



# Integrating next-generation sequencing into clinical oncology: strategies, promises and pitfalls

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## ABSTRACT

We live in an era of genomic medicine. The past five years brought about many significant achievements in the field of cancer genetics, driven by rapidly evolving technologies and plummeting costs of next-generation sequencing (NGS). The official completion of the Cancer Genome Project in 2014 led many to envision the clinical implementation of cancer genomic data as the next logical step in cancer therapy. Stemming from this vision, the term ‘precision oncology’ was coined to illustrate the novelty of this individualised approach. The basic assumption of precision oncology is that molecular markers detected by NGS will predict response to targeted therapies independently from tumour histology. However, along with a ubiquitous availability of NGS, the complexity and heterogeneity at the individual patient level had to be acknowledged. Not only does the latter present challenges to clinical decision-making based on sequencing data, it is also an obstacle to the rational design of clinical trials. Novel tissue-agnostic trial designs were quickly developed to overcome these challenges. Results from some of these trials have recently demonstrated the feasibility and efficacy of this approach. On the other hand, there is an increasing amount of whole-exome and whole-genome NGS data which allows us to assess ever smaller differences between individual patients with cancer. In this review, we highlight different tumour sequencing strategies currently used for precision oncology, describe their individual strengths and weaknesses, and emphasise their feasibility in different clinical settings. Further, we evaluate the possibility of NGS implementation in current and future clinical trials, and point to the significance of NGS for translational research.

## FROM GENES TO GENOMES AND GENOMICS

The concept of precision oncology is not exactly a new one. Since the development of hormonal therapies for breast cancer 40 years ago,<sup>1</sup> their efficacy was determined based on the predictive value of specific hormone receptor expression patterns. The development of specific targeted therapies, including trastuzumab for breast cancer<sup>2</sup> or

imatinib mesylate for patients with chronic myeloid leukaemia,<sup>3</sup> was seen as validation of a molecularly targeted approach and initiated the pursuit of target-driven cancer remedies. Enabled by the possibilities of large-scale high-throughput next-generation sequencing (NGS) technologies along with the computational resources and tools to store, process and analyse the data, more powerful methods for the characterisation of individual patients and tumour types became available. Following the first sequenced human cancer genome,<sup>4</sup> NGS led to a better understanding and characterisation of many cancers, resulting in definition of new subtypes, development of biomarkers and establishment of novel therapeutic targets, and culminating in the completion of The Cancer Genome Atlas project (TCGA; <http://cancergenome.nih.gov>).<sup>5</sup> Additional ongoing NGS endeavours include Therapeutically Applicable Research to Generate Effective Treatments (TARGET) for paediatric, and International Cancer Genomics Consortium (ICGC; <https://dcc.icgc.org>) for adult cancers. TCGA and ICGC will generate comprehensive whole-genome sequencing (WGS) data from ~25 000 tumours. Databases such as Catalogue of Somatic Mutations in Cancer (COSMIC) offer curated information on somatic mutations from more than one million tumour samples.<sup>6 7</sup> NGS data from these large consortial research efforts uncovered a number of recurrent genomic aberrations across several tumour types.<sup>8 9</sup> In addition, these studies also identified a ‘long tail’ of rare but in many cases actionable mutations.<sup>10 11</sup> Some of the knowledge acquired from genome and transcriptome sequencing has already been translated into clinical practice and supported the molecular subtyping of breast cancer<sup>12</sup> or identified novel and targetable genetic alterations in lung cancer<sup>13 14</sup> and began influencing our

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understanding of other cancer entities.<sup>15</sup> Higher molecular resolution achieved by NGS made increasingly evident that most tumours harbour complex genomic changes including large-scale chromosomal rearrangements described depending on their origin as chromothripsis<sup>16</sup> or as chromoplexy.<sup>17</sup> In light of intratumoural and intertumoural heterogeneity, it is currently unknown how these various events may influence the response to targeted treatments tailored to single driver mutations. Additionally, besides the identification of classical driver and passenger mutations,<sup>18</sup> the occurrence of weak tumour-promoting mutations during tumour evolution has to be considered.<sup>19</sup> The next important step to practical application will depend on a better functional characterisation of the individual genetic alterations.<sup>20</sup> Comprehensive genomic data thus empower a broad avenue of translational research projects, many of them driven by individual research groups<sup>21–22</sup> but also performed within collaborative efforts such as the Cancer Target Discovery and Development (CTD<sup>2</sup>).

