Precision Medicine in Stroke

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Preface

In this book, we intend to provide the reader with a comprehensive coverage of the state of the art of Precision Medicine in stroke. The first chapters are dedicated to the basic and current concepts regarding precision medicine and the rationale for its application in stroke medicine. The second part of the book addresses current use of precision medicine in ischemic and hemorrhagic stroke. Monogenic stroke disease, pharmacogenomics, and acute stroke treatment are the areas that have been most influenced by the application of precision medicine to stroke. Advanced brain imaging methods have started to help us to know more about individual thresholds to brain ischemia and to personalize therapeutic time windows for endovascular treatments. The third part of the book explores future applications of precision medicine in stroke. A review of the ongoing studies and of the different biomarkers that are being studied is provided. The expectation is that the use of different types of biomarkers will in the future enhance early stroke diagnosis and estimation of prognosis that will allow an adapted treatment for each patient. The fourth part of the book provides an in-depth exposition of how different interdisciplinary areas like artificial intelligence, molecular biology, and genetics are contributing to this area. Also, a description of the different tools used in the interdisciplinary areas and that can be applied to further enhance the study of precision medicine in stroke is given. Concepts regarding registry-based stroke research and how it can be used to contribute to precision medicine research are also provided.

Finally, Dr. Louis Caplan provides a very needed reflection regarding the differences and similarities between personalized and precision medicine and reminds us that our main objective is to know and help the individual that presents in front of us.

With this book, we intend to provide the reader with a comprehensive coverage of where we currently stand regarding precision medicine in stroke and to show how future stroke care may be influenced by it. If after reading this book or some of its chapters some of the readers become motivated or interested to contribute even more to the development and application of stroke medicine to stroke, we will have managed to achieve the ultimate objective that led us to edit and write this book. Advancement and application of precision medicine to stroke medicine will

hopefully lead to a better and individualized care of stroke patients, which will contribute to reduce the burden of stroke.

We would like to thank our colleagues that contributed with their time and expertise to this book.

Lisbon, Portugal — Ana Catarina Fonseca
Lisbon, Portugal — José M. Ferro

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Part I

Precision Medicine

Introduction

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José M. Ferro

José was a 77-year Caucasian old male, retired, active and fully independent, with hypertension and diabetes, on aspirin, statin, amlodipine, valsartan and carvedilol. One night, after having dinner, he experienced the sudden onset of left hemiparesis, facial asymmetry and speech disturbance. His wife called the national emergency number 112. The paramedics transported him to the reference hospital offering hyperacute stroke treatment, where he arrived 1:32 after symptom's onset. He scored 15 in the NIHSS. CT showed no early infarct signs (ASPECTS 10). CT angiography showed a M1 left MCA occlusion. The patient had no contraindications for rtPA. So, while preparing the endovascular procedure, rtPA bolus was started with no improvement. Mechanical thrombectomy successfully opened the artery in single catheter pass. The patient immediately improved to a NIHSS of 4, with no aphasia, mild right upper limb paresis and minimal lower limb weakness. He is admitted to the stroke unit. On the second hospital, he developed fever. He had clinical and radiological signs of pulmonary infection. He received paracetamol and antibiotics for 7 days and low-molecular-weight heparin, in prophylactic dosage, for prevention of deep venous thrombosis of the lower limbs. The search for the cause of stroke included carotid and vertebral ultrasound, which showed bilateral <50% heterogeneous, partly calcified carotid stenosis. The echocardiogram disclosed a dilated left atrium and left ventricular hypertrophy. 24-Hour Holter monitoring identified paroxysmal atrial fibrillation for 4:43 h. At this stage, the patient continued statin and antihypertensive and was prescribed a direct anticoagulant. Rehabilitation was started. The patient was discharged on day 7 after admission,

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with the diagnosis of ischemic stroke, of cardioembolic cause, scoring 3 points on the NIHSS and achieving grade 2 on the modified Rankin scale.

