As the complexity and intricacies of surgical procedures continue to increase, so too do the demands that are placed on the tools and the techniques that a surgeon employs to get the job done. One of the most advanced technologies in some of today’s toolkits is the robot. Indeed, robotic-assisted surgery has gained a lot of press in recent years – and not all of it positive. But, any negative perceptions of the value of robotic-assisted surgery are not dampening the appetite for research and innovation in this ever-expanding area, and one recent study has demonstrated its value in assisting soft tissue surgery (1).

The research team at the Children’s National Health System in Washington DC, used its biocompatible, 3D tracking software to guide soft tissue surgery with precision. What makes this system special? The researchers note that current robust tracking methods are utilized in surgeries of rigid tissues – such as bone – but soft tissue surgery hasn’t yet received any similarly effective tracking aids due to its malleable nature.

The investigators’ system has already previously shown its mechanized capabilities, operating in tools such as the STAR – an autonomous surgical robot that we’ve previously highlighted as Image of the Month (2) – but the researchers’ recent publication highlights its possible effectiveness and accuracy in assisted robotics.

Using near-infrared fluorescence markers applied to the tissue surface, and two specialized cameras, the software has been shown to track surgical tools and targets in 3D with no marker loss and near millimeter accuracy – even when blood and tissue obscure the surgical site.

Although the system’s limitations mean that robust tracking is only possible in tissue of around 1.3 mm thickness and limited to speeds of 1 mm/s, the team believes that with more iterative work, their findings could have wider implications for real-time tracking of deformable targets in surgical environments. But only time will tell if the rise of the surgical assistant machines will make a mark and become robust enough to use clinically in soft tissue operations. WA

Reference
NoBody’s Fool

Advanced mass spectrometry uncovers a tiny protein with important implications

Meet NoBody (non-annotated P-body dissociating polypeptide): a new, functional human microprotein, discovered and characterized by biochemists using cutting-edge gene sequencing and proteomics technologies at Yale University (1).

The team examined myeloid leukemia cells, first removing larger proteins, then using liquid chromatography-mass spectrometry (LC-MS) based proteomics to uncover the amino acid sequence of each of the more than 400 microproteins that remained. Having discovered a veritable treasure trove of previously unknown proteins, the researchers set out to find out their function (if any) in the body.

“Evolutionary sequence conservation is a great way to help you find function because if the protein sequence of a gene doesn’t change, that probably means it is doing something important and changes to the sequence would be detrimental,” explains co-senior author Alan Saghatelian. “Sarah Slavoff (co-senior author) decided to dig into the function of the gene for these microproteins, and through functional proteomics, linked NoBody to proteins that had been characterized in mRNA decay.” In other words, NoBody helps to recycle genetic material in the cell.

Recent advances in gene sequencing have enabled detection of the small open reading frames (smORFs) that encode microproteins like NoBody, Saghatelian says. “Without RNA-sequencing we could only have found about a third of smORFs. In addition, mass spectrometers have gotten so much more sensitive that it really allows us to dig a lot deeper into the proteome to find them. Ten years ago, the same experiment might have yielded 10–20 percent of what we are seeing today.”

Saghatelian says that the discovery of NoBody has major implications: “First, it points to the fact that digging into the biology of microproteins is going to lead to new biological insights, and in some cases we will learn something about a disease, which we can use to develop new treatments.” Second, he says, it has contributed to understanding the regulation of mRNA decay. “mRNA levels are used to do so much in biology, but most of this is considered from a production viewpoint (which is to say, transcription). However, degradation of particular mRNAs will be important too. The field has tons of room to grow – there is still a lot we don’t understand.”

The lab is now focused on finding and characterizing more microproteins, as well as building better technology to identify the most interesting ones to study. “In terms of biology and the relation to disease, my feeling is that we’re only at the beginning,” concludes Saghatelian. JC

Reference
**A Battery Pill to Swallow**

A new stomach acid-powered cell powers ingested electronics for longer

Ingestible electronic pills have become an increasingly popular endoscopic imaging tool amongst gastroenterologists ever since its introduction to the clinic 15 years ago (1). As ingestible clinical devices get smaller and increasingly complex, the challenge has quickly become how to power the necessary electronics – and how to do so for useful lengths of time and without the risk of toxicity. A team of investigators from Massachusetts Institute of Technology, Brigham and Women’s Hospital, and KTH Royal Institute of Technology turned to the gastrointestinal (GI) tract for a solution and developed an ingestible cell that “harvests” energy from the stomach, small intestine and colon (2).

