“We lack effective therapies for stroke,” says David Hess, Professor and Chairman of the department of neurology at Augusta University. Hess believes the answer lies with stem cell therapy – and, to that end, a recent paper shares the results of a phase II trial of multipotent adult progenitor stem cells in ischemic stroke patients (1). Although the findings showed that the treatment was safe over a 90-day period, there was no significant improvement versus a placebo. Despite the results, the team remain hopeful. We spoke with Hess to find out why.

Why focus on progenitor cells?

I believe cell therapy is the third wave of therapeutics – after small molecules and biologicals. As there was some evidence that cell therapy was effective against myocardial infarction in the heart, we thought it could be effective against stroke in the brain. Other than tissue plasminogen activator and mechanical thrombectomy – a very effective device-based therapy – there are few efficacious treatment options.

Can you share more details of the trial?

The cells were very safe and well tolerated – which was initially a hurdle we had to overcome – and there was a trend to improved outcomes and reduced infections, especially in patients treated less than 36 hours after their stroke. In keeping with their immunomodulatory effect, the cells reduce the acute neuroinflammatory response that increases tissue injury after stroke, and prevent splenic and immune exhaustion – all of which fit our overall hypothesis; unfortunately, we expanded the time window to 48 hours, so we had many patients treated late. We believe this may have blunted the effect.

What’s next?

Hints from the phase II trial suggest that treating patients under 36 hours will indeed lead to better functional outcomes at three and 12 months. But before these cells could ever be approved for routine human use, we will need to demonstrate their safety and efficacy, which we plan to do in two late-phase trials – one taking place in Japan and the other taking place in North America and Europe.

Reference

Immune Activation and Autism

Finding the genetic pathways that drive autism spectrum disorder

May 2017

According to previous research (1), pregnant women who undergo immune activation by contracting a severe infection within their first two trimesters may stand an increased risk of their child developing autism spectrum disorder (ASD). But how are the two factors linked? To understand the connection, a new study aimed to investigate a possible pathway in mouse models (2).

“We were particularly interested in understanding to what extent maternal immune activation (MIA) would induce changes in the developing brain that are similar to, or overlapping with, changes found in the postnatal ASD brain,” says Tiziano Pramparo, lead investigator and assistant researcher in the department of neuroscience at University of California, San Diego. “Finding a certain degree of overlap would provide indirect evidence that there are shared etiological routes between MIA and ASD pathophysiology, thus potentially suggesting an increased risk in those pregnancies with a history of prenatal infections and hospitalization.”

The team began their investigation by looking into cortical development – and if MIA caused genetic changes in the processes behind it. After reanalyzing prior data on the topic (3), the researchers noted that alterations in developmental genes were similar to young ASD brains. After digging further and conducting their own study on mouse models, the investigators discovered an even closer link. “We identified the dysregulation of genes involved in protein production, and specifically those involved in the cap-dependent translation initiation gene,” says Pramparo.

“When we analyzed which set of genes were commonly altered in the cortical tissue between the MIA rodents and ASD postmortem brains, we found that the strongest signal was for genes with translation initiation functions – specifically EIF4E,” Pramparo says. “If one wanted to speculate and jump to conclusions, we could say that MIA-induced effects due to prenatal infections may lead to dysfunction of the machinery regulating the production of proteins, and this alteration is one of the potential causes underlying ASD.”

Pramparo appears confident enough to speculate, and has already applied for additional funds to investigate whether the pathway can be targeted with a specific blocker to ameliorate – or even reverse – any behavioral and cellular abnormalities induced by MIA.

With the beginnings of a success story in hand, Pramparo and his team plan to use the same prenatal model systems to discover any overlapping effects of Zika infections on genes that are important for cortical development – and whether certain types of Zika infections during pregnancy could also lead to an increased risk of ASD.

References

A Winning Combination?

Engineering more effective antibody-drug conjugates

May 2017

Antibody-drug conjugates (ADCs) are an emerging cancer treatment with a lot of promise – by combining the targeting ability of an antibody with a compounds cell-killing ability, it’s possible to create a highly selective – and effective – therapeutic. We spoke to Christoph Rader, part of a team from The Scripps Research Institute who have engineered antibodies using selenocysteine residues, dubbed selenomabs, which they hope will improve the utility of ADCs.

Why try to optimize ADCs?

We wanted to develop new homogeneous antibody-drug conjugates (ADCs) to overcome the current limitations of heterogeneous ADCs, which are based on randomly conjugating the drug molecule to the lysine or cysteine residues of the antibody molecule. The random conjugation results in a mixture of ADC species, with different pharmacokinetic and pharmacodynamic properties. Ideally, a homogenous ADC constitutes a single species, and has highly defined and reproducible pharmacological properties. When thinking about clinical utility, it’s a bit like comparing monoclonal and polyclonal antibodies.

Where does selenocysteine fit in?

To generate homogeneous ADCs, unique chemical reactivity has to be built into the antibody. Selenocysteine – also known as the 21st natural amino acid – has unique chemical reactivity that allows selective drug conjugation in the presence of the other >1,000 amino acids in the antibody molecule. And our team has a decade of experience working with selenocysteine, especially when it comes to engineering antibodies with selenocysteine residues.

In our current study, we built on our knowledge to make highly potent and stable selenomab-drug conjugates for models of human breast cancer and multiple myeloma.

The conjugation chemistry had to be tailored to the high reactivity of the selenocysteine residue to ensure selectivity and stability, so we collaborated closely with organic chemists. We also improved the expression of selenomabs by better harnessing the natural selenocysteine incorporation machinery of mammalian cells, which allowed us to achieve the yields necessary to carry out extensive biochemical characterization of selenomab-drug conjugates, along with in vitro and in vivo activity studies.

How did the new selenomab-drug conjugates perform?

Despite their lower drug-to-antibody ratio (DAR), the activity of our selenomab-drug conjugates outperformed the gold standard ado-trastuzumab emtansine (Kadcyla) both in vitro and in vivo.

What’s next?