### THE STATE OF CLINICAL TRIALS IN PRECISION ONCOLOGY

Compared to our knowledge and understanding of the cancer genome and its complexity, our current clinical methodologies of implementing precision oncology into clinical practice are lagging behind. Encouraging results from non-randomised pilot studies using molecular profiling other than NGS to assign patients to a molecularly guided therapy<sup>23–24</sup> were followed by case reports on the successful identification of exceptional responders by NGS in molecularly unstratified trials.<sup>25–27</sup> The long-held clinical suspicion that targeting an actionable and highly predictive molecular alteration depends on the cellular context has recently been confirmed in patients with *BRAF* V600 mutations,<sup>28</sup> having connotative implications for the design of basket trials. Additional limitations of a basket trial design have been demonstrated by a phase II study in thoracic tumours (CUSTOM), which accrued only 2 out of its 15 preplanned treatment arms.<sup>29</sup> Rising enthusiasm for precision oncology was partially curbed by the results of the French SHIVA trial, the first randomised, controlled, phase II study using molecular profiling (including NGS) and molecularly matched treatment in patients with advanced solid tumours.<sup>30</sup> While this study may be pitted against a rapid integration of precision medicine into clinical practice, several criticisms were expressed regarding its statistical design, biological rationale and clinical implications.<sup>31</sup> First, this trial was stratified to three therapeutic arms, with the majority of patients receiving either hormonal therapy or mTOR inhibitors, which display only limited activity as single agents outside from their specific indications. Second, the assignment to some of the targeted treatments was based on an inappropriate biological rationale. Third, two recent meta-analyses found that molecularly targeted therapies do indeed lead to better

outcomes when compared with non-targeted therapies. A comprehensive analysis of 570 phase II, single-agent studies showed that those using personalised approach reported better outcomes and fewer toxicities.<sup>32</sup> In addition, when considering registration trials for 58 Food and Drug Administration (FDA)-approved anticancer drugs from 1998 to 2013, the use of a personalised strategy was independently associated with higher response rates and improved progression-free and overall survival.<sup>33</sup> However, the findings of the SHIVA trial underline the need for a better characterisation of molecular drivers before assigning patients to targeted therapy in order to observe a clinical benefit. This result should also prompt a thorough reconsideration of the way clinical trials are conducted and analysed in the era of precision medicine. Innovative developments include pioneering adaptive clinical trial designs based on molecular rather than histological stratification as well as implementing computational algorithms based on the entirety of individual patient data,<sup>34</sup> the latter being supported by comprehensive collaborative efforts such as ICGCmed (<http://icgcmec.org/>) or CancerLinQ.<sup>35</sup> This paradigm shift leads to a unique outlook for precision medicine, in which diverse data (eg, clinical, molecular, pharmacological) are obtained and analysed in real-time, enabling rapid-learning algorithms to perform and learn from countless N-of-1 trials, and quickly extrapolate the obtained results to other patients. Eventually, precision oncology has to be combined with current effective treatment strategies by vigorous clinical testing and verification. Clinical trials need to master the ingrained limitations of precision oncology in targeting rare molecular alterations across different tumour entities. Their strategies include the development of novel matching algorithms,<sup>36</sup> the use of randomised adaptive designs (I-SPY 2, BATTLE-2), targeted NGS gene panels in advanced solid tumours (NCI-MATCH, IMPACT 2) and off-target comparators (NCI-MPACT). Others focus on alterations in a specific tumour type (ALCHEMIST trials and Lung-MAP) or a specific molecular alteration across tumour types (CREATE)<sup>37</sup> to ensure the accrual of sufficient number of patients. Despite these concerted efforts, larger collaborations and data repositories will be needed to address less common histological or molecular subtypes, which otherwise might elude a statistical analysis. One alternative is the retrospective identification of markers of exceptional response through NGS (eg, in National Cancer Institute 'Exceptional Responders' study, NCT02243592), or the prospective accrual and analysis of an even greater number of patients with advanced cancer that have a potentially actionable genomic variant (ASCO sponsored TAPUR trial, NCT02693535). In conclusion, the premise of precision oncology, namely to deliver the right treatment for the right patient in the right dose and at the right time remains to be validated in clinical trials which have to adapt to the changing understanding of tumour biology. We

need to foster novel approaches to precision medicine in order to evaluate our therapeutic algorithms and gather the necessary evidence within longitudinal research programmes.

