

FROM THE MAKERS OF **BioCentury** and **nature** 

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# 2012 gets a new deal

### By Kai-Jye Lou, Staff Writer

*SciBX*'s second annual comprehensive analysis of public-private partnerships and early stage venture financing activity reveals that government institutions and organizations are taking a significant role in forming and funding partnerships, and California-based companies are leading the way in public-private partnership activity. On the financing front, biotech startups in the San Francisco Bay Area took in the lion's share of early stage venture dollars despite a year-over-year decline in worldwide early stage venture investments in biotechs.

The specific business areas that public-private partnerships (PPPs) focused on in 2012 closely mirrored 2011, with cancer taking the top spot both years (*see* Figure 1, "Business areas covered by 2012 public-private partnerships"). PPPs in the infectious diseases space—the second most active area in 2011—moved down a spot to third and swapped positions with PPPs working in diagnostics and pharmacogenetics (*see* Figure 1.I and Figure 1.II).

Of the 68 PPPs in the diagnostics and pharmacogenetics space, 31 are pursuing cancer-related projects and 11 are working in infectious diseases.

For early stage venture financings by business area, cancer also took the top spot both years. Between the two years, there was a noticeable drop in financing activity for companies working in endocrine and metabolic diseases (*see* Figure 1.III and Figure 1.IV).

Although business areas were relatively unchanged, a detailed look at the data shows regional shifts in PPP activity from 2011 to 2012. For example, Europe narrowed the gap in PPP activity with the U.S. The continent had 4% fewer PPPs than the U.S. in 2012. In 2011, Europe had 13% fewer PPPs (*see* Figure 2, "Regional breakdown of public-private partnerships in 2012 and 2011").

The European numbers were driven in part by a more than 60% uptick in disclosed PPPs involving U.K.-based companies and institutions, plus a jump in activity from the European Commission's Seventh Framework Program and Europe's **Innovative Medicines Initiative** (IMI).

Overall, the U.S. continued as the leader in the PPP landscape in 2012, with U.S. institutions and companies involved in about two-thirds of the 387 disclosed PPPs for the year.

#### Wild West

The 2012 U.S. regional data also highlight California's strong showing in both PPP activity and early stage venture financing (*see* Figure 3, "Further regional breakdown of public-private partnerships").

California companies and institutions combined to make the state the overall U.S. leader in PPP activity in 2012, followed by Massachusetts

## **COVER STORY**



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For the year, biotechs worldwide raised \$959.2 million in disclosed seed and series A financings, a 25% dip from the \$1.3 billion raised from such financings in 2011. Those numbers are likely to change somewhat as time passes because seed and series A financings often are not disclosed in the year they are completed.

California-based biotech startups founded last year were the clear leaders in seed and series A financings. These startups raked in at least \$158.9 million and accounted for four of the five largest series A rounds—a big improvement over 2011, when California-based startups raised \$60.5 million and accounted for only one of the top five series A rounds (*see* Table 1, "Largest series A financing rounds for companies founded in 2012").

Six of the seven 2012 California startups that received venture financing are based in the San Francisco Bay Area.

U.K.-based startups founded in 2012 came in a distant second, raising \$29.2 million in disclosed seed and series A financings for the year.

The largest series A round in 2012—\$49 million—went to Massachusetts-based **Histogenics Corp.**, a regenerative medicine company focused on cartilage repair. The company was founded in 2000, and the financing was designed to recapitalize the company following its acquisition of ProChon Biotech Ltd. in May 2011.

#### Across the pond

Regional data out of Europe showed that the U.K. was again the leader in PPP activity in 2012. U.K.-based companies signed on with 49 PPPs, and U.K.-based institutions were involved in 43 (*see* Figure 3.II).

Table 1. Largest series A financing rounds for companies founded in 2012. Four of the largest series A rounds for biotechs founded in 2012 went to companies based in the San Francisco Bay Area. Among the 23 companies founded in 2012 that disclosed venture financing that year, there were 16 U.S. companies, 3 based in the U.K. and 1 each based in Australia, Belgium, Russia and Switzerland. The largest series A round in 2012 was \$49 million and raised by Histogenics Corp., a Massachusetts-based regenerative medicine company that develops products for cartilage repair. The company was founded in 2000 and thus was excluded from this table. Source: BCIQ: BioCentury Online Intelligence

Company	Business area	Location	Amount raised (\$M)
Global Blood Therapeutics Inc.	Hematology	South San Francisco, Calif.	40.7
MyoKardia Inc.	Cardiovascular disease	San Francisco, Calif.	38.0
Allakos Inc.	Inflammation	San Francisco, Calif.	32.0
Labrys Biologics Inc.	Neurology	San Francisco, Calif.	31.0
Aclaris Therapeutics Inc.	Dermatology	Malvern, Pa.	21.0
BioMotiv LLC	Cancer; cardiovascular disease; neurology	Cleveland, Ohio	21.0

### **COVER STORY**

On the industry side, Europe also showed a shift in PPP activity, with the U.K.'s **GlaxoSmithKline plc** replacing France's **Sanofi** as the most active company in 2012. GSK disclosed 15 PPPs last year, and 7 of these involved the infectious diseases space, in contrast to just 2 of 6 in 2011.

Notable PPPs for GSK in 2012 include its participation in IMI's NewDrugs4BadBugs program to fund late-stage trials of pharma-backed antibiotic compounds<sup>1</sup> and a partnership with not-for-profit **The Centre for Drug Research and Development** to support research at academic institutions across Canada.<sup>2</sup>

In Asia, both Japan and China showed a pattern of PPP activity nearly identical to that of 2011, with Japanese companies and Chinese institutions showing the most activity (see Figure 3.III). Companies in Japan continued to partner with an even mix of institutions within the country's own borders and abroad, whereas Chinese institutions typically found partners abroad.

Shenzhen-based **BGI** was the single most active Asian entity on the PPP front, with nine disclosed genomics-related partnerships in 2012.

Singapore's **Agency for Science**, **Technology and Research** (A\*STAR) was the second most active entity in the Asia region, with a string of eight deals primarily related to the development of diagnostic tools and genomics research.

#### **Government relations**

Among public funders, governments stepped up their PPP activity in 2012, with the **NIH** and the European Commission taking the top two spots and IMI and A\*STAR tying in fifth place (*see* **Table 2**, "**Leaders in the number of public-private partnerships**"). China's BGI took the third spot, and the **Bill & Melinda Gates Foundation** took fourth.

PPPs receiving direct support from national governments and/or government-run institutions also accounted for the largest PPPs by value, with an aggregate budget expected to exceed \$1.6 billion over the next 7 years (*see* Table 3, "Top public-private partnerships in 2012 by value").

The largest of these involves more than a dozen biopharma companies partnering with national governments, nongovernmental organizations and international health organizations to eliminate or Table 2. Leaders in the number of public-private partnerships.The NIH and European Commission took the top positions withrespect to the number of disclosed public-private partnerships (PPPs)in 2012. The U.K.'s GlaxoSmithKline plc took the top spot on thecompany side, whereas Sanofi and Pfizer Inc. shared the secondspot. Excludes deals that only involved IP transfer.Source: BioCentury Archives

Institute	Number of PPPs
NIH (includes the National Cancer Institute and National Institute for Allergy and Infectious Diseases)	17
<b>European Commission</b> (via the Seventh Framework Program)	16
BGI	9
Bill & Melinda Gates Foundation	8
Agency for Science, Technology and Research (A*STAR)	7
Harvard University	7
Innovative Medicines Initiative	7
University College London	7
The University of Texas (includes The University of Texas Medical Branch, The University of Texas MD Anderson Cancer Center and The University of Texas Southwestern Medical Center)	7
Company	Number of PPPs
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	15
Pfizer Inc. (NYSE:PFE)	11
Sanofi (Euronext:SAN; NYSE:SNY)	11
AstraZeneca plc (LSE:AZN; NYSE:AZN)	10
Johnson & Johnson (NYSE:JNJ)	10

control 10 neglected tropical diseases by 2020. The partners expect to provide more than \$785 million to support R&D and strengthen drug distribution and implementation programs.

