Personalized Medicine

Federico Goodsaid, Felix Frueh, and Michael E. Burczynski

Contents

Abstract

Personalized medicine (PM) has grown in both clinical importance and in cultural recognition and popularity over the last two decades. In this chapter we will first address the different public perceptions of the term "personalized medicine," with the ultimate goal of identifying the definition(s) of PM which best reflect how it is currently prosecuted within modern drug development. A brief historical context will then be provided, followed by a discussion of the general tenets of personalized medicine as employed within the pharmaceutical industry and in clinical practice. The chapter concludes with some tables and a small number of case studies that highlight the PM concept in both oncology and rare diseases and a consideration of what the future holds for the field.

F. Goodsaid · F. Frueh
Opus Three LLC Life Sciences, Pescadero, CA, USA
e-mail: federico@opusthree.com; felix@opusthree.com

M. E. Burczynski (✉)
Department of Translational Medicine, Global R&D, Teva Pharmaceuticals, Frazer, PA, USA
e-mail: tedburczynski@gmail.com

F. J. Hock, M. R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-56637-5_47-1

Personalized Medicine: One Concept, Many Approaches

A great deal of excitement and hope has been generated by simply articulating the concept of "personalized medicine" within modern society, but this same excitement has also left open the possibility for oversimplification, misinterpretation, and – to some extent – even confusion regarding what PM really means today. For this reason it is useful to first consider the gamut of possible definitions for personalized medicine and then narrow down the definition to the most reasonable and practical one that pharmaceutical companies employ today. The pursuit of personalized medicines has revolutionized our industry's shared quest to create novel medicines that are more precise for treating ailments of patients around the globe. But what is PM, at its core?

Personalized medicine can take many forms, but for the purposes of this chapter, it is sufficient to consider three main categories of PM: (1) truly "individualized" therapies – literally, where a single medicine is created for a single patient; (2) "precision" medicines, which are medicines meant for all patients but which are accompanied by some sort of test to determine the overall likelihood of a beneficial effect; and finally (3) "targeted" medicines which are medicines specific to a single molecular target present within individuals and which can be robustly tested to identify those patients who should or should not receive the medicine.

We will briefly call the reader's attention to the extremely innovative and recently emerging "individualized PM" therapies first. We'll then focus the remainder of the chapter on the latter PM approaches of "precision medicine" and/or "targeted medicine" which are much more commonly pursued in drug development today. The reader should keep in mind that there is no consensus on exactly which of these definitions best reflect the idea of PM today, and we draw these distinctions only for the purpose of presenting the overall topic of PM in clearest fashion.

PM as Individualized Medicine

For the uninitiated, the entire notion of PM may immediately invoke a futuristic state in which a single patient afflicted with a disease might be diagnosed with high accuracy and a customized pill subsequently created which treats that single, particular patient with perfect precision. This is the most literal definition of personalized medicine, in which a truly "personalized" therapy including the necessary chemistry or biology would then be manufactured or synthesized in real time to address that individual patient's specific needs. While this territory has been well-tread in the plots of modern-day biomedical thriller novelists like Michael Crichton, Douglas Preston, and Michael Ransom . . . this is not (yet?) the norm we are living in today. It is likely to remain unrealistic for quite some time, despite the advancements in clinical sciences and in technology (e.g., 3D printing, CRISPR, and other technologies) which are just starting to illustrate how this future state may indeed 1 day evolve.

Despite the limitations, there are a small but growing number of truly personalized (individualized) medicines that have recently been approved by the FDA and/or are being developed today in the form of experimental therapies quite literally tailored to individual patients. While these emerged from academic translational laboratories in research hospitals initially, they have found their way into both the clinic and into pharmaceutical development. The best example of a truly individualized medicine comes from the groundbreaking field of immuno-oncology.

Immuno-oncology is beyond the scope of any single textbook or chapter or section . . . but in an oversimplified nutshell, it is the synthesis of the fields of immunology and oncology for the express purpose of reinvigorating the patient's own immune system to kill the tumor cells present in the host. While several classical monoclonal antibody-based IO therapies have been approved to date (Yervoy, Opdivo, and others). . . the FDA's approval of Yescarta® (axicabtagene ciloleucel,

Kite Pharmaceuticals), a chimeric antigen receptor therapy (known as CAR-T) for the treatment of adult patients with relapsed or refractory large B-cell lymphoma, officially ushered in the era of individualized therapy.

The CAR-T approach uses genetic engineering and adoptive transfer of a patient's own T-cells back into the host to attack the very tumors which initially evaded that patient's immune system and flourished in its presence. This complex and laborious research approach was the first of its kind to be evaluated as therapeutic at large pharmaceutical companies. The development process was used to determine whether it could be adapted out of the research hospital setting and used in standard clinical medicine environments and made available to patients with responsive forms of disease.

While CAR-T is technically a procedure, rather than a standardly administered medication, it is nonetheless the best example to date of how PM can actually refer to a truly individualized medicine concept. By its very nature, CAR-T can only work if it is used to create and express chimeric antigen receptors in the patient's own T-cells. Thus any future treatments based on an analogous approach – namely, of removing a patient's cells, genetically engineering them, and transferring them back into the patient, will constitute additional examples of individualized therapies in the years to come.

As will be discussed in the next section, however, in the vast majority of cases, PM does not refer to a single therapy being developed for a single individual, but instead reflects an approach in drug development to create medicines that will work well in specific subsets of patients.

PM as Precision Medicine

While we may still be a long way from routinely experiencing the futuristic scenarios of individualized medicines described above, recent progress has provided exciting new interventions, insights, and knowledge toward a state where we will no longer be satisfied with a one-size-fits-all approach to medicine: a state the pharmaceutical industry has started to accept and is embracing as illustrated by the rapidly increasing number of personalized medicines reaching the market.

