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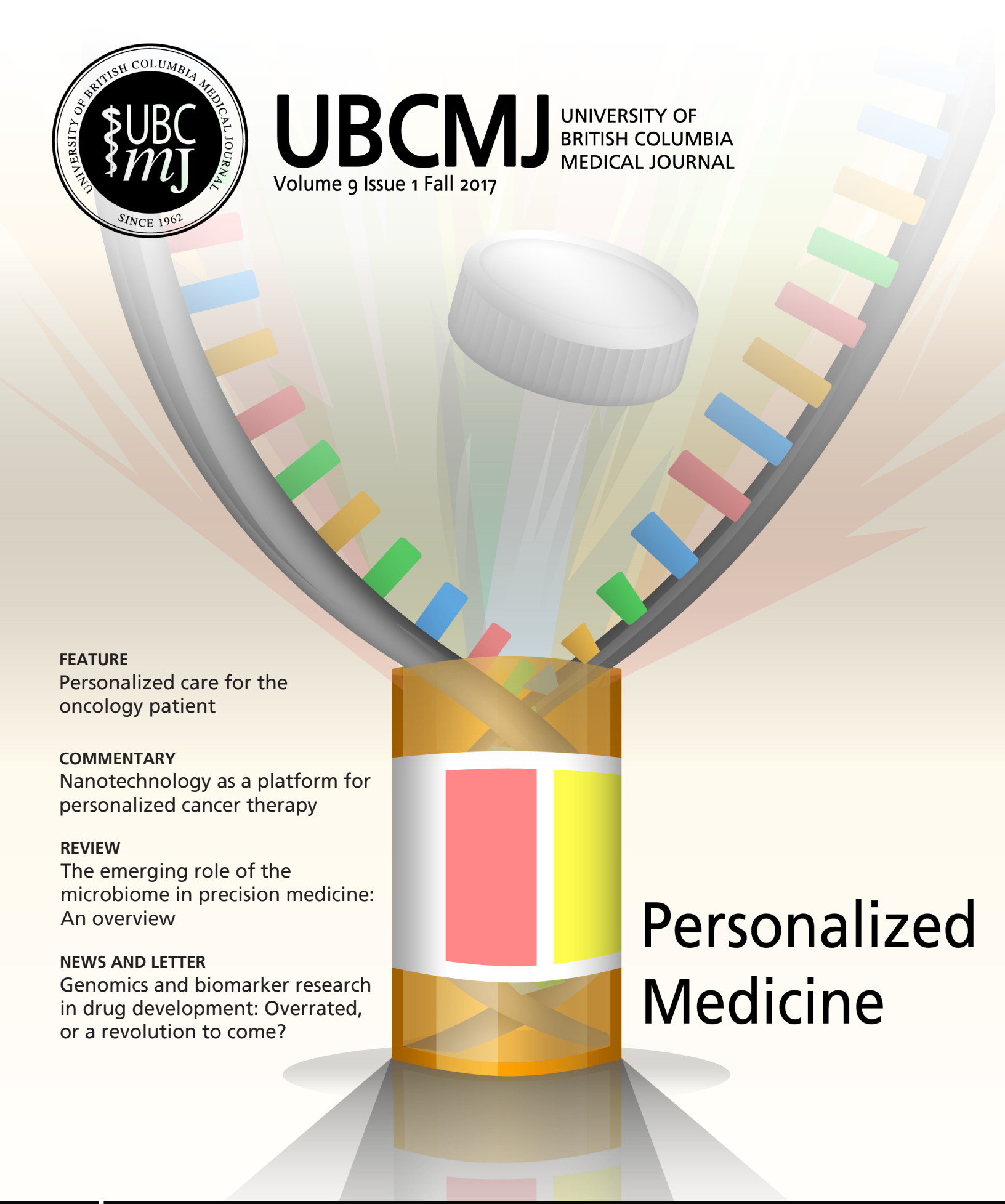

Personalized Medicine

THE UNIVERSITY OF BRITISH COLUMBIA

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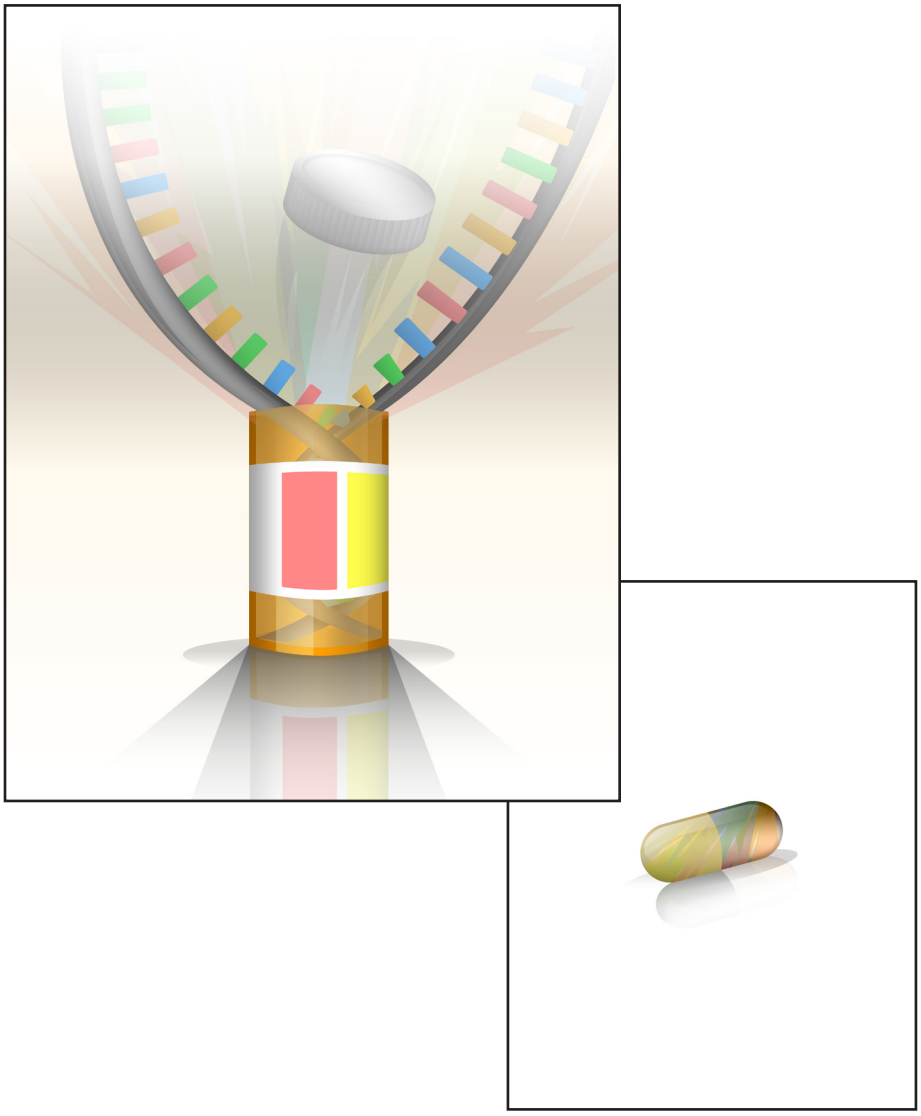
On the cover



It is commonly understood that no two people are alike. While we have guidelines to help us treat patients with a standard of care, scientific and technological advances are showing us that what's best for one patient may not be ideal for another.

In this issue we discuss the topic of personalized medicine and the exciting developments that it may bring to the practice of medicine.

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Personalized medicine

Heidi Britton[1]; Alvin Qiu[2]
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To some clinicians, the concept of personalized medicine may not be a new one. When evidence–based data emerge that support stratifying patients to potentially improve outcomes, medicine has historically adapted practice and protocols accordingly.

However, with the development of next generation sequencing and other technologies, an era of molecular–level precision medicine is emerging, and at some institutes, has already arrived. The Human Genome Project increased the affordability of sequencing technologies, spurring exploratory genomic research, and led to the development of applied clinical genomics. Patient–specific imaging, gene expression, metabolites, proteins, lipids, and other biomarkers offer potential new means of guiding and fine–tuning treatment and prevention in specific diseases.

Cancer is one such disease, and precision medicine is often used as a synonym for the buzzword "oncogenomics", or characterization of cancer–related genes. Massively parallel sequencing technologies have made clinical genetic testing more accessible to clinicians, and allowed for further exploratory and clinical research. Rather than a "minimum hit" phenomena to oncogenesis, cancer is now thought to be a highly complex disease, involving multiple signalling pathways, heterogeneous mutation patterns, and unique genes in every individual. In BC, eligible patients can be referred for genetic testing of the *BRCA1/BRCA2* tumour suppressor genes, with mutations predictive of breast, ovarian, and several other cancers, and associated with 5-10% of all breast cancers.[1] Circulating tumour DNA (ctDNA) is found in around 50% of patients with stage I cancer and is being actively investigated for its potential utility in detecting and even quantifying occult early–stage or relapsing disease.[2-4] Featured in this issue, is an article by researchers at the BC Cancer Agency, where close to 900 cancer patients have been evaluated through the BC Cancer Agency's Personalized Oncogenomics program.

There are many other avenues of precision medicine at earlier stages of development. Pharmacogenetics approaches provide genetic information, which can be utilized in choosing patient–specific medications and dosing regimens. Substantial fundamental work attempts to bring stratification to the individual, aiming to distinguish cells in an individual by their surface chemistries, and to design methods for targeted drug or gene delivery tailored to that individual.

With vast data output, current and future challenges include data interpretation, data storage, how to interpret variants of unknown clinical significance, and proving causality given such a complex system. Targeted gene screens or exome sequencing are currently preferred over whole genome sequencing for their speed, cost–effectiveness, and their capability to reduce discovery of variants of unknown significance. The exome is 1-2% of the genome, yet contains around 85% of Mendelian disease–causing pathogenic variants and many disease–predisposing variants.[5] Mathematicians, computer scientists, biostatisticians, and basic and clinical researchers are addressing these challenges, and success will depend largely on data sharing and collaboration—e.g., through consortia such as the Cancer Genome Atlas.

To move a technology from bench to bedside, scientific validity must be rigorously proven and coherent public policy and guidelines developed. Regulatory boards, such as the US Center for Disease Control, are developing frameworks to evaluate clinical genetic tests focusing on protection of patient confidentiality, clinical and analytical validity, data ownership, and critically, how to manage accidental genetic findings that are deemed significant but do not affect treatment, prevention, or outcome.[6]

This UBCMJ issue includes a microcosm of the breadth of 'personalized medicine': Coutin & Nislow discuss cell–to–cell heterogeneity as a critical consideration in patient variation to drug response, while Lo presents a method to supplement traditional biochemical methods for diagnosing dyslipidemias with targeted lipid sequencing panels. Shopsowitz discusses the challenges of targeting cancer cells with nanoparticle gene therapy, with the ultimate aim the provision of highly personalized treatments based on the patient's specific mutations and cell surface markers. Kosyakovsky reviews emerging research on the microbiome in health and disease. Altogether, these contributors present some of the most active areas of research in precision medicine, ranging from the fundamental science to the front lines of clinical practice.

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Personalized care for the oncology patient

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Cancer is a disease of alterations within a person's genome leading to unregulated growth.[1] By understanding these growth pathways, it is possible to use this information to select drugs that might be beneficial for treatment. Chemotherapy, in general, is therapy that targets various growth mechanisms; furthermore, oncologists have been using personalized chemotherapy care for over a decade. An early example is the use of trastuzumab in HER2–positive metastatic breast cancer that has transformed the treatment of this type of cancer.[2] More recently, advances in gene sequencing technology have seen genomic tumour profiling become more accessible. The paradigm of molecular targeted therapy became reality with the use of imatinib in chronic myeloid leukemia following the discovery of the BCR–ABL fusion.[3] However, the information gained from most genomic profiling technology remains largely experimental and invalidated.

Genomic technology has advanced through the use of next generation sequencing technology. The concept of profiling tumours and normal tissues in clinical settings is not new and has progressed to the point where such profiling has now entered routine clinical care in several jurisdictions including the Memorial Sloan Kettering Cancer Centre[4] and the use of the OncoPanel at the British Columbia Cancer Agency (BCCA) genomics lab (www.ccgenomics.ca). Many clinical–and consumer–based tests use panels that target specific mutations. Panels are populated with mutation or gene targets that are selected based on their known clinical relevance. This provides limited information, but is quick and reliable. Whole genome sequencing (WGS) is more detailed, looking at the entire genome instead of a specific site of a known mutation. While WGS provides more information, data analysis is more time–consuming. WGS also yields variants that might not yet be described and would therefore have unknown significance.

In addition to the OncoPanel, the BCCA and Genome Sciences Centre also enrolls patients into an ongoing clinical trial known as the Personalized OncoGenomics Program (POG). This analyzes whole genome and transcriptome sequencing from fresh tumour samples to provide detailed genomic reporting in the hopes of providing personalized treatment (Figure 1). Understanding the genomic biology, using transcriptome sequencing integrated with genome data, will substantially enhance the sensitivity of detecting actionable alterations and will hopefully reveal therapeutic targets that remain cryptic to panel sequencing. In addition, germline testing will provide a more efficient means of identifying familial risk factors for developing cancer based on hereditary panels, resulting in improved counseling for prophylactic strategies.

The goal of these personalized tests is to gain a detailed understanding of the pathways that can drive a cancer's growth, leading to the identification of biomarkers that can aid in treatment decisions (predictive factors) or estimate a person's survival with the disease (prognostic factors). The research community has continued to

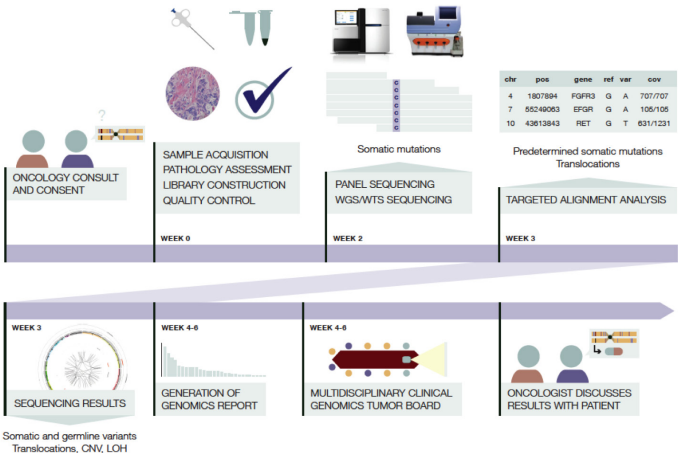

Figure 1 | Personalized OncoGenomics Timeline

provide novel markers that are being validated prospectively in clinical trials. Many of these biomarker–driven trials are breaking away from the hypothesis model of tumour site specificity and moving towards biomarker specificity.[5] One example is the upcoming Canadian Profiling and Targeted agent Utilization tRial (CAPTUR), a phase II basket trial, hypothesizing the presence of a molecular marker will predict the response to target therapy, for patients with incurable metastatic solid tumours. If a tumour has a genomic variant known to be a target of, or to predict sensitivity to, a Health Canada–approved anticancer drug these patients will be eligible to treated with targeted therapies based on the genomic variant and the specific tumour type (Figure 2). The clinical trial protocols will then be used to determine if a person's cancer is responding to treatment.

The caveat to this explosion of bioinformatics is that the majority of discoveries remain largely non–validated. Mutation in or expression of one growth pathway can prove to be predictive in one tumour site, but might not hold true in another. The site of disease biopsy is also key, as a metastatic lesion will have a different profile from primary tissue. Another unfortunate consequence of this technology's progression has led to direct–to–consumer commercial marketing for genetic testing. This is done largely out of context to a patient's history and a

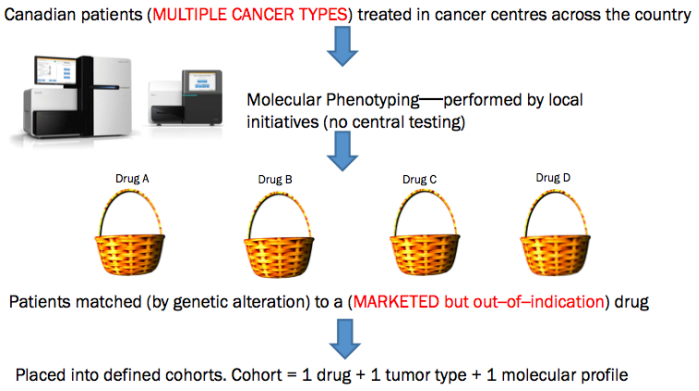

Figure 2 | CAPTUR Study Design

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report is generated and left to the unsuspecting physician to interpret. The disease process is made up of both genetic and environmental risks and genomic information obtained without the proper context tends to be confusing and misleading for both physician and patient.

Given that most of these genomic data are largely experimental and should be analyzed in the context of a person's disease process, the use of consumer testing should be discouraged at present. Efforts to validate markers for therapeutics should be done in the context of clinical trials, and the data are largely hypothesis generating rather than hypothesis testing. While the goal of personalized medicine based on sequencing appears to be attainable, treatment decisions based on non-validated genomic information should be done with an extensive discussion with patients about the pitfalls of using non–validated markers.