In contrast, the top 5 PPPs without direct support from government agencies have an aggregate budget of about \$490 million.





### **COVER STORY**

**Table 3. Top public-private partnerships in 2012 by value.** Two of the five largest 2012 public-private partnerships (PPPs) without direct government support were valued at \$100 million or more. PPPs that include grants, awards and/or other types of direct funding support from national governments and government institutions are included in the 2012 list but ranked separately from those without such support. Only 128 of the 387 PPPs reported in 2012 had disclosed dollar amounts. Excludes deals that only involved IP transfer. *Source: BioCentury Archives* 

		Disclosed
Companies	Institutions	(\$M)
PPPs without direct support from national governments and/or governm	ent institutions	
Aeras	Bill & Melinda Gates Foundation	220
BioMotiv LLC	University Hospitals	100
Merck & Co. Inc. (NYSE:MRK)	California Institute for Biomedical Research	90
Pfizer Inc. (NYSE:PFE)	Cystic Fibrosis Foundation	58
Shire plc (LSE:SHP; NASDAQ:SHPG)	Fondazione Telethon	22
PPPs with direct support from national governments and/or government	institutions	
Abbott Laboratories (NYSE:ABT); AstraZeneca plc (LSE:AZN; NYSE:AZN); Bayer	Bill & Melinda Gates Foundation; Drugs for Neglected	>785
AG (Xetra:BAYN); Becton Dickinson and Co. (NYSE:BDX); Bristol-Myers Squibb	Diseases initiative; Lions Clubs International	
Co. (NYSE:BMY); Eisai Co. Ltd. (Tokyo:4523; Osaka:4523); Gilead Sciences Inc.	Foundation; Mundo Sano; The Children's Investment	
(NASDAQ:GILD); GlaxoSmithKline plc (LSE:GSK; NYSE:GSK); Johnson &	Fund Foundation; The World Bank; United Arab	
Johnson (NYSE:JNJ); Merck & Co.; Merck KGaA (Xetra:MRK); Novartis AG	Emirates government; U.K. government; U.S. government;	
(NYSE:NVS; SIX:NOVN); Pfizer; Sanofi (Euronext:SAN; NYSE:SNY)	World Health Organization	
Emergent BioSolutions Inc. (NYSE:EBS); GlaxoSmithKline; Kalon Biotherapeutics	The Texas A&M University System; U.S. Department of	400
LLC; Lonza Group Ltd. (SIX:LONN); Novartis	Health and Human Services	
AstraZeneca; GlaxoSmithKline	Innovative Medicines Initiative	281.6 <sup>A</sup>
None yet	U.S. Department of Defense; U.S. Department of	108
	Veterans Affairs	
Not applicable	Duke University; NIH; The Scripps Research Institute	31 <sup>B</sup>
APoflecte current budget, but program is estimated to utilize up to £600 million (\$755.3 m	aillion) over the payt 7 years BPoflacts initial funding, but progr	am is

<sup>A</sup>Reflects current budget, but program is estimated to utilize up to €600 million (\$755.3 million) over the next 7 years. <sup>B</sup>Reflects initial funding, but program is estimated to receive \$186 million or more over the next 6 years.





**2012 and 2011.** Number of disclosed public-private partnerships (PPPs) worldwide in the respective year. Data includes double counting as some PPPs involve companies and/or institutions from more than one region. Values refer to the actual number of companies or institutions. Total number of disclosed PPPs is 387 for 2012 and 241 for 2011.

### Infectious influences

The disconnect that *SciBX* reported in 2011 between PPP activity and early stage venture financing for companies in the infectious diseases space<sup>3</sup> continued in 2012.

In aggregate, infectious disease companies raised \$70.9 million in disclosed seed or series A financing for 2012, down from \$107.1 million in 2011. Three companies did not disclose amounts raised. Only one infectious disease company founded in 2012—**Sequoia Vaccines Inc.**—received series A financing. The size of the round was not disclosed.

Nevertheless, the largest PPPs in 2012 by disclosed funding levels are in infectious diseases.

Of the top 5 such partnerships supported by national governments and/or government-run institutions, 4 are targeting infectious diseases and currently have an aggregate budget that is expected to exceed \$1.5 billion over the next 7 years.

The largest PPP without direct government support is between the not-for-profit biotech **Aeras** and the Bill & Melinda Gates Foundation. The foundation will provide the biotech with up to \$220 million in grants over 5 years to support the development of vaccines for tuberculosis.

The disconnect between PPP activity and venture financing may change going forward as the FDA implements legislation passed last year in the U.S.

# COVER STORY



**Figure 3. Further regional breakdown of public-private partnerships.** Regional breakdown of companies and institutions involved in public-private partnerships (PPPs) in the top five U.S. states (I), top five European countries (II) and top five Asian countries (III). Values refer to the actual number of companies or institutions. Data includes double counting as some PPPs involve companies and/or institutions from more than one state and/or country.

The Generating Antibiotics Incentives Now (GAIN) Act, which came into effect Oct. 1, 2012, provides added exclusivity for antibiotics and earmarks antibiotics for Priority Review.<sup>4,5</sup>

The act also mandates the creation of a pathogen-focused antibacterial drug development pathway and may remove some of the impediments to financing antibiotic drug development.

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#### REFERENCES

- 1. Cain, C. *BioCentury* **20**(23), A1–A4; June 4, 2012
- 2. Haas, M.J. SciBX 5(25); doi:10.1038/scibx.2012.645
- 3. Lou, K.-J. SciBX 5(3); doi:10.1038/scibx.2012.59

- 4. Usdin, S. BioCentury 20(47), A1-A7; Nov. 19, 2012
- 5. Cain, C. SciBX 5(46); doi:10.1038/scibx.2012.1198

### COMPANIES AND INSTITUTIONS MENTIONED

Aeras, Rockville, Md. Agency for Science, Technology and Research, Singapore BGI, Shenzhen, China Bill & Melinda Gates Foundation, Seattle, Wash. The Centre for Drug Research and Development, Vancouver, British Columbia, Canada GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K. Histogenics Corp., Waltham, Mass. Innovative Medicines Initiative, Brussels, Belgium National Institutes of Health, Bethesda, Md. Sanofi (Euronext:SAN; NYSE:SNY), Paris, France Sequoia Vaccines Inc., St. Louis, Mo.

### **TARGETS & MECHANISMS**

# Zelboraf dosing rework

By Tim Fulmer, Senior Writer

Novartis Institutes for BioMedical Research and University of California, San Francisco researchers have shown that simply altering the dosing regimen of Zelboraf is sufficient to prevent the development of drug resistance in mouse models of melanoma.<sup>1</sup> The researchers now plan to test an intermittent dosing regimen of Zelboraf in patients with melanoma.

Zelboraf vemurafenib (PLX4032) is a small molecule BRAF inhibitor marketed by **Roche** and **Daiichi Sankyo Co. Ltd.** to treat metastatic melanoma in patients expressing the V600E BRAF mutation. That mutation occurs in about 60% of melanomas and 7% of all cancers.<sup>2</sup> By inhibiting BRAF, Zelboraf blocks downstream activation of the MAPK pathway, which otherwise would trigger tumor cell proliferation.

About half of treated patients develop resistance to the drug in six to nine months. The mechanisms of Zelboraf resistance in tumors are still being worked out, although preclinical research has suggested multiple possible scenarios. These include the development of secondary mutations in BRAF, presence of activating mutations in downstream nodes of the BRAF pathway and activation of alternative cell survival pathways.<sup>3-5</sup>

To better understand the mechanisms of Zelboraf resistance, University of California,

San Francisco (UCSF) researchers collaborated with researchers at the Novartis Institutes for BioMedical Research (NIBR) to develop an animal model of BRAF inhibitor–resistant melanoma.

