Precision Medicine: Enabling Healthcare Progress in the Twenty-First Century

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2.1 Introduction

The genomic revolution marked the beginning of the twenty-first century in biology and medicine. Completion of the Human Genome Project in 2003 brought a flood of discoveries that transformed biology. The Human Genome Project also fostered technological innovations that enabled decoding the entire genetic information in health and disease, leading to the concept of personalized genomic medicine and the foundation of the precision medicine movement [1]. By 2012, the new label "precision medicine" gained momentum [2] and since then it has been increasingly used by key opinion leaders in scientific headlines and journal articles [3].

A decisive seal of approval to the movement was given when the President of the United States launched the "Precision Medicine Initiative" in 2015 with the intent to merge genomic, biological, behavioral, environmental, and other data on individuals to identify drivers of health that might support personalized healthcare decision-making [4]. Given the enormous potential and promise for new medical breakthroughs based on this "emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person," several other countries have developed dedicated precision medicine programs on a national scale. For example, the United Kingdom initiated the sequence of 100,000 genomes from National Health Service patients, and China announced the "China Precision Medicine Initiative" in 2017. In Europe, Nordic region countries and Switzerland have proposed road maps for similar initiatives [5, 6].

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In the twentieth century, the growing impact of the scientific method on clinical practice led to the concept of evidence-based medicine, wherein clinical decision-making is based on evidence obtained from randomized controlled trials [7]. The first randomized controlled clinical trial was conducted in 1946 and demonstrated the efficacy of streptomycin for treating tuberculosis [7]. This was followed by a period of rapid methodological progress in the design and analysis of clinical trials as well as observational studies. Randomized clinical trials have provided strong scientific evidence on useful interventions, thanks to double-blind treatment application and tests for treatment associations with clinical outcomes. Contrasting with evidence-based medicine's empirical associations, precision medicine in the twenty-first century strives for developing a new taxonomy of human disease that results from the convergence of scientific data obtained through multi-"omics" approaches, advanced imaging, and information technology. Although precision medicine was conceptualized based on the power of genomics, its overarching aim is to enable a new era of medicine that integrates molecular, physiological, behavioral, and environmental data on individuals. According to this non-reductionist perspective, treatment selections should be based on molecular biomarkers as well as on demographic and physiological measurements, comorbid conditions, individual patient preferences, and lifestyle.

This chapter illustrates current state-of-the-art applications of the precision medicine concept, from new trends in genetic diagnostics and the advent of RNA therapeutics, gene therapy, and genome editing to breakthroughs in cancer treatment and the microbiome as a new research frontier.

2.2 New Trends in Genetic Diagnosis

At present, the catalog of Mendelian or rare genetic disorders is still far from complete. Clinical application of high-throughput DNA sequencing technologies (whole-exome and whole-genome sequencing) to cases undiagnosed by conventional approaches currently enables the identification of new disease/genetic associations at a rate of approximately 260 per year [8]. However, DNA sequence alone is not sufficient for diagnosis of all patients due to two main limitations. First, the clinical relevance of many gene variants remains unknown and, second, the clinical impact of variation in noncoding regions of the genome is still poorly understood. By combining DNA sequencing with additional technologies such as metabolomics and transcriptomics, the discovery rate of new genetic based diagnosis is predicted to increase to approximately 500 per year in the near future. For example, detection of abnormal levels of particular metabolites in serum using recently developed mass spectroscopy techniques (which expanded by at least 100-fold the ability to detect small molecules in circulation) complements the interpretation of sequence variants found in genes that encode enzymes involved in a particular metabolic pathway [9]. Application of RNA sequencing can also help to shed light on the possible pathogenicity of variants of unknown significance identified through DNA sequencing by revealing transcriptional alterations [10].