As stated previously, the other definitions or views on PM include "precision medicine," which is a more technical view on delivering highly precise interventions to patients that are calculated based on a set of predefined variables (e.g., biomarkers, likelihood ratios of outcomes, etc.), or "targeted medicine" which refers to the use of therapeutic intervention for which the specific target is known, is present in a patient, and can be assayed using a test to determine the suitability of the therapy for that patient (there are many examples for this type of PM). These approaches are highly similar conceptually, and the main difference between them is that precision medicine ultimately relies on a test that gives a range of likely responses to a therapy across a population of patients, whereas a targeted medicine relies on a test that determines whether a patient should or should not be a candidate for therapy. Ultimately the key aspect common to both of these PM approaches is that they combine a patient's (measurable) characteristics (most often biomarkers) with the choice of a therapy to increase the likelihood of a positive outcome in the patient, both from the perspective of safety and efficacy.

How has PM impacted drug development? The pharmaceutical industry has been facing a challenge with the fact that new technologies capable of stratifying patients into subgroups with specific characteristics that render them more or less susceptible to response or harm are rapidly becoming available. The era of developing a chemical for the average patient population of which as many as 80% may derive no or limited benefit is coming to an end, which means that the market is becoming more segmented, but also bigger: diseases are no longer just described as "breast cancer" but as Her2-positive, ER-negative

breast cancer, indicating that there are subtypes of disease with molecular markers that can be used to better characterize the disease and may resemble markers useful for targeted or individualized therapy, i.e., PM. Obviously this led to a significant and still present concern that PM by creating subpopulations may decrease the size of the market and that it will be more challenging to develop new blockbuster therapies. This is largely accurate, but at the same time new opportunities have been created for the development of therapies that are more likely to succeed as the appropriate patient population can be more easily identified and, therefore, the chance of response to the therapy can be increased. In addition, the introduction of a truly personalized medicine into a highly fragmented market should in theory result in a greater share of the market than introduction of yet another "me-too" therapy. Ultimately several examples have illustrated that PM can be a powerful way to decrease the development time and cost for new therapies and a solid incentive for developing therapies in existing areas of medical need.

In addition to the challenges posed by discoveries that may be useful to identify responders, nonresponders, and/or patients at risk based on the presence or absence of certain markers, the field has provided new insights into targets and our understanding of disease and mechanisms leading to disease. Drugs have been developed targeting phenotypes with specific underlying genetic causes (e.g., Vertex CFTR) that wouldn't have been possible if not for a much deeper understanding of the molecular mechanisms causing the disease. The definitions and views on PM described above are therefore intricate to the goal of better understanding disease and using this knowledge in drug discovery and development. It is reasonable to assume that such understanding will provide the foundation of future drug development and that, therefore, it will become increasingly difficult for pharmaceutical manufacturers to ignore the scientific and clinical advancements that enable personalized medicine and the development of new therapies based on such knowledge.

Similar to drug development – or perhaps ahead of it – clinical practice has been impacted by the advent of new technologies allowing a better characterization of patients and disease. Based on increasingly accurate molecular diagnostics, the assessment of disease has become significantly more precise. On the one hand, this has allowed us to identify therapies that are more likely to benefit patients. On the other hand, it has created a situation where certain patient groups are now left with few therapeutic options: if it can be predicted that a therapy has no or only a very limited chance to improve a patient's ailment, it may leave the patient (and physician) without a reasonable option for treatment. Although this may be a scientifically sound argument, it leaves the compassionate aspect of treatment largely out of consideration. In addition, most therapies including targeted therapies do not portray a black-and-white response profile: therefore, there is always a (small) chance that a patient may benefit in the absence of a positive marker signal. Clinical practice is significantly more complicated than drug development from this perspective: how much better off are patients with the availability of PM? The dissection of disease in ever-finer subtypes is shining a light of how much (or little) we really understand about disease and the underlying pathophysiology. There is a transition phase – we are in the middle of it – where the gap between knowing whether or not a patient has a chance to respond to a certain treatment is ahead of the availability of treatments addressing all subtypes of disease.

Still, the knowledge that a patient has a greater likelihood of responding to a certain therapy is invaluable. Not only does it provide assurance to the treating physician that a treatment decision can be made based on up-to-date accurate information, but it also provides the rationale for payers to cover increasingly expensive therapies. It would not be feasible to reimburse therapies that cost many thousands of dollars if the response to no-response profile could not be improved. Taken together, the pharmaceutical industry has an opportunity to benefit from significant scientific advancements to develop more precise

therapeutic options, which at the same time are more likely to be paid for if they address a truly unmet medical need. Patients benefit from a more precise assessment (diagnosis) of their disease and will have more and better treatment options that are addressing the underlying pathophysiology, and payers have a better foundation for making coverage and reimbursement decisions.

The Path from One Gene–One Drug to Many Genes–One Drug

The past decade has seen the concurrent development – and success – of more targeted therapeutic approaches and molecular diagnostics, leading to better, more precise assessment of disease and therapeutic interventions. Precision medicine has transformed therapeutic alternatives in oncology and other clinical areas, driven by the rapid evolution of molecular diagnostics based on a more detailed understanding of disease and disease mechanism (pathophysiology).

The practice of medicine has always focused on specific phenotypic characteristics of each patient to determine how to treat them. However, the first demonstration of precision medicine requiring molecular diagnostics was the application of molecular diagnostics in the pharmacogenomics of drug metabolism enzymes (https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079849.pdf). Mutations in the genes for these enzymes lead to incorrect dosing for many therapeutic drugs across multiple clinical areas (Phillips et al. 2001). The Critical Path for Innovation documents issued by the FDA in 2004 (http://wayback.archive-it.org/7993/20180125035500/https://www.fda.gov/downloads/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/UCM113411.pdf) both acknowledged and encouraged the development of molecular diagnostic tests for drug metabolism enzyme mutations which would lead to accurate dosing. The list for therapies which the FDA recognizes today (https://www.fda.gov/drugs/scienceresearch/ucm572698.htm) as requiring testing to determine accurate dosing includes over 90 entries. Over the next decade, a second set of molecular diagnostics was developed, reflecting the development of targeted therapies in oncology (https://www.fda.gov/drugs/scienceresearch/ucm572698.htm) (Table 1).

