Precision Medicine for Molecularly Targeted Agents and Immunotherapies in Early-Phase Clinical Trials

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ABSTRACT: Precision medicine in oncology promises the matching of genomic, molecular, and clinical data with underlying mechanisms of a range of novel anticancer therapeutics to develop more rational and effective antitumor strategies in a timely manner. However, despite the remarkable progress made in the understanding of novel drivers of different oncogenic processes, success rates for the approval of oncology drugs remain low with substantial fiscal consequences. In this article, we focus on how recent rapid innovations in technology have brought greater clarity to the biological and clinical complexities of different cancers and advanced the development of molecularly targeted agents and immunotherapies in clinical trials. We discuss the key challenges of identifying and validating predictive biomarkers of response and resistance using both tumor and surrogate tissues, as well as the hurdles associated with intratumor heterogeneity. Finally, we outline evolving strategies employed in early-phase trial designs that incorporate omics-based technologies.

KEYWORDS: precision medicine, early-phase trials, immunotherapy

SUPPLEMENT: Pharmacodynamic Biomarkers of Response and Resistance

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Introduction

The overriding principle of precision medicine in oncology is the matching of molecular, genomic, and clinical data with the underlying mechanisms of specific therapeutics to provide more rational and effective anticancer strategies. However, despite the remarkable progress made in the understanding of novel drivers of oncogenic processes, success rates for the approval of oncology drugs remain low with substantial fiscal consequences.[1–3] The chasm between significant discoveries in laboratory-based medical research and the subsequent development of successful therapeutics in the clinic has often been referred to as the "Valley of Death."[4,5] More creative and innovative strategies are clearly required to bridge this gap, and key to this will be the use of novel drug development trial strategies and the incorporation of predictive biomarkers of response earlier in this process.

Here, we review recent technological advances in precision medicine and the use of predictive biomarkers of response and resistance in clinical trials of targeted therapies and immunotherapies. We also discuss the use of tumor and surrogate tissues for biomarker studies and innovative modern trial designs that may accelerate and enhance the process of drug development.

Key Technologies in Precision Medicine

Rapid advances in technology with decreasing costs and improved throughput are now enabling the collection of large amounts of information on different cancer "omic" landscapes. The criteria for the use of omics-based predictors in clinical trials have recently been published by the US National Cancer Institute.[6,7] We develop these recommendations further by proposing how they may be incorporated earlier in the oncology drug development process.

Next-generation sequencing. There are a range of next-generation sequencing (NGS) techniques and platforms currently in routine use in the clinical arena (Table 1). Targeted exome sequencing of a panel of genes for "hotspot mutations" selected according to their relevance to specific cancers is the most common molecular profiling tool utilized at present. There are several advantages with this approach, such as being more cost- and time-effective, as well as requiring more manageable bioinformatic and computational requirements with regard to data storage and analysis.[8]

NGS uses massively parallel sequencing arrays to interrogate DNA coding regions (whole-exome sequencing [WES]) or the entire eukaryotic genome (whole-genome sequencing [WGS]). WGS offers the most comprehensive strategy for

Table 1. Evolution of sequencing technologies showing the advantages and limitations of each strategy.[86–93]

GENOME SEQUENCING METHODS			
TYPE	**METHOD**	**ADVANTAGES**	**LIMITATIONS**
First generation sequencing			
Sanger sequencing	Amplification of specific genes by PCR followed by sequencing based on capillary-based methods. Capture of signal: fluorescence-based imaging.	– Reduced costs. – Poor quality nucleic acid material could be used.	– High DNA input: micrograms – Insensitive to alterations that occur in an allele frequency lower than 20%. – Limited breadth beyond a few genes. – Unable to detect rearrangements or DNA copy number changes.
Next generation sequencing or massively parallel sequencing technologies			
DNA-Seq RNA-Seq ChIP-Seq Methyl-Seq	**Technology** **Amplicon sequencing*: Enrichment of targeted genes by PCR. – Advantages: Requires less DNA input. – Limitations: Potentially can bias the observed allele fraction. Higher bias in calling copy number. **Hybrid capture*: Probes are designed with homology to gene of interest and bind cDNA. – Advantages: More reliable copy number detection. – Limitations: Higher depth of sequencing required. Bioinformatic support required.	– Low cost per sequenced base. – Increased sensitivity and scalability. – High throughput. – Capability to detect multiple type of genome alterations: rearrangements, amplifications.	– Moderate DNA input: nanogram. – Quality of material to be used. – MPS technologies remain highly sophisticated: – Bioinformatic support required. – Urgent necessity of effective archival storage mechanisms. – Assay validation.
	Whole genome sequencing	– Copy number and re/arrangements with 30–60 fold depth of coverage. – Non coding regions could be also characterized.	– Applicability for routine diagnosis still challenging – Archival formalin-fixed paraffin embedded tumour used is problematic. – Amount of information generated without clinical impact.
	Targeted sequencing (whole exome and panel specific) • WES – higher depth of sequence coverage. • Targeted sequencing – higher depth of sequence coverage.	– Higher coverage of selected regions with less raw information. – Archival and frozen tissue can be safely used. – Re-arrangement detection will be limited.	– Limitations of panel size. – Difficulties in copy number description.
Emerging sequencing technologies			
	Single-molecule sequencing or direct detection of nucleotide signal No amplification or template used. Sequencing by synthesis or degradation.	– Low cost. – Potential to increase throughput and sequence quality.	– Output remains poorly characterized. – Archival FFPE tumour DNA should be tested.

