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By *Joanne Kotz, Senior Editor*

A team from **GlaxoSmithKline plc** and the **Structural Genomics Consortium** has identified the first selective and potent histone demethylase inhibitor and shown it dampens the macrophage inflammatory response.¹ The pharma now is exploring the therapeutic applications of the inhibitor in autoimmune and inflammatory diseases.

Histone methyltransferases add methyl groups to histone lysine residues, which can then be removed by histone demethylases. These two enzyme classes work in concert to regulate gene transcription.

Academics and companies have identified histone methyltransferase inhibitors, suggesting the enzyme class is generally druggable. However, whether potent and selective inhibitors could be developed against histone demethylases has remained an open question.

Previous efforts, including work at SGC, identified histone demethylase inhibitors that had either *in vitro* selectivity or potency—but not both—and none had sufficient cellular activity, said Udo Oppermann, a group leader in epigenetics and inflammation at the SGC and professor in molecular biology and deputy director of the Institute of Musculoskeletal Sciences at the **University of Oxford**. Oppermann was a coauthor on the study.

The SGC leads a public-private partnership, which includes GSK, three other pharmas and additional academic groups, that is developing open-access chemical probes that modulate epigenetic proteins.

The GSK-SGC team set out to identify a potent and selective inhibitor of the histone demethylase jumonji domain containing 3 (JMJD3; KDM6B), which removes methyl groups specifically from trimethylated lysine 27 on histone 3 (H3K27me3).

Proinflammatory stimuli such as lipopolysaccharide (LPS) induce expression of JMJD3 in macrophages. The histone demethylase then participates directly in activating the transcription of more than half of the LPS-induced genes.^{2,3}

First, the team obtained a weakly active hit against JMJD3 via a high throughput screen of GSK's library of two million compounds. The researchers then used structure-based design to optimize the hit to the lead molecule, GSK-J1, which inhibited JMJD3 with an IC_{50} of 60 nM.

In addition to its potency, the molecule also had good selectivity—it showed little to no activity against a panel of about 10 other jumonji histone demethylases.

GSK-J1 did show activity against a very closely related histone demethylase called lysine-specific demethylase 6A (KDM6A; UTX).

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Like JMJD3, UTX catalyzes the demethylation of H3K27me3.

In LPS-stimulated primary human macrophages from healthy volunteers, a prodrug of GSK-J1 with improved cell permeability blocked the expression of half of the LPS-induced cytokines, including tumor necrosis factor- α (TNF- α). Subsequent experiments showed that inhibiting both JMJD3 and UTX was required for GSK-J1's activity.

Finally, the team asked whether GSK-J1 had activity in a disease context. In macrophages obtained from patients with rheumatoid arthritis (RA), the prodrug blocked TNF- α production.

Data were published in *Nature*.

"GSK-J1 is the first truly selective jumonji inhibitor and hence represents an important step in demonstrating the tractability of the jumonji family for drug discovery. It is also the first selective H3K27 demethylase inhibitor reported and as such provides a chemical tool to further explore the biology and therapeutic potential of H3K27 demethylases," said Laurens Kruidenier, first author on the paper and lead biologist in GSK's immuno-inflammation EpiNova DPU.

"This is the first paper that shows it is possible to make inhibitors with high affinity and specificity" against jumonji histone demethylases, agreed Kristian Helin, director of the Biotech Research & Innovation Centre at the University of Copenhagen and CSO of EpiTherapeutics ApS.

EpiTherapeutics is targeting histone methyltransferases and histone demethylases in cancer. Helin told *SciBX* that EpiTherapeutics has undisclosed inhibitors of other jumonji histone demethylases with potency and selectivity profiles that are similar to those of GSK-J1.

"Dual pharmacological inhibition of JMJD3 and UTX results in the amelioration of a wide array of important proinflammatory cytokines

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that includes TNF- α . The mechanistic evaluation of JMJD3/UTX inhibitors in animal models of inflammation" will be an important next step, said Jose Lora, senior director of preclinical sciences at epigenetics drug discovery company **Constellation Pharmaceuticals Inc.**

Casting a wide net

"GSK continues to drive focused validation efforts with GSK-J1 in a variety of *in vitro* and *ex vivo* disease models, internally and through

its collaborations," wrote Kruidenier and Rab Prinjha, head of the EpiNova DPU, in a joint email to *SciBX*.

These studies will include determining the differential effects of GSK-J1 versus existing anti-inflammatory treatments including anti-TNF- α antibodies.

Kruidenier and Prinjha said GSK also will compare GSK-J1 with the pharma's bromodomain

and extra terminal domain (BET) inhibitor, another epigenetic target that regulates inflammation.

In 2010, GSK reported in *Nature* the identification of an inhibitor of BET bromodomains and showed that the compound blocked a subset of the macrophage inflammatory response that did not include TNF- α .⁴

Histone demethylases and bromodomains are not the only epigenetic families that GSK is exploring. In 2011, the pharma

partnered with **Epizyme Inc.** to target an undisclosed set of histone methyltransferases.⁵

In addition to these internal and collaborative efforts, Kruidenier and Prinjha said that "GSK-J1 was developed in partnership with the SGC with a view to enabling evaluation by the wider scientific community. GSK-J1 is being made available [through the SGC] to researchers who wish to investigate its role in chromatin biology in their own therapeutic areas."

GSK declined to disclose the patent status of the work.

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REFERENCES

1. Kruidenier, L. *et al. Nature*; published online July 29, 2012; doi:10.1038/nature11262
Contact: David M. Wilson, GlaxoSmithKline plc R&D, Stevenage, U.K.
e-mail: david.m.wilson@gsk.com
2. De Santa, F. *et al. Cell* **130**, 1083–1094 (2007)
3. De Santa, F. *et al. EMBO J.* **28**, 3341–3352 (2009)
4. Nicodeme, E. *et al. Nature* **468**, 1119–1123 (2010)
5. Bouchie, A. & Fulmer, T. *BioCentury* **20**, A1–A5; Jan. 23, 2012

COMPANIES AND INSTITUTIONS MENTIONED

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EpiTherapeutics ApS, Copenhagen, Denmark

Epizyme Inc., Cambridge, Mass.

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.

Structural Genomics Consortium, Oxford, U.K.

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"GSK-J1 is the first truly selective jumonji inhibitor and hence represents an important step in demonstrating the tractability of the jumonji family for drug discovery."

—**Laurens Kruidenier,**
GlaxoSmithKline plc



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Isis takes on myotonic dystrophy

By Lev Osherovich, Senior Writer

Researchers at the **University of Rochester** and **Isis Pharmaceuticals Inc.** have come up with an efficient way to treat symptoms of myotonic dystrophy type 1 in mice using systemically delivered antisense oligonucleotides.¹ The finding has fueled an early stage development deal between Isis and **Biogen Idec Inc.**

Myotonic dystrophy type 1 (DM1) is a dominantly inherited condition that causes progressive muscle weakening and is the most common type of muscular dystrophy, affecting about 1 in 8,000 people. It is caused by spontaneous expansion of a trinucleotide repeat sequence in an untranslated region of the mRNA encoding dystrophin protein kinase (DMPK; DM1). The resulting transcript is abnormally large and interferes with the activity of nuclear proteins that are critical for normal muscle function.