In oncology, the relationship between molecular diagnostics and precision medicine was triggered by the approval of trastuzumab (https://www.accessdata.fda.gov/drugsatfda_docs/appletter/1998/trasgen092598L.pdf) targeting overexpression of the Her2 gene. Whether measuring genomic overexpression or genomic variants for patient selection, these tests identified patients likely to benefit from targeted therapies. Targeted therapies initially led to the concept that precision medicine was all about "one gene–one drug" (Jørgensen 2013). This simplified view of precision medicine kept corresponding molecular diagnostics simple in platform, analysis of results, and validation. Technologies (Brooks 1982; Luthra and Medeiros 2006) for the platforms of these molecular diagnostics predated precision medicine and drove a strictly incremental adoption of targeted therapies. Several studies (https://www.nature.com/articles/537S106a; https://www.nytimes.com/2010/06/15/health/15canc.html) published over the first decade of precision medicine questioned the therapeutic value of targeted therapies with companion diagnostics identifying patients with "one gene–one drug" molecular diagnostics.

Sanger sequencing for DNA (Cheong and Caramins 2014) was available at the onset of precision medicine. While it was the original platform for much of the knowledge available at the time about DNA sequences, it was also slow, expensive, and not suitable for molecular diagnostic applications. Next-generation sequencing (NGS) (Loewe 2013) developed throughout the first decade of precision medicine and transformed the speed, cost, and molecular diagnostic value of DNA sequences. For example, NGS results have allowed pathologists and oncologists in Molecular Tumor Boards optimizing therapeutic opportunities for their patients to look not only at single gene expression and mutations

Table 1 Targeted therapies and biomarkers in oncology in the FDA table of pharmacogenomic biomarkers in drug labeling

Drug	Biomarker	Drug	Biomarker
Abemaciclib (1)	ESR (hormone receptor)	Letrozole	ESR, PGR (hormone receptor)
Abemaciclib (2)	ERBB2 (HER2)	Mercaptopurine	TPMT
Ado-trastuzumab Emtansine	ERBB2 (HER2)	Midostaurin (1)	FLT3
Afatinib	EGFR	Midostaurin (2)	NPM1
Alectinib	ALK	Midostaurin (3)	KIT
Anastrozole	ESR, PGR (hormone receptor)	Neratinib (1)	ERBB2 (HER2)
Arsenic trioxide	PML-RARA	Neratinib (2)	ESR, PGR (hormone receptor)
Atezolizumab	CD274 (PD-L1)	Nilotinib (1)	BCR-ABL1 (Philadelphia chromosome)
Avelumab	CD274 (PD-L1)	Nilotinib (2)	UGT1A1
Belinostat	UGT1A1	Niraparib	BRCA
Blinatumomab	BCR-ABL1 (Philadelphia chromosome)	Nivolumab (1)	BRAF
Bosutinib	BCR-ABL1 (Philadelphia chromosome)	Nivolumab (2)	CD274(PD-L1)
Brentuximab vedotin	ALK	Nivolumab (3)	Microsatellite instability, mismatch repair
Brigatinib	ALK	Obinutuzumab	MS4A1 (CD20 antigen)
Busulfan	BCR-ABL1 (Philadelphia chromosome)	Olaparib	BRCA
Cabozantinib	RET	Olaratumab	PDGFRA
Capecitabine	DPYD	Omacetaxine	BCR-ABL1 (Philadelphia chromosome)
Ceritinib	ALK	Osimertinib	EGFR
Cetuximab (1)	EGFR	Palbociclib (1)	ESR (hormone receptor)
Cetuximab (2)	RAS	Palbociclib (2)	ERBB2 (HER2)
Cisplatin	TPMT	Panitumumab (1)	EGFR
Cobimetinib	BRAF	Panitumumab (2)	RAS
Crizotinib (1)	ALK	Pazopanib (1)	UGT1A1
Crizotinib (2)	ROS1	Pazopanib (2)	HLA-B
Dabrafenib (1)	BRAF	Pembrolizumab (1)	BRAF
Dabrafenib (2)	G6PD	Pembrolizumab (2)	CD274 (PD-L1)
Dabrafenib (3)	RAS	Pembrolizumab (3)	Microsatellite instability, mismatch repair
Dasatinib	BCR-ABL1 (Philadelphia chromosome)	Pertuzumab (1)	ERBB2 (HER2)
Denileukin diftitox	IL2RA (CD25 antigen)	Pertuzumab (2)	ESR, PGR (hormone receptor)
Dinutuximab	MYCN	Ponatinib	BCR-ABL1 (Philadelphia chromosome)
Durvalumab	CD274 (PD-L1)	Rasburicase (1)	G6PD
Enasidenib	IDH2	Rasburicase (2)	CYB5R
Erlotinib	EGFR	Ribociclib (1)	ESR, PGR (hormone receptor)
Everolimus (1)	ERBB2 (HER2)	Ribociclib (2)	ERBB2 (HER2)

(*continued*)

Table 1 (continued)

