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THIS WEEK

ANALYSIS

COVER STORY

1 CRISPR in the liver

Although the therapeutic utility of CRISPR-based approaches has yet to be demonstrated, venture dollars are flowing into new companies developing the platform. Proof of concept may come faster than expected as new findings show that a CRISPR-based therapeutic can correct a mutation in adult mice with genetic liver disease.

TARGETS & MECHANISMS

5 Reversing (heart) failure in Friedreich's ataxia Heart failure accounts for over half the deaths in patients with Friedreich's ataxia, but there are no therapies for the neurodegenerative disease. Now, a team of French researchers has shown that i.v. *frataxin* gene therapy could prevent or reverse heart failure in a mouse model of the disease.

7 Leveling cancer with IL-15

Findings that link high IL-15 levels in tumors with increased local T cell proliferation and favorable patient outcomes position the cytokine as a robust prognostic and complement ongoing efforts to develop IL-15 as a therapeutic in colorectal cancer and other malignancies.

9 Mired in miR-25

New evidence that miR-25 inhibition can help treat heart failure contradicts earlier results. Although the current study shows that blocking miR-25 restores SERCA2A—and thus improves cardiac contractility—discrepancies between the studies need to be resolved before a therapy can be developed.

THE DISTILLERY

12 This week in therapeutics

Treating type 2 diabetes with a DPP-4 peptide vaccine; inhibiting Nav1.6 to alleviate optic neuritis; using cysteine dietary supplementation to prevent neurotoxicity associated with Huntington's disease; and more...

18 This week in techniques

Detection of *Plasmodium falciparum* infection with ultrasound-based separation of red blood cells; bloodbased DNA methylation signature for progressive supranuclear palsy and frontotemporal dementia; loss-offunction *SMARCA4* mutations associated with small-cell carcinoma of the ovary, hypercalcemia type; and more...

INDEXES

- 20 Company and institution index
- 20 Target and compound index

CRISPR in the liver

By Amy Donner, Senior Editor

Despite the fact that the therapeutic utility of CRISPR-based approaches has yet to be demonstrated, venture dollars keep flowing into new companies developing the platform. But proof of concept may come faster than expected as new findings show that a CRIPSR-based compound can correct a mutation in adult mice with genetic liver disease.¹

Last year, multiple independent teams adapted a newly identified bacterial defense system to create a platform for site-directed genome editing.²⁻⁷ The platform relies on a synthetic single guide RNA (sgRNA) to target the activity of the CRISPR (clustered, regularly interspaced short palindromic repeats)-associated bacterial endonuclease Cas9—the system is referred to in short as CRISPR-Cas9.

Cas9 introduces a double-strand break in genomic DNA in a location designated by complementary interactions between the sgRNA and the DNA target, which triggers DNA repair and genome editing.

Because editing specificity is driven by the sequence of the sgRNA, the CRISPR-Cas9 system is simpler and cheaper than other genomeediting tools, such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which use engineered, DNA-binding proteins to site-specifically target DNA.

CRISPR-Cas9 has been applied in embryos to engineer mouse and nonhuman primate models of human disease.^{8,9} The platform also has been used to engineer complex mouse models by simultaneously introducing multiple gene mutations into embryonic stem cells.^{10,11}

The first foray into therapeutic application occurred last year, when **Editas Medicine** was founded to use CRISPR-Cas9 to correct genomic defects in undisclosed diseases. Editas raised \$43 million and brought together 5 scientific cofounders who spearheaded the conversion of the bacterial defense system to a programmable genome-editing tool.

Last week, the first CRISPR-Cas9 patent for genome-editing applications was assigned to the **Broad Institute of MIT and Harvard** and the **Massachusetts Institute of Technology** (MIT). The patent covers CRISPR-Cas9 systems engineered to work in eukaryotic cells, especially human cells, and methods of using these systems. For research use, the technology will be available to anyone on a nonexclusive basis. The Broad Institute did not disclose further licensing details.

Today, **CRISPR Therapeutics**, which aims to translate CRISPR-Cas9 into medicines with the potential to cure human genetic diseases, emerged from stealth mode. CRISPR Therapeutics was founded by **Versant Ventures** and raised \$25 million.

Like Editas, CRISPR Therapeutics has not yet disclosed the specific targets or indications it will pursue.

COVER STORY



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Charpentier is a professor at the Laboratory for Molecular Infection Medicine at **Umeå University**, the **Helmholtz Centre for Infection Research** and **Hannover Medical School**.

Anderson is an associate professor of chemical engineering and medical engineering and science at MIT and an intramural member of **The David H. Koch Institute for Integrative Cancer Research at MIT**.

Porteus is an associate professor of pediatrics at the **Stanford University School of Medicine**, and Cowan is an associate professor of stem cell and regenerative biology at **Harvard University** and **Massachusetts General Hospital**.

Mello is chair of molecular medicine and co-director of the RNA Therapeutics Institute at the **University of Massachusetts Medical School** and a **Howard Hughes Medical Institute** (HHMI) investigator.

Crisp solution

Anderson and his team have now for the first time used CRISPR-Cas9 therapeutically. The researchers chose tyrosinemia type I for their proof-of-concept studies.

Tyrosinemia type I is a genetic disorder caused by deficiency in fumarylacetoacetate hydrolase (FAH), an enzyme required to break down the amino acid tyrosine. In the absence of this enzyme activity, toxic metabolites accumulate and cause liver or kidney failure. The disease affects about 1 in 100,000 people worldwide.

According to Hao Yin, a postdoctoral associate at the David H. Koch Institute and one of two lead authors on the study, because the disease is the direct result of the presence of an aberrant version of only one gene, using a mouse model of the disease containing a mutated version

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COVER STORY

of the gene offered a straightforward way to establish proof of concept. "The phenotype mimics the human situation. It is caused by a point mutation, and correction can produce stable protein and stable mRNA, which are easy to detect even if editing occurs only in a small portion of cells," said Yin.

The team's CRISPR-Cas9 therapeutic was composed of a vector expressing a *Fah*-targeting sgRNA and *Cas9* along with a second molecule—the *Fah*-correcting donor DNA. Tail vein injection of the therapeutic prevented weight loss, whereas mutant mice receiving saline, *Fah*-correcting DNA alone or empty vector alone rapidly lost 20% of their body weight.

The therapeutic decreased liver toxicity as demonstrated by decreased liver enzyme activity compared with controls and improved liver histology. The CRISPR-Cas9 therapeutic also increased the amount of spliced *Fah* mRNA to up to 36% of wild-type levels and yielded Fah⁺ hepatocytes.

The authors estimated that the therapeutic corrected the *Fah* gene mutation in about 1 in 250 liver cells, which was sufficient to alleviate disease symptoms.

"It is important to consider whether you have to correct every cell or just a small percentage of cells. For tyrosine metabolism, you don't need the protein made in all the cells, so in this indication, a fractional correction is sufficient," said Art Krieg, SVP and CSO of **Sarepta Therapeutics Inc.** He formerly was CEO of **RaNA Therapeutics Inc.**, CSO of **Pfizer Inc.**'s Oligonucleotide Therapeutics Unit and has more than 20 years of experience with oligonucleotide-based therapies.

Off-site editing activity¹²⁻¹⁵ was not a problem in the study. In cultured cells, genome editing did not lead to detectable cutting at computationally predicted off-target sites.

The therapeutic was well tolerated. After three months, the treated mice had stable body weight, no signs of hyperplasia in the liver and no detectable tumors, which could be induced by off-target editing.

Data were published in *Nature Biotechnology*. The team also included researchers from **Oregon Health & Science University** and the **Skolkovo Institute of Science and Technology**.

"We believe that showing that the CRISPR-Cas9 system can correct a disease mutation and phenotype *in vivo* is the first step to turn it into therapy," said Anderson. "These observations on the CRISPR-Cas9 system, in combination with recent advances in nucleic acid delivery technology, make us optimistic that this technology can be used therapeutically."

"The paper is very important with regard to *in vivo* applicability," said Rodger Novak, CEO of CRISPR Therapeutics.

"The paper is very important with regard to *in vivo* applicability."

-Rodger Novak, CRISPR Therapeutics "The principle of being able to achieve delivery and a level of editing that was efficient and accurate enough to lead to phenotypic disease correction is mind blowing," said Jennifer Doudna. "Even a month ago, we might not have imagined

that this kind of application was possible. The discovery of this platform technology was reported in the scientific literature less than two years ago."

Doudna is a professor of molecular and cell biology and chemistry at the **University of California**, **Berkeley**, a faculty affiliate of physical biosciences of the **Lawrence Berkeley National Laboratory** and an HHMI investigator. She also is a scientific cofounder of Editas.

Special delivery

Delivery and off-target activity are still the primary challenges for any CRISPR-Cas9-based therapeutics. Indeed, the authors of the new paper acknowledged that they stacked the deck in their favor choosing a disease of the liver, in which delivery is less of a challenge and high-efficiency editing is not necessary to alter the disease.

"Hydrodynamic tail vein injection is great for delivery in mice, but it is not clinically relevant," noted Krieg.

Novak agreed that delivery is a challenge that remains to be overcome. He noted that in the study, hepatocytes with the gene correction expanded and repopulated the liver at the expense of hepatocytes with the mutation.¹⁶ He said that the selective pressure that increased the number of healthy cells mitigated the low editing efficiency.

In other indications and in other tissues, the selective advantage will not be there to help, he added.

The authors are planning to address delivery. Yin expects that the efficiency of targeting in hepatocytes can be improved with formulations that better allow delivery of nucleic acids to liver, including adeno-associated viruses or nanoparticles.

Another issue Krieg pinpointed was that CRISPR-Cas9 systems carry a greater risk of off-target activity than other oligonucleotide-based platforms.

But Novak expects that off-target concerns will be manageable. "The sgRNA is critical in determining the amount and effect of off-target activity. For some sgRNAs, with the most sensitive methods available we don't see any off-target activity and with some you see only a few sites. The design of the sgRNA

"It will be very important for the field to develop unbiased methods to evaluate offtarget cleavage that do not rely on computational predictions."

> -Rachel Haurwitz, Caribou Biosciences Inc.

is extremely simple, so we can choose the ones with the best activity and with a beneficial ratio of on-off target activity."

He said that the two important considerations for off-target activity are the number of off-target sites and the location of those sites.

"For a good sgRNA, yielding only two to three additional doublestranded breaks somewhere in the genome, we need to determine what kind of genetic elements are impacted. More research is needed to understand this," said Novak.

Rachel Haurwitz, president and CEO of **Caribou Biosciences Inc.** added, "It will be very important for the field to develop unbiased methods to evaluate off-target cleavage that do not rely on computational predictions."

Caribou was founded in 2011 and is using CRISPR-Cas9 technology to develop R&D tools.

The therapeutic road

According to Krieg, pursuing *ex vivo* applications with the editing platform looks to be the best strategy for the short term.

