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Keys to the CAR

By Tracey Baas, Senior Editor

Although companies, investors and academics are all racing to develop chimeric antigen receptor-based T cells for cancer, numerous basic science questions still surround the technology. The way forward will likely involve combining chimeric antigen receptors with antibodies, ligands or other small molecules to add specificity and safety to current therapies.

Last week's deal between **Pfizer Inc.** and **Cellectis S.A.** to develop chimeric antigen receptor (CAR) T cell therapies for up to 15 targets is the latest in a series of collaborations aiming to translate the technology to the clinic.

Among the most high-profile CAR deals are **Novartis AG's** partnership with the **University of Pennsylvania** in 2012 and **Celgene Corp.'s** partnership with **bluebird bio Inc.** and the **Baylor College of Medicine** in 2013, both to develop and commercialize cancer immunotherapies.

In addition, **Juno Therapeutics Inc.** was formed in early 2014 with a \$120 million financing to commercialize immunotherapeutic discoveries by scientists at three institutions: **Memorial Sloan-Kettering Cancer Center (MSKCC)**, the **Fred Hutchinson Cancer Research Center** and the **Seattle Children's Research Institute**.

In March, five MSKCC trials involving CD19-specific CAR T cells were halted after two treatment-related deaths. Although the trials resumed after the FDA approved MSKCC's amended protocols, the incident shone a light on the many unknowns that still surround the space.

A think tank convened by *SciBX* has put together a road map for tackling basic science questions and hurdles for clinical implementation of T cell-based therapies.

The think tank, comprising academic, clinical, biotech, pharma and VC stakeholders, discussed priorities for the field at the *SciBX* Summit on Innovation in Drug Discovery and Development in Boston. In addition, key opinion leaders interviewed by *SciBX* before and after the summit added their thoughts and concerns to the road map.

Summit participants identified three research areas in which work is needed to enable innovation in T cell-based immunotherapy: achieving on-target, on-tissue specificity of T cells; attaining the right balance between persistence and safety of T cells; and figuring out how T cells enter and interact with the ever-shifting tumor microenvironment.

As with many cancer therapies, eliminating on-target, tumor-specific tissue while sparing normal tissue emerged as a top priority. Although a target might be mutated or more highly expressed on tumor tissues, even trace amounts on normal cells can lead to serious side effects.

The potential to hit the right target on the wrong cell also makes it difficult to find a balance between T cell persistence and safety. The

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greater the persistence, the more likely patients will not relapse. But with greater persistence, there is an increased chance that T cells would ultimately find and destroy normal tissues with low levels of antigens or antigen sequence motifs.

Finally, summit participants agreed that strategies need to either prevent an immunosuppressive tumor microenvironment from developing or block the environment from shutting down T cell activity.

The good news is that the three issues could have a common solution: combining traditional CARs with secondary effectors such as antibodies, ligands or other small molecules, delivered with or expressed by the CAR-based T cells. The effectors would provide an additional mechanism to fine-tune specificity, a built-in safety switch to neutralize the engineered cells if adverse events emerge or an immunostimulatory capability to overcome the generally immunosuppressive microenvironment (see Figure 1, “Building a better T cell”).

For example, tackling tumor specificity might require using T cells engineered to contain multiple cell-identifying mechanisms that distinguish tumor cells from nontumor cells. Several approaches, including the addition of inhibitory CARs (iCARs) or chimeric co-stimulatory receptors (CCRs), already are in development.

Specific strategies to control persistence and improve the safety profile of engineered T cells include incorporating suicide genes in the cells or using transient expression systems of mRNA that is intrinsically short lived.

The panel said that incorporating immunostimulatory adjuvants into treatment regimens—or designing T cells that present or secrete these molecules—could help T cells counter the immunosuppressive effects of tumor microenvironments.

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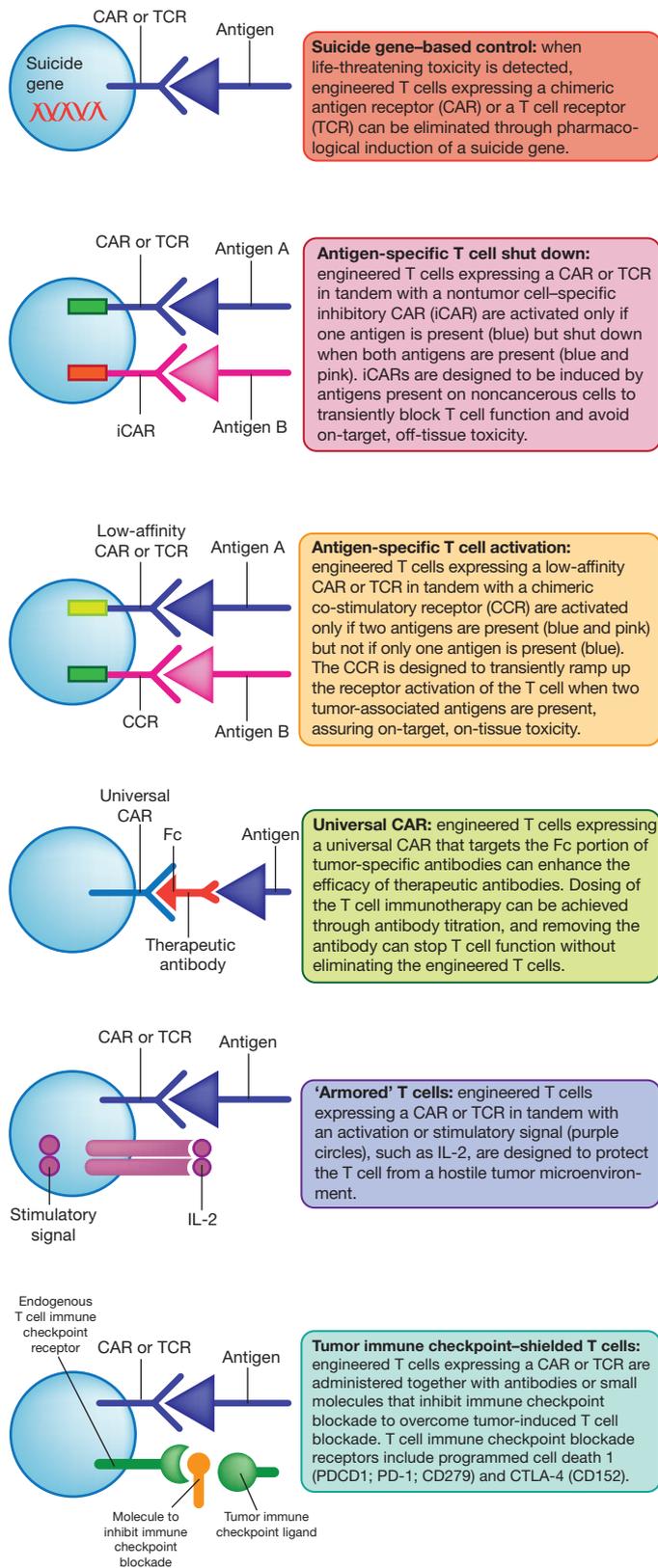


Figure 1. Building a better T cell. Various engineering strategies can be used to design T cells to engage tumor-associated antigens and kill tumor tissue while sparing normal tissue.

Beyond the scientific hurdles, the CAR space is grappling with both IP challenges and the commercial viability of the treatments—the products are individualized and thus are not off the shelf.

In 2012, **St. Jude Children's Research Hospital** filed suit against UPenn in the U.S. District Court for the Eastern District of Pennsylvania alleging breach of materials transfer agreements regarding CAR technology. UPenn counterclaimed that its cell constructs are different from St. Jude's. Novartis and Juno have filed motions on behalf of their respective partners, UPenn and St. Jude.

Although the legal issues will eventually be sorted out, commercial issues also need to be addressed. The panel said that allogeneic cells should continue to be explored as a platform for making CAR- or T cell receptor (TCR)-based T cell therapies more readily available as off-the-shelf solutions.

In addition, the panel said that companies with autologous T cell therapies should take advantage of the existing infrastructure for transfusion medicine such as bone marrow transplantation. The method is still the most widely used form of cell therapy and could help flesh out the therapeutic potential and challenges in developing other cell-based therapies.

“There will most likely be more than one path to success, with strategies needing to be tuned for each different type of cancer,” said think tank member Renier Brentjens, director of cellular therapeutics and a medical oncologist at MSKCC and a scientific cofounder of Juno.

In addition to Brentjens, the *SciBX* Summit think tank consisted of panelists Stewart Abbot, Gwen Binder-Scholl, Bruce Booth, Aya Jakobovits and respondent Madhusudan Peshwa.

Abbot is executive director of integrative research at Celgene. Binder-Scholl is EVP at **Adaptimmune Ltd.** Booth is a partner in the life sciences group at **Atlas Venture**. Jakobovits was president and CEO of cancer immunotherapy company **Kite Pharma Inc.** and is now a venture partner at **OrbiMed Advisors LLC**. Peshwa is EVP of cellular therapies at **MaxCyte Inc.**

Eye on the target

Immunotherapeutic T cells are engineered to express either a CAR or TCR that interacts with a tumor-associated antigen (*see Figure 2, “Receptor expression and engagement of antigen”*). The T cells become activated when they encounter antigen-expressing tumor cells. The result is elimination of the malignant cells and expansion and proliferation of the T cells to provide persistent protection.

In 2010 and 2011, adoptive T cell therapies grabbed headlines when second-generation CARs were used to treat CD19⁺ leukemias and lymphomas and showed unprecedented success in small clinician-led trials at the **National Cancer Institute** (NCI), UPenn and MSKCC.¹⁻⁴

These second-generation molecules were more potent than their predecessors because they included two T cell signaling domains rather than one.

In December 2013, UPenn and **The Children's Hospital of Philadelphia** published results from a Novartis-backed clinical trial of CD19-targeting T cells in 54 adults and children with intractable leukemia. In that trial, 19 of 22 pediatric patients with acute lymphoblastic leukemia (ALL) experienced complete remission (CR). Out of those, 5 relapsed during the following 20 months.⁵

Among 32 adult patients with chronic lymphocytic leukemia (CLL), 7 had CR.⁶

Figure 2. Receptor expression and engagement of antigen. (I) T cell receptors (TCRs) consist of a heterodimer of α - and β -chains that associate with the γ -, δ -, ϵ - and ζ -chains of the CD3 complex. The TCR recognizes processed antigenic peptides presented by major histocompatibility complex class I (MHC) molecules on the surface of a tumor cell. The targets can thus be antigens derived from intracellular or extracellular proteins as long as they are processed and displayed as MHC complexes.

