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Cancer Precision Medicine: Why More Is More and DNA Is Not Enough

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Abstract
Every tumour is different. They arise in patients with different genomes, from cells with different epigenetic modifications, and by random processes affecting the genome and/or epigenome of a somatic cell, allowing it to escape the usual controls on its growth. Tumours and patients therefore often respond very differently to the drugs they receive. Cancer precision medicine aims to characterise the tumour (and often also the patient) to be able to predict, with high accuracy, its response to different treatments, with options ranging from the selective characterisation of a few genomic variants considered particularly important to predict the response of the tumour to specific drugs, to deep genome analysis of both tumour and patient, combined with deep transcriptome analysis of the tumour. Here, we compare the expected results of carrying out such analyses at different levels, from different size panels to a comprehensive analysis incorporating both patient and tumour at the DNA and RNA levels. In doing so, we illustrate the additional power gained by this unusually deep analysis strategy, a potential basis for a future precision medicine first strategy in cancer drug therapy. However, this is only a step along the way of increasingly detailed molecular characterisation, which in our view will, in the future, introduce additional molecular characterisation techniques, including systematic analysis of proteins and protein modification states and different types of metabolites in the tumour, systematic analysis of circulating tumour cells and nucleic acids, the use of spatially resolved analysis techniques to address the problem of tumour heterogeneity as well as the deep analyses of the immune system of the patient to, e.g., predict the response of the patient to different types of immunotherapy. Such analyses will generate data sets of even greater complexity, requiring mechanistic modelling approaches to capture enough of the complex situation in the real patient to be able to accurately predict his/her responses to all available therapies.

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Introduction

Advances in next-generation sequencing (NGS) technologies are providing opportunities for genomic precision medicine and the promise of personalised medicine to impact clinical practice in oncology. Personalised approaches, based on the characterisation of an individual's cancer genome, hold potential to reveal clinically actionable molecular targets and are providing new hope for stratifying treatment strategies. These approaches have become a standard feature of cancer diagnostics and treatment. In particular, there is now a large number of NGS-based multi-gene panels available comprising gene variants with relevance to a range of cancers [1]; for example, prognostic or predictive gene panels such as the Prosigna Breast Cancer Prognostic Gene Signature Assay (FDA approved) and OncoDx, for analysis of the activity of genes that can affect how a cancer is likely to behave and respond to chemotherapy.

Although only known and well-characterised biomarkers are included in such analyses, these approaches still only provide a treatment option for patients who happen to carry the selected markers – typically only a small fraction of patients. But even among the patients who carry such markers, again only a sub-fraction will respond due to the fact that additional genetic variants which impact drug response are likely to be present in the tumour or the individual. Cancers are generally multigene disorders; the same gene may be damaged by different individual mutations or epigenetic changes, leading to the same proliferative effect. For the majority of patients, restricting molecular investigations to just a few genomic regions runs the risk of missing important information.

Tumours arise by evolutionary processes from the normal cells of the body, generating cells able to escape the exquisite regulation of cell growth in normal cells. Each tumour is therefore the result of an evolutionary process, and just as in organismal evolution, there are many different combinations of changes that can occur. Tumours therefore differ due to many reasons. They arise in patients with different genomes, from cells with different types of genetic and epigenetic backgrounds, even in the same tissue, usually through random processes. Furthermore, specific changes often occur randomly in only a subset of tumour cells, which propels heterogeneity (with further changes occurring under the selective pressure of the therapy).

To account for inter-tumour heterogeneity and the myriad of different alterations that may arise in tumour cells, a deep molecular analysis is essential to adequately reflect the tumour's complexity. Such a deep molecular analysis is also particularly important when we are dealing with rare cancers (up to 22% of all new cancer diagnoses [2]), or cancers of unknown origin (2% of tumours, [3]), since there is no body of knowledge on likely therapy success, as is the case for the more common cancer types. Transfer of results from one cancer type to another remains a challenge, illustrated by the example of BRAF-mutated non-melanoma cancers [4]. Overall, however, the response rate of cancer patients to the drugs they receive is on average lower than for any other disease area [5], illustrating the desperate need for improved knowledge of which tumour patients will respond to which drug.

Results from the recently concluded MOSCATO 01 trial, which investigated the utility of high-throughput gene sequencing for improving clinical outcomes for advanced/metastatic cancer patients, revealed that an improved progression-free survival (PFS) ratio could be achieved in a third of patients treated with a targeted therapy [6]. Furthermore, the MOSCATO trial relied on screening at the DNA level only, and did not consistently integrate RNA information, which would have leveraged the number of cases showing medical benefit from an integrative precision medicine approach. These results are comparable to other retrospective analyses, including one meta-analysis that showed the potential benefit of a precision medicine approach [7, 8]. However, it should be noted that the only randomised trial to date failed to show the benefit of a targeted approach as compared to treatment according to the doctor's choice [9], even when a third of the patients crossing over to targeted treatment exhibited an improved PFS ratio [10].

