in the mouse model. ***SciBX*** **7(41); doi:10.1038/scibx.2014.1213** **Published online Oct. 23, 2014**	Unpatented; model available under materials transfer agreement	Santos, M. *et al. Cancer Res.*; published online Sept. 24, 2014; doi:10.1158/0008-5472.CAN-14-1218 **Contact:** Jesus M. Paramio, The Research Center for Energy, Environment and Technology (CIEMAT), Madrid, Spain e-mail: jesusm.paramio@ciemat.es
Nonhuman primate model of β-amyloid (Aβ)-induced Alzheimer's disease (AD)	Nonhuman primates receiving intracerebroventricular injections of Aβ oligomers could be useful as models for evaluating new treatments for AD. Brains of nonhuman primates that received Aβ oligomer injections to the lateral ventricle showed hallmarks of human AD, including astrocyte and microglia activation, synaptic loss, hyperphosphorylation of microtubule-associated protein-τ (MAPT; tau; FTDP-17) and neurofibrillary tangle formation. Next steps include testing whether agents known to have neuroprotective properties in rodents can prevent the AD-like pathology in the brains of the Aβ oligomer–injected nonhuman primates. ***SciBX*** **7(41); doi:10.1038/scibx.2014.1214** **Published online Oct. 23, 2014**	Unpatented; licensing status not applicable	Forny-Germano, L. *et al. J. Neurosci.*; published online Oct. 8, 2014; doi:10.1523/JNEUROSCI.1353-14.2014 **Contact:** Fernanda G. De Felice, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil e-mail: felice@bioqmed.ufrj.br **Contact:** Douglas P. Munoz, Queen's University, Kingston, Ontario, Canada e-mail: doug.munoz@queensu.ca
Drug delivery			
Cytosolic delivery of antibody mimics using anthrax toxin protective antigen (PA) and the N-terminal domain (LF_N) of the anthrax toxin lethal factor (LF)	Cell culture studies suggest the PA/LF_N system could enable cytosolic delivery of small antibody mimics to inhibit intracellular oncoproteins and help treat cancer. The delivery system uses PA to enable the delivery of LF_N-conjugated molecules into cells. In a human leukemia cell line, a BCR-ABL tyrosine kinase–targeted small antibody mimic conjugated to LF_N inhibited intracellular BCR-ABL kinase activity versus a nonfunctional conjugate and induced apoptosis. Next steps include developing a cleavable linker to allow bioactive cargo to be released from the carrier upon entry into the cytosol and testing the system in animal cancer models. ***SciBX*** **7(41); doi:10.1038/scibx.2014.1215** **Published online Oct. 23, 2014**	Patent application filed; available for licensing	Liao, X. *et al. ChemBioChem*; published online Sept. 22, 2014; doi:10.1002/cbic.201402290 **Contact:** Bradley L. Pentelute, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: blp@mit.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Hydrogel delivery system for controlled delivery of bupivacaine	A hydrogel delivery system could enable controlled delivery of the generic anesthetic bupivacaine with few side effects. The delivery system uses chitosan-coated poly(lactic-*co*-glycolic acid) (PLGA) microparticles loaded with bupivacaine and embedded within pluronic F-127, a thermoresponsive gel. *In vitro*, the hydrogel-embedded microparticles released their bupivacaine cargo in a controlled manner over seven days. *In vitro*, the chitosan-coated microparticles lacked cytotoxicity against bone marrow mesenchymal stem cells and caused less proinflammatory cytokine release from macrophages than uncoated PLGA microparticles. Next steps include GMP manufacturing of the hydrogel delivery system and evaluation in large-animal models of pain. ***SciBX* 7(41); doi:10.1038/scibx.2014.1216** **Published online Oct. 23, 2014**	Patent application filed; unavailable for licensing	Taraballi, F. *et al. J. Pharm. Sci.*; published online Sept. 29, 2014; doi:10.1002/jps.24190 **Contact:** Ennio Tasciotti, Houston Methodist Research Institute, Houston, Texas e-mail: etasciotti@houstonmethodist.org