COVER STORY

Doudna also likes *ex vivo* applications because it derisks the platform. "You can ensure correct targeting before putting the cells back into the patient," she said.

Otherwise, she saw multiple paths forward. "People will try to target the liver and maybe the blood, where you can do the targeting *ex vivo*. Another attractive target is the eye, where delivery options such as injection can be more efficient than for other tissue types."

For nucleic acid therapeutics, "there are many *ex vivo* applications that one could consider, and CRISPR-Cas9 could be promising for *ex vivo*," said John Maraganore, CEO of **Alnylam Pharmaceuticals Inc.**, which develops RNAi therapeutics.

Alnylam is focused on *in vivo* applications of RNA therapeutics to the liver. "It's not that we can't get delivery to other cell types and tissues, but with the liver we have a very robust set of preclinical and now clinical data that give us great confidence that we can build our pipeline. The liver is going to keep us busy for a very long time," said Maraganore.

Feng Zhang, a scientific cofounder of Editas, would not discuss the company's plans but said that in general CRISPR therapies should be best suited to diseases like HIV, in which turning down gene expression is desirable, and diseases like sickle cell or hemophilia, in which repairing a mutation is the goal.

Zhang is a core member at the Broad Institute and an investigator at the **McGovern Institute for Brain Research at MIT**.

CRISPR Therapeutics will primarily focus on *ex vivo* therapeutic applications. "We know how to deliver into hematopoietic stem cells with high efficiency," Novak said. He did not disclose more specific plans but estimated that an IND submission is about two to three years away. CRISPR Therapeutics also will develop delivery systems so it can eventually pursue *in vivo* applications.

The study's findings are patented, and discussions with pharmaceutical companies for licensing and partnering are ongoing.

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TARGETS & MECHANISMS

Reversing (heart) failure in Friedreich's ataxia

By Michael J. Haas, Senior Writer

Heart failure accounts for over half the deaths in patients with Friedreich's ataxia, but no therapies exist to treat this neurodegenerative disease or its associated cardiomyopathy. Now, a team of French researchers has shown that i.v. *frataxin* gene therapy could prevent or even reverse heart failure in a mouse model of Friedreich's ataxia.¹

AAVLife, a new company founded by several team members, has licensed the findings and will test dosing and safety of direct cardiac injection of the gene therapy in healthy pigs.

Friedreich's ataxia (FRDA) is caused by inherited loss-of-function mutations in *frataxin* (*FXN*; *FRDA*), which encodes a mitochondrial protein involved in the assembly of iron-sulfur clusters that are essential to mitochondrial energy production.

FXN deficiency leads to degeneration of the spinal cord, resulting in progressive loss of motor function in the limbs, scoliosis, impaired vision and hearing, and speech problems. FXN deficiency also causes iron overload in cardiac mitochondria and impairs bioenergetics, thus contributing to heart failure.

There are no disease-modifying therapies to treat FRDA. Instead, disease management can include physical therapy to aid motor control, surgery to correct scoliosis and angiotensin-converting enzyme (ACE) inhibitors or other drugs that treat heart failure.

Thus, a team led by Hélène Puccio set out to test whether an adenoassociated virus serotype 10 (AAV10) vector encoding human *FXN* could treat cardiomyopathy in conditional *Fxn* knockout models of FRDA.

Puccio is director of **Institut National de la Santé et de la Recherche Médicale** (INSERM) research and head of the Department of Translational Medicine and Neurogenetics at the **Institute of Genetics and Molecular and Cellular Biology**.

In 2001, a group led by Puccio developed a conditional *Fxn* knockout mouse model—*Fxn^{-/-}* mice are nonviable because of embryonic lethality— that recapitulated key features of human FRDA, including cardiomyopathy and large sensory neuron dysfunction.²

In 2012, a team led by Ronald Crystal, chair of genetic medicine and a professor of internal medicine at **Weill Cornell Medical College**, showed that AAV10 exhibited significant tropism for the heart and liver—and to a lesser degree the dorsal root ganglia (DRG)—of nonhuman primates.³

The two groups connected after Crystal began collaborating on a different AAV-related project with Patrick Aubourg, who was familiar with Puccio's work on the *Fxn* knockout models.

Aubourg is co-director of INSERM's research unit at Le Kremlin-Bicêtre and head of pediatric neurology at the **University Hospital of Bicêtre** and the **University of Paris-Sud**.

The new team was led by Puccio and included Crystal and Aubourg. The group showed that prophylactic i.v. injection of the AAV10-*FXN* therapy in the mouse models, before the onset of cardiomyopathy, prevented the mitochondrial proliferation and iron accumulation in cardiomyocytes, left

ventricular hypertrophy (LVH), loss of cardiac function and death from heart failure that were seen in untreated mice.

In models showing the first signs of cardiomyopathy, the gene therapy decreased mitochondrial proliferation and levels of iron deposits in cardiomyocytes, decreased LVH and cardiac fibrosis and increased cardiac function and survival compared with no treatment.

"Our study demonstrates that frataxin-deficient cardiomyocytes have severe mitochondrial dysfunction but retain the capacity to recover rapidly once their levels of frataxin are normalized," Aubourg told *SciBX*. This in turn suggests "that the cardiomyocytes of Friedreich's ataxia patients are still present and could be rescued" with the gene therapy.

The team included a researcher from the **University of Strasbourg**. Data were reported in *Nature Medicine*.

Heartfelt delivery

Last year, Aubourg, Puccio and Crystal cofounded AAVLife to develop the AAV10-*FXN* gene therapy. Last week, the company raised \$12 million in a series A round to help fund a Phase I trial. AAVLife expects to begin the trial in 2015.

"This is an exciting therapy that we would want to see in the clinic," said Guy Miller, chairman and CEO of **Edison Pharmaceuticals Inc.** "Even though it doesn't affect CNS symptoms of Friedreich's ataxia, reversing the cardiomyopathy is reason enough to move it forward. On a scale of 1-10, we give it a 20."

Edison has two compounds in the clinic to treat FRDA. Vatiquinone (EPI-743), an oral small molecule targeting NAD(P)H dehydrogenase quinone 1 (NQO1; QR1) that augments endogenous glutathione biosynthesis, is in Phase IIb testing. EPI-A0001, a coenzyme Q10 analog that enhances electron flux and increases ATP synthesis, is in Phase IIa trials.

Susan Perlman agreed. "Improving cardiac function would be a great help in increasing energy levels and endurance in these patients, which would certainly improve their quality of life," she said.

Perlman is a clinical professor of neurology, director of the Ataxia Center and director of the **Huntington's Disease Society of America**'s Center of Excellence at the **University of California, Los Angeles**.

"The study results are impressive and highlight a promising therapeutic approach for a difficult disease," but the findings would need to be confirmed in larger animals such as pigs, whose hearts are similar in size to humans, said Hina Chaudhry, an associate professor of medicine and director of cardiovascular regenerative medicine at the **Icahn School of Medicine at Mount Sinai**.

Chaudhry also is the founder of **VentriNova Inc.**, which has VN-100, an adenoviral vector encoding *cyclin A2* (*CCNA2*), in preclinical development to treat myocardial infarction (MI).

Chaudhry and Perlman also said that additional studies will have to determine the safety of the therapy.

Perlman wanted to see follow-on studies of the treated mice to examine the effects of the therapy on tissues outside the heart. "Viral vector-based therapies have a risk of carrying their gene payloads to cells and tissues where they might prove harmful," she said.

Roger Hajjar agreed that the effects of overexpressing *FXN* in liver and other organs were unclear. Also, "AAV10 is a relatively new vector and gene therapy; investigators have little experience in its use in the heart," he said. "Its safety profile and efficacy as a vector in cardiac tissue need to be established."

TARGETS & MECHANISMS

Thus, he would have liked to see the team use an empty AAV10 vector or one encoding a nontherapeutic marker protein as a control in the mouse models.

Hajjar is a professor of medicine in cardiology at **Mount Sinai Hospital** and director of the Cardiovascular Research Center at the Icahn School of Medicine. He also is a cofounder and scientific advisory board member of **Celladon Corp.**

Celladon and **AmpliPhi Biosciences Corp.** are developing Mydicar (AAV1/SERCA2a), a recombinant AAV vector encoding *ATPase Ca⁺⁺ transporting cardiac muscle slow twitch 2 (ATP2A2; SERCA2A)* that is in Phase II testing to treat heart failure and in preclinical development to treat hypertension. Celladon also has CDN1163, a small molecule targeting SERCA enzymes, in preclinical development to treat diabetes and cardiovascular and neurological diseases.

Hajjar and Perlman said that the benefits of the gene therapy in treating cardiomyopathy in patients with FRDA clearly outweighed any potential risks associated with the vector or overexpression of *FXN* outside the heart.

"Since *frataxin* is expressed at low levels in all cell types, we are not worried about off-target effects," said Amber Salzman, cofounder and CEO of AAVLife.

Crystal added that AAVLife plans to test the efficacy and safety of direct cardiac injection—not i.v. injection—of the therapy in pigs. "The issue will be whether we can genetically modify 40%–50% of cardiomyocytes, which we think will be sufficient" to prevent or treat cardiomyopathy in FRDA, he said.

The team will explore the number, size and spatial separation of direct cardiac injections of the AAV10-*FXN* therapy necessary to modify that fraction of cells in healthy pigs because there are no pig models of FRDA, he said.

Salzman added that the studies in pigs will compare two types of direct cardiac injection—into the myocardium and into coronary veins—to determine which route can modify the desired 40%–50% of cardiomyocytes.

"Our concern is that coronary injection will not get us there because the therapy will pass through the heart and get taken up by the liver," she said. "We don't want to have an overload of the vector in the liver because that could present a safety risk at high doses."

Conversely, Chaudhry said that "as long as excess *frataxin* expression in the liver and other organs isn't a problem, I think i.v. injection would be the way to go with this therapy" because direct cardiac injection is more complicated and invasive than i.v. injection.

In addition, she said, using a cardiac-specific promoter with the AAV10-*FXN* therapy could enhance the safety of i.v. injection by preventing unwanted expression of the gene outside the heart.

All the nerve

In addition to investigating the safety and delivery routes of the AAV10-*FXN* gene therapy, Perlman and Miller wanted to know whether it could be adapted to treat the neurological symptoms of FRDA.

"The team reported that the AAV10 vector not only has tropism for the heart but also for specific cell types in the CNS, including the DRG, that are among the primary sites of neuropathology in the disease," said Jennifer Farmer, executive director of the **Friedreich's Ataxia Research Alliance** (FARA). "The team also reported that they were able to detect expression of the *FXN* transgene in several CNS cell types in the mouse models."

FARA provided funding for the Nature Medicine study.

"I hope that similar benefits with a gene therapy targeting the spinal cord or cerebellar structures of the CNS can be demonstrated" in mouse models with neurological symptoms of FRDA, Perlman said.

"A key question now is how to develop an analogous vector that would target the CNS to ameliorate neurological symptoms of Friedreich's ataxia," Miller said.

