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Application of personalised medicine to solid tumours: opportunities and challenges

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Summary

Personalised medicine is an emerging model that will revolutionise our current healthcare system. In the last decade, several genomic aberrations were discovered that are now used as predictive markers for treatment with targeted therapeutics. The technological advances in the last few years, such as the development of high resolution DNA microarrays or second generation sequencers, have led to a dramatic increase in the number of ongoing genomic profiling studies. These studies, in turn, are leading to an enormous number of detected genomic aberrations whose biological interpretation is still pending. This review will provide an overview on the current state of personalised medicine in cancer. Discussion of the use and development of the various technologies will help us to understand the opportunities and challenges that arise when novel technologies are implemented.

Key words*: personalised medicine; cancer genomics; sequencing; array-CGH; genomic aberrations*

Introduction and overview

Personalised medicine or healthcare is an emerging model using novel methods of molecular analysis to improve our management of a patient's disease or even a predisposition toward a disease. The fundamental aim of personalised medicine is individualisation of each patient's treatments for und thus optimisation of medical care, outcome, side effects and costs. According to a recently published report by *PricewaterhouseCoopers*, personalised medicine is creating a booming market which is projected to grow by 11% annually [1]. This new healthcare also creates new challenges for pharmaceutical companies, since this development will shift the business model from the blockbuster drug towards a more collaborative model combining specialised therapies and molecular diagnostics. In informal terms, personalised medicine can be described as *"the right treatment for the right person at the right time"*.

One of the main principles which form the rationale for personalised medicine is assessment of the individualised risk of a given disease. In a very simple manner, this has been in use for many years in several applications, e.g. by calculation of the Framingham Risk Score for the prediction of future coronary heart disease events, or by analysis of the family history [2]. The latter represents results from a shared genomic background and the environmental influences. First degree relatives share half of their genomic information and thus their disease history is frequently used to estimate susceptibility to a given disease. In the last decade the use of genomic information from the germ line for risk assessment and thus for preventive health care has significantly increased. The presence of single or defined sets of single nucleotide polymorphisms (SNPs) has been associated with the risk of given diseases or reported as predictive for a certain therapy [3–4]. SNPs are single base DNA sequence variations occurring between members of a species or between paired chromosomes of an individual. SNP association studies have been boosted by the development of SNP microarrays, which allow simultaneous detection of up to 900,000 different SNPs of an individual's DNA. These studies have identified hundreds of sequence variants that are associated with the risk of a specific disease, such as breast cancer [5], prostate cancer [6], diabetes [7], tuberculosis [8] and many more [9]. This information has been capitalised on by companies offering the service of analysing a specific set of SNPs to estimate the probability of developing a particular disease. The company *23andMe* (California) provides an SNP microarray test starting at USD 207.00 and, based on the findings, it offers information on susceptibility for 215 diseases and conditions (e.g. drug responses). The same technology is used for the AmpliChip450®, the first pharmacogenetic microarray test approved by the FDA in 2005 and manufactured by Roche. This test classifies the patient on the basis of SNP profiles of the cytochrome P450 (CYP) genes *CYP2D6* and *CYP2C19* into poor, intermediate, extensive or ultrarapid metaboliser. This information can then be used by the clinicians to adapt the dose specifically for therapeutics that are metabolised by these two enzymes [10].

These examples are only intended to illustrate the versatility of the field of personalised medicine. To cover all aspects would go beyond the scope of this review. We will focus on personalised medicine based on cancer genomics and especially on the advances that have been made in the last decade due to the development of novel technologies such as array comparative genomic hybridisation (aCGH) and next-generation sequencing (NGS). However, it is of

the utmost importance to understand the opportunities and challenges that will be encountered when novel technologies are implemented. For this purpose, we will address the experience gained using transcriptomic analyses for personalised medicine in cancer.

