Personalized Medicine in the Genomics Era

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Summary

Personalized medicine (also termed individualized medicine, precision medicine, genomic medicine or personalized genomics) increasingly contributes to recent advances in the understanding of the pathogenesis as well as the prevention and the management of different diseases. In this context, genome-wide association studies and next-generation sequencing, including exome and whole genome sequencing as well as pharmacogenomics and novel cancer therapy strategies are expected to improve medical decision-making with respect to the individualized diagnosis, prevention and treatment of diseases. Major advances have been realized recently, especially in sequencing technologies and their applications in pharmacogenomics as well as in cancer treatment. Numerous limitations and obstacles remain to be overcome, however, before personalized medicine can be widely implemented in clinical practice.

Key words: array analyses, next generation sequencing, exome sequencing, whole genome sequencing, pharmacogenomics, immune checkpoint blockade, genomically/ non-genomically matched tumors, cancer genomics, personalized cancer medicine, practical limitations and obstacles.

Introduction

Molecular and cell biology are not only integral part of basic biomedical research but are also increasingly translated into patient care. In recent years, DNA sequencing, including whole genome sequencing (WGS), and omics analyses have identified genetic markers and signatures which allow to predict the individual disposition for a specific disease, its prognosis and natural course as well as its response/ resistance to therapy. Several major global research efforts have been launched and were in part completed during the last decades. These include the international human genome organization project that established the complete sequence of the human genome almost 20 years ago (1, 2). Further, the international haplotype map project was initiated in 2005 to identify, based on genome-wide association studies (GWAS) in different ethnic populations, single nucleotide polymorphisms (SNPs) and their association with specific human diseases and individual phenotypic characteristics, respectively (3, 4).

In 2007 the US Department of Health and Human Services launched the Personalized Health Care Initiative (PHCI) that aims to accelerate the development of gene-based health information technologies that transform the practice of medicine towards individualized patient care. In 2015 a precision medicine initiative was proposed in the US that is aimed at the enhanced use of genomic information to improve the diagnosis and treatment of human diseases (5). The initial focus was on cancer with the longer-term perspective to include a broader range of diseases. In this context, genomic medicine is beginning to transform health care and requires an increasing understanding of genomic medicine by clinicians (6).

In the following, four aspects of personalized medicine are selected and will be addressed in some detail:

1. Tools for the Implementation of Genomics in Personalized Medicine

With the rapid advances in biotechnology, genetics and genomics, molecular genetic profiling is aimed to guide the clinical management related to the prevention, diagnosis and therapy of diseases. Genetic testing has focused on 3 types of genetic variation: (1) disease-causing mutations that have a major effect on gene function and are associated with relatively rare inherited diseases which follow an autosomal dominant, autosomal recessive or X-linked inheritance pattern; (2) genetic variants with a limited effect on gene function, frequently single nucleotide polymorphisms (SNPs), which are associated with an increased risk of complex diseases involving more than one gene or gene-environment interaction; (3) pharmacogenetic variants, frequently also SNPs, that affect drug response.

Genetic testing can provide valuable information that has an impact on patient management and may allow tailored interventions in personalized medicine. The appropriate use of genetic testing should be coordinated with the qualified genetic counseling of patients/ family members (including family history) that is based on the expertise in genetics, the logistics of testing as well as the familiarity with the associated ethical, legal and psychosocial issues. It can be performed with the goal to diagnose or exclude a specific genetic disease or to predict a disease risk in children/ adolescents (7) or adults as well as preimplantation or prenatal testing, using specimens obtained by chorionic villous sampling or amniocentesis and more recently by sequencing of cell-free fetal DNA from maternal plasma. There are typically three possible outcomes from

genetic testing: a causative mutation is identified, a causative mutation is not identified, a variant of uncertain significance (VUS) is identified. The clinical validity of a genetic test depends on the penetrance of a disease-causing mutation (likelihood to develop the disease) and its expressivity (phenotypic or clinical variation such as severity, age of onset or disease progression). While for example the penetrance of a mutation in the APC gene is very high with virtually all individuals with a mutation in this gene will develop features of familial adenomatous polyposis (FAP). By contrast, only about 30 % of individuals with the APOE e4/e4 genotype are at risk for developing Alzheimer's disease. In terms of expressivity, a mutation in the HFE gene is very variable with some individuals developing features of severe iron overload (hereditary hemochromatosis with liver cirrhosis) at a young age while others will never show symptoms of iron overload, even in the absence of treatment.