### TARGETED PANEL SEQUENCING VERSUS WHOLE-EXOME SEQUENCING VERSUS WHOLE-GENOME SEQUENCING

NGS is the technology that makes precision oncology in its current form possible. Based on massively parallel sequencing of DNA with subsequent data processing and sequence alignment, NGS permits the simultaneous analysis of multiple genetic aberrations, including single nucleotide variants (SNVs), small insertions/deletions (indels) as well as copy number variants (CNVs) or complex genomic rearrangements. Although nowadays sequencing-by-synthesis is the predominant sequencing technology in use, multiple technologies and platforms have been developed and are commercially available.<sup>38</sup> The scope of available strategies for cancer sequencing ranges from targeted gene panels encompassing several thousand base calls through whole-exome sequencing (WES) analysis of the ~22 000 human protein-coding genes (40–50 million bases) to WGS across all 3.3 billion bases of the human genome. NGS found its first clinical application in germline testing for known monogenic and rare diseases by targeted panels<sup>39</sup> while it was shown that WES is ideally suited for the diagnosis of suspected novel Mendelian diseases,<sup>40 41</sup> where it shows a diagnostic accuracy of 25–30%.<sup>42</sup> The clinical potential of NGS started to influence oncology as soon as 2011, when the feasibility of integrating WGS, WES and transcriptome sequencing into oncological decision-making was demonstrated.<sup>43</sup> Since then, a plethora of precision oncology programmes and clinical trials using NGS have been developed and all NGS methods have been used successfully in a clinical setting. Here we describe the advantages and limitations of the available NGS

strategies with regard to their usefulness in clinical practice (table 1).

### Targeted panel sequencing

The NGS panel assays allow for a rapid and reliable identification of the most common and defined aberrations for precision oncology and range from panels of 20 to more than 500 genes. Targeted panels rely on amplicon-based or hybridisation capture-based NGS, which show consistent results in detecting SNVs and indels in a range of clinical applications.<sup>44 45</sup> Albeit amplicon-based methods have a simpler workflow, they might not be suitable for analysing larger panels or exomes.<sup>46</sup> Targeted panels offer the advantage of high depth as well as high overall exon coverage (>99%). The depth of coverage represents the number of times a specific base has been sequenced and aligned to the reference genome whereas exon coverage indicates the overall percentage of individual genes (exons) spanned by at least one sequencing read. These two variables are crucial factors for the consistent calling of sequence variations.<sup>47</sup> Most targeted panels have an average depth of coverage of 500× and more, here surpassing WES and WGS applications by an order of magnitude. Insufficient exon coverage in guanine-cytosine (GC)-rich or repetitive regions is less frequently observed in targeted panel NGS and can be resolved by Sanger sequencing. NGS cancer panels thus provide a rapid and cost-effective tool with high accuracy, analytical sensitivity and specificity for the detection of SNVs, indels and selected translocations, and can be quickly adapted in a clinical setting.<sup>48 49</sup> Owing to the higher depth of coverage, they also offer a lower threshold for uncovering intratumoural heterogeneity and changes with low variant allele frequency, which are inherent to many cancer types,<sup>50</sup> albeit only within preselected cancer genes. Obvious limitations of targeted panels include low sensitivity for detecting chromosomal

**Table 1** Comparison of tumour DNA sequencing strategies

	Targeted panels	Whole exome	Whole genome
Pro	<ul style="list-style-type: none"> <li>▶ High depth of coverage</li> <li>▶ Readily standardisable</li> <li>▶ Rapid interpretation for clinical use</li> <li>▶ Low costs</li> <li>▶ Easy clinical implementation</li> </ul>	<ul style="list-style-type: none"> <li>▶ Detection of unknown variants</li> <li>▶ Detection of CNVs</li> <li>▶ Research applications</li> <li>▶ Feasible in clinical routine</li> <li>▶ Low price/performance ratio</li> </ul>	<ul style="list-style-type: none"> <li>▶ Comprehensive assessment of cancer genomes</li> <li>▶ Highest resolution of genomic alterations</li> <li>▶ SNVs in enhancer/promoter and ncRNA regions</li> <li>▶ Decreasing costs</li> <li>▶ Subject to future studies</li> </ul>
Contra	<ul style="list-style-type: none"> <li>▶ Limited, 'peephole' observations</li> <li>▶ Limited value for research</li> <li>▶ Limited assessment of complex aberrations</li> </ul>	<ul style="list-style-type: none"> <li>▶ Not fully comprehensive</li> <li>▶ Lower CNV resolution</li> <li>▶ Amplification or exon capture necessary</li> <li>▶ High bioinformatic effort</li> <li>▶ Demanding clinical interpretation</li> <li>▶ Time-consuming workflow</li> </ul>	<ul style="list-style-type: none"> <li>▶ Uncertain value for clinical interpretation</li> <li>▶ Most expensive</li> </ul>

CNV, copy number variant; ncRNA, non-coding RNA; SNV, single nucleotide variant.