Although the precision oncology approach still remains an unproven hypothesis [11], existing data suggest that benefits can definitely be achieved for a subset of patients. Since this proof-of-principle has been conducted, the extension of these benefits to a larger population still remains to be achieved, but as we argue here, deeper molecular analyses will have to be part of this process. An improvement in molecular diagnostics is an inevitable part of this quest to improve patient outcome.

As sequencing costs fall [12], more inclusive approaches to analyse cancer patients, comprising a deeper analysis on the genomic and transcriptomic levels, e.g. whole exome and whole genome sequencing and including both patient and tumour, are now feasible, with the scope to provide more accurate and comprehensive information on an individual's cancer, and offering a potentially more powerful precision medicine approach.

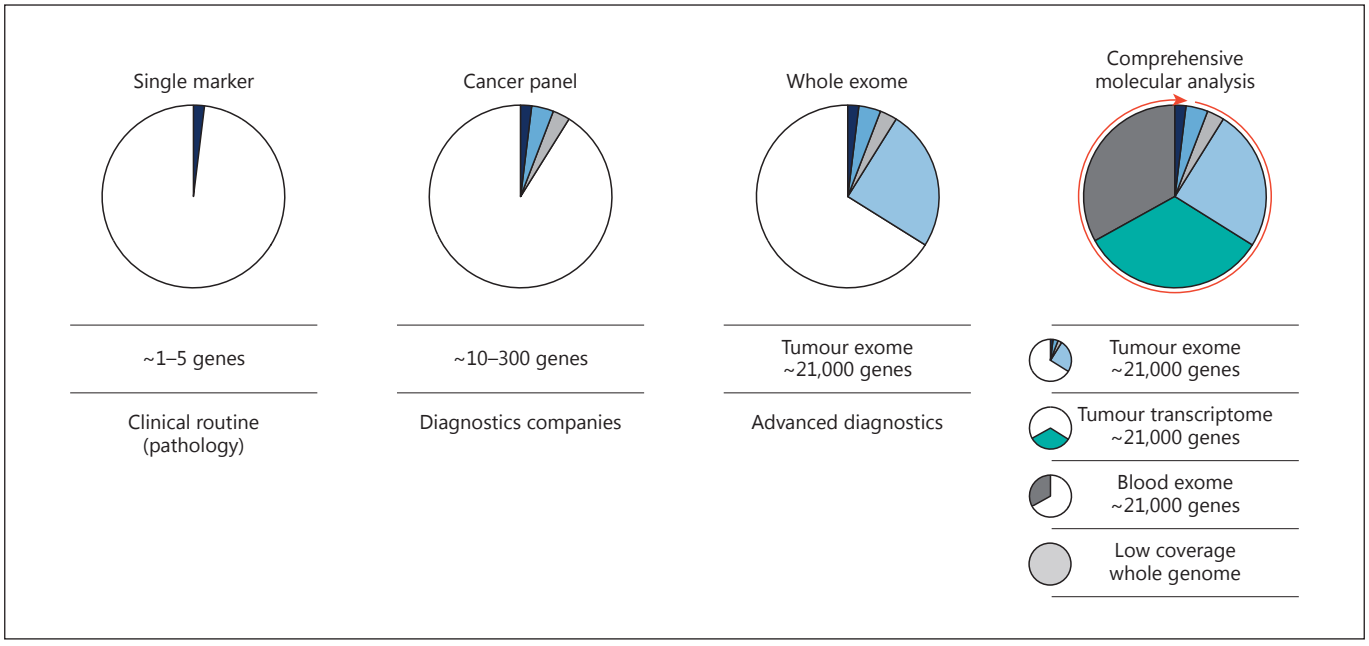

Fig. 1. From single markers to comprehensive molecular analysis. Schematic comparison of the range of the different approaches for molecular characterisation of tumour and patient. The approaches taken span a continuum from sequencing a limited number of tumour genes (a gene panel) to analysis of the whole exome to combined analysis of both patient and tumour using both genome and transcriptome information.

But what are the real benefits of such a comprehensive approach? Do we actually gain more relevant information through deeper analysis of both the patient and their tumour?

Here, we evaluate the therapy-related information obtained from analysis at these different levels, considering a range of commercially available NGS panels with targeted gene sets for detection of cancer-relevant gene variants. A comparison of information gained is made to that generated by a comprehensive molecular analysis encompassing both patient and tumour (deep exome of tumour and patient in combination with deep transcriptome of the tumour and additional low-coverage genome analysis).