tumor genomic analysis; however, it is currently limited in its routine clinical applicability because of cost and turnaround time for sequencing and analysis. The sequencing of exomic regions with WES may represent a more practical technique to use routinely in the clinic. Drilon et al demonstrated that WES technology was able to identify actionable genomic alterations in a further 65% of non-small cell lung cancers (NSCLCs), which had originally tested "negative" for mutations by non-NGS methods.[9] Different groups have also now incorporated WES into their patient selection strategies in clinical trial units.[8] Ultimately, the choice of technique used is likely to be driven by the research hypothesis in question.

WES of patient tumors may also enable us to understand the underlying biological mechanisms underpinning clinical responses and resistance. Wagle et al performed WES on a patient with anaplastic thyroid carcinoma who had an exceptional response to everolimus and identified a mutation in tuberous sclerosis complex 2 (*TSC2*) as a potential oncological driver.[10] TSC2 is a negative regulator of the mammalian target of rapamycin pathway and a potentially druggable target. Furthermore, WES of tumor biopsies obtained upon disease progression may identify novel mechanisms of acquired drug resistance, as observed with a patient with relapsed chronic lymphocytic leukemia who acquired a mutation in Bruton's tyrosine kinase (BTK) that limits drug binding after initial successful treatment with the recently approved BTK inhibitor ibrutinib.[11]

Transcriptomics. Similar technologies applied to RNA sequencing have enabled the profiling of expressed components of the genome, termed the cancer transcriptome. This technology has been utilized for the detection of novel oncogenic fusion genes at a fraction of the cost of WGS, for

example, with echinoderm microtubule-associated protein-like 4–anaplastic lymphoma kinase (*EML4-ALK*) gene fusions in NSCLC.[12–14]

Furthermore, transcriptome analysis has the added benefit of detecting microRNAs (miRNAs) and noncoding RNAs (ncRNAs), as well as providing gene expression information.[15] This provides abundant data for potential biomarker discovery, as shown by miRNA gene signatures developed to clarify tissue-of-origin in patients with carcinomas of unknown primary,[16] which may subsequently direct treatment options.

Whole transcriptome RNA sequencing on single cells is emerging as a powerful tool to study both clonal diversity and cancer progression.[17] Ramsköld et al successfully undertook whole transcriptome RNA sequencing on circulating tumor cells (CTCs) isolated from patients with melanoma, enabling the comparison of data with primary melanocytes, permitting the identification of putative response and resistance mechanisms.[18] Similar studies undertaken in CTCs isolated from patients with breast cancer have identified novel targets contributing to metastases.[19]

Epigenetics. Epigenetics broadly covers the inheritable changes to gene expression that are not directly due to changes in nucleotide sequences. Importantly, genomic methylated tumor DNA fragments can now be inexpensively sequenced using NGS techniques.[20,21] Furthermore, the landscape of posttranslational modification of histones that is critical for the regulation of chromatin structure and gene expression can be assessed with chromatin immunoprecipitation with NGS.[22] Integrating epigenomic analyses such as these together with gene expression data has linked oncogenic drivers with the disruption of epigenetic regulation, thereby identifying clinically actionable targets such as enhacer of zeste 2 polycomb repressive complex 2 subunit (EZH2).[23,24]

Proteomics. Despite the wealth of information provided by genomic and transcriptomic analyses, cancer proteins are highly dynamic molecules and are subject to extensive functional regulation and posttranslational modifications. Proteomics is a rapidly evolving field of the study of whole protein repertoire of a defined entity. The first draft of the human proteome, as defined by mass spectroscopy, has now been published,[25] and the technologies are currently being utilized in deciphering cancer proteomes.[26]

Another interesting area of development is pathway-specific post-translational profiling. Andersen et al undertook phosphoprofiling of 375 nonredundant phosphoinositide 3-kinase (PI3K) pathway-relevant phosphopeptides and identified a putative biomarker for PI3K pathway overactivity that predicted for sensitivity to an AKT inhibitor.[27] Similar techniques are also being utilized to study the cancer ubiquitinome[28] and are projected to set a new standard in elucidating biochemical mechanisms of ubiquitin (Ub)-driven signaling systems in cancer.[29] For example, genome sequencing studies have described a mutation in cullin-RING Ub ligase adaptor protein speckle-type POZ protein in ~10% of prostate cancers. Interrogation of the Ub landscape of these mutant proteins revealed impaired ubiquitylation within a subset of proteins, including oncogenic effectors in a dominant-negative fashion.[30]

Looking to the future, advanced omics technologies together with computational techniques will enable the assessment of signaling pathway and network aberrations that will greatly facilitate the selection of drug combinations likely to benefit specific patients. Early-phase trials provide the ideal setting for the development of new research technology applications for clinical oncology.