Analysis of the transcriptome for personalised medicine in cancer

During the last decade, genome-wide gene expression analysis has become a commonly used tool of the research community. These analyses were performed by use of so-called RNA expression microarrays (also called cDNA microarrays or GeneChips®, depending on the manufacturer). This technology allows simultaneous measurement of the expression levels of large numbers of genes. As with all experiments, the correct setup, including appropriate selection ("input material") and standardised preparation of the samples prior to hybridisation on the microarray as well as consistent post-hybridisation processing (such as washing and scanning of the microarrays) are essential if the findings are to be reliable and reproducible. Further, and in contrast to other classic experiments in the laboratory, the data analysis (including biostatistics and bioinformatics) in microarray expression experiments dramatically impacts on the results [11]. This is especially the case when the number of analysed variables far exceeds the number of samples. Despite this challenge, the novel technology provided a unique opportunity to identify molecular markers that might be used for diagnosis, prognosis or as predictor of clinical outcome. In many cases these markers were not single genes, but were composed of a combined expression pattern of multiple genes. However, most of these so-called multi-gene prognostic and predictive signatures could not be independently validated and were never used in a clinical environment. The MammaPrint® (Agendia) was one of the very few exemptions: this RNA microarray is based on a 70-gene expression signature for dichotomous classification of nodal negative breast cancer patients into low and high risk of distant recurrence [12]. This classification is the basis for deciding whether breast cancer patients will either receive (high risk) or not receive (low risk) chemotherapeutic treatment. This test was cleared by the FDA in 2007 for nodal negative breast cancer patients with tumours smaller than 5 cm and represents, after the AmpliChip450®, the second microarray test approved by the FDA. At present this assay is undergoing further validation in a prospective multi-centre phase III European clinical trial called MINDACT (Microarray In Node negative and 1–3 positive lymph node Disease may avoid ChemoTherapy) in which breast cancer patients with up to three positive lymph nodes are included [13]. In contrast to the MammaPrint® assay, the Oncotype DX (Genomic Health) analyses a 21-gene signature by use of real-time PCR [14]. It is used for prediction of risk recurrence in ER positive lymph node negative breast cancer patients. Based on the expression levels of the genes, the breast cancer patient is classified into low, intermediate or high risk of recurrence. The result is similar to that from the MammaPrint® test: in case of 'low risk', the patient does not receive adjuvant chemotherapy. The meaning of the 'intermediate risk' group is further assessed in the large clinical trial called TAILORx (Trial Assigning IndividuaLised Options for Treatment (Rx)). Interestingly, the gene lists of these two tests (MammaPrint® and Oncotype DX®) which have a very similar aim, only share one gene. However, both tests interrogate the same three pathways that chiefly impact on breast cancer outcome to a large degree: proliferation, ER and HER2 [15]. Other commercially available tests for prediction of clinical outcome are the Theros Breast Cancer IndexSM (BCI) (Biotheranostics) and the MapQuant DX by Ipsogen (reviewed in [16]). The Theros BCI is also used for risk prediction in ER positive patients, by the use of a combinatorial assay of a 2-gene ratio comprising the genes *HOXB13* and *IL17R1*, and a molecular grade index composed of the expression of five genes [17]. Similar to the Oncotype DX, the Theros BCI uses real-time PCR and allows for the use of FFPE material. The MapQuant DX is an Affymetrix microarray based assay with the aim of reclassifying histological grade 2 tumours into grade 1 or grade 3. This gene signature comprises 97 genes, proliferation genes being the main component [18].

In breast cancer, gene expression microarray studies have also been used to define the so-called intrinsic molecular subtypes. The most prominent was published by Perou and Sorlie, and classifies breast cancer samples into four different subtypes with a distinct clinical outcome [19–20]: one chiefly ER positive group, called luminal; and three chiefly ER negative groups, called basal-like, HER2 and normal-like. Later on, the luminal group was further divided into luminal A and B. Although the existence and prognostic significance of these groups has been confirmed by others, this classification has proved of little use in a clinical setting since its discovery did not lead to reconsideration of the existing or development of novel therapeutic strategies. At present stratification of breast cancer samples into these molecular subtypes is controverted. In recent studies, up to half of the HER2 clinically positive cases (as ascertained by immunohistochemistry and FISH) could not be assigned to the HER2 group [21]. However, higher concordance is achieved if subtype classification models (SCMs) are used instead of the above mentioned prediction models, which are based on hierarchical clustering [22]. Further, none of the microdissected breast cancer specimens with more than 90% tumour content could be assigned to the normal breast-like group, suggesting that the existence of this group might be an artifact due to contamination with normal tissue [21, 23].