Drug	Biomarker	Drug	Biomarker
Everolimus (2)	ESR (hormone receptor)	Rituximab	MS4A1 (CD20 antigen)
Exemestane	ESR, PGR (hormone receptor)	Rucaparib (1)	BRCA
Fluorouracil (2)	DPYD	Rucaparib (2)	CYP2D6
Fulvestrant (1)	ERBB2 (HER2)	Rucaparib (3)	CYP1A2
Fulvestrant (2)	ESR, PGR (hormone receptor)	Tamoxifen (1)	ESR, PGR (hormone receptor)
Gefitinib	EGFR	Tamoxifen (2)	F5 (factor V Leiden)
Ibrutinib (1)	Chromosome 17p	Tamoxifen (3)	F2 (prothrombin)
Ibrutinib (2)	Chromosome 11q	Thioguanine	TPMT
Imatinib (1)	KIT	Trametinib (1)	BRAF
Imatinib (2)	BCR-ABL1 (Philadelphia chromosome)	Trametinib (2)	G6PD
Imatinib (3)	PDGFRB	Trametinib (3)	RAS
Imatinib (4)	FIP1L1-PDGFRA	Trastuzumab (1)	ERBB2 (HER2)
Inotuzumab ozogamicin	BCR-ABL1 (Philadelphia chromosome)	Trastuzumab (2)	ESR, PGR (hormone receptor)
Irinotecan	UGT1A1	Tretinoin	PML-RARA
Lapatinib (1)	ERBB2 (HER2)	Vemurafenib (1)	BRAF
Lapatinib (2)	ESR, PGR (hormone receptor)	Vemurafenib (2)	RAS
Lapatinib (3)	HLA-DQA1, HLA-DRB1	Venetoclax	Chromosome 17p

associated with targeted therapies, but to genes in molecular pathways related to target genes in oncology (Haslem et al. 2018). Decision-making by Molecular Tumor Boards is now based not only on that which is as certain as companion diagnostics but also on that which is less certain, on sequencing information which can contribute to the accuracy of this therapeutic decision-making.

The Path from Solid Tumor Biopsy Specimens to Liquid Biopsy Specimens

Accessibility to specimens that can be used to measure a patient's individual characteristic is the foundation of precision medicine. In oncology, such specimen is most often based on solid tumor biopsies. For the most part, therapeutic decision-making is possible with these specimens. However, there are tissues (such as lung tissue) for which solid tumor biopsies are challenging (Esposito et al. 2017) to obtain for molecular diagnostic therapeutic decision-making in oncology patients. In addition, tumor biopsy specimens are also difficult to use to monitor response and recurrence in oncology.

Therefore, liquid biopsies, i.e., the use of blood as a specimen to identify markers that are representative of a specific pathophysiology (e.g., circulating nucleic acids), have been used for prognostic applications in oncology over two decades. Figure 1 shows the different permutations for indications/uses, signal sources, and platforms for liquid biopsies.

Today, there is one FDA-approved product (https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150047B.pdf) for EGFR-targeted therapeutic decision-making using cell-free DNA and qPCR. However, liquid biopsies are subject to fundamental limitations. For example, the sensitivity for detection of cell-free DNA from tumors as a fraction of total plasma cell-free DNA can be challenging, and the pipeline software for the accurate identification of variants can be difficult to calibrate, leading to very low congruence for same patient-paired samples and differences between platforms (Torga and Pienta 2018).

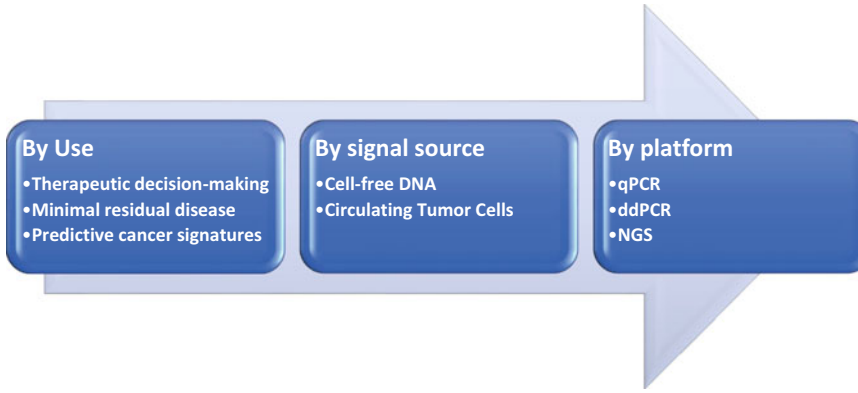


Fig. 1 The new frontier in precision medicine for oncology: liquid biopsies. Different uses, signal sources, and platforms define the products currently developed or reaching the market

Prognostic and Predictive Testing in Oncology: Case Study for Oncotype Dx

Precision medicine has been enabled by molecular diagnostics with prognostic and predictive indications. Oncotype DX is a case study for product development in these areas which illustrates the value of these tests for cancer patients and the complexity of product development, regulatory review, and product acceptance strategies to make them viable.

As of 2018, over 400 peer-reviewed papers reference this assay platform. The Oncotype DX® assay (https://preview.ncbi.nlm.nih.gov/pubmed/?term=Oncotype+DX) was developed to optimize the selection of adjuvant systemic therapy for patients with estrogen receptor (ER)-positive, lymph node-negative breast cancer. The Oncotype DX result is presented as the recurrence score which is a continuous score that predicts the risk of distant disease recurrence.

Implementation of the Oncotype DX assay has led to a change in how chemotherapy is utilized in patients with early stage, estrogen receptor (ER)-positive, lymph node-negative breast cancer (Mamounas et al. 2018). This test generates a recurrence score between 0 and 100, which correlates with probability of distant disease recurrence. Patients with low-risk recurrence scores (0–17) are unlikely to derive significant survival benefit with adjuvant chemotherapy and hormonal agents derived from using adjuvant hormonal therapy only. Conversely, adjuvant chemotherapy has been shown to significantly improve survival in patients with high-risk recurrence scores ($\geq$31). Patients at highest risk of recurrence are prescribed systemic treatment. Low-risk patients avoid adverse events from therapies unlikely to influence their survival.