The management of patients for whom genetic testing reveals an increased risk for a disease may include a more aggressive screening or a screening initiated at an earlier age than in the general population, counseling of lifestyle modifications, initiation of pharmacological or surgical interventions and the individual consideration of disease-related ethical, legal and psychosocial issues.

The genetic variations can be detected by different strategies:

(1) **Specific single gene tests** that aim to detect mutations (point mutations, nonsense mutations, frameshift mutations, deletions or insertions) in the coding region of a gene known to be associated with heritable diseases (**Fig. 1 and Fig. 2**), such as factor V Leiden, cystic fibrosis, familial breast cancer, hemophilia B, beta-thalassemia, hereditary retinoblastoma, or FAP.

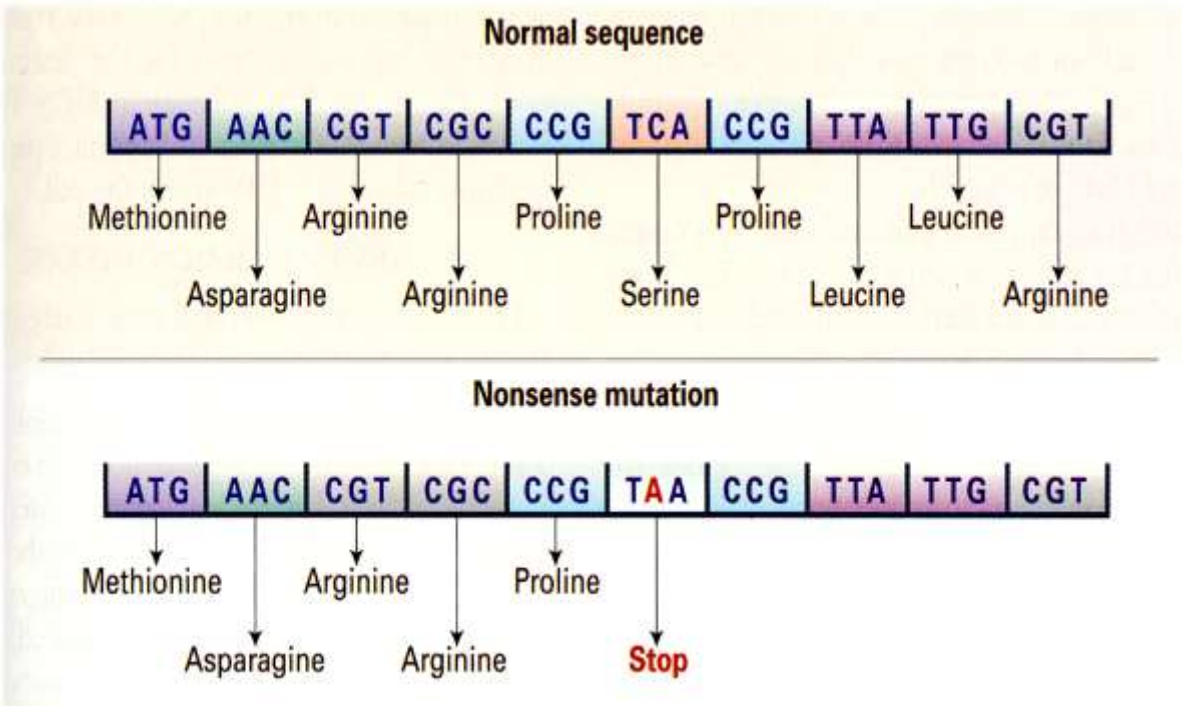

Fig. 1: Principle of nonsense mutation: TCA to TAA mutation results in a TAA stop codon and a truncated protein.