Similar to breast cancer, several distinct molecular subtypes with prognostic significance have been identified for lung cancer. There was very little correlation between the different gene expression signatures used for the classification. In a recent report, Subramanian et al compared the 16 most relevant studies in non-small cell lung cancer (NSCLC) from 2002 until 2009 and concluded that none of the studies succeeded in showing improvement in predictive power over and above known risk factors [24]. Further, the authors conclude that most of the studies contained serious problems, such as unfocused study design and inappropriate data analysis, and that much more attention has to be given to statistical validation and re-

producibility [24]. However, the discovery and approval of targeted therapeutics in lung cancer, such as gefitinib and crizotinib for *EGFR* mutated and *EML4-ALK* translocated NSCLC, respectively, and *BRAF* mutation in melanoma, has shifted the attention from gene expression signatures/studies towards the development of novel therapeutic strategies based on the presence of genomic aberrations.

Genomic aberrations as predictive markers

The technological advance in cancer genomics

In 1914, Theodor Heinrich Boveri first hypothesised that cancer could be a chromosomal disease [25–26]. Nearly 100 years later, it is generally accepted that cancer is caused and driven by a sequential accumulation of genomic changes (so-called mutations) in cancer-relevant genes [27–31]. Analogous to the Darwinian evolutionary process, cancer development is thought to be based on the acquisition of genetic variability by mutations followed by the natural selection that acts on the resulting phenotype (reviewed in [32]). These mutations may affect only one or more nucleotides (so-called small scale mutations) or even larger segments with an effect on the structure of a chromosome, such as is the case for deletions, amplifications and translocations [32–35]. The effect on the protein level of genomic mutations, such as deletions, insertions or substitutions, can be multifaceted. Most of the detected mutations (so-called silent mutations) have no effect on the protein level; others can lead, for example, to a truncated protein with an aberrant function or to a constitutively active protein. Up to the present, thousands of such genomic aberrations have been reported and listed [36]. However, the impact of these mutations on cancer development has not yet been completely elucidated. For this purpose, it was intended to classify each somatic mutation in a cancer cell genome either as a driver mutation (i.e., a mutation that is causally implicated in oncogenesis and has been selected for) or a passenger mutation, which is not causally involved in cancer development (reviewed in [32]). This classification and the estimated number of driver mutations required for cancer development have been challenged and are the subject of controversy in the literature [37–39].

Until the technological advances in recent decades, the findings of genomic aberrations with clinical (predictive) impact were chiefly restricted to haematological malignancies. This was mainly due to the restrictions imposed by the analyses that were required for the discovery of novel structural chromosomal aberrations: classic cytogenetic analyses, such as karyotyping, depend on the availability of high-quality preparations of metaphase chromosomes. In epithelial tumours the chromosome morphology required for metaphase analysis is often poor, yielding only partial and poor-quality karyotypes. It is thus not unexpected that the first and most prominent example of a predictive genomic aberration in cancer applies to chronic myelogenous leukaemia (CML): the discovery (1960) and description (1973) of the Philadelphia chromosome, a reciprocal translocation between chromosome 9 and 22 [40]. This translocation results in the *BCR-ABL* gene fusion that leads to the expression of a chimaeric protein, BCR-ABL tyrosine kinase. In 1996, Druker et al demonstrated the use of a specific inhibitor (hereafter known as imatinib) against the kinase activity of this BCR-ABL protein [41]. Nowadays this translocation is detected by fluorescence in-situ hybridisation (FISH) or polymerase chain reaction (PCR) and used as a predictive marker for treatment of CML patients with imatinib mesilate (Glivec®). This exemplary case is regarded as a milestone in personalised medicine. The discovery of the *ERBB2* (*HER2*) gene amplification with overexpression of the protein is an analogous milestone in epithelial cancers. In 1989, Hudziak et al. first described the monoclonal antibody 4D5 against the extracellular domain of the HER2 protein. This antibody was later modified and is now known as trastuzumab [42]. Testing for this aberration to select breast cancer patients for treatment with trastuzumab is nowadays diagnostic routine.