The first generation of TCRs generally had low affinity and specificity for their targets

because the immune system naturally avoids making high-affinity TCRs against self-proteins to prevent autoimmune reactions. Thus, second-generation TCRs used endogenous, tumor-targeting TCRs as a starting point and were then optimized for potency and cancer antigen affinity.

(II) Chimeric antigen receptors (CARs) are synthetic receptors made up of a single-chain antibody variable fragment (scFv) linked through a hinge domain to one or more T cell signaling domains capable of activating the T cell response. CARs can target tumor-associated protein, carbohydrate or glycolipid antigens presented in an MHC-independent manner on the outside of cells.

The first generation of CARs included only one T cell-activating signaling molecule and showed limited antitumor effects owing to suboptimal T cell stimulation and proliferation.

The real breakthroughs occurred with second-generation CARs, which consist of two T cell signaling domains that provide enhanced activation of T cells and produce better T cell expansion, proliferation and persistence than first-generation CARs. (Figure based on Figure 1 in Kershaw, M.H. *et al.*, *Nat. Rev. Cancer* **13**, 525–541; 2013.)

Although the collective clinical results for second-generation CARs are impressive, the CD19-targeting T cells eradicate normal and malignant B cells indiscriminately. As a result, patients require lifelong immunoglobulin replacement therapy.

Thus, improving target specificity was a central talking point at the *SciBX* Summit.

“Target specificity can be seen as a precarious balance that is attained or lost due to the designed sequence of the CAR, TCR or T cell signaling molecules and the variability of patient-specific target expression on both cancer and normal cells,” said Abbot. “Right now, we want to make the T cell and target interaction more potent. But in doing so, we have to worry about the antigen being expressed on critical normal tissues.”

One idea is to pursue targets that were uncovered during decades of mAb development.

“You’d want to take another look at antibodies that target antigens expressed at low levels on cancer cells that showed limited success when incorporated into antibody conjugates—where the conjugate was not enough to supply a kick to eliminate the tumor cells,” said Thomas Schuetz, a consultant in the life sciences group at Atlas Venture. “That might be where a CAR-based T cell could supply not only cytotoxic T cell function but also proliferation and expansion of the therapeutic T cells.”

“People want to know what the right target is. If other studies, such as those with antibodies, have validated the target already, that’s a good lead to follow,” added Malcolm Brenner, a professor of molecular and human genetics and director of the Center for Cell and Gene Therapy at Baylor.

According to Booth, one of the big questions in the space is whether the technology can break into solid tumors effectively. “To date, there’s

not been any solid evidence in support of it,” he said. “Finding suitable tumor antigens that can be targeted safely with engineered T cells is the key to that.”

Brenner has had success developing CAR T cells against a known antibody target—a neuroblastoma-related disialoganglioside called GD2. In an investigator-led trial at Baylor, 6 of 11 patients with refractory or relapsed neuroblastoma had responses to the GD2-specific CAR T cell therapy, including 3 CRs.⁷

A moving target

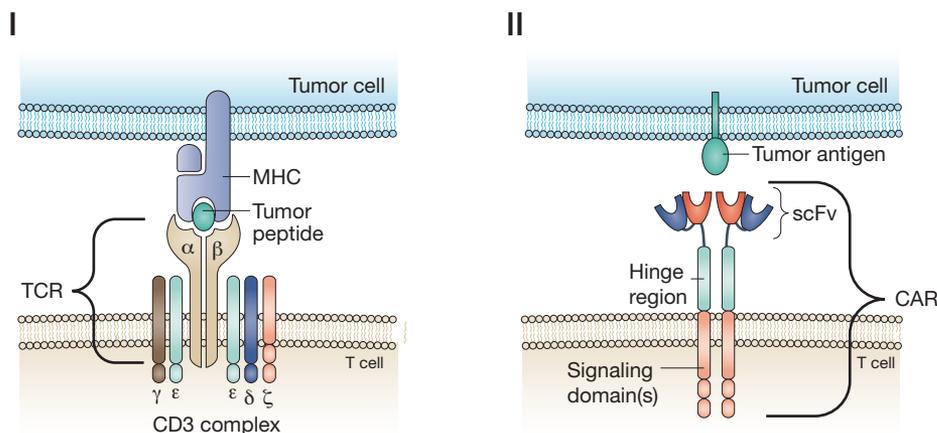
T cell therapeutics may themselves induce changes in a tumor by applying selective pressure that facilitates downregulation of the target cancer antigen or causes proliferation of tumor cells that lack the target cancer antigen. In some cases, these changes result from altered conditions in the tumor microenvironment.

In all cases, the result can be populations of cancer cells that do not express the original antigen target.

“Antigen escape—changes in expressed antigens—is a real problem that needs to be considered. If the tumor cell stops producing the target, there will be incomplete killing,” said Dario Campana, a professor of pediatrics at the **National University of Singapore**.

Carl June’s group highlighted this concern in a study using CD19-targeting CAR T cells to treat two patients with ALL.⁸ CR is ongoing in 1 patient at 11 months’ post-treatment. The other patient initially had CR but relapsed after about 2 months because escape variants appeared that did not express the CD19 antigen.

June is a professor in the Department of Pathology and Laboratory Medicine at the **Perelman School of Medicine at the University of Pennsylvania** and director of the translational research program at



the **Abramson Family Cancer Research Institute at the University of Pennsylvania**.

One solution for CAR-based therapies that no longer recognize cancer cells is a second set of T cell therapeutics focused on a second target expressed by the escaped T cells. Alternatively, combinations of CAR-based T cells could be used as a first regimen, before antigen escape occurs.

“We think antigen escape can be dealt with by using a CAR fleet—an infusion of CAR T cells that have several targets,” said June. “We have seen this strategy work in preclinical trials, but we haven’t yet tried it in clinical trials. For ALL, one possibility might be the co-infusion of CAR T cells redirected to precursor-B acute lymphoblastic leukemia specificities, such as CD22, in addition to CD19.”

The National University of Singapore and St. Jude are taking a different tack by using T cells expressing a universal CAR designed to cause antibody-dependent cell cytotoxicity.⁹ The CAR does not target a cancer-associated antigen and instead targets the Fc portion of antibodies that bind to cancer-associated antigens.

The result is a tool that can target many different antigens by selecting an alternative tumor-targeting antibody without requiring researchers to redesign the CAR T cells.

Combinatorial CARs

Many opinion leaders who attended the summit said that combinatorial strategies provide the best option so far for controlling T cell activity and suggested that T cells should be designed to interact with two antigens instead of one.

There are two ways to go about the two-pronged attack. One is T cell cytotoxicity that is activated upon encountering two antigens. The other involves blocking cytotoxicity when the T cells encounter one tumor antigen and a normal cell self-antigen.

“A single CAR or TCR going after a single antigen is just a little too hopeful,” said Brentjens.

An MSKCC team has included in its T cell-based therapeutics—in addition to the traditionally engineered CAR—an iCAR that fuses a single-chain variable fragment (scFv) to a T cell inhibitory signaling domain (see **Figure 1, “Building a better T cell”**). When both antigens are present, T cell cytotoxicity is blocked.¹⁰

Michel Sadelain, director of MSKCC’s Center for Cell Engineering and a scientific cofounder of Juno, has designed T cells expressing two different receptors—a CAR and a co-stimulatory CAR—that ramp up T cell function when encountering on-target cells while sparing off-target cells.¹¹

UPenn researchers have designed T cells that express one CAR with one T cell signaling domain and another CAR with a second T cell signaling domain.¹² When both CARs are engaged by antigen, the T cells are activated.

“These strategies are important first steps to provide a molecularly encoded control for T cell-based therapeutics,” said Abbot. “Finding tumor-associated antigens or combinations of antigens, with limited display on crucial healthy tissues, is key.”

He said that despite the possibilities provided by combination strategies, “from a clinical perspective, what we need first is validation of top-ranked antigens and, more importantly, clinical outcomes that recapitulate the type of efficacy that has been seen with CD19 CAR-based therapies.”

Avoiding collateral damage

According to Seth Ettenberg, head of oncology biologics at the **Novartis Institutes for BioMedical Research**, no single model exists to reveal everything that needs to be known about toxicity or durable responses to therapy.

“Humanized mice allow you to begin to ask questions using the ultimate cells your therapy will reside in, but questions regarding toxicity and even ultimate dissection of the underlying biology may in some cases be very different than the human setting,” Ettenberg said.

“The immunodeficient mice, while least representative, clearly allow the distinction of candidate therapies and the fastest and most amenable approach to look at human tumors,” he added. “So data from each of these models can help guide selection and a better understanding of the therapy.”

Two different strategies are being employed to provide an added layer of safety when first moving to patients: suicide genes and transient CARs.

A suicide gene engineered in the T cell can be chemically induced, which kills the transferred T cells and shuts down the immunotherapy. The first clinical study using CAR T cells incorporating such a safety feature was started in March by researchers from the NCI to treat pediatric patients with osteosarcoma and other blastomas of non-neural origin.

The NCI researchers are using GD2-targeting CAR T cells that incorporate **Bellicum Pharmaceuticals Inc.**’s CaspaCIDE safety switch technology. The technology consists of an inducible *caspase-9* (*CASP9*; *MCH6*) gene that can be activated with the small molecule activator AP1903. AP1903-mediated induction of *CASP9* leads to rapid destruction and elimination of T cells engineered with the suicide gene. The CaspaCIDE technology has been used to selectively eliminate harmful immune cells in patients receiving stem cell transplants and to block acute graft-versus-host disease (GvHD).¹³

Another way to improve safety is to use T cells with transient CAR expression.

“Some groups are considering using electroporated mRNA to establish transient CAR expression in T cells,” said Abbot. “This might make a good tool to determine potential toxicities without the challenges of long-term persistence of CAR T cells. If anything goes wrong, the problem is short lived.” The downside, said Abbot, is that transient CARs may have muted efficacy.