Progress in Developing Predictive Biomarkers in Early Phase

The Phase I trial setting is ideally placed for the testing of biomarkers that have strong scientific rationale and preclinical evidence to predict for antitumor efficacy, but they are yet to be validated in the clinic.[31] These early-phase trials may determine if the biomarker can be robustly detected and measured for use in correlative studies in the clinic. Ideally, some of these enrichment biomarkers will eventually evolve into predictive biomarkers of response following further analytical validation and clinical qualification.

To date, the majority of validated biomarkers have been identified through retrospective subgroup analyses of large randomized Phase III clinical trials in unselected populations.[32] An example of how the application of predictive biomarkers of response changes with time and technology is illustrated by the CRYSTAL Phase III clinical trial of cetuximab (Erbitux; ImClone/Merck/Bristol–Myers Squibb) in patients with metastatic colorectal cancer (mCRC). This study was designed to evaluate the addition of cetuximab to chemotherapy in the first-line treatment of patients with epidermal growth factor receptor (EGFR)-positive mCRC but was designed before the emergence of robust data on the predictive role of *KRAS* mutation status on cetuximab treatment of mCRC.[33] The initial retrospective analyses of clinical samples using NGS revealed that *KRAS* mutations in codons 12 and 13 were associated with a lack of antitumor response to cetuximab in patients with mCRC.[33,34] Subsequent work using the more sensitive beads, emulsion, amplification, and magnetics (BEAM) technology not only confirmed the initial analyses but also suggested that additional *KRAS* mutations, as well as mutations in *NRAS*, may be associated with reduced clinical benefit to cetuximab therapy in mCRC.[35]

The development of vemurafenib (Zelboraf®, Genentech) for patients with melanoma harboring *BRAF* mutations is a good case in point, neatly illustrating the parallel development of a successful anticancer treatment alongside a companion diagnostic. A dose escalation Phase I trial was initially conducted in an unselected patient population and showed antitumor responses in 11 of the 16 patients with BRAF mutations but none in *BRAF* wild-type patients. The subsequent expansion was restricted to patients with melanoma whose tumors

harbored the BRAF V600E mutation as ascertained by the Roche polymerase-chain reaction assay (Cobas 4800, Roche), and showed responses in 26 of the 32 patients with *BRAF*-mutant tumors.[36] On the basis of these clinical data and preclinical studies, a Phase III enrichment trial, which compared vemurafenib to standard chemotherapy, was undertaken in previously untreated patients with *BRAF* V600 mutation metastatic melanoma.[37] This resulted in the Food and Drug Administration (FDA) approval of vemurafenib as first-line treatment for *BRAF* V600-mutant metastatic melanoma in 2011, alongside the Cobas 4800 BRAF V600 Mutation Test diagnostic test and provided a model for the rigorous codevelopment of biomarkers tests in early-phase trials.

Similarly, for the ALK-inhibitor crizotinib (Xalkori, Pfizer), the Phase I trial selected patients by means of fluorescence in situ hybridization (FISH) with the use of an ALK break-apart probe. In the dose escalation phase of the trial, two patients with NSCLC had drastic antitumor responses, prompting large-scale prospective screening for NSCLC with *ALK* rearrangements and enrollment onto an expanded molecular cohort.[38] As several lines of evidence suggested that ROS proto-oncogene 1 (ROS1) was also a therapeutic target of crizotinib, FISH was also used to identify *ROS* rearrangements in NSCLC tumors, and these patients were subsequently enrolled in a further expansion cohort of a Phase I crizotinib study where potent antitumor activity was observed.[39] Newer techniques, including transcriptome analysis, have further identified novel oncogenic fusions of ALK, RET, and ROS, which may be sensitive to ALK inhibitors,[39] and the expansion cohorts of early-phase trials would be the ideal setting to explore tumors with rare molecular drivers. In these two examples of biomarker-driven early-phase trials, preliminary efficacy in both cases was observed in the Phase I trial expansion cohorts, exemplifying the potential of such approaches to generate hypotheses for confirmatory testing in later phase clinical trials.

Many centers are now routinely molecularly characterizing patients referred for early-phase trials either with WGS/WES or targeted sequencing of mutational hotspots.[8,40] Retrospective analyses of patients treated in a Phase I setting at the MD Anderson Cancer Centre have shown that targeted agents matched with tumor molecular alterations were associated with improved outcomes compared with nonmatched therapy in patients with advanced cancers.[41] The investigators recently published their experience from the prospective screening of 2,000 patients with advanced cancer with genomic profiling.[42] The particular genomic analysis utilized changed over time and was dictated by improvements in technology and a greater understanding of key molecular drivers of disease. Promisingly, 39% of patients were found to have at least one somatic mutation in a potentially actionable gene, although only 11% of these patients were eventually enrolled onto clinical trials of targeted agents for various reasons. A number of obstacles to genotype-matched treatment were identified including patients being lost to follow-up, patient choice for treatment elsewhere, declining performance status, time taken for molecular analysis (median 31 days), and the lack of genotype-matched trials. These results are important for highlighting the potential for large-scale patient testing to impact modern Phase I units in terms of patient selection, and future work should aim to optimize the promise it brings (Fig. 1). While NGS studies in clinical trials currently focus on somatic aberrations, it is envisioned that the interrogation of patient germline DNA for potential drivers of cancer will also be important in the future.

