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RNA TRANSLATED

UNIVERSITY OF MICHIGAN CENTER FOR RNA BIOMEDICINE



CENTER FOR
RNA BIOMEDICINE
UNIVERSITY OF MICHIGAN

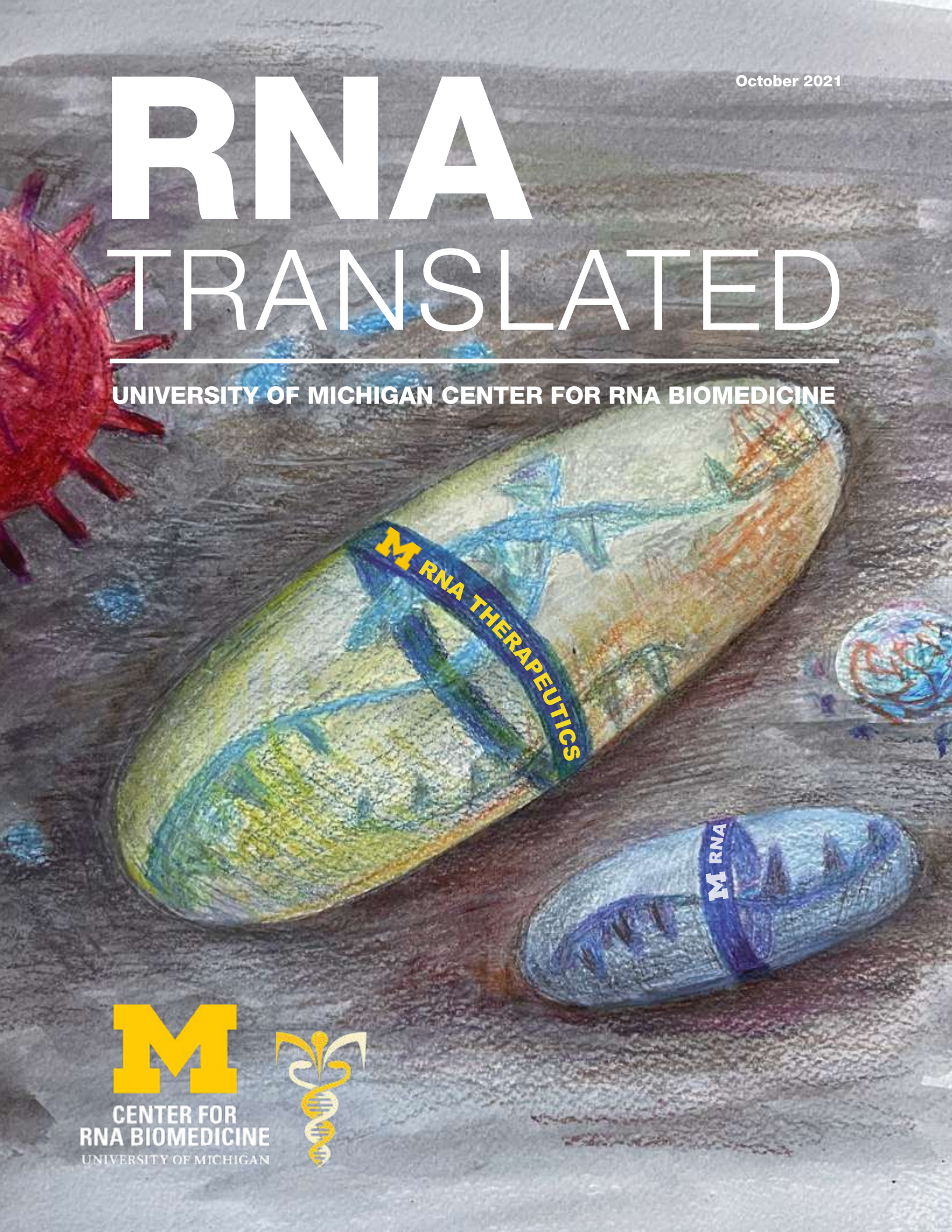




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U-M CENTER FOR RNA BIOMEDICINE’S MISSION AND LEADERSHIP

The University of Michigan Center for RNA Biomedicine seeks to:

- Promote and develop cross-disciplinary collaborations on RNA across campus.
- Mentor the next diverse generation of RNA scientists in an equitable and inclusive way.
- Enrich the U-M's intellectual and training environment around RNA biomedicine.
- Leverage and promote the strengths of the U-M RNA community, ranging from single cell and single molecule biophysics to RNA therapeutics, and across RNA mediated diseases such as cancer, neurodegeneration, and viral infection.
- Provide a central organizational structure to help recruit and develop common resources, including collaborative research grants and shared equipment, as well as domestic and international researchers.

Strategic Advisory Board

The Strategic Advisory Board consists of U-M leaders from four different colleges and schools. This Board makes strategic recommendations about the Center’s goals and orientations.

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The Executive Committee consists of eight U-M faculty from the College of LSA, the Medical School and the School of Public Health. This committee supports the implementation of the mission of the Center.

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Molecular, Cellular, and Developmental Biology, College of LSA

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From the Co-Directors



Mats Ljungman, Ph.D., Professor of Radiation Oncology and of Environmental Health, Medical School

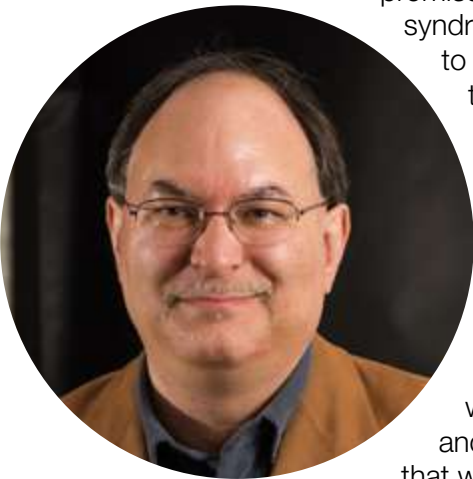
We are thrilled to present the 2021 issue of *RNA Translated*. Last year’s theme was “The Year of the RNA Virus” and it covered the innovative efforts of U-M researchers to study the SARS-CoV-2 virus and its infection cycle to come up with novel treatment options. We alluded to the clinical trials that had begun with a variety of vaccine approaches and the hope was that these vaccines could become the way out of the pandemic. As it turned out, the most effective approach to defeat the RNA virus is to use an RNA vaccine!

To celebrate these successes of RNA therapeutics to protect human lives across the globe, we are taking a closer look at the tremendous potential of RNA therapeutics to impact human health. In this issue of *RNA Translated*, you will learn about clinicians and scientists at U-M who use antisense oligonucleotides (ASOs) to treat spinal muscular atrophy (SMA) and Dravet syndrome, and researchers who explore the potential of small molecules to target microRNAs (miRNAs) that cause cancers. We also highlight efforts by U-M researchers to utilize CRISPR technology for precision targeting to knock-out or restore a gene function as well as using CRISPR to specifically kill cancer cells.

Although the CRISPR field is very young, it is bursting with excitement and promise that it will soon be used on a large scale to cure many genetic human syndromes. The Nobel Prize in Chemistry in October 2020 was awarded to Emmanuelle Charpentier and Jennifer Doudna for their elucidation of the CRISPR system and for their insight into harnessing this system for precision gene editing. A watershed study published just this summer reported on the first systemic use of CRISPR in people to cure a devastating disease caused by a gene overexpressed in liver. That no major side effects were found in these patients was highly welcome news for the CRISPR field.

For U-M to play a leadership role in this new era of medicine, we are in the process of building M-RNA Therapeutics. With this new initiative we aim to leverage the U-M research strengths in RNA biomedicine and nanoparticle delivery sciences to assemble a world class resource that will convert research innovations into clinical treatments through RNA therapeutics.

It is truly a thrilling time for RNA biology and RNA therapeutics!



Nils G. Walter, Ph.D., Francis S. Collins Collegiate Professor of Chemistry, Biophysics and Biological Chemistry, Professor of Chemistry, Professor of Biophysics, College of Literature, Science, and the Arts

RNA THERAPEUTICS

RNA, an obscure acronym to most of the world only a year and half ago, has been front page news since early 2020. A tiny RNA virus, known as SARS-CoV-2, has made a big name for itself as it rages globally, destroying millions of lives, disrupting all habits and routines, and forcing global and individual adaptation. Economic systems, employment, sustainability—all have become uncertain while the pandemic further reveals systemic social inequities. This RNA virus has turned the world as we knew it upside down.

Out of this chaotic pandemic came an RNA vaccine that gives us the power to fight back against the deadly virus. Over five decades of RNA research led up to an RNA vaccine breakthrough at exactly the time it was most needed. We owe immense gratitude to the many scientists who have contributed to RNA and related science fields and the development of this novel technology to create the vaccines that are saving countless lives around the world.

Another RNA research breakthrough was acknowledged in the fall of 2020, when the Nobel Prize in Chemistry was bestowed “for the discovery of a genome editing method.” Known as CRISPR (pronounced ‘crisper’), this RNA-guided genome editing tool was developed less than 10 years ago and is already routinely used in biomedical research labs.

RNA therapeutics research is moving fast in many fields from rare genetic diseases to cancer, Alzheimer’s, and other neurodegenerative diseases. Clinical trials are ongoing, and we anxiously await results. We are on the cusp of breakthroughs that will change medical practices and paradigms forever.

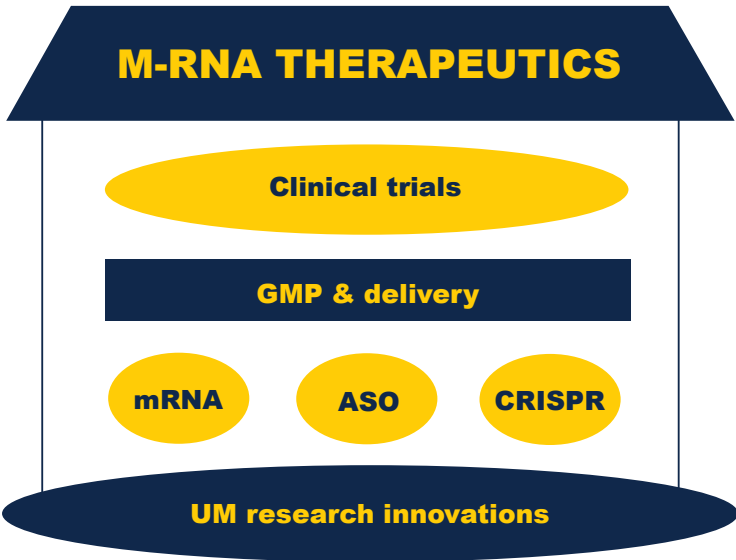
This is why, this year, we are launching a new initiative, M-RNA Therapeutics, with the goal of positioning the University of Michigan as a leader in this field.

Our annual magazine, *RNA Translated*, includes 18 interviews from University of Michigan (U-M) scientists and scholars, who tell some of the story of this research at U-M and beyond. This is, in a way, the continuation of the RNA research story we started last year, with our first magazine titled “2020, the year of the RNA virus.”

The U-M RNA scientific community is advancing foundational research and translating it into novel highly effective therapies. Collectively, we are quickly moving from bench to bedside, successfully replacing treatments with cures. In some cases, it has been less than five years since RNA-based cures have been transforming the lives of patients and their families. Only a large university that supports and synergizes many fields of research can provide the environment for such an enterprise. It is a privilege for the Center for RNA Biomedicine to play a catalytic role in it.

M-RNA Therapeutics at the University of Michigan

**An initiative to make the University of Michigan
a world leader in RNA therapeutics, spearheaded by
the Center for RNA Biomedicine and the Biointerfaces Institute**



Fueled by the COVID-19 mRNA vaccine success and the recent Nobel Prize in Chemistry for the discovery and elucidation of the bacterial CRISPR systems that can be harnessed for genome editing, RNA therapeutics has garnered considerable interest from academia, the pharmaceutical industry, and the public at large. To capitalize on this great momentum, the Center for RNA Biomedicine and the Biointerfaces Institute plan to leverage the strength of University of Michigan (U-M) research in RNA biomedicine and nanoparticle sciences to build a world-class resource that will convert U-M foundational research innovations into RNA-based clinical treatments.

Together, we envision establishing a pipeline of RNA therapeutics reaching from the research lab to the clinic in five to ten years. Our therapies will include mRNA for vaccines against a multitude of diseases, antisense oligonucleotides (ASOs) for the suppression of undesired genes, and CRISPR for genome editing of defective genes and for precision targeting of cancer cells. M-RNA Therapeutics will also offer these technologies for laboratory research such as gene knockouts and screens.

M-RNA Therapeutics requires the unmatched breadth of scientists at the U-M who come together and spark synergies rooted in their diverse areas of expertise, skills, and interests. Fostering and supporting this community is at the heart of the Center for RNA Biomedicine’s mission. “With the inauguration of the Center for RNA Biomedicine in 2016, we anticipated the enormous potential that therapies based on either using RNA

as a drug itself or targeting it with a drug would have in the future. The world has finally caught up with us, and we are now seeking to further enhance our capabilities through M-RNA Therapeutics,” says Nils Walter, co-Director of the Center and the Francis S. Collins Collegiate Professor of Chemistry, Biophysics and Biological Chemistry, named after a former faculty of the University of Michigan and current champion of mRNA vaccines as Director of the National Institutes of Health.

The Biointerfaces Institute is an important partner for us to spearhead M-RNA Therapeutics, connecting biomedicine with engineering and translating biologics into deliverable drugs. Joerg Lahann, Wolfgang Pauli Collegiate Professor of Chemical Engineering, Director of the U-M Biointerfaces Institute and Member of our Strategic Advisory Board, shares a vision where biomedicine and engineering can and must come together to tackle these challenges: “I foresee many areas of partnership as we study nucleic acids for delivery, as novel delivery platforms, and as technologies for immunotherapy. Together, we can drive this research at U-M, and we need it as a scientific field, as a research University, and as a nation.”

A recent landmark study published in *The New England Journal of Medicine* describes a clinical trial where CRISPR mRNA was directly injected as lipid nanoparticles into the blood of patients suffering from a devastating condition driven by the overexpression of a disease-causing gene in the liver. “This study shows that this type of delivery was safe and that it successfully reversed the symptoms of these patients. This opens the door for the treatments of thousands of genetic human syndromes using nanoparticle delivery of CRISPR. With M-RNA Therapeutics, Michigan Medicine would have the opportunity to be a leader in this exciting and rapidly emerging field,” explained Mats Ljungman, Professor of Radiation Oncology and co-Director of the Center for RNA Biomedicine.

To build M-RNA Therapeutics into a world-leading effort, we plan to leverage existing resources to hire additional leaders in this field while fundraising to rapidly seed new projects and provide the necessary infrastructure. Recruitment of faculty in RNA therapeutics is already actively ongoing through support from the University’s Biosciences Initiative to the Center for RNA Biomedicine. We are also lobbying to house M-RNA Therapeutics and core faculty at a joint location on campus.

We are currently working to partner with biomedical and pharmaceutical companies, fundraise through philanthropy, and submit proposals for federal funding. M-RNA Therapeutics is thus poised to become a vibrant innovation hub with state-of-the-art technologies that will propel the translation of RNA-based research ideas into clinical therapies.



To highlight the RNA biomedical revolution that is under way, we interviewed 18 University of Michigan (U-M) scientists and scholars who represent examples of this research at the U-M and beyond. These narratives are organized in three sections following the M-RNA therapeutics thrust: mRNA vaccines, ASOs and CRISPR. mRNA vaccines produce non-harmful cellular proteins that trigger an immune response that will be remembered when the real pathogen presents itself, ASOs are small RNAs that inhibit or stimulate a gene to compensate for a defective one, and CRISPR technology allows scientists to cut and replace specific sections of DNA or RNA. CRISPR is also now routinely used in research labs to study gene functions. These leading technologies are quickly becoming common therapies and research practices, transforming medical practice and expanding biomedical research opportunities.

mRNA vaccines

The extraordinary result of decades of RNA research: COVID-19 mRNA vaccines

Vaccination relies on our body’s reaction to a pathogen. As a defense mechanism, the immune system not only produces antibodies that destroy invaders, but it also remembers them. This natural phenomenon is key to the health and survival of humans and other species.

A traditional vaccine is an inoculation with a pathogen that has been weakened either through heat or other techniques. The pathogen in the vaccine triggers the immune system to produce antibodies to eliminate it. And, very importantly, the immune system will remember how to fight the pathogen should it present itself again. This preparedness saves precious time, and effectively prevents the development of a disease caused by this pathogen. Traditional vaccines have been very effective against infectious diseases like rabies, measles, and smallpox.

However, over billions of years, certain pathogens such as viruses have developed strategies to circumvent detection by antibodies. For example, viruses move quickly into host cells, reproduce very fast, and change form often through mutations, challenging the immune system cells that chase them. Viral variants can become so different from their original form that they are no longer recognized by the immune system. This is why the formula for the flu vaccine needs to be updated every year, and COVID-19 vaccines might need to be reformulated to protect against emerging variants.

How different is the mRNA COVID-19 vaccine from traditional vaccines?

SARS-CoV-2, the RNA virus that causes COVID-19, has characteristic spike proteins on its surface. These spikes allow the virus to bind to cells and enter them. The mRNA vaccine contains an engineered segment of RNA that when delivered into cells using lipid nanoparticles, orders cells to make the viral spike protein. As with a traditional vaccine, the immune system detects the spike protein on the surface or outside of these cells and produces antibodies that bind to it. When the actual SARS-CoV-2 virus invades the body, the immune system recognizes the spike proteins and quickly delivers antibodies. Unable to latch onto host cells, the viral infection is halted.



Louis Pasteur, a French chemist and microbiologist, is known for several major biomedical discoveries, including vaccination.

In 1878–1879, Pasteur discovered how to attenuate the strength of a pathogen and to use it in small doses to trigger an immune response that will be remembered by the body. Pasteur’s vaccination method is still the most common form of vaccination and includes a long list of recommended vaccines for U.S. children, including those for measles, mumps, rubella, varicella (chickenpox), and some types of influenza (flu).



A contemporary of **Alfred Nobel** (1833–1896), Pasteur (1822–1895) would no doubt have been awarded The Prize for his contributions to humankind. However, the Nobel’s prestigious prizes were first bestowed only in 1901.

Two mRNA vaccines for COVID-19 received FDA emergency use authorization in December 2020: the Pfizer-BioNTech vaccine (December 11) and the Moderna Therapeutics one (December 18), based on evidence from clinical trials that showed, respectively, 95% and 94% effectiveness within two weeks after the administration of two vaccine doses. On August 23, 2021, the FDA approved the Pfizer-BioNTech COVID-19 Vaccine, marketed as Comirnaty, for the prevention of severe COVID-19 disease in individuals 16 years of age and older.

The concept behind the COVID-19 mRNA vaccine is simple, but it has taken decades of RNA research to be developed and implemented. As Dr. Melissa Moore, Chief Scientific Officer at Moderna Therapeutics, explained during a March 2021 webinar organized by the U-M Center for RNA Biomedicine: “If it had been COVID-17, not COVID-19, we would not have had the vaccine so fast.”

Several biotechnological challenges needed to be resolved. First the RNA sequence of SARS-CoV-2 had to be known. Then, the scientists had to determine which part of the RNA sequence was responsible for the spike protein, and how the RNA structure influenced translation of the spike protein. Once the mRNA could be engineered, the challenge remained to deliver it into cells. Moderna’s and Pfizer’s mRNA vaccines are delivered by lipid nanoparticles that utilize the natural way lipids are transported in the body. The making of these lipid nanoparticles is what Dr. Moore affectionately calls “the secret sauce.”

“It does not matter which vaccine you get,” said Moore, “What matters is that all vaccines are clinically tested for safety and that they are administered quickly before the virus can mutate. If we wait too long, we might need booster vaccinations against variants,” she added.

Thanks to decades of RNA research and the dedication of thousands of RNA scientists, millions of vaccinated people can now feel strongly protected from COVID-19.

RNA vaccines against cancers

RNA vaccines have also been studied for treating cancers. The vaccine principles remain the same, but the targeted “pathogens” are the tumor cells rather than an external invader. Since each tumor carries unique mutations, the vaccine must be personalized for each patient. Once the tumor is surgically removed, the RNA of malignant cells is sequenced to identify specific mutated targets. Then a synthesized messenger RNA can be injected to tell cells which proteins to make. The immune system will detect and attack the cells that have the malignant proteins and will remember them if future growths occur. Clinical trials for RNA vaccines against different cancers are on the way or about to be approved.

ASOs (antisense oligonucleotides)

RNA therapy compensates for a gene loss of function

The first cures

Most genetic diseases are caused by either the over expression or under expression of a gene or a set of genes, a process that is controlled in part by DNA and RNA. RNA regulation mechanisms have been a focus of interest over the last 50 years, in the hope of harnessing them to cure these diseases. One approach is to use antisense oligonucleotides (ASOs) that are short (20-base long) RNA or DNA molecules that can bind to an mRNA in the cell and block it from being translated into a protein or alter its function in some programmable way. ASOs can be engineered to target specific RNAs. This simple and natural property of nucleic acids can be harnessed to target, suppress or alter defective genes.

Following are three examples of application of ASO therapy in rare genetic diseases. These studies demonstrate the incredible potential of mRNA-targeting ASOs for future therapies, with certain patients responding well to ASO therapy. With these novel technologies, there is hope for a cure for thousands of patients with severe epilepsy syndromes. The foundational science that supports these advances is delivering new insights at a fast pace. Collaborations between basic scientists, bioengineers, clinicians and, importantly, patient families, are the key to this medical revolution.

“New RNA-based therapies are totally changing the life of my patients. This is truly amazing,” said Dr. Erin Neil, a pediatric neurologist at Michigan Medicine who treats patients born with spinal muscular atrophy (SMA). “The babies with SMA we see today do not look at all like what they would have only five years ago, and it is mind-boggling to actually see the impact of biomedical research on our patients. I’m very grateful for all the work of these scientists!”

Patients with SMA suffer from proximal muscular weakness affecting their legs, arms and torso. They have difficulty breathing, coughing, and swallowing. There are four different types of SMA with varying degrees of symptom severity and disease progression. SMA does not affect a person’s ability to think, learn, and build relationships with others. It is recognized in approximately one in 11,000 births in the United States and can affect any race or gender. 85% of newborn babies are currently screened for SMA in 38 U.S. states. Systematic newborn screening for SMA started in March 2020 in Michigan where, on average, 12 babies are born every year with the disease. The FDA approval of RNA-based therapies for SMA made it possible to put this disease on the newborn screening panel.



Erin Neil Knierbein, D.O., Assistant Professor of Pediatrics, Child Neurology, Neuro-muscular Medicine (Psychiatry and Neurology), Medical School

SMA is a rare genetic disease that is caused by a defect in the survival motor neuron 1 gene (*SMN1*) that causes the loss of an important piece, exon 7, during the splicing process. As a result, there is no production of SMN1 protein, which causes motor-neurons to die off from the time of conception. The body also has an *SMN2* gene that produces about 10% of the total required SMN protein level for normal muscular development. This *SMN2* gene can be targeted by therapies to produce more SMN proteins. However, patients with a loss of function of *SMN1* also have various degrees of defects in *SMN2*, with disparities between patients in the number of copies of the gene. The greater the number of *SMN2* genes, the better the therapy outcomes. About one in every 50 Americans is a genetic carrier of SMA, and in most clinical cases, affected children inherit one copy of the defective gene from each parent.

Over the last five years, RNA research has delivered three FDA approved therapeutic options. “It’s amazing to be able to know, through newborn screening, that these babies have SMA within a week of their birth, although they do not show any symptoms. Once screened, these patients come to Michigan Medicine for additional testing, and we can start treatment as soon as we have the green light from the state’s children’s special healthcare insurance,” said Dr. Neil. “This can go very fast, which is what these patients need.”

The sooner a treatment is given, the sooner the motor neurons can be rescued, and outcomes can dramatically be improved. Although none of these medications is a cure, and what is lost cannot be recovered, these therapies stabilize the muscular deterioration. In itself, stabilization is a success for a medical condition that will deteriorate over time. Dr. Neil reports patients who can sit and rollover, and feed without a tube; none of these would have been possible for them only five years ago, when there was no therapy option.

“The babies with SMA we see today do not look at all like what they would have only five years ago, and it is mind-boggling to actually see the impact of biomedical research on our patients.”

In December 2016, the RNA drug nusinersen (Spinraza) became the first FDA-approved therapy for SMA. It is an antisense oligonucleotide (ASO) therapy in which a small-sized single-stranded nucleic acid binds to the *SMN1* gene and prevents exon 7 from being spliced out. This allows for *SMN2* gene to compensate and produce the necessary SMN proteins needed for muscle development (see figure 1). Nusinersen is delivered through a series of four injections in the spine, over a span of nine weeks, and every four months thereafter for life. “This was the first medication that changed the lives of my patients,” said Dr. Neil. “For the most part, this medication is well tolerated, but the physical injection can be challenging due to the anatomy of the patients with muscular atrophy.”

