

Towards Precision Medicine in the Clinic: From Biomarker Discovery to Novel Therapeutics

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Abstract

Precision medicine continues to be the benchmark to which we strive in cancer research. Seeking out actionable aberrations that can be selectively targeted by drug compounds promises to optimize treatment efficacy and minimize toxicity. Utilizing these different targeted agents in combination or in sequence may further delay resistance to treatments and prolong antitumor responses. Remarkable progress in the field of immunotherapy adds another layer of complexity to the management of cancer patients. Corresponding advances in companion biomarker development, novel methods of serial tumor assessments and innovative trial designs act synergistically to further precision medicine. Ongoing hurdles such as clonal evolution, intra and intertumor heterogeneity and varied mechanisms of drug resistance continue to be challenges to overcome. Large-scale data-sharing and collaborative networks using next generation sequencing platforms promise to take us further into the cancer 'ome than ever before, with the goal of achieving successful precision medicine.

The Precision Medicine Rationale

Oncology research has evolved in parallel with improved understanding of the cancer genotype and phenotype ushering in a new age of **precision medicine** (see Glossary for this and all terms in bold). This is colloquially termed “the right drug, for the right patient, at the right time”. These biological therapies target specific abnormalities within the cancer cell genome, proteome, immunome and/or “any-ome” that are involved in cancer initiation, development, and survival [1,2]. In stark contrast to the historic “one-size-fits-all” chemotherapy approach, precision medicine combines individual patient characteristics with their tumor genomic landscape to enable matching with molecularly targeted agents and immunotherapeutics to maximize treatment efficacy and minimize toxicity.

As insights into the genomic and immunomic complexity of cancer increase with the aid of **next generation sequencing** (NGS) and other sophisticated platforms, daunting challenges continue to hinder the promise of precision medicine to find the “right drug”. These include **clonal evolution** encompassing tumor cell aberrations that alter and accumulate over time and **tumor heterogeneity** reflecting (epi)genetic differences between tumors (**intertumor heterogeneity**) and indeed within the same tumor specimen (**intratumor heterogeneity**). Additionally, logistical hurdles, such as small and underpowered molecular-specific cohorts, biostatistical obstacles and the need for innovative trial designs to incorporate modern translational studies further impede progress [3]. Alongside our improved identification of the “right patient” and the “right time”, there has been a tenacious attempt to find surrogate **biomarkers** of disease response and modern noninvasive methods of serial tumor monitoring. The emergence of

immuno-oncology and its rapid integration into standard of care treatment for several different tumor types has added another level of complexity to personalized medicine [4 – 6].

In an effort to accelerate progress, the USA recently launched the Precision Medicine Initiative and with it, a million-patient, multimillion-dollar longitudinal cohort study. The future of personalized oncology is bright, but solving the challenges that hamper its progression is a slow endeavor, often fraught with disappointment. This review sets out to address the challenges and barriers to achieving universal precision medicine in oncology, and conceivable solutions to overcome them. We focus on the challenges in interpreting genomic and epigenomic abnormalities, discuss how such aberrations change through clonal evolution and how this gives rise to intratumor and intertumor heterogeneity. We discuss the promise, pitfalls and opportunities surrounding the use of NGS, surrogate markers of disease response, **theranostics**, and novel clinical trial designs.

Deciphering aberrations within the cancer cell

While the turn of the century has seen an exponential growth in the collection of cancer “omics” data (genomics, interactomics, transcriptomics, proteomics and metabolomics), it remains a struggle to differentiate “passenger” aberrations that do not impact cell function from significant “driver” abnormalities to which the cancer cell is addicted for growth and survival [7]. Single tumor biopsies can often underestimate the number of driver events in part due to intratumor heterogeneity and intertumor variations, potentially leading to sample bias. Furthermore, aberrant events that are more frequent within the tumor are easier to isolate and may give the illusion of clonal dominance, whereas low frequency driver aberrations can often be missed or

overlooked. To date, there has been some success in prohibiting tumor growth when pursuing actionable aberrations, including imatinib (Gleevec®, Novartis) for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, gefitinib (Iressa®, AstraZeneca) for epidermal growth factor receptor (EGFR)-mutant lung cancer, and crizotinib (Xalkori®, Pfizer) for anaplastic lymphoma kinase (ALK)-positive lung cancer, to name a few.

Clonal somatic aberrations found within all cancer cells have been acquired early in tumorigenesis [8]. The tumor genomic landscape further evolves over time as demonstrated by the Darwinian successive acquisition of beneficial aberrations, resulting in superior “fitter” clones that eventually surpass their parent clones [9, 10]. Branched evolution results in multiple distinct subclones due to the deterministic outgrowth of superior and better-adapted cell groups. These can develop as a result of direct exposure to cancer therapies (**selection pressure**) resulting in treatment resistance [11 – 14]. Certain tumors express increased genomic instability such as melanomas, tobacco-exposed lung cancers or cancers with DNA repair defects including microsatellite instability are predisposed to developing excessive subclonal aberrations [13]. **Figure 1** represents an example of clonal evolution with resultant inter and intratumor heterogeneity.

Although treatments aimed at clonal driver aberrations can result in tumor responses, highlighting the importance of identifying these early events, subclonal abnormalities may also need to be targeted to avoid or delay the emergence of resistance to therapies [15]. McGranahan and colleagues have shown that although mutations in known driver genes occurred early in cancer evolution, later subclonal actionable aberrations were also identified [16]. These included

BRAF (V600E), *IDH1* (R132H), *PIK3CA* (E545K), *EGFR* (L858R), and *KRAS* (G12D) aberrations, which may ultimately hinder the efficacy of molecularly targeted agents. Over 20% of *IDH1* mutations in glioblastomas and approximately 15% of PI3K-AKT pathway aberrations across all tumors were subclonal. RAS-MAPK pathway aberrations are less likely to be subclonal than PI3K-AKT network gene abnormalities. There is also context dependency between tumors, for example, loss of the tumor suppressor gene *PTEN* is a clonal driver event in triple negative breast cancer, but often subclonal in prostate tumors [17, 18].

Clonal evolution can give rise to intertumor and intratumor heterogeneity when selection pressures from the host's immune system, peritumoral microenvironment and/or anticancer therapy result in vastly divergent molecular landscapes between the primary tumor and associated metastases as well as variances within a single tumor specimen itself [14,19]. A greater understanding of this respective inter and intratumor heterogeneity provides plausible reasons for the observation of differential treatment responses at different disease sites and novel mechanisms of resistance [20]. It has also led to concerns about conventional methods of (epi)genomic analysis using archival tissue, which may not truly be reflective of the current state of disease after multiple lines of anticancer therapies. Additionally, a single specimen from one biopsy site may potentially mislead the physician's choice of treatment due to intratumor heterogeneity [21], as pictorially represented in **Figure 2**. Several large trial-based efforts are ongoing to advance our understanding of inter and intratumor heterogeneity, such as TRACERx in non-small cell lung cancer [NCT01888601] and BEAUTY in breast cancer [NCT02022202].