The pill-sized powerhouse applies basic galvanic principles, using safely-ingestible metals as the anode and cathode, and taking advantage of surrounding gastric and intestinal ‘juices’ as the electrolytes. The team’s bio battery-based device has already endured a six-day voyage through the GI-tract of a pig, and used the harvested energy to measure central body temperature and wirelessly transmit the data to the investigators.

The researchers believe their bio-galvanic cell has the potential (pun intended) to provide power to the next generation of ingestible electronics. And moving on from simply measuring gut statistics, the long-lasting power-source could find itself monitoring cardiac activity, for example. *WA*

**Video of the Month**

The field of optogenetics – the use of light-responsive proteins to control events in the cells of living tissue – has gained popularity as a research tool over the past few years, but has been somewhat limited by the need to use heavily-modified non-native proteins. In recent research (1), Klaus Hahn, Nikolay Dokholyan, and Onur Dagliyan (The University of North Carolina) share a new approach that uses a “switch” to make a native protein light-responsive but otherwise intact – “just the way nature made it” (2).

When the blue circle appears in the video below, fibroblasts are being irradiated, which activates a guanine exchange factor (PA-Vav2) and inhibits its substrate, Rac1, resulting in a change in cell morphology.

Credit: The University of North Carolina at Chapel Hill/Video by Onur Dagliyan.

**Reference**


Reference

Scar Wars

Is transdifferentiation the solution to scarless wound healing?

Psychologically, scars can act as a reminder of a painful or traumatic event. Physiologically, scar tissue exhibits compromised biomechanical properties when compared with uninjured skin (1). Reversing scarring – or preventing its formation in the first place – remains a desirable but elusive goal.

For a time, researchers worked on the principle that reducing or eliminating myofibroblasts would result in scarless wound healing – unfortunately to no avail. But a team of cross-institutional scientists have now discovered that myofibroblasts can transdifferentiate into adipocytes when hair follicles are present (2), which could mean that wound healing can be manipulated with scar-free results.

Various studies on wound healing in mouse models have found that hair follicles often regenerate at the site of large wounds. The new research delved into the process that leads to the generation of relatively normal skin in mice, and found that adipocytes around hair follicles were physiologically normal. The team hypothesized that hair follicles may be able to induce “ordinary” adipocytes and confirmed that hair follicles expressed the signaling molecules (for example, bone morphogenic protein, BMP) that trigger transdifferentiation of myofibroblasts into adipocytes in wound healing.

Keen to show that the findings are translatable into humans, the group also exposed human keloid fibroblasts (in culture conditions) to BMP and human hair follicles with similar results. Clearly translation of the findings into potential clinical approaches will demand further research and time, but the direct injection of signaling molecules and the development of cream formulations are both being considered (3). Interestingly, outside of wound healing, the researchers indicate that regeneration of fat cells could also pave the way for a new anti-aging treatment… But we’ll need to wait to see which comes first.

WA

“Miami, We Have a Problem”

A Florida-based team provides the first quantitative evidence for the role of CSF in spaceflight-induced ocular changes

Since that “one small step,” mankind has made giant leaps forward in space science. Today, astronauts regularly check in and out of the International Space Station (ISS), and the time they spend there is becoming longer and longer. But extended spaceflight brings with it a specter: visual impairment due to intracranial pressure (VIIP) syndrome, giving space agencies another vital mission… to characterize the syndrome and to figure out how to protect their astronauts from it.

VIIP is thought to result from microgravity-induced fluid shift, with symptoms being reported by up to two-thirds of astronauts during or after space flight (1), (2). But to date, the actual etiology of VIIP syndrome has not been defined. Now, a team from the University of Miami who have been studying changes in eye shape and cerebrospinal fluid (CSF) volume in astronauts, have provided the first quantitative evidence for a direct role of CSF in spaceflight-induced ocular changes (Figure 1) (3). Noam Alperin, Professor of Radiology and Biomedical Engineering at University of Miami Miller School of Medicine, and lead author of the study, tells us more…

Why?

Our group has been investigating the CSF system for a long time, and we’d developed a method to measure intracranial pressure (ICP) non-

Reference

Invasively by magnetic resonance imaging (MRI). In 2010, I received a call from NASA, “Miami, we have a problem.”

**How?**

We installed a protocol in their MRI scanner that’s located near the Houston Space Center. For four years, we studied astronauts before and after space flights, collecting data from short-duration and long-duration astronauts. The algorithm we’ve developed to assess morphological changes provides a quantitative measure, and is much more accurate, reliable and reproducible than previous methods which involved “eyeballing the eyeball.”