Is More, More?

While the term precision medicine implies a molecular analysis of tumours, the depth of this analysis can vary quite significantly, extending from the testing of a few genetic alterations (e.g., KRAS, BCR-ABL1 translocation, BRCA1/2, BRAF V600E), through sequencing of the coding regions of a smaller or larger number of genes within "cancer panels" or analysis of the whole exome (the coding regions of the genome) of the tumour and/or patient, to what we consider as the currently optimal comprehensive molecular tumour analysis: a very deep molecular characterisation of both patient and tumour, comprising deep whole exomePlus (exome and known regulatory/cancer-relevant regions/) plus low-coverage whole genome, allowing more sensitive detection of copy-number changes (CNVs) and regions of loss of heterozygosity (LOH) than exome analysis alone, plus (very importantly) deep transcriptome sequencing of the tumour (Fig. 1).

The identification of gene variants with diagnostic, prognostic, or treatment response relevance (biomarkers) has undoubtedly revolutionised cancer research and care, providing a means of stratifying patients who carry these markers. Testing for a RAS mutation has become standard of care in colorectal cancer and guides the use of EGFR inhibitors as part of routine clinical practice [13]. Multi-gene panels, with focused or customised sets of gene variants provide an extended scope in comparison to single gene markers, ranging from tens to hundreds of genes. The extended gene set provides a higher coverage of disease relevant variants without high costs and analysis demands, and the targeted nature of the gene set af-

fords the possibility of sequencing at high coverage, ensuring high-confidence variant detection and detection of mutations only present in a smaller subset of the tumour cells. Nevertheless, panels are by and large insufficient to address the complexity of the tumour, potentially missing changes that may be critical for determining the optimal treatment strategy for patients. Mutations in EGFR, for example, indicate sensitivity to EGFR therapies in lung cancer, and secondary alterations, such as overexpression of MET or *EGFR* T790M mutations, have been described to induce resistance [14].

As we increase the scope of the analysis and move beyond panels to whole exome and whole transcriptome sequencing, further information on the individuality of a tumour is gained. Sequencing the exome provides a knowledge baseline that covers the gene variants within the most well-characterised (~2%) fraction of the genome. Moreover, approximately 85% of mutations causally linked with diseases are located in the exome [15]. Deep whole-exome sequencing therefore reduces the risk of overlooking cancer-relevant genomic alterations in the tumour. Low-coverage whole-genome analysis in addition to whole-exome analysis, provides a sensitive means for the detection of copy-number alterations, especially focal deletions and regions of LOH, which is superior to whole exome analysis but still at affordable sequencing costs. Complete genome sequencing at the 100× or higher coverage required (due to the relatively low purity and the heterogeneity of many tumour samples), could in theory replace the combination of low coverage genome and deep exome for the analysis of the tumour and patient. However, this approach is currently still not cost-effective, considering the lack of easily identifiable relevant variants in the genome outside of the coding and known regulatory regions. As sequencing costs drop, this is likely to change – even in the medium term – considering the development of new sequencing instruments (e.g., by Illumina) that are set to propel us into the era of the 100 dollar genome within the next 3–10 years (see www.illumina.com).

Even if we broaden the scope of the analyses then do we still have enough information to identify relevant disease-associated variants? If exome analysis is restricted to only tumour sample(s), genetic changes can be identified through comparison to the reference human genome. However, the human reference genome represents an average genome with polymorphisms whose allele frequencies are known within different populations. When the specific personal genetic background of the patient is unknown, it is challenging to discriminate between tumour-specific variations and germline polymorphisms (i.e., the patient's genome). A complex mixture of genetic information is generated that impedes high-confidence selection of tumour-specific variants, with impacts for identification of actionable variants (i.e., those variants associated with known targeted therapy and/or patient prognosis and/or response to any therapy), potentially compromising the success of treatment. The inclusion of a non-tumour control exome (from the same patient) used as the personal reference baseline, allows discrimination of somatic and germline mutations, greatly reducing the risk of misinterpreting the results [16]. In the study by Jones et al. [16], the authors evaluated the potential to misinterpret germline alterations as somatic when conducting tumour only testing, using a 111-gene panel and exome sequencing of a set of 815 tumour-normal pairs from 15 different tumour types. This analysis revealed a false-positive discovery rate of 31 and 65%, respectively. They also reported that in 3% of patients, a change was suspected as somatic but in fact was a germline alteration in a cancer-predisposing gene. Not analysing a patient's personal genome can therefore lead to misreading germline variants as somatic, potentially compromising the interpretation of the tumour sequence data. On the other hand, it is clear that additional analyses will be needed to understand the functional implications of each variant found in the tumour or of rare damaging variants in the personal genome. Furthermore, genetic counselling must be offered to the patient in case of incidental findings related to heritable predispositions.