Over the past decade, this test and others on this platform have opened genomic-based personalized cancer care for breast cancer in the USA. It is now widely utilized in various parts of the world (Giuliano et al. 2017; Jaafar et al. 2014; Rouzier et al. 2013). Together with several other genomic assays, Oncotype DX has been incorporated into clinical practice guidelines on biomarker use to guide treatment decisions. The assay has been validated for use in the prognostication and prediction of degree of adjuvant chemotherapy benefit in both lymph node-positive and lymph node-negative early breast cancers (Siow et al. 2018). Clinical studies have consistently shown that the Oncotype DX has a significant impact on decision-making in adjuvant therapy recommendations and appears to be cost-effective in diverse health-care settings.

Oncotype DX has succeeded, not only as a transformational tool for oncology patients but also as the most successful commercial platform, by far, in this class. This success, however, has been achieved independently of regulatory approval in the USA (Ross et al. 2008). Genomic Health considered the development of the algorithm used to determine the test score

proprietary. A similar assay (MammaPrint) developed by Agendia received approval by the FDA (Brandão et al. 2018). Agendia agreed to submit its platform for approval by the FDA and to share the development data for its algorithm. After this approval, the FDA proposed a draft IVDMIA guidance (later withdrawn) to address these types of assays (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079148.htm; https://www.alston.com/-/media/files/insights/publications/2010/08/recent-fda-actions-on-ldts-and-genetic-testing-whe/files/kazon_bna_art_8_25_10/fileattachment/kazon_bna_art_8_25_10.pdf).

Oncotype DX and MammaPrint are important case studies on the power of molecular diagnostics to transform cancer treatments and perhaps even transform regulatory policy: they redefined the relationship between successful analytical and clinical development and validation and its adoption by oncologists throughout the world for the benefit of their patients.

The Power of Precision Medicine in Rare Disease Therapeutic Development

Precision medicine has found one of its most successful applications in rare disease therapeutic product development. A rare disease often is originally defined through its clinical symptoms. In the absence of other information about the molecular mechanism of a rare disease, DNA sequencing data can empirically show germline mutations which correlate with the clinical symptoms associated with a rare disease (Bacchelli and Williams 2016). This empirical approach can be followed, as it would in oncology, to identify enrichment biomarkers or companion diagnostics for patient selection.

Cystic Fibrosis

Rare diseases for which molecular mechanisms have been exhaustively studied open novel applications for precision medicine. For example, cystic fibrosis is a rare disease (30,000 patients in the USA) (https://www.cff.org/What-is-CF/About-Cystic-Fibrosis/) for which the molecular mechanism is well established. Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (https://www.cff.org/What-is-CF/Genetics/Types-of-CFTR-Mutations/). Mutations in CFTR affect gating and other structural features and the number of active copies in the membrane (https://cftr2.org/).

There are two types of biomarkers associated with these mutations. These mutations can be used as patient selection markers in clinicals studies for targeted therapies (Merk and Schubert-Zsilavecz 2013). From a regulatory perspective, these mutations could develop into CDx tests, but over 95% of CF patients in the USA have been genotyped for CFTR mutations by the Cystic Fibrosis Foundation (Wiencek and Lo 2018), and the FDA has also cleared (https://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf) the Illumina CFTR Clinical Genotyping NGS Test on the MiSeq Dx to identify any previously unknown variants in these patients.

Cystic fibrosis may represent an extreme opportunity for precision medicine as there may be as little as a single individual associated with a specific CFTR mutation (sporadic or familial mutations). Clinical study designs focused on individual CFTR mutations are not possible for most CFTR mutations (as they wouldn't be for any other such disease in which sporadic mutations occur). Therefore, a clinical study design strategy consistent with this limitation is one where patients with multiple CFTR mutations are combined into patient populations with a shared mutational class. While this strategy has succeeded in designing clinical studies for therapeutic products such as Kalydeco (Moran 2017; Linsdell 2017), it has still not been broadly accepted by the FDA for label expansion.

The second type of biomarker associated with these mutations is the in vitro electrophysiological measurement of chloride transport in cells isolated from CF patients (Cholon and Gentzsch 2018). This test is used to quickly identify patients who are likely to benefit from Kalydeco and other CF therapies (https://www.fda.gov/Drugs/NewsEvents/ucm559051.htm) and may be

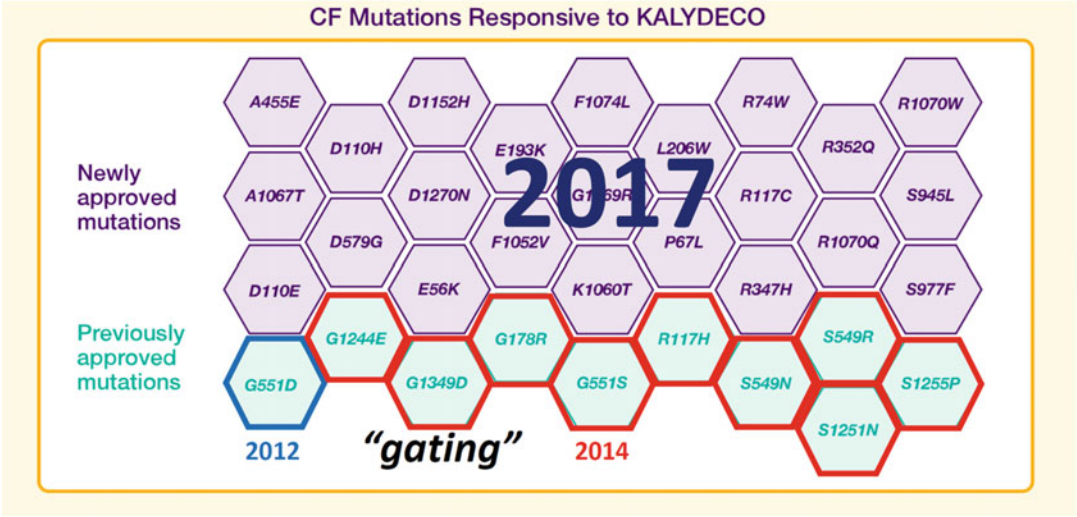


Fig. 2 Cystic fibrosis mutations responsive to Kalydeco

viewed as a surrogate for a specific phenotype of the disease. Importantly, this test has also led to Kalydeco label expansion claims for 23 mutations represented in a population of at least 900 patients (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm559212.htm). This unique precedent at the FDA could potentially lead in the future to the application of such surrogate tests for the initial approval of new therapies for rare diseases (Fig. 2).