The team was led by Martin McMahon, professor of cancer biology at UCSF and co-leader of the Developmental Therapeutics Program and UCSF's Hellen Diller Family Comprehensive Cancer Center, and Darrin Stuart, senior research investigator at NIBR.

The first step was generating xenograft mice with subcutaneous melanoma tumors derived from a patient expressing the V600E BRAF mutation. The animals then were treated daily with Zelboraf. After about 56 days of dosing, drug-resistant tumors developed in 2 of 10 animals.

One of those tumors was then resected, subdivided and reimplanted into a new cohort of mice, which were treated twice daily with Zelboraf to generate a model of Zelboraf-resistant melanoma.

Immunoblot analysis of the mouse tumors showed that Zelborafresistant tumors had substantially higher levels of BRAF mRNA and BRAF protein than drug-sensitive tumors, suggesting high BRAF levels contributed to Zelboraf resistance.

Curiously, when Zelboraf was discontinued in mice with drugresistant melanomas, the tumors regressed within 10 days after drug withdrawal. That result suggested Zelboraf-resistant tumors were at a growth disadvantage in the absence of drug.

Thus, although continuous Zelboraf dosing might select for drugresistant cells, intermittent dosing could create a tumor environment disadvantageous to the development of drug-resistant cells. Indeed, mice continuously dosed developed lethal Zelboraf resistance within 100 days. In contrast, none of the mice on an intermittent dosing schedule developed drug resistance over the course of 200 days.

The authors concluded that intermittent dosing of Zelboraf "could serve to eliminate the fitness advantage of the resistant [melanoma] cells and delay the onset of drug-resistant disease."

The findings were published in Nature.

McMahon told *SciBX* that his lab will now collaborate with the UCSF Melanoma Center to test intermittent Zelboraf dosing in the clinic. He declined to provide a timeline for starting the study.

Zelboraf's label recommends a twice-daily 960 mg dose until disease progression or unacceptable toxicity occurs. The label advises against reducing the twice-daily dose below 480 mg.

McMahon said that the UCSF team also will look into whether the dosing findings in melanoma can be generalized to other cancers with mutated BRAF, such as lung and thyroid cancer.

NIBR will continue to explore alternative dosing schedules as a strategy

for overcoming drug resistance in patients with cancer, Stuart told *SciBX*. He declined to provide further details on therapeutic compounds being tested or specific cancer indications.

The mouse models developed in the *Nature* paper "provide a unique opportunity to study the dynamics of tumor cell populations and the evolution of resistance," added Stuart.

After Zelboraf, the most advanced BRAF inhibitor is **GlaxoSmithKline plc**'s dabrafenib (GSK2118436). The small molecule is in registration to treat advanced or metastatic melanoma and in Phase III testing to treat

solid tumors in patients with V600E BRAF mutations.

Last June, GSK presented Phase I/II data at the **American Society** of **Clinical Oncology** (ASCO) meeting showing that dabrafenib plus a MEK inhibitor prolonged progression-free survival and decreased secondary malignancies compared with Zelboraf or dabrafenib alone in patients with melanoma. MEK mediates downstream BRAF signaling.

GSK did not respond to requests for comment.

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#### REFERENCES

- Das Thakur, M. et al. Nature; published online Jan. 9, 2013; doi:10.1038/nature11814
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  Fielde M. F. Scheld, M. F. Scheld, M. C. 200, 200, 200, 2010 (2010)
- 2. Flaherty, K.T. et al. N. Engl. J. Med. 363, 809-819 (2010)
- 3. Nazarian, R. *et al. Nature* **468**, 973–977 (2010)
- 4. Wagle, N. et al. J. Clin. Oncol. 29, 3085–3096 (2011)
- 5. Jänne, P.A. et al. Nat. Rev. Drug Discov. 8, 709–723 (2009)

### COMPANIES AND INSTITUTIONS MENTIONED

American Society of Clinical Oncology, Alexandria, Va. Daiichi Sankyo Co. Ltd. (Tokyo:4568; Osaka:4568), Tokyo, Japan GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K. Novartis Institutes for BioMedical Research, Emeryville, Calif. Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland University of California, San Francisco, Calif.

"[Intermittent dosing of Zelboraf] could serve to eliminate the fitness advantage of the resistant [melanoma] cells and delay the onset of drug-resistant disease."

—Das Thakur et al., Novartis Institutes for BioMedical Research

### TOOLS

## ANALYSIS

# **Rejuvenating T cells**

### By Tracey Baas, Senior Editor

Two Japanese teams have used induced pluripotent stem cells to regenerate antigen-specific T cells in patients whose immune systems have been depleted.<sup>1,2</sup> **Megakaryon Corp.** is developing and commercializing the technology of one group to produce induced pluripotent stem cell-derived human T cells.

T cells play an important role in the host response to viral infections and cancer, but chronic exposure to viral or cancer antigens can drive T cells into an exhausted state in which they lose their effector function and capacity to proliferate.

Multiple groups have tried to expand patients' antigen-specific T cells *ex vivo* in order to boost levels of antigen-responsive T cells for adoptive immunotherapy to help patients mount a response to a virus or cancer.<sup>3</sup> However, these expanded T cells have not been effective due to loss of function.

The most successful studies occurred in patients with advanced melanoma treated with CD8<sup>+</sup> T cell adoptive immunotherapy, in which eradication of tumors correlated with increased presence of stem cell–like CD8<sup>+</sup> T cells.<sup>4</sup>

These results collectively suggest that using induced pluripotent stem (iPS) cell technology to generate large numbers of antigen-specific CD8<sup>+</sup> T cells would enhance the effects of adoptive immunotherapy.

Several groups have generated T cell-derived

iPS cells,<sup>5-8</sup> but not much is known about differentiating these cells back into antigen-specific T cells.

In the new studies, two Japanese groups used mature, antigenspecific CD8<sup>+</sup> T cells and reprogrammed them into iPS cells (*see* Figure 1, "Adoptive T cell-based immunotherapy strategies"). These iPS cells showed typical characteristics of pluripotency such as embryonic stem cell (ESC)-like morphology, capacity for teratoma formation, endogenous expression of the transcription factors used in reprogramming and disappearance of the T cell markers CD3 and CD8.

The Tokyo group, led by Hiromitsu Nakauchi, used mature HIV p27 (nef)-specific CD8<sup>+</sup> T cells obtained from a patient infected with HIV-1 to produce iPS cells. The other group, led by Hiroshi Kawamoto, used a melanoma patient–derived T cell line expressing the melanoma epitope melan-A (MLANA; MART1) to produce iPS cells.

Nakauchi is professor and director at the Center for Stem Cell Biology Regenerative Medicine at the Institute of Medical Science at **The University of Tokyo**. Kawamoto did the research when he was team leader at the laboratory for lymphocyte development at the **RIKEN Research Center for Allergy and Immunology**. He now is a professor of immunology at the Institute for Frontier Medical Sciences at **Kyoto University**.

Each group first showed that the antigen specificity encoded in the genomic DNA of the parent mature T cells was conserved in the reprogrammed iPS cells. The teams then differentiated their iPS cells into mature CD8<sup>+</sup> T cells by coculturing with mouse feeder cells.

The resultant CD8<sup>+</sup> T cells exhibited the same antigen specificity as the original T cells. CD8<sup>+</sup> T cells generated from the patient with HIV

showed antigen-specific killing activity against nef, whereas the CD8<sup>+</sup> T cells generated from the melanoma cell line showed antigen-specific killing activity against melan-A.

Nakauchi's team further showed that the redifferentiated T cells exhibited a high proliferating capacity and re-elongation of their telomeres, suggesting the cells were rejuvenated.

Both studies were published in Cell Stem Cell.

### Getting ready for the clinic

Both teams now plan to test the safety and efficacy of the regenerated antigen-specific human T cells in animal models of cancer or infectious diseases.

Kawamoto thinks the method will be best suited for treating metastatic cancers.