We are particularly interested in the development of ADCs that carry more than one kind of drug. Using drug surrogates, we previously developed conditions that allow us to orthogonally conjugate two different drugs to cysteine and selenocysteine with high precision (2). We are now using this technology to conjugate drugs to these “thio-selenomabs”. We also plan to continue our work on improving the expression of selenomabs to facilitate the translation of selenomab-drug conjugates and thio-selenomab-drug conjugates into clinical trials.

References

Reuse, Recycle, Regenerate

Two repurposed drugs prevent neurodegeneration in mice – and both are already in clinical use

May 2017

“As a neurologist, I was appalled by how little we could do to treat neurodegenerative diseases in humans, compared to our extraordinary advances in other branches of medicine,” recalls Giovanna Mallucci, a researcher with the UK Medical Research Council (MRC).

Mallucci has spent 15 years attempting to solve the riddle of neurodegeneration – trying to better understand why brain cells die, and looking for potential treatments. The hard work finally seems to be paying off with the discovery of two repurposed drugs that have been shown to prevent neurodegeneration in mice (1).

The MRC team initially tested 1,040 compounds in worms, before taking their most promising candidates forward to mouse studies. Using mouse models of prion disease and frontotemporal dementia (FTD), they identified two drugs that prevented signs of brain cell damage and restored memory in the FTD model, and reduced brain shrinkage in both models.

Crucially, the two compounds are already being used in humans – trazodone is already licensed for use in depression and the other, dibenzoylmethane, is already under investigation for its anticancer properties. “It means we can go ahead and do a clinical trial, without first dealing with years of toxicology testing and phase I/II trial,” says Mallucci. “It also means we can avoid spending years generating possible candidates – these ones are ready to go.”

Responses so far from both the scientific community and the public have been very positive, says Mallucci, and the team has received many queries about upcoming studies. “We’ve yet to secure funding, so we expect it to take around a year before we move to clinical trials,” she adds.

Reference
Technique Out of Left Field

Using left-handed DNA to better monitor and control PCR

May 2017

Biomedical engineers at Vanderbilt University have designed a new handheld device that uses left-handed DNA (L-DNA) to monitor and control the molecular reactions that take place in PCR. The L-DNA is fluorescently tagged, and provides information on the reactions taking place – a spectrophotometer detects the varying levels of fluorescence in the sample. Based on the hybridization state of the DNA, the device adjusts its thermal cycling, allowing it to adapt to variations in the reaction and compensate for errors.

The aim of the innovation is to improve the reliability of PCR and shrink the required equipment down to improve accessibility. Dubbed “adaptive PCR,” the approach removes the need for thermal calibrations and cycling programs and reduces the impact that environmental conditions have on the success of the reaction. Its small size also means it can be transferred easily from the lab to the clinic.

Reference

Lab-Grown Bone

Engineered biomaterial supports donor blood marrow

May 2017

Bone marrow transplants are not pleasant for patients. Irradiation and, in some cases, cytoreductive therapy are required to prepare the patient by killing host stem cells – a process with significant potential side effects, ranging from nausea and fatigue to a loss of fertility.

Could lab-grown bone hold the key? Researchers at the University of California San Diego have developed a

Figure 1. Engineered bone with functional marrow. Image credit: Varghese lab, UC San Diego.
synthetic biomaterial that matures into functional bone (1) with hematopoietic activity with the introduction of donor bone marrow cells (see Figure 1). Implanted beneath the skin, the biomimetic matrix provides support for the donor cells to grow without competition from host cells, so drugs and radiation dosing are not required. So far, the implant has shown promise in mice; after four weeks, the engineered bone tissue contained both host and donor blood cells – and after six months, the host/donor mix was still present in the bloodstream, indicating that the new bone marrow remains functional.

There are limitations – the implant would not be suitable for the treatment of malignant diseases that require the elimination of cancerous cells. But the authors do see its potential in treating other bone-marrow diseases, such as aplastic anemia, where there is an insufficient supply of blood cells. And they also believe the engineered bone could be used as a platform to study hematopoiesis and the impact of disease.

Reference

Here Be Dragons

Antimicrobial peptides unearthed in Komodo dragon plasma provide the inspiration for potential new antibiotics

May 2017

Komodo dragons have dirty mouths. It’s not that they swear like sailors, but rather that their saliva is teeming with pathogenic bacteria. And though dental hygiene (or lack thereof) is no longer thought to be the only source of its deadly bite (the discovery of venom glands has fueled a little-known debate in reptile research circles...), the monitor lizard does need to be capable of recovering from nasty septic wounds inflicted by competing dragons, which is thought to have led to a particularly robust immune system.

Could dragon’s blood, just like in some legends, be a source of medicine – and better understanding their role – has ramped up of late because of growing concerns over antimicrobial resistance.

Using one of the peptides they discovered as inspiration, the team then designed a synthetic peptide they dubbed DRGN-1. The compound significantly enhanced healing when testing on mice with infected wounds, by promoting the clearance of polymicrobial infection (2).

We’re some way off Komodo monitor-derived therapeutics – but, if ever a new pharmaceutical company wishes to target a certain (fantasy-board-game-playing, Game-of-Thrones-watching) subset of the population, “Dragon Drugs Inc” is a sure winner...

References
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The Extraocular Muscles in ALS: A Research Riddle

Could the preservation of the eye muscles in ALS provide a new outlook on a deadly disease?

By Anton Tjust

May 2017

Amyotrophic lateral sclerosis (ALS) is a devastating condition. An incurable neurodegenerative disease, it is characterized by a progressive loss of upper and lower motor neurons, leading to complete paralysis and eventually death through respiratory failure, usually within three to five years of symptom onset. Currently, the only treatment available is riluzole, but this drug only extends the lifespan of ALS patients by an average of two to three months.