Multiple genetic changes occur within tumours as a result of genomic instability or external environmental factors such as UV-radiation or mutagens in cigarette smoke, which may contribute to cancer initialisation or/and progression. We need to know whether the changes exhibited at the DNA level are also having an effect on the function of a particular gene(s). Sequencing of the tumour transcriptome (e.g., RNAseq) enables identification of those cancer gene variants that are actually expressed in the tumour cells and will therefore be most likely to affect cellular functions [17]. Gene expression levels and transcriptome profiling also provide insight into the activity of prognostic, diagnostic, or pharmacogenomic marker genes, equivalent to immunohistochemistry-based assays, which help doctors to better understand the pathology of an individual tumour or even classify a cancer of unknown origin. Most importantly, without transcriptome analysis of the tumour, we completely ignore the effects of epigenetic and other regulatory processes. Recessive oncogenes can, for example, be

functionally inactivated by epigenetic processes (e.g., promoter methylation) as well as by deleterious mutations. Activation of pathways can drive tumour development even if no "classical" mutations are detectable, and specific alternative transcript forms in cancer genes, such as oncogenic isoforms (e.g., ALK^{ATI} [18]) formed by alternative initiation site usage, alternative splicing or RNA editing [19], can significantly affect the biology of the tumour. Many forms of cancer are associated with gene fusions promoting tumorigenesis. Gene fusions typically result from chromosomal translocations, deletions, or inversions but, for technical reasons, those cancer-relevant events are usually much easier to identify in RNAseq experiments (e.g., [20]).

Transcriptome analysis offers many advantages in the field of personalised medicine. Recent advances have shown that gene expression signatures can be powerful prognostic or diagnostic tools. Such signatures have, for example, been derived for predicting response to standard therapies in colorectal cancer [20] and for stratifying breast cancer patients [21].

A recent hope for therapy breakthrough has been reflected by the developments and successes in immunotherapy now regularly administered in cutaneous melanoma and lung cancer. However, response rates to these treatments are still highly variable and associated costs extremely high, necessitating the use of predictive biomarkers. PD-L1 expression has been weakly associated with response, but even patients lacking PD-L1 expression can be responsive to immunotherapy (in melanoma) [22]. In certain tumour types, samples tend to carry a high mutational burden with a hypermutator phenotype induced by external sources such as UV radiation or by loss of DNA damage response components, such as microsatellite instable tumours (MSI-high) in colon cancer. MSI-high tumours have shown excellent response to immunotherapy, likely due to the fact that the high mutation load generates neoantigens that mark the tumour cells as foreign, to then be targeted by the activated immune system [23]. Potential biomarkers for immunotherapy response include the use of mutation load and gene signatures to improve prediction outcome [24]. The combination of genome and transcriptome information represents an added value that allows assessment of many types of biomarkers, again showing the strength of an integrative approach.

As sequencing costs continue to drop, it is less and less justifiable to base the treatment of cancer patients on partial, superficial information. A combined analysis using genomics and transcriptomics enables derivation of highly detailed molecular information on somatic and germline variants, structural variants, and expression deregulation, providing orders of magnitude more information than other methods available. The combination of DNA and RNA-based analyses complement each other, serving as mutual controls for verifying potential findings [17] and increasing the sensitivity of variant detection, a feature especially important for analysis of low-purity tumours [25]. In particular, identification of variants affecting RNA processing, eventually leading to oncogenic isoforms (e.g., EGFR viii/ERBB2 ΔEx16), is close to impossible using genome-based analyses in isolation [26], since the effect is only visible when considering the gene structure, as determined by RNAseq. Similarly, potential effects of RNA editing are inherently only detectable by RNA-based analyses. In one study, genomic amplifications were observed for the *ALK* oncogene in 10% of the non-small cell lung cancer patients tested, but none of them were positive for ALK expression, as assessed by immunohistochemistry [27]. Thus, without information on the transcriptome, ALK inhibitors may be erroneously considered as a therapeutic option.