This apparently simple clinical vignette is a typical example of a patient with ischemic stroke successfully treated by thrombectomy. However, if we look closely, it is much more than that. The vignette describes the demographic, biological and health features which are components of the unique individual of José: he is a male, 77 years old, retired, active, independent, hypertensive and diabetic. We know the medications he was on. He could, if needed, include hundreds of other variables from his health and other records and databases to describe him in much more detail. The clinical and imagiological features of the disease are reported and scores quantify the severity of the stroke. He has no contraindications to rtPA and presents a large vessel occlusion, making him a candidate for thrombectomy. Results of vascular and cardiac ancillary procedures unveil several "abnormal" results. The chain and process of care are detailed: recognition of symptoms, reaction, transportation, hospital admission, confirmation of the diagnosis, hyperacute care, treatment of complications, secondary prevention, rehabilitation and discharge. At almost each step of the chain, his condition changes from the previous one, adding more information on the description of his uniqueness. Would he be a different person, with a different type and severity of stroke, and the choices at each step of the process would also probably be different.

Along the process of care, the management of the patient depends on key dichotomous decisions, in order to apply or not several logistic (e.g. transport and admission), diagnostic and therapeutic interventions, which can result in benefit or harm to the patient. Each intervention and step of care contributes to the main desired outcome (complete cure, full return to previous health and functional status) and a multitude of secondary outcomes. Before deciding for each intervention, can we predict with robustness and confidence if the intervention will result in benefit or harm to the patient and if the desired outcomes will be reached?

At the current stage of knowledge, many of the clinical decisions related to therapeutic interventions are supported by scientific evidence, whose quality varies from high to very low. Such evidence was generated by experimental studies, often randomised controlled trials and meta-analysis of randomised controlled trials. The results of these studies are presented for the whole group of participants, without considering its individual variability. Large randomised controlled trials and meta-analysis with thousands of participants can have enough statistical power to analyse a priori identified subgroups, described by the presence and absence of one or two variables. However, they almost never have the statistical power to analyse subgroups defined by combinations of variables, such as those which describe José. Furthermore, subjects are enrolled in clinical trials following the trial protocol inclusion and exclusion criteria. These criteria are chosen to maximise the possibility of finding a significant result on the efficacy outcomes and avoiding severe adverse events, thus protecting patient safety. Inevitably, many patients with the disease and the intervention being tested are left out of the trial. Therefore, the results of a trial cannot be applied to individuals not meeting the inclusion and exclusion criteria of a trial. In the case of José, that would imply at least that he

complies with the inclusion-exclusion criteria of randomised controlled trials evaluating the benefit of rtPA, thrombectomy, admission to a stroke unit and heparin to prevent deep venous thrombosis in acute stroke and of direct anticoagulants for secondary prevention of stroke in patients with atrial fibrillation.

For several other clinical decisions we do not have evidence from randomised clinical trials (e.g. should the patient be transported in a seated position or lying down?). In this situation, it is often claimed "we need a randomised clinical trial". Some of these trials are really needed, are feasible and must be done. But for several other clinical questions, especially in less common disease (e.g. rare types or causes of stroke), such trials are not feasible, because of the huge number of required participants and centres involved, the corresponding high cost of launching such a trial and the difficulty of finding a sponsor or agency to fund the study. Other trials will be considered unethical, because the component of care to be tested is already implemented in current practice, and there is no equipoise regarding its efficacy and safety.

When we do not have evidence from clinical trials, how can we help José? We can rely on results from observational studies, which however have a high risk of bias to evaluate interventions. In fact, in daily clinical practice, interventions are not prescribed randomly, and in general, more severe patients tend to receive more intervention and more aggressive ones. Even if we adjust for all baseline imbalance between groups, we will not be able to control for the implicit information due to clinical experience and the emotional factors that lead to prescription of an intervention to an individual patient.

If observational information is not available, we had to decide based on clinical experience and opinions. Clinical experience accumulates in individual doctors, in those more talented, with a peculiar expertise or with higher leadership, such as heads of clinical units and opinion leaders but also in clinical organisations, such as clinical units, medical societies and expert consensus. Clinical experience and opinion have several biases, including cognitive and emotional factors, that lead to the registration and evocation of some cases and events but not of others and are also influenced by intellectual conflicts of interest with previous opinions and convictions. Nevertheless, clinical experience is a very valuable and useful natural human intelligence creation, which from the accumulation of information from numerous clinical cases produces recognition patterns for almost instantaneous diagnosis and binary questions for rapid management decisions.

