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By *Chris Cain, Senior Writer*

Over the last decade, numerous compounds that activate glucokinase have advanced into the clinic to treat diabetes, but the approach has faced safety challenges because of, amongst other issues, high rates of adverse hypoglycemic events. Now, researchers from **Amgen Inc.** have identified compounds that instead target glucokinase regulator, opening the door to a potentially safer strategy for modulating hepatic glucokinase activity.¹

Glucokinase (GCK; GK) catalyzes the conversion of glucose into glucose-6-phosphate, the first step of glycolysis, and is a key regulatory component for maintaining homeostatic levels of glucose in humans. Most of the enzyme is concentrated in the liver, but it also plays a key role in glucose sensing in the pancreas.

Since **Roche** scientists described the first allosteric GK activator a decade ago,² diverse classes of compounds have been synthesized and advanced into the clinic as therapies to reduce blood glucose levels in diabetes.³

Although activating GK has been clearly shown to reduce blood glucose levels in patients with diabetes, controlling the effect so as not to tip the balance and cause adverse hypoglycemic events has turned out to be a tall order.⁴

To address this, companies have designed compounds that only partially agonize GK or have increased selectivity for the liver. A liver-selective GK activator was shown to reduce the occurrence of hypoglycemia in animal models.⁵ At least eight GK activators remain in the clinic (*see Table 1, “Glucokinase activators in clinical development”*).

Now, Amgen has taken a different tack to address the problem by targeting not GK but glucokinase regulator (GCKR; GKR), a GK regulatory subunit present in the liver.

GKR inhibits GK in hepatocytes by sequestering the protein in an inactive complex in the nucleus—disrupting the GKR-GK interaction relieves this sequestration. This process is naturally regulated by sugars such as fructose-6-phosphate and fructose-1-phosphate, which bind GKR and strengthen or weaken its interaction with GK, respectively.

To tap into this regulatory mechanism, Amgen scientists performed an *in vitro* screen to identify compounds that could disrupt the GK-GKR interaction and then eliminated compounds that bound GK directly. This led to the identification of a thiophene sulphanomidopiperazine, AMG-1694, which was further optimized to bind GKR and promote GK-GKR dissociation at a concentration of 7 nM.

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Crystal structures of AMG-1694 in complex with GKR and sorbitol-6-phosphate, which stabilizes the complex, showed that it bound to a site on the protein distinct from the GK-GKR interface or its sugar-binding site.

David Lloyd, a principal scientist at Amgen and a corresponding author on the paper, told *SciBX* that identifying this compound was a challenge. "It's important to note that the hit rate for the screen was extremely low," he said. "In fact, we only obtained two weak hits in the related screen. Careful biochemical follow-up determined they bound to GKR, and this was indeed a surprise. It was at this point we realized we had something different." Amgen principal scientist Clarence Hale was also a corresponding author on the paper.

In liver sections taken from normal or diabetic rats dosed with AMG-1694, GK was dose-dependently translocated out of the nucleus and blood glucose levels were reduced only in the diabetic rats, whereas both normal and diabetic rats dosed with a direct activator of GK had reductions in blood glucose.

To test the effect of GKR disruption in mouse models, a related compound with improved pharmacokinetics in mice, AMG-3969, was synthesized. In three mouse models of obesity, the compound dose-dependently reduced blood glucose concentration, but it had no effect in normal mice. It also did not affect levels of triglycerides, which was a concern because human genetic studies have suggested that inhibiting GKR may raise triglyceride levels.

Results were published in *Nature*.

Leveraging the liver

Although a thorough mechanistic explanation for how the GKR-binding compounds lower blood glucose remains to be elucidated, their liver-specific, high blood glucose concentration-dependent activity sets them apart from GK activators.

Brian Miller, an associate professor of chemistry at **Florida State University**, told *SciBX*, "In principle, targeting GKR is advantageous because to the best of our knowledge GKR-mediated regulation of GK is exclusive to the liver. As such, a GKR therapeutic would solve the liver specificity issues that are currently haunting the remaining GK activator programs."

He added, "Therapeutic intervention in GK activity by targeting GKR is not expected to impact the cooperativity of GK kinetics, which is quite important in whole-body glucose homeostasis. All the direct GK activators reduce or eliminate this important functional characteristic of GK."

Amgen's Lloyd emphasized that targeting GKR enhances GK activity only at high glucose concentrations and not when glucose levels are normal. "A crucial difference between a GKR disruptor and a liver-specific GK [activator] is that the GKR [disruptor] promotes the actions of GK in the physiological range of glucose actions," he said.

"A crucial difference between a GKR disruptor and a liver-specific GK [activator] is that the GKR [disruptor] promotes the actions of GK in the physiological range of glucose actions."

—David Lloyd, Amgen Inc.

Earlier this year, Miller published the crystal structure of the mammalian GK-GKRP complex.⁶ Also this year, **Boehringer Ingelheim GmbH** published the structure of inactive GKRP bound to fructose-6-phosphate.⁷ **Boehringer Ingelheim** has not disclosed any programs targeting GKRP.

Matthew Vander Heiden, an assistant professor of biology at the Koch Institute for Integrative Cancer Research at the **Massachusetts Institute of Technology**, told *SciBX* that he was impressed that the researchers identified a compound that potently disrupts a protein-protein interaction. “Everyone says, ‘Well, it’s a protein-protein interaction, so it’s not druggable’, but this is an example that shows that is not always the case.”

He added that he wanted to see more detail of how the compound disrupts GK-GKRP interaction. “If a small molecule can do this, it is suggestive that an endogenous molecule could also engage this mechanism.”

Lloyd said that working to understand this mechanism is among his team’s next steps. “We are very interested in how the GKRP disruptors have altered or restored

the metabolic physiology of the liver. Along these lines, we wish to investigate the outcome of hepatic glucose with these compounds. We are also interested in the structural release of GK.”

Amgen spokesperson Kristen Davis said that this is an early research-stage program and did not disclose further development plans. The company has filed a patent covering GKRP-binding compounds and did not disclose their availability for partnering.

“In principle, targeting GKRP is advantageous because to the best of our knowledge GKRP-mediated regulation of GK is exclusive to the liver.”

—**Brian Miller,**
Florida State University

Table 1. Glucokinase activators in clinical development. Despite the discontinuation of some programs from large pharmas, including **Merck & Co. Inc.** (NYSE:MRK), **Takeda Pharmaceutical Co. Ltd.** (Tokyo:4502), **AstraZeneca plc** (LSE:AZN; NYSE:AZN) and **Roche** (SIX:ROG; OTCQX:RHHBY), at least eight glucokinase activators remain in clinical development.

Source: *BCIQ: BioCentury Online Intelligence*

Company	Product	Development
Advinus Therapeutics Ltd.	GKM-001	Phase I/II
Array BioPharma Inc. (NASDAQ:ARRY)	ARRY-403 (formerly AMG151) ^A	Phase II
Daiichi Sankyo Co. Ltd. (Tokyo:4568)	DS-7309	Phase I
Pfizer Inc. (NYSE:PFE)	PF-04937319	Phase II
Roche	HMS5552 (formerly RO5305552)	Phase I
Teijin Pharma Ltd.	TMG-123	Phase I
TransTech Pharma Inc.	GKI-399 (TTP399)	Phase I/II
Zyus Cadila Group (NSE:CADILAH; BSE:532321)	ZYGK1	Phase I

^AThis year, **Amgen Inc.** (NASDAQ:AMGN) returned all rights to AMG151 to Array BioPharma.

In August, Amgen discontinued development of its clinical-stage GK activator, ARRY-403 (formerly AMG151) and returned rights to partner **Array BioPharma Inc.**

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Array BioPharma Inc. (NASDAQ:ARRY), Boulder, Colo.
Boehringer Ingelheim GmbH, Ingelheim, Germany
Florida State University, Tallahassee, Fla.
Massachusetts Institute of Technology, Cambridge, Mass.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland

Riding the integrin wave in thrombosis

By Benjamin Boettner, Associate Editor

The use of platelet integrin $\alpha_{2b}\beta_3$ inhibitors in thrombosis, while clinically very effective, is associated with a high risk of bleeding that has limited their use. Now, researchers at the **University of Illinois at Chicago** have applied their new insights into the integrin-signaling mechanism to develop an integrin $\alpha_{2b}\beta_3$ inhibitor that suppresses arterial thrombosis without triggering bleeding in mice.¹

Next, the team will assess the inhibitor's therapeutic efficacy, toxicity and pharmacological profiles in animal models of thrombosis.

Integrin $\alpha_{2b}\beta_3$ (GPIIb/IIIa; CD41/CD61) is a transmembrane adhesion protein expressed on the surface of platelets. Following injury, it is activated by thrombin, which makes it accessible to clotting factors such as fibrinogen and von Willebrand factor (vWF). This results in platelet adhesion to the blood vessel, followed by platelet aggregation and ultimately thrombus formation.