Clinically, the disease can present itself with intriguing variability. It may manifest at any age, but often occurs in people who are middle-aged or older. Subsequent survival is also variable, with some patients surviving under a year, and a small proportion living for decades. When the patient experiences the first symptoms, they are mostly restricted to a single extremity – for example, the right hand. The disease most commonly presents itself distally with a mixture of upper and lower motor symptoms, such as weakness and loss of dexterity. This is later followed by a progression into more proximal muscles, appearance of the same symptoms in the ipsilateral leg, and eventually, involvement of all voluntary muscles in the body. In about 25 percent of the cases, the disease manifests in the bulbar region, first affecting muscles of articulation and mastication. Another variable trait in ALS is the degree of cognitive involvement. Some patients retain all of their premorbid personality and functions, and some patients develop an outright frontotemporal lobe dementia.

ALS and the eye

Despite the various ways in which ALS can present itself, nearly all ALS patients have one thing in common: you don’t encounter them in the ophthalmologist’s office. With some notable exceptions (1), the extraocular muscles are seemingly preserved in most ALS patients, even until the terminal stage. Notably, eye movements and blinking are usually the last modes of communication available to terminal ALS patients (2). But why?

That simple question presents an area of study with huge potential; understanding the underlying mechanisms for eye motility sparing in ALS could provide new insights into how the progress of ALS could be slowed down in more vulnerable muscles.

From an evolutionary perspective, extraocular muscles and their motor neurons are ancient companions that pre-date the advent of terrestrial life on Earth. Extraocular muscles are present and innervated according to a principally similar system in lampreys, whose ancestors (Cambrian cyclostomes) diverted from what would evolve into jawed fish (gnathostomata) between 460 and 535 million years ago. This implies that extraocular muscles are at least that old. The muscles of the trunk, gills and fins of Cambrian fish have since evolved into muscles of terrestrial locomotion, anti-gravity balance, breathing and grasping, but the extraocular muscles still serve (though with greater performance) the same basic function of orienting the gaze, just as they did half a billion years ago.
Exploring the mysteries of eye motility

In my recent doctoral thesis, I explored the sparing of eye motility, using histological studies of the extraocular muscles of ALS patients and the most commonly used mouse model for ALS. Although ALS is a motor neuron disease and therefore frequently studied from the perspective of the central nervous system, skeletal muscles are also important players in its progression. During embryonic development and after the initial establishment of the neuromuscular junction—the specialized synapse that forms between muscle fibers and motor neuron axons—muscle fibers provide the innervating motor neuron with neurotrophic factors, such as glial cell-derived neurotrophic factor (GDNF). The neurotrophic factors are retrogradely transported along the axon back to the nerve cell body in the central nervous system, promoting neuronal survival signaling. The relationship between muscle fibers and motor neurons is critical during embryonic development, where 30–50 percent of all motor neurons projecting to muscles in the limbs and trunk are lost to apoptosis in favor of those motor neurons that are more successful in establishing contact with many muscle fibers. In contrast, muscle fibers in the extraocular muscles appear to provide maturing motor neurons with more generous amounts of neurotrophic factors, and therefore a much larger proportion of motor neurons escape apoptosis—a fact that also explains the exceptionally small motor unit sizes (the relationship between the number of muscle fibers controlled by a single neuron) present in the extraocular muscles.

Studies have shown that deterioration of the contact between muscle fibers and motor neurons, starting at the so-called neuromuscular junction, is an early manifestation of ALS that precedes the actual loss of motor neurons. Therefore, adaptations and maladaptions that take place at the level of the neuromuscular junction could play a large role in the progression of ALS. A good example of this is the observation that when GDNF is overexpressed in the muscles of ALS animal models, it leads to a prolonged survival of the animals, whereas overexpression in glial cells located much closer to the motor neurons in the spinal cord has no such effect. Further examples of this relationship between muscle fibers and motor neurons are conditional knockout mice where ablation of satellite cells, resident stem cells of muscle tissue, leads to impairment in the re-establishment of neuromuscular junction following nerve injury (3).

Satellite studies

Satellite cells are present in all muscles of the body, and are normally in a resting

Asking the Right Questions

The relative resistance of extraocular muscles in the context of ALS, when viewed on its own, seems like a pathophysiological oddity. But if we take a broader view of the numerous degenerative diseases being studied across different areas of medical research, a very different picture emerges.

In different neurodegenerative diseases, monogenetic, dominantly inherited sub-forms of diseases have been identified, where a seemingly ubiquitous (or near-ubiquitous) gene product mysteriously exerts its pathogenic effect mainly on a specific type of cell. For example, whereas hexokinase 1, an enzyme responsible for the phosphorylation of glucose, is present in most tissues in the body, specific mutations in the gene that codes for it can lead either to a dominantly inherited retinitis pigmentosa or, in the case of another missense mutation, a recessive form of Charcot-Marie-Tooth disease. Mutations in the gene coding for superoxide dismutase 1 are responsible for approximately six percent of ALS cases. But despite the pancellular ubiquitous nature of this enzyme (with the highest concentrations actually found in the liver), the mutated form of the protein seems to primarily affect motor neurons in the CNS.

Again, in retinal disease, mutations in the gene coding for bestrophin-1 leads to vitelliform macular dystrophy, a progressive retinal disease that mostly spares the rods. Interestingly, bestrophin-1 seems to be important in chloride ion shuttling in different types of epithelia throughout the body (such as in the airways and colon), not just in the retina. Apparently, in the complex constellation of genes and proteins that sustain our cells, certain cellular processes have become more robust and tolerant to flaws in some cell types than in others. W

It is reasonable to look at any disease based on how the patient presents him or herself and ask yourself: “What is wrong with organ X in patient Y?” However, as researchers and physicians, we are looking for solutions to problems. Sometimes we should look for answers from the other end of the tube and ask ourselves, “If this cellular problem is so ubiquitous in my patient, what are cells X and Y doing right that cell Z is doing wrong?”
state. In response to training or injury, they can become activated, causing them to proliferate and generate new myonuclei for growing and regenerating muscle fibers. Satellite cells in extraocular muscles differ from other satellite cells in several regards. Compared with other satellite cells, they maintain a heightened expression of several developmental transcription factors and have been shown to proliferate and produce new myonuclei more efficiently than limb muscle satellite cells when engrafted into muscle tissue. It has also been proposed that they are more abundant, and in a more continuous state of activation, when compared with satellite cells in limb muscles (4). Interestingly, in vitro studies involving satellite cells from limb muscle biopsies of ALS patients and satellite cells from the most commonly used mouse model for ALS have shown that their growth performance in vitro is impaired compared to satellite cells derived from unaffected patients. Could satellite cells in limb muscles become worn out in a protracted disease course?