The discovery of novel genomic aberrations in carcinomas notably increased with the development of comparative genomic hybridisation (CGH) in 1992 [43]. This technology obviated the need for metaphases from tumour material by using fragmented and fluorescent-labelled reference (normal) and sample (tumour) DNA, followed by competitive hybridisation to normal metaphases. This procedure allowed the detection of DNA copy number gains (amplifications) and losses (deletions) with a resolution of 10 Mb. This method was then further developed by hybridisation of the labelled DNA fragments on microarrays instead of normal metaphases. The current generation of high-resolution microarrays contains up to one million different oligonucleotide probes, each of them specific for a region in the genome. This technology allows the detection of gene copy number transitions and therefore the detection of deletions and amplifications at a resolution of only a few kb. However, resolution at a single base level can only be achieved by sequencing. In the last few years, several companies have developed instruments of the so-called second generation of sequencing. In contrast to the classic Sanger sequencing, this technology (also known as next-generation sequencing (NGS) or massive parallel sequencing) allows the parallelisation of the sequencing, thereby producing millions of sequences in a single run. A further advantage of this technology is its versatile area of applications: whole genome, full exome, transcriptome or even the microRNAome can be efficiently sequenced. The dramatic reduction in costs for this previously very expensive technology has led to its widespread use. Nowadays, companies offer whole-genome sequencing services for less than USD 5,000, which has led to a dramatic increase in the number of ongoing genomic profiling studies as well as in the number of cancer samples used in these studies. Indeed, the Cancer Genome Atlas and the International Cancer Genome Consortium have started to sequence hundreds of genomes per cancer type. The bottleneck of the generated whole genome profiles is not only bioinformatic and biostatistical analyses, but more and more their biological interpretation. The whole genome sequencing of a smoker's primary non-small cell lung cancer (NSCLC) and a cell line derived from a melanoma revealed 50,000 and 33,000 somatic mutations respectively [44–45]. Although these examples are extreme, since both samples originate

from tumours that have been exposed to mutagens for a long time, they are intended to illustrate the complexity of such genomes and the challenges that have to be faced in reliably identifying driver mutations.

Increasing number of predictive genomic aberrations in solid tumors

In the last few years several genomic aberrations have been identified that are regarded as clinically relevant, since their presence influences drug treatment. The US Food and Drug administration (FDA) lists 22 medicaments used in oncology whose label section includes pharmacogenomic information [46]. In lung cancer, especially NSCLC, major advances have been made in selecting patients for novel targeted therapeutics. The most recent example is the approval of crizotinib, a small-molecule dual inhibitor against the kinases of the proteins MET and ALK. This inhibitor has recently been approved for patients whose tumours harbour an *ALK* rearrangement (chiefly *EML4-ALK* gene fusion). Further, EGFR inhibitors, such as gefitinib and erlotinib are chiefly effective if the EGFR protein is constitutively active due to a mutation in the *EGFR* gene. However, many *EGFR* mutated lung cancers become resistant to EGFR inhibitors because of additional genomic aberrations, such as the secondary T790M mutation of the *EGFR* gene or *MET* gene amplification