"Only immunotherapy will be capable of completely getting rid of metastatic cancers, while conventional methods such as surgery, chemotherapy or radiation cannot," he said.

> "I think they will next need to investigate the efficiency and the ease of generating antigenspecific T cells from a patient's blood and establish the safety of the therapy by using *in vivo* models," said Keiichi Fukuda, professor at the **Keio University School of Medicine**. His laboratory was one of the first to generate iPS cells from human circulating T cells.<sup>7</sup>

> Because contamination with nonhuman cellular products could potentially induce immunorejection, "for clinical applications, a xeno-free iPS cell culture condition is desirable," he noted.

Kawamoto agreed that the mouse feeder cells will need to be removed. "A method to induce human early hematopoietic progenitors from iPS cells by a feeder-free system has already been established by **Cellular Dynamics International Inc.**," he noted. "Our team is also developing a system that induces T cell progenitors from early hematopoietic progenitors in feeder-free condition based on a method that we reported in *Science* in 2010. By combining the two ideas, we are now setting up a complete feeder-free and xeno-free system." Kawamoto published a paper on differentiating murine hematopoietic progenitors into T cells.<sup>9</sup>

Cellular Dynamics markets the iCell platform for differentiating iPS cells into homogeneous, functional cell types. The company also markets human iPS cell-derived iCell cardiomyocytes, endothelial cells, neurons and hepatocytes, and has iCell hematopoietic cells in development.

Nakauchi thinks excluding xenogeneic materials may not be an absolute requirement for clinical trials. "We already have GMP-quality mouse feeder cells," he said.

He did say that because of tumorigenic risk from residual undifferentiated cells, "for the first-in-man clinical study, and to ensure safety, we are planning to install a suicide-gene system that can be activated by administration of a drug like anciclovir."

Previous studies have shown that by incorporating expression of herpes simplex virus thymidine kinase (HSV-tk) in ESCs, any teratoma formation can be eliminated by dosing with ganciclovir, a drug that targets HSV-tk.<sup>10,11</sup>

Kawamoto agreed that introducing a suicide gene was a good idea and said his group was thinking of doing the same with its system.

"Only immunotherapy will be capable of completely getting rid of metastatic cancers, while conventional methods such as surgery, chemotherapy or radiation cannot." *—Hiroshi Kawamoto, Kyoto University* 

# TOOLS

Figure 1. Adoptive T cell-based immunotherapy strate-

**gies.** Two teams reprogrammed antigen-specific CD8<sup>+</sup> T cells (orange cells) using Yamanaka factors and SV40 large T antigen to produce induced pluripotent stem (iPS) cells (purple cells). When cocultured with mouse feeder cells, the iPS cells differentiated into mature, antigen-specific CD8<sup>+</sup> T cells (blue cells). These cells could be expanded using the T cell growth factor IL-2, and the result was a source of antigen-specific T cells that showed longer telomeres, greater effector function and higher proliferating capacity than antigen-specific T cells that did not go through the iPS cell stage (orange cells).



Standard adoptive T cell-based immunotherapy involves

expanding CD8<sup>+</sup> T cells *ex vivo* with IL-2. The cells can be infused back into the patient to help them mount a response to a virus or cancer, but the approach has shown limited efficacy. (Figure based on Figure 1B in ref. 12.)

#### Stepping into stem cells

Megakaryon was founded in September 2011 by a group including **iPS Academia Japan Inc.** and **iCELL Inc.**, which are providing seed capital and are making their IP available to Megakaryon.

iPS Academia Japan manages patents and other IP related to iPS cell technology developed by Shinya Yamanaka of Kyoto University and colleagues. iCELL was established to manage the IP related to ESC and iPS cell technologies developed by Nakauchi and colleagues.

Yamanaka, who won last year's Nobel Prize in medicine for his discovery that mature cells can be reprogrammed to become pluripotent, serves on Megakaryon's scientific advisory board.

Megakaryon is headquartered in Japan and plans to establish a U.S. presence to support the development and commercialization of its ESCand iPS cell-derived products.

The company's most advanced project relates to iPS cell-derived platelets. Platelets obtained from blood donations cannot be frozen and have a shelf life of only five days after collection. Platelet shortages could be avoided by using human iPS cells to produce large quantities of platelets.

The company plans to start clinical studies within two years.

According to Genjiro Miwa, representative director of Megakaryon, "The pipeline also includes T cell and red blood cell projects, which are about two years behind the platelet project."

A patent application has been filed by the University of Tokyo for the work of the Nakauchi team. The RIKEN Research Center for Allergy and Immunology has patented the work by Kawamoto's team, and it is available for licensing.

Baas, T. *SciBX* 6(5); doi:10.1038/scibx.2013.107 Published online Feb. 7, 2013

#### REFERENCES

- Nishimura, T. *et al. Cell Stem Cell*; published online Jan. 3, 2013; doi:10.1016/j.stem.2012.11.002
  Contact: Hiromitsu Nakauchi, The University of Tokyo, Tokyo, Japan e-mail: nakauchi@ims.u-tokyo.ac.jp
  - e-mail: nakaucni@ims.u-tokyo.ac.jp
- Vizcardo, R. et al. Cell Stem Cell; published online Jan. 3, 2013; doi:10.1016/j.stem.2012.11.006
  Contact: Hiroshi Kawamoto, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan e-mail: kawamoto@rcai.riken.jp
- 3. June, C.H. J. Clin. Invest. 117, 1466–1476 (2007)
- 4. Rosenberg, S.A. Nat. Rev. Clin. Oncol. 8, 577-585 (2011)
- Brown, M.E. *et al. PLoS ONE* 5, e11373; published online June 29, 2010; doi:10.1371/journal.pone.0011373
- 6. Loh, Y.-H. et al. Cell Stem Cell 7, 15–19 (2010)
- 7. Seki, T. et al. Cell Stem Cell 7, 11–14 (2010)
- 8. Staerk, J. et al. Cell Stem Cell 7, 20-24 (2010)
- 9. Ikawa, T. et al. Science **329**, 93–96 (2010)
- 10. Rong, Z. et al. J. Biol. Chem. 287, 32338-32345 (2012)
- 11. Martz, L. SciBX 5(34); doi:10.1038/scibx.2012.890
- 12. Crompton, J.G. et al. Cell Stem Cell 12, 6-8 (2013)

#### COMPANIES AND INSTITUTIONS MENTIONED

Cellular Dynamics International Inc., Madison, Wis. iCELL Inc., Tokyo, Japan iPS Academia Japan Inc., Kyoto, Japan Keio University School of Medicine, Tokyo, Japan Kyoto University, Kyoto, Japan Megakaryon Corp., Tokyo, Japan RIKEN Research Center for Allergy and Immunology, Yokohama, Japan The University of Tokyo, Tokyo, Japan

# TOOLS

# Sequencing single B cells

### By Lauren Martz, Staff Writer

Researchers from **The University of Texas at Austin** have developed a high throughput method for sequencing individual B cell immunoglobulin receptors that keeps the link between their paired heavy and light variable chains intact.<sup>1</sup> The technique increases the efficiency of identifying disease biomarkers and designing therapeutic antibodies but requires modifications to increase throughput and accuracy.

B cell receptors help the immune system detect antigens and trigger antibody production. An individual harbors a repertoire of B cell receptors specific to a range of antigens to which the person has been previously exposed. Sequencing each B cell receptor, which consists of

a variable heavy  $(V_{\rm H})$  and a variable light  $(V_{\rm L})$  chain, in a patient could be useful for generating therapeutic vaccines and antibodies as well as for designing disease diagnostics.

But this would be a challenging task for most sequencing methods because of the need for large quantities of B cell DNA as starting material. Because each B cell has a unique B cell receptor, pooling B cells results in loss of the original  $V_{H}$ : $V_{L}$ pairing of individual B cell receptors.

Existing single cell-based sequencing approaches, such as single-cell PCR, could preempt this problem, but they typically are complex, expensive and time consuming.