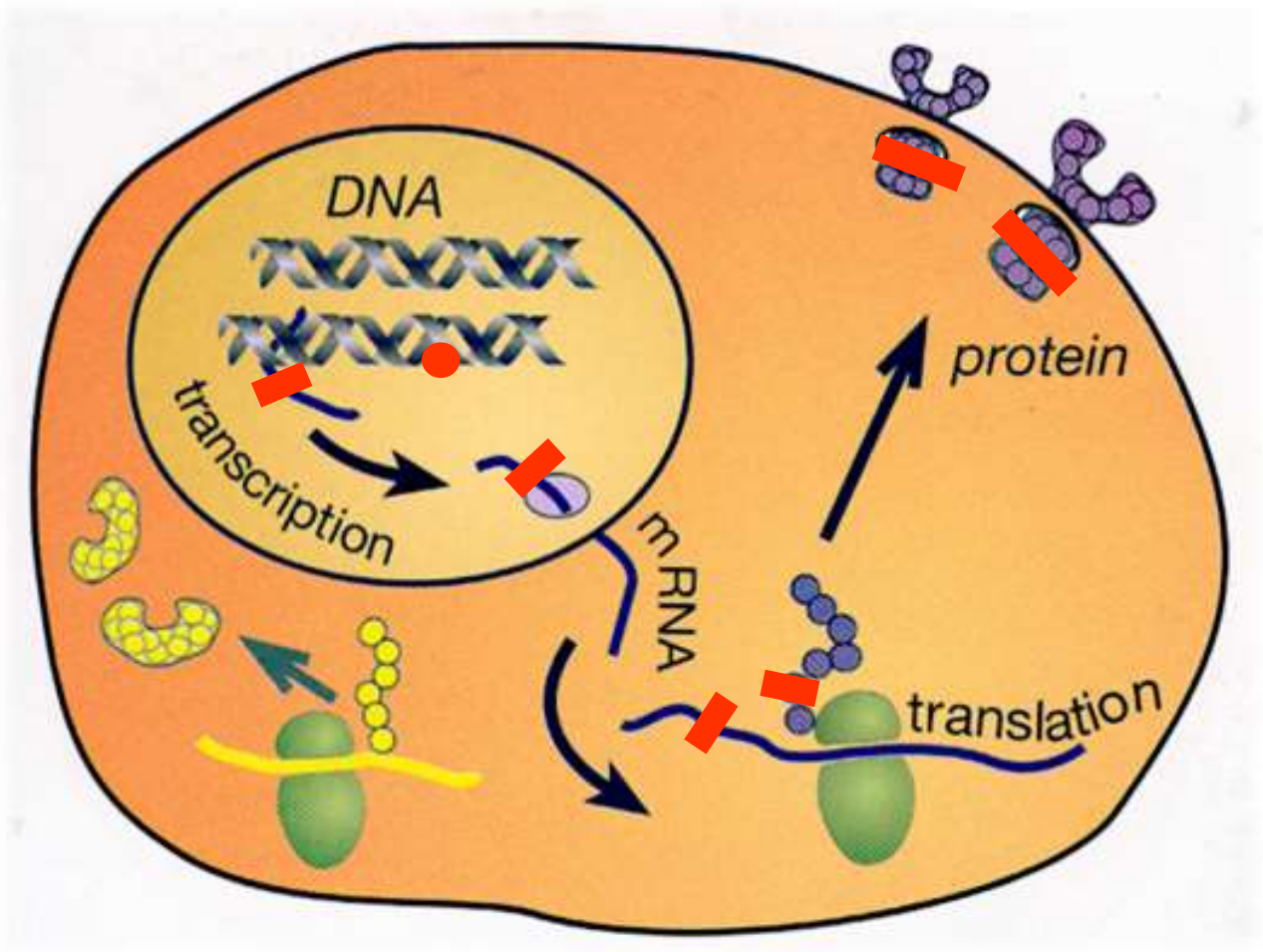

Fig. 2: Principle of gene expression and effect of a mutation (red ball: stop codon) as cause of truncated proteins that result in hereditary diseases.

(2) **Specific gene panels** that provide DNA sequence information for multiple genes that cause the same phenotype, such as epilepsy, autism, hereditary deafness or intellectual deficits.

(3) **Genotyping panels** that include SNPs (**Fig. 3**) that have been associated with common complex genetic diseases such as type 2 diabetes mellitus, autoimmune diseases and others (see GWAS above).