In our research group, we asked ourselves – what role might satellite cells play in the resilience of extraocular muscles to ALS? Our results (both published and as of yet unpublished) (5) (6) suggest that the previously reported abundance and continuous activation of satellite cells in extraocular muscles might have been overstated, and that only a small portion of the extraocular muscle, close to the tendon, maintains an increased pool of satellite cells. We found that the majority of the muscle belly contains generally low numbers of satellite cells, and the majority of them are not in an active state. Further analysis of extraocular muscles from ALS patients revealed similar results. Analysis of limb muscles revealed more dynamic changes, with varying numbers of satellite cells in limb muscles of different ALS patients. Importantly, however, satellite cell numbers in ALS patients typically varied between normal and high levels compared with normal elderly sedentary individuals and did not appear to wear out or decrease in numbers during a protracted disease course [Figure 1]. Rather, those muscle fibers in the most severely affected limbs that still retained contact with a motor neuron tended to increase in size together with an increase in the number of satellite cells and myonuclei associated with them.

Figure 1. Fluorescence images of a cross-sectioned extraocular muscle (left column) and a cross-sectioned limb muscle (right column) from an ALS patient. Satellite cells (white arrows) and other myogenic stem cells (white arrowheads), are identified by their molecular markers Pax7 (red) and NCAM (yellow) and presented in relation to cellular membranes (grey – top row) and to cell nuclei (blue – bottom row). It seems that satellite cells are still present in both extraocular muscles and limb muscles at the very terminal stage of the disease.
Therefore, it seems that satellite cells, while possibly affected at a level that can be demonstrated in culture conditions, are able to perform well enough in their native niche within the muscle. By extension, the distinguishing traits of satellite cells in extraocular muscles does not appear to be a key element in the sparing of eye motility in ALS.

**Making connections**

In both the animal model [Figure 2] and in ALS patients, there is a maintained presence of terminal axons at the muscle fiber endplate, in contrast to limb muscles, where large portions of the muscle fibers lose axonal contact (7)(8). Loss of axonal contact is an early manifestation of ALS, which suggests that the protective mechanisms present in the extraocular muscles are influencing disease progression at a relatively early stage. However, my preliminary, unpublished data suggest

![Figure 2. Fluorescence images of neuromuscular synapses, in one extraocular muscle (left column) and one limb muscle (right column) in an ALS mouse model. The contact between motor axon (green) and motor endplate (red) is retained in extraocular muscles (bottom left), whereas the contact is broken and disappears in limb muscles as the disease progresses (bottom right).](image)
that not all fiber types in the extraocular muscles are preserved. Slow-type muscle fibers, which in the extraocular muscles are innervated at several points along the length of the fiber rather than at a single point in the middle (as is usual for muscle fibers) appear to be affected by ALS, decreasing in size and proportion in terminal patients. We believe that eye motility is maintained not because of a general sparing of all fiber types in the extraocular muscles, but rather because of compensatory mechanisms elicited by one or several other of the specific fiber types present in the extraocular muscles.

A powerful tool

Other investigators have tried to answer the question of differential vulnerability in ALS by comparing the transcriptomes of vulnerable motor neurons with spared motor neurons. One such study (9) has revealed that oculomotor neurons natively express higher levels of several growth factors. And the factor IGF-II, seems to exert a neuroprotective effect on spinal cord motor neurons cultured in vitro, and when overexpressed through an injected viral vector [Figure 3], extends the lifespan of the most commonly used ALS mouse model by around 10 percent (10). Also, besides the presence of neurotrophic factors and other growth factors, the intense activity in oculomotor neurons has also lead to numerous evolutionary adaptations to sustain the constant activity and stresses of ion cycling that takes place with each depolarization cycle. Such adaptations include a different composition of GABA-receptors with more powerful inhibitory responses, as well as a lessened susceptibility to glutamate excitotoxicity, a mechanism that has been recognized as an important contributor to ALS pathophysiology since the early 1990s.

Future studies will hopefully answer the question of whether the sparing of eye motility in ALS is a result of the summation of many factors that happen to be beneficial in delaying the deleterious processes of ALS, or whether more specific traits present in the oculomotor neurons have the coincidental effect of delaying ALS. Nevertheless, studying selective sparing is a powerful tool for researchers in different fields, as it encourages us to learn from biological systems that already, through pre-existing tools, have a solution to the problems we face.

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References

In Perspective

The Missing Piece of the Puzzle

Patients are a central component of medical research – so why aren’t their voices always heard? As a fellow of the European Patients’ Academy, I invite you to begin a proper two-way dialog with your research participants – you may be surprised when you both learn something new...

By Marleen Kaatee

May 2017

A few years ago, medical researchers in many fields began to realize that they faced a significant knowledge gap: they didn’t know what patients actually experienced. But how could they find out? Talking to patients was an obvious solution, but most patients don’t understand how biomedical science works. There’s no common language between the laymen and the laboratory, and it’s hard for either group to determine what the other may find useful.

Though it seemed like an insurmountable obstacle at first, the European Union decided to step in. Through the Innovative Medicines Initiative and the European Federation of Pharmaceutical Industries and Associations, the EU launched a massive five-year initiative to train patient advocates in all aspects of medicine research and development. The result? EUPATI – the European Patients’ Academy.

EUPATI offers a number of services, but its most recognizable is its “school.” The Patient Expert Training Course is a 14-month program that includes both e-learning modules and face-to-face events.

After they graduate, students become EUPATI fellows and serve as resources for both patients and researchers.