In severe cases, the disease causes paralysis and respiratory problems. There are no disease-modifying or symptomatic therapies for DM1, leaving palliative physical therapy as the only option for patients.

Prior cell culture and mouse studies by the Rochester team and other academics have suggested that eliminating trinucleotide-expanded DMPK transcripts could alleviate DM1.²

Indeed, the Rochester team, led by Professor of Neurology Charles Thornton, had previously shown in a mouse model of DM1 that disrupting the structure of the expanded RNA sequence that causes DM1 halted the progression of disease and reversed muscle weakening.³

Despite these positive data, eliminating the toxic transcript that causes DM1 has been challenging *in vivo*. The most straightforward approach to targeting the transcript is to use a sequence-specific antisense oligonucleotide (ASO). However, it is difficult to knock down genes in muscle cells because high metabolic and transcriptional activity in these cells leads to high levels of many transcripts.

As a result, prior efforts to knock down repeat-expanded *DMPK* transcripts in DM1 mice required a complex, high-dose regimen of injections into affected muscles.⁴

In the new study, Thornton and collaborators at Isis led by C. Frank Bennett, SVP of research, set out to solve the delivery and dosing challenges.

The team constructed an ASO that hits its target in a variety of muscle tissues after a single subcutaneous injection. Moreover, the ASO persisted within the muscle cells and prevented further RNA buildup months after the initial treatment.

“This is one of the more robust demonstrations of using antisense oligonucleotides to target muscle tissue,” said Bennett. “Historically we’ve thought of muscle tissue as being on the lower end of responsiveness to antisense technology. Here we show that doses that could be used in a clinical setting can actually be effective.”

Single shot

Bennett and Thornton first ran cell culture screens for ASOs that knocked down the expression of a model transcript bearing the DM1-associated trinucleotide repeats. The most potent hits were then tested in

a mouse model expressing a model transcript with the DM1-associated trinucleotide repeats.

In cell culture and mice, ASOs decreased levels of the toxic RNA compared with a scrambled-sequence control ASO and alleviated the nuclear accumulation of proteins trapped by the aberrant transcript.

Mice receiving a single subcutaneous injection of the most potent ASOs showed better function in a variety of muscle groups than animals given scrambled control. The effect of the ASOs on muscle strength lasted for months after dosing ceased, suggesting that the molecule remains in the nucleus for a lengthy period.

Thornton said subcutaneous injection of the ASOs proved surprisingly effective and represented a leap beyond prior tissue-specific RNA knockdown strategies tried by his team.

“This was a whole-body treatment,” said Thornton. “All of the muscles of the mouse were corrected by a single injection into the skin. This is the biggest difference compared with prior approaches.”

He noted that DM1 affects a range of muscle tissue groups and displays an unpredictable course of progression in many patients. Rather than try to treat it in each stricken muscle group, Thornton said it would be better to have a systemic therapy.

The high potency of the ASOs may be due to the manner in which the antisense molecules find and destroy their target, said Bennett.

The company’s ASOs work by flagging target transcripts for destruction by RNase H, a cellular enzyme that ordinarily helps degrade excess or abnormal mRNAs.

“Our drugs work through RNase H, which recognizes an RNA-DNA heteroduplex,” said Bennett. “We get cleavage of the target RNA by RNase H.”

Bennett said that RNase H is especially abundant in the nuclei of muscle cells. Because the DM1-associated transcript also accumulates in the nucleus, all that is needed to trigger the RNA’s destruction is a few molecules of ASO to help connect the RNase H enzyme to its target.

Thornton previously used morpholinos, a class of synthetic RNA-binding molecule, to prevent the toxic effects of trinucleotide-expanded RNA.³ Morpholinos are RNA-like molecules that bind to sequence-matched nucleic acid targets but do not necessarily cause the degradation of their targets.

He said ASOs are likely a better therapeutic class.

“By changing our strategy from trying to block the toxic RNA and insulating it to getting rid of it by cleavage, we gained a huge potency advance,” said Thornton. “Now even the relatively small amount of material that goes into muscle cells is effective.”

Results were published in *Nature*.

Dollars and antisense

In June, Isis granted Biogen rights to ASO therapeutics for DM1 in exchange for \$12 million up front and up to \$59 million in preclinical and clinical milestones.

“This is one of the more robust demonstrations of using antisense oligonucleotides to target muscle tissue. [...] Here we show that doses that could be used in a clinical setting can actually be effective.”

—C. Frank Bennett,
Isis Pharmaceuticals Inc.

Bennett said Isis is now screening for variants of the ASOs with even greater potency than the ASOs used in the study. He did not disclose a timetable for clinical development of the compounds.

Meanwhile, Dutch biotech **Prosensa B.V.** is pursuing its own ASO strategy for DM1. In 2009, Thornton collaborated with Prosensa and researchers at **Radboud University Nijmegen Medical Centre** to show that the biotech's ASOs could reduce DM1-like pathology in the same mouse model used in the Isis study.

Compared with Isis' ASOs, Prosensa's technology uses a different backbone chemistry and relies on other cellular nucleases besides RNase H to cleave target mRNAs, said CBO and SVP of business development Luc Dochez.

Dochez said it is too early to say how the two approaches stack up against one another and that a real comparison "would require PK/PD studies in humans."

To maximize the activity of Prosensa's ASOs in muscle tissues, Dochez said the company is designing a preclinical candidate ASO fused to a muscle cell-targeting peptide. The program and its lead product, PRO135, is available for out-licensing or partnering.

Dochez noted that some patients with DM1 exhibit neurological symptoms, so it may also be desirable to hit expanded DMPK transcripts in the CNS, though it is not yet clear how to do so.

"A muscle-targeting peptide could be a way to overcome the challenges

of systemic delivery," said Dochez. "With muscle-targeting delivery, we could address the myotonia in the muscle, but there's also a CNS component in the more severe forms of the disease, which poses an additional challenge. But even addressing part of the disease could be a big leap."

Isis has filed for patents covering the findings described in the *Nature* paper.

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REFERENCES

1. Wheeler, T.M. *et al. Nature*; published online Aug. 2, 2012; doi:10.1038/nature11362
Contact: Charles Thornton, University of Rochester, Rochester, N.Y.
e-mail: charles_thornton@urmc.rochester.edu
2. Muntoni, F. & Wood, M.J. *Nat. Rev. Drug Discov.* **10**, 621–637 (2011)
3. Wheeler, T.M. *et al. Science* **325**, 336–339 (2009)
4. Mulders, S.A.M. *et al. Proc. Natl. Acad. Sci. USA* **106**, 13915–13920 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Weston, Mass.
Isis Pharmaceuticals Inc. (NASDAQ:ISIS), Carlsbad, Calif.
Prosensa B.V., Leiden, the Netherlands
Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
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CXCR2 antagonists in breast cancer

By Lauren Martz, Staff Writer

A team at the **Memorial Sloan-Kettering Cancer Center** has shown that CXC chemokine receptor 2 antagonists sensitized tumors to chemotherapy in mouse models of metastatic breast cancer.¹ The team is working on additional preclinical studies with the intention of moving the antagonists into clinical trials for breast cancer.

Resistance to chemotherapy often occurs in breast cancer as a result of a small percentage of cancer cells that develop a survival advantage and show a more aggressive, metastatic phenotype.