Duchenne Muscular Dystrophy

The molecular defect in Duchenne muscular dystrophy is a defective dystrophin molecule (Hoffman 1989). This is a very large protein, transcribed from multiple exon sections (Gao and McNally 2015). Exon-skipping therapy is a structural strategy to partially correct the defect in dystrophin by splicing out exon 51 from transcription of the complete dystrophin molecule (Yokota et al. 2007). The resulting (shorter) dystrophin molecule does not have the full activity of normal dystrophin, but shows improved performance compared to the version with a detective exon 51 (Lim et al. 2017). Patients with this exon 51-skipped version have improved outcomes in their physiology and life expectancy. There are other defective exons transcribed into dystrophin for which this same strategy can be applied (Lee et al. 2017).

The exon 51-skipping therapy has been available throughout the past decade. However, its regulatory approval pathway throughout this period highlights the major challenges faced in the development and approval of novel precision therapies for rare diseases. There are two broad areas in which this challenge has had a major impact on how – and how long – this regulatory pathway has been drawn:

1. Acceptance by the FDA of a dystrophin activity as a surrogate biomarker for exon-skipping therapies
2. Clinical study designs which reflect both the small patient populations available for rare disease clinical development

To this date, a standard endpoint in clinical trials for the approval of rare disease therapies associated with at the FDA is the 6-minute walk (Hamuro et al. 2017). This surrogate reflects the regulatory need to show an improvement in how a patient feels, functions, or survives. A decade ago, this surrogate biomarker was required by the FDA to show therapeutic efficacy in Duchenne muscular dystrophy. This surrogate, however, is

unable to capture accurately the immediate mechanistic effects of exon-skipping therapies in Duchenne muscular dystrophy. The FDA now allows the use of dystrophin activity as an alternative surrogate to show efficacy in this disease.

As with other rare diseases, clinical study designs in Duchenne muscular dystrophy are challenged by the limited number of patients available, the broad heterogeneity in disease symptoms, and the difficulty in the delivery of therapies such as exon skipping to their specific tissue targets. Studies to test the efficacy of exon-skipping therapies have been crippled by their heterogenous results (Randeree and Eslick 2018). Notwithstanding the broad range of therapeutic responses in these studies, the FDA approved the first indication for exon 51-skipping therapy in 2016 (Syed 2016).

Precision Medicine Biomarkers

Precision medicine biomarkers start from two main sources:

- Biological hypotheses associated with the therapeutic target
- Empirical data obtained from early clinical studies with the proposed therapy

A biological hypothesis for a targeted therapy leads to biological pathways where specific patient selection biomarkers can be initially proposed (Jones and Libermann 2007). Early clinical studies with a limited number of patients, such as Phase 2a studies, can be used to prioritize possible patient selection biomarkers (Le Tourneau et al. 2008). In precision medicine applications, these biomarkers are detected using primarily genomic (variants or expression-level measurements) (Lin et al. 2017) or immunohistochemistry (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm094002.htm) platforms. As shown above, genomic platforms for patient selection biomarkers have evolved from those assays which genotype single genes to those which genotype multiple genes through NGS. Immunohistochemistry platforms continue to be important for semiquantitative assessments of cell surface proteins.

Cell sorting is required for liquid biopsy assays for circulating tumor cells (CTCs) (Alix-Panabières and Pantel 2013). This platform has been in use for several decades (Krebs et al. 2011). Basic cell-sorting technology is mature, but derivative technologies required for accurate selection and detection of specific CTC types have been developed over this period (Andree et al. 2018). The original parameter measure in CTC liquid biopsies was cell enumeration for nonspecifically or specifically labeled cells. More recently, NGS sequencing for DNA isolated from CTCs has also been considered as a possible liquid biopsy platform (Dawson 2018).

Digital devices are used in precision medicine to assess the physiological status of patients with neurodegenerative diseases like multiple sclerosis (Marziniak et al. 2018). Devices as simple as smartphones can be used either as substitutes for paper-based PROs and CROs or as real-time physiological biomarkers (Shah et al. 2016). These digital devices can measure real-time physiological biomarkers such as gait or other measures of activity. These parameters can be measured at baseline and then followed throughout treatment, providing precision measurements of therapeutic efficacy.