Thus, George Georgiou and colleagues at the University of Texas at Austin set out to design a new single-cell approach that would preserve the original  $V_{\rm H}$ : $V_{\rm L}$  pairing while increasing throughput and lowering cost.

Georgiou is chair of engineering and professor of molecular genetics and microbiology at the university. The team also included coinvestigator Andrew Ellington, professor of chemistry and biochemistry at the University of Texas at Austin, and researchers from the **University of Houston, Charité University Medicine**, the **German Rheumatism Research Center** and **The University of Chicago**.

The team's strategy involved depositing B cells onto high-density well plates with the goal of having at most one cell per well with a 95% probability.

The plates were then covered and incubated with lysis solution and magnetic beads to release and capture the mRNA from the individual cells, respectively.

The team used RT-PCR followed by linkage PCR to amplify these genetic materials and sequenced the linked heavy and light chains' most variable regions using the MiSeq high throughput sequencing platform from **Illumina Inc.** 

To show that the method could sequence endogenous B cell receptor  $V_H:V_L$  pairs, the team sequenced three different patient B cell subpopulation repertoires: paired  $V_H$  and  $V_L$  sequences of immunoglobulin-

"Heavy-chain sequences are important because they can be used as markers for antibodies. These are useful for applications such as tracking B cells, monitoring for minimal residual disease or diagnostics. But having both chains together allows you to reconstruct monoclonal antibodies produced by the B cells to really get at their functions." *—Harlan Robins*,

Adaptive Biotechnologies Corp.

expressing B cells from a healthy donor, plasmablasts from a healthy donor immunized against tetanus toxin and peripheral memory B cells from a healthy donor after influenza immunization. The sequencing strategy identified thousands of paired  $V_{\rm H}$ : $V_{\rm L}$  sequences, including those of known controls that were included in cell samples.

To validate the identified sequences and show that the sequencing data are useful for identifying and designing therapeutic antibodies, Georgiou and colleagues expressed 10 of the paired sequences from the tetanus toxin-immunized donor as immunoglobulin in human embryonic kidney cells. The resulting antibodies all bound tetanus toxin, showing the sequences were paired accurately and suggesting they might be useful as functional antibodies.

Results were published in Nature Biotechnology.

### Sequencing applications

This single-cell method not only could help sequence B cells more accurately than traditional high throughput approaches but also should offer

cost and speed advantages over other singlecell sequencing methods.

There are, however, technological hurdles as additional modifications are needed to further increase throughput and decrease the error rate.

"Heavy-chain sequences are important because they can be used as markers for antibodies. These are useful for applications such as tracking B cells, monitoring for minimal residual disease or diagnostics. But having both chains together allows you to reconstruct monoclonal antibodies produced by the B cells to really get at their functions," said Harlan Robins, cofounder of **Adaptive Biotechnologies Corp.** 

For example, he said, "the paired sequences have the clinical potential of rapidly improving vaccine design and identifying neutralizing antibodies. Right now, identifying therapeutic

antibodies requires a hefty set of experiments. You can also create a set of mono- or polyclonal antibodies from a person who has had a response to a pathogenic challenge in order to develop a strong therapeutic."

Adaptive is developing immunoSEQ, a sequencing service capable of generating sequences of the immunoglobulin heavy chain and T cell receptors (TCRs).

The method developed by Georgiou's team could sequence thousands of paired heavy and light chain regions in 10 hours over 4 days for about \$550. Using single-cell PCR to sequence the same number of pairs would take more than 10 weeks and would cost \$25,000.

Guy Hermans, principal scientist at **Ablynx N.V.**, said that compared with single-cell sequencing approaches for pairing heavy and light chains, "the benefits of the technology include maintaining the correct pairing with high throughput using low reagent and labor costs."

Ablynx CSO Andreas Menrad added that the new method has the potential to process small samples, which could help identify T or B cells infiltrating solid tumors or inflammatory lesions.

Ablynx's Nanobody platform involves the design of antibody-derived therapeutics based on only the heavy chain of immunoglobulin.

"A huge advantage for this new approach is that the complicated part of the assay does not have to be done with live cells at the sites of the early steps," added Robins. "This is important because it could allow a pharma company doing a clinical trial to just put the samples on the plates at the trial sites and then run the sequencing experiments later."

One important drawback, he said, is that the throughput of the current approach is not that high. Thus, Robins thinks the logistics of scaling up the approach could be difficult.

"For this technology, it requires you to have far less than one cell per well. If wells with more than one cell result, you end up with false positives and incorrect cross-pairings. Therefore, there may be a limit to how quickly you can scale it up."

He suggested that more data could be obtained simply through preparation and sequencing of additional plates.

Robins said another issue is that the researchers used as active controls B cells that were spiked with immortalized IM-9 lymphoblast cells. Those control cells "look different than the other cells, and the assays tend to favor those cells, making the results look better than they are," he said. "It is possible that this could lead the researchers to get some false positives."

Hermans noted that once the sequences are determined, the group also does not have a method in place to screen the antibodies for antigen binding. "The bottleneck of the approach is the processing and validation of the downstream sequencing results," he said.

William Robinson, associate professor of medicine in the division of immunology and rheumatology at the **Stanford University School of Medicine** and cofounder of **Atreca Inc.**, told *SciBX*, "Incomplete variable region sequences are obtained, and thus complete antibodies are not characterized in a high throughput fashion." He said that to address

the problem, synthesis of additional primers, additional PCR reactions and additional sequencing reactions will be required to obtain complete variable region sequences.

Atreca's Immune Repertoire Capture technology uses barcoding to generate full-length heavy and light chain variable region sequences.

The incomplete variable regions are "only a short-term problem," noted Robins. "High throughput sequencing is evolving so quickly it should be able to catch up in a few years."

Georgiou told *SciBX* that patent applications covering the technology have been filed. His team has not yet licensed the technology and has not determined how to move forward commercially.

#### Martz, L. SciBX 6(5); doi:10.1038/scibx.2013.108 Published Feb. 7, 2013

#### REFERENCES

 DeKosky, B.J. *et al. Nat. Biotechnol.*; published online Jan. 20, 2013; doi:10.1038/nbt.2492
Contact: George Georgiou, The University of Texas at Austin, Austin, Texas e-mail: gg@che.utexas.edu

#### COMPANIES AND INSTITUTIONS MENTIONED

Ablynx N.V. (Euronext:ABLX), Ghent, Belgium Adaptive Biotechnologies Corp., Seattle, Wash. Atreca Inc., San Carlos, Calif. Charité University Medicine, Berlin, Germany German Rheumatism Research Center, Berlin, Germany Illumina Inc. (NASDAQ:ILMN), San Diego, Calif. Stanford University School of Medicine, Stanford, Calif. The University of Chicago, Chicago, Ill. University of Houston, Houston, Texas The University of Texas at Austin, Austin, Texas