Detection of Cancer-Relevant Alterations: A Comparative Analysis

More sequencing should equate to more data, but is this clinically relevant? To further substantiate the power of the different approaches, we evaluated their ability to detect cancer-relevant variations using published data for 20 tumour types from two large cancer genome characterisation studies: the Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC). This selection of 20 cancer types across several tissues provides the highest overlap among standard molecular cancer data analysis, comprising somatic mutations, gene expression, gene fusion and CNV data, for a total of 3,736 donor tumours. For this comparative analysis, we assessed the target regions of widely used commercially available cancer panels: the Agilent ClearSeq Comprehensive Cancer panel, the Illumina TruSight Tumor 170 cancer sequencing panel, the IonTorrent Ion AmpliSeq™ Comprehensive Cancer Panel, and the FoundationMedicine One test, as well as whole-exome sequencing and whole-exome and transcriptome sequencing (Table 1). The data were processed as follows: Structural rearrangements were only considered when both breakpoints affect genes, likely resulting in gene fusions. Gene fusion data were downloaded from http://54.84.12.177/PanCan-

Table 1. Overview of genetic alterations reported in the 20 TCGA/ICGC cohorts and matched to the target regions of the different panels/tests evaluated

Panel/test	Gene affected by	Total number	Cancer-relevant genes	Clinically relevant genes
ClearSeq comprehensive cancer panel	Mutation	7,553	5,959	4,788
– Mutations in 151 genes	Amplification	0	0	0
	Deletion	0	0	0
	Gene fusion	0	0	0
	TSG downregulated	0	0	0
	Oncogene upregulated	0	0	0
Ion AmpliSeq comprehensive cancer panel	Mutation	16,624	11,821	5,267
– Mutations in 409 genes	Amplification	0	0	0
	Deletion	0	0	0
	Gene fusion	0	0	0
	TSG downregulated	0	0	0
	Oncogene upregulated	0	0	0
TruSight Tumor 170 cancer panel	Mutation	6,962	6,219	4,996
– Mutations in 151 genes	Amplification	306	253	166
– Fusions in 55 genes	Deletion	0	0	0
– Amplifications in 59 genes	Gene fusion	482	216	149
	TSG downregulated	0	0	0
	Oncogene upregulated	0	0	0
FM_{one}	Mutation	12,704	10,008	5,427
– Mutations in 315 genes	Amplification	963	639	190
– Amplifications in 315 genes	Deletion	2,221	1,555	665
– Deletions in 315 genes	Gene fusion	135	135	117
– Fusions in 28 genes	TSG downregulated	0	0	0
	Oncogene upregulated	0	0	0
Whole-exome sequencing	Mutation	352,908	19,934	5,657
– Mutations in 21,000 genes	Amplification	129,632	1,854	195
– Amplifications in 21,000 genes	Deletion	150,225	3,328	670
– Deletions in 21,000 genes	Gene fusion	0	0	0
	TSG downregulated	0	0	0
	Oncogene upregulated	0	0	0
Whole exome + transcriptome	Mutation	352,908	19,934	5,657
– Mutations in 21,000 genes	Amplification	129,632	1,854	195
– Amplifications in 21,000 genes	Deletion	150,225	3,328	670
– Deletions in 21,000 genes	Gene fusion	18,562	1,225	266
– Expression of 21,000 genes	TSG downregulated	131	131	25
	Oncogene upregulated	8,375	8,375	983

Cancer-relevant genes were defined using the Cosmic Cancer gene census (http://cancer.sanger.ac.uk/census) and its annotation for oncogenes and tumour suppressor genes (TSG). Clinically relevant genes were defined according to the most recent BROAD Institute Target file (as of 14th February, 2015). See main text for criteria used for defining alterations.

FusV2/ for 14 cancer types. Data were further processed selecting only coding mutations, CNVs as amplifications if copy-number was larger than 6 or *log*(segment mean) >3, and as deletions if copy-number = 0 or *log*(segment mean) < (–2). Gene expression data were considered per tumour type and significantly up-/down-regulated genes were defined by a per gene z-score of >3 or <–3, respectively. As a null model, we assumed that each test identifies each of the reported alterations with 100% sensitivity (if the alteration was covered by the test).

Next, we annotated the "*cancer-relevant genes*" (Table 1) using the Cosmic Cancer gene census (downloaded

on March 29, 2017, http://cancer.sanger.ac.uk/census) and its definition of oncogenes and tumour suppressor genes. The identified cancer-relevant genes comprised any alteration found in a gene with reported relevance to cancer; information which provides a comprehensive view of the tumour pathology and that may or may not have directly clinical relevance. The information gained could, however, lead to a different interpretation of the tumour biology and stimulate further investigation that, at some point, could lead to novel treatment routes.

