



## Hot Topic

## Review of precision cancer medicine: Evolution of the treatment paradigm

Apostolia M. Tsimberidou<sup>a,\*</sup>, Elena Fountzilias<sup>b</sup>, Mina Nikanjam<sup>c</sup>, Razelle Kurzrock<sup>c</sup><sup>a</sup> The University of Texas MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics, Houston, TX<sup>b</sup> Department of Medical Oncology, Euromedica General Clinic, Thessaloniki, Greece<sup>c</sup> Center for Personalized Cancer Therapy and Division of Hematology and Oncology, UC San Diego Moores Cancer Center, San Diego, CA, USA

## ARTICLE INFO

## Keywords:

ctDNA  
 Personalized  
 Precision  
 Molecular profile  
 Matched therapy  
 Genomic landscape

## ABSTRACT

In recent years, biotechnological breakthroughs have led to identification of complex and unique biologic features associated with carcinogenesis. Tumor and cell-free DNA profiling, immune markers, and proteomic and RNA analyses are used to identify these characteristics for optimization of anticancer therapy in individual patients. Consequently, clinical trials have evolved, shifting from tumor type-centered to gene-directed, histology-agnostic, with innovative adaptive design tailored to biomarker profiling with the goal to improve treatment outcomes. A plethora of precision medicine trials have been conducted. The majority of these trials demonstrated that matched therapy is associated with superior outcomes compared to non-matched therapy across tumor types and in specific cancers. To improve the implementation of precision medicine, this approach should be used early in the course of the disease, and patients should have complete tumor profiling and access to effective matched therapy. To overcome the complexity of tumor biology, clinical trials with combinations of gene-targeted therapy with immune-targeted approaches (e.g., checkpoint blockade, personalized vaccines and/or chimeric antigen receptor T-cells), hormonal therapy, chemotherapy and/or novel agents should be considered. These studies should target dynamic changes in tumor biologic abnormalities, eliminating minimal residual disease, and eradicating significant subclones that confer resistance to treatment. Mining and expansion of real-world data, facilitated by the use of advanced computer data processing capabilities, may contribute to validation of information to predict new applications for medicines. In this review, we summarize the clinical trials and discuss challenges and opportunities to accelerate the implementation of precision oncology.

## Background

The rapidly expanding body of knowledge about the roles of genomics and the immune system in cancer has enabled the development of therapies targeted to specific molecular alterations or other biologic characteristics, such as those implicated in immune suppression. However, genomics has also revealed a complicated reality about malignancies that requires a major shift in the therapy paradigm: away from tumor type-centered and toward gene-directed, histology-agnostic treatment, which is individualized for each patient on the basis of biomarker analysis. This paradigm shift is reflected by the emergence of precision medicine trials with innovative design [1–21]. Next-generation sequencing (NGS) of advanced cancers has demonstrated that genomic alterations do not fall neatly into categories defined by the tumor organ of origin. Furthermore, metastatic tumors harbor tremendously complex and individually unique genomic and immune landscapes [22,23]. Therefore, in order to target malignancies with

“precision,” treatment needs to be personalized.

Historically, phase II and III oncology clinical trials have measured outcomes histologically, but histological assessment cannot always capture the effects of gene-targeted agents or immunotherapy. Precision medicine approaches analyze patients' circulating DNA (liquid biopsy), as well as immune markers and other biologic features, to assess efficacy and make treatment decisions. Genomic biomarkers have been the most successful to date, but other biomarkers, including protein assays and transcriptomics, are being developed and tested [13,24,25]. Several molecular alterations have been identified using sequencing and high-throughput technologies and have led to the approval of targeted agents by the Food and Drug Administration (FDA) [26,27]. Importantly, in recent years, the precision medicine paradigm has embraced immunotherapy and its interaction with genomics, as genomic characteristics, such as mismatch repair gene defects, are critical predictors of checkpoint blockade response [28–30].

Herein, we review the rapid evolution of precision medicine in

\* Corresponding author at: The University of Texas MD Anderson Cancer Center, Department of Investigational Cancer Therapeutics, Unit 455, 1515 Holcombe Boulevard, Houston, TX 77030, USA.

E-mail address: [atsimber@mdanderson.org](mailto:atsimber@mdanderson.org) (A.M. Tsimberidou).