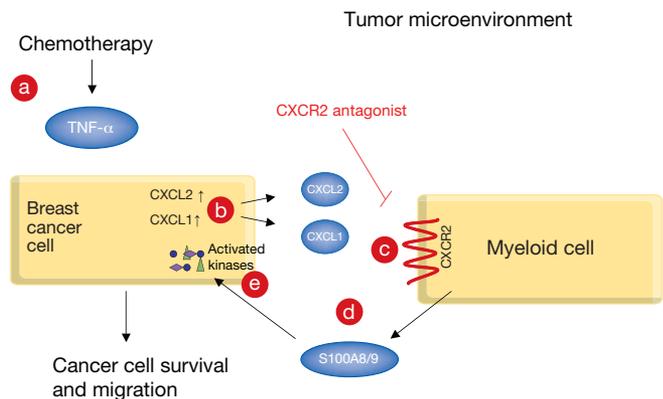


Figure 1. Breast cancer survival network. Chemotherapy results in a burst of paracrine factors near breast tumors that upregulate survival pathways in the cancer cells, causing chemotherapeutic resistance and metastasis. The process begins when chemotherapy induces the release of tumor necrosis factor- α (TNF- α) [a] from stromal cells in the breast, including endothelial cells. As a result, chemokine CXC motif ligand 1 (CXCL1; GRO; MGSA) and CXCL2 (MIP2) are upregulated in breast cancer cells [b] by mechanisms including TNF- α release. The release of CXCL1 and CXCL2 attracts CXC chemokine receptor 2 (CXCR2; IL8RB)-expressing stromal cells from the tumor microenvironment to the tumor. Specifically, CXCR2-expressing, complement receptor 3 (CR3; CD11b)⁺/lymphocyte antigen 6 complex locus G (LY6G; GR1)⁺ myeloid cells accumulate near the tumor [c]. The myeloid cells release paracrine factors including S100 calcium binding protein A8 (S100A8; calgranulin A; Mrp8) and S100A9 (calgranulin B; Mrp14) [d], which activate kinases such as MAP kinase 3 (MAPK3; ERK-1), MAPK1 (ERK-2), MAPK and ribosomal protein S6 kinase 70 kDa polypeptide 1 (RPS6KB1; S6K1) on the breast cancer cells [e]. Activation of the cancer-associated kinases promotes cancer cell survival and metastasis.

CXCR2 antagonists prevent CXCR2-expressing myeloid cells from homing to cancer cells, blocking release of S100A8 and S100A9 and subsequent kinase signaling that promotes cancer survival. Blocking CXCR2 could reduce chemotherapy resistance and breast cancer metastasis.

The challenge has been identifying the factors that confer the survival advantage and figuring out how to target them in combination with chemotherapy to prevent resistance, tumor relapse and metastasis.

In 2005, the Sloan-Kettering team, led by Joan Massagué, found that expression of chemokine CXC motif ligand 1 (CXCL1; GRO; MGSA) and CXCL2 (MIP2) on breast cancer cells was associated with risk of relapse and metastasis to the lungs.²

Building on that work, the group set out to determine the mechanism that linked CXC ligand expression on tumors to chemotherapeutic resistance, with the hope of identifying molecular targets to prevent resistance.

Massagué is chairman of the Cancer Biology and Genetics Program and director of the Metastasis Research Center at Memorial Sloan-Kettering. He is also a **Howard Hughes Medical Institute** investigator. The team included researchers from **Cancer Research UK**.

The team found that CXCL1 and CXCL2 were amplified in 7.5% of human primary breast cancer tissues tested and 19.9% of metastases, suggesting both chemokines were associated with invasiveness. In mouse xenograft models of metastatic breast cancer, small hairpin RNA against CXCL1 and CXCL2 decreased mammary tumor growth and metastasis compared with shRNA control.

Although the levels of CXCL1 and CXCL2 were elevated in the breast cancer tissue, the levels of their receptors, including CXC chemokine receptor 2 (CXCR2; IL8RB), were low. That suggested the prosurvival effects of the ligands might be the result of their actions on tissues surrounding the tumor.

Thus, the team set out to determine whether the ligands were altering the activity of cells within the tumor microenvironment.

Indeed, in the same mouse models, CXCL1 and CXCL2 knockdown decreased levels of complement receptor 3 (Cr3; Cd11b)⁺/lymphocyte antigen 6 complex locus G (Ly6g; Gr1)⁺ myeloid cells, which express higher levels of CXCR2 than other stromal cells, in the tumor microenvironment. Moreover, coculture of the tumor and Cd11b⁺/Gr1⁺ myeloid cells prevented the tumor cells from undergoing chemotherapy-induced apoptosis. That suggested the Cd11b⁺/Gr1⁺ myeloid cells were mediating the chemotherapy resistance of the tumor cells.

A subsequent series of experiments revealed a mechanism whereby CXCL1 and CXCL2 bound CXCR2 on nearby Cd11b⁺/Gr1⁺ myeloid cells, which triggered the secretion of two survival factors, S100 calcium binding protein A8 (S100a8; calgranulin A; Mrp8) and S100a9 (calgranulin B; Mrp14). Those survival factors then acted on the tumor to promote survival in the presence of chemotherapy (see Figure 1, “Breast cancer survival network”).

Finally, the group tested whether it might be possible to block the entire mechanism using CXCR2 antagonists in combination with chemotherapy. In a mouse breast cancer xenograft model, doxorubicin plus a CXCR2 antagonist lowered metastatic burden and tumor growth better than doxorubicin alone.

The results were published in *Cell*.

“The tumor-promoting effects of the tumor microenvironment have been gaining attention, and the CXCL/CXCR2 axis seems to be one of the key signals in the tumor microenvironment,” Ijichi Hideaki told *SciBX*. He is a research associate in the Department of Gastroenterology at **The University of Tokyo Hospital**.

(Continues on p. 7)

Screening for immunogenic cell death

By Kai-Jye Lou, Staff Writer

French researchers have used a fluorescence-based screening platform to identify small molecules that induced immunogenic cell death in mouse tumors.¹ Next, the group plans to test the best hits in a Phase I/II trial to treat locally invasive head and neck cancers in combination with chemotherapy.

In 2005, Guido Kroemer and colleagues first showed that the anthracycline chemotherapeutic doxorubicin could induce immune-mediated death of cancer cells in addition to the known process of cell death through DNA damage.² The immunogenic process was later

(Continued from "CXCR2 antagonists in breast cancer," p. 6)

"Combination of conventional chemoreagents, which target tumor cells directly, and modulators of the tumor microenvironment might be a promising strategy for synergistic treatment of cancer," he added.

Next steps

Before CXCR2 antagonists can be tested in combination with chemotherapeutics in the clinic, researchers agree that extensive preclinical evaluation of efficacy will be required. It will also be necessary to address a few potential adverse effects.

The Sloan-Kettering study "used mostly xenograft models, and they did not examine CXCR2-antagonizing effects on genetically engineered models," said Hideaki. "If they could also see consistent results as well as prolonged overall survival using these models, it would strengthen their findings."

Indeed, Massagué told *SciBX* his team's next steps include extensive preclinical analysis with different CXCR2 inhibitors and chemotherapeutics for different durations. "Patient-derived grafts and transgenic models need to be tested side by side with xenografts to understand the full gamut of efficacy of these CXCR2 inhibitors in reducing breast metastasis," he said.