The alterations (e.g., mutations, amplifications, deletions, gene fusions, expression changes) covered by each of the panels/test were surveyed. This initial analysis revealed an increase in the number of alterations in accordance with the scope of the analysis (Table 1). Because our focus here was on those alterations with potential clinical relevance, we also annotated "*clinically relevant genes*" (Table 1), based on the most recent BROAD Target file: http://archive.broadinstitute.org/cancer/cga/sites/default/files/data/tools/target/TARGET_db_v3_02142015.xlsx. As the scope of the analysis increased from the focused cancer panels to the more comprehensive analysis reached by whole exome and whole exome combined with transcriptome analysis, we observed a moderate (~10%) increase in the number of cancer-relevant mutations (Table 1). The addition of transcriptome analysis increased the number of alterations detected in clinically relevant genes by 20%, in comparison to results from genomic analysis only.

How does this relate to the information per patient? We compared the coverage provided by each panel/test in terms of number of clinically relevant alterations per patient (derived from the TCGA and ICGC data sets) (Fig. 2). This analysis revealed that the targeted cancer panels could identify clinically relevant alterations in around half of the patient data sets surveyed, whereas the extended molecular analysis approach (whole exome and transcriptome) succeeded in at least three-quarters of the patients. When assessing all approaches in combination, however, no alterations in clinically relevant genes were detected within a quarter of the patients. It is likely that these patients are suffering from cancers that are driven by alterations that are, so far, not well enough understood or lie outside of the analysed exome and thus are not informative for any clinical action as yet.

Due to differences in the aberrational landscape of cancer types, we then evaluated the distribution of these clinically relevant alterations across tumour types (Fig. 3). For tumours with a high mutational load, the different approaches show a similar fraction of samples with relevant

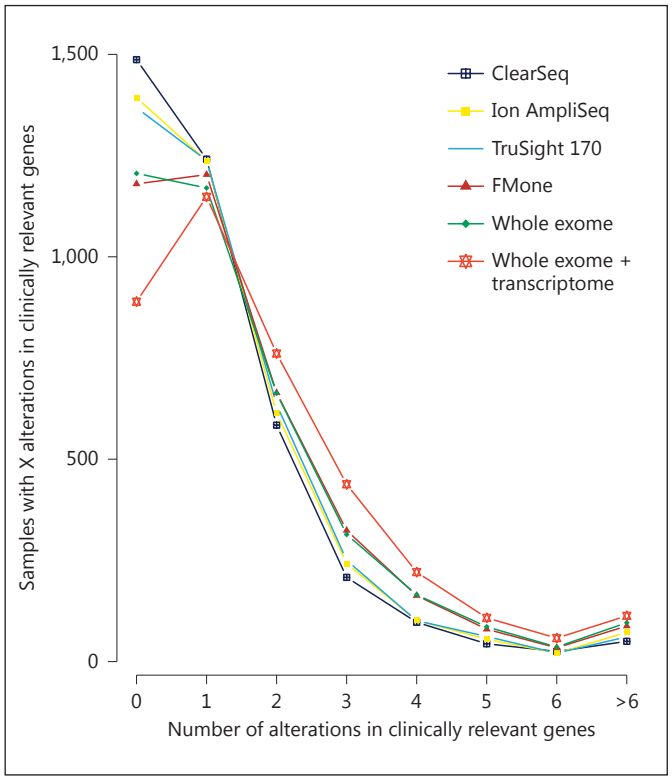

Fig. 2. Distribution of alterations in clinically relevant genes counted per patient. The coverage provided by each panel/test (see key) in terms of number of clinically relevant alterations per patient is depicted. The *x* axis shows the number of alterations in clinically relevant genes. The *y* axis shows the specific number of patients with that number of alterations. Data sets used comprise published omics data on 20 tumour types from two large cancer genome characterisation studies, the Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), totalling 3,736 donor tumours. Alterations covered include somatic mutations, gene expression, gene fusion and copy-number variation data.

alterations. For those cancers, the drivers are typically somatic mutations such as *KRAS* in colorectal adenocarcinoma (COAD-US and READ-US, [28]) or frequent mutations in *PTEN* or *PIK3CA* in uterine endometrial carcinoma (UCEC-US [29]). In such cases, panels are informative for a significant fraction of the relevant alterations; however, treatment-relevant information or rare events such as amplifications of *ERBB2* or gene fusions in *ALK* [20] are missed by panel sequencing. In terms of ability to detect clinically relevant alterations, analysis of the whole exome combined with transcriptome analysis provided a clear advantage over the other approaches for clear cell renal cell carcinoma (KIRP-US), a carcinoma in which cells