CNVs and complex genomic rearrangements. Since targeted panels examine genes with a known functional role in cancer pathogenesis, they have a higher chance to find clinically relevant alterations, and lower probability to detect unknown or only marginally pathogenic variations. Targeted gene panels also reduce the complexity, duration and costs of bioinformatical and clinical interpretation and can therefore easily be implemented in clinical trial protocols. In fact, nearly all ongoing clinical trials of precision oncology rely on targeted gene panel NGS for the detection of actionable molecular targets. Most targeted panel tests can be performed on formalin-fixed paraffin-embedded (FFPE) tissue<sup>51</sup> which reduces the logistic and ethical burden of performing biopsies to obtain fresh tissue specimen. On the other hand, differences in FFPE sample preparation and processing can have substantial effects on the outcome of NGS. FFPE is known for being a major source of sequence artefacts, for example, by DNA cross-linking or cytosine deamination,<sup>52</sup> which can be dealt with through bioinformatical or biochemical methods.<sup>53</sup> Results from FFPE NGS-based panel sequencing have been shown to match up to results obtained from fresh frozen tissue.<sup>54</sup> Sequencing of whole exomes from FFPE might also be feasible.<sup>55</sup> However, the sample age, fixation protocol and storage method seem to affect the integrity of NGS results from FFPE tissue<sup>56</sup> and should thus be taken into account, especially when interpreting rare or low-frequency SNVs obtained from old FFPE samples. Another factor that should be taken into consideration when assessing targeted panels from tumours without matched normal tissue is the chance of misinterpreting a pathogenic germline variant or reporting a false-positive somatic alteration.<sup>57</sup>

Taken together, targeted gene panels may provide a fast and cost-efficient way to obtain a somatic tumour profile with a reasonable number of actionable alterations for rapid clinical interpretation. These alterations are situated in well-defined cancer-related genes for which a targeted therapy is in many cases available and are therefore ideally suited for the selection and stratification of patients in clinical trials. Several targeted gene panels for somatic characterisation of solid tumours are commercially available, and are best suited for standardisation and validation in a routine laboratory without any significant bioinformatical support. They are limited by their omission of the vast majority of genomic information leading to non-detection of complex genomic aberrations or mutations in genes outside of the preselected panel. Targeted panel sequencing is therefore restricted to 'peephole' observations and offers only limited answers to the existing research questions.

### Whole-exome sequencing

WES targets approximately 1% of the whole genome. As most currently known disease-relevant mutations arise in protein-coding regions, WES can detect up to 85% of disease-causing mutations. Using appropriate exon

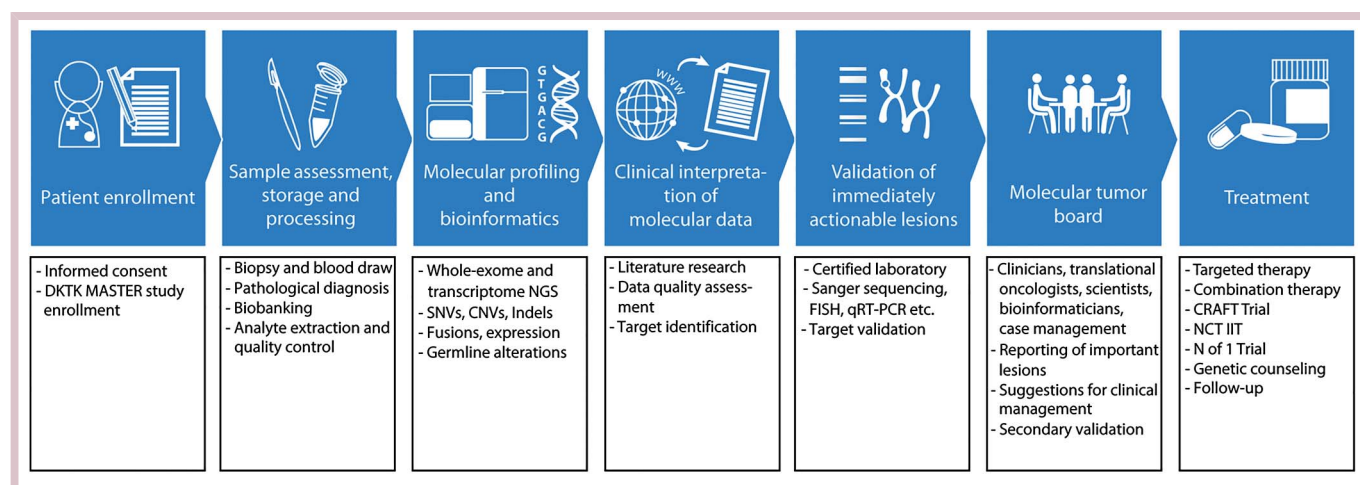
capture and enrichment protocols, WES is becoming less expensive and offers a higher depth of coverage (100–150×) than WGS in up to 95% of exons. This percentage is comparable to and more cost-effective than WGS, leading to WES (often combined with transcriptome sequencing) being used as the main sequencing strategy in some clinical settings.<sup>58</sup> Aforementioned differences between amplicon-based and capture-based methods are more pronounced with regard to WES, and are reflected in their on-target rates and uniformity of coverage.<sup>59</sup> In comparison to targeted panels, the clinical interpretation of WES is more time consuming due to the amount of generated data. Similar constraints for preanalytical sample preparation and preservation as to targeted panel sequencing apply.<sup>60</sup> Nevertheless, WES from FFPE tissue has been shown to deliver results comparable to fresh frozen samples in a clinically relevant timeframe.<sup>55</sup> In brief WES does not offer the same depth of coverage as targeted panels and may completely overlook about 5–10% of the exons.<sup>47</sup> Yet, the overall sensitivity of WES increases greatly with the use of paired germline testing, which can unequivocally discriminate between somatic and germline alterations.<sup>61</sup> Limitations of the clinical utility of WES stem from its high reliance on several technological platforms introducing possible biases without the possibility of rapid extrinsic validation. These range from differences between exome capture methods,<sup>62–64</sup> NGS sequencing platforms<sup>65</sup> to the variability of bioinformatical pipelines.<sup>66</sup> With the availability of WES in a clinical setting, the identification of germline and somatic variants of unknown significance also causes misperceptions among physicians as well as their patients regarding their presentation and interpretation.<sup>67 68</sup> On the other hand, detection of hitherto unknown variants is one of the major advantages of WES over targeted panel-based sequencing as previously unrecognised cancer genes might be discovered, prompting novel associations and hypotheses. Not only does WES provide some limited insights into non-exonic sequences,<sup>69</sup> many exome capture kits nowadays also target miRNAs, untranslated regions and selected conserved non-coding sequences, further increasing the translational value of WES in basic and clinical research. A compromise between targeted panels and WES has been suggested, consisting of reporting only a limited panel of genes from WES for routine clinical use, whereas additional data may be used for research and released on request to clinicians. We believe that the future of precision oncology lies in the detailed molecular characterisation of a large number of patients, and a prospective linking of NGS with individual clinical data. This, however, cannot be accomplished by targeted panel sequencing since the latter does not assess many sporadic genomic aberrations and completely fails to detect novel or unique associations. Owing to its decreasing costs, high reproducibility across experienced centres<sup>70</sup> and a manageable amount of generated data suitable for rapid