<https://doi.org/10.1016/j.ctrv.2020.102019>

Received 9 January 2020; Received in revised form 22 March 2020; Accepted 24 March 2020

0305-7372/© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

oncology and, in particular, the challenge and opportunity that genomic science has revealed *vis-à-vis* the need for “N-of-1” treatments. This treatment model does not conform to either canonical trial design or clinical practice, which seek to find commonalities between patients and treat them alike; instead, its goal is to provide optimized individualized treatment for each patient on the basis of biomarker analysis.

## History

Survival improvement with gene- or immune-directed therapy was accelerated by several major discoveries. In particular, the introduction of imatinib mesylate (Abl tyrosine kinase inhibitor) for patients with Philadelphia chromosome [t(9;22)]-positive chronic myelogenous leukemia producing the enzymatically aberrant Bcr-Abl [31,32] resulted in near-normal life expectancy for patients with this previously fatal leukemia.

In 2001, the human genome was sequenced [33]. Although this milestone represented an arduous and tremendously expensive endeavour, both the price and time required for sequencing have decreased precipitously, with technology advancing in a manner unparalleled in human history. A plethora of first- and second-generation precision medicine trials have since been conducted (Tables 1 and 2). They include, but are not limited to, the first pan-histology biomarker-driven trial using mostly protein markers [1], the prospective molecular profiling of patients with advanced cancer in the phase I clinical trials setting (IMPACT trial) [2,4], the SHIVA randomized trial [5], trials assessing customized combinations [6,12], and trials including transcriptomics [13].

## Innovative clinical trial designs for precision medicine

Traditionally, oncology trials are drug-centered, aiming to identify common attributes among patients (e.g., their tumor type or, more recently, a shared genomic abnormality) and fit them into a trial with a specific drug regimen. The large variability in genomic subgroups, microenvironment, baseline characteristics, comorbidities, and other covariates resulted in tumor-specific clinical studies encompassing a tremendously heterogeneous population in histology-specific, gene-agnostic trials. Phase III randomized trials were often critical for regulatory approval of a novel agent/regimen, especially since the anti-tumor activity of a new drug/regimen was frequently only marginally better than the comparator arm (usually, conventional therapy), perhaps because the regimen was effective in only a small subgroup of the diverse population represented by any specific histology.

### *Basket, umbrella, platform, octopus, and master protocols*

More recently, basket designs have emerged that target a common genetic defect [27]. The 75% objective response rate noted across tumor types with larotrectinib, which targets *NTRK* fusions, best exemplifies the potential of the basket gene-directed, histology-agnostic model, though other single-gene targets have proven much less responsive [27]. Umbrella trials involve a single histology and different treatments based on the genomic alterations in patient subgroups [34]. Other trial designs include platform trials, which use a single analytic technique, such as NGS, to identify genomic or other biomarkers in tumors with multiple histologies; octopus trials (also referred to as “complete phase I trials”) that have multiple arms testing different combinations featuring a particular drug; and master protocols, which encompass trials with several histologic arms (previously, “broad phase II trials”) or multiple platform, basket, or umbrella trials or sub-trials [2–4,6]. Randomization has also evolved, with the emergence of Bayesian adaptation, which allows dynamic modifications of randomization based on small numbers of patients and real-time outcomes.

### *From drug-centered to patient-centered studies*

The ultimate goal of precision medicine is an individualized, patient-centered (rather than drug-centered) trial based on the best available biomarkers. In “N-of-1” trials, each patient’s treatment is considered separately on the basis of molecular, immune, and other biologic characteristics. These trials involve customized drug combinations tailored to individual patients [12]. Determining efficacy in “N-of-1” trials requires assessing the “strategy” of matching patients to drugs, rather than treatments, which differ from patient to patient.

### *Real-world data*

With advanced computer data “processing” capabilities, real-world registries and data mining are expanding. Two drug approvals by the FDA were based, at least in part, on such data: pembrolizumab for any solid tumor with a mismatch repair gene defect (<https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm5600470.htm>) and palbociclib for male breast cancer (<https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm635276.htm>). The stunning possibility exists that real-world data, if confirmed to accurately portray the anticipated results of prospective trials, will dramatically accelerate the drug approval process.

### **Genomic and other biomarkers**

Genomics has been the cornerstone of precision medicine studies. Beyond genomics, RNA and protein profiling, with proteins being the effectors of signaling, also appear to be important in mediating biologic impact. Interestingly, matching patients to drugs on the basis of genomics has proven more effective in improving outcome than matching on the basis of protein assays, perhaps for technical reasons [24]. Despite the current practical limitations, protein and transcript assays may provide essential information when integrated with genomics [13]. Recently, panels that incorporate immune signatures, based on DNA, RNA, and/or proteins, have also gained clinical significance [35].