**When?**

We saw that most astronauts developed VIIP to a certain severity by six months. From studying short-duration astronauts who have been in spaceflight for two weeks, we know that VIIP starts after a much longer duration than this – I would say after several months of time in space and we expect that the longer the flight, the worse the deformations.

**What’s next?**

We’re already starting to use our approach to study glaucoma, and we’ve done a lot of work that will hopefully be published soon. We think our method of measuring CSF volume is a consistent way to assess the balance between the eye and the brain. We’ll also continue working with NASA to examine the effects of “head down tilt” on the globe of the eye. In this study, subjects will spend 30 days in bed with a head-down tilt of six degrees to simulate the movement of fluids from the legs to the head, and we’ll measure and quantify the deformation that occurs.

**Reference**


Only Tau Will Tell...

Could tau be a useful biomarker of concussion?

With a bestselling novel and a Hollywood film to its credit (1), the subject of sports-related cranial trauma is finally receiving some long-overdue attention. American football is notorious for repeated concussion, and with the combined worth of National Football League teams being nearly $75 billion (2), safety of its stars – and protecting them from the probability of long-term neurological symptoms – is of great interest. To that end, researchers at the National Institutes of Health (NIH) have been investigating diagnostic markers to not only detect cases of concussion, but also to provide insight into prognosis and recovery (3).

Tau proteins have previously been linked to axonal damage after traumatic brain injuries (4) and, therefore, have potential as biomarkers of concussion. Indeed, the NIH team discovered that higher levels of tau observed six hours after a sport-related concussion correlate with an extended recovery period – findings that may eventually play a vital part in determining when an athlete can resume play.

There is a complicating factor, though; athletes generally have higher tau concentrations than non-sport playing controls, which must be taken into account. The researchers suggest that the elevated base level of tau may be caused by the general physical exertion of sports in conjunction with the increased blood-brain barrier permeability that occurs during sports-related activities. Although this natural elevation seems benign, it remains an important factor to consider – both for research into the association between tau and brain injury, and for any eventual diagnostic use.

Embracing Robotics with Heart

A biomimetic, pneumatic sleeve that ‘hugs’ the pericardium could support failing cardiac function

“We were investigating new methods in dynamic actuation, and the integration of pneumatic actuators into a soft robotics platform showed promise for achieving complex motions like that exhibited by the heart,” says Frank A. Pigula, co-corresponding author and cardiothoracic surgeon.

“The actuators are based on the McKibben air muscle (pneumatic artificial muscle) and the myocardial helical band morphology,” says Pigula. “And the development of new materials and control systems in the area of soft robotics offered new opportunities.”

Designing the device was an iterative process that required in vitro and in vivo experimentation to finalize, according to Pigula. The current version of the soft cardiac sleeve has been tested on pig hearts; when cardiac output was reduced to approximately 45 percent of baseline activity, the device was able to recover the porcine heart to almost full activity (97 percent).

Reference
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The device is comprised of a biomimetic elastomer matrix, embedded with pneumatic actuators that compress and twist to mimic contractions of the heart. “The actuators are based on the McKibben air muscle (pneumatic artificial muscle) and the myocardial helical band morphology,” says Pigula. “And the development of new materials and control systems in the area of soft robotics offered new opportunities.”

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The team believes the cardiac sleeve could have long-term viability in sustaining cardiac strength, but further iterations will be needed. To that end, Pigula says they’re going to work on design optimization and attachment methodology, as well as other translational applications: “This technology could be suitable for a range of other assist applications (both implantable and external), and our groups will continue to explore these avenues.” WA

Reference

Biting Back Against Malaria
Are we close to a vaccine against the mosquito-borne killer?

Almost half the planet is at risk of contracting malaria (1), and although prevention methods have helped quell the number of people who have contracted it from previous years, our battle with the deadly disease seems to be one-sided. Now, research documenting the successful clinical test of a potential malaria vaccine indicates a chance to turn the tables.

The research team, based at the Center for Infectious Disease Research in Seattle and the University of Washington, created an attenuated vaccine of Plasmodium falciparum (Pf GAP3KO) – the most common cause of malaria – through precise genetic deletion of three genes expressed in the pre-erythrocytic stage. The objective was to create sporozoites that were virulent enough to induce an immune reaction but innocuous enough to cause no adverse effects. During the trial, the Pf GAP3KO strain was introduced, via mosquitoes, into 10 human volunteers who endured 150-200 bites each to ensure infection. Despite the mosquitoes’ best efforts, all volunteers subsequently tested negative for blood-stage malaria, and developed antibodies against P falciparum, which could confer immunity against infection by the wild-type.