Targeting the Immune System

One of the benefits of incorporating biomarker enrichment strategies earlier in clinical trials is that it allows for the continuous refinement of technologies. Programmed death-ligand 1 (PD-L1) expression has emerged as a potential predictive biomarker for PD-1-directed therapy. Multiple, distinct, companion assays for PD-L1 positivity have been developed, but there is as yet no comparison, standardization, or prospective validation of these assays between different pharmaceutical companies.[43] It is clear that patients who have PD-L1 overexpression, based on different assays with varying cutoffs, tend to have more robust responses to anti-PD-1/anti-PD-L1-directed therapy.[44,45] Herbst et al published findings from a Phase I study that demonstrated the importance of PD-L1 expression on tumor-infiltrating immune cells when predicting for response to MPDL3280A (PD-L1 inhibitor).[46] The story is, however, far from complete, as robust responses have also been reported in some patients with low levels of expression of these markers, thereby complicating the issue of PD-L1 as an exclusion biomarker.[46,47] Given the complexity of the tumor–immune system interface and feedback loops involved, it is likely that PD-L1 expression on tumor cells and/or the tumor-immune infiltrate represent only part of the predictive model necessary for selecting patients predisposed to respond to immunotherapy, and much work is ongoing in this area.

Less is known about the use of programmed death-ligand 2 (PD-L2), which is also targeted by anti-PD-1 therapy as a predictive biomarker of response.[48] It is known to be expressed on both tumor cells and immune cell infiltrates, albeit less frequently than PD-L1, and often in tumors that also express PD-L1. To date, PD-L2 expression has not been found to independently predict for response to anti-PD-1 therapy.[48] Another issue to consider is the potential dynamic expression of predictive biomarkers, including PD-L1 and PD-L2, that may change with disease state,[49,50] infection and inflammation,[48,51,52] and previous treatments.[53,54] The majority of studies of predictive biomarkers have used archival tissue samples, and greater work is needed to determine whether archival samples can be substituted for fresh tumor specimens collected contemporaneously.

Mutational burden is now showing promise in predicting response to novel immunotherapeutics. For example, tumor