These studies are underway.

Potential side effects need to be evaluated as well, said Hideaki.

"Since CXCR2 is known to be involved in the host resistance to bacterial and fungal infections and the wound healing response, antagonizing CXCR2 might cause certain disadvantages," he said. "This should be carefully investigated *in vivo* using multiple genetically engineered cancer models to clarify not only the therapeutic effects but also certain adverse effects."

He added that humans have an additional CXCR1 and CXCR2 ligand that mice lack, IL-8 (CXCL8), which is involved in many aspects of different diseases. Because most *in vivo* functional evidence of the CXCR2-antagonism effect was shown using mouse models, the researchers should be careful about this difference between humans and mice, he said.

At least two CXCR2 antagonists have already been evaluated in clinical safety studies, although according to Hideaki "no trials have been performed in cancers."

shown to be mediated by multiple factors involved in apoptosis and autophagy.³⁻⁶

Based on those mechanistic findings, Kroemer and colleagues decided to design an automated epifluorescence microscopy-based screening platform that could detect several hallmarks of immunogenic cell death and thus be used to identify new compounds that trigger this process in a human osteosarcoma cell line.

First, the researchers validated the new screening platform using a library of 120 marketed cancer therapies. Based on the readouts, the most potent compounds included several known inducers of immunogenic cell death, including doxorubicin, daunorubicin and mitoxantrone. Two other top hits were drugs known to have immunogenic side effects: vincristine and vinorelbine.

Next, the researchers used the platform to screen for new inducers of immunogenic cell death. Of the top 10 hits, 4 were small molecule cardiac glycosides, including digoxin and digitoxin, which are marketed to reduce arrhythmias in patients with heart failure.

(Continues on p. 8)

CXCR2 antagonists in the clinic include **AstraZeneca plc's** AZD5069, which is in Phase II testing to treat chronic obstructive pulmonary disease (COPD), and **Dompe Farmaceutici S.p.A.'s** Reparixin, which is in Phase II testing for graft and renal transplant rejection. The companies declined to comment.

Markers

In addition to the potential therapeutic applications of the findings, the team thinks the results have important implications for breast cancer diagnosis and prognosis.

Massagué told *SciBX* his team has confirmed a strong association between S100A8 and S100A9 and resistance to perioperative chemotherapy. "This could lead to useful markers of resistance and relapse in the future."

He added, "Since S100A8 and S100A9 are easy to detect in serum and tissues, these markers might also be useful for developing clinical assays in the future."

Massagué said Sloan-Kettering has filed a provisional U.S. patent application covering the work, and the IP is available for licensing.

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REFERENCES

- Acharyya, S. *et al. Cell*; published online July 6, 2012; doi:10.1016/j.cell.2012.04.042
Contact: Joan Massagué, Memorial Sloan-Kettering Cancer Center, New York, N.Y.
 e-mail: massaguj@mskcc.org
- Minn, A.J. *et al. Nature* 436, 518-524 (2005)

COMPANIES AND INSTITUTIONS MENTIONED

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Cancer Research UK, London, U.K.
Dompe Farmaceutici S.p.A., Milan, Italy
Howard Hughes Medical Institute, Chevy Chase, Md.
Memorial Sloan-Kettering Cancer Center, New York, N.Y.
The University of Tokyo Hospital, Tokyo, Japan

The researchers then tested digoxin *in vivo* as it is the more commonly used drug. In mice, digoxin stimulated immunogenic cancer cell death and elicited a protective antitumor immune response.

Finally, the researchers did a retrospective analysis of patients with various carcinomas receiving chemotherapy and found that those also on digoxin had greater survival rates than patients not on digoxin.

The findings were published in *Science Translational Medicine*. Kroemer is a professor in the faculty of medicine at the **University Paris Descartes**, director of the apoptosis, cancer and immunity unit at **Institut National de la Santé et de la Recherche Médicale** (INSERM), hospital practitioner at **Georges Pompidou European Hospital** and director of the Metabolomics facility at the **Gustave Roussy Institute**.

“The most striking result from this study was that the group was also able to correlate their preclinical data with retrospective patient data,” said Ignacio Melero, a professor of immunology and senior consultant at the University Clinic of Navarra at the **University of Navarra**. “Because the chemotherapies and cardiac glycosides assessed in the retrospective analysis have already seen decades of patient use and have established safety profiles, it should be easy to test the combinations in the clinic.”

The use of cardiac glycosides in the cancer setting had been suggested by past research, though the mechanisms underlying their anticancer effects in those studies were unclear.⁷

“The greatest potential of this platform is in identifying and evaluating smart combination therapeutic regimens because it allows one to screen for compounds that are immunostimulatory but not necessarily very cytotoxic. For example, one could envision combining a very potent, very cytotoxic agent with a less cytotoxic but immunostimulatory agent from the screen to improve the presentation of tumor antigens to the host immune system,” said Estuardo Aguilar-Cordova, CEO of **Advantagene Inc.**

Advantagene’s lead compound is ProstAtak, which is being tested in combination with radiation therapy in a Phase III trial in patients with newly diagnosed prostate cancer. ProstAtak is an adenovirus vector encoding herpes simplex virus thymidine kinase (HSV-tk) coadministered with an antiherpetic drug. The therapy induces immunogenic cell death in tumor cells but the vector itself also creates a danger signal that further stimulates the immune system.

Standard of care complement

Subgroup analysis of the retrospective cancer patient data suggests Aguilar-Cordova has the right idea.

In that analysis, digoxin did not confer additional survival benefit in patients treated with chemotherapy that induced immunogenic cell death. Therefore, Kroemer thinks the cardiac glycosides are best suited for use with cancer therapies that do not induce immunogenic cell death, such as cisplatin.

“When you look at the wide use of cisplatin, you see it is quite efficient in reducing tumor mass but also inefficient in conferring any long-term benefit to the patient,” Kroemer told *SciBX*. “Our idea is that if we could combine cisplatin with other agents to make it more immunogenic, we may be able to improve long-term patient outcomes.”

The cardiac glycosides and other molecules that induce immunogenic cell death could also complement existing antigen-specific cancer

vaccines, adoptive cell transfer therapies, and drugs that non-specifically stimulate T cells, such as Yervoy ipilimumab, added Pierre Coulie.

“Traditional cancer vaccine and adoptive transfer therapies generally target only a few tumor antigens,” said Coulie. “Molecules that induce immunogenic cell death could give the host immune system an opportunity to react against more tumor antigens, including individual mutated antigens, and thus mount a stronger antitumor immune response.”

Coulie is a professor of immunology and leader of the Human Tumor Immunology group at the **de Duve Institute** and the **Université Catholique de Louvain**.

Bristol-Myers Squibb Co. markets Yervoy, a human mAb against CTLA-4 (CD152) receptor, to treat unresectable or metastatic melanoma.

Kroemer said his group is developing additional cancer cell lines to use with the screening platform and is trying to make the technology compatible with all cancer cell lines. In addition, the group is modifying the screening platform to identify target-specific compounds that also induce immunogenic cell death.

Aguilar-Cordova added that it is important to increase the throughput of future iterations of the screening platform.

As for the lead cardiac glycoside screening hits, Kroemer said the group plans to start enrolling patients with locally invasive head and neck cancers in an investigator-led Phase I/II trial in 2013.