clinical interpretation, WES and transcriptome sequencing currently represent a 'gold standard' when it comes to implementing precision oncology in an academic setting. Necessary infrastructure for clinical WES should be readily available at large comprehensive cancer centres, and a clinical sequencing programme can be set up using a designated workflow (figure 1) with a turnaround time of less than 6 weeks from sample processing to clinical interpretation.

### Whole-genome sequencing

In comparison to WES, WGS offers the advantage of being able to uncover changes in regulatory regions such as promoters and enhancers, and detect intronic or intergenic rearrangements. Although WGS currently offers lower depth of coverage than WES, it does not necessitate exon capture or other enrichment strategies, thus introducing less bias in sample preparation and sequencing. At a sufficiently high coverage rate, WGS may even outperform WES in terms of percentage of successfully sequenced exons (exon coverage).<sup>73</sup> Although sensitivity, break-point detection and absolute copy number estimation decline with decreasing depth of coverage, the detection of CNVs with WGS instead of WES results in a much higher resolution.<sup>47</sup> This again stresses the need for a consistent WGS platform, which offers sufficient depth of coverage (average 30–60×) over a majority of genomic regions.<sup>71 72</sup> A perfect NGS method would ideally provide uniform coverage across the genome without any sequence-dependent variation. However, all NGS technologies exhibit inconsistencies (low coverage or missing sequence data) in regions of

high-GC and low-GC content and long tandem repeats.<sup>74</sup> Owing to the sheer quantity of sequence data, this turns into a perceptible source of bias in WGS compared with other sequencing strategies. There are also significant differences between the different WGS platforms regarding their genomic coverage as well as the sensitivity and comprehensiveness of the individual variant calls.<sup>75–77</sup> The identification of variants at low allelic frequencies due to intratumoural heterogeneity or cancer aneuploidy requires a greater depth of coverage,<sup>78</sup> an obstacle which can partly be addressed by bioinformatical methods.<sup>79</sup> Low frequency of some molecular alterations within a sample also brings up the question of their driver status and their actionability, which cannot be resolved by NGS alone given the spatial and temporal limitations of current technologies. Another WGS-specific limitation stems from the prevalence of repetitive regions and occurrence of pseudogenes in the human genome. Short read lengths of NGS often lead to alignment errors<sup>80</sup> and consequently WGS mutation calling pipelines have to be methodically benchmarked and WGS data analysis necessitates the development of specific guidelines.<sup>72</sup> Having said this, WGS is the platform offering the most comprehensive and unbiased examination of the cancer genome and leads to the discovery of novel mutations<sup>81</sup> or classifications. For example, mutational signatures based on WGS and WES have been successfully used to identify possible subsets of patients with gastric cancer who might respond to PARP inhibitors.<sup>82 83</sup> WGS allows for the identification of driver mutations in non-coding regions such as untranslated regions,<sup>84</sup> promoters,<sup>85</sup> enhancers<sup>86</sup>