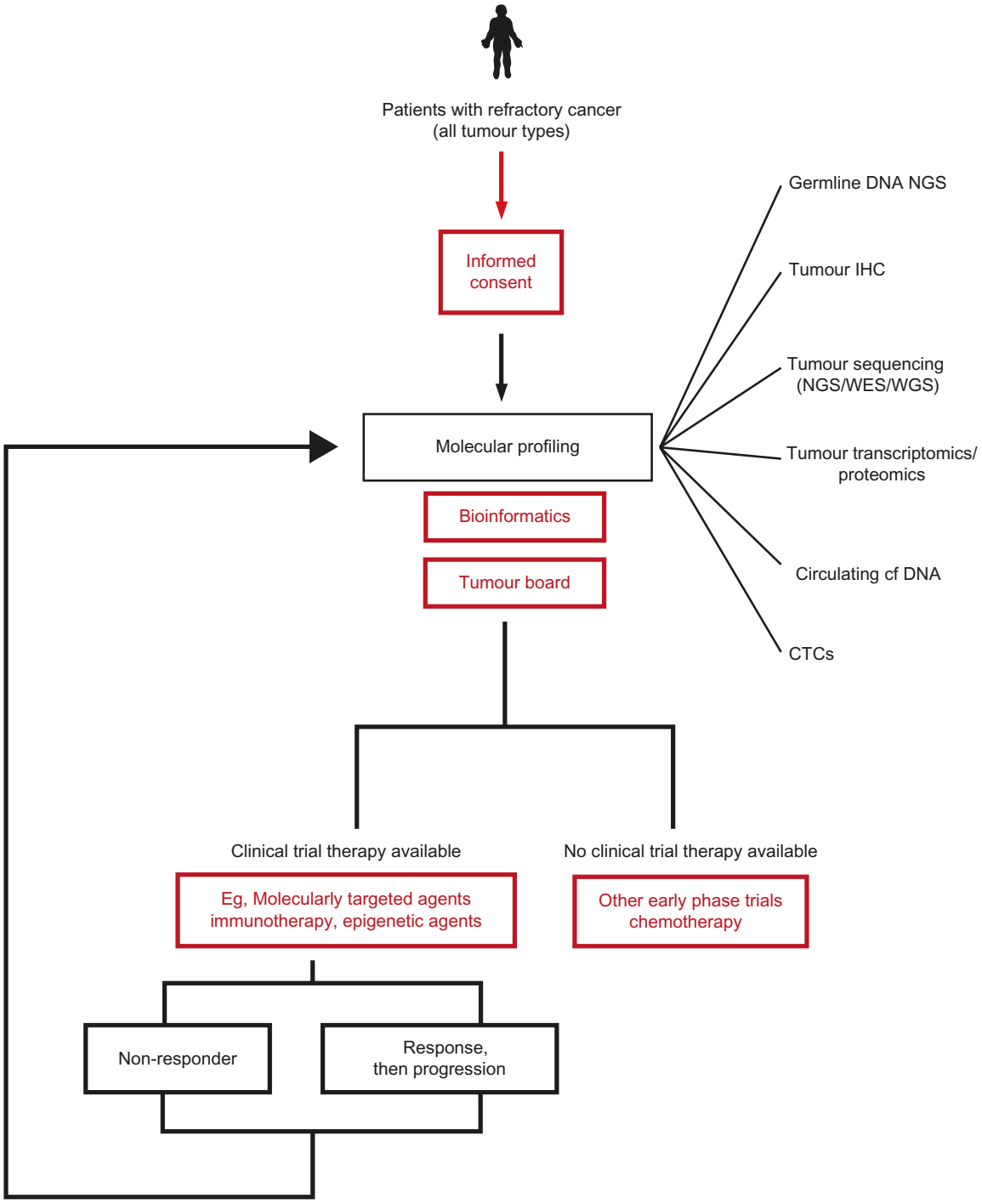

Figure 1. Roadmap of how patients are referred, matched, and enrolled onto different types of early-phase trials depending on their molecular profiles. Patients with refractory cancers who provide their consent will undergo molecular profiling of tumor and surrogate tissues as illustrated above.
Abbreviations: NGS, next-generation sequencing; WES, whole-exome sequencing; WGS, whole-genome sequencing; IHC, immunohistochemistry; cfDNA, circulating cell free DNA; CTCs, circulating tumor cells.

types that have higher mutational burdens have been associated with better response rates to immune checkpoint inhibition, in particular, melanoma and NSCLC. The hypothesis is that the increased frequency and burden of mutations are more likely to provoke an initial immune response, which the tumor learns to evade.[55] Checkpoint inhibition with anti-CTLA4 and/or anti-PD-1 then releases the brakes on the immune system resulting in a response.[56,57] Nonsynonymous mutations (NS-Ms), where DNA-base alterations result in a different amino acid being coded, and hence a different protein, appear to be important. This is in contrast to synonymous mutations that do not affect protein transcription and hence result in the original protein being transcribed. The NS-Ms, therefore, result in new nonself-antigens or neoantigens being

expressed, which are more likely to result in immune detection and activation. This has been shown in a study of patients with advanced NSCLC treated with pembrolizumab, where patients with >178 NS-Ms were found to have a higher durable clinical benefit rate (85%), in contrast to patients with <178 NS-Ms (14%). The higher nonsynonymous mutation burden correlated strongly with improved objective response rate (ORR) and progression-free survival (PFS). This correlation was less evident when the total mutational burden (nonsynonymous and synonymous mutations) was considered, highlighting the importance of nonsynonymous mutations and novel protein formation in inducing an effective immune response.[58]

In addition, some specific mutations appear to be more important than others. In both NSCLC and melanoma, candidate neoantigens have been identified that bind with high affinity to MHC class I receptors, which appear to strongly predict for antitumor responses to both CTLA4 and PD-1 treatments beyond that of mutational burden alone. Patients with NSCLC treated with pembrolizumab whose tumors had high candidate neoantigen burden had significantly improved outcomes compared to controls (median PFS 14.5 months versus 3.5 months; $P = 0.002$).[58,59]

Recent studies have also shown high response rates in patients with mismatch-repair deficiency (MRD). In a Phase II study of pembrolizumab in patients with colorectal cancer, 4/10 (40%) of patients with MRD CRC (colorectal cancer) achieved immune-mediated responses, compared to 0/18 (0%) mismatch-repair proficient (MRP) patients with CRC. A further 5/7 patients with non-CRC MRD had antitumor responses. All other efficacy measures were substantially improved in the MRD cohorts compared to patients with MRP. WES revealed a mean of 1,782 mutations per MRD tumor compared to 73 per MRP tumor, respectively. Likewise, with regard to immunogenic mutations, there were a mean of 578 mutation-associated neoantigens in the MRD tumors, in contrast to 21 in patients with MRP.[60] Other DNA repair pathway defects may also synergize with immunotherapy. For example, *BRCA1/2*-mutated breast and ovarian carcinomas have been previously shown to have higher nonsynonomous mutation burdens compared to *BRCA* wild-type tumors,[61] raising the intriguing possibility that *BRCA1/2* mutations may also predict responses to immunotherapy.