“Such cancers are usually treated with cisplatin and radiotherapy, so we would combine the classical therapies with a cardiac glycoside,” he told *SciBX*. “For the primary endpoints, we plan to measure infiltration of tumor by T cells and also therapeutic efficacy.”

The Gustave Roussy Institute has filed two patent applications covering methods and compounds to induce immunogenic cell death. The work is available for licensing.

Lou, K.-J. *SciBX* 5(31); doi:10.1038/scibx.2012.808
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REFERENCES

- Menger, L. *et al. Sci. Transl. Med.*; published online July 18, 2012; doi:10.1126/scitranslmed.3003807
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- Casares, N. *et al. J. Exp. Med.* **202**, 1691–1701 (2005)
- Obeid, M. *et al. Nat. Med.* **13**, 54–61 (2007)
- Panaretakis, T. *et al. Cell Death Differ.* **15**, 1499–1509 (2008)
- Ghiringhelli, F. *et al. Nat. Med.* **15**, 1170–1178 (2009)
- Apetoh, L. *et al. Nat. Med.* **13**, 1050–1059 (2007)
- Prassas, I. & Diamandis, E.P. *Nat. Rev. Drug Discov.* **7**, 926–935 (2008)

COMPANIES AND INSTITUTIONS MENTIONED

Advantagene Inc., Auburndale, Mass.
Bristol-Myers Squibb Co. (NYSE: BMY), New York, N.Y.
de Duve Institute, Brussels, Belgium
Georges Pompidou European Hospital, Paris, France
Gustave Roussy Institute, Villejuif, France
Institut National de la Santé et de la Recherche Médicale, Villejuif, France
Université Catholique de Louvain, Brussels, Belgium
University of Navarra, Pamplona, Spain
University Paris Descartes, Paris, France

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				
Autoimmune disease	Jumonji domain containing 3 (JMJD3; KDM6B); lysine-specific demethylase 6A (KDM6A; UTX)	An <i>in vitro</i> and cell culture study identified a compound that inhibited the histone demethylases JMJD3 and UTX and that could help treat autoimmune diseases. <i>In vitro</i> , the small molecule GSK-J1 inhibited JMJD3 and UTX with nanomolar potency and with selectivity over other histone demethylases. In primary human macrophages, a prodrug of GSK-J1 with increased cell permeability decreased the expression of half of the lipopolysaccharide (LPS)-induced cytokines, including tumor necrosis factor- α (TNF- α), compared with an inactive control compound. Next steps include exploring the therapeutic effects of GSK-J1 in <i>in vitro</i> and <i>ex vivo</i> disease models. At least five companies market TNF- α blockers for autoimmune disease and other indications (<i>see Hitting histone demethylases, page 1</i>).	Patent and licensing status undisclosed; available for collaborations	Kruidenier, L. <i>et al. Nature</i> ; published online July 29, 2012; doi:10.1038/nature11262 Contact: David M. Wilson, GlaxoSmithKline plc R&D, Stevenage, U.K. e-mail: david.m.wilson@gsk.com
SciBX 5(31); doi:10.1038/scibx.2012.809 Published online Aug. 9, 2012				
Cancer				
Breast cancer	Chemokine CXC motif ligand 1 (CXCL1; GRO; MGSA); CXCL2 (MIP2); CXC chemokine receptor 2 (CXCR2; IL8RB)	<i>In vitro</i> and mouse studies suggest antagonizing CXCR2 could prevent resistance to chemotherapy and metastasis in breast cancer. In primary human breast cancer and metastasis samples, CXCL1 and CXCL2 amplifications were associated with metastasis and led to overexpression of the cancer survival factors, particularly following chemotherapy. In two mouse models of breast cancer metastasis to the lung, a CXCR2 antagonist plus chemotherapy decreased tumor growth and metastatic burden compared with either treatment alone. Next steps include testing various CXCR2 antagonists with various chemotherapies in preclinical breast cancer models. At least five companies have CXCR2 antagonists in clinical and preclinical development for pulmonary indications or for prevention of transplant rejection (<i>see CXCR2 antagonists in breast cancer, page 6</i>).	Patent application filed; available for licensing	Acharyya, S. <i>et al. Cell</i> ; published online July 6, 2012; doi:10.1016/j.cell.2012.04.042 Contact: Joan Massagué, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: massaguj@mskcc.org
SciBX 5(31); doi:10.1038/scibx.2012.810 Published online Aug. 9, 2012				