Many of the genes conferring either sensitivity or resistance to specific therapeutics are components of the PI3K or the RAS/RAF pathway. This is not surprising, since these interconnected pathways are downstream of the (trans)membrane receptors, i.e. inhibition of an upstream protein cannot be successful if a downstream kinase is constitutively activated due to the presence of a mutation. This is for example the case of the *KRAS* mutation, which has also been found in up to 40% of colorectal cancers [47–48]. Similarly to lung cancer, patients with colorectal adenocarcinomas harbouring this mutation do not respond to anti-EGFR therapies. The comparable mechanism is observed with the *BRAF* gene: usually, patients whose gastrointestinal stromal tumours of the stomach (GISTs) harbour specific mutations in the *CKIT* or *PDGFRA* gene are subjected to therapies involving the kinase inhibitors imatinib or sunitinib. Again, however, this therapy is only effective if the *BRAF* gene does not harbour the V600E mutation. Intriguingly, this activating mutation has become a success story in malignant melanoma. Approximately two thirds of these tumours harbour this activating *BRAF* mutation. In a recently published clinical study, Flaherty et al. demonstrated that the majority of melanoma patients with this mutation (V600E) responded to vemurafenib, an inhibitor specific for the serine-threonine kinase of the BRAF protein [49]. However, resistance to BRAF inhibitors emerges, and in up to 30% of the patients treated secondary tumours (non-melanoma skin cancers) have been described. Very recently it was reported that the majority of these secondary tumours (21 out of 35 in this particular study) harboured *RAS* mutations, predominantly affecting the *HRAS* gene [50–51]. Further, up to 25% of malignant melanomas harbour an *NRAS* mutation. In most of the cases this mutation leads to constitutive activation of the RAS signalling pathway. Thus far, however, no clinical trials with NRAS inhibitors have been started.

These examples illustrate the complexity of personalised cancer medicine. Depending on the site of the mutation, a mutated gene can either be interpreted as a negative or a positive predictor for a specific targeted therapeutic. It is noteworthy that the above mentioned mutations were all discovered with the first generation of sequencers, most of them even before the human genome was sequenced for the first time. In the meantime, hundreds of cancer genomes have been sequenced and even more will follow [42]. It will be exciting to observe how this wealth of data and results can be translated into clinically relevant information.

Outlook and concluding remarks

The discoveries from the genomic analyses conducted in the last two decades now impact daily on diagnostic routine. For some tumour types, such as NSCLC, standard diagnostic procedure requires the analysis (sequencing) of several genes (hotspots) to determine the optimal treatment. Findings from large scale sequencing projects will sooner or later find their way into the diagnostic departments. Further, many recurrent mutations that are now being discovered are present at low or very low prevalence (1–5%), but in different tumour types. These facts will prompt increasing demand for sequencing requests in the near future, and thus it is not surprising that most of the manufacturers of the second generation of sequencers (NGS) are now offering bench-top sequencers, such as GS Junior (454, Roche), MiSeq (Illumina) or the Ion Torrent system (life technologies). The accessibility of genomic profiling technologies will also impact on the design of clinical studies. In the near future it may be necessary to include whole genome sequencing of cancer samples from patients enrolled in clinical studies, especially if targeted therapeutics are being used. The resulting wave of new molecular data will most probably identify sub-cohorts of patients who specifically benefit from the therapy interrogated. On the other hand, the comprehensive genomic analyses in the context of clinical trials bring new challenges: the increase in data complexity and its analysis may lead to delay in the approval of the drugs. In addition, the traditional way of conducting clinical trials in a stepwise manner (i.e., phase I–III trials) is not suited to small subpopulations of cancers with rare but well-druggable genetic alterations, calling for new clinical trial concepts.

Similar to other experiments in the laboratory, correct classification and selection of the input material before profiling is a crucial step. Pathology departments with their large archives of formalin fixed, as well as fresh frozen tumour samples are assuming a major role in the research area of personalised cancer medicine and in decision-making on how these technologies are to be implemented into clinical and diagnostic routine [52]. Proper classification of the material followed by macro- or microdissection is a central issue in defining the optimal material and enriching tumour tissue for genomic analyses. Further, tumor heterogeneity, a phenomenon that pathologist observe every day, has its origin at the genomic level: tumours can consist of distinct clonal tumour populations. We and others have recently shown that these clonal populations can harbour

population-specific genomic aberrations [53–54]. Although the technological advances introduced in this review are promising, a test for diagnostic routine must still fulfill one important criterion: it must be applicable to the material that is available in pathology departments. In most patients, cancer diagnosis is made on small, formalin-fixed biopsies or cytological specimens. Performing comprehensive and technically advanced genomic profiling on these specimens remains a major challenge.

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