Because of its key role in platelet adhesion and aggregation, integrin $\alpha_{2b}\beta_3$ has long been considered a prime target for disrupting thrombus formation. Indeed, there are three inhibitors on the market: **Johnson & Johnson** markets the mAb ReoPro abciximab to treat angioplasty and stroke and to accompany percutaneous coronary interventions, and **Merck & Co. Inc.** sells Integrilin eptifibatid, a cyclic heptapeptide derived from

a snake venom protein, and the nonpeptide antagonist Aggrastat tirofiban. Both Merck drugs are marketed to treat coronary arterial indications and as a companion therapeutic for percutaneous coronary intervention.

Although the inhibitors are routinely used to prevent thrombosis during coronary interventions, and about 8 million patients worldwide have been treated with these inhibitors,² the elevated bleeding risk they carry has limited their broader application.

The high bleeding risk results from inhibition of both platelet-vessel adhesion and platelet-platelet aggregation at wound sites.

Xiaoping Du, a principal investigator in the Department of Pharmacology at the University of Illinois at Chicago, and colleagues at the university have developed an integrin $\alpha_{2b}\beta_3$ inhibitor that does not cause an increase in bleeding risk. Instead of focusing on preventing integrin $\alpha_{2b}\beta_3$ activation altogether, the team used its insights into the regulation of integrin $\alpha_{2b}\beta_3$ signaling to develop an inhibitor that targeted platelet aggregation but not the initial platelet adhesion to the blood vessel wall.

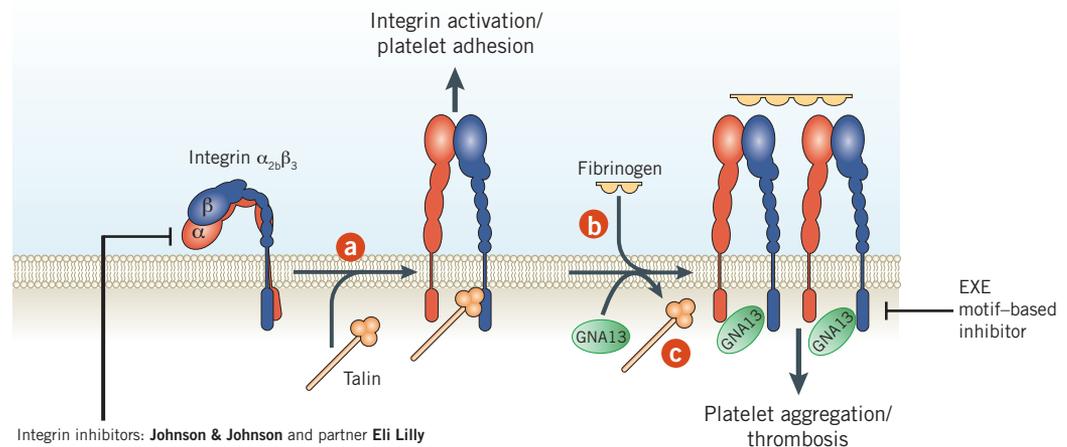
Combining biochemical and genetic approaches, the team first worked out the sequence of regulatory events guiding integrin $\alpha_{2b}\beta_3$ activity during clotting.

“Given its functionally selective action on integrin, the approach can be seen as the holy grail in the search for an antithrombotic drug.”

— Edward Plow,
Lerner Research Institute
at the Cleveland Clinic

Figure 1. Selective inhibition of integrin $\alpha_{2b}\beta_3$ in thrombosis.

Findings published in *Nature* reveal two waves in integrin $\alpha_{2b}\beta_3$ (GPIIb/IIIa; CD41/CD61) regulation associated with distinct phases of the blood clotting process. During integrin activation by thrombin [a], talin binds to a region of integrin $\alpha_{2b}\beta_3$ that contains an EXE motif. This first regulatory wave triggers platelet adhesion to blood vessels. Following activation, integrin $\alpha_{2b}\beta_3$ becomes accessible to clotting factors such as fibrinogen [b]. This second wave is associated with platelet aggregation. During platelet aggregation, integrin $\alpha_{2b}\beta_3$ -fibrinogen complexes are maintained but talin is displaced by guanine nucleotide binding protein (G protein) α_3 (GNA13) [c]. Inhibiting GNA13 binding with a competitive, membrane-tethered, EXE motif-based peptide prevents platelet aggregation and thrombosis without increasing the risk of bleeding. Marketed integrin $\alpha_{2b}\beta_3$ inhibitors interfere with ligand binding and formation of integrin $\alpha_{2b}\beta_3$ -fibrinogen complexes. (Figure based on Box 2 in Shattil, S.J. *et al.*, *Nat. Rev. Mol. Cell Biol.* **11**, 288–300; 2010.)



Integrin inhibitors: **Johnson & Johnson** and partner **Eli Lilly and Co.**'s mAb ReoPro abciximab, which targets integrin $\alpha_{2b}\beta_3$ (CD41/CD61) and integrin $\alpha_{2b}\beta_3$, is marketed to treat angioplasty and to accompany percutaneous coronary interventions and is in Phase II to treat stroke. **Merck & Co. Inc.** markets Integrilin eptifibatid, a cyclic heptapeptide derived from a snake venom protein, and the nonpeptide antagonist Aggrastat tirofiban to treat coronary arterial indications and as a companion therapeutic for percutaneous coronary intervention. Both drugs target integrin $\alpha_{2b}\beta_3$.

In a first wave of signaling, integrin $\alpha_{2b}\beta_3$ is activated by the talin adaptor protein, which binds to the integrin β_3 (GPIIIa; CD61) moiety of the protein complex and triggers platelet adhesion. In a second wave, another adaptor protein, guanine nucleotide binding protein (G protein) α_{13} (GNA13), replaces talin on the integrin β_3 moiety. This step is associated with platelet aggregation (see Figure 1, “Selective inhibition of integrin $\alpha_{2b}\beta_3$ in thrombosis”).

Du and his team identified a binding motif on the C terminus of integrin β_3 , the EXE motif, that mediated GNA13 binding, leading to the hypothesis that a synthetic peptide containing the EXE motif could selectively disrupt GNA13 binding and, thus, platelet aggregation.

In vitro, a membrane-tethered hexapeptide containing the EXE motif blocked integrin spreading on fibrinogen. In mouse models of both laser-induced, arteriolar and FeCl₃-induced, occlusive carotid artery thrombosis, the peptide was as effective as Integrilin in preventing thrombus formation but did so without triggering any bleeding.

“We believe that this type of antithrombotic agent may represent the next generation of antithrombotic therapy, which could not only be very effective but also safer,” Du said.

Edward Plow, chair of the Department of Molecular Cardiology at the **Lerner Research Institute at the Cleveland Clinic**, told *SciBX* that “given its functionally selective action on integrin, the approach can be seen as the holy grail in the search for an antithrombotic drug.”

Results were published in *Nature*.

Breaking the wave

Although the selective inhibitors described by Du and his team provide a potential boon for targeting integrin in thrombosis and other related indications, further development will hinge on their safety profiles and their efficacy vis-à-vis other well-established antithrombotic therapeutics.

According to Du, “The use of the inhibitor described in our study may be expanded to indications like stroke, where hemorrhage is as much of a risk as thrombosis. Thus, our inhibitor may have additional therapeutic benefits.”

Plow, however, cautioned that “so far the inhibitor’s effects have only been shown in mice. Earlier $\alpha_{2b}\beta_3$ integrin antagonists also looked good in initial animal models, and only in clinical studies did bleeding become obvious.”

He added that evaluation of the candidates in additional mouse models of thrombosis using larger experimental groups, and then in canine and primate models, will provide answers to this question.

In addition, it will be important to assess the EXE motif-based inhibitor’s toxicity profile, including on- and off-target toxicity and other side effects, and measure its pharmacological properties in preclinical models, Du said.

“The use of the inhibitor described in our study may be expanded to indications like stroke, where hemorrhage is as much of a risk as thrombosis. Thus, our inhibitor may have additional therapeutic benefits.”

—Xiaoping Du,
University of Illinois at Chicago

Du told *SciBX* that his team has prioritized such follow-up studies. “It is important to determine how other β integrin subunits will be impacted by the inhibitor *in vivo*. Indeed, the study showed that the EXE motif is present also in the related integrin β_{1a} , β_{1d} , β_5 , β_6 and β_7 subunits, which may lead to the suppression of other integrin-related processes outside of the platelet compartment,” he said.

Du added that inhibition of more than one integrin complex may be beneficial for treating thrombotic conditions and for reducing inflammation associated with thrombosis.

“Many substances released from platelets during coagulation are proinflammatory and, conversely, activated leukocytes and neutrophil extracellular traps promote thrombus formation. Leukocyte integrin signaling is critically important for inflammation,” said Du.

With regard to the clinical potential of the new inhibitor, Plow said, “The factors ultimately deciding the inhibitor’s fate will be its clinical potential in comparison with antithrombotic therapeutics that presently dominate the market, including P2Y₁₂ antagonists, and whether they can be produced economically as is the case for P2Y₁₂ antagonists.”

Sanofi markets Plavix/Iscover clopidogrel acetylsalicylic acid, a P2Y₁₂ (adenosine diphosphate receptor) antagonist, to treat cardiovascular indications and stroke. **Boehringer Ingelheim GmbH** markets Aggrenox, a modified-release aspirin plus dipyridamole, for the same indications. Also to treat thrombosis, **Bayer AG**, **The Medicines Co.** and **Mitsubishi Tanabe Pharma Corp.** market the thrombin inhibitors Recludan lepirudin, Angiomax bivalirudin and Arganova argatroban, respectively, and **Eisai Co. Ltd.** markets Warfarin.