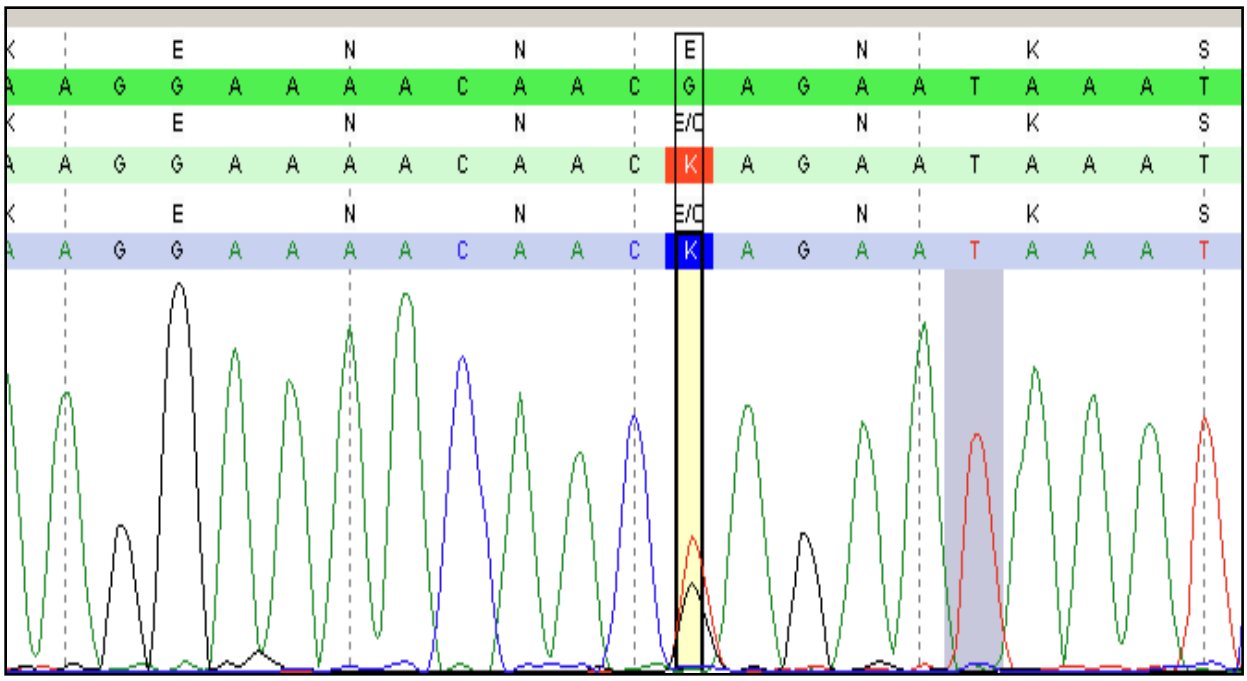

Fig. 3: Automated DNA sequencing with identification of a single nucleotide polymorphism in the position indicated (one allele G black, one allele T red as compared to GG black or TT red in both alleles).

(4) **Whole genome or exome sequencing** that allows through high-throughput platforms, also referred to as 'next-generation sequencing (NGS)', the sequencing of the genome (protein-coding as well as regulatory/ non-coding regions) or of the exome (protein-coding regions). The sequencing encompasses the whole genome rather than a specific disease gene and provides information about the individual's genetic variation with a wide range of findings that may be unrelated to the indication for the test (secondary findings) as well as VUS.

(5) **Microarrays, fluorescence *in situ* hybridization and high resolution chromosome analysis** are used to detect genetic alterations at the level of an entire or of a segment of a chromosome. These methods allow the detection of aneuploidies, gene translocations and deletions of chromosomal regions. They are typically used for the prenatal diagnosis of aneuploidies (abnormal number of chromosomes, e.g., trisomy 21 (**Fig. 4**), the classification of hematological malignancies and the evaluation of major unexplained congenital anomalies in children.