Drug platforms			
Clustered, regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing for designing antibiotic, RNA-guided nucleases	Mouse, moth and cell culture studies suggest CRISPR-Cas9 genome editing could be used to develop new treatments against antibiotic-resistant bacterial infections. CRISPR-Cas9 genome editing was used to design RNA-guided nucleases that target antibiotic resistance and virulence factor genes in bacteria. In culture, bacteriophage- or bacterial conjugation–mediated delivery of these RNA-guided nucleases resulted in selective killing of targeted bacteria. In wax moth larvae infected with chloramphenicol-resistant bacteria, bacteriophage-mediated delivery of these nucleases led to improved survival versus that seen with control bacteriophage. In a mouse model of bacterial skin infection, bacteriophage-mediated delivery of these nucleases decreased the proportion of targeted bacteria compared with untargeted bacteria. Next steps include scaling the technology to target other pathogens, putting the RNA-guided nucleases into bacteriophage systems with broad host range and testing the technology in other *in vivo* models (*see* **Programmable sensitivity, page 8**). ***SciBX* 7(41); doi:10.1038/scibx.2014.1217** **Published online Oct. 23, 2014**	Patent application filed for findings from both studies; licensing status undisclosed	Citorik, R.J. *et al. Nat. Biotechnol.*; published online Sept. 21, 2014; doi:10.1038/nbt.3011 **Contact:** Timothy K. Lu, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: timlu@mit.edu Bikard, D. *et al. Nat. Biotechnol.*; published online Oct. 5, 2014; doi:10.1038/nbt.3043 **Contact:** Luciano A. Marraffini, The Rockefeller University, New York, N.Y. e-mail: marraffini@rockefeller.edu **Contact:** David Bikard, Pasteur Institute, Paris, France e-mail: david.bikard@pasteur.fr
DEP domain containing 6 (DEPTOR; DEPDC6) inhibition to enhance differentiation of embryonic stem (ES) cells	Cell culture studies suggest DEPTOR inhibition could help promote *in vitro* differentiation of ES cells into desired cell types. In mouse ES cells, shRNA against *Deptor* decreased markers of pluripotency and increased markers of endodermal or ectodermal differentiation compared with control shRNA. Next steps include characterizing the effects of DEPTOR inhibition on differentiation of human ES cells into specific cell types. ***SciBX* 7(41); doi:10.1038/scibx.2014.1218** **Published online Oct. 23, 2014**	Patent and licensing status undisclosed	Agrawal, P. *et al. J. Biol. Chem.*; published online Sept. 25, 2014; doi:10.1074/jbc.M114.565838 **Contact:** Robert E. Hughes, Buck Institute, Novato, Calif. e-mail: rhughes@buckinstitute.org **Contact:** Deepak A. Lamba, University of Washington, Seattle, Wash. e-mail: dlamba@buckinstitute.org
Prevention of immunogenicity to therapeutic proteins by co-treatment with *O*-phospho-L-serine (OPLS)	Cell culture and mouse studies suggest OPLS could prevent immunogenicity against protein therapeutics. In a mouse model of hemophilia A, pretreatment with factor VIII plus OPLS decreased the antibody response induced by a subsequent factor VIII challenge compared with factor VIII plus dexamethasone pretreatment. In hemophilia A model mice, injection with dendritic cells cultured with factor VIII plus OPLS resulted in lower serum levels of anti–factor VIII antibodies than injection of dendritic cells cultured with factor VIII alone. Next steps include studies to assess OPLS in combination with other therapeutic proteins and clinical testing. OPLS is a research compound. ***SciBX* 7(41); doi:10.1038/scibx.2014.1219** **Published online Oct. 23, 2014**	Patented; available for licensing and partnering	Fathallah, A.M. *et al. J. Pharm. Sci.*; published online Sept. 29, 2014; doi:10.1002/jps.24173 **Contact:** Sathy V. Balu-Iyer, University at Buffalo, Buffalo, N.Y. e-mail: svb@buffalo.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Imaging			