The functional validation of each genomic aberration is important and may be achieved through different approaches, including the analysis of relevant cell lines, *in vivo* mouse “**avatars**” such as patient-derived xenografts, genetically engineered mouse models (GEMMs) or **organoid** cultures (miniature 3-dimensional tissue forms) [22, 23]. Additional genomic databases and libraries such as the Catalogue of Somatic Mutations In Cancer (COSMIC) compile somatic aberrations along with their functional relevance and are useful publicly available sources of data [24]. Stratifying the functional importance of identified aberrations takes into account predicted bioinformatic algorithms and preclinical data to allow ranking of these aberrations into levels or tiers, as eloquently detailed by Van Allen and colleagues [25]. Tier 1 refers to a molecular alteration that has been robustly validated in early phase or Phase III clinical trials, Tier 2 defines the aberration as being identified as significant in single and/or underpowered Phase I/II trials, Tier 3 aberrations have potential functional impact suggested by preclinical studies and finally, Tier 4 aberrations are predicted to have significance but without supporting clinical or preclinical data.

Next generation sequencing, liquid biopsies and theranostic biomarkers panels

Expanding the breadth of precision medicine requires further progress in a number of key areas. Among these, we discuss the utility of NGS and associated obstacles, as well as the development of liquid biopsies and theranostics for use in the clinic. High-throughput NGS methods for detecting cancer gene aberrations comprise ‘**OMIC technologies**, such as the sequencing platforms of DNA Seq, RNA Seq, CHIP Seq and Methyl Seq. They involve **whole genome sequencing (WGS)** and **whole exome sequencing (WES)**. WES focuses upon the coding regions of the genome, whereas WGS is a comprehensive assessment involving all nucleotides of

an individual's DNA, but it is often limited by its increased, albeit improving, fiscal burden, enormity of data returned and slow turnaround time [26]. To date, large-scale collaborative and pioneering 'OMIC databases such as the International Cancer Genome Consortium (<http://www.icgc.org/>) [27], Cancer Genome Atlas (<http://cancergenome.nih.gov/>) and Cancer Cell Line Encyclopedia [28] have identified several hundred cancer-driver mutations and varied genomic aberrations across multiple cancer types, often uncovering potential targetable abnormalities [8, 29, 30].

Similar to DNA interrogation, technologies applied to RNA sequencing have advanced our understanding of the transcriptome and allowed the identification of important microRNAs and non-coding RNAs [31]. RNA Seq has been additionally useful in detecting oncogenic fusion proteins such as *EML4-ALK* in non-small cell lung cancer (NSCLC) [32, 33], differentially expressed transcripts between tissues and the discovery of single nucleotide variations. It has been also successful in elucidating the tissue-of-origin in patients with carcinomas of unknown primary [34]. Beyond NGS and RNA Seq, further decoding of the functions and interactions of highly dynamic and closely linked cancer proteins has led to a whole science of proteomics, metabolomics and interactomics [35]. Finally, as well as genetic abnormalities, epigenetic changes such as post-translational histone modification and DNA hypermethylation are also likely to be functionally important in the development and progression of cancer [36 – 38]. These are subject to intensive research and have led to the development of epigenetic agents.

Serial 'OMIC assessments allow for a dynamic interaction with the tumor as it potentially changes over time. Longitudinal metachronous assessments from sequential tumor biopsies or

other biomarkers allow repeated analysis to decipher the tumor status during treatment and upon progression to assess for differences [39, 40]. This vantage point allows strategic alterations in therapy, uncovers novel mechanisms of acquired drug resistance and aids in the interpretation of underlying mechanisms of clonal evolution, metastasis and cancer differentiation.

Despite the great potential of NGS, the data that emerge from such analyses are ultimately only as good as the source tissue. Inter and intratumor heterogeneity may result in molecular characteristics of analyzed archival tumor biopsies that do not accurately represent the current tumor (epi)genome. There is also often further uncertainty over the preservation techniques of formalin and paraffin that can damage DNA integrity. The most obvious solution involves multiple, fresh serial tumor biopsies from different parts of the primary cancer itself, as well as many secondary metastases to minimize sampling bias and to paint a comprehensive molecular canvas of the tumor's genomic landscape. Such biopsies are however invasive, costly, logistically challenging, and importantly may potentially result in harm to patients. Better strategies of obtaining fresh contemporary tissue for NGS analysis to enable rational matching with targeted therapies, monitoring of treatment response and the detection of emerging resistant subclones are thus essential.

An alternative strategy to the use of multiple successive tumor biopsies is the utility of "liquid biopsies" which appear to be a promising and viable alternative. Circulating tumor cells (CTCs) are sloughed from primary and metastatic tumor cells into the bloodstream, which can then be isolated and analyzed by NGS technology [41]. Comparative genomic analyses of CTCs,

primary tumors and metastatic deposits in colorectal and prostate cancer have expressed concordance, supporting their potential application as an adjunct to tumor analysis [42 – 44].

Circulating cell-free DNA (cfDNA) are derived from cancer cells following apoptosis and/or necrosis and harbor genetic alterations reflective of the parent cell [45]. These can also be isolated and sequenced to identify mutational changes in “real-time” prior to radiological disease progression with high sensitivity [46 – 48]. cfDNA are often detected in the absence of CTCs [49] and can be detected in other bodily fluids such as urine and saliva, with high concordance reported [50, 51]. The *EGFR* T790M mutation was detected in serum and urine cfDNA, despite being unidentified in the tumor biopsy potentially due to intratumor heterogeneity or perhaps inferior sampling [52]. Furthermore in the Phase I/II TIGER-X study (NCT01526928), the antitumor response to the *EGFR* mutation-specific inhibitor rociletinib (Clovis Oncology) was comparable regardless of which NSCLC patient tissue the T790M mutation was identified in. The Phase I AURA study of the *EGFR* mutation-specific inhibitor osimertinib (AZD9291, AstraZeneca) in *EGFR* T790M mutant NSCLC utilized serial cfDNA assessments to reveal distinct molecular subtypes emerging, including the gain of another resistant *EGFR* mutation C797S and development of alternative resistance mechanisms [53].

The use of liquid biopsies continues to strive to overcome current challenges and limitations. cfDNA is currently only applicable in tumors where the exact driver mutation is already known, such as *EGFR*. The use of next generation sequencing when applied to cfDNA is in an early stage of development with promising results reporting identification of tumor mutations in almost 70% of patients [48]. cfDNA analyses may also be limited by the tumor tissue to normal

tissue cfDNA ratio [54]. Standardization and optimization of bioinformatics analyses and validated software programmes are essential to ensure cross-study comparisons going forward.