When I was first diagnosed with primary sclerosing cholangitis (PSC), a rare liver disease, I couldn’t find much accessible information in my native language. I knew right away that I wanted to do more to help tackle my disease – and what I figured out is that we needed to change a lot from the patient’s perspective. Researchers and medical professionals can access academic papers and contact colleagues, but what can a recently diagnosed patient with no scientific training do? We need to be brought into the fold, so to speak, because we have a lot to learn to really understand our own diseases and the research surrounding them – but we also have a lot to teach healthcare professionals who have never experienced these diseases themselves. Collaboration is key, and if patients are to be equal partners at the negotiation table, we need to understand things at a professional level. With that in mind, I asked around, and someone told me about the Patient Expert Training Course, which was quite new at the time. I applied right away and was accepted.

Through my education, I’ve become a much better participant in my own health care, as well as an advocate for the research and treatment of others.

Expert education

The program kicks off with six online modules that students work through from home – a convenient arrangement, as learning can be scheduled around treatment and other activities! The e-learning system also includes a forum where students can ask questions. When I took the course, there were three specialists assigned to each module, so we had access to researchers, pharmaceutical representatives and patient advocates who could answer our questions 24/7. While working on the e-learning component of the course, students also have
two face-to-face sessions that encompass four consecutive full days of training with experts – mine took place in Barcelona. One especially interesting aspect was the role-play sessions; we pretended to be in a situation within the European Medical Agency’s Pharmacovigilance Risk Assessment Committee meeting; one person played the patient, another a relative, a third the regulatory representative, and so on. That kind of hands-on training gave us a really good feel for the different positions people hold and their duties within their organizations. It also made me a better participant in research groups, and it gave me the ability to anticipate and address potential areas of miscommunication before problems arise.

Getting started

In the beginning, it was a bit daunting because I wasn’t familiar with the vocabulary that the experts use (especially as I wasn’t working in my mother tongue). I think the medical field may use more abbreviations than any other! But the e-learning modules really helped me to get a grip on the things I found most difficult. They were so well-organized that I could walk myself through them one step at a time – and for patients who want to know more, every chapter includes not only a self-test, but also extra reading in case you want to learn about a topic in greater depth.

I think the format of the course really helps students feel like they’re part of a group, despite the fact that most of it is held online. Even though I was alone in my room, the modules made learning easy – and I always had the opportunity to ask questions, which was great. The forum was my favorite part of the course; I really appreciated being able to get clarification when I was confused, and I liked the fact that I could sometimes help other students who were struggling with questions of their own. In fact, I had a better experience with the online forum than I did attending university classes in person, because the commitment of the experts was obvious. Whenever I asked anything, I always got an answer within 24 hours!

A toolbox for training

EUPATI offers an excellent online toolbox (eupati.eu), which allows patients to search for any topic – and the resources are all available in seven languages, with more on the way. So even people who can’t commit to the whole training course can prepare themselves as a patient representative by going through the toolbox. Of course, for patients who do want the whole package, all of the expert training course modules are available for download – you don’t get the “group feeling” or the real-time access to experts if you go through the course yourself, but other than that you can study as much as you like on your own time.

My advice to researchers and laboratory medicine professionals is to be a little more sensitive to patients’ needs – whether experts or otherwise. You can point your patients or research participants to the training toolbox and other resources and encourage them to educate themselves. You can also make yourselves accessible to them, so that they feel comfortable coming to you with questions. You never know when you may both learn something new! Another key way to help is by treating patients and patient advocates as equal partners at the negotiation table. Even if they don’t have a pile of medical degrees, they’re certainly well-versed in their own conditions!

The most important thing is to get patients involved from day one. As a researcher, you might have a brilliant idea for something to study – but you might find that it’s not a priority for the patients themselves. By working as a team from the start, you can encourage patients to add their views to your own ideas and learn things that – as a non-patient – you’d have no other way of knowing. For instance, I once had a conversation with 17 other PSC patients; one of them mentioned that he had a milk intolerance... and all the other 16 said they had the same issue! I was telling the story to a researcher and his eyes started to twinkle. He said, “Marleen, I need to know these things. I’ve been in the lab for 30 years – and I’ve never met a PSC patient!” For me, that was pretty shocking. But it just goes to show that professionals and patients can actually help one another identify research priorities and get all of the stakeholders involved in the work.
In my experience, if you invest a little time in these kinds of activities, it will come back to you tenfold by making your collaborations and studies much easier—and often much better as well.

**The communication challenge**

There’s also great value in interacting with patients outside the research context. Many organizations have Facebook pages, which basically means that you have indirect access to those patients and their caregivers 24/7. You can see trends as they happen. For instance, the only possible treatment for PSC is a liver transplant. In the PSC groups on social media, you can read about the difficulties friends and family members face when their loved ones are on the waiting list; you see people deteriorate without having the ability to intervene. But on the other hand, when a patient gets “the call” or has an “anliversary” (marking the anniversary of a transplant), it’s a very special experience—and everyone in the group celebrates alongside them.

You also find out about the “little things” by interacting regularly with patients. To give you another PSC example, there is “the itch.” Most PSC patients suffer tremendously from itching, but there’s no way to cure it. Anti-itching medication exists, of course, but it doesn’t significantly affect the cholestatic itch. So if you are a researcher who wants to study the itch, talking to patients first will help you understand exactly which itch they want to have studied and how and why it impacts their lives. Not only will that give you more information for your work and potentially improve your chances of finding funding, but it might also add some motivation from a personal angle.

I think there’s a lot of value in talking to patients about your research—but I think there’s equal value in making your work accessible to them after it’s complete. When you publish the results of your study, you can add a “lay version” so that the participants in your trial, and the disease community at large, can also find out what you discovered. It makes everyone part of a larger community, and I think that’s very

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**Do you know a suitable patient for expert training?**

**Who?** Patients with chronic or lifelong conditions, caregivers of such patients, or employees and volunteers with patient organizations. Participants must live in the European Region, speak English, and have an interest in and a desire to be involved with medical research.