As we continue to understand more about tumour biology, more therapies will be developed and treatments will be tailored for patients. This learning has extended beyond the oncology arena and will undoubtedly affect other areas of medicine, leading to a paradigm shift in how we treat patients in the future.

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The dark matter of personalized medicine: Non–genetic variation

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Abstract

Personalized medicine in its current iteration was developed with the goal of making an individual's genome sequence a source of useful, usable information. However, genome–based decisions require more data, an under–appreciated fact in a world where genomic sequencing is becoming increasingly more prevalent. The genotype of an individual is necessary, but not sufficient, for our understanding of how our genome interacts with our environment to translate into phenotype. For example, the variability in how genetically identical cells respond to the same stimulus is a universal feature of biological systems that limits treatment effectiveness.[1] To realize the promise of personalized medicine fully, we need to understand this variability. This requires a model that accounts for the variability observed within individuals in the human population. Here we outline the need to 1) understand (predict) how cell–to–cell variation impacts treatment outcomes, and 2) identify methods to modulate this variability to maximize treatment effectiveness.

When genomic sequencing becomes entirely common, what's next? Within the next decade, many humans with the resources to do so will have their genome sequenced. This information could provide the individual and their doctors with detailed disease risk profiles, drug sensitivity predictions, and recommendations for maximizing wellness. The vision that powered the initial sequencing of the human genome may be realized.[2] The ideal near term future is clear: billions of individual human genomes leveraged into accurate and actionable predictions, leading to longer, healthier lifetimes.

Much remains to realize this scenario—there are numerous well–documented limitations that need to be overcome for scientists to deconvolute the genome into significant components, random components, and everything in–between. These limitations range from healthcare system costs to the challenges of inferring the role of genetic variation in complex traits.[3,4] There is also a concern of selection bias, where the genetic variation among large and wealthy demographics are overrepresented, leading to an underestimation of total genetic variation in humans.[5] For the purposes of this discussion, we assume these are difficult but tractable problems that can be solved over the next decade.

Here, we restrict our discussion to the consequences of cell–to–cell variation within the individual and how it pertains to personalized medicine. We suggest that characterizing the cell–to–cell variation in response to drug treatment across a large population of individuals is an essential, overlooked principle for predictive modelling of treatment outcome. Further, we highlight the potential for treatments that modulate the variability of the biological system for maximizing beneficial treatment outcomes and patient wellness.

The genome is only a subset of the actionable chemical information that a human can provide. Genetic variability holds special standing in personalized medicine. Due to the relative permanence of the information it contains, the genome is an obvious place to look for predictors of disease, drug responsiveness, and wellness. It is convenient to correlate one or more individual genetic variants with a disease or health outcome across an otherwise diverse population. This concept was initially validated in the pilot phase of the 1,000 genomes project that demonstrated the average human walks around with at least 50-100 disease–implicated genetic variants.[6] This finding has since been greatly extended with the recent deep sequencing of 10,000 human genomes, observing an average of approximately 57 single–nucleotide variants per kilobase.[7] Knowing one's genetic information is already actionable today, in that it allows for the patient and medical practitioner to establish treatments to curb or prevent premature health loss. Commercial services may be used to identify risk for Parkinson's disease, late–onset Alzheimer's, celiac disease, and others. Over time, it is possible the scientific community will extract all the actionable knowledge out of our genomes. What possibilities exist beyond this? In principle, any differences in the chemical identity of an individual, even beyond genome variants, that exist long enough to be both measured and acted upon could inform individual treatment.

To identify which parts of this information are valuable predictors of treatment outcome, we and others are attempting to quantitatively measure the cell–to–cell heterogeneity in drug response across a large population. We take an inventory of the cell's contents immediately before and after drug treatment, and look for common differences. At present, this process is prohibitively complex and too costly to use on an individual basis. We therefore leverage the simpler and more resource–efficient discovery system, budding yeast. In this pared–down model of human biology, less time, money, and human energy are required to test a biological hypothesis. We can measure a single cell's reaction to each of a few thousand drugs across thousands of genetically identical cells and provide a complete picture of the non–genetic variation in drug response in this biological system. Thanks to the extensive evolutionary conservation between yeast and human, and by leveraging previously developed techniques, we can extend this to a panel of thousands of biomedically relevant genotypes in order to understand how genotype affects non–genetic variation.[8,9] This will allow us to learn which non–genetic elements of the cell's chemical identity are useful predictors for a given treatment.

With a detailed understanding of how cell–to–cell variability impacts the response to treatment, it may be possible to design drugs that cause less of a variable response. 'Combination therapy', involving a drug targeting the mechanisms that underlie cell–to–cell variability with another that is a disease–specific treatment, should

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lead to a more predictable, overall more effective, response.[10] A promising example of this in practice is the sensitization of cells to tumor necrosis factor–related, apoptosis–inducing ligand (TRAIL). Co–administering Sorafenib (among others) reduced the variability in the timing of cell death, demonstrating that co–drugging may reduce cell–to–cell variability in drug response.[11] Drugs that target the cell–to–cell variability pathways are also less likely to suffer from the dose–dependent limits of monotherapy.[12]

The large–scale characterization of human genetic variation has demonstrated that our genomes are useful predictors of treatment outcome. In the years to come, as many more genomes are sequenced, the predictors we already have found will be improved, and new predictors presently limited in power will become actionable. The next challenge will be finding a means to predict how the non–genetic component of variation in cellular response will impact treatment outcome. There has been much work describing the processes and types of chemical changes in a cell that outlive a single cell–cycle: DNA methylation, nuclear organization, protein post-translational modification (particularly histones), inheritance of nuclear and cytoplasmic RNA species. However, what features can generally be manipulated to maximize positive outcomes during treatment are still unknown.

The future of personalized medicine will greatly benefit from identifying which mechanisms drive the variability of responses to a given treatment. We suggest that studying cell–to–cell variability within the individual, across a large population of individuals, will identify many drivers of non–genetic variation. Developing drugs that target these drivers will then allow all of us to alter non–genetic variation in our favour. One of the principle maxims of personalized medicine may be achieved: the right drug, at the right dose, at the right time, for each individual.

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The promise of personalized medicine: A business–focused perspective

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In 1997, Harvard Business Professor Clayton Christensen published the book, The Innovator's Dilemma, a landmark publication that coined the term "disruptive innovation."[1] The book crystallized a new entrepreneurial approach that, many credit, led to the internet revolution and the rise of the tech giants that dominate the technology–driven world today. The essence of disruptive business theory is that large incumbent businesses are often outcompeted and replaced by competitors with product offerings that are so cheap, and of such low apparent usefulness and quality, that initially they aren't considered threats by the companies that are eventually destroyed.[2] Examples of disruption abound: currently, many areas of manufacturing are poised to be disrupted by 3–D printer technology.[3] Initially considered useless toys, 3–D printers are now being used to print stem cell–derived biological tissue and reusable superalloy rocket motors. Christensen's insights gained him exalted status within the tech sector and millions of entrepreneur disciples.

In 2009, after a series of battles with chronic and acute illness, Christensen published a second book, The Innovator's Prescription, describing the medical industry as a complex interconnected web of third–party health insurers, medical professionals, and regulatory bodies.[4] The author recast the practice of medicine as three interconnected businesses, separating diagnosis, treatment, and communication into a "Solution Shop", "Process Business", and "Managed Network", respectively.

The solution shop: This "business" comprises the diagnostic activity of healthcare workers. Once a problem is diagnosed, a doctor can prescribe a course of treatment to cure the patient. Because the doctor cannot control external and unknown risk factors, the uncertainty associated with this activity generally necessitates a fee–for–service pricing model.

The process business: After diagnosis, a course of treatment can begin. In this business model a "material" is taken in and undergoes a well–studied process that adds value to it. In this case, a patient is treated and cured of disease or ailment. Generally, process businesses operate on a pay–for–outcome model, but this is not the case for healthcare.

The managed network: This part of the healthcare business facilitates communication between experts, and also to patients suffering from chronic diseases, spreading state–of–the–art information about medical practices to interested parties. This type of business usually operates by a fee–for–membership model.

The deconvoluted business model accentuates how each segment of the healthcare market informs and guides the other by a circular feedback mechanism. Currently, doctors are primarily responsible for diagnosis and treatment and the two are often blended together in an iterative cycle until the problem is solved. It is apparent that optimized diagnosis and networking practices would lead to more efficient treatment delivery, and that small diagnostic improvements could have magnified effects in terms of efficient expenditures and the pricing of care. The high value of improved diagnosis and networking, because of its magnified effect on the cost of treatment, makes it an attractive target for disruptive innovation.

The future of medicine in a personalized world

It is obvious to an astute reader that personalized or precision medicine is primarily, in the parlance of Christensen's theory, an improvement to the Managed Network and Solution Shop diagnostic business models. Legions of entrepreneurs and investors are eager to implement disruptive personalized diagnostic technologies that they envision will allow doctors to track the health of individual patients accurately enough to eventually implement a fee–for–outcome model on the process, or treatment side, of the tripartite medical system. Though this dubious future is at best a long way off, personalized approaches to medicine are progressing towards improved diagnostic success along three main trajectories: bioinformatics, personalized diagnostics, and big data analytics. These three broad areas, and how they are poised to change the way we diagnose disease, are described below for the interested reader.

Bioinformatics

Bioinformatics is the study of molecular biology using modern computational methods. In medicine, these technologies are being applied to large data sets of human genome sequences in an effort to extract meaningful links between a patient's health and their genetic makeup. This powerful technique promises, in the near term, to have significant impact in the area of pharmacogenetics, early disease diagnosis and treatment, and personalized chemotherapy, among others.[5]

Personalized diagnostics

Fully implementing a personalized approach depends on the collection of mass amounts of data in order to understand an individual's specific healthcare needs in a meaningful way. Two main avenues of progress are being vigorously investigated. The first is the comprehensive evaluation of individuals through a suite of diagnostic tests to measure genetic and other biomarker data across thousands to millions of variables. These data can inform bioinformatic models and be cross–referenced to existing databases to evaluate a patient's health against the current body of published medical knowledge. The second disruptive personalized diagnostic approach is the use of extremely inexpensive point–of–care, or patient–operated diagnostic devices to persistently monitor health metrics over time.[6] A well–known example of this type of device is the home electrocardiogram machine.[7]

Big Data Analytics

Distinct from bioinformatics is a second computer–aided diagnostic analysis with a much larger scope: big data analytics.[8] This personalized approach expands the breadth of information used to diagnose disease to include all available data. Big data personalization will correlate

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everyday data on a patient's purchases, movement, heart rate, sleep schedule, social media activity, and other metrics with medical data gathered through traditional bioinformatics. In this scheme, low–quality data can be used to accurately track individual and population–level health and inform genetic and biomarker diagnostic data sets.

The power of change—getting involved

Changing population demographics, as Canadians age, guarantees that fundamental changes are coming to the medical system in this country. Personalized approaches are an attractive avenue towards maintaining a sustainable healthcare system. The pace of progress in the quest for effective, widely available, personalized medicine depends on the participation of medical professionals from all segments of the industry—and doctors in particular. Rather than threatening the existing system, these disruptive changes can help preserve healthcare in the face of spiraling, unsustainable cost increases.[9]

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The emerging role of the microbiome in precision medicine: An overview

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Abstract

The advent of precision medicine has promoted an influx of research relating to the identification of patient–specific factors, both genetic and acquired, which could be targeted and manipulated in the context of disease. The microbiome is a particularly good example of a potential target of these measures, as it represents a diverse array of unique, modifiable factors, known to play an important role in both normal human physiology and the development of pathology. Given this, the contribution of the microbiome to human disease, as well as the potential utilization of microbial modulation in prevention and treatment, is a burgeoning area of study. In this review, we summarize the recently established correlations between intestinal microbial dysbiosis and disease pathogenesis in the fields of cardiology, oncology, psychiatry, and immunology, highlighting the specific organisms that have been identified as potential therapeutic targets. However, the practicality and potential harms involved in screening for dysbiosis and manipulating the microbiome need to be carefully assessed before these findings can truly be applied to the world of personalized medicine.

Introduction

The role of the microbiome in human health has been one of the most rapidly evolving fields of medical research in the past several decades. With commensal bacteria making up a staggering 57% of our total body cell count by recent estimates, the microbiome is increasingly being recognized as a distinct organ.[1] The impact of commensal flora, ranging from the widely studied intestinal microbiota to the organisms lining the respiratory and genitourinary tracts, has been studied across many fields of medicine. These microorganisms have been found to have an incredible number of interactions with every body system, from neuroendocrine effects on the central nervous system to the stimulation and modulation of our immune systems.[2] The more we understand about the interaction of the microbiome and its host environment, the clearer it becomes that these microorganisms play an integral role in the development and maintenance of normal physiological functions.

Given our emerging understanding of the importance of these interactions to the healthy functioning of the human body, there inevitably comes the question of the role of the microbiome in disease. Can the interplay between the host and microbiome have an adverse impact on overall health? Where does one draw the line between "healthy" gut flora interactions and pathogenic behaviour? What role does microbial dysbiosis, or the imbalance of microbial composition in favour of more harmful organisms, play in disease pathogenesis?

The remainder of this review will serve as an update on the progress of our understanding of the role of the intestinal microbiome, the most thoroughly researched portion of our commensal flora, in various diseases. While the gut microbiome has been found to play a role in nearly every field of medicine, we will specifically focus on its involvement in the fields of cardiology, immunology, oncology, and psychiatry, as well as the evidence surrounding the correlation between specific bacteria and the initiation and progression of illness. Finally, we will consider the potential for microbial manipulation—whether one day, the knowledge of a patient's specific microbial balance may play a role in the prevention and treatment of disease.

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Role of microbiota in various fields of medicine

Cardiology

The role of the intestinal microbiota in cardiovascular disease has been a point of great interest in recent years. These studies have investigated variances in microbial composition in patients with cardiac risk factors (including obesity, diabetes, hypertension, and dyslipidemia) and have also utilized animal models to determine to what extent the manipulation of flora could modulate the course of their diseases. One study found that the bacterial phyla Bacteroidetes (including Bacteroides and Prevotella) and Firmicutes were disproportionately represented in the flora of obese subjects (both mice and humans), proposing a mechanism by which these specific bacteria were capable of increased energy harvesting from the diet. Even more strikingly, they found that over time, gut–sterilized mice transplanted with a sample of the flora from obese individuals developed significantly more body fat than their lean–transplanted counterparts.[3]

Similar findings were established in the context of hypertension. Hypertensive patients were found to have significant overgrowth of Prevotella and Klebsiella species; furthermore, transplantation of the hypertension–associated microbiota into germ–free mice was also found to significantly raise blood pressure, demonstrating a more causal role for these microorganisms in the pathophysiology of the disease.[4] There have been several studies aiming to characterize the precise mechanism by which these specific bacteria impact metabolism, implicating a set of pro–inflammatory metabolic functions that may contribute to atherogenesis. On the other hand, there is also evidence that the fat–metabolizing properties of certain phyla (particularly studied with Lactobacillus–containing probiotics) may actually provide an atheroprotective effect.[5] Thus, in further understanding the role of different bacteria in the pathogenesis of (and protection from) atherogenesis, we may develop strategies to specifically modulate each patient's microbiome for both the prevention and treatment of cardiovascular disease.

Psychiatry

There has been a great deal of interest surrounding the potential role of the microbiome in the pathogenesis of psychiatric conditions. There have been many studies demonstrating the physiologic

connection between the microbiome and the mind through the relationship between the enteric and central nervous systems. It has been hypothesized that psychiatric pathology could be fueled by neuroendocrine dysregulation secondary to bacterial production of neurotransmitters. The pioneering studies in this field aimed to characterize the difference in microbial composition between healthy subjects and those with mental illness. These studies illuminated several Lactobacillus and Bifidobacterium species, often depleted in patients with depression and anxiety, as key players in the body's stress response via the modulation of the hypothalamic–pituitary axis (HPA) through the vagus nerve.[6] A more direct link demonstrating the effect of microbial dysbiosis on mental illness was demonstrated in a murine model. Transplanting the microbiome from depressed human patients into rats was found to induce significant behavioural changes, including an increase in depressive symptoms (as measured by the sucrose preference test, in which a decreased level of voluntary sucrose ingestion is interpreted as anhedonia) as well as anxiety–like behaviours (measured through validated experimental methods to gauge anxiety in rats, such as the elevated plus maze and the forced swim test).[7] These results raised the interesting possibility that the altered microbiome itself may be a direct player in the pathogenesis of psychiatric disease, as opposed to simply reflecting a by–product of the disease state itself.

In an attempt to apply these findings to the clinical world, there have been several recent studies demonstrating the positive effect of microbial modulation on mental health. Probiotics containing the key contributory bacteria Lactobacillus rhamnosus (often depleted in anxiety and depression) were initially tested in murine models and were found to decrease anxiety/depressive behaviours, likely by inducing changes in GABA receptor expression in the hippocampus and prefrontal cortex.[8] Follow-up human trials with a probiotic containing a separate Lactobacillus strain (L. casei strain Shirota) demonstrated a decrease in salivary cortisol as well as the physical manifestations of anxiety in academically stressed medical students, and another study demonstrated that a mixed Lactobacillus/Bifidobacterium probiotic cocktail was able to decrease cognitive reactivity (including rumination and aggression) to sad mood in healthy subjects, suggesting a possible role for probiotics in the prevention of depression in its early stages.[9-10] However, the human trials of probiotics have so far been limited in scope, and there has yet to be a systematic trial definitively demonstrating the role of probiotics in addressing or preventing mental illness. Nonetheless, the data so far have been supportive of a causal relationship between intestinal bacterial composition and mental health. With a greater understanding of the specific organisms responsible for contributing to these diseases, this research may enable microbial modulation (including the probiotic supplementation of protective bacteria) to become an alternative treatment modality in psychiatric illness.