Extensive work and larger scale trials will be needed to investigate the full potential of Pf GAP3KO, but these preliminary results represent new hope for a successful malaria vaccine. WA

Reference
Measuring the Microbiome

Untangling the complex web of relationships between humans and the trillions of microbes who share our bodies is a daunting task, but novel application of modern analytical techniques at least gives us a chance.

By Liam M Heaney, postdoctoral scientist in the Department of Cardiovascular Sciences, University of Leicester, UK.

The symbiotic relationship between humans and microbes is important for maintaining good health. And according to mounting evidence, dysfunctional relationships could increase susceptibility to disease (1). Here, I will use the example of trimethylamine [N-oxide] (TMAO), a molecule mediated through metabolism of dietary components by gut microbes, to illustrate the complexity of the microbiome.

TMAO can be measured in biofluids and, in 2011, was found to be elevated in the plasma of patients diagnosed with coronary artery disease (2). Later, it was demonstrated to be elevated in patients at higher risk of major adverse cardiac events (for example, stroke, myocardial infarction) within three years (3). Most systemically circulating TMAO is formed by metabolism of dietary components, such as L-carnitine and free choline, by the gut microbiota (4).

These molecules are readily available in red meat and dairy, and TMAO has been identified as a possible mediator in the link between red meat and cardiovascular disease. But the relationship is complex. Paradoxically, TMAO is present in relatively high quantities in fish, yet populations with seafood-rich diets are considered at lower risk of heart disease than other western populations (5). We, and others, are attempting to unravel the relationship between diet, TMAO and heart disease.

TMAO is a non-volatile small molecule (molecular weight 75.11), and liquid chromatography-mass spectrometry (LC-MS) methods have been developed to measure circulating concentrations in plasma and serum, and excreted concentrations in urine. Though previous methods have predominantly employed multiple reaction monitoring on triple-quadrupole MS systems, our lab has developed a protocol employing the quadrupole-traveling wave-time of flight setup on a Waters Synapt G-2S instrument (6). The inclusion of a dilution step, using an isotopically labeled internal standard (D9-TMAO), allows a highly specific and selective analysis of samples with accurate quantification. Additionally, the inherent ability for selected/multiple reaction monitoring measurements using LC-MS allows for simultaneous analysis of other molecules related to gut microbial metabolism, without loss of sensitivity or selectivity. For example, analyses may include additional molecules, such as L-carnitine, choline, betaine and ß-butyrobetaine, allowing an improved understanding of the dynamics and kinetics of these molecular/metabolic relationships.

Using these methods, we have shown that elevated levels of TMAO are associated with poor prognosis in acute hospitalizations of heart failure (7) and myocardial infarction (8). These
experiments support previous data from gene knockout mice models, which showed that high levels of TMAO induced atherosclerosis (9) and worsened conditions associated with heart failure (for example, left ventricular ejection fraction) (10). Interestingly, we (and others) have also reported a strong correlation between circulating TMAO levels and markers of renal dysfunction. It is crucial that we ascertain whether elevated TMAO levels cause increased cardiovascular risk, or whether elevated TMAO is a side effect of renal dysfunction (11). In the latter case, increases in TMAO may be a surrogate biomarker for severity of cardiovascular/renal disease, rather than a direct cause. I’m confident that ongoing studies into the nature of these relationships will give us the evidence we need to establish ground rules and overcome problems experienced in end-stage renal disease patients undergoing haemodialysis, (2016).

Whether TMAO acts as a direct toxin on human cardiac/renal tissue or exists merely as a surrogate biomarker, this small molecule offers valuable prognostic information for a range of cardiovascular conditions, and we hope eventually to see it in clinical use.

Reference

We Must Guide POCT

Why it’s important to establish ground rules and quality for point-of-care testing

By Xavier Navarro, Area Manager and POCT Coordinator at Laboratori de Referència de Catalunya, Barcelona, Spain.

Point-of-care testing (POCT) should not be a new topic for laboratory medicine discussions but in some institutions, it is. And, those who are unaware of the potential problems of poor global quality management of POCT need reminding about it regularly, particularly as people who are not laboratory medicine specialists may perform the measurements!

Clearly, there are very good reasons for using POCT. For example, it reduces turnaround time (TAT) or “vein to brain time” (time from result availability to action taken), and it is advantageous in reducing unnecessary blood drawing (in intensive care and neonatal intensive care units). It also minimizes handling or transporting samples to the laboratory, improves patient care by reducing hospital visits and, therefore, unnecessary journeys.

I would say that, above all, any decision to implement POCT should be guided by a desire to improve patient care, eliminate problems caused because the laboratory cannot improve TAT, and overcome problems experienced with laboratory processes that prove difficult or impossible to improve. But, it is important to ensure that the quality of POCT is of the same high standard as those tests performed in the laboratory.