Crystal agreed that "the challenge with the nervous system is getting gene therapy into the right cells—especially the spinal cord and cerebellum. But we are working on a CNS-specific version of the gene therapy."

Those studies will use a neurological mouse model of FRDA developed by the team, Aubourg said. "We will also use nonhuman primates to assess the best and safest route of vector delivery to target the populations of neurons in DRG, the spinal cord and cerebellum that are affected by the disease."

But first, he said, the team is completing dose-response studies of the gene therapy in the cardiomyopathic mouse models, planning the studies of the therapy in healthy pigs and planning studies to confirm the cardiac tropism of the AAV10 vector in nonhuman primates.

Crystal and Farmer said that FARA would assist with patient recruitment once the therapy is ready for the clinic.

According to Aubourg, INSERM and **Cornell University** have filed a patent application covering the findings reported in *Nature Medicine*.

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TARGETS & MECHANISMS

Leveling cancer with IL-15

By Kai-Jye Lou, Senior Writer

Initial excitement about using IL-15 as a therapeutic in cancer has been tempered by substantial delivery and efficacy issues. Now, data from an **Institut National de la Santé et de la Recherche Médicale**–led team showing that IL-15 stimulates T cell proliferation inside tumors suggests that the cytokine could help boost the potency of checkpoint inhibitors and other immunotherapies that harness T cells to attack cancer cells.¹

The data also highlight IL-15 as a potential prognostic biomarker for predicting patient outcomes in colorectal cancer and other malignancies.

IL-15 is an immunostimulatory cytokine that promotes the proliferation of various immune cell populations by activating a dimeric receptor complex on immune cells composed of subunits shared with the IL-2 receptor.

According to Peter Rhode, VP of R&D at cancer company **Altor BioScience Corp.**, interest in developing IL-15 as an immunotherapy for cancer was first sparked over five years ago, when the cytokine was viewed as a potential successor to IL-2-based immunotherapies.

Although both cytokines activate similar downstream pathways because of the shared receptor subunits, IL-15 was perceived as a safer alternative to IL-2 based on animal data suggesting decreased toxicity.²

"Our work provides one mechanism explaining the difference in T cell levels we see in patient tumors that translate into differences in clinical outcomes."

> — Jérôme Galon, Institut National de la Santé et de la Recherche Médicale

However, according to Thomas Waldmann, chief of the metabolism branch at the **National Cancer Institute** and co-discoverer of IL-15, concerns related to translation of animal data to humans and delivery difficulties hampered its clinical development. His group and other groups are still working to resolve those problems.

One approach has been to engineer molecules that combine IL-15 and the IL-15 receptor α -chain (IL-15RA) to increase the potency of the immune response.

One of Altor's lead compounds, ALT-803, is an IL-15-mutant agonist complexed to an Fc fusion protein of IL-15RA. The product is in Phase I/II trials for metastatic melanoma and hematologic malignancies.

Now, a large-scale association study of colorectal cancer tumors led by Jérôme Galon at the Institut National de la Santé et de la Recherche Médicale (INSERM) has provided patient data highlighting the importance of IL-15 to patient outcomes and the proliferation of various immune cell populations in tumors.

Galon is a research director at INSERM and head of the institute's Laboratory of Integrative Cancer Immunology.

Galon's aim was to unravel the complex roles of cytokines in regulating the immune response inside tumors that helps control their growth.

His team had previously shown that the number of infiltrating cytotoxic and memory T cells in tumors could predict patient outcomes with greater accuracy than conventional tumor-staging approaches.³⁻⁶

Higher densities of those immune cells at the centers and invasive margins of tumors correlated with better disease outcomes and increased survival, especially in colorectal cancer.

However, the underlying biological factors influencing the levels of these infiltrating immune cell populations in tumors were poorly

understood. Several genomic and expression changes in cytokines had been linked to colorectal cancer tumor progression and recurrence, whereas other cytokines showed no association.

That led Galon and colleagues to design a comprehensive analysis of cytokines in a large number of colorectal cancer tumors to "The results suggest that IL-15-based therapies could be effective in providing antitumor activity, at least for those patients who have reduced IL-15 expression at the tumor site."

> —Peter Rhode, Altor BioScience Corp.

identify which cytokines might be altered and what role they play in the intratumoral immune reaction.

The researchers assessed expression of 59 cytokines or their receptors in samples from multiple cohorts of patients with colorectal cancer. They found that patients with tumors containing an *IL-15* gene deletion had lower levels of B and T cell proliferation in the tumor microenvironment than patients without the deletion.

In samples from patients with high *IL-15* expression, increased densities of proliferating T and B cells were seen in both the invasive margins and center of the tumor compared with those in samples from patients with low *IL-15* expression.

Overall, patients with low IL-15 levels—including those without an *IL-15* deletion in the tumor—had increased risk of tumor recurrence and decreased survival compared with patients with high IL-15 levels.

The researchers also showed that in colorectal cancer tissue samples, incubation with IL-15 induced proliferation of T cell populations.

Mechanistically, the results suggest that IL-15 stimulates T cells and thus confirm the cytokine's role as a key mediator of the immune response in the tumor microenvironment. Practically, the data position IL-15 as a robust predictor of patient outcomes in colorectal cancer.

Results were published in Science Translational Medicine.

Beyond prognostics

Although Galon's study establishes IL-15 as a prognostic biomarker in colorectal and other cancers, it also provides new arguments to further develop the therapeutic potential of the cytokine.

According to Galon, the study suggests that IL-15 might be the missing mechanistic link for correlations seen in the clinic between T cell levels and patient outcomes.

"Our work provides one mechanism explaining the difference in T cell levels we see in patient tumors that translate into differences in clinical outcomes," he said.

Galon added that the results could help identify potential patient populations that would respond to IL-15-based immunotherapies. These could include patients with tumors containing a defect in IL-15

TARGETS & MECHANISMS



Figure 1. Bolstering cancer immunotherapy with IL-15. (I) IL-15 is an immunostimulatory cytokine that typically is found pre-bound to the IL-15 receptor α -chain (IL-15RA) on antigen-presenting cells (APCs) such as dendritic cells. These APCs display IL-15 to a heterodimeric receptor complex on T and NK cells to induce their proliferation.

The receptor complex is composed of the IL-2 receptor β -chain (CD122; IL2RB) and IL-2 receptor γ -chain (CD132; IL2RG). IL-15 also inhibits apoptosis pathways in the various leukocyte populations, resulting in more persistent immune cells.

Candidate therapies that stimulate IL-15 activity can potentially bolster the efficacy of cancer immunotherapies.

One potential combination is with checkpoint inhibitors such as the anti-programmed cell death 1 (PDCD1; PD-1; CD279) mAbs, which block the interaction between PD-1 and programmed cell death 1 ligand 1 (CD274 molecule; PD-L1; B7-H1). The combination could yield a larger and more persistent pool of immune cells available to attack cancer cells and thus could result in a more robust antitumor response.

(II) Without IL-15 stimulation, fewer immune cells are around to attack cancer cells after treatment with a checkpoint inhibitor.

or those who have lower-than-average numbers of infiltrating T cells, he said.

Rhode agreed that the findings could support an IL-15-targeted therapy for specific patient populations.

"The results suggest that IL-15-based therapies could be effective in providing antitumor activity, at least for those patients who have reduced IL-15 expression at the tumor site," he said.

In addition, the results further strengthen IL-15's position relative to IL-2.

"Both cytokines seem to act through the same receptor but trigger a different response, with IL-15 inducing a stronger proliferative response than IL-2," Rhode told *SciBX*.

Novartis AG markets Proleukin aldesleukin IL-2 for metastatic melanoma and renal cell carcinoma (RCC). Eisai Co. Ltd. markets Ontak denileukin diftitox for cutaneous T cell lymphoma (CTCL). Proleukin and Ontak both carry black box warnings for serious adverse effects such as capillary leak syndrome.

Moreover, according to both Galon and Rhode, the cytokine's ability

to stimulate T cell proliferation suggests that IL-15 immunotherapies might work synergistically with various cancer immunotherapies, such as those that block immune checkpoint proteins (*see* Figure 1, "Bolstering cancer immunotherapy with IL-15").

Immune checkpoint proteins, such as CTLA-4 (CD152) and programmed cell death 1 (PDCD1; PD-1; CD279), act as regulatory controls to dampen excessive T cell activation and prevent autoimmunity. However, tumor cells are known to express checkpoint proteins to evade the host immune system.

"Checkpoint inhibitor therapies initiate a T cell attack against cancer cells. Adding IL-15 can help boost the proliferation of these T cells," said Galon.

Bristol-Myers Squibb Co. markets the anti-CTLA4 mAb Yervoy ipilimumab for metastatic melanoma. Yervoy is the first checkpoint inhibitor to reach the market.

The two most advanced anti-PD1 mAbs are nivolumab from Bristol-Myers and partner **Ono Pharmaceutical Co. Ltd.** and MK-3475 from **Merck & Co. Inc.**

(Continues on p. 9)

TARGETS & MECHANISMS

Mired in miR-25

By Lauren Martz, Staff Writer

Preclinical data showing that microRNA-25 inhibition can help treat heart failure directly contradict a 2013 paper suggesting that decreased levels of the miRNA provoke the condition.^{1,2} Although the new study shows that blocking microRNA-25 restores levels of the SERCA2A calcium uptake channel—and thus improves cardiac contractility—the discrepancy with the earlier result needs resolving before a microRNA-25-based therapy can be developed.

The two studies zeroed in on microRNA-25 (miR-25) by different routes.

The new study, headed by Mark Mercola at the **University of California, San Diego**, found miR-25 by focusing on the connection between intracellular calcium and heart muscle contraction and screening for miRNA inhibitors of *SERCA2A* (*ATPase Ca*⁺⁺ *transporting cardiac muscle slow twitch 2*; *ATP2A2*).

The findings were published in Nature.

By contrast, last year's study, headed by Leon De Windt at **Maastricht University**, pinpointed miR-25 by searching for post-transcriptional regulators of cardiomyocyte hypertrophy in mouse models of heart failure.

That study found that inhibiting miR-25 in healthy mice led to cardiac dysfunction and increased the animals' susceptibility to heart failure. In addition, inhibiting miR-25 in mice with heart failure exacerbated the disease.

Those data were published in Nature Cell Biology.

Mercola is a professor of bioengineering at the University of California, San Diego and a professor and director of the Muscle Development and Regeneration Program at the **Sanford-Burnham** **Medical Research Institute**. De Windt is a professor of molecular cardiology at Maastricht University.