### *Genomics*

Given the advances in NGS technologies and the large number of laboratories in the US that perform Clinical Laboratory Improvement Amendments (CLIA)-certified NGS, optimization of the accuracy, reproducibility, and standardization of sequencing methods; variant annotation; and data interpretation is critical. Guidelines for the validation of NGS panels [36] and the interpretation and reporting of genomic variants have been developed [37]. Although whole-genome sequencing is not yet the standard practice in the clinic, the FDA has approved two NGS panels that include hundreds of genes [38].

Most genomic sequencing involves tissue, but blood-derived circulating tumor DNA (ctDNA), circulating tumor cells [39], and exosomes [40] are increasingly used, with the latter two reflecting the contents of live cells.

### *Blood-derived cell-free DNA analysis*

Clinical-grade ctDNA testing, which is non-invasive and reflects tumor heterogeneity (because tumor DNA may be leaked into the bloodstream from multiple metastatic lesions), is increasingly being used to select anti-cancer therapy and to monitor subclone dynamics during treatment [41,42]. The discordance noted in some cases between results of ctDNA testing and tumor tissue genotyping analysis [43] could reflect technical issues but might be attributable to the following biologic reasons: (i) tumor NGS measures genomics in the small piece of tissue biopsied while ctDNA assesses shed DNA from multiple sites; (ii) ctDNA is associated with tumor load and can be detected at low levels.

**Table 1**  
Examples of Precision Medicine Trials: Design and Outcomes.

Year First/Last author	Trial name	Trial type	No of pts screened (N)	Proportion of pts. matched	Biomarker(s)	Outcome	Institute(s)	Comments
<b>Diverse treatment-refractory tumor types</b>								
2010 [1] Von Hoff D Penny R	Bisgrove	Prospective, navigational	86	77%	IHC, FISH, microarray	27% of 66 matched pts had a PFS2/PFS1 ratio* $\geq 1.3$ (95% CI, 1.7% to 38%; $p = 0.007$ ).	US (9 sites)	
2012 [2] Tsimberidou A Kurczok R	IMPACT, first cohort	Registry type, Navigational	1144	15%	PCR-based genomics, 9 genes	Matched vs unmatched RR, 27% vs. 5% ( $p < 0.0001$ ), TTF: median, 5.2 vs. 2.2 mos ( $p < 0.0001$ ) OS: median, 13.4 vs. 9.0 mos ( $p = 0.017$ )	MD Anderson Cancer Center	
2014 [3] Tsimberidou A Berry D	IMPACT, second cohort	Registry type, navigational	1276	11%	PCR-based genomics, 18–50 genes	Matched vs unmatched RR, 11.9% vs. 5% ( $p < 0.0001$ ), PFS: median, 3.9 vs. 2.2 mos, ( $p = 0.001$ ); OS: median, 11.4 vs. 8.6 mos ( $p = 0.04$ )	MD Anderson Cancer Center	2-month landmark analyses, matched therapy group: OS, responders 30.5 months vs. 11.3 months for non-responders ( $p = 0.01$ ).
2017 [4] Tsimberidou AM Kurczok R	IMPACT, third cohort	Registry type, navigational	1436	27%	PCR-based genomics and NGS, 11 to 182 genes	Matched vs unmatched Higher rates of ORR ( $p = 0.0099$ ), TTF ( $p = 0.0015$ ), and OS ( $p = 0.04$ ) PFS not improved with matched therapy ( $p = 0.41$ )	MD Anderson Cancer Center	
2015 [5] Le Toumeau Paolletti X	SHIVA	Prospective, randomized	741	13%	Targeted NGS, ~50 genes		Institut Curie, 8 French sites	~80% of patients received single- agent hormone modulators or everolimus
2016 [6] Schwaederle M Kurczok R	PREDICT	Registry type	347	25%	NGS, 182 or 236 genes	Matched vs unmatched Higher rates of SD $\geq 6$ months/PR/CR ( $p = 0.02$ ) and PFS ( $p < 0.04$ ). Higher matching scores correlated with better OS: 15.7 vs 10.6 mos ( $p = 0.04$ )	University of California San Diego	
2016 [7] Wheler JJ Kurczok R	MD Anderson Personalized Cancer Therapy Initiative	Prospective, navigational	500	24%	NGS, 236 genes	Higher matching scores correlated with higher rates of SD $\geq 6$ months/PR/CR ( $p = 0.024$ ), TTF ( $p = 0.0003$ ), and OS ( $p = 0.05$ )	MD Anderson Cancer Center	
2016 [8] Stockley TL Bedard PL	IMPACT/ COMPACT	Prospective	1893	5%	Hot spot panel, 23 genes	Matched vs unmatched Higher ORR: 19% vs 9%, ( $p = 0.026$ ).	Princess Margaret, Canadian centers	
2017 [9] Massard C Soria JC	MOSCATO	Prospective	1035	19%	Targeted NGS, 40–75 genes; aCGH; RNAseq	PFS2/PFS1 ratio* was $> 1.3$ in 33% (63/193) of patients	Institut Gustave Roussy	251 patients enrolled; 230 were treated; however, how many were screened pre-enrollment is unknown
2018 [10] Hainsworth JD Kurczok R	MyPathway	Prospective, Phase 2 basket	251	Not available	Genomic testing via any CLIA lab	Matched patients, ORR: All, 23% HER2-altered, 38% BRAF-altered, 43% RR = 13% (23 of 182 treated)	Multiple sites, Genentech	
2019 [11] Tredan O Blay JY	Profiler	Prospective	2579	6%	NGS, 69 genes		Four institutes (France)	
2019 [12] Sicklick J Kurczok R	I-PREDICT	Prospective, navigational	149	49%	NGS, 315 genes; ctDNA; PDL1 IHC	Higher matching scores correlated with increased rates of SD $\geq 6$ months/PR/CR: 50% vs 22.4% ( $p = 0.028$ ), PFS ( $p = 0.0004$ ), and OS ( $p = 0.038$ )	University of California San Diego and Avera	First trial to administer customized combination therapy (“N-of-1” matching)
2019 [13] Rodon J Kurczok R	WINTHER	Prospective, navigational	303	35%	NGS, 236 genes; transcriptomics	Higher matching scores correlated with longer PFS ( $p = 0.005$ ) and OS ( $p = 0.03$ )	Five countries (Spain, Israel, France, Canada, US)	First solid tumor trial to include transcriptomics
<b>Specific tumors—Lung</b>								
2011 [14] Kim ES Hong WK	BATTLE	Prospective, adaptive, randomized	255	Not available	11 biomarkers	8-week disease control rate, 46%	MD Anderson Cancer Center	It is unclear how many patients were screened before consent