In my view, laboratory professionals should see POCT as an extension to lab work and subject it to the same quality standards. Consequently, there is no need to reinvent the wheel, just use your established standards. Here are some of the basic items we have implemented within our POCT quality standards in Barcelona:

• take care of the patient
• create and lead a team
• analyze, simplify and document all processes
• select and evaluate POCT analyzers
professionals from both healthcare
multiskilled, comprising a variety of
including:

assure global POCT quality
learn from errors allowing
continuous quality improvement.
All of the above are simply what
we’ve been doing for a long time!

Undoubtedly, every new POCT
scenario should be developed with
the aim of improving healthcare and
patient benefits while maintaining
outstanding reliability of every point-of-
care measurement. Far from facing these
tasks as individuals, it needs a strong
team led by an experienced laboratory
professional as POCT coordinator. I
suggest that the POCT coordinator
has very important responsibilities, including:

selecting and leading a team of
trained and strategic professionals
deciding which global quality
assurance protocols will be
applied. He or she will monitor
internal quality control, external quality assurance,
process performance indicators, quality system assessments, and
implement further improvements as necessary
assuring access to important
information allowing efficient and
effective use of systems. He
or she will demonstrate a deep
knowledge of every test procedure
(identification and preparation
of patient, how to obtain and
manage samples, analytical
process, validation, etc.)
establishing training plans for
personnel (subjects, timing,
assessment of acquired
knowledge, etc.)
assuring that all testing conforms
to legal requirements.

Hopefully, your team will be
multiskilled, comprising a variety of
professionals from both healthcare
(nurses, physicians, clinical laboratorians)
and from other disciplines too (such as
information technology, administration,
and others). This will provide a unique
opportunity to approach POCT subjects
from several inspiring points of view.

In that context, clinical laboratory
professionals will have a vital role in
POCT team education and training
by providing well-structured and
easily understandable documentation
and instructions for using POCT
devices. This kind of education is
necessary because there is a general
lack of laboratory specific education for
healthcare professionals, as well as a false
assumption of “simplicity” of POCT
devices, and an incorrect, general belief
that whatever value obtained from a
measuring system is true. So, laboratory
professionals must publish clear, visual,
and easy to read operating instructions.
These must be available at the POCT
site, allowing others to use POCT
devices safely, with the highest quality
throughout the measuring process,
which will ensure reliable results, avoid
errors and help protect patient safety.

Clearly, the participation of laboratory
professionals in selecting and evaluating
POCT devices will help to ensure that
hardware is fit for purpose. Importantly,
device evaluation must be to Clinical
and Laboratory Standards Institute
(CLSI) guidelines – this is mandatory,
and so is observing global quality
assurance regulations such as ISO9001,
ISO15189, ISO22870 or any others
(CAP, Joint Commission, etc). Finally,
a well-structured quality assessment
system is essential to enable continuous
improvement and to contribute to
patient safety.

By following the above and applying
global quality standards here in
Barcelona, we’re achieving great things
with POCT – and those are the same
standards we’ve always used as laboratory
medicine professionals.

Beyond Cancer Imaging

Exploring the new world of theranostics that integrates cancer diagnosis with treatment

By Zaver M. Bhujwalla

At the interface of diagnosis and therapy, theranostic imaging is showing real promise in the rapidly expanding field of precision medicine. As we know, when administering cancer therapy, it is critical to minimize damage to normal tissue. Molecular imaging provides this crucial support by identifying disease-specific targets, contributing to the design of agents against these targets, visualizing their delivery, and monitoring their therapeutic effect. And now that genomic and proteomic profiling can provide an extensive “fingerprint” of each tumor, it is possible to design personalized cancer theranostics which minimize damage to normal tissue. The importance of these advances must not be underestimated; the effective application of theranostic agents may achieve the goal that has remained elusive for so many malignancies – a cure for cancer.

So, let’s look at how we can define a theranostic. It is the integration of a diagnostic and therapeutic agent to deliver a highly-targeted treatment.

If we focus first on diagnosis, this is categorized according to the imaging modality that is used and the cancer-associated target that is being exploited. Several technologies that span bench to bedside – such as magnetic resonance imaging/spectroscopy, positron emission tomography, single photon emission computerized tomography, and optical imaging for intra-operative imaging –
are applicable to theranostic imaging. But there is also a rapid expansion of innovative nanoplatforms for theranostics, too, which are based on the application of nanotechnology to imaging modality, therapeutic cargo, or target. Examples include liposomes, nanoparticles, micelles and viral vectors that display imaging reporters, and deliver either conventional therapy or nucleic acid-based interventions, such as complementary DNA or small interfering RNA. There is an increasing trend to combine imaging modalities and, as a result, the nanoplatforms may carry multi-modal imaging reporters. Innovative strategies such as pH-responsive micelles that show pH-dependent demicellization below pH 6.5 have also been developed.