**Figure 1** DTKM MASTER is an example of whole-exome and transcriptome sequencing-based precision oncology programme. Following patient consent and study enrolment, biopsies are taken and processed in a certified laboratory using standardised protocols and storage methods. Pathological diagnosis and tumour cell content are validated by an independent pathologist. After DNA and RNA isolation and library preparation, NGS on an Illumina HiSeq 2500 platform is performed. Bioinformatical analysis is followed by data curation and validation of putative molecular targets. Following discussion in a molecular tumour board meeting, further enrolment in clinical trials and other personalised treatment strategies are recommended. CNV, copy number variant; FISH, fluorescence in situ hybridisation; NGS, next-generation sequencing; NCT IIT, National Center for Tumour Diseases investigator initiated trial; qRT-PCR, quantitative real-time polymerase chain reaction; SNV, single nucleotide variant.

and other intergenic regulatory sequences associated with carcinogenesis.<sup>87</sup> Structural variants and chromosomal rearrangements resulting in oncogenic fusions may be surprisingly common in some cancer entities,<sup>88</sup> such as prostate cancer,<sup>17 89</sup> and can be efficiently detected by WGS.<sup>90 91</sup> In addition, WGS can reliably detect genomic integration sites of pathogenic viruses, such as human papillomavirus in cervical cancer,<sup>92</sup> which are a possible source of additional genomic instability and copy number alterations. Apart from the identification of variants of unknown significance in the coding regions of the genome, interpretation of WGS is restricted by the lack of knowledge about the functional impact of mutations in non-coding regions or chromosomal aberrations.<sup>10</sup> Furthermore, both WGS and WES may identify mutations in splicing sites, leading to alternative transcripts with unknown and possibly oncogenic functions.<sup>93</sup> Some of the difficulties in assessing the functional relevance of several genomic alterations may be resolved by integrative analysis of the transcriptome. Although it might be too early to embrace WGS in a clinical setting, this strategy provides a solid foundation for comprehensive analysis of cancer and offers novel insights into cancer biology leading to a better diagnosis and therapy in the future.

### Limitations of NGS in precision oncology

Despite the many benefits that NGS-based methods brought about in the last decade, we would like to point out the well-known as well as some less obvious general limitations of all currently used strategies (table 1). First, there are good reasons why NGS will not replace standardised and well-evidenced histopathological diagnoses. All our currently clinically applied diagnostic, prognostic and predictive tools are rooted in the histological examination of the tumour. Although NGS can in many cases help identify and subtype different cancer entities, it should be used in addition and not instead of an accurate pathological evaluation. Second, NGS from tumour biopsies can only assess DNA and RNA changes in a small subset of tumour cells at a given timepoint, thus providing low temporal and spatial resolution of the whole tumour. This problem can be tackled from many different angles, such as by achieving a better spatial resolution through novel techniques,<sup>94</sup> single-cell sequencing, serial analysis of circulating cell-free nucleic acids or tumour cells,<sup>95 96</sup> or by pragmatically focusing on actionability of individual targets via functional studies. Ex vivo functional testing or assessment of circulating cell-free DNA<sup>97–99</sup> might also offer some solutions as to which alterations might be crucial for tumour growth or make a particularly good drug target. It is notable, though, that many of the solutions addressing the limitations of NGS are themselves based on NGS. In addition to the DNA and RNA alterations accessible by NGS, most cancers often engage in post-translational, epigenetic and metabolic adaptations to facilitate their growth and metastasis. Addressing the epigenome,

proteome and metabolome may thus become the next great challenge of precision oncology. Third, development of necessary software tools for analysis and clinical interpretation of 'big data' generated by NGS to support clinical decision-making is still lagging behind the existing hardware equipment for their computation, management and storage. Moreover, large bioinformatical effort is necessary in order to make data obtained on diverse NGS platforms and analysed by different bioinformatical pipelines and algorithms directly comparable. Success of NGS and precision oncology hinges therefore largely on effective communication and professional collaboration between all parties involved.