For diagnostic decisions on diagnostic procedures, i.e. which tests or sequence of tests should be performed in an individual patient, there is an additional methodological problem. Almost all studies evaluating diagnostic tests looked at outcomes such as sensitivity, specificity and accuracy or compared a new diagnostic method with the currently used "gold standard". Very few studies investigated if performing or not a diagnostic test changes the outcome of the patient or causes harm, including anxiety while waiting for performing the test or for or from the test results.

In summary, to be able to help José and other patients, by advising and prescribing diagnostic and therapeutic interventions which benefit them and do not produce arm, we have now at our disposal accumulated knowledge from clinical trials,

meta-analysis of clinical trials, observational studies and clinical experience, and we must integrate all this information and use it judiciously.

Currently we can make a detailed description of individuals using thousands of variables. We also have robust information to make assumption for groups of patients regarding several important clinical questions. Unfortunately we are yet quite weak to identify which individuals will benefit most from an intervention or even more to detect those who will be harmed or experience significant adverse events.

Precision medicine is the next step in the scientific approach to the practice of clinical medicine. Precision medicine's final aim is to identify for each disease a tailored diagnosis and therapy for each individual. Such an aim can be achieved through a collection of a multitude of clinical, biological and imaging data points and by the synthesis of individualised data into clinical usable end points. Refinement of statistical approaches, namely using artificial intelligence techniques, will allow the individualised prediction of recurrence risk, medication effects and recovery [1, 2]. A leap forward will be the integration of precision medicine with public health and population health management [3].

Precision medicine is already a reality in clinical practice in oncology, haematology and rheumatology, and in other specialities managing inflammatory conditions and monogenic diseases. Slower, but already significant, progresses have been achieved in other chronic non-communicable diseases, including stroke [1, 2, 4].

This book's main purpose is to cover the current and future applications of precision medicine in stroke. The book is introduced by a definition of precision medicine and its need to improve stroke care. Specific chapters will describe the state of the art of precision medicine in monogenic causes of stroke, pharmacogenetics of drugs commonly used to treat or prevent ischemic stroke and acute stroke imaging. Two other chapters will detail how precision medicine is already used in the management of haemorrhagic stroke, including subarachnoid haemorrhage. The future applications of precision medicine in stroke are focused in two chapters dealing, respectively, with early stroke diagnosis and prognosis determination.

The implementation of precision medicine needs an interdisciplinary approach. Advances in genetic and molecular biology will be pivotal to identify new diagnostic markers and therapeutic targets. The construction of big data banks and the analysis of big data will be done mostly using artificial intelligence technique and other advanced statistical methods. The book includes three chapters detailing these issues. The final chapter by Prof. Louis Caplan offers a broad perspective of the care of the individual person, and discusses the differences and overlaps between the concepts of personalised and precision medicine [5, 6].

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

2

Maria Carmo-Fonseca

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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Do We Need Precision Medicine in Stroke?

3

Ana Catarina Fonseca

Precision medicine is broadly defined as tailored diagnosis and therapy for each individual patient [1]. From an initial standpoint where treatment for a disease was needed and therefore a "one-size-fits-all" approach was used, we are now changing to a tailored approach with treatment suited for the particular characteristics of each individual.

This change in paradigm has been possible due to changes that occurred during the last decade. Widely available genetic and functional assays have revolutionized diagnostic and therapeutic options across various disciplines [2] and this sweeping change is also starting to impact stroke diagnosis and treatment and will further continue in years to come.

The term stroke encompasses a syndrome with a range of different manifestations that can affect distinctive parts of the brain and may be caused by diverse mechanisms. Rather than being a single disease, stroke is actually the common endpoint of several diseases. It is important to identify the underlying mechanism accurately in individual patients in order to choose the best treatment approach and reduce the risk of recurrence [3].

Currently, small attention is paid to different phenotypes and similar subtypes of stroke tend to be treated in a similar way in randomized clinical trials. Clinical trials take into account a normal distribution of the population and do not consider specificities of each individual. Therefore, we know that there is a part of the population that will not respond to treatment indicated by the current treatment guidelines. Results from clinical trials give the same prescription to patients with the same diagnosis. Among the patient group that receive this treatment four main groups can be defined: patients in which the drug will be toxic and not beneficial, patients in which the drug is toxic but beneficial, patients in which the drug is not toxic and not

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beneficial, and patients in which the drug is not toxic but is beneficial. Knowing exactly in which group each patient fits would greatly improve stroke care. Some patients may suffer harm and therefore we need better ways to identify those who might benefit the most.