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Melanoma	BRAF; MEK	<p><i>In vitro</i> studies suggest MEK inhibitors could help treat melanomas carrying the BRAF L597 mutation. In melanoma tissue samples lacking BRAF V600E and other common oncogenic mutations, genomic sequencing identified mutations in BRAF exon 15 including L597 mutations. In human cells with BRAF L597 mutations, a MEK inhibitor decreased oncogenic signaling compared with no inhibitor. One patient with metastatic melanoma who responded to the MEK inhibitor TAK-733 in a Phase I trial was subsequently shown to carry an L597 mutation in BRAF. Next steps could include testing MEK inhibition in animal models of BRAF L597 mutant melanoma.</p> <p>Takeda Pharmaceutical Co. Ltd. has TAK-733 in Phase I testing for solid tumors.</p> <p>At least six other companies have MEK inhibitors in clinical and preclinical testing to treat various cancers.</p> <p>SciBX 5(31); doi:10.1038/scibx.2012.811 Published online Aug. 9, 2012</p>	Patent and licensing status unavailable	<p>Dahlman, K.B. <i>et al. Cancer Discov.</i>; published online July 13, 2012; doi:10.1158/2159-8290.CD-12-0097 Contact: William Pao, Vanderbilt University School of Medicine, Nashville, Tenn. e-mail: william.pao@vanderbilt.edu</p>
Melanoma	Mdm4 p53 binding protein homolog (MDM4; MDMX)	<p>Mouse and cell culture studies suggest antagonizing MDM4 could help treat melanoma. In a panel of 54 primary cutaneous and metastatic melanomas, MDM4 was overexpressed in about 65% of tumor tissues. In mice, a stapled peptide antagonist of MDM4 decreased tumor growth compared with vehicle. In melanoma cell culture, the stapled peptide increased the anticancer effect of the BRAF inhibitor Zelboraf vemurafenib compared with vehicle. Next steps include improving the pharmacological properties of the stapled peptide and screening for small molecule MDM4 antagonists.</p> <p>Roche, Chugai Pharmaceutical Co. Ltd. and Daiichi Sankyo Co. Ltd. market Zelboraf to treat melanoma. It is also in Phase I testing for colorectal cancer and Phase II testing for thyroid cancer.</p> <p>SciBX 5(31); doi:10.1038/scibx.2012.812 Published online Aug. 9, 2012</p>	Patent pending; available for licensing	<p>Gembarska, A. <i>et al. Nat. Med.</i>; published online July 22, 2012; doi:10.1038/nm.2863 Contact: Jean-Christophe Marine, Center for Human Genetics, Leuven, Belgium e-mail: jeanchristophe.marine@cme.vib-kuleuven.be</p>
Cardiovascular disease				
Cardiomyopathy	Mammalian target of rapamycin complex 1 (mTORC1); lamin A/C (LMNA)	<p><i>In vitro</i> and mouse studies suggest inhibiting mTORC1 could help treat laminopathies including dilated cardiomyopathy. In two independent studies using mouse models of LMNA mutation-associated cardiomyopathy, mTORC1 inhibitors restored autophagy and decreased disease progression and cardiac dilation compared with no treatment or vehicle. Next steps include testing rapamycin or rapamycin analogs in human patients.</p> <p>SciBX 5(31); doi:10.1038/scibx.2012.813 Published online Aug. 9, 2012</p>	<p>Findings in first study unpatented; licensing status not applicable</p> <p>Patent application filed covering findings in second study; available for licensing from Columbia Technology Ventures</p>	<p>Ramos, F.J. <i>et al. Sci. Transl. Med.</i>; published online July 25, 2012; doi:10.1126/scitranslmed.3003802 Contact: Brian K. Kennedy, Buck Institute, Novato, Calif. e-mail: bkennedy@buckinstitute.org Contact: Matt Kaeberlein, University of Washington, Seattle, Wash. e-mail: kaeber@uw.edu</p> <p>Choi, J.C. <i>et al. Sci. Transl. Med.</i>; published online July 25, 2012; doi:10.1126/scitranslmed.3003875 Contact: Howard J. Worman, Columbia University, New York, N.Y. e-mail: hjw14@columbia.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Diabetes	Free fatty acid receptor 1 (FFAR1; GPR40)	Mouse and <i>in vitro</i> studies have identified GPR40 agonists that could help treat type 2 diabetes. <i>In vitro</i> , a mesylpropoxylated derivative of 4-benzyloxydihydrocinnamic acid showed greater potency and specificity for GPR40 than the GPR40 agonist TAK-875. In mice, the lead compound had higher oral bioavailability than TAK-875. Next steps could include additional preclinical testing of lead compounds from this study. Takeda Pharmaceutical Co. Ltd. has TAK-875 in Phase III testing to treat type 2 diabetes. Connexios Life Sciences Pvt. Ltd. has the GPR40 agonists CNX-011-326 and CNX-011-67 in preclinical development for type 2 diabetes. SciBX 5(31); doi:10.1038/scibx.2012.814 Published online Aug. 9, 2012	Patent and licensing status unavailable	Christiansen, E. <i>et al. J. Med. Chem.</i> ; published online June 25, 2012; doi:10.1021/jm3002026 Contact: Trond Ulven, University of Southern Denmark, Odense, Denmark e-mail: ulven@sdu.dk
Endocrine disease	Pyroglutamylated RFamide peptide receptor (QRFP; GPR103)	Mouse and <i>in vitro</i> studies suggest a GPR103 agonist could increase food intake to help treat anorexia. Chemical synthesis and <i>in vitro</i> testing identified a lead peptidomimetic that had more potent GPR103 agonist activity and a longer half-life than a peptide fragment of the receptor's endogenous ligand. In normal mice, the lead peptidomimetic increased food intake during a two-hour period compared with the peptide fragment. Ongoing work includes optimization of the lead peptidomimetic. SciBX 5(31); doi:10.1038/scibx.2012.815 Published online Aug. 9, 2012	Unpatented; available for licensing or partnering	Neveu, C. <i>et al. J. Med. Chem.</i> ; published online July 16, 2012; doi:10.1021/jm300507d Contact: Jérôme Leprince, Institut National de la Santé et de la Recherche Médicale (INSERM), Mont-Saint-Aignan, France e-mail: jerome.leprince@univ-rouen.fr
Infectious disease				
HCV; dengue fever	Phospholipase A ₂ group IVA cytosolic calcium- dependent (PLA ₂ G4A; cPLA2- α)	<i>In vitro</i> studies suggest inhibiting PLA ₂ G4A could help treat HCV infection and dengue fever. In human hepatoma cell-based assays of HCV and dengue viral infectivity, a PLA ₂ G4A inhibitor decreased the production of infectious particles and the intracellular infectivity of each virus compared with no treatment. Planned work includes testing PLA ₂ G4A inhibition in animal models of HCV and/or dengue virus infection. SciBX 5(31); doi:10.1038/scibx.2012.816 Published online Aug. 9, 2012	Patented by Twincore GmbH; available for licensing	Menzel, N. <i>et al. PLoS Pathog.</i> ; published online July 26, 2012; doi:10.1371/journal.ppat.1002829 Contact: Thomas Pietschmann, Twincore GmbH, Hannover, Germany e-mail: thomas.pietschmann@twincore.de
Malaria	Glucose-6- phosphate dehydrogenase (g6pd)	<i>In vitro</i> studies identified <i>Plasmodium falciparum</i> g6pd inhibitors that could help treat malaria. In a high throughput screen of about 350,000 compounds, benzothiazinones were the most potent inhibitors of <i>P. falciparum</i> g6pd, an enzyme required for parasite proliferation and propagation. In culture, the lead compound inhibited growth of both chloroquine-resistant and chloroquine-sensitive <i>P. falciparum</i> strains with subnanomolar IC ₅₀ values. Next steps include testing the compounds <i>in vivo</i> . SciBX 5(31); doi:10.1038/scibx.2012.817 Published online Aug. 9, 2012	Patent application filed covering the lead series; available for licensing	Preuss, J. <i>et al. J. Med. Chem.</i> ; published online July 19, 2012; doi:10.1021/jm300833h Contact: Lars Bode, University of California, San Diego, La Jolla, Calif. e-mail: lbode@ucsd.edu Contact: Anthony B. Pinkerton, Sanford-Burnham Medical Research Institute, La Jolla, Calif. e-mail: apinkerton@sanfordburnham.org Contact: Katja Becker, Justus Liebig University Giessen, Giessen, Germany e-mail: katja.becker@uni-giessen.de