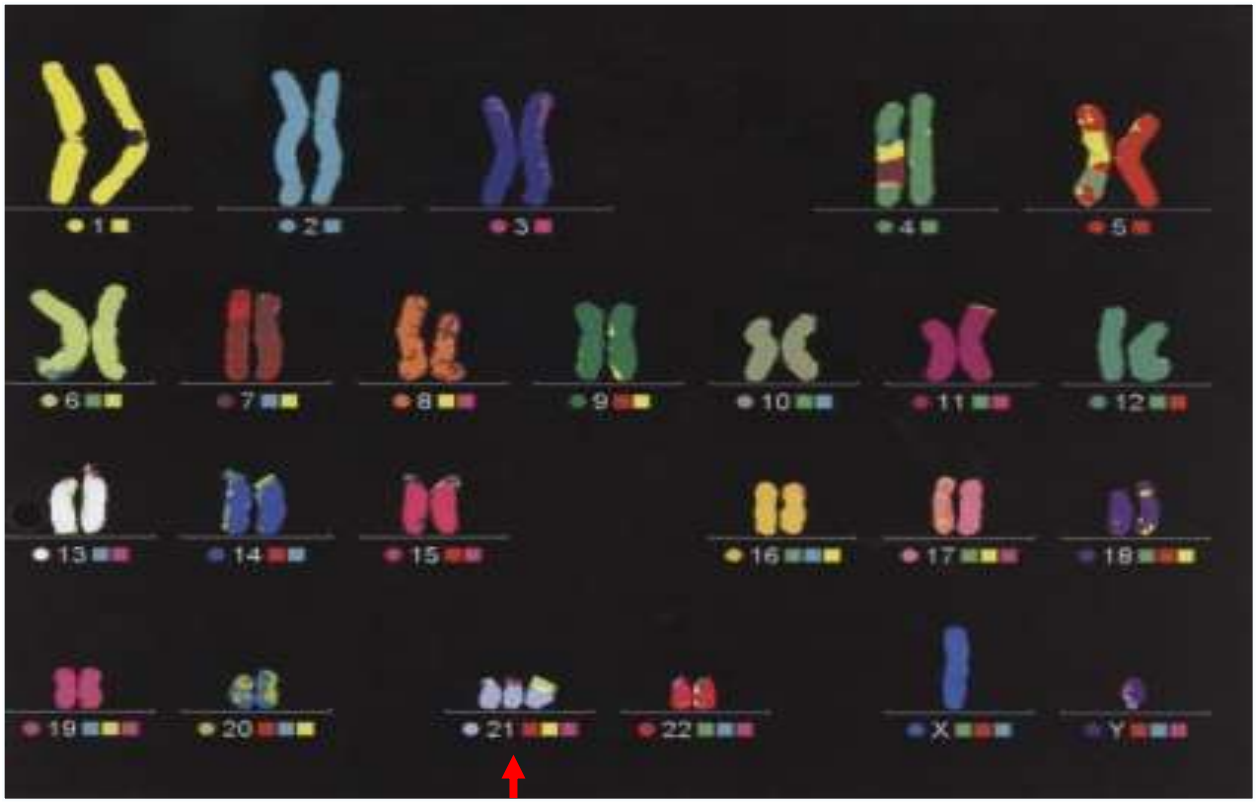

Fig. 4: Karyogram of a male (XY) patient with trisomy 21.

In recent years, DNA sequencing technologies have greatly improved to the point that it has become feasible to sequence an individual's entire genome at lower cost, faster and more accurate. Manual or automated sequencing based on the methods developed by Sanger, Maxam and Gilbert as 'conventional, traditional or first generation sequencing' allows to determine the sequence of DNA fragments up to 500-900 bases. By comparison, NGS, also termed 'high-throughput sequencing', 'deep sequencing' or 'second-generation sequencing', is based on the parallel sequencing of multiple DNA fragments which results in a much higher speed and amount of sequence data generated at significantly lower cost. 'Third generation sequencing' is similar to NGS but uses single DNA molecules rather than amplified DNA as template thereby potentially eliminating errors in the DNA sequence introduced during the amplification process. This technology is currently still under development and is generally not yet clinically available.

The source of DNA traditionally is double-stranded nuclear DNA from cells or tissues, including formalin-fixed, paraffin-embedded pathology samples. Further, 'liquid biopsies' are rapidly emerging as an important and minimally invasive adjunct to standard biopsies, in particular cell-free DNA (cfDNA), circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) from blood (8).

Genomic technologies and understanding of genomic variants are increasingly moving from laboratory or research to clinical applications, focusing on targeted gene panels, designed around specific disease entities, e.g., breast or ovarian cancer, cardiomyopathies or developmental abnormalities.

2. Pharmacogenomics

The response to pharmacologic interventions shows a tremendous interindividual variability with plasma drug levels that can vary more than 1'000-fold between 2 individuals with approximately the same weight. Factors that contribute to this variability are among others drug-drug interactions, drug-food interactions, gender, age, pregnancy and renal or liver diseases. In addition genetic factors are likely to play a major role because the individual response to a given drug is highly reproducible. Pharmacogenomics address the role of various components of the genome on the response to a drug (9). These include genetic sequence variants, structural alterations of chromosomes, e.g., translocations, epigenetic variants, mainly related to changes in the DNA methylation status, the histone modification (methylation, acetylation) or alterations in noncoding RNAs and telomere length, and variations of the expression level of relevant genes. The genetic variations can be inherited through the germline or can be acquired.