Du said that a patent application covering the EXE motif-based inhibitor has been filed in the U.S. and Europe by the University of Illinois at Chicago, and the team is looking for partners that can facilitate the development of the inhibitors.

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Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Mitsubishi Tanabe Pharma Corp. (Tokyo:4508), Osaka, Japan
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
University of Illinois at Chicago, Chicago, Ill.

Getting to the root of periodontitis

By Michael J. Haas, Senior Writer

Current periodontitis therapies do not target the underlying immune response that drives the disease. A team from the U.S. and Brazil is looking to change that and has shown that microparticles loaded with chemokine CC motif ligand 22 recruited T_{reg} cells to the gingiva and slowed periodontitis progression in mice and dogs.¹

Future studies will need to determine whether the microparticle-based therapy can help treat patients with more advanced disease who already have substantial loss of tooth-supporting alveolar bone.

Periodontitis results from a chronic inflammatory response to bacterial plaque and tartar deposits on the teeth, and the condition damages the gingiva (gums) and alveolar bone, leading to tooth loss. Treatment involves lengthy scaling and root-planing procedures to remove the deposits and may include local use of antibiotics to kill the bacteria and/or systemic treatment with low doses of the generic antibiotic doxycycline to inhibit tissue-damaging matrix metalloproteinases (MMPs) in the gingiva.

In devising a strategy to actually modulate the disease, a team led by Steven Little connected two key pieces of information. The first was a series of studies in patients with periodontitis²⁻⁴ and disease models⁵ that showed that insufficient numbers of T_{reg} cells in the gingiva may enable the chronic inflammation that drives disease progression.

The second piece came from a study that showed that chemokine CC motif ligand 22 (CCL22) secreted by ovarian tumors recruited T_{reg} cells to the tumor microenvironment, thus enabling immune evasion by the tumors.⁶

Little and colleagues put the two pieces together and hypothesized that tissue-specific delivery of CCL22 could recruit T_{reg} cells and help treat diseases involving destructive inflammation.

Last year, Little's team took the first step in investigating this hypothesis by showing that poly(lactic-co-glycolic) acid (PLGA) microparticles loaded with mouse Ccl22 released their cargo and recruited T_{reg} cells to the injection site in the hind leg muscles of normal mice.⁷

The microparticles did not migrate far from the injection site because they were too large to be taken up by phagocytic cells or cross the vascular endothelium.

Now, Little's team has tested the PLGA-based technology in animal models of periodontitis. "It is the most prevalent disease of destructive inflammation, and it has a massive market size, making it an obvious target," he told *SciBX*.

Little is chair of chemical and petroleum engineering and a faculty fellow in the Department of Bioengineering and Department of Immunology at the **University of Pittsburgh**. He also is a fellow at the university's **McGowan Institute for Regenerative Medicine** and cofounder of **Qrono Inc.**, a company that develops long-acting,

injectable formulations of drugs. Qrono was not involved in the PLGA studies.

In mouse models of periodontitis, saline solution of the Ccl22-loaded PLGA microparticles injected into the gingiva recruited T_{reg} cells, thereby lowering levels of proinflammatory cytokines and MMPs and decreasing alveolar bone loss compared with injections of empty microparticles.

The team also delivered the microparticles as a dry powder to subgingival pockets—spaces between the gums and teeth that deepen as periodontitis progresses—in dog models of the disease. That mode of administration would be simpler and less painful than injection and thus more likely to be used in the clinic.

The dry powder microparticles decreased pocket depth, gingival inflammation and alveolar bone loss compared with empty microparticles.

The powder was well retained in the subgingival pockets and did not dissipate when the dogs ate or drank, which Little said was probably because saliva hydrated the powder and allowed it to swell and remain in place.

Collectively, the findings suggest that CCL22-loaded microparticles applied as dry powder to subgingival pockets could treat periodontitis in patients, Little said. "We expect that this would most likely be given as an adjunct to normal scaling and root-planing procedures that are performed by a clinician

during regular maintenance of the disease," he added.

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

The team included Gustavo Garlet, an associate professor of biological sciences at the **University of São Paulo's** School of Dentistry.

Back to the bone

Little said that his team has run safety studies of the CCL22-loaded microparticles in an undisclosed animal model and observed no side effects, even at a dose 40-fold higher than that required for a therapeutic effect. Ongoing work includes testing the microparticles in a second, undisclosed model.

"Since periodontal tissue destruction is ultimately inflicted by the host inflammatory response, a therapy that effectively modulates that response should have advantages over the current standard of care," said George Hajishengallis, professor of microbiology at the **University of Pennsylvania's** School of Dental Medicine. "Local, topical delivery of CCL22-loaded microparticles could be a promising approach."

He added that "complete inhibition of inflammation may not be required, as the homeostatic mechanisms of the host may operate effectively when excessive inflammation is under control" as a result of the therapy.

Hajishengallis agreed with Little that the microparticles were likely to complement, not replace, the current standard of care in periodontitis because removing bacterial plaques remains a critical aspect of treatment.

"Bacteria cause inflammation, which fosters more bacterial growth, which in turn can cause even more inflammation," said Hajishengallis. "The whole thing is a vicious cycle."

"Local, topical delivery of CCL22-loaded microparticles could be a promising approach."

— George Hajishengallis,
University of Pennsylvania

Hajishengallis thinks that the microparticles could deliver other host-modulating therapeutics to treat periodontitis, including regulatory chemokines, lipoxins and resolvins, complement inhibitors and integrin antagonists such as EGF-like repeats and discoidin I-like domains 3 (DEL1; EDIL3).^{8,9}

However, Takuro Yuge, senior manager of R&D strategic planning at **Kaken Pharmaceutical Co. Ltd.**, thinks that the microparticle technology would have to be supplemented.

“Host immune responses are important drivers of periodontitis, and the recruitment of T_{reg} cells by CCL22 may be effective at preventing the production of proinflammatory cytokines in the periodontal tissues that initiate alveolar bone resorption,” he said. “But most patients with periodontitis already have alveolar bone loss and require therapies that promote bone formation, not just prevent its resorption.”

“Regulation of homeostasis with T_{reg} cells may not be enough to increase bone formation in patients with significant bone deterioration,” said Katsumi Nogimori, Kaken’s executive corporate officer and deputy chief officer of R&D.

Kaken’s KCB-1D is in Phase III testing to treat periodontitis. The company has not disclosed the target or therapeutic modality of the therapy.

But Little disagreed, noting that his team’s mouse model studies showed that the Ccl22-loaded microparticles upregulated multiple factors involved in bone growth and regeneration, including bone morphogenetic protein 4 (Bmp4), Bmp6, transforming growth factor- β (Tgfb; Tgfb β), collagen type I α 2 (Col1a2) and several osteoblast transcription factors.

“I think that these findings suggest that recruiting T_{reg} cells promotes not only a prohomeostatic environment but a proregenerative one as well,” he said. “Thus, our treatments could indeed promote bone regeneration in patients who already have significant alveolar bone loss.”

The team has not yet tested that possibility because it would require a lengthy and expensive preclinical study, he said. “We aren’t ruling out

“Regulation of homeostasis with T_{reg} cells may not be enough to increase bone formation in patients with significant bone deterioration.”

**—Katsumi Nogimori,
Kaken Pharmaceutical Co. Ltd.**

this kind of study,” but the team is currently considering the best way to translate the technology to the clinic.

Little said that the team also is testing the technology in models of other diseases that involve destructive inflammation. “We have recently obtained very promising data in animal models that suggest these microparticle formulations could stave off

transplant rejection and even induce tolerance of the transplant,” he said.

The University of Pittsburgh has patented the findings, and the IP is available for licensing or partnering, Little said.

He added that the NIH’s **National Institute of Dental and Craniofacial Research** and the **Wallace H. Coulter Foundation** funded the *PNAS* study.

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National Institute of Dental and Craniofacial Research, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
Orono Inc., Pittsburgh, Pa.
University of Pennsylvania, Philadelphia, Pa.
University of Pittsburgh, Pittsburgh, Pa.
University of São Paulo, Bauru, Brazil
Wallace H. Coulter Foundation, Miami, Fla.

Naïve human iPS cells

By Lauren Martz, Staff Writer

The inability to generate fully undifferentiated human induced pluripotent stem cells *in vitro* has dogged the development of stem cell-based platforms because the residual lineage bias of the cells most likely contributes to the inefficiency and inconsistency of current differentiation protocols. An Israeli team thinks it has solved the problem with the optimization of a small molecule and cytokine cocktail capable of maintaining human cells in a more fully undifferentiated state.¹

The group expects that the naïve cells will be useful in generating somatic cells for research and clinical applications and for creating new animal models, although it still remains unclear whether the cells provide an advantage over conventionally derived induced pluripotent stem (iPS) cells.