In May 2019, the second SMA drug, onasemnogene abeparvovec (Zolgensma) was approved in the U.S. It is a viral vector-based gene therapy in which the shell of a common virus (adenovirus) delivers an engineered copy of the *SMN2* gene to the motor neuron cells. Rather than a series of life-long injections, onasemnogene abeparvovec is administered through a one-time intravenous injection. The therapy is time-sensitive and must be given before the age of two. After that, the child is likely to have antibodies to the adeno-associated viral vector that is used. These patients have to be on a daily steroid, prednisone, which lowers their immune response. This presents the disadvantage of delaying the administration of vaccines. Still, this therapy

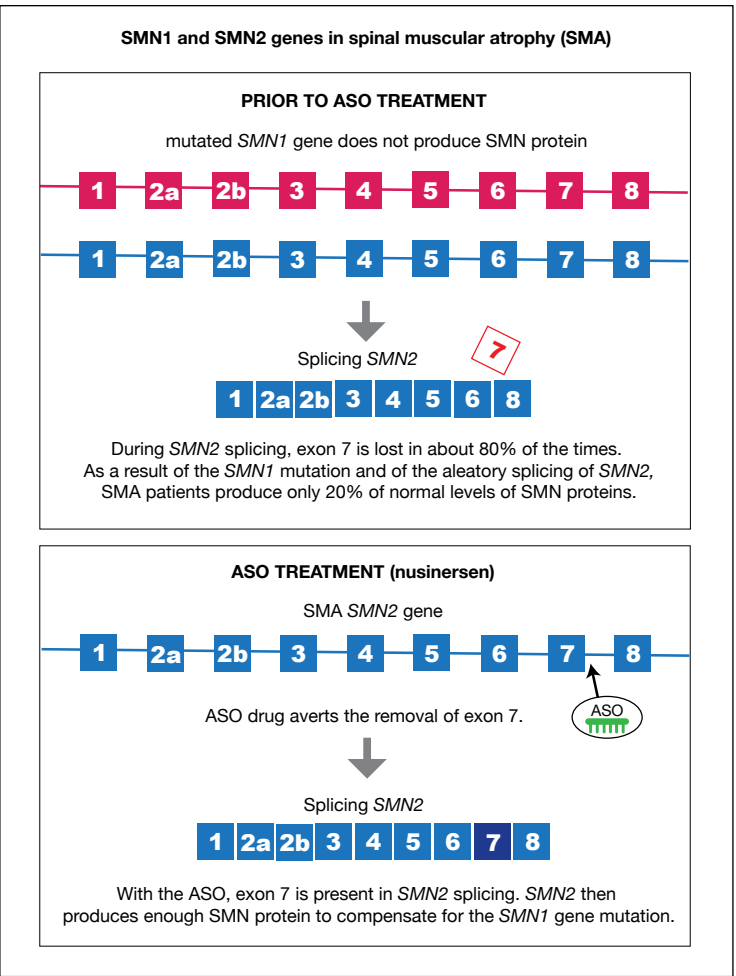


Figure 1: ASO (nusinersen) treatment for SMA patients

insurance in the state of Michigan, the extremely high cost of these three therapies is covered for patients with Medicaid. If the patients have a private insurance, the physicians request and obtain approval from the insurance company. Nusinersen costs \$125,000 per dose, which amounts to \$750,000 for the first year, and \$375,000 per year for life. The one-time gene therapy, onasemnogene abeparvovec, costs \$2.15 million for the one dose. Risdiplam costs about \$340,000 per year.

The SMA patients’ families are in general highly involved in the treatment of their children. “Families are wonderful advocates for their children. It is a lot of work for these families, and I’m in awe of many of my patients and their families,” commented Dr. Neil. SMA patients’ families have founded [Cure SMA](#), a support and advocacy organization. “This is an excellent science-based organization that is extremely supportive of families,” said Dr. Neil. “They organize a yearly conference and have a ‘welcome care package’ for newly diagnosed families.” In collaboration with Cure SMA, Dr. Neil is currently designing a survey of SMA patients’ parents to better understand how they make decisions about treatments. She expects these results to further inform practitioners and policymakers about the family concerns with these patients.

“Over the last four and half years, we have had three new medication options to treat SMA. This is a huge medical breakthrough!” concluded Dr. Neil. “The field of pediatric neurology is very exciting, and it is extraordinary to be able to help these patients and their families.”

offers the remarkable advantage of being a one-time injection in the bloodstream and constitutes a life-long treatment.

The third and most recent treatment, risdiplam (Evrysdi), was approved by the FDA in August 2020. Risdiplam is a small-molecule splicing modifier designed to boost *SMN2* mRNA expression in order to increase the functional SMN protein production in muscle. It functions similarly to the ASO drug by promoting the production of SMN2 proteins to compensate for the defective *SMN1*. Risdiplam can be prescribed to SMA patients who are two months of age and older and is given daily orally in a liquid form for life.

As a pediatric neurologist, Dr. Neil helps parents make decisions around choosing the right treatment for their child. In this context, she regrets that the clinical trials for these three drugs did not use comparable units and criteria. “We don’t have head-to-head comparisons as to how patients have responded to treatments, but it is wonderful that they all have used physical clinical outcomes which are very meaningful for a patient. These drugs are all very effective, and this is thrilling. Before this, there was nothing. All we could do was symptomatic care.”

Thanks to the Children’s Special Health Care Services (CSHCS), a Medicaid supplemental insurance in the state of Michigan, the extremely high cost of these three therapies is covered for patients with Medicaid. If the patients have a private insurance, the physicians request and obtain approval from the insurance company. Nusinersen costs \$125,000 per dose, which amounts to \$750,000 for the first year, and \$375,000 per year for life. The one-time gene therapy, onasemnogene abeparvovec, costs \$2.15 million for the one dose. Risdiplam costs about \$340,000 per year.

Dravet syndrome



Lori L. Isom, Ph.D., Chair, Department of Pharmacology, Maurice H. Seevers Collegiate Professor of Pharmacology, Professor of Molecular and Integrative Physiology, Professor of Neurology, Medical School

Dr. Lori Isom is a neuroscientist at Michigan Medicine who studies Dravet syndrome, a severe genetic disease characterized by hard-to-control seizures and a broad spectrum of symptoms including autistic traits, sleep cycle disorders, developmental delays, and cognitive impairments. Dravet syndrome is estimated to affect 35,000 patients in the world. There is no effective drug to treat these children who have a much higher (up to 20%) risk of sudden unexpected death in epilepsy (SUDEP) than patients with other forms of epilepsy. SUDEP happens mostly at night and during sleep.

Epilepsy results from dysregulations in neuronal firing—a process that is initiated by voltage-gated sodium channels encoded by nine genes of the *SCN* gene family. The majority of Dravet syndrome cases are caused by variants in the *SCN1A* gene that encodes the voltage-gated sodium channel Nav1.1. As a sodium cell biologist, Dr. Isom knew that *SCN1A* was expressed not only in the brain but also in the heart. Work from her laboratory showed that mice modeling *SCN1A*-linked Dravet syndrome have cardiac arrhythmias with characteristics similar to sodium channel-linked Long QT syndrome. In this syndrome, patients have heartbeat arrhythmia episodes with possible nocturnal sudden death during sleep. Dr. Isom and her colleagues came up with the hypothesis that *SCN1A* haploinsufficiency, which plays an important part in Dravet syndrome, could also cause cardiac arrhythmias, and that the combination of seizures and cardiac arrhythmias may have a role in the mechanism of SUDEP.

To test her idea, Dr. Isom collaborated with Dr. Jack Parent, a neurologist, epileptologist, and stem cell biologist also at Michigan Medicine, whose laboratory generates induced pluripotent stem cells (iPSCs) from Dravet syndrome patients. Using cardiac myocytes differentiated from these cells, Drs. Isom and Parent confirmed their previous hypothesis from mouse models. In the Dravet syndrome patient cardiac cells they grew, they saw the electrical signature of what a fatal arrhythmia would look like. They were able to predict that the girl who had donated her skin cells may be at risk for a severe arrhythmia episode, and recommended that the child and her family consult with a cardiologist. “When the cardiologist confirmed her heart condition, it was a very powerful moment,” recalled Dr. Isom. This was the first time a connection had been made between Dravet syndrome and cardiac arrhythmia.

Dr. Louis Dang, M.D., Ph.D., a pediatric neurologist and epileptologist also in Dr. Parent’s lab, is exploring the possibility of using ASOs to increase protein production from the non-mutant healthy gene so that it compensates for the deficiency. Instead of producing 50% of the protein, the copy of the healthy gene, or allele, may produce up to 90% of the required protein level which should suffice to restore normal behavior in brain cells.

In 2018, the U-M team was contacted by Stoke Therapeutics, a biotech company co-founded by Dr. Isabel Aznarez, a leader in the field of antisense oligonucleotide (ASO) therapy. Stoke Therapeutics focuses particularly on therapeutic strategies to upregulate protein expression using ASOs. Upregulation is a compensation strategy that can naturally occur in the brain and other tissues. This ASO technique takes advantage of a process that happens spontaneously in nature.

Dravet syndrome is caused in most patients by *SCN1A* gene variants in one of the alleles. The RNA made from the mutant allele is degraded by the cell and therefore will not produce a protein. The Stoke Therapeutics team used an upregulating ASO

technique that allows the *SCN1A* allele to overproduce, bringing the protein amounts to normal levels. The hope is that, unlike anti-seizure drugs that address only the epileptic symptoms, the ASO treatment will cure the entire Dravet syndrome pathology.

Dr. Isom’s group used a mouse model of *SCN1A*-linked Dravet syndrome to test this idea. They injected the Stoke Therapeutics ASO directly into the mouse brain two days after birth. The team was able to successfully increase *SCN1A* mRNA and Nav1.1 protein levels from 50% to 100%, which saved 97% of the tested mice from seizures and SUDEP. “From a single dose, we protected them out to three months,” said Dr. Isom. “This result was incredibly exciting and predicted that this ASO might be effective in human patients!”¹

Following this remarkable success, Stoke Therapeutics proceeded to safety trials in non-human primates, and, in 2020–2021, two human clinical trials were approved for children from two to 18 years of age. These studies, named “Monarch” and “Swallowtail,” started with a single dose in August 2020, followed by multiple dose trials in February 2021. Michigan Medicine is one of the sites taking part in this study. The potential impact of this study is tremendous, not only for Dravet patients and their families, but also for curing many other genetic diseases that affect millions of people.

Genetic epilepsy



Miriam Meisler, Ph.D., Myron Levine Distinguished University Professor, Professor of Human Genetics, Professor of Neurology, Medical School

In 1995, Dr. Miriam Meisler, a geneticist and neurologist at Michigan Medicine, started studying the *SCN8A* gene, which encodes the Nav1.6 protein that is a constituent of the sodium channel. It was the last of the 10 genes of the sodium channel family to be discovered. Sodium channel proteins have different functions and sites of expression in the body. One is the SCN5A protein expressed in the heart and critical for a functional heart beat, several others are expressed in peripheral neurons for sensation and movement, and four (*SCN1A*, *SCN2A*, *SCN3A* and *SCN8A*) are expressed in the brain where they trigger neuron activity. Mutations in the sodium channel genes result in severe multi-symptom pathologies. *SCN1A* mutations cause Dravet syndrome, and *SCN2A* mutations have been found in autistic children. *SCN8A* contains approximately 2,000 amino acids and rare mutations in it result in either a loss of function, resulting in mental disabilities, or a gain of function that causes seizures. Since 2012, 300 patients with epilepsy have been found to have an *SCN8A* gene mutation.

Patients with Dravet syndrome with a loss of function mutation of *SCN1A* have only 50% of the required channel activity. Similarly, patients with *SCN8A* loss of function are lacking 50% of NAV1.6 activity. Underproduction of *SCN8A* results in mental disabilities.

Gain of function mutations of *SCN8A* result in elevated stimulation of neurons that can provoke seizures. At the University of Arizona, a geneticist whose daughter suffered from seizures uncovered this new disorder. Determined to find a cure, he sequenced his entire family’s DNA, and eventually identified a mutation in his daughter’s *SCN8A*

¹ Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome, Zhou Han, Chunling Chen², Anne Christiansen, Sophina Ji, Qian Lin, Charles Anumonwo, Chante Liu, Steven C. Leiser, Meena, Isabel Aznarez, Gene Liao and Lori L. Isom, *Science Translational Medicine* 26 Aug 2020; Vol. 12, Issue 558, eaaz6100, DOI: 10.1126/scitranslmed.aaz6100

gene. In 2012, he contacted Dr. Meisler to find out whether this mutation could be causing the seizures. The U-M team did a series of functional studies, beginning in cultured cells, where they found that the sodium channel was not closing properly. They observed the same result in neurons, and then in mice with the patient mutation inserted into their genome. From these studies, the scientists could confirm that the patient had an *SCN8A* gain of function mutation. The *SCN8A* protein was active but dysfunctional, either opening too soon or closing the sodium channels too late. The resulting elevated channel activity excessively stimulated the neurons, leading to seizures.

This research and other studies have revealed the genetic causes of epilepsy syndromes that occur in the first few months of a child’s life. It is now common practice to sequence newborn babies’ DNA and screen for mutations in sodium channel genes as soon as seizures begin. Early detection allows for early therapy that reduces seizures and may limit developmental damage.

The genetics of sodium channel pathology also offer a potential pathway to a cure. However, it is difficult to design a drug that specifically inhibits *SCN8A* without also affecting the expression of other sodium channel genes. ASO therapy is an alternative genetic approach to accomplish downregulation that may allow much greater precision in targeting.

“The beauty of these genetic approaches is that they are absolutely specific.”

“The beauty of these genetic approaches is that they are absolutely specific. With a sodium channel-targeted drug, you may affect several of the 10 sodium channel proteins, resulting in a lot of side effects. RNA therapeutics allow you to target only one gene, and that’s where we want to go,” said Dr. Meisler.

Another advantage of ASOs is that they are taken up directly by neurons without the need for a delivery vector as they become distributed in the cerebrospinal fluid. However, repeated injections in the brain or spinal cord are intrusive, and scientists are actively researching alternative modes of delivery. It is time-consuming to first identify the right ASO to target a particular mutation on the defective gene and then to determine the correct dose without degrading too much of the mRNA. In mice studied at U-M, ASOs were degraded after six to seven weeks, and re-injection was required. Improved stabilization of the ASOs constitutes another area of research.

In 2017, Dr. Meisler began a collaboration with Ionis Pharmaceuticals. The company tested many ASOs to eventually find a few that knocked down the *SCN8A* mRNA without toxic effects in normal mice. At U-M, the team tested these ASOs on their *SCN8A* mutant mice and found that seizures were prevented for as long as the ASO remained present.² The next step will be to test this approach on primates and, hopefully, move to a clinical trial. With an ASO therapy already in clinical trial for Dravet syndrome for *SCN1A* loss of function, it is encouraging to note that the *SCN8A* ASO

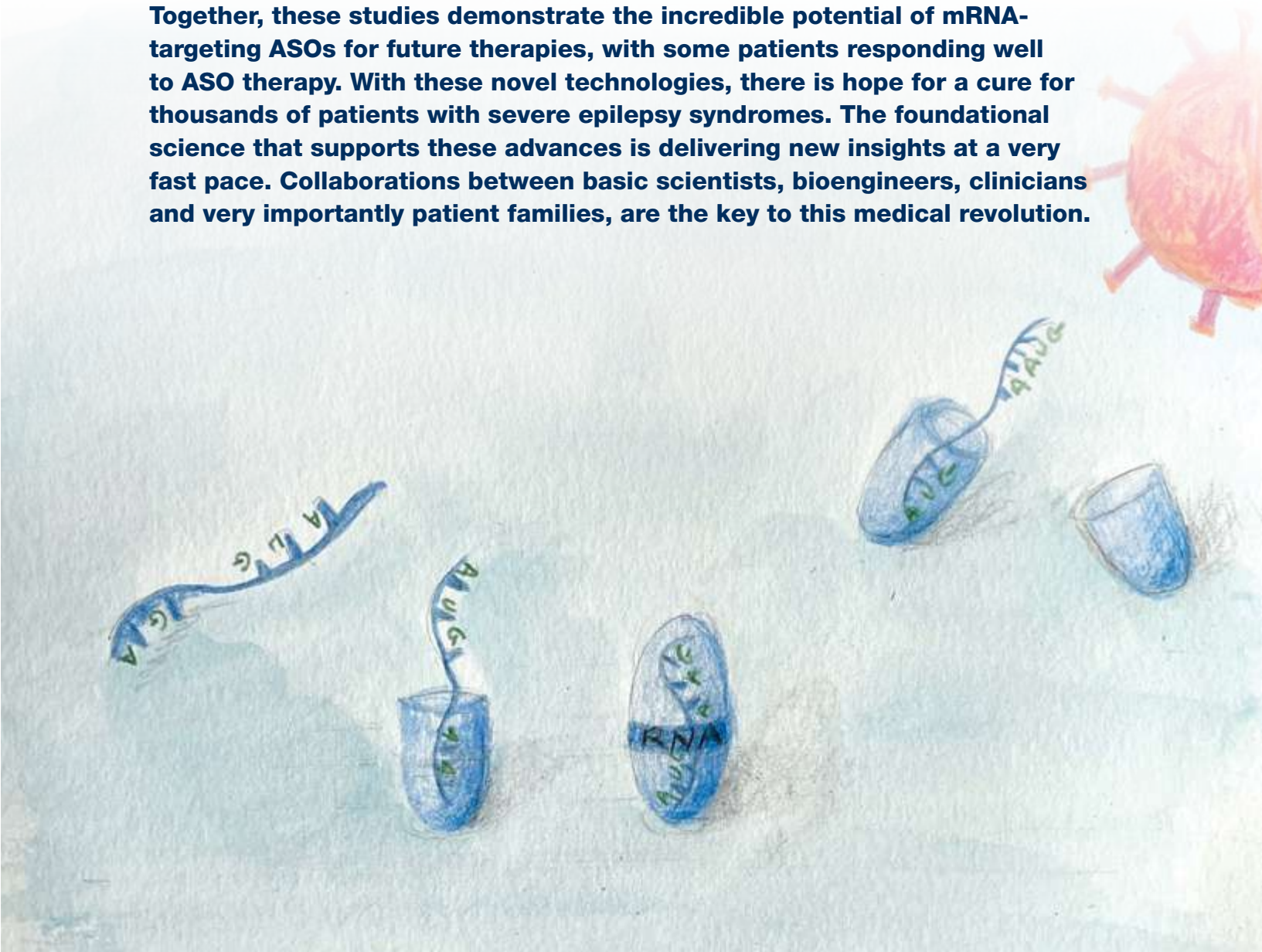
²Lenk GM, Jafar-Nejad P, Hill SF, Huffman LD, Smolen CE, Wagnon JL, Petit H, Yu W, Ziobro J, Bhatia K, Parent J, Giger RJ, Rigo F, Meisler MH. Scn8a Antisense Oligonucleotide Is Protective in Mouse Models of SCN8A Encephalopathy and Dravet Syndrome. *Ann Neurol*. 2020 Mar;87(3):339-346. doi: 10.1002/ana.25676. Epub 2020 Feb 6. PMID: 31943325; PMCID: PMC7064908

also has a positive impact in Dravet patients. Thus, discoveries about one sodium channel can bring insight into the others, and a treatment for one type of sodium channel defect might be also helpful for developing treatments for other sodium channel defects.

RNA therapy in ophthalmology

At U-M Kellogg’s Eye Center, Dr. Abigail Fahim, an ophthalmologist (see page 31), uses RNA technology in a Phase I/II clinical trial run by ProQR Therapeutics. This clinical trial is for patients with a retinal degeneration caused by mutations in a gene called *USH2A*. Patients with these mutations present with retinitis pigmentosa, a slowly progressive blinding disease, part of the Usher syndrome, which includes both retinitis pigmentosa and hearing loss. Mutations in exon 13, a section of *USH2A*, are commonly responsible for this disease. The goal is to use RNA therapeutics to bind to the splice site of exon 13, and thereby bypass exon 13 altogether. This therapy is delivered with an intravitreal injection into the eye, a common procedure performed in the ophthalmology clinic.

Together, these studies demonstrate the incredible potential of mRNA-targeting ASOs for future therapies, with some patients responding well to ASO therapy. With these novel technologies, there is hope for a cure for thousands of patients with severe epilepsy syndromes. The foundational science that supports these advances is delivering new insights at a very fast pace. Collaborations between basic scientists, bioengineers, clinicians and very importantly patient families, are the key to this medical revolution.



Deciphering the role of non-coding RNAs

The Human Genome project revealed that only 1% of our genome actually codes for proteins. However, RNA species are synthesized from many other sequences in the genome and these RNAs are collectively referred to as non-coding RNAs. These non-coding RNAs have been found to regulate the expression of protein-coding genes while perturbations in these RNAs can cause diseases. How could we then restore or mimic the role of these RNAs to treat these diseases?

The vast unknown territory of non-coding RNAs

In 1968, scientists described the human genes as an alternation of sequences of nucleic acids. The sequences that code for proteins, called exons (short for “expression”), are usually short and constitute only 1% of the total sequence information of the genome. Between exons are intervening sequences called “introns” that are non-coding sequences. Following RNA synthesis, introns are removed in a process called splicing. Introns, together with exons (the genes), make up about 19% of the total human genome.

The rest of the genome used to be considered “junk” because no one knew what it was used for. This large uncharted territory has attracted many scientists who now understand that our cells synthesize RNA from a large number of these non-coding sequences located between genes, and that these RNAs play important roles in cell functions. A large effort is ongoing to decipher the many functions of these non-coding RNAs to potentially target them for therapeutics.

Over the last two decades, several approaches have been attempted. In one of them, microRNAs (miRNAs) have been developed as drugs that could directly regulate the expression and function of coding and non-coding RNAs. Other studies have focused on developing a complementary nucleotide sequence (small interfering RNA – siRNA) to target the non-coding RNAs to restore normal function of cells.

It is, however, a challenge to use miRNAs or siRNAs for therapy because they may target several different RNAs, leading to off-target effects. The introduction of siRNA or miRNAs into the body can also trigger an immune response, although recently these concerns have been addressed through novel chemical modification strategies.

Dr. Amanda Garner, a medicinal chemist at U-M, has been studying miRNAs whose overexpression causes diseases, and small molecules that could block those miRNAs. Her team has developed an assay to determine the activity of many small molecules against miRNAs, as well as compounds made from living organisms for their ability to affect the activity of miRNAs. They have identified several novel families of compounds that can target miRNAs and block their overexpression, with a particular focus on those that can cause tumors. However, for Garner, this is “the old view” that has largely proved unsuccessful in drug discovery. “We have tried to target miRNAs for a long time, but this approach was too simplistic and we needed to think about this differently,” she said. “We started with some assumptions, but science is about solving problems, not just validating your given hypotheses. The field of RNA-targeted drug discovery is new in many ways, and we had to change our thought process.”

“We have to embrace the complexity of RNA!”

Collaborating with experts in RNA structure, chemistry, chemical biology, biochemistry and biophysics, Garner’s team is exploring the complexity of RNA and discovering how much more there is to learn while going from the test tube to the patient. “We’re still at the beginning of our understanding of RNA and its ability to be regulated by RNA-binding proteins,” said Dr. Garner. “There are many kinds of RNAs with many functions in the cell, and we need scientists with different areas of expertise to come together to take on this challenge.”

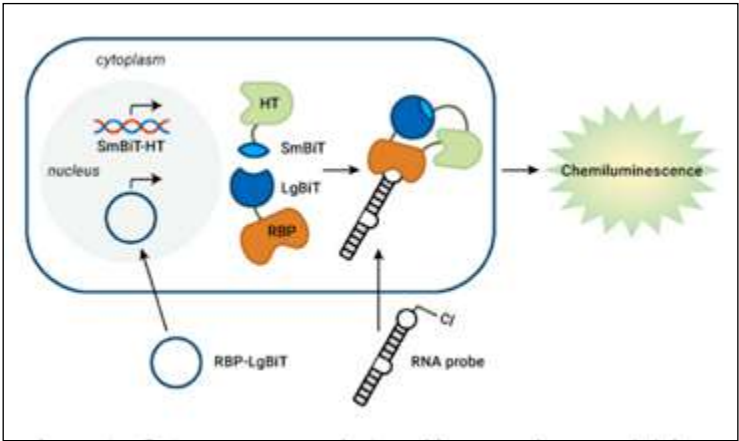


Amanda Garner, Ph.D., Associate Professor of Medicinal Chemistry, Medicinal Chemistry College of Pharmacy, Former member of the Center for RNA Biomedicine Executive Committee

Armed with deep knowledge acquired from extensive study of small molecule compounds, Garner is reorienting her problem-solving approach, remaining open-minded while mapping out cellular RNAs (messenger RNAs, miRNAs and lncRNAs) and their interactions with proteins and small molecules. Her team is currently developing techniques for screening that can be broadly applicable and shared with other scientists. “It would be great if we could all use the same techniques so we can better collaborate and compare our results,” Garner said.

One of these techniques, catalytic enzyme-linked click chemistry assay (cat-ELCCA), is a biochemical assay that the Garner lab has used to identify inhibitors of miRNAs. Another of these techniques is named RiPCA, short for RNA interaction with Protein-mediated Complementation Assay, that is a cell-based assay for detecting RNA-protein interactions. The development of RiPCA is part of the lab’s continued evolution of working towards deciphering the best methods for identifying cell-active and physiologically-relevant RNA-targeted small molecules. For Garner, small molecules have proved to be very valuable tools to study the complexity of biological systems, which she hopes will extend to elucidating RNA functions. She is also enthusiastic about the development of siRNAs into therapeutics with some of them starting to be approved for rare diseases like hereditary ATTR amyloidosis, acute hepatic porphyria and primary hyperoxaluria type 1.

Biologics are drugs produced from living organisms or containing components of living organisms. These drugs pave the way to identify useful RNA therapeutics targets. For example, the understanding gained from the development of nusinersen for spinal muscular atrophy in children (see page 9) has been applied to develop novel compounds. Nusinersen is injected in the spine, a very invasive procedure that needs to be repeated every four months. Only last year, a small molecule (risdiplam) was approved to deliver a similar treatment, this time in an oral form. “This is a dramatic improvement for these patients,” said Dr. Garner.



Schematic of RiPCA, a system designed by Amanda Garner’s lab to study RNA-protein interactions in live cells.