Mercola's team sought regulators of SERCA2A because previous work from collaborators at the **Icahn School of Medicine at Mount Sinai** and **Celladon Corp.** and from other academic labs tied *SERCA2A* expression to heart failure. Those studies showed that restoring *SERCA2A* expression with adeno-associated virus (AAV) vector-mediated gene therapy can improve cardiac function in animal models of heart failure and patients with heart failure.^{3,4}

The patient study was a Phase I/II trial by Celladon and the Icahn School of Medicine on the company's Mydicar (AAV1/SERCA2a), an AAV vector encoding *SERCA2A*. Mydicar significantly reduced cardiovascular events and met the primary endpoint of reducing a composite of outcome measures in patients with advanced heart failure. The product has breakthrough designation from the FDA for reducing hospitalizations in patients with heart failure and is in Phase IIb testing.

SERCA2A mediates calcium ion uptake in cardiomyocytes, which is necessary for normal contraction of the heart muscle. During heart failure, decreased SERCA2A activity impairs calcium uptake and prevents normal cardiac contractions.

However, the resulting decrease in cardiac contractility is only one component in the complex process of heart failure; thus, replacing *SERCA2A* via gene therapy only addresses part of the cause of the heart dysfunction.

In addition, gene therapy allows limited control over the level of protein expression and does not work for all patients because of immunological variability in the population.

Roger Hajjar—the Mount Sinai collaborator on the study told *SciBX*, "One of the problems with using AAVs is that half the population has neutralizing antibodies against AAV1 and has to be excluded from the studies."

William Marshall added that even when patients can be treated

(Continues on p. 10)

(Continued from "Leveling cancer with IL-15," p. 8)

Nivolumab is in Phase III testing for metastatic melanoma, nonsmall cell lung cancer (NSCLC) and RCC. Ono submitted an NDA in Japan for nivolumab to treat melanoma last December.

MK-3475 is in Phase III trials for advanced melanoma, for which it has breakthrough therapy designation from the FDA. It is in Phase II/III testing for NSCLC and Phase I/II trials for RCC. Merck began submission of a rolling BLA for MK-3475 to treat advanced melanoma in January.

Galon told *SciBX* that his group plans to develop mouse models to evaluate how manipulating IL-15 signaling affects tumor growth. He said that some of these studies will include evaluation of IL-15 in combination with checkpoint inhibitors.

INSERM has filed a patent covering an immune gene signature for predicting prognosis of patients with cancer and for tumor classification. The signature includes IL-15.

Lou, K.-J. *SciBX* 7(16); doi:10.1038/scibx.2014.449 Published online April 24, 2014

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Altor BioScience Corp., Miramar, Fla. Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y. Eisai Co. Ltd. (Tokyo:4523), Tokyo, Japan Institut National de la Santé et de la Recherche Médicale, Paris, France Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J. National Cancer Institute, Bethesda, Md. Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland

Ono Pharmaceutical Co. Ltd. (Tokyo:4528), Osaka, Japan

TARGETS & MECHANISMS

with AAV-mediated gene therapy, they are generally limited to one dose. "With a gene therapy, you really only get one shot on goal, and then the immune system is able to prevent the virus from delivering its cargo."

Hajjar is director of the Cardiovascular Research Center at the Icahn School of Medicine and a professor of medicine in cardiology at **Mount Sinai Hospital**. He is also a cofounder and scientific advisory board member of Celladon. Marshall is CEO of **miRagen Therapeutics Inc.**

miRagen's antimiR-208, a modified short nucleic acid sequence targeting miR-208, is in preclinical testing for heart failure. "miR-25 is par

Mercola and colleagues wanted to find an alternative way of controlling SERCA2A that would avoid the drawbacks of gene therapy. Because miRNA dysregulation had been implicated in heart failure pathology,⁵ the researchers thought an miRNA might be responsible for the downregulation of *SERCA2A*.

miRNAs silence gene expression by binding the mRNA seed region, a 2–8 base pair stretch in the 5' untranslated region, to prevent translation.

The team performed a whole-genome

miRNA screen in human embryonic kidney cells and identified miR-25 as the most potent miRNA at targeting and downregulating *SERCA2A*.

In cardiomyocytes, miR-25 effectively disrupted calcium transport by delaying calcium uptake kinetics in cardiomyocytes.

The researchers examined myocardial samples from five patients with severe heart failure and found higher miR-25 levels in the left ventricles than in tissue from five control hearts from patients without contractile dysfunction.

Next, the team tested the effect of modulating miR-25 levels *in vivo*. In healthy mice, gene transfer of *miR-25* decreased Serca2a levels and caused a greater decline in cardiac function compared with what was seen using miR-92a, a miRNA in the same family as miR-25 that shares its seed region.

Conversely, injection of an anti-miR-25 oligonucleotide increased Serca2a levels in wild-type mice compared with injection of a scrambled anti-miRNA. Anti-miR-25 had no effect on Serca2a levels or cardiovascular parameters in Serca2a^{-/-} knockout mice.

Finally, the group assessed the effects of the anti-miRNA in a mouse model of heart failure.

In mice with established heart failure induced by transverse aortic constriction for 3 months, anti-miR-25 improved cardiac function at 4.5 and 5.5 months. The anti-miR-25 also restored left ventricular ejection fraction to normal levels and improved survival, and it increased SUMOylated Serca2a levels and decreased cardiac fibrosis compared with control anti-miRNA.

Together, the data suggest that miR-25 upregulation during heart failure causes SERCA2A suppression and, consequently, contractile dysfunction. The team also concluded that blocking miR-25 can reverse the decline in heart function.

"miR-25 is part of a family of molecules with conserved seed regions. This means that multiple miRNAs may be binding to the same regulatory motif on SERCA2A, and the selectivity of the molecules for miR-25 over other family members could affect the results." —William Marshall, miRagen Therapeutics Inc.

The group included researchers from the University Medical Center Utrecht, ICIN Netherlands Heart Institute and Gwangju Institute of Science and Technology.

miR-25 conflict

Several researchers told *SciBX* that the first step should be to reconcile the results with the conflicting data from the Maastricht study.

According to Mercola, the discrepancies may be due to the different disease stages analyzed by the two groups. Whereas his team examined miR-25 in samples from patients with advanced heart failure, De Windt's

team investigated changes during the early stages of pressure overload.

Mercola added that data from the Maastricht team suggest that "we might not want to block miR-25 in people who do not yet have clear signs of heart failure."

"Since heart failure results from various diseases, we will need to learn more about what types and states of disease are likely to benefit," he said.

Marshall suggested that the conflicting data could be due to the different anti-miR-25 molecules used by each team. "miR-25 is part of a family of molecules with conserved seed regions. This means that multiple miRNAs

may be binding to the same regulatory motif on *SERCA2A*, and the selectivity of the molecules for miR-25 over other family members could affect the results."

De Windt told *SciBX* that his team plans to repeat the mouse experiments. "This happens all the time in science, and it is our responsibility to sort it out."

He also pointed out some aspects of the work in the *Nature* paper that may contribute to the inconsistent results.

"First, the [Sanford-Burnham] team screened for miRNAs targeting *SERCA2A* in human embryonic kidney cells. While it was a smart idea to screen for miRNAs against the target, miRNAs behave completely differently in different cell types. Most of the miRNA candidates identified in their screen are not even present in the heart," he said.

De Windt added that Mercola's team drew conclusions from a small number of human hearts and animals and suggested that replication in larger cohorts would strengthen the results.

Despite the tissue data from patients with advanced heart failure, De Windt commented that the mouse model used in the *Nature* paper was not very severe. "The left ventricular ejection fraction only decreases by 6% during heart failure relative to healthy mice. In healthy human hearts, the ejection fraction is about 60% of the heart's blood volume per pump, and this fraction is reduced by about half in heart failure. The experiments should be repeated in a model that more closely mimics the extent of heart failure in patients," he said.

Translational resolution

De Windt told *SciBX* that his team now plans to develop a cardiac-specific *miR-25* knockout mouse model. He said that would give a definitive answer about how the miRNA is involved in mouse heart failure.

But Krisztina Zsebo, president and CEO of Celladon, said that

TARGETS & MECHANISMS

mouse models of heart disease and the effects of polynucleotide therapeutics including viral vectors in mice do not generally translate well to humans.

"In mice, the effects of i.v. injection of antisense oligonucleotides are unlikely to translate to effects in large animal models due to the difference in blood volume. The small blood volume in mice allows a high concentration of the nucleic acid that you just can't achieve in large animal models," she said.

Indeed, Mercola's next step is to evaluate miR-25 in heart failure models in larger animals such as pigs, dogs, sheep or nonhuman primates.

However, delivery of oligonucleotides to the heart in larger species presents its own challenges.

Neil Gibson, CSO of **Regulus Therapeutics Inc.**, said that delivering oligonucleotides to specific tissues presents an opportunity for advancement in the field.

"Single-stranded oligonucleotides in saline can be used as therapeutics to target miR-25 to treat heart failure, for example, but the majority of the oligonucleotide tends to go to limited places in the body such as the liver or macrophages. Delivery to the heart or other less-accessible cell types is a challenge that still needs a lot of work," he said.

"Active investigation is ongoing to determine the best type of antimiRNA delivery strategy for the heart. Our company has found that conjugating oligomers to a ligand that engages cell-specific receptors effectively targets hepatocytes. It may be possible to identify receptors with cardiac-specific expression to enrich oligomer accumulation in the heart to treat heart failure," he added.

Regulus' RG-101, a ligand-conjugated anti-miRNA targeting miR-122, is in Phase I testing to treat HCV.

Antisense vs. AAV

Celladon's Zsebo is not convinced that an oligonucleotide-based approach will upregulate *SERCA2A* as effectively as gene therapy, despite the fact that anti-miR-25 may affect multiple dysregulated pathways in heart failure, including SERCA2A.

"SERCA2A is downregulated at both the transcriptional and translational levels in heart failure. Antisense oligonucleotides only block the translational downregulation," she said. She added that the effects of antisense oligonucleotides are very short lived compared with the effects of gene therapy as they have half-lives of only 10–12 hours. The effects of AAV-mediated gene therapy may last longer than four years.

However, Marshall said that antisense therapeutic formulation methods can achieve high stability and extend half-lives *in vivo*. Although miRagen's antisense molecules have not been tested in humans, he expects that, based on rat studies, patients could be dosed every few weeks or every month.

Mercola told *SciBX* that there are two principal advantages to an miR-25 approach. "Pharmacological intervention can be titered or even halted depending on the needs for managing a patient's disease, and miR-25 might block expression of other proteins besides SERCA2A that control calcium handling and contractility."

Sanford-Burnham has filed a patent application covering the work. The IP is available for licensing.

Martz, L. *SciBX* 7(16); doi:10.1038/scibx.2014.450 Published online April 24, 2014

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Icahn School of Medicine at Mount Sinai, New York, N.Y. ICIN Netherlands Heart Institute, Utrecht, the Netherlands Maastricht University, Maastricht, the Netherlands miRagen Therapeutics Inc., Boulder, Colo. Mount Sinai Hospital, New York, N.Y. Regulus Therapeutics Inc. (NASDAQ:RGLS), San Diego, Calif.