Although this requires further validation and study, the development of predictive biomarkers of response to immunotherapy is hugely exciting and can potentially be explored in expansion cohorts of future early-phase trials or basket studies. A neoantigen burden cut off for nonsynonymous mutational burden level may potentially be used to enrich Phase I trials with patients more likely to benefit from immunotherapy. Furthermore, the neoantigen burden may also provide a target for future drug development by focusing on combination treatments that increase the burden of tumor neoepitopes and thus synergize with immune checkpoint blockade.[55]

Tissue Analysis in Precision Medicine

In recent years, much research has focused on the molecular characterization of tumor tissue, as well as noninvasively obtained surrogate tissues, such as CTCs, circulating cell-free plasma DNA (cfDNA), and miRNAs/ncRNAs, for different applications in precision medicine.

Tumor tissue. Advances in NGS and bioinformatics have revealed the extent of spatial and temporal diversity mediated by branched cancer clonal evolution.[62–65] Going forward, this raises challenging issues for precision medicine. First, the use of single tumor biopsy samples is likely to grossly underestimate intratumoral heterogeneity.[66,67] In addition, obtaining multiple tumor biopsies from different metastatic sites at sequential time points is logistically challenging, there is an urgent need for surrogate biomarkers to provide such temporal information, and cfDNA may form part of the solution.

A single drug is unlikely to be adequate in treating a genetically heterogeneous tumor, as clones occurring at low allele frequencies are not readily detectable at diagnosis, but may ultimately contribute to therapeutic failure and poor outcomes. Hence, identifying low-frequency events present in tumors before commencement of therapy, as well as emerging events that subsequently occur may ultimately influence patient outcomes. Prospective studies such as the Lung TRACERx (TRAcking nonsmall cell lung Cancer Evolution through therapy [Rx]; NCT01888601) and DARWIN II (Deciphering Antitumor Response With INtratumor Heterogeneity; NCT02183883) studies aim to explore this by studying both tumor samples and cfDNA and are anticipated to provide a wealth of information resulting in a more adaptive approach to clinical practice and the design of modern combination therapeutic approaches.[68]

One emerging mechanism fueling tumor diversity and subclonal evolution is genomic DNA cytosine deamination catalyzed by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) family members.[69] Deregulation of these enzymes causes a general mutator phenotype that manifests with the acquisition of diverse and heterogeneous subclonal driver events. Strikingly, in head and neck cancers and NSCLC, over 85% of subclonal mutations in *PIK3CA* occurred in an APOBEC context.[70] This may not only guide the choice of subsequent lines of targeted therapy, but APOBEC inhibition itself may represent a new class of drug target aimed at limiting tumor evolution, adaptation, and drug resistance.[69,70] Furthermore, an intriguing hypothesis is that "APOBEC-high" tumors are also likely to have higher levels of DNA damage and may be amenable to a synthetic lethal approach analogous to PARP inhibitor treatment of *BRCA1/2* mutation cancers,[71] which again can potentially be explored in clinical trials.

Circulating tumor cells. These are cells that are shed from primary tumors and metastatic deposits into the blood stream, which can be captured and isolated using a range of technologies.[72] Comparative genomic analyses of CTCs,

primary tumors, and metastases in patients with colorectal or prostate cancer have confirmed that mutations found in CTCs resemble those detected in both the primary tumor and metastases if sensitive deep-sequencing technologies are applied, supporting its use as real-time "liquid biopsies."[73,74] CTC enumeration together with LDH levels has been shown to be a potential surrogate marker for survival in patients with advanced prostate cancer treated within the COU-AA-301 trial with either abiraterone and prednisolone or prednisolone alone.[75] Sophisticated molecular profiling of CTCs is now feasible, and this is likely to be of significant clinical utility as it may reflect tumor evolution within an individual, particularly under selection pressures of systemic therapies. Nevertheless, multiple challenges, such as issues of intrapatient variability in the molecular characterization of CTCs, will need to be addressed in the future.

Circulating free DNA. cfDNA fragments harboring genetic alterations derived from tumor cells can be identified using highly sensitive purification and sequencing methods. Sequential targeted exome sequencing and WES of cfDNA are now utilized to identify changes in the tumor mutational landscape in "real time." A further advantage of cfDNA is that they are frequently detected in the absence of CTCs.[76]

More recently, targeted sequencing of serial cfDNA specimens was shown to detect driver mutations at low allele frequencies with high sensitivity.[77] This potentially enables the longitudinal monitoring of clonal dynamics on treatment and the early detection of resistant mutations prior to radiological disease progression.[77,78] Real-time incorporation of such information into early-phase trials may also provide interesting insights into the diversity of underlying biological mechanisms through which tumors acquire drug resistance. This approach is illustrated by the targeted sequencing of plasma cfDNA samples from patients with NSCLC treated within the Phase I AURA study of the EGFR mutation-specific inhibitor AZD9291 (AstraZeneca). All samples were positive for the T790M mutation before treatment, but upon developing AZD9291 resistance, three distinct molecular subtypes emerged.[79] One cohort of patients retained the EGFR T790M mutation while gaining a further EGFR mutation (C797S); a second cohort lost the T790M mutation but retained the original EGFR activating mutation, while a third cohort retained the EGFR T790M mutation but acquired other means of resistance.[79] Such data are key to the early rational development of new hypotheses of combination strategies to overcome resistance mechanisms.