Dual-modality immuno–single-photon emission computed tomography (immuno-SPECT) and near-infrared fluorescence (NIRF) imaging probes to guide prostate cancer surgery	A dual-modality probe called RDC018 that enables imaging with immuno-SPECT and NIRF could help guide surgical resection of prostate tumors. RDC018 is a tetrapeptide conjugated to an NIR fluorophore and labeled with the radionuclide ^{111}In. In mouse xenograft models of metastatic prostate cancer, injection of a bispecific antibody that binds tumor cells and RDC018 followed by injection of RDC018 enabled imaging of the primary tumor and metastatic lesions with immuno-SPECT and NIRF imaging. In the mouse xenograft model, the approach also enabled image-guided surgical resection of metastatic tumor nodules in bone. Next steps could include evaluating RDC018 for dual-modality imaging in additional tumor models. *SciBX* 7(41); doi:10.1038/scibx.2014.1220 Published online Oct. 23, 2014	Patent and licensing status unavailable	Lütje, S. *et al. Cancer Res.*; published online Sept. 24, 2014; doi:10.1158/0008-5472.CAN-14-0594 **Contact:** Susanne Lütje, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands e-mail: susanne.lutje@radboudumc.nl
Markers			
Seven-antibody composite biomarker panel to predict recurrent focal segmental glomerulosclerosis (rFSGS) after kidney transplantation	Studies in human samples suggest a signature of seven pathogenic antibodies could help predict rFSGS after kidney transplantation, which can lead to graft rejection. In patient serum samples taken before kidney transplantation, an ELISA for a panel of seven antibodies predicted rFSGS with 92% accuracy, with anti-CD40 antibodies having the strongest predictive power. In mice, CD40-blocking antibodies prevented proteinuria and rFSGS-associated symptoms induced by injection of an autoantibody isolated from patients. Next steps include validating the biomarker panel in a prospective clinical trial in kidney transplant recipients. *SciBX* 7(41); doi:10.1038/scibx.2014.1221 Published online Oct. 23, 2014	Patent application filed covering use of the antibody panel and new targets for drug design and therapy for rFSGS; available for licensing	Delville, M. *et al. Sci. Transl. Med.*; published online Oct. 1, 2014; doi:10.1126/scitranslmed.3008538 **Contact:** Minnie M. Sarwal, University of California, San Francisco, Calif. e-mail: minnie.sarwal@ucsf.edu **Contact:** Jochen Reiser, Rush University Medical Center, Chicago, Ill. e-mail: jochen_reiser@rush.edu
Single-cell immune signatures from mass cytometry to predict clinical recovery from surgery	Single-cell immune signatures generated via mass cytometry could help predict recovery outcomes in patients following surgery. In whole-blood samples from 26 patients taken after primary hip arthroplasty, mass cytometry showed that the signal transducer and activator of transcription 3 (STAT3), cAMP responsive element binding protein 1 (CREB1; CREB) and NF-κB pathways are differentially activated after surgery compared with presurgical baselines. The results were used to define immune signatures that accounted for 40%–60% of the observed interpatient variability in recovery from fatigue, functional hip impairment and pain. Next steps could include validating the immune signatures in larger patient cohorts. *SciBX* 7(41); doi:10.1038/scibx.2014.1222 Published online Oct. 23, 2014	Patent and licensing status unavailable	Gaudillière, B. *et al. Sci. Transl. Med.*; published online Sept. 24, 2014; doi:10.1126/scitranslmed.3009701 **Contact:** Garry P. Nolan, Stanford University School of Medicine, Stanford, Calif. e-mail: gnolan@stanford.edu **Contact:** Martin S. Angst, same affiliation as above e-mail: ang@stanford.edu **Contact:** Brice Gaudillière, same affiliation as above e-mail: gbrice@stanford.edu

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Targets and compounds