### TRANSCRIPTOME SEQUENCING

Profiling signatures based on mRNA expression are being rapidly introduced into clinical cancer management. Beyond prognostic molecular classification,<sup>100</sup> recent results of the prospective TAILORx<sup>101</sup> and MINDACT<sup>102</sup> studies successfully validated the premise of mRNA expression profiles as useful clinical tools for predictive therapeutic stratification of patients with breast cancer. These multigene profiling assays use qRT-PCR-based or microarray-based platforms to assess mRNA expression. With regard to the genomic assessment of cancer, measuring gene expression in addition to DNA alterations might prove biologically significant and clinically useful. In the context of clinical cancer genome sequencing, RNA sequencing should be considered and performed in parallel to WES or WGS. Transcriptome sequencing provides synergistic information regarding allele-specific expression, information on transcribed gene fusions and expression levels of cancer-specific genes.<sup>103</sup> Besides mRNA, several non-coding RNA (ncRNA) species including miRNA, siRNA, piRNA and lncRNA can be detected by RNA sequencing, which increases the chances of RNA-based biomarker discovery and development. The downsides of transcriptome sequencing include its requirement for fresh tissue along with high variability of RNA expression levels depending on intrinsic factors such as cell type, cell cycle phase or cell viability. The quantification of RNA is also susceptible to biases stemming from low quantities of cancer cells, heterogeneity within the tumour sample, and inadequate or unattainable baseline expression reference. Transcriptome sequencing adds another layer of data, increases the burden of clinical interpretation, and is not easily standardisable in clinical laboratories. To circumvent some of these difficulties, targeted panel strategies for RNA sequencing have been developed.<sup>104</sup> Nevertheless, transcriptome sequencing provides a deeper insight into the complex cancer biology and will be followed by epigenome, proteome and metabolome assessment.

### Resolving tumour heterogeneity

Even the most sensitive and specific high-throughput assays deliver only a snapshot of the given specimen at a

specific point in the evolution of cancer and patients history.<sup>105</sup> Integrative analyses of large cohorts provide general information about mutation rates and tumour heterogeneity and while offering into clonal events driving cancer progression. Nevertheless, the underlying genetic background and the selective pressure of patients' individual medical histories and treatments invariably lead to much wider heterogeneity than we are able to assess with our current methods, and might be the main cause of treatment failure due to evolutionary adaptation.<sup>106 107</sup> As our sequencing data are based on limited tumour sampling, we should not forget the fact that we are assessing only a small fraction of the individual tumour. Moreover, genomic heterogeneity increases in metastatic disease, and may further evolve by monoclonal and polyclonal seeding as well as metastasis-to-metastasis spread.<sup>108</sup> Owing to the plethora of possible origins for genomic heterogeneity and its far-reaching consequences,<sup>109</sup> it seems likely that precision oncology will have to consider and cope with intratumoural heterogeneity as one of its major limitations. There are some reasons for cautious optimism, though. Mathematical modelling predicts that most cancers harbour 5–8 driver mutations, thus limiting the number of possible (and druggable) culprits.<sup>8</sup> In addition, there might be a limited set of highly essential genes required for human cancer cell proliferation<sup>110 111</sup> although specific differences between different tumour types may exist.<sup>111</sup> The problem of intratumoural heterogeneity may be addressed by various methods. An unbiased systems biology approach may be used to rapidly identify and target possible synergistic and vital cellular pathways. Meticulous dissection of signalling pathways in a differing cellular background will be necessary to elucidate underlying pathogenic mechanisms. Functional genomics may thus help us to understand the multifaceted landscape of tumour-specific alterations with the aim of comprehensively characterising and understanding the heterogeneity of individual tumours. The relentlessly innovative pursuit of answers to research questions raised by NGS-generated 'big data' will not only drive our current and future cancer research efforts, it will also, at some point, influence our daily clinical decisions.

### PERSONALISED NGS-BASED CLINICAL TRIAL DESIGNS

The best clinical evidence in current medical practice was obtained through meticulously designed and thoroughly performed randomised, controlled and preferably blinded phase III clinical trials. In clinical oncology, even statistically well-performed phase III trials have to cope with the schism between statistically significant and clinically meaningful results. With the ascent of precision medicine and the acknowledgment of increasing biological complexity, many current protocol designs are destined to become inadequate. Although many early clinical advances in precision oncology have been made

in entities that are considered to be molecularly homogeneous, it is highly unrealistic to expect most solid tumours to present a single actionable mutation and nicely segregate into subtypes and thus treatment arms.<sup>112</sup> Targeted therapies provide a large number of therapeutic options but in many cases, only a subset of patients will actually respond to targeted treatments, thus trading known common drug toxicities for a very small benefit. Characterising response markers retrospectively and identifying exceptional responders is one possible strategy of precision oncology.<sup>113</sup> However, it does not provide a prospective validation necessary for high-level clinical evidence. Early on, prospective clinical trials using either 'umbrella' or 'basket' designs were developed to study molecularly targeted therapies.<sup>114</sup> The strategy of umbrella trials is to screen for a preselected number of molecular aberrations within a large cohort of patients who are then assigned to a targeted treatment in a follow-up study. Histology-agnostic basket trials focus on specific molecular alterations and enrol patients with same molecular targets to receive the corresponding drug. Successful clinical implementation of basket and umbrella trials as well their limitations have been illustrated recently<sup>115</sup> and will present challenges to regulatory authorities responsible for drug approval.<sup>116</sup> Novel study protocols based on Bayesian statistics were thus developed and include adaptive designs which enable an ongoing modification of the clinical trial based on knowledge acquired through the trial itself.<sup>117</sup> Such efforts are currently being pursued at many institutions worldwide and may be exemplified by the Continuous ReAssessment with Flexible exTension (CRAFT) trial design of the German Cancer Consortium (DKTK). Given the increasing amount of genomic data and resulting combinatorial complexity, we have to venture beyond the conventional basket and umbrella approaches.<sup>118</sup> The complexity of personalised cancer therapy is rooted both in the large number of possibly druggable molecular aberrations due to intratumoural and intertumoural heterogeneity, which results in increasingly oversized screening cohorts, and in the need for rational drug combination and sequencing. However, given the increasing amounts of targeted agents, the number of potential drug combination and conceivable sequential treatments increases exponentially, thus making any a priori stratification for conventional clinical trial design impossible.