If we take a look at targets, cancer theranostics involve the delivery of a therapeutic cargo to tumor targets that can be non-invasively imaged, and therefore cancer-associated cell surface antigens provide useful targets. For example, 20 to 30 percent of breast cancers express the human epidermal growth factor receptor (HER2). Trastuzumab, a humanized monoclonal antibody directed against the extracellular domain of HER2, was a breakthrough treatment for this population of patients. Good responses are also seen with the dual tyrosine kinase inhibitor lapatinib, which inhibits EGFR/ErbB1 and HER2/ErbB2. However, a large percentage of patients develop drug resistance due to adaptation of signaling pathways; hence, theranostic imaging is an attractive option for identifying and monitoring the outgrowth of drug-resistant tumors. Theranostic imaging can also exploit prostate-specific membrane antigen, a type II integral membrane protein expressed abundantly on the surface of androgen-independent, advanced prostate cancer, and CD44, a transmembrane glycoprotein that is important in metastasis and in stem-like breast, prostate, pancreatic, ovarian and colorectal cancers.

Most tumors, however, do not express cancer-specific cell surface antigens; developing theranostic tools for these malignancies therefore requires investigators to mine other aspects of the tumor: metabolism, angiogenesis, inflammation, tumor microenvironment (TME), and stromal cell receptors. The physiological environment of the tumor is usually characterized by hypoxia, acidic extracellular pH, and substrate deprivation; none of these features are typical of non-malignant tissues. Similarly, the TME – comprising the extracellular matrix, cancer-associated fibroblasts, adipocytes, pericytes, vascular and lymphatic endothelial cells, and multiple immune cells such as tumor-associated macrophages – also tends to differ from the non-cancerous extracellular environment. These points of difference provide opportunities for theranostic imaging.

In my view, a major challenge is the need to rapidly translate and clinically implement the most promising theranostic agents. Quantitative image analysis, the cost of synthesizing theranostic agents, immune responses to these agents, challenges with good manufacturing synthesis, difficulties in obtaining US Food and Drug Administration/European Medicines Agency/Institutional Review Board approval, and the cost of clinical trials are some of the challenges in this field. Despite these challenges, innovations in theranostics occurring at the interface of chemistry, molecular biology, and imaging will provide major advances in the field of cancer treatment.
Chasing Checkpoints

Therapeutic modulation of checkpoint signals is bringing new treatments to cancer patients – and the industry is scrambling to find more.

By Frédéric Triebel

Checkpoint inhibitors have been identified as one of the most exciting areas of progress in the industry (1) – and with good reason. A huge amount of research from academia and industry is focusing on the potential of checkpoint inhibitors for treating cancer – and a handful of therapies have been launched. What is a checkpoint inhibitor? Many cancers produce mutant proteins that are recognizable as “foreign” by the immune system. Often, however, tumor cells also have the ability to push molecular buttons – “checkpoints” – that inhibit anti-tumor immunity. But when one door closes another opens; the elucidation of checkpoint-mediated inhibition has led to a new class of therapies called checkpoint inhibitors designed to block tumor-mediated inhibitory signals, thus allowing the immune system to mount a more effective anti-tumor response.

The potential of immunotherapy in cancer treatment has been discussed for well over one hundred years. In the 1880s, William Coley published a paper describing how he’d injected cancer patients with streptococcal cultures and observed tumor regression in some cases – but he didn’t know why. What we have today that Coley didn’t is a better understanding of immunotherapy, how T-cells work and how cancer evades the body’s natural immune system – although there is still a long way to go (2).

Inhibiting inhibition

I’ve spent most of my career focusing on human immunology and it has been interesting to see how the times have changed. Back when I entered the field, immunology was little more than a subspecialty of infectious disease. During my fellowship in the 1970s, I was very focused on human tumor-infiltrating lymphocytes (TILs – implicated in killing tumor cells), which was quite unique at the time given that the vast majority of immunologists were working with mouse cells. I cloned human T cell receptors and elucidated how they could recognize two different determinants, the antigenic peptide and the major histocompatibility complex. In the 1990s, my group at Institut Gustave
Roussy (France) discovered that 10-30 percent of TILs from different metastatic human tumors were of identical specificity, showing that they had recognized a tumor-specific peptide and multiplied locally (clonotypic expansion). This in turn suggested that disinhibiting this population could generate an anti-tumor response, and led to my work on checkpoint inhibitors.