Immunology

Given that the intestinal microbiome is involved in countless interactions with its host's immune system, the presence of a relationship between microbial composition and immunologic disease is unsurprising. The most established correlation has been in the context of inflammatory bowel disease (IBD). Organisms such as B. fragilis, segmented filamentous bacteria, and mucosal–adherent E. coli have been implicated in disease progression, primarily through toxin–mediated mucosal barrier disruption and pro–inflammatory mucosal invasion.[11-12] Treatments targeted at favourably shaping the flora have already been trialled in mouse models; treatment with low–dose penicillin in early life, aimed to specifically target the harmful segmented filamentous bacteria, was found to protect against the development of drug–induced colitis.[12] There has been a selection of other postulated IBD treatments targeted to work by similar mechanisms, including other specific antibiotics (including metronidazole), probiotics, and fecal transplant.[13] At this point in time, there has been no clear therapeutic advantage elucidated in human trials; however, there is considerable room for advancement in this field. Using a more precise approach and considering the specific factors in each patient's microbial profile may enable us to make more targeted efforts to therapeutically modulate the microbiome. Additionally, more research into the role of these microbial–modulating measures in healthy subjects may help identify those at risk for IBD development and ultimately lead to clinical benefit through prevention.

Oncology

The role of the intestinal microbiota in the pathogenesis of cancer is less well characterized and is still in the early stages of correlational studies. However, there have been several studies which have demonstrated the potential of the microbiome to promote a pro–inflammatory state, which in turn is known to be a predisposing factor for carcinogenesis. One notable example is hepatocellular carcinoma (HCC), which has been long known to have a significant association with chronic hepatic inflammatory changes and fibrosis. Given that the hepatic circulation receives the majority of its blood supply from the intestinal venous system, the liver is exposed to a high concentration of gut microbial by–products, including pro–inflammatory bacterial antigens and toxins.[14] An animal study conducted in mice with chronic hepatic injury demonstrated that exposure to common bacterial ligands (including lipopolysaccharide) suppressed apoptosis and promoted further proliferation in HCC tumours.[15] Furthermore, intestinal sterilization during hepatocarcinogenesis reduced overall tumour size by up to 70%, demonstrating the potential role of microbiological modulation in suppressing HCC development. Additionally, there have been many studies linking intestinal microbial architecture with colorectal cancer (CRC), implicating organisms such as S. bovis, B. fragilis, E. faecalis, and E. coli as being disproportionately represented in these patients' microbiomes.[16-17] Moreover, these populations have also been directly implicated in carcinogenesis; certain E. coli populations harbouring a DNA–damage–associated pks mutation, enriched in patients with CRC, have been found to promote colon adenocarcinoma proliferation.[18-19] Similar correlational studies have been done for pancreatic, lung, and squamous cell carcinomas. While there is still much more to be discovered before these study findings can be applied for therapeutic intent, this research is certainly laying the grounds for the exploration of targeted, microbe–specific approaches that may contribute to the prevention (or even treatment) of multiple forms of cancer.

Discussion

The balance of the microbiome has been shown to play a critical role in both the maintenance of health and the progression of disease across many fields of medicine. The key question that remains is whether this knowledge can be applied towards the prevention and management of these diseases. Analyzing each patient's microbial profile in order to identify contributory dysbiosis could offer new targets for therapy, as well as an opportunity to identify those at risk for developing disease. Microbial modulation may represent an entirely new frontier in the

expanding world of precision medicine, providing a new lens with which to guide individualized patient care.

However, much more research needs to be done before this approach could become a reality in the clinical world. To begin with, we need a more complete understanding of the specific microbial imbalances that may have a causal (or protective) role in disease before we can develop targeted treatments to address these factors. As previously discussed, the microbiome plays a critical role in normal physiology and disease prevention, so any intervention aimed at re–shaping the microbial balance could run the risk of disrupting its normal functions. Antibiotic treatments will need to be carefully considered, as the potential risk of impairing normal floral function (or more significantly, of enabling the notorious C. difficile infection) could outweigh the potential benefits of intervention. Furthermore, there is the issue of identifying patients whose microbial composition puts them at risk for various diseases. It is unclear which factors would prompt a physician to assess a patient for intestinal dysbiosis, and moreover, as of now, the tools with which this could be done (including genetic sequencing analysis from fecal samples) are unfamiliar, expensive and of questionable accuracy.[20] We would need to develop robust guidelines for identifying patients who would benefit from screening, as well as to standardize the analysis of microbial risk factors, whether this involves holistic sequencing of the entire microbial architecture or screening for biomarkers of pathogenic bacteria. Ultimately, this entire process would need to be subjected to the rigorous scrutiny of cost–effectiveness, as with every screening tool.

Conclusion

In short, as attractive as the microbiome is as a novel therapeutic target, there is still a significant amount of research that needs to be done before translating our basic understanding of its role in pathogenesis into clinical practice, both in identifying actionable microbial targets as well as in making these measures a practical reality. Nonetheless, given the strong evidence which has been gathered so far, we may well be looking at a future where a combination of antibiotics, probiotics, and specific microbial–targeted therapies could form its own unique branch of personalized medicine.

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Distinguishing neuromyelitis optica spectrum disorder from multiple sclerosis using magnetic resonance imaging techniques

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Abstract

Neuromyelitis optica spectrum disorder (NMOSD) is a rare neuroinflammatory central nervous system disorder, characterized by astrocytopathy with secondary demyelination. NMOSD and multiple sclerosis (MS) have overlapping clinical manifestations, making NMOSD clinically challenging to distinguish. A highly specific serum antibody test is available that can distinguish NMOSD from MS, but it is not very sensitive to NMOSD. The similarity of NMOSD clinical and imaging features to those of MS, and the lack of awareness about NMOSD among physicians could lead to misdiagnosis. Distinguishing NMOSD from MS is important as prognosis and treatment options differ. Here, we will discuss myelin water imaging, an advanced quantitative magnetic resonance imaging technique to explore the pathology of lesional and normal–appearing tissues of MS and NMOSD. We will also review machine learning methods that automatically distinguish between the two diseases. Both techniques are actively being studied at the University of British Columbia.

Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is a rare central nervous system disorder that typically presents with optic neuritis, longitudinally extensive transverse myelitis, and area postrema clinical syndrome.[1] It is characterized by an antibody–mediated attack on water channels expressed on astrocytes and in the ensuing inflammatory response, secondary demyelination occurs.[1] Due to similar clinical manifestations, NMOSD was thought to be a subtype of multiple sclerosis (MS), which is an autoimmune disorder of the brain and spinal cord characterized by edema, inflammation, demyelination and axonal damage, resulting in impaired saltatory conduction.[2] Examples of these overlapping clinical symptoms include vision loss, weakness in extremities, fatigue and sensory dysfunction. However, NMOSD has emerged as a distinct disorder from MS since the discovery of serum aquaporin 4 immunoglobulin G antibodies (AQP4–IgG) in 2004.[3,4] This autoantibody marker is very specific (97-99%) to NMOSD; however, it is not as sensitive (59-76%).[4] This means that some patients who test seronegative for AQP4–IgG may still have NMOSD. Therefore, other imaging biomarkers or differentiation techniques are highly desirable to classify NMOSD from MS. The similar clinical and imaging features of NMOSD and MS, and the lack of awareness about NMOSD among physicians could lead to misdiagnosis, especially if the patient is AQP4–IgG seronegative with the presence of brain lesions. Differentiating NMOSD from MS has crucial implications to prognosis and treatment because standard MS therapy, such as interferon-beta, may worsen NMOSD and increase relapses.[5]

To better understand the different pathology of NMOSD and MS, magnetic resonance techniques can be used. Magnetic resonance imaging (MRI) is a non–invasive medical imaging tool that utilizes a strong magnetic field to align or anti–align magnetic moments of protons (termed spins), mainly from water, and use radio frequency pulses to manipulate the spins, generate signal, and produce images with high spatial resolution.[6] Conventional MRI is frequently used in diagnosis and clinical management of NMOSD and MS. It is useful for visualizing lesion distributions in space and time. However, the limitations of this imaging modality include low specificity to pathological processes, low sensitivity to diffuse damage in the normal-appearing white matter, and limited association with clinical status in both MS and NMOSD.[7] Therefore, there is significant ongoing research into using advanced MRI techniques to better understand pathological processes such as demyelination and axonal loss in disease–specific tissues. Here, we discuss myelin water imaging, which can be used to investigate the differences in pathological processes in both lesional and normal–appearing tissues of NMOSD and MS, as well as machine learning approaches that are used to automatically distinguish between NMOSD and MS for early and accurate diagnosis of NMOSD. Both techniques are currently actively studied at the University of British Columbia.[8-13]

Myelin Water Imaging

Myelin is a fatty substance that envelops the axon and enables saltatory conduction, allowing increased conduction velocity.[14] Damage to myelin slows the transmission of information sent along the axon and causes the exposed axon to degenerate. Therefore, a measurement of myelin content *in vivo* can be useful in better understanding demyelinating diseases such as MS and NMOSD.

Myelin water imaging (MWI) is an advanced, quantitative MRI technique that was developed and pioneered by Dr. Alex MacKay's group at the University of British Columbia in 1994.[15] MWI uses multi–echo T2 relaxation measurements to quantify the fraction of signals originating from three major water compartments in healthy human brain: a long T2 (~2 sec) from cerebrospinal fluid, an intermediate T2 (~60-80 msec) from intra– and extracellular water, and a short T2 (~15-20 msec) from water trapped between the myelin bilayers.[6,15,16] The myelin water fraction (MWF) is defined as the ratio of water between myelin bilayers to the total water content.[6] Furthermore, the MWF has been validated as a marker for myelin content by histopathological analysis using a myelin–specific stain in postmortem human brain tissues.[17] Since its validation, many studies have focused on MWF to better understand MS and NMOSD pathology.

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In 2016, Jeong et al. compared the MWF in periventricular white matter lesions in 27 relapsing–remitting MS and 20 AQP4 IgG-positive NMOSD patients, as periventricular white matter is commonly affected in both conditions.[18] They found that the mean MWF in MS periventricular lesions (4.06±2.69%) was significantly lower than in NMOSD periventricular lesions (6.18±3.15%) (p=0.002).[18] Furthermore, they found that 59.4% of the MS lesions, compared to 33.3% of the NMOSD lesions, had severe (≥75%) myelin loss (p=0.001), thereby suggesting that there is more severe demyelination in MS than in NMOSD.[18] However, Jia et al.[19] noted that the data must be interpreted with caution, as MWF was reported to vary among different lesion types in MS.[20]

Additionally, Matthews et al. found several normal-appearing white matter regions with significant MWF reduction in the MS cohort over one year; however, this change was not seen in NMOSD or controls.[21] Therefore, there were widespread neurodegenerative changes in MS but not NMOSD cohorts, which may support the clinical finding of progression in MS but little or no progression in NMOSD.[21]

In contrast to studies done by Jeong et al. and Matthews et al., a combined transcranial magnetic stimulation (TMS) and MWI study showed lower TMS recruitment curve slopes and lower MWF in the corticospinal tract in NMOSD, compared to MS and controls. This suggested greater damage in NMOSD than in MS.[13] The conflicting results may be due to small sample size and different patient population, particularly since the disease course of MS is very heterogeneous.

Currently, there is active research focusing on myelin water imaging at the University of British Columbia. For example, Combes et al. investigated potential diffuse myelin changes in NMOSD by computing z-score MWF maps from a MWF atlas that was created from healthy control data.[8] This illuminated how much MWF in NMOSD deviated from the normal.[8] They found that the volume of abnormal MWF in MS (378±542 voxels, p=0.001) and NMOSD (126±205 voxels, p=0.1) were higher than in healthy controls (33±65 voxels).[8] Furthermore, lesion volume was significantly correlated with volume of abnormal MWF (p=0.02) and average normal–appearing white matter z-score (p=0.009) in MS; however, this pattern was not detected in NMOSD.[8] In the future, further longitudinal studies with larger datasets investigating the difference in MWF in brain and spinal cord lesions and normal–appearing white matter are warranted.

Finally, even though MWF has been shown to correlate with histological stains of myelin,[17] there are some confounding factors that may affect *in vivo* measurement of MWF. For example, after myelin damage in MS and NMOSD, macrophages clear myelin debris from the injury site. If the myelin debris is not cleared efficiently, it may affect MWF. Animal studies have shown that myelin debris may affect MWF;[22-24] however, this has not been studied in human tissues.

Machine Learning Approach

Machine learning-based pattern recognition techniques have advantages over human observation, such as their ability to take a large number of variables into consideration and improve classification with consistency.[25-32] Additionally, this predictive approach of using machine learning can help to better differentiate NMOSD and MS in a way that it is reproducible and interpretable. Recently, this technique has gained much popularity in psychiatric disorders[26,27] and neurodegenerative disorders such as Alzheimer's disease,[25,28] traumatic brain injury,[29] and clinically isolated syndromes.[30]

In 2015, Eshaghi et al. used a machine learning algorithm on support vector machines, and found that the average accuracy to differentiate NMOSD and MS was 88% using both conventional and advanced imaging techniques.[31] Here, white matter lesion load, normal–appearing white matter integrity, and functional connectivity were the most important factors for distinguishing between NMOSD and MS.[31] A limitation of this study was that it was conducted at a single centre, thereby failing to ensure generalizability of its model to a global patient population.

Therefore, in 2016, Eshaghi et al. used a random–forest, which is another machine learning algorithm for classification, from two sites, using only conventional imaging techniques.[32] They found that the cortical thickness, volume, and surface area measures resulted in average accuracy, sensitivity and specificity of 74%, 77% (i.e., 77% of true MS cases were classified as MS), and 72% (i.e., 72% of true NMOSD cases, without MS, were correctly classified), respectively, to distinguish between MS and NMOSD.[32] When they combined thalamic volume, the most discriminating gray matter measure, with white matter lesion volume, it resulted in higher average accuracy, sensitivity, and specificity of 80%, 85% and 76%, respectively between NMOSD and MS, in two sites.[32] Given these results, a machine learning approach that automatically differentiates NMOSD from MS would be advantageous as the main method for differentiating between the two diseases. In comparison, the AQP4-IgG serum test method is highly specific (97-99%) but not as sensitive (59-76%).[4]

Currently, researchers from the University of British Columbia are developing a machine learning algorithm to distinguish NMOSD from MS as well.[9] The aim is to use a machine learning approach based on artificial neural networks, called deep learning, to determine if the patterns of brain MRI lesions and measures derived from diffusion tensor imaging (DTI) can automatically discriminate between NMOSD and MS.[9] DTI is a quantitative MRI technique that measures characteristics of water diffusion and it is influenced by—but not specific to—myelin content, axonal damage, inflammation or edema.[6] Preliminary results on 82 NMOSD and 52 MS patients have shown that deep learning can achieve an accuracy rate of 81%, thereby demonstrating the potential of deep learning to distinguish NMOSD from MS using patterns of brain lesions and diffusion tensor imaging metrics.[9] A limitation of the machine learning method is that it is not a hypothesis–driven approach. Instead, it reveals relationships upon receiving large amounts of information, but it cannot guarantee these are causal relationships. In the future, the machine learning approach can be further studied to differentiate NMOSD with and without AQP4-IgG.

Conclusion

Although conventional imaging is useful in the diagnosis and clinical management of neuroinflammatory diseases such as NMOSD and MS, it faces limitations including low specificity to pathological processes, and limited association with clinical status in MS and NMOSD. Quantitative MR measures can provide valuable information on pathology and can be used to develop machine learning algorithms to facilitate earlier and more accurate diagnosis of NMOSD automatically. Myelin water imaging can successfully detect the differences in myelin content between lesional and normal–appearing NMOSD and MS tissues. Therefore, MWI can provide insight on disease progression and treatment efficacy. Furthermore, machine learning–based pattern recognition techniques using gray matter measures alone or in combination with white matter lesion load, as well as DTI metrics, have

the potential to distinguish NMOSD from MS automatically. All these techniques may facilitate earlier and accurate differential diagnosis in clinical practice.

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Visual hallucinations in patients receiving intravitreal anti–VEGF agents in northern British Columbia: Prevalence and characteristics

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Abstract

Objectives Visual hallucinations, also known as Charles Bonnet Syndrome, are sometimes experienced by patients with poor vision. The aim of this study was to determine the prevalence and characteristics of visual hallucinations in adult patients receiving intravitreal anti–vascular endothelial growth factor (VEGF) treatment for macular degeneration, diabetic retinopathy, and retinal vein occlusion (RVO).

Study Design Cross-sectional survey.