### THE DISTILLERY

### 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	Summarv	Licensing status	Publication and contact information
Cancer		·	Ū	
Bladder cancer	IL-20; IL-20 receptor-α (IL20RA); cyclin- dependent kinase inhibitor 1A (p21, Cip1) (CDKN1A; CIP1)	Patient sample and cell culture studies suggest inhibiting IL-20 could help treat muscle-invasive bladder cancer. In human bladder cancer tissue, mRNA levels of <i>IL-20</i> and its receptor <i>IL20R1</i> were greater than those in healthy tissue. In cell-based assays, IL-20 stimulation increased migration and invasion of bladder cancer cell lines and expression of the cell cycle inhibitory protein CDKN1A compared with vehicle control administration. In the IL-20-stimulated cells, small interfering RNA against CDKN1A or IL20R1 prevented the increases in invasion and migration. Next steps could include developing pharmacological inhibitors. Novo Nordisk A/S's NN8226, a neutralizing mAb against IL- 20, is in Phase II testing to treat rheumatoid arthritis (RA).	Patent and licensing status unavailable	Lee, SJ. et al. J. Biol. Chem.; published online Dec. 27, 2012; doi:10.1074/jbc.M112.410233 <b>Contact:</b> Sung-Kwon Moon, Chungju National University, Chungbuk, South Korea e-mail: sumoon66@dreamwiz.com
		<i>SciBX</i> 6(5); doi:10.1038/scibx.2013.109 Published online Feb. 7, 2013		
Cancer	Baculoviral IAP repeat- containing 2 (BIRC2; cIAP1); x-linked inhibitor of apoptosis (XIAP)	Mouse and cell culture studies identified bicyclic octahydropyrrolo[1,2- <i>a</i> ]pyrazine–based inhibitors of cIAP1 that could help treat cancer. In a human breast cancer cell line, the most potent compound inhibited cell growth at nanomolar concentrations. In a mouse xenograft model of human breast cancer, the most potent compound caused dose-dependent tumor regression. Researchers did not disclose next steps, which could include testing the lead inhibitor in models of additional cancer indications. Takeda Pharmaceutical Co. Ltd. has the lead cIAP1 inhibitor in preclinical development. At least eight companies have compounds that inhibit IAP proteins in Phase II testing or earlier to treat various cancers.	Patent application filed covering use in cancer; licensing status undisclosed	Hashimoto, K. <i>et al. J. Med. Chem.</i> ; published online Jan. 8, 2013; doi:10.1021/jm301674z <b>Contact:</b> Tomoyasu Ishikawa, Takeda Pharmaceutical Co. Ltd., Kanagawa, Japan e-mail: tomoyasu.ishikawa@takeda.com <b>Contact:</b> Bunnai Saito, same affiliation as above e-mail: bunnai.saito@takeda.com
		<i>SciBX</i> 6(5); doi:10.1038/scibx.2013.110 Published online Feb. 7, 2013		
Cancer	Tankyrase TRF1- interacting ankyrin- related ADP-ribose polymerase (TNKS)	An SAR study identified TNKS inhibitors that could help treat cancer. Rational design studies led to a series of compounds that blocked two substrate-binding pockets formed by TNKS dimers. <i>In vitro</i> , the best of those compounds inhibited TNKS with an IC <sub>50</sub> value of 8 nM and prevented wingless-type MMTV integration site (WNT) pathway signaling in cell culture. Next steps include designing and testing more biologically stable derivatives of high-affinity TNKS inhibitors. <b>SciBX 6(5): doi:10.1038/scibx.2013.111</b>	Patent and licensing status undisclosed	Bregman, H. <i>et al. J. Med. Chem.</i> ; published online Jan. 14, 2013; doi:10.1021/jm301607v <b>Contact:</b> Xin Huang, Amgen Inc., Cambridge, Mass. e-mail: hxin@amgen.com
	<b>, , , ,</b>	high-affinity TNKS inhibitors. SciBX 6(5); doi:10.1038/scibx.2013.111 Published online Feb. 7, 2013		

Indication	Target/ marker/ pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	Mixed lineage kinase 4 (KIAA1804; MLK4); <i>K-Ras</i>	An <i>in vitro</i> and mouse study suggests inhibiting MLK4 could help treat a subset of colorectal cancers with mutant <i>K-Ras. In vitro</i> , mutant variants of MLK4 had greater kinase activity than the wild-type enzyme. In xenograft mouse models of colorectal cancer driven by mutant <i>K-Ras</i> , knockdown or knockout of <i>MLK4</i> decreased tumor formation compared with no knockdown or knockout. Next steps include developing small molecule inhibitors of MLK4 and testing them in cell-based and xenograft mouse models of <i>K-Ras</i> -mutant colorectal cancer.	Patented; licensing status undisclosed	Martini, M. <i>et al. Cancer Res.</i> ; published online Jan. 14, 2013; doi:10.1158/0008-5472.CAN-12-3074 <b>Contact:</b> Alberto Bardelli, University of Torino, Torino, Italy e-mail: alberto.bardelli@unito.it
		<i>SciBX</i> 6(5); doi:10.1038/scibx.2013.112 Published online Feb. 7, 2013		
Leukemia	B cell lymphoma 2 (BCL-2; BCL2)	Mouse and cell culture studies suggest BCL-2 inhibitors could be useful for selectively killing leukemia stem cells. In mice engrafted with human chronic myelogenous leukemia (CML) stem cells, the pan-BCL-2 inhibitor sabutoclax decreased stem cell burden and increased stem cell sensitivity to the tyrosine kinase inhibitor Sprycel dasatinib compared with vehicle. In CML stem cell–enriched primary acute myelogenous leukemia (AML) samples, compared with non-stem cell–enriched AML samples, two related BCL-2 inhibitors, ABT-263 and ABT-737, caused selective increases in cell death. Next steps could include a clinical trial of BCL-2 inhibitors in combination with other antileukemia therapies. Bristol-Myers Squibb Co. markets Sprycel to treat acute lymphoblastic leukemia (ALL) and CML. Abbott Laboratories and Roche's Genentech Inc. unit have ABT-263 in Phase I/II testing or earlier to treat various cancers. ABT-737 is a research reagent from Abbott. Oncothyreon Inc.'s sabutoclax is in preclinical development to treat cancer. At least seven other companies have BCL-2 inhibitors in Phase II testing to treat various cancers including leukemia and lymphoma. <b>SciBX 6(5); doi:10.1038/scibx.2013.113</b> <b>Published online Feb. 7, 2013</b>	Patent and licensing status unavailable	Goff, D.J. et al. Cell Stem Cell; published online Jan. 17, 2013; doi:10.1016/j.stem.2012.12.011 Contact: Catriona H.M. Jamieson, University of California, San Diego, La Jolla, Calif. e-mail: cjamieson@ucsd.edu Lagadinou, E.D. et al. Cell Stem Cell; published online Jan. 17, 2013; doi:10.1016/j.stem.2012.12.013 Contact: Craig T. Jordan, University of Rochester Medical Center, Rochester, N.Y. e-mail: craig_jordan@urmc.rochester.edu
Endocrine/meta	bolic disease	,		
Diabetes	Growth hormone- releasing hormone receptor (GHRHR); GHRH	<i>In vitro</i> and mouse studies suggest pancreatic islet cells pretreated with GHRHR agonists and transplanted into the adrenal gland could help treat diabetes. In cell culture, a potent GHRH analog increased the viability and proliferation of rat islet cells compared with vehicle control. The viability was further increased by coculture with adrenal cells. In a mouse model of type 1 diabetes, transplantation of analog-preconditioned islets into the adrenal gland rapidly decreased blood glucose levels compared with transplantation into the standard kidney capsule. Next steps could include optimizing the GHRH analogs for clinical use. At least four companies have GHRHR agonists in development stages ranging from preclinical to marketed for various indications.	Patent applications pending; exclusively licensed to Biscayne Pharmaceuticals Inc.; may be available for collaborations or partnerships	Schubert, U. <i>et al. Proc. Natl. Acad. Sci.</i> <i>USA</i> ; published online Jan. 23, 2013; doi:10.1073/pnas.1221505110 <b>Contact:</b> Andrew V. Schally, University of Miami Miller School of Medicine, Miami, Fla. e-mail: andrew.schally@va.gov

