Dear friends of RIRC,

As we pass Thanksgiving Weekend and move into December, the Covid pandemic rages on, and RIRC operations, like all others, continue to be impacted. There have been so many issues and challenges this year that I've failed to adequately communicate to all of you. We've posted some news on our website, but under normal circumstances, you would be receiving the Fall Newsletter right now. This will be delayed I'm afraid. We'll get material to our wonderful friends, Rob and Janet Johann, so they can put together a proper newsletter soon.

In the meantime, I’m writing this 2020 update, which I'll post on our website, about our activities and progress as we come to the end of what will be an unforgettable year for sure!

Since we don't have to fit this into a defined number of pages in a newsletter pdf, this will be a bit less carefully edited, more "chatty" perhaps, and will include both the usual newsletter items (update on research) and also some more human interest items. In addition to putting this on our website, we'll send out some of the individual articles in Facebook posts.

RIRC research during the Pandemic

The first fantastic news is that RIRC has been able to continue our spinal cord injury research uninterrupted even during March-May when almost all of the rest of UCI's research was completely shut down. The reason we were allowed to continue is that our research was considered to be critical by university officials, in the same high priority category as research on Covid. We are very grateful for that because it recognizes that even in the midst of a horrible pandemic, other disorders are still recognized as important. The "critical research" designation also allowed us to complete some very exciting long-term research projects with spinal cord injured animals, which would otherwise have been interrupted, and would have had to re-start from the beginning.

Although we were able to complete ongoing research projects, we were not allowed to start any new ones during the time of the complete shut-down in March-June (termed Stage I). The university was allowed to move to Stage II in mid-June, allowing a re-start of research more generally, but we did have to limit the number of staff on site to a level of 30% of what occupancy would normally be. We're still in Stage II in December as the case numbers surge, and we're hoping that we won't have to return to Stage I limits.

Limiting occupancy to 30% has definitely been challenging, but we've been able to keep everyone working by rotating schedules and creating ways for work to be done remotely. Kelly Matsudaira even set up a histology lab in her family room at home (picture below). I'm not sure how happy Kelly’s husband Richard was about this. But doing steps in histology processing in PJ bottoms and slippers is certainly more efficient (no hazardous chemicals of course). Many thanks Kelly and Richard!

Fortunately, due to everyone’s amazing dedication and cooperation with crazy scheduling, we've been able to complete ongoing studies and launch several new ones. So, as we move into December following Thanksgiving weekend, we are thankful and optimistic for the future.
It’s been hard on our mental health that we haven’t been able to connect with everyone in the lab every day in person. It’s amazing how much we all miss this, as we carefully manage our schedules to avoid over-crowding and “physically distance” to comply with university guidelines. But we have learned how to read each-other’s eyes. Okay, enough whining--we’re doing well and managing to continue exciting projects.

Those of us who are able-bodied have experienced inconveniences and isolation from friends and family due to Covid, but we know that those of you living with SCI experience this constantly because of the challenges of getting out and about even under normal circumstances. We hope all of you are keeping safe, even though this means even more isolation. Please know, our hearts go out to all of you, if only virtually, and we really miss connecting with you in person!

Thanksgiving without the Turkey Trot

We woke up on Thanksgiving morning with the feeling that we had over-slept. Looked around, and then said "Oh..." On so many prior Thanksgivings, we were up, dressed warmly, and heading to Yorba Linda before 6:00AM to meet up with the wonderful Plymouth Rock and Run team to join thousands of runners, many in costume, waiting for the start of the races. Last year was incredible, with everyone trying to stay dry because of the rain. There were lots of large garbage bags re-purposed as rain gear! Thanksgiving morning 2020 felt incomplete for sure—quiet but not the usual fun. And we haven’t seen Fran Lopes in person since spring! I hope all of you out there who are able actually did something Thanksgiving morning before celebrating with Thanksgiving dinner. Hopefully, by Thanksgiving 2021, the pandemic will have resolved and the wonderful Plymouth Rock & Run Turkey Trot will again be possible.