Sanford-Burnham Medical Research Institute, La Jolla, Calif. University of California, San Diego, La Jolla, Calif. University Medical Center Utrecht, Utrecht, the Netherlands

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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
Cancer				
Breast cancer	Homeobox C10 (HOXC10)	<i>In vitro</i> and mouse studies suggest decreasing <i>HOXC10</i> methylation could help treat breast cancers resistant to aromatase inhibitors and other estrogen-depleting therapies. In human breast cancer cell lines, silencing <i>HOXC10</i> via histone and DNA hypermethylation was associated with resistance to estrogen deprivation. In an estrogen-deprived mouse xenograft model of breast cancer, silencing <i>HOXC10</i> expression increased tumor growth compared with unmodified <i>HOXC10</i> expression. In patients with breast cancer who received aromatase inhibitor therapy, recurrent tumors had lower HOXC10 protein levels than primary tumors. Future studies could include testing aromatase inhibitors and DNA hypomethylating agents in mouse models of breast cancer. Celgene Corp., Pfizer Inc. and Nippon Shinyaku Co. Ltd. market Vidaza azacitidine, a hypomethylating agent that inhibits DNA methyltransferase, to treat myelodysplastic syndrome (MDS). Celgene and Pfizer market Vidaza to treat acute myelogenous leukemia (AML). Otsuka Pharmaceutical Co. Ltd., Eisai Co. Ltd. and Johnson & Johnson market Dacogen decitabine, a hypomethylating agent that inhibits DNA methyltransferase, to treat MDS and AML. Celgene has CC-486, an oral formulation of azacitidine, in Phase II trials to treat MDS and Phase I testing to treat solid tumors.	Patent and licensing status unavailable	Pathiraja, T.N. <i>et al. Sci. Transl. Med.</i> ; published online March 26, 2014; doi:10.1126/scitranslmed.3008326 Contact: Steffi Oesterreich, Women's Cancer Research Center at the University of Pittsburgh, Pittsburgh, Pa. e-mail: oesterreichs@upmc.edu
		<i>SciBX</i> 7(16); doi:10.1038/scibx.2014.451 Published online April 24, 2014		
Breast cancer	X-box binding protein 1 (XBP1)	Studies in mice and patient samples suggest inhibiting the XBP1 branch of the unfolded protein response could help treat triple- negative breast cancer (TNBC). In mouse xenograft models of TNBC, shRNA against <i>XBP1</i> decreased tumor growth, lung metastasis and tumor relapse compared with control shRNA. In two independent TNBC cohorts, an expression signature encompassing 96 XBP1-regulated genes was associated with decreased relapse-free survival. Next steps include establishing preclinical and genetic models to validate the XBP1 pathway as a therapeutic target.	Patent application filed; unavailable for licensing	Chen, X. <i>et al. Nature</i> ; published online March 23, 2014; doi:10.1038/nature13119 Contact: Laurie H. Glimcher, Weill Cornell Medical College, New York, N.Y. e-mail: lglimche@med.cornell.edu
		SciBX 7(16); doi:10.1038/scibx.2014.452		

Published online April 24, 2014

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Cancer	7,8-Dihydro- 8-oxoguanine triphosphatase (NUDT1; MTH1)	<i>In vitro</i> , cell culture and mouse studies have identified inhibitors of MTH1 that could help treat cancer. MTH1 cleaves and neutralizes oxidized nucleotides, which can accumulate and cause DNA damage. In cancer cell lines, shRNA against <i>MTH1</i> induced cell death, whereas control shRNA did not. In cell lines, a series of small molecule MTH1 inhibitors, including the (<i>S</i>) stereoisomer of Xalkori crizotinib, inhibited MTH1 with nanomolar potency, induced DNA damage and killed cancer cells at micromolar concentrations without also killing untransformed cells. In multiple mouse models of cancer, including Ras-mutant cancers, the inhibitors decreased tumor growth compared with vehicle. Next steps include optimizing compounds for additional preclinical testing. Pfizer Inc. markets the c-Met receptor tyrosine kinase and anaplastic lymphoma kinase (ALK) inhibitor Xalkori, the (<i>R</i>) stereoisomer of crizotinib, to treat ALK fusion–positive non– small cell lung cancer (NSCLC).	Patent applications filed for both studies; available for licensing	Huber, K.V.M. <i>et al. Nature</i> ; published online April 2, 2014; doi:10.1038/nature13194 Contact: Giulio Superti-Furga, Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria e-mail: gsuperti@cemm.oeaw.ac.at Gad, H. <i>et al. Nature</i> ; published online April 2, 2014; doi:10.1038/nature13181 Contact: Thomas Helleday, Karolinska Institute, Stockholm, Sweden e-mail: thomas.helleday@scilifelab.se
		SciBX 7(16); doi:10.1038/scibx.2014.453 Published online April 24, 2014		
Cancer	CD248 endosialin (TEM1)	Mouse studies suggest a DNA plasmid encoding a TEM1– tetanus toxoid (TT) fusion peptide could be used as a cancer vaccine. TEM1 is overexpressed in the vasculature and stroma of many human tumors. In multiple mouse xenograft models of human cancer, vaccination with a DNA plasmid encoding Tem1-TT resulted in decreased tumor angiogenesis and growth compared with vaccination using a DNA plasmid encoding Tem1 or TT alone. Next steps include optimizing the vaccine's potency and designing a Phase I trial to evaluate it.	Patented in the U.S.; patent application filed in the EU; available for licensing	Facciponte, J.G. <i>et al. J. Clin. Invest.</i> ; published online March 18, 2014; doi:10.1172/JCI67382 Contact: Andrea Facciabene, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa. e-mail: facciabe@mail.med.upenn.edu
		SciBX 7(16); doi:10.1038/scibx.2014.454 Published online April 24, 2014		
Cancer	G protein-coupled receptor 39 (GPR39); smoothened (SMO)	<i>In vitro</i> studies suggest agonizing GPR39 could inhibit the hedgehog pathway to help treat cancer. A cell-based screen for hedgehog pathway inhibitors identified cyclohexyl-methyl aminopyrimidines (CMAPs) that inhibited the pathway downstream of SMO. In mouse cells with an active hedgehog pathway, siRNA targeting <i>Gpr39</i> attenuated CMAP-induced loss of cellular viability. In the mouse cell lines, overexpression of <i>Gpr39</i> increased CMAP-induced loss of cellular viability compared with wild-type expression. Next steps include identifying specific hedgehog-dependent cancers that express GPR39. Heptares Therapeutics Ltd. has a GPR39 agonist in preclinical testing to treat diabetes.	Patent status undisclosed; available for licensing	Bassilana, F. <i>et al. Nat. Chem. Biol.</i> ; published online March 16, 2014; doi:10.1038/nchembio.1481 Contact: Sarah J. Luchansky, Novartis Institutes for BioMedical Research, Cambridge, Mass. e-mail: sarah.luchansky@novartis.com Contact: Rishi K. Jain, same affiliation as above e-mail: rishi.jain@novartis.com
		<i>SciBX</i> 7(16); doi:10.1038/scibx.2014.455 Published online April 24, 2014		

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Colorectal cancer	IL-15	Patient studies suggest IL-15 could be useful for treating colorectal cancer. In a cohort of patients with colorectal cancer, carriers of an IL-15 deletion had lower IL-15 expression and showed lower levels of B and T cell proliferation in the tumor microenvironment than patients without the deletion. Carriers of the <i>IL-15</i> deletion also showed increased risk of tumor recurrence and decreased survival. Next steps include developing mouse models to evaluate the therapeutic use of IL-15 alone and in combination with checkpoint inhibitors. Altor BioScience Corp. has ALT-803, an IL-15-mutant agonist complexed to an Fc fusion protein of IL-15 receptor α -chain (IL-15RA), in Phase I testing to treat melanoma and preclinical development for other cancers. Cytune Pharma S.A.S. has CYP0150, a fusion protein of IL-15 and IL-15RA, in preclinical development for cancer (<i>see</i> Leveling cancer with IL-15 page 7).	Patent application filed covering gene signature for cancer prognosis and tumor classification that includes IL-15; available for licensing and partnering	Mlecnik, B. <i>et al. Sci. Transl. Med.</i> ; published online March 19, 2014; doi:10.1126/scitranslmed.3007240 Contact: Jérôme Galon, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France e-mail: jerome.galon@crc.jussieu.fr
		SciBX 7(16); doi:10.1038/scibx.2014.456 Published online April 24, 2014		
Non–small cell lung cancer (NSCLC)	PTK7 protein tyrosine kinase 7 (PTK7; CCK4)	Studies in mice and patient samples suggest inhibiting PTK7 could be useful for treating lung adenocarcinomas. In primary human lung adenocarcinoma samples, compared with normal lung tissue, PTK7 was overexpressed. In a mouse xenograft model of lung adenocarcinoma, shRNA against <i>PTK7</i> decreased tumor growth compared with control shRNA. Next steps could include screening for and evaluating pharmacological PTK7 inhibitors in mouse models of lung adenocarcinoma.	Patent and licensing status unavailable	Chen, R. <i>et al. Cancer Res.</i> ; published online March 20, 2014; doi:10.1158/0008-5472.CAN-13-2775 Contact: E. Alejandro Sweet-Cordero, Stanford University, Stanford, Calif. e-mail: ascor@stanford.edu
		<i>SciBX</i> 7(16); doi:10.1038/scibx.2014.457 Published online April 24, 2014		
Sarcoma	Glycogen dependent kinase 3 (GSK3)	Zebrafish and cell culture studies suggest GSK3 inhibition could help treat embryonal rhabdomyosarcoma (ERMS). In human ERMS cells, GSK3 inhibitors identified from a screen of about 40,000 compounds decreased growth compared with vehicle. In zebrafish with ERMS tumors, a GSK3 inhibitor suppressed tumor growth, depleted tumor-propagating cells and prevented tumor cell self-renewal, whereas vehicle did not. Next steps could include testing GSK3 inhibitors in mouse models of ERMS.	Patent and licensing status unavailable	Chen, E.Y. et al. Proc. Natl. Acad. Sci. USA; published online March 24, 2014; doi:10.1073/pnas.1317731111 Contact: David M. Langenau, Massachusetts General Hospital, Boston, Mass. e-mail: dlangenau@mgh.harvard.edu Contact: Xu Wu, Harvard Stem Cell Institute, Cambridge, Mass.
		Published online April 24, 2014		e-mail: xwu@cbrc2.mgh.harvard.edu
Cardiovascula	ar disease			
Cardiovascular disease	Adenosine A ₁ receptor (ADORA ₁)	<i>In vitro</i> studies identified a biased ADORA ₁ agonist that could help treat cardiovascular diseases without common side effects. ADORA ₁ agonists have cardioprotective effects but can cause bradycardia as an on-target adverse effect. A biased ADORA ₁ ligand designed by linking adenosine—an orthosteric agonist— with a positive allosteric modulator of ADORA ₁ signaling had a potent agonist effect without also inducing bradycardia- associated signaling. In rat cardiomyoblasts expressing Adora ₁ , the agonist conferred better protection from ischemia than either compound alone and did not decrease isolated rat atrial heart rate. Next steps include demonstrating efficacy across a broader range of cardiovascular models. Bayer AG has the ADORA ₁ partial agonist BAY 1067197 in Phase I/II testing to treat heart failure.	Findings unpatented; unavailable for licensing	Valant, C. <i>et al. Proc. Natl. Acad. Sci.</i> <i>USA</i> ; published online March 11, 2014; doi:10.1073/pnas.1320962111 Contact: Arthur Christopoulos, Monash University, Parkville, Victoria, Australia e-mail: arthur.christopoulos@monash.edu Contact: Peter J. Scammells, same affiliation as above e-mail: peter.scammells@monash.edu
		<i>SciBX 1</i> (16); doi:10.1038/scibx.2014.459 Published online April 24, 2014		