Isabelle Rivière, director of the cell therapy and cell engineering facility at MSKCC and a scientific co-founder of Juno, agreed. “Some investigators are looking at transient CAR-expressing T cells as a tool for the preliminary validation of novel target antigens. Others are exploring safety switches and combinatorial targeting strategies to achieve selective tumor targeting. It would also be extremely useful to have means to modulate T cell numbers and persistence.”

“More information about how and which T cells survive in the body is needed,” said Booth. “Should persistence be considered the ultimate goal? Successful studies with examples of complete remission have shown that the T cells stick around, and that may be telling us something.”

“Results from UPenn have demonstrated that persistence of CAR T cells in patients is a hallmark of successful therapy,” noted Ettenberg. He added that it would be hard to get the benefits of transient CARs without sacrificing potency.

Peshwa was not ready to consign transient CARs to basic research. He said that transient CAR T cells, generated using mRNA electroporation,

might be persistent enough to battle cancer while showing additional benefits not related to persistence.

The shifting tumor microenvironment

As more information is being uncovered about how T cells interact with the tumor microenvironment, it is becoming clear that initial T cell success can be dampened as tumor microenvironment conditions change during the course of treatment.

Solid tumors create their own microenvironment. They are heterogeneous, structurally complex and can recruit a variety of cell types, including fibroblasts, immune inflammatory cells and endothelial cells, through production and secretion of stimulatory growth factors and cytokines.¹⁴

Hematological cancers also may form tumors that can be found within the microenvironment of bone marrow or secondary lymphoid organs.

The cancers can employ a host of mechanisms that can occur concurrently within the microenvironment to tamp down the immune response and reduce the effectiveness of immunotherapy.¹⁴⁻¹⁶

CARs might need to be combined with checkpoint inhibitors such as molecules that target programmed cell death 1 (PDCD1; PD-1; CD279) or CTLA-4 (CD152).

An alternative strategy being tested is to convert suppressive checkpoint signals found on T cells into stimulatory signals.

For example, T cells have been engineered to express an extracellular checkpoint receptor, PD-1, linked to an intracellular CD28. The transmembrane complex enhances T cell function in the presence of the tumor-associated checkpoint ligand programmed cell death 1 ligand 1 (PD-L1; B7-H1; CD274) rather than suppressing T cell function.¹⁵

“Right now, many of the clinical trials using engineered CAR T cells in blood cancers are simultaneously analyzing the tumor microenvironment and the transferred T cells to determine the potential for cells to be functionally suppressed and to understand the mechanisms by which they are suppressed,” Stanley Riddell told *SciBX*. “This may provide insights into appropriate combinatorial therapies.”

Riddell is a member of the Clinical Research Division at the Fred Hutchinson Cancer Research Center and a professor of oncology at the **University of Washington**. He also is a scientific cofounder of Juno.

Novartis has already put itself at the ready by acquiring CoStim Pharmaceuticals Inc. and its portfolio of late-stage discovery programs focused on immune checkpoint proteins, including PD-1. Novartis said that the deal will provide the potential to combine checkpoint inhibitors with targeted therapies in the pharma’s pipeline, including CAR immunotherapies.

Manipulating cytokine profiles also can be used to enable T cells to resist and change the tumor microenvironment. T cells expressing IL-12 have been shown to have enhanced antitumor function, resist immunosuppression by T_{reg} cells and change myeloid cell composition within the tumor microenvironment from immunosuppressive to immunostimulatory.

Make it work

As the scientific issues are being sorted out, manufacturing and clinical protocol issues also require attention.

“In reality we’re really working with a Model A Ford,” said Brentjens. “We as clinician scientists don’t want to generate false hope. The

technology works as a proof of principle, ALL is okay, but we need to more fully develop the technology to treat other types of cancer.”

The panelists and key opinion leaders at the Summit said that the first order of business should be figuring out where cell-based therapies fit into current treatment regimens.

“Once safety profiles have improved, T cell therapeutics might be used earlier rather than later in treatment to avoid the accumulation of multiple mechanisms of resistance by tumor cells after several relapses,” said Gianpietro Dotti, an associate professor of medicine at Baylor.

“It will most likely be cancer specific and dependent on what options are available to the patient. For instance, for kids with leukemia there is a 90% success rate using current chemotherapy regimens,” he continued. “That would not be the instance to move the treatment up unless we clearly demonstrate a therapeutic advantage, reduced toxicities and costs compared to conventional treatments.”

Regardless of when T cell therapies get used, Campana said that such treatments need to be part of a physician’s thought process “before cancer patients start their first day of cancer treatment. Right now autologous T cells are the adoptive cell of choice, and sometimes it is a challenge to obtain functional T cells from people with cancer that are undergoing treatment. T cells could be banked before any type of treatment begins.”

Once CAR-based T cells become more prominent as a treatment option, T cell manipulation and manufacturing is going to have to be streamlined and more automated.

Novartis is arguably the furthest along in creating manufacturing infrastructure for CAR T cells. In 2012, the pharma bought **Dendreon Corp.**’s Morris Plains, N.J., immunotherapy manufacturing facility.

Bruce Levine, an associate professor in cancer gene therapy and director of the Clinical Cell and Vaccine Production Facility at UPenn, is training Novartis personnel at the Morris Plains site to engineer CAR T cells.

“Penn will conduct early phase clinical trials not only targeting CD19 but additional tumor targets in solid tumors. Novartis will conduct later-phase and pivotal clinical trials at its Morris Plains facility, and then, following FDA approval, commercial production will take place at this facility as well,” said Levine.

Novartis also agreed to provide \$20 million to establish the Center for Advanced Cellular Therapies at UPenn to co-develop CAR-based therapies. The effort is being led by Carl June.¹⁷

Borrowed T cells

In addition, the panel recommended further exploration of allogeneic cells as a platform for making CAR- or TCR-based T cell therapies more readily available as off-the-shelf solutions.

Researchers had first focused on autologous T cells because they wanted to avoid the possible complications of the body attacking the T cells (host-versus-graft disease) or of the T cells attacking host tissues (GvHD) that could accompany the nonself allogeneic T cells.

If a balance can be found that allows allogeneic T cells to proliferate in the body without attacking the host, off-the-shelf CAR T cells could be a possibility. One donor could provide T cells that could be used to engineer CAR-based T cells for multiple recipients without any manufacturing lag time. Off the shelf is considered the Holy Grail for cell-based therapies.

“With low-dose irradiation, patients are already prepped for allogeneic T cell transfer,” noted Peshwa. “Indeed, allogeneic T cells have been shown to eventually be rejected but no graft-versus-host disease develops.”

Brenner has first-hand experience setting up clinical trials using allogeneic T cells. “We’ve had some good experience in using allogeneic T cells with donor CD19 T cells and have seen no problem with graft-versus-host disease. These types of therapies aren’t that far away from reality. Maybe in the next year or so,” he said. “I always thought that allogeneic, CAR-based T cells wouldn’t work, but now the data is showing it might be a possibility.”

Fighting the IP fight

Even as scientific and clinical progress continue, it still remains murky who owns the IP to the various components or procedures needed to create engineered, CAR-based T cells.

While Novartis and Juno are jostling over who owns the rights to CAR T cells that target CD19, MSKCC is moving forward with a clinical study of CARs in collaboration with UPenn. The study is designed to see which of the two centers has a superior CAR.

“Cross-licensing and sleeper patents are some intricacies that are going to have to be worked out,” said Booth. “Right now researchers are working with reagents or methodologies that they might not be able to get access to, but they are proceeding with a pragmatic wait-and-see mentality.”

Binder-Scholl agreed. “IP is limiting in the field,” she said. “The engineered T cell therapy can be considered a process, but there will be IP for the target, IP for the receptor placed on the cells, IP for the technology to produce the T cells and IP for the technology to expand the T cells.”

Adaptimmune has chosen to carve out its niche in TCRs. In June, the company partnered with **GlaxoSmithKline plc** to co-develop Adaptimmune’s lead immunotherapy—a TCR-based T cell targeting cancer/testis antigen 1B (CTAG1B; NY-ESO-1).

“IP is incredibly complicated and far from resolved. Basically, lawyers are going to have to put on the armor and get ready to fight it out in a really complicated IP situation,” said Booth. “They are going to be making a lot of money from IP battles.”

“I’d like to believe that CAR therapy competitors will be rational and cross-license relevant IP rather than fight it out, but that’s not been the history of biotech,” noted Booth. “Even in the antibody space, smart cross-licensing only happened after years of fighting and stacks of lawyer bills.”

“But this is going to be what is necessary to advance these types of therapies,” said Booth. “We’re not talking pills for the rest of your life—we’re talking about a bona fide cure.”

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COMPANIES AND INSTITUTIONS MENTIONED

Abramson Family Cancer Research Institute at the University of Pennsylvania, Philadelphia, Pa.
Adaptimmune Ltd., Abingdon, U.K.
Atlas Venture, Cambridge, Mass.
Baylor College of Medicine, Houston, Texas
Bellicum Pharmaceuticals Inc., Houston, Texas
bluebird bio Inc. (NASDAQ:BLUE), Cambridge, Mass.
Celgene Corp. (NASDAQ:CELG), Summit, N.J.
Cellectis S.A. (Euronext:ALCLS), Paris, France
The Children’s Hospital of Philadelphia, Philadelphia, Pa.
Dendreon Corp. (NASDAQ:DNDN), Seattle, Wash.
Fred Hutchinson Cancer Research Center, Seattle, Wash.
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Juno Therapeutics Inc., Seattle, Wash.
Kite Pharma Inc. (NASDAQ:KITE), Los Angeles, Calif.
MaxCyte Inc., Gaithersburg, Md.
Memorial Sloan-Kettering Cancer Center, New York, N.Y.
National Cancer Institute, Bethesda, Md.
National University of Singapore, Singapore
Novartis AG (NYSE:NVS; SIX:NOVN), Basel Switzerland
Novartis Institutes for BioMedical Research, Cambridge, Mass.
OrbiMed Advisors LLC, New York, N.Y.
Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pa.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Seattle Children’s Hospital, Seattle, Wash.
St. Jude Children’s Research Hospital, Memphis, Tenn.
University of Pennsylvania, Philadelphia, Pa.
University of Washington, Seattle, Wash.