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Inflammation				
Inflammation	<i>APAF1 interacting protein (APIP)</i>	<i>In vitro</i> and genetic sequencing studies suggest reducing <i>APIP</i> expression could help treat systemic inflammatory response syndrome (SIRS), which is a whole-body inflammatory state that can occur in response to infection. In human lymphoblastoid cells, a genetic screen showed that the rs514182 SNP near <i>APIP</i> was associated with decreased expression of the methionine salvage pathway enzyme and increased cell death upon exposure to <i>Salmonella</i> . In patients with SIRS, the SNP was associated with 50% lower death rate than that seen in patients without the SNP. Next steps include developing compounds that modulate methionine salvage pathways. SciBX 5(31); doi:10.1038/scibx.2012.818 Published online Aug. 9, 2012	Findings unpatented; licensing status not applicable	Ko, D.C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 25, 2012; doi:10.1073/pnas.1206701109 Contact: Samuel I. Miller, University of Washington, Seattle, Wash. e-mail: millersi@uw.edu
Neurology				
Alzheimer's disease (AD); cognitive dysfunction	β -Site APP-cleaving enzyme 1 (BACE1); amyloid precursor protein (APP)	Human genetic, cell culture and <i>in vitro</i> assay studies suggest the A673T substitution in <i>APP</i> may protect against AD and normal cognitive decline. In multiple elderly AD and non-AD cohorts, human genetic and cognitive performance studies showed that the A673T mutation in <i>APP</i> was associated with greater conserved cognition than that in noncarriers and suggested the mutation may protect against AD and normal cognitive decline. In an <i>in vitro</i> BACE1 cleavage assay, mutant A673T <i>APP</i> was processed 50% less efficiently than wild-type <i>APP</i> . Next steps could include developing A673T mutant <i>APP</i> mouse models. SciBX 5(31); doi:10.1038/scibx.2012.819 Published online Aug. 9, 2012	Patent and licensing status unavailable	Jonsson, T. <i>et al. Nature</i> ; published online July 11, 2012; doi:10.1038/nature11283 Contact: Kari Stefansson, deCODE genetics ehf, Reykjavik, Iceland e-mail: kstefans@decode.is
Stroke	NMDAR	<i>In vitro</i> and mouse studies have identified a hydrogen sulfide (H ₂ S)-releasing NMDAR antagonist that could help treat stroke. H ₂ S is neuroprotective but can be neurotoxic at higher concentrations due in part to NMDAR activation. In human neuroblastoma cells and murine primary cortical neurons subjected to oxygen-glucose deprivation followed by reoxygenation, S-memantine, a compound that combines a slow-releasing H ₂ S donor and the NMDAR antagonist memantine, increased viability compared with either parent compound alone. In a mouse model of global cerebral ischemia and reperfusion, S-memantine decreased infarct volume and increased survival and neurological function compared with either parent compound. Next steps include synthesizing more candidate compounds. Forest Laboratories Inc., H. Lundbeck A/S, Daiichi Sankyo Co. Ltd. and Merz GmbH & Co. KGaA market Axura memantine to treat Alzheimer's disease (AD). SciBX 5(31); doi:10.1038/scibx.2012.820 Published online Aug. 9, 2012	Patent application filed covering new compound; available for licensing	Marutani, E. <i>et al. J. Biol. Chem.</i> ; published online July 19, 2012; doi:10.1074/jbc.M112.374124 Contact: Fumito Ichinose, Massachusetts General Hospital, Charlestown, Mass. e-mail: fichinose@partners.org

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Ophthalmic disease				
Blindness	<i>Nicotinamide nucleotide adenylyltransferase 1 (NMNAT1)</i>	Studies with human tissues identified multiple <i>NMNAT1</i> mutations that could help diagnose Leber's congenital amaurosis (LCA). Previous <i>Drosophila</i> studies have shown that <i>Nmnat1</i> deficiency leads to degeneration of photoreceptor cells. In four independent studies, genomics analyses identified associations between multiple <i>NMNAT1</i> SNPs and LCA in patients who did not harbor mutations in known LCA-associated genes. In fibroblasts and red blood cells from the patients with LCA, functional analyses showed that the <i>NMNAT1</i> SNPs restricted the protein's enzymatic activity. Ongoing work includes further investigations of the effects of the SNPs on NMNAT1 function and the role of the mutant proteins in LCA. SciBX 5(31); doi:10.1038/scibx.2012.821 Published online Aug. 9, 2012	Patent and licensing status unavailable for findings in first and second studies	Koenekoop, R.K. <i>et al. Nat. Genet.</i> ; published online July 29, 2012; doi:10.1038/ng.2356 Contact: Rui Chen, Baylor College of Medicine, Houston, Texas e-mail: ruichen@bcm.edu
			Findings in third study unpatented and available for partnering	Perrault, I. <i>et al. Nat. Genet.</i> ; published online July 29, 2012; doi:10.1038/ng.2357 Contact: Josseline Kaplan, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France e-mail: josseline.kaplan@inserm.fr Contact: Jean-Michel Rozet, same affiliation as above e-mail: jean-michel.rozet@inserm.fr
			For findings in fourth study, patent application filed in China by BGI; available for licensing and partnering	Falk, M.J. <i>et al. Nat. Genet.</i> ; published online July 29, 2012; doi:10.1038/ng.2361 Contact: Eric A. Pierce, Massachusetts Eye and Ear Infirmary, Boston, Mass. e-mail: eric_pierce@meei.harvard.edu
				Chiang, P.-W. <i>et al. Nat. Genet.</i> ; published online July 29, 2012; doi:10.1038/ng.2370 Contact: Ming Qi, Zhejiang University School of Medicine, Hangzhou, China e-mail: ming_qi@urmc.rochester.edu
Blindness; retinitis	Unknown	Mouse studies suggest a photosensitive compound could help treat retinitis pigmentosa (RP). In retinas from mouse models of RP, the compound induced differing levels of retinal ganglion cell (RGC) firing in response to near-UV and blue-green light, whereas no wavelength of light induced RGC firing in untreated controls. In the mouse models, intraocular injection of the compound increased both the response of pupils to light and light avoidance behaviors compared with no treatment. Future studies could include investigating the compound's effect on visual acuity in mouse models of RP. SciBX 5(31); doi:10.1038/scibx.2012.822 Published online Aug. 9, 2012	Patent and licensing status unavailable	Polosukhina, A. <i>et al. Neuron</i> ; published online July 26, 2012; doi:10.1016/j.neuron.2012.05.022 Contact: Richard H. Kramer, University of California, Berkeley, Calif. e-mail: rhkramer@berkeley.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Other				
Cushing's disease	Cytochrome P450 family 11 subfamily B polypeptide 1 (CYP11B1)	<i>In vitro</i> studies identified CYP11B1 inhibitors that could help treat Cushing's disease. In hamster fibroblasts expressing human CYP11B1, a cyclopropyl-based inhibitor derived from three reference compounds showed greater potency and selectivity than the reference compounds and Metopirone metyrapone, which is known to inhibit CYP11B1. In cell-based assays, the compound inhibited both human and rat CYP11B1. Next steps could include testing the compound in disease models. Novartis AG markets Metopirone, an inhibitor of endogenous adrenal corticosteroid synthesis, to treat Cushing's disease. SciBX 5(31); doi:10.1038/scibx.2012.823 Published online Aug. 9, 2012	Patent and licensing status unavailable	Yin, L. <i>et al. J. Med. Chem.</i> ; published online July 12, 2012; doi:10.1021/jm3003872 Contact: Rolf W. Hartmann, Saarland University and Helmholtz Institute for Pharmaceutical Research Saarland, Saarbruecken, Germany e-mail: rolf.hartmann@helmholtz-hzi.de Contact: Qingzhong Hu, same affiliation as above e-mail: q.hu@mx.uni-saarland.de
Various				
Cancer; thrombosis	Not applicable	<i>In vitro</i> and mouse studies suggest preventing formation of neutrophil extracellular traps (NETs) could help prevent thrombosis in patients with cancer. In neutrophils isolated from three different mouse models of cancer, stimulation with a platelet-activating factor generated more thrombosis-inducing NETs than stimulation in neutrophils isolated from control mice. In a mouse model of breast cancer, NETs were associated with the formation of pulmonary thromboses. In this model, lipopolysaccharide (LPS) stimulation resulted in greater NET production than that in tumor-free mice and induced a prothrombotic state. Next steps include screening for therapeutics with anti-NET effects. SciBX 5(31); doi:10.1038/scibx.2012.824 Published online Aug. 9, 2012	Patent application filed; available for licensing	Demers, M. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 23, 2012; doi:10.1073/pnas.1200419109 Contact: Denisa D. Wagner, Immune Disease Institute, Boston, Mass. e-mail: wagner@idi.harvard.edu