Methods Participants with poor vision were recruited from an anti–VEGF injection clinic for treatment of age–related macular degeneration (AMD), diabetic retinopathy, and RVO. Anti–VEGF agents included bevacizumab, ranibizumab, and aflibercept. Patients were screened for visual hallucinations, and vision was tested (best corrected visual acuity and contrast sensitivity).

Results 122 patients (mean age 75.3 years) were screened in a period of 6 weeks. 49 were male (40.2%). Diagnoses included AMD (n=92; 75.4%), diabetic retinopathy (n=21; 17.2%), and RVO (n=17; 13.9%). The prevalence of Charles Bonnet syndrome was 6.6% (n=8). Hallucinations usually involved images of people, were brief (<30 s-10 mins), and were associated with dim lighting (n=6). Poor visual acuity (p=0.002) and contrast sensitivity (p=0.001) were associated with visual hallucinations.

Conclusions Patients who see an ophthalmologist for treatment of eye diseases (macular degeneration, diabetic retinopathy, and RVO) can experience visual hallucinations that do not have a mental illness genesis. Patients will benefit from increasing health care professionals' awareness of Charles Bonnet syndrome, as hallucinations can be associated distinctly with poor visual acuity and contrast sensitivity, rather than secondary to mental illness.

Abbreviations CBS – Charles Bonnet Syndrome; VEGF – vascular endothelial growth factor; AMD – age-related macular degeneration; RVO – retinal vein occlusion

Introduction

Charles Bonnet Syndrome (CBS) was first described by Swiss philosopher Charles Bonnet in the 18th century. He noted visual hallucinations in his grandfather, who was blind secondary to cataracts.[1] Bonnet described three key elements still used in modern clinical practice: patients with CBS experience visual hallucinations with preserved insight, have low vision secondary to eye disease, and have intact cognition.[1-3] CBS is commonly experienced by elderly patients, between 70-85 years old[3,4], who have poor vision, yet clinicians and patients remain largely unaware of the diagnosis. The pathogenesis of CBS is uncertain, though two main theories exist. The "release theory" suggests that the visual cortex receives abnormal signals from a lesion in the visual pathway, leading to hallucinations.[5] Alternatively, the "deprivation theory" suggests that the visual association cortex produces images due to a reduction in sensory input.[3,5]

Reported visual hallucinations can be quite varied in their description, but commonly experienced hallucinations do not last more than a few minutes and include patterns, faces, objects, figures, and animals.[3] Hallucinations can be in colour or greyscale, and images can be moving or stationary. Other characteristics remain under study, as current studies report contradicting results regarding the effects of sex, living arrangements, light, time of day and other factors on the prevalence of CBS. Advanced age and low visual acuity have been shown to be risk factors for CBS, especially in patients who have advanced macular degeneration (AMD).[6,7] Poor contrast sensitivity is another known risk factor for CBS.[8] Other eye diseases such as glaucoma, diabetic retinopathy, RVO, and cataracts are seen in patients with CBS.[2,3,5,7-9]

The prevalence of CBS in elderly patients is reported to be anywhere from 0.5-40%.[1,5,10] The high variance in rates of CBS can be attributed to the association of visual hallucinations and mental illness. Case reports have noted that patients can feel distress, and many do not seek medical advice for fear of diagnosis with mental illness or neurodegenerative diseases such as Alzheimer's dementia.[11] As much as 60% of patients experience confusion during visual hallucinations and 33% were fearful of impending insanity.[5] As such, it is important to clarify with patients that visual hallucinations are not always related to cognitive dysfunction.

The purpose of this study will be to determine the prevalence of visual hallucinations in patients receiving intravitreal anti–vascular endothelial growth factor (VEGF) treatment in Prince George, British Columbia. It will also be possible to determine characteristics of any hallucinations experienced, such as description, onset, and triggering factors. This information can aid in our current understanding of CBS.

Methods

This clinic–based study was undertaken in Prince George, a community in northern British Columbia. This population was chosen for sampling convenience (proximity to researchers, as well as common eye pathologies among participants). Participants over 18 years of age receiving treatment for AMD, diabetic retinopathy, and RVO were recruited through an injection clinic. All participants were receiving anti–VEGF agents (bevacizumab, ranibizumab, or aflibercept). Anti–VEGF agents help to preserve vision by preventing the formation of leaky blood vessels and

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edema. Over a period of 6 weeks, 122 patients gave informed consent to participate in the survey. Ethics approval was obtained from UBC Behavioural Research Ethics Board (ID=H15-02003).

Each patient was given a one–to–one short introduction to Charles Bonnet Syndrome. Patients were informed that people with poor vision like themselves can experience visual hallucinations, and that these hallucinations might not necessarily be caused by mental illness. Hallucinations were defined as concrete images without a stimulus. Thus, other visual disturbances such as scintillations, illusions, and distortions were excluded based on history. Patients who screened positive for visual hallucinations were asked further questions to describe their experiences. A standardized questionnaire explored the content of hallucinations, as well as onset, duration, frequency, triggers, and temporal relation to anti–VEGF treatment. All questionnaires were verbally administered by the same study researcher.

Patients were asked about their general medical health and were screened for a history of hypertension, stroke, migraines, diabetes mellitus, depression, schizophrenia, Parkinson's disease, and dementia. Diagnoses of ocular pathologies and best corrected visual acuity (binocular) were obtained directly from the patients' charts.

Finally, binocular contrast sensitivity testing was undertaken using a validated iPad contrast sensitivity test created by Ridgevue.[12] An iPad with retinal display was placed one metre away from the patient with the room lights off. The auto–brightness was turned off, and the brightness was adjusted to the middle of the scale. Each page of the test consists of two letters of equal contrast, and the contrast of subsequent pages decreases by 0.1 log units. Testing ended when the patient missed both letters on a given contrast page. Contrast sensitivity was scored as 0.05 x total number of correct letters.

A stepwise multiple linear regression analysis was used to develop a model for predicting Charles Bonnet Syndrome from vision code, contrast sensitivity, age of patients, RVO, diabetic retinopathy, cataracts, AMD and patient sex. Statistical significance was defined as p–value≤0.05. Best corrected visual acuities were stratified based on overall functionality (Canadian National Institute of the Blind vision code 0 = 20/20 to 20/69; vision code 1+ = 20/70 or worse).[7] Characteristics of visual hallucinations were tabulated.

Table 1 | Prevalence of Charles Bonnet Syndrome, demographics.

	Number (%)	Number with Hallucinations (%)
Total Clients	122 (100)	8 (6.6)
Age		
>80	47 (38.5)	6(75)
65-80	54 (44.3)	2 (25)
<65	19 (15.6)	0
Sex		
Male	49 (40.2)	3(37.5)
Female	73 (59.8)	5(62.5)
Ocular Pathology*		
AMD	92(75.4)	7(87.5)
Cataracts	3(2.5)	0
Glaucoma	8(1.6)	1(12.5)
Diabetic Retinopathy	21(17.2)	0
Retinal Vein Occlusion	17 (13.9)	1(12.5)

*Patients can have more than 1.

Table 2 | Correlations between Charles Bonnet Syndrome and predictive factors.

	Charles Bonnet Syndrome	P value
Vision Code 1+ (20/70 or worse)	0.25	0.002
Contrast sensitivity	-0.31	0.001
Sex of Patient	0.02	0.411
AMD	0.15	0.047
Cataracts	-0.04	0.322
Diabetic Retinopathy	-0.12	0.105
Retinal Vein Occlusion	-0.012	0.449

Results

Out of 122 clients, 49 were male (40.2%). Average age of participants was 75.3 years. Diagnoses included AMD (n=92; 75.4%), diabetic retinopathy (n=21; 17.2%), and RVO (n=17; 13.9%). Out of 122 clients, 8 met the diagnostic criteria for Charles Bonnet Syndrome (prevalence rate of 6.6%). Demographics of the patient cohort is shown in Table 1. Higher vision code (poor visual acuity; p=0.002) and poor contrast sensitivity (p=0.001) were significant predictors of Charles Bonnet Syndrome. The one–predictor model was able to account for 6% of the total variance in Charles Bonnet Syndrome, F(1, 117) = 8.01, p<.01, R2=0.06, 95% CI [0.02, 0.14]. Further correlations between Charles Bonnet Syndrome and predictive factors can be found in Table 2.

In the cohort which screened positive for visual hallucinations (n=8), the quality of the hallucinations were explored (see Table 3). The most common hallucination experienced was that of people and faces (n=7). There was a mixture of chromatic and greyscale, although most participants had stationary hallucinations (n=7). Hallucinations tended to be brief (<30 seconds to 10 minutes), and often occurred in situations with dim lighting (n=6). The onset of hallucinations ranged from 1+ months to several years. Very few participants (n=2) had previously discussed their experiences with a physician.

Discussion

The findings in this study are congruent with past studies, and confirmed that visual hallucinations are not uncommon in patients with low visual acuity. The overall prevalence rate was 6.6%, which is lower than the rate of 18.8% seen in a recent large CBS cohort study.[7] The high variance in rates of CBS can be attributed to the association of visual hallucinations and mental illness, and it is possible that some patients were hesitant to disclose. Past studies reported a prevalence of CBS to be anywhere from 0.5 to 40%.[1,5,10]

The findings in this study also suggest that visual hallucinations are associated with both poor visual acuity and poor contrast sensitivity, which is consistent with past studies.[6-8] These findings also support one of the proposed etiologies of CBS, known as deprivation or deafferentation theory. Poor visual acuity and poor contrast sensitivity can contribute to sub–threshold visual input, which causes the visual association cortex in the brain to produce images—visual hallucinations.[3,5] Although the underlying etiology of CBS remains unclear, the finding that there was no difference in the prevalence of CBS in each eye pathology suggests that the vision loss itself plays a bigger role in the etiology of visual hallucinations.

By exploring the quality of visual hallucinations in our small cohort of 8 people, a wide variety of visual hallucinations were seen. All 8 patients confirmed that they experienced hallucinations prior to starting anti–VEGF treatment. The most common hallucination experienced was that of people and faces, and usually these hallucinations were stationary.

Table 3 | Characteristics of hallucinations experienced in Charles Bonnet Syndrome.

Hallucination Characteristics	Patient 1 82 yo F	Patient 2 78 yo M	Patient 3 84 yo F	Patient 4 84 yo F	Patient 5 72 yo M	Patient 6 89 yo F	Patient 7 84 yo M	Patient 8 83 yo F
Ocular pathology	AMD, glaucoma	AMD	AMD	AMD	AMD	AMD, detachment 25 years ago	AMD	Central RVO
Visual Acuity in best eye (score)	20/50	20/400	Hand motions	20/25	20/40	20/200	20/50	20/100
Contrast sensitivity	0.65	N/A	0.9	1.5	1.5	1.1	1.4	1.05
Description of hallucinations	People	People and faces (cartoonish)	Shapes	People (elf–like)	People (sometimes familiar)	Faces only	People (sometimes half body)	People (half body)
Onset	2-3 years ago	1 year ago	6 months ago, have stopped	1+ months ago	3-4 months ago	5 years ago. Stopped 1 year ago	6 months ago	6 months ago
Duration	1-2 mins	1 min +	2-3 mins	<30 seconds	3-10 mins	2-3 minutes	4-5 seconds	Few seconds to few minutes
Frequency	1-2x per week	Up to 10 per day	2-3 per day	Twice	Unable to quantify	1 per month	1 every 2 weeks	1 every 3-4 weeks
Chromatic or Greyscale	Both	Colour	Colour	Colour	Greyscale	Greyscale	Greyscale	Both
Moving or Stationary	Stationary	Stationary	Stationary	Stationary	Moving	Stationary	Stationary	Stationary
Triggers	Dim lighting	When trying to focus	Dim lighting	None	Dim lighting	Dim lighting	Dim lighting	Dim lighting
Recreational Drug Use	Oral marijuana (hallucinations 1 year before use)	No	No	No	No	No	No	No
Discussed with others previously	No	With physician	No	With daughter	With physician, wife	No	No	Family members
Degree of distress	Initially, none now	None	Initially, none now	A little distress at time	Some distress	None	None	None

Hallucinations tended to be brief (minutes in duration). Dim lighting was a notable trigger in our CBS cohort, which supports deprivation theory. Because these patients had been experiencing hallucinations for an extended period of time (months to years), they did not report significant distress when hallucinations recurred. However, all patients reported feeling relieved when they were reassured that visual hallucinations can be a consequence of their vision loss. Interestingly, very few participants had previously discussed their experiences with a physician. This suggests that there is still a stigma with mental illness.

Research limitations

Although efforts were made to screen for other causes of visual hallucinations, including mental illness, it is possible that some patients who met the criteria for Charles Bonnet Syndrome might in fact have another underlying cause of hallucinations other than poor visual acuity and contrast sensitivity. As discussed previously, it can be difficult to determine the true prevalence rate of CBS due to the inherent stigma of mental illness. As a result, it is possible that some patients were reluctant to disclose that they were actually having visual hallucinations. It was hoped that this reluctance would be minimal due to the time spent in explaining CBS to each patient.

In hindsight, it would have been beneficial to explore whether or not visual hallucinations preceded low or reduced vision. In addition, this study had a relatively small sample size, a very specific patient population, and is only limited to northern British Columbia.

Conclusion

The findings in this study are congruent with past studies, and confirmed that visual hallucinations are common in patients with low visual acuity. Many elderly patients with poor visual acuity can experience hallucinations, and thus it is important to increase the awareness of Charles Bonnet syndrome in the medical community in order to improve patient care. Healthcare providers have a great capacity to inform patients that visual hallucinations are not always associated with mental illness. In addition, appropriate referrals can be made to other healthcare professionals to rule out other conditions that can cause visual hallucinations.

There appears to be an association between poor contrast sensitivity and visual hallucinations. Although the etiology of CBS remains unclear, it is possible that contrast sensitivity can play an important role in the etiology of visual hallucinations (deprivation theory). Currently, there is limited research in this field, and future research endeavours might be beneficial in furthering our understanding of visual hallucinations. It would also be beneficial to further explore patient perspectives on visual hallucinations in the future.

Footnotes and disclosure

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From targeted to pinpoint: The implementation of pharmacogenomics in clinical oncology

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Abstract

The numerous technological and pharmacological advances of the 20th century triggered hope throughout the oncological community that cancer would be systematically eliminated. However, since the advent of targeted therapies and the hope elicited from such triumphs as imatinib, the pace of progression has slowed to a crawl. Moving forward, cancer researchers and clinicians must continue to identify the major somatic and germline mutations that underscore the pathogenesis of neoplasia and should seek to capitalize on ever–improving DNA sequencing capability with the goal of devising new therapies that achieve higher cure rates, minimize recurrence, and enhance the tolerability of widely used regimens.

How we got here

The 20th century represented one of history's first significant leaps forward in the war on cancer.[1] Sidney Farber's achievement of a partial remission from leukemia through the administration of a novel therapeutic in 1947 signalled to the world that the seemingly unbeatable scourge could, at the very least, be kept at bay.[2] The remainder of the century, regrettably, was marked by mere occasional steps forward, with researchers frequently baffled by infuriating forms of resistance.[3] One notable example of such resistance surrounded trastuzumab (Herceptin), which was originally studied for the management of HER–2 positive breast cancer in 1987. After approval, this drug's benefit was impeded by numerous modes of resistance, including SRC tyrosine kinases, a common mechanism of cancer's resistance to numerous medications.[3] Unquestionably, such feats as the utilization of comprehensive screening programs and aggressive prevention campaigns have improved outcomes for thousands of patients in the modern era.[4,5] In fact, the National Cancer Institute reported that the cancer death rate declined by 23% between 1991 and 2016.[6] However, cancer's pharmacological therapy boasted only minor enhancements. Thus, researchers acknowledged the necessity of a paradigm shift in the field: the blunt tool that targeted all the body's actively dividing cells, conventional chemotherapy, would no longer suffice. The flaws of this practice, ranging from crippling side effects to inadequate remission rates, are well–documented.[7-9] As such, researchers attempted to combat the various neoplastic conditions from a different perspective—by identifying the very pathogenetic mutations that underlie the abnormal growth of cells. With this lofty goal, cancer's first targeted therapy, rituximab (Rituxan) was approved for the treatment of medically intractable B–cell non-Hodgkin lymphoma (NHL) in 1997 (Table 1).[10] Rituximab, which remains a part of the primary regimen for treatment of NHL to this day, contributes to the vastly improved 5–year survival rate for the condition, which now exceeds 90% in low–risk cases.[10] Since this initial discovery, many more targeted therapies have been approved for the management of a wide array of cancers, often targeting aberrant growth factors, angiogenesis, and apoptosis responses within tumour cells. Perhaps most notably, imatinib (Gleevec), blocking the overly active BCR–Abl tyrosine kinase produced by a 9-22 chromosomal translocation, was approved in 2001 for the treatment of chronic myelogenous leukemia (CML).[10] This advancement fostered an increase in 5–year survival for CML from 31% in 1993 to nearly 90% today.[10] While imatinib, rituximab, and other so–called targeted therapies have given oncologists hope to one day offer effective, side effect–minimizing therapies for all cancer types, the pace of progress has once again slowed, with many malignancies still requiring conventional chemotherapy to attempt to slow the rate of growth. As such, many still question how to systematically investigate and implement pharmacogenomics in the new generation of oncology.