Speed of precision diagnosis is critical particularly in childhood disorders, since corrective therapies have their greatest efficacy when used early. In this regard, the recently developed ability to sequence fetal genomic DNA in the mother's circulation, which is used to screen for chromosomal aneuploidy as well as to diagnose specific defects in fetal genes [11], underscores the potential for in utero precision medicine. In a recently reported case, diagnosis of adrenal hyperplasia led to in utero fetal hormonal replacement therapy, anticipating in utero screening for genetic diseases for which there are treatment options [9].

2.3 The Advent of RNA Therapeutics, Gene Therapy, and Genome Editing

In 2000, Francis Collins, the then director of the genome agency at the National Institutes of Health, suggested, "Over the longer term, perhaps in another 15 or 20 years, you will see a complete transformation in therapeutic medicine" [12]. Indeed, by 2020, breakthroughs in RNA therapeutics, gene therapy, and genome editing have reinforced the vision and aspiration for the precision medicine movement.

Spinal muscular atrophy (SMA) used to be one of the most common genetic causes of infant mortality and a major cause of childhood morbidity due to muscle weakness, until innovative drugs changed the disease outcome for the first time [13]. SMA is caused by deletions or loss-of-function mutations in the *SMN1* gene. However, the human genome has a highly homologous gene called *SMN2*, which differs from *SMN1* by 11 nucleotides but has an identical coding sequence. One of the altered nucleotides weakens a splice site, resulting in skipping of exon 7 in the messenger RNA (mRNA). Approximately 80–90% of the transcripts derived from the *SMN2* gene skip exon 7, leading to a protein product that is rapidly degraded. Thus, forcing the inclusion of exon 7 in *SMN2* mRNA should suffice to produce a fully functional SMN protein that compensates for loss of the *SMN1* gene [14]. Nusinersen (Spinraza®), the first drug that received approval for treatment of SMA, is an antisense oligonucleotide that is administered intrathecally and increases SMN protein concentration by modifying the splicing of the *SMN2* mRNA. Another approved splicing modifier is Evrysdi™ (risdiplam), a small molecule that is administered orally and is the first medicine for SMA that can be taken at home. An alternative recently approved treatment strategy for SMA is gene replacement therapy. Onasemnogene abeparvovec (Zolgensma®) is an adeno-associated viral vector-based gene therapy designed to deliver a functional copy of the *SMN1* gene to the motor neurons through a single intravenous infusion [13].

In addition to children with SMA, several other patients affected by incurable diseases are benefiting from specific gene and RNA therapies. Namely, Luxturna® is a gene therapy for an inherited retinal disease that leads to progressive visual loss and ultimately total blindness. The disease is caused by biallelic loss-of-function mutations in the *RPE65* gene that destroy the ability of retinal pigment epithelium cells to react to light. The drug is applied intraocularly by a subretinal injection and

consists of an adeno-associated viral vector that carries a functional copy of the *RPE65* gene [15].

Excitement over potentially curative gene therapy options for nonmalignant hematological disorders is also growing. For the X-linked bleeding disorders hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency), near-to-complete correction has been achieved in patients injected with engineered recombinant adeno-associated virus vectors that deliver the functional sequence of the defective coagulation factor gene to the liver [16]. More recently, a gene therapy for transfusion-dependent beta-thalassemia received regulatory approval [17]. In this case, hematopoietic stem cells are collected from the peripheral blood, maintained in culture in the laboratory (i.e., ex vivo), and modified by transduction with an engineered lentiviral vector carrying the therapeutic DNA sequence; next, the corrected stem cells are infused intravenously into the patient, who was previously treated with myeloablative chemotherapy [18]. The success seen in beta-thalassemia motivated efforts to extend the therapy to sickle cell patients [17].