**What?** A 14-month training course consisting of six e-learning modules (250 study hours) and two five-day face-to-face meetings. The course is fully funded by IMI and EFPIA.

**Where?** The online lessons can be taken in the participant’s home. The face-to-face meetings are conducted in Barcelona.

**When?** Applications must be received by March 31, 2017. The course itself runs from September 2017 to December 2018, with face-to-face meetings in March and September 2018.

**How?** Patients can apply at: eupati.eu/third-cycle-apply-now

**Why?** “As a researcher, not having to explain what a Petri dish is, or what pharmacovigilance is, makes communication with patients much easier. It’s also more interesting for research and healthcare professionals to talk to informed patients for additional insight into the diseases they’re studying.”

“I think the biggest benefit for me as a patient is that I now know the vocabulary. I understand what the professionals are talking about. And if I have a question, I know how to find an answer.”
beneficial.

I would go as far as to say that, if you don’t have a patient expert on your team – both to help guide your work and to help you make it accessible to other patients – you’re missing a whole array of opportunities to advance medicine.

**Finding – and working with – patient experts**

The best way to begin your search for a patient expert is to consult with the patient organizations in your country – or, if there aren’t any, the international associations. For instance, for liver diseases, we have the European Association for the Study of the Liver. It always starts with finding a patient organization, because then you can contact them to ask for a patient panel; request support with writing a lay version of your research; get assistance seeking funding for your project. The opportunities are almost limitless!

A word of caution: you need to make sure you’re actually speaking with the patients themselves; sometimes, patient organizations provide people who claim to know everything about a particular disease, but don’t actually have it themselves. In the Netherlands, we call those people “office patients.” If you’re not a patient yourself, you don’t know what it’s like to have a chronic illness, no matter how much experience you have.

I always start by asking researchers, “What can I do for you?” They are always very surprised, because the old-school approach is for the patient to dictate what he or she wants, and the researchers to try to cater to it. I turn it around by asking what I can do to help them. The first thing they say most of the time is that they need money – and I say, “That’s important, but let’s not talk money right now. If we have a good enough plan, the money will come.” Then they say, for instance, that they want to do research, but don’t know where to find a patient population. That I can help with! “Did you know that I help moderate a Facebook group of 400 patients and caregivers?” Or they’ll tell me what they want to research – and it turns out to be something that isn’t a high priority for most patients. “Our research priorities are actually X, Y and Z. Here are the results of a pan-European survey we conducted in six languages.” When researchers know what’s really bothering patients – whether it’s something as obvious as transplant success rates or as subtle as needing too much sleep – it enables them to focus their work so that it has the greatest benefit for the patient community.

Of course, patients and professionals are attacking diseases from two very different angles. We can’t always expect the two groups to have identical goals, but we can encourage open communication by asking researchers and clinical professionals to make their work accessible to patients, and by asking patients to educate themselves as much as possible in how to be a useful participant in the research. If we can learn to meet in the middle and treat one another as equals, we’ll be well on our way to defeating these challenging conditions.

Marleen Kaatee is the founding President of PSC Patients Europe and a fellow of the EUPATI Patient Expert Training Course.
There is little doubt that the future of medicine is gene editing. But right now, the focus is on figuring out how to get there as safely and effectively as possible. An approach at the forefront of our gene-editing endeavors is CRISPR (clustered regularly interspaced short palindromic repeats)/Cas – and since its debut in 2013 (1)(2)(3), research has been booming. Although CRISPR/Cas9 shows much promise therapeutically, improvements and tailored modifications are needed before it hits the clinic. For example, the Cas9 endonuclease – which binds and cuts DNA at specific locations as dictated by a short guiding RNA (sgRNA) sequence (Figure 1) – could benefit from optimization; the most commonly used orthologue (derived from Streptococcus pyogenes – SpCas9) weighs in at a mighty 4.10 kbp and 1,368 amino acids, and is too big to be packaged into a single adeno-associated virus (AAV) vector along with its sgRNA sequence. It can be split over more than one AAV vector, but this can come at the cost of reduced endonuclease activity (4). One alternative is Cas9 from Staphylococcus aureus (SaCas9); it’s around 1 kbp smaller than SpCas9 and can be packaged into a single AAV vector. Unfortunately, the number of targetable genes is predicted to be limited by a much less frequently occurring protospacer-adjacent motif (PAM) – a 2–6 base pair DNA sequence that acts as an essential targeting component of the system.

Given the apparent lack of “good” choice, a team in South Korea decided to seek alternative Cas9 orthologues – with promising results. In their recently published study (5) they characterized Cas9 from Campylobacter jejuni (CjCas9), demonstrated its use for in vitro and in vivo gene editing, and showed that CjCas9 targeted to Vegfa or Hif1a could reduce choroidal neovascularization (CNV) in a mouse model of age-related macular degeneration (AMD; See Box – Summary of Key Results). Seokjoong Kim, Research Director of Toolgen, and Sung Wook Park, one of the lead authors on the paper, tell us more.

What inspired your study?

Seokjoong Kim: I’m a molecular biologist, and over the last 10 years I’ve been working to develop gene editing tools such as transcription activator-like effector nuclease (TALENs) and CRISPR. When we were ready to move into the clinical translation of CRISPR technology, I found AAV very attractive because it has been clinically proven to deliver genes very efficiently and safely. I wanted to combine a CRISPR/Cas9 system with AAV, but I quickly found out that the typical Cas9 system from Streptococcus pyogenes is simply too big. We started to use Cas9 systems from different species, but decided to focus on CjCas9 because it was the smallest we could find in the literature and databases. In collaboration with The Institute for Basic Science in Seoul, we then performed a full characterization of CjCas9, including its PAM sequence and the optimal size of the sgRNA. We showed that we were able to support efficient gene editing in vitro and in vivo with CjCas9. Our initial trial was in muscle, but we
**Summary of Key Results**

- At 2.95 kbp and 984 amino acid residues, CjCas9 is around 30 percent smaller than SpCas9

- Cleaving of human genomic DNA in vitro by CjCas9 was found to be more specific than SaCas9 with no reduction in efficiency

- In vivo, CjCas9 was shown to induce targeted mutations in three genes: – In mouse myotubes, CjCas9 induced targeted mutations at the Rosa26 locus. – In mouse retina, targeted mutations were induced in Rosa26, Vegfa and Hif1a in retinal pigment epithelium (RPE) cells

- In a laser induced CNV mouse model, the team found that targeting Vegfa and Hif1a each reduced the relative CNV area by over 20 percent (see Figure 2). Hif1a encodes hypoxia-inducible factor alpha (HIF-1α), a transcription factor that activates the transcription of VEGF-A.