### This week in therapeutics (continued)

*SciBX* 6(5); doi:10.1038/scibx.2013.114 Published online Feb. 7, 2013

### This week in therapeutics (continued)

HematologyMyeloproliferative disorderSolute carrier family 2 facilitated	<i>In vitro</i> and mouse studies suggest inhibiting GLUT1 could help prevent weight loss associated with myeloproliferative disorder. In a mouse model of myeloproliferative disorder, adipose tissue atrophy, glucose consumption by leukocytes	Patent and licensing status unavailable	Gautier, E.L. et al. J. Exp. Med.;
Myeloproliferative Solute carrier disorder family 2 facilitated	In vitro and mouse studies suggest inhibiting GLUT1 could help prevent weight loss associated with myeloproliferative disorder. In a mouse model of myeloproliferative disorder, adipose tissue atrophy, glucose consumption by leukocytes	Patent and licensing status unavailable	Gautier, E.L. et al. J. Exp. Med.;
glucose transporter member 1 (SLC2A1; GLUT1)	and inflammation in bone marrow were greater than what was seen in control mice. In three mouse models of the disease, small hairpin RNA-mediated knockdown of Glut1 in the bone marrow decreased adipose tissue loss compared with no knockdown. Next steps could include identifying and testing GLUT1 inhibitors in animal models.		published online Jan. 14, 2013; doi:10.1084/jem.20121357 <b>Contact:</b> Laurent Yvan-Charvet, Columbia University, New York, N.Y. e-mail: ly2159@columbia.edu
	<i>SciBX</i> 6(5); doi:10.1038/scibx.2013.115 Published online Feb. 7, 2013		
Hepatic disease			
Liver disease Fibroblast growth factor 7 (FGF7)	Mouse studies suggest FGF7 could help activate liver progenitor cells (LPCs) to treat liver diseases. LPCs proliferate in response to liver injury and are believed to contribute to regeneration. In three mouse models of liver injury and in human patients with liver disease, LPCs expressed FGF7. In mice, inducing Fgf7 overexpression shortly after liver injury or after establishment of chronic liver disease increased LPC activation and decreased liver injury compared with no overexpression. Next steps could include testing FGF7 or its derivatives in mouse models of liver disease. Swedish Orphan Biovitrum AB markets Kepivance palifermin, a truncated form of FGF7 that acts as a keratinocyte growth factor receptor (KGFR; FGFR2; CD332) agonist, to treat oral mucositis. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.116 Published online Feb. 7, 2013	Patent and licensing status unavailable	Takase, H.M. <i>et al. Genes Dev</i> ; published online Jan. 15, 2013; doi:10.1101/gad.204776.112 <b>Contact</b> : Tohru Itoh, The University of Tokyo, Tokyo, Japan e-mail: itohru@iam.u-tokyo.ac.jp
Infectious disease			
Bacterial Not	In vitro studies identified a short membrane-disrupting	Patent and licensing	McGrath D.M. et al. Proc. Natl. Acad
infection applicable	peptide that could help treat bacterial infections. <i>In vitro</i> , the <sub>D</sub> (KLAKLAK) <sub>2</sub> peptide caused lipid bilayer disruption, was active against drug-resistant strains of Gram-negative bacteria and inhibited <i>Pseudomonas aeruginosa</i> biofilm growth with an MIC <sub>50</sub> of 600 µg/mL. Next steps include conducting good laboratory practice (GLP) toxicology in rodents and primates and testing in models of severe infections. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.117 Published online Feb. 7, 2013	status undisclosed	Sci. USA; published online Jan. 23, 2012; doi:10.1073/pnas.1221924110 Contact: Wadih Arap, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: warap@mdanderson.org Contact: Renata Pasqualini, same affiliation as above e-mail: rpasqual@mdanderson.org Contact: Richard L. Sidman, Harvard Medical School, Boston, Mass. e-mail: richard_sidman@hms.harvard.edu
HBV; HDV Solute carrier family 10 sodium/ bile acid cotransporter family member 1 (SLC10A1)	<i>In vitro</i> and cell culture studies suggest antagonizing SLC10A1 on host cells could help prevent HBV and HDV infection. <i>In vitro</i> mass spectrometry analysis and interaction studies showed that SLC10A1 bound to the envelope proteins of HBV and HDV. In cultured hepatocytes, small interfering RNA- mediated knockdown of <i>SLC10A1</i> decreased HBV and HDV infection compared with no knockdown. In an immortalized hepatocyte cell line that normally cannot be infected with HBV or HDV, expression of <i>SLC10A1</i> permitted viral infection. Next steps could include using SLC10A-expressing cell lines to screen for anti-HBV or anti-HDV prophylactics. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.118	Patent and licensing status unavailable	Yan, H. <i>et al. eLife</i> ; published online Nov. 13, 2012; doi:10.7554/eLife.00049 <b>Contact:</b> Wenhui Li, National Institute of Biological Sciences, Beijing, China e-mail: liwenhui@nibs.ac.cn

Indication	Target/ marker/ pathway	Summary	Licensing status	Publication and contact information
HCV	HCV NS3/4A protease complex	In vitro studies identified a macrocyclic acyl sulfonamide– based inhibitor of HCV NS3/4A protease that could help treat drug-resistant HCV infection. In a panel of enzyme inhibition assays, the compound inhibited the NS3/4A protease from 13 HCV strains, including 6 with known resistance mutations, at nanomolar and subnanomolar concentrations. The inhibitor also showed no activity in a panel of seven off-target proteases and had low cytotoxicity in a human hepatocyte cell line. Next steps could include testing the lead inhibitor in models of HCV infection. Vertex Pharmaceuticals Inc. markets Incivek telaprevir, a small molecule HCV NS3/4A protease inhibitor, to treat HCV infection. Merck & Co. Inc. markets Victrelis boceprevir, a small molecule HCV NS3/4A protease inhibitor, for the indication. Boehringer Ingelheim GmbH's faldaprevir, an oral HCV NS3/4A protease inhibitor, is in Phase III testing to treat HCV infection. At least six other companies have inhibitors of the HCV NS3/4A protease in Phase III testing or earlier to treat HCV infection. SciBX 6(5); doi:10.1038/scibx.2013.119 Published online Feb. 7, 2013	Patent and licensing status unavailable	O'Meara, J.A. <i>et al. J. Biol. Chem.</i> ; published online Dec. 27, 2012; doi:10.1074/jbc.M112.439455 <b>Contact:</b> Jeff A. O'Meara, Boehringer Ingelheim Ltd. R&D, Laval, Quebec, Canada e-mail: jeff.omeara@boehringer-ingelheim.com
Neurology				
Neurology	Macrophage colony- stimulating factor 1 (CSF1; M-CSF); IL-34; colony- stimulating factor 1 receptor (CSF1R; C-FMS; CD115)	Mouse and cell culture studies suggest increasing CSF1R signaling could protect neurons to help treat brain injury and neurodegenerative diseases. In cultured neurons, the CSF1R ligands CSF1 or IL-34 decreased excitotoxic injury compared with saline. In a mouse model of chemically induced neurodegeneration, CSF1 delivered systemically two or six hours after the chemical insult decreased neurodegeneration compared with saline. Next steps could include testing the therapeutic effect of CSF1 in preclinical models of brain injury or neurodegenerative diseases. SciBX 6(5); doi:10.1038/scibx.2013.120 Published online Feb. 7, 2013	Patent and licensing status unavailable	Luo, J. <i>et al. J. Exp. Med.</i> ; published online Jan. 7, 2013; doi:10.1084/jem.20120412 <b>Contact:</b> Tony Wyss-Coray, Stanford University School of Medicine, Stanford, Calif. e-mail: twc@stanford.edu
Various				
Autoimmune disease; diabetes	Not applicable	Mouse studies suggest enteric microbiota from males could help protect females from type 1 diabetes. In a mouse model of nonobese type 1 diabetes, oral transfer of enteric microbiota from adult male mice to young female mice led to testosterone-dependent decreases in the markers and incidence of type 1 diabetes compared with oral transfer of enteric microbiota from adult female mice or no transfer. Next steps include determining how commensal bacterial consortia regulate testosterone levels and how alterations in testosterone levels attenuate autoimmunity. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.121 Published online Feb. 7, 2013	Unpatented; available for partnering from The Hospital for Sick Children <b>Contact:</b> Arlene Yee, The Hospital for Sick Children, Toronto, Ontario, Canada phone: 416-813-8858 e-mail: arlene.yee@sickkids.ca	Markle, J.G.M. <i>et al. Science</i> ; published online Jan. 17, 2013; doi:10.1126/science.1233521 <b>Contact:</b> Jayne S. Danska, The Hospital for Sick Children Research Institute, Toronto, Ontario, Canada e-mail: jayne.danska@sickkids.ca