Zoomentia (I've become a Zoombie)

Many people think that because we’re working remotely and not in the lab or office as much as usual, we must have more free time. Nothing could be further from the truth! I am on Zoom for hours almost every day. There are some positives; I don’t have to wear anything dressy below the waist; I don’t have to fly to Washington DC 3-4 times each year; and we can put meetings together involving participants in the next room or anywhere in the world. One thing we’ve been doing is inviting scientists from other institutions to our lab meetings, which has been a lot of fun for all.

The downside is Zoom fatigue, which is a real thing. I can attend in-person meetings and conferences for 8-10 hours non-stop but a few hours on Zoom, and I’m a Zombie (or Zoombie). I’ve started calling the state of mind “Zoomentia” (Zoom-induced dementia), because I definitely feel demented at the end of these calls.
CIRM Funding renewed through passage of Proposition 14

Great news for California! Proposition 14, which provides $5.5 billion for regenerative medicine research, was approved by the voters! This funding will allow the California Institute for Regenerative Medicine (CIRM) to continue operating for another decade or more. Of note, $1.5 billion, which is half of the total amount provided in the original Proposition 71, will be dedicated to research on diseases and disorders of the nervous system. In addition to the research on stem cells, which was the focus of Proposition 71, Proposition 14 includes approaches involving genetic modifications and other technologies under the umbrella of "regenerative medicine". It's expected that calls for grant proposals will be issued as early as January.

And yes, our research using viral vectors to deliver gene-modifying cargoes is the type of research that falls under the regenerative medicine umbrella.

RIRC Research News

Much of our research over the year has been focused on further development of viral vector technologies to deliver gene-modifying cargoes to enable axon regeneration after spinal cord injury. In consideration of eventual therapeutic application, we've continued to assess how knocking down PTEN for long periods of time affects nervous system function. The reason is that vector-based strategies to knock down PTEN to enable regeneration aren't like drugs that you can stop taking; the vectors remain active for months and the gene cargoes they carry continue to be expressed.

The good news is that we're continuing to find that long-term vector-mediated knockdown of PTEN in the way that enables regeneration doesn't have major negative consequences. In early 2020, we published a major paper in the scientific journal Experimental Neurology in which we show that deleting PTEN in the cortex doesn't cause any abnormal brain activity such as seizures (https://doi.org/10.1016/j.expneurol.2019.113098). We did find that some intracellular signaling pathways involved in synaptic plasticity were altered in neurons lacking PTEN. This may mean that functional benefits of regeneration due to PTEN knockout might be enhanced by restoring PTEN expression after regeneration has been achieved.

Another major paper that has just been accepted in the scientific journal Cerebral Cortex is our first study using the amazing new technology of retro-AAV. This paper is the first report in a scientific journal of what we told you about in our fall newsletter last year (Anatomy 101, Spinal connections Number 32). The main take-home is that injections of retro-AAV into the spinal cord lead to the delivery of gene-modifying cargoes to the majority of nerve cells in the cortex that give rise to the corticospinal tract. We're just completing several studies to test whether this approach leads to even more recovery than is seen with deletion of PTEN by injecting AAV into the cerebral cortex.

Finally, we are continuing to focus a major part of our effort on studies of possible therapies for chronic spinal cord injury, what we call the Chronic Injury Project. One example is testing whether targeting PTEN can induce regeneration and recovery in the chronic injury setting. As you can imagine, these studies take a long time to complete and are very expensive because the first step is to create spinal cord injuries in rats and then maintain the animals for months until the injuries are chronic. It's very difficult to get funding from NIH for such long-term studies because the work goes slowly and grant reviewers like experiments that get quick results. We are extremely grateful to Bob Yant and his company, Cure Medical, for their extremely generous donations to support the Chronic Injury Project.
**Dr. Mariajose Metcalfe receives NIH award**