- To investigate the potential side effects of the partial knockouts of Vegfa and Hif1a in RPE cells, cone function was measured by full-field electroretinography. Photopic and flicker response were not significantly decreased, but the size of the opsin-positive area was reduced when Vegfa was targeted.

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What are your most important findings?

**SK:** We are pleased that we have been able to develop the Campylobacter jejuni CRISPR-Cas9 system and show that AAV-mediated delivery can support very efficient gene editing in the eye – we saw editing efficiencies of 30–60 percent. Fortunately, the dose efficiency of gene editing was enough to show some phenotypic changes, and we were happy to see that targeting Vegfa and Hif1a in the eye with CRISPR gene editing could change the phenotype in a mouse model.
Mice injected with \textit{AAV-CjCas9} targeted to Rosa26, Vegfa or Hif1a were treated at day 42 post-injection to induce CNV; one week later they were analyzed. 

\textbf{a.} Representative images showing the area of laser-induced CNV (stained with isolectin B4). Scale bar, 200 mm. 

\textbf{b.} Graph showing the relative CNV area. Error bars indicate SEM. (n=17–18). One-way ANOVA and Tukey's post hoc tests, *P<0.05; **P<0.01; ***P<0.001; NS, not significant; SEM, standard error of the mean. Adapted from (5).
of laser-induced CNV.

**SWP:** The delivery method is really quite different. Most gene therapy relies on AAV2, which is typically delivered through subretinal injection, which inevitably causes damage to the retinal layer. We had already experience in using AAV9 for targeting retinal pigment epithelium (RPE) cells, and we delivered the CjCas9 AAV9 viral vectors via intravitreal injection. We believe intravitreal injection is a much better delivery method because we limit damage to the retinal neurons. Also, simply injecting the viral vector into the vitreal cavity can increase efficiency because more cells in the retina or RPE can be transduced and edited.

**Any surprises or challenges along the way?**

**SK:** When I initially chose the study, I thought that the production of AAV would be well established because there had been so many trials – so I was surprised at how inefficient and expensive it is, even at the research level! With the ongoing work on AAV-mediated gene therapy in the US and Europe, I hope that in the near future we will have more established and optimized protocols and processes for AAV production.

**SWP:** It isn’t proven in well-established studies, but there is some evidence that targeting the Vegfa gene can cause problems, so we needed to check the side effects. There was some local opsin decrease but there were no constant functional decreases in photoreceptor response. So at the present time, we believe that targeting Vegfa is still a viable option.

**When do you expect to move into human trials?**

**SK:** It’s hard to say! We believe we can perform some large animal studies within this year – and we hope that we’ll be able to prepare enough data to file an investigational new drug (IND) application in 2018. One thing for us to consider is the fact that we are currently targeting Vegfa and Hif1α – but these are not actually defective genes in AMD and DR. We do believe that targeting these genes – and others that we are working on – could be a viable option for long-term AMD or DR therapy, but we’re not sure if regulatory bodies will be comfortable with targeting non-defective genes with CRISPR. Therefore, we are also interested in targeting other genetic diseases in the eye that might be more easily accepted by regulatory bodies and society.

**SWP:** We also have to consider the hurdles to overcome before getting to trials. As well as the necessary larger animal and safety trials, we need to find an effective human sequence and prove that it can be edited with the CRISPR-CjCas9 system. Hif1α is quite conserved between the mouse and human genomes, so we are thinking of targeting this in clinical trials. Additionally, because anti-VEGF inhibits VEGF therapy is a well-established treatment regimen for AMD and DR, we’ll need to demonstrate how effective CRISPR is compared with existing therapies. These diseases usually wax and wane over time, so multiple repeat injections of anti-VEGF agents are needed; we hope that our genome editing approach might be able to downregulate VEGF and other factors to below the threshold level that causes disease.

**Where do you hope your work will take you?**

**SWP:** Using the advantage of the small size of CjCas9, we might be able to think about combinational therapy approaches where we target dual genes with a single AAV system. We haven’t tried this yet, but we are looking into the possibility of different approaches.

**SK:** There is no limit to the genes you can edit with the CRISPR-Cas9 system. VEGF is a key target for modulating vascularization, firstly because it is one of the very important factors in the process, but also because it can be inhibited extracellularly by current therapy models like monoclonal antibodies. We’re also looking at genes for intracellular or nuclear proteins that cannot be easily modulated by antibodies or small molecules. Looking forward, we want our technology to bring new hope to patients who are losing sight.

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**References**

Cracking Cancer’s Code

Sitting Down With… George Calin, Professor, Department of Experimental Therapeutics, and Co-Director, The RNA Interference and non-coding RNA Center, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, USA.

May 2017

How did you get into studying the role of RNA in cancer?