cfDNA has also been detected from other bodily fluids, for example, urine and saliva. In a study of six patients with non-Langerhans cell histiocytosis known to harbor *BRAF* V600E mutations, 100% concordance was demonstrated between tumor and urine cfDNA, while 85% concordance was observed between plasma cfDNA and urine cfDNA.[80] Separately, in a cohort of patients with head and neck cancers, Wang et al found that patient saliva was preferentially enriched for tumor DNA.[81] In the future, for patients who are unable to undergo tumor biopsies because of issues of accessibility or other reasons, cfDNA from surrogate tissues may provide a noninvasive alternative for identifying predictive biomarkers of response.[80]

Clinical Trial Designs Utilized in Precision Medicine

Modern early-phase clinical trials have been critical in recent cancer drug development successes, involving both molecularly targeted agents and immunotherapies.[82] Although the primary endpoint for all Phase I trials remains the establishment of both safety and tolerability of novel drugs or combinations, the incorporation of innovative biomarker-driven trial designs has enabled key questions and hypotheses to be tested early prior to large and costly late-phase trials. It is therefore important that early-phase trials should evolve to include both pharmacokinetic and pharmacodynamics profiling to confirm mechanisms of drug action, as well as the identification of patient subpopulations who may potentially benefit using genomic and molecular testing with analytically validated predictive biomarker assays (Fig. 1). Furthermore, early-phase clinical trials represent an opportunity to explore and begin the clinical qualification of key enrichment biomarkers before more rigorous confirmatory assessments are undertaken in larger studies.

Adaptive designs. Adaptive designs use data gathered as the trial progresses for changing some aspect of the trial and/or its statistical analysis procedures midstream without undermining the integrity of the trial.[83] In the early clinical trial setting, an adaptive study design may provide a framework for the codevelopment of drugs and companion diagnostics, with the identification of relevant biomarkers and their subsequent clinical qualification. Increasingly, the expansion stage of Phase I trials allows a seamless strategy for the preliminary evaluation of efficacy that may be restricted to a particular subpopulation defined by biomarker status. Using a modified Simon stage-2 design, futility-type rules may be applied within each indication to suspend enrollment in that indication if there were no responders observed by a certain enrollment number.

A good example of this design is the Phase I study for MPDL3280A, which allowed for tumor-specific cohorts and biomarker (PD-L1 positive)-enriched cohorts (NCT01375842). Patients in the initial PD-L1-positive urothelial bladder cancer cohort showed noteworthy responses to MPDL3280, leading to further expansion to include adequate numbers of biomarker-positive and -negative patients. The final analyses of 207 patients with metastatic urothelial bladder cancer showed that patients with high expression of PD-L1 on tumor-infiltrating immune cells had an objective response rate (ORR) of 43%, compared to an ORR of 11% in patients with low expression, suggesting the potential of immune cell PD-L1 expression levels as a biomarker that will be further interrogated in randomized trials.[47] Data from this early-phase trial led to the

FDA granting breakthrough designation for MPDL3280 in bladder cancer.

Umbrella and basket designs. Newer NGS technologies are now identifying larger cohorts of patients with relatively rare mutations. Enrolling them to a trial involving a single tumor and molecular subtype may prove challenging for patient accrual, and therefore, this has led to the development of new designs in clinical trials, in particular umbrella and basket trials (Tables 2 and 3).

Umbrella trials enroll patients with a specific tumor histology but have separate arms involving different driver aberrations found in a particular tumor type. A pertinent example of an umbrella trial is the UK National Lung Matrix Trial that is currently ongoing. This trial involves multiple molecularly targeted treatments against different subtypes of NSCLC. Patient tumors will first be molecularly profiled using a hotspot panel within the Cancer Research UK Stratified Medicine Programme 2 study to determine their treatment arm of the trial, depending on the specific driver mutations that are detected. The trial is adaptive in design and has been designed with flexibility in mind, so as to potentially add or remove treatment arms as new data come to light.

Basket trials are studies that include multiple tumor histologies that share a common genetic aberration. The vemurafenib basket trial is such a study where patients with BRAF V600 mutations were treated with vemurafenib regardless of primary histology. Each arm of the trial had a specific histology and was analyzed separately in the context of a Simon 2-stage design to allow for early stopping if no efficacy was seen. This study was noteworthy for showing preliminary efficacy in BRAF V600-mutated NSCLC, Erdheim–Chester disease, and Langerhans'-cell histiocytosis; but also for highlighting that tumor lineage might influence drug sensitivity as underscored by the lack of responses in colorectal cancers harboring the same driver mutation.[84]

Both umbrella and basket trial designs provide efficiency in being able to conduct multiple cohorts within a study, rather than having to run multiple separate trials. A newer type of trial design is a mixture of approaches undertaken with umbrella and basket trials. The forthcoming NCI-MATCH study aims to enroll 3,000 patients of any cancer histology to undergo tumor biopsies to detect a driver mutation in one of 143 selected genes. If a mutation is detected, then the patient will be allocated to a specific treatment known to have efficacy against that mutation. These treatments include crizotinib (Pfizer) for ALK rearrangements or ado-trastuzumab emtansine (Roche) for HER2 amplification. The primary endpoint will be objective response and six months PFS in each arm compared to historic controls. Each arm will

Table 2. Ongoing umbrella trials illustrating the ability of the trial design to incorporate multiple biomarker stratified cohorts for each specific cancer.