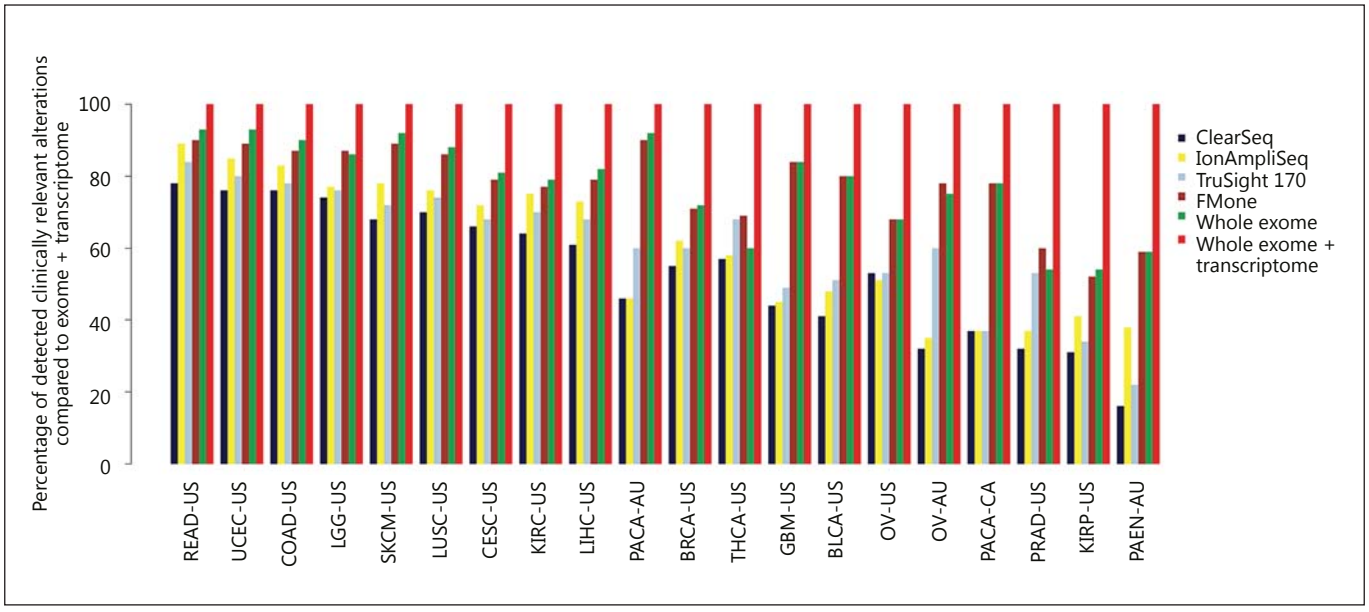

Fig. 3. Distribution of clinically relevant alterations detected per cancer type. Bars show the percent of alterations in clinically relevant genes detected by the different panels/tests as a percentage compared to whole exome + transcriptome analysis. Colours depict the different approaches (see legend). Data sets used comprise published omics data on 20 tumour types from two large cancer genome characterisation studies, the Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), totalling 3,736 donor tumours. BLCA-US, bladder urothelial carcinoma; BRCA-US, breast invasive carcinoma; CESC-US, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD-US, colon adenocarcinoma; GBM-US, glioblastoma multiforme; KIRC-US, kidney renal clear cell carcinoma; KIRP-US, kidney renal papillary cell carcinoma; LGG-US, brain lower grade glioma; LIHC-US, liver hepatocellular carcinoma; LUSC-US, lung squamous cell carcinoma; OV-AU, ovarian serous cystadenocarcinoma; OV-US, ovarian serous cystadenocarcinoma; PACA-AU/PAEN-AU, pancreatic cancer endocrine neoplasms; PACA-CA, pancreatic cancer; PRAD-US, prostate adenocarcinoma; READ-US, rectum adenocarcinoma; SKCM-US, skin cutaneous melanoma; THCA-US, thyroid carcinoma; UCEC-US, uterine corpus endometrial carcinoma.

have been reported to show strong hypomethylation [30]. Interestingly, we observed the highest load of upregulated oncogenes in this tumour entity affecting clinically relevant genes in a third of the patients. Another interesting example is prostate adenocarcinoma (PRAD-US), for which addition of RNAseq doubles the number of alterations detected in comparison to the DNA-based only and panel approaches (Fig. 3). In this cancer type, molecular characterisation did not reveal any clear driver alteration in around 26% of cases, but these tumours are suspected to be driven by as yet unknown abnormalities, potentially reflected by altered expression levels [31]. For the majority of cancer types, the panels were able to detect approximately 50–60% of relevant alterations, whereas the more comprehensive analysis integrating exome and transcriptome analysis identified alterations in clinically relevant genes in 70–80% of cases. However, this still leaves us lacking relevant information on up to half, and at best, a quarter of patients (Fig. 2, 3).