It was entirely down to chance. I was born in Romania, which at that time was a communist country. The study of genetics was frowned upon, but I was lucky to have thegeneticist Dragos Stefanescu as a mentor; he taught me cytogenetics but also how to do good science. I wanted to study abroad, so I wrote 120 letters to 120 scientists all over the world – and ended up moving to Italy to Massimo Negrini’s laboratory, where I studied molecular biology and molecular genetics before moving to the United States. I was at Kimmel Cancer Center in Philadelphia with the famous geneticist Carlo Croce, when we discovered a link between microRNAs and human cancer. It was the first time a link had been made between any type of non-coding RNA (ncRNA) and a human disease. But the initial paper was rejected by a famous journal in just 12 hours. Attached was a simple note saying that our discovery was random, and because it wasn’t proven that microRNAs were important in cancer, our work would be better suited to a smaller journal. Today, that paper has probably been cited more than 4,000 times, and there are over 30,000 papers in PubMed on microRNAs or long ncRNAs and cancer. That discovery opened the door, both for me and for many other scientists. But much of it was actually luck.

In 1992, when I was a young student in Romania, the first scientist who helped me write a research essay was Thomas Cech, who received a Nobel Prize for his work with RNA and his discovery of ribozymes. It’s funny to think that now, 25 years later, I am working in the field that Thomas popularized: the role of RNAs in biology, and in pathology in general.

How did you find success as a translational researcher?

I don’t consider myself successful – yet. I don’t think I can consider my work successful until we see a drop in cancer mortality, either through an ncRNA therapeutic or through somehow targeting ncRNAs.

My collaborators and I have focused a lot of effort on developing microRNA therapeutics but, until now, we’ve only seen limited trials. I think the next 5–10 years will be a time in answering the question of whether these new types of drugs (or potentially drug targets) can be used in therapeutics, or for the development of biomarkers. Clever ideas are great, but we have to use them to deliver a meaningful change to the patient, either through better diagnoses or therapeutics.

Gleevec is a good example of the power of science and medicine working together to turn around the natural history of a deadly cancer. Chronic myeloid leukemia before Gleevec was fatal. Now, there are patients surviving for a very long time. And that’s what I hope to achieve using microRNAs or anti-microRNAs, whether in cancer or other diseases.
What advice would you give to other researchers aiming for the clinic?

That’s simple – never give up! If you’re a fundamental scientist, collaborate with clinicians, and clinician-scientists, and experts on drug delivery, statisticians, and bioinformaticians, because that’s how you get a strong team. You need a big team and you need to put in a lot of passionate work – several years, if not decades.

Also, you often need to work hard to find funding, because it’s always limited, as there are always more ideas than there is money to go around. Other than that, your path may be very different depending on your institution, and country, and so on. But I would urge all scientists to consider how they might be able to apply their discoveries to patients from the earlier phases of their research!

You aim to produce the first complete catalog of human ncRNAs. How close are you?

Day by day, I think I am getting further away! The genome is complex, and there have been many surprises. A colleague at Thomas Jefferson, Isidore Rigoutsos, and I recently had a paper accepted on the topic of human- and primate-specific ncRNAs that have no homology with other species. Essentially, these transcripts represent a fingerprint of humanity, which could have big implications for research – many scientists, including me, spend a lot of time and money using mouse models of human cancers. But as geneticists, we know that mouse models don’t reproduce human cancers very well. Without a deep and meticulous understanding of the genome, and how it functions, I don’t believe we will make big advances in developing new biomarkers and therapeutics for cancer. I hope our new ncRNA research will help to bridge that gap. We are heading in the right direction, but it’s going to take a long time. Luckily, the journey is fascinating and full of unexpected turns, which is great – it keeps life interesting!

When you look back at your career, would you do anything differently?

I don’t think there’s anything I would change. My path has led me to one of the biggest cancer centers in the world, where I’m lucky to have great collaborators and colleagues. But so much of my success is down to other people that I cannot thank them enough. It’s important to thank my mentors, because without them I wouldn’t be where I am. We should all thank our fellows more as well – no matter how clever you are, how good your ideas are, or how much funding you bring, you are in debt to the people in your lab who work to make your ideas a reality. So I’d like to thank both those who came before and those who came after for making my work possible.
Sitting Down With

Cracking Cancer’s Code

Sitting Down With... George Calin, Professor, Department of Experimental Therapeutics, and Co-Director, The RNA Interference and non-coding RNA Center, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, USA.

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In the developed world, extreme prematurity is the leading cause of death in neonates (1). And for those who do survive, complications related to organ immaturity are common. Some of these problems may be directly caused by the move from the womb to the outside world – for example, breathing gas instead of liquid is related to issues with lung development (2).

New approaches are needed to give premature infants a better start in life – and a host of problems could be addressed by returning infants to the womb – or, more accurately, a womb-like environment. We spoke to Alan Flake, a fetal surgeon at the Center for Fetal Diagnosis and Treatment at Children’s Hospital of Philadelphia, who is developing an extra-uterine support device (3) that has so far seen success with fetal lambs (see Figure 1).

How did you become involved with the project?

I am a fetal surgeon, and my research career has been dedicated to developing new treatments for the fetus. The extreme premature infant is simply a fetus that is born too soon. My fellow, Emily Partridge, wanted to try this strategy and I agreed as it was in line with the goals of my laboratory.

Where does the idea for a womb-like device stem from?

It’s actually a very old idea; investigators have been trying to do this for 60 years but none have succeeded. If you view the premature infant as a normal fetus that is delivered early, then finding a way to extend gestation by building a device that replicates the environment of the womb is an obvious approach.

What difficulties were associated with the creation of a womb-like environment?

Many! But whenever we have encountered an obstacle, we have solved it by turning to normal fetal physiology for inspiration. For instance, the key to obtaining a sterile fluid environment was to develop a closed system that exchanged amniotic fluid (like the womb).

To achieve normal fetal circulation, we also had to solve the problems associated with using the umbilical cord, such as vascular spasm. Only by using the cord as the vascular interface can you achieve normal placental blood flow, which allowed us to maintain normal fetal delivery, with normal fetal oxygen saturations. The next obstacles we’ll need to overcome will be regulatory.

When do you anticipate the first human studies?

We are initiating a pre-clinical animal trial that the FDA has assisted in designing. Once this is complete we can apply for an IDE (Investigational Device Exemption) from the FDA for first in human studies. We are also designing a clinical device to initiate these studies. We anticipate that we will be able to move forward to our first human studies in two to three years.

References