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Endocrine/me	tabolic disease			
Diabetes	Dipeptidyl peptidase-4 (DPP- 4; CD26)	Mouse studies suggest a DPP-4 peptide vaccine could help treat type 2 diabetes. DPP-4 increases degradation of glucagon-like peptide-1 (GLP-1), which normally enhances insulin secretion and insulin sensitivity. In mice, a DPP-4 vaccine candidate was shown to induce DPP-4-specific antibody titers and decrease DPP-4 in plasma and increase GLP-1 levels compared with the vaccine's protein carrier alone. In two mouse models of diabetes or in mice fed a high-fat diet, the vaccine delayed diabetes onset, decreased postprandial glucose levels and increased insulin sensitivity. Next steps could include testing the long-term effects of the vaccine and evaluating it in additional diabetes models.	Patent and licensing status unavailable	Pang, Z. et al. Proc. Natl. Acad. Sci. USA; published online March 17, 2014; doi:10.1073/pnas.1322009111 Contact: Hironori Nakagami, Osaka University, Osaka, Japan e-mail: nakagami@gts.med.osaka-u.ac.jp
		SciBX 7(16); doi:10.1038/scibx.2014.460 Published online April 24, 2014		
Gaucher's disease	Glucosidase-β bile acid 2 (GBA2)	Mouse studies suggest inhibiting GBA2 could help treat type 1 Gaucher's disease. In a mouse model of Gaucher's disease, <i>Gba2</i> knockout resulted in decreased disease severity compared with parental controls with no <i>Gba2</i> alteration. Next steps include testing GBA2 inhibitors on animal models of Gaucher's disease. <i>SciBX</i> 7(16): doi:10.1038/scibx 2014.461	Patent and licensing status undisclosed	Mistry, P.K. <i>et al. Proc. Natl. Acad. Sci.</i> <i>USA</i> ; published online March 17, 2014; doi:10.1073/pnas.1400768111 Contact: Mone Zaidi, Icahn School of Medicine at Mount Sinai, New York, N.Y.
		Published online April 24, 2014		e-mail: mone.zaidi@mountsinai.org
Obesity	Iroquois homeobox 3 (IRX3)	<i>In vitro</i> and mouse studies suggest decreasing <i>IRX3</i> expression could help treat obesity. In mice, sequencing and circular chromosome conformation capture studies showed that obesity-associated regions of the <i>fat mass and obesity associated (Fto)</i> gene bound the <i>Irx3</i> promoter and enhanced <i>Irx3</i> expression. In mice, <i>Irx3</i> knockout decreased weight and body fat content compared with no alteration. In human brain tissue samples, SNPs associated with increased body mass index were associated with increased IRX3 expression. Next steps could include identifying pharmacological compounds that downregulate <i>IRX3</i> .	Patent and licensing status unavailable	Smemo, S. <i>et al. Nature</i> ; published online March 12, 2014; doi:10.1038/nature13138 Contact: Marcelo A. Nóbrega, The University of Chicago, Chicago, Ill. e-mail: nobrega@uchicago.edu
		SciBX 7(16); doi:10.1038/scibx.2014.462 Published online April 24, 2014		
Obesity	Ubiquitin D (UBD; FAT10)	Mouse studies suggest inhibiting FAT10 could help treat obesity. In mice, knockout of <i>Fat10</i> led to smaller adipocyte size and less weight gain than no alteration. In the knockout mice, energy expenditure during the day increased compared with that of wild-type controls. Next steps include developing therapeutic strategies to mimic the <i>Fat10</i> knockout phenotype.	Unpatented; available for partnering	Canaan, A. <i>et al. Proc. Natl. Acad. Sci.</i> <i>USA</i> ; published online March 24, 2014; doi:10.1073/pnas.1323426111 Contact: Allon Canaan, Yale School of Medicine, New Haven, Conn. e-mail:
		SciBX 7(16); doi:10.1038/scibx.2014.463 Published online April 24, 2014		allon.canaan@yale.edu
Inflammation				
Inflammation	Nav1.6 (PN4; SCN8A)	In vitro and mouse studies identified a Nav1.6 inhibitor that could help treat optic neuritis, which is often an initial sign of multiple sclerosis (MS). A series of voltage-dependent sodium channel modulators was designed based on an indazolyloxadiazolyl scaffold. In rat hippocampal slices, the optimal inhibitor targeting Nav1.6 bound the channel with an IC ₅₀ value of about 13 μ M and produced about 47% neuroprotection at 50 μ M under oxygen-glucose deprivation conditions. In a mouse model of MS, the inhibitor prevented loss of retinal nerve cells and decreased optic neuritis symptoms compared with vehicle. Next steps could include testing the inhibitor in additional preclinical models of optic neuritis or MS.	Patent and licensing status unavailable	Browne, L. <i>et al. J. Med. Chem.</i> ; published online March 6, 2014; doi:10.1021/jm401881q Contact: David L. Selwood, University College London, London, U.K. e-mail: d.selwood@ucl.ac.uk
		<i>SciBX</i> 7(16); doi:10.1038/scibx.2014.464 Published online April 24, 2014		

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Musculoskele	tal disease			
Bone repair; osteoporosis	Hypoxia-inducible factor 1α (HIF1A; HIF1α); notch 1 (NOTCH1)	Mouse studies suggest stimulating expansion of a vascular endothelial vessel subtype in bone could help treat fractures or age-dependent osteoporosis. In mice, immunohistochemical analysis and lineage tracing showed that a specific type of branched, proliferative vasculature in a portion of the bone was associated with osteoprogenitor cells. In aged mice suffering from a loss of this specific type of vasculature and bone mass, activation of Hif1a transcriptional activity led to expansion of this vasculature type and increased both osteoprogenitor cell numbers and bone mass compared with vehicle. In mice, inactivation of notch signaling in endothelial cells decreased endothelial cell proliferation and bone formation compared with what was seen in wild-type controls. In mice with inactivated notch signaling, recombinant noggin (Nog), a secreted bone morphogenetic protein (Bmp) antagonist induced by Notch1, improved vascularization and formation of bone. Next steps could include developing targeted approaches for activating HIF1A or NOTCH1 signaling in bone.	Patent and licensing status unavailable	Kusumbe, A.P. <i>et al. Nature</i> ; published online March 12, 2014; doi:10.1038/nature13145 Ramasamy, S.K. <i>et al. Nature</i> ; published online March 12, 2014; doi:10.1038/nature13146 Contact: Ralf H. Adams, University of Muenster, Muenster, Germany e-mail: ralf.adams@mpi-muenster.mpg.de
		<i>SciBX</i> 7(16); doi:10.1038/scibx.2014.465 Published online April 24, 2014		
Neurology				
Ataxia	Frataxin (FXN; FRDA)	Mouse studies suggest <i>FXN</i> gene therapy could help prevent or treat heart failure in patients with Friedreich's ataxia. In presymptomatic mouse models of Friedreich's ataxia, an i.v. injected adeno-associated virus serotype 10 (AAV10) vector encoding <i>FXN</i> prevented the onset of left ventricular hypertrophy, loss of cardiac function and death from heart failure that were observed in untreated mice. In symptomatic mouse models of Friedreich's ataxia, the AAV10- <i>FXN</i> gene therapy decreased multiple symptoms, including left ventricular hypertrophy and cardiac fibrosis, and increased cardiac function and survival compared with no treatment. Ongoing work includes testing the dose response of the gene therapy in mice (<i>see</i> Reversing (heart) failure in Friedreich's ataxia, page 5).	Patented by Cornell University and Institut National de la Santé et de la Recherche Médicale (INSERM); licensed to AAVLife	Perdomini, M. <i>et al. Nat. Med.</i> ; published online April 6, 2014; doi:10.1038/nm.3510 Contact: Hélène Puccio, Institute of Genetics and Molecular and Cellular Biology, Illkirch, France e-mail: hpuccio@igbmc.fr
		SciBX 7(16); doi:10.1038/scibx.2014.466 Published online April 24, 2014		
Huntington's disease (HD)	Cystathionase (CTH)	Studies in mice and human samples suggest dietary supplementation with cysteine could help prevent neurotoxicity in patients with HD. The enzyme CTH generates cysteine from cystathionine. In mouse models of HD and in patient tissue samples, CTH levels were lower in HD-relevant brain regions than those in healthy mice or samples from healthy subjects. In a transgenic mouse model of HD, a cysteine-enriched diet delayed motor abnormalities, partially reverted loss of brain weight and extended survival, whereas a normal diet did not. Next steps could include investigating additional cysteine- related therapies in animal models. <i>SciBX</i> 7(16); doi:10.1038/scibx.2014.467	Unpatented; licensing status not applicable	Paul, B.D. <i>et al. Nature</i> ; published online March 26, 2014; doi:10.1038/nature13136 Contact: Solomon H. Snyder, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: ssnyder@jhmi.edu

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Pain	MicroRNA let-7b (MIRLET7B; LET-7B); toll-like receptor 7 (TLR7); transient receptor potential A1 (TrpA1)	Cell culture and mouse studies suggest inhibiting extracellular MIRLET7B could help treat pain. In mouse dorsal root ganglion neurons, the pain-inducing chemical formalin increased Mirlet7b secretion compared with vehicle. In these neurons, Mirlet7b was shown to bind to Tlr7 and result in subsequent activation of Trpa1, a cation channel associated with inflammatory pain. In mouse models of formalin-induced inflammatory pain, pretreatment with a Mirlet7b-inhibiting oligomer decreased pain-related behaviors compared with pretreatment using a scrambled control oligomer. Planned work includes identifying whether other miRNAs activate nociceptive neurons.	Unpatented; unlicensed	Park, CK. <i>et al. Neuron</i> ; published online April 2, 2014; doi:10.1016/j.neuron.2014.02.011 Contact: Ru-Rong Ji, Duke University Medical Center, Durham, N.C. e-mail: ru-rong.ji@duke.edu
		Published online April 24, 2014		