Due to the variation in their response to drugs, doses can be effective in some patients, ineffective in others or even cause adverse drug reactions (ADR) which are an important cause of hospital admissions and in-hospital mortality (10). Most ADRs are caused by a too strong known or intended effect of a drug (type A ADR) while others are unrelated to a drug's pharmacological action (type B ADR). Pharmacogenomics aim to define the genetic mechanisms underlying the variable drug response with the attempt to improve drug efficacy and reduce ADRs thereby optimizing drug prescribing in clinical practice. Conceptually, pharmacogenomics includes pharmacokinetics and pharmacodynamics. Pharmacokinetics define the variability in drug concentration due to drug transport and metabolism (absorption, distribution, tissue localization, biotransformation and excretion) while pharmacodynamics describe the variability of drug action due to variability of the individual's therapeutic response to a drug (drug affinity, drug activity at the site of action which is often a receptor). There are 2 phases of xenobiotic metabolism that are controlled by several hundred drug metabolizing enzymes: (1) drug modifications (phase I metabolism) by addition of polar groups to lipophilic molecules by oxidation, reduction or hydrolysis to facilitate water-solubility. These reactions are predominantly catalyzed by cytochrome P450 superfamily of mixed function oxidases (CYPs). (2) Drug conjugation (phase II metabolism) to form readily excretable, non-toxic substances. An example is thiopurine-S-methyltransferase (TPMT) that is involved in the metabolism of azathioprine and 6-mercaptopurine.

Important single gene variants affecting pharmacokinetics are involved in prodrug metabolism and in the metabolism of drugs with a narrow therapeutic range. A pharmacologically inactive prodrug requires bioactivation to be therapeutically effective. Clinically important examples for prodrugs are codeine that is bioactivated to morphine by CYP2D6 and the antiplatelet drug clopidogrel that is bioactivated by CYP2C19. While most genetic variants are associated with a partial or complete loss-of-function there are also gain-of-function variants resulting in an excess drug response. Examples include CYP2C19*17 resulting in bleeding during clopidogrel therapy and CYP2D6 duplications resulting in an excess narcotic effect of codeine, including respiratory arrest. A clinically important example for drugs with a narrow therapeutic range is 6-mercaptopurine that is bioinactivated by TPMT and xanthine oxidase. Loss-of-function TMPT variants are associated with a reduced bioinactivation of 6-mercaptopurine and an increased bone marrow toxicity. DPYD variants similarly

may cause an increased toxicity of the chemotherapeutic agent 5-fluorouracil and other fluoropyrimidines, such as capecitabine. Loss-of-function variants can also be mimicked by drugs that inhibit the drug metabolism pathways, e.g., different proton pump inhibitors or allopurinol. Further, drug transporters may be another cause of a variable drug concentration and action. Most clinically relevant pharmacogenetic traits affect the pharmacokinetics (9).

The effect of a pharmacogenomically guided therapy on the clinical outcome has been analyzed in numerous retrospective as well as prospective studies, e.g., for azathioprine or 6-mercaptopurine, warfarin or clopidogrel. To date, however, the uptake of pharmacogenomics in clinical practice is still limited and awaits the results from trials comparing the effect of pharmacogenomically guided to conventional drug use in terms of clinical outcomes. Further, the PREemptive Pharmacogenomic Testing for Preventing Adverse Drug Reaction study of the EU's Ubiquitous Pharmacogenomics study group is evaluating a pre-emptive pharmacogenomic testing strategy in 12 genes to reduce ADRs to related 43 target drugs (11). Worldwide, large personalized medicine programs including extensive genotyping or WGS have been initiated with the aim to optimize the personalized drug use and patient management. The results of these programs are expected in the near future and should be the basis for the uptake of pharmacogenomics in clinical practice, resulting in the optimal therapy of the individual patient.