One major step forward was thought to be the completion of the human genome project in 2003, which elucidated thousands of novel biomarkers that have since been targeted therapeutically. Furthermore, next–generation sequencing has substantially lowered costs and reduced the required time for screening of mutations within either tumour or host DNA.[11]

Where we are now

A prevalent thrust in oncology today is to utilize comprehensive analyses of the tumour's and the patient's DNA to predict a drug's efficacy and safety.[12] The implementation of pharmacogenomics into the clinic must differentiate between two forms of genetic mutation: somatic and germline.[13]

Somatic (tumour) mutations represent the driving force behind oncogenesis.[14] One example of present relevance is the anaplastic lymphoma kinase (ALK) rearrangement that underlies some forms of non-small cell lung cancer (NSCLC) and can now be precisely targeted with such drugs as crizotinib (Xalkori) and ceritinib (Zykadia).[13] Although these medications did show promise, crizotinib, for example, prolonged survival amongst advanced NSCLC by only 4.7 months as compared to conventional chemotherapy.[15] As such, clinicians and researchers alike must once again face the question of how to enhance this benefit.

One significant component to treatment success is the minimization of treatment–related adverse events. Germline (patient) mutations are major determinants of the pharmacodynamic handling of medications and, as such, play a critical role in identifying patients at high risk of developing serious untoward effects that contribute to treatment discontinuation.[12] To illustrate this, we turn to estrogen–dependent breast cancer, in which treatment with aromatase inhibitors may be limited by severe musculoskeletal pain.[16] Studies have identified single nucleotide polymorphisms (SNPs) in the T–cell leukemia 1A (TCL1A) gene that are significantly associated with this adverse event.[17] Theoretically, such genetic markers, which exist for chemotherapy and targeted therapies alike, could be investigated at the start of cancer treatment to predict those individuals who will discontinue treatment due to intolerability, sparing them unnecessary harm.[12] However, the thorough assessment of such markers through randomized controlled trials (RCTs) would be required before clinical implementation.[13]

The barriers

The most obvious factor to be considered is, as always, cost. While some genetic modifications occur in the vast majority of tumours, others are far less common.[12] A recent cost–effectiveness analysis

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Table 1 | Timeline of Significant Advancement in Cancer Targeted Therapy. Derived from American Society of Clinical Oncology[26]

Year	Significance	Drug	Target	Indication
1997	First FDA-approved targeted cancer drug	Rituximab	CD20	B-cell non-Hodgkin Lymphoma (NHL)
1999	First FDA-approved targeted anti-breast cancer drug	Trastuzumab	HER2	HER2+ breast carcinoma
2001	Fastest approval in FDA history; first drug to target the Philadelphia chromosome	Imatinib	BCR-Abl	Chronic myelogenous leukemia (CML)
2001	First effective c-Kit blocker	Imatinib	c-Kit	Gastrointestinal stromal tumour (GIST)
2003	First FDA-approved targeted anti-lung cancer drugs	Gefitinib, Erlotinib	EGFR	Non-small cell lung cancer (NSCLC)
2004	First FDA-approved anti-angiogenic drug	Bevacizumab	VEGF	Colorectal, lung, ovarian, kidney cancers
2005	Additional colon cancer targeted therapies	Cetuximab, Panitumumab	EGFR	Colorectal cancer
2010	First proven survival benefit for advanced melanoma	Ipilimumab	CTLA4	Metastatic melanoma

on the utilization of ALK testing in advanced NSCLC found that the process was inefficient due to high medication costs and marker infrequency.[18] As many oncologists attest, ALK testing and subsequent targeted treatment portends a significant mortality benefit and, as such, should remain a standard of care.[15,19] However, the cost of such interventions must be considered.

The major barrier in anti–neoplastic drug development is no longer the identification of potential targets, which has been vastly enhanced through advances in molecular technology, but rather differentiating key mutations that underlie the pathogenesis (driver mutations) from non–clinically relevant alterations (passenger mutations). Although stratifying these may be difficult, the silence of the mutation, its location, and its frequency will assist with the demarcation.[12]

Each time a potential biomarker is identified, it must be thoroughly assessed from toxicity, efficacy, and economic perspectives. The only viable means of providing this evidence are large–scale RCTs. This has now emerged as a major bottleneck in the field, since identification of potentially beneficial loci has accelerated with advances in sequencing technology.[11] The concept of thorough analysis of each of these potential sites is daunting. As part of the solution, the American Society of Clinical Oncology implemented the CancerLinQ initiative in 2015 to facilitate collection of global data from patient care in real time, enabling the production of relevant hypotheses to be investigated in an efficient manner.[12,20]

Where we go next

One component of oncology's future will require no significant intervention from researchers—the cost of genomic sequencing and its turnaround time will continue to decrease.[21] Since 2007, when the cost to sequence an entire genome was approximately $10 million, the expense has plummeted to below $1,500 in 2015.[22] Although cost–effectiveness analyses on the feasibility of widespread implementation of genomic sequencing in all aspects of healthcare remain controversial,[23] the application to cancer therapeutics would significantly reduce costs.[24] Ultimately, genomic sequencing will be incorporated into the regular practice of medical oncologists. This process is well underway in British Columbia, under the direction of the B.C. Cancer Agency's Personalized Oncogenomics (POG) program.[25] The hope is that such advances will hasten the development of novel medications.

As noted, the future of oncology will, inevitably, be greatly influenced by RCTs. However, sole reliance on this method of analysis is impractical given cost and time restrictions. Therefore, observational trials, despite their inherent potential for biases and confounders, will play an important role in classifying real–world risks and benefits.[13]

Finally, it must be acknowledged that the future of oncology lies within the sequences of the germline and somatic mutations that are identified. The importance of obtaining specimens from hosts and tumours and of contributing to the developing global database of cancer DNA must be impressed upon all developing clinicians and clinical researchers.[20] Only in this way will we draw closer to the seemingly mythical concept of cancer eradication.

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Nanotechnology as a platform for personalized cancer therapy

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Abstract

While chemotherapy has done wonders to save and prolong lives, it can cause harmful side effects in many patients and has limited efficacy in certain cancers. Newer, personalized approaches to cancer therapy look to target specific molecular characteristics of an individual's cancer cells, with the aim of improving cure rates and reducing side effects. To achieve this goal, it is vital to integrate the abundant molecular information now readily obtained from cancers—e.g., their mutational landscapes and gene expression profiles—with relevant therapeutic strategies. Nanotechnology is a powerful tool that is being studied extensively for this purpose. This article will describe key areas where nanotechnology may enable personalized approaches to cancer treatment, along with future directions and challenges in the field.

When people think of nanotechnology, they likely imagine things that are very small. However, from a molecular perspective, the nanoscale is in fact rather large. Small molecule drugs—the mainstay of medical therapy for the past 100+ years—are sub-nanometer in size: roughly 3 million ibuprofen molecules can fit into a 100 nm cube.[1] While there has been some debate over the exact size range that constitutes a nanoparticle, the FDA appears to have settled on a definition of materials with at least one dimension between 1-1000 nm.[2] Compared to traditional pharmaceuticals, nanoparticles are thus a big stepup in size, allowing for therapeutics with increased complexity and functionality. Nanoparticles can achieve this through diverse designs: shape, size, composition, and surface chemistry can all be modified to optimize performance (Figure 1).[3] In the context of cancer therapy, nanoparticles are being engineered to target and destroy tumours by delivering drugs or biologics, as well as through direct cytotoxic activity.

Chemotherapies tend to have significant toxicity in many cell types other than the cancer cells they are intended for; nanoparticle encapsulation of chemotherapy drugs can enhance tumor localization and mitigate off–target effects. The first clinically approved example of this concept was Doxil—a liposomal nanoparticle formulation of doxorubicin, first approved by the FDA in 1995.[4] Compared to free doxorubicin, Doxil has demonstrated similar overall efficacy to free doxorubicin, but greater tumor accumulation and reduced cardiotoxicity; it is approved for indications in multiple cancers including breast and ovarian.[5] Doxil is not specifically targeted to cancer cells, but relies on passive accumulation: by virtue of their size, nanoparticles have a tendency to accumulate in tumors due to the relatively high permeability of tumor vasculature coupled with poor lymphatic drainage.[6] Other nanoparticle formulations relying on passive accumulation have been approved for clinical use, including albumin–nanoparticle bound paclitaxel (Abraxane), liposomal vincristine (Marqibo), and liposomal irinotecan (Onivyde).[4] Of these, Abraxane is likely the most successful with nearly $1 billion in annual sales and approval for use in treating non–small cell lung cancer, late–stage pancreatic cancer, and metastatic breast cancer. Improved nanoparticle delivery to tumors is expected to be achieved by adding targeting ligands (e.g., antibodies) to the nanoparticle surface that recognize specific cancer markers. This concept, which is often referred to as active targeting, is being extensively researched in preclinical studies, and several targeted systems are currently being investigated in Phase I-III clinical trials.[4]

An emerging area of nanoparticle research is the delivery of delicate biological cargo. Nucleic acids are of considerable interest for cancer therapy, as they can be used to replace defective genes (via DNA or mRNA) or to silence the expression of oncogenes (e.g., via short interfering RNA). However, as drug candidates, nucleic acids suffer from several drawbacks, including rapid degradation in the blood and an inability to enter most cells. In this context, nanoparticles may act like artificial viruses that can transport nucleic acids and "infect" cancer cells to deliver a payload. The most commonly studied nanoparticles for this application are lipid–based, with several different systems now in clinical trials to treat diverse cancers.[4] Other more exotic materials are also being investigated pre–clinically, such as gold nanoparticles with dense nucleic acid shells and nanoporous silica particles.[7,8] The ultimate vision for nanoparticle gene therapy is to provide highly personalized treatments based on the patient's specific mutations (targeted by the nucleic acid) and cell surface markers (targeted by ligands attached to the nanoparticle surface).

Combining multiple drugs to target a particular cancer is a commonly used treatment strategy.[9] By packaging different therapeutics into a single nanoparticle, they can reach cancer cells at the same time at a specific ratio, irrespective of their individual pharmacokinetics. This can be important, as the precise ratio of two or more drugs can

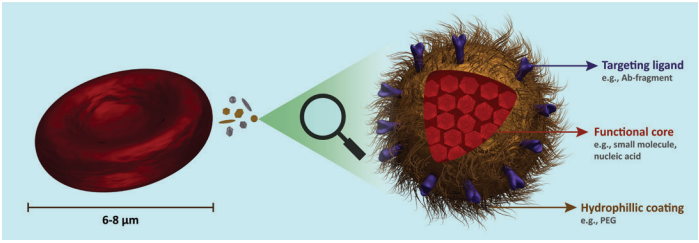

Figure 1 | Schematic illustration of biomedical nanoparticles. Nanoparticles are typically about 1/100th the diameter of a red blood cell, and can be formed with various shapes, sizes, and compositions. Nanoparticles can be made from organic and inorganic materials, with common examples being lipids, biodegradable polymers, silica, gold, and silver. The right side of the diagram shows a zoomed-in view of a typical nanoparticle design. The interior of the particle is often loaded with an active payload—for example, a small-molecule drug or nucleic acid. The outer surface of the nanoparticle is typically coated with a hydrophilic polymer layer—most often polyethylene glycol (PEG)—to improve particle stability and prolong circulation. Lastly, the outer surface of the particle may also be decorated with specific ligands to target cancer cells or other cells of interest. Examples include transferrin, folic acid, and antibodies directed against HER2.

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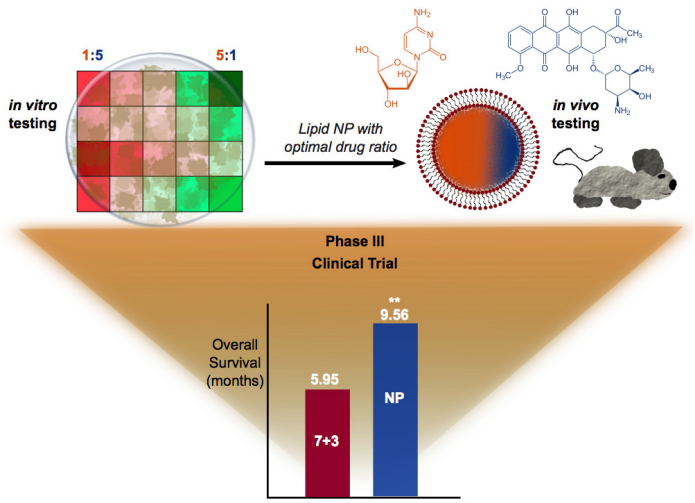

Figure 2 | Nanoparticles from bench to bedside. Drug combinations may show antagonistic, additive, or synergistic effects depending on their ratio; Celator pharmaceuticals is developing nanoparticles that deliver precise synergistic ratios of therapeutics to treat cancer.[10] The process begins by testing a given drug combination in vitro against a number of cancer cell lines (top left; red = antagonism, green = synergy). Nanoparticles are then designed to contain the optimal synergistic drug ratio to maximize effect, and are tested in animal models (top right). A recent phase III trial of the lipid nanoparticle formulation CPX-351 (Vyxeos), containing a 5:1 ratio of cytarabine and daunorubicin, showed superior efficacy compared to 7+3 (a standard regimen of the same two drugs) for the treatment of secondary acute myeloid leukemia in older patients (bottom).[11]

have a profound impact on their combined effect.[10] A recent phase III trial showed promise for using a nanoparticle formulation to co–deliver cytarabine and daunorubicin to patients aged 60-75 with high–risk acute myeloid leukemia.[11] The nanoparticle studied—called Vyxeos—contains a 5:1 ratio of the two active drugs (cytarabine and daunorubicin), which was previously shown to maximize synergy in vitro.[12] The phase III study demonstrated a significant 3.6 month improvement in overall survival, along with a superior response rate and no increased toxicity, compared to the control group receiving a standard regimen of the same two drugs (Figure 2). In addition to controlling drug ratios, preclinical data have suggested that nanoparticles can also be used to modulate the timing of drug release at the tumor site, which may further help to maximize the effect.[13,14]

Despite promising clinical and preclinical results, the vision of nanomedicines that exclusively target cancer cells remains elusive. A recent review article calculated that a median of 0.7% of administered nanoparticle doses reach solid tumors in mouse studies published over the past decade, with active particle targeting via surface ligands increasing this number to just 0.9%.[6] However, the range of efficiencies reported in the review was highly variable, and according to industry experts, tumor accumulation is thought to be upwards of 10% for the best–performing nanomedicines in humans.[15] Several barriers have been identified that impede tumor targeting: for example, upon contact with blood, proteins adsorb to the nanoparticle surface, which can both interfere with active targeting and promote phagocytosis by the mononuclear phagocyte system.[16] Nanoparticle shape, size, surface charge and chemistry all appear to play a role, and delineating the complex interplay between these factors and the biological milieu is extremely difficult. Other challenges include optimizing extravasation, tumor penetration, and particle uptake/drug release at the cancer site. Given that nanoparticles are frequently taken up by phagocytic immune cells, some researchers have suggested exploiting this property for cancer immunotherapy.[17] Other new approaches include nanoparticles that can respond to external stimuli (e.g., radiation directed at the tumor) to release their cargo or cause direct damage through heat or free radical generation. While this sounds futuristic, some of these systems have already entered clinical trials: e.g., hafnium oxide nanoparticles designed to amplify the effect of radiation therapy within tumors (NBTXR3) are being tested in a phase II/III trial for soft–tissue sarcoma.[18]

Nanotechnology currently plays a niche role in the overall landscape of cancer therapy, but there are indications that this will change. A recent analysis by scientists at the FDA showed that there has been a steady increase of drug product submissions containing nanomaterials over the past 30 years, with the largest fraction (40%) being for cancer indications.[2] Furthermore, the overall success rate for drugs containing nanomaterials—i.e., the fraction of new drug approvals relative to the number of investigational new drug submissions—is 15%, which is comparable to the success rate for biologics. It is also notable that many of the nanomedicine products currently undergoing clinical trials have been developed by startups and smaller pharmaceutical companies; if the field continues to prove itself with further success stories, larger pharmaceutical companies will likely become more involved. Continued advances in our basic understanding of the bio–nano interface are leading to improvements in rationally designed systems, and creative new designs are published nearly every day; some of these small designs will hopefully have a big impact on patient care.

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First, do no harm: The role of cannabis education in response to the opioid crisis

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Abstract

Recent years have seen unprecedented levels of accidental opioid overdose–related deaths in British Columbia. In response, the College of Physicians and Surgeons released guidelines to reduce over–prescribing of opioids. Unfortunately, many Canadians continue to suffer with chronic pain, and offering suitable treatment alternatives is a priority. Since 1999, the courts have recognized patients' rights to use cannabis for therapeutic purposes (CTP). Recently, the government tasked physicians as gatekeepers to CTP. However, there is a need for greater educational opportunities on CTP for clinicians engaged in pain management to ensure that lack of knowledge is not a barrier to accessing a potentially effective therapy with a safety profile that is superior to opioids.