A step towards truly personalised clinical trials might lie in the implementation of oncological N-of-1 trials,<sup>119</sup> but this approach encounters scepticism and may not provide high-level evidence in this setting.<sup>120</sup> National and international collaborative projects which plan to integrate genomic data with individual patients' health records, such as ASCO's CancerLinq, AACR's Project GENIE or ICGCmed, all follow a bold assumption which, in theory, should equal an amalgamation of hundreds of thousands N-of-1 trials. Nevertheless, incorporation of genomic, epigenomic, transcriptomic and

proteomic characterisation of tumours will invariably lead to a level of intricacy inaccessible to established trial designs. A simplification of the occurring molecular conundrum might be achieved, for example, by analysing combinations of somatic aberrations and identifying larger networks of key molecular changes spanning different genes, pathways and tumour types.<sup>121 122</sup> Rational inclusion of immunotherapy and drug combinations based on actionable network nodes into clinical trial designs may address the insufficiency of current monotherapies in achieving durable responses. Finally, a better functional knowledge of the underlying tumour biology will possibly enable us to devise novel, rational and adaptive therapeutic tactics to outmanoeuvre the ever-evolving and therapy-evading cancer in future clinical trials.

### THE FUTURE OF PRECISION ONCOLOGY

As the cost of sequencing is predestined to fall even further, sequencing of cancer genomes in a clinical setting will become more common. NGS-enabled precision oncology will add another layer of complexity to our daily clinical decision-making. Looking at the diversity of cancer genomes and their phenotypes, we will have to generate high-quality NGS data and integrate them with histopathological and clinical findings, followed by appropriate clinical trials. Faced with the implications of the data volume exceeding the human cognitive capacity for processing and interpretation in a meaningful and timely manner, the need for rapid-learning computer-based systems in clinical oncology should be acknowledged.<sup>34 123</sup>

The implementation of computer-based self-learning algorithms, such as IBM's Watson Oncology, is currently being tested at some institutions.<sup>124 125</sup> At the other end stands a competent healthcare provider whose role is to implement and carry out the treatment decision as well as face its results together with the patient. While the majority of physicians in large comprehensive cancer centres regularly interpret NGS-based panels and discuss them with their patients, a quarter of them have low confidence in their genomic knowledge.<sup>126</sup> One possibility to evade some challenges of a comprehensive WGS and WES assessment is to select and filter only a predefined set of genomic data to the clinician and the patient.<sup>127</sup> The value of reporting genetic variants with limited clinical significance has to be routinely discussed while guidelines for interpretation and reporting of incidental findings in clinical exome and genome sequencing are being developed.<sup>68 128</sup>

About 150 years ago, the lifework of Gregor Mendel,<sup>129</sup> titled 'Experiments on Plant Hybridization', was published. In this work, Mendel postulated and validated many rules of heredity which enabled the development of genetics as a scientific discipline. After being ignored by the scientific community of the time, Mendel allegedly proclaimed: 'My time will come'. It was not the case until after his death when other scientists began to

incorporate his findings into genetics and subsequently apply them to other fields such as medicine. With the development of NGS and the rapid availability of individual genomic information, implementation and application of genetic data should not be slowed down by ignorance and dismissal. Fortunately, many cancer centres around the world have started trials and programmes of precision oncology. We should embrace this novel paradigm as it will most probably shape and enhance our understanding of tumour biology and cancer therapy in the decades to come. There is an urgent need to evaluate and prospectively validate our most promising approaches to precision oncology. This effort relies on a continued participation and collaboration of clinical oncologists, cancer researchers, computational biologists, bioinformaticians and, most importantly, patients. Seamless clinical implementation of precision oncology needs support from regulatory and ethical authorities, and can only be successful with sufficient resources and long-term commitment from national and international funding agencies.

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