PI3K δ inhibition: solid immunotherapy

By Michael J. Haas, Senior Writer

Until now, inhibition of phosphoinositide 3-kinase- δ in cancer has been limited to leukemias and lymphomas because the isoform is only expressed on immune cells. A new finding that links the kinase to T cell responses in a range of solid tumor types could expand use of the inhibitors and launch a new class of immunotherapies.¹

The team behind the study is planning a clinical trial of an undisclosed phosphoinositide 3-kinase- δ (PI3K δ) inhibitor in solid tumors.

PI3K δ is one of four PI3K isoforms that play key roles in cell growth, differentiation and survival. The PI3K α and PI3K β isoforms are expressed in nearly all cell types and have been targeted in solid tumors, whereas the PI3K γ and PI3K δ isoforms are found primarily on immune cells.

Several companies are developing inhibitors of PI3K δ for hematological malignancies, autoimmune diseases and inflammatory indications in which the isoform is overactive (see Table 1, “A peek at PI3K δ inhibitors”).

In a series of studies over the last decade, the team’s principal investigators, Bart Vanhaesebroeck and Klaus Okkenhaug, studied a mutant, catalytically inactive form of PI3K δ and showed the subtype to be important for the differentiation, expansion and normal function of T and B cells.²⁻⁵

Vanhaesebroeck is a professor of cell signaling at the **University College London Cancer Institute**, and Okkenhaug is group leader in the

laboratory of lymphocyte signaling and development at the **Babraham Institute**.

Vanhaesebroeck told *SciBX* that for the new study, he and his colleagues initially thought that because mice with mutant Pi3k δ have compromised immune responses, they might be more prone to developing solid cancers than wild-type mice.

However, when the researchers established tumors in mutant Pi3k δ mice with cells from breast, lung and other solid tumors, they saw the opposite of what they expected. The mutant mice developed smaller primary tumors and fewer metastases and survived longer than wild-type mice given the same treatment.

In addition, PI-3065—a PI3K δ inhibitor from **Roche’s Genentech Inc.** unit—decreased growth of breast and pancreatic tumors in wild-type mice and increased survival compared with vehicle.

PI-3065 was originally developed by Piramed Ltd., which was acquired by Roche in 2008.

Next, the team investigated the link between PI3K δ inhibition and its effects on the immune system in solid tumors and focused on several T cell populations that have known roles in antitumor immunity.

In mice, mutant Pi3k δ moderately reduced the activity of tumor antigen-specific Cd8⁺ T cells but inactivated T_{reg} cells that ordinarily eliminate Cd8⁺ T cells. Because Pi3k δ deficiency had a larger impact on T_{reg} cells than on the Cd8⁺ T cells, the net result was a gain in antitumor immunity.

Furthermore, in mice with pancreatic tumors, PI-3065 decreased the number of T_{reg} cells in draining lymph nodes and increased the number of Cd8⁺ T cells in primary tumors and in draining lymph nodes.

The team concluded that Pi3k δ suppression enhanced antitumor immune responses in the mice.

Table 1. A peek at PI3K δ inhibitors. According to a study in *Nature* by Ali *et al.*, inhibition of phosphoinositide 3-kinase- δ (PI3K δ) or the PI3K catalytic subunit δ -polypeptide (PI3KCD; p110 δ) could treat solid tumors by modulating T cell responses. At least 12 companies are developing PI3K δ -specific inhibitors or dual PI3K γ and PI3K δ inhibitors for hematological malignancies, autoimmune disease and inflammation that could be repurposed to treat solid cancers.

Source: *BCIQ: BioCentury Online Intelligence*

Company	Product	Description	Status
Gilead Sciences Inc. (NASDAQ:GILD)	Idelalisib	Small molecule inhibitor of PI3K δ	In registration for chronic lymphocytic leukemia (CLL) and non-Hodgkin’s lymphoma (NHL)
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502)/ Infinity Pharmaceuticals Inc. (NASDAQ:INFI)	IPI-145	Oral inhibitor of PI3K γ and PI3K δ	Phase III for CLL; Phase II for NHL, lymphoma, rheumatoid arthritis (RA) and inflammation; Phase I for hematological malignancies and autoimmune diseases
Rhizen Pharmaceuticals S.A./TG Therapeutics Inc. (NASDAQ:TGTX)	TGR-1202	PI3K δ inhibitor	Phase I/II for CLL, B cell lymphoma and hematological malignancies
Amgen Inc. (NASDAQ:AMGN)	AMG 319	Small molecule inhibitor of PI3K δ	Phase I for hematological malignancies
Incyte Corp. (NASDAQ:INCY)	INCB40093	PI3K δ inhibitor	Phase I for B cell lymphoma
Gilead Sciences	GS-9820	PI3K δ inhibitor	Phase I for lymphoma
Rhizen Pharmaceuticals	RP6530	Dual PI3K γ and PI3K δ inhibitor	Phase I for hematological malignancies; preclinical for B cell lymphoma and T cell lymphoma
	RP6503	Dual PI3K γ and PI3K δ inhibitor	Preclinical for asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and inflammation
Exelixis Inc. (NASDAQ:EXEL)/ Merck & Co. Inc. (NYSE:MRK)	XL499	Selective inhibitor of PI3K δ	Preclinical for cancer and inflammation
Pathway Therapeutics Inc./MEI Pharma Inc. (NASDAQ:MEIP)	PWT143	PI3K δ inhibitor	Preclinical for cancer
Xcovery Holding Co. LLC	X-339	Selective inhibitor of the p110 δ isoform of PI3K	Preclinical for CLL

The team included researchers from the **Medical Research Council's** Harwell campus, **Queen Mary University of London** and Roche's Genentech and Piramed units who assisted with the PI-3065 experiments.

Results were published in *Nature*.

Taken together, the team's findings suggest that PI3K δ inhibitors could treat solid tumors by "tipping the immune system balance in favor of the tumor-targeting CD8⁺ T cells," Vanhaesebroeck told *SciBX*.

The team is also elucidating the mechanism by which PI3K δ deficiency deactivates T_{reg} cells in the cancer models and has a manuscript under review showing that PI3K δ is expressed in some solid tumors, in which it appears to play a role in tumor cell invasion.

Swaroop Vakkalanka, president of **Rhizen Pharmaceuticals S.A.**, agreed that the findings support the potential of PI3K δ inhibitors to treat solid tumors and said that the *Nature* report begins to shed light on precisely how PI3K δ inhibition exerts its therapeutic effects in those cancers.

He added that his company has unpublished preclinical data showing the efficacy of a dual PI3K γ and PI3K δ inhibitor in solid tumors. Rhizen will decide whether to develop this compound and its PI3K δ -specific inhibitor for solid tumors after conducting additional preclinical studies, he told *SciBX*.

Delivering delta inhibitors

According to Vanhaesebroeck, PI3K δ inhibitors could be used as immunotherapies in combination with other cancer therapies—including surgery and radiation—or even as adjuvants to cancer vaccines.

"We know from clinical trial data that PI3K δ inhibitors are safe and well tolerated by cancer patients, some of whom have received the inhibitors for up to three years," he said. "Stimulating the immune system with something that we already know is safe will open a lot of doors for treating solid tumors."

Lori Friedman, senior director of translational oncology at Genentech and coauthor of the *Nature* study, agreed. But to identify which patients might benefit from PI3K δ inhibitor therapy, she said that "clinical researchers will need to analyze a variety of solid tumor types to understand which ones rely on T_{reg} cells for evading immune responses."

In addition, they will need to investigate how PI3K δ inhibition affects the balance of T_{reg} and CD8⁺ T cell responses in patients, she said.

Vakkalanka added that using PI3K δ inhibitors in combination with other cancer therapies could have additive or synergistic effects that lower the effective doses and thereby provide an added margin of safety for each therapy.

However, he also wanted to see preclinical and clinical studies combining a PI3K δ inhibitor with immunotherapies that enhance T cell activity against cancer, such as antibodies against programmed cell death 1 (PDCD1; PD-1; CD279). "A PI3K δ inhibitor could support the effectiveness of those therapies in treating cancer by inactivating T_{reg} cells and exerting a broad anti-inflammatory effect," he said.

Because activating mutations in PI3K α frequently occur in solid tumors, the *Nature* findings also point to the possibility that inhibiting both PI3K α and PI3K δ could have therapeutic advantages over inhibiting only PI3K δ , Friedman said.

Indeed, Genentech is interested in determining whether its small molecule PI3K inhibitor pictilisib (GDC-0941; RG7321) has an immunomodulatory effect via PI3K δ inhibition in patients with solid tumors, in addition to having a direct effect on tumor growth via PI3K α inhibition, Friedman told *SciBX*.

Pictilisib is in Phase II testing to treat breast cancer and non-small lung cell cancer (NSCLC). The compound inhibits all four PI3K isoforms with nanomolar potency and is more potent against the PI3K α and PI3K δ isoforms.⁶

However, Vakkalanka said that inhibiting both PI3K α and PI3K δ would not be desirable because the safety profiles of pan-PI3K inhibitors are not as good as those of isoform-specific inhibitors. Thus, he cautioned that finding inhibitors specific for the PI3K δ isoform would be important for retaining a good safety profile.

Vanhaesebroeck agreed. "Immune cells express the α isoform as well as the δ isoform, and some literature studies suggest that inhibiting both isoforms severely compromises the function of immune cells," he said. This means that "hitting too many PI3K isoforms might override the immunotherapeutic effects" of inhibiting PI3K δ .

Vanhaesebroeck said that the team is planning a clinical trial of an undisclosed PI3K δ inhibitor to treat an undisclosed solid tumor type. "We want to look for early evidence that PI3K δ inhibition has an immunomodulatory effect on solid tumors," he told *SciBX*.

The findings are not patented or licensed, he said.

Genentech declined to disclose the development status of PI-3065.

Haas, M.J. *SciBX* 7(25); doi:10.1038/scibx.2014.726
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Babraham Institute, Cambridge, U.K.
Genentech Inc., South San Francisco, Calif.
Medical Research Council, Harwell, U.K.
Queen Mary University of London, London, U.K.
Rhizen Pharmaceuticals S.A., La Chaux-de-Fonds, Switzerland
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
University College London Cancer Institute, London, U.K.