In vitro, human iPS cells have been shown to be rather heterogeneous and to exhibit varying residual lineage characteristics—even when taken from the same patient.² Residual lineage bias of iPS cells is reflected in DNA methylation patterns, transcriptional profiles with upregulated lineage commitment genes and X-chromosome inactivation.^{3,4}

These varying genetic and epigenetic properties of iPS cell lines most likely are the reason why existing protocols for their differentiation into somatic lineages can be inefficient and inconsistent.

In addition, and although human pluripotent stem cells, albeit of limited stability, have been generated in a primed state *in vitro* using transgene expression, mouse pluripotent stem cells have been shown to exhibit an earlier, more undifferentiated state *in vitro*.⁴ This raises the question of how far human pluripotent stem cell dedifferentiation can be taken.

“The question of whether a ground state actually exists in human pluripotent stem cells and not just in cells from rodents is very interesting,” said Sheng Ding, a professor of pharmaceutical chemistry at the **University of California, San Francisco** and a senior investigator at the university’s **Gladstone Institute of Cardiovascular Disease**.

Now, Jacob Hanna and colleagues at the **Weizmann Institute of Science** have developed a method for generating human iPS cells in a naïve ground state and maintaining them undifferentiated by using a combination of small molecules.

Hanna is a principal investigator at the Weizmann Institute. The paper also included researchers from the **Tel Aviv Sourasky Medical Center** and the **Tel Aviv University Sackler Faculty of Medicine**.

The team generated human iPS cells with doxycycline-inducible expression of a basket of transgenes known to promote pluripotency: *OCT4*, *SOX2*, *KLF4* and *c-Myc* (*MYC*).

Next, the group screened for a combination of small molecules and cytokines in culture that maintained cell pluripotency after the removal of doxycycline and, thus, doxycycline-induced expression of the transgenes.

The researchers found a handful of small molecules and cytokines that maintained the cells in a naïve state. The small molecules included 2i/LIF and inhibitors of p38 mitogen-activated protein kinase (p38 MAPK; MAPK14), c-jun N-terminal kinase (JNK), rho-associated coiled-coil containing protein kinase (ROCK) and protein kinase C (PKC). The cytokines included fibroblast growth factor 2 (FGF2) and transforming growth factor- β (TGFB; TGF β).

The naïve iPS cells maintained pluripotency through more than 50 passages when cultured with medium containing those small molecules and cytokines, and the cells formed dome-shaped colonies similar to those created by naïve mouse pluripotent stem cells.

The group cultured typical, primed iPS cells, human embryonic stem cells (ESCs) and cells derived directly from human blastocyst inner cell masses in the medium. In each case, the team generated pluripotent cells with a naïve, undifferentiated phenotype. The group also reprogrammed human fibroblasts to iPS cells under the culture conditions and generated cells with a naïve phenotype.

The team also saw similarities between medium-derived, naïve human iPS cells and naïve mouse pluripotent stem cells, which further suggested that the human cells were truly undifferentiated.

Moreover, the naïve human iPS cells had upregulated pluripotency genes and downregulated lineage commitment genes compared with primed human iPS cells. The naïve cells also had high single-cell cloning efficiency.

Finally, the group injected labeled, naïve human iPS cells into mouse morulas, which were then grown in female mice for seven to eight days. *Ex vivo*, the embryos had signs of chimerism, and the human cells incorporated into the different tissues during early organogenesis. These findings further suggest

a highly undifferentiated cell state.

Results were published in *Nature*.

“If this process of maintaining naïve iPS cells is reproducible and can be applied to other species such as pigs, cows and nonhuman primates, one could imagine genome manipulation of these types of animals to develop disease models [as] very valuable for research.”

**—Sheng Ding,
University of California,
San Francisco**

Process benefits

Compared with previous attempts to maintain ground-state pluripotency in human stem cells, the Weizmann team’s work may help with generating transgene-independent, stable, naïve human iPS cells.

“Previous attempts to generate naïve human iPS cells relied on the use of transgenes that integrate into the genome. Not only do these transgenes encode powerful oncogenes that themselves drive cancer in other contexts, but the integrations themselves can be oncogenic and preclude clinical applications,” said Alejandro De Los Angeles, an MD and PhD candidate at **Harvard Medical School**.

Matthew Vincent, director of business development at **Advanced Cell Technology Inc.**, added that “a very elegant aspect of this approach is that rather than worrying about picking the best iPS cell induction strategy to modify, they solved the final steps, and their approach can be applied to a whole range of induction techniques to predictably stabilize the cells.”

He added, “They specifically tested the approach on pluripotent stem cells formed with transgene induction techniques. It would be

interesting to see whether this approach also applies to other induction techniques such as chemical, protein, microRNA and mRNA. I expect that it will.”

Advanced Cell has an ESC-derived retinal pigment epithelium cell therapy in Phase I testing to treat dry age-related macular degeneration (AMD) and Stargardt macular dystrophy.

Ding said that it was good that the researchers reported stability over many passages. “The issue of stability is critical for people working on this question. Without genetic manipulation, the chemical cocktail in which you culture the cells usually decreases the cell count with each passage, and the culture is not very stable. If, indeed, these conditions maintain long-term passage, they would overcome an important hurdle,” he said.

According to Vincent, “Stem cells should be able to be passaged over and over again. They should be an inexhaustible cell source, but generally this is not the case for iPS cells. They experience replicative gene senescence over time and eventually develop genetic instability.”

He added, “There is still a relatively large greenfield opportunity out there for whoever develops an iPS cell technique that gives good stability without the replicative capacity issues.”

Chris Parker, VP and chief commercial officer at **Cellular Dynamics International Inc.**, said that it will be “interesting to see if they can grow large quantities of the cells while maintaining the undifferentiated state. For any applications for these cells, we will need a lot of them for practical use.”

Cellular Dynamics markets the iCell platform for differentiating iPS cells into homogeneous, pure target cell types. The company also markets human iPS cell-derived cardiomyocytes, endothelial cells, neurons and hepatocytes, and it expects dopaminergic neurons to be available in 1Q14.

Hanna added that the naïve cells are also more amenable to genetic manipulation than primed iPS cells.

De Los Angeles did note that the Weizmann team’s process involves complicated culture conditions.

“The culture conditions used to maintain these cells are complex, and this may impede the study of the mechanisms regulating naïve pluripotency and the widespread application of this technology,” he said.

Emile Nuwaysir, COO of Cellular Dynamics, added, “These cells are at this time a little harder to culture and handle and are less stable than typical iPS cells.”

De Los Angeles did note that it may be possible to simplify the culture conditions to achieve even better results. He also thinks that it would be good to develop clinical-grade, xeno-free human pluripotent stem cell growth media to move the approach toward therapeutic and commercial applications.

Parker told *SciBX*, “We need to look for replication and reproduction of the approach to see if other groups can get the same cell types. Replication is so often not possible.”

***In vitro* applications**

The real question is what the new iPS cells allow researchers to accomplish that could not be done before. Hanna said that the new approach could lead to iPS cells that are more homogeneous, grow faster and have single-cell cloning efficacy that makes them more amenable to genetic manipulation.

“We will be interested in seeing whether the cells that start in a more naïve state can be used to produce somatic cell lines with better properties. Are they more mature or do they have different functions?” asked Parker. “To date, this hasn’t been documented.”

Hanna agreed. He said that his team’s next step is studying these cells and protocols for differentiation into various somatic cell lineages. These studies should show whether the new naïve cell type leads to improvements in quantity or quality of the resulting somatic cell products.

“There are certain cell types such as blood cells and germ cells that have been very difficult to make using conventional human pluripotent stem cells. It will be worth exploring whether the described naïve human pluripotent stem cells or future, more fully naïve pluripotent stem cells will enhance access to these elusive lineages,” said De Los Angeles.

Ding was less sanguine that the new approach would yield improved downstream cell products. “It is very unclear if there will be benefits for *in vitro* pluripotent stem cell applications,” he said. “Mouse embryonic cells at a naïve state and at a later pluripotent state have both been used for *in vitro* applications with no real advantage of using one particular state over another.”

Parker and Nuwaysir said that they would consider adopting the new technology if the findings are validated and if the approach is scalable and cost effective.

“We are quick to adopt any improvements that make the process of deriving cells from iPS cells better. If improvements in efficacy or

cell product properties are conceivable, we would certainly look into adopting the technology to move it forward,” said Parker.

De Los Angeles also thinks the technology has commercial potential. “I believe that naïve human pluripotent stem cells, either those described in the current study or future versions, will gradually replace the current human pluripotent stem cells,” he said.

Knockout animals

One key property of naïve iPS cells that is absent from primed human iPS cells is the ability to form germline-competent chimeras. This means that when iPS cells are injected into a blastocyst, the foreign cells are able to differentiate along with the host germ cells and are incorporated into all tissues through development. This property indicates that the cells have full pluripotent potential and allows whole-animal genetic manipulation.

“Besides rodents, no other species has embryonic stem cells that contribute to chimerism,” said Ding. “In mice and rats, embryonic stem cells can be manipulated, then put back into blastocysts for

“There are certain cell types such as blood cells and germ cells that have been very difficult to make using conventional human pluripotent stem cells. It will be worth exploring whether the described naïve human pluripotent stem cells or future, more fully naïve pluripotent stem cells will enhance access to these elusive lineages.”