Today, immunology is a vibrant area of research, and cancer immunotherapy, in particular, continues to go from strength to strength. At first, much attention focused on blocking the CTLA-4 checkpoint, which had benefits in certain groups of patients, but responses were not universal and there could be significant toxicity in some cases. So far, there has only been one FDA approval of a CTLA-4 checkpoint inhibitor: ipilimumab is a monoclonal antibody that was first approved in 2011 for treating skin melanoma – and was the first new treatment for metastatic melanoma in more than a decade. The treatment, however, can have serious side effects (3), which has spurred drug developers to look for alternative options.

Today, a lot of checkpoint inhibitor activity has focused on programmed death 1 (PD-1). PD-1 is located on T cells while PD-L1 is found on normal cells to prevent them from being attacked by T cells. PD-L1 is also found on some cancer cells. Drugs targeting PD-1/PD-L1 have been approved by the FDA in recent years – atezolizumab, nivolumab and pembrolizumab – have good response rates, but side effects can still be serious for some patients. Many more PD-1/PDL-1 drugs are being developed, but the industry is also looking to develop drugs that interfere with other checkpoints, such as lymphocyte activation gene-3 (LAG-3), killer immunoglobulin like receptor (KIR), and T-cell immunoglobulin and mucin domain-3 (TIM-3) (4). Of these, LAG-3 is receiving growing interest. A number of pharma companies are investigating LAG-3; Novartis, Merck, Regeneron, Boehringer-Ingelheim and Bristol-Myers Squibb all have LAG-3 products in the clinic. BMS’ anti-LAG-3 has been in the clinic for more than three years, and the company started five new large clinical trials last
Business LAG

Academic research is pretty competitive and you have to fight for your ideas. From 1990 until 2002, my group was the only one publishing on LAG-3. At times like this, when you really believe in the potential of something, it’s important to be resilient. I knew that if the immune system could recognize tumors, then it would be an important advance – potentially we could wipe out even large tumor masses. And the results I was seeing with LAG-3 were compelling.

During the 1990s, I was director of a unit at INSERM – the Institut national de la santé et de la recherche médicale in Paris, France. INSERM gave the LAG-3 patents to Serono, but Sereno were not focused on moving it forward at the time. I wanted to get things moving so I quit my immuno-oncology professorship at the university with the aim of getting the patents back and starting up a new company – Immutep. Along the way, I learned just how inefficient the biotech world is. More than three years of negotiations were required to licence the patent back from Serono, and obtaining Series A funding from investors required immense effort. Series A was the last tranche raised; funding rounds B and C were never forthcoming, and a cash-strapped Immutep was forced to license some antibody products to GlaxoSmithKline and Novartis – after all, without money, you can’t accomplish anything.

But it turned out well in the end. Immutep was acquired by Prima Biomed in 2014 – and today I’m the Chief Scientific and Medical Officer. At the time, Marc Voigt, CEO of Prima Biomed, wasn’t looking for LAG-3, specifically – he was simply interested in promising innovation focused on immuno-oncology, but the GSK and Novartis deals, as well as the advanced clinical development and potential of another product, caught his eye and validated the work that we were doing at Immutep. Today, my work with LAG-3 continues at Prima Biomed and the two licensed products are progressing well in clinical trials. We also have a third product in development – IMP321 for metastatic breast cancer and metastatic melanoma. Notably, we are the first company to have a Chinese-made biological enter clinical trials in Europe and the first one presenting – just very recently – an agonist antibody to LAG-3.

We’ve only just begun

We have only scraped the surface of the potential of checkpoint inhibitors for drug development. Many people in the industry still don’t really understand T cell or dendritic cells (and explaining the science to investors is always a challenge), but they do understand durable tumor regression and how important it is. The excitement and expectations for the field are having a very positive impact in terms of greater funding, even for more challenging approaches to immunotherapy, such as CAR-T cells. Indeed, the widely reported successes with CAR-T therapies have also helped to put immunology in the spotlight.

All of that said, I think immunology still deserves yet more attention. Many of the chronic diseases that the industry is desperately trying to develop treatments for can be approached from an immunological perspective. In many cases, disease is caused because of immune system regulation. Of course there are other factors that play a role too, such as genetics and external influences, but in general I believe that the full potential of immunology has yet to be uncovered and many new discoveries are waiting to be made.