3.1 Acute Ischemic Stroke Treatment

Currently acute ischemic stroke treatment is similar in all patients. Intravenous thrombolysis (IV rtPA) with 0.9 mg/kg alteplase is given to all patients that present within 4.5 h of ischemic stroke independently of stroke etiology if all inclusion and exclusion criteria are fulfilled [4]. Established blood pressure and glucose limits that should be maintained as well as alteplase dosages are similar in all individuals independently of their personal history, extension of white matter disease, or presence of microbleeds. The major risk of using intravenous thrombolysis is intracranial hemorrhage that may be fatal in some patients. Knowing which patients are at a higher risk of this complication and if different dosages of alteplase adapted to each individual could potentially contribute to further enhancement of the benefit while reducing risk would be valuable.

The randomized clinical trials that aimed to expand the current therapeutic time window of 4.5 h in patients with IV rtPA that had a stroke at wake-up or unknown time of onset, using advanced imaging methods, like the EXTEND [5] and WAKE-UP [6] trials, have further helped to personalize acute ischemic stroke treatment. They take into account that tissue resistance to ischemia may vary from individual to individual. Another possible treatment strategy in acute ischemic stroke to enhance individualized treatment could be to optimize the brain tissue protection responses to ischemia. There are two main mechanisms of protection: collateral circulation and hypoxia-inducible factor (HIF) response [7]. These responses to cerebral ischemia vary from individual to individual and may be dependent on genetic and environmental factors. A better understanding of these mechanisms may move us further towards the goals of precision medicine, enabling physicians to customize treatments using personalized time windows for each patient and to use specific treatments that could enhance the endogenous responses to ischemia [8]. Individualization of treatment will ultimately contribute to increasing the number of patients that will be able to receive treatment and to maximizing the benefit/risk balance.

3.2 Diagnostic Strategies

Recently, similar considerations have been done regarding diagnosis evaluation of covert brain infarction (CBI) [9]. Different phenotypes may warrant different diagnostic approaches. For example, a patient with a lacune and severe white matter hyperintensities might primarily benefit from blood pressure control because the

pathophysiology is probably hypertensive small vessel disease. Patients with multiple cortical CBIs without signs of white matter disease might benefit from long-term rhythm monitoring to detect subclinical atrial fibrillation which could be treated with oral anticoagulation. However for patients with multiple CBIs in one vascular territory noninvasive angiography of the supra-aortic vessels and revascularization or best medical treatment of large-artery stenotic disease might be the best option [10].

3.3 Stroke Prevention

Individual response to antithrombotics, statins, and even drugs commonly used to treat blood pressure and diabetes mellitus is often tested through a trial-and-error approach. Statins are among the most effective and widely used drugs for the primary and secondary prevention of cardiovascular disease and its complications. However, studies show that there is considerable individual variation in the low-density lipoprotein cholesterol (LDL) reduction at all doses of simvastatin, atorvastatin, and rosuvastatin [10]. Variability in drug response exists regardless of the measured phenotype, and genetic variability may be a contributing factor [11]. Novel approaches to pharmacogenetics have extended statin pharmacogenetic observations to new phenotypes and candidate genes [11]. In the future, genotyping could be used to routinely guide statin treatment. This could help to improve the benefit while reducing the risk of adverse events.

Currently ischemic stroke secondary prevention is broadly divided into two main groups: cardioembolic and noncardioembolic etiology. Cardioembolic strokes are treated with anticoagulants. For anticoagulation resumption after stroke from cardioembolic stroke, there is a balance of benefit/risk that needs to be taken into account: although the risk of early recurrent ischemic stroke is high, early oral anticoagulation is suspected to increase the risk of potentially harmful intracranial hemorrhage, including hemorrhagic transformation of the infarct [12]. Clinical trials are currently being conducted to try to determine what is the best time to start anticoagulation after ischemic stroke [12]. Most of these trials take into account the size of the infarct or the severity of the neurological deficit to randomize patients. However, it is comprehensible that patients may present with individual peculiarities that may further influence their risk of early stroke recurrence or hemorrhagic transformation of the infarct and influence this benefit/risk balance. Also, we know that the higher the CHADS2-VASC score a patient with atrial fibrillation presents, the higher the probability of having a stroke. However, currently all patients are equally treated with anticoagulants. More data is needed to know if this different stroke risk can be addressed with different treatment strategies.