The Path from Enrichment Biomarkers to Companion Diagnostics

Clinical study designs in precision medicine for therapeutic product approval at the FDA make use of proposals for enrichment biomarkers (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM332181.pdf) for patient selection to maximize therapeutic effects in the selected patient population. Patient selection biomarker proposals often start with a biological hypothesis linked to the targeted therapy. The major hurdle with these initial proposals is the limited number of patients available for validation of these biomarkers in

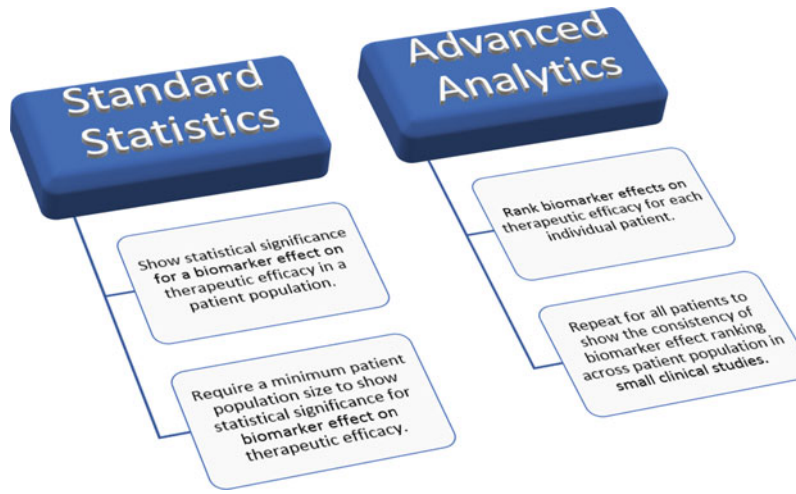
preliminary (e.g., Phase 2a) studies with a novel therapy. Standard statistical methods face challenges in ranking biomarkers tested in these preliminary studies because of the limited number of patients in these preliminary studies. This is one of the most difficult steps in the development of therapeutic products for precision medicine. In particular, a decision *how* to include biomarker candidates into clinical trial designs is critical: for the most part, it is advisable that specimens are collected to ensure the analysis of markers at least retrospectively, but if the evidence is strong enough, the marker(s) can be used for stratification, in which case the first steps toward the development of a companion diagnostic has been taken.

In order to address the issue of limited number of samples, advanced analytics platforms such as KEM® (Ariana Pharma) (http://www.arianapharma.com/2017/10/anavex-life-sciences-reports-pk-and-pd-data-from-phase-2a-trial-of-anavex2-73-in-mild-to-moderate-alzheimers-disease-patients/) focus on ranking the biomarkers tested in preliminary studies. The platform does not focus on a standard statistical significance for specific biomarker results but rather measures the link between observed therapeutic effects and different biomarkers included in a preliminary study. The tool then ranks all biomarkers across all patients in a study and allows a quick assessment about the homogeneity of biomarker effects across small patient populations:

This approach can allow one to select a potential companion diagnostic biomarker accurately from preliminary (including pilot) studies such as Phase 1 in patients or Phase 2a studies. Subsequently, a new study, e.g., a Phase 2b study, can be designed and tailored toward demonstrating the therapeutic efficacy in the newly selected patient population. Simultaneously, this study can then also serve as validation of the test as a companion diagnostic.

Conclusion

Personalized medicine has already had a significant impact in today's pharmaceutical development and patient care. By better characterizing patients and disease through sophisticated diagnostics and the use of advanced statistical tools and clinical trial designs, we are now routinely optimizing therapies. Personalized medicine can lead to the development of truly individualized medicines, as is the case with CAR-T therapies, or it can lead to the development of precision and/or targeted medicines, which increase the likelihood that therapies will work in specific patients. Personalized medicine is also no longer an oncology-delimited consideration, and there are now many examples of these approaches having success in many other therapeutic areas. Ever-improving methods for detecting genetic alterations, biomarkers of



many different types, and other measurable characteristics are enabling cutting-edge translational research in these disease areas that will ultimately lead to personalized therapies for years to come. The question of whether to pursue a personalized medicine approach in drug development has evolved in the past few years, growing from an esoteric possibility into an absolutely critical-path consideration for every new target and associated therapeutic candidate entering pharmaceutical development today. Our ability to embrace and implement personalized medicine approaches in pharmaceutical development will be a key determinant of our collective ability as drug developers to address truly unmet medical needs of patients in the future.

References and Further Reading

Alix-Panabières C, Pantel K (2013) Circulating tumor cells: liquid biopsy of cancer. Clin Chem 59(1):110–118

Andree KC, Mentink A, Zeune LL, Terstappen LWMM, Stoecklein NH, Neves RP, Driemel C, Lampignano R, Yang L, Neubauer H, Fehm T, Fischer JC, Rossi E, Manicone M, Basso U, Marson P, Zamarchi R, Loriot Y, Lapierre V, Faugeroux V, Oulhen M, Farace F, Fowler G, Sousa Fontes M, Ebbs B, Lambros M, Crespo M, Flohr P, de Bono JS (2018) Towards a real liquid biopsy in metastatic breast and prostate cancer: diagnostic LeukApheresis increases CTC yields in a European prospective multi-center study (CTCTrap). Int J Cancer. https://doi.org/10.1002/ijc.31752

Bacchelli C, Williams HJ (2016) Opportunities and technical challenges in next-generation sequencing for diagnosis of rare pediatric diseases. Expert Rev Mol Diagn 16(10):1073–1082

Brandão M, Pondé N, Piccart-Gebhart M (2018) Mammaprint™: a comprehensive review. Future Oncol. https://doi.org/10.2217/fon-2018-0221

Brooks JJ (1982) Immunohistochemistry of soft tissue tumors: progress and prospects. Hum Pathol 13(11):969–974

Cheong PL, Caramins M (2014) Approaches for classifying DNA variants found by Sanger sequencing in a medical genetics laboratory. Methods Mol Biol 1168:227–250

Cholon DM, Gentzsch M (2018) Recent progress in translational cystic fibrosis research using precision medicine strategies. J Cyst Fibros 17(2S):S52–S60

Dawson SJ (2018) Characterizing the cancer genome in blood. Cold Spring Harb Perspect Med. https://doi.org/10.1101/cshperspect.a026880. pii: a026880

Esposito A, Criscitiello C, Trapani D, Curigliano G (2017) The emerging role of "Liquid Biopsies," Circulating tumor cells, and circulating cell-free tumor DNA in lung cancer diagnosis and identification of resistance mutations. Curr Oncol Rep 19(1):1