One in five Canadians live with chronic pain (CP).[1] It is associated with an increased risk of co–morbid psychological illnesses and mortality, as well as decreased quality of life.[2,3] Additionally, the costs incurred from CP are staggering. In 2010, it was estimated that CP costs the Canadian healthcare system more than $6 billion dollars annually. Productivity costs related to job loss and sick days were estimated at $37 billion.[4]

In the 1990s, opioids emerged as a primary treatment for CP, due in part to increased marketing efforts by pharmaceutical manufacturers of novel opioid analgesics such as oxycodone (OxyContin) that purportedly improved the "efficiency and quality of pain management… without unacceptable side–effects".[5] These campaigns contributed to significant increases in the prescription of opioids; for example, oxycodone prescriptions increased by 850% between 1991 and 2007.[6] OxyContin was pulled from the market in 2012 and was replaced with a non-crushable and non–chewable capsule called OxyNEO in order to decrease misuse. The discontinuation of OxyContin created a void for dependent users, which was subsequently filled by an influx of illicit opioids and cheap generic opioids.[5] In April 2016, Dr. Perry Kendall, British Columbia's Provincial Medical Health Officer, declared a public health emergency in response to the increasing number of overdoses occurring in British Columbia. The majority of these overdoses were the direct result of opioid use.[7]

Although they are widely prescribed for pain relief, opioid therapies are controversial as they pose a risk for dependence and potential for fatal overdose due to tolerance and drug interaction.[8,9] Despite the risks, opioids continue to be prescribed to Canadians at a high rate. In 2015, physicians wrote 53 opioid prescriptions for every 100 people in Canada.[10] As such, Canada ranks second in the rate of opioid prescription of all developed countries.[11] In British Columbia, prescription of strong opioids saw an increase of 50% from 2005 to 2011, and in 2016, the College of Physicians and Surgeons of British Columbia released standards and guidelines in an attempt to reduce over-prescribing of opioids.[12] The standards state that non–pharmacologic and non–opioid analgesics (e.g., nonsteroidal anti–inflammatory drugs) are preferred for the treatment of chronic non–cancer pain and that the potential benefit of long-term opioid treatment is modest with significant risks. Nevertheless, opioids still play an important role in pain management in certain patient populations. For example, the Fraser Health Authority recommends the use of opioids in patients with advanced illnesses and in patients with cancer and non–cancer debilitating pain that is refractory to non–opioid medications.[13]

For some patients, cannabis may be a suitable alternative to opioid analgesics. Cannabis is a complex therapeutic agent that possesses psychoactive, analgesic, and anxiolytic capabilities. It has been posited that cannabis not only modulates pain signaling, but may also improve psychological aspects implicated in pain perception, such as mood and sleep.[14,15] In contrast to opioid analgesics, cannabis has a relatively low risk of dependence and no risk of fatal overdose.[16] Many patients report using cannabis effectively to treat their pain, and 30% of patients report substituting opioid medication with cannabis.[17,18] Findings from a recent review provide evidence of the efficacy of cannabis for pain. Of 38 published randomized clinical trials, 71% concluded that cannabinoids had empirically demonstrable and significant pain relieving effects.[19] Furthermore, a 2017 report produced by the National Academies of Sciences, Engineering, and Medicine (NASEM) stated that there is conclusive evidence that CTP is an effective treatment for chronic pain. The NASEM report also concluded that using cannabis is associated with specific harms, including worsening respiratory function, acute cognitive impairment, and risk of developing a substance use disorder.[20]

Since 1999, the Canadian courts have recognized the rights of patients to access CTP under Health Canada's Marihuana Medical Access Program.[21] The government program has gone through several iterations and is now the Access to Cannabis for Medical Purposes Regulations, which authorizes physicians to provide medical documentation allowing patients to access CTP from government-authorized producers of cannabis. Currently, there are well over 100,000 CTP patients registered in the government program, and this number is expected to increase to 400,000 over the next few years.[21,22] The incoming Cannabis Act will likely increase access to CTP, as patients who formerly experienced barriers finding a physician to authorize CTP will be able access cannabis outside of the medical system.

However, despite its apparent promise as an analgesic, the College of Family Physicians of Canada (CFPC) guidelines recommend CTP as a last resort in light of a paucity of research on the effectiveness and long-term consequences of using cannabis to treat pain, as well as concerns over misuse.[23] Given the current opioid crisis, the good

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safety profile of cannabis, and the dozens of studies reporting cannabis as effective for pain relief, this stance by the CFPC seems unduly conservative. Indeed, although physicians are integral to the process of patients acquiring CTP, physicians may perceive themselves as lacking the necessary knowledge about benefits, harms, indications, and appropriate treatment plans pertaining to CTP. Researchers from McGill University recently conducted a national survey aimed at determining the educational needs pertaining to CTP among physicians. They concluded that there was a clear need for education on the use of CTP, proper dosage, and the creation of effective treatment plans. In addition, it was concluded that the inclusion of CTP in physician practices would likely increase with additional education. Specifically, survey results called for peer–reviewed summaries with a preference for online education.[24] Past research suggests that continuing medical education interventions directed in a family practice setting are effective and directly influence patient outcomes.[25] Finally, increasing CTP educational opportunities in medical school could play an important role in producing future cohorts of physicians who are more comfortable with CTP and its value as treatment option in CP.

Many questions regarding CTP still need to be answered, but the therapeutic potential of cannabis in the treatment of CP and other conditions is encouraging. As the number of individuals using cannabis increases, governing bodies must update their recommendations with emerging research findings. Specifically, it is imperative that barriers to researching CTP are altered so that it can be studied more effectively; this can be achieved by developing more diverse funding networks, reclassifying cannabis, and improving standards of research methodology pertaining to cannabis.[20] Additionally, greater educational opportunities pertaining to CTP should be made available to improve standard–of–care and to provide greater treatment options for patients. Cannabis must be subject to the same risk–benefit analysis as other medications, and an important aspect of that is appropriate training for the healthcare professionals tasked with authorizing its use.

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Machine doctor (MD): The threat to human medical doctors' job security from deep learning

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Abstract

The age of "Big Data" has arrived with the liberation of large amounts of digital and analog data. The data can be used to help deep learning algorithms develop sophisticated layers of computerized "neural networks" that generate increasingly accurate clinical gestalt refined over numerous iterations. Combined with the simultaneously advancing clinical capabilities of computers, autonomous "machine doctors" might begin to offer financial and operational advantages over human medical care. However, taking up such a technology should be weighed against the machine's lack of ethical transparency and inability to improve health equity.

Introduction

The exponential growth of computing power has ushered in the era of "Big Data". This term took off around 2010 and is defined by voluminous data in the order of exabytes spanning various fields such as social media, blogs, videos, and images generated at an astounding hourly rate of petabytes.[1-3] Combined with the deep learning technology that has become vastly more powerful since 2006,[4] the replacement of human physicians by machine doctors (MDs) might be near.

Deep Learning—a more "organic" learning method for machines

Each refinement of a medical trainee's diagnostic or therapeutic plan improves some cognitive shortcomings. The iteratively selective reinforcement increases or decreases the weight of certain clinical features in the learner's mind. These key factors and their associated weights form the essence of a learner's neural network.[5]

This learning process has been adapted for machines. After being exposed to large amounts of unsorted data, the machine can derive an optimal solution by proposing several factors that are iteratively optimized in number, quality, weight, and layers to arrive at the most accurate solution possible.[5,6] For example, attempting to classify television by including objects with eight edges, a shiny screen, and a cord might inadvertently include computers. The subsequently corrected criteria might include the lack of a mouse or keyboard.

The accurate processing of non–traditional data such as images, speech patterns, sentiment, and languages can be combined with the data from clinical trials, electronic health records, social media, and personal devices to unlock the full power of deep learning algorithms.[7-21]

Current medical advances with deep learning

One medical application of deep learning comes from the "C-Path" algorithm. After training with several hundred pathology slides, the algorithm achieved an 89% accuracy in independently diagnosing breast cancer, and even determined novel prognostic features.[22]

Another algorithm, based on the GoogleNet Inception v3 Convoluted Neural Network (CNN), was trained with 129,450 images to determine "keratinocyte carcinomas versus benign seborrheic keratosis; and malignant melanomas versus benign nevi."[23] The CNN performed as well as 21 board–certified dermatologists in both tasks.[23]

The famous Watson supercomputer created by IBM is yet another example of machine learning. It has been used to assist the world–renowned oncologists at the Memorial Sloan Kettering Cancer Center by directly interpreting medical charts to understand a case and synthesizing the latest research to devise the optimal course of antineoplastic therapy.[24]

Deep learning in medicine leading to computerized clinicians

Experts have already suggested that machines will soon compete against pathologists and radiologists.[25,26] Even though no direct displacement has taken place yet, the famous Silicon Valley investor Vinod Khosla suspects that even Siri might provide a more accurate diagnosis than an average family doctor in 10–15 years.[27]

A clinician's job begins with holding an appropriate conversation for an accurate history. After successfully identifying the scene,[28] objects,[29,30] and human subject(s),[31] a MD can conceivably begin to elicit history with pre–determined questions. It can then transcribe the audio input for accurate analysis of denotations, as well as sociocultural or emotional connotations.[32-34] During the conversation, the machine can also adjust its questions around the human sentiments identified.[35-36] Even though machines cannot yet hold a conversation, current technologies already take orders in natural language forms [37] and might be conversational by the year 2020.[38]

Analysis of waveforms from the heart and lungs are well under way.[39-41] Although the engineering community has only recently moved beyond computer aids for palpation to automated palpation,[42-45] defining the appropriate pressure resistance to delineate a mass or elicit tenderness will be relatively straightforward.

Combining these ongoing advances in computerized clinical skills with the abstract reasoning abilities from deep learning algorithms might soon yield machines capable of autonomously proposing personalized diagnoses and management plans for each patient encounter. This will be the era of autonomous MDs.

The threat to human physicians' jobs—pros and cons

The MDs might threaten the job security of modern physicians who practice and train in an environment that de–emphasizes humanity in favour of routine biomedical diagnostic or therapeutic approaches.[46-50] Without the need for costly and prolonged training, the MD workforce can scale up much more cheaply and quickly;[51] unbridled by humans' lifestyle, location, or income preferences, MDs can aid underserved areas; freed from emotional and physiological fatigue, MDs' work hours can overshadow that of human doctors'. Patients can also

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receive the most up–to–date care, thanks to patch updates that can be automatically disseminated and installed.

The transition to robotic services has already started in surgery, where an independent suturing machine recently outperformed expert surgeons.[52] In medical laboratories also, automation has replaced postdoctoral researchers in routine tasks,[53] leading to a 30–fold decrease in operational cost.[54]

The disadvantages of adopting MDs, however, extend beyond losing control over the machines[55] or sacrificing therapeutic relationships with human doctors. The long–term cost saving requires large upfront investments into infrastructures. Whereas machines excel in repetitive tasks, they might flounder in novel situations without humans' reasoning ability.[56] At least in the near future, machines will also require help from human innovators to improve their algorithms,[27] leading Khosla to suggest that there is "still need to leverage the top 10 or 20% of doctors (at least for the next two decades) to help [the] bionic software get better at diagnosis."[27] The age–old concern around the lack of ethical principles guiding machines remains valid,[57] especially because MDs' opaque decision trees preclude the possibility to understand—let alone influence—the machines' ethical principles.[58] Such a technocratic advance also misses opportunities to tackle social determinants through proactive policies[59] or combat the perverse political and economic incentives that continuously drive health inequity.[60,61]

Conclusion

The collision between "Big Data" and deep learning might very well usher in MDs that are capable of forming personalized clinical decisions for routine diagnoses and therapeutic plans. Even though the practical advantages and the mechanistic framing of modern medicine make machine replacement of human doctors a financially and logistically attractive alternative, further developments ought to consider the machines' inaccessible ethical framework and inability to improve health equity.

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Meeting the needs of persons with dementia: Challenges facing speech–language pathologists

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Abstract

Persons with dementia are the fastest growing clinical population within the speech–language pathologist's scope of practice. In Canada, there are currently 546,000 individuals living with dementia. Providing adequate care to this growing population presents challenges to speech-language pathologists. In this paper, two challenges are presented. The first challenge concerns the lack of speech–language pathology services available for persons with dementia. The second challenge concerns the lack of education surrounding the need for speech–language pathology services for persons with dementia.

Persons with dementia (PWD) represent a significant segment of the Canadian and global population. Worldwide, nearly 46.8 million individuals are living with dementia.[1] In Canada, that number is currently 546,000, which is expected to increase by 66 % before the year 2031.[2] The World Health Organization has named dementia as the leading cause of disability and dependency among older adults.[1] Dementia is a progressive neurological condition that is defined as, "a significant cognitive decline from a previous level of performance in one or more cognitive domains" (p. 605).[3] The cognitive decline must interfere with an individual's independence in everyday activities.

Within the field of speech–language pathology, individuals with communication–associated dementia problems are the fastest growing clinical population.[4] Communication issues affect PWD due to an impairment to the central executive system, affecting working memory and episodic memory.[5] Examples of communicative tasks adversely affected by dementia range from responding appropriately to comments, to holding in mind the topic of conversation.[5] Speech–language pathologists (SLPs) can provide a variety of communication interventions for PWD to address a wide range of goals, such as: promoting meaningful social participation; decreasing frequencies of responsive behaviours; improving abilities to perform activities of daily living; or, supporting the expression of needs and wants. For example, an SLP could target the stimulation of spared cognitive processes, by using spaced retrieval training or the creation of memory books.[6-9] Alternatively, an SLP might target the communicative functioning of PWD through environmental modifications, such as communication partner education or validation therapy.[10,11]

Providing adequate speech–language pathology services to PWD in Canada faces some challenges. In this paper, two such challenges are presented. First, PWD are an under–served population by way of speech–language pathology services. Second, education surrounding the benefits and efficacy of speech–language pathology services for PWD is minimal.

Denying service to a significant segment of the population

Despite the size of the clinical population and available treatment options, an alarming number of PWD do not receive SLP services for their communicative needs. A survey by Hopper et al. (2007) attempted to shed light on the nature of SLP service delivery for individuals with dementia in the Canadian context.[12] The survey found that 60 % of respondents agreed that while PWD may benefit from SLP services, caseload demands prevent providing services. Moreover, 76.3 % of respondents identified the following as one of the top barriers to services: other patients with more acute concerns have priority. This suggests that although most SLPs agree that our services may benefit PWD, SLPs are unable to provide services due to other more acute conditions, such as dysphagia, receiving priority.

It has been ten years since Hopper et al. (2007) conducted their survey, and there is no further evidence that service provision for individuals with dementia has changed in Canada.[12] In fact, in the past ten years, the number of people living with dementia has only increased. As long as dementia services remain a low priority, thousands of individuals in Canada will continue to be underserved by SLPs.

Receiving the appropriate education and knowledge

Universities with SLP academic programs, such as the University of British Columbia, are making great strides to introduce educational components surrounding services for PWD. However, many academic speech–language programs often provide only minimal preparation for service delivery for PWD.[4] Beyond academia, practicing SLPs have voiced that a lack of knowledge regarding how to treat PWD can impede their provision of adequate service.[12] With little service delivery in the field, it can be difficult for SLP students to gain hands–on experience with PWD during practicums.

In addition to education for students and practicing SLPs, our colleagues from other disciplines could also use additional training. Another barrier to service for PWD stems from a lack of referrals from other professionals.[12,13] Other health workers may not be aware of what SLPs can offer for PWD, and therefore, do not refer our services.[12] Similarly, individuals receiving a dementia diagnosis are unlikely to know to ask for a referral for SLP services.

In conclusion, adequate service provision for PWD requires addressing both SLP caseload limitations and the lack of education surrounding the need for SLP services. The Speech–Language and Audiology Canada (SAC) Code of Ethics states that the value of professionalism entails "seek[ing] to advance the quality and provision of professional services through advocacy, public education," and, to "work collaboratively with members of both their own profession and other professions in the interest of delivering the best quality of care" (p. 2).[14] To abide by these principles and deliver the best quality of care to PWD, much headway is needed to increase the availability of SLP services for Canadians with dementia.

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Opportunities and challenges in using targeted next–generation sequencing for the diagnosis of dyslipidemias

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Abstract

Dyslipidemias are disorders in lipid metabolism that can jeopardize one's metabolic and cardiovascular health. Historically, dyslipidemias were diagnosed using biochemical findings such as abnormal levels of lipoproteins in the blood. Development of many cases of dyslipidemias are due to underlying genetic factors, but older methods of DNA sequencing were too slow and costly to be practically used in the clinic. Targeted next–generation sequencing (NGS) offers a unique opportunity to determine genetic diagnoses of inherited dyslipidemias more efficiently and at lower costs compared to older methods. NGS approaches to diagnosing dyslipidemias have been validated in several studies demonstrating the ability to create gene panels that can accurately diagnose patients that in some cases went undetected by other clinical guidelines. Advancements in NGS technologies provide new opportunities for the routine incorporation of a patient's genetic information into clinical care for dyslipidemias.