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			
Impact of receptor tyrosine kinase (RTK) ligands on sensitivity of human tumor-derived cell lines to kinase inhibitors	<p>Analysis of the impact of RTK ligands on sensitivity of human tumor-derived cell lines to kinase inhibitors could guide combination therapy to prevent drug resistance. In about half of <i>BRAF</i> mutant melanoma cell lines, the RTK ligand hepatocyte growth factor/scatter factor (HGF/SF) induced c-Met proto-oncogene (MET; HGFR) expression and resistance to the <i>BRAF</i> inhibitor Zelboraf vemurafenib. In these cell lines, co-treatment with the MET kinase inhibitor Xalkori crizotinib prevented resistance to Zelboraf. In patients with <i>BRAF</i> mutant melanoma, increased plasma HGF/SF levels correlated with poorer survival outcomes than normal HGF/SF levels. Next steps include analyzing the effects of RTK ligands on larger numbers of kinase-activated, human tumor-derived cell lines.</p> <p>Zelboraf, from Daiichi Sankyo Co. Ltd., Chugai Pharmaceutical Co. Ltd. and Roche, is marketed to treat melanoma, in Phase I testing for colorectal cancer and in Phase II testing for thyroid cancer. Xalkori from Pfizer Inc., which also inhibits anaplastic lymphoma kinase (ALK), is marketed to treat non-small cell lung cancer (NSCLC) and is in Phase I testing to treat solid tumors.</p> <p>SciBX 5(31); doi:10.1038/scibx.2012.825 Published online Aug. 9, 2012</p>	Patent and licensing status undisclosed	<p>Wilson, T.R. <i>et al. Nature</i>; published online July 4, 2012; doi:10.1038/nature11249</p> <p>Contact: Jeffrey Settleman, Genentech Inc., South San Francisco, Calif. e-mail: settleman.jeffrey@gene.com</p>
Stromal and cancer cell coculture system to uncover drug resistance mechanisms and guide combination therapy	<p>A stromal and cancer cell coculture system could help uncover drug resistance mechanisms and guide combination therapy. In cocultures of <i>BRAF</i> mutant melanoma cell lines and human stromal cell lines, stromal cell secretion of hepatocyte growth factor/scatter factor (HGF/SF) led to c-Met proto-oncogene (MET; HGFR) activation and resistance to <i>BRAF</i> inhibition. In the cocultures, HGF/SF-neutralizing antibodies or the MET inhibitor Xalkori crizotinib could block this resistance. In patients with <i>BRAF</i> mutant melanoma, HGF/SF secretion from stromal cells was associated with poorer response to <i>BRAF</i> inhibitors ($p < 0.05$) than if there was no secretion. Next steps could include using the coculture system to uncover mechanisms of drug resistance in additional cancers.</p> <p>Roche, Daiichi Sankyo Co. Ltd. and Chugai Pharmaceutical Co. Ltd. market the <i>BRAF</i> inhibitor Zelboraf vemurafenib for <i>BRAF</i> mutant melanoma. The inhibitor is also in Phase I testing for colorectal cancer and Phase II testing for thyroid cancer.</p> <p>Pfizer Inc. markets Xalkori, which also inhibits anaplastic lymphoma kinase (ALK), for non-small cell lung cancer (NSCLC). It is also in Phase I testing to treat solid tumors.</p> <p>At least three companies have mAb antagonists of HGF/SF in clinical testing in cancer.</p> <p>SciBX 5(31); doi:10.1038/scibx.2012.826 Published online Aug. 9, 2012</p>	Patent application filed covering combination <i>BRAF</i> and HGF/SF inhibition; licensing status undisclosed	<p>Straussman, R. <i>et al. Nature</i>; published online July 4, 2012; doi:10.1038/nature11183</p> <p>Contact: Todd R. Golub, Broad Institute of MIT and Harvard, Cambridge, Mass. e-mail: golub@broadinstitute.org</p>

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug delivery			
Intracellular delivery of therapeutic small interfering RNA with carbonate co-oligomers	Carbonate co-oligomers for intracellular delivery of therapeutic siRNA could help treat a range of diseases. <i>In vitro</i> , co-oligomers of guanidinium-rich carbonate monomers readily formed stable, noncovalent complexes with siRNA. In a human keratinocyte cell line transfected with vectors encoding fluorescent proteins, several co-oligomer–siRNA complexes selectively decreased fluorescent protein expression compared with free siRNA or complexes delivering scrambled siRNA. Ongoing work includes developing the technology for intradermal and/or topical delivery of therapeutic siRNA to treat undisclosed diseases. SciBX 5(31); doi:10.1038/scibx.2012.827 Published online Aug. 9, 2012	Patented by Stanford University; licensing status undisclosed	Geihe, E.L. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online July 30, 2012; doi:10.1073/pnas.1211361109 Contact: Paul A. Wender, Stanford University, Stanford, Calif. e-mail: wenderp@stanford.edu
Nanoparticle vaccines targeted to the large intestine	Nanoparticle vaccines targeted to the large intestine could help protect against mucosal infections. Poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulating vaccinia virus antigens and adjuvants were coated with a methacrylate-based polymer to facilitate delivery of the vaccine to the large intestine and prevent premature uptake in the small intestine. In mice, oral delivery of the methacrylate-coated nanoparticle vaccines protected against intestinal and vaginal mucosal vaccinia virus challenge and induced both CD8 ⁺ T cell responses and vaccinia virus-specific antibody responses. Oral delivery of uncoated nanoparticle vaccines or nanoparticles that release the vaccine in the small intestine failed to confer protection and trigger an immune response. Next steps could include testing the delivery method with other vaccines. SciBX 5(31); doi:10.1038/scibx.2012.828 Published online Aug. 9, 2012	Patent and licensing status unavailable	Zhu, Q. <i>et al. Nat. Med.</i> ; published online July 15, 2012; doi:10.1038/nm.2866 Contact: Jay A. Berzofsky, National Cancer Institute, Bethesda, Md. e-mail: berzofsk@helix.nih.gov
Systemic delivery of antisense oligonucleotide therapy for myotonic dystrophy type 1 (DM1)	A study in mice suggests subcutaneous injection of an antisense oligonucleotide could help treat DM1. In a mouse model of DM1, subcutaneous injection of antisense oligonucleotide targeting sequences derived from DM1-associated mutant dystrophin myotonia protein kinase (DMPK; DM1) led to lower accumulation of nuclear transcripts than subcutaneous injection of saline. Antisense oligonucleotide-treated mice had better muscle function in a variety of muscle groups than saline-treated controls. Next steps include selection of a preclinical candidate that maximizes mutant DMPK1 knockdown in other mouse models of DM1. Partners Isis Pharmaceuticals Inc. and Biogen Idec Inc. have compounds related to this study in discovery-stage development for DM1. Prosensa B.V. has antisense oligonucleotides targeting DMPK in preclinical development for DM1 (<i>see Isis takes on myotonic dystrophy, page 4</i>). SciBX 5(31); doi:10.1038/scibx.2012.829 Published online Aug. 9, 2012	Patents related to this technology held by Isis Pharmaceuticals; unavailable for licensing	Wheeler, T.M. <i>et al. Nature</i> ; published online Aug. 2, 2012; doi:10.1038/nature11362 Contact: Charles A. Thornton, University of Rochester, Rochester, N.Y. e-mail: charles_thornton@urmc.rochester.edu

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