**—Alejandro De Los Angeles,
Harvard Medical School**

whole-animal and germline manipulation. If this process of maintaining naïve iPS cells is reproducible and can be applied to other species such as pigs, cows and nonhuman primates, one could imagine genome manipulation of these types of animals to develop disease models [as

“We need to look for replication and reproduction of the approach to see if other groups can get the same cell types. Replication is so often not possible.”

— **Chris Parker,**
Cellular Dynamics
International Inc.

very valuable for research.”

Nuwaysir noted that “the cells developed by this process were actually able to form human-mouse chimeras. This is a fundamentally different property that did not exist in iPS cells in the past and raises a lot of new questions about how the cells may be used.”

Ding added, “These chimeras may have important implications for the development of humanized animals. The field of

humanizing animals is growing because it is very helpful in studying human disease processes, but at this time it is mainly limited to reconstituting specific organs or tissues with human cells.”

“This team suggests that it may be possible to incorporate human cells into animals on the whole-system level using the ground-state iPS cells, which could lead to improved mimics of the human system,” he said.

One problem, said Ding, is the difficulty in confirming that these human cells are in fact naïve and have chimeric potential.

“Proving that the cells are in this state would require putting them into human blastocysts to see if they incorporate into the tissues, but this will never be possible for humans from an ethical perspective,” he

said. “The closest way to confirm that this really is a naïve state is to apply the culture conditions to nonhuman primate cells and to see if the naïve monkey cells can contribute to chimerism and integrate into the germline in those animals.”

Hanna said that the Weizmann Institute has filed a patent application, and the IP is available for licensing. He expects there could be avenues for commercialization of either the cells themselves or the growth conditions.

Martz, L. *SciBX* 6(45); doi:10.1038/scibx.2013.1282

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This week in therapeutics

THE DISTILLERY brings you this week's most essential scientific findings in therapeutics, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in therapeutics includes important research findings on targets and compounds, grouped first by disease class and then alphabetically by indication.