Disorders of lipid metabolism, otherwise known as dyslipidemias, are among the strongest risk factors for atherosclerosis and coronary artery disease (CAD).[1] About 2.4 million Canadians have CAD, and it is a leading cause of death in Canada.[2,3] Lipids are transported in the body inside lipoprotein particles that exist in various forms in the body such as low–density lipoproteins (LDL) and high–density lipoproteins (HDL). Each of these particles serves a distinct role in the body, and dysregulation of these lipoproteins can result in a number of clinical manifestations. For example, familial hypercholesterolemia (FH) is one of the most well–studied dyslipidemias and results in abnormally high LDL–C levels that can contribute to development of atherosclerosis and premature cardiovascular disease.[4] FH is an autosomal dominant trait that is typically caused by a heterozygous mutation in the LDLR, APOB, or PCSK9 genes and is just one example of numerous types of dyslipidemias that have a clear genetic basis (Table 1).[5] Therefore, being able to readily obtain genetic information about a patient can improve the diagnosis and management of dyslipidemias. Next–generation sequencing (NGS) is a broad term that encompasses DNA sequencing technologies that have emerged post–Sanger sequencing; while a technical comparison of sequencing technologies is beyond the scope of this review, Sanger sequencing is the original method of DNA sequencing and is limited by the fact that it can only analyze a single DNA fragment at a time. On the other hand, NGS can analyze multiple DNA fragments in parallel with exponentially more data being produced at a fraction of the time and cost.[6] While both methods still serve some purpose in clinical research today, for most purposes NGS is thought to have much greater potential for offering the ability to incorporate genetic information into routine clinical care.[7] Targeted NGS is a type of sequencing where one determines the presence of variants in a predetermined set of genes, as opposed to sequencing the whole genome, and it is easier to sift through the data for clinically actionable results. It also provides greater power to detect rare variants in relevant genes.[8] This commentary will discuss the recent advancements in using targeted NGS for diagnosing dyslipidemias and considerations for the implementation of this technology in clinical care.

Currently, the diagnosis of dyslipidemias is based primarily on clinical and biochemical findings, such as abnormal LDL, HDL, or triglyceride (TG) levels in the plasma, family history, and physical examination.[9] Management of dyslipidemias involves both pharmacological and behavioural interventions, depending on the risk level of the patient.[10] For example, guidelines published by the Canadian Cardiovascular Society (CCS) in 2016 recommend that patients with LDL–C >5 mmol/L and an additional risk factor such as atherosclerosis receive statin drug therapy.[10] Previous studies have demonstrated that genetic variations in specific genes (Table 2) are closely associated with abnormal lipid levels, but a patient's risk level in many guidelines is determined using metrics that do not take into account genetic factors, such as the Framingham Risk Score.[9,11] If genetic information was made more readily available to clinicians through NGS, guidelines may be updated with genetic criteria that may better assess a patient's risk level. For example, studies have demonstrated that calculating a genetic risk score using variants identified in previous genome–wide association studies can be used as an independent predictor for development of cardiovascular disease.[12] DNA sequencing is already being used to supplement biochemical methods of diagnosis of certain dyslipidemias such as chylomicronemia syndrome.[9,13] Johansen et al.[14] and Sadananda et al.[15] have both used

Table 1 | Select dyslipidemias and associated clinical features.

Dyslipidemia	Common Genetic Basis	Clinical Features
Familial hypercholesterolemia (FH)	Autosomal dominant mutations in LDLR, APOB, or PCSK9 genes	Xanthomas, arcus corneae, premature CAD
Familial defective apo B-100	Autosomal dominant missense mutation in apoB-100	Xanthomas, arcus corneae, premature CAD
Tangier disease	Autosomal recessive mutation in gene coding for ABCA1	Peripheral neuropathy, hepatosplenomegaly
Apolipoprotein A1 deficiency	Mutations in APOA1 gene	Atypical xanthomas, corneal opacification, premature CAD
LCAT deficiency	Autosomal recessive mutations in LCAT gene	Corneal opacification, hepatosplenomegaly
Chylomicronemia syndrome	Mutations in gene coding for LpL	Failure to thrive, anorexia, nausea, vomiting, pancreatitis

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Table 2 | Example monogenic dyslipidemia phenotype and associated genes.

Phenotype	Gene	Chromosome
High LDL-C	LDLR, APOB, PCSK9, STAP1, APOE, LDLRAP1, LIPA, ABCG5, ABCG8	19, 2, 1, 4, 19, 1, 10, 2, 2
Low LDL-C	MTTP, APOB, PCSK9, SAR1B, ANGPTL3	4, 2, 1, 5, 1
High HDL-C	CETP, LIPC, SCARB1	16, 15, 12
Low HDL-C	ABCA1, LCAT, LPL	9, 11, 16
High TG	APOC2, APOE	19
Low TG	APOC3	11

targeted NGS to diagnose dyslipidemias—in one instance they were able to make a genetic diagnosis in 35.9% of patients with low HDL and identified 21 novel variants related to HDL levels.[15] The results of these studies are significant as they are some of the first to validate targeted lipid NGS panels with the correct subset of genes to establish diagnoses of dyslipidemias. Another advantage of identifying genetic causes of dyslipidemias in the clinic is the opportunity to conduct genetic screening in family members. This is beneficial as it may allow for early diagnosis and management of patients whose disease may otherwise not have been apparent—it has also been demonstrated to have significant cost savings in UK FH patient populations.[16]

It is clear that we now possess a comprehensive understanding of the genetic basis of dyslipidemias to make meaningful clinical decisions based on genetic data and are approaching the technical capabilities to offer this testing in the clinic. It is important that we approach the incorporation of genetic information into routine clinical practice with realistic expectations, as challenges to diagnosing dyslipidemias still exist. The diagnosis of polygenic dyslipidemias, disorders due to multiple genetic variants, remains complex as individuals often have many common variants that each individually contribute very little to overall lipoprotein levels and thus make it difficult to identify causal variants.[17] Another caveat to consider is that although NGS may identify variants, providers may not know whether a variant is of clinical significance. Despite these challenges, one should remain optimistic about the implementation of NGS into clinical care in part due to the concerted efforts of regulatory bodies in facilitating the adoption of these technologies. In recent years, there has been the development of various standards for identifying causal variants, such as guidelines published by the American College of Medical Genetics, to help clinicians utilize genetic information.[18] In conclusion, advancements in our understanding of the genetic architecture of dyslipidemias suggest that incorporation of genetic information into clinical care would greatly improve the diagnosis and management of these conditions. NGS technologies provide the technical capabilities for clinicians to quickly obtain sufficient amounts of genetic information at a reasonable cost to inform care. This approach has been validated in numerous studies demonstrating the ability to both diagnose dyslipidemias and identify new causal variants. For these reasons, the incorporation of genetic information about lipid metabolism into routine practice ought to be seen as essential for providing quality care for patients in the future.

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Tip–toeing into the world of genomics: Ethics of gene sequencing in clinical medicine

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Hippocrates is often credited with being the first to separate patients into groups and individualizing treatments based on each group's predicted response.[1] This ideology is known as "personalized medicine," and has in recent times seen a renaissance due to modern advancements in biotechnology. In 1990, the National Human Genome Research Institute (NHGRI) announced the commencement of the Human Genome Project to sequence and map out 100% of the genetic information of a human being, which is commonly referred to as the human genome.[2] The project was completed on April 14th, 2003, almost 13 years later. At the present date, we can now sequence a human genome in as little as 26 hours.[3] The cost of sequencing a genome has also dropped significantly from $100M in 1990 to approximately $1000 USD in 2015 (Figure 1). Genomic sequencing has become so fast and affordable that sequencing a patient's genome to make clinical decisions is well within reach. Many gene loci are implicated in disease, and some varieties of gene loci respond to treatments with differing efficacy. By tailoring therapies to a patient's genetic code, we have the potential to create better management guidelines that inform more appropriate clinical decisions at the point of care. However, with access to abundant amounts of genetic information one must be careful with what information they uncover. Accidentally finding markers for diseases that have no effective means of prevention or treatment can do harm to patients without improving their health outcomes. Also, if patients are to have their genomes sequenced to better their health outcomes in the future, one must wonder who holds ownership of this genetic information and how this information will be stored and used. Lastly, making genomic sequencing a medical norm could have unforeseen consequences both socially and economically that may impact all of us and how we live our lives. Considering the above, much thoughtful planning is warranted when implementing genome screening programs.

Personalized medicine informed by genomics already exists today in modern clinical practice. For example, a patient with a particular family history can raise flags to prompt genetic testing. An example of this could be a female patient with several family members who have had breast and ovarian cancers that were diagnosed early on in their lifetimes. A referral to medical genetics could unveil that the patient is a carrier for mutations in the *BRCA1* and *BRCA2* genes. Carriers of mutations in these tumor–suppressing genes are known to be at a significantly increased risk for breast and ovarian cancers.[4] Screening these patients and intervening is particularly useful, as there is evidence that early screening and interventions such as bilateral prophylactic oophorectomy and mastectomy in *BRCA1* and *BRCA2* mutation–positive individuals decreases their incidence of cancer.[5]

When genomics yields results by screening for a few gene loci, it can be all too tempting to screen the entire genome to see what other mysteries lie in our DNA and how we can take control of our destinies to improve health outcomes. This temptation is reaffirmed by the commitment of $25 million USD over five years towards four projects on genomic screening in infants by the National Institute of Child Health and Development and the NHGRI.[6] One of the projects, named the BabySeq project, is currently underway at Brigham and Women's Hospital and at Boston Children's Hospital. Half of the 240 recruited newborns will be placed into control groups that will receive the current standard heel prick blood test that screens for approximately 30 heritable and treatable diseases, while the other half will receive full genome sequencing.[6] This project may find exciting new genes that correlate with disease and response to medical treatments, which can provide deeper insights into clinical judgements and improve patient outcomes. However, what happens with pathological genomic data that may be uncovered by genomic sequencing that we cannot change the outcome of? For example, the genomic sequencing of an infant could show that they carry the autosomal dominant huntingtin (HTT) gene for Huntington's disease (HD), a rare but devastating neurodegenerative disease that has an average life expectancy of 10 to 15 years from its onset of symptoms.[7] This is not particularly useful information since there are no known means of preventing HD, and thus this can cause unnecessary anxiety and suffering for the patient. Some parents who suspect that their child may have the HTT gene have looked into screening their child for it to inform their future financial decisions, such as not putting money aside for a college fund if their child is expected to develop HD.[8] The current guidelines from the Huntington's Disease Society of America have strongly opposed screening children for the above psychological and social harms and lack of benefits.[9]

It is difficult to reconcile established guidelines of the past with new advances in genomics. In the guidelines provided by the American

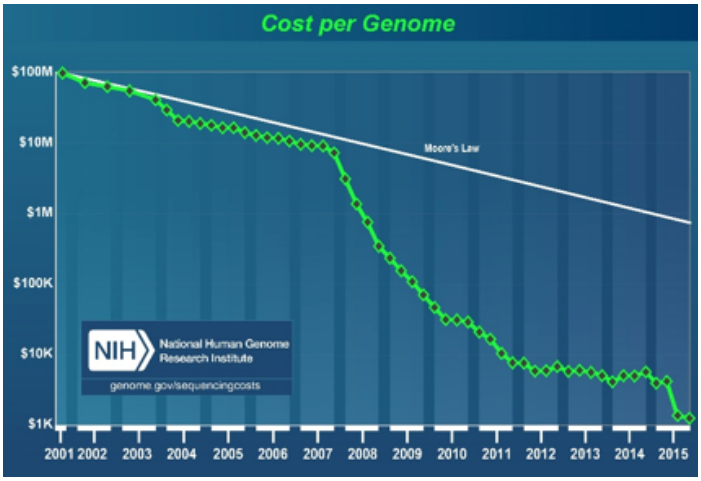

Figure 1 | Cost of sequencing a genome from 2001-2015. Moore's law here approximates the cost savings involved with the doubling of computing power every year which is the standard in the computer hardware industry. The rapidity of decline in costs of genomic sequencing relative to Moore's law highlights its massive success.[18]

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College of Medical Genetics and Genomics (ACMG) it is mentioned that child screening for adult–onset diseases is consistently cautioned against in the literature. However, the ACMG also argues that these policies are difficult to reconcile with the realities of genome sequencing in this transitional period of adopting genomic medicine. They argue that these practices are inconsistent with respecting a parent's right to make decisions about their children's health, and genomic results may have implications on the lives of the parents and family. Healthcare providers have an obligation to inform parents and the child, when appropriate, about these potential implications. This statement suggests an important consideration in the era of genomic medicine; after sequencing a child for a primary indication, it becomes relatively easy for a laboratory to report a limited number of variants for conditions that could be medically important to that child's future or to the rest of the family. The ACMG also mentions that one possible solution would be to restrict the sequencing and omit genes associated with diseases for which there is no available treatment.[10] On the contrary, it could be distressful for a patient who had their entire genome sequenced only to find out that they had a gene variant omitted in their screen that resulted in them presenting with a debilitating disease later in life. Therefore, guidelines must be set out and clearly expressed to both the healthcare team and the patient stipulating why this information would be omitted and possibly leave the option open for the patient to sequence genes for such diseases when they come of age and if they choose to.

Another nuanced issue regarding genomic sequencing is the opportunity to uncover genomic data that has uncertain clinical implications. For example, if a genomic screen were to uncover that a patient carried the recently discovered *CLU* and *PICALM* genes and were at a slightly increased risk for developing Alzheimer's disease, what would the physician do?[11] Some may argue that certain patients would want to know this information as they may choose to live their lives differently given a different life expectancy or quality of life. However, there are no known means of primary prevention for Alzheimer's disease and the patient may never present with the disease as its association with Alzheimer's is only a correlation. Thus, complete genome sequencing may be over–diagnosing patients, again leading to unnecessary worry for the patient and potentially causing unnecessary follow–up testing and inappropriate use of healthcare resources. Once more, patients should retain the right to know this information should they choose to. However, it is important to note that patient education has a critical role to play in genomic screening, so that patients are able to fully understand and appreciate the consequences of learning this information about themselves and its usefulness in their goals of care. In addition to the issues of what one may uncover by collecting these data, another big question is who owns these data? The most natural answer would be that the individual who provides the tissue sample owns the data related to it. However, with current precedent there are many barriers for patients to access their medical information due to the lack of user-friendly Electronic Medical Records.[12] Therefore, once a person's genome is sequenced, anonymized, and stored by a Large–Scale Genome Sequencing and Analysis Centre it is conceivable that patients will not be able to access their genomic data and must rely on Direct–to–Consumer genome sequencing services such as Navigenics and 23andMe for their genomic information.[13]

Lastly, as genome sequencing becomes the norm in medicine, its technology and knowledge will spread broadly. DNA sequencing technology is currently widely available commercially, and anybody can use it.[14] The prospect of open access to genomic screening could potentially raise concerns with how it is used. Its usage is difficult to regulate and thus there may be parts of the world that decide to use genomic sequencing to discriminate individuals based on their genomic background. Insurance companies could charge high premiums or even refuse to insure individuals who are at high risk for certain conditions.[8] Before a patient submits their genetic samples to be sequenced, it is critical that companies make it clear to patients what rights to their own genome that they are revoking and how their genomic data will be used and who it will be shared with. Legislation already exists to protect people from such genetic discrimination in developed countries like the United States, but this may not be the case in other countries. In Guangdong, China, three civil servant candidates claimed to be discriminated against during a recruitment process for carrying a gene associated with thalassemia, which is a common gene carried in approximately 11% of the Guangdong province.[15] The court ruled that thalassemia is a disease and thus it is legal for employers to use it as one of their requirements for recruitment, despite the plaintiff being only carriers for the gene of the disease. This example is one of many around the world illustrating that although legislation protecting against genomic discrimination likely exists, its enforcement may vary from region to region.[16,17]

The medical profession looks to alleviate suffering and do right by their patients. Impassioned by the drive to create better medicine for patients, medical research has created technological innovations to uncover many different medical conditions, and causes for them. However, by doing so we may be unknowingly creating ethical quagmires. Before making genomic sequencing commonplace in medicine, some thought should be given to how it could affect our lives socially, economically, legally, as well as medically. Moving forward, it may be prudent to design genome screening programs that "black–box" genes that may cause more harm than good should that gene be known to the patient. This black–box status could be removed if the patient expresses that they wish to know the status of such genes; however, this process should be accompanied with the guidance of a health professional who can explain the benefits and risks of knowing such information. In this way, patient autonomy can still be respected while mitigating potential risks. Also, as a part of their medical record, a patient's genetic information should be readily available to them in a user–friendly format. Apps and mobile platforms that are secure and allow the patient to view their genome can potentially fill this void in the future. In concordance with current precedent surrounding the genomic screening of minors, patients making decisions regarding their genomic information should also be of a legal decision–making age. Such a genome screening program should also be evaluated often, so that it may keep up with the rapid pace of technological growth while still providing quality care. Biotechnology has vastly improved over the past few decades by leaps and bounds, and is expected to continue growing in the future. However, when it comes to implementing break-through technologies such as genomic screening in the clinical setting, we may be better off tip-toeing our way into the world of genomics.

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Genomics and biomarker research in drug development: Overrated, or a revolution to come?

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As our understanding of physiology and pathophysiology continues to evolve, the domains of pharmacological research and drug development are seeing parallel increases in the sophistication of their methodology. Such expanding knowledge is expected to form the foundation of many future medical advances, slated to propel us toward a veritable era of personalized medicine in which treatments are tailored to unique patient subgroups at a molecular and genetic level.[1] Although the prospect of monumental advances in medical treatment might well be looming on the horizon, the current climate of genomic and biomarker research in drug development reveals a picture of mixed success. To truly usher in an era of personalized medicine, the utility and drawbacks of biomarker research must be understood, regulatory requirements must become consistent at national governing levels, and the information gained from such research must have a clinically meaningful impact to foster advances in treatment.