Altogether, gene replacement therapies currently approved and in development use engineered viruses to deliver an extra DNA sequence that replaces the defective gene. Thus, this treatment strategy is restricted to autosomal recessive disorders, which are caused by biallelic loss-of-function mutations. Treating autosomal dominant diseases often requires silencing the expression of the mutated gene, which encodes a toxic protein. A very promising gene-silencing approach is RNA interference (RNAi), which involves the delivery into cells of short synthetic double-stranded RNAs (called small interfering RNAs or siRNAs) that activate a cellular enzymatic machine to degrade the mutant mRNA. Because siRNAs can in principle downregulate any human mRNA, they should be ideal to eradicate the expression of disease-causing genes. A breakthrough was achieved in 2018 with the first-ever siRNA product (patisiran) approved as a therapy [19]. Onpattro® (patisiran) is being used to treat adult patients with familial polyneuropathy caused by transthyretin-mediated amyloidosis [20]. This autosomal dominant, progressive, multisystemic, and life-threatening disease is caused by mutations in the gene encoding transthyretin (TTR). The mutant TTR protein accumulates as amyloid in peripheral nerves, heart, kidney, and gastrointestinal tract giving rise to polyneuropathy and cardiomyopathy. The drug is a double-stranded small interfering RNA encapsulated in a lipid nanoparticle for delivery to hepatocytes. By specifically binding to a conserved sequence region common to mutant and wild-type TTR mRNA, patisiran causes its degradation via RNA interference and subsequently a reduction in serum TTR protein levels and tissue TTR protein deposits [19, 20].

As an alternative to gene replacement and RNA-targeted therapies, the CRISPR gene-editing tool holds great promise in treating a wide range of genetic disorders. In 2020, Emmanuelle Charpentier and Jennifer Doudna were awarded the Nobel Prize in Chemistry for developing the prokaryotic CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) immune system into a simple easy-to-use programmable gene-editing tool with a guide RNA molecule that directs the bacterial Cas9 endonuclease to cleave sequence-specific regions of DNA. Just 8 years

after their groundbreaking paper [21], CRISPR has transformed molecular biology research and is pushing medicine to enter a new age. The hype surrounding CRISPR is certainly high, but the first results from ongoing clinical trials suggest that real cures are already taking shape. For example, patients with either beta-thalassemia or sickle cell disease were successfully treated with an "ex vivo" therapy that uses CRISPR to modify hematopoietic stem cells. When reintroduced to the patient, the edited stem cells establish a permanent supply of red blood cells containing fetal hemoglobin. Despite the difference in oxygen affinity when compared to adult hemoglobin, fetal hemoglobin is still functional in adults and can rescue the defect caused by the mutations [22].

More recently, CRISPR gene editing was performed directly in the human body. The CRISPR therapy EDIT-101 was injected in the eye to treat congenital blindness caused by a mutation in the CEP290 gene [23]. EDIT-101 uses a construct containing an adeno-associated viral vector with two guide RNAs to identify the location of the mutation, combined with DNA encoding the Cas9 enzyme under a promoter specific to photoreceptor cells [24]. Another CRISPR treatment, NTLA-2001, was injected intravenously to treat patients with familial polyneuropathy caused by transthyretin-mediated amyloidosis [25]. NTLA-2001 consists of lipid nanoparticles designed to deliver to the liver a guide RNA specific to the disease-causing gene and messenger RNA that encodes the Cas9 protein. Preclinical data showed robust and long-lasting transthyretin reduction following knockout of the *TTR* gene in vivo [25].

In conclusion, therapeutic genome editing has reached the clinic, with several applications under development at rapid pace. This represents a profound opportunity to change healthcare for many patients, but there are still many challenges ahead. Further developments in regulatory action are also critical to ensure that the new technology is used safely and responsibly [26].

2.4 Precision Oncology

Genomics has revolutionized cancer research and transformed our understanding of how cancer arises. Thus, oncology was expected to benefit the most in the near term from the precision medicine approach [4]. Indeed, in recent years cancers have been reclassified based on the mutations that drive the disease, a multitude of new drugs were developed that target specific molecular features of the tumor, and constant technological advances have expanded the ability to characterize cancer patients beyond sequencing the tumor DNA.