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This week in techniques

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

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

Approach	Summary	Licensing status	Publication and contact information
Assays & screens			
Detection of <i>Plasmodium falciparum</i> or Trypanosome infection with ultrasound-based separation of red blood cells (RBCs)	An ultrasound-based separation technique could help detect low levels of parasitemia. The densities of RBCs infected with <i>P. falciparum</i> are known to be lower than those of uninfected RBCs. A handheld device was constructed that exposed a human blood droplet to ultrasonic waves to induce density-based separation of infected RBCs and allow for sample extraction for microscopic analysis. Compared with no separation, the technique increased infected cell levels in samples by over 100-fold and enabled detection of <i>Trypanosoma cyclops</i> and the ring stage of <i>P. falciparum</i> at levels corresponding to 6 parasites/mL and 25 parasites/µL. Ongoing work includes optimizing the technology for <i>P. falciparum</i> detection.	Patented by the University of Glasgow; licensed to SAW Dx Ltd.	Bourquin, Y. <i>et al. Angew. Chem. Int. Ed.</i> ; published online March 26, 2014; doi:10.1002/anie.201310401 Contact: Jonathan M. Cooper, University of Glasgow, Glasgow, U.K. e-mail: jon.cooper@glasgow.ac.uk
	SciBX 7(16); doi:10.1038/scibx.2014.469 Published online April 24, 2014		
<i>Ex vivo</i> model of HIV latency to screen latency-reversing agents	An <i>ex vivo</i> model of latent HIV in patient T cells could help predict clinical efficacy of latency-reversing agents. In a coculture of T cells from patients latently infected with HIV and healthy controls, latency-reversing compounds previously shown to be effective <i>in vitro</i> failed to induce spreading of the virus to noninfected cells, indicating that they do not reverse viral latency <i>ex vivo</i> . In latently infected cells from patients on antiretroviral therapy, all tested compounds failed to reverse latency as measured by HIV mRNA transcription except bryostatin-1, which is toxic <i>in vivo</i> . Next steps include identifying new agents or combinations that reactivate latent HIV in patient cells.	Unpatented; licensing status not applicable	Bullen, C.K. <i>et al. Nat. Med.</i> ; published online March 23, 2014; doi:10.1038/nm.3489 Contact: Robert F. Siliciano, The Johns Hopkins University School of Medicine, Baltimore, Md. e-mail: rsiliciano@jhmi.edu
	SciBX 7(16); doi:10.1038/scibx.2014.470 Published online April 24, 2014		
Four-gene recombination proficiency score to guide the use of DNA- damaging cancer therapies	Studies in patient tumor samples have identified a four-gene expression signature that could help determine prognosis and guide treatment for various cancers. The signature quantifies the efficiency of DNA repair pathways and is based on the mRNA levels of four DNA repair-associated genes— <i>RAP1 interacting factor homolog</i> (<i>RIF1</i>), <i>X-ray repair complementing defective repair in Chinese</i> <i>hamster cells 5</i> (<i>XRCC5</i> ; <i>KU80</i>), <i>PARP1 binding protein</i> (<i>PARPBP</i> ; <i>PARI</i>) and <i>RAD51 homolog</i> (<i>RAD51</i>). In tumors from patients with non-small cell lung cancer (NSCLC) treated with surgery plus DNA-damaging, platinum-based chemotherapy, a low signature score was associated with increased patient survival. Next steps include further characterizing the molecular responses associated with downregulation of the four genes.	Provisional patent application filed; available for licensing	Pitroda, S.P. et al. Sci. Transl. Med.; published online March 26, 2014; doi:10.1126/scitranslmed.3008291 Contact: Phillip P. Connell, The University of Chicago, Chicago, Ill. e-mail: pconnell@radonc.uchicago.edu
	SciBX 7(16); doi:10.1038/scibx.2014.471 Published online April 24, 2014		
Disease models			
Humanized mice to model chronic HBV infection	Mice with humanized immune and liver cells could be used to model chronic HBV infection. In mice with engrafted human hepatocytes and leukocytes, inoculation with HBV ⁺ patient serum caused chronic liver fibrosis and inflammation. In sera from 75% of the mice, detectable viral DNA persisted for 4 months. In the model, combined inoculation with the HBV ⁺ sera and an anti-HBV antibody prevented infection. Next steps include using the model to identify HBV therapeutics.	Patent application filed covering the humanized mouse models; licensing discussions under way	Bility, M.T. <i>et al. PLoS Pathog.</i> ; published online March 20, 2014; doi:10.1371/journal.ppat.1004032 Contact: Lishan Su, The University of North Carolina at Chapel Hill, Chapel Hill, N.C. e-mail: lsu@med.unc.edu Contact: Moses T. Bility, same affiliation
	SciBX 7(16); doi:10.1038/scibx.2014.472 Published online April 24, 2014		as above e-mail: moses_bility@med.unc.edu

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Drug platforms			
Mice with directed integration of human immunoglobulin transgenes to produce chimeric antibodies with fully human variable domains	Transgenic mice that produce chimeric antibodies with fully human variable domains could be used to discover therapeutic antibodies. In mouse embryonic stem cells, the entire human immunoglobulin variable gene repertoire was inserted into the corresponding mouse loci, and endogenous mouse variable genes were silenced. The resulting transgenic mice had functional immune systems that when injected with a human CD40 ligand (CD40LG; CD40L; CD154) antigen or a <i>Staphylococcus aureus</i> α -hemolysin (aHL) antigen produced antibodies with neutralization capacity comparable to or more potent than that of the anti-CD40L antibody 5C8 or anti-aHL antibody KBSA301. Next steps could include testing eight different antibodies produced by the transgenic mice in animal models of disease. Biogen Idec Inc. has 5C8 in Phase I testing to treat systemic lupus erythematosus (SLE). Kenta Biotech Ltd. has KBSA301 in Phase I/II trials to treat pneumonia and Phase I testing to treat <i>Staphylococcus</i> infection. SciBX 7(16); doi:10.1038/scibx.2014.473	Patented; available for partnering	Lee, EC. <i>et al. Nat. Biotechnol.</i> ; published online March 16, 2014; doi:10.1038/nbt.2825 Contact: Allan Bradley, Kymab Ltd., Cambridge, U.K. e-mail: abradley@kymab.com
 .	Published online April 24, 2014		
Markers			
Blood-based DNA methylation signature for progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD)	Studies in human samples have identified a blood-based methylation signature that could be useful for diagnosing PSP and FTD. In peripheral blood samples from 171 patients who have FTD or PSP and 185 unaffected controls, analysis of genome-wide DNA methylation patterns identified a methylation signature clustered within the 17q21.31 locus of the human genome that was associated with disease status. The 17q21.31 locus contains genes for which mutations are known to associate with risk for neurodegenerative tauopathies, including PSP and FTD. Next steps could include validating the DNA methylation signature in a larger patient cohort.	Patent and licensing status unavailable	Li, Y. <i>et al. PLoS Genet.</i> ; published online March 6, 2014; doi:10.1371/journal.pgen.1004211 Contact: Giovanni Coppola, University of California, Los Angeles, Calif. e-mail: gcoppola@ucla.edu
	SciBX 7(16); doi:10.1038/scibx.2014.474 Published online April 24, 2014		
Loss-of-function SWI/ SNF-related matrix- associated actin- dependent regulator of chromatin subfamily a member 4 (SMARCA4; BRG1) mutations associated with small- cell carcinoma of the ovary, hypercalcemia type (SCCOHT)	Genetic studies suggest loss-of-function mutations in <i>SMARCA4</i> could help diagnose SCCOHT. Mutations in the chromatin remodeling gene <i>SMARCA4</i> were identified in patients from three families using whole-genome sequencing, and the association was validated in an additional affected family, preserved tumor samples and a SCCOHT cell line. Loss of <i>SMARCA4</i> was also detected in 38 of 43 SCCOHT tumor samples but only 1 of 139 samples of other ovarian tumors. In an independent analysis of 12 SCCOHT samples, exome sequencing identified <i>SMARCA4</i> mutations in all tumors, and immunohistochemistry studies on 9 of the samples showed decreased expression of the protein compared with that in samples from healthy controls. In nonsmall cell lung cancer (NSCLC) cells lacking <i>SMARCA4</i> , ectopic expression of the gene decreased tumor growth. In a separate study, 9 of 12 SCCOHT tumor and patient germline tissue samples had <i>SMARCA4</i> protein expression. Next steps include identifying strategies to treat patients with the mutations. <i>SciBX</i> 7(16); doi:10.1038/scibx.2014.475 Published online April 24, 2014	For first study, findings unpatented; licensing status not applicable Patent application filed for findings in second study; available for licensing Patent and licensing status unavailable for findings in third study	Witkowski, L. et al. Nat. Genet.; published online March 23, 2013; doi:10.1038/ng.2931 Contact: William D. Foulkes, McGill University, Montreal, Quebec, Canada e-mail: william.foulkes@mcgill.ca Jelinic, P. et al. Nat. Genet.; published online March 23, 2014; doi:10.1038/ng.2922 Contact: Douglas A. Levine, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: levine2@mskcc.org Ramos, P. et al. Nat. Genet.; published online March 23, 2014; doi:10.1038/ng.2928 Contact: Jeffrey M. Trent, The Translational Genomics Research Institute, Phoenix, Ariz. e-mail: jtrent@tgen.org Contact: David G. Huntsman, The University of British Columbia, Vancouver, British Columbia, Canada

e-mail: dhuntsma@bccancer.bc.ca

INDEXES

Company and institution index

Α

AAVLife Alnylam Pharmaceuticals Inc. Altor BioScience Corp. AmpliPhi Biosciences Corp.

В

Bayer AG Biogen Idec Inc. Bristol-Myers Squibb Co. Broad Institute of MIT and Harvard

С

Caribou Biosciences Inc. Celgene Corp. Celladon Corp. **Cornell University CRISPR** Therapeutics Cytune Pharma S.A.S.