Prospective precision medicine studies currently underway propose to explore surrogate biomarkers, intra and intertumor heterogeneity, as well as clonal and subclonal evolution in response to different treatments via tumor samples, CTC enumeration and cfDNA analysis to devise novel individualized and adaptive management strategies and are listed in **Table 1**.

Another major challenge to precision medicine is the dearth of predictive biomarker panels for use in conjunction with molecularly targeted agents and other therapies to guide the rational matching of patients with antitumor therapeutics. Where possible, it is therefore essential to advance the development of both drug and predictive biomarker in parallel. This field of theranostics – using diagnostic testing to rationally select targeted therapies – relies on biomarker assays being analytically validated and appropriately clinically qualified. The BRAF inhibitor, vemurafenib (Zelboraf®, Genentech) was developed and approved with its companion diagnostic PCR assay (Cobas 4800®, Roche) following clinical validation in a Phase III trial (55, 56]. Similar theranostic development scenarios were observed with the ALK inhibitor crizotinib (Xalkori®, Pfizer) and its ALK break-apart probe for fluorescence in situ hybridization (FISH) [57], imatinib (Gleevec®, Novartis) and BCR/ABL-positive chronic myeloid leukemia [58], and trastuzumab (Herceptin®, Genentech) in ERBB2-positive breast cancers [59]. **Figure 3** shows different treatment approaches for the molecularly targeted agents often using companion predictive biomarkers to improve patient outcomes.

Designing early phase precision medicine trials

The current drug development process is lengthy, ineffective and costly. In early phase trials, disappointingly, less than 10% of drugs are successfully approved for eventual use in the general cancer population [2,60]. There is thus renewed impetus to alter the design of clinical trials to evolve with the demands of precision medicine. Here we discuss novel trial designs, innovative targeting strategies and the need for new yardsticks of response.

Historically, Phase I trials have focused on conventional safety and tolerability endpoints to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLT) by enrolling patients with advanced cancers using a classical 3+3 dose escalation trial design. Such a trial strategy, which was originally designed for cytotoxic chemotherapies, is painstakingly slow and may result in large proportions of patients treated at subtherapeutic dose levels. Compared to chemotherapies, novel molecularly targeted agents may also be well tolerated without DLTs observed during the first cycle of therapy. As many targeted therapies are oral drugs taken on a regular basis until disease progression, in contrast to chemotherapies given over a limited number of cycles, the former are often associated with intolerable low-grade chronic toxicities that restrict their long-term use [2, 61]. Such findings are often only detected in late Phase III registration trials, requiring dose modifications and interruptions of the Phase I MTD [62].

There are a number of novel strategies to improve the current drug development paradigm [63]. Phase I patient populations now often include mutation-focused expansions where possible. For example, **basket trials** assess single or a family of genomic abnormalities using NGS and other platforms for treatment with matched targeted therapies, regardless of tumor origin (**Table 1**).

Alternatively, **umbrella trials** assess a number of pre-specified genetic aberrations with matched targeted agents, usually involving specific tumor types (**Table 1**). Novel precision medicine clinical trial designs include accelerated titration design and model-based designs using continual reassessment methods, which allow the exploration of a greater number of dose levels and more patients to be treated at active therapeutic levels [64 – 66].

An essential aspect of patient care is the ability to track tumor biology through the treatment journey. We propose the following biomarker-driven strategies to evade resistance mechanisms and prolong antitumor treatment responses: (1) switch approaches, (2) combination regimens, and (3) priming/herding strategies. These are illustrated in **Figure 3**.

Biopsy-driven treatment as in **Figure 3A** alters targeted therapies according to multiple tumor biopsy analysis results. Switch strategies use real-time NGS analysis of tissue or liquid biopsy samples to detect early signs of drug resistance and to direct the switch to an alternate antitumor agent that can potentially overcome relevant subclonal outgrowth(s) (**Figure 3B**). Combination therapies with two or more directed agents that target horizontal and vertical pathways involved in cancer development and survival to delay or prevent tumor outgrowth and resistance by preventing potential bypass routes (**Figure 3C**). For example, the combination of trametinib (MEK inhibitor) and dabrafenib (BRAF inhibitor) in metastatic malignant melanoma improved patient outcomes from single agent therapy with a progression free survival advantage from 7.3 months to 11.4 months [67]. It may also be theoretically possible to herd cancer cells down particular molecular pathways and thereby artificially drive clonal evolution through predictable

paths using multi-scale mathematical modeling, so as to select for certain subclones that can then be targeted (**Figure 3D**) [68 – 70].

Finally, there is a pressing need to improve the assessment of clinical trial endpoints. Rather than continue to rely on overall survival and progression-free-survival to assess targeted agent efficacy, the use of intermediate endpoint biomarkers and advanced functional imaging modalities are essential, along with a move away from antiquated traditional response assessments. Indeed, tumor reduction may be misleading as a measure of response, especially with immunotherapeutics, and alternative considerations should be pursued.

Precision Medicine in Immuno-Oncology

Precision medicine for **immunotherapies**, while having the same over-arching principles, harbors several major differences to strategies for molecularly targeted agents. Biologic targeted therapies directly inhibit cancer aberrations, whereas immunotherapies harness the immune system and are thus an indirect form of cancer treatment.

While ‘OMIC technology has driven precision medicine in targeted therapy, the same may not be said of immunotherapy, as *bona fide* driver aberrations have not been found to correlate robustly with responses [71]. Immunotherapy has led to superior patient benefit in cancers previously resistant to both chemotherapy and targeted therapy strategies, such as advanced squamous cell NSCLC, renal cell carcinoma and bladder cancers. However, even in these tumor groups, only approximately one in five patients truly appear to benefit from immunotherapy [4,72]. This implies an urgent need for better predictive biomarkers of response and resistance so

as to identify those who will benefit and to exclude patients who will not, avoiding potential drug toxicities and unnecessary financial burden. PD-L1 expression is the most advanced predictive biomarker for PD-1 and PD-L1 inhibitors and is already approved as a companion diagnostic for use with pembrolizumab (Merck) and nivolumab (BMS) [5]. However, there is much debate on PD-L1 as a biomarker, as selected patients without PD-L1 expression have also been found to benefit from immunotherapy, and thus, completely excluding PD-L1 negative patients from immune checkpoint inhibitor therapy would be unfair and unethical [73]. There is currently interest in monitoring PD-L1 expression changes on tumor cells at different treatment time points, as well as investigating its significance on tumor-infiltrating lymphocytes and correlating such data with patient outcomes [74]. Inter and intra-tumor heterogeneous expression of PD-L1 has been reported with variations of approximately 25% [75, 76], resulting in sampling errors and discrepancies between core biopsies and surgical specimens reported in one study of up to 48% [77]. This heterogeneity, as discussed previously, remains an ongoing challenge. Mutational burden has also been associated with improved patient outcomes to immunotherapy with a higher **nonsynonymous mutation** load, that is a greater volume of functional genomic aberrations, associated with improved objective response rate and progression-free survival [78].