(continued on next page)

Table 1 (continued)

Year First/Last author	Trial name	Trial type	No of pts screened (N)	Proportion of pts. matched	Biomarker(s)	Outcome	Institute(s)	Comments
2014 [15] Kris MG Bunn PA	Lung cancer mutation consortium	Prospective	1537	17%	Multiplex genotyping, 10 genes	Improved OS with matched vs unmatched therapy (p = 0.006)	14 US sites	
2016 [16] Aisner D Kwiatkowski DJ	Lung Cancer Mutation Consortium II	Prospective	904	12%	NGS, minimum of 14 genes	Improved survival with matched therapy (p < 0.001)	16 sites	
2016 [17] Papadimitrakopoulou V Herbst RS	BAATTLE-2	Prospective, adaptive, randomized	334	Non-applicable	ALK, FISH, EGFR, and KRAS Sanger sequencing	KRAS alterations: longer PFS without erlotinib (p = 0.04); KRAS wild-type tumors: longer OS on erlotinib (p = 0.03)	MD Anderson Cancer Center	
<b>Specific tumors—Breast</b>								
2012 [18] Esserman LJ Hylton N	I-SPY 1	Neoadjuvant, correlative	237	Non-applicable	IHC	pCR differs by subset	Multiple US sites	Aim was to develop biomarkers of response to conventional therapy
2015 [19] Andre F Bonneton H	SAFIRO1/UNICANCER	Prospective	423	13%	Sanger sequencing (2 genes: PIK3CA and AKT); aCGH	Matched group, ORR 9%	18 centers in France	
2016 [20,21] Park JW Berry DA Rugo HS Esserman LJ	I-SPY 2	Phase 2 adaptive design, neoadjuvant	Non-applicable	Non-applicable	IHC, Mammprint	Improved pCR rates in 2 study arms with drug addition: HER2+, hormone receptor-negative: neratinib plus standard therapy (N = 115) vs standard therapy (N = 78): 56% vs 33% Triple-negative: veliparib plus carboplatin (N = 72) with standard therapy vs standard therapy (N = 44): 51% vs 26%	Quantum-Leap Healthcare (US sites)	Results for 2 arms of I-SPY-2 study available
<b>Specific tumors—Gastric</b>								
2019 [120] Lee J WK Kang	VICTORY	Prospective	772	14%	NGS, IHC, PDL1, MMR and EBV status	Improved PFS and OS with matched vs unmatched therapy (p < 0.0001)	Republic of Korea	The trial included 10 phase II trials that operated independently (based on eight biomarkers)

\*PFS2/PFS1 ratio is defined by the PFS on the trial versus the PFS on the therapy immediately preceding the trial; in general, PFS is shorter with every subsequent therapy.