Successful innovative treatment strategies have emerged, such as chimeric antigen receptor T-cell (CAR-T) therapy. This technique involves an ex vivo genetic modification of the patient's T cells to recognize the B-cell protein CD19. Once the modified T cells are returned to the patient, they bind to and destroy B cells expressing CD19. The first CAR-T product approved in 2017 (tisagenlecleucel; Kymriah®) resulted in a remission rate of 81% in pediatric and adolescent patients with refractory or relapsed B-cell acute lymphoblastic leukemia [27]. A second CAR-T

product (axicabtagene ciloleucel; Yescarta®) was also approved in 2017 for use in relapsed or treatment-resistant large B-cell lymphoma [28].

Another innovative treatment approach that is attracting much attention is the use of mRNA to develop cancer vaccines [29]. Comprehensive catalogs of all acquired somatic mutations in individual tumors are being explored to determine which mutations may be particularly potent vaccination targets as they can create neoantigens that are not subject to central immune tolerance. Using machine learning approaches to reliably predict highly immunogenic epitopes expressed in each cancer in order to design and manufacture a vaccine unique for each patient, two studies reported exciting immunologic and clinical results after treatment of melanoma patients [30, 31]. Although one study administered the neoantigens in the form of peptides [30] and the other administered mRNAs [31], in both cases vaccination expanded preexisting neoantigen-specific T-cell populations and induced a broader repertoire of new T-cell specificities in cancer patients.

Despite the availability of several drugs targeting specific molecular features of tumors, only a minority of cancer patients currently benefit from targeted therapies. Three recent studies assessed how comprehensive molecular profiles of the tumor improve the outcome of patients with incurable recurrent and/or metastatic cancer [32–34]. The first study included analysis of circulating tumor DNA (ctDNA) to guide therapy [32], the second study included drug combinations [33], and the third study included RNA sequencing in addition to DNA analysis [34]. The authors of the first study found a high concordance rate between mutations identified in ctDNA and in the tumor tissue. An actionable molecular alteration was identified in 41 patients (41%), among whom 11 patients (11%) were treated with a matched therapy. Four patients experienced an objective response, which represents 36% of the treated patients and 4% of the whole included population. The second study focused on several actionable molecular alterations to propose drug combinations (including immunotherapy) under the premise that simultaneous targeting of more than one molecular alteration in the tumor may delay relapse. The study enrolled 149 patients, of which 73 were treated with matched therapy. Seventeen patients experienced an objective response, which represented 23% of the patients treated with matched therapy and 11% of the whole cohort. The third study identified 158 patients (52%) with an actionable molecular alteration, and 69 patients received matched therapy determined on the basis of DNA alterations. An additional 38 patients received therapy selected on the basis of changes in RNA expression. Twelve patients experienced an objective response, which represented 11% of the patients treated with matched therapy and 4% of the whole cohort. The clinical benefit of precision medicine approaches for pediatric patients with refractory/relapsed/progressive malignant disease was also recently assessed [35]. For a cohort of 525 children, 28% were candidates to receive targeted therapies. Although overall survival was the same for all participants, the 20 children with targets ranked highest in priority had a median progression-free survival of 204 days, compared with 114 days for all other 505 patients [35].

In striking contrast to the small number of patients that benefited from targeted therapies in the above studies, an inhibitor of the neurotrophic tropomyosin receptor kinase (NTRK), larotrectinib, was associated with durable objective responses across

a wide range of cancers (75%) independent of their location [36]. Both larotrectinib and another inhibitor of the same target, entrectinib, are currently used in the clinic for any advanced solid tumor with a NTRK gene fusion, in adults and children. Why larotrectinib and entrectinib induce such high response rates, regardless of which NTRK gene fusion is being targeted, is still unknown. NTRK gene fusions are unusual, occurring in less than 1% of common cancers, but they are found much more frequently in rare cancers, such as secretory breast cancers and infantile fibrosarcoma. Although these drugs will only help a small number of people, they demonstrate the value of continued drug development targeting oncogenic molecules.