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Autoimmune disease				
Autoimmune disease	Bromodomain containing 2 (BRD2); BRD4	<p>Cell culture and mouse studies suggest inhibiting the BET bromodomains of BRD2 and BRD4 could be useful for treating autoimmune disease. In cultured human T cells, a small molecule BET bromodomain inhibitor decreased expression of proinflammatory cytokines and differentiation of inflammation-associated T helper type 17 (Th17) cells compared with vehicle. In chromatin immunoprecipitation studies of murine T cells, the BET bromodomain inhibitor prevented the binding of BRD2 and BRD4 to the IL-17 locus. In mouse models of rheumatoid arthritis (RA) and multiple sclerosis (MS), the BET bromodomain inhibitor decreased inflammation and autoimmunity compared with vehicle control. Next steps could include preclinical development of BET bromodomain inhibitors for autoimmune indications.</p> <p>At least seven companies including Constellation Pharmaceuticals Inc. have BET bromodomain inhibitors in preclinical through Phase II development for oncology and other indications including inflammatory diseases.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1283 Published online Nov. 21, 2013</p>	Patent and licensing status undisclosed	<p>Mele, D.A. <i>et al. J. Exp. Med.</i>; published online Oct. 7, 2013; doi:10.1084/jem.20130376</p> <p>Contact: Jose M. Lora, Constellation Pharmaceuticals Inc., Cambridge, Mass. e-mail: jose.lora@constellationpharma.com</p>
Cancer				
Acute myelogenous leukemia (AML)	Glutamate-cysteine ligase catalytic subunit (GCLC); glutathione peroxidase 1 (GPX1)	<p><i>In vitro</i> studies suggest inhibiting glutathione metabolism could help treat AML. In CD34⁺ leukemia stem cells, compared with normal CD34⁺ hematopoietic cells, levels of both glutathione pathway regulatory proteins and oxidized glutathione were increased. In the leukemia stem cells, the natural products parthenolide and piperlongumine, which inhibit glutathione metabolism targets including GCLC and GPX1, depleted glutathione and induced cell death when given as monotherapy. In primary AML specimens, cytarabine plus parthenolide synergistically induced cell death. Next steps could include testing glutathione metabolism inhibitors in animal models of AML.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1284 Published online Nov. 21, 2013</p>	Patent and licensing status unavailable	<p>Pei, S. <i>et al. J. Biol. Chem.</i>; published online Oct. 2, 2013; doi:10.1074/jbc.M113.511170</p> <p>Contact: Craig T. Jordan, University of Colorado Denver, Aurora, Co. e-mail: craig.jordan@ucdenver.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	HER2 (EGFR2; ErbB2; neu); PD-1 receptor (PDCD1; PD-1; CD279)	<p>Mouse studies suggest inhibiting PD-1 could enhance the antitumor effects of HER2 chimeric antigen receptor (CAR) T cell therapies. In HER2 transgenic mice with HER2⁺ sarcoma or breast carcinoma tumors, HER2-targeting CAR T cells plus an anti-PD-1 antibody decreased tumor growth and increased long-term survival compared with HER2 CAR T cells or anti-PD-1 antibody alone, and the combination did not induce autoimmunity. In the mice receiving the combination therapy, the enhanced antitumor effects were attributed to increased function of the HER2 CAR T cells and decreased tumor infiltration of myeloid-derived suppressors. Next steps could include testing the combination therapy in clinical trials.</p> <p>At least six companies have PD-1 antibodies to treat several cancer indications in development stages from preclinical to Phase III.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1285 Published online Nov. 21, 2013</p>	Patent and licensing status unavailable	<p>John, L.B. <i>et al. Clin. Cancer Res.</i>; published online July 19, 2013; doi:10.1158/1078-0432.CCR-13-0458</p> <p>Contact: Phillip K. Darcy, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia e-mail: phil.darcy@petermac.org</p>
Cancer	Uridine phosphorylase 1 (UPP1)	<p>Cell culture studies identified UPP1 inhibitors that could be useful for improving the efficacy of 5-fluorouracil (5-FU) chemotherapy. In a human colon cancer cell line, the 2 lead inhibitors increased the antiproliferative effect of 5-FU compared with vehicle as measured by a 6- and 12-fold decrease in IC₅₀ values. Next steps include evaluating the combination therapy in additional cancer cell lines to help determine for which indications it could be most effective.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1286 Published online Nov. 21, 2013</p>	Patent application filed; available for licensing from the Pontifical Catholic University of Rio Grande do Sul Technology Transfer Office	<p>Renck, D. <i>et al. J. Med. Chem.</i>; published online Oct. 16, 2013; doi:10.1021/jm401389u</p> <p>Contact: Luiz A. Basso, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil e-mail: luiz.basso@pucrs.br</p> <p>Contact: Diogenes S. Santos, same affiliation as above e-mail: diogenes@pucrs.br</p>
Lung cancer	Neurotrophic tyrosine kinase receptor 1 (NTRK1; TrkA)	<p><i>In vitro</i> and mouse studies suggest TrkA inhibitors could help treat a subgroup of patients with lung cancer. In tumor samples from 91 patients with lung cancer, 3 patients carried <i>NTRK1</i> gene fusions. In noncancerous cells, expression of the gene fusions caused constitutive activation of TrkA, the protein encoded by <i>NTRK1</i>, and induced proliferation, anchorage-independent growth and tumor development when implanted in mice.</p> <p>In fusion-expressing cells, TrkA inhibitors including ARRY-470 blocked TrkA autophosphorylation and cell proliferation. Next steps include a clinical trial using Trk inhibitors in biomarker-selected patients.</p> <p>Array BioPharma Inc. has ARRY-470 in preclinical testing for cancer.</p> <p>Nerviano Medical Sciences s.r.l. has PHA-848125, an inhibitor of cyclin dependent kinases (CDKs) and TrkA, in Phase II testing to treat cancer.</p> <p>Amgen Inc. and Tesaro Inc. have the dual TrkA and anaplastic lymphoma kinase (ALK) inhibitor TSR-011 in Phase I/II testing to treat non-small cell lung cancer (NSCLC).</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1287 Published online Nov. 21, 2013</p>	Patent application filed; licensing status undisclosed	<p>Vaishnavi, A. <i>et al. Nat. Med.</i>; published online Oct. 27, 2013; doi:10.1038/nm.3352</p> <p>Contact: Robert C. Doebele, University of Colorado Denver School of Medicine, Aurora, Co. e-mail: robert.doebele@ucdenver.edu</p> <p>Contact: Pasi A. Jänne, Dana-Farber Cancer Institute, Boston, Mass. e-mail: pasi_janne@dfci.harvard.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Melanoma	Receptor tyrosine kinase-like orphan receptor 1 (ROR1); ROR2	<i>In vitro</i> and mouse studies suggest inhibiting ROR2 could help treat invasive melanoma. Proliferative, ROR1 ⁺ , noninvasive melanoma cells can phenotypically switch to a ROR2 ⁺ , invasive phenotype. In noninvasive melanoma cells, small interfering RNA targeting ROR1 increased ROR2 levels and invasion compared with control siRNA. In mice with BRAF-mutant melanoma cells with low sensitivity to the BRAF inhibitor Zelboraf vemurafenib, siRNA targeting ROR2 sensitized cancer cells to vemurafenib. Next steps include developing a ROR2 inhibitor. Daiichi Sankyo Co. Ltd., Chugai Pharmaceutical Co. Ltd. and Roche market Zelboraf to treat melanoma. SciBX 6(45); doi:10.1038/scibx.2013.1288 Published online Nov. 21, 2013	Unpatented; unlicensed	O'Connell, M.P. <i>et al. Cancer Discov.</i> ; published online Oct. 8, 2013; doi:10.1158/2159-8290.CD-13-0005 Contact: Ashani T. Weeraratna, The Wistar Institute, Philadelphia, Pa. e-mail: aweeraratna@wistar.org
Cardiovascular disease				
Thrombosis	Integrin β_3 (GPIIIa; CD61); guanine nucleotide binding protein (G protein) α_{13} (GNA13)	Mouse studies suggest selectively inhibiting CD61 interactions with GNA13 could help prevent thrombosis with fewer bleeding side effects than marketed CD61 antagonists. In mouse thrombosis assays, a peptide inhibitor that selectively blocked binding of Gna13 to the intracellular C terminus of Cd61 suppressed occlusive thrombosis as effectively as the integrin inhibitor Integrilin eptifibatide, but unlike Integrilin the peptide did not cause secondary bleeding. Next steps include assessing the inhibitor's subunit specificity toward other β -integrins as well as toxicity and pharmacology in animal models. Merck & Co. Inc. markets the integrin $\alpha_{2b}\beta_3$ (GPIIb/IIIa; CD41/CD61) inhibitors Integrilin and Aggrastat tirofiban and Johnson & Johnson markets the CD41/CD61 mAb ReoPro abciximab to prevent thrombosis in cardiovascular indications (<i>see Riding the integrin wave, page 4</i>). SciBX 6(45); doi:10.1038/scibx.2013.1289 Published online Nov. 21, 2013	Patent application filed; available for licensing	Shen, B. <i>et al. Nature</i> ; published online Oct. 27, 2013; doi:10.1038/nature12613 Contact: Xiaoping Du, University of Illinois at Chicago, Chicago, Ill. e-mail: xdu@uic.edu
Dental disease				
Periodontitis	Chemokine CC motif ligand 22 (CCL22)	Animal studies suggest CCL22-loaded microparticles could help treat periodontitis. In mouse models of the indication, gingival injection of polymer microparticles loaded with mouse Ccl22 recruited T _{reg} cells to the injection site and decreased gingival levels of proinflammatory cytokines, levels of matrix metalloproteinases and loss of alveolar bone compared with injection of empty microparticles. In dog models of periodontitis, topical application of CCL22-loaded microparticles to subgingival pockets decreased pocket depth, gingival inflammation and alveolar bone loss compared with empty microparticles. Ongoing work includes preclinical safety testing of the CCL22-loaded microparticles (<i>see Getting to the root of periodontitis, page 6</i>). SciBX 6(45); doi:10.1038/scibx.2013.1290 Published online Nov. 21, 2013	Patented by the University of Pittsburgh; available for licensing or partnering	Glowacki, A.J. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Oct. 28, 2013; doi:10.1073/pnas.1302829110 Contact: Steven R. Little, University of Pittsburgh, Pittsburgh, Pa. e-mail: srlittle@pitt.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Endocrine/metabolic disease				
Diabetes	Glucokinase (GCK; GK); glucokinase regulator (GCKR; GKR)	<p><i>In vitro</i> and mouse studies identified compounds that could help treat type 2 diabetes by binding GKR and disrupting its interaction with GK. A high throughput screen and additional structural optimization identified a compound that bound GKR and promoted the disassociation of the GK-GKR complex with an IC₅₀ of 7 nM. In three diabetic mouse models, a structurally related compound decreased blood glucose levels compared with vehicle. The compound did not reduce glucose levels in normal mice. Next steps include studying the effect of these compounds on the metabolic physiology of the liver.</p> <p>At least nine companies have GK activators in Phase II or earlier development to treat type 2 diabetes. In October, Amgen Inc. returned rights to the GK activator ARRY-403 (formerly AMG151) to Array BioPharma Inc. The compound had completed Phase II testing in type 2 diabetes (see <i>Glucokinase alternative</i>, page 1).</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1291 Published online Nov. 21, 2013</p>	Patent application filed; licensing status undisclosed	<p>Lloyd, D.J. <i>et al. Nature</i>; published online Nov. 13, 2013; doi:10.1038/nature12724</p> <p>Contact: David J. Lloyd, Amgen Inc., Thousand Oaks, Calif. e-mail: dlloyd@amgen.com</p> <p>Contact: Clarence Hale, same affiliation as above e-mail: chale@amgen.com</p>
Porphyria	Proteasome; uroporphyrinogen III synthase (UROS)	<p>Mouse and cell culture studies suggest proteasome inhibitors could be useful for treating congenital erythropoietic porphyria, which is caused by destabilizing mutations in <i>UROS</i>. In human erythrocyte cell lines carrying mutations in <i>UROS</i> that destabilize the protein, the proteasome inhibitor Velcade bortezomib partially protected the synthase from premature degradation. In mice carrying analogous <i>Uros</i> mutations, Velcade decreased disease severity compared with saline. Next steps could include evaluating other proteasome inhibitors in the preclinical models and testing various dosing protocols.</p> <p>Takeda Pharmaceutical Co. Ltd. and Johnson & Johnson market Velcade to treat multiple myeloma (MM) and mantle cell lymphoma (MCL).</p> <p>Amgen Inc. and Ono Pharmaceutical Co. Ltd. market the selective proteasome inhibitor Kyprolis carfilzomib to treat MM.</p> <p>At least 10 companies have proteasome inhibitors in Phase III testing or earlier to treat various cancers.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1292 Published online Nov. 21, 2013</p>	Patent and licensing status unavailable	<p>Blouin, J.-M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 21, 2013; doi:10.1073/pnas.1314177110</p> <p>Contact: Emmanuel Richard, University of Bordeaux Segalen, Bordeaux, France e-mail: emmanuel.richard@u-bordeaux2.fr</p>
Infectious disease				
Ebola virus	Ebola glycoprotein GP1; Ebola glycoprotein GP2	<p>Primate studies suggest combining anti-Ebola antibodies plus interferon-α (IFNα; IFN-α) could help treat Ebola virus. In cynomolgus and rhesus macaques, ZMAb—a combination of three murine antibodies that target GP1 and GP2—plus DEF201, an adenoviral vector-delivered IFN-α, protected 75%–100% of animals when given 3 days after infection. In cynomolgus macaques, DEF201 beginning 1 day postinfection plus ZMAb beginning 4 days postinfection protected 50% of animals. Next steps include ongoing safety and toxicology studies for ZMAb and clinical testing of ZMAb and DEF201 by Defyrus Inc.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1293 Published online Nov. 21, 2013</p>	MABs for Ebola and use of interferon to treat infections patented; ZMAb licensed exclusively to Defyrus; DEF201 available for licensing for filovirus indications	<p>Qiu, X. <i>et al. Sci. Transl. Med.</i>; published online Oct. 16, 2013; doi:10.1126/scitranslmed.3006605</p> <p>Contact: Gary P. Kobinger, Public Health Agency of Canada, Winnipeg, Manitoba, Canada e-mail: gary.kobinger@phac-aspc.gc.ca</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Fungal infection	IL-12; natural killer p30 receptor (NKp30; NCR3; CD337)	<p>Patient and cell culture studies suggest IL-12 can increase NKp30 expression and enhance anticryptococcal immunity. In patients with HIV/AIDS, NKp30 expression was lower than that in healthy individuals. In NK cells from HIV-infected individuals, IL-12 stimulated NKp30 expression and anticryptococcal activity. Next steps include identifying the fungal ligand for NKp30 and elucidating downstream pathways.</p> <p>OncoSec Medical Inc. has a DNA plasmid coding IL-12 in Phase II testing to treat various cancers.</p> <p>Profectus BioSciences Inc. has DNA-encoded IL-12 in Phase I testing to treat HIV/AIDS.</p> <p>Intrexon Corp. also has DNA-encoded IL-12 in Phase II or earlier testing to treat various cancers.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1294 Published online Nov. 21, 2013</p>	Patent filed; available for licensing or partnership via Innovate Calgary at the University of Calgary	<p>Li, S.S. <i>et al. Cell Host Microbe</i>; published online Oct. 16, 2013; doi:10.1016/j.chom.2013.09.007</p> <p>Contact: Christopher H. Mody, University of Calgary, Calgary, Alberta, Canada e-mail: cmody@ucalgary.ca</p>
Malaria	<i>Plasmodium falciparum</i> phosphoethanolamine N-methyltransferase (PfPMT)	<p>Cell culture studies suggest inhibiting PfPMT could help prevent <i>P. falciparum</i> infection and transmission. In <i>P. falciparum</i> cultures, knockout of PfPMT prevented the generation of mature gametocytes, a critical step in malaria transmission. In red blood cell-cultured <i>P. falciparum</i>, a PfPMT inhibitor increased death compared with no treatment and limited growth of gametocytes depending on their developmental stage. Next steps include synthesizing analogs of the inhibitor and testing them in mouse models of malaria.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1295 Published online Nov. 21, 2013</p>	Unpatented; available for licensing from the Yale School of Medicine Office of Cooperative Research	<p>Bobenchik, A.M. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 21, 2013; doi:10.1073/pnas.1313965110</p> <p>Contact: Choukri Ben Mamoun, Yale School of Medicine, New Haven, Conn. e-mail: choukri.benmamoun@yale.edu</p>
Inflammation				
Inflammatory disease	p38 mitogen-activated protein kinase (p38 MAPK; MAPK14); tumor necrosis factor- α (TNF- α)	<p><i>In vitro</i> studies identified dibenzoxepinone-based p38 MAPK inhibitors that could help treat inflammatory diseases. In an <i>in vitro</i> assay, the most potent member of a compound series inhibited p38 MAPK with an IC₅₀ of 1.6 nM. In human whole blood, the same compound inhibited TNF-α release with an IC₅₀ of 125 nM. Next steps could include evaluating the lead inhibitor in animal models of inflammatory disease.</p> <p>At least four companies have p38 MAPK inhibitors in Phase II testing or earlier to treat various autoimmune or inflammatory diseases.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1296 Published online Nov. 21, 2013</p>	Patent and licensing status unavailable	<p>Baur, B. <i>et al. J. Med. Chem.</i>; published online Oct. 17, 2013; doi:10.1021/jm401276h</p> <p>Contact: Stefan A. Laufer, Eberhard Karls University of Tuebingen, Tuebingen, Germany e-mail: stefan.laufer@uni-tuebingen.de</p>
Musculoskeletal disease				
Osteoporosis	NADPH oxidase 4 (NOX4)	<p>Studies in mice and in human samples suggest inhibiting NOX4 could help treat osteoporosis. In an ovariectomized mouse model of osteoporosis, knockout or inhibition of Nox4 with GKT137831 increased trabecular bone density compared with vehicle or no knockout and prevented bone loss. In bone material from patients with osteoporosis, NOX4 levels were higher than those in healthy controls. Next steps include clinical testing of NOX4 inhibitors to treat osteoporosis.</p> <p>Genkyotex S.A.'s NOX1 and NOX4 inhibitor, GKT137831, is in Phase II testing to treat diabetic retinopathy and preclinical testing to treat liver and pulmonary fibrosis.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1297 Published online Nov. 21, 2013</p>	Patent application filed by Goethe University Frankfurt and Genkyotex; licensing status undisclosed	<p>Goetsch, C. <i>et al. J. Clin. Invest.</i>; published online Oct. 15, 2013; doi:10.1172/JCI67603</p> <p>Contact: Katrin Schröder, Goethe University Frankfurt, Frankfurt, Germany e-mail: schroeder@vrc.uni-frankfurt.de</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Neurology				
Amyotrophic lateral sclerosis (ALS)	Chromosome 9 open reading frame 72 (C9orf72)	<p>Studies in patient samples and mice suggest antisense oligonucleotides targeting the hexanucleotide repeat in <i>C9orf72</i> could help treat some patients with ALS. GGGGCC repeats in <i>C9orf72</i> represent the most common genetic cause of ALS. In postmortem brain tissue from patients with ALS or various types of neurons derived from ALS patient fibroblasts carrying the repeats, RNA foci formed and sequestered RNA-binding proteins, including heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), purine-rich element binding protein A (PURA) and adenosine deaminase RNA-specific B2 (ADARB2). These RNA foci disrupted normal gene splicing and transcription and induced glutamate-mediated excitotoxicity. In the ALS neurons or tissues, antisense oligonucleotides targeting <i>C9orf72</i> or the repeat expansions suppressed RNA foci formation and partially corrected expression of the dysregulated genes. They also decreased glutamate-induced excitotoxicity compared with scrambled antisense oligonucleotides. In normal mice, <i>C9orf72</i> antisense oligonucleotides were well tolerated and did not cause any symptoms of ALS. Next steps include selecting the best antisense oligonucleotide for clinical development. Isis Pharmaceuticals Inc. was involved with each manuscript and has antisense oligonucleotide candidates in preclinical development to treat ALS.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1298 Published online Nov. 21, 2013</p>	For all three studies, patent applications filed by Isis Pharmaceuticals covering antisense oligonucleotides; unavailable for licensing	<p>Sareen, D. <i>et al. Sci. Transl. Med.</i>; published online Oct. 23, 2013; doi:10.1126/scitranslmed.3007529 Contact: Robert H. Baloh, Cedars-Sinai Medical Center, Los Angeles, Calif. e-mail: robert.baloh@csmc.edu</p> <p>Donnelly, C.J. <i>et al. Neuron</i>; published online Oct. 16, 2013; doi:10.1016/j.neuron.2013.10.015 Contact: Jeffrey D. Rothstein, The Johns Hopkins University, Baltimore, Md. e-mail: jrothstein@jhmi.edu Contact: Rita Sattler, same affiliation as above e-mail: rsattler1@jhmi.edu</p> <p>Lagier-Tourenne, C. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online Oct. 29, 2013; doi:10.1073/pnas.1318835110 Contact: John Ravits, University of California, San Diego, La Jolla, Calif. e-mail: jravits@ucsd.edu Contact: Don W. Cleveland, same affiliation as above e-mail: dcleveland@ucsd.edu</p>
Ataxia	Enhancer of zeste homolog 2 (EZH2)	<p><i>In vitro</i> and mouse studies suggest inhibiting EZH2 could help treat ataxia telangiectasia. In <i>ataxia telangiectasia mutated (Atm)</i>-deficient mice, intracerebellar injection of lentiviruses expressing <i>Ezh2</i> small hairpin RNA decreased neuronal cell death compared with injection of lentiviruses expressing control shRNA and improved ataxic behavioral abnormalities. Next steps include testing either EZH2 inhibition in the model or a combination of drugs that depress histone methylation while enhancing acetylation.</p> <p>Epizyme Inc. and Eisai Co. Ltd. have EPZ6438 (E7438), a selective inhibitor of EZH2, in Phase I/II testing to treat lymphoma and non-Hodgkin's lymphoma (NHL). Constellation Pharmaceuticals Inc., Novartis AG and GlaxoSmithKline plc have EZH2 inhibitors in preclinical development to treat cancer.</p> <p>SciBX 6(45); doi:10.1038/scibx.2013.1299 Published online Nov. 21, 2013</p>	Findings unpatented; unavailable for licensing	<p>Li, J. <i>et al. Nat. Neurosci.</i>; published online Oct. 27, 2013; doi:10.1038/nn.3564 Contact: Karl Herrup, The Hong Kong University of Science and Technology, Hong Kong, China e-mail: herrup@ust.hk Contact: Jiali Li, Rutgers University, Piscataway, N.J. e-mail: jlili@dls.rutgers.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Spinal cord injury (SCI)	Not applicable	Rat studies suggest deep brain stimulation could be useful for promoting recovery of motor function following SCI. In a rat model of SCI-induced hind limb motor impairment, excitatory deep brain stimulation (DBS) of the mesencephalic locomotor region restored hind limb function during walking and swimming exercises to near-preinjury levels. In animals with SCI-induced hind limb functional paralysis, DBS restored some basic hind limb motor function. Next steps include determining the intensity of stimulation needed to restore voluntary control of motor function without forcing motor activity.	Unpatented; licensing status not applicable	Bachmann, L.C. <i>et al. Sci. Transl. Med.</i> ; published online Oct. 23, 2013; doi:10.1126/scitranslmed.3005972 Contact: Lukas C. Bachmann, University of Zurich, Zurich, Switzerland e-mail: bachmann@hifo.uzh.ch
SciBX 6(45); doi:10.1038/scibx.2013.1300 Published online Nov. 21, 2013				