We’re delighted to announce that Dr. Mariajose Metcalfe has received a new two year NIH award for a novel combinatorial strategy using viral vectors to enable regeneration after SCI. As many of you know, there are two major factors that prevent regeneration after SCI: 1) Lack of ability of adult CNS neurons to grow (intrinsic); 2) The inhibitory barriers at the injury site (extrinsic). PTEN inhibition addresses the first, enabling substantial growth of mature neurons, but growth across a lesion site is still impeded. One well-known growth-blocking molecule is chondroitin sulfate proteoglycan (CSPG), which is expressed by reactive astrocytes that well off the lesion site.

Mariajose’s project will take a novel approach that combines our viral vector strategy to knock down PTEN, which enables neurons to grow with a second viral vector-based strategy to express an enzyme called chondroitinase (ChASE), which degrades CSPG. For more details on the science, see Anatomy 101 below.

**Jennifer Yonan receives NIH pre-doctoral fellowship**

Congratulations to Jen Yonan for being awarded an NIH pre-doctoral fellowship for her dissertation research on induction of growth of mature neurons due to PTEN deletion. Jen wrote the proposal in late 2019 and funding started in April 2020. This will cover her fellowship and provide some direct support for her research.

Jen also presented her work at the semi-annual Cold Spring Harbor Meeting on Synapses and Circuits in September.

At our annual "Grad Day" for the Department of Anatomy & Neurobiology on December 2, Jen placed second in the competition for "best presentation". She also received an award for "best question" asked of another presenter. Way to go Jen!
Os Steward receives NINDS Landis Award for Outstanding Mentorship

I’m thrilled and honored to let everyone know that I received a very unique award from The National Institute for Neurological Disorders and Stroke (NINDS), one of the institutes comprising NIH. https://start.emailopen.com/public1/viewlpauto.aspx?id1=cryptz2%3aMadP89fKbcCeBhgl4eLDMVA%3d%3d&id2=1123&id3=221153&id4=&id5=0e06ec61789641a9b3b0d72029bdea900yntrDquj0VWMUf.400752754%40emailopen.com

The Landis Award, named after previous NINDS Director Story Landis, isn’t a grant for research; instead, it is to recognize individuals who have been outstanding mentors over their career. The award is a huge honor, and includes adding $100,000 to an ongoing grant, which can be used to support future trainees. This will be a huge boost to what can be accomplished with the grant.

The Landis Mentorship Award is given annually, but for different career levels on a rotating basis (senior, intermediate, and junior investigator), so eligibility for nomination of people at each level happens only once every 3 years. The Award is given based on nominations and letters of support from previous trainees. I was hugely honored to be nominated and grateful to my current and previous trainees who were responsible for my nomination and wrote letters of support.

RIRC trainees accepted to graduate and medical school

The technical staff at RIRC is anchored by incredibly skilled people like Kelly Matsudaira, Jamie Mizufuka (now Jamie Dam) and Ardi Gunawan, but also includes people who have recently graduated from college and are taking a gap year to get additional research experience before applying to graduate or medical school. Working with us, they expand their technical skills but also play a major role in our research productivity.

Kiara Quinn        Anuradha Gore

In August 2020, Kiara Quinn, who had been responsible for testing spinal cord injured mice and rats over the year since graduating from UCLA, started in the PhD program in Biomedical Engineering at Johns Hopkins University. This this is one of the top programs in the country. Anuradha Gore, who had been working with Kelly on neurohistology over the year, started in medical school at the University of Iowa, also a top medical school in the country. Hearty congratulations to Kiara and Anuradha, and thank you for your dedicated work at RIRC!
We’re delighted to have recruited two new technician trainees to pick up where Kiara and Anuradha left off. Daisy Gallardo has joined our staff and will work on neurohistology with Kelly Matsudaira. Daisy is applying to graduate school to pursue a PhD in Neuroscience and we are hoping that she will be accepted into the PhD program at UCI. Kevin Sanchez has joined us to help with the live animal studies under the supervision of Marijose Metcalfe. Kevin plans to apply to PhD programs next year. Welcome Daisy and Kevin!