Perspectives

Genomic and transcriptomic sequence analysis is undoubtedly opening up many opportunities for precision medicine. Here, we show that, as expected, an integrative approach using a combination of genomic and transcriptomic sequencing analyses significantly outperforms less comprehensive methods in the identification of clinically relevant changes. It is essential to broaden analysis to include all genes in both the tumour and patient germline, to consider the effects of CNVs and LOHs, and it is very important to incorporate information on the transcriptome; otherwise, we are in danger of missing clinically relevant information. In the analysis conducted here, only half of the changes in clinically relevant genes were detected using panel approaches. A strategy based on a deep molecular analysis of every tumour and patient germline, will be able to identify appropriate targeted drugs in an increasing number of cases, hence providing

attractive options with a chance of achieving medical benefit, further strengthening the case for an overdue switch to a precision medicine first strategy in tumour drug therapy. This would allow us to circumvent, whenever possible, treating patients first with often highly toxic, highly mutagenic chemotherapeutics, which have the potential to interfere with the action of the immune system, a key element in, for example, the natural defence of the body against tumours, and the basis of immune therapy, one of the best anti-tumour tools we have available at the moment.

In identifying more, directly or indirectly, targetable changes with higher accuracy (often already validated in appropriate clinical trials), such a deeper analysis offers the chance to identify additional (and more relevant) treatment options than the more restricted approaches on offer. Deeper knowledge of the biology of tumour and patient will therefore often be able to identify new, potentially life-saving therapies. At worst it will add no new knowledge beyond that provided, at slightly lower cost, with the more limited tools used today on a more regular basis.

Although the combined approach integrating DNA and RNA analyses provides a more advanced and robust strategy for selecting the optimal treatment for cancer patients, it is clearly not the endpoint in the increasingly detailed molecular characterisation of tumour and patient. For example, inclusion of protein data has the potential to provide additional, relevant information. Proteins and their modifications, including phosphoproteomics data, play a significant role in regulating complex biological processes (e.g., [32, 33]) and therefore will provide key information on the results of post-transcriptional regulation in biological networks (e.g., [34]). Spatially resolved sequencing analysis would allow the identification of intra-tumour heterogeneity and its consideration in the selection of the optimal therapy or combination of therapies. Tumour sub-clones harbour different alterations and therefore might react differently to a given drug, with the potential development of resistant sub-clones. Appreciation of this heterogeneity is highly relevant when it comes to treatment monitoring. Cell-free DNA and circulating tumour cell analyses have been developed in recent years capable of following cancer-specific alterations in blood with high sensitivity. Such approaches enable monitoring of tumour burden and heterogeneity and determination of whether a sub-clone expands due to the selective pressure of the treatment [35].

It is clear that a more comprehensive analysis approach enables the detection of more actionable variants, but it is the translation of this information into better outcomes for cancer patients that will really make the difference. The main challenge resides in the interpretation of the data. While a single known alteration is often easily translated into a treatment decision by the doctor, a typical genomic/transcriptomic analysis will reveal several alterations plus a larger number of previously undescribed alterations of as yet unknown significance. Those data have to be interpreted and prioritised according to relevance, and tailored to the needs of a molecular tumour board. An even more difficult enterprise is the interpretation of several and/or contradictory findings. With advanced complex data, new routes for decision-making must be developed. In this respect, advanced computational approaches can help to judge the right treatment decision. Statistical and correlative approaches using pattern matching and machine learning exploit available data on treatment outcome using a variety of clinical, patient and molecular data to infer potential treatment options for a new patient. These approaches are, however, of a correlative nature and depend on vast amounts of prior data. In contrast, mechanistic models based on pre-existing known information on the biological networks in every human cell (virtual patient models), the molecular data generated for the individual tumour and patient, as well as molecular information on the action and targets of drugs, enable us to process data on every patient as unique, allowing predictions in a situation in which every tumour (and every patient) is different [36, 37]. This approach is extremely powerful as it opens up the possibility of making an assumption not only on drugs that have been used in the past but also on any drug approved for medical use for which the molecular mechanism of drug action is known. The outcome of such an approach will be a quantitative prediction of the effect of each drug or a drug combination on the proliferation of the tumour cell.

We believe that an improved and controlled chain of processes in the mode of tumour analysis will leverage the precision and true personalisation of cancer treatment, starting with a comprehensive molecular analysis, followed by advanced data interpretation using virtual patient models.

Over 140 years ago at the Charité, Berlin, Virchow started the age of cellular pathology. Today, we are poised at the start of molecular pathology as the basis for value-based precision medicine to predict the optimal individual treatment in silico.

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Disclosure Statement

H.L. is founder of and advisor to Alacris Theranostics GmbH, which aims to develop "virtual patient" models for use in therapy choice and drug development. All other authors except D.T.R. are co-affiliated to Alacris Theranostics GmbH.

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