3. Personalized Cancer Treatment based on Molecular Tumor Profiling

Malignancies arising from the same organ are traditionally subclassified by histological techniques that provide the basic information for the prediction of the prognosis and the choice of treatment. Histology can be supplemented by immunohistochemistry and fluorescence *in situ* hybridization analyses that allow the subtyping of some tumors and their molecularly directed treatment. Clinical examples are estrogen-receptor (ER) positive breast cancers (12) and their treatment with anti-estrogens (endocrine therapy) and breast cancers with amplification of the human epidermal receptor 2 (HER2; (13)) and their treatment by anti-HER2 strategies, including the monoclonal antibodies trastuzumab, pertuzumab, trastuzumab emtansine and the small molecule tyrosine kinase inhibitors (TKIs) lapatinib, neratinib and pazopanib, all of which interfere with the tyrosine kinase (TK) signalling cascade and thereby with cell proliferation, migration, invasion and survival (14). Treatment-relevant biomarkers have recently been identified also in other cancers (15).

The extensive genomic investigation of human cancers in recent years revealed their enormous complexity (16) that makes it difficult to exploit the novel information for a clinically relevant therapeutic strategy (17). Many approaches exist to define alterations in the cancer genome, epigenome, transcriptome and proteome. NGS has increasingly entered clinical research and patient management. Genomic biomarkers can have a prognostic relevance and can predict the response or resistance of a tumor to a particular therapeutic strategy. An example for positive predictive biomarkers are activating epidermal growth factor receptor (EGFR) mutations in lung adenocarcinoma that predict a response to EGFR-directed TKIs. Negative predictive biomarkers are activating KRAS mutations in colorectal cancer that are associated with a resistance to EGFR-directed monoclonal antibodies, e.g., cetuximab and panitumumab.

Between 2006 and 2018 34 targeted drugs or drug combinations were approved for the

first-line treatment of advanced malignancies. Among these, 16 were approved coupled with genomic biomarkers, such as an activating BRAF or EGFR mutation in patients with melanoma or non-small-cell lung cancer (NSCLC), the anaplastic lymphoma kinase (ALK) translocation in patients with NSCLC, a HER2 amplification in breast cancer patients and others (17). Other drugs or drug combinations were approved without being coupled to a genomic biomarker or are coupled to a nongenomic biomarker, e.g., ER positivity in patients with breast cancer (**Table 1**).

Table 1: FDA-approved, genomically and nongenomically matched targeted therapies (selection) (17).

Genomically Matched Tumors	**Genomic Biomarker**	**Drugs**	**RR***
Breast cancer	HER2 amplification	Lapatinib & Letrozole	28%
Non-small-cell lung cancer	Activating BRAF mutation	Dabrafinib & Trametinib	63%
	Activating EGFRmutation	Erlotinib	65%
		Gefitinib	70%
	ALK translocation	Ceritinib	73%
		Crizotinib	65%
Melanoma	Activating BRAF mutation	Dabrafenib	52%
		Trametinib	22%

*RR: response rate

NonGenomically Matched Tumors	**Genomic Biomarker**	**Drugs**	**RR***
Breast cancer	ER positivity	Aromatase inhib. & Abemaciclib	59%
		Aromatase inhib. & Ribociclib	53%
Hepatocelluar carcinoma.	None	Sorafenib	2%
Melanoma	None	Ipili	11%
		Nivol	40%
		Ipili & Nivol	60%
		Pembro	34%
Renal cell carcinoma	None	Cabozantinib	33%
		Ipili & Nivol	42%
Thyroid cancer	None	Sunitinib	28%
		Sorafenib	12%

*RR: response rate

To the group of nongenomically matched targeted therapies also belongs the novel immune checkpoint blockade strategy (18). The concept is based on the elimination of inhibitory signals of T-cell activation which allows tumor-reactive T cells to overcome regulatory mechanisms resulting in an effective antitumor response (19). The negative co-stimulatory molecules CTLA4 and PD-1 physiologically attenuate T-cell

activation and thereby protect the host from autoimmunity. For the immune checkpoint blockade monoclonal antibodies against CTLA4 (ipilimumab), PD-1 (nivolumab, pembrolizumab) and PD-Ligand 1 (atezolizumab, avelumab, durvalumab) were developed and have been FDA-approved for the treatment of different tumors (**Table 2**). To date, they are mostly applied for the treatment of melanoma, non-small-cell lung cancer, clear-cell renal carcinoma and squamous cell carcinoma of the head and neck (18).

Table 2: FDA-approved immune checkpoint blockade therapies (18).