In the future, immunotherapy will be increasingly broadly applied, not only in oncology and autoimmune disease, but also in neurodegenerative diseases, such as Parkinson’s and Alzheimer’s. There is also potential in less obvious conditions, such as atherosclerosis. Many chronic conditions would benefit from approaches that are more fundamental than merely treating symptoms. For example, could the use of statins for high blood pressure eventually be replaced by an immunology-based approach to the underlying problem?
The Heart of Translation

Sitting Down With... Douglas L. Mann, Lewin Professor and Chief, Cardiovascular Division, Washington University School of Medicine in St. Louis, USA.

What inspired you to study medicine – and focus on cardiology?

I tried a number of different careers before I settled on medicine – I initially wanted to study music and then English. And though medicine was never Plan A it appealed because it allowed me to combine a love of science knowledge and the personal aspects of interacting with people.

During medical school, I actually hated cardiology. But on the first day of my residency, I found myself putting a pacemaker in a patient who had undergone a cardiac arrest, and I immediately fell in love with the field. I enjoyed the acuity of cardiology as a subspecialty and liked how elegant the physiology was – even though the technology wasn’t very sophisticated at the time. I then segued into understanding how the heart worked, first by learning ventricular mechanics, then cell and molecular biology. It was a series of steps that led me to pursue a career in cardiology, but the first day of my internship sparked the fire.

What are the biggest research challenges that cardiologists face?

There are many. The area that we have studied in the lab and the clinic is the role of inflammation in the heart. We have tried to understand the biology of the failing heart by specifically focusing on the role of the inflammatory response. Inflammation is a double-edged sword; you need it for repair, but when activated to an excessive amount – which can happen during surgery – it can lead to deleterious consequences that promote heart failure, cardiac remodeling, contractile dysfunction, and fibrosis. However, without inflammation the heart cannot repair itself following cardiac injury. Developing therapies that minimize the deleterious effects of inflammation without interfering with the beneficial effects of inflammation is extremely difficult. And trying to intervene at the right point in time with a suitable therapeutic agent has proven to be a difficult undertaking – for us, as well as other labs around the world.

How do you define translational science?

I’m the editor of a new open-access journal called JACC: Basic to Translational Science. The editorial board has chosen to define translation science as anything that leads to a new discovery that benefits patients afflicted with cardiovascular disease, beginning with studies at the bench and all the way up to Phase II B clinical trials in humans. I envision translational science as the ‘arc of discovery’ from the inception of the idea to the proof of concept in clinical trials. This definition also encompasses the idea of “reverse translation” of which the discovery of PCSK9 is a great example. Reverse translation refers to using cohort population studies to identify a candidate gene that is associated with a disease trait through GWAS, then creating animal models in the lab to understand how the gene mutation actually works, followed by designing drugs that target the defect. I believe that translational science can...
have a very broad definition; if you try to narrow it down to just one a single rigid definition, you may miss out on new ways to develop therapies.

**How difficult is clinical market translation in cardiac medicine?**

Very difficult for many reasons! To start with, a lot of investment is going into innovative therapies rather than ‘me-too’ drugs because the market has become saturated. The failure rate for new therapies is extremely high, which may scare away investors who are risk averse. Second, because we have been very successful in developing therapies that affect meaningful clinical cardiovascular outcomes, the expectation is that all new drugs will have a similar impact. Simply improving quality of life or extending life by six months with a new and novel drug is no longer enough to gain FDA approval. Additionally, the translational path for developing new cardiovascular drugs and devices is inherently fragile because of the need to work with sick cardiac patients.

**Where do you see cardiac therapies heading?**

I think the future will always rely on identifying novel pathways that can be targeted safely with drugs, devices, and new biologics. The exciting part about translational medicine today is that the scientific approach is much broader because of the development of all of the “omic” technologies, as well as the ability to perform computational modeling for rationale drug design. The ability to identify new targets and develop new therapies has never been greater.

However, a current issue that’s going to become an even bigger challenge in the future is how to develop new therapies more efficiently to reduce the cost of therapies for patients and society. It is unrealistic to expect companies to spend billions of dollars developing new therapies only to realize that patients or governments can’t or won’t pay for them when they finally reach the market. Therefore, translational science will not only be about developing new therapies, but also about coming up with therapies that are affordable – and that will require a rethink of the clinical phase of development as well. But let’s consider this a “glass half-full” statement, rather than a “glass half-empty” statement! We as a community need to stay positive; there is plenty of room for cardiac translational science to grow in the future. We just need to be aware of the challenges that lie ahead – and learn to deal with them as they develop. Cardiovascular medicine remains the number one cause of death worldwide; my view is that we need more translational science, not less!