Another field in which we are progressively seeing a change in the "one-size-fits-all" approach regards secondary stroke prevention of noncardioembolic strokes. According to the European Stroke Organization guidelines, clopidogrel is a first-line antiplatelet for secondary stroke prevention [13, 14]. However, it is known that

there is significant heterogeneity across individuals regarding clopidogrel metabolism. Clopidogrel is an inactive prodrug that needs hepatic bioactivation by several enzymes, including CYP2C19 [15]. The prodrug is converted via a two-step process involving several cytochrome P450 (CYP) enzymes to an active metabolite. This resulting active metabolite irreversibly inhibits the platelet ADP receptor, P2Y12 [15]. Genetic variants that diminish the activity of the enzyme will cause shunting of the prodrug to the esterase-mediated degradation pathway to form inactive metabolites. This will lead to decreased levels of the active metabolite and less inhibition of platelets, leading to a greater risk of cardiovascular events. While the *1 allele of CYP2C19 has full enzymatic activity, the *2 (rs4244385) variant is the most common of the reduced-function variants and produces a complete loss of enzymatic activity resulting in a lower amount of active metabolite and attenuated clopidogrel-induced platelet inhibition [15]. With the *2 allele, a gene-dose effect is seen, where an increasing number of reduced-function alleles results in a decreasing amount of platelet inhibition. Apart from *2, other loss-of-function variants exist. These variants are rare but produce similar enzymatic defects as the *2 allele [15].

The Food and Drug Administration (FDA) agency requires a black-box warning for reduced effectiveness in persons who are poor metabolizers of clopidogrel. However, testing for CYP2C19 genotype to aid clinical management is not currently routinely done. For carriers of *2, one potential treatment strategy could be to consider higher doses of clopidogrel than usually used. Genetic variants affecting clopidogrel metabolism may identify nonresponders and reduce side effects, but these approaches have not yet been widely adopted in clinical practice.

Studies like the CHANCE [16] and POINT [17] clinical trials showed that patients with high-risk TIA (ABCD2 >3) or NIHSS <4 benefit from short-term dual-antiplatelet therapy with clopidogrel and aspirin. However, in the POINT study compared to the CHANCE trial, major hemorrhage occurred significantly more frequently in patients treated with clopidogrel + aspirin than in the placebo + aspirin arm. Also, one of the reasons why the POINT study was prematurely stopped was because the prespecified safety threshold for major hemorrhage had been crossed in the clopidogrel + aspirin arm in the interim analysis. One possible explanation was that CHANCE only included Chinese patients and these are known to have non-function allelic variants of CYP2C19 that may decrease the risk of bleeding.

Another important question is whether all stroke subtypes presenting with TIA or minor stroke should receive dual-antiplatelet therapy, or whether it is more effective in some stroke subtypes [3]. A subgroup analysis in the CHANCE trial showed an apparently greater effectiveness of dual-antiplatelet therapy in people with intracranial stenosis than in those without stenosis [18]. In the SOCRATES trial, ticagrelor was comparably effective to aspirin in early secondary prevention, but was more effective than aspirin in a subgroup analysis in patients with large artery stroke [3, 19]. This suggests that more intensive antiplatelet regimens are particularly effective in patients with TIA and stroke due to large artery stenosis. However, there is some uncertainty regarding the use of dual-antiplatelet therapy with aspirin and clopidogrel in patients with lacunar stroke. These patients were included in the CHANCE and POINT trials. However, the SPS3 study showed that in long-term

prevention after lacunar stroke, dual-antiplatelet therapy was associated with no additional reduction in ischemic events, but with a significantly increased risk of bleeding [3, 20]. More data is needed to know which specific patients benefit the most from certain interventions.

3.4 Conclusions

The examples that were given only scratch the surface of possible applications of precision medicine in stroke. Application of precision medicine to stroke will improve the benefit of current treatments while minimizing their risk. It will also allow the development of treatments aimed at specific targets.

In order to further proceed, one of the challenges will be the collection of standardized clinical, biological (genomic, serological, and novel), and imaging data [21]. This will need cerebrovascular expertise on big data approaches to clinically relevant paradigms. Stroke precision medicine will also need experienced and rational valuation of these multiple data points and appropriate statistical methodology to obtain individualized decision-making [21].

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