New Comprehensive Clinical Spine Program at UCI Medical Center

In 2019, Dr. Michael Oh, a highly-respected spine neurosurgeon from the University of Pittsburgh, joined our Department of Neurosurgery. One of his goals is to develop an infrastructure on the clinical side for a new Comprehensive Spine Center at UCI. This Center will coordinate all of the different specialties involved in the treatment of people with disorders of the spine, and who have suffered a spinal cord injury. In addition to creating an even higher level of care, the new center will provide the infrastructure for expanded human subjects research and new clinical trials to test promising therapies.

Dr. Oh brought together key stakeholders including Os Steward, Dr. Nitin Bhatia (Chair, Department of Orthopaedics) and Dr. Frank Hsu (Chair, Department of Neurosurgery) to develop the formal proposal, which will be presented to the leadership of UCI Health and the School of Medicine with the goal of obtaining 5 year strategic funding for program coordination, public outreach and education.

Os Steward elected as incoming President of the Society for Neuroscience

Another honor for Os this year was to be elected as incoming President of the Society for Neuroscience (SFN). This is our field's main professional society, with membership over 35,000, which encompasses the entire field of neuroscience. Os will serve on the SFN Council and Executive Committee as President Elect, then will serve one year as President and then one year as Past President.
In Memorium

It is with great sadness that we report that Madeline Swinden, wife of Jim Swinden, passed away on June 12, 2020. Madeline's obituary, published in the LA times, is here: https://www.legacy.com/obituaries/latimes/obituary.aspx?n=madeline-martin-swinden&pid=196473021

Jim and Madeline have been friends of RIRC since the very beginning, lending their support in multiple ways, including being directly involved in the founding of RIRC. Soon after Christopher Reeve suffered a spinal cord injury in a horseback riding accident 1995, Jim Swinden saw a Barbara Walters interview with Chris, and was so moved that he immediately told his mother, Joan Irvine Smith about it. Joan and Jim, both being equestrians, connected with the story, and Joan was particularly touched by the fact that Christopher never blamed his horse. This was the initial step that led Joan to work with the University to establish a new research center at UCI that would focus on spinal cord injury research. To make this happen, Joan and the Joan Irvine Smith & Athalie R. Clarke Foundation made a challenge gift of $1 million, and then Joan spent weeks working to contact Christopher Reeve to convince him allow his name to be associated with the Center.

Madeline was very active as a volunteer leader in many other important programs at UCI. For many years, she served as President and the driving force of the School of Medicine Research Associates. She also served as a member of the Susan Samuela Integrated Health Institute Board of Advisors and as a member of the UCI School of Medicine Department of Neurology Advisory Board.
After a spinal cord injury (SCI), complex pathological interactions lead to a cascade of secondary damage that results in the blockade of axonal growth. Our work and that of other labs establishes that regeneration can be induced by targeting intrinsic growth capacity but regeneration is still impeded by the growth-hostile environment of the damaged spinal cord. Several critical molecules that impede axon regeneration in the injury territory have been identified, but one of the most important is a molecule called chondroitin sulfate proteoglycan (CSPG) produced by reactive astrocytes at the injury site, which creates an especially powerful stop signal for growing axons. In addition to blocking regeneration, CSPG also forms an extracellular structure called the peri-neuronal net, which stabilizes synaptic connections in the mature brain and spinal cord. The peri-neuronal net forms a scaffold around synapses sort of cementing them in place; this can impede formation of new connections by regenerating axons. Based on these properties of CSPGs, scientists believe that blocking or degrading CSPGs may be a good strategy for addressing the extrinsic factors that impede regeneration at the injury site and impede formation of replacement connections once axons get beyond the injury.