Managing meningitis

By Tracey Baas, Senior Editor

SHX Vaccines is taking a new approach to vaccinating against meningitis—preventing meningococci from binding to host cells in the first place. The company's university collaborators have shown proof of concept for the approach in human tissue explants, including efficacy against serotype B—the bacteria's most common but most intractable subgroup.¹

The next step is finding the *Neisseria meningitidis* epitopes that interact with their newly identified human receptor—CD147 (basigin Ok blood group; BSG; EMMPRIN).

N. meningitidis is a Gram-negative bacterium that exists exclusively in humans. The microorganism typically resides in the nasopharyngeal mucosa, where it has no effects. Problems arise when meningococci enter the bloodstream, at which point the bacteria can disseminate to various tissues and result in potentially lethal meningitis.

N. meningitidis serogroup B is a common cause of bacterial meningitis and sepsis in infants in Europe and in the U.S.

Licensed meningococcal vaccines use a polysaccharide fragment found in the bacterial outer coating of serogroups A, C, W and Y to induce a protective immune response. However, serogroup B's outer coating resembles human neural cell tissue and therefore does not induce an immune response, thus precluding a polysaccharide vaccine strategy.

There are two vaccines for serogroup B in registration, and both have breakthrough therapy designation from the FDA. The vaccines—LP2086 from **Pfizer Inc.** and Bexsero from **Novartis AG**—use isolated protein fragments found on the outer surface of serogroup B bacteria that can induce an immune response. Bexsero is approved in Europe, Canada and Australia.

In April, **GlaxoSmithKline plc** announced its purchase of Novartis' vaccine business, with Bexsero being part of the deal.

Stéphane Hugué, CEO of SHX Vaccines, said that it is unlikely that Bexsero or LP2086 will be sufficient to provide protection from all B strains. In addition, patients still would require a different vaccine against the other serotypes.

Now, a French team has developed a different strategy—inhibit any serotype of *N. meningitidis* from adhering to human endothelial cells in the first place.

The first step was finding the precise cellular adhesion receptor that interacts with meningococcal pili. A series of cell line studies pinpointed CD147 as the receptor. CD147 has broad tissue distribution and surface exposure, and its expression is enhanced at sites of meningococcal adhesion.

In human endothelial cells, CD147-specific siRNA or an anti-CD147 mAb decreased meningococcal adhesion compared with control siRNA or a control protein. Results were consistent when using meningococcal strains belonging to serogroups A, B, C and W.

The next order of business was finding out which bacterial pilus components interacted with CD147. A cell-based competition assay

showed that *N. meningitidis* major pilin PilE (pilE) and *N. meningitidis* type IV pilus assembly protein (pilV) were the culprits.

Indeed, mutant meningococci that lacked pilE or pilV failed to adhere to human endothelial cells.

To investigate the role of pilE and pilV in colonization *in vivo*, the team used humanized SCID mice engrafted with human skin containing functional human blood vessels. The model allows the study of meningococci infection because the bacteria only colonize human cells and not mouse cells.

In the mouse model, wild-type meningococci massively colonized the human dermal vasculature, whereas mutant bacteria that lacked pilE or pilV failed to colonize.

Similar results were seen using *in situ* meningococcal infection models of fresh human frontal brain tissues obtained from deceased normal individuals. Wild-type meningococci developed microcolonies adjacent to CD147⁺ cells, whereas meningococci lacking pilE or pilV colonized poorly. Pretreatment with the antibody significantly decreased adhesion of bacteria to human brain vessels compared with pretreatment using control antibodies.

Results were published in *Nature Medicine*.

The sticky details

It remains unclear how pilE and pilV act in concert to initiate bacterial adhesion to CD147.

Ongoing work by the team includes identifying the precise pilE and pilV epitopes required for interaction with CD147. The research could allow the development of pilin-targeted antibodies and lead to new vaccines for meningococcal infection.

Guillaume Duménil, group leader at the **Paris Cardiovascular Research Center**, wanted to see additional experiments in the mouse model. Duménil's laboratory developed the humanized SCID mice engrafted with human skin to study *N. meningitidis* infection.

"The team shows that bacteria lacking pilV or pilE do not colonize the human vasculature, but I would like to see in future experiments that blocking of CD147 also impedes colonization," Duménil noted. "This would have more fully substantiated CD147 as the endothelial receptor for *N. meningitidis*."

"The team's *in vitro* experiments also hinted that CD147 interacts with multimeric forms of the pilins rather than monomeric forms, so having a crystal structure of the pilin-CD147 interaction could also be key,"

added Duménil. "With a crystal structure in hand, the precise pilE and pilV epitopes required for interaction with CD147 could be determined. Knowing the epitopes would allow sequence homology studies among different serotypes and strains and might give some indication of what type of broad coverage could be achieved using pili-specific antibodies or vaccine strategies."

David Stephens said that exploring the direct blocking of bacterial pilV or pilE could be attractive as an adjuvant therapy to complement antibiotics.

Standard of care for people who are suspected to have *N. meningitidis* disease is the immediate delivery of intramuscular or i.v. antibiotics

"Knowing the epitopes would allow sequence homology studies among different serotypes and strains and might give some indication of what type of broad coverage could be achieved using pili-specific antibodies or vaccine strategies."

—Guillaume Duménil,
Paris Cardiovascular Research
Center

and hospital admission. “There have been a lot of failed attempts at adjuvant therapies to block host inflammatory events or host targets in meningococcal sepsis,” said Stephens. “A lot of the failures have to do with the rapid onset of meningococcal sepsis and the inflammatory cascade that has already been launched before reaching medical care.” Stephens is VP for research at **Emory University’s** Woodruff Health Sciences Center, chair of the university’s Department of Medicine and chief of medicine at **Emory Healthcare**.

A prophylactic vaccine that targets a wide range of invasive *N. meningitidis* would alleviate these problems.

“Antibodies that prevent pilus-CD147 interactions could provide a potential novel path to a broad-based meningococcal vaccine that prevents systemic disease,” said Stephens.

“We are sponsoring ongoing work by the Paris team that will determine the specific pili epitopes that interact with CD147 in order to develop bacteria-specific antibodies for meningococcal serogroups A, C, W and Y, as well as multiple strains of serogroup B,” said Huguet. “Our ultimate goal is to use epitope-antibody structural information to develop vaccines.”

SHX Vaccines was founded in March 2012 to develop antibodies and vaccines for *N. meningitidis* infection.

Findings in the new study are patented by the **Institut National de la Santé et de la Recherche Médicale** (INSERM) and **University Paris Descartes**. The IP is licensed to SHX Vaccines.

Baas, T. *SciBX* 7(25); doi:10.1038/scibx.2014.727
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COMPANIES AND INSTITUTIONS MENTIONED

Emory Healthcare, Atlanta, Ga.

Emory University, Atlanta, Ga.

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

Institut National de la Santé et de la Recherche Médicale, Paris, France

Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland

Paris Cardiovascular Research Center, Paris, France

Pfizer Inc. (NYSE:PFE), New York, N.Y.

SHX Vaccines, Paris, France

University Paris Descartes, Paris, France



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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				
Adrenocortical adenoma	Protein kinase cAMP-dependent catalytic- α (PRKACA)	DNA sequencing of patient samples and <i>in vitro</i> studies suggest inhibiting PRKACA could treat adrenocortical carcinoma. DNA sequencing of patient tumors and matched normal tissues identified mutations in about 50% of patients with corticotrophin-dependent Cushing syndrome, which is caused by excessive cortisol production by adrenocortical adenomas. <i>In vitro</i> , disease-associated mutations increased PRKACA's kinase activity compared with wild-type PRKACA. Next steps could include identifying selective PRKACA inhibitors. SciBX 7(25); doi:10.1038/scibx.2014.728 Published online June 26, 2014	Patents pending on biomarkers and therapeutics for corticotrophin-dependent Cushing syndrome; licensing status undisclosed	Sato, Y. <i>et al. Science</i> ; published online May 23, 2014; doi:10.1126/science.1252328 Contact: Seishi Ogawa, Kyoto University, Kyoto, Japan e-mail: sogawa-ky@umin.ac.jp Contact: Yukio Homma, same affiliation as above e-mail: homma-uro@umin.ac.jp
Cancer	BRAF	<i>In vitro</i> and patient studies suggest combining BRAF inhibitors with autophagy inhibitors such as chloroquine could help treat BRAF-mutant cancers. In astrocytoma cells with the BRAF V600E activating mutation, autophagy was greater than that in cells with wild-type BRAF. In the BRAF-mutant cancer cells, chloroquine induced cell death and was synergistic with the BRAF inhibitor Zelboraf vemurafenib. In a patient who developed Zelboraf resistance, Zelboraf plus chloroquine led to stable disease for over 16 months and decreased tumor growth compared with Zelboraf alone or in combination with chemotherapy. Next steps include additional preclinical studies to understand the role of autophagy in BRAF-mutant cancers. Roche, Chugai Pharmaceutical Co. Ltd. and Daiichi Sankyo Co. Ltd. market Zelboraf to treat melanoma. SciBX 7(25); doi:10.1038/scibx.2014.729 Published online June 26, 2014	Patent status not applicable; unlicensed	Levy, J.M.M. <i>et al. Cancer Discov</i> ; published online May 13, 2014; doi:10.1158/2159-8290.CD-14-0049 Contact: Jean M. Mulcahy Levy, University of Colorado Denver, Aurora, Colo. e-mail: jean.mulcahy-levy@childrenscolorado.org
Cancer	c-Myc (MYC); phosphoribosyl pyrophosphate synthetase 2 (PRPS2)	Mouse studies suggest inhibiting PRPS2 could help treat cancers driven by MYC. In a <i>Myc</i> -dependent mouse model of Burkitt lymphoma, high rates of protein and nucleotide synthesis were linked through translational upregulation of Prps2, a rate-limiting enzyme for the synthesis of purines. In the model, knockout of <i>Prps2</i> delayed tumor initiation and prolonged survival without affecting other cell types. Next steps include antagonizing PRPS2 in additional MYC-driven tumor types and developing a specific inhibitor of PRPS2. SciBX 7(25); doi:10.1038/scibx.2014.730 Published online June 26, 2014	Patent application filed by the University of California, San Francisco; licensed to Effector Therapeutics Inc.	Cunningham, J.T. <i>et al. Cell</i> ; published online May 22, 2014; doi:10.1016/j.cell.2014.03.052 Contact: Davide Ruggero, University of California, San Francisco, Calif. e-mail: davide.ruggero@ucsf.edu