Tumors	**Therapeutic agents**
Gastric and gastroesophageal carcinoma	Pembro
Head and neck squamous cell carcinoma	Nivo, Pembro
Hepatocellular carcinoma	Nivo
Hodgkin lymphoma	Pembro
Melanoma	Ipili, Nivo, Pembro
Merkel cell carcinoma	Ave
MSI-high, MMR deficient solid tumor/ CRC*	Nivo, Pembro
Non-small cell lung cancer	Atezo, Durva, Ipili, Nivo, Pembro
Renal cell carcinoma	Durva, Ipili, Nivo. Pembro
Urothelial carcinoma	Atezo, Ave, Durva, Nivo, Pembro

*MSI: microsatellite instability; MMR: mismatch repair; CRC: colorectal carcinoma.

Overall, genomic sequencing of human tumors has greatly contributed to our understanding of cancer. However, many challenges remain that require the close collaboration of basic scientists, clinical scientists and clinicians to translate these data into the development of practical preventive measures, clinically useful diagnostic tools as well as novel effective anticancer therapies.

4. Benefits, Obstacles and Limitations of Personalized Medicine

In addition to clinical and environmental factors, the advances in biotechnology, genetics and genomics increasingly provide a molecular genetic profile that will eventually become an integral part of the individualized management of different medical conditions. Examples include pharmacogenomics (see above) that determine the individual's drug

metabolism and may result in dose modifications of medicines or the detection of genomic or non-genomic biomarkers that predict effective therapeutic interventions in cancer (see above). These examples may be associated -among others- with a reduced exposure to medications of lower efficacy or higher toxicity, with reduced healthcare costs, improved patient satisfaction and compliance. At present, most available biomarker assays have an impact on the clinical practice of oncology. These include identification of biomarkers in asymptomatic individuals with early malignancies, resulting in a reduction of more aggressive, toxic and costly therapies and a greater number of individuals being cured from their disease. Gene expression profiling in other cases helps to stratify the need for therapy or the type of therapy (see above).

While the acceptance of the concept of personalized medicine together with the momentum for its implementation in clinical practice is in general increasing, the practical use still is limited to selected examples where the identification of specific biomarkers or genetic tests are entering medical decision-making. This is due to numerous factors, such as the relatively high cost of testing, reimbursement issues, the lack of reliable predictive biomarkers for most clinical conditions and the lack of clear therapeutic alternatives based on genetic results. Further, most clinicians lack a detailed knowledge and expertise in genetic risk predictions and genetic counseling. Last but not least, a number of concerns exist about the accuracy, interpretation and value of genetic testing, especially of direct-to-consumer testing (DTC) and about the fact, that prospective studies of the predictive accuracy of genetic testing are not available, precluding its use for effective counseling or reliable decision-making. This also holds true for the influence of non-genetic factors, e.g., race and life-style, on the interpretation of genetic results.

Conclusions and Perspectives

Recent advances in cell and molecular biology allowed an increasingly detailed understanding of the pathogenesis of many human diseases. With the rapid development of novel molecular, genetic/ epigenetic, microbiological and biochemical analyses it is now possible to identify on the one hand disease-related genetic alterations by various tools, including specific single gene tests, specific gene panels, genoptyping panels, whole genome or exome sequencing as well as chromosomal analyses. On the other hand, based on array technologies, thousands of genes, RNA species, proteins or metabolites can be analyzed simultaneously to yield disease-specific profiles (‘signatures’). These tools are increasingly entering clinical practice and will contribute to the implementation of genomics in personalized medicine.

Clinically most interesting areas of personalized medicine are pharmacogenomics as well as cancer treatment. The advances in these areas are expected to result in personalized pharmacogenomically-guided drug therapy that is associated with an increased therapeutic efficacy and a reduction of ADRs. Similarly, tremendous progress has been made in personalized cancer medicine based on the extensive genomic investigation of human cancers. This led among others to the establishment of genomic biomarkers that have prognostic relevance and the power to predict the response or resistance of a tumor to a particular therapeutic strategy.

Taken together, genomics research has made major advances in recent years and holds the promise of the increasingly personalized management of patients in the future in which the individual’s information or genetic profile will guide clinical decisions with respect to disease prevention, diagnosis and therapy. Many obstacles and limitations need to be

overcome, however, before personalized medicine will become clinical routine.

Conflict of interest
The author declares no conflict of interest.

Financial disclosure
The author has no financing to disclose.

Acknowledgment. The excellent secretarial support of Mrs. Mariette Gutgsell is gratefully acknowledged.

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