UMBRELLA TRIALS					
TRIAL	**SETTING**	**N**	**BIOMARKERS/ARM**	**TREATMENT**	**DESIGN**
Alchemist[94]	Adjuvant Non-squamous NSLCC	6000–8000	EGFR ALK Dual Wild type	Erlotinib vs plbo Crizotinib vs plbo Registry (Nivolumab vs plbo expected)	Phase III
Focus 4[95]	Metastatic CRC SD afer 16weeks 1st line chemo	~1500	BRAF PIK3CA/PTEN RAS All WT Non stratified	BRAFi + EGFRi +/– MEKi vs plbo PIK3CAi +/– MEKi vs plbo AKTi + MEKi vs plbo HER1,2,3 inhib vs plbo Capecitabine	Phase II–III
I-SPY2[96]	Neoadjuvant breast	Up to 120 per arm	N/A	Multiple arms testing (up to 12) experimental treatments added to paclitaxel standard of care Includes: Neratinib, Veliparib, AMG38, MK2206, Pertuzumab and trastuzumab, TDM1	Phase II
Lung-MAP[97]	Squamous NSCLC, 2nd line	~5000	PI3KCA CDK4/6 or CCDN1/2/3 FGFR1/2/3 HGF/c-MET No genetic alteration	Taselisib vs Docetaxel Palbociclib vs Docetaxel AZD 4547 vs docetaxel Riloutumumab plus erlotinib vs erlotinib MEDI4736 vs docetaxel	Phase II–III
National Lung MATRIX[98]	Refractory NSCLC	410	FGFR TSC or LKB1 PI3KCA/PTEN/AKT Rb+P!6/CDK4/CCND1/KRAS MET/ROS NF1/NRAS EGFR+T790M Biomarker negative	AZD4547 AZD2014 AZD5363 Palbociclib Crizotinib Docetaxel+seltmetinib AZD9291 MEDI4736	Phase II Bayesian adapative

Abbreviation: Plbo, placebo.

Table 3. Ongoing basket trials illustrating the ability of each biomarker specified trial design to incorporate multiple tumor-specific cohorts.

BASKET TRIALS				
TITLE	**BIOMARKER**	**HISTOLOGY**	**TREATMENT**	**DESIGN**
Single drug studies				
VE-BASKET[99]	BRAF V600 mut	NSCLC Ovarian CRC Cholangiocarcoma Breast Myeloma Other	Vemurafenib (+/- Cetuximab in CRC)	Phase II Simon 2 stage, 19 patients per arm
CREATE[100]	ALK or MET	Anaplastic large cell lymphoma Inflammatory myofibroblastic tumour Papillary renal cell Clear cell sarcoma Alveolar soft part sarcoma Alveolar rhabdomosarcoma	Crizotinib	Phase II 582 patients total
Multiple drug/multiple molecular alteration				
NCI-MATCH[101]	ALK or ROS1 BRAF V600E/K Other BRAF EGFR HER2 activation EGFR T790M HER2 amplification NF2 cKIT	Any histology	Crizotinib Dabrafenib + trametinib Trametinib Afatinib Afatinib AZD9291 TDM1 VS6063 Sunitinib	Multiple single arm Phase II Adaptive design

proceed as a Simon 2-stage design, with early cessation of the arm if no activity has been observed. The ultimate goal is to test up to 26 different drugs in 1,000 matched patients. Like the UK National Lung Matrix Study, this trial has been designed with flexibility in mind and has the ability to add new cohorts as the trial progresses to allow for advances in scientific knowledge.[85]

Conclusion

We have now truly entered the era of precision medicine, driven by modern technological advances that have uncovered critical data on different cancer "omic" landscapes. These have led to the development of predictive biomarkers of response and resistance that are being exploited in clinical trials of both molecularly targeted agents and immunotherapies. We envision that the continued uncovering of biological insights into different cancers through the interrogation of both tumor and surrogate tissues within clinical trials with modern and innovative study designs will further accelerate the delivery of novel antitumor agents and impact patients with different molecular subtypes of cancers.

Author Contributions

Conceived and designed the review: JL, SH, TY. Wrote the first draft of the manuscript: JL, SH, TY. Contributed to the writing of the manuscript: JL, SH, DR, TY. Agree with manuscript results and conclusions: JL, SH, DR, TY. Jointly developed the structure and arguments for the paper: JL, SH, TY. Made critical revisions and approved final versions: JL, SH, DR, TY. All authors reviewed and approved of the final manuscript.

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