This week in therapeutics (continued)

Indication	Target/marker/pathway	Summary	Licensing status	Publication and contact information
Cancer	Epidermal growth factor receptor (EGFR); insulin-like growth factor-1 receptor (IGF1R; CD221)	<p>Mouse studies suggest a bispecific antibody against EGFR and IGF1R could help treat cancer. The antibody has a Fab portion that binds to and inhibits EGFR and IGF1R and an Fc portion that enhances antibody-dependent cytotoxicity. In multiple mouse xenograft models of cancer, the bispecific antibody decreased tumor growth and increased survival compared with vehicle or monospecific antibodies against EGFR or IGF1R. Next steps include further optimizing the antibody and performing affinity maturation on the IGF1R-binding arm.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.731 Published online June 26, 2014</p>	Patent and licensing status undisclosed	<p>Schanzer, J.M. <i>et al. J. Biol. Chem.</i>; published online May 19, 2014; doi:10.1074/jbc.M113.528109 Contact: Juergen M. Schanzer, Roche Diagnostics GmbH, Penzberg, Germany e-mail: juergen_michael.schanzer@roche.com</p>
Cancer	Epidermal growth factor receptor (EGFR); tumor necrosis factor receptor superfamily member 9 (TNFRSF9; 4-1BB; CD137)	<p>Studies in mice and human samples suggest sequential treatment with anti-EGFR and anti-CD137 antibodies could help treat EGFR⁺ tumors. In EGFR⁺ mouse xenograft and syngeneic tumor models, the anti-EGFR mAb Erbitux cetuximab increased the number of Cd137⁺ NK cells in blood, spleen and tumors, and subsequent treatment with BMS-663513, an anti-CD137 antibody, increased tumor regression and prolonged survival compared with either antibody alone. In samples from patients with head and neck cancer, Erbitux increased the number of CD137⁺ NK cells by 4%–15% compared with pretreatment numbers. Next steps include designing a clinical trial of anti-EGFR plus anti-CD137 antibodies. Eli Lilly and Co., Bristol-Myers Squibb Co. and Merck KGaA market Erbitux to treat colorectal and head and neck cancer. Amgen Inc. markets the anti-EGFR mAb Vectibix panitumumab to treat colorectal and other types of cancer. BMS-663513 is in Phase I trials to treat solid tumors. Pfizer Inc.'s anti-CD137 antibody, PF-05082566, is in Phase I trials to treat cancers including non-Hodgkin's lymphoma (NHL).</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.732 Published online June 26, 2014</p>	Patent and licensing status undisclosed	<p>Kohrt, H.E. <i>et al. J. Clin. Invest.</i>; published online May 16, 2014; doi:10.1172/JCI73014 Contact: Holbrook E. Kohrt, Stanford University Medical Center, Stanford, Calif. e-mail: kohrt@stanford.edu</p>
Cancer	S100 calcium binding protein A8 (S100A8; calgranulin A; MRP8); S100A9 (calgranulin B; MRP14)	<p><i>In vitro</i> and mouse studies identified myeloid-derived suppressor cell (MDSC)-depleting peptides that could help promote antitumor immunity. Immunosuppressive MDSCs in the tumor environment help tumors evade immune detection. Phage-display studies identified two peptides targeting MDSC-expressed S100A8 and S100A9. In a mouse model of cancer, i.v. injection of either peptide fused to mouse IgG2b depleted MDSCs in blood and spleen, delayed tumor development and decreased tumor size compared with injection of a control peptide-antibody fusion or no treatment. Next steps include developing a therapeutic targeting human MDSCs. Active Biotech AB and Ipsen Group have tasquinimod, a small molecule that binds S100A9, in Phase III testing to treat prostate cancer.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.733 Published online June 26, 2014</p>	Patent application filed covering the peptide-antibody fusions; licensing discussions under way	<p>Qin, H. <i>et al. Nat. Med.</i>; published online May 25, 2014; doi:10.1038/nm.3560 Contact: Larry W. Kwak, The University of Texas MD Anderson Cancer Center, Houston, Texas e-mail: llkwak@mdanderson.org</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Head and neck cancer; esophageal cancer	Retinoid X receptor (RXR); retinoic acid receptor- γ (RARG)	<p><i>In vitro</i> and mouse studies suggest combining RXR and RARG agonists could help prevent oral and esophageal squamous cell carcinomas. In a mouse model of oral carcinogenesis, an RARG agonist plus the RXR agonist Targretin bexarotene decreased the number of malignant lesions and their severity compared with the RARG agonist alone or no treatment. The agonists also decreased markers of cancer and oxidative stress compared with no treatment. Next steps could include evaluating the combination strategy in additional cancer models.</p> <p>Eisai Co. Ltd. markets Targretin to treat cutaneous T cell lymphoma (CTL).</p> <p>Kowa Co. Ltd. has the oral RARG and RXR agonist peretinoin in Phase III testing to treat liver cancer.</p> <p>Io Therapeutics Inc. has the RXR agonist IRX4204 in Phase II or earlier testing to treat various cancers.</p> <p>Roche and Clementia Pharmaceuticals Inc. have the RARG agonist palovarotene in Phase II testing to treat musculoskeletal indications.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.734 Published online June 26, 2014</p>	Patent and licensing status unavailable	<p>Tang, X.-H. <i>et al. Proc. Natl. Acad. Sci. USA</i>; published online June 3, 2014; doi:10.1073/pnas.1404828111</p> <p>Contact: Lorraine J. Gudas, Weill Cornell Medical College, New York, N.Y. e-mail: ljpgudas@med.cornell.edu</p>
Solid tumors	BRAF; smoothened (SMO)	<p>Studies in patient samples and cell culture suggest BRAF and SMO inhibitors could be useful for treating ameloblastomas, which are rare, typically benign tumors that form in the jaw. Whole-transcriptome sequencing of ameloblastoma samples from 28 patients showed that 11 had <i>SMO</i> mutations and 13 had <i>BRAF</i> mutations, all of which were primarily activating mutations. In an immortalized mouse ameloblast-lineage cell line that expressed one of the identified activating mutations in <i>Smo</i>, the hedgehog pathway inhibitor Trisenox arsenic trioxide decreased hedgehog pathway activity compared with vehicle. In a human ameloblastoma cell line that expressed one of the identified activating mutations in <i>BRAF</i>, the BRAF inhibitor Zelboraf vemurafenib blocked proliferation with an IC_{50} value of 0.19 μM. Next steps could include developing animal models of ameloblastoma and using them to evaluate drugs that inhibit the hedgehog pathway or BRAF.</p> <p>Teva Pharmaceutical Industries Ltd., H. Lundbeck A/S and Nippon Shinyaku Co. Ltd. market Trisenox to treat acute promyelocytic leukemia (APL).</p> <p>Roche, Daiichi Sankyo Co. Ltd. and Chugai Pharmaceutical Co. Ltd. market Zelboraf to treat melanoma.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.735 Published online June 26, 2014</p>	Patent and licensing status unavailable	<p>Sweeney, R.T. <i>et al. Nat. Genet.</i>; published online May 25, 2014; doi:10.1038/ng.2986</p> <p>Contact: Robert B. West, Stanford University Medical Center, Stanford, Calif. e-mail: rbwest@stanford.edu</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Dental disease				
Dental tissue repair	Transforming growth factor- β 1 (TGFB1)	<p>Mouse and cell culture studies suggest photoactivation of latent TGFB1 could help regenerate teeth. In a rat pulp-dentin healing model, low-power laser treatment increased tertiary dentin volumes compared with no treatment. Pharmacological or genetic inhibition of Tgfb1 signaling prevented the response, suggesting the treatment effect results from the release of latent Tgfb1. In cultured mouse dental stem cells treated with a low-power laser, pharmacological inhibition of Tgfb1-releasing reactive oxygen species or transforming growth factor-β receptor 1 (Tgfr1; Alk5) blocked the cells from differentiating into dentin-forming odontoblasts. Next steps include improving the approach to generate more organized dentin and determining an optimal treatment regimen for human studies.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.736 Published online June 26, 2014</p>	Patent application filed; available for licensing	<p>Arany, P.R. <i>et al. Sci. Transl. Med.</i>; published online May 28, 2014; doi:10.1126/scitranslmed.3008234 Contact: David J. Mooney, Harvard School of Engineering and Applied Sciences, Cambridge, Mass. e-mail: mooneyd@seas.harvard.edu</p>
Gastrointestinal disease				
Colitis	Not applicable	<p><i>In vitro</i> and rat studies suggest synthetic disialyl hexasaccharides could help prevent necrotizing enterocolitis in infants. Human milk oligosaccharides including disialyllacto-<i>N</i>-tetraose (DSLNT) contribute to the gut health of breastfed infants but are difficult to synthesize. In a neonatal rat model of necrotizing enterocolitis, the synthetic disialyl hexasaccharides disialyllacto-<i>N</i>-neotetraose (DSLNnt) and α2-6-linked disialyllacto-<i>N</i>-tetraose (DSLNT) protected rats from necrotizing enterocolitis with potency comparable to that of human milk oligosaccharides. Next steps include testing the synthetic hexasaccharides in additional animal models.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.737 Published online June 26, 2014</p>	Patent and licensing status unavailable	<p>Yu, H. <i>et al. Angew. Chem. Int. Ed.</i>; published online May 21, 2014; doi:10.1002/anie.201403588 Contact: Xi Chen, University of California, Davis, Calif. e-mail: xiichen@ucdavis.edu</p>
Infectious disease				
Bacterial infection	Guanosine 5'-diphosphate 3'-diphosphate (ppGpp); guanosine 5'-triphosphate 3'-diphosphate (pppGpp)	<p><i>In vitro</i> studies suggest inhibiting ppGpp and pppGpp could help treat bacterial infections by eliminating biofilms. <i>In vitro</i>, an innate defense peptide from bacteria including <i>Pseudomonas aeruginosa</i>, <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> bound to and induced degradation of (p)ppGpp, which are involved in the bacterial stress response and biofilm formation. In culture, the peptide led to dispersal or death of multiple types of bacteria in established biofilms, whereas overproduction of (p)ppGpp increased biofilm formation and conveyed resistance to the peptide. Next steps include identifying the optimal dose and developing antibodies for use in combination with the peptide.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.738 Published online June 26, 2014</p>	Patent application filed; available for licensing	<p>de la Fuente-Núñez, C. <i>et al. PLoS Pathog.</i>; published online May 22, 2014; doi:10.1371/journal.ppat.1004152 Contact: Robert E.W. Hancock, The University of British Columbia, Vancouver, British Columbia, Canada e-mail: bob@hancocklab.com</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Bacterial infection	Unknown	<p>Cell culture studies suggest an aromatic polyketide from <i>Clostridium beijerinckii</i> could be useful for treating bacterial infections. In a panel of bacteria, the peptide called clostrubin inhibited growth of methicillin-resistant <i>Staphylococcus aureus</i> (MRSA), vancomycin-resistant <i>Enterococcus</i> (VRE) and several strains of mycobacteria with higher potency than that of ciprofloxacin. Next steps could include developing optimized clostrubin analogs.</p> <p>Ciprofloxacin is a generic fluoroquinolone antibiotic.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.739 Published online June 26, 2014</p>	Patent and licensing status unavailable	<p>Pidot, S. <i>et al. Angew. Chem. Int. Ed.</i>; published online May 14, 2014; doi:10.1002/anie.201402632</p> <p>Contact: Christian Hertweck, Hans Knoell Institute, Jena, Germany e-mail: christian.hertweck@hki-jena.de</p>
Bacterial infection; meningitis	Basigin Ok blood group (BSG; EMMPRIN; CD147); <i>Neisseria meningitidis</i> type IV pilus assembly protein (pilV); <i>N. meningitidis</i> major pilin PilE (pilE)	<p>Cell culture, mouse and <i>ex vivo</i> tissue studies suggest blocking the interaction between pilin subunits pilV or pilE and their receptor CD147 could help prevent or treat meningococcal infections. In human endothelial cell cultures, CD147-specific siRNAs or antibodies decreased adhesion of <i>N. meningitidis</i> compared with control siRNA or antibodies. In mice grafted with human skin containing functional blood vessels, <i>N. meningitidis</i> with pilV or pilE colonized the human vasculature, whereas <i>N. meningitidis</i> lacking the pilin subunits did not. In <i>ex vivo</i> human brain tissue, wild-type <i>N. meningitidis</i> colonized CD147⁺ endothelial cells, leptomeningeal cells and cortical brain vessels. Next steps include identifying specific pilE and pilV epitopes that interact with CD147 to develop targeted antibodies. SHX Vaccines is financing the studies(see Managing meningitis, page 10).</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.740 Published online June 26, 2014</p>	Patented; licensed to SHX Vaccines	<p>Bernard, S.C. <i>et al. Nat. Med.</i>; published online June 1, 2014; doi:10.1038/nm.3563</p> <p>Contact: Sandrine Bourdoulous, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France e-mail: sandrine.bourdoulous@inserm.fr</p>
Coronavirus	Coronavirus NSP6 protein (nsp6)	<p>Cell culture studies suggest targeting nsp6 could help treat coronavirus infection. In a chemical screen using normal human lung cell lines, a small molecule called K22 inhibited replication caused by a pathogenic strain of human coronavirus with an IC₅₀ value of 0.7 μM. In human airway epithelial cells, K22 decreased replication of various coronavirus strains including Middle East respiratory syndrome coronavirus (MERS-CoV) compared with vehicle. In culture, coronavirus strains with nsp6 mutations were resistant to K22, suggesting the compound targets that protein. Next steps include optimizing the activity of K22 and elucidating the biological function of NSP6.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.741 Published online June 26, 2014</p>	Unpatented; licensing status not applicable	<p>Lundin, A. <i>et al. PLoS Pathog.</i>; published online May 29, 2014; doi:10.1371/journal.ppat.1004166</p> <p>Contact: Edward Trybala, University of Gothenburg, Gothenburg, Sweden e-mail: edward.trybala@microbio.gu.se</p> <p>Contact: Volker Thiel, Kantonale Hospital St. Gallen, St. Gallen, Switzerland e-mail: volker.thiel@vetsuisse.unibe.ch</p>