A better understanding of the development of tumor **neoantigens**, their recognition by the immune system and evolution over time has given us the opportunity for further personalization of immunotherapeutics with the development of cancer vaccines and cell therapies [79, 80]. Identifying increased absolute and subpopulation lymphocyte counts as well as intratumor heterogeneity of neoantigens has been associated with increased sensitivity to immune checkpoint inhibition and improved overall survival [81, 82].

329

330 While much research focus has been on immune checkpoint inhibitors, there are several other
331 individual-focused immuno-oncology strategies. Adoptive T-cell therapy is the process whereby
332 specific T-cells are identified, expanded and infused into the patient, with T-cell receptor
333 technology (TCR) allowing recognition of proteins expressed within cancer cells. The changes to
334 the immune system as a result of the host's immune response and the tumor's effects is known as
335 **immunoediting**. Immunoediting of T-cell mediated neoantigens using adoptive T-cell therapy
336 has shown durable responses by overcoming some of the resistance mechanisms of tumors, such
337 as loss of neoantigen expression [80]. This would be the epitome of precision medicine in
338 immuno-oncology; however, this technology is still in the early phases of clinical testing, and
339 much work is still required to confirm its safety and efficacy [83]. Several challenges exist in
340 getting T-cells to reach the tumor to effect cytotoxicity; one potential solution is the use of
341 **chimeric antigen receptor (CAR) T cells**. CARs are modified autologous T-cells that are
342 genetically modified with synthetic receptors made of signaling elements and antigen binding
343 domains of T-cell and B-cell receptors respectively [84]. This allows the CAR to target the
344 antigen it has been designed for. Disappointingly, suppression of CARs within the tumor
345 microenvironment has resulted in a lack of efficacy in clinical trials. This has led to the
346 development of "armored" CARs, which are protected from innate destruction in the tumor
347 microenvironment [85].

348

349 Modern clinical trial designs for immunotherapies have taken a different route to those for
350 targeted agents. Several early phase immunotherapy trials are large "all-comer" studies, which
351 incorporate multiple expansion cohorts, resulting in patient numbers ranging from several

hundred to over a thousand patients [73, 86]. This clearly has implications on the time, resources and cost of running such large clinical trials.

Concluding Remarks

Precision medicine, the act of utilizing specific antitumor therapies against molecularly matched cancers, is expected to become the paradigm of future cancer medicine. To date, despite high expectations, precision medicine has disappointingly only improved patient survival in severally molecularly-driven cancers [87 – 89]. Despite advances in modern biotechnologies, including NGS platforms and theranostic companion biomarkers, as well as innovative clinical trial designs, challenges persist, such as clonal evolution resulting in intratumor and intertumor heterogeneity [9] (**Outstanding questions box**).

In order to address such challenges, rational combination regimens of targeted agents and immunotherapies, as well as switch and priming biomarker design strategies should be pursued to delay the development of drug resistance and improve antitumor responses (**Outstanding Questions Box**). In the future, further advances in ‘OMIC-technology and bioinformatics will enhance the detection of low-frequency events occurring before and during treatment (**Outstanding Questions Box**). In addition, advances in non-invasive imaging techniques and liquid biopsies detecting cfDNA will also permit real-time assessments and identify the emergence of resistant clones at earlier time points. Finally, more widespread adoption of contemporary clinical trial designs will also accelerate drug and companion predictive biomarker development in parallel, while critically improving patient outcomes in cancer medicine [1] (**Outstanding Questions Box**).

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Figure 1. Branched clonal evolution with resultant intratumor and intertumor heterogeneity

- Graphic of branched clonal evolution with resultant intratumor and intertumor heterogeneity. Selection pressures and genomic instability can lead to subclonal tumor outgrowths and development of further aberrations, both driver and passenger
- Single biopsies from single sites can result in imprecise management decisions with molecularly targeted therapies that may not target other tumor sites due to intertumor heterogeneity.