Gao QQ, McNally EM (2015) The dystrophin complex: structure, function, and implications for therapy. Compr Physiol 5(3):1223–1239

Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, Weaver DL, Winchester DJ, Hortobagyi GN (2017) Breast cancer-major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin 67 (4):290–303

Hamuro L, Chan P, Tirucherai G, AbuTarif M (2017) Developing a natural history progression model for Duchenne muscular dystrophy using the Six-Minute Walk Test. CPT Pharmacometrics Syst Pharmacol 6(9):596–603

Haslem DS, Chakravarty I, Fulde G, Gilbert H, Tudor BP, Lin K, Ford JM (2018) Nadauld LD precision oncology in advanced cancer patients improves overall survival with lower weekly healthcare costs. Oncotarget 9(15):12316–12322

Hoffman EP (1989) Human molecular genetics and the elucidation of the primary biochemical defect in Duchenne muscular dystrophy. Cell Motil Cytoskeleton 14(1):163–168

Jaafar H, Bashir MA, Taher A, Qawasmeh K, Jaloudi M (2014) Impact of oncotype DX testing on adjuvant treatment decisions in patients with early breast cancer: a single-center study in the United Arab Emirates. Asia Pac J Clin Oncol 10(4):354–360

Jones J, Libermann TA (2007) Genomics of renal cell cancer: the biology behind and the therapy ahead. Clin Cancer Res 13(2 Pt 2):685s–692s

Jørgensen JT (2013) Companion diagnostics in oncology – current status and future aspects. Oncology 85(1):59–68

Krebs MG, Sloane R, Priest L, Lancashire L, Hou JM, Greystoke A, Ward TH, Ferraldeschi R, Hughes A, Clack G, Ranson M, Dive C, Blackhall FH (2011) Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol 29(12):1556–1563

Le Tourneau C, Vidal L, Siu LL (2008) Progress and challenges in the identification of biomarkers for EGFR and VEGFR targeting anticancer agents. Drug Resist Updat 11(3):99–109

Lee T, Awano H, Yagi M, Matsumoto M, Watanabe N, Goda R, Koizumi M, Takeshima Y, Matsuo M (2017) 2′-O-methyl RNA/ethylene-bridged nucleic acid chimera antisense oligonucleotides to induce dystrophin exon 45 skipping. Genes (Basel) 8(2):67

Lim KR, Maruyama R, Yokota T (2017) Eteplirsen in the treatment of Duchenne muscular dystrophy. Drug Des Devel Ther 11:533–545

Lin CH, Lin E, Lane HY (2017) Genetic biomarkers on age-related cognitive decline. Front Psych 8:247

Linsdell P (2017) Structural changes fundamental to gating of the cystic fibrosis transmembrane conductance regulator anion channel pore. Adv Exp Med Biol 925:13–32

Loewe RP (2013) Combinational usage of next generation sequencing and qPCR for the analysis of tumor samples. Methods 59(1):126–131

Luthra R, Medeiros LJ (2006) TaqMan reverse transcriptase-polymerase chain reaction coupled with capillary electrophoresis for quantification and identification of bcr-abl transcript type. Methods Mol Biol 335:135–145

Mamounas EP, Russell CA, Lau A, Turner MP, Albain KS (2018) Clinical relevance of the 21-gene recurrence score® assay in treatment decisions for patients with node-positive breast cancer in the genomic era. NPJ Breast Cancer 4:27

Marziniak M, Brichetto G, Feys P, Meyding-Lamadé U, Vernon K, Meuth SG (2018) The use of digital and remote communication technologies as a tool for multiple sclerosis management: narrative review. JMIR Rehabil Assist Technol 5(1):e5. https://doi.org/10.2196/rehab.7805

Merk D, Schubert-Zsilavecz M (2013) Repairing mutated proteins – development of small molecules targeting defects in the cystic fibrosis transmembrane conductance regulator. Expert Opin Drug Discovery 8(6):691–708

Moran O (2017) The gating of the CFTR channel. Cell Mol Life Sci 74(1):85–92

Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W (2001) Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. JAMA 286(18):2270–2279

Randeree L, Eslick GD (2018) Eteplirsen for paediatric patients with Duchenne muscular dystrophy: a pooled-analysis. J Clin Neurosci 49:1–6

Ross JS, Hatzis C, Symmans WF, Pusztai L, Hortobágyi GN (2008) Commercialized multigene predictors of clinical outcome for breast cancer. Oncologist 13(5):477–493

Rouzier R, Pronzato P, Chéreau E, Carlson J, Hunt B, Valentine WJ (2013) Multigene assays and molecular markers in breast cancer: systematic review of health economic analyses. Breast Cancer Res Treat 139(3):621–637

Shah KN, Hofmann MR, Schwarzkopf R, Pourmand D, Bhatia NN, Rafijah G, Bederman SS (2016) Patient-reported outcome measures: how do digital tablets stack up to paper forms? A randomized, controlled study. Am J Orthop (Belle Mead NJ) 45(7):E451–E457

Siow ZR, De Boer RH, Lindeman GJ, Mann GB (2018) Spotlight on the utility of the Oncotype DX® breast cancer assay. Int J Womens Health 10:89–100

Syed YY (2016) Eteplirsen: first global approval. Drugs 76(17):1699–1704

Torga G, Pienta KJ (2018) Patient-paired sample congruence between 2 commercial liquid biopsy tests. JAMA Oncol 4(6):868–870

Wiencek JR, Lo SF (2018) Advances in the diagnosis and management of cystic fibrosis in the genomic era. Clin Chem 64(6):898–908

Yokota T, Pistilli E, Duddy W, Nagaraju K (2007) Potential of oligonucleotide-mediated exon-skipping therapy for Duchenne muscular dystrophy. Expert Opin Biol Ther 7(6):831–842