Various

Cancer; <i>Mycobacterium</i>	DNA; topoisomerase II (TOP2)	<i>In vitro</i> studies identified analogs of the natural product chartreusin that could serve as lead compounds to help treat cancer or mycobacterial infection. Chartreusin binds DNA and inhibits TOP2. Chemical synthesis, biosynthesis, SAR and <i>in vitro</i> testing of chartreusin analogs identified several lead compounds that inhibited proliferation of human colon cancer, melanoma and/or chronic myeloid leukemia (CML) cell lines at low micromolar to high nanomolar GI ₅₀ values. Another lead compound inhibited growth of <i>M. vaccae</i> at a low micromolar minimum inhibitory concentration. Planned work includes optimizing the lead compounds to treat cancer or mycobacterial infection.	Patented by the Hans Knoell Institute; available for partnering and licensing	Ueberschaar, N. <i>et al. J. Am. Chem. Soc.</i> ; published online Oct. 21, 2013; doi:10.1021/ja4080024 Contact: Christian Hertweck, Hans Knoell Institute, Jena, Germany e-mail: christian.hertweck@hki-jena.de
SciBX 6(45); doi:10.1038/scibx.2013.1301 Published online Nov. 21, 2013				

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This week in techniques

THE DISTILLERY brings you this week's most essential scientific findings in techniques, distilled by *SciBX* editors from a weekly review of more than 400 papers in 41 of the highest-impact journals in the fields of biotechnology, the life sciences and chemistry. The Distillery goes beyond the abstracts to explain the commercial relevance of featured research, including licensing status and companies working in the field, where applicable.