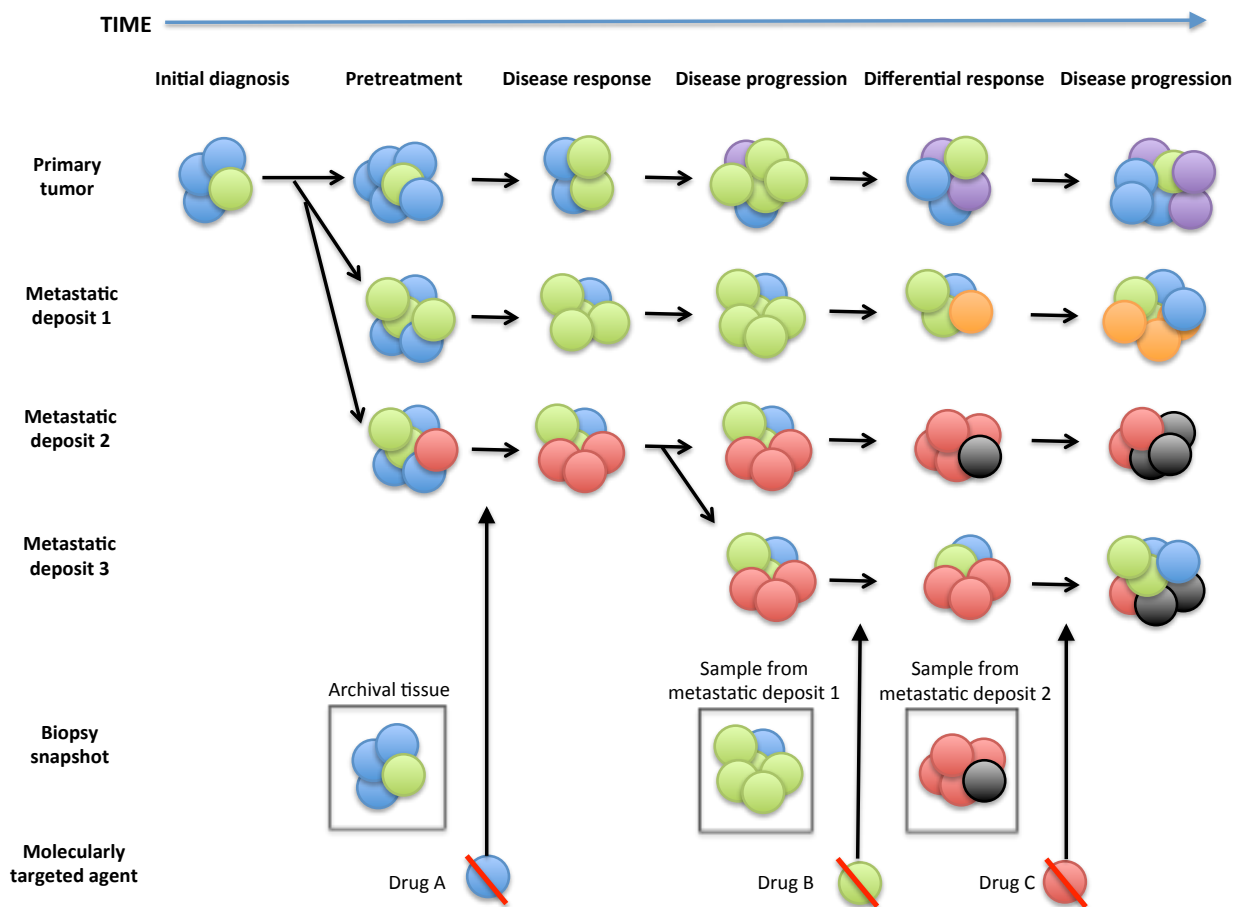


Figure 2. Tumor mass made up of distinct clones and subclones. This leads to three very different biopsy specimens, as depicted in separate boxes 1 – 3. This highlights the importance of multiple biopsies from different locations within a single tumor specimen to get a truer reflection of the underlying complexity

Intratumor heterogeneity with resultant differential biopsy snapshots

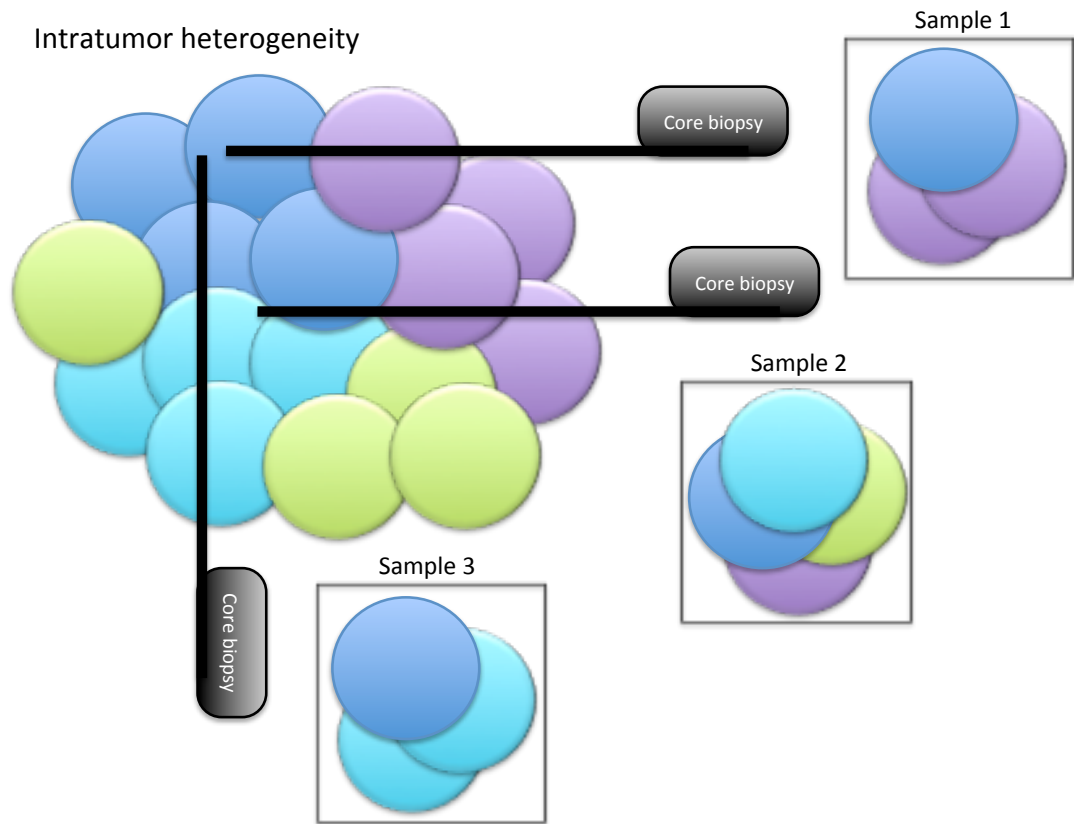


Figure 3. Potential precision medicine treatment strategies to improve tumor response to molecularly targeted agents: a) biopsy-driven; b) switch; c) combination; and d) priming

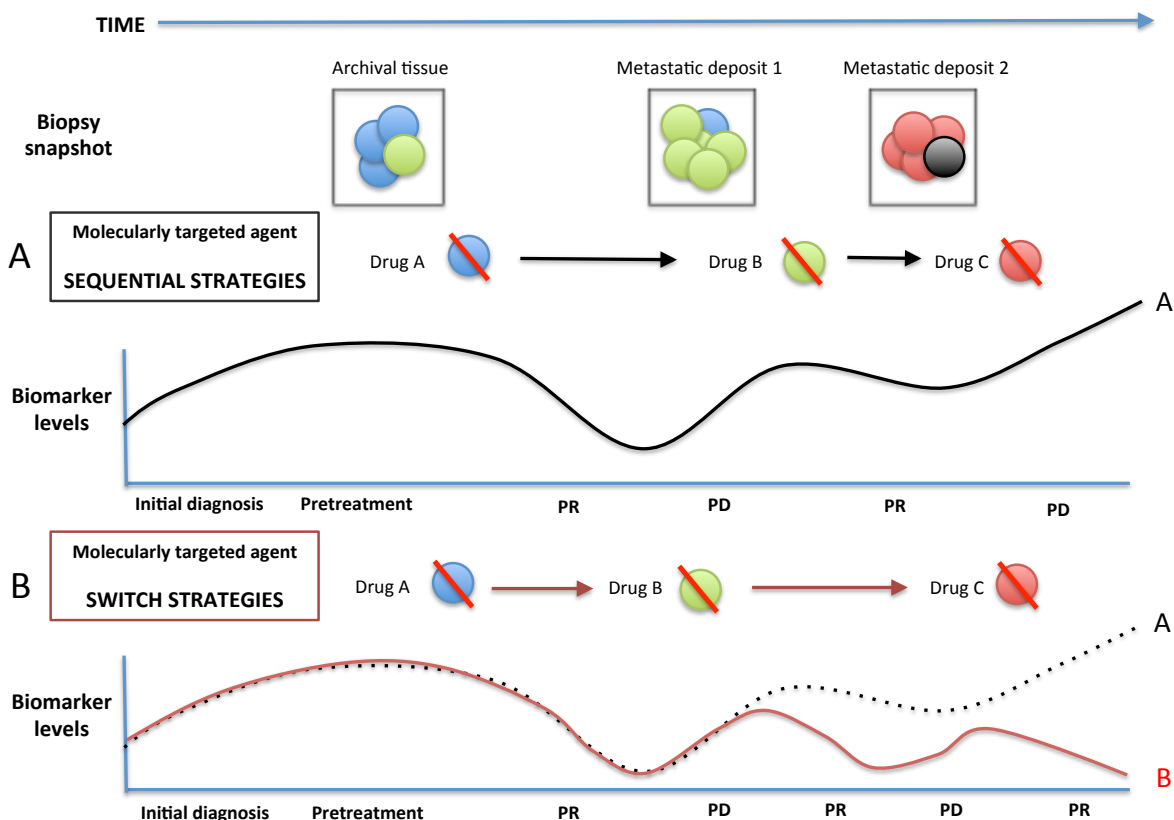
Four examples of precision medicine treatment approaches guided by sequential tumor biopsies, clinical and radiological progression decisions and/or biomarker assessment