RNA therapy for cancer

Glioma tumors “choke” in their debris with no coming back



Maria Castro, Ph.D., Richard C Schneider Collegiate Professor of Neurosurgery, Professor of Cell & Developmental Biology, Medical School

Drs. Castro and Lowenstein, Professors of neurosurgery and cellular biology at Michigan Medicine, have dedicated their careers to the study of glioma tumors that cause the most common and lethal form of brain cancer. High grade gliomas progress extremely rapidly and are typically fatal within a couple of years. Low grade gliomas progress at a slower rate and the survival rate is five to 12 years. In the low grade form of brain cancer, the patients are usually younger and otherwise healthy, but the tumor always comes back, and when it does, it is very aggressive. Castro, Lowenstein and their collaborators have looked for a cure that would not only destroy the gliomas, but also prevent their recurrence. They searched for natural biological processes that could be manipulated to fight gliomas, one of them being autophagy.

Autophagy is the mechanism by which cells remove unnecessary or dysfunctional components during homeostasis and also maintain metabolism during nutritionally challenging periods. In low grade gliomas, a mutation of *IDH1* gene massively upregulates the pathway that controls the autophagy machinery, keeping autophagy very active, which promotes growth and survival.³

Dr. Castro and her team worked with the Joerg Lahann’s group (see article p. 21) to engineer a nanoparticle that encodes and delivers a short hairpin RNA (shRNA) that inhibits the expression of the autophagic protein ATG7. The nanoparticle was tested in a mouse model of low grade glioma by injection into the blood stream. These nanoparticles had the capability of reaching the tumor site where they delivered the shRNA causing the down regulation of the ATG7 protein and subsequently the suppression of autophagy. This led to a reduction in tumor growth and elicited an immune response memory that prevented new tumor growth. In this study, 60% of the mice survived the glioma and its recurrence. “We know that these tumors will always come back in patients with low-grade gliomas, but we do not know when. If their immune system is already trained to identify the tumor as soon as it comes back, they will have a much better chance of preventing the tumor from recurring. It appears that suppressing the autophagy pathway creates an immune memory, and this could be a very effective approach for these patients,” concluded Dr. Castro.

³ Núñez FJ, Mendez FM, Kadiyala P, Alghamri MS, Savellieff MG, Garcia-Fabiani MB, Haase S, Koschmann C, Calinescu AA, Kamran N, Saxena M, Patel R, Carney S, Guo MZ, Edwards M, Ljungman M, Qin T, Sartor MA, Tagett R, Venneti S, Brosnan-Cashman J, Meeker A, Gorbunova V, Zhao L, Kremer DM, Zhang L, Lyssiotis CA, Jones L, Herting CJ, Ross JL, Hambardzumyan D, Hervey-Jumper S, Figueroa ME, Lowenstein PR, Castro MG. IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. *Sci Transl Med*. 2019 Feb 13;11(479):eaaq1427. doi: 10.1126/scitranslmed.aag1427. PMID: 30760578; PMCID: PMC6400220

Collagen is everywhere and is quite unique



Pedro Lowenstein, M.D., Ph.D., Richard C Schneider Collegiate Professor of Neurosurgery, and Professor of Cell and Developmental Biology, and Bio-medical Engineering, Medical School

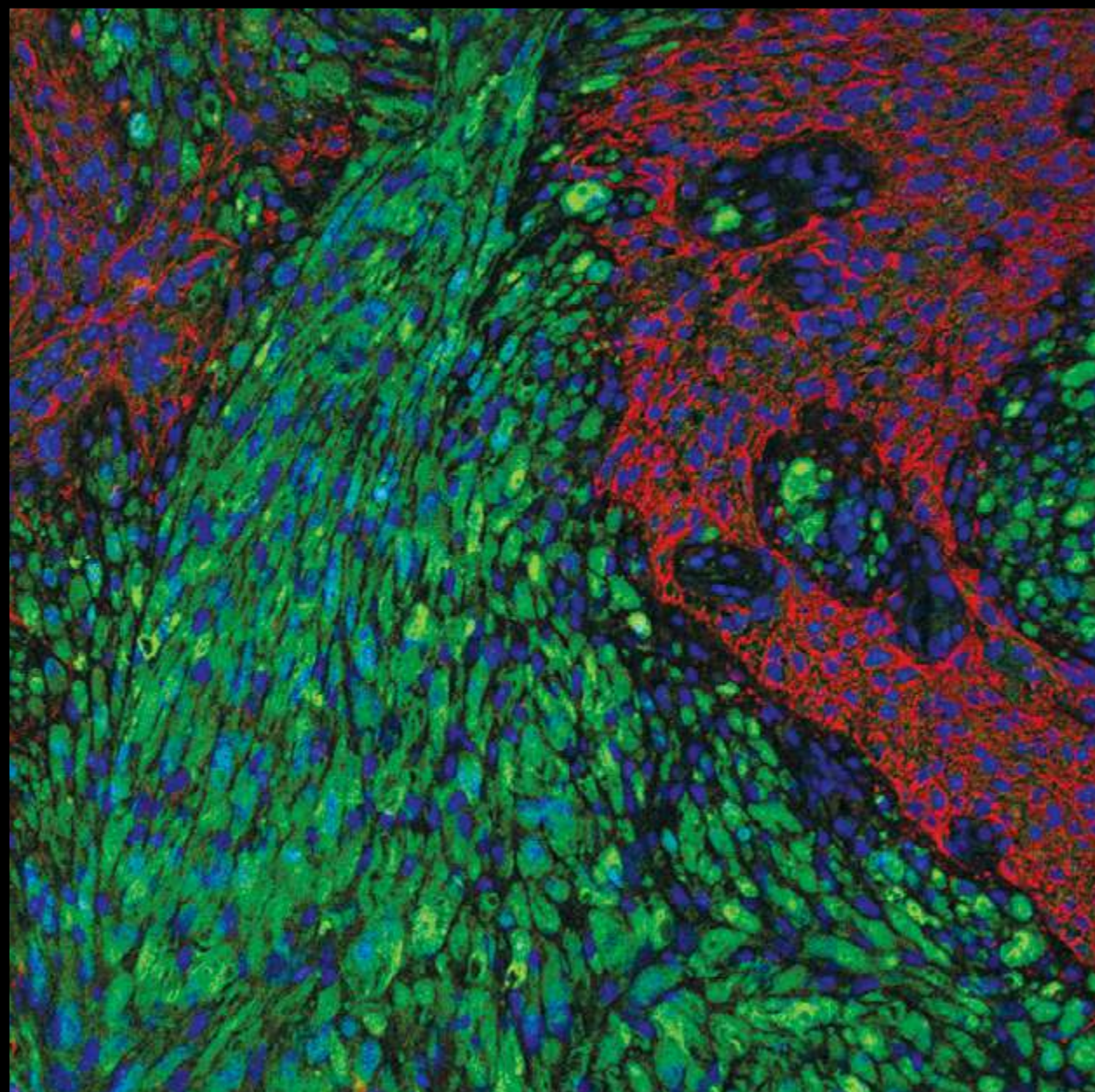
Although a glioma can be successfully removed surgically, it is nearly impossible to excise all of the cells that might have dispersed into the neighboring tissues. Without additional therapy, these cells will grow back into tumors and eventually overwhelm the patients’ brains. Dr. Lowenstein and his team are studying the area at the interface between tumors and healthy cells to better understand the infiltration process of glioma cells into healthy tissues. In one study, they are investigating collagen, a familiar protein to the cosmetics industry because among many functions, it confers plasticity to tissues. Most research emphasis has gone into understanding how changes in stiffness affect tumor growth, as tumors prefer stiffer surfaces to grow on. However, collagen also acts on up to four different receptors that affect the intracellular signaling pathways affecting tumor growth and progression.

Collagen is well known as the principal protein in the extra-cellular matrix that holds the cells together. As such, it provides elasticity to the tissues. However, Dr. Lowenstein and his team were quite surprised to find that large amounts of collagen are also present inside tumor cells. When they used an shRNA to knock down the expression of collagen in tumor cells, the scientists observed major changes in the tumors: they became much less malignant and aggressive, and the experimental mice survived significantly longer. In addition, the immune response against the glioma was enhanced. The fact that collagen replaces itself much faster in tumors than in other parts of the body suggests that it plays an important role in tumor cells and therefore could be a good protein to target for treatment. However very little is still known about collagen’s intracellular role, and the mechanisms by which it stimulates tumor growth. Collagen is a complex protein made of three helical domains that interact with four different types of receptors in the brain. Lowenstein’s team is studying these receptors and preliminary results show that collagen could, through the receptors, signal the tumor malignancy to other tumor cells.

“It is exciting to use novel nanotechnology to develop potentially therapeutic procedures to abolish collagen expression in tumor cells, and thus capitalize on a basic science finding and turn it into a potential therapy for patients with brain tumors,” said Dr. Lowenstein.

Because of its unique structure and characteristics, collagen is a very good candidate for precise targeting by RNAs or CRISPR Cas9 novel technologies and therapeutics. Since gliomas are local and never metastasize outside the brain, shRNA or CRISPR Cas9 contained in a vector could be effectively injected during surgery, after excision of a tumor. “Local delivery is a very attractive option. It’d be relatively easy to inject nanoparticles or viral vectors that would target the tumor surface or the collagen receptors during surgery,” added Dr. Castro.

The delivery of RNA therapeutics, including CRISPR, is one of the challenges that are being solved very quickly, as demonstrated in the following article on lipid nanoparticles.



Live image from an explant culture of an experimental glioblastoma. Tumor cells in green move along collagen fibers, guided in their invasive path by astrocytes (in red). Credits: A. Comba and P. Lowenstein

A container to deliver drugs

Once a cellular process and localization have been identified for treatment, the next step is to deliver the drug to the desired area and across cell membranes. This challenge has inspired many studies in the field of chemical engineering, bringing interdisciplinary teams together to advance promising novel technologies.



Joerg Lahann, Ph.D., Wolfgang Pauli Collegiate Professor of Chemical Engineering, and Professor, Biomedical Engineering, Macromolecular Science & Engineering, and Materials Science & Engineering; Director, University of Michigan Biointerfaces Institute

Dr. Joerg Lahann, a Professor of Chemical Engineering and Director of the University of Michigan Biointerfaces Institute, has been particularly interested in the RNA drug delivery challenge over the last six years. His team has designed several types of synthetic protein nanoparticles that are about one tenth micron in size, or, on average, only about one hundredth the size of a cell. These synthetic protein nanoparticles can be compared to a box that protects its cargo, labeled with tags that control its journey through the body. The synthesized proteins serve as building blocks and confer natural protein-like properties to the delivery machinery. For example, these proteins can cross the barrier that protects the brain against pathogens or toxins. Once injected into the blood stream, many of these nanoparticles end up in the liver where enzymes quickly degrade the synthesized proteins, limiting possible side-effects. The polymers bring stability and greater longevity to the entire complex. Synthetic protein nanoparticles are well suited for sustained circulation such as in the blood stream.

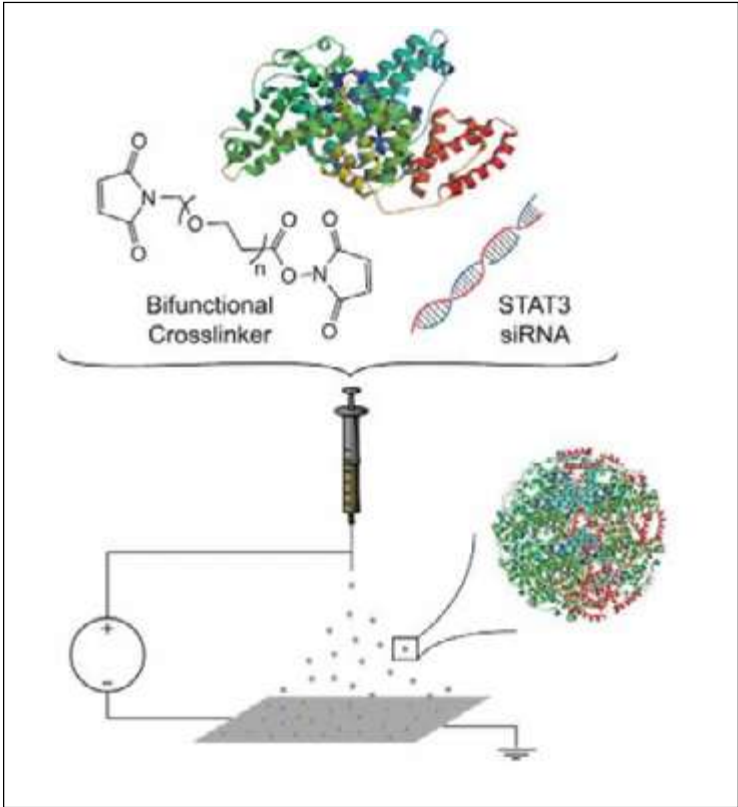
Because proteins naturally interact with biotherapeutics, Dr. Lahann and his team thought of using synthetic protein nanoparticles to deliver nucleotide-based systems. Their first attempt was with small interfering RNAs (siRNAs) in a collaboration with Drs. Maria Castro, R. C. Schneider Collegiate Professor of Neurosurgery and Professor of Cell and Developmental Biology and Pedro Lowenstein, Richard C. Schneider Collegiate Professor of Neurosurgery, Professor of Cell and Developmental Biology (see articles pages 18–19). The goal of this study was to treat the most common and fast-growing form of brain tumor, glioblastoma, with an siRNA that would inhibit STAT3, a protein driving multiple signaling pathways related to tumor progression and evasion of the immune system.⁴ One of the challenges was to deliver the siRNA across the blood-brain barrier.

The solution was to create a synthetic protein nanoparticle mixed with a tumor penetrating peptide (iRGD) and to load it with the siRNA. The nanoparticle was injected into the tail vein of mice who had glioblastoma. The technology proved to be quite successful with good targeting of the brain tumors, and clear reduction of STAT3 and phosphorylated STAT3 which confirm efficient delivery and uptake of the siRNA. As a result, the mouse survival rate was dramatically improved: seven out of eight mice survived while all of the control mice died after 28 days. “It is truly rewarding to see a technology that has been developed by students in the lab have such a pronounced effect, so far in mice, on a horrible disease, such as brain cancer,” said Dr. Lahann.

Still focusing on the STAT3 pathway, the team is now considering this technique for delivering siRNAs targeting a variety of cancer pathways. “The ability to efficiently deliver RNA bedside therapeutics to the brain tumor microenvironment using nanoparticles will expand the armamentarium and should enable us to treat and prolong the survival of brain tumor patients in the near future,” said Dr. Castro.

⁴ Gregory, J.V., Kadiyala, P., Doherty, R. et al. Systemic brain tumor delivery of synthetic protein nanoparticles for glioblastoma therapy. *Nat Commun* 11, 5687 (2020). <https://doi.org/10.1038/s41467-020-19225-7>

The Lahann lab has also developed a multi-compartment nanoparticle that can deliver different therapeutics, each placed in its own compartment within the box. A benefit of the compartments is to separate drugs that could react with each other. Compartments can also be engineered to release each payload independently according to different factors such as the intracellular pH concentration, or before and after entering a cell. Since it is likely that the most successful treatment for glioblastoma will be achieved through a combination of therapeutics, the compartmented box could be the perfect delivery container. “For example, to treat glioblastoma, we could put an siRNA in one compartment and paclitaxel, a chemotherapeutic drug, in another one, and release each separately. This is an excellent application for this technology, and we already have good results with this approach,” said Dr. Lahann.



Encouraged by these successful results, the team is interested in using synthetic protein nanoparticles to deliver plasmids (a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand) for gene therapy. They are engaged in a collaboration with Intergalactic Therapeutics, a small biotech company. Plasmids present additional challenges due to their size, which is much larger than siRNAs. The Lahann team is developing a new set of delivery modalities, and initial *in vitro* experiments in liver cells already show how certain modifications in the synthetic protein nanoparticle formula work well for gene therapy.

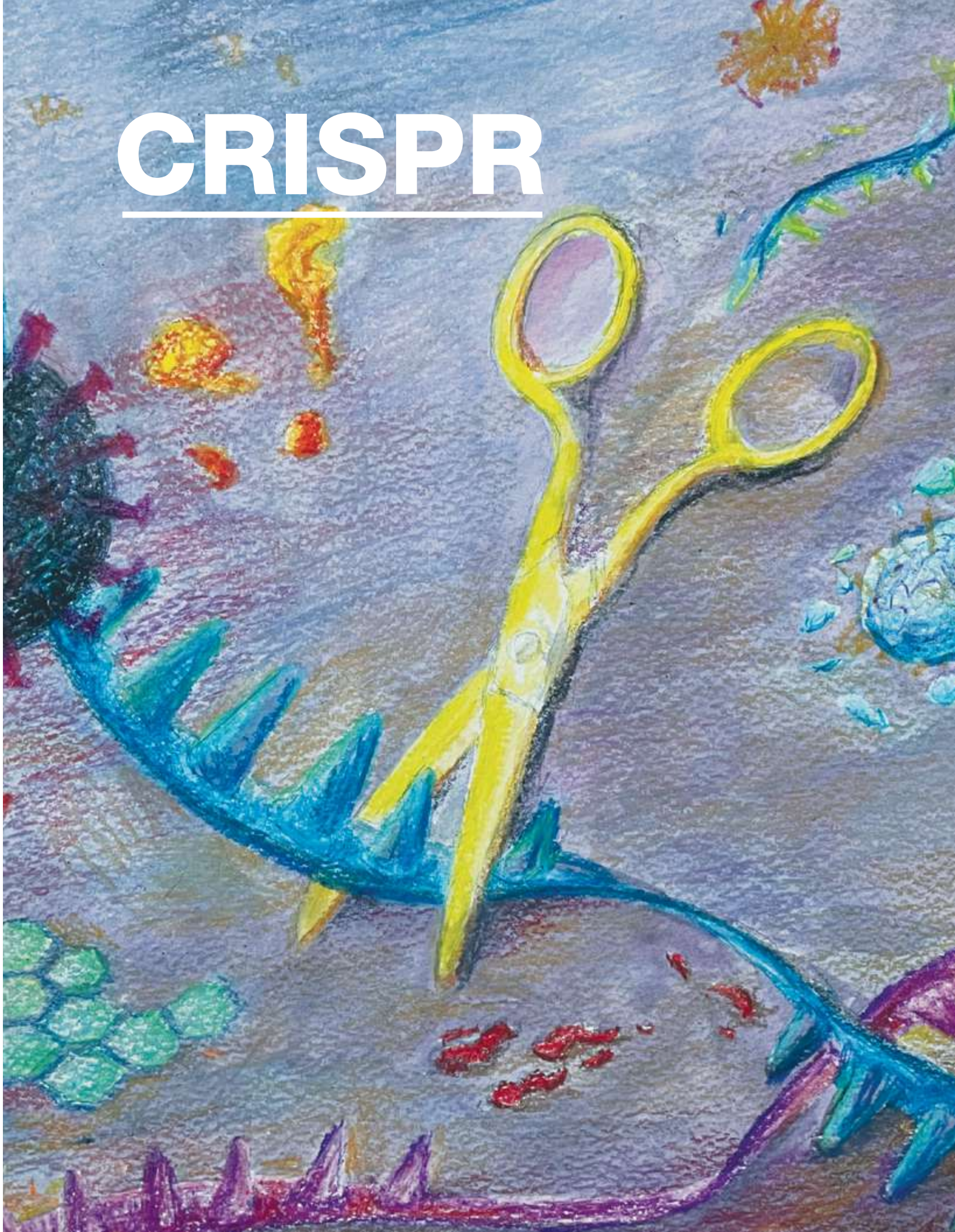
“Our lab has moved an immune mediated gene therapy viral platform to the clinic to treat glioblastomas. The possibility of expanding this technology using nanoparticles to deliver plasmids encoding immune stimulatory molecules could provide an excellent non-invasive therapeutic modality,” said Dr. Lowenstein.

This figure describes the process of making the particles used for delivery to the brain tumors. These nanoparticles are made of protein, a synthetic stabilizer, and the therapeutic payload, here siRNA against STAT3. The process to manufacture the particles is called electrohydrodynamic jetting. See footnote 4, p. 21.

Synthetic protein nanoparticles could also be used to deliver mRNAs and CRISPR Cas9. “We may have to change the proteins, or the way we engineer the particles, but, theoretically, I don’t see why we could not do this for mRNA or CRISPR,” said Dr. Lahann.

This technology has great potential for the delivery of many different therapeutics that could treat a range of diseases, including cancers in the lungs and liver. “We can engineer these nanoparticles for stability, size, composition, and compartments. We have a much better understanding of how to change the nanoparticles, and that’s what we need to move into clinical applications,” concluded Dr. Lahann.

CRISPR



The 2020 Nobel Prize in Chemistry



A 6:00 a.m. phone call woke up Jennifer Doudna. It was another Californian sunny morning, but the sun shone brighter for her on October 7, 2020, when an East Coast journalist asked her for thoughts on the Nobel Prize in Chemistry. She was fully awake when she heard that her dream came true: she was the co-laureate of the most prestigious award possible! And while the reporter revealed to Doudna the Swedish announcement, another call came in from Europe, this time from Emmanuelle Charpentier, the other co-laureate.

In the fall of 2020, while the greatest pandemic ever known to humanity was raging—and we cannot emphasize enough that it is a pandemic caused by an RNA virus and mRNA vaccines are now our greatest weapons against it—Charpentier and Doudna were awarded the highest prize in science bestowed in recognition of a contribution “for the greatest benefit of humankind.” These two women scientists are changing the course of human history, with “the development of a method for genome editing.” This method is founded upon decades of RNA research that was launched in the 1950s with the discovery of RNA.

Charpentier and Doudna collaborated and equally contributed to further understand and harness a natural phenomenon in which RNA and proteins associate to become “genetic scissors” that can cut DNA at a precise location. Called CRISPR (pronounced “crisper”) for clustered regularly interspaced short palindromic repeats, “this technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true,” the Nobel Committee said in announcing the prize.

It usually takes decades for such a tremendous impact from a discovery to reveal itself. However, the 2020 Nobel Prize in Chemistry was awarded for a discovery published only in 2012—one which is already changing the story of humanity.

With the following interviews and testimonials from University of Michigan scientists and medical practitioners, we further demonstrate the tremendous impact of this discovery on genetic and RNA research and therapeutics. CRISPR has quickly found its place in biomedical labs where it is used to investigate gene functions and gene regulations. It is starting to be used in therapies to destroy or replace defective genes that cause many serious diseases. Clinical trials are on the way, and this is only the beginning. The biomedical revolution is happening, launching humanity into the next chapter of its history.

1986

Yoshizumi Ishimo, a student at Osaka University in Japan, noticed five DNA repeats separated by “spacers” in *E. coli* bacteria.

2002–2003

During this period, Francisco Mojica, a graduate student at the University of Alicante in Spain, discovered that one of those spacer sequences matches a viral genetic sequence. As he learned about Ishimo’s findings, he started to realize that these repeats had an important role. He called them CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats).

Mojica kept searching and found that bacteria with CRISPR spacer sequences were immune from infection by a virus that had the same sequence, while bacteria without the spacer did get infected. When new viruses came along, the bacteria that survived were able to incorporate some of that virus’s DNA and thus create, in its progeny, an

acquired immunity to that new virus.

At about the same time, Ruud Jansen, from Utrecht University in The Netherlands, was studying similar repeats in tuberculosis bacteria, and he and Mojica corresponded.

Jansen published an article about genes that are associated with the repeat, using Mojica’s name CRISPR

to describe the repeat. He called these genes “CRISPR-associated” or “Cas.”

Eugene Koonin, from the U.S. National Center for Biotechnology Information, showed that the Cas system incorporated a piece of the viral DNA into the bacteria’s own DNA, creating an adaptive immunity memory.



Marie Curie



Emmanuelle Charpentier



Jennifer Doudna



Barbara McClintock

Women “for the greatest benefit of humankind”

No doubt, Emmanuelle Charpentier and Jennifer Doudna, like many women scientists, are quite fond of and inspired by Marie Curie who received the Nobel Prize twice for her discoveries in the field of radioactivity. She was the first woman to win a Nobel Prize in 1903, the first person and the only woman to win it twice (1903 and 1911), and the only person to win the Prize in two scientific fields, first in Physics and then in Chemistry, a field particularly dear to Alfred Nobel himself.

As a graduate from the Pierre and Marie Curie Institute in Paris, at the heart of the Latin Quarter, Emmanuelle Charpentier became a scientist in the aura of Marie Curie’s great achievements. Charpentier then received her Ph.D. from the Pasteur Institute.

As for Jennifer Doudna, a Harvard graduate who grew up surrounded by Hawaiian flora, she was greatly inspired by another woman Nobel Prize laureate, Barbara McClintock from Cold Spring Harbor Laboratory, who was awarded The Nobel Prize in Physiology or Medicine 1983 “for her discovery of mobile genetic elements.”

2005

Philippe Horvath and Rodolphe Barragou, a French team of scientists, confirmed Mojica and Koonin’s findings. They were also able to engineer sequences that could be added to a bacteria, replicating the natural way bacteria develop an immune response. These findings were published in *Science* in 2007.

2006

Jennifer Doudna investigated an enzyme known as Dicer that snips a long piece of RNA into short fragments. Dicer seeks out an mRNA molecule with a matching sequence, then uses a scissors-like enzyme to chop it up. Doudna discovered how to reengineer Dicer and published the method in *Science*.

2008

July, in Berkeley, CA, the first annual CRISPR meeting was attended by only 35 scientists.

In 2008, Luciano Marraffini and Erik Sontheimer of Northwestern University in Chicago, showed that the CRISPR system targets the DNA of the invading virus—rather than working through RNA interference. They suggested that the CRISPR complex could be used as a gene editing tool.

2010–2011

Emmanuelle Charpentier discovered the role of the tracer-RNA (tracrRNA) that takes long strands of RNA to make the small guide RNAs, and also plays a role in holding the CRISPR complex together.

March 2011, at a microbiology conference in Puerto Rico, Charpentier and Doudna entered into a collaboration.

2011–2012

Charpentier and Doudna further discovered that the tracrRNA keeps the CRISPR complex together. They engineered a CRISPR complex where they fused the tracrRNA and the CRISPR RNA (crRNA) to make a single-guide RNA which binds to a protein (Cas9) that cuts the DNA in a precise location. The possibility of using this technique for gene editing was recognized.

June 28, 2012, the results are published in *Science*. This publication supports the 2020 Nobel Prize in Chemistry’s nomination.

CRISPR biology

Over billions of years, bacteria and viruses have developed a love-hate relationship. On average, bacteria are about 140 times larger than viruses, but viruses can still threaten bacteria. Viruses have only one goal: to reproduce, as fast as possible. They do so by taking over host cells where they hijack the resources they need to reproduce. As a result, the host cells are usually depleted and killed.

Bacteria fight off these tiny persistent invaders by developing adaptive immune response strategies. One defense strategy is to prevent the reproduction of the virus by cutting its RNA or DNA. Another is to take a small segment of a viral genome and incorporate it within its own DNA, as a form of preparedness against future viral attacks.⁵ These biological strategies are implemented with CRISPR systems.

In nature, when a bacterium detects a viral DNA, it can produce an RNA that matches the sequence of the invading virus. This RNA associates with a protein and forms an enzyme, also called a CRISPR-associated system, that is guided by the RNA along the viral DNA until it eventually finds its matching sequence. Once positioned, the complex cleaves the strand on each side of the targeted area and the virus has been defeated.

In 2012, Drs. Charpentier and Doudna discovered how to harness CRISPR-Cas9 system to induce RNA-guided precision cuts at predetermined sites in the genome. This discovery ushered in a new era of genome editing to potentially cure a variety of genetic diseases. Mimicking nature, scientists can engineer different CRISPR systems. This technique is modular, which is very appealing to scientists who can adapt the tool to their needs.

The guide RNA is the “programmed” part of the system responsible for bringing the Cas9 protein to a specific area of DNA, while the Cas9 proteins can be compared to an engine, with different proteins conferring different properties to the system. Cas9 is the CRISPR protein that has been the most studied and used so far. The protein families of Cas12 and Cas13 can be used to target and cut specific sequences in RNA. These are used for example in diagnostic screening platforms. Cas3 is described as a “shredder” that edits large areas (see page 29). Since only a small slice of all microorganisms has been screened for CRISPR activity, additional Cas systems will undoubtedly be discovered that will provide an even more extensive CRISPR tool box to be used for therapeutics.

CRISPR therapeutics are still in their infancy, and scientists are working hard to improve the CRISPR complex’s precision in targeting genes. They are looking for techniques to increase the efficiency at which CRISPR edits cells as well as ways to avoid off-target effects or adverse immune responses.

⁵ For more information on viruses, see *RNA Translated, 2020, the year of the RNA virus.*

CRISPR technology

“MI” machinery improves CRISPR Cas9

CRISPR Cas9 is a very powerful system for precision targeting of specific sequences in the genome. It makes a cut and allows the cell to repair the break or replace a mutated sequence with a supplied wild-type DNA sequence using recombination, a procedure called “knock-in.”



Jifeng Zhang, Ph.D., Research Associate Professor, Internal Medicine, Medical School

Several technical aspects of CRISPR are still challenging and many labs are exploring ways to improve the efficiency of the genome editing. The knock-in efficacy rate is still low with a substantial number of off-target editing events. Of equal concern, the on-target insertion or deletion (indel) rates are often higher than those of the desired correction. Other challenges include the development of efficient and safe *in vivo* delivery system as well as methods to circumvent adverse immune responses. These issues are currently under intense investigation because of the potential of the CRISPR technology to cure a number of genetic diseases as well as cancer is enormous.

Research Associate Professors Jifeng Zhang and Jie Xu in the Department of Internal Medicine at the Medical School, have tackled some of these problems by developing a CRISPR Cas9 variant, fondly called meticulous integration Cas9, or “miCas9” (also a dual reference to Michigan). This CRISPR variant allows a very precise on-target gene editing while maximizing the editing success rate.

DNA double-strand breaks (DSB) are usually repaired through two primary pathways. The most common form is non-homologous end joining (NHEJ) repair that connects the two loose ends of the DNA break. This type of repair sometimes introduces errors that leave scars at the target site that are often not beneficial. The alternative is the homology directed repair (HDR) that occurs only in cells that have replicated their genome and have a homologous sister chromatid that can be used in the repair event. HDR is used with CRISPR to replace a mutated gene sequence by supplying a short piece of a homologous wild-type DNA sequence. The balance between NHEJ and HDR determines the outcome of the gene editing application.



Jie Xu, Ph.D., Research Associate Professor, Internal Medicine, Medical School

In 2016, Drs. Jifeng Zhang and Jie Xu and their collaborators discovered that a chemical compound called RS-1, an activator of RAD51 and a key player in the HDR pathway, significantly improves CRISPR mediated gene knock-in rates.⁶ They then went on to search for a molecule that could recruit many more RAD51 proteins to further increase the chance of HDR occurrences. They found a 36 amino acid peptide, which they named Brex27, that is part of RAD51 binding protein BRCA2. Fusion of this small motif to spCas9, a Cas9 derived from *Streptococcus pyogenes*, led to the creation of miCas9. In comparison to spCas9, miCas9 satisfactorily addresses efficacy and safety deficiencies by reducing the ways that other enzymes introduce mistakes. It is the first nuclease that can achieve this to the best of our knowledge.⁷

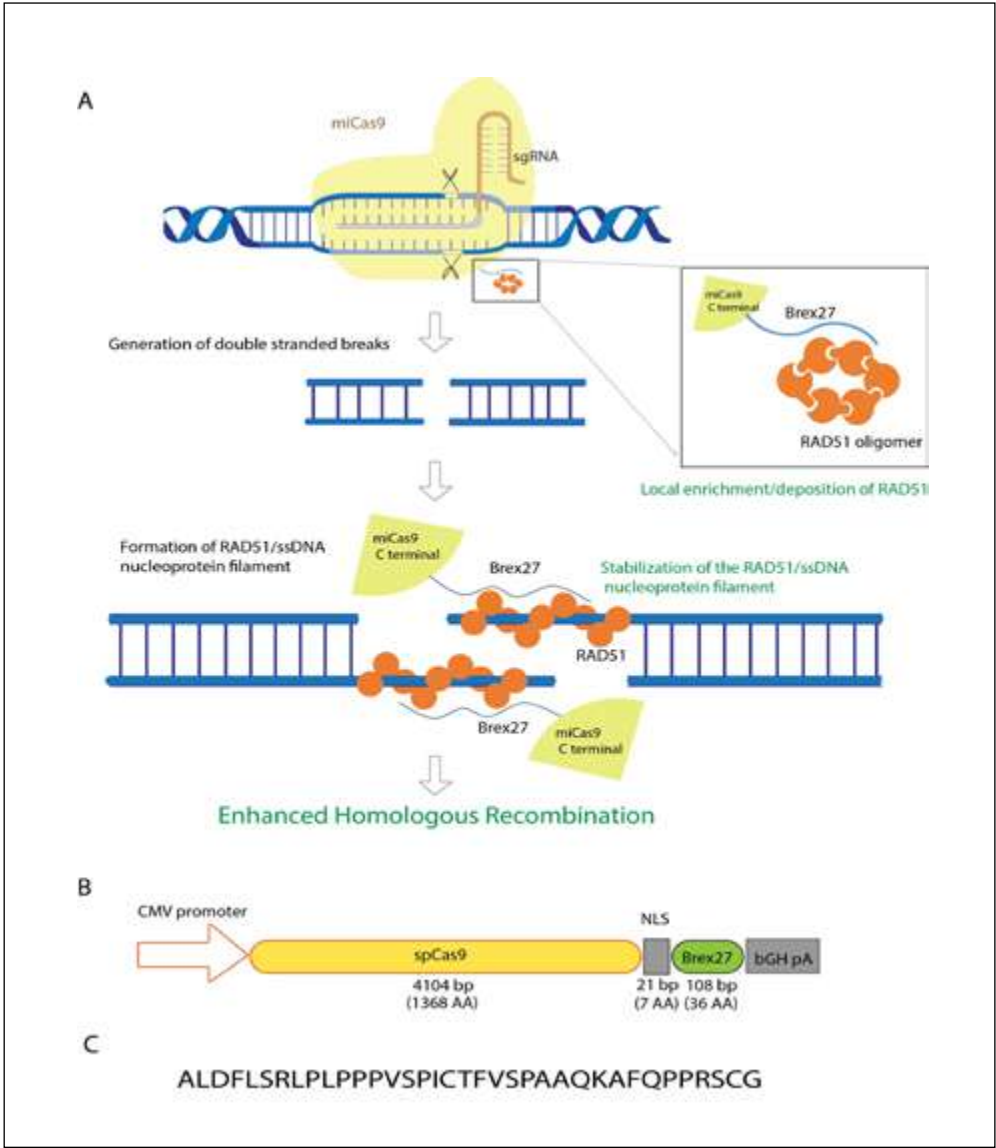
⁶ Song, J., Yang, D., Xu, J. et al. RS-1 enhances CRISPR/Cas9- and TALEN-mediated knock-in efficiency. *Nat Commun* 7, 10548 (2016). <https://doi.org/10.1038/ncomms10548>

⁷ Ma, L., Ruan, J., Song, J. et al. MiCas9 increases large size gene knock-in rates and reduces undesirable on-target and off-target indel edits. *Nat Commun* 11, 6082 (2020). <https://doi.org/10.1038/s41467-020-19842-2>

“The small size of Brex27 is advantageous,” said Dr. Xu. “Unlike some other fusion motifs to Cas9, adding Brex27 increases the size of spCas9 only by 2%. This is an important aspect because when it comes to *in vivo* delivery of therapeutic biologics, ‘size matters’ and any ‘room saving’ helps. In this regard, Brex27 is the smallest effective HDR promoting motif to date.”

The team believes that miCas9 may find broad applications in gene editing research and therapeutics. “miCas9 could be used to correct mutations in genes” said Dr. Zhang, while Xu added “for example, the mutated gene that causes cystic fibrosis.” Drs. Zhang and Xu are looking forward to testing miCas9 in animal models.

The team will continue to improve miCas9, seeking to strengthen the binding between Brex27 and the RAD51 protein to increase the recruitment of RAD51. The rationale is that the more RAD51 proteins are present, the less editing errors can occur. “There are many possible applications for miCas9 and hopefully miCas9-version 2 will further enhance the efficacy and safety of the CRISPR machinery,” concluded Dr. Xu.



This figure shows mi-Cas9 steps. Brex27 is a filament made of 36 amino acids from the BRCA2 protein that recruits RAD51 to the repair site. Courtesy Dr. Zhang.

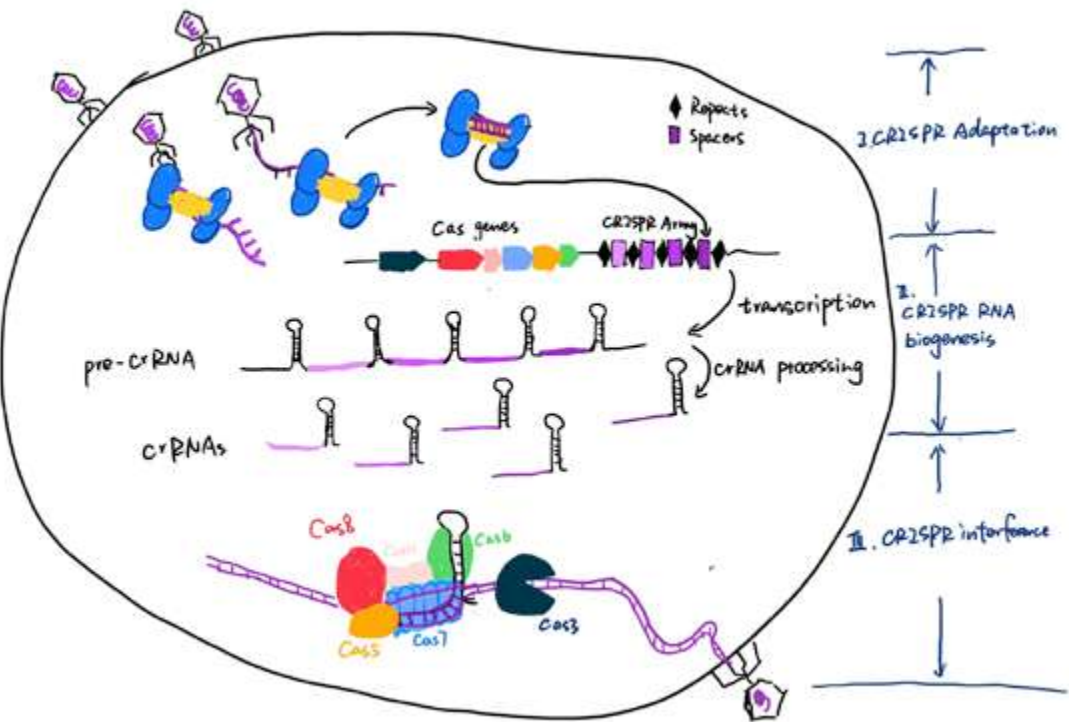
The Cas casting

In the microbial world, CRISPR complexes come in many shapes and forms, and scientists have only explored the capabilities of a few of these proteins in a limited number of microorganisms. The best understood so far are Cas3, Cas6, Cas9 and the families of Cas12 and Cas13. Each of these complexes has its own characteristics.

Dr. Yan Zhang, a biological chemist at the Medical School, is particularly interested in the CRISPR-Cas3 systems that represent more than half of all the bacterial CRISPR-Cas systems. Cas3 is a nuclease and helicase fusion enzyme that can break up longer sections of the genetic material as it goes along the DNA. It is comparable to a “DNA shredder with a motor.” The activity of Cas3 results in engineered human cells with targeted large chromosomal deletions. This Cas3 technology could be a powerful tool for exploring non-coding elements, removing integrated viral genomes, and interrogating structural variants impacting human diseases. This research is a collaboration between Dr. Yan Zhang and Dr. Ailong Ke from Cornell University, who brings complementary expertise in biochemistry and structural biology.



Yan Zhang, Ph.D., Assistant Professor, Biological Chemistry, Medical School



A sketch of CRISPR-Cas3 at work as an adaptive immune system in bacteria. The Zhang lab studies CRISPR-Cas biology, mechanism and application, and recently developed CRISPR-Cas3 into a “DNA shredder” technology for creating large genomic deletions in human cells. Artwork by Renke Tan, a graduate student in Dr. Yan’s lab.

CRISPR to screen and test gene functions



Sami Barmada, M.D., Ph.D.,
Angela Dobson
Welch and Lyndon
Welch Research
Professor, Associate
Professor, Neurology,
Medical School

CRISPR is quickly expanding the possibilities for molecular biology research and is becoming an essential tool to study the role of a specific gene or set of genes. A CRISPR system can target a particular gene and cut it so that when it is incorrectly repaired, it fails to produce a protein (“knocking out”). Once cut, it is also possible to replace the targeted section with a homologous piece of DNA that adds a fluorescent tag to the protein it encodes. With these techniques, scientists can explore the function and localization of a particular gene within cells. This understanding of the biology is foundational to the development of drugs and therapies that can precisely target a defective gene or pathways.

“CRISPR gives us outstanding flexibility for manipulating gene expression and assessing gene functions *in vivo*,” said Dr. Sami Barmada, a neurologist at Michigan Medicine who studies frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and other neurodegenerative diseases. “There are many clever and innovative applications of CRISPR, including turning on or off genes with lights or magnets—the possibilities are tremendous.”

“There are many clever and innovative applications of CRISPR, including turning on or off genes with lights or magnets—the possibilities are tremendous.”

“Knock outs”

Dr. Barmada’s lab conducted a small-scale screen of 12 genes responsible for RNA methylation. Using CRISPR Cas9, the scientists targeted each of these genes in primary neurons isolated from rodent brains, knocking them out one at a time, and determined whether these manipulations prevented neurodegeneration in models of ALS and FTD. In the process, they identified at least one candidate gene that significantly prolongs neuronal survival in mice when it is knocked out via CRISPR. This study was spearheaded by Michael McMillan, a Ph.D. candidate. Following up on this, Dr. Barmada’s team is looking into the mechanisms by which RNA methylation affects neurodegeneration in ALS and FTD, and whether this pathway may be targeted by genetic means or drugs to slow the disease in animals and, eventually, humans.

Similarly, Dr. Peter Todd, also a neurologist at Michigan Medicine (see page 35), uses CRISPR to study whether the loss of certain genes influences toxicity in models of ALS. If knocking out one of these modifier genes suppresses toxicity, targeting that gene with a small molecule inhibitor could be an effective therapy.

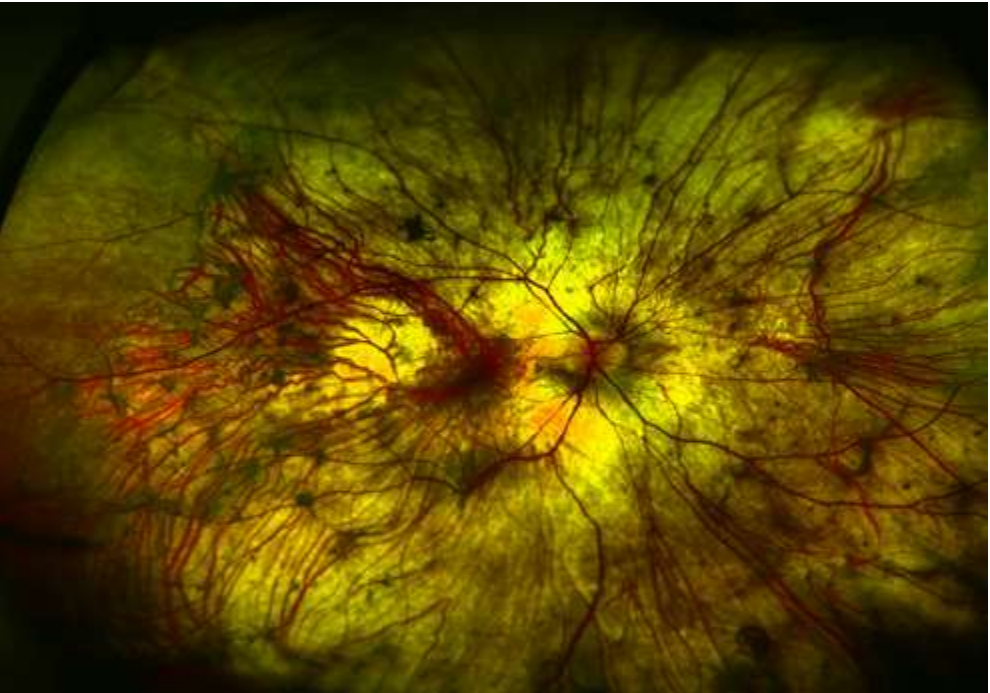
Dr. Donna Martin, a pediatrician and a geneticist at Michigan Medicine, works with stem cells at different stages of differentiation. She is particularly interested in the role of the *CHD7* gene that is involved in CHARGE syndrome pathology (see page 36). Her team observes cellular behaviors while controlling for the various levels of CHD7 protein. In collaboration with investigators at Case Western Reserve University in Cleveland and Washington University in St. Louis, they used CRISPR Cas9 to correct the mutated copy of the *CHD7* gene in cells from CHARGE syndrome patients. They obtained two sets of cells that are identical (isogenic cell lines) except for one single

base pair change in the DNA. Most often, scientists have to use publicly available normal controls, or compare across cell lines. With these CRISPR Cas9 edited cells, Dr. Martin’s team can now perform more rigorous comparative genome-wide studies.



Abigail T. Fahim, M.D., Ph.D.,
Assistant Professor,
Ophthalmology and
Visual Sciences
Kellogg Eye Center,
Medical School

Ophthalmic research is another area where CRISPR quickly became a favorite tool for functional studies. In her lab, Dr. Abigail Fahim, an ophthalmology researcher and clinician at Michigan Medicine who specializes in inherited retinal diseases, studies choroideremia, a very rare genetic disease that causes blindness and for which there is no cure. The choroideremia gene *CHM* plays an important role in the secretion and transport of proteins throughout the cell. Her team is studying how the defective choroideremia gene affects neighboring cells and cell death. By knocking out the choroideremia gene with CRISPR in induced pluripotent stem cells, she replicates the disease *in vitro*. Like Dr. Martin does with CHARGE syndrome cells, Dr. Fahim generates two controlled cell lines that she compares to study the impact of the missing gene that causes choroideremia. The choroideremia gene CRISPR knockouts are done in collaboration with the Human Stem Cell and Gene Editing Core, a U-M state-of-the-art biomedical facility. “CRISPR is a very convenient way to knock out genes,” she said. “We can get two perfectly identical cell lines, with only one variation, the knocked-out gene.”



Fundus photo (back of the eye) of a choroideremia patient.

Repairs and tags

When a DNA strand has been cut with CRISPR, specialized machinery in the cell attempts to repair the break. If synthesized pieces of DNA homologous to the sequences near the break are injected into the cell, they may be used by the repair machinery to fix the broken DNA strand (see miCas article, p. 27). The Barmada lab uses homology-directed repair to label cellular proteins with fluorescent or multi-functional tags, allowing them to ‘see’ these proteins using a microscope. Whereas before they would have had to use antibodies to detect proteins important for disease, CRISPR enables scientists to track these proteins and their behavior in living cells in real time and in unprecedented detail. “Assessing protein activity in live cells was and is a thorny problem, but now we can actually see these proteins using CRISPR to tag them,” explained Dr. Barmada. “It’s incredible and exceptionally helpful for what we want to do.” So far, the team has studied six disease-related proteins with this approach and patented one cell line that may prove invaluable for the development of new therapeutics for neurodegenerative diseases.

Mastering this technique paves the way for several innovative applications. For example, proteins can be labeled with photoconvertible tags, which can be used to measure how quickly a protein is made or degraded. Other tags make it possible to purify a protein and examine what it interacts with. “CRISPR-based tools add a whole level of flexibility that was difficult, if not impossible, to appreciate just a few years ago,” commented Dr. Barmada. “Once you have the ability to tag these proteins, it opens up a lot of doors.”

“CRISPR-based tools add a whole level of flexibility that was difficult, if not impossible, to appreciate just a few years ago.”

Making neurons and mutated cell lines

The Barmada lab also adapted CRISPR as an efficient means of creating mature neurons from human stem cells. Conventional approaches for differentiating stem cells into neurons can take 6-8 weeks or even longer. However, the directed expression of master transcription factors can considerably shorten this, while also generating more uniform and reproducible cultures of neurons. Using CRISPR and homology-directed repair, the team inserted a cassette allowing the inducible expression of transcription factors for creating forebrain neurons or motor neurons, the two types of cells affected in FTD and ALS, in only two weeks. “This approach is much more straightforward and robust than traditional methods and reduces quite a bit of the variability typical of stem cell differentiations,” added Dr. Barmada. “Although this technique involves some work upfront, the quality of cells, time savings and cost benefits are well worth it.”

These examples demonstrate well the power and versatility of CRISPR system to advance genetic research and create a new pool of knowledge that will be foundational for developing drugs and effective therapies.

CRISPR to model brain development and genetic epilepsy syndromes



Jack Parent, M.D., William J Herdman Professor of Neurology, Research Professor, Michigan Neuroscience Institute; Director of the Human Stem Cell and Gene Editing Core (HSCGE), Medical School

Dr. Jack Parent is a neurologist and stem cell specialist at Michigan Medicine who has extensively developed and applied CRISPR-based technologies to model genetic epilepsies and study brain development in cells. Dr. Parent has collaborated with many U-M scientists including in studies of genetic epilepsy syndromes, with Dr. Isom on Dravet syndrome (see page 12), and with Dr. Martin on CHARGE syndrome (see page 36). “This is what I love about Michigan, it is very collaborative,” said Dr. Parent.

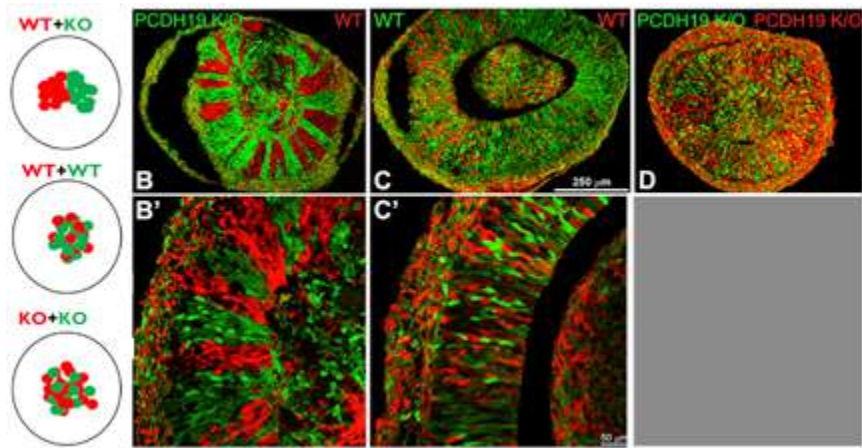
In Dr. Parent’s lab, scientists model what happens in the neurons of a patient who suffers from an epilepsy syndrome, using a variety of techniques. In one of them, they start with reprogramming blood or skin cells from patients to develop stem cells. From these, they grow cortical neurons that can be edited with CRISPR Cas9 to either make an epilepsy model or correct a mutation. They can control the production of a cell and at the same time knock out a gene from a stem cell that will eventually become a neuron. They can then observe the genes that trigger epilepsy and other symptoms, in a dish.

With CRISPR, this team has also created two cell lines that differ by only one gene mutation. In cases where there is no patient specimen available, they can use CRISPR to knock out one gene and grow two cell lines, one with and one without the expression of the encoded protein. This technique, used by Dr. Martin to study CHARGE syndrome and by Dr. Fahim in eye diseases, allows one to rigorously compare cells and establish the role played by a specific gene and its encoded protein.

Another technique used in Dr. Parent’s lab is CRISPR interference (CRISPRi) that suppresses the expression of a gene rather than cutting and editing it. They use a so-called dCas9 (deactivated Cas9) that cannot cut the DNA but represses the expression of the gene by enzymatically modifying the DNA or surrounding chromatin. Dr. Parent’s team is currently using this technique to screen nearly 20,000 genes to identify novel genes that are involved with brain malformations during brain development.

The Parent lab also developed a technique to create three-dimensional human brain organoids that allow investigators to study specific brain regions. They generate the organoids from genetic epilepsy patients to determine how brain development is altered, or in some cases use CRISPRi to suppress (“knock down”) selected genes in these brain organoids to make a focal lesion and study its impact on the brain development in a dish. They apply these techniques to study rare developmental brain diseases such as polyhydramnios, megalencephaly, symptomatic epilepsy syndrome (PMSE), and X-Linked Clustering Epilepsy (XCE).

XCE is one of the most common forms of epilepsy. It occurs in about one in 20,000 births. XCE is caused by the loss of function of the gene that produces protocadherin-19 (PCDH19) protein, which allows neuron interactions during development. This disorder is X-linked and only occurs in females when one of the two X chromosomes is affected, producing cells that are either PCDH19-negative or PCDH19-positive and that cannot interact. If the single X chromosome in males carries the mutation, the individual is not sick as other genes can compensate for the



Abnormal cell sorting in human female brain organoids; X-linked clustering epilepsy brain organoid models (WT: wild type; KO: knock out). Courtesy Dr. Parent.

deficiency. In a new collaboration with Ionis Pharmaceuticals, the team is using antisense oligonucleotides (ASOs) to suppress the gene that has the normal copy. The cells then have no PCDH19 protein at all and may behave normally.

Like Dr. Barmada, this team also uses CRISPR to add sequences to genes to generate proteins with fluorescent markers that permit the study of the production of proteins and localization inside cells.

“CRISPR is just getting better and better,” commented Dr. Parent. “Scientific advances are likely to be more important in activating and suppressing multiple genes, rather than editing them.”

Dr. Parent is the Director of the University of Michigan (U-M) Human Stem Cell and Gene Editing (HSCGE) core facility. Created in 2015, the HSCGE core provides a wide range of services in stem cell and gene editing technologies to support the neuroscience research community at the U-M and beyond. It offers state-of-the-art resources and expertise for human pluripotent stem cells (hPSCs) for basic research, drug discovery, and cell therapy. Since its creation, about 30 U-M faculty and 15 external users have benefited from this core research facility.

Dr. Parent, along with Dr. Lori Isom, partnered with nearly a dozen other institutions on the NIH-funded Epilepsy Center Without Walls project that ran from 2014 to 2020, bringing clinical and basic researchers to study SUDEP. Since last fall, Drs. Parent and Isom have co-directed another Epilepsy Center Without Walls project known as EpiMVP. This NIH-funded program gathers six institutions to study the functional effects of mutations in a set of epilepsy genes using stem cell, rodent, and zebrafish models. Partners from Cornell University, Northwestern University, UCSF, and St Jude’s Children’s Hospital, led by U-M, are using the models to understand genetic variants of uncertain significance (VUS). The results from this multi-institution collaboration should distinguish which mutations actually cause a given severe epilepsy syndrome from those that are benign, and help physicians with treatment plans.

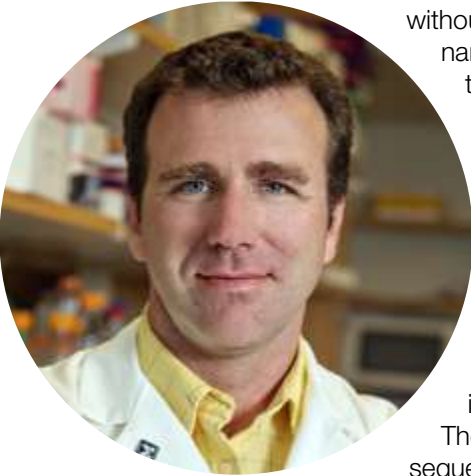
Each day, these novel techniques are bringing foundational research results closer to the bedside. It is also the hope that these technologies, themselves, can be used as therapies, or, even better, definitive cures.

Toward gene therapy: not cutting, just delivering

Most genetic diseases are caused by either the over-expression or under-expression of a gene or a set of genes, a complex process that is controlled by epigenetic reprogramming and activity of transcription factors. As with ASOs, scientists are looking for ways to harness the RNA guiding and delivery capability of a CRISPR system to stimulate or inhibit specific gene expression, without actually editing the gene. Two teams are following this path to try to cure two rare genetic diseases: fragile-X syndrome and CHARGE syndrome.

CRISPR to reactivate genes

In 2014–2015, a form of CRISPR was developed that could activate or inhibit genes without cutting them. Called endonuclease deficient Cas9 (dCas9, also nicknamed “dead Cas”), it lost its endonuclease activity (the cutting machinery) through a designed mutation, while preserving its capability to bind to an RNA and be directed to a specific DNA target. A dCas9 system can be fused with transcriptional activators that alter gene expression through epigenetic reprogramming, either inhibiting (CRISPRi) or activating (CRISPRa) transcription, without changing the DNA sequence. Other proteins are being studied that could perform similarly to Cas9, for example the dCas12a system can target several genes at the same time.



Peter Todd, M.D., Ph.D., Bucky and Patti Harris Career Development Professor of Neurology, and Professor of Neurology, Medical School

Dr. Todd, a neurologist at Michigan Medicine, studies fragile-X syndrome, a monogenetic condition that is the most common cause of autism and intellectual disability. It affects about 1 in 4,000 people, more often boys. The disease is caused by an abnormally long repeat at the beginning of a DNA sequence on the X chromosome. This repeat becomes unstable and enlarges over generations. It eventually causes the DNA to become methylated, which in turn switches off gene expression, impacting the production of RNA and encoded proteins.

In the lab, the Todd group has successfully used a dCas9 system fused to a transcriptional activator (CRISPRa) to target the abnormal repeat and turn the gene back on. They were able to target that gene very selectively, yielding a fifty-fold increase in RNA levels from what it was in the off state. “These results are very encouraging,” commented Dr. Todd. “If we can reactivate genes that were inactivated, we’re hoping that it would treat the disease.”⁸

In 2016, Dr. Todd’s lab used dCas9 in human cell lines, and then in neurons derived from embryos that had the fragile-X repeat. dCas9 was first programmed to target the abnormal repeat, but it turned out that this sequence is not unique in the human genome. Since its targeting could not be selective enough, the scientists adopted another strategy to activate the gene in stem cells. They obtained the same results at the progenitor cell stage, which is the second differentiation level for cells that will become neurons. Now at the neuronal level, the team is investigating techniques that would keep gene expression on and boost production of the protein over a long period of time.⁹

⁸ Targeted Reactivation of FMR1 Transcription in Fragile X Syndrome Embryonic Stem Cells, Jill M Haenfler, Geena Skariah, Caitlin M Rodriguez, Andre Monteiro da Rocha, Jack M Parent, Gary D Smith, Peter K Todd, *Front Mol Neurosci.*, 2018 Aug 15;11:282. doi: 10.3389/fnmol.2018.00282

⁹ A native function for RAN translation and CGG repeats in regulating fragile X protein synthesis. Rodriguez, C.M., Wright, S.E., Kearse, M.G. et al. *Nat Neurosci* (2020). <https://doi.org/10.1038/s41593-020-0590-1>

Another research group at MIT is investigating the possibility of modifying DNA to suppress inhibiting methylation to make the gene more active.¹⁰

“All these effects have some possible therapeutic benefits, but they are not as permanent as with cutting,” said Dr. Todd. However, cutting a gene requires a very high level of precision that is yet not consistently achieved with CRISPR.

CRISPR to further stimulate the one healthy gene



Donna Martin, M.D., Ph.D., Chair, Department of Pediatrics, Ravitz Foundation Endowed Professor of Pediatrics and Communicable Diseases, Professor of Human Genetics, Medical School

Dr. Donna Martin has focused her research on CHARGE syndrome, a genetic disease that affects hearing, vision, and presents facio-cranial malformations with possible congenital heart defects. In addition to cognitive developmental issues, psychiatric symptoms can be associated with autistic traits, and aggressive and obsessive-compulsive behaviors. CHARGE syndrome affects about one in 10,000 persons. It is caused by the loss of one of the two copies of the gene *CHD7* that acts in a broad way to regulate the expression of other genes in cells.

Dr. Martin’s team uses dCas9 that is hooked up to an epigenetic enzyme that activates gene expression (CRISPRa). The dCas9 complex selectively targets the wild-type copy of the gene and promotes it to produce more CHD7. So far, Dr. Martin’s team has demonstrated that, with CRISPRa, it is indeed possible to upregulate *CHD7* in cells. Their next step is to test this technique on mouse models of the CHARGE syndrome.

Unfortunately, individuals with the CHARGE syndrome exhibit multiple developmental defects that may be challenging to reverse even when restoring adequate CHD7 expression. For example, if the heart did not form normally, correcting CHD7 expression cannot reverse this defect. It is still not clear how the psychiatric manifestations are associated with *CHD7* deficiency, and if they could benefit from CHD7 correcting treatments. “If it could be effective and given *in vivo*, it would be amazing for these patients and their families!” added Dr. Martin.

Dr. Martin’s ultimate goal is to develop therapies for CHARGE syndrome patients. “If we can upregulate that normal copy of *CDH7* in specific cells, at specific times, and without severe side effects and if it is indeed effective in influencing how the cells are functioning, then it could reduce the suffering of these patients,” she concluded.

¹⁰Rescue of Fragile X Syndrome Neurons by DNA Methylation Editing of the FMR1 Gene, X Shawn Liu, Hao Wu, Marine Krzisch, Xuebing Wu, John Graef, Julien Muffat, Denes Hnisz, Charles H Li, Bingbing Yuan, Chuanyun Xu, Yun Li, Dan Vershkov, Angela Cacace, Richard A Young, Rudolf Jaenisch, *Cell*. 2018 Feb 22;172(5):979-992.e6. doi: 10.1016/j.cell.2018.01.012

CRISPR to cut and cure

KLIPPING tumors



Mats Ljungman, Ph.D., Professor of Radiation Oncology and of Environmental Health, Medical School, Co-Director of the Center for RNA Biomedicine

In his quest for a precision cancer therapy, Dr. Mats Ljungman, Professor of Radiation Oncology and co-director of the Center for RNA Biomedicine, is exploring whether CRISPR technology could be used to selectively attack cancer cells. While most efforts involving CRISPR are focused on genome editing, the CRISPR machinery could also be used as a molecular weapon to slice up chromosomes of cancer cells. Research has shown that chromosomes may undergo a “catastrophic” event early in the process of carcinogenesis causing multiple breakages. While many cells die in such events, some of them repair the damaged chromosomes in ways that give them the power to multiply faster and to form tumors. Such chromosome rearrangements bring into close proximity pieces of chromosomes that are normally far apart. The formation of chromosome rearrangements is unique to cancer cells and is observed across all forms of cancer. “This is our Achilles’ heel, right there,” said Dr. Ljungman, “and we could use CRISPR to specifically target these chromosome rearrangement junctions and cut tumor DNA strands similarly to what is done with radiation therapy, but without affecting normal cells.”

Dr. Ljungman and his team are using a modified version of CRISPR that requires two guide RNAs, instead of the usual one, and two dCas9 complexes. The cutting of the DNA to kill the cancer cell is then left to an endonuclease that is added to the machinery but needs to homodimerize in order to become active. By designing a pair of guide RNAs to specifically bind to both sides of a chromosome rearrangement junction in the cancer cell, the CRISPR machinery can be brought in and activated at the site of the to-be-cut location on the DNA. Dr. Ljungman and his research team, which includes Dr. Huibin Yang, Radhika Suhas Hulbatte, Natalie Gratsch and Lauren Hertzelt, have generated proof-of-concept for this approach in multiple cancer cell lines both in cell culture and *in vivo*.

Cancer “Mars shot”

Dr. Ljungman formulated his idea in 2016, at the time when the National Cancer Institute launched the Cancer Moonshot initiative, with Congress authorizing \$1.8 billion in research funding over seven years. During a weekly lab meeting, Ljungman presented his ideas and joked that “this project is shooting for Mars!” He named the approach Precision KLIPP Therapy — “KLIPP” means either “cut” or “opportunity” in Swedish— and hired Dr. Huibin Yang to head up the project. It took two years to design all the CRISPR reagents needed and to create cancer cell lines that could turn on these reagents at will to study the effects on the cancer cells. “The day when Huibin showed me the images of two bright dots in the nucleus of the targeted cells was exhilarating! After two long years of dedicated hard work, we had our answer— we got our CRISPR machine to cut the DNA of the cancer cell!”



That tumors harbor hundreds of chromosome rearrangements in their genomes has been known for over a hundred years. Yet, no cancer-directed therapy has so far attempted to exploit this common hallmark of cancer cells. “We are now entering a very exciting period where genome sequencing and CRISPR technology make it possible to identify and precisely target these cancer-specific rearrangements,” Dr. Ljungman



KLIPP TEAM

From left to right: Mats Ljungman, Radhika Suhas Hulbatte, Huibin Yang, Lauren Hertzner and Natalie Gratsch. Photo: Elisabeth Paymal

explained. “Furthermore, rapid advances in the development of delivery systems, such as lipid nanoparticles used to deliver mRNA for COVID vaccinations, opens the door for future clinical applications for Precision KLIPP Therapy.”

Dr. Ljungman and his team started the testing of their technique on colon cancer cells. Encouraged by their early successful results, they then turned to bladder cancer and partnered with Dr. Phillip Palmbos, Assistant Professor of Internal Medicine, who is a clinician-scientist specializing in bladder cancer. “Localized bladder cancer is a major clinical problem. As an initial test site for Precision KLIPP Therapy, the bladder confers a great advantage for delivery in that the CRISPR reagents can be instilled at high concentration directly into the bladder without the risk of systemic exposure. This is important in the early testing of this approach since unwanted side effects of the CRISPR reagents in other organs have not been ruled out,” Dr. Palmbos explained. The recent publication in *The New England Journal of Medicine*¹¹ of the first systemic in-human treatment with CRISPR to specifically knock out a disease-causing gene in the liver of patients showed that it is a safe therapy. It opens the door for many more genome editing treatments involving CRISPR. “This study is very encouraging and represents a watershed moment for the CRISPR field and its clinical applications,” added Dr. Ljungman.

The KLIPP team has generated CRISPR mRNA in large quantities *in vitro* that will be used for these studies, and they have shown that the CRISPR complex can be expressed in the nucleus of the cancer cells grown in culture. They are now taking the next step to target tumors *in vivo*. The Ljungman group has teamed up with Dr. Anna

Schwendeman, Professor of Pharmaceutical Sciences, who has extensive expertise in nanoparticle development both from her time in industry as well as at the University of Michigan. Dr. Schwendeman and her team are currently developing lipid nanoparticle delivery systems for the Precision KLIPP Therapy that will be used in pre-clinical testing for bladder cancer. “With the success of lipid nanoparticle delivery of mRNA vaccines against COVID-19, there is tremendous interest in using these techniques for delivery of CRISPR mRNA for the treatment of a whole range of diseases,” Dr. Schwendeman said.

Precision KLIPP Therapy is directed to attack structural targets in chromosomes that are common and unique to cancer cells. “Our approach always targets chromosome rearrangement junctions, regardless of the type of cancer. Therefore, our treatment could be considered as a ‘universal targeting treatment,’” Dr. Ljungman added. Tumor heterogeneity is a major obstacle for most cancer therapeutics because of the emergence of resistant clones. Here, as well, Dr. Ljungman thinks that there are ways for Precision KLIPP Therapy to get around treatment resistance. “Chromosome rearrangements occur early in tumorigenesis and will be common in most clones that grow out and should therefore be targetable. However, when resistant clones grow out, we will biopsy them and whole genome sequencing would allow for the identification of new chromosome rearrangement junctions that can be targeted. In this way, there is always hope for the patient even if the tumor comes back.”

“In this way, there is always hope for the patient even if the tumor comes back.” —Mats Ljungman, Ph.D.

To keep this project moving forward, Dr. Ljungman has written 30 grant applications during the last three years and has been fortunate to obtain internal pilot funding from M-Cubed, KickStart and MTRAC. However, this funding only covers part of what is needed for the project. “With new and risky projects, the burden of proof is on the investigator and the reviewers should be critical. I have learned a lot from the reviewer’s comments and suggestions,” Dr. Ljungman acknowledged. The KLIPP team received some great news on a recent R21 application to NCI for the development of the Precision KLIPP Therapy for bladder cancer and this support should allow to further investigate this therapeutic approach.

While bladder cancer is the initial focus for Dr. Ljungman and his team, they have also partnered with other oncologists: Dr. Karen McLean, Associate Professor of Obstetrics and Gynecology, to target ovarian cancer, Drs. Maria Castro and Pedro Lowenstein, Professors of Neurosurgery, to target glioblastoma, and Dr. Erika Newman, Associate Professor of Pediatric Surgery, to treat pediatric neuroblastoma. In addition, collaborations with Dr. Joerg Lahann, Professor of Chemical Engineering, and Dr. Nils Walter, Professor of Chemistry, are ongoing for the development of novel nanoparticle delivery systems and for single molecule microscopy studies, respectively. “The University of Michigan offers great opportunities to collaborate from bench to bedside and I am very fortunate to be able to work with these talented and dedicated colleagues,” Dr. Ljungman said.

The KLIPP team is currently working on their first manuscript describing proof-of-concept for the Precision KLIPP Therapy approach. They have filed a patent for the technology with the University of Michigan Tech Transfer Office.

¹¹ CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis, Julian D. Gillmore, M.D., Ph.D., Ed Gane, M.B., Ch.B., Jorg Taubel, M.D., Justin Kao, M.B., Ch.B., Marianna Fontana, M.D., Ph.D., Michael L. Maitland, M.D., Ph.D., Jessica Seitzer, B.S., Daniel O’Connell, Ph.D., Kathryn R. Walsh, Ph.D., Kristy Wood, Ph.D., Jonathan Phillips, Ph.D., Yuanxin Xu, M.D., Ph.D., et al., *The New England Journal of Medicine*, June 26, 2021, DOI: [10.1056/NEJMoa2107454](https://doi.org/10.1056/NEJMoa2107454)



Left to right: Radhika Suhas Hulbatte, Huibin Yang, Lauren Hertzner and Natalie Gratsch in Mats Ljungman’s lab. Photo: Elisabeth Paymal

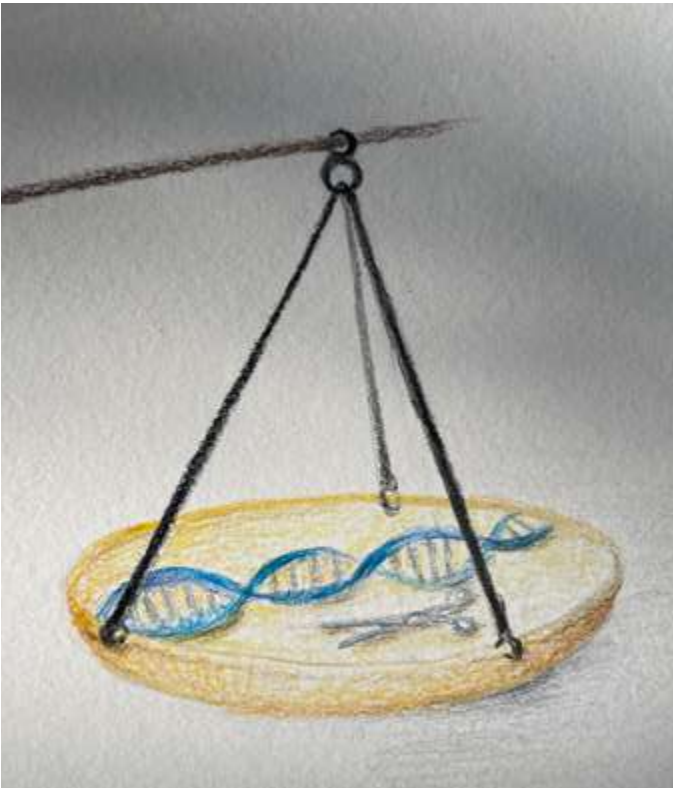
CRISPR targets the eye

Dr. Fahim (see page 31) is also involved with a multi-site clinical trial in Phase I/II, run by Editas Medicine. The study seeks to cure Leber Congenital Amaurosis (LCA), a very rare inherited eye disorder that causes severe vision loss at birth. LCA is found in one to two out of every 100,000 newborns. The CEP290 gene is the most common cause of LCA: a common mutation in CEP290 creates a splice site that incorporates unwanted sequence into the gene, and hence into the finished protein product. The clinical trial uses CRISPR to knock out the splice site and provoke a simple DNA repair. This therapy is delivered surgically, with an injection underneath the retina where it gets absorbed over the next day. “CRISPR is perfect for this disease because if a few base pairs of intron sequence are lost in the repair process, it should not matter,” explained Dr. Fahim. Although it is difficult to know how many cells will be effectively edited in a patient, if this treatment proves successful, it could be a permanent cure.

“With these clinical trials, we’re happy to be able to have more options for our patients, although this is the very beginning of these therapies.”
—Abigail Fahim, M.D., Ph.D.

Dr. Fahim and her U-M colleagues select clinical trials to bring to Kellogg based on research data from animal models that show evidence of safety as well as plausibility and efficacy. “With these clinical trials, we’re happy to be able to have more options for our patients, although this is the very beginning of these therapies,” said Dr. Fahim.

Practice and Ethics of Human Genome Editing



Genome editing offers great hope for millions of patients and their families who suffer from incurable devastating diseases. Rare genetic diseases alone affect an estimated 25 to 30 million Americans. Recent clinical trial successes in several of these patients pave the way for other cures. However, hopes for cures have been disappointed in the past. Breakthroughs must still be viewed cautiously, considering there are no guarantees with a brand-new technology.

Human genetic selection is integral to the history of humankind, be in the form of social rules, taboos or religious proscriptions on who we can and cannot have children with. These rules aim to regulate who we are and the society we live in. And, at times, genetic selection has been used to commit horrifying human rights violations. Vivid images come to mind: racial supremacy, genocides, forced sterilization, or “playing God” to name a few. The latest developments in genome editing revive the ghosts of these eras, at a time when our society is facing deep social injustice and when the values associated with science are being questioned.

Certain genetic diseases are more frequent in particular ethnicities, as for example Tay-Sachs disease among Jewish people of Ashkenazi descent, cystic fibrosis among Caucasians, and sickle cell disease among people of African descent.¹² Curing these diseases with highly sophisticated technologies brings up important questions around social justice and racism.

There is also a definite tension between the extraordinary therapeutic relief that gene editing can bring to patients and the dangers of developing and implementing techniques that can have horrifying consequences. Although eugenics debates are not new, gene editing developments require us to think about these issues again, and in pressing terms.

In response, the scientific community has globally risen to advocate for transparency and ethical regulations for the development and practice of human genome editing, at national and international levels. Leaders in the field of CRISPR development have immediately rallied to advocate for policy and regulations of genome editing.

In the following article, we briefly describe what the issues are and how some of them are currently addressed. We hope to inform and support engagement and dialog between the different stakeholders.

¹² *The Troubled Dream of Genetic Medicine; Ethnicity and Innovation in Tay-Sachs, Cystic Fibrosis, and Sickle Cell Disease*, Keith Walloo and Stephen Pemberton, Johns Hopkins University Press, 2006-04-20

The ethical framework

Genes exist in all living organisms and contain the information needed to specify traits. Gene editing is an irreversible process that permanently modifies a gene’s expression and the function of its encoded protein. It can be performed either on germline cells, which are the cells that will transmit genes to the next generation of individuals, or on somatic cells that do not transmit genes to offspring. The gene editing goals can be therapeutics, to cure or to prevent diseases, or enhancement of appearance and performance. For many stakeholders, these crucial distinctions provide a framework to discuss genome editing ethics in humans. We focus here on gene editing for therapy rather than enhancement.



J. Scott Roberts, Ph.D., Professor, Health Behavior & Health Education Co-Director, Dual-Degree (MPH/MS) Program in Public Health and Genetic Counseling Director, ELSI Research Training Program, School of Public Health

The discussion is complex with multiple dimensions. In an interview, Dr. Scott Roberts, a Professor of Health Behavior and Health Education in UM’s School of Public Health, and an expert in the ethical, legal, and social implications of advances in genomic science and technology, introduced the following ethical issues, all of great importance.

All medical procedures should receive a patient’s informed consent. However, embryos, infants, and children rely on their family’s decision. Some patients or their parents do not understand well the risks associated with a procedure, and their hopes might influence their judgment. Of course, future generations who would inherit an edited gene on a germ line cannot give consent.

Equity and social justice are of concern since genome editing techniques are still extremely costly and rare, limiting who can access them and benefit from them.

What are the goals of the genome editing? Are there therapeutic alternatives? Genome editing research may be the best hope to cure patients with certain diseases. To stop such research would raise another set of ethical issues.

Who is to give final approval for the practice of genome editing? Historically, the governance of genome editing has been left to experts who can understand the science but might be less aware of broad societal implications. There is a recommendation for bridging the gap between scientists and the general population.

Considering the intricacy of each of these points, Dr. Roberts added that “there is a real tension between rapidly advancing science to benefit patients as soon as possible and implementing proper regulations.”

Jesse Gelsinger (1982–1999)

In 1999, 17-year-old Jesse Gelsinger, a patient with a genetic disease called ornithine transcarbamylase (OTC) deficiency received a gene therapy treatment that killed him. Several ethical points had been abused and his death created a massive public reaction against genome editing. First, Gelsinger had not been properly informed of the risks of the experimental therapy. Second, the impact of his disease was relatively manageable, and, third, the lead scientist had a conflict of interest while he encouraged his 17-year-old patient to take the experimental treatment. This tragic case alerted the general publics about some of the dangers of unethical practice with gene therapy.



Melissa Creary, Ph.D., M.P.H., Assistant Professor of Health Management and Policy Senior Director, Office of Public Health Initiatives, American Thrombosis & Hemostasis Network, School of Public Health

Genome editing involves many stakeholders, including patients and their families, disease advocacy associations, the public and private sectors, religious groups, federal funders like NIH, and representatives in research and science. These stakeholders have different perspectives that need to be brought together.

Social justice in health

As a research funding agency, the NIH plays an important role in supporting diversity and equity, including assurance that human subjects research is conducted with diverse populations. For example, in cancer, genetic variants have been studied mostly in white European people, and there is a knowledge gap about cancer risk in other populations. This bias has consequences for preventive medicine recommendations. “Issues of social justice are very important to keep in mind also with genome editing,” added Dr. Roberts. “In the past, research has not been as inclusive as it should have been.”

Dr. Melissa Creary, an Assistant Professor of Health Management and Policy in U-M’s School of Public Health and a health equity scholar, recognizes that scientists who are curing sickle cell disease with CRISPR are making very important discoveries that can and should benefit everyone. She also views this biomedical revolution as an extraordinary opportunity for these scientists to think deeply about their role in society and the values they support.

In some cases, health equity, defined as social justice in health, has become an engine to give access to treatments to patients who would not otherwise have access to it. While this is a good thing, Dr. Creary questions what happens when social justice becomes profitable and intertwined with business, research funding, and engaging with a population that is very disadvantaged. “Although there is an attempt at addressing social justice via this technology, the sickle cell disease population has had generations of injustice that will affect access to technological advances and that still need to be addressed. We have to think of this technology in both broader historical and longitudinal context and how society disadvantages these populations to begin with. Racism is generational, cumulative and embodied, and cannot be fixed with a thin layer of justice via technology.”

Stakeholder dialogs and engagement

To help scientists deeply think about these issues, and integrate them into their practice, the NIH created the Ethical, Legal, and Social Implications (ELSI) program. This interdisciplinary program was launched in 1990 at the beginning of the Human Genome project that was led by former U-M Professor Francis Collins. This program aims to inform and engage scientists with ethics. “When scientists partner with these ethics and society scholars, they all learn from each other,” said Dr. Roberts, who directs the ELSI program at U-M. “This program offers a very helpful framework for scientists and clinicians who are confronted with these difficult questions. To integrate such a program with professional licensing and continuing education is a great way to keep engaging scientists in the ethics debates.”

Daniel Thiel, a Ph.D. candidate in the School of Public Health and an ELSI scholar, explained that there is a risk of losing control over the use of genome editing that will create more social injustice. He also warned about the potential rise of a eugenics market for substandard quality genome editing services comparable to what is currently happening with stem cells for which there is a lack of quality control standards.



Daniel Thiel, Ph.D. Candidate, Sociology/Health Management & Policy, School of Public Health and College of LSA, ELSI Scholar

“I wish for more productive exchanges between experts and the public in these debates,” said Thiel, who has been studying how CRISPR is discussed in the media. “I’ve found that the public has not been included in these discussions, and we’re very good at delaying these debates. However, it takes a lot of time to have a social conversation, and the science of CRISPR is moving extremely fast.”

“It takes a lot of time to have a social conversation, and the science of CRISPR is moving extremely fast.”

Dialogs between scientists, stakeholders, patients, advocates on either side, and policy makers are important to inform and decide which kind of society we want to live in. Our individual and societal values are enmeshed with the genome editing possibilities brought about by CRISPR technologies. We all are stakeholders in the society we live in and, as parents and citizens, we have important and difficult decisions to make to shape the world we want to live in.

Research Ethics and Compliance at the University of Michigan

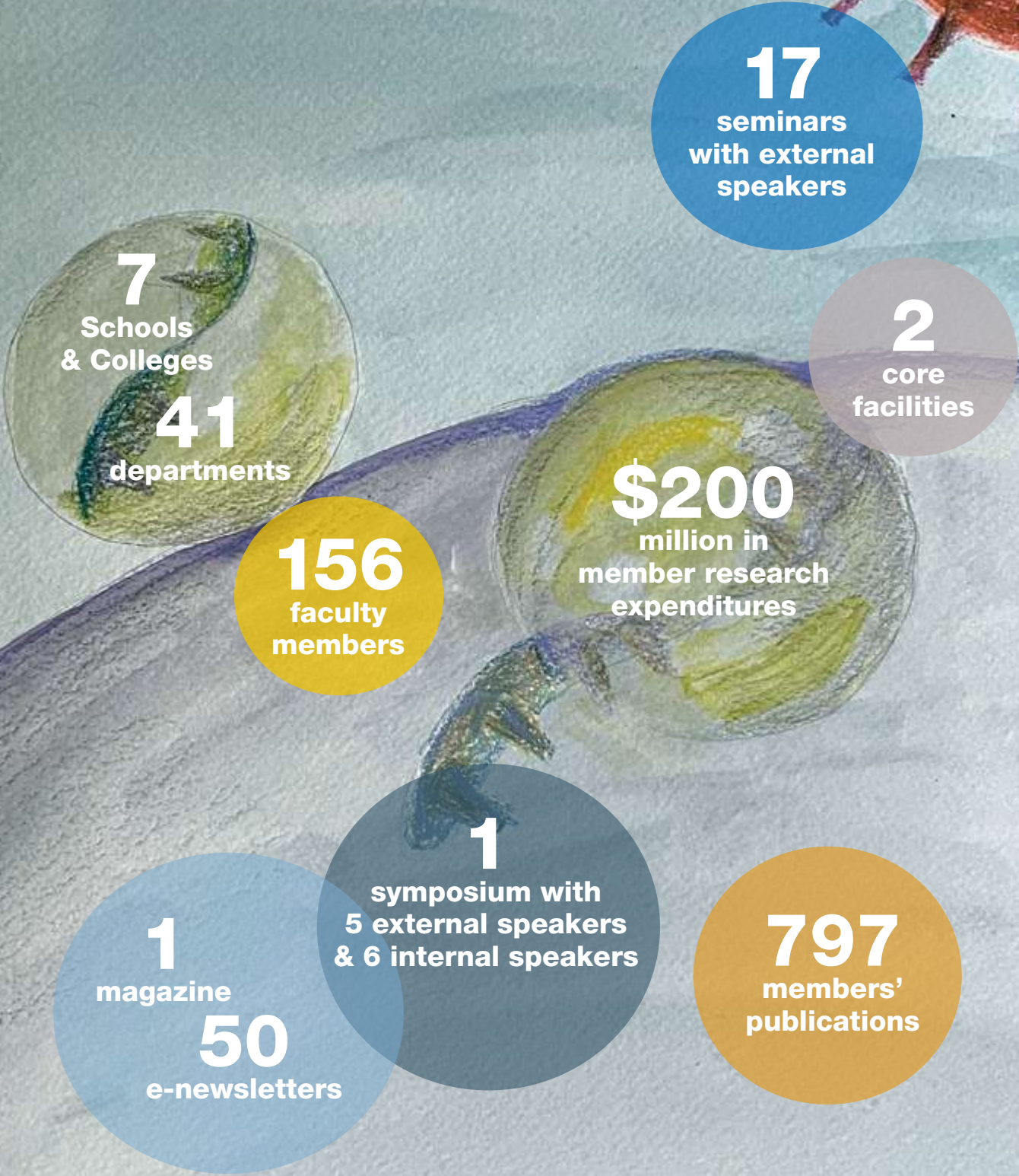
The University of Michigan Office of Research has a dedicated service for Research Ethics and Compliance with a Human Research Protection Program (HRPP) that follows federal agencies’ rules. Six Institutional Review Boards (IRBMED) of the UM Medical School oversee human subjects research conducted at the Medical School and Michigan Medicine. IRBs are mandated to include community members.

In June 2021, the Association for the Accreditation of Human Research Protection Program (AAHRPP) has re-accredited the University of Michigan’s Human Research Protections Program (HRPP). This accreditation indicates that an organization follows rigorous standards for ethics, quality, and protections for human research. With the AAHRPP seal, the U-M is recognized among the world’s most respected, trustworthy research organizations.

The U-M Center for Bioethics in Social Sciences in Medicine provides a forum to study deliberative democracy and fosters deep, qualitative and sustained engagement between medical practice and various publics.

University of Michigan Center for RNA Biomedicine

2020–2021 REPORT





Ian Hall, a graduate student in Sarah Keane's lab, Department of Chemistry, College of LSA. Photo: Elisabeth Paymal

Center for RNA Biomedicine in Numbers

July 1, 2020–June 30, 2021

PEOPLE

156 RNA faculty members
Male: 69%; female: 31%

ACROSS CAMPUS

Schools/colleges: 7
Departments: 41

LEADERSHIP

2 Co-Directors
8 Executive Committee members
11 Strategic Advisory Board members
14 Student and Postdoc Council members

RESEARCH FACILITIES

2 Research core facilities:
Bru-seq Lab and SMART Center

FUNDING

RNA faculty are involved
in raising about \$200M/year
in research expenditures.

HIRING

6 Department chairs & search
committees collaborating on
Biosciences faculty recruitment

SCIENTIFIC PUBLICATIONS

797 total publications from all the
faculty members

SEMINARS

17 external speakers
70 participants in average
attendance

GRANT WRITING SUPPORT

1 *Grant Sprint* has brought together
13 PIs across 6 institutions.

COMMUNICATION

1 annual magazine
50 newsletters

SUPPORT FROM
THE RNA SOCIETY

The U-M RNA Student & Postdoc
Council was awarded an “RNA
Salon Grant.”

The “RNA Collaborative Seminar
Series,” initiated and led by the
Center, is promoted by The RNA
Society (website and Twitter).
As of June 2021, it connected 20
RNA research centers and has
hosted bi-weekly seminars with
about 150 participants attending
each seminar.

We invite you

To join us and over 2,600
followers on Twitter
(@umichrna).

To read our weekly newsletter,
“The RNA Transcript,” that
reaches over 1,000 RNA fans,
with an opening rate
averaging 40%. [Subscribe here.](#)

To visit our website,
rna.umich.edu, that received
over 14,000 users during the
2020–2021 academic year.

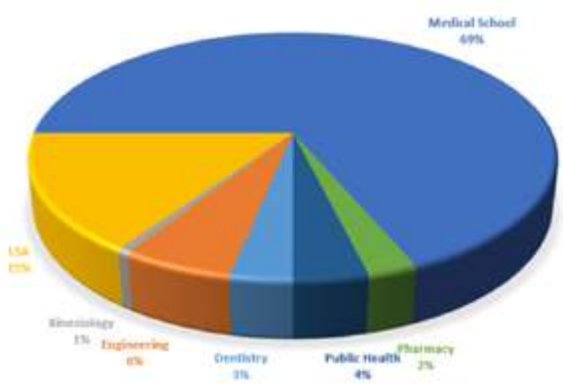
U-M CENTER FOR RNA BIOMEDICINE

156 FACULTY MEMBERS

from 7 Schools and Colleges
across 41 departments

Carlos Aguilar, Biomedical, College of Engineering
Huda Akil, Psychiatry, Medical School
Benjamin Allen, Cell & Developmental Biology, Medical School
Joshi Alunkal, Internal Medicine, Medical School
Anthony Antonellis, Human Genetics, Medical School
Brian Athey, Computational Medicine and Bioinformatics, Medical School
Sara Aton, Molecular, Cellular, & Developmental Biology, College of LSA
Ebrahim Azizi, Internal Medicine, Medical School
Ryan Bailey, Chemistry, College of LSA
James Bardwell, Molecular, Cellular, & Developmental Biology, College of LSA
Sami Barmada, Neurology, Medical School
Scott Barolo, Cell & Developmental Biology, Medical School
Stuart A. Batterman, Environmental Health Sciences, School of Public Health
Markus Bitzer, Internal Medicine, Medical School
Alan Boyle, Computational Medicine and Bioinformatics, Medical School
Charles Brooks, Chemistry and Biophysics, College of LSA
Margit Burmeister, Computational Medicine and Bioinformatics, Medical School
Mark A. Burns, Chemical Engineering, College of Engineering
Laura Buttitta, Molecular, Cellular, & Developmental Biology, College of LSA
Dawen Cai, Cell & Developmental Biology, Medical School
Sally Camper, Human Genetics, Medical School
Maria Castro, Neurosurgery, Medical School
Matt Chapman, Molecular, Cellular, & Developmental Biology, College of LSA
Grace Chen, Internal Medicine, Medical School
Vivian Cheung, Pediatrics, Medical School

Arul Chinnaiyan, Pathology, Medical School
Michael Cianfrocco, Biological Chemistry, Medical School
Justin Colacino, Environmental Health Sciences, School of Public Health
Catherine Collins, Molecular, Cellular, & Developmental Biology, College of LSA
Kathleen Collins, Internal Medicine, Medical School
Analisa DiFeo, Pathology, Medical School
Gregory Dressler, Pathology, Medical School
Monica Dus, Molecular, Cellular, & Developmental Biology, College of LSA
Eric Fearon, Internal Medicine, Medical School
Eva Feldman, Neurology, Medical School
Claudia Figueroa-Romero, Neurology, Medical School
Aaron Frank, Chemistry and Biophysics, College of LSA
Peter Freddolino, Biological Chemistry, Medical School
George Garcia, Medicinal Chemistry, College of Pharmacy
Amanda Garner, Medicinal Chemistry, College of Pharmacy
Scott Gitlin, Internal Medicine, Medical School
Thomas Glover, Human Genetics, Medical School
Daniel Goldman, Biological Chemistry, Medical School
Stephen Goutman, Neurology, Medical School
Yuanfang Guan, Computational Medicine & Bioinformatics, College of Engineering
Johann Gudjonsson, Dermatology, Medical School
Erdogan Gulari, Chemical Engineering, College of Engineering
Deborah Gumucio, Cell & Developmental Biology, Medical School
Gary Hammer, Internal Medicine, Medical School



Members’ repartition across Schools and Colleges

Sue Hammoud, Human Genetics, Medical School
Alfred O. Hero, Electrical Engineering and Computer Science, College of Engineering
Gerry Higgins, Computational Medicine and Bioinformatics, Medical School
Zhonggang Hou, Biostatistics, School of Public Health
Shigeki Iwase, Human Genetics, Medical School
Ursula Jakob, Molecular, Cellular, & Developmental Biology, College of LSA
Paul Jenkins, Medicinal Chemistry, College of Pharmacy
Hui Jiang, Biostatistics, School of Public Health
Andrew Johnston, Dermatology, Medical School
Alon Kahana, Ophthalmology & Visual Science, Medical School
Sundeep Kalantry, Human Genetics, Medical School
Hyun Min Kang, Biostatistics, School of Public Health
Sarah Keane, Chemistry and Biophysics, College of LSA
Evan Keller, Urology, Medical School
Tom Kerppola, Biological Chemistry, Medical School
Jeffrey Kidd, Human Genetics, Medical School

Anthony King, Psychiatry, Medical School
Steven King, Internal Medicine, Medical School
Jacob Kitzman, Human Genetics, Medical School
Raoul Kopelman, Chemistry, College of LSA
Markos Koutmos, Chemistry and Biophysics, College of LSA
Kristin Koutmou, Chemistry, College of LSA
Steven Kregel, Pathology, Medical School
Matthias Kretzler, Internal Medicine, Medical School
Chandan Kumar-Sinha, Pathology, Medical School
Steve Kunkel, Pathology, Medical School
Katsuo Kurabayashi, Mechanical Engineering, College of Engineering
Kenneth Kwan, Human Genetics, Medical School
Roland Kwok, Obstetrics and Gynecology, Medical School
Adam Lauring, Internal Medicine, Medical School
Jun Hee Lee, Molecular & Integrative Physiology, Medical School
Jun Li, Human Genetics, Medical School
Yongqing Li, Surgery, Medical School
Jiandie Lin, Cell & Developmental Biology, Medical School
Jie Liu, Computational Medicine and Bioinformatics, Medical School
Mats Ljungman, Radiation Oncology, Medical School
Pedro Lowenstein, Neurosurgery, Medical School
Andrew Ludlow, Kinesiology, School of Kinesiology
Janine Maddock, Molecular, Cellular, & Developmental Biology, College of LSA
Anna Mapp, Chemistry, College of LSA
David Markovitz, Internal Medicine, Medical School
Richard McEachin, Biostatistics, School of Public Health
Miriam Meisler, Human Genetics, Medical School
Daniela Mendonca, Biologic and Materials Sciences & Prosthodontics, School of Dentistry
Gustavo Mendonca, Biologic and Materials Sciences & Prosthodontics, School of Dentistry
Rajasree Menon, Computational Medicine and Bioinformatics, Medical School
Ryan Mills, Computational Medicine and Bioinformatics, Medical School
Stephanie Moon, Human Genetics, Medical School

John Moran, Human Genetics, Medical School
Deepak Nagrath, Biomedical Engineering, College of Engineering
Sunitha Nagrath, Chemical Engineering, College of Engineering
Jayakrishnan Nandakumar, Molecular, Cellular, & Developmental Biology, College of LSA
Nouri Neamati, Medicinal Chemistry, College of Pharmacy
Melanie Ohi, Cell & Developmental Biology, Medical School
Gilbert Omenn, Computational Medicine and Bioinformatics, Medical School
Akira Ono, Microbiology, Medical School
Edgar Otto, Internal Medicine, Medical School
Ximena Paez-Colasante, Neurology, Medical School
Nallasivam Palanisamy, Pathology, Medical School
Bruce Palfey, Biological Chemistry, Medical School
Meeyoung Park, Neurology, Medical School
Stephen Parker, Computational Medicine and Bioinformatics, Medical School
Daniel Peltier, Pediatrics, Medical School
Bambarendage (Pini) Perera, Environmental Health Sciences, School of Public Health
Brian Pierchala, Biologic and Materials Sciences & Prosthodontics, School of Dentistry
Sethuramasundaram (Sethu) Pitchiaya, Urology, Medical School
Ping Qiu, Internal Medicine, Medical School
Kaushik Ragunathan, Biological Chemistry, Medical School
Indika Rajapakse, Computational Medicine and Bioinformatics, Medical School
Rajesh Rao, Ophthalmology & Visual Science, Medical School
Diane Robins, Human Genetics, Medical School
Brandon Ruotolo, Chemistry, College of LSA
Russell Ryan, Pathology, Medical School
Maureen Sartor, Computational Medicine and Bioinformatics, Medical School
Santiago Schnell, Molecular & Integrative Physiology, Medical School
Laura Jean Scott, Biostatistics, School of Public Health
Audrey Seasholtz, Biological Chemistry, Medical School
Jiaqi Shi, Pathology, Medical School
Lyle Simmons, Molecular, Cellular, & Developmental Biology, College of LSA

Janet Smith, Biological Chemistry, Medical School
Ryan Spengler, Internal Medicine, Medical School
Cristiane Squarize, Periodontics & Oral Medicine, School of Dentistry
Jeanne Stuckey, Biological Chemistry, Medical School
Chitra Subramanian, Surgery, Medical School
Michael Sutton, Molecular & Integrative Physiology, Medical School
Andrew Tai, Internal Medicine, Medical School
Alice Telesnitsky, Microbiology and Immunology, Medical School
Muneesh Tewari, Internal Medicine, Medical School
Peter Todd, Neurology, Medical School
Raymond Trievel, Biological Chemistry, Medical School
Lam Cheung Tsoi, Dermatology, Medical School
David Turner, Biological Chemistry, Medical School
Michael Uhler, Biological Chemistry, Medical School
Sarah Veatch, Biophysics, College of LSA
John Voorhees, Dermatology, Medical School
Nils G. Walter, Chemistry, College of LSA
Lisha Wang, Pathology, Medical School
Stanley Watson, Psychiatry, Medical School
Max Wicha, Internal Medicine, Medical School
Andrzej Wierzbicki, Molecular, Cellular, & Developmental Biology, College of LSA
Thomas Wilson, Pathology, Medical School
Trisha Wittkopp, Ecology & Evolutionary Biology, College of LSA
Chao-Yie Yang, Internal Medicine, Medical School
Jianzhi Zhang, Ecology & Evolutionary Biology, College of LSA
Jifeng Zhang, Internal Medicine, Medical School
Yan Zhang, Biological Chemistry, Medical School
Xiang Zhou, Biostatistics, School of Public Health



Two core facilities are affiliated with the Center for RNA Biomedicine, the Bru-seq Lab and the Single-Molecule Analysis in Real-Time (SMART) Center.

The Bru-seq Lab

The Bru-seq Lab team has developed and offers four RNA sequencing techniques that measure the rate of both synthesis and degradation of RNA molecules over time: Bru-seq, BruChase-seq, BruUV-seq, and BruDRB-seq. These techniques are also used to map transcription start sites and enhancer elements. In addition, they can assess the transcription elongation rates of all expressed genes.

Central to these innovative techniques is the labeling of the RNAs. This is achieved with bromouridine (“Bru”) which replaces uridine in the RNA sequence. The labeled RNA can then be isolated from the total RNA and mapped using next-generation sequencing technology. The RNA sequencing is performed at the University of Michigan [Advanced Genomics Core](#) facility.



Michelle Paulsen, Bru-seq Lab manager, at the bench. Photo: Elisabeth Paymal

The Bru-seq Lab biostatisticians have also developed a custom-designed analysis pipeline that compares different aspects of gene expression between samples.

The Lab offers an all-inclusive service, starting with cells and ending with basic data analysis. In addition, users have access to an exclusive pipeline to perform further data analysis. Areas of expertise are RNA isolation, cDNA library preparation, and sequencing data analysis.

In 2020, the Bru-seq Lab processed and sequenced 410 RNA samples, its highest yearly number of sequences ever, in spite of the COVID-19 three-month shut down. At the end of August 2021, the lab had already processed and sequenced 381 samples.

The Bru-seq Lab serves researchers from the University of Michigan and other

institutions in the United States and around the world. In 2020–2021, the lab has collaborated with many U-M’s researchers on their Bru-seq projects, and has also achieved several Bru-seq projects of its own.

In addition, the Bru-seq Lab has an ongoing collaboration with Pfizer for which it has prepared and sequenced over 200 Bru-seq and BruUV-seq samples.

The Bru-seq Lab contributes to the ENCODE project

The Encyclopedia of DNA Elements (ENCODE) Consortium

The Bru-seq Lab is a contributor to the Encyclopedia of DNA Elements ([ENCODE](#)) Consortium, an international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI). The goal of ENCODE is to build a comprehensive list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active. The project is currently in its fourth phase.

As a mapping center for the ENCODE consortium, the Bru-seq Lab has spearheaded a large-scale cell collection effort. The main purpose of this project is to be able to reliably compare samples across different sequencing platforms. Since the cells were all grown under the same conditions, in the same place and at the same time, any variabilities between labs and their cell growth protocols would be negated and lead to a more robust dataset.

Cell lines grown for the ENCODE project

16 cell lines were grown in duplicate for a total of 32 separate cell growths/collections. Each cell collection needed a minimum of 200 million cells, but most had more. The estimated total number of cells grown is close to 10 billion.

- Cell lines used:
- HCT116 - colon cancer
 - K562 - CML
 - GM12878 - lymphoblast
 - Panc-1 - pancreatic cancer
 - PC-3 - prostate cancer
 - MCF-7 - breast cancer
 - HepG2 - liver carcinoma
 - IMR90 - fetal lung fibroblast
 - A673 - Ewing’s sarcoma
 - Caco-2 - colon cancer
 - MCF10A - breast epithelial
 - Calu-3 - lung cancer
 - PC-9 - lung cancer
 - OCI-LY7 - B-cell lymphoma
 - HUVEC - umbilical vein endothelial cells
 - HMEC - mammary epithelial cells

- Sequencing techniques using these cell lines:
- ATAC-seq
 - ChIA-PET
 - Hi-C
 - RNA-seq
 - DNase-seq
 - Pro-seq
 - Bru-seq

The Single Molecule Analysis in Real Time (SMART) Center

The pandemic affected nearly all aspects of our lives, and research at core facilities like the Single Molecule Analysis in Real Time (SMART) Center was not spared. Disrupted project timelines, uncertainty about facility access, and tightening of financial belts contributed to sharp declines in usage of the SMART Center. For the last eighteen months, the core has striven to continue serving users with social distancing and building access restrictions, often relying on core staff to collect data from drop-off samples when users were unable to do so. Throughout this year’s hardships, SMART has remained committed to supporting use of high-resolution fluorescent imaging and other single molecule methods, and all instruments have been kept operational and available to groups across campus.



The Single Particle Tracker (SPT) microscope at the SMART Center, shown here, is being updated to meet evolving research needs. A suite of new components, including new cameras, lasers, temperature control for both sample heating and cooling, and microfluidic flow controls enable an array of multiplexed imaging techniques. Photo: Elisabeth Paymal

Staff at the core have also used this time to build toward the future, focusing on improvements to instruments and analysis pipelines, and redoubling dedication to supporting advanced imaging and quantitative analysis. The SMART Center is currently updating several microscopes to meet the evolving needs of the U-M research community and continue to provide broad access to cutting-edge single molecule and super-resolution imaging technology.

Of particular interest to the RNA community, the SMART Center will soon offer sub-cellular spatial -omic imaging (e.g., MERFISH for transcriptomics)—to be validated in conjunction with Biosciences Initiative-funded Single Cell Spatial Analysis Program (SCSAP) at U-M. In support of this effort, new cameras, lasers, and perfusion fluidics will allow faster, brighter imaging and multiplexed super-resolution imaging of up to 12 labels (e.g., allowing for 1000s of RNA targets via MERFISH), as well as application support for custom acquisition protocols and analysis. All instruments continue to be accessible during this upgrade process, and staff are available for consultation and brainstorming either in person or via Zoom.

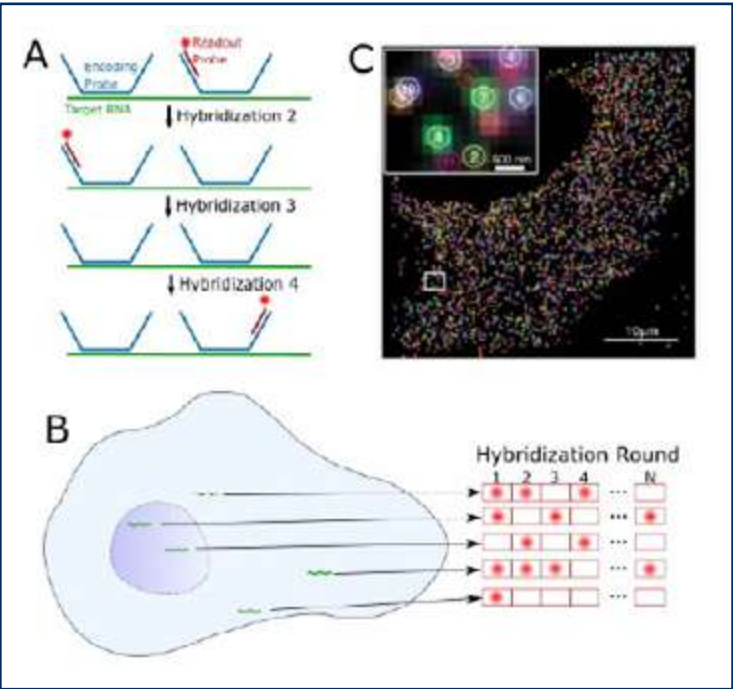
MERFISH application spotlight:

Mapping RNA expression with sub-cellular spatial transcriptomics

The spatial distribution of RNA expression can reveal and influence cell type, organization, interactions, and functions. Spatial transcriptomics quantifies both RNA abundance and localization in cells and tissues, providing a visual key for understanding local RNA processing, roles, and regulation.

Image-based approaches like MERFISH or seqFISH are highly multiplexed variations of single molecule FISH, simultaneously localizing the position of up to thousands of species of RNA with resolution down to 10s of nanometers. Similar to smFISH, target RNA molecules are hybridized against many short fluorescently labeled oligonucleotides, giving both specificity and amplification of the readout signal. In contrast to traditional smFISH, though, the probe oligonucleotides comprise a ‘barcode’ that uniquely identifies a multiplicity of target RNA species when imaged over several rounds of hybridization. The multiplexed error-robust encoding allows hundreds or thousands of RNA species to be identified and localized with only about a dozen hybridization cycles.

The instrumentation used in these RNA imaging techniques can also be applied to other methods alone (e.g., sequential immunohistochemistry) or in combination (e.g., to examine co-localization of protein targets with RNA species).



Multiplexed RNA Imaging and Quantification using MERFISH. A) The sample is sequentially incubated with a library of encoding probes that hybridize against target RNA. The sequence of encoding probes uniquely identifies the RNA species. In sequential rounds of hybridization, readout probes are introduced which bind to specific readout sequences on the encoding probes and are imaged to identify which RNA molecules have been stained with that sequence. The fluorescent probes are then photobleached or cleaved away, and the next readout probes introduced. B) The binary sequence of fluorescent signals for each RNA location in the image stack constitutes a barcode unique for each RNA species, allowing identification and localization of thousands of species of RNA in only a handful of rounds of hybridization. C) An example MERFISH multiplexed RNA detection. (Image C is reproduced with permission from The American Association for the Advancement of Science.)

Our 2020–2021 RNA Innovation Webinar Series

This pandemic year has been an unexpected opportunity to further strengthen our local, national, and international network of RNA scientists. We offered 17 one-hour Zoom webinars, our *RNA Innovation Seminar Series*, where RNA scientists presented their latest research on a broad range of topics, from foundational biology to potential therapeutics. These seminars fostered new insights and synergies for potential collaborations. Our members were given the opportunity to meet individually with presenters and explore possible partnerships.

Shifting from an in-person to a virtual format, we invited more international speakers and expanded our reach to a non-UM attendance. Our seminars were attended on average by over 70 participants, over half of them being non-U-M affiliates. They were faculty (average 23), postdoctoral fellows (average 23), students (average 18), and other stakeholders (average 7) who engaged through Q&A and chat. Our invited speakers were 42% female, 58% male, and our Rising Scholars series included 5 speakers from underrepresented minorities.

The speakers were selected based on their research topics to balance the range of science presented in the series. Part of the mission of the Center is to hire Biosciences Initiative-supported RNA faculty, and the seminars were an effective way to connect with potential candidates. In keeping with the University and Center’s values, Diversity, Equity and Inclusion (DEI) was an important consideration in the speaker selection process. The presenters were introduced and selected by our co-directors and executive committee members.

Taking advantage of the international virtual stage, we partnered with 21 RNA Centers across the globe to launch the bi-monthly RNA Collaborative Seminar Series. Our center hosted three such webinars, including a presentation by Melissa Moore, Chief Scientific Officer at Moderna Therapeutics, who explained the incredibly fast timeline for the development of the mRNA vaccine. Dr. Moore’s presentation was attended by almost 1,500 participants.

Speakers

- Andrey Krasilnikov, Ph.D.**, Associate Professor of Biochemistry and Molecular Biology, Center for RNA Biology, Pennsylvania State University, “Structures of eukaryotic RNases MRP/P RNP reveal RNA-driven protein remodeling” (September 21, 2020)
- Hiroaki Suga, Ph.D.**, Professor, Department of Chemistry, Graduate School of Science, The University of Tokyo, Japan, “Genetic code reprogramming that revolutionizes the discovery processes of peptide drug leads” (September 28, 2020)
- Chase Weidmann, Ph.D.**, Postdoctoral Research Scholar in the laboratory of Ben Major, Department of Cell Biology & Physiology, Washington University School of Medicine in St. Louis, “Defining functional hubs in RNA-protein interaction networks” (October 5, 2020)
- Gene Yeo, Ph.D.**, Professor, Cellular and Molecular Medicine, University of California, San Diego, “Systematic discovery of molecular and cellular functions of RNA binding proteins” (October 19, 2020)
- Aleksandra Filipovska, Ph.D.**, Professor, The University of Western Australia Centre for Medical Research, “Regulation of the mitochondrial transcriptome in health and disease” (October 26, 2020)
- Michelle Hastings, Ph.D.**, Professor, Cell Biology and Anatomy; Director, Center for Genetic Diseases, Rosalind Franklin University of Medicine and Science, “Splice-switching antisense oligonucleotides for the treatment of disease” (November 16, 2020)
- John Mattick, Ph.D.**, Professor of RNA Biology, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia, “RNA at the epicenter of cell and developmental biology” (December 7, 2020)
- Narry Kim, Ph.D.**, Professor, School of Biological Sciences, Seoul National University, Founding Director, RNA Research Center Institute for Basic Science, “RNA-based regulation of viruses” (December 14, 2020)
- Elena Conti, Ph.D.**, Director, Max Planck Institute of Biochemistry, “The RNA exosome complex: the Dr Jekyll and Mr Hyde of RNA degradation” (January 25, 2021)
- Jeffrey L. Twiss, M.D., Ph.D.**, Professor of Biological Sciences, University of South Carolina, “Regulating the axonal proteome through mRNA transport and translation” (February 1, 2021)
- Karla Neugebauer, Ph.D.**, Professor of Molecular Biophysics and Biochemistry and of Cell Biology, Yale School of Medicine, “Dynamics of co-transcriptional pre-mRNA processing and assembly of related biomolecular condensates” (February 15, 2021)
- James Nuñez, Ph.D.**, HHMI Hanna Gray Fellow, University of California, San Francisco, “Programmable transcriptional memory by CRISPR-based epigenome editing” (March 15, 2021)
- Natoya Peart, Ph.D.**, Postdoctoral Researcher – Carstens Lab/Lynch Lab Department of Medicine/Department of Biochemistry and Molecular Biophysics, University of Pennsylvania, “Direct binding of ESRP1 to regulated transcripts is required for position-dependent splicing regulation” (April 5, 2021)
- Jailson (Jay) Brito Querido, Ph.D.**, Postdoctoral Scientist, MRC Laboratory of Molecular Biology, Cambridge, UK, “Structural insights into mRNA translation initiation in humans” (April 19, 2021)
- Olivia Rissland, Ph.D.**, Assistant Professor, RNA Bioscience Initiative, Department of Biochemistry & Molecular Genetics, University of Colorado School of Medicine, “Regulating the Regulators: Control of RNA Binding Proteins during Embryogenesis” (May 3, 2021)
- Thomas Martinez, Ph.D.**, Postdoctoral Fellow, Salk Institute for Biological Studies, “Annotation and characterization of human protein-coding small open reading frames” (May 17, 2021)
- Yousuf Khan, Ph.D.**, Knight-Hennessy Scholar, NSF fellow, Stanford University, “CCR5 as a model to examine reporter assays in evaluating translational phenomena” and **Mike McMillan, Ph.D. candidate**, Cellular and Molecular Biology, University of Michigan, “Intersection between RNA methylation and TDP43-mediated toxicity in ALS” (June 14, 2021)

How the timing was just right for developing a COVID-19 mRNA vaccine



Melissa Moore, Ph.D., Chief Scientific Officer, Moderna Therapeutics

Over 1,450 people attended our webinar with Melissa Moore, Chief Scientific Officer at Moderna Therapeutics, on March 3rd, 2021. In her presentation titled “A timely confluence: The backstory of Moderna’s COVID-19 vaccine,” Dr. Moore explained how the mRNA vaccine works, and how it was developed in record time of just 45 days. To put an end to this pandemic, she strongly encouraged the audience to get vaccinated, with any vaccine available, as soon as possible.

While we were all stunned by the rapid onslaught of the COVID-19 pandemic, the release of a clinically tested mRNA vaccine in less than a year was just as astounding, sounding almost too good to be true. Yet the speed at which this innovative way of making a vaccine became possible was based on decades of cutting-edge RNA research that were ready to be taken to the next level.

Dr. Moore’s talk followed the chronology of the many scientific and technological developments that undergirded Moderna’s pandemic preparedness. By the end of 2019, Dr. Moore and collaborators had already published two major scientific articles. One explains how RNA structure controls the production of proteins,* and brings insight into the proper design of the mRNA. The other** relates to the development of mRNA-filled fat globules (termed LNPs, lipid nanoparticles), and addresses the “packaging for delivery” of the mRNA. Concurrently, clinical trials for other mRNA therapeutics were ongoing, providing the necessary data for the development of an mRNA vaccine. In 2016, Moderna had the foresight to invest in a new production plant, which was operational by 2019. As such, Moderna was ready, scientifically and technically, to produce an mRNA vaccine when in early January 2020, the genetic sequence of SARS-CoV-2, the virus that causes COVID-19, was released.

Regarding new virus variants, Dr. Moore explained that, so far, they all share the same spike protein of the original SARS-CoV-2 virus, which is the protein that the vaccine uses to provoke the immune response against the virus. If mutations should change, Moderna is poised to use the same now-proven technique to recode the mRNA and develop boosters or vaccines within only a few weeks.

Dr. Moore confirmed that the scientific principles regarding the mRNA vaccines of Moderna and Pfizer/BioNTech are similar. It is the LNP “packaging” of the mRNA that differs.

This live virtual webinar event was a tremendous success. Of the over 1,450 people attending, 20% were international. Faculty, postdocs and students represented 75% of the audience. The engagement was remarkable with more than 300 questions sent to Dr. Moore.

The Center is very grateful to Dr. Moore for her extraordinary contribution to the field of RNA therapeutics and for her virtual presentation at the University of Michigan. This webinar was organized and hosted by the U-M Center for RNA Biomedicine within the [RNA Collaborative Seminar Series](#).

* “mRNA structure regulates protein expression through changes in functional half-life,” D. Mauger, J. Cabral, V. Presnyak, S. Su, D. Reid, B. Goodman, K. Link, N. Khatwani, J. Reynnders, M. Moore, and I. McFadyen, *PNAS*, November 26, 2019 116 (48) 24075-24083; first published November 11, 2019; <https://doi.org/10.1073/pnas.1908052116>

** “Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines,” K. Hassett, K. Benenato, E. Jacquinet, A. Lee, A. Woods, O. Yuzhakov, S. Himansu, J. Deterling, B. M. Geilich, T. Ketova, C. Mihai, A. Lynn, I. McFadyen, M. Moore, J. Senn, M. Stanton, Ö. Almarsson, G. Ciaramella, L. Brito, *Molecular Therapy Nucleic Acids*, Volume 15, 15 April 2019, Pages 1–11, <https://doi.org/10.1016/j.omtn.2019.01.013>



March 25–26, 2021

5th ANNUAL RNA SYMPOSIUM
“PROCESSING RNA”



Registration
[rna.umich.edu/](https://rna.umich.edu/2021-symposium/)
[2021-symposium/](https://rna.umich.edu/2021-symposium/)

Schedule

Thursday, March 25

11:00–11:05 / Welcome

11:05–12:00 / Keynote 1
Tracy Johnson, Ph.D.
University of California – Los Angeles
“RNA Splicing, Chromatin Modification,
and the Coordinated Control of
Gene Expression”

12:00–12:10 / Short break

12:10–1:05 / Keynote 2
Kevin Weeks, Ph.D.
University of North Carolina
“Structure-Based Discovery of New
Functions in Large RNAs”

1:05–1:35 / **Data Blitzes**
Cathy Smith, Graduate Student
“Splicing Disruption”
Daniel Peltier, Clinical Lecturer
“LncRNA-mediated regulation”
Yan Zhang, Assistant Professor
“CRISPR-Cas3 engineering”

1:35–2:30 / Keynote 3
Feng Zhang, Ph.D.
Massachusetts Institute of Technology
“Exploration of Biological Diversity to
Discover Novel Molecular Technologies”

Friday, March 26

11:00–11:05 / Welcome

11:05–12:00 / Keynote 4
Brenda Bass, Ph.D.
University of Utah
“Distinguishing Self and Non-self dsRNA
in Vertebrates and Invertebrates”

12:00–12:10 / Short break

12:10–1:05 / Keynote 5
Christopher Lima, Ph.D.
Sloan-Kettering Institute
“Mechanisms that Target RNA for Destruction”

1:05–1:35 / **Data Blitzes**
Meredith Purchal, Graduate Student
“Pseudouridinylation”
Adrien Chauvier, Postdoctoral Fellow
“Riboswitch-mediated transcription”
Shannon Wright, Graduate Student
“Toxic RAN translation”

1:35–2:25 / **Panel discussion with the
keynote speakers**, hosted by Sara Aton
Department of Molecular, Cellular, and
Developmental Biology and Markos Koutmos,
Departments of Biophysics and Chemistry

2:25–2:30 / Closing

“Processing RNA,” our 5th Annual Symposium

With over 550 participants, this two-day symposium held March 25th and 26th, virtually gathered the large RNA research community from the University of Michigan (U-M), the US, and around the world.

The symposium opened with remarks by Nils Walter, co-director of the U-M Center for RNA Biomedicine, who reminded the attendees of the importance of RNA research for society, as demonstrated by the COVID-19 RNA vaccine. Mats Ljungman, co-director of the U-M Center for RNA Biomedicine, emceed day two.

Five distinguished keynote speakers presented on various RNA processes: Brenda Bass, Ph.D., University of Utah; Tracy Johnson, Ph.D., UCLA; Christopher Lima, Ph.D., Sloan Kettering Institute; Kevin Weeks, Ph.D., University of North Carolina; and Feng Zhang, Ph.D., MIT.

Six U-M junior and early career scientists presented their research in a data blitz format: Adrien Chauvier, Ph.D., Postdoc and Research Assistant; Daniel Peltier, M.D., Ph.D., Clinical Lecturer; Meredith Purchal, Graduate Student; Cathy Smith, Graduate Student; Shannon Wright, Graduate Student; and Yan Zhang, Ph.D., Assistant Professor.

The panel discussion provided inspiring recommendations to trainees and mentors, from life/work balance and time management, to networking, and the importance of trusting your scientific passion (see below).

The MiSciWriters graciously blogged about this symposium. [Read the blogs.](#)

This event was supported by the University of Michigan Biosciences Initiative. The data blitzes were co-sponsored by Lexogen.

See the [detailed program](#).

ADVICE TO TRAINEES AND MENTORS

At the panel discussion of our 5th Annual Symposium, we asked the keynote speakers for advice for trainees and mentors. Brenda Bass from the University of Utah, Tracy Johnson from UCLA, Chris Lima from Sloan Kettering Institute, Kevin Weeks from University of North Carolina, and Feng Zhang from MIT drew from their personal experiences and shared best practices to become successful scientists. The discussion was led by U-M faculty Sara Aton (Molecular, Cellular, and Developmental Biology) and Markos Koutmos (Chemistry and Biophysics), both members of the Center’s Executive Committee.

PASSION: “Follow your passion” is a well-known recommendation, but it can take different meanings over time as you advance in your career. For example, a certain science might really speak to you and be really exciting, but you might find yourself alone on this path. In such a case, your passion might best be the leader. The advice is to take ownership of your path, and when it is not clear, trust that your passion will make things happen.

Passion is contagious and a mentor’s excitement can transfer to their mentees. A good connection between a mentor and a mentee is critical for the exchange of information, trust and respect. There is a unique relationship between each mentor and mentee. Similarly, each trainee has his/her own way of thinking, and in order to best support them, it is the responsibility of the mentor to take the time to understand how each mentee processes information. There is no one perfect formula, but leading by example and staying empathetic always apply.

MENTORING: As future leaders, trainees must learn to inspire and manage people. While passion comes from within, most universities offer mentorship training programs, and part of their training should include mentoring others. If trainees are to become leaders one day, they will need to build the interpersonal skills necessary to navigate conflicts and manage teams.



Top row, left to right: Markos Koutmos, Sara Aton, Chris Lima, Kevin Weeks; bottom row, left to right: Feng Zhang, Tracy Johnson, and Brenda Bass

COLLABORATION: Collaboration is key in RNA research where different disciplines must come together to explore the complexity of RNA biology. Scientists must reach outside their labs and connect with other collaborators. Another recommendation is to stay curious, and to keep learning outside your field of expertise.

COMMUNICATION: Communicating your science and research is an important responsibility. In addition to professional publications, a scientist must write grants and promotional pieces. Mentors need to help trainees develop these skills, as well as encourage them to take workshops on communicating science, and practice by talking to non-scientists—friends, family—about their research.

You also have to be deliberate about career moves. As early as the third year of graduate studies, you can start thinking about strategies to be attractive to companies or desired academic labs and build a network.

TIME MANAGEMENT: Time management is a well-known recurring challenge. Academia can at times create additional professional pressure for activities that do not directly relate to one’s research interests. Academic service can be very demanding, and the advice is to pick one or two activities that are of deep interest and close to your own values, and politely decline others. The ability to say no, when necessary, is critical for success. It is particularly important for Assistant Professors who need to prioritize their research to receive tenure. Another piece of advice is to clearly identify the things that only you can do, and delegate others.

KNOW THYSELF: It takes a tremendous amount of courage to be a scientist. By definition, the life of

a scientist is one of successive failures, and of learning from these to advance research. The quest for funding can be a Sisyphean process, and repeated rejections are exhausting. It is therefore crucial not only to be very motivated by your own research, but to also balance work with hobbies and personal passions. You also need to take the time to know yourself. If you are not happy, changes are in order. The compromises that you need to make have to be carefully pondered. A complementary notion to “know thyself” is to practice empathy and keep considering the point of view of others, i.e. take the time to know others as well as yourself!

As these recommendations were provided, they also became great reminders to us all to “walk the walk.” Peer pressure, deadlines, competition, and pandemics are all stressors that can derail anyone from following these best practices and intentions. Reminder prompts such as the word “No!” on a post-it note can be a visual reminder that it is OK, and sometimes necessary, to say “no.” Taking one day off of work each week can be a good practice to identify and respect personal priorities and establish a work/life balance. Ultimately, each person is responsible for identifying their needs and creating a structure to thrive.

If all these recommendations might feel like a tall order, there was something quite memorable in the delivery of these messages. The panelists are all accomplished scientists who grew up in parallel and have experienced first-hand all the challenges that scientists face. On “stage,” they were demonstrating collaboration and a collegial, supportive attitude, radiating a warmth and friendliness that kept the audience smiling. And this attitude might be the best advice of all!

Grant Sprints

A new way to write grant proposals that is more efficient, effective and fun: the *Grant Sprints*. By creating a space and time for innovative thinking, *Grant Sprints* facilitate the concept, ideation, and writing process while delivering strong grant proposals. Designed at the University of Michigan by Ann Verhey-Henke, Center for Socially Engaged Design, Grant Sprints help faculty reconnect with their passion and feel again the excitement of collaborations and discovery. The first of its kind “virtual” *Grant Sprints* was facilitated in October–November 2020, consisting of 13 Pls from six different institutions resulting in an NSF Biology Integration Institutes Program submission.



What do participants think of the *Grant Sprints*?

“Our grant is now focusing on a direction that we didn’t expect. Through our discussions, multiple ideas for new experiments emerged. I also learned a lot more about what my collaborators are able to accomplish and we can all leverage this shared expertise to improve our own work. Finally, everyone bought into the now-joint mission of writing our first grant together, so no one person has to do all the heavy lifting.”

Faculty Hiring

The University of Michigan recognizes that RNA research is an important field of research and wishes to further expand and strengthen its research expertise and training capability of the next generation of RNA scientists. To this end, through funding from the Biosciences Initiative, the Center for RNA Biomedicine has been charged to hire five tenure-track faculty over five years, starting in 2018. These RNA scholar faculty can be at any levels of their career development and are hired in collaboration with a departmental host.

This faculty search is also a wonderful opportunity for the Center for RNA Biomedicine’s members to further collaborate with hiring committees from six departments that are particularly engaged in RNA research: Biological Chemistry, Biomedical Engineering, Cell and Developmental Biology, Human Genetics, Life Sciences Institute, and Molecular, Cellular, and Developmental Biology.

Our first recruitment cycle (2018–2019) led to the successful hire of one junior faculty, Stephanie Moon, Ph.D., who started on January 1st, 2020, in the Department of Human Genetics. Moon’s research interests are in RNA degradation and stress granules which play an important role in degenerative diseases. She is the recipient of several awards, and, in 2020, with Nils Walter, she received a prestigious Chan Zuckerberg Initiative (CZI) Collaborative Pairs Pilot Project Award.

Even though the pandemic forced restrictions on recruiting, we are pleased to announce our second RNA faculty scholar hire, Chase Weidmann, Ph.D., Assistant Professor in the Department of Biological Chemistry of the Medical School, beginning September 1st, 2021. Weidmann’s interests are in understanding how alterations in RNA-binding protein profiles, a cell’s “RBPome,” confer deleterious activities onto noncoding RNAs in human disease, especially in cancer. Weidmann’s scientific journey story is on page 60.

We are thrilled to be able to hire scientists who share and practice our values of scientific excellence and collaborations, as well as of diversity, equity, and inclusion.

Exploring unknown territories

Chase Weidmann had three goals for a career: do something “cool,” improve the lives of other people, and, of course, be able to support himself. He found the “cool” in high school science classes and he quickly realized that biology and biotechnology offer vast uncharted territories to explore. One such uncharted area was the academic system itself, as he sometimes struggled to navigate his way through college. Still, he was very glad to find that stipends and resources would enable him to continue exploring in graduate school. He pursued his doctoral research at the University of Michigan (U-M) where now, a decade later, he is joining the RNA faculty community as an Assistant Professor in the Department of Biological Chemistry and as the second hired RNA Scholar Faculty of the Center for RNA Biomedicine.

From his first encounter with math, science, and technology in a Wisconsin high school, Weidmann generally knew that science was what he should study, but he had little idea about how to turn this interest into a career. Which scientific field could both captivate his interest and lead him to his second goal of helping people and positively impacting society? As a young undergraduate at the University of Rochester in New York, he was introduced to the wonders of RNAs: their roots in the origins of life, their critical role in the translation of proteins, and indeed their great therapeutic potential.

RNA-protein interactions naturally became his first rotation topic when he joined the University of Michigan for his doctorate studies, in 2009. He explored two vastly different areas of cellular biology in his rotations, namely in cellular trafficking and protein chaperones, but he quickly realized that RNA remained his true passion. He completed his doctorate dissertation on mechanisms of messenger RNA regulation by the RNA-binding proteins

Pumilio and Nanos, with Professor Aaron Goldstrohm in the U-M Department of Biological Chemistry. Weidmann explained that at the time, another class of RNA, long non-coding RNAs (lncRNAs), constituted its own vast uncharted territory that was quickly becoming the new frontier of RNA research. “People used to think that non-coding DNA was just junk, then it turns out that most of it is actually transcribed into RNAs. Even if only a small percent of these transcripts are functional, this represents a vast untapped ocean of potential therapeutic targets. Obviously lncRNA research will make a huge impact in the understanding and curing of diseases like cancer.”

As a postdoctoral fellow at the University of North Carolina – Chapel Hill, Department of Chemistry, in Kevin Weeks’s lab, Weidmann dove into new research on lncRNAs, and became specifically interested in the way they interface with protein to accomplish cellular functions or, in the case of cancer cells, promote deadly metastases. While many smaller non-coding RNAs can form tight stable structures, many lncRNAs contain unstructured “noodle-like” domains that can interact with a number of different proteins. In a cancer cell, unstructured lncRNA domains in the wrong place at the wrong time can bridge together proteins where they should not, contributing to the further proliferation and metastasis of tumorous cells.

For studies of lncRNA function and dysfunction, Weidmann developed in-cell probing assays that use chemical compounds to mark RNAs either according to their structures or their protein binding networks. These technologies help pinpoint the parts of lncRNAs responsible for their activities. With the help of the Center for RNA Biomedicine SMART Center (see page 52), Weidmann plans to visually track these lncRNA-protein assemblies to understand how they can wreak havoc on gene expression.

At the U-M Department of Biological Chemistry, Weidmann is now building his lab to further research RNA-protein pathways that could eventually become therapeutic targets. Weidmann’s hope is that new treatments might be developed with the help of other U-M experts and collaborators. “RNA has such a big footprint, it will be very easy to find collaborations at U-M, especially with the support of the Center for RNA Biomedicine.”



Mentoring

Weidmann is grateful for the effective and generous mentoring he has received all along his career. Mentoring has helped him navigate academia and build his confidence. Late to begin his career in research, his undergraduate advisor, Elizabeth Grayhack, took a chance on Weidmann and gave him his first independent project in her own laboratory. Starting in the lab, he fondly recalled the amazing lab tech who taught him to not rush and plan his time. Early in Weidmann’s graduate studies at Michigan, Professor Aaron Goldstrohm frankly laid out for him the milestones to become a principal investigator. “A lot of my success has been with the luck of meeting the right person at the right time. Mentorship is very important; working hard is of course also key, but it is only a small portion of the success equation.” Those experiences have been very determinant for Weidmann who is highly motivated to become a mentor himself and help students and junior scientists avoid the mistakes he made. Already, one of his recommendations to students is to develop the confidence to reach out to faculty and other potential mentors, as he feels he missed out on too many

opportunities due to “imposter syndrome.” Weidmann feels that actively engaging students who might have a hard time reaching out is critically important in addressing challenges in diversity, equity and inclusion (DEI) in academia, and he hopes to make a difference through his leadership and with hiring opportunities in his laboratory and as a faculty member.

Weidmann recognizes that we have reached a historical moment when people are reconsidering the values by which they live. He believes in “living our best lives and in doing our best work,” which he thinks can be better achieved with flexible hybrid working and learning models, as well as with leveraging the technologies that enable them. Weidmann imagines many innovative ways to deliver science. He is interested in exploring alternative approaches to train the next generation of students, while still preserving mentor-mentee, one-on-one, and small group opportunities, whether it be through dedicated teaching assistants in the classroom or on-demand virtual office hours.

Weidmann feels deeply rooted in the mid-west and is delighted to be back. During his graduate school, he got married in Ann Arbor to another biologist and (almost) mid-westerner, and they both appreciate that U-M is conveniently located halfway between their respective hometowns. Even in hobbies, Weidmann has a restless mind: he is a big fan of video and board games that present various levels of complexity, story-driven narratives, and opportunities for both collaborative and competitive play. More recently, he began acting as game master for his first Dungeons & Dragons roleplaying campaign, something he finds stimulates his creativity and on-the-fly thinking in unexpected ways. Gamification, as both a teaching tool and as a way to harness gamers’ problem-solving ability, is increasingly being applied to scientific questions, including for understanding RNAs. It is no surprise: creativity, curiosity, and perspective shifts are key skills to solve scientific problems and will no doubt serve well on the next scientific frontier.



Three takeaways from a scientific journey: it's interesting, it's meaningful, and it's fun!



Dr. Sethuramasundaram (Sethu) Pitchiaya was born in the hot and humid city of Chennai, on the Bay of Bengal in India. Formerly called Madras, this large metropolis is renowned for its heritage art and architecture, hand-woven silk and cotton fabrics, and delicious spicy food. His parents were originally from small towns in the state, but they moved to the city to provide the best education possible for their children. Starting at his Chennai high school, Dr. Pitchiaya identified three milestones in his journey towards RNA research.

Science is interesting

Since his childhood, Dr. Pitchiaya has been passionate about two things: science and sports. He has competitively practiced several sports and served as his high school's sports captain. But his passion and curiosity for sciences started with his physics, chemistry, and math teachers. They were extremely engaging, taught him how to efficiently solve problems, and strongly encouraged him to ask questions without inhibitions, which, to this day, he considers almost more important than to give answers. "When you are trying to understand a topic, your intellectual curiosity drives you to ask a lot of questions, and this process can be more interesting and enriching than the answers themselves. People should remember that there is no such thing as a stupid question. Our curiosity is what drives creativity, growth and change." He still cherishes and encourages this attitude in everything he does and everyone he mentors.

Science is meaningful

Dr. Pitchiaya was selected into the prestigious Industrial Biotechnology program at Anna University, in Chennai, following his undergraduate placement tests. "I could not pass on such a remarkable

opportunity! This program was offered only by a few colleges in India back then." He fondly recalls how the faculty were making the field of genetic engineering attractive and exciting for their students. Many of his professors had been educated in the U.S. or Europe and were very dedicated to their trainees, instilling their passion for science in their students. Eager to test out the concepts he only learned in books and lectures, Dr. Pitchiaya interned at the National Center for Biological Sciences (NCBS) in Bangalore during his summer breaks, where he completed two internships in ion channel biology and electrophysiology. Initially, this first foray into hands-on research reminded him of cooking, as Dr. Pitchiaya said jokingly, "we typically mixed ingredients, heated or cooled the mixture for a certain period and checked out the results on agar or agarose gels. Just short of eating them!" However, he quickly realized that the proteins he was researching determined whether cells "thrive or die" and the proper function of many such proteins were in fact crucial indicators of crop yield. He was also fascinated that concepts on capacitance, resistance and electric fields, which he learned in physics and electrical engineering classes, could also be applied to solve biological questions. Dr. Pitchiaya is highly appreciative of the many conversations he had with his undergraduate research mentor, Dr. Matthew K. Matthew – these discussions made him realize that science was indeed meaningful.

Science is fun

As a component of his undergraduate curriculum and drawn by his interests in applying engineering principles to biology, Dr. Pitchiaya started a semester-long research project at NCBS, in the laboratory of Dr. Yamuna Krishnan, who is currently at the University of Chicago. Under her mentorship, Dr. Pitchiaya ventured into DNA nanotechnology and started engineering 3-D DNA structures, which were promising drug delivery candidates and could enable DNA computing. His main takeaway from that period is that research is fun. "Science is like solving a puzzle, you need to put pieces together and connect dots!" This is also when Dr. Pitchiaya realized that he wanted to pursue research and enter a Ph.D. program.



Dr. Sethuramasundaram (Sethu) Pitchiaya in the lab.

Immersing in the scientific experience

Inspired by his past research experience, Dr. Pitchiaya was interested in pursuing a Ph.D. in nucleic-acid nanotechnology and synthetic biology. He was naturally drawn to the laboratory of Dr. Nils Walter for graduate training, as he was working on a very innovative project on DNA nano-devices (called DNA spiders) and catalytic RNAs, the forebearers of all life forms. In the Walter lab, he was presented with an opportunity to study gene regulatory microRNAs by developing tools to visualize them within native cellular confines. "I was instantly sold on the idea as I thought the project would provide a glimpse at nature's gene regulatory circuits, lessons from which could be applied to synthetic biology." Ever since, he has been hooked on studying RNA and gene regulation. "It was during my graduate training that I got my first glimpse into the entire scientific process: identifying a problem, mapping out the strategy and research methodology for answering the question, conducting the research and drawing meaningful conclusions from the research endeavor. Interesting, meaningful and a whole lot of fun—overall so gratifying!"

Dr. Pitchiaya then pursued a brief postdoctoral stint at the SMART Center in the Department of Chemistry. There, he learned how to build state-of-the-art equipment that allows the study of molecular behaviors inside cells in real-time (see page 52). He also engaged with many of the Center's users and

collaborated with several researchers across various disciplines. Dr. Pitchiaya's interests in long non-coding RNA (lncRNA) biology led him to join the team of Dr. Arul Chinnaiyan, a pioneer in precision oncology, to study lncRNA function in cancer as an AACR-Bayer prostate cancer research fellow. "In Dr. Chinnaiyan's lab, I learned to employ an integrated approach of combining high-resolution microscopy tools with high-throughput sequencing and classical molecular and cellular biology assays to arrive at unified models of lncRNA function in cancer progression." Dr. Pitchiaya continued as an independent research track faculty in the Michigan Center for Translational Pathology (MCTP), directed by Dr. Chinnaiyan. He began applying his cross-disciplinary expertise and developing tools that measure RNA and protein levels within individual cells at high-throughput to broadly understand the mechanisms by which mammalian cells regulate gene expression and how gene dysregulation leads to cancer. During this time, he received multiple prestigious and competitive awards, namely a NCI-SPORE career enhancement award, a PCF young investigator award, and a DoD Idea Development award to understand the impact of gene expression heterogeneity in tumor biology.

Space, time, variations and RNA regulation

Dr. Pitchiaya is now a tenure-track Assistant Professor in the Departments of Urology and Pathology of the U-M Medical School, and a

member of the Rogel Cancer Center and the Center for RNA Biomedicine. His lab will work towards understanding the mechanisms by which RNA metabolism is regulated, especially in the context of stress response, and probing the molecular basis of heterogeneity in cancer. He will particularly focus on the impact of dynamic biomolecular organization, such as those occurring within membraneless organelles, on RNA regulation and cell fate. He says that “the collaborative environment and the expanse of resources available at U-M is incredible” and will help furthering his research.

Dr. Pitchiaya will leverage the resources of the SMART center, the Advanced Genomics Core and the Bru-seq Lab, along with developing novel spatial omics technology for his research. “We’ll work at the interface of foundational science and translational research where we’ll apply our basic understanding of gene regulatory mechanisms to treat diseases,” he added.

“I truly appreciate the support and independence that both Dr. Walter and Dr. Chinnaiyan provided during my graduate and postdoctoral training. And I’m glad to give a big shout out to the departments of Urology, Pathology, Cancer Center and MCTP for supporting science and scientists, such as me, especially through difficult times induced by the pandemic.” In addition to thanking his mentors for their guidance and encouragement, Dr. Pitchiaya is deeply grateful for his parents’ support. He expressed his immense appreciation for two strong women in his life: his mother who taught him the prowess of determination by tirelessly making sure that the family’s needs were tended to at any situation, and his best friend and wife, Dr. Visha Krishnan, for her warmth and compassion that make him see the glass half full, and smile at life. Outside of work, Dr. Pitchiaya and his wife love traveling, and enjoy learning about local cultures and traditional cuisines.



Dr. Pitchiaya visiting Mahabalipuram, India. It is known for its temples and monuments built by the Pallava dynasty in the 7th and 8th centuries.

RNA Skill Share

As the RNA scientific community has grown, so have the number of techniques and experimental approaches. While new research avenues are becoming available, branching into new lines of inquiry can be difficult.

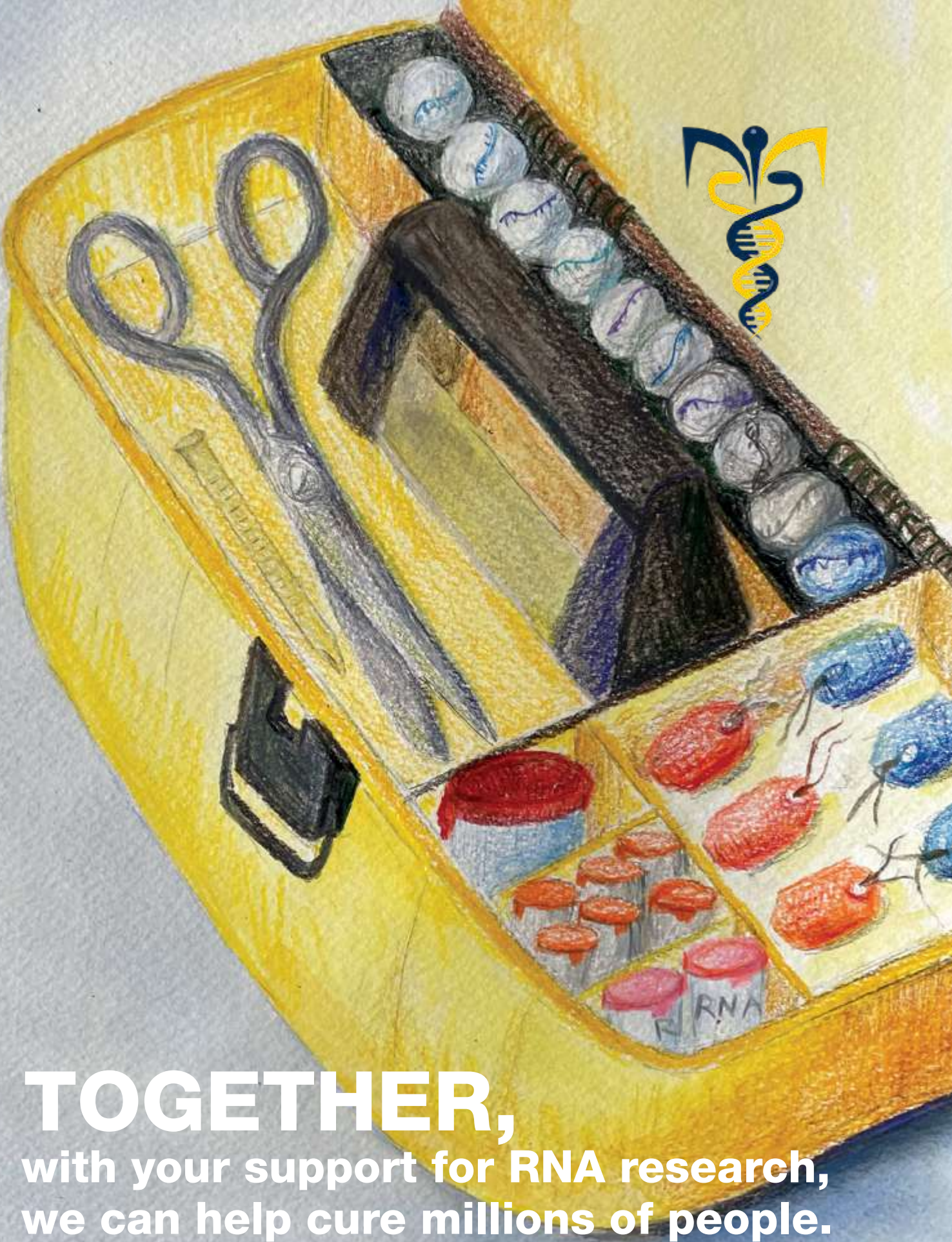
Thus, the RNA Skill Share was created. Simply put, the RNA Skill Share is a publicly accessible directory of researchers who have knowledge and expertise they are willing to share free of charge to enhance research efforts across the RNA community.

Current Skill Share Topics

- Single Molecule FRET
- Polysome Profiling
- Computational Techniques (Aspects of RNA sequencing and Statistical Modeling)
- RNA Sequencing (Experimental Approaches)
- RNA Transcription
- RNA Extraction Methods
- RNA Structural Analysis

In addition to the RNA skill directory, in 2021, we launched a video demo series. The first video is about total RNA extraction, and is available [here](#).

RNA Skill Share is led by the Center’s Student and Postdoc Council.



TOGETHER,
with your support for RNA research,
we can help cure millions of people.

RNA research is changing the face of medicine

It has been barely ten months since mRNA vaccines for COVID-19 were developed and they illustrate the tremendous success and impact that RNA therapeutic research has on human health and society. Decades of RNA research has led to this breakthrough which is further opening the door to many other therapeutic discoveries (see page 7).

“I think the new vaccines are just the start of using RNA as therapeutics. There is huge potential for growth and a lot of research is being done.”

—Heather A. Carlson, Ph.D., Chair of the Department of Medicinal Chemistry, Director of the Interdepartmental Program in Medicinal Chemistry, Professor of Medicinal Chemistry, Biophysics, and Chemistry

Unlike many traditional therapies, RNA therapeutics can tackle the root of a disease (the gene and its utilization) rather than treat only the symptom. Development of these treatments relies on the deep understanding of foundational RNA biology, a process that requires collaborations between scientists from different fields.

With the potential to cure millions of patients, it is urgent to further support and promote RNA therapeutics. The earlier the diagnostic of a genetic disease can be made, the sooner the treatment can be delivered, and the better the results, with possibly long-lasting and totally life-changing results for the patients and their families.

ASOs and gene therapy are already practiced for certain genetic diseases as, for example, spinal muscular atrophy, or SMA (see page 9). Thanks to systematic screening at birth in many U.S. states, pediatricians can learn that a baby has the SMA mutation as soon as two days after birth, allowing the physicians to start an RNA-based treatment at seven days of age, dramatically improving the outcomes.

Another example is Dr. Lori Isom’s research on ASOs to cure Dravet syndrome patients (see page 12). This research is now moving from the lab to clinical trials with the potential to completely change the lives of these patients and their families.

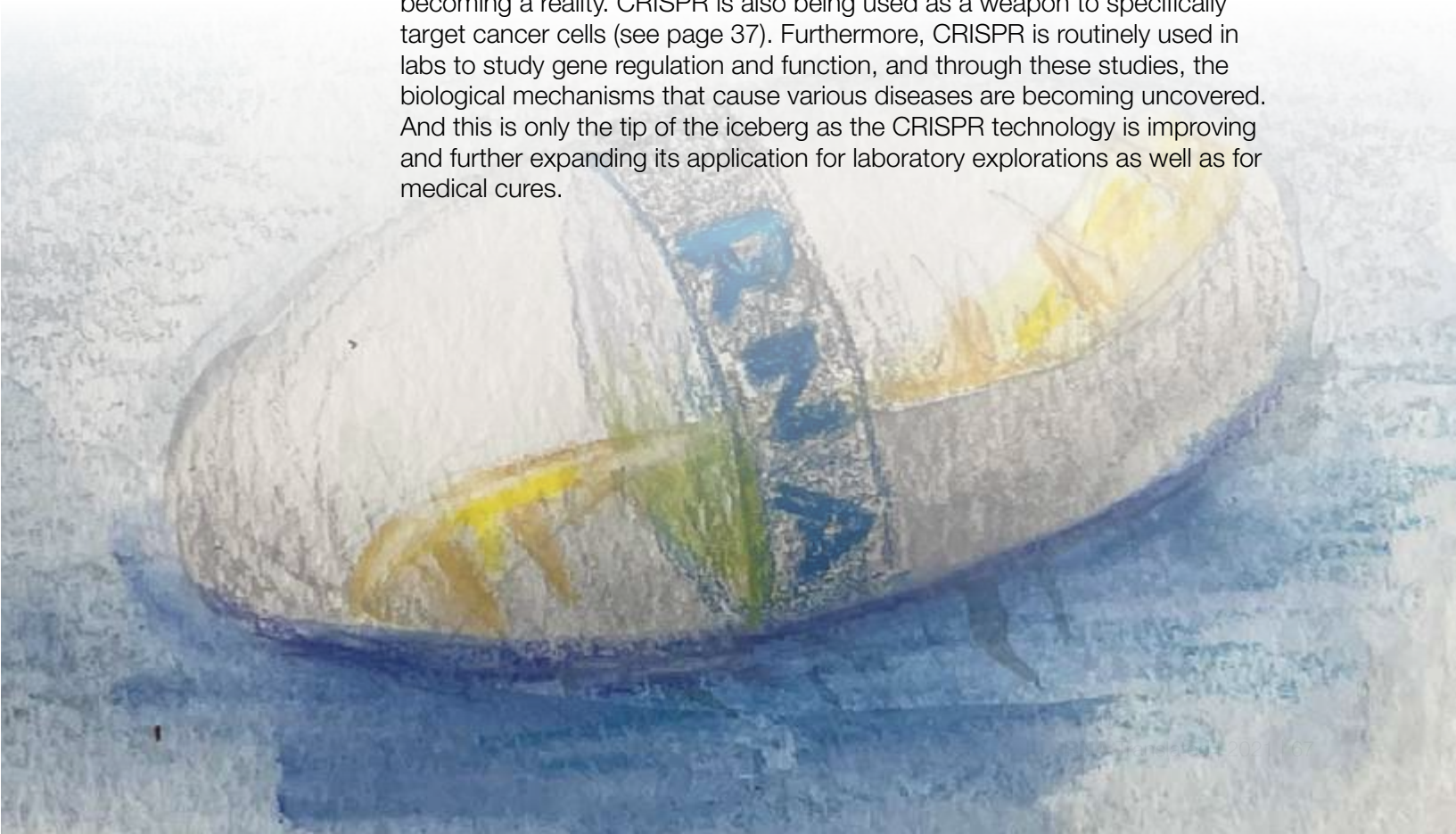
“RNA is a fantastic idea! Genome editing is permanent, and this means taking a very high risk. With RNA therapy (ASOs), it is instead reversible but you’re still getting to the base of the disease. If we can save the life of one of these little kids, it’s worth an entire career.”

—Dr. Lori Isom, Ph.D., Chair, Department of Pharmacology, Maurice H. Seevers Collegiate Professor of Pharmacology, Professor of Molecular and Integrative Physiology, Professor of Neurology

Patients and physicians work hand-in-hand with biomedical scientists.

To be successful, these cures require collaborations between many stakeholders: scientists, physicians, patients and their families, and policy makers to name a few. In addition to offering support groups and providing guidance for new patient families, patients’ organizations are very helpful with preparing for human trials and collecting family genetic history. U-M scientists are deeply engaged in these collaborations and contribute to the scientific and medical advisory boards of such patient organizations. For example, Dr. Martin (page 36) is on the board of the Charge Syndrome Foundation, Drs. Isom (page 12) and Parent (page 33) on the board of the Dravet Syndrome Foundation, and Dr. Todd (page 35) at the National Fragile-X Foundation.

With CRISPR, the possibility of curing thousands of genetic diseases is becoming a reality. CRISPR is also being used as a weapon to specifically target cancer cells (see page 37). Furthermore, CRISPR is routinely used in labs to study gene regulation and function, and through these studies, the biological mechanisms that cause various diseases are becoming uncovered. And this is only the tip of the iceberg as the CRISPR technology is improving and further expanding its application for laboratory explorations as well as for medical cures.



Support **M**-RNA Therapeutics

The impact of these rapid biomedical research advances on patients and on society are well understood at the U-M Center for RNA Biomedicine and at the U-M Biointerfaces Institute. Together, we are seeking your support to realize a vision of an M-RNA therapeutics at Michigan that will strengthen U-M research in the three leading-edge areas of the ongoing biomedical revolution: mRNA vaccines, ASO therapies, and CRISPR. With your help, we could hire outstanding faculty and scientists, purchase state-of-the-art equipment and create new labs, and further explore innovative ideas and their applications.

Why would you support the U-M Center for RNA Biomedicine?

At the University of Michigan (U-M) Center for RNA Biomedicine, we demonstrate that through scientific collaborations we gain a deeper understanding of the many roles RNA plays in cellular biology. Synergies between experts are key to accelerate discoveries and innovations.

Understanding RNA is highly challenging because it requires studying a wide range of processes and phenomena, from within single cells at nanometer scales to complex interactions between the 60 trillion cells of the human body. RNA research needs a broad group of experts from biology to engineering, computational science to medicine, to share ideas, data, and techniques to lay the foundations for the therapies of the future. RNA research also requires state-of-the-art equipment and highly sophisticated techniques.

The mission of the U-M Center for RNA Biomedicine is to support the RNA scientific community with these multiple challenges. We are the largest academic RNA research center in the US.

Together, our core members constitute a self-identified group of over 150 faculty scientists who lead cutting-edge investigations to understand the basic biology of RNAs, at nano-molecular, cellular and organism levels. Their scientific findings have the potential to translate into therapies for yet non-curable diseases.

Together, these faculty synergize their knowledge, skills and enthusiasm for scientific discoveries. The Center fosters their passion and supports their scientific inquiries by facilitating meetings, identifying funding opportunities, and organizing events.

Together, these faculty train and mentor the next generation of scientists in innovative and rigorous thinking (see Drs. Weidmann and Pitchiaya's interviews, pages 60 and 62). They mentor students and colleagues to be leaders with strong passion and ethics. The Center includes a Student and Postdoc Council that reaches out to young scientists and contributes to their curriculum and extra-curricular experience.

Together, our members experiment and practice novel techniques. They push the limits of leading-edge technology at the Center's two core facilities where they can further develop and test innovative concepts (see Bru-seq Lab and SMART Center articles, pages 50–53).

A dynamic Center with broad connections

Established across seven Schools and Colleges of the University of Michigan, the Center for RNA Biomedicine is well integrated within the U-M and has a remarkable track record both in funding and in attracting, training and retaining leaders in RNA research. The Center and its outstanding faculty are well-recognized and renowned in the field of RNA research and biomedical sciences, nationally and internationally. Several of our faculty are elected members of the National Academy of Sciences and of other prestigious scientific societies.

In 2016, the U-M Center for RNA Biomedicine received initial seed funding from the U-M Taubman Institute under Dr. Eva Feldman's leadership. Four years later, the Center was awarded a Tier 1 Grant from U-M President M. Schlissel under the Biosciences Initiative for \$10M over five years.

Our faculty raises an average of \$200M per year in research funding from federal grants and Foundation awards.

Our students and postdocs are recruited by the best private biomedical firms as well as by highly-ranked public academic and research institutions.

U-M knows how to do research

The University of Michigan is the largest public research university in the US.

U-M's entire research expenditures are \$1.62 billion, and about half of these are in the biosciences, with activity in medicine, pharmacy, dentistry, public health, nursing, engineering, kinesiology, biology, psychology, computer science, chemistry and physics.

For decades, the U-M has successfully encouraged and fostered cross-disciplinary collaborations, an approach to doing research that is required to innovate in biomedicine.



For more information on how to support RNA research and the University of Michigan Center for RNA Biomedicine, please contact Martina Jerant (mjerant@umich.edu).

The Center for RNA Biomedicine is supported by generous funds provided by the University of Michigan Biosciences Initiative, Alfred Taubman Medical Research Institute, Office of the Vice President for Research (OVPR), Michigan Center for Translational Pathology, Protein Folding Diseases Initiative, Rogel Cancer Center, the University of Michigan Department of Internal Medicine, University of Michigan Endowment for Basic Sciences, and the College of Literature, Science, and the Arts. The Center's RNA Student & Postdoc Council was awarded an "RNA Salon" grant from The RNA Society.

Thank you for your support!





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