### This week in therapeutics (continued)

### THE DISTILLERY

#### 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 & scree	ns		
High throughput sequencing strategy for pairs of immunoglobulin heavy $(V_H)$ and light $(V_1)$ variable regions	A method to sequence paired $V_{\rm H}$ and $V_{\rm L}$ regions could help improve the design of vaccines, therapeutics and diagnostics. Sequencing methods usually do not provide any information on which $V_{\rm H}$ and $V_{\rm L}$ chains are paired. The new approach involves depositing, lysing and capturing mRNA from single B cells in high-density microwell plates. This mRNA is reverse transcribed, amplified as a pair by linkage PCR and then sequenced. The strategy identified variable region pairs for three types of B cells and was faster, less expensive and able to identify more paired sequences than single-cell RT-PCR. Next steps include optimizing the approach to increase throughput ( <i>see</i> Sequencing single B cells, page 9).	Patent application filed; licensing status available from The University of Texas at Austin	DeKosky, B.J. <i>et al. Nat. Biotechnol.</i> ; published online Jan. 20, 2013; doi:10.1038/nbt.2492 <b>Contact:</b> George Georgiou, The University of Texas at Austin, Austin, Texas e-mail: gg@che.utexas.edu
	Published online Feb. 7, 2013		
Localized intracellular proteome analysis using a spatially restricted enzymatic tag	Localized intracellular proteome analysis could be used as a discovery tool to map the protein components of organelles in diseased cells. To label proteins present in a specific cellular compartment, ascorbate peroxidase enzyme was linked to a mitochondrial matrix localization sequence and expressed in cultured human cells. Ascorbate peroxidase-mediated biotinylation followed by mass spectrometry analysis identified 495 mitochondrial matrix proteins, 94% of which had previously been associated with mitochondria. Next steps include using the approach to study the composition of additional organelles. Applications to disease could include characterizing the proteome of mitochondria in genetic disorders linked to mitochondrial dysfunction. <b>SciBX 6(5); doi:10.1038/scibx.2013.123</b> <b>Published online Feb. 7</b> , 2013	Patent application filed; available for licensing	Rhee, HW. <i>et al. Science</i> ; published online Jan. 31, 2013; doi:10.1126/science.1230593 <b>Contact:</b> Alice Y. Ting, Massachusetts Institute of Technology, Cambridge, Mass. e-mail: ating@mit.edu
Disease models	3		
Humanized mouse model for graft-versus-host disease (GvHD) prevention by $T_{reg}$ cells	A humanized mouse model for GvHD could be useful for testing the therapeutic potential of cultured human $T_{reg}$ cells. Previous studies in mice have suggested that adoptive transfer of allogeneic $T_{reg}$ cells could prevent GvHD. In the new study, human $T_{reg}$ cells were cultured <i>ex vivo</i> and transferred into mice with a humanized immune system. Mice receiving an immune cell fraction enriched with $T_{reg}$ cells had lower levels of proinflammatory cytokines and better survival than controls receiving nonspecific immune cells. Next steps could include scaling up production of $T_{reg}$ cells in preparation for clinical trials. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.124 Published online Feb. 7, 2013	Patent and licensing status unavailable	Zheng, J. et al. Sci. Transl. Med.; published online Jan. 16, 2013; doi:10.1126/scitranslmed.3004943 Contact: Wenwei Tu, The University of Hong Kong, Hong Kong, China e-mail: wwtu@hku.hk Contact: Yu-Lung Lau, same affiliation as above e-mail: lauylung@hkucc.hku.hk

### THE DISTILLERY

### This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Clustered, regularly interspaced short palindromic repeats (CRISPR) RNA editing system to modify genomic DNA	Four independent teams developed CRISPR-derived genome editing systems that could be used to modify genomic DNA in diverse biological systems. CRISPR is a bacterial adaptive immunity system that uses host-expressed nucleases and RNA repeats to cleave foreign DNA. To adapt this system to cleave and edit genomic DNA, the four teams designed a DNA vector that expressed a bacteria-derived CRISPR-associated nuclease together with guide RNAs that contained CRISPR features and homology to host genes. Two teams used the system to induce site- specific insertions and deletions in multiple genomic loci in cultured mouse and human cells, whereas a third team developed a system to cleave and edit specific sites within the zebrafish genome. The fourth team engineered a system to modific endogenous genomes in two distinct bacterial species. Next tens include	Patent application filed for findings in first study; licensing status undisclosed Findings in second study unpatented; licensing status	Jinek, M. <i>et al. eLife</i> ; published online Jan. 29, 2013; doi:10.7554/eLife.00471 <b>Contact:</b> Jennifer Doudna, University of California, Berkeley, Calif. e-mail: doudna@berkeley.edu Hwang, W.Y. <i>et al. Nat. Biotechnol.</i> ; published online Jan. 29, 2013; doi:10.1038/nbt.2501
	characterizing and optimizing the specificity of the approach. <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.125 Published online Feb. 7, 2013	not applicable Patent application filed for findings in third study; licensing status	General Hospital, Charlestown, Mass. e-mail: jjoung@partners.org Contact: JR. Joanna Yeh, same affiliation as above e-mail: iyeh l@partners.org
		undisclosed Patent application filed for findings in fourth study; licensing status undisclosed	Cho, S.W. <i>et al. Nat. Biotechnol.</i> ; published online Jan. 29, 2013; doi:10.1038/nbt.2507 Contact: Jin-Soo Kim, Seoul National University, Seoul, South Korea e-mail: jskim01@snu.ac.kr
			Jiang, W. et al. Nat. Biotechnol.; published online Jan. 29, 2013; doi:10.1038/nbt.2508 Contact: Luciano A. Marraffini, The Rockefeller University, New York, N.Y. e-mail: marraffini@rockefeller.edu Contact: David Bikard, same affiliation as above e-mail: dbikard@rockefeller.edu
Pluripotent cell-specific inhibitors (PluriSIns) to reduce the tumorigenic risk of stem cell- based therapies	PluriSIns that are selectively toxic to human pluripotent stem cells could reduce the tumorigenic risk of stem cell–based therapies. Residual undifferentiated stem cells in stem cell–derived cell therapies can lead to teratoma formation. A screen of about 50,000 small molecules identified 15 PluriSIns that were cytotoxic to human embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells but not to differentiated cells derived from these stem cells. In a mixture of differentiated and undifferentiated stem cells injected into mice, none of the cell mixtures pretreated with the lead PluriSIn developed teratomas, whereas all vehicle-treated cell mixtures did. Next steps include examining the ability of PluriSIns to reduce the risk of teratoma formation <i>in vivo</i> . <i>SciBX</i> 6(5); doi:10.1038/scibx.2013.126 Published online Feb. 7. 2013	Two patent applications filed; available for licensing through Yissum, the technology transfer company for The Hebrew University of Jerusalem, and Roche	Ben-David, U. <i>et al. Cell Stem Cell</i> ; published online Jan. 9, 2013; doi:10.1016/j.stem.2012.11.015 Contact: Nissim Benvenisty, The Hebrew University of Jerusalem, Jerusalem, Israel e-mail: nissimb@cc.huji.ac.il
Imaging			
Endomicroscopy capsule for diagnosing upper GI tract disease	A disposable, tethered endomicroscopy capsule could be used to provide 3D microscopic images of the upper GI tract to help diagnose disease. The capsule is swallowed, can be moved up and down with a tether and uses optical frequency domain imaging to collect data and construct 3D images. In healthy individuals and those with known Barrett's esophagus, the pill collected images for about six minutes, and the images were then used to identify the upper GI disease. Next steps include testing the capsule in additional patients and optimizing the capsule for data collection.	Patent application filed; licensed to NinePoint Medical Inc.	Gora, M.J. et al. Nat. Med.; published online Jan. 13, 2013; doi:10.1038/nm.3052 Contact: Guillermo J. Tearney, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. e-mail: gtearney@partners.org
	Published online Feb. 7, 2013		

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