3A. Sequential targeted treatments based upon tumor biopsy results

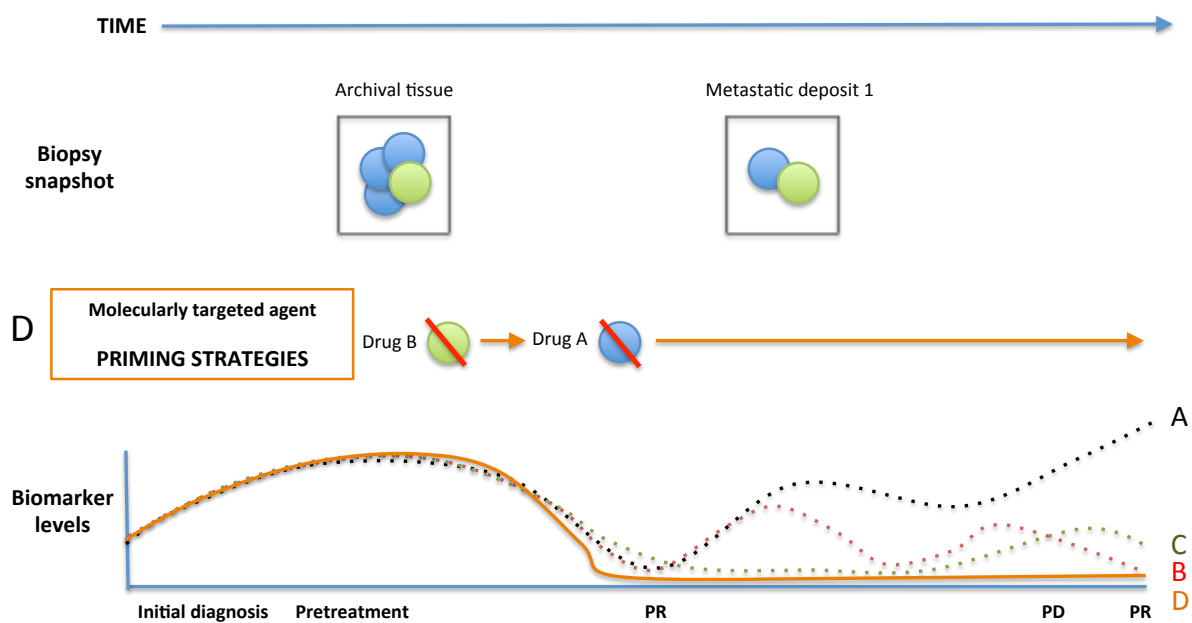
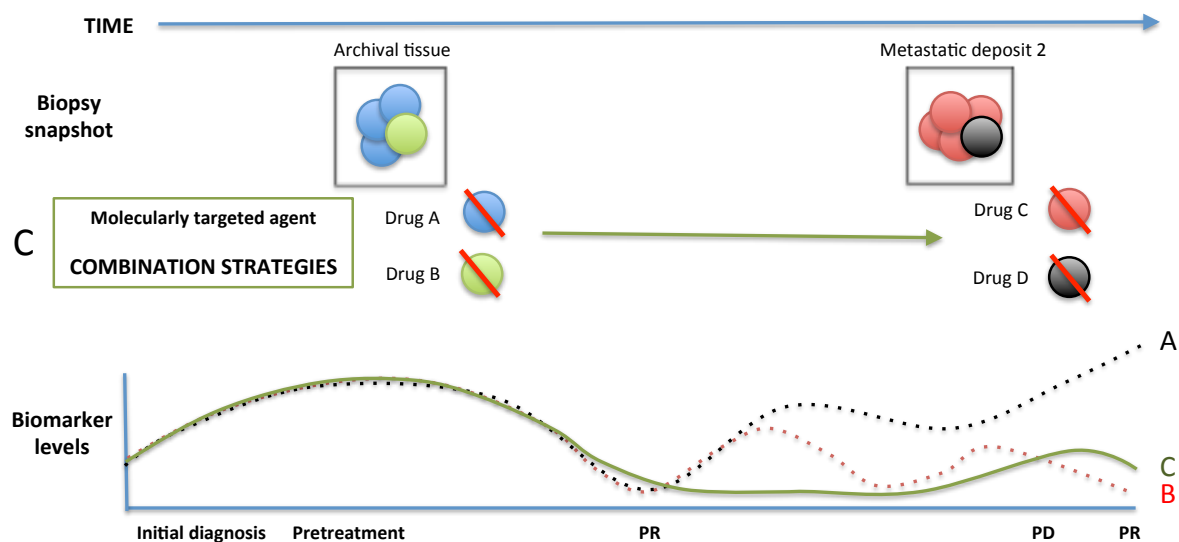
3B. Targeted treatment switch strategies led by measured biomarker assessments

3C. Targeted therapy combination therapy led by biomarker assessment and/or biopsy results from NGS or other platforms

3D. Priming approach purposefully encourages tumor differentiation into specific and actionable subclones that can then be exposed to molecularly targeted agents