How can precision oncology therapies help more people with cancer? Possibly, we are still too limited in the ability to identify dominant oncogenic drivers and in the targeted drug armamentarium. In this regard, the paradigm in precision oncology is to take into account the molecular and cellular features of a tumor as well as those of its microenvironment and additional traits of the individual, such as genetics and lifestyle, to create a tailor-made treatment [37]. In addition to new technology to detect more molecular biomarkers, real-world data in the rapidly expanding electronic health records may further assist in identifying new options for targeted treatments. Cloud-based machine learning systems are already helping clinicians to devise more effective treatment plans for cancer patients [38].

Finally, it is important to emphasize that even the most common forms of standard cancer treatment, i.e., surgery, chemotherapy, and radiotherapy, are improving all the time and becoming increasingly precise. For example, the new generation of linear accelerators have a built-in magnetic resonance imaging scanner that allows to closely monitor the patient anatomy and adapt the treatment plan to reduce the risk of radiation side effects [39]. Moreover, radiation oncologists are studying how to adapt treatments to the molecular profile of each individual. An association was recently found in breast cancer patients between radiation side effects and variants of two genes linked with circadian rhythm [40]. Whether radiation doses can be optimized based on molecular signatures of radiation sensitivity is currently under investigation [39].

2.5 A New Frontier in Precision Medicine: The Microbiome

In recent years, advances in genome sequencing technologies and metagenomic analysis resulted in an explosion of studies on the human microbiome. Most importantly, microbiome research is changing our perception of human biology in health and disease [41]. It is estimated that over 10,000 species of microorganisms, including bacteria, fungi, protozoa, and viruses, are present in the human body. Tremendous variation in a person's microbiome occurs depending on diet, medication, age, stress levels, or disease. The microbiome is also implicated in the direct biotransformation of drugs. In particular, bacterial drug metabolism is a general mechanism through which the microbiome in the gastrointestinal and reproductive tracts, and perhaps even within diseased tissue, alters drug response [41]. Namely, variation in the efficacy of cancer immunotherapeutic drugs was linked with differences in the gut microbiome [42–44].

Therapeutic manipulation of the microbiome is a rapidly advancing field [41]. One approach to microbiome modification is to selectively deplete strains with undesirable activities, such as those that act on drugs to form toxic metabolites. Another is to introduce engineered strains into the host as live bacterial therapeutics. Two recent studies described engineering *Escherichia coli* to express genes that could complement absent host functions in human metabolic diseases caused by genetic mutations [45, 46]. Other efforts aim to genetically edit bacteria that are actively colonizing the human body. Progress toward precision modification of the human microbiome holds great potential as a novel approach for managing certain human diseases.

2.6 Conclusions and Outlook

Precision medicine explicitly prioritizes the individualization of patient care through mechanistic reasoning and integration of distinct methodologies. How to advance knowledge for precision medicine is fundamentally different from evidence-based medicine, which focuses on population-based studies. Population-based data will certainly remain important for understanding health and disease, but should no longer be considered as sufficient. A major challenge for precision medicine will be to integrate molecular data with aspects of lifestyle and environment, as promised in its definition. Success will depend on constant development of new technology, and approaches for rapid incorporation of the evolving medical knowledge into clinical practice. The ability to measure, store, share, and analyze health-related data is rapidly expanding. Electronic health records will provide a dynamic overview of health outcomes at various stages of life. Increased availability of personal devices such as smartphones, activity monitors, and wearable GPS units, as well as electronic data capture tools to monitor behavior and exposure to environmental cues, offers unprecedented opportunities for behavioral interventions and real-time assessment of individual health. Progress in artificial intelligence will be transformative. Improved machines will acquire better medical images. Advanced "multi-omics" technologies will provide comprehensive molecular profiles of individuals. Engineered tissues and organs will enable mechanistic dissection of biological pathways driving disease and innovative drug design. In conclusion, the current precision medicine movement is just starting to reveal how medical knowledge and healthcare will develop in the future.

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