A biomarker has been defined broadly by the World Health Organization as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[2] The use of biomarkers in medical research is certainly not new, although with the more recent advent of human genome–wide association studies (GWAS),[3] novel biomarkers have found increasing use within the field of genomics and pharmacogenomics. A typical GWAS evaluates potential correlations between genetic variants, usually single nucleotide polymorphisms (SNPs), and certain phenotypic outcomes, such as the occurrence of disease or the response to a pharmacological agent.[4,5] In the context of a GWAS, a SNP could be considered a genetic biomarker, although biomarkers can take many other forms, including serving as surrogate endpoints in clinical trials.[6] The use of biomarkers, such as LDL cholesterol, as surrogate endpoints for more meaningful clinical outcomes, such as death from cardiovascular disease, is often a convenient and cost–effective alternative in research settings.[6] However, biomarkers must be used cautiously, as they can be misleading. For instance, in a study using rhythm control as a surrogate endpoint for decreased cardiovascular morbidity in arrhythmia patients, a select group of anti–arrhythmia agents was approved that was subsequently shown to increase mortality among certain patient subgroups.[7] Moreover, the utility of genetic biomarkers such as SNPs might be called into question, as they are often associated with relatively small effect sizes for the phenotypic trait of interest.[8,9] Despite their potential to make research more targeted and efficient, it must therefore be remembered that biomarkers are contributing but one piece to the ever–complicated puzzle of biological pathways.

Recognizing a progressive decline in new drug development, in 2004 the United States Food and Drug Administration (FDA) launched its "Critical Path Initiative", a series of proposals to increase the development of novel pharmaceutical agents.[10] Chief among these included the integration of biomarkers into pharmacological research.[10] To encourage collaboration among government, academia, and industry, the FDA also introduced formal guidelines and a regulated approach to biomarker qualification for drug research.[11,12] These initiatives have spurred the creation of a list of qualified biomarkers that might be used in various stages of drug development, including pre–clinical and clinical trials.[13] As an example, the Predictive Safety Testing Consortium (PSTC) has submitted a series of early–stage nephrotoxicity biomarkers that can serve as alternatives to traditional later–stage markers, such as creatinine and glomerular filtration rate.[12] The European Medicines Agency (EMA) has adopted a similar formalized approach to biomarker qualification, but there currently exists no formal process in Canada for biomarker recognition.[14] For its part, Health Canada has published a guidance document in which it encourages the submission of biomarker information for the purposes of pharmacogenomics research and drug development;[15] however, the actual qualification and recognition of such biomarkers has not been regulated as thoroughly in Canada as in other jurisdictions. The impact that this might have on the scope of biomarker utilization in Canada remains to be seen, as the legal and regulatory requirements might have difficulty keeping pace with the technological advancements that are made in genomics and biomarker research.

The utility of biomarkers, particularly in the realm of drug development, ultimately depends upon the extent to which they are able to influence meaningful clinical outcomes, including patient morbidity and mortality. At present, the use of biomarkers in pharmacogenomics research has been largely concentrated in the field of oncology.[8] For example, there exist several large databases of cancer cell line information correlated with pharmacological profiles of various antineoplastic drugs, including the Cancer Cell Line Encyclopedia and the US National Cancer Institute (NCI–60) panel, among others.[16] In certain cases, the use of pharmacogenomic biomarker information has undoubtedly led to improved clinical outcomes, as with the FDA approval of trastuzumab (Herceptin) for HER2 receptor–positive breast cancer patients, with accompanying guidelines that HER2 status must be established prior to treatment initiation.[8] In many other cases, however, the clinical implications of published drug–biomarker interactions remain uncertain. The FDA maintains a list of drug–biomarker interactions that are currently included in official drug labels,[17] although it has been reported that the majority of these interactions are of questionable significance, as they are not supported with guidelines on incorporation into clinical decision–making.[8] Due to the uncertain clinical significance surrounding many genetic biomarkers, some researchers have proposed that a paradigm shift towards phenome–wide association studies (PheWAS) might be beneficial.[4] Through PheWAS, starting from the standpoint of genetic variants and searching for all correlated phenotypic outcomes, a better appreciation of the pleiotropic effects of many previously discovered genetic biomarkers might be obtained.[4] It is foreseeable that such

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complementary approaches of GWAS and PheWAS could serve to make biomarkers more robust clinical tools, capable of contributing to better guidelines on how drug–biomarker interactions might be incorporated into patient care decisions.

With the establishment of large–scale research initiatives such as the Precision Medicine Initiative from the National Institutes of Health (NIH), it is anticipated that PheWAS with sample sizes of one million or more participants might soon become a reality.[18] When considering how genetic information from large pools of volunteers might be combined with the vast data sets contained within electronic medical records (EMRs), the exciting potential of PheWAS becomes apparent. For instance, utilizing such information as EMR billing codes and past medical history as markers for phenotypic traits, we might begin to appreciate the true pleiotropy of many genetic variants, which might be associated with diverse and unexpected phenotypic outcomes.[19] As an example, early phenome–wide association studies found that genetic variants such as the HLA–B27 and CTLA4 genes were associated with numerous diverse autoimmune diseases, suggesting a potential common underlying biological pathway among seemingly distinct pathophysiological processes.[18] As similarities among diverse disease entities are discovered, it is possible that the process of drug development might shift toward one of drug repositioning.[19] Through this strategy, drugs that were originally developed to target one molecular pathway might find novel uses in other clinical situations as we begin to uncover the pleiotropic effects of many genetic biomarkers and the roles they might play in multiple disease processes. As an example, cyclin–dependent kinase 4 (CDK4) inhibitors, originally marketed as antineoplastic drugs, have also been found to be beneficial treatment agents in rheumatoid arthritis due to the shared molecular pathways between the disease and certain forms of cancer.[20] As the realm of PheWAS continues to expand, it is inevitable that many more such similarities will be elucidated, and the discovery of novel uses for existing drugs might perhaps become just as important as the development of completely new agents.

Looking back on the previous two decades of the twenty–first century, during which time human genome–wide association studies became feasible and the FDA launched its "Critical Path Initiative", one might pose the question of whether genomic and biomarker research is living up to its potential. Are more revolutionary drug–biomarker interactions akin to the classic trastuzumab–HER2 receptor interaction waiting to be discovered, whether through new drug development or drug repositioning? Or is the genetic biomarker approach too simplistic, failing to capture the true reality of complex pathophysiological processes, to be clinically useful? To cast a definitive verdict might be premature. It must be remembered that genomic and biomarker research remains in its infancy, and new research paradigms such as phenome–wide association studies present intriguing possibilities for shedding light on the current shadows of our knowledge, offering promise that the revolution we have been awaiting is still to come.

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CRISPR/Cas9: The Brave New World of Genetic Engineering

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In the last decade, breakthroughs in genetic engineering have produced a new genetic editing tool called CRISPR/Cas9—a technology so robust that it can modify DNA sequences in virtually any living organism with incredible precision.[1-4] Adapted from an ancient microbial defense system, CRISPR/Cas9 can be programmed to remove, edit, and insert new genetic material—from large gene multiplexes to a single DNA base–pair.[2-5] CRISPR/Cas9 has been heralded for its simplicity, inexpensiveness, and array of applications in biology, biotechnology, and medicine.[1] The explosive rise of CRISPR/Cas9 in research and the media has yielded intense excitement and controversy worldwide.[2-5] As this genetic engineering revolution evolves faster than regulations to control it, the question becomes: how can we wield a technology powerful enough to remodel the human genome, yet simple enough to be performed by an undergraduate student?

The CRISPR/Cas9 saga began with the discovery of an ancient immune system that protects bacteria against infections by viruses.[2,6-7] Within a unique prokaryotic gene locus, small pieces of viral DNA were discovered to be incorporated into the bacterial genome.[8] This gene locus became known as Clustered Regularly Interspaced Palindromic Repeats (CRISPR).[8] It is now known that CRISPR–associated (Cas) genes function as an "adaptive" immune system that can sample viral DNA and integrate it into the CRISPR locus.[6] These stored pieces of viral DNA serve as a record of past infection to help bacteria detect and destroy this invader in future infections.[2,9] Upon reinfection, CRISPR combines an RNA template (derived from its own viral DNA copy) with CRISPR RNA and DNA–cleaving enzymes to create a "targeted missile" that scavenges the cell for viral DNA matching the template and subsequently destroys these foreign invaders by DNA degradation.[4,9]

What captivated researchers about this prokaryotic CRISPR/Cas system was its ability to make targeted double–stranded breaks (DSBs) in DNA at precise locations—for virtually any DNA sequence.[10,11]Previous research had shown that DSBs could stimulate genome editing by harnessing the natural ability of DNA to repair itself.[2,3] When a DSB occurs, DNA can detect and repair this break in two ways: by joining the split ends back together, or by introducing a new DNA fragment that is homologous to the sequences at each end of the break.[1,9] The "non–homologous" end joining (NHEJ) mechanism fuses DNA without any repair template. The error–prone NHEJ repairs DNA by inserting or deleting several base pairs while joining broken ends, leading to a frameshift mutation or gene knockout.[9,12] On the other hand, if an exogenous DNA repair template is present, DNA can instead use the homology–directed repair (HDR) mechanism to insert a foreign DNA segment.[9] Thus, if the RNA–guided CRISPR/Cas system could be modified to recognize a specific sequence of DNA, such as a genetic mutation, then a DNA break at this mutation site could stimulate genome editing through natural DNA recombination events.[1]

A significant breakthrough in 2012 was the invention of a tool that reprogrammed CRISPR/Cas9 to cut any desired DNA sequence in the prokaryotic genome.[13,14] This groundbreaking invention was credited to Jennifer Doudna and Emmanuelle Charpentier, who used the Cas9 protein with a CRISPR system derived from Streptococcus pyogenes to engineer a molecule that could split DNA at precise locations of their choosing.[1,13] Within months of this development, Siksnys et al. discovered that CRISPR/Cas9 could be applied to other prokaryotes, including S. thermophiles.[6] By January 2013, Feng Zhang of the Broad Institute was the first to adapt CRISPR/Cas9 to edit human cells, thus unlocking the potential of CRISPR/Cas9 in the eukaryotic genome.[15]

Though recombinant DNA technologies were first developed in the 1970s, CRISPR/Cas9 has transformed genetic engineering because of its precision, effectiveness, and relative affordability.[1-4] Other recent gene editing technologies like Zinc Finger Nucleases and transcription activator-like effector nucleases (TALEN) rely upon proteins to recognize DNA sequences, and have proven difficult to implement with precision.[10,16] On the other hand, CRISPR/Cas9 uses RNA–guided enzyme complexes to target DNA and achieve site–specific DNA cleavage, making it far more accurate and effective.[10,17] Rather than having to rebuild "hardware" each time scientists want to target a gene of interest, CRISPR/Cas9 acts like "software" that can easily be programmed and reprogrammed to target multiple genes.[2] Also, CRISPR/Cas9 is naturally multiplexable, meaning that CRISPR arrays can target multiple different DNA sequences simultaneously.[15]

The ease of programming CRISPR/Cas9 set the stage for a tidal wave of new genetic research.[4,18] In 2012, there were 126 papers published on CRISPR. Last year alone, this number reached 2,155 publications.[18] The ability to modify the genome, its epigenetic contexts, and its transcripts in eukaryotic cells has yielded a myriad of developments in basic science, biotechnology, and medicine.[1] Many researchers believe that CRISPR/Cas9 has the potential to cure many genetic diseases, starting with single–gene mutations like sickle–cell anemia and cystic fibrosis.[5] Using viral vectors, CRISPR/Cas9 can spread cell changes in vitro back to organisms in vivo.[10] For instance, a PCKS9 gene knockout using CRISPR/Cas9 introduced into the mouse liver was shown to lower blood cholesterol by 40%.[19]

The promise of CRISPR/Cas9 to edit human cells triggered a battle to commercialize the CRISPR/Cas9 technology.[18] Doudna and the team at UC Berkeley were first to file a CRISPR/Cas9 patent in May 2012, while Zhang and the Broad Institute of Harvard and MIT filed their initial patent claim in December 2012.[14,18] After Zhang adapted the CRISPR technology to eukaryotic cell lines, the Broad Institute filed 11 more patents claiming that it invented the CRISPR/Cas9 system for human use.[18] The Broad Institute then paid the U.S. Patent and Trademark Office (USPTO) to fast–track the review process on its claims. Many scientists were surprised when the USPTO began to approve Broad Institute patents in April 2014 before its decision on UC Berkeley's claim.[18]

A legal battle ensued between the researchers, their academic institutions, and private corporations who had already invested over a billion dollars into this CRISPR technology—all to answer the question, who owns CRISPR/Cas9?[18] In February 2017, the U.S. Patent Trial and Appeal Board ruled that the patent claims of the Broad Institute did not conflict with those of UC Berkeley, and the USPTO patent decisions would stand.[18] Thus, the UC Berkeley team would own the intellectual property and licensing rights for aspects of CRISPR/Cas9 in prokaryotic cells, while the Broad Institute won these rights in eukaryotic cells.[18]

With this patent decision, the Broad Institute would control the central patent for commercial uses of CRISPR in plant and animal cells, including agriculture and medicine.[14,18] While both groups agreed that academic institutions could freely conduct research with their technology, U.S. companies in biotechnology and pharmaceutical industries may have to pay licensing fees to both parties due to the nature of their patents.[18]

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Beyond commercial interests, the patent decision created divisions within the scientific community, since both parties desired the academic credit and prestige associated with invention of the CRISPR/Cas9 technology.[18] While the UC Berkeley and Broad Institute patent battle over CRISPR/Cas9 continues in Europe, it is estimated that over 860 CRISPR patents exist worldwide, with more added each day.[14,20]

Intellectual property aside, an imperative question is: who controls CRISPR–Cas9 research? Serious ethical issues have been raised by scientists and the public about advances in genetic engineering.[21] A mix of excitement and fear surrounds the possibility of gene therapy in humans to treat disease. But what if this technology is applied controversially, such as for human enhancement and creating "designer humans"?[2] In 2015, an international consortium of research organizations from the U.S., the U.K., and China called for a moratorium on making heritable modifications to the human genome.[21] Back in 1975, a moratorium on genetic engineering declared at a gene–editing summit in California was largely respected.[7] Yet, researchers and scientific groups have no regulatory authority to prevent misuse of CRISPR/Cas9.[7] Even if such regulatory mechanisms existed in one country, these regulations may differ from country to country, thus requiring cooperation on a global scale.

Since the CRISPR/Cas9 tool was developed five years ago, research advances in genetic engineering are far outpacing the policies and regulations surrounding this technology. In August 2016, human trials of CRISPR/Cas9–modified cells in lung cancer patients began at Sichuan University in China.[22] CRISPR/Cas9 co–inventor Doudna cautions that rushing the implementation of CRISPR/Cas9 in humans before its biochemical functions are optimized may lead to unintended consequences.[2] But the promise of lucrative profits and economic gain may override the calls of many scientists to proceed with caution. Research and Markets forecasts that the CRISPR and Cas genes market will reach over $4 billion USD by 2025.[23]

In a landmark February 2017 decision, the U.S. National Academy of Science and National Academy of Medicine announced its support for gene editing in viable human embryos.[24] This advisory group endorsed heritable gene alterations aimed to treat serious diseases where no reasonable alternatives exist.[24] The announcement came less than two years after the requested international moratorium on human gene editing. Just last year, scientists claimed that it would be "irresponsible to proceed [with human gene editing]" until the risks and societal impacts were better understood.[24] While the current endorsement limits the use of genetic engineering to therapeutic applications only, some worry that this decision will "open the floodgates" to controversial applications like eugenics in the future.

In March 2017, weeks after the US advisory group announced its support for therapeutic uses of human genetic engineering, a team of Chinese scientists published the first report of CRISPR/Cas9 manipulations in normal human embryos.[25,26] Published in Molecular Genetics and Genomics, this report documented a mixed success rate in treating mutations causing beta-thalassemia and favism in viable human embryos.[25,26] Meanwhile, other researchers are harnessing the CRISPR/Cas9 technology for experimental treatments to cure certain cancers, blindness, and other genetic conditions as early as this year.[24] While the topic of human embryo editing remains controversial, the scientific communities in most countries are still debating the ethical implications surrounding widespread applications of CRISPR/Cas9 in human gene therapies.[27]

CRISPR/Cas9 has launched scientists and society into a brave new world of genetic engineering. Adapted from an immune system of bacteria that has existed for billions of years,[6,10] CRISPR/Cas9 has been engineered into a genetic editing tool applicable in any organism.[1-2] While CRISPR/Cas9 shows promise in biotechnology, agriculture, and medicine, the ethical implications of this technology are hotly debated.[1,27] Should we use gene editing in traits that can be inherited and spread within the population? Where do we draw the line between prudent and frivolous uses of genetic engineering? The scientific community must acknowledge the responsibility that comes with the power to edit the human genome. Scientists and society must engage in democratic discourse on how best to harness the breakthrough that is changing humankind—macroscopically and microscopically.

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