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Malaria	<i>Plasmodium falciparum</i> schizont egress antigen-1 (Pfsea-1)	Mouse and cell culture studies suggest inhibiting Pfsea-1 could help protect against malaria infection. In cocultures of red blood cells (RBCs) and three strains of <i>P. falciparum</i> , anti-Pfsea-1 antibodies decreased parasite growth by 58%–74% compared with control sera. In <i>in vitro</i> assays, an anti-Pfsea-1 antibody prevented <i>P. falciparum</i> schizonts from leaving infected RBCs. In mice, vaccination with recombinant sea-1 from <i>P. berghei</i> plus an adjuvant stimulated an immune response against the antigen and resulted in increased survival following a <i>P. berghei</i> challenge compared with vaccination using adjuvant alone. Next steps include trials in nonhuman primates and then a Phase I trial. SciBX 7(25); doi:10.1038/scibx.2014.742 Published online June 26, 2014	Patent pending; available for licensing	Raj, D.K. <i>et al. Science</i> ; published online May 23, 2014; doi:10.1126/science.1254417 Contact: Jonathan D. Kurtis, Brown University, Providence, R.I. e-mail: jonathan_kurtis@brown.edu
Inflammation				
Inflammation	Inducible nitric oxide synthase 2 (NOS2; iNOS); JAK kinase (JAK); signal transducer and activator of transcription 1 (STAT1)	Cell culture studies have identified a saccharin derivative that could be useful for treating interferon (IFN)-mediated inflammation. In a murine macrophage cell line, the lead derivative inhibited lipopolysaccharide (LPS)-induced production of proinflammatory factors with an IC ₅₀ value of 9.61 μM. The compound decreased LPS-induced production of proinflammatory factors by downregulating iNOS to inhibit IFN-mediated activation of the JAK/STAT1 pathway. Next steps include identifying a specific disease indication to target with the identified inhibitors. SciBX 7(25); doi:10.1038/scibx.2014.743 Published online June 26, 2014	Unpatented; licensing details available from the University of Colorado Technology Transfer Office Contact: Darcey Miller, University of Colorado, Denver, Colo. e-mail: darceymiller@cu.edu	Csakai, A. <i>et al. J. Med. Chem.</i> ; published online May 24, 2014; doi:10.1021/jm500409k Contact: Hang Yin, University of Colorado at Boulder, Boulder, Colo. e-mail: hubert.yin@colorado.edu
Musculoskeletal disease				
Osteoporosis	Tryptophan hydroxylase 1 (TPH1; TPH)	Rat and <i>in vitro</i> studies identified ursolic acid derivatives that could be useful for treating osteoporosis. <i>In vitro</i> , the lead derivative was shown to bind TPH1 with micromolar affinity. In ovariectomized rats, oral treatment with the compound increased markers of bone formation and improved bone microarchitecture with efficacy comparable to that of injectable parathyroid hormone (PTH). Next steps could include optimizing the lead derivative and evaluating it in additional models of osteoporosis. SciBX 7(25); doi:10.1038/scibx.2014.744 Published online June 26, 2014	Patent and licensing status unavailable	Fu, H.-J. <i>et al. J. Med. Chem.</i> ; published online May 20, 2014; doi:10.1021/jm5002293 Contact: Xian-Mei Zhou, Nanjing University of Chinese Medicine, Nanjing, China e-mail: xianmeizhou@aliyun.com Contact: Jian-Xin Li, Nanjing University, Nanjing, China e-mail: lijxnju@nju.edu.cn

This week in therapeutics (continued)

Indication	Target/marker/ pathway	Summary	Licensing status	Publication and contact information
Neurology				
Parkinson's disease (PD)	Parkin (PARK2); ubiquitin specific peptidase 30 (USP30)	Cell culture and fly studies suggest inhibiting USP30 could help treat PD. In a mitochondrial degradation assay using cultured human dopaminergic neurons, USP30 inhibited PARK2-mediated mitophagy, a process whereby damaged mitochondria get degraded that is impaired in PD. In the cultured human dopaminergic neurons, <i>USP30</i> -targeting siRNA rescued PD-associated impairments in mitophagy, whereas control siRNA did not. In a fruit fly model of PD, <i>usp30</i> knockdown improved motor function and increased both mitophagy and survival compared with no alteration. Next steps include evaluating USP30 inhibition in mammalian models and identifying pharmacodynamic markers of USP30 inhibition. SciBX 7(25); doi:10.1038/scibx.2014.745 Published online June 26, 2014	Patent and licensing status undisclosed	Bingol, B. <i>et al. Nature</i> ; published online June 4, 2014; doi:10.1038/nature13418 Contact: Baris Bingol, Genentech Inc., South San Francisco, Calif. e-mail: bingol.baris@gene.com
Transplantation				
Graft-versus-host disease (GvHD)	IL-21; inducible T cell co-stimulator (ICOS); CD40; CXC chemokine receptor 5 (CXCR5)	Mouse studies suggest inhibiting the effects of T follicular helper cells could help treat chronic GvHD and associated bronchiolitis. In a mouse model of chronic GvHD, transplantation of T cells deficient in CXCR5 improved bronchiolitis symptoms by increasing pulmonary function and decreasing antibody and collagen deposition in the lungs compared with transplantation of wild-type T cells. In the model, antibodies against IL-21, ICOS or CD40, which are all involved in T cell IL-21 production, improved bronchiolitis symptoms compared with a control antibody. Next steps could include determining the most effective strategy to block T follicular helper cell function. SciBX 7(25); doi:10.1038/scibx.2014.746 Published online June 26, 2014	Patent and licensing status unavailable	Flynn, R. <i>et al. Blood</i> ; published online May 12, 2014; doi:10.1182/blood-2014-03-562231 Contact: Bruce R. Blazar, University of Minnesota, Minneapolis, Minn. e-mail: blaza001@umn.edu