This week in techniques includes findings about research tools, disease models and manufacturing processes that have the potential to enable or improve all stages of drug discovery and development.

Approach	Summary	Licensing status	Publication and contact information
Computational models			
Analysis of adverse events database to identify mechanisms underlying side effects	<i>In silico</i> and mouse studies suggest analysis of adverse events database can identify new targets that separate beneficial therapeutic effects from adverse events. In the FDA Adverse Event Reporting System (FAERS) database, incidence of myocardial infarction (MI) was lower in patients taking Avandia rosiglitazone and Byetta exenatide than patients taking Avandia alone. Network interaction analysis showed that pathways activated by Byetta and Avandia intersected at plasminogen activator inhibitor 1 (SERPINE1; PAI1). In a mouse model of diabetes, Avandia and Byetta normalized Pai1 levels and decreased clotting dynamics compared with Avandia alone. Next steps include identifying pathway interaction nodes for other drug combinations and testing in preclinical models. The peroxisome proliferation-activated receptor- γ (PPARG; PPAR γ) agonist Avandia and the glucagon-like peptide-1 receptor (GLP-1R; GLP1R) agonist Byetta are marketed to treat diabetes by GlaxoSmithKline plc and Bristol-Myers Squibb Co., respectively. SciBX 6(45); doi:10.1038/scibx.2013.1302 Published online Nov. 21, 2013	Patent application filed; available for licensing	Zhao, S. <i>et al. Sci. Transl. Med.</i> ; published online Oct. 9, 2013; doi:10.1126/scitranslmed.3006548 Contact: Ravi Iyengar, Icahn School of Medicine at Mount Sinai, New York, N.Y. e-mail: ravi.iyengar@mssm.edu
Disease models			
Mouse model of hemolytic anemia induced by malaria drugs	A mouse model of hemolytic anemia could be useful for evaluating the tolerability of antimalarial compounds. Marketed antimalarial 8-aminoquinoline (8-AQ) compounds cause hemolytic anemia in patients with inherited glucose-6-phosphate dehydrogenase (G6PD) deficiency. In immunocompromised mice engrafted with human G6PD-deficient erythrocytes, compared with mice engrafted with normal erythrocytes, the 8-AQ compound primaquine increased hemolysis and anemia. Next steps include collaborating with Medicines for Malaria Venture, a philanthropic organization, to test the tolerability of malaria therapeutic candidates in the model. SciBX 6(45); doi:10.1038/scibx.2013.1303 Published online Nov. 21, 2013	Unpatented; licensing status not applicable	Rochford, R. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online Oct. 7, 2013; doi:10.1073/pnas.1310402110 Contact: Rosemary Rochford, SUNY Upstate Medical University, Syracuse, N.Y. e-mail: rochforr@upstate.edu
Drug platforms			
A vaccine based on a stabilized variant of respiratory syncytial virus (RSV) F protein	A stabilized variant of RSV F protein could be used as a vaccine antigen to prevent RSV infection. RSV F was modified with C-terminal trimerization domains, cysteine pairs and cavity-filling hydrophobic substitutions to generate stable RSV F trimers that resembled a conformation of the protein adopted before virus-cell interaction. In mice, vaccination with modified RSV F provided neutralizing activity eight times greater than that achieved with vaccination using an RSV F antigen that adopted a conformation seen after virus-cell fusion. In rhesus macaques, vaccination with the modified RSV F provided neutralizing activity 70–80 times greater than that seen with vaccination using the postfusion-like RSV F. Next steps include moving the modified version of RSV F into GMP production for clinical trials. At least nine companies have therapeutics or vaccines in development to treat RSV. SciBX 6(45); doi:10.1038/scibx.2013.1304 Published online Nov. 21, 2013	The prefusion-stabilized RSV F glycoproteins as vaccine antigens are patented; available for licensing through the NIH's Office of the Director	McLellan, J.S. <i>et al. Science</i> ; published online Nov. 1, 2013; doi:10.1126/science.1243283 Contact: Peter D. Kwong, National Institutes of Health, Bethesda, Md. e-mail: pdkwong@nih.gov Contact: Barney S. Graham, same affiliation as above e-mail: bgraham@nih.gov

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Naïve human stem cell medium for maintenance of human induced pluripotent stem (iPS) cells in a naïve state	<i>In vitro</i> studies identified culture conditions to maintain human iPS cells in a naïve state. Culture of primed human iPS cells, embryonic stem cells (ESCs) or blastocyst-derived cells in naïve human stem cell medium generated pluripotent stem cells with features of naïve mouse iPS cells including a preinactivation X chromosome state, upregulation of pluripotency genes and downregulation of lineage commitment genes, and it produced decreases in DNA methylation compared with culture of primed human iPS cells alone. Mouse embryos injected with human naïve iPS cells and grown in female mice showed chimerism and incorporation of the human cells during organogenesis. Next steps include assessing properties of cells derived from naïve iPS cells (<i>see Naïve human iPS cells, page 8</i>).	Patent application filed; available for licensing	Gafni, O. <i>et al. Nature</i> ; published online Oct. 30, 2013; doi:10.1038/nature12745 Contact: Jacob H. Hanna, Weizmann Institute of Science, Rehovot, Israel e-mail: jacob.hanna@weizmann.ac.il Contact: Noa Novershtern, same affiliation as above e-mail: noa.novershtern@weizmann.ac.il Contact: Rada Massarwa, same affiliation as above e-mail: rada.massarwa@weizmann.ac.il
Small molecule target discovery using forward genetics	<i>In vitro</i> , cell culture and mouse studies suggest a high throughput small hairpin RNA screening platform could be useful for identifying the targets of lead compounds. A cell culture screen for small molecule inhibitors of acute lymphoblastic leukemia growth identified STF-11804, which inhibited tumor growth at nanomolar concentrations in a range of tumor lines. In a genomewide screen for shRNAs that enhance STF-118804 activity, shRNA against nicotinamide phosphoribosyl transferase (NamPRT; NAMPT) increased STF-118804 sensitivity compared with shRNAs against other targets. <i>In vitro</i> , STF-118804 inhibited NAMPT activity. Next steps include optimization and preclinical development of STF-118804 for hematological malignancies.	Stanford University has pending patents on STF-118804; University of California has filed patents on use of its shRNA screening platform for target identification; both sets of patents available for licensing	Matthey, C.J. <i>et al. Chem. Biol.</i> ; published online Oct. 31, 2013; doi:10.1016/j.chembiol.2013.09.014 Contact: Michael L. Cleary, Stanford University School of Medicine, Stanford, Calif. e-mail: mcleary@stanford.edu
Imaging			
Optical metabolic imaging for diagnosing cancer and assessing treatment response	Optical metabolic imaging could help diagnose cancers and assess response to treatment. The method measures the fluorescence intensities and lifetimes of autofluorescent metabolic coenzymes. In cultured cell lines, optical metabolic imaging of untransformed human breast cells and various malignant human breast cancer subtypes revealed distinguishing differences in the basal metabolic profiles of the various cell types. In cell culture and mouse xenograft models of human breast cancer, optical metabolic imaging revealed metabolic differences in cancer cells that responded to Herceptin trastuzumab versus nonresponsive cells or cells treated with a control IgG. Next steps include validating the approach against standard clinical approaches to assess patient response and classify breast cancer subtype. Roche's Genentech Inc. unit markets Herceptin, a humanized mAb against HER2 (EGFR2; ErbB2; neu), to treat breast and gastric cancers.	Patent and licensing status available from Vanderbilt University	Walsh, A.J. <i>et al. Cancer Res.</i> ; published online Oct. 15, 2013; doi:10.1158/0008-5472.CAN-13-0527 Contact: Melissa C. Skala, Vanderbilt University, Nashville, Tenn. e-mail: m.skala@vanderbilt.edu

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KCB-1D	7	kinase	8,15	ReoPro	4,13	U			
KLF4	8	PAI1	18	Rho-associated coiled-coil		UPP1	12		
Kyprolis	14	Parthenolide	11	containing protein kinase	8	Uridine phosphorylase 1	12		
		PD-1	12	RO5305552	3	Uroporphyrinogen III synthase	14		
L		PD-1 receptor	12	ROCK	8	UROS	14		
Lepirudin	5	PDCD1	12	ROR1	13	V			
		Peroxisome proliferation-		ROR2	13	Velcade	14		
M		activated receptor- γ	18	Rosiglitazone	18	Vemurafenib	13		
MAPK14	8,15	PF-04937319	3	RSV F protein	18	von Willebrand factor	4		
Matrix metalloproteinase	6	PfPMT	15	S		vWF	4		
MMP	6	PHA-848125	12	SERPINE1	18	W			
MYC	8	Piperlongumine	11	Sorbitol-6-phosphate	2	Warfarin	5		
N		PKC	8	SOX2	8	Z			
NADPH oxidase 4	15	Plasminogen activator		STF-11804	19	Zelboraf	13		
NamPRT	19	inhibitor 1	18	T		ZMAb	14		
NAMPT	19	<i>Plasmodium falciparum</i>		Talin	5	ZYGK1	3		
Natural killer p30 receptor	15	phosphoethanolamine		TGFB	7,8				
NCR3	15	N-methyltransferase	15	TGF β	7,8				

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