\*\*Only studies published as manuscripts, not just as abstracts, included.

**Abbreviations:** aCGH = array comparative genomic hybridization, ASCO = American Society of Clinical Oncology, CLIA = clinical laboratory improvement amendment, cDNA MA = cDNA microarray, CGP = comprehensive genomic profiling, CR = complete remission, ctDNA = circulating tumor DNA, FISH = fluorescence in situ hybridization, IHC = immunohistochemistry, mos = months, NGS = next-generation sequencing, ORR = overall response rate, OS = overall survival, pCR = pathological complete response, PCR = polymerase chain reaction, PFS = progression-free survival, PR = partial remission; pts = patients, RR = response rate, RRP = reverse phase protein array, SD = stable disease, TTF = time to treatment failure.

**Table 2**  
Selected ongoing studies of precision medicine.

Year started	Trial name	Trial type	Cancer type	Biomarker	NCT number	Institute(s)	Comment
2010 [20,21]	I-SPY 2	Prospective randomized	Neoadjuvant breast cancer	IHC, MammaPrint	NCT01042379	Quantum-Leap Healthcare, US sites	Ongoing study with preliminary results (see Table 1)
2012 [121]	SPECTA-Color	Registry type	Advanced colorectal cancer	NGS/IHC	NCT01723969	European hospitals	
2013	MPACT	Prospective	Advanced cancer	NGS	NCT01827384	NCI, US sites	
2014 [122]	ALCHEMIST	Prospective	Early stage non-small cell lung cancer	Direct sequencing, FISH, CLIA certified genotyping	NCT02194738	NCI, US sites	
2014 [34]	Lung-MAP	Prospective	Advanced squamous cell lung cancer	NGS	NCT02154490	NCI, US sites	
					NCT02785913		
					NCT02965378		
					NCT02785939		
					NCT02785952		
					NCT02926638		
					NCT02766335		
2014 [123]	AURORA	Registry type	Metastatic breast cancer	NGS/RNAseq	NCT02102165	Institut Jules Bordet, Brussels, Belgium, European hospitals	
2014 [124]	Signature	Prospective	Advanced cancers	Variable	CT02187783	Novartis, multiple sites	
2014 [10]	MyPathway	Prospective	Advanced cancers	Genomic testing	NCT02186821	Genentech, US sites	
2014	IMPACT2	Prospective, randomized	Metastatic cancer	Genomic testing	NCT02091141	MD Anderson Cancer Center	
2014 [125]	Pangea	Prospective	Gastro-esophageal adenocarcinoma	Tumor biomarker profiling/cell-free DNA	NCT02213289	University of Chicago	
2015 [126-129]	NCI-MATCH	Prospective	Advanced cancers	NGS	NCT02465060	NCI, US sites	
2015 [12]	I-PREDICT	Prospective navigational	Advanced cancers including treatment-naïve patients	CGP	NCT02534675	UC San Diego	Ongoing study with preliminary results (see Table 1)
2016	DART	Prospective	Rare cancers	NGS correlational testing: whole genomic, transcriptome, liquid biopsy (ctDNA), and immune signature	NCT02834013	SWOG/NCI, multiple US sites	
2016 [130]	TAPUR	Prospective	Advanced cancers	Genomic analysis or IHC	NCT02693535	ASCO, US sites	
2016	DRUP	Prospective	Advanced cancers	NGS	NCT02925234	Netherlands	
2017	Pediatric MATCH	Prospective	Pediatric advanced Cancers	CLIA-certified molecular testing	NCT03155620	NCI-COG, US sites	
2018	Columbia University N-of-1 Clinical Trials	Prospective	Metastatic cancer	Computational strategies (OncoTarget and OncoTreat)		Columbia University	

**Abbreviations:** aCGH = array comparative genomic hybridization, ASCO = American Society of Clinical Oncology, CGP = comprehensive genomic profiling, CLIA = Clinical Laboratory Improvement Amendments, COG = Children's Oncology Group, FISH = fluorescence in situ hybridization, IHC = immunohistochemistry, NCI = National Cancer Institute, NGS = next-generation sequencing, RNA seq = RNA sequencing; SWOG = Southwest Oncology Group.