Abbreviations: PR: Partial response; PD: Progressive disease



Trial name	Precision Medicine Trial type	Investigational agent & target if known	Inclusion criteria	Target recruitment (n)	Sponsor / Country	NCT identifier
ALCHEMIST Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial	Umbrella	To facilitate accrual to adjuvant Intergroup studies, E4512 (ALK fusion mut & crizotinib) & A081105 (erlotinib & EGFR mut)	Operable Non-squamous NSCLC (Stage IB-IIIa)	6000 – 8000	NCI / ECOG-ACRIN Cancer Research Group	NCT02194738, NCT02193282, NCT02201992
AURORA Aiming to understand molecular aberrations in metastatic breast cancer	Other	Biopsy (repeat), biomarker & NGS analysis	Metastatic breast cancer	1300	Breast International Group (BIG) UK / Europe	NCT02102165
BATTLE-2 Biomarker-Integrated Targeted Therapy Study	Umbrella	AZD6244 + MK-2206 Erlotinib + MK-2206 Sorafenib	Stage IIIB or IV NSCLC progressed on first line treatment	450	MD Anderson Cancer Centre/NCI USA	NCT01248247
DARWIN II Deciphering Antitumor Response and Resistance With Intratumor heterogeneity	Other To assess if intra-tumor heterogeneity is associated with PFS	Alectinib: ALK/RET gene rearrangement MPDL3280A: no actionable mut & PD-L1+ or PDL1- after 1 line of chemo Trastuzumab emtansine: HER2 amplification Vemurafenib: BRAFV600	Relapsed NSCLC	119	Roche UK	NCT02314481
Exceptional Responders study	Other Hypothesis generation for reasons for exceptional response to cancer treatment	Biopsy, biomarker & NGS analysis	Patients with a complete response or partial response of at least 6 months where expected ORR typically <10%	300	NCI USA	NCT02243592, NCT02496195
FOCUS4	Umbrella	BRAF + EGFR +/- MEK inhibitor: BRAF mut Aspirin: PI3KCA mut AKT + MEK inhibitors: KRAS or NRAS mut HER1, 2, 3 inhibitor: All wild type with PTEN expression Capecitabine: nonstratified	Metastatic colorectal cancer post standard 1 st line therapy	4500-5500	MRC/CRUK UK	ISRCTN Clinical Trials Register 9006156
GEMM Genomics-Enabled Medicine for Melanoma	Umbrella	Molecularly matched targeted agents vs physician's choice of therapy	BRAF wild-type Stage IIIa-IV metastatic melanoma after immunotherapy	136	NCI USA	NCT02094872

I-SPY2 Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis 2	Umbrella Neoadjuvant and Personalized Adaptive Novel Agents to Treat Breast Cancer	AMG 386 +/- trastuzumab AMG 479 (Ganitumab) + metformin ABT-888 Ganetespib Neratinib PLX3397 + paclitaxel Pembrolizumab + paclitaxel Trastuzumab + pertuzumab T-DM1 + pertuzumab MK-2206 +/- trastuzumab Comparator arm (paclitaxel + doxorubicin + cyclophosphamide +/- trastuzumab)	Neoadjuvant breast cancer	1200	QuantumLeap Healthcare Collaborative USA, Canada	NCT01042379
Lung-MAP Lung Cancer Master Protocol	Umbrella	AZD4547: FGFR1, FGFR2, FGFR3 Docetaxel: comparator arm Erlotinib +/- rilutimumab: HGF/c-MET MED14736 (durvalumab): no active drug-biomarker option Nivolumab +/- ipilimumab: no active drug-biomarker option Palbociclib: CDK4/6, CCND1,2 & 3 Taselisib: PI3KCA expression	Recurrent advanced squamous NSCLC	10000	SWOG/NCI USA	NCT02154490
MATCH Molecular Analysis for Therapy Choice	Basket	Afatinib: HER2; EGFR mut AKT inhibitor AZD5363: Akt mut Binimetinib: NRAS mut in codon 12, 13, or 61 Crizotinib: MET amp/exon 14 del; ALK trans; ROS1 trans / inv Dabrafenib (+ trametinib): BRAF V600 Dasatinib: DDR2 S768R, I638F or L239R mut Defactinib: NF2 inactivating mut FGFR inhibitor AZD4547: FGFR1-3 amp, mut or trans Nivolumab: Mismatch repair deficiency Osimertinib (AZD9291): EGFR T790M mut Palbociclib: CCND1, 2, or 3 amp + Rb expression by IHC PI3Kbeta inhibitor GSK2636771: PTEN mut, del, expression, loss Sunitinib maleate: cKIT exon 9,11,13 or 14 mut Taselisib: PTEN loss; PI3K mut or amp without RAS mut Trametinib: BRAF V600 (with dabrafenib); BRAF fusion or non-V600; NF1 mut; GNAQ or GNA11 mut Trastuzumab emtansine: HER2 amp Vismodegib: SMO or PTCH1 mutation	Solid tumors & lymphoma post progression on standard therapy	5000	NCI / ECOG-ACRIN USA	NCT02465060
MPACT Molecular Profiling-based Assignment of Cancer Therapy	Basket	Everolimus: PI3K pathway defect MK-1775 (Wee1 inhibitor) + carboplatin: DNA pathway repair defects Temozolomide + veliparib (ABT-888; PARP inhibitor): DNA repair pathway defects Trametinib DMSO: Ras/Raf/Mek pathway mut	Advanced solid tumors	180	NCI USA	NCT01827384

MyPathway	Basket	Erlotinib Trastuzumab + Pertuzumab Vemurafenib Vismodegib	Refractory metastatic cancer with mut or gene abnormalities predictive of response to [below]	500	Roche/Genentech USA	NCT02091141
National Lung Matrix Trial	Umbrella	AZD4547 (FGFR inhibitor) AZD2014 (MTORC1/2 inhibitor) AZD5363 (AKT inhibitor) AZD9291 (EGFRm+ T790M+ inhibitor) Crizotinib (ALK / MET / ROS1 inhibitor) MEDI4736 (anti-PDL1) Palbociclib (CDK4/6 inhibitor) Selumetinib (MEK inhibitor) + doctaxel	Stage IIIB or IV NSCLC	2000	CRUK UK	NCT02664935
SAFIRO2 Efficacy of Targeted Drugs Guided by Genomic Profile in Metastatic NSCLC Patients	Umbrella	Targeted therapy substudy 1 AZD2014 (mTOR inhibitor) AZD4547 (FGFR inhibitor) AZD5363 (AKT inhibitor) AZD8931 (HER2, EGFR inhibitor) Erlotinib (standard maintenance for squamous NSCLC) MEDI4736 (PD-L1 inhibitor) Pemetrexed (standard maintenance for non-squamous NSCLC) Selumetinib (MEK inhibitor) Vandetanib (VEGFR, EGFR inhibitor) Immune substudy 2 Maintenance MEDI4736: no actionable genomic alterations vs standard maintenance	Advanced NSCLC (vs standard of care)	650	UNICANCE R France	NCT02117167
SIGNATURE program	Basket	LEE011: CDK4/6, cyclin D1/3 or p16 gene alterations Ceritinib (LDK378): ALK or ROS1 alterations BGJ398: FGFR gene alterations	Metastatic cancer refractory to standard therapy	100 per trial	Novartis USA	NCT02187783, NCT02186821
TAPUR Targeted Agent and Profiling Utilization Registry	Basket	Axitinib: VEGFR mut, amp, overexpression Bosutinib: Bcr-Abl, SRC, LYN, LCK mut Cetuximab: KRAS, NRAS & BRAF wildtype Crizotinib: ALK, ROS1 & MET mut Dasatinib: Bcr-Abl, SRC, KIT, PDGFRB, EPHA2, FYN, LCK, YES1 mut Erlotinib: EGFR mut Olaparib: Germline or somatic BRCA1 / BRCA2 inactivating mut; ATM mut or del Palbociclib: CDKN2A/p16 loss; CDK4 & CDK6 amp Pembrolizumab: POLE/POLD1 mut Regorafenib: RET, VEGFR1, VEGFR2, VEGFR3, KIT, PDGFR-beta, RAF-1, BRAF mut/amp Sunitinib: CSF1R, PDGFR, VEGFR mut Temsirrolimus: mTOR or TSC mut Trastuzumab + pertuzumab:	Advanced solid tumors, multiple myeloma and B-cell non-Hodgkin lymphoma	1030	ASCO USA	NCT02693535