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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
Drug delivery			
<i>Ex vivo</i> GI organoids to model cancer progression and evaluate driver genes	<i>Ex vivo</i> and mouse studies suggest GI organoid cultures could help model colorectal cancer (CRC). Primary GI organoids were produced by growing mouse GI explants containing epithelial and mesenchymal components in an air-liquid interface culture system. In primary GI organoids, combinations of <i>adenomatous polyposis coli (Apc)</i> knockout, mutant <i>K-Ras (Kras)</i> expression, <i>p53</i> knockdown or <i>SMAD family member 4 (Madh4; Smad4; Dpc4)</i> knockdown led to oncogenic transformation that recapitulated histological human CRC subtypes. All four modifications together resulted in the most severe colorectal adenocarcinoma phenotype. In <i>Apc</i> knockout organoids, <i>miR-483</i> overexpression was sufficient to cause dysplasia in culture and tumor formation in mice after subcutaneous transplantation, suggesting <i>miR-483</i> acts as a driver gene for CRC. Next steps include evaluating function and temporal requirements of additional driver gene candidates and extending the findings to human organoids.	Patent and licensing status undisclosed	Li, X. <i>et al. Nat. Med.</i> ; published online May 25, 2014; doi:10.1038/nm.3585 Contact: Calvin J. Kuo, Stanford University School of Medicine, Stanford, Calif. e-mail: cjkuo@stanford.edu
SciBX 7(25); doi:10.1038/scibx.2014.747 Published online June 26, 2014			
Drug platforms			
Crystal structure of the prokaryotic NavM sodium channel to model human Nav1.1 (SCN1A)	The crystal structure of the prokaryotic NavM sodium channel could aid the rational design of Nav1.1-targeting compounds to treat various human diseases including epilepsy. In <i>in vitro</i> assays, the generic antiepileptic drug lamotrigine inhibited both NavM and human Nav1.1 with comparable potency. The X-ray crystal structure of NavM in complex with a known Nav1.1 antagonist revealed that the compound bound to the top of the pore cavity and blocked the sodium channel without causing a conformational change. Researchers did not disclose next steps, which could include using the crystal structure to develop new Nav1.1-targeted agents.	Patent status undisclosed; available for partnering	Bagn�eris, C. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 21, 2014; doi:10.1073/pnas.1406855111 Contact: Barbara A. Wallace, University of London, London, U.K. e-mail: b.wallace@mail.cryst.bbk.ac.uk Contact: David E. Clapham, Boston Children's Hospital, Boston, Mass. e-mail: dclapham@enders.tch.harvard.edu
SciBX 7(25); doi:10.1038/scibx.2014.748 Published online June 26, 2014			
Generation of human induced pluripotent stem (iPS) cell-derived retinal progenitor cells	Human iPS cell-derived retinal progenitor cells could be useful for developing cell therapies to treat degenerative retinal conditions. The retinal progenitor cells were generated by reprogramming human fibroblasts into iPS cells with non-integrating plasmid vectors encoding various transcription factors followed by withdrawal of fibroblast growth factor 2 (FGF2) from the iPS cell culture medium for two days and subsequent culturing in a pro-neuronal medium. The resulting retinal progenitor cells could be differentiated into all the various retinal cell types, including retinal ganglion cells and photoreceptor precursors. Next steps could include further characterizing the function of fully differentiated retinal cell types generated from the iPS cell-derived retinal progenitor cells.	Patent and licensing status unavailable	Reichman, S. <i>et al. Proc. Natl. Acad. Sci. USA</i> ; published online May 27, 2014; doi:10.1073/pnas.1324212111 Contact: Olivier Goureau, Institut National de la Sant�e et de la Recherche M�edicale (INSERM), Paris, France e-mail: olivier.goureau@inserm.fr
SciBX 7(25); doi:10.1038/scibx.2014.749 Published online June 26, 2014			

This week in techniques (continued)

Approach	Summary	Licensing status	Publication and contact information
Minimal saponin adjuvant with high activity and reduced toxicity	<p>Mouse studies suggest a minimal saponin adjuvant could help improve immunotherapy and vaccine efficacy. The saponin natural product QS-21 Stimulon can enhance antigen immunogenicity but is chemically unstable and can cause toxicity. In mice, a synthetic, minimal version of QS-21 that lacks a branched trisaccharide domain and an aldehyde substituent induced antibody responses that were greater than or comparable to those induced by unmodified QS-21 while showing lower toxicity. Next steps include elucidating the mechanism of action and evaluating the <i>in vivo</i> efficacy of antigen combinations with the minimal saponin.</p> <p>Agenus Inc., GlaxoSmithKline plc and Johnson & Johnson are evaluating QS-21 as an adjuvant in Phase III trials.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.750 Published online June 26, 2014</p>	Patent application filed; available for licensing from Memorial Sloan-Kettering Cancer Center	<p>Fernández-Tejada, A. <i>et al. Nat. Chem.</i>; published online June 1, 2014; doi:10.1038/nchem.1963</p> <p>Contact: Derek S. Tan, Memorial Sloan-Kettering Cancer Center, New York, N.Y. e-mail: tand@mskcc.org</p> <p>Contact: Jason S. Lewis, same affiliation as above e-mail: lewisj2@mskcc.org</p> <p>Contact: Govind Ragupathi, same affiliation as above e-mail: ragupatg@mskcc.org</p>
Near-infrared (IR)-based amplification of antibody-functionalized, chemotherapy-loaded nanocarriers plus radiation therapy (quadripeutics) to treat aggressive, therapy-resistant cancer	<p>Cell culture and mouse studies showed that a therapeutic combination called quadripeutics could help treat aggressive, therapy-resistant head and neck squamous cell carcinoma (HNSCC). Quadripeutics technology combines four clinically validated therapeutic components: encapsulated drugs, colloidal gold nanoparticles, near-IR laser pulses and X-rays. In cell culture, antibody-functionalized gold nanoparticles and drug-loaded nanocarriers self-assembled and were locally activated with a near-IR pulse and X-rays to induce drug release and cell death. In multiple mouse models of HNSCC, quadripeutics decreased tumor growth compared with chemotherapy plus radiation therapy. Next steps include clinical trials in the U.S. and Europe to test quadripeutics in head and neck cancers.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.751 Published online June 26, 2014</p>	Multiple patents covering the method and laser clinical system device filed; licensing discussions under way with Mechanical Drugs Inc.	<p>Lukianova-Hleb, E.Y. <i>et al. Nat. Med.</i>; published online June 1, 2014; doi:10.1038/nm.3484</p> <p>Contact: Dmitri O. Lapotko, Rice University, Houston, Texas e-mail: dl5@rice.edu</p>
Targeted gene editing of human hematopoietic stem cells (HSCs)	<p>Targeted gene editing of human HSCs could be useful for treating genetic diseases such as X-linked severe combined immunodeficiency (SCID-X1). The approach uses mRNA electroporation and integrase-defective lentiviral vectors to deliver zinc finger nucleases (ZFNs) and donor DNA templates into CD34⁺ human umbilical cord blood cells, and it uses targeted integration of a GFP cassette into <i>adeno-associated virus integration site 1 (AAVS1)</i> or <i>IL-2 receptor γ-chain (CD132; IL2RG)</i>. In a mouse model of SCID-X1, the approach restored the ability to reject allogeneic human breast cancer cells. In HSCs isolated from a patient with SCID-X1, the strategy was used to replace the disease-causing, mutant <i>IL2RG</i> with a functional copy of the gene. Next steps could include assessing the long-term safety of the targeted gene-editing approach in animal models.</p> <p>SciBX 7(25); doi:10.1038/scibx.2014.752 Published online June 26, 2014</p>	Patent and licensing status unavailable	<p>Genovese, P. <i>et al. Nature</i>; published online May 28, 2014; doi:10.1038/nature13420</p> <p>Contact: Luigi Naldini, San Raffaele Scientific Institute, Milan, Italy e-mail: luigi.naldini@hsr.it</p>

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N. meningitidis major pilin 10,16
 PilE 10,16
N. meningitidis type IV pilus assembly protein 10,16
 Nav1.1 19
 NOS2 17
 Nsp6 16
 NY-ESO-1 7

P

p110 δ 8
 p53 19
 Palovarotene 14
 Panitumumab 13
 Parathyroid hormone 17

PARK2	18	pppGpp	15	S100A8	13	factor- β 1	15
Parkin	18	PRKACA	12	S100A9	13	Trisenox	14
PD-1	3,9	Programmed cell death 1	3,9	Saccharin	17	Tryptophan hydroxylase 1	17
PD-L1	6	Programmed cell death 1 ligand 1	6	Saponin	20	Tumor necrosis factor receptor superfamily member 9	13
PDCD1	3,9	Protein kinase cAMP-dependent catalytic- α	12	SCN1A	19	U	
Peretinoin	14	PRPS2	12	Signal transducer and activator of transcription 1	17	Ubiquitin specific peptidase 30	18
PF-05082566	13	PTH	17	<i>SMAD family member 4</i>	19	USP30	18
Pfsea-1	17	PWT143	8	<i>Smad4</i>	14	Ursolic acid	17
Phosphoinositide 3-kinase- δ	8	Q		SMO	17	V	
Phosphoribosyl pyrophosphate synthetase 2	12	QS-21 Stimulon	20	Smoothened	14	Vancomycin	16
PI-3065	8	Quadripeutics	20	STAT1	17	Vectibix	13
PI3K	8	R		T		Vemurafenib	12,14
PI3K α	8	RARG	14	T cell receptor	3		
PI3K catalytic subunit δ -polypeptide	8	Retinoic acid receptor- γ	14	Targretin	14	X	
PI3K β	8	Retinoid X receptor	14	Tasquinimod	13	X-339	
PI3KCD	8	RG7321	9	TCR	3	XL499	8
PI3K δ	8	RP6503	8	Tgfr1	15	Z	
PI3K γ	8	RP6530	8	TGR-1202	8	Zelboraf	12,14
Pictilisib	9	RXR	14	TNFRSF9	13	ZFN	20
piE	10,16	S		TPH	17	Zinc finger nuclease	20
piIV	10,16	S100 calcium binding protein A8	13	TPH1	17		
<i>Plasmodium falciparum</i> schizont egress antigen-1	17			Transforming growth factor- β receptor 1	15		
ppGpp	15			Transforming growth			

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