### Blood-derived circulating tumor cell (CTC) analysis

The presence of CTCs, which are epithelial tumor cells, has been independently associated with worse survival in several types of cancer [44–46]. For example, in a prospective, multicenter, double-blind study, the number of CTCs in patients with untreated metastatic breast cancer correlated with shorter progression-free survival (PFS) and overall survival (OS) [44]. CTCs may also be a predictive biomarker for chemotherapy and immunotherapy [45,47]. However, the use of CTCs in clinical practice has not been fully established [48]. Finally, serial CTC analyses might enable real-time surveillance of the disease. A comparative study of five prospective randomized phase III trials in 6081 patients with metastatic castration-resistant prostate cancer assessed the prognostic value of CTCs compared to prostate-specific antigen [49]. CTC  $\geq 0$  at baseline and at week 13 from treatment initiation was associated with OS. The investigators demonstrated that CTC monitoring was a robust and meaningful response endpoint for early-phase clinical trials in this setting [49].

### Transcriptomics

Transcriptomics refers to the study of RNA transcripts and their function. Transcriptomic analysis is performed using high-throughput technologies, including microarrays and RNA sequencing and it is a potentially valuable tool, particularly when there is discrepancy between genomic alterations and gene expression. Transcriptomics are utilized to identify prognostic and predictive gene expression signatures [50,51], to explore miRNAs and their role in mRNA regulation [52,53] and to identify the tissue of origin in cancer of unknown primary [54–56]. The first solid tumor precision medicine trial to use transcriptomics in the clinic—WINTHER—compared RNA expression in tumors to that in adjacent normal tissue and demonstrated that transcriptomics increased the number of patients that could be matched to therapy [13]. Comparing tumor to normal tissue from the same patient may be necessary because of the large inter-patient variability in normal RNA expression. Other investigators have also used transcriptomics to select targeted treatments in patients with advanced solid tumors [57,58]. Challenges that prevent extensive use of transcriptomic biomarkers are degradation and fragmentation of RNA in formalin-fixed, paraffin-embedded tissue samples, complexity of required bioinformatic analysis of profiling data and low reproducibility of the results.

### Proteomics

Proteomic analysis using immunohistochemical and other assays of tumors from patients with refractory metastatic cancer led to the identification of molecular targets that could guide therapeutic decisions and was associated with longer PFS compared to the patients' PFS with their prior therapy (using patients as their own controls) [1]. Proteomic assays are used in clinical practice to identify prognostic or predictive biomarkers for targeted treatments (hormone receptor expression, HER2 overexpression, ALK expression). However, the weaker correlation of proteomic markers, compared to genomic markers, with clinical outcomes suggests that technical issues should be addressed [24]. In a meta-analysis of phase 1 clinical trials of small molecules that used a genomic biomarker vs. those that used a protein biomarker, the median response rate was 41% vs. 25%, respectively ( $p = 0.05$ ) [24]. Ongoing studies with targeted therapies include correlative analyses using peripheral blood and tumor tissue to identify proteomic biomarkers of response or resistance to treatment (LEEomic, NCT03613220 and BABST-C, NCT03743428).

### Immunotherapy and cellular therapy

By reactivating the innate immune antitumor response, immunotherapy has provided a major breakthrough in oncology

treatment [28,59]. Several novel approaches are currently being explored: checkpoint blockade, oncolytic viruses, cell-based products, modified cytokines, CD3-bispecific antibodies, vaccine platforms, and adoptive cell therapy [60].

### Checkpoint blockade

There are seven FDA-approved checkpoint inhibitors: ipilimumab, pembrolizumab, nivolumab, avelumab, cemiplimab, durvalumab, and atezolizumab. Selected patients with advanced disease have remarkable response, including durable complete remission (CR). Despite the significant benefit noted in patients with diverse tumor types treated with checkpoint inhibitors, approximately 80% of patients across cancers do not experience beneficial effects. In the era of precision medicine, genomics, transcriptomics and other technologies are employed for the identification of biomarkers that predict benefit from immunotherapy. Interestingly, biomarkers predicting checkpoint inhibitor responsiveness are genomic: high tumor mutational burden (TMