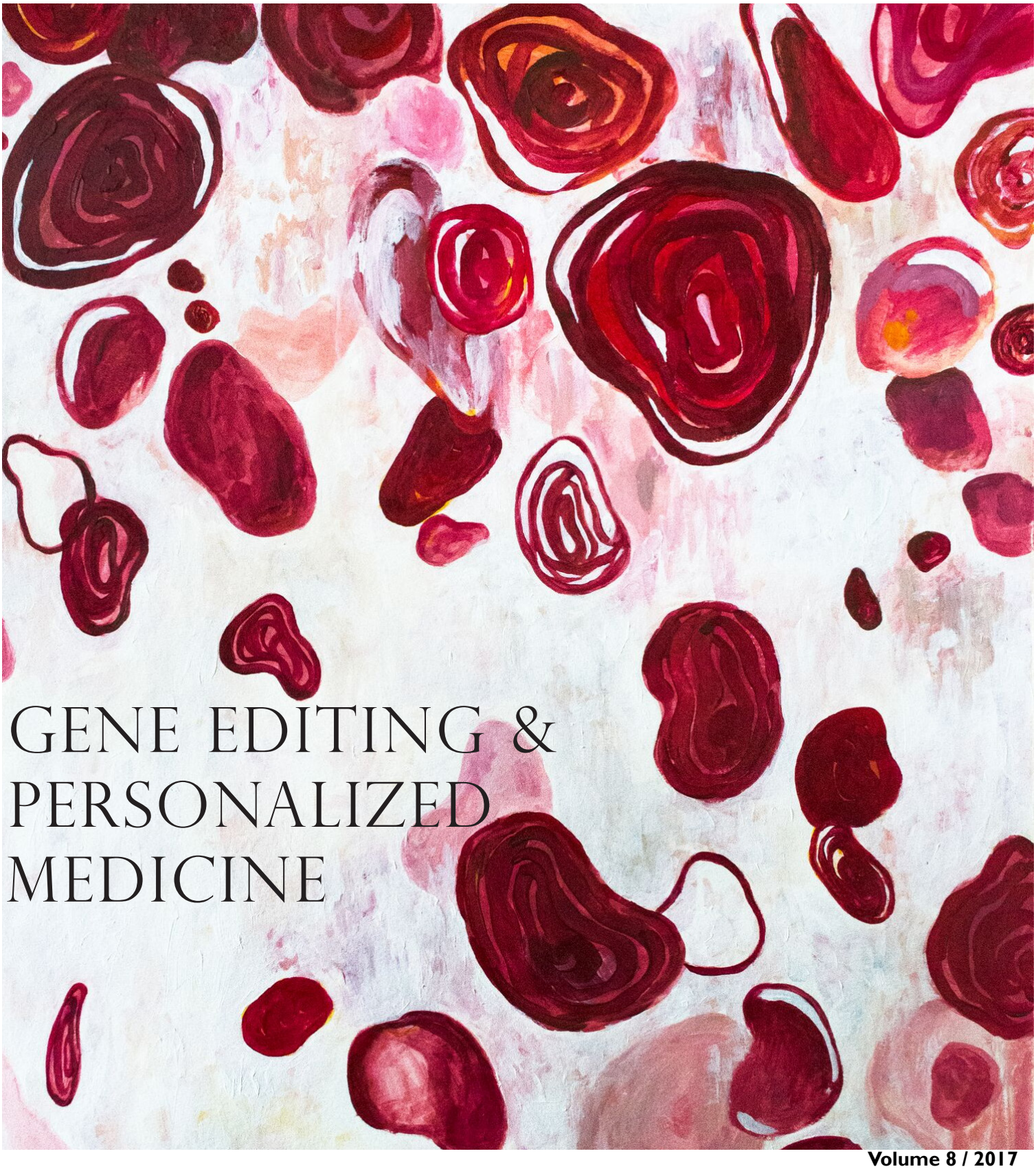
HEALTH SCIENCE INQUIRY

A publication platform for graduate students to discuss, discover, and inquire...



GENE EDITING & PERSONALIZED MEDICINE

Volume 8 / 2017

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Cover Design

Life as a Blood Cell *Sheryl Yang*

About the Artist: *Sheryl obtained a BSc in Psychology from the University of Toronto in 2015.*

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Special Thanks to Our 2017 Partnering Journals:

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LETTER FROM THE CO-EDITORS-IN-CHIEF

Dear Readers,

It is with great pleasure that we present the 8th annual issue of Health Science Inquiry on Gene Editing and Personalized Medicine.

With tremendous recent developments in gene editing technology, there is great potential for advancement in personalized medicine. Through this issue, we have welcomed commentaries on the clinical application of personalized medicine, future perspectives on the role of genome editing, and commentaries on conducting responsible science and the ethical implications of new technologies in the field of genome editing.

With staff, contributing authors, and artists from across Canada, HSI continues to serve as a national platform for student involvement and discussion. We are continually impressed by the insightful submissions that we receive from Canadian graduate and medical students. We are also deeply grateful to our partner journals, Journal of Personalized Medicine, Canadian Medical Association Journal, and The Journal of Medicine & Philosophy, for their support and commitment to student development.

In addition to our Main Submissions, HSI features News Articles and expert testimony on cutting-edge research and novel findings in the field of Gene Editing and Personalized Medicine. We also publish relevant career information and regular blog posts on various topics related to scientific discovery and graduate student life, which you can find on our website, healthscienceinquiry.com. HSI is also dedicated to promoting creative expression within the research community by providing a platform for graduate students to display related artwork and to promote a different side of the research world through art and creative expression.

Finally, we would like to thank our dedicated staff for their work on this year's issue. The HSI team, consisting of 45 graduate and medical students from different disciplines across the country, represents a broad Canadian voice. We are proud to create this open forum and hope that this publication inspires discussion among readers, authors, and peers.

Sincerely,

Tracy Moreira-Lucas and Tanya Miladinovic
Co-Editors-in-Chief

NEWS ARTICLES

News Reporters from HSI's Editorial Team investigated various issues in Gene Editing and Personalized Medicine

The ethics of genetic engineering

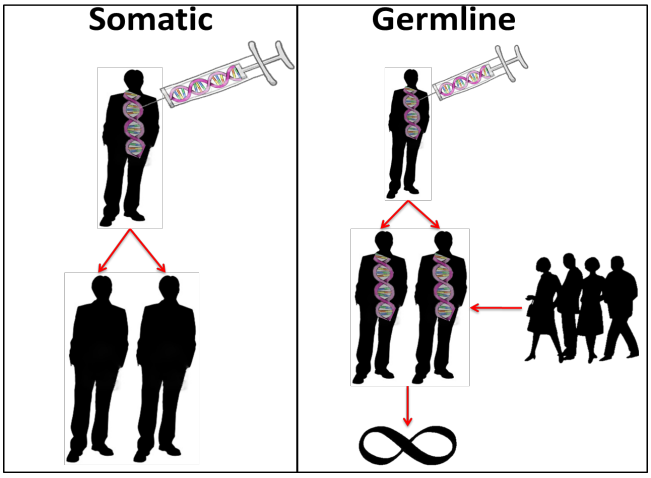
By Logan Townsend

Although still controversial, genetically modified foods have been grown and made commercially available for decades. More recently, and probably more controversially, various forms of human genetic modifications now exist. Basically, scientists alter the genetic makeup of a person, usually by injecting a virus that carries a particular gene. Once implanted, the virus will insert genes into the recipient's genome, thereby altering the recipient's DNA. This method could conceivably be used to 'fix' hereditary defects and genetic mutations, or for more superficial reasons.

It is important to appreciate that there are different types of human genetic engineering, including somatic and germline (1). Somatic engineering affects only the individual receiving treatment whereas germline engineering will affect the individual, their progeny, and all subsequent offspring. Simply put, genetic-engineering could alter a single person or their entire lineage.

Because others have the freedom to do what they want to their own bodies (hence cosmetic surgery, tattoos, and doctor assisted death), I suspect there are fewer objections to somatic engineering. The controversial crux of genetic-engineering probably comes from the manipulation of DNA in a way that will influence all subsequent offspring. Humans don't want strangers infringing upon their own rights...or genes; to paraphrase a classic line, your rights end at the beginning of my telomere.

There are many objections to human genetic-engineering, and one is that it is unnatural. Philosopher David Hume (2) said there is no word more ambiguous and equivocal than the definition of 'nature,' and if we cannot define 'natural' we cannot define 'unnatural.' John Stuart Mill (3) thought nature "...means the sum of all phenomena...including not only all that happens, but all that is capable of happening. Nature, then ... is a collective name for all facts, actual and possible." Similarly, Mark Sagoff (4) gives a more modern description of nature, "Everything in the universe. Everything technology produces has to be completely natural because it conforms to all of nature's laws and principles." By these definitions, genetic-engineering would certainly be natural.



But Anthony van der Schaaf (5) realizes that when people say 'unnatural' they could actually mean 'supernatural' and object that we are 'playing God'. However, even most theologians agree that God expresses himself in all forms of creation (6), which I would argue must include genetic-engineering. We could also take genetic-engineering to be an expression of human free-will. Thus, genetic-engineering is either an expression of God's will or it is the result of God giving us free will (6), but either way bio-engineering wouldn't be violating God's will.

But maybe the best response to the God objection, coming from Van Der Schaaf (5), is that "humans do not possess the powers of God, so we are really only playing God." In other words, people aren't worried that we have

the abilities of God, because we obviously don't, but rather we are cognitively unable to understand the powers we do have. With this in mind, to paraphrase Van Der Schaaf, if someone says that we should not 'play God' by fiddling with DNA, their real concern is that humans are too ignorant or deluded to understand the implications and ramifications of heritable human genetic-engineering... and unfortunately this would be much harder to refute. ■

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6. Vander Schaaf A. *Frankenfood meets the gastronome: A philosophical analysis of some ontological and axiological aspects of the genetic modification of food* [PhD thesis]. University of Guelph; 2010.

Logan Townsend

Logan is a first year PhD student at the University of Guelph. His research relates to metabolic contributors to the development of diabetes. Specifically he focuses on liver and adipose tissue and the effects of interleukin-6. In his spare time he enjoys rock climbing and mountain biking.

The CRISPR/Cas9 system in gene editing of cancer stem cells

By Manreet (Sonia) Padwal

It has been well documented that cancer development is a result of multiple mutations within tumor cells. Unfortunately, current treatments do not guarantee complete removal of the tumor and the cancer may reappear. Much existing literature suggests that a small population of cells within the tumor has the ability to induce tumor formation and growth (1). This small population of cells, referred to as cancer stem cells, seem to arise from the accumulation of mutations in normal stem cells over a long period of time. Opposing theories still suggest all cells have the ability to initiate tumorigenesis (Branavan Manoranjan. Conversation with Manreet Padwal. 2016 Dec 15*), but the stem cell theory has been well established in leukemia and solid malignancies (1).

With accumulating evidence in support of the cancer stem cell theory, scientists are investigating an approach to specifically target the tumor-initiating stem cell (1). Current therapies are centered around treating the bulk of tumor cells and fail to treat the tumor-initiating stem cell population. If any of the stem cells survive, the cancer may reoccur (1), which is why emphasis has shifted to direct targeting of the tumor stem cell population. Dr. Sheila Singh of McMaster University is leading research in investigating the most frequent form of brain cancer: glioblastoma. Her research focuses on identifying tumor stem cell markers and generating a customized approach to target and eliminate these specific cells. Branavan Manoranjan, an MD/PhD student in Dr. Singh's lab, notes that "markers that identify stem cells in the brain have not been concretely identified and this ultimately comes down to methodology."

Gene editing technology is a powerful tool applicable to genome editing in a range of pathologies (2). Investigators are beginning to use a more simple and versatile CRISPR/Cas9 gene editing system to identify new cancer stem cell markers. Dr. Chitra Venugopal, a Research Associate in Dr. Sheila Singh's lab, is using CRISPR/Cas9 to map out the tumor landscape in human glioblastoma by altering genes to understand their role in tumor formation. "With the ease of CRISPR/Cas9, everyone is transitioning" (Dr. Chitra Venugopal. Conversation with Manreet Padwal. 2016 Dec 15*). According to Manoranjan, "a study like this gives us an idea of what genes are expressed at that particular time point and therefore we can create a customized therapy for that target."

Current investigators have identified the expression of CD133 as a marker of cancer stem cells and a potential therapeutic target (3). Dr. Venugopal is trying to develop a more selective approach to identify CD133-positive cells and is using a drug or antibody to target these cells. Researchers are also investigating the possibility of using the patient's immune cells to target cancer antigens. To this end, the CRISPR/Cas9 system can be used to edit the immune cell genome to better recognize antigens on the tumor (4). Dr. Venugopal discusses the potential of using immune cells to recognize antigens such as CD133 as a means of specifically targeting tumor-initiating stem cells. "The ultimate goal would be to design an immune cell to hit multiple antigens at the surface," says Manoranjan.

A Chinese team is already using the CRISPR/Cas9 gene editing technique to engineer immune cells to knock out PD-1, a gene cancer cells take advantage of that acts to dampen the immune response, ultimately resulting in less detection of cancer cells. Thus, deletion of PD-1 would create more active immune cells and boost the immune system in fighting cancer more effectively (5). Although scientists are still far from perfecting this treatment, the CRISPR/Cas9 gene editing technique is making headway and shows promise in the field of cancer research. With further investigation, CRISPR technology will allow scientists to modify the immune system to directly target cancer stem cells, potentially leading to a strategy to treat and prevent the progression of cancer. ■

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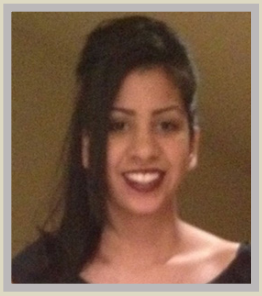

Manreet (Sonia) Padwal

Sonia is a PhD candidate at McMaster University with a BSc in Life Sciences. Her doctoral research focuses on elucidating the role of matrix metalloproteinases and WNT signalling in the development of peritoneal membrane injury. Sonia is also involved in clinical research where her primary objective involves understanding how to better protect and preserve the peritoneal membrane for patients on peritoneal dialysis. She is also passionate about the visual arts and has submitted her work to local galleries and events.

Developing CRISPR-Cas9 as a therapeutic tool to treat inherited genetic disorders

By Daniel Robinson

Generating site specific genetic modifications with relative ease has long been a desire of researchers for a variety of reasons, ranging from the study of specific proteins in biochemical pathways, to researching important regulators in development. Of particular interest to the medical field, the ability to correct genetic abnormalities within a person's genome would, for the first time, offer viable cures to fix genetic diseases.

With the recent development of clustered, regularly interspaced, short palindromic repeats (CRISPR) into a usable technology, scientists throughout the world now have the opportunity to generate specific gene modifications. Known as CRISPR-Cas9, this system uses engineered RNAs in combination with nucleases to generate these genetic modifications within the genome of a specific host organism (1).

One attractive application of CRISPR-Cas9 is its use in the development of therapies to correct genetic disorders. At The Hospital for Sick Children in Toronto, Dr. Ronald Cohn has been doing just that - studying the extent to which CRISPR-Cas9 can be used as a therapeutic tool to treat inherited genetic disorders.

When asked about the benefits of CRISPR-Cas9 as a genome editing tool over other technologies (such as TALEN and Zinc Finger Nucleases), Dr. Cohn explains that "CRISPR technology is overall more precise, easier to use and also cheaper" (2). Given the advantages of this system and its ability to easily and precisely edit a genome, Dr. Cohn adds that it is "a game changer as we are now for the first time able to conceptualize how to actually fix gene mutations" (2).

In recently published work, Dr. Cohn showed that CRISPR-Cas9 can directly correct genes in cultured muscle stem cells from patients affected by Duchenne Muscular Dystrophy (DMD) (3). Dr. Cohn explains that his research efforts are focused "on removing duplications in the dystrophin gene as a means to restore the full length, wild type protein". Because DMD is caused by an incorrect expression of the dystrophin protein, using CRISPR-Cas9 to fix the defects and correctly express the dystrophin protein could become a novel way of treating this disease.

Further, Dr. Cohn and his research team successfully "developed a new methodology of correcting a splice site mutation independent of homology directed repair" (2) on the DMD muscle stem cells. In essence, Dr. Cohn and his lab were able to successfully correct the genetic mutation in the human DMD muscle stem cells, which allowed for the production of full length and functional dystrophin.

Despite the current progress towards developing CRISPR-Cas9 as a therapeutic tool, there is still work that remains to assure its safety for use in patients. Precisely, Dr. Cohn warns that "we don't know enough about potential off target effects and [if] there might be an immune reaction toward the protein Cas9" (2). A series of optimizations with in vivo experiments in animal models must also be performed to optimize the efficacy of this system.

The work performed in Dr. Cohn's lab has provided a big step in establishing the use of CRISPR-Cas9 technology as a method to correct genetic abnormalities within an affected person's genome. Doing so would directly fix the inherent cause of the genetic disorder instead of treating the symptoms – all while providing an improved quality of life in affected persons and reduced financial and time strains on the Canadian healthcare system. ■

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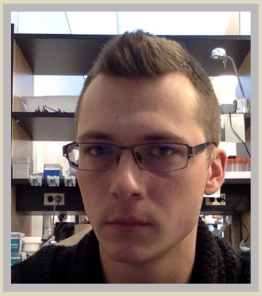

Daniel Robinson

Daniel received his B.Sc. (hons.) in Biochemistry at the University of Manitoba where he studied the conformational changes in the Glycerol Facilitator protein using ^{19}Fluorine Nuclear Magnetic Resonance. He is currently in his Master's at the University of Ottawa and will shortly attempt a transfer exam to fast-track into the PhD program to continue his work on studying the implications of specific elongation factors involved in myogenesis. During his free time, Daniel likes to stay active by jogging, cycling, and going to the gym.

Utilizing genomic tools to characterize the tumour landscape of glioblastoma

By Puja Bagri

Revolutionary innovations in next-generation DNA sequencing technologies have given way to significant advances in our understanding of cancer genomics and tumour biology. New developments in these methods are increasing the speed and efficiency of genome sequencing, while reducing the associated costs. This has allowed for tremendous growth in cancer research, providing novel approaches to characterize the genomic tumour landscape, and leading to better therapeutic options.

Cancers result from accumulative genomic alterations; thus, understanding the genome of cancer patients can provide a thorough blueprint of an individual's cancer cells and offer improved insight for diagnosis and therapy. A collaboration between Dr. Sheila Singh at McMaster University and Drs. Jason Moffat and Sachdev Singh from University of Toronto entails research using cutting-edge new technologies and a patient-centered approach to better understand a fatal form of brain cancer, glioblastoma (GBM). GBM is the most common primary brain tumour in adults, accounting for 80% of all malignant brain tumours (1). Incidents of GBM are most common in adults over the age of 40, and it is estimated there are 13, 000 new cases diagnosed every year in North America (2). The multi-model strategy currently being used to treat GBM includes surgery, chemotherapy, and radiation, but fails to provide effective protection due to the resistance of the tumour cells (3). The ability of the tumour cells to invade and infiltrate healthy surrounding tissue makes complete eradication nearly impossible and tumour recurrence inevitable. Due to recurrent, therapy-resistant tumours, patients have an average survival time of only 12-15 months, and with less than 10% of patients surviving beyond 5 years (4).

Research being conducted in Dr. Sheila Singh's lab focuses on what causes GBM tumour recurrence. They are utilizing cutting-edge genomic tools such as next-generation RNA sequencing (RNA-seq), proteomics and CRISPR (clustered regularly interspaced short palindromic repeats) to characterize the tumour landscape of recurrent GBM, and will be developing immunotherapeutic modalities targeting novel markers of GBM. As described by Dr. Sheila Singh's graduate student, Chirayu Chokshi, the ultimate goal of this project is to generate a "translational pipeline from initial target discovery (through target validation and exploration of mechanism), [and] develop new biotherapeutics against novel cancer targets, conduct preclinical testing in our advanced patient-derived animal model of treatment-resistant GBM, and finally, translate these findings into early clinical trails to provide hope for future GBM patients".

Although great progress has been made in understanding the genomic abnormalities involved in GBM tumourigenesis due to large-scale molecular profiling efforts, there are still many challenges preventing the development of a successful treatment. According to Chokshi, the futility of current GBM treatments is due to the heterogenous nature of this disease. Even though molecular profiling of GBM has allowed scientists to identify potential therapeutic targets, no single driver mutation can explain GBM tumourigenesis or be targeted to treat all patients. Hence, the molecular diversity of GBM is what makes it so difficult to treat. Not only is there great intra-tumour (within each patient) heterogeneity, there is also significant patient-to-patient (inter-tumour) heterogeneity as well.

For these reasons, the Singh lab is interested in applying a patient-specific approach for treating GBM. Their strategy is to target the cells causing tumour recurrence by utilizing a patient-derived xenograft model, which will allow for the discovery of driver mutations. Using RNA-seq, they plan to track GBM cell populations that undergo clonal evolution as a result of selective pressures exerted by standard treatments, and identify the cellular composition of the population causing tumour recurrence in their in vivo model. This will allow them to determine the intracellular pathways that drive GBM relapse in individual patients, which can then be targeted during therapy. This approach is very promising and has the potential to help prevent tumour recurrence.

While there are still several challenges due to the molecular complexity of GBM, genomic tools

are giving scientists hope that viable treatments will soon be available. The Singh lab believes that the power of genomic tools extends past the ability to characterize GBM tumours, and will eventually lead to better disease outcomes for patients. ■

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Puja Bagri

Puja is a 1st-year PhD candidate in the Department of Medical Sciences at McMaster University. She began her MSc in the McMaster Immunology Research Centre in 2014 and transferred to the PhD stream in 2016. Her research focuses on studying the in vivo mechanisms by which female sex hormones regulate genital tract immune responses to sexually transmitted viral infections such as HSV-2.

Modifying the microbiome:A gateway to treat Type 2 diabetes?

By Sarah Trottier & Logan Townsend

Type 2 diabetes (T2D) is a fast-growing epidemic, and although lifestyle and genetics are common contributors to its causality, recent research now shows that the bacteria living inside of our digestive tract (i.e. the gut microbiome) is another important factor. Indeed, the composition and diversity of the microbiome differs between obese and lean individuals with T2D (1), but even between diabetics and non-diabetics regardless of obesity (2). This has lead researchers to pursue a deeper understanding of the pathophysiological events linking the microbiome to host metabolism, in hopes of uncovering new potential therapeutic options.

For example, one study involved transplanting gut bacteria from typical mice into germ-free mice (mice without any gut bacteria) and observed a drastic increase in body weight within only 10 days (3). Part of the reason for this is that some microbiome compositions fa- cilitate greater caloric extraction from the diet, thus lead- ing to an increase in fat storage (4). This is exemplified by germ-free mice, which are resistant to obesity and T2D, even when fed a high-fat "western" diet (4).

It is well-known that exercise can prevent and treat T2D; interestingly, data from rodents shows a ro- bust effect of exercise on the microbiome, which may be contributing to the positive effects of exercise. Willem Peppler, a doctoral student at the University of Guelph, says there are specific bacterial strains, namely Bifidobacterium and Akkermansia muciniphila, that improve intestinal health, and that these strains are increased with exercise. But Peppler also notes that it is difficult to determine if there is a consistent effect of physical fitness on the microbiome in every individual. While there are a lot of data assessing the effect of exercise training on the microbiome in rodents, there are virtually no data in humans. Given that there are differences in the microbiome between individuals based on diet, geographical location, and other factors, it would be beneficial to determine whether the effects of exercise training observed in rodent models apply to humans as well.

Even if we can affect the human microbiome in positive ways through exercise, the microbiome tends to to remain relatively stable within a few years after birth, and seems to only temporarily respond to select stimuli (like exercise, diet, and antibiotics). Promisingly, metabolic benefits were observed when gut bacteria were transplanted from lean donors into recipients with metabolic syndrome (2). But while the transplantation of microbiomes may work in humans, this procedure remains controversial... and icky. These results raise the intriguing question of whether it is possible to engineer an 'ideal' microbiome that would be capable of harvesting sufficient energy and nutrients from the diet, but make one resistant to obesity and T2D. For example, we could reduce the quantity of Erysipelotrichaceae and Turicibacteraceae, two bacterial families associated with obesity and gut inflammation. Or it may be as simple as engineering greater microbial diversity, as this is decreased in obese and diabetic individuals. Despite probiotics and prebiotics being commercially available for decades, research into the relationship between host and microbiome is relatively young, but there are already extremely promising results. Who knows, one day we may all be popping pills full of gut germs to try and stay healthy!☐

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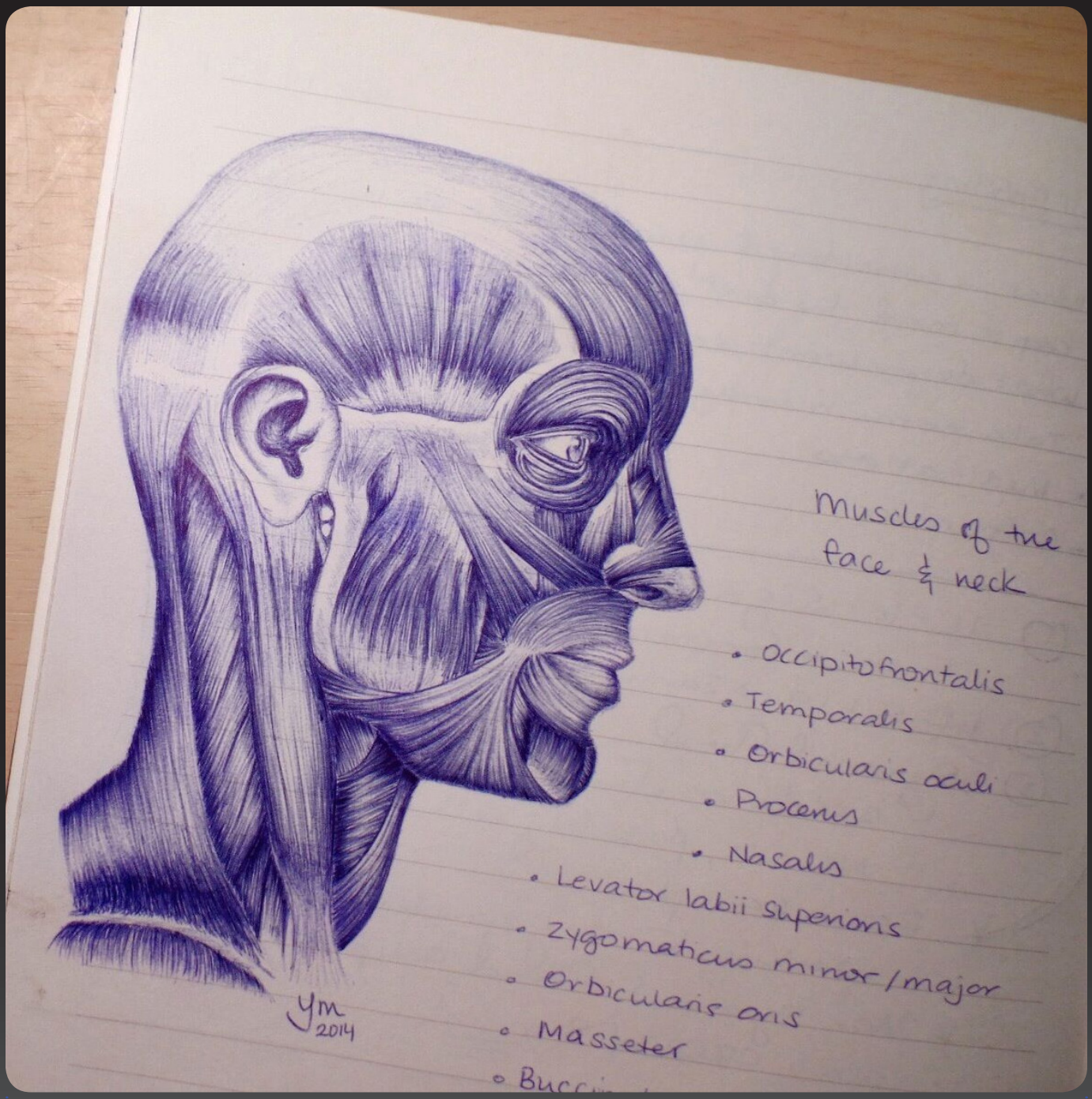
Sarah Trottier

Sarah is currently completing her MSc degree at the University of Guelph. She previously received a bachelor's degree in Health Promotion at Laurentian University. Her interest in exercise physiology and nutrition has inspired her current research focussing on the additive effects of dairy protein and exercise training on body weight management.

Logan Townsend

Logan is a first year PhD student at the University of Guelph. His research relates to metabolic contributors to the development of diabetes. Specifically he focuses on liver and adipose tissue and the effects of interleukin-6. In his spare time he enjoys rock climbing and mountain biking.



Facial Muscles *Yasmeen Mezil*

About the Artist: *Yasmeen is a PhD candidate in the Child Health and Exercise Medicine Program at McMaster University. Her research focuses on the effects of exercise on muscle and bone health in girls and women. Alongside her research, Yasmeen works as a Teaching Assistant in the Education Program in Anatomy, where she teaches anatomy to Occupational Therapy students. During the late hours of her class preparations, she finds herself gravitating towards the empty space of her notes and doodling the muscles of the neck and bones of the elbow. Her newfound use of ballpoint pen has given her a new favourite medium, one of which is versatile, beautiful, and not to mention, very convenient for the grad student. Yasmeen has also submitted professional illustrations for research projects at McMaster University and St. Josephs Hospital and her work can be found on Instagram @ymanatomy or Twitter @ OsteoMyo.*

MAIN SUBMISSIONS

Call for Submissions

In October 2016, HSI sent out a call for submissions to graduate students at Canadian universities across Canada asking them to submit short (700-800 word) commentaries on various topics related to Gene Editing and Personalized Medicine under one of the following sub-themes:

1. "-omics" technologies in personalized medicine: clinical applications
2. The role of genome editing in health care: future perspectives
3. Conducting responsible science: the safety and ethical implications of genome editing

Review and Judging Process

Beginning in March 2017, each submission was reviewed by two HSI Reviewers who critically assessed each commentary and provided feedback to the authors regarding its content and structure. After receiving their feedback, authors were given three weeks to revise their submission and resubmit to the journal. Our team of Senior Editors reviewed each revised commentary, and using information from the feedback given to them and additional editorial staff input, made a final publication decision. Each submission was then reviewed and scored twice by a team of independent judges not affiliated with HSI.

The Top Three Articles

The highest scoring submission for each sub-theme were provided with the opportunity to have their articles forwarded via expedited review for possible publication in one of our partner journals, Journal of Personalized Medicine, Canadian Medical Association Journal, and the Journal of Medicine & Philosophy. We received some outstanding submissions, and the editorial team highly commend the authors for their achievement. After tabulating the results, we are pleased to announce the top 3 submissions for the 2017 issue of Health Science Inquiry:

1. The Predictive Power of Omics: Clinical Applications
   Moushumi Nath, Xinwen Zhu

2. Heritable Genome Editing: Technological Innovation for Future Perspectives in Healthcare
   Meghan Lofft

3. In Utero Gene Therapy: A Brave New World of Designer Babies?
   Spandana Amarthaluru, Michael Aw, John-Paul Oliveria

Main Submission

Cut, Repair, Stitch – How Close Are We to Molecular Surgery Performed Using Genome Editing Techniques?

Yichi (Tony) Zhang,[a] Aaron MacCosham[b]

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Introduction

We are currently on the cusp of being able to perform molecular surgery, where nucleases cut out disease mutations, and correct nucleotide sequences can be stitched back together to repair genes (1). Molecular surgery has the potential to facilitate treatment or completely cure diseases with a genetic basis. This has been made possible due to the evolution of genomic editing with the development of zinc finger nucleases (ZFNs), then transcription activator-like effector nucleases (TALENs), and finally clustered regularly interspaced short palindromic repeat (CRISPR)-associated Cas9 nucleases (1). All three methods enable their respective nucleases (either FokI or Cas9) to introduce double-strand breaks (DSBs) in DNA at specific genomic loci. Afterwards, DNA repair occurs via either non-homologous end-joining (NHEJ) or homology-directed repair/homologous recombination (HDR/HR) (1). While key differences between these techniques exist (Table 1), the simplicity, reproducibility, and affordability of CRISPR/Cas9 have made it the most commonly used technique. This is mainly due to the use of guide nucleotides for Cas9, compared with the protein engineering that is required for the other two methods. Therefore, this review will focus on CRISPR/Cas9 in terms of its impact on clinical research, its limitations and potential solutions, and clinical applications

Table 1: Comparison of the three main genome-editing platforms.

	ZFN	TALEN	CRISPR/Cas9
Nuclease	FokI	FokI	Cas9
Construction	Difficult. Protein engineering required for every target.	Moderate. Protein engineering, complex cloning methods required.	Easy. 20-nucleotide guide RNA required. Easily re-targeted using simple cloning procedures.
Required Components	Two ZFNs needed around the target sequence.	Two TALENs needed around the target sequence.	Guide RNA complementary to the target sequence with one Cas9.
Multiplexing	Challenging.	Challenging.	Possible.
Feasibility of genome-wide screens	Challenging.	Challenging.	Possible.
Affordability	Very costly and time consuming.	Affordable, but time consuming.	Very affordable.
Targeting constraints	Difficult to target non-guanosine-rich sequences.	5' targeted base must be a tyrosine for each TALEN monomer.	Targeted sequence must precede a protospacer-associated motif (PAM) NGG sequence.
***Ex vivo* delivery**	Relatively easy through electroporation and viral transduction, for example.	Relatively easy through electroporation and viral transduction, for example.	Relatively easy through electroporation and viral transduction, for example.
***In vivo* delivery**	Relatively easy since ZFN expression cassettes are small enough to be contained in viral vectors.	Difficult, as each TALEN and the repetitive nature of DNA-encoding TALENs are large, so unwanted recombination occurs in lentiviral vectors.	Moderate. The most commonly used Cas9 from *S. pyogenes* bacteria is large and imposes problems packaging for viral vectors. Smaller orthologs exist.

of genomic editing to humans.

The future of genomic editing in scientific research – challenges and innovative solutions

Genomic editing has already facilitated the development of new disease models and the identification of novel drug targets. For instance, the applicability of disease studies on animal models greatly improved when genome editing was used to efficiently create primate models that closely resemble humans (2). To identify novel therapeutic targets for drug development, screening studies using CRISPR/Cas9 are being conducted to identify specific pathways used by diseased cells (3).

Despite recent advances, the issues of efficacy, safety, delivery, and ethics continue to prevent genome editing from being applied clinically. In terms of efficacy, NHEJ-mediated DNA repair efficiently cuts and stitches diseased genes at any point in the cell cycle, but if insertions are required then repair could be slowed, as HDR is only available during certain cell cycle phases. Safety is a concern, especially for CRISPR/Cas9, due to off-target mutations that result from Cas9 nucleases occasionally cutting non-specifically. This presents dangers to humans if harmful mutations are introduced, even at a low rate (4). Another challenge involves the delivery of editing components to all cells, while avoiding immune response, should viruses be used for delivery. Lastly, due to the ability of this technology to genetically alter individuals and their offspring, many ethical issues are raised regarding editing human embryos (1,5).

In order to address the above-mentioned challenges, NHEJ-mediated ligation of DNA templates could be used to increase the rate of recombination if Cas9 is modified to generate sticky ends in DNA (5). Many solutions are being researched to address the off-target effects of Cas9. For example, using a Cas9 nickase mutant to simultaneously nick both DNA strands, fusing inactive Cas9 to FokI, and using a small-molecule-triggered Cas9, all increase the specificity of cutting individually and in combination (6,7). The delivery of these additional components further stresses the necessity of non-viral delivery systems, as viral vectors are small. Therefore, nanoparticle- and lipid-based delivery systems are under development (8,9). Policymakers evaluating the ethics of this technology should be particularly concerned about the dangers of off-target effects associated with CRISPR/Cas9. However, many patients suffering from incurable diseases like Duchenne Muscular Dystrophy (DMD) are desperate for a cure. Therefore, policymakers should take the patient's wishes into consideration as well. Currently, only four countries have approved the use of genome editing on human embryos, a process known as germline editing. Governments are understandably reluctant to move forward with germline editing for fear of genetic testing to enhance human performance, and opening the debate regarding when and if human embryos are considered living humans.

Clinical applications of CRISPR/Cas9 and other gene editing methods to humans

As previously mentioned, genomic editing has yet to be translated to clinical application. However, it is very close to being used to treat infectious diseases like HIV. In fact, genomic editing of $CD4^+$ T cells to generate cells resistant to HIV infection has already reached phase II clinical trials (10). Highly publicized cancer trials using CRISPR/Cas9 are also underway. However, many currently incurable diseases like DMD and cystic fibrosis require germline editing, and that is where genome editing can have the greatest impact in the near future (1,2).

Conclusion

The tremendous excitement around genome editing and the idea of molecular surgery – taking any disease, removing causative mutations, and stitching DNA back together to leave the cell unharmed – is warranted given recent progress. However, issues around efficiency, safety, delivery, and ethics must still be resolved. The ethics of using genomic editing on human embryos needs to be addressed carefully. Given that only four countries have approved germline editing using CRISPR/Cas9, policymakers are understandably reluctant moving forward. However, the success of current clinical trials may convince regulatory bodies to use genome editing to its full potential. ■

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Main Submission

An Overview of CRISPR – From Germs to Giants

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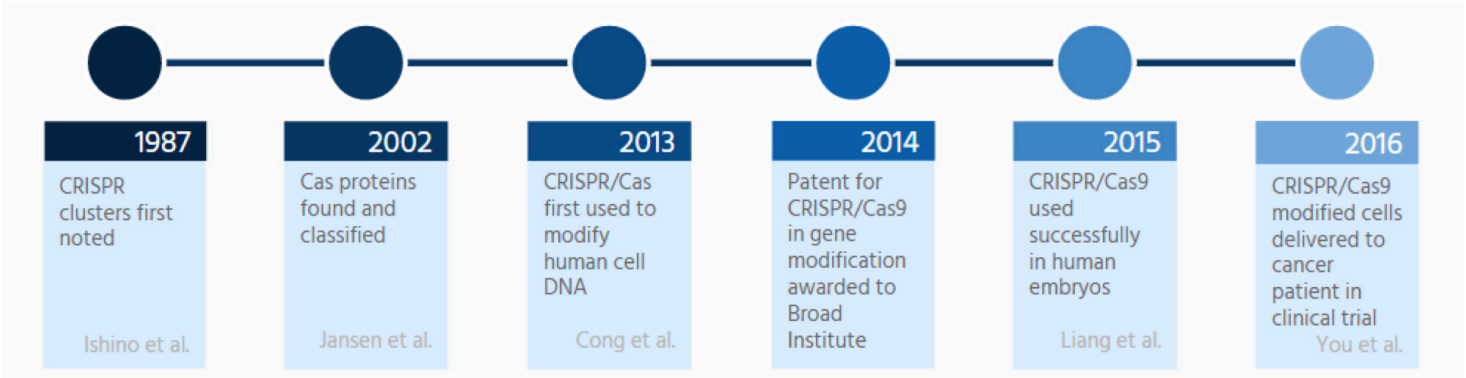
Introduction

In 1987, Yoshizumi Ishino and his colleagues noticed a set of regularly repeating genomic sequences within *E. coli* DNA (1,2). While repeats in DNA were common, these repeats were separated by different, irregular sequences. Other researchers began noticing the same oddity in all kinds of bacteria, and academic interest grew (1). The body of literature on CRISPR, or clustered regularly interspersed short palindromic repeats, grew over the course of the next 15 years (Figure 1), but its function was not fully understood (3). By 2002, proteins that regularly interacted with CRISPR DNA segments (Cas proteins) had been identified (1). In 2005, various research teams discovered that the gaps between the regular repeats matched up to extracellular sequences, suggesting that bacterial cells could record DNA from previous viral invaders (1). With the finding that archaea were protected from viruses whose genome matched with sequences between CRISPR segments, a picture of a primitive bacterial defense mechanism started to emerge (1).

CRISPR's gene editing capabilities in bacteria were soon discovered, and were applied to alter mammalian DNA (Figure 1) (4). CRISPR was able to overcome many problems with existing gene-altering methods. Meganucleases, for example, are very sequence-specific, but difficult to engineer correctly (5). Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are more straightforward to synthesize, but ZFNs lack accuracy, and the complex and time-consuming engineering process of TALENs discourages their use (2,5). The aim of this paper is to provide an overview of the mechanism of CRISPR and its current and potential applications, as well as explore some of the bioethical considerations of the technology.

Mechanism of CRISPR

The CRISPR/Cas system works in three stages (Figure 2A). First, segments of the invading viral DNA are integrated into the CRISPR array as spacer sequences, which act as genomic records of encountered infections (3). Next, these sequences are transcribed and processed into CRISPR RNA (crRNA), which guides Cas proteins to viral sequences complementary to the crRNA sequence (3). Lastly, crRNA forms a multiprotein complex that cleaves viral DNA, allowing for bacterial immunity against the virus (2,3). There are three types of CRISPR systems, but researchers have been particularly interested in the type II CRISPR/Cas9 system (2,3). CRISPR/Cas9 has been modified into the CRISPR technology known today. In prokaryotes, Cas9 is an



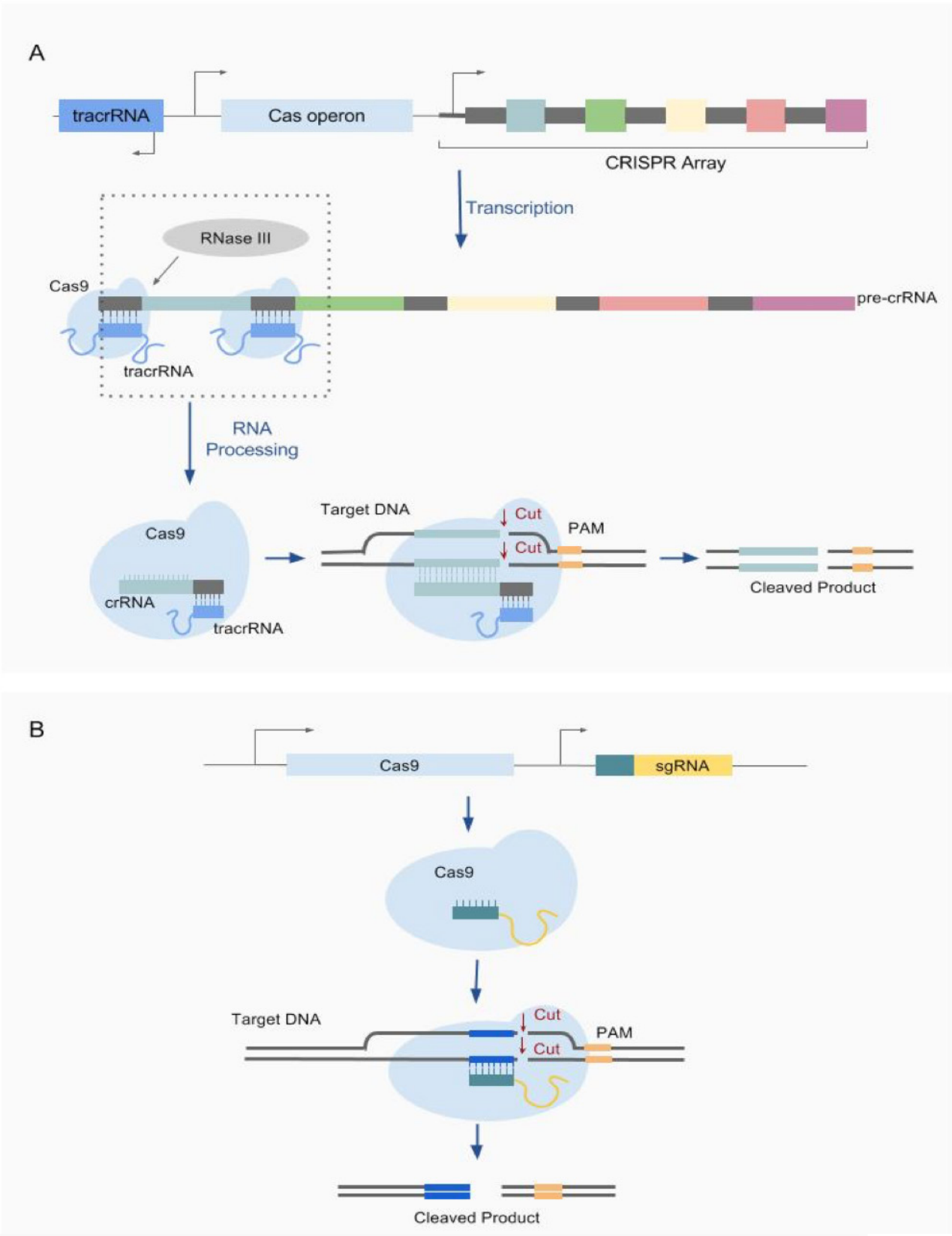


Figure 2: CRISPR/Cas mechanisms. (A) CRISPR/Cas mechanism as a bacterial "immune" response to viral DNA. Adapted from (3,2). (B) Genetically engineered CRISPR/Cas9 mechanism used as a genetic editing technique. Adapted from (2).

RNA-mediated DNA endonuclease that forms a complex with crRNA:trans-activating RNA (tracrRNA) to cleave and form double-stranded breaks based on the presence of protospacer adjacent motifs (PAMs) on the viral DNA (Figure 2A) (2). By genetically engineering the crRNA:tracrRNA duplex into a single guide RNA (sgRNA) and including PAM in target sequences, researchers can program the CRISPR/Cas9 system to cleave any desired sequence (Figure 2B) (2,3). Cleaved sequences can then be repaired by non-homologous end joining (NHEJ) or homology directed repair (HDR), resulting in gene knockouts or gene corrections (2).

Applications in CRISPR Technology

Gene therapy is closer to reality than ever before, primarily due to the specificity and simplicity of CRISPR. For genetic conditions affecting a single protein, like cystic fibrosis, treatments are already being developed, with promising results *in vitro* (6). Some see CRISPR functioning as a primary preventative measure in disease. For example, it could sterilize all mosquitoes that carry malaria, or alter chemokine receptors expressed on CD4+ T lymphocytes to prevent HIV from spreading (6). This technology is developing quickly; countries are increasingly approving CRISPR experimentation on human embryos, a short step away from clinical applications (6). It has been incorporated into gene therapy and germline editing for both genetic diseases and cancer. In 2015, Liang *et al.* were the first to use CRISPR to edit genes in human embryos, specifically to treat β-thalassemia, a hemoglobinopathy resulting from an inherited human β-globin gene mutation (7). Moreover, an ongoing clinical trial conducted by You *et al.* (2016) has used CRISPR-modified immune cells as a treatment for patients with aggressive lung cancer (8).

Bioethical Considerations of CRISPR

CRISPR technology has been popularized due to its low complexity and cost (9). It has potential as a treatment for various diseases, but this raises ethical and safety concerns. Using CRISPR in germline editing risks causing heritable and unpredictable genetic mutations with unknown side effects (9). Before including CRISPR as a therapeutic intervention, further development of the system is required, as well as a stronger understanding of its effects on human genetics. Furthermore, the possibility of germline editing for genetic enhancement of physical and intellectual traits leads us to question where we should stop manipulating the human genome (9).

CRISPR has also created conflict over patent ownership. Jennifer Doudna (UC Berkeley) and Feng Zhang (Broad Institute of Harvard and MIT) have been engaged in legal battles with each other since 2014 over the ownership of CRISPR genome editing (9). Despite Zhang winning the patent for use in eukaryotic cells in 2017, there are still ongoing European patent battles, and CRISPR continues to advance beyond what existing patents cover (10). CRISPR's patent owner could have a stake in all therapies that emerge from one of the most remarkable discoveries of the last 100 years (9). Placing financial ownership of a scientific revolution in the hands of one individual or one institution could lead to monopolization of all resulting CRISPR treatments for the coming decades.

Conclusion

Despite CRISPR's advantages over existing gene editing technologies, concerns about its use remain. While effective in bacteria and small mammals, CRISPR's accuracy in human gene targeting is not as well studied; targeting an incorrect gene could cause unpredictable mutations and side effects. Furthermore, as a technology that could one day allow for purely aesthetic genome modification, the scientific community must ask itself where the boundary lies in terms of what we can – and should – change. As with many advances in science, we must define our own limits. ■

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List of Abbreviations

Cas – CRISPR associated proteins
crRNA – CRISPR ribonucleic acid
CRISPR – clustered regularly interspersed short palindromic repeats
HDR – homology directed repair
HIV – human immunodeficiency virus
NHEJ – non-homologous end joining
PAM – protospacer adjacent motif
sgRNA – single guide ribonucleic acid
TALEN – transcription activator-like effector nucleases
tracrRNA – trans-activating ribonucleic acid
ZFN – zinc finger nuclease

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Michelle is a 5th year Bachelor of Science (honours) candidate at McMaster University, majoring in molecular biology and genetics. In 2016, she joined McMaster's Cardio-Respiratory Research Lab to complete her honours thesis and co-op work term. Her work involves investigating the immunobiology of allergic asthma, mainly focusing on B cell biology.

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Main Submission

Next Steps in Cancer Therapy: Integration of Gene Editing in Inducible Pluripotent Stem Cells

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When Paul Berg, the father of genome engineering, received the Noble Prize in 1980 for inserting the lambda phage gene into monkey Simian 40 oncogenic virus, a revolutionary era began. Since then, several tools have been created to optimize the manipulation of DNA for targeted gene editing, such as adenoviral transduction vectors, zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the new and simple clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9). Of these, TALENs and CRISPR/Cas9 are the predominant tools for clinical cancer therapy used with injectable differentiated cells. We foresee future intersections between genome editing and stem cell-based techniques that will pave the way for the introduction of universal donor stem cells (UDSCs) in the treatment of cancer.

Today, many cancer researchers are continuing Berg's path of gene editing while exploiting cancer hallmarks. Cancer manifests itself through sustaining proliferative signaling, inhibiting the activity of growth suppressors, impairing DNA repair and apoptotic mechanisms, deregulating normal epigenetic patterns, inducing chemo-resistance, and enhancing excessive angiogenesis, leading to uncontrollable invasion and metastasis (1). Many have used CRISPR/Cas9 to manipulate cancer-related genes in human-derived cells and human cell lines to understand lymphoma, lung cancer, and various other types of cancers. Findings from these cell-based genome-editing studies warrant the application of genetically engineered cells in a clinical setting (2,3).

These cell-based discoveries led to the introduction of genetically engineered cell therapy in clinical trials for the treatment of cancer. The first application of this method used genetically engineered immune cells to target and attack cancerous cells. One success story is the trial for the treatment of two leukemic infants. This group injected donor-derived TALEN engineered T cells into the cancer patients, while concurrently using immunosuppressive chemotherapy to avoid immune rejection of the injected cells designed to attack the cancer cells (4). Furthermore, an ongoing lung cancer clinical trial using CRISPR, headed by Dr. Lu You, utilizes a similar strategy of immune cell enhancement to provide targeting of cancer cells (5). If this trial succeeds, it will further emphasize the significance of gene-edited injectable cell therapy in developing future clinical cancer treatments. However, the concurrent immunosuppressant usage in the conducted studies points to the persistent risk of immune rejection of the injected cells.

Attempts to minimize the risk of immune rejection have used inducible pluripotent stem cells (iPSCs) in integration with gene editing for cancer therapy. In this method, the patient's somatic cells are collected, de-differentiated into self-renewing iPSCs that can be genetically modified, and then re-differentiated into any cell type of interest for injection into the same patient. This approach avoids the risk of immune rejection of the immunotherapy. However, the generation of iPSCs and the reprogramming into fully differentiated immune cells faces its own challenges. It can be costly, time-consuming, and highly variable in epigenetic status, genomic stability, and the pluripotency potential of differentiating into various cell types. Moreover, autologous immune cell rejection may still occur due to unpredicted alterations in the surface antigens of the iPSC-derived immune cells (6).

To further bypass the challenges of immune rejection when using personalized iPSCs, groups are currently employing the CRISPR/Cas9 system to generate UDSCs that are devoid of antigens typically targeted by the immune system (6). Future success in such studies will lead to the generation of UDSC line banks to readily provide countless possibilities of differentiated cells containing the desired genetic modification, specifically designed for each patient. Moreover, UDSC lines can be generated using various human stem cell types, such as iPSCs and bone marrow-derived multipotent progenitor cells (6,7).

Interestingly, while several groups are comparing TALEN and CRISPR independently, others are combining them to develop inducible-CRISPR methods, which can promote genetic modification at multiple loci following iPSC stimulation. The application of these technologies is crucial for mechanistic interrogation of complex and pleiotropic genetic mutations that are often observed in cancer genetics (8,9). Additionally, efforts have been undertaken to improve CRISPR/Cas9 technology for use in the clinical field to address the off-target concerns and to investigate the integration of the entire Cas9 plasmid construct into the targeted genomic loci (10). In the future, these improvements can be applied to UDSCs, leading to more precise and patient-specific genetic modifications proceeding the injection.

The era in which gene editing applications link the basic science field to the clinical world is approaching faster than anticipated. TALEN has already been successfully used in donor T cells for the treatment of leukemia. Simultaneously, CRISPR/Cas9 is now recognized as one of the simplest, easiest, and most efficient gene editing technologies. Today, the CRISPR/Cas9 system is being tested to treat lung cancer for the first time in humans. We envision the integration of TALEN and CRISPR/Cas9 with personalized iPSCs, to thereby generate UDSCs, as the next promising step towards enhancing cancer therapy. ■

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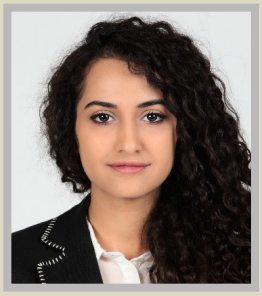

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Main Submission

CRISPRs to Treat, Understand, and Prevent Disease

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Genome editing technology has the potential to revolutionize healthcare. Development of the clustered regularly interspaced short palindromic repeats (CRISPR) technology has advanced targeted genome editing through engineered nucleases. Referring to CRISPRs, National Geographic states, "No scientific discovery of the past century holds more promise..." (1). In this review, the future perspectives of CRISPR genome editing in health care will be discussed; specifically, how this tool may be used to treat, understand, and prevent diseases.

Since its characterization in 1990 (2), CRISPR/Cas9 technology has revived the field of gene therapy due to its affordability, simplicity of use, accuracy, reproducibility, and application to a wide array of cell types (3). This technology improves upon earlier gene therapies, such as transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs). These earlier methods depended on protein/DNA recognition for target specificity, whereas the CRISPR/Cas9 technology uses ribonucleotide complex formation to bind to its targets, permitting more simplicity in target design and access to diverse genomic locations (3).

CRISPR systems are adapted from bacterial immune systems. The original CRISPR/Cas9 system is composed of Cas9, a single polypeptide nuclease that cleaves DNA, and a single guide RNA (sgRNA) that guides Cas9 to the genomic target site. Double-strand cleavage occurs near a protospacer adjacent motif (PAM) sequence, often one to five nucleotides downstream of the target. The cell responds by imperfectly repairing the break, producing either insertions or deletions (indels) at the target DNA site. If that target is a gene, the indels result in the knockout of the gene. In addition to producing knockouts, this tool may be used to create knock-ins, providing additional possibilities for gene therapy (3).

There are two categories of future use for CRISPRs to treat or prevent human disease: 1) changing somatic cells to treat an individual, and 2) changing germline cells so a disease is not inherited. In both categories, cells are extracted from a patient and genes are edited *ex vivo* using CRISPR/Cas9 techniques before being re-implanted back into the person.

As an example of treating somatic cells, the first human CRISPR/Cas9 trial is underway to treat immune disorders, like metastatic non-small cell lung cancer. In this trial, CRISPR/Cas9 is used to knock down the gene coding for a protein called PD-1 in cancer patient immune cells. Knocking down PD-1 activates the body's immune system so it can fight the cancer (4).

Plans are underway to use similar approaches to treat other diseases. HIV genomes integrated into human immune cells have been deleted using CRISPR/Cas9 technology. The hope is to completely eliminate the HIV virus and its effects in patients. Rather than knocking out a gene, cells extracted from patients with sickle cell disease will be treated with CRISPR/Cas9 methods to replace defective genes with functional ones (5).

With germline cells, CRISPR/Cas9 can modify the genome of cells involved in sperm and egg production, thereby affecting successive generations. Currently, CRISPR/Cas9 is being used to produce transgenics in model organisms, from plants to primates, for a wide range of applications including agriculture and health research (6). For example, in our own research, we are using CRISPR/Cas9 to develop models of heart disease in zebrafish. Models like ours, and others, can then be used to study the molecular mechanisms and physiological impact of the genomic alterations. These models can then act as a tool for high-throughput screening to eventually develop new therapeutics or gene editing strategies. Such precision therapies will protect patients from harmful side effects and reduce the rising cost of disease worldwide (6).

While generating transgenic model organisms is common, 40 countries currently oppose germline modification in humans (7). Many researchers are concerned that these interventions are dangerous, as we may not

witness the side effects until years later (7). We share these concerns; we need to ensure CRISPR/Cas9 knockout efficiency is very high (and hence the risk of "off-target" effects is very low) before approving its use in humans. The level of acceptable efficiency remains a subject of debate and involves determining whether to treat terrible diseases now with the possible risk of unanticipated effects later. To minimize such risks, researchers are improving the CRISPR/Cas9 efficiency of precise genome editing. Even now, CRISPR specificity is being improved by developing CRISPR/Cas9 nucleases with modified PAM specificities (8) and engineered sgRNA structures (9).

CRISPRs modify the genome with great efficiency and safety, and will therefore revolutionize genetic medicine. Approaches are likely to evolve as CRISPRs become applicable to wider targets and more diverse genome editing functions, including large insertions, deletions, and specific base-pair changes, essentially fixing disease mutations. While the technology exists to modify the human genome, there are incredible ethical issues that must be addressed to maximize the positive impact of the technology (1). Through the application of CRISPRs to treat, understand, and prevent disease, healthcare advances will reduce the global burden of diseases and people will lead longer and healthier lives. ■

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Main Submission

Vectors Have Both Magnitude and Direction: Considerations for Viral versus Non-Viral Vectors in Gene Therapy

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Introduction

In February 2017, a Canadian clinical team introduced functional genes into a patient to treat Fabry disease, a rare genetic disorder characterized by abnormalities in lysosomal storage, which can ultimately lead to life-threatening kidney and heart complications (1). This case received worldwide media attention as the first recorded gene therapy for the disease. Gene therapy is characterized by the therapeutic delivery of nucleic acids into a patient's cells, in order to induce functional changes into the genetic code (2). The patient's medical team, led by Dr. Aneal Khan, used a lentivirus to insert altered genes into stem cells harvested from the patient's bone marrow (1). The cells were subsequently injected back into the patient, and the patient underwent careful immune monitoring to ensure that the lentiviral method of gene therapy did not cause a systemic inflammatory response. This clinical breakthrough prompted discussion in the scientific community regarding the possible challenges with different gene therapy vectors. Somatic gene therapy treatments can deliver DNA into nucleated cells through recombinant viruses or non-viral DNA complexes (3). Both options possess significant advantages and challenges, which require further investigation.

Considerations Regarding Viral Vectors

Viral vectors have been a source of controversy since September 1999, when an 18-year-old patient died of complications in a viral-based clinical trial for gene therapy (4). Jesse Gelsinger was injected with an adenoviral vector carrying a functional gene in order to treat ornithine transcarbamylase deficiency. He died of immune-related complications four days after injection, with subsequent investigations from the Food and Drug Administration concluding that the trial was in violation of research ethics. However, viruses remain the most common vector in gene therapy (Figure 1), as viruses efficiently introduce their genetic material into host cells with the goal of replication (4,5). Limitations of viral vectors include insertional mutagenesis, difficulty in production, as well as immunogenicity due to the patient's immune response (6). Depending on the location within the host's genome, mutations can have varying effects on the cell. For example, lentivirus-based viral vectors, such as that used in the Fabry trial, possess the risk of augmenting cancer, as lentiviruses can spontaneously insert sequences at unplanned locations in genes involved in apoptosis or cellular replication (7). This was evident in a retroviral gene therapy trial in 2002, in which

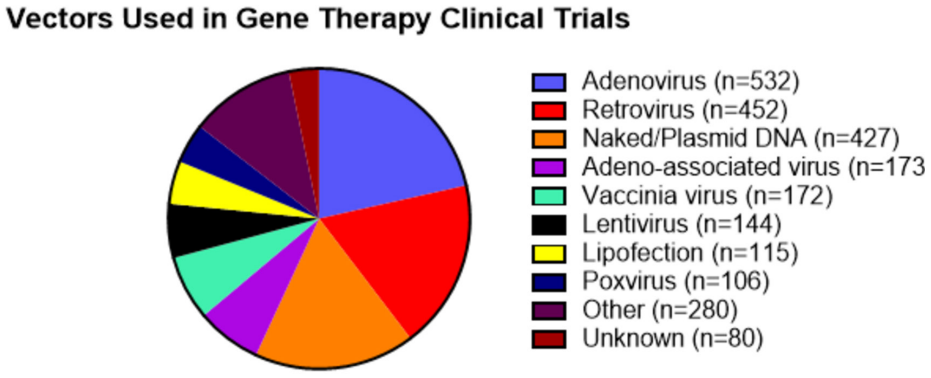

Figure 1: Prevalence of vectors used in gene therapy clinical trials. Adapted from (5).

four patients unfortunately developed leukemia following their lentiviral treatment (7).

Considerations Regarding Non-Viral Vectors

As a result, non-viral gene therapies have gained attention from researchers as a potential alternative. Non-viral vectors are comprised of synthetically produced biological particles, in which the plasmid DNA (pDNA) carrying therapeutic genes is encapsulated or bound to a synthetic chemical compound (8). Upon delivery, the vector is then released at the target site in order to induce changes in the genome. Examples of non-viral vectors include lipoplexes, inorganic nanoparticles, and the injection of naked DNA directly into the host cell (9). In contrast to viral-derived vectors, non-viral systems are relatively easy to mass-produce, and the risk for inflammatory complications is significantly lower (4,7). Furthermore, non-viral vectors pose advantages; in addition to pDNA, they are also capable of delivering synthetic compounds, such as short interfering RNA. However, limitations of non-viral vectors include decreased extracellular stability of the delivery complex, reduced internalization and cellular trafficking of the vector, and unsustainable expression of the therapeutic gene. Ultimately, while recent technological breakthroughs have attempted to mediate these challenges, the transfer efficacy of non-viral gene therapies remains greatly reduced in comparison to viral vectors (4). Further research must be conducted in order to increase the transfer efficacy and bioavailability of non-viral vectors.

Alternative Vectors

When faced with viral and non-viral options for vectors, recently developed "hybrid vectors" also remain a viable option for gene therapy (4,10). Hybrid vectors are comprised of a viral vector, which is conjugated to a synthetic biocompatible polymer, resulting in ablation of the native virus and enhanced transduction towards host cells (10). While this option could still elicit a potential immunologic response to the viral constituents, the risk of inflammatory complications is significantly decreased. One relatively promising example of a hybrid vector uses adeno-associated viruses to encapsulate potent genes in a bacteriophage capsid, and offers sustained gene expression (4). However, depending on the type of hybrid vector, the production process can be cumbersome (10). It is also important to note that alternative vectors may pose an additional risk of oncogenesis, depending on the vector used, the therapeutic gene, and the cell type targeted (11).

Conclusions

In conclusion, both viral and non-viral vectors offer significant advantages and obstacles in effective gene therapy (Table 1). It is important for clinical researchers to tailor vectors to specific applications of gene therapy, in addition to considering alternative options such as hybrid vectors. While cases such as the recent Fabry disease trial present the promising capabilities of gene therapy, the technology is not without risks that must be carefully considered. Further research must be conducted in order to develop an "ideal" gene therapy vector that balances transduction efficiency with the safety profile and ease in production of the vector. ■

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Table 1: A comparison of viral, non-viral, and alternative vectors.

Vectors	Advantages	Limitations
Viral	High transduction efficiency Sustained transgene expression	Insertional mutagenesis Immunogenicity Difficulty in production
Non-viral	Low toxicity and immunogenicity Relatively easy to mass-produce	Reduced extracellular stability Low transduction efficiency and specificity Limited duration of transgene expression
Alternative	Sustained transgene expression Decreased risk of inflammatory complications	Possible immunogenicity and insertional mutagenesis Complicated production

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Main Submission

New Developments in Human Embryonic Research Put Pressure on the 14-Day Rule

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Since 1979, the "14-day rule" has stood as a regulatory and legal limit on *in vitro* human embryo growth. It stipulates that human embryos cannot be maintained *in vitro* past 14 days of development. The limit was first proposed following the ethical discussion surrounding the first *in vitro* human fertilization in 1970 at the University of Cambridge (1). Since then, it has been adopted by many countries, either as scientific guidelines or set into legislation, as has been done in Canada. Recent developments in human embryonic research are beginning to test that rule for the first time, both by the maintenance of embryos up to 13 days for the first time, and by the creation of embryo-like structures, to which the 14-day rule may not apply.

The 14-day barrier was selected as that is the point of human development at which germ layer differentiation begins and the primitive streak – the early site of gastrulation and the site that later becomes the spine – becomes apparent. At this point an embryo is a blastocyst, barely visible to the naked eye. Before 14 days, approximately 50% of embryos at this stage are sloughed off by the uterus – a high attrition rate – and embryos can fuse together or split into twins, indicating that the embryo may not yet be a discernible individual. Once the primitive streak develops at 14 days, the embryo is considered an individual, as it can no longer fuse with another embryo or split into twins.

The 14-day rule sought to strike a balance between the need to ascribe value to human embryos without abolishing the scientific investigation of viable human embryos. It is also just one of many regulations governing human embryonic and genetic research. Nonetheless, the 14-day rule is not without critique, both from those who believe that it places too little value on the moral status of an embryo, and from those who believe it is an arbitrary obstacle to important human developmental research.

Until last year, the rule existed without any practical methodological challenge. It was only in 2016 that two scientific groups in the United States and the United Kingdom attained the capacity to grow self-organizing human embryos for 13 days *in vitro* – for the first time coming up against the barrier of the 14-day limit (2,3). Never before had researchers been able to extend their *in vitro* embryonic growth further. In addition, human embryonic stem cells were recently induced to develop features characteristic of later developmental stages, including primitive streaks and distinct germ cell layers; however, those were not intact whole embryos, and as such, did not violate the 14-day rule (4).

In addition to this immediate methodological challenge to the 14-day limit, more taxing ethical and legal challenges will soon emerge. Early this year, a report on the implications of synthetic human entities with embryo-like features (SHEEFs) cast doubt on the ability of the simple 14-day rule to effectively direct research utilizing these or similar structures (5). SHEEFs are not a new concept, but they will soon become a new reality. They are embryo-like assemblies of cells created from pluripotent human stem cells. These could potentially be viable embryo-like cell clusters created from induced pluripotent stem cells, or they could be structures distinct from embryos, but displaying similar features of development (4).

Adult human cells have yet to be induced to form a SHEEF; however, this work has progressed further with mouse cells, where researchers have maintained combinations of cultured embryonic and trophoblast stem cells up to 6.5 days with comparable development to maternally-developing embryos (6). In addition, research with human pluripotent stem cells induced from adult fibroblasts and grown *in vitro* has led to the development of organoids, including livers shown to be functional upon implantation and growth in animal models (7).

The applicability of the 14-day rule to SHEEFs depends on the definition of "embryo." Is a "gastruloid" created from human embryonic stem cells that exhibits three germ layers but no other familiar structural pat-

tering an embryo? Is a structure apparently indistinguishable from a human embryo but created with human pluripotent stem cells induced from adult tissue also an embryo, and as such, subject to the same structural protections?

This technology is clearly in its infancy; however, the implications are that these current embryo-like cell clusters could one day be sustained *in vitro* – or *in vivo* in the case of induced cells – to a point of development where more complex human features would develop. These complex human features, like a nervous or circulatory system, offer great potential for scientific investigation. However, the 14-day rule was designed to protect a biological individual from experimentation, and since these features are distinctly human, this may spark ethical controversy.

Recently, several groups have spoken out in favour of amending or extending the 14-day rule, both to accommodate the progress of human embryonic research and to account for the difficulty of defining SHEEFs (5,8). To some it seems absurd to have allowed the 14-day rule to stand, only for the rule to possibly be broken by researchers (9). Without the 14-day rule, what would stand as a new limit? A modified 14-day rule would need to take into account the new practical reality of growing human embryos beyond 14 days. It would also be valuable to include direction for research into SHEEFs and other structures that have similarities but do not conform to the structural definition of a human embryo.

It is inevitable that novel discoveries, as well as ethical, social, and legal challenges, will soon arise from research into viable human embryos *in vitro* and SHEEFs. It would be prudent for these concerns to be addressed shortly – before researchers advance beyond the limits and imagination of current ethical frameworks. ■

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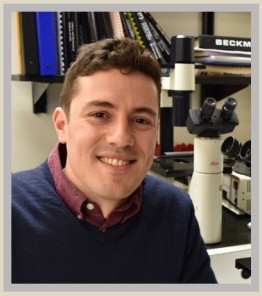

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Main Submission

CRISPR and TALEN: Facilitating Tailored Genomes of the Future

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Introduction

Genome modification allows scientists to directly manipulate gene expression through the utilization of novel genetic editing techniques. Notable techniques include clustered interspaced short palindromic repeats (CRISPR) and CRISPR-associated systems (Cas9), and transcription activator-like effector nucleases (TALEN). This article will discuss the strengths and limitations in both design and execution of the aforementioned techniques, and their applicable utility.

CRISPR

The CRISPR method contains viral genetic information within spacer DNA sequences, obtained following viral exposure (1). Cas genes code an endonuclease and helicase protein complex, which initiates double-stranded breaks in highly specific regions in the genome utilizing the spacer sequences as "guiding machinery." These breaks ultimately inactivate the gene of interest, rendering the viral infection ineffective (1). Thus, archaeal and bacterial cells are able to store viral genetic information within their spacer sequences as an adaptive mechanism to avoid viral infiltration (2).

In order to selectively replace a gene of interest, three main components need to be added into a cell: the Cas9 protein, guiding RNA (gRNA) specific for the gene of interest, and single-stranded DNA (ssDNA) containing the desired genetic information (3). When all three components are present, Cas9 will cut the gene at a particular location and replace it with the ssDNA. Cellular machinery then reverse-transcribes the gene segment. The double-stranded breaks created by Cas9 are repaired through non-homologous end-joining or homology-directed repair, thereby incorporating the added genetic information (4).

TALEN

TALEN has a DNA-binding domain and a nuclease effector domain. The DNA-binding domain is composed of 18 subunits, known as transcription activator-like effector (TALE) repeats, each recognizing one DNA base. Every repeat consists of 33 or 34 amino acids, and the 12th and 13th amino acids are known as the repeat variable diresidue (RVD), which varies between each TALE (5). There are 4 common RVDs, each preferentially binding to one of the four DNA bases, and each TALE is directly engineered to target one particular base. There are 2 sets of 18 TALEs bound to opposite sides of the target DNA region, hence one complete DNA-binding domain is able to recognize a 36 base-pairing sequence (6). Thus, the DNA-binding domain can be engineered to specifically target known sequences in the human genome.

Regarding its nuclease activities, TALEN utilizes Fok1 enzymes adjoined to each end of the TALE repeats. Two Fok1 enzymes will line up at opposite sites of the target DNA sequence, dimerize, and cleave out a particular section of nucleotides. Since site recognition is carried out by the highly accurate TALE repeats, which are engineered to target very specific sequences, this mechanism is applicable to manipulate any sequence in the human genome (7). Other endonucleases, such as EcoR1, are restricted to cut sequences 5 to 6 base pairs long. Therefore, TALEN has been proven to be a great tool in applications that require flexibility (8).

Applications

The field of genetics and genomics is undergoing a transformative phase with the emergence of genome modification technologies, which have unique advantages and drawbacks. For example, when comparing efficiency and ease of design, the CRISPR technique excels over TALEN. However, in terms of specificity and accuracy, the TALEN technique excels over CRISPR.

CRISPR is a more efficient genome editing technique (9), since the CRISPR system only requires the creation of a single gRNA sequence that is both significantly smaller in size and can recognize multiple loci (6). This efficiency makes CRISPR highly suitable for time-sensitive genetic

manipulations. An example of this would be in agriculture, where changing crop-growing conditions requires quick adaptability; thus, the CRISPR system could be used to quickly and efficiently modify the genome of plants to be able to withstand poor growing conditions.

However, the CRISPR system is prone to off-target activity, resulting in lower accuracy and specificity for targeted genome modifications. This is attributed to the gRNA found in Cas9 proteins, which contributes to the specificity and regulation of the CRISPR/Cas9 system (2). This gRNA can accommodate up to 5 mismatched base pairs per target site, making CRISPR prone to off-target activity (2). This is a concern, especially in the context of operations that require a high degree of accuracy. Alternatively, TALEN is a technique that excels in both accuracy and specificity. The DNA binding domain can be engineered to recognize a 36 base pair-long sequence, which is probably unrepeated in the human genome, thus significantly reducing potential errors (10). In addition, TALEN designs are very flexible, albeit lengthy, which may allow this technique to be applied to any sequence in the human genome. TALEN has been successfully implemented to correct genetic mutations that cause disease, engineer stably modified human stem cells, and hone the immune system to combat cancer (8).

As expected with many novel technologies, TALEN also has limitations, notably in its ease of design. At a single target site, 36 subunits (18 on each side) are required to be assembled, with each subunit consisting of 33-34 amino acids. In other words, it would be necessary to design a different DNA-binding domain of more than 1000 amino acids for each target site, which can be a highly tedious and technical process (6).

Conclusion

Currently, genome editing technologies are nowhere near perfect. However, with time, they are sure to be utilized in novel high-risk procedures. For operations demanding high accuracy, such as human genome editing, it would be best to use TALEN. However, for cruder genetic engineering, CRISPR would be a more effective technique. Nevertheless, gene therapy is an evolving field, and extensive investigation into both techniques must be conducted in order to elucidate their efficacious utility in varying fields of science and bioengineering. ■

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List of Abbreviations

CRISPR – clustered regularly interspaced short palindromic repeats

Cas9 - CRISPR-associated systems

gRNA - guiding RNA

RVD - repeat variable diresidue

TALE - transcription activator-like effector

TALEN - transcription activator-like effector nuclease

ssDNA – single-stranded DNA

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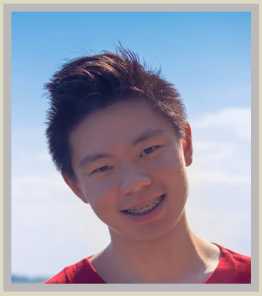

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Main Submission

Clinical Application of Omics Technology in the History of Primary Lactose Intolerance

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Primary lactose intolerance (PLI) refers to a group of symptoms associated with lactose maldigestion, which arise due to a physiological decline in the activity of the enzyme lactase after weaning (1). Although Hippocrates was the first to describe the symptoms of PLI, it took 2000 years to recognize that these symptoms are due to an intolerance of lactose, which is the main sugar in milk (1). During the early 1970s, it was discovered that PLI is an inherited trait (1); however, the genetics behind the hereditary nature of PLI have puzzled many scientists. Due to the rapid development of "omics" technologies, from the 1990s until the present date, researchers have made strides toward understanding the genetic and epigenetic factors of PLI (1-4). The use of omics technology has helped to both identify PLI genotypes and determine its true prevalence in populations. Furthermore, many nutrigenomic and personalized nutrition studies use PLI gene variants to examine the association between dairy and disease risk. Results from these types of studies help in developing personalized dietary recommendations tailored to one's genotype to enhance health outcomes (5).

The early 1990s saw the discovery of state of the art omics technology, which led to the identification of the lactase gene. Despite this, the lactase gene is not associated with PLI prevalence around the world. In 2002, PLI genotypes were identified using another sophisticated omics technique that matched the history of PLI in nine families with a map of gene variants surrounding the lactase gene (4). These genotypes were associated with lactase activity levels, PLI symptoms, and dairy intake (6). This turning point in the history of PLI allowed for quantification of the true prevalence of PLI around the world (6). It also confirmed that PLI is our ancestors' trait, and that the ability to consume milk in adulthood is an acquired genetic trait (1). Furthermore, this discovery provided a new tool to differentiate between PLI and secondary lactose intolerance (1).

Genotypes of PLI are associated with different activity levels of the enzyme lactase (4). However, based on the sucrase-lactase ratio, these genotypes were grouped into two traits: PLI and lactase persistence (LP; the ability to consume milk in adulthood) (7). In 2016, data from the Toronto Nutrigenomics and Health Study showed that those who possessed a genotype associated with intermediate lactase activity levels also had lower dairy intake and high risk of suboptimal vitamin D concentrations (8). These findings were similar to the association observed in individuals with the PLI genotype, but to a lesser extent. Inadequate vitamin D intake has been a main concern associated with the dairy-free diet followed by those with PLI (7). Since tests used in a clinical setting don't differentiate between PLI, intermediate PLI, or LP (9), these results support the use of PLI genetic information to diagnose intermediate PLI genotypes, and examine this group for risks associated with low dairy intake.

In the presence of low activity levels of the enzyme lactase, lactose is digested by colonic bacteria (1). Relatively few studies have looked at the effect of dairy consumption on colonic bacteria in individuals with PLI. This is mainly due to the burden of symptoms associated with dairy consumption in these individuals, and the limited capability of techniques that can examine the effect of dairy on the broad spectrum of colonic bacteria (1). This was no longer an issue after the development of omics techniques, which allowed for measurement of changes in the thousands of bacterial species that reside in the colon. Using these techniques in a clinical trial, it was determined that lactose consumption in individuals with PLI led to an increase in the abundance of 4 types of good colonic bacteria, a decreased lactose intolerance symptom score, and increased tolerance to milk (10). This is in agreement with one of the dietary recommendations outlined for individuals with lactose intolerance, which encourages those with PLI to continue consuming dairy in small amounts to increase milk tolerance (7). Recently, using multiple "Omics" technologies, multiple

factors were found to play a role in the epigenetics of PLI and LP, including dairy ingestion, age, and ethnicity (2,3). This is in agreement with the "gene-culture co-evolution" theory, which states that LP is a result of a genetic changes introduced after humans adopted dairy as part of their diet (1,6).

Developments in omics technologies have played a key role in understanding the effect of PLI genotypes on dairy consumption, as well as identifying intermediate PLI trait. Furthermore, omics technologies are currently being used to examine the effect of lactose intake in individuals with PLI. Future omics research examining PLI might develop promising personalized dietary recommendations that aim to help in decreasing PLI symptoms, as well as increasing milk tolerance and intake in those with PLI and intermediate PLI. These recommendations will also help both groups to consume the recommended number of servings of dairy per day. ■

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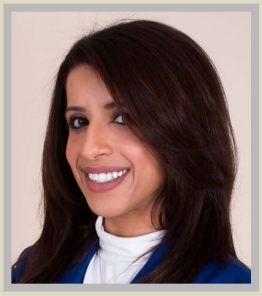

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Main Submission

A Lens Into Mass Cytometry: A Multifaceted Technology Used to Delve Deep into Single-Cell Analysis

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Introduction

Modern bioanalytical chemistry has enabled us to simultaneously detect multiple cellular markers, including proteins, genes, or small molecules within or on the surface of individual cells (1). These markers can be objectively measured and used as an indicator of disease state (1). Mass cytometry, or cytometry by time of flight mass spectrometry (CyTOF), is a versatile technology used for multiplexed single-cell analysis. Compared to its predecessor, flow cytometry, CyTOF allows for simultaneous measurement of more biomarkers with greater precision. These developments allow for exciting applications within the clinical, pharmaceutical, and research fields. In this article, we describe the methods underlying CyTOF, as well as discuss its emerging applications and limitations.

How Does Mass Cytometry Work?

Before understanding how mass cytometry works, it is important to understand the fundamental concepts of its predecessor, flow cytometry. In flow cytometry, cells of interest are labeled with fluorochrome-conjugated antibodies and measured with lasers. Fluorescent labels are excited at various wavelengths, which allows for the detection and quantification of up to 20 cellular markers (2). Mass cytometry involves the labeling of cells with metal-tagged (e.g. lanthanide earth metals) antibodies for the detection and quantification of up to 50 cellular markers (2). Simultaneously exploring more parameters per cell allows for a better understanding of complex cellular systems and signalling pathways, contributing to our current understanding of immunology and stem cell

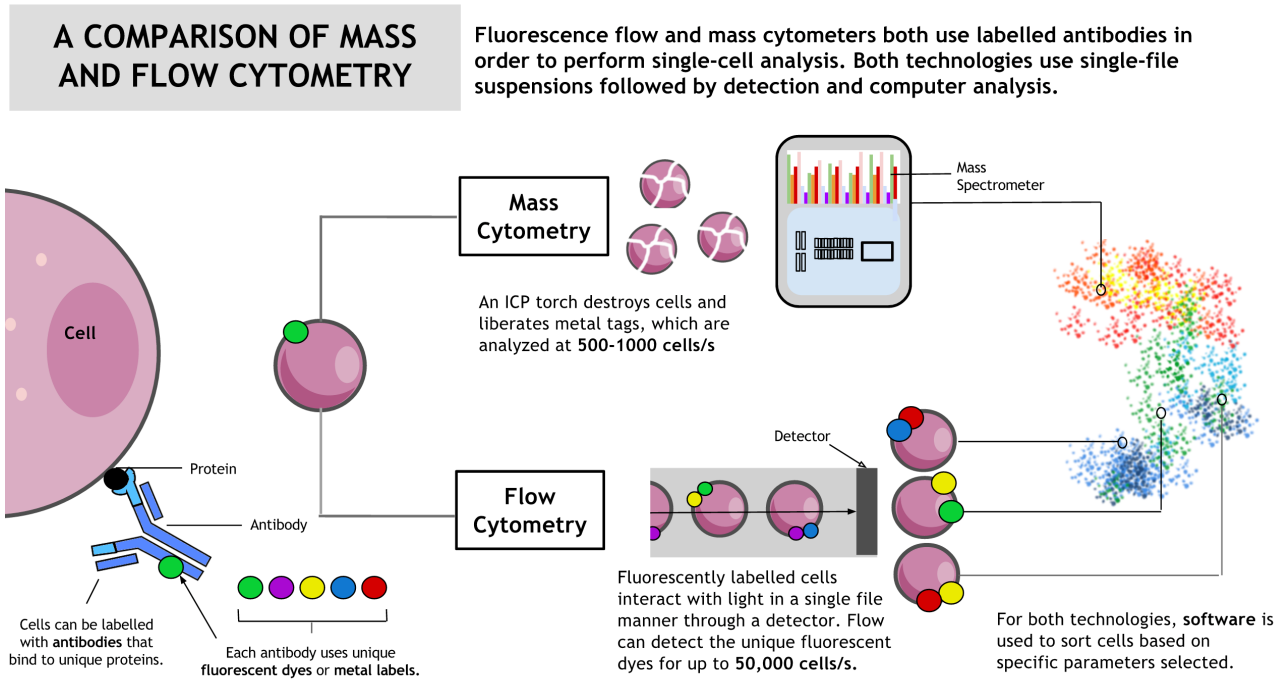

Figure 1: A comparison of mass cytometry and flow cytometry. Adapted from (2).

biology. Furthermore, CyTOF utilizes high temperature plasma to nebulize cells into a "cloud" of elemental ions, which contains the lanthanide metals that can be electronically analyzed based on mass and charge (Figure 1) (2). Quantifying cells based on mass and charge allows us to circumvent fluorochrome light detection overlap and autofluorescence, limitations of flow cytometry, thereby enabling the measurement of significantly more biomarkers for each cell (2).

Applications Of Mass Cytometry

Mass cytometry is a ground-breaking technological advancement in science, as it allows for highly detailed single-cell profiling that can be used to track progression of disease, determine specific immune signatures of patients, and assess the success of therapies (3,4). Currently, the measurement of gene expression levels and mass spectrometric analysis of human serum are used to identify biomarkers for the aid in diagnosis of a wide array of diseases. However, gene expression levels do not always correlate with protein levels in serum. Mass cytometry, a more sensitive technology, overcomes these inconsistencies by quantifying protein levels produced by single cells (5). Furthermore, mass cytometry shows promise for clinical application at the patient bedside (4). This technology allows clinicians to monitor immune cell signatures in patient blood samples in order to determine diagnostic and prognostic patterns, and to identify potential therapeutic targets (4). For example, a study by Fragiadakis *et al.* (2015) used mass cytometry to assess pre-operative immune cell distributions and intracellular signalling responses amongst a pool of patients undergoing hip arthroplasty (4). Quantitative analysis of intracellular signalling pathways that are vital to surgical recovery, such as pSTAT4, pCREB, and pNF-κB, were compared to successful post-operative immune profiles (4). Studies found that pre-operative immune states were predictive of successful surgical outcomes, suggesting that pre-operative implementation of mass cytometric analysis can be used as a screening tool to predict successful hip arthroplasty (4). However, a limitation of the study is that the patient cohort had minimal comorbidities and underwent the same surgical procedure, thereby reducing generalizability of the results to more heterogeneous patients with complex comorbidities. Lastly, although mass cytometry has tremendous potential in clinical applicability, the technology generates complex data sets, and scientists are still exploring statistical algorithms to better translate findings to clinical relevance, which is currently lacking consistency in present literature (6,7).

On a scientific scale, mass cytometry allows for cellular barcoding, which improves the efficiency and sensitivity of single-cell analysis; this allows researchers to better understand shifts and functional potential of specific cellular subsets and subphenotypes (5). Capitalizing on the utility of cellular barcoding to increase the efficiency in the acquisition of cells from multiple patients and/or cells from different compartments (e.g. spleen, blood, and bone marrow) significantly increases the throughput of patient samples (8). Specifically, metal-labelled cell barcoding (MCB), explored by Bodenmiller *et al.* (2012), uses a binary combination of seven different lanthanide ions to get 128 different combinations of lanthanide elements, increasing the combinations of lanthanide ions that can be used for barcoding (9). Thus, in this case, a barcode channel would be a combination of lanthanide metals conjugated to a functionalized antibody specific to a cellular marker. This high-content, high-throughput screening with MCB can be useful for clinical trials investigating novel therapeutics, pre-clinical testing of drugs, and *in vivo* and *in vitro* mechanistic investigation of human disease (8).

Limitations Of Mass Cytometry

Despite many advantages, a notable limitation with mass cytometry is the low throughput of cells, with roughly 30% of cells reaching the detector; this is significantly less than in flow cytometry, where 95% of cells reach the detector (5). The decreased throughput of mass cytometry poses severe constraints when detecting rare populations of cells, as low cellular recovery may lead to inaccurate quantitation. Another issue is the speed of analysis, where CyTOF processes 1000 cells per second, while flow cytometry processes 50 000 cells per second (5). However, this higher throughput results in a drift in signal intensity over time, increasing sampling bias when a large dataset is analysed (5). Using samples with a large number of cells can reduce signal drift, and using adequate quality controls within experimental systems can account for variations between samples. Finally, a limitation of mass cytometry is the inability to recover cells for further analysis, as cells are ionized into a "cloud" (2). However, this limitation can be circumvented by employing fluorescent-activated cell sorting (FACS) to isolate cells for functional assays on specific cell populations of interest (2).

The Future Of Mass Cytometry

The future of mass cytometry, particularly its clinical applications, has great promise in progressing scientific discovery (8). There is potential to develop computational methods for analyzing individual cells that could be used to predict alterations in cellular behaviour over time, and in different locations throughout the body. Mass cytometry could further enable time-dependent measurements at the single-cell level in complex heterogeneous tissue environments like malignant tumours. Specifically, the use of multiplexed ion-beam imaging (MIBI) has exciting potential with its use of secondary ion mass spectrometry, using antibodies tagged with elemental metal

reporters to visualize intact tissue slices (8). MIBI is similar to CyTOF, where both have the ability to measure surface and intracellular proteins on a single cell level; however, MIBI provides more specific information regarding cell interaction, cell morphology and localization within tissues (10). Specifically, MIBI uses secondary ion mass spectrometry (SIMS), which analyzes the secondary ejected ions from a solid surface, originating from a focused primary ion beam. MIBI allows clinicians to analyze up to 100 targets on tissue sections simultaneously, making it very effective within the heterogeneous cell populations often found in diseased tissues (e.g. malignant biopsies) (11). In addition to quantifying specific cells, MIBI provides high definition images showing cell morphology and localization (9). Overall, the implications of CyTOF and MIBI can be applied to visualize solid tissues from diseased states, including the central nervous system, bone marrow, spleen, and synovium, compartments relevant in inflammatory diseases (10).

Conclusion

Taken together, the utility of CyTOF is very promising, and scientists are merely at the infancy of clinical innovation and discovery by employing this technology. Although CyTOF does have a low throughput and slow speed of analysis, few experiments yield large quantities of arguably high quality data in clinical and research settings. Mass cytometry establishes a framework for time-dependent measurements at the single-cell level in complex tissue environments, with technological limitations being circumvented by both metal barcoding and the creation of computer analysis software. Overall, CyTOF technology will be crucial in elucidating the health status of patients through understanding the behaviour and distribution of individual cells, which will ultimately guide patient-specific treatment regimens of disease states based on cellular profiles (7). ■

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List of Abbreviations

CyTOF – cytometry by time of flight mass spectrometry

STAT3 – signal transducer/activator of transcription 3

CREB – cAMP response element binding protein

NF-kB – nuclear factor kappa-light-chain-enhancer of activated B cells

ICP – inductively coupled plasma

FCB – fluorescent cell barcoding

MCB – mass cell barcoding

MIBI – multiplexed ion-beam imaging

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Matthew is a 3rd year honours Bachelor of Health Sciences candidate at McMaster University, who has demonstrated a keen interest in the field of immunology. Joining McMaster's Cardio-Respiratory Research Lab in 2016, his work revolves around the investigation of therapeutic interventions in managing allergic asthma, as well as the general investigation behind allergen immunotherapy and its role in clinical practice.

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Main Submission

Heritable Genome Editing: Technological Innovation for Future Perspectives in Healthcare

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*Selected as Top Article

The year is 2017. Western civilization has become the epitome of a technology-driven era, with advances in medical science revolutionizing our treatment of disease. In 2011, genome editing was named "Method of the Year" by *Nature Methods,* and harnessing the CRISPR/Cas9 system to bring precision to this practice earned it the title of "Breakthrough of the Year" by *Science* in 2015 (1). However, legislations and policymaking have struggled to keep up with the rapid expansion of genomic diagnostics and treatment in clinical practice, and the growing disconnect between these two fundamental aspects of our healthcare system creates a barrier to translating knowledge into tangible health benefits for patients. As with any approach to a complex problem, there lies a challenge in defining a particular need. What aspect holds most promise to facilitate the application of our knowledge?

I believe that prevailing ethical issues are the foundation of this disconnect. Arguments on procreative liberty, public health benefit, and disability rights are nothing new to bioethical literature in this realm, though they have been left largely unexplored past the hypothetical. Now, with the tangible benefit of genomic interventions gaining increasing attention from successful *in vitro* studies, what was once hypothetical has become reality. Challenging public, clinical, and political populations alike to explore this potential is a much-needed influence to promote future health perspectives and human well-being in an emerging era of precision medicine.

In February 2017, the United States National Academy of Sciences (NAS) and National Academy of Medicine (NAM) released a report titled "Human Genome Editing: Science, Ethics and Governance" that supports this notion. An international panel of experts addressed the need to explore current capabilities of human genome editing and propose updated regulations that advocate for careful consideration over total prohibition (1). Recommendations on topics of heritable changes and where to draw the line on what would be considered enhancement invite the most scrutiny. The concept of gene editing that radiates beyond an individual in their lifetime to a whole future lineage has been a longstanding qualm for regulatory legislations and, as such, has considerable international variation (Figure 1) (1,2).

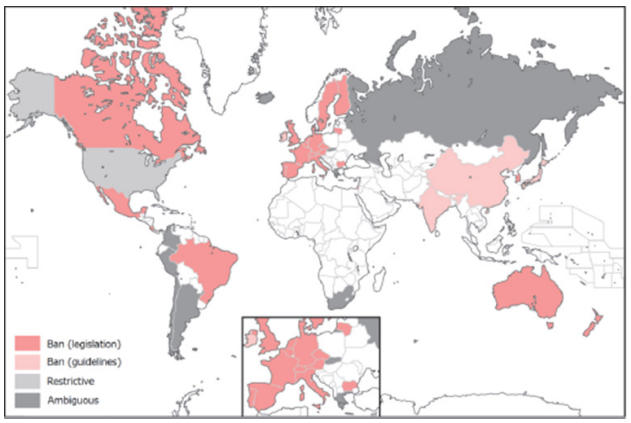

Figure 1: The regulation of heritable human genome editing varies extensively worldwide. Red represents countries with strict legal prohibition, while dark grey represents those that are ambiguous in their policies (2).

Take, for example, the widely popularized news story in mid-2016 about the first live-born "three-parent baby." The eye-catching title describes a 36-year-old woman who carries a mutation in some of her mitochondrial DNA (mtDNA), an independent genome exclusive to these tiny organelles, inherited through the maternal cell line (1,3). Though unaffected herself, the mutations manifested into a lethal form of Leigh syndrome in all of her six naturally conceived children (3). Mitochondrial replacement therapy (MRT), a form of *in vitro* fertilization, allowed healthy mtDNA from a donor "third parent" to replace mutated mitochondria in the mother's oocyte, resulting in a healthy pregnancy and birth with no complications (3).

However, because this technique is not approved in the United States, and would violate Canadian laws, the MRT procedure took place in Mexico, where regulations are more accommodating (3,4). Cases like this exemplify the need to establish international norms; MRT does not alter the nuclear genome, but does create a new set of heritable genetic material that would never occur naturally, hence designating it to be classified as a heri-

table genetic change. If a lengthy, legal process could be streamlined both with updated legislation and state of the art technology like CRISPR/Cas9, it would diminish a monumental barrier to accessible healthcare for similar patients who are quasi-eligible for life-changing genetic treatments, yet unable to afford international intervention.

Varying barriers to MRT and other types of germline gene therapy stem from regulations addressing the sensitive topic of genetic ailments debilitating enough to warrant their elimination in the cell line of an individual – an unsavory concept for many ethicists and rights activists in the disability community (5). Disability rights have seen milestone successes, and concerns of a slippery slope to past eugenic practices arise from the idea of heritable genome editing providing a "cure" for disability (6). The logic behind each opposing side is sound, though I argue that these ideas of social acceptance regarding disease or disability and social acceptance of heritable genome editing do not have to be mutually exclusive. Advanced technology can now provide the possibility to prevent an outcome parents might reasonably want to avoid, and it does not follow that these individuals would value the lives of existing people with disability or disease to a lesser extent (7).

Changing any standard of care in medicine is a multidimensional, carefully dissected process – and for good reason. This being said, many norms in our society today were at one point inconceivable ideas. The Human Genome Editing (2017) report gives explicit recognition to how human genome editing can effectively transform the treatment of disease. These discussions are needed to facilitate the next step: inviting international governing bodies and jurisdictions to embrace an attitude of forward-motion thinking for future perspectives that will most benefit the evolution of medical treatment. ■

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Meghan Lofft

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The personal side to genetic testing

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Genetic testing for cancer susceptibility is a premier example of personalized medicine (1). Owing to the development of next-generation sequencing, genetic testing is rapidly evolving from phenotype-driven sequential testing to multiplex testing. This new process has significantly reduced cost and increased the efficiency of gene sequencing (2). Multiplex testing uses whole genome sequencing to generate information on predefined target genes. Genetic cancer panels are an example of how multiplex testing can be applied clinically to offer individual risk information (3). Panel-based testing groups similar genes either by cancer type or level of associated cancer risk. This grouping technique reduces the potential for information overload, while still providing sufficient information for meaningful informed consent (4). With the growing number of identified cancer susceptibility genes, along with potential time and cost savings of multiplex testing, it is anticipated that panel-based testing will be incorporated into routine clinical care in the near future. While the clinical application of this form of testing should be guided in part by medical outcomes such as assessment of mortality and morbidity, it should also include broader outcomes including personal utility, which comprises effects that are personal, psychological, and social.

Panel-based genetic testing increases the likelihood of detecting an affected individual's disease-causing mutation and provides a more complete genetic evaluation (3). However, there are risks and limitations, as some genes have not yet been sufficiently studied, leaving their cancer risk unclear. There are also moderate-penetrance mutations, such as *CHEK2*, for which the optimal management protocols regarding screening and cancer prevention are unknown (5). Finally, research to provide risk estimates for individuals who may have multiple moderate-penetrance mutations is lacking. Therefore, test findings can be confusing and anxiety-provoking for patients, without apparent benefit. Providers need direction on how to guide individuals who receive genetic results in the absence of clear risk approximation, when there are incidental findings and optimal management is unknown.

Any new test, drug, or procedure typically undergoes a comprehensive assessment, including an evaluation of analytic validity (e.g. characteristic of the test; precision and reliability of the measurement of the assay), clinical validity (e.g. the ability of a test to predict the trait or condition in question), and clinical utility (e.g. improved measurable medical outcomes). However, small sample sizes and low incidence rates of many hereditary cancers have resulted in limited research demonstrating improved survival or health outcomes as a consequence of interventions informed by a genetic predisposition. For example, research on age-specific risk for many genes is inadequate. Consequently, recommendations regarding preventive measures are uncertain.

In light of limited evidence for clinical utility, some have argued that the evaluation of benefits of panel-based testing in clinical practice should incorporate personal utility (6,7). Personal utility is defined as genomic information that informs and guides personal understanding, decision-making, and behavioural responses to genetic test results (8). Access to genetic information can increase an individual's sense of control, allowing them to advocate for health-related decisions (6), reinforce adherence to clinical recommendations, and inform reproductive decisions (7). Personal utility can include important outcomes, such as greater compliance with screening recommendations or the initiation of risk-reducing behaviours. Personal utility can also include emotional effects (e.g. comfort or distress), collective effects (e.g. impacting family dynamics or stigmatization), and cognitive effects (e.g. increased disease comprehension). For example, individuals tested for Huntington's disease, a life-limiting illness with no preventative or therapeutic options, may experience personal utility from such a test through the conferred psychological, social, and practical benefits (7,9).

Personal utility is an important concept to consider when discussing the advantages and drawbacks of genetic testing. However, the subjective and multidimensional nature of this construct presents challenges in terms of measurement. One study on panel-based genetic testing for breast cancer susceptibility measured personal utility with a 12-point scale and found that perceived utility was modest after pre-test counselling (4). However, there was a significant increase in perceived utility after testing, which was highest among those receiving a positive genetic test result (4). This type of study underscores the importance of exploring the construct of personal utility in genetic testing, especially for those tests with uncertain clinical utility due to limited research.

When considering the clinical relevance of panel-based testing, personal utility is a criterion that should be weighed in the context of clinical utility, validity, cost-effectiveness, and health service delivery (10). There is a lack of recognition of the significance of this outcome, and empirical data is required to elucidate this concept. Panel-based genetic testing is a practical form of genomics and its full benefit will be undermined if we ignore the impact on the person. ■

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Main Submission

It's In Your Genes: Recent Considerations in Germline versus Somatic Gene Therapy

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Introduction

Although the notion of a "designer baby" seems to be dAs of February 14th 2017, the National Academy of Sciences endorsed the use of germline therapy in certain well-defined cases, resulting in immediate responses of public controversy and discussion (1). In its public release, the National Academy of Sciences compounded the findings of 22 scientists over the past year, consolidating the research of leading experts in both science policy and genetics. While this was not a change in legislation, and in fact opposes pro-life legislation prohibiting FDA approval of genetically modifying embryos, the released guidelines were a significant step towards making germline gene therapy more available (1). Ultimately, this may lead to policy reform in North America and Europe for both somatic and germline gene therapies.

Both somatic and germline gene therapies involve therapeutic delivery of nucleic acids into a patient's cells, in order to induce functional changes into the genetic code (Figure 1) (2). The process of somatic gene therapy only affects individual body cells and cannot be passed to offspring (3). In contrast, germline gene transfer involves genetic modification of tissues that are inherited from one generation to the next. As such, the germline technology carries certain ethical issues beyond those of somatic gene therapy. Somatic therapy has often been favoured by legislators due to its minimal risks and relative transiency. Over 600 clinical trials involving somatic gene therapy are currently running in the United States, targeting immunodeficiencies, cystic fibrosis, and clotting pathologies (4). However, germline therapy continues to be prohibited in Canada, the United States, and most of western Europe, due to the ethical issues associated with manipulating future generations, and the unknown consequences that may arise (4).

Current Challenges

There are several risks associated with germline gene therapy, particularly as research in germline therapy has been stagnant due to restrictions in funding; these restrictions prevent further information on the

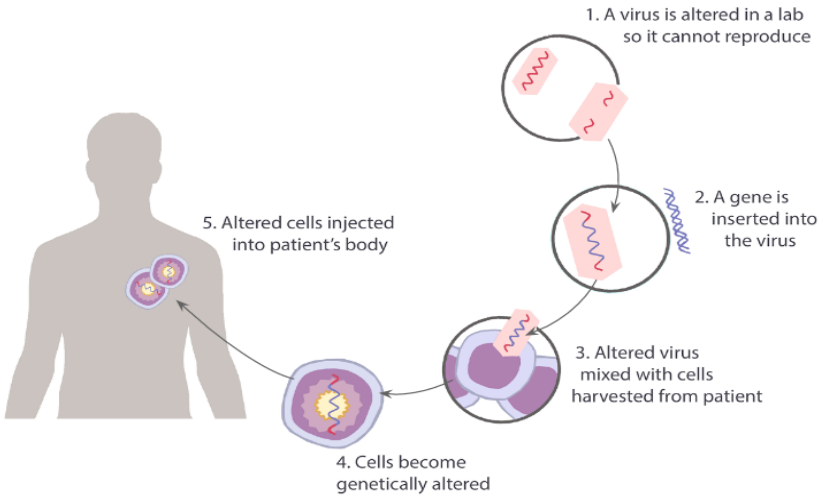

Figure 1: Simple model of the gene therapy process.

future consequences of germline gene therapy from being explored. Foremost, germline gene therapy has demonstrated the difficulty in transferring genetic vectors into spermatocytes or oocytes (5). While these complications can be easily detected in animal models, this may prove to be logistically difficult in human embryos. Multiple or partial gene copies could not only prove to be embryonic lethal, but could also remain dormant and be passed onto future generations to magnify any possible complications (5). This poses particular risk for polygenic diseases, which do not fit simple Mendelian disease models. In addition, germline gene therapy could pose risks as a platform for eugenics (6), or could be used to select physical characteristics that are unrelated to health. With steps being taken to make germline gene therapy more available, the response from the public has predominantly focused on the ever-present risk of "designer babies." While the National Science Agency panel explicitly stated that this technology not be legislated unless its sole purpose was health-related, the classification of "health-related reasons" is often contested (1,6). For example, there is a current stakeholder controversy regarding the use of genetic technology regarding disability (7). As such, any potential applications of germline gene therapy would require extensive regulation and appraisal.

Potential Advantages

Nonetheless, germline gene therapy remains highly promising due to its clinical applications. Not only can germline gene therapy treat single-gene diseases in individual patients, it also has the potential to completely remove a disease from the population (5,8). This would not only ensure public health in a manner similar to vaccines and population-based interventions, but also reduce the long-term health costs related to treating the disease. Over 24 million people in the United States alone are affected by autoimmune diseases with a heritable component, with treatment options often characterized by symptom management rather than providing an outright cure (9).

Conclusions

The recent developments from the National Academy of Sciences may inspire international reform regarding the genetic editing of gametes. As the United States government does not currently support federal funding for germline gene therapy, it is important for the research field to gain further awareness (5). The regulatory suggestions from the National Academy of Science are a significant step in allowing narrow, well-defined subsets of germline applications to be investigated for clinical potential. However, future steps, such as financial and political support from major North American government parties, are necessary for progress in gene therapy. ■

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Table 1: Disorder prevalence for single-gene disorders in live infant births in the United States.

Autosomal dominant	
Huntington's disease	1 in 15,000
Hereditary spherocytosis	1 in 5,000
Marfan syndrome	1 in 4,000
Neurofibromatosis type I	1 in 2,500
Autosomal recessive	
Galactosemia	1 in 57,000
Lysosomal acid lipase deficiency	1 in 40,000
Mucopolysaccharidoses	1 in 25,000
Phenylketonuria	1 in 12,000
X-linked	
Hemophilia	1 in 10,000
Duchenne muscular dystrophy	1 in 7,000

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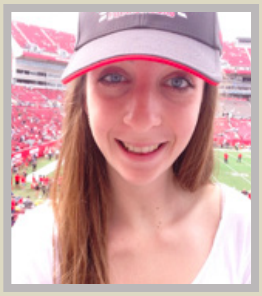

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Main Submission

Query into the Future of Gene Editing: Possibilities and Apprehensions

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Since the discovery of 22,300 protein-coding genes by the Human Genome Project, geneticists have generated tools to manipulate DNA using engineered nucleases such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) (1-3). Concurrent advancements in stem cell research have allowed for de-differentiation of somatic cells back to a pluripotent state, known as induced pluripotent stem cells (iPSCs), which can then be re-differentiated into any cell type (4). This review is focused on the safety and ethical concerns of the intersection between gene editing and iPSCs to create genetically corrected injectable cells for therapeutic purposes. To emphasize the importance of genetically engineered injectable cells, we will discuss the application of this approach for treatment of chronic disorders, prevalent in the western world.

Although the use of genetic engineering allows for site-specific genetic alterations (3), future consequences of this approach remain in juvenile stages. Older gene editing techniques, such as transcription activator-like effector nuclease (TALEN) and adeno-associated virus (AAV), present with limitations of non-specific site targeting, cytotoxicity, and low vector transfer efficiency. In one case of heart disease, AAV vectors caused fever and muscle spasm in patients, emphasizing the importance of performing prior safety trials (5). Fortunately, the new CRISPR/Cas9 has been revolutionizing the field by its simplicity, low toxicity, and high efficiency. The ability to simultaneously deliver multiple single-guided RNAs using CRISPR/Cas9 has allowed for editing genes in polygenetic forms of diabetes and heart disease (6). The first clinical trial is currently being conducted using CRISPR/Cas9 in a small human lung cancer population, in which the safety of these methods will be monitored.

The employment of iPSCs in gene editing allows for the introduction of desired cell-specific genetic alterations (4). However, even less is known regarding future consequences of this approach. Risks associated with using stem cells for gene editing include the type of cells used, the procurement, culturing, the level of manipulation, and site of injection. These risk factors may lead to tumourigenesis, immune activation, and bio-transmission of pathogens. Limitations in safety databases, such as low numbers of treated patients and limited long-term follow-ups, leads to a lack of scientific understanding of the long-term consequences (7). Currently, scientists are investigating ways to manage these risks. To better address the immune rejection issues of iPSCs, scientists are investigating the use of CRISPR/Cas9 to form universal donor stem cells (UDSCs), which lack antigens that are usually targeted by the immune system (8).

With these limitations in perspective, scientists are monitoring the application of gene-edited iPSC-derived injectable cells in cultures and small clinical trials. This novel approach has been applied to treatment of monogenetic cardiovascular disease and type I and II diabetes (T1D and T2D, respectively). One recent study corrected phospholamban-dependent cardiomyopathy and generated human PSC-derived cardiomyocytes (9). Genetically engineered iPSCs have also been applied to T1D, a disease resulting from the autoimmune destruction of pancreatic β-cells. It has been proposed that by differentiating β-cells from UDSCs, the immune attack can be bypassed (8). Concordantly, genome editing has been used in T2D, a disease that results in peripheral tissue insulin resistance and pancreatic β-cell exhaustion. Furthermore, genome-edited iPSCs have been used to show that the haploinsufficiency of key insulin-related genes is sufficient for early exhaustion-induced β-cell death, identifying targets for gene correction (10). Moreover, we propose that these methodologies may be applied to re-inject iPSC-derived hematopoietic stem cells, genetically engineered to be less pro-inflammatory, to dampen the inflammation and insulin resistance in peripheral tissues.

The findings of these studies, which reaffirm the importance of this field, need to be cautiously assessed at every stage. The obscurity in future consequences

of genome editing also challenges the Hippocratic oath of non-maleficence. If genome editing is the future direction of medicine, there needs to be a consensus on the extent of its clinical application. There is the likelihood of this technique being first available to the wealthiest, and used for purposes of self-improvement instead of the treatment of life-threatening illnesses. Critics argue that the fiscal load of genome editing will not allow its advancement into medical practice. However, the cumulative lifetime financial and medical burden of chronic disorders begs for an alternative approach that may not only manage these diseases but treat them. Therefore, before genome editing reaches this capacity, there needs to be an open dialogue for establishing governmental policy framework, patents, and regulations that are systematically monitored.

Today, the application of genetic engineering on iPSCs is an attractive approach that removes the drawbacks of donor genetics. While there remain several safety and ethical issues with this method, the discoveries achieved for cardiomyopathies and diabetes from this scientific intersect cannot be disregarded. Therefore, it is important to further navigate the ethical and safety concerns through immediate strategic actions by scientists and governing bodies, and to accelerate this treatment for these highly prevalent and chronic diseases. ■

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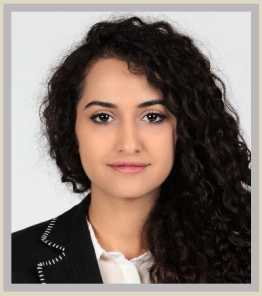

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The Predictive Power of Omics: Clinical Applications

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*Selected as Top Article

Introduction

Scientia potentia est: "knowledge is power."

This simple idea drives medical decision-making. To maintain the health and well-being of their patients, physicians must acquire information on environmental and biological factors that may predict disease risk, progression, and treatment effectiveness. However, traditional clinical tools are limited in their ability to capture information, usually relying on only a handful of readouts. For example, questionnaires or blood tests can be used to reveal certain parameters, such as anxiety level or red blood cell count, but not the totality of the condition. Enter the "omics" revolution: the advent of technologies that aim to capture complete information about some aspect of physiological systems. These omics technologies, including various high-throughput DNA and RNA sequencing platforms, as well as mass spectrometry analyses of protein and metabolite levels, allow for the holistic consideration of the complex networks and interactions that form and sustain each individual (1,2). Patient-specific data can be used in computational models that predict disease onset, disease progression, and treatment effectiveness, thus facilitating medical decision-making (Figure 1) (3,4). The value of omics in healthcare is therefore the predictive power it provides.

The Predictive Power of Omics in:

Disease Risk and Onset

Omics can be applied to assess both disease risk and onset. Genomics, for example, can be used to predict the lifetime risk of a disease. Currently, physicians collect information on family history and may screen for a select number of genetic risk factors. In contrast, genomics has the potential to identify all possible genetic risk factors for any disease (3). This increases the likelihood of capturing rarer genetic risk factors or genetic variants that increase risk to lesser extents, providing a more accurate risk assessment. Additionally, other omics technologies could be used to predict disease onset (5,6). The power of the combined omics analysis was demonstrated by the Snyder Lab at Stanford University. An integrative Personal Omics Profile (iPoP) was produced for one individual over the course of 14 months through recurrent measurements of transcriptomics, proteomics, and metabolomics. This study identified that the subject had an elevated risk of developing diabetes, and observed increases in blood glucose concentrations that signaled the onset of the disease; following diet changes, exercise, and low doses of aspirin, the glucose levels returned to normal (5). Regular monitoring of generally healthy individuals with omics-based technologies

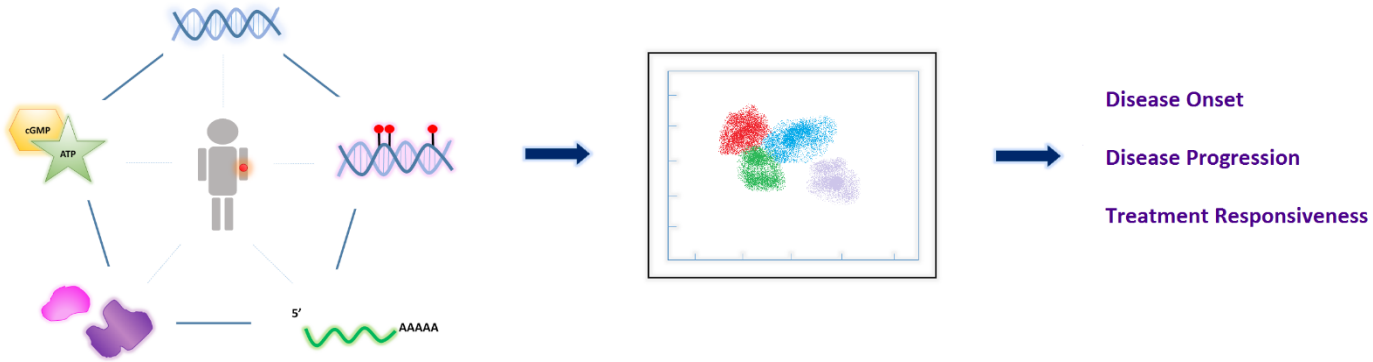

Figure 1: Information acquired through different omics technologies (genomics, epigenomics, transcriptomics, proteomics, metabolomics, and more) can be used in computational models to accurately predict different health parameters.

could generate data that herald the onset of disease, allowing early diagnosis and early intervention, which are both crucial to treatment success.

Prognoses

Prognostic factors include progression of disease severity, likelihood of survival, and survival time. Accurate predictions of these factors have important roles in medical decision-making, affecting treatment and end-of-life decisions (7). One omics-based test currently applied in making prognoses is the Oncotype DX test (2). This test examines the gene expression profile of a patient's breast cancer tumour in order to predict the likelihood of breast cancer recurrence, information that can aid in treatment selection. Individuals with low risk of recurrence are treated with endocrine therapy alone. In contrast, individuals with high risk of recurrence are treated with both endocrine therapy and chemotherapy. This way, individuals with low risk of recurrence are pre-empted from unnecessary and highly invasive treatments with minimal therapeutic effects. A meta-analysis of medical decision-making reveals that more than 1/3 of physicians have omitted chemotherapy in the treatment of breast cancer patients based on results obtained from the Oncotype DX test (2). Therefore, omics can assist physicians and patients in making informed decisions by facilitating accurate prognoses.

Treatment Effectiveness

Not all treatments are equally effective for all patients; what cures one individual might only cause adverse side effects in another individual with an ostensibly similar ailment. Omics-based techniques can help healthcare professionals formulate optimal treatment strategies for each patient by allowing for better characterization of the biochemical underpinnings of diseases and improved prediction of the effects of pharmacological intervention (8). Genomic sequencing of tumours can identify the exact mutations responsible for an individual's cancer, so that the appropriate pathways may be targeted. Selection of drugs for treatment should be guided by pharmacogenomics, a relatively new field whose development is based on the availability and continued improvement of genome sequencing techniques. Pharmacogenomics examines how genetic differences between individuals underlie differential responses to drugs. For instance, dosing strategies for commonly used drugs such as warfarin are adjusted according to genetic polymorphisms affecting the patient's drug-metabolizing enzymes (9). Even more recently, developments in epigenomics have sparked interest in the modulation of drug effects by epigenetic mechanisms. Knowing a patient's genetic and epigenetic background will therefore be crucial to picking treatment strategies that are most likely to succeed.

Limitations and Conclusions

Current barriers to the implementation of omics approaches in clinical practice are mainly logistical in nature. Although powerful, many of the methods of data collection and analysis are time consuming and resource-intensive. Conducting reliable analyses of these large data sets is an additional hurdle, as the study of systems and computational biology is still in its infancy and the processing of omics data is computationally intensive (8). Nevertheless, as the costs of omics technologies decrease and more holistic research is conducted, current trends suggest the eventual widespread use of omics in personalized medicine (3).

The ability of omics to capture the totality of one aspect of physiological systems in a hypothesis-free manner facilitates the accurate prediction of disease onset, progression, and treatment responsiveness. This high predictive power sets it apart from traditional methods of treatment that require a number of guess-and-check points. Omics will be an important influence on the healthcare system, necessitating large collaborative efforts. These efforts will yield highly accurate healthcare-related prediction models, which will have tremendous influence on medical decision-making and individual healthcare. ■

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Main Submission

In Utero Gene Therapy: A Brave New World of Designer Babies?

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Introduction

Although the notion of a "designer baby" seems to be distant science fiction, with the advent of genome-editing technologies such as CRISPR/Cas9, "designer babies" may soon become a reality. In 2015, the United Kingdom's parliament authorized the conception of the world's first genetically modified embryo (Figure 1) (1). Given that many countries including Canada, Mexico, and Australia have banned *in utero* genetic editing, this legislation is a milestone and has been met with great apprehension. Many fear this legislation will create a slippery slope promoting the normalization of "designer babies," wherein genetic modification is used not only for medical purposes, but also for the selection of desired traits to improve the "quality" of an embryo. Accordingly, this article will explore the potential applications of this technology as well as the pertinent ethical considerations.

Applications of *In Utero* Gene Therapy

Proponents of *in utero* genetic modification boast its vast potential in treating genetic disorders. Although seemingly foreign, gene therapy has already been employed postnatally in humans to treat disorders such as hemophilia B, a bleeding disorder caused by a genetic defect resulting in a lack of coagulation factor IX (FIX) (2,3). In 2011, Nathwani *et al.* employed an adenovirus-associated vector to incorporate the functional FIX gene into target human cells *in vivo*. A single infusion in adult

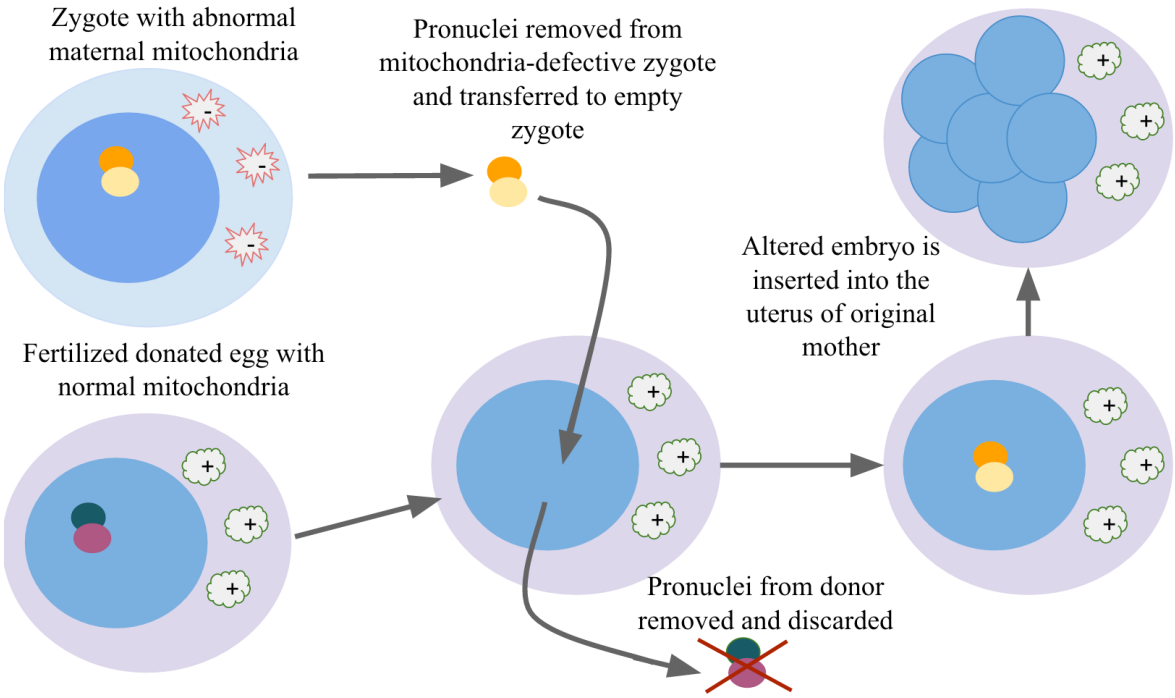

Figure 1: This genetic modification performed on the United Kingdom's first tripartite zygote allowed for the removal of defective maternal mitochondrial DNA that may have resulted in diseases, including fetal muscular dystrophy, as well as heart, kidney and liver failure. This genetic modification involves exchanging the defective maternal mitochondrial DNA with that of another female. Adapted from (10).

hemophiliacs increased long-term expression of the FIX gene, with no long-lasting toxicity (3). These results prompted discussion of using *in utero* gene therapy to treat hemophilia A, a genetic deficiency of coagulation factor VIII (FVIII) (4). Current treatments for hemophilia A involve bi-monthly intravenous infusions. Unfortunately, the annual cost of these treatments can exceed $300,000, and some patients become immunologically intolerant to the FVIII treatment, rendering it ineffective (4). The physical and financial burdens associated with this condition can therefore negatively impact the quality of life of these individuals (4).

Despite stigma surrounding prenatal genetic modification, *in utero* gene therapy may be safer and more efficacious than postnatal alterations, as cells *in utero* replicate more readily, are more responsive to genetic alterations, and early introduction of FVIII may reduce the likelihood of developing an immune response against treatment (4,5). As such, *in utero* modifications can confer cost-reducing benefits to those with genetic disorders. However, fetal development is sensitive and errors can be debilitating or fatal, as demonstrated by the phocomelia epidemic caused by the consumption of thalidomide by pregnant women for morning sickness. Nevertheless, the United Kingdom's bold leap to legalize *in utero* genetic modifications is a pivotal step towards developing a safe method for treating genetic disorders.

Ethical Considerations for *In Utero* Gene Therapy

It must be noted that current technology does not afford us the capacity to create "super-humans," per se. Furthermore, the selection of "superior" traits is often merely selection against negative traits. However, despite the apparent benefits, *in utero* gene therapy must still be accompanied with ethical consideration to address potential outcomes of selecting "superior" traits. Firstly, there are potential consequences to the fetus itself, which are nearly impossible to predict with embryonic alteration (6). Is it reasonable to subject fetuses to *in utero* modifications, even for the purpose of medical treatment, if there may be unforeseeable and inheritable outcomes? Considering the vast number of individuals affected by genetic disorders such as hemophilia A, the use of *in utero* gene therapy to treat these disorders without exploration of the long-term consequences could elicit widespread biological repercussions which will be propagable to future generations (7).

Furthermore, some claim that parents do not have the right to select traits in offspring on account of fetal autonomy (8). Although parents can significantly shape the characteristics of offspring through environmental factors (e.g. registering children for sports classes), do they have the right to modify these traits on a molecular level? Many argue that genetic selection of desired traits facilitates an unwarranted level of parental genetic autonomy (8).

Furthermore, given that *in utero* gene therapy is a relatively new practice, its associated costs may facilitate a new form of social hierarchy between those who can and cannot afford access to this technology, thereby extending socioeconomic divisions into genetic divisions that will intensify with future generations (7). By extension, the selection of "desired" traits, such as intelligence and athleticism, will provide this "designer" generation unethical advantages over those that are conceived naturally, as they may be more biologically predisposed towards "superior" traits conferring success (9).

Conclusion

Currently, genome editing technologies are nowhere near perfect. However, with time, they are sure to be utilized in novel high-risk procedures. For operations demanding high accuracy, such as human genome editing, it would be best to use TALEN. However, for cruder genetic engineering, CRISPR would be a more effective technique. Nevertheless, gene therapy is an evolving field, and extensive investigation into both techniques must be conducted in order to elucidate their efficacious utility in varying fields of science and bioengineering. ■

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List of Abbreviations

FIX	Coagulation Factor IX
FVIII	Coagulation Factor VIII

Spandana Amarthaluru

Spandana is a 4th year Bachelor of Health Sciences (Honours) student at McMaster University. She began working with the McMaster Cardio-Respiratory Lab in September 2015 under Dr. Gail Gauvreau and John-Paul Oliveria. Her work with the lab focuses on the role of IgE and B cells in allergic asthma.

Michael Aw

Michael is a 2nd year undergraduate student in the Bachelors of Health Sciences (Honours) program at McMaster University. His association with the McMaster Cardio-Respiratory laboratory began in 2016 as a summer intern. His research interests are catered towards allergic diseases, in particular the pathology of allergic asthma.

John-Paul Oliveria

John-Paul is a 4th year Doctor of Philosophy candidate in the Department of Medical Sciences at McMaster University. He began his MSc in the McMaster Cardio-Respiratory Research Lab in 2011 and transferred to his PhD in 2013. His research focuses on the biology of B cells (IgE+ B cells and regulatory B cells) and the pathogenesis of allergic asthma. In the fall of 2017, he will be commencing a post-doctoral scholar appointment at Stanford University.

Hubris *Joseph Bencic*

About the Artist: *Joseph uses surreal and abstract imagery to explore themes like human psychology and perception through his art. He has exhibited in Toronto and Vancouver. His current series is about sigils, words or symbols of power. "Hubris" is an attempt at simplicity, paring down all unnecessary forms and images so that the message is all that remains. Joseph's work can be seen at http://josephbencic.wix.com/artist, and on instagram or facebook.*

ASK AN EXPERT

Experts in the field delved deep into various issues regarding Gene Editing and Personalized Medicine

Ronald F. Carter, MSc, DVM, PhD, FCCMG

Professor Emeritus, Dept of Pathology & Molecular Medicine, McMaster University

"Do you believe that N-of-1 trials (a clinical trial in which a single patient is the entire trial), are appropriate to evaluate the effectiveness of genomic therapies for rare and/or common genetic disorders? Do gene-editing/targeting therapies have the potential to expedite the advancement of personalized medicine?"

We can now affordably detect the molecular mutations that cause thousands of rare genetic disorders, and drugs can be designed to specifically target protein dysfunctions caused by individual mutations. Further, DNA editing techniques like CRISPR bring the promise of actually correcting genetic mutations in the human body. It is all promise, however, until we can show that personalized drugs and gene editing deliver better outcomes. How can we safely and efficiently evaluate and adopt such personalized treatment modalities, when it often costs at least $1 billion and takes many years to achieve licensing and funding for a single new drug?

The first issue is how to demonstrate safety and efficacy for novel, personalized treatments engineered specifically for truly rare genetic disorders. Drug trials typically enroll hundreds to thousands of participants before approval for use is obtained; for rare diseases, there will never be enough participants to meet the usual requirements. With some syndromes, there are literally only a handful of affected people worldwide; how few studies and participants are enough? Is one person sufficient, if the disease is devastating, very few people have it, and no other effective treatment is known? I would argue that it could be permitted if conditions were met to help us mitigate and accept adverse risk. These conditions include: a) no known alternate effective therapy; b) a severe disease outcome is expected; c) cell culture studies show evidence of cause and effect mechanisms for improvement of cellular function; d) cell culture and animal model studies show limited expectation of unacceptable toxicity; e) dosage escalation can be attempted; f) defined endpoints with measurable outcomes are set, and g) there is a legally binding framework addressing issues of informed consent and management of adverse outcomes. Any implementations must also address the fact that the cost per patient will be utterly unaffordable on a large scale if the process of drug development, testing, approval, and production remains anything like what we have now.

Gene editing also beckons as a very effective intervention for genetic disease. Gene editing techniques come with two levels of potential implementation and impact: a) somatic corrections limited to the life and scope of an individual under treatment, and b) germline corrections that become hereditary and might alter allele frequencies in the general population. Gene editing is in its infancy and we certainly need to demonstrate the safety of the technology, especially for germline editing. However, it is important to note that in biological and evolutionary terms, gene editing of somatic and germline tissues may have opposite impacts. If it is effective and broadly used for a large number of genetic conditions, somatic editing would help more patients to survive, but also to potentially pass on disease alleles to their children. In contrast, germline editing could reduce the frequencies of pathogenic DNA sequences in populations and thereby improve reproductive fitness over time. This is a critical point, because when better care gives people with genetic disease the longevity to reproduce, then inevitably the burden of genetic disease in the population increases over time. Germline editing, however, raises huge concerns about the appropriateness of altering the human genome.

While laudable and desirable on an individual basis, the more healthcare helps people with disease, the more people live with disease. Paradoxically, generations from now, despite our ethical and moral concerns, we may have to embrace the most invasive and ethically troubling version of genetic engineering in order to preserve our own ability to reproduce. If we truly want to focus on optimal health of populations, maybe we should learn from somatic editing in order to perfect germline gene editing. Of course, ethical, moral, medical, legal, scientific, and cultural perspectives should fuel vigorous debate on the merits and risks of such an approach. We will need to decide if gene editing is worth all the costs. ■

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Dr. Ronald F. Carter

Dr. Carter is the Director of Laboratory Genetic Services for the Hamilton Regional Laboratory Medicine Program, which has a catchment of 2.3 million people in the central south region of Ontario. He is also Head of the laboratory cancer genetics service, which provides diagnostic cancer genetic testing (cytogenetic and molecular test services, including hereditary cancer syndromes). His research is primarily based upon collaborative studies involving clinical correlations of diagnostic or prognostic genetic markers.

Ask an Expert

Renaud F. Boulanger, MSc; Veronique Fraser, MSc, RN; Lori Seller, MSc, RT

Ethics advisors, Centre for Applied Ethics, McGill University Health Centre

Ethics guidelines and gene editing technology: the challenges of enforceability and legitimacy

"Our intelligence creates problems that our intelligence cannot handle. Come back Socrates, we're sorry about the hemlock." Richard Gordon, 1993

Recent advances in genetic technology have given rise to considerable excitement and debate. Genome editing tools such as CRISPR/Cas9 have made modifying the human genome more efficient, accurate, and less expensive. Gene editing presents promise for advancements in human health while simultaneously raising significant ethical concerns about its proper use and potential for misuse (1). For instance, should a line be drawn between genetic therapy and genetic enhancement? If so, where should the line be drawn – and who gets to decide?

One response to the development and implementation of health innovations has been the creation of ethical guidelines. These fall into two broad categories: guidelines for research and development, and guidelines for practice. Research ethics guidelines are particularly effective, as they tend to be highly enforceable through an established ethics oversight system that can put a halt to research projects. For instance, the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) have established consensus on the scientific and ethical conduct of clinical trial research (2). Failure to adhere to the ICH Good Clinical Practice guidelines may jeopardize the registration of the investigational agent under study, possibly costing millions of dollars in lost revenues to product developers.

Ethics guidelines are also important outside the context of research: they provide ethical parameters on complex issues, such as the allocation of resources during pandemics, organ donation, and decision making at the end-of-life. However, these guidelines tend to lack the enforceability mechanisms of research ethics. This can present notable challenges, such as when a product that was submitted to considerable scrutiny and regulation in the research phase enters the market and is used off label. The case of neuro-enhancing drugs is a case in point (3). Thus, research ethics guidelines, however robust, could never fully protect against the use of gene editing technologies for genetic enhancement once these technologies are approved.

In the absence of strict enforceability, ethics guidelines for practice are more likely to benefit from effective uptake if they are perceived to be legitimate, i.e. reflecting a shared acceptance of moral authority. Transparency and public engagement are increasingly accepted as two necessary conditions for ensuring the legitimacy of guidelines. This trend can be observed in the recent joint report of the National Academy of Sciences, National Academy of Medicine, National Academies of Sciences, Engineering, and Medicine, and Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations (4). By dedicating a full chapter to public engagement, the report sets the stage for a more open public debate.

In addition to transparency and public engagement, articulation of the principles that are to be upheld is essential to the development of effective ethics guidelines. Principles provide a framework for ethical discussion and analysis, which can then help illuminate the implications of specific decisions (5). For example, ethical analysis could look at the ethical implications of the use of gene editing technologies, such as inequity in access and outcome. This analysis need not rely on distinctions between therapy and enhancement. The National Academies' report proposes seven such principles they claim are "universal in nature" and built on established international and national guidelines and norms: 1) promoting well-being; 2) transparency; 3) due care; 4) responsible science; 5) respect for persons; 6) fairness; and 7) transnational cooperation. Following further public debate, these principles could be adopted in ethics guidelines on the use of gene editing technologies.

The need for ethical guidance and oversight in the use of genetic technologies is not new, and it is encouraging that the response to addressing this need

has been proactive, rather than reactive to scandals, as has often been the case in the history of research ethics. Back in 1975, the Asilomar Conference gathered scientists and other stakeholders to discuss the use of genetic technology (6), a conversation that continues to this day. Going forward, the development of effective ethics guidelines for gene editing technologies will depend greatly on their degree of enforceability in the form of regulations and legislation, and on their recognized legitimacy stemming from transparency, public engagement, and principle-based justifications. It is incumbent not only on bioethicists, but also on scientists, policy makers, government officials, and the broader public to explore mechanisms to increase enforceability and legitimacy of future ethics guidelines for gene editing technologies. ■

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Renaud F. Boulanger

Renaud is a professional ethicist at the Centre for Applied Ethics, McGill University Health Center (MUHC) and a founding member of the Save the Children UK Research and Evaluation Ethics Committee. He has also provided consultation services to the Bill & Melinda Gates Foundation and worked for the International Development Research Centre. Outside of his work for the MUHC Research Ethics Board, Renaud writes primarily on the ethics of humanitarian research, turberculosis research, and community engagement. He is an executive editor for BioéthiqueOnline and holds an MSc in Bioethics from McGill University.

Veronique Fraser

Veronique has graduate degrees in nursing and bioethics from McGill University and completed a fellowship in clinical and organizational ethics at the Centre for Clinical Ethics and the Joint Centre for Bioethics at the University of Toronto. She has a background in emergency/trauma and community nursing and currently works as a professional ethicist at the Centre for Applied Ethics, McGill University Health Centre (MUHC). She has published on global health ethics and her recent research interests include medical aid in dying and the professionalization of bioethics.

Lori Seller

Lori has a Masters in Philosophy with a specialization in Biomedical Ethics from McGill University. She has been a professional ethicist at the McGill University Health Center (MUHC) since 2011. Her work involves clinical ethics consultation, teaching, policy development and co-chairing the MUHC Research Ethics Board. Her research interests include end of life issues and medical aid in dying.

Ask an Expert

Andrew Emili, PhD

Donnelly Centre, University of Toronto

Disease 'interactome' maps that may one day point to new diagnostics

MoGen faculty member Andrew Emili uses powerful mass spectrometers to peer deep into the hidden inner workings of human cells, looking for signatures of disease. To combat the scourge of disease, he believes that we need to develop more effective diagnostic and prognostic tools, which depends in turn on the discovery of more informative "markers" of early stage pathology before irreversible tissue damage is accrued. That's one major reason why his group develops and uses proteomic methods to probe the protein makeup of healthy and diseased human cells in detail.

Every cell in the human body expresses a unique array of proteins — molecules that physically associate with each other in a dynamic and cell type-specific manner to perform intricate biochemical functions — that allow cells to do what they are meant to do. For example, interconnections between certain proteins in cardiac muscle cell are critical for the heart to beat in a regular manner. In skin cells, a different repertoire of proteins interact to detect and repair chromosomal DNA damage following exposure to ultra violet radiation and other genotoxins. In the liver, another set of proteins come together to metabolise stored energy supplies in response to blood borne signaling cues.

Given this intricacy, it should come as no surprise then that human disease can often be traced back to the failure of such proteins to interact in the way they were meant to. Along this line of reasoning, it stands to reason that identifying which are proteins and connections are normally present, and how these go awry in clinical conditions, is likely essential to alleviate or, one day, even cure disease.

But here's the rub: while researchers in MoGen and other laboratories affiliated with the UofT are making progress in understanding which gene products are mutated or otherwise perturbed in diseases like cancer, neurodegeneration and cardiovascular disorders, we still don't have a clue as to how this ultimately impacts human cellular networks. That's because most molecular interaction studies have been restricted to simple cell model systems, primarily because of a lack of suitable tools to probe more complex clinical samples in a native pathophysiological context. This means that clinicians and researchers alike remain in the dark about when faulty associations arise in a particular disorder, or how to detect and treat such anomalies. A useful analogy is to think of the problem as being presented with a box of parts for a new IKEA furniture set that is missing the assembly instructions - you see some bolts, screws and holes, and intuitively know there is a basic relationship between the components, but its not clear what goes where (failure to solve this properly can have catastrophic consequences).

What is urgently needed then is an experimental approach for systematically mapping cellular circuits in patient-derived specimens. One of my lab's goals, therefore, is to create and deploy innovative new methods to reveal normal and diseased protein interaction networks so we can spot the faulty links critical to patient outcomes. These protein maps can also be used to find both signatures of early stage pathology, and mechanistic indicators of how advanced a particular disorder is. The most informative biomarkers hold great promise for diagnosing diseases much earlier than we currently do now, ideally even before any tissue damage appears, which means a physician will be able to prescribe treatments to nip a condition in the bud before it progresses irrevocably to late stage disease, improving outcomes for the patient while reducing the burden on our health care system.

How do we achieve this? Over a decade ago, my lab started building global protein interaction maps for simple models, most notably microbes like yeast and *E.coli*. Despite gene conservation, there are of course major differences compared to humans, limiting the value of such networks for clinical inference. While a given protein may interact with three other proteins in yeast, its ortholog may have four or more binding partners in humans. So while knowledge of a model organism's protein networks can give clues, one really needs to study patient speci-

mens directly to discover the most clinically relevant associations or perturbations. For various ethical and practical reasons, investigating the inner workings of human tissue directly remains challenging, but my group is generating protein maps for healthy and diseased human cells using our newly developed techniques. Our platform depends on traditional approaches, like biochemical fractionation, in combination with modern cutting edge technologies, like mass spectrometry, to detect the physical connections of thousands of human protein assemblies. Once defined, individual complexes linked to disease progression can be rapidly measured across an array of clinical samples to establish correlation and, potentially causality, with patient outcomes.

Our major focus currently is the networks involved in neurological disease, but we also plan to study connectivity alterations in cancer and heart disease. Our long-term goal is to generate high quality interaction datasets that other biomedical researchers can use to understand the spectrum of altered protein associations that occur in a human clinical setting, and to use these data to devise better tests and drugs to 'correct' such errors. It's a grand vision, and won't happen overnight. Human cell biology is incredibly complicated at the molecular level. Nevertheless, this is why this kind of transformative research is so exciting, and fundamentally so important — it provides essential information to understand how cells work at the most basic level, and is necessary for the development of precision medicine. ■

Dr. Andrew Emili

Dr. Emili is a PI in the Donnelly Centre at the University of Toronto. He is internationally recognized as a leader in mapping protein interactions and macromolecules on a proteome-scale. Dr. Emili's group develops and applies innovative high-throughput methods to characterize protein networks and complexes of broad biomedical significance, publishing global interaction maps of unprecedented quality, scope and resolution.

Ask an Expert

Karen J. Jacob, Alice Virani

"With the recent advents of gene-editing technologies, how can we establish ethical guidelines to prevent the exploitations of these technologies in applications that are outside of conventional medicine?"

The genetic technology known as CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated system 9) has revolutionized the field of gene editing and generated much excitement in the scientific community. While previous gene editing technologies have been resource-heavy and required significant specific expertise, CRISPR/Cas9 is relatively quick, simple, inexpensive, and widely available. The CRISPR/Cas9 system, naturally found as part of the bacterial immune system, recognizes foreign DNA and removes it with unparalleled precision and ease (1). Scientists have already adapted this technology in the laboratory to eliminate disease in animal models (2) and to edit human cells (3).

In humans, CRISPR/Cas9 can be used to edit somatic cells of the body, changing the genetic information in a subset of cells in a living human. There has been promising research in somatic CRISPR/Cas9 applications, such as its recent use in repairing a mutation in hematopoietic stem and progenitor cells (4,5). In fact, clinical trials using CRISPR/Cas9 to alter immune cells for the treatment of cancer are already underway (6,7).

This technology can also be used to edit human germline cells. Editing the genome of egg or sperm cells differs significantly from somatic cells, as it produces a change that would be in every cell of the resulting individual. This would result in a human whose genome has been edited even before birth, and these changes would be heritable and passed on to the next generation.

While somatic applications of CRISPR/Cas9 are advancing rapidly, the use of CRISPR/Cas9 in human germline cells has been much more controversial, and has essentially been put to a halt. The potential for misuse of this technology in the prenatal setting, and concerns over safety and unknown risks, has prompted the scientific community to call for a world-wide moratorium on the use of CRISPR/Cas9 in human reproductive cells, while international discussion and guideline development ensues (8).

While much of the hope associated with this technology is centered on application to genetic syndromes, CRISPR/Cas9 could theoretically be used outside the realm of conventional medicine for enhancement or even cosmetic purposes, not just to alter a disease gene that runs in the family.

The moratorium on the use of CRISPR/Cas9 in germline cells comes, in part, from fear of exploitation of the technology; it is meant to provide time for guidelines and regulations to be in place before the technology is developed for non-conventional use. Discussion within the scientific and ethics communities, as well as research on stakeholder perspectives, including experts in the field, patients and families influenced by genetic disease, and the public, are essential in creating robust policy that takes all perspectives into account. Ethical considerations include social consequences, potential stigmatization of groups with disabilities, issues of access across different levels of socioeconomic status, and the exploitation of vulnerable individuals who might wish to pursue a cure. On the other hand, putting this technology on hold also means it will be difficult to learn more about it. Such fears led the United Nations Convention of Biodiversity to reject a moratorium on the application of CRISPR/Cas9 for gene drive (the ability of a gene to be inherited more frequently than Mendelian genetics would dictate, increasing its prevalence in the population) (9).

CRISPR/Cas9 gene editing technology is undoubtedly here to stay, and the technology is expanding. We are already seeing discoveries of variants of the CRISPR/Cas9 system, like the CRISPR/Cpf1, the CRISPR/CasX and the CRISPR/CasY systems (10,11). In addition, while a worldwide moratorium may be laudable, it has no legislative power or regulation on a global scale. As such, focused attention to build appropriate and meaningful policy, built on the foundations of international discussion and stakeholder perspectives, can lead us in the right direction. ■

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Karen J. Jacob

Karen Jacob holds a master's in Medical Genetics and has recently received a master's in Genetic Counselling from the University of British Columbia. Her broad research interests have led her to author academic publications in the fields of molecular biology, medical genetics and biomedical ethics and policy. Her most recent research has focused on elucidating policy recommendations for the use of CRISPR-Cas9 gene editing technology in human germline cells.

Alice Virani

Dr. Alice Virani is a clinical assistant professor in the Department of Medical Genetics at the University of British Columbia. Her research interests relate to the many ethical and social issues inherent within clinical practice and research in genetic and genomic medicine.

Self Portrait *Tanya Miladinovic*

About the Artist: *Tanya is a senior PhD candidate at McMaster University in the Medical Sciences program, specializing in breast cancer research. Her love of art manifests in her lab work and she uses her research as a platform for creative expression through microscopy and innovative experimental design. She has been creating art on different mediums since childhood and began painting more regularly over the past year as an outlet for creativity beyond that afforded by everyday life in the lab. Inspiration for her work emanates from her unyielding obsession with sunsets. This piece embodies the concept of Science and Art existing on a continuum. She has exhibited at various locations in the GTA. Her work can be viewed at www.paintedbytan.wixsite.com/paintedbytan.*

SPOTLIGHT ON CAREERS

Pedrum Mohammadi-Shemirani interviewed professionals in the field of Gene Editing and Personalized Medicine

Interview with Dr. Viraj Mane: Manager at Ontario Genomics

By Pedrum Mohammadi-Shemirani

Dr. Viraj Mane is a Manager for Business Development & Research at Ontario Genomics (OG), where he focuses on their water & forestry portfolios. He received his PhD in Human & Molecular Genetics from Baylor College of Medicine. After graduating, he went on to secure post-doctoral positions in immunobiochemistry and nanotechnology. After realizing academia might not be for him, he transitioned into the private sector, where he worked as a technical analyst for the United States Department of Defense. Upon relocating to Toronto, he initially secured a position at the MaRS Innovation Office focusing on technology commercialization for discoveries coming out of hospitals & universities. He was then connected to his current position at OG, where he is more focused on project management and business development. He is primarily responsible for presenting the OG mandate to businesses that are looking for solutions but unfamiliar with genomics. He facilitates mutually beneficial partnerships among academic researchers, businesses, and end users; identifies sources of project funding; and provides consulting services to enhance clients' products using genomic tools.

Simultaneously, Dr. Mane has been designing and developing a diagnostic medical device. Beginning with independent research, he came up with an idea, reviewed literature, searched patents, applied for grants, and created a prototype on his own time and money. He was eventually able to partner with a hematology director at the children's hospital in Washington, DC to conduct validation studies of the device. Ultimately, after rounds of patent rejections and appeals, this multi-year process has resulted in an issued patent for his invention.

1. How did your graduate education prepare you for your career?

I always tell graduate students when applying for non-academic jobs that it's not too important to dwell on specific techniques you've learned, since anyone can be taught a technique. You need to emphasize your other abilities, like a commitment to delving into the details of a project while still thinking about the problem at a high level. For instance, if your research is looking at a particular molecular pathway, you should still be thinking about its role in disease, the healthcare costs of the disease, quality of life for patients, and so on.

At the same time, even though I haven't been required to learn specific lab techniques in my current role, it is still vital that I understand them since I'm often interfacing with scientists. However, even if you may not have worked directly with a technique or technology, your knowledge of basic jargon will enable you to read the literature and come up to speed much faster.

Similarly, processing information effectively is one of the best skills PhDs can claim to have. You may not be able to remember details about everything, but you should know how to access information efficiently. Through your vast experiences appraising evidence in science, you will likely know how to sift through different sources, pick the best ones, and synthesize your findings accordingly to inform your experiments. These are the same skills used in strategic planning and project management. You might not know exactly how to make a project succeed, but you maximize your chances of success by collecting the best information to make the most informed decision.

2. What is your average day/week like? Can you describe a current project you are working on?

There is a lot of variation from day-to-day, or even week-to-week, which is an aspect I enjoy a lot. But there are general activities that I'm often working on:

I reach out to companies, whom I have sometimes only spoken to briefly, so they can better understand the OG mandate and what we might be able to do for them. Or, I might be helping other companies that have already expressed interest move to the next steps. This involves brainstorming ideas to see what their next 6-12 months might look like, and, if all goes well, how we might leverage that into a multi-year project to benefit them.

We often look for different funding opportunities to put together joint projects for companies and academic researchers who may have never otherwise partnered. As an extension, we help administer larger-scale multi-year research grants through Genome Canada (a parent organization to OG) as well.