D

David H. Koch Institute for Integrative Cancer Research at MIT

Е

Edison Pharmaceuticals Inc. Editas Medicine Eisai Co. Ltd. 8,12

F

Friedreich's Ataxia Research Alliance

G

Gwangju Institute of Science and Technology

н

Hannover Medical School Harvard University Helmholtz Centre for Infection Research Heptares Therapeutics Ltd. Howard Hughes Medical Institute Huntington's Disease Society of America

I

· · · · · · · · · · · · · · · · · · ·	
Icahn School of Medicine	
at Mount Sinai	5,9
ICIN Netherlands Heart	
Institute	10
Institut National de la	
Santé et de la Recherche	
Médicale	5,7,16
Institute of Genetics	
and Molecular and	-
Cellular Biology	5
J	
Johnson & Johnson	12
К	
Kenta Biotech Ltd.	19

1	L Lawrence Berkeley National Laboratory	3	t
5,16	M Magatriaht Linivaraity	0	c
_ 4	Massachusetts General	9	Ā
7,14	Massachusetts Institute	2	Ā
6	of Technology McGovern Institute for	1	۶ ۱
14	Brain Research at MIT Merck & Co. Inc.	4 8	F
19 8	miRagen Therapeutics Inc. Mount Sinai Hospital	10 6,10	a A
1	N National Cancer Institute	7	Å
3 12	Nippon Shinyaku Co. Ltd. Novartis AG	12 8	ŀ
6,9 6 16	Ono Pharmaceutical Co. I td.	8	e
1	Oregon Health & Science	2	ŀ
14	Otsuka Pharmaceutical	3	1
	Co. Ltd.	12	4
2	Pfizer Inc. 3,1	2,13	E
-	R ReNA Therepouties Inc.	0	E
5 1	Regulus Therapeutics Inc.	11	E
8,12	S Sanford Burnham Madiaal		E
	Research Institute	9	(
6	Sarepta Therapeutics Inc. SAW Dx Ltd.	3 18	c ł
	Skolkovo Institute of Science and Technology	3	(
10	Stanford University School	2	(
2	U	L	(
2	Umeå University University Hospital of Bicâtre	2	(
2 13	University Medical	10	(
0	Center Utrecht University of California,	10	(
, 2	Berkeley University of California,	3	(
5	Los Angeles University of California	5	(
	San Diego	9	(
5,9	University of Massachusetts	10	(i
10	Medical School University of Paris-Sud	2 5	Ŗ
7 16	University of Strasbourg	5	0
,7,10	V VentriNova Inc.	5	(
5	Versant Ventures	1	0
	W Weill Cornell Medical		(
12	College	5	I
19	Torget and compound in	dor	[r
	5C8	19	[

7,8-Dihydro-8-oxoguanine triphosphatase	13	Dipeptidyl peptidase-4 DNA methyltransferase DPP-4	15 12 15
Α		_	10
α -Hemolysin	19	E	
AAV1	9	EPI-743	5
AAV1/SERCA2a	6,9	EPI-A0001	5
AAV10	5,16	E	
ACE	5	БАН	2
Adeno-associated		Fat mass and obesity	2
virus serotype 10	5,16	associated	15
Adenosine A ₁ receptor	14	EAT10	15
ADORA ₁	14	Formalin	17
aHL	19	Frataxin	5 16
Aldesleukin IL-2	8	FRDA	5 16
ALK	13	Fto	15
ALI-803	7,14	Fumarylacetoacetate	10
Anaplastic lymphoma	10	hydrolase	2
kinase	13	FXN	5.16
Angiotensin-converting	-		0,.0
enzyme	5	G	
AntimiR-208	10	G protein–coupled	
Aromatase	12	receptor 39	13
ATP ATP	6,9	GBA2	15
Al Pase Catt transporting	0.0	GLP-1	15
cardiac muscle slow twitch 2	6,9	Glucagon-like peptide-1	15
Azacitidine	12	Glucosidase-β bile acid 2	15
В		Glutathione	5
B7-H1	8	Glycogen dependent	
BAY 1067197	14	kinase 3	14
Bmp	16	GPR39	13
Bone morphogenetic protein	16	GSK3	14
BRG1	19	н	
Bryostatin-1	18	HIF1α	16
C		HIF1A	16
C		HIF1A Homeobox C10	16 12
C c-Met receptor tyrosine	13	HIF1A Homeobox C10 HOXC10	16 12 12
C c-Met receptor tyrosine kinase Cas9	13 1	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α	16 12 12 16
C c-Met receptor tyrosine kinase Cas9 CC-486	13 1 12	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α	16 12 12 16
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4	13 1 12 14	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α	16 12 12 16 7 14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCK4 CCNA2	13 1 12 14 5	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 IL-15 receptor α -chain	16 12 12 16 7,14 7 14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCK4 CCNA2 CD122	13 1 12 14 5 8	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α-chain IL -15RA	16 12 16 7,14 7,14 7,14 7 14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132	13 1 12 14 5 8 8	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α-chain IL-15RA IL-2 receptor	16 12 12 16 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132 CD152	13 1 12 14 5 8 8 8	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α-chain IL-15RA IL-2 receptor IL-2 receptor	16 12 12 16 7,14 7,14 7,14 7,14 7,8
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132 CD152 CD154	13 1 12 14 5 8 8 8 19	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α -chain IL-15RA IL-2 receptor IL-2 receptor β -chain IL-2 receptor γ -chain	16 12 12 16 7,14 7,14 7,14 7,14 8 8
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132 CD152 CD154 CD248 endosialin	13 1 12 14 5 8 8 8 19 13	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α -chain IL-15RA IL-2 receptor IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 RB	16 12 12 16 7,14 7,14 7,14 7,14 7,14 8 8 8 8
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132 CD152 CD154 CD248 endosialin CD26	13 1 12 14 5 8 8 8 19 13 15	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 Receptor γ -chain IL2RB IL2RB	16 12 16 7,14 7,14 7,14 7,14 7,14 8 8 8 8 8 8
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule	13 1 12 14 5 8 8 19 13 15 8	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α IL-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 Receptor γ -chain IL2RB IL2RB IL2RG Ipilimumab	16 12 16 7,14 7,14 7,14 7,14 8 8 8 8 8 8 8 8
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279	13 1 12 14 5 8 8 8 19 13 15 8 8	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RG Ipilimumab Iroguois homeobox 3	16 12 16 7,14 7,14 7,14 7,14 8 8 8 8 8 8 8 15
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand	13 1 12 14 5 8 8 8 19 13 15 8 8 19	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3	16 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L	13 1 12 14 5 8 8 8 19 13 15 8 8 19 19	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3	16 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG	13 1 12 14 5 8 8 8 19 13 15 8 8 19 19	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7 8 8 8 8 8 15 15
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163	13 1 12 14 5 8 8 8 19 13 5 8 8 19 19 19 6	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301	16 12 12 16 7,14 7,14 7,14 7 7,14 7 7,14 7 8 8 8 8 8 8 8 8 15 15
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40LG CDN1163 Clustered, regularly	13 1 12 14 5 8 8 8 19 13 5 8 8 19 19 19 6	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 <i>KU80</i>	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG CDN1163 Clustered, regularly interspaced short	13 1 12 14 5 8 8 19 13 15 8 8 19 19 19 6	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1 5	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD274 molecule CD279 CD40 ligand CD40L CD40LG CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1 5 1	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α-chain IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1 5 1 32	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α-chain IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL-2 RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40LG CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1 5 1 36 1 13 6	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α-chain IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL-2 RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA-25	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4	13 1 12 14 5 8 8 19 13 5 8 8 19 19 6 1 5 1 31 6 1 5 1 31 6 8 5	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α-chain IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL-2 RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 Cyclin A2 Over a constant of the	13 1 12 14 5 8 8 19 13 5 8 8 19 19 19 6 1 5 1 3 16 8 5 1	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α-chain IL-15RA IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-208 miR-208	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 Cyclin A2 CYP0150 Curatebicanees	13 1 12 14 5 8 8 19 13 5 8 8 19 19 19 6 1 5 1 3 16 8 5 4 2	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α-chain IL-15RA IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122 miR-208 miR-25 miP.920	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 <i>Cyclin A2</i> CYP0150 Cystathionase	13 1 12 14 5 8 8 19 13 5 8 8 19 19 19 6 1 5 1 3 16 8 5 4 16	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α-chain IL-15RA IL-2 receptor β-chain IL-2 receptor γ-chain IL-2 receptor γ-chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122 miR-208 miR-25 miR-92a MIRI ET7P	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 Cyclin A2 CYP0150 Cystathionase D	13 1 12 14 5 8 8 19 13 5 8 8 19 19 19 6 1 5 1 3 16 8 5 14 16	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I IL-15 IL-15 receptor α-chain IL-2 receptor β-chain IL-2 receptor β-chain IL-2 receptor γ-chain IL-2 receptor γ-chain IL2RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122 miR-208 miR-25 miR-92a MIRLET7B	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 Cyclin A2 CYP0150 Cystathionase D Dacogen	13 1 12 14 5 8 8 19 13 5 8 8 19 19 19 6 1 5 1 3 16 8 5 14 16 12	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 receptor γ -chain IL-2 RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122 miR-208 miR-25 miR-92a MIRLET7B MK-3475 MTH1	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14
C c-Met receptor tyrosine kinase Cas9 CC-486 CCK4 CCNA2 CD122 CD132 CD152 CD154 CD248 endosialin CD26 CD274 molecule CD279 CD40 ligand CD40L CD40L CD40LG CDN1163 Clustered, regularly interspaced short palindromic repeats Coenzyme Q10 CRISPR Crizotinib CTH CTLA-4 Cyclin A2 CYP0150 Cystathionase D Dacogen Decitabine	13 1 12 14 5 8 8 8 9 13 15 8 8 9 9 9 9 6 1 5 1 3 16 8 5 14 16 12 12	HIF1A Homeobox C10 HOXC10 Hypoxia-inducible factor 1 α I L-15 IL-15 receptor α -chain IL-15RA IL-2 receptor β -chain IL-2 receptor β -chain IL-2 receptor γ -chain IL-2 receptor γ -chain IL-2 receptor γ -chain IL-2 RB IL2RG Ipilimumab Iroquois homeobox 3 IRX3 K KBSA301 KU80 L LET-7B M MicroRNA let-7b MicroRNA let-7b MicroRNA let-7b MicroRNA-25 miR-122 miR-208 miR-25 miR-92a MIRLET7B MK-3475 MTH1 Mvdicar	16 12 12 16 7,14 7,14 7,14 7,14 7,14 7,14 7,14 7,14

INDEXES

Ν		PD-L1	8	RIF1	18	U	
NAD(P)H dehydrogenase		PDCD1	8	6		UBD	15
quinone 1	5	PN4	15		15	Ubiquitin D	15
Nav1.6	15	Programmed cell death 1	8		15	N.	
Nivolumab	8	Programmed cell death 1		SERCAZA	6,9	V	_
Nog	16	ligand 1	8	SMARCA4	19	Vatiquinone	5
Noggin	16	Proleukin	8	SMO	13	Vidaza	12
Notch 1	16	PTK7	14	Smoothened	13	VN-100	5
NOTCH1	16	PTK7 protein tyrosine		SWI/SNF-related matrix-		X	
NQO1	5	kinase 7	14	associated actin-dependent		X-box binding protein 1	12
NUDT1	13	0		regulator of chromatin	10	X-rav repair complementing	•=
0		Q OP1	5	Sublamily a member 4	19	defective repair in Chinese	
Ontok	0		5	т		hamster cells 5	18
Ontak	0	R		TEM1	13	Xalkori	13
Р		RAD51	18	TLR7	17	XBP1	12
PARI	18	RAD51 homolog	18	Toll-like receptor 7	17	XRCC5	18
PARP1 binding protein	18	RAP1 interacting factor		Transient receptor		X	
PARPBP	18	homolog	18	potential A1	17	Y	
PD-1	8	Ras	13	TrpA1	17	Yervoy	8
		RG-101	11	Tyrosine	2		



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