Previous studies injected the bacterial enzyme chondroitinase ABC (ChASE) to break down CSPGs and have shown enhanced axonal regeneration and neuroplasticity in vivo, and functional recovery following spinal cord injury in rats. Some studies have injected the enzyme directly into the nervous system, but this strategy has the disadvantage that the enzyme doesn’t remain active for very long and is degraded over the course of days.

Another approach has been to inject a viral vector that carries a gene cassette that expresses ChASE. For example, Dr. Elizabeth Bradbury and colleagues at Kings College London have used a lentiviral vector (derived from the virus that causes HIV) to deliver a mammalian-compatible ChASE gene to the spinal cord. When this vector is taken up by cells at the injury site, the cells express and release ChASE over a long time period. One disadvantage is that lentiviruses induce innate and adaptive immune responses that could limit the effectiveness of the transgene.

Adeno-associated virus (AAV) vectors are derived from a non-pathogenic replication deficient parvovirus that have the ability to transduce nerve cells and present low innate immunity (see Anatomy 101, Spinal Connections 31 for the basic biology of AAV vectors). Our studies using AAV/shPTEN in rats and retro-AAV/Cre in mice show that AAVs have robust transduction efficiency in the brain that persists for months without any apparent negative consequences. This vector system is an ideal platform to express ChASE in the spinal cord as a combinatorial approach with retrogradely transported-AAV against shPTEN (retro-AAV/shPTEN).
We’ve previously addressed the importance of targeting neuron-intrinsic mechanisms by knocking down PTEN, showing regeneration and enhanced recovery of skilled motor function. However, all studies involving interventions to enhance intrinsic growth capacity indicate that the injury continues to be a huge barrier to regenerating axons. Accordingly, a combinatorial strategy that activates intrinsic mechanisms and also targets the growth inhibitory molecules at the injury site is an obvious next step. This combinatorial approach is possible because our technique to deleting PTEN (and thus activating growth) uses retrogradely transported AAVs (retro-AAVs), which are injected into the spinal cord near the injury site. This is of course exactly the same area to be targeted by interventions to reduce growth inhibitory molecules.

Building on this extensive prior literature, the funded project will test an innovative combinatorial approach to simultaneously target intrinsic and extrinsic factors that impede axonal growth after spinal cord injury. To assess this, we will test two sets of AAVs injected above and below the injury. One of the AAVs will knock-down PTEN (retro-AAVshPTEN), that targets intrinsic factors promoting regeneration of cut axons; and the other AAV will express ChASE (AAV-ChASE), that targets CSPGs that contribute to the growth-hostile environment present after SCI and promote sprouting of spared axons. A gene therapy method of transgene delivery with injections performed under the same procedure, where host cells themselves express the transgene, bypasses the need for repeated, invasive administration of treatment.

People often ask whether we collaborate with other scientists. The answer is “yes” and this is a perfect example. Dr. Liz Muir from the University of Cambridge, who created a version of ChASE that could be expressed by human cells, generously provided us with the DNA that expresses ChASE that we used to engineer a new AAV/ChASE vector. Dr. Muir and Dr. Bradbury have also been very generous with technical advice to help us get the approach going. Thank you to both Dr. Muir and Bradbury!

*Giving in the year of Covid*

Finally, I come to giving. I'm actually writing this on Giving Tuesday. Every year I emphasize how donations to RIRC are so important, and this is even more true this year because our most important fundraiser (Turkey Trot) was cancelled. However, there's also an enormous need to help people in need due to job loss, and so many other things resulting from the pandemic.

This year, Steward family giving will try to do our part to help others in need. At the same time, people living with spinal cord injury are also in need of new treatments and cures. Many of you have been amazingly generous in supporting particular research projects, but we also need "un-restricted" donations to allow totally innovative ideas to be tested.

So, after you've considered contributing to help those who have been impacted by Covid, if you have additional capacity, please consider giving to RIRC research programs.