One of my proudest accomplishments has been getting the Ministry of Environment and Climate Change to understand our mandate better and to engage with us more, which may also lead to a new joint research project. I want to have an open dialogue with them, so we can ask them questions regarding environmental regulations or approvals. This allows us to deliver high-quality answers and solutions to our clients, which helps them adjust their development strategy and time frames. It's not easy for businesses to get feedback like that, so this is a value proposition that we can now start providing to our clients.

One example of our partnerships was investigating tools to improve the surface area for beneficial bacteria to grow in wastewater, so they can provide natural filtration functions. This biological treatment alternative is particularly useful for remote communities that can't afford mechanical treatment plants or chemically treated water. We secured some of our own funds for this project as well as three external sources, and it has been doing well so far. A more recent project involved a team that wanted to develop DNA reagents (aptamers) to detect pathogenic oocysts in water to determine whether it is potable or needs to be treated.

3. What is your favourite and least favourite part about the job?

My favourite is the diversity of tasks. We can't control which companies might find us, so they can range from established businesses to small companies or even startups. As a result, we use a common set of tools but we're always being asked to do different things. The variability means we are applying our skillset differently based on a client's background and particular goals.

I also like that management is encouraging of professional growth and taking initiative. They've given me more freedom compared to previous positions, and I really appreciate that they've put that trust in me.

The downsides are limited compared to other positions I've had. One of my earlier positions had a lot of reporting requirements, so I ended up spending a significant portion of time capturing all my activities into weekly and monthly reports. There is a bit of administrative work in every job, since all organizations need to keep records, but there is not as much of it at OG in my experience.

4. What is the current demand for MSc or PhD students in your field?

At OG, I believe all the other managers have PhDs. In fact, over half of our office has a PhD. It might not be a requirement, but it is definitely noticed by our board and our partners that the due diligence and level of review we provide is thorough and unique. We are a team of scientists who transitioned into more business-oriented roles but still have that analytical rigor earned from our previous education.

In terms of the larger industry, it depends on the specific area. In the innovation ecosystem, like many offices in the MaRS tower, there are a lot of MSc and PhD students. This is because you have to understand the technology to know if it is truly innovative or just duplicative of existing technologies. A scientist might approach you believing their invention to be an improvement, but you now have to read highly technical patents to be able to evaluate the technical and commercial merits of their claim at a deeper level.

With that being said, you must determine for yourself if graduate school is the best way to get where you want to go. There is an opportunity cost associated with graduate school, and it's important to look at your options to make an informed decision. If you are unable to commit to a PhD it might be possible to still build technical skills by other means, such as free online courses, seminars, weekend coding camps, blogging, etc.

5. Do you have any advice for current graduate students who would like to envisage a similar career-path?

My advice would be to take the time to learn outside of traditional academia. Your resume talks about your academic skills, but in business it helps to build a portfolio of your actual work. This portfolio should demonstrate that you have initiative, and show things you have done in areas of interest outside of your research. In my case, I had no formal business training, but I was passionate

about innovation and entrepreneurship. I took advantage of opportunities to attend courses and seminars on these subjects, and I put the knowledge to practical use in my ongoing journey of developing a clinical device.

However, your portfolio pieces don't have to be successes, and can be anything that demonstrates your interest, initiative, and commitment. As an example, you might have written a blog post about biotechnology and conducted research into a particular company to evaluate their product's novelty in the market. The subject material doesn't even have to be related directly to your research. If there is some aspect of business you are passionate about, there is no reason you can't build a body of knowledge in that on your own time. It may not be the same quality as a professional market report, but it still shows that you put yourself out there and people will credit you for making the attempt.

People are looking for what makes you different from other candidates, so you've got to give yourself an edge.

Interview with Dr. Angela McDonald: Manager at McKinsey & Company

By Pedrum Mohammadi-Shemirani

Dr. Angela McDonald is an engagement manager at McKinsey & Company, a global management consulting firm, where she specializes in the pharmaceutical, medical device, and biotechnology sectors. She completed her PhD in Biomedical Engineering at the University of Toronto in the Institute of Biomaterials and Biomedical Engineering. Her thesis was related to regenerative medicine, and trying to understand gene regulatory networks that drive cell fate in different stem cell populations.

Using a team approach, McKinsey specializes in helping clients solve complex problems and capture opportunities. As an engagement manager, Dr. McDonald primarily manages and coordinates the on-the-ground team, which typically consists of 2-5 consultants. She ensures everyone has the resources needed to complete their tasks and integrates each individual workstream into cohesive recommended actions for the client. She also meets with senior leadership and partners at the firm who guide the team's problem-solving approach. In addition, Dr. McDonald manages the relationship with the client, being the primary point-of-contact for the sponsor from the client's organization.

1. How did your graduate education prepare you for your career?

I think it helped two-fold. The first aspect was the problem-solving skills you develop in graduate school. You learn to be very analytical but pragmatic in your problem-solving during a PhD, which is something I use every day here. The teams are often multi-disciplinary with people from all types of backgrounds, and the PhD training really helps give me a solid analytical base.

The second aspect comes from the fact that I work in the same area as my PhD, so I'm able to apply my background knowledge. As a result, I'm able to have thoughtful conversations with the scientists and R&D departments about the work they are doing and understand it at a deeper level, or participate in work related to a medical device or therapeutic molecules technical properties and feasibility. There are many PhDs who work in areas unrelated to their thesis though, so while they might not use their content knowledge they will still use their analytical skills.

2. What is your average day/week like? Can you describe a current project you are working on?

I'll paint the average week. All of my work is done in the US or Europe. Not everyone at McKinsey travels each week but my Monday mornings begin at the airport, where I'm catching my flight to wherever I'm working for that week, usually in Texas, Boston, New York, or Paris. During this time, I'm usually calling my team members in different time zones, and getting everything organized for the week.

From Monday to Thursday, we are with the client. I might be having meetings with them, problem-solving sessions with the team and our partners, sorting out logistics, and providing support for my team. The days on-site will end around 7PM, at which point we will eat dinner together and I will do a few more hours of work in the evening.

On Thursday, I fly back to Toronto. My Fridays are spent at the Toronto office catching up with people here, and on calls with my team and different clients.

Even though I'm not in academia anymore, I still like to be close to science. One of the coolest projects I did was for a client in Europe. They have an innovative medical device that could potentially be used for a bunch of applications, but the client wasn't sure what the best route was. I spoke with lots of different surgeons to understand the problem areas in their procedures, and if there was any room for this device to reduce complications. In the end, we came up with 10 potential applications based on doctors' unmet needs that had reasonable market potential, which we presented to the client. The client went back to the lab to conduct additional

tests to narrow the list based on the technical feasibility of each application. Now I'm going back to help them think through the development and commercialization model to get their product to the clinic.

3. What is your favourite and least favourite part about the job?

My favourite part of the job is all of the learning, which is what I enjoyed about graduate school too. At McKinsey, for 6 weeks I might be working on applications of new polymer technology, then switch to ophthalmology and the major innovations in its therapeutics, followed by vaccines and the technology platforms being used to improve efficacy, or neuroscience and its most promising areas for developing Alzheimer's drugs. You're constantly thinking about new technologies and therapeutic areas, and what markets might look like with these advances.

Everyone definitely works very hard here, but I personally don't dislike that. I would have to say my least favourite part of the job is when I have to wake up at 4:30AM on Monday morning to catch a flight. We don't do that every week, but on some occasions it's necessary.

4. What is the current demand for MSc or PhD students in your field?

We hire a lot of advanced professional degrees, which include PhD degree holders. We value the strong analytical skills you develop in these graduate school programs, and we are always looking for smart people who can conceptualize and analyze complex problems.

The traditional track to business consulting is graduating with an undergraduate degree and entering into a business analyst role, or an advanced professional degree into an associate role. MSc students usually come in at the business analyst level, but we hire lots of Master's students as well.

5. Do you have any advice for current graduate students who would like to envisage a similar career path?

I'd say to just get out there and talk to people. Start building your network. Talk to people who are working as consultants to really understand what the job is. I know it took me a while to wrap my head around what it means to be a consultant. The interview process is pretty rigorous and it takes effort to prepare for, so you should evaluate whether it's something you want to do. Once you've made your decision, you can put all your effort into the interview. A portion of the interview is a case interview, where you will be presented with a business problem. This might be a foreign concept to students from science backgrounds, so it's helpful to meet people who you can practice with and learn from. McKinsey knows not everyone is familiar with case studies so we have prep materials available (http://www.mckinsey.com/careers/interviewing) and recruiters are willing to coach candidates as well.

Interview with Dr. Kripa Raman: Research Associate at Cornerstone Research Group

By Pedrum Mohammadi-Shemirani

Dr. Kripa Raman completed her PhD in Medical Sciences from McMaster University, having written a thesis on the discovery and validation of blood-based RNA markers to differentiate between subtypes of stroke and predict their prognosis. Prior to this, she received a BSc in Molecular Biology and Genetics with a minor in Biochemistry, also from McMaster University. She is currently a research associate at Cornerstone Research Group, a consulting firm in Burlington that specializes in evidence-based market access solutions for pharmaceutical and biotechnology products.

1. How did your graduate education prepare you for your career?

My thesis projects involved big data, which required me to deepen my understanding of statistics and introduced me to R, a programming language for statistical computing and graphics. The ability to understand and write code is very valuable in my current position. More generally, my graduate education gave me opportunities to fine-tune my ability to critically evaluate and discuss scientific literature, conduct literature reviews, and to answer complex questions. All of these are skills I use on a regular basis at Cornerstone.

2. What is your average day/week like? Can you describe a current project you are working on?

Each week is different depending on the project requirement. This week I've spent the first few hours of the day catching up on readings. Then I switch to setting up and programming an economic model. Intermittently through the day I meet with managers and/or clients to discuss issues, present progress, and garner feedback.

One of my first projects at Cornerstone was a market scan for a pharmaceutical product. This involved the study and interpretation of events and trends that could influence the market (i.e., disease epidemiology, current management of treatment, emerging competitors, etc.). This big picture view can help a company better understand their customers, and identify competitors' vulnerabilities.

Initially the project was a bit daunting since there was such a wide scope, and it was a new disease area. Actually, it was similar to starting a thesis! This project was also my first exposure to health technology assessment bodies (i.e., Canadian Agency for Drugs and Technologies in Health (CADTH) and the National Institute for heath and Care Excellence (NICE)). These organizations are responsible for evaluating the efficacy and cost effectiveness of new health product. Now I'm working on developing clinical and economic tools to convey the importance of new products to agencies like CADTH.

3. What is your favourite and least favourite part about the job?

During my PhD I was surprised how much I enjoyed the data analysis and programming aspect of my research. Luckily, I have had ample opportunities to use these skills in my job, and even to do some new types of programming as well. I also really enjoy collaborating with clients, and the variety in projects.

My least favourite aspect is writing and editing large reports, but it's an important and necessary part of most jobs.

4. What is the current demand for MSc or PhD students in your field?

In general, the job market in health economics seems pretty good. The cost of healthcare is a hot topic right now, which makes cost effectiveness and budget impact analyses that much more important for pharmaceutical and biotechnology companies trying to get reimbursement.

In terms of educational background, the majority of my co-workers have graduate degrees (MSc or PhD). The job requires the ability to critically evaluate scientific literature, which is a skill often developed in grad school.

5. Do you have any advice for current graduate students who would like to envisage a similar career path?

I would suggest learning more about the role of government and health technology agencies. Reading some of the drug recommendations by CADTH, for example, will help you to become familiar with the clinical evaluations and the economic language.

Interview with Dr. Laurence MacPhie: Partner at Bereskin & Parr LLP

By Pedrum Mohammadi-Shemirani

Dr. Laurence MacPhie is a partner at Bereskin & Parr LLP, a leading Canadian intellectual property law firm. He received his undergraduate degree in Biochemistry from the University of Waterloo and his PhD in Human Genetics at Oxford University, where his thesis work focused on elucidating the genetics behind complex traits, particularly identifying the genes influencing ADHD, dyslexia, and other cognitive disorders. During his studies, he began to notice a gap between scientists working in genetics and others working on the legal and social issues surrounding the emergence of new genetic technologies. This spurred him to return to Canada and complete his law degree at the University of Toronto. He now works as a patent agent and lawyer specializing in the life sciences, and has lectured on various topics relating to commercialization and patent law at Canadian universities.

1. How did your graduate education prepare you for your career?

My graduate education provided a great foundation for a career in patent law. Patent lawyers and patent agents typically have at least an undergraduate level degree in science or engineering. However, graduate-level research can provide a deeper understanding of the scientific process that comes from developing a research program and carrying out experiments. That background is very useful for understanding new technologies and working on patent applications – even if the new technology is in a different field than your previous research.

I also find that inventors appreciate talking to someone who has spent time in a lab and understands how difficult it can be to carry out research and produce data to support an invention. A graduate degree also gives you certain amount of credibility with clients and can help establish expertise in a particular area.

2. What is your average day/week like? Can you describe a current project you are working on?

Generally, I spend 50% of my time dealing with the Canadian patent office on behalf of foreign companies, often from Europe, the United States, or Japan, helping those companies secure patent rights in Canada. I spend the other 50% of my time helping Canadian clients, such as biotechnology companies, or hospitals and universities engaged in technology transfer, draft patent applications and/or create a patent strategy that is suitable for them.

The specifics of each strategy are highly dependent on the type of client and technology they are seeking to commercialize. For instance, the right approach for a start-up company working on a medical device might not be suitable for a large pharmaceutical company or an inventor at a university whose research suggests a new diagnostic test. As a result, I spend a lot of time talking to clients to identify their particular needs and considering different options for pursuing patent protection.

Patent lawyers often get to work on a wide variety of different technologies. One of our major international clients specializes in enzymes suitable for commercial and industrial applications, including heat-stable enzymes originally isolated from thermophilic bacteria. These kinds of enzymes can be found in a variety of products, such as laundry detergents, where they have to be capable of tolerating extreme heat and denaturing conditions without losing their function.

We have also been involved from the beginning helping a Canadian biotechnology company obtain intellectual property for a peptide with anti-cancer properties. The peptide is now in clinical trials and has shown positive results in patients with solid tumors. Patent protection is very important for companies trying to develop therapeutic agents and can help secure the necessary funding for running clinical testing and R&D.

3. What is your favourite and least favourite part about the job?

I enjoy working at the interface between scientific research and commercialization. Many of our clients have really interesting technology or are working on cutting-edge research and development. As a patent lawyer, I am no longer involved in research, but I still get to work with inventors on scientific projects and try to move that research into the commercial arena.

My least favourite part of the job is the administrative tasks that are an essential part of running a law practice. Patent law involves a lot of critical deadlines that require careful time management, as well as working late or on weekends. It can be a demanding profession but is also very rewarding.

4. What is the current demand for MSc or PhD students in your field?

There is always a demand for good people in intellectual property and patent law. The field isn't growing as fast as it used to, but it is unlikely to diminish in importance over the years.

Law school is a significant investment of time and money, but is necessary if you wish to practice law. Law school can also open doors to practice areas other than intellectual property. I know lawyers with graduate degrees in the sciences who went to law school with the intention of practicing patent law, but are now working as criminal lawyers or corporate lawyers.

Becoming a patent agent is a separate qualification from becoming a lawyer and allows you to represent clients before the Canadian Patent Office. It is not necessary to be a lawyer in order to become a patent agent, but you must have trained in the field for at least two years before writing a series of exams to qualify for the certification. The field is competitive and there are typically only a small number of companies or firms looking to train patent agents each year, so it largely requires a graduate degree, such as a PhD, and a healthy dose of luck.

Some larger companies may also be looking for people with a technical background to help manage their intellectual property or become intellectual property specialists. Companies often recruit for those types of jobs internally or may look externally to try and find someone with a strong technical background (such as a graduate degree) and some legal or patent experience.

5. Do you have any advice for current graduate students who would like to envisage a similar career path?

Graduate school is a great time to start networking and gain exposure to as many different opportunities as possible. A graduate degree is a good indication of technical competence, but having some additional experience relevant to law and/or commercialization will help set you apart. One suggestion I have is to try to get involved in any capacity with your university's technology transfer office. The field of intellectual property is somewhat obscure and any kind of concrete experience working with patents is valuable, either on a law school application or a resume.

Furthermore, experience with entrepreneurship or in a business environment is great for people interested in patent law. Entrepreneurship is not for everyone, but experience helping run a small business or trying to launch a new company (even if not a tech company) is a useful addition to the technical education provided by a graduate degree.

Interview with Dr. Alexis Carere: Manager at Ontario Genomics

By Pedrum Mohammadi-Shemirani

Dr. Alexis Carere is a postdoctoral research fellow and genetic counsellor in the Genetic and Molecular Epidemiology Laboratory, led by Dr. Guillaume Paré, in the Department of Pathology and Molecular Medicine at McMaster University, Hamilton, Canada.

Her previous education includes an ScD in Epidemiology from the Harvard T.H. Chan School of Public Health, an MS in Genetic Counseling from the University of North Carolina at Greensboro, an MA in Philosophy from Western University, and an HBSc in Genetics from Western University.

Her current position comprises both clinical and research roles. On the clinical side, she provides genetic assessment and counselling of patients with suspected hereditary cardiovascular disease at the Hamilton General Hospital Lipids Genetics Clinic. Meanwhile, her primary academic research is focused on using genetic epidemiology and statistical genetics methods, such as Mendelian randomization, to identify novel causal blood biomarkers for Alzheimer's disease.

1. How did your graduate education prepare you for your career?

In some ways my graduate training was very specific: I have a Master of Science in Genetic Counselling, and I am now a board-certified genetic counsellor; I have a Doctor of Science in Epidemiology, and I am now a postdoctoral fellow in Epidemiology. That said, my graduate training has also turned out to be interrelated and complementary. My experience in genetic counselling and clinical care has informed my work in epidemiology, and given me the ability to carve out a research space for myself that is unique. My training in epidemiology and biostatistics has helped me be a better genetic counsellor, because I am able to critically evaluate the relevant science that forms the basis of our profession, in order to provide evidence-based care. I also have a Master of Arts in Philosophy, and that has been foundational for me as I think about and address (in both patient care and research) the bioethical issues inherent to medical genetics.

2. What is your average day/week like? Can you describe a current project you are working on?

Every day and every week is a little bit different! Some days I am in clinic, so I am seeing patients with suspected genetic disease (in my current setting, that means patients with a history of cardiovascular disease – heart attack or stroke – that is strikingly young, recurrent, or seems to run in the family). Other days I am working with a team to review the results of our exome sequencing research, in which we try to identify the molecular causes of some of the cases we see in the clinic. In addition to evaluating individual cases, we are always trying to improve the way this research is done, and thinking about how best to integrate genetic sequencing into clinical care. I am also often conducting epidemiological research, which means performing statistical analyses on the computer, and then writing up the results of the analyses as scientific papers. Right now I'm working on an analysis of blood biomarkers for Alzheimer disease. Being a postdoctoral fellow involves a lot of hours spent in front of the computer, designing and tweaking analyses, and trying to figure out how best to present your findings to the research community.

3. What is your favourite and least favourite part about the job?

My favourite part is working with patients, and helping them to engage with genetics and genetics research. My second favourite part is attending scientific conferences, where I get to meet lots of cool people and learn the newest science. My least favourite part is probably endless revisions of papers, both before submission and after review. Meeting journal formatting requirements can be really tedious, and trying to stay true to the paper

you want to write while addressing reviewer suggestions can sometimes be a challenge. At least on the other end of it all, there is usually a publication, so it is well-rewarded work!

4. What is the current demand for MSc or PhD students in your field?

Genetic counsellors in Canada and the United States are required to train through an accredited program, and to pass their country's certification exam, so the demand for specific training is high within the profession. More generally, the job market for genetic counsellors is excellent in both countries (many counsellors move back and forth between the two during training and employment, or even work remotely across borders). You don't need a doctorate to be a genetic counsellor; on the other hand, the past few years have seen an increase in genetic counsellors obtaining doctorates, and an increase in the career opportunities open to them. The neat thing is that a genetic counsellor could go in a lot of different directions with doctoral training – molecular genetics, psychology, epidemiology, counselling, etc. – which each open up different career paths. There are opportunities throughout academic research, clinical care, and industry.

5. Do you have any advice for current graduate students who would like to envisage a similar career path?

Talk to a genetic counsellor! In fact, talk to different genetic counsellors, working in different industries or settings. We used to talk about "traditional" versus "non-traditional" genetic counselling jobs, but that's really an outdated way of looking at things. Today, becoming a genetic counsellor doesn't have to mean signing up to provide reproductive genetic counselling to expectant couples (unless you want to!), so you may get a narrow view of the profession if you only speak with prenatal counsellors, for example. As it pertains to your interests, try contacting and even shadowing someone who works in a prenatal or pediatric clinic; someone who primarily sees adult patients or works on a research study; someone who works in a laboratory or industry setting; or someone who works in public health outreach (e.g., education). Prepare for your application and interviews by delving into the genetic counselling literature and recent genetics news to see what's happening at the edges of the profession, where there is so much new and exciting growth.

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