		HER2 amp emurafenib + Cobimetinib: BRAF V600E mut Vismodegib: PTCH1 del or inactivating mut				
TRACERx Tracking NSCLC Evolution through therapy (Rx)	Other To study NSCLC evolutionary genomic landscape between primary and metastatic sites & intratumor heterogeneity	Biopsy (repeat), biomarker & NGS analysis	Patients with early stage I-IIIa NSCLC eligible for surgery	842	CRUK UK	NCT0188860 1
WINTHER Worldwide Innovative Networking Therapeutics	Basket	Treatment with matched targeted therapies available on the market or on clinical trials	Metastatic cancer with available histologic normal counterpart	200	WIN consortiu m France, Spain, Israel, USA, Canada	NCT0185629 6

641 Table 1. Currently recruiting major precision medicine trials (alphabetical order)

642

643 Abbreviations (alphabetical): amp: amplification; ASCO: American Society of Clinical Oncology; CRUK: Cancer

644 Research United Kingdom; del: deletion; ECOG-ACRIN: Eastern Cooperative Oncology Group (ECOG) and

645 American College of Radiology Imaging Network (ACRIN); EGFR: epidermal growth factor receptor 1;

646 EGFRm+: EGFR mutant; HER2: human epidermal growth factor receptor 2; inv: inversion; MRC: Medical

647 Research Council; mut: mutation; NGS: next generation sequencing; NCI: National Cancer Institute; NSCLC:

648 non small cell lung cancer; ORR: overall response rate; PD-L1: programmed cell death ligand 1; PFS:

649 progression free survival; SWOG: South Western Oncology Group ; trans: translocation; UK: United Kingdom;

650 USA: United States of America; WIN: Worldwide Innovative Network

651

652 TRENDS BOX

653

654 Precision medicine in oncology has been advanced by the discovery and development of
655 sophisticated and modern next generation sequencing technologies

656

657 Clonal evolution, intertumor and intratumor heterogeneity are important challenges to address to
658 achieve success in precision medicine

659

660 Studies to modulate the cancer mutational landscape by applying artificial selection pressures or
661 altering the tumor microenvironment are ongoing

662

663 Experience with immunotherapies and our knowledge of their effects on the cancer cell continue to
664 advance, revealing the importance of neoantigens and the need for robust predictive biomarkers of
665 response and resistance

666 OUTSTANDING QUESTIONS BOX

- 667 1. Can we develop realistic cancer evolution models to assess sensitivity to molecularly
668 targeted agents?
- 669 2. How can the development of validated companion biomarkers for molecularly targeted
670 agents and immunotherapies be optimized?
- 671 3. Can we control, restrain or predict clonal evolution, intertumor and intratumor
672 heterogeneity?
- 673 4. What degree of functional validation is required to ensure that cancer aberrations identified
674 by next generation sequencing are clinically significant?
- 675 5. What proportion of tumors harbor multiple driver aberrations that are challenging to
676 address with precision medicine strategies?

677 GLOSSARY

678 **Avatars:** a mouse implanted with cells or tissue freshly extracted from a human being, to test drug
679 therapies for an individual patient or to study a disease process

680 **Basket trials:** test the effect of a single drug on a single mutation in a variety of cancer types; can also
681 screen multiple drugs across many cancer types.

682 **Biomarker:** a characteristic that is objectively measured or evaluated as an indicator of normal biological
683 processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention

684 **Chimeric antigen receptor (CAR) T cells:** artificial engineered T cell receptors, which graft specificity
685 onto an immune effector cell.

686 **Clonal evolution:** the process of acquisition of genomic/epigenomic aberrations in multicellular
687 organisms, such as a tumor

688 **Clonal mutation:** Aberration that exists in the vast majority of the tumor cells

689 **Epigenetic:** changes that occur in gene expression if cellular phenotype due to mechanisms other than
690 changes in the DNA sequence

691 **Immunoediting:** changes in the **immunogenicity** of tumors due to the anti-tumor response of the
692 immune system, resulting in the emergence of immune-resistant variants.

693 **Immunogenicity:** the ability of a substance to provoke a humoral and/or cell-mediated immune response
694 in the body

695 **Immunotherapy:** the prevention or treatment of disease with agents that stimulate the host's immune
696 response.

697 **Intertumor heterogeneity:** (epi)genetic differences between two tumors

698 **Intratumor heterogeneity:** (epi)genetic differences within the same tumor specimen

699 **Mutagenesis:** process by which the genetic information of an organism is changed in a stable manner,
700 resulting in a mutation. It may occur spontaneously in nature, or as a result of exposure to mutagens

701 **Neoantigens:** antigens encoded by tumor-specific mutated genes.

702 **Next generation sequencing:** non-Sanger-based high-throughput DNA sequencing technologies.

703 **Nonsynonymous mutation:** point mutation in a protein-coding region that alters the amino acid sequence
 704 of a tumor, including missense, nonsense, splice site and indel mutations.

705 **‘OMIC technology:** informal field of study in biology ending in -omics, such as genomics, proteomics or
 706 metabolomics.

707 **Oncogene:** gene with the potential to cause cancer

708 **Organoid:** three-dimensional organ-bud grown in vitro with realistic micro-anatomy and comparable
 709 genomic landscape to parent tissue of origin

710 **Precision Medicine:** "an emerging approach for disease treatment and prevention that takes into
 711 account individual variability in genes, environment, and lifestyle for each person" [definition
 712 National Institutes of Health (NIH)]; “a form of medicine that uses information about a person’s
 713 genes, proteins, and environment to prevent, diagnose, and treat disease” [definition of National
 714 Cancer Institute (NCI)].

715 **Selection pressure:** The extent to which organisms possessing a particular characteristic are
 716 either eliminated or favored by environmental demands.

717 **Subclonal mutation:** an aberration that exists in only a subset of the tumor cells

718 **Theranostic:** coined to define ongoing efforts to combine diagnostic and therapeutic capabilities into a
 719 single agent.

720 **Tumor suppressor gene:** gene that protects a cell from one step on the path to cancer. When mutated,
 721 can cause a loss or reduction in its function and the cell can progress to cancer, usually in combination
 722 with other genetic changes.

723 **Umbrella trial:** test the impact of different drugs on different mutations often in a single cancer type.

724 **Whole Exome Sequencing:** a technique for sequencing all the coding genes in a genome (known as the
 725 exome)

726 **Whole Genome Sequencing:** a laboratory process that determines the complete DNA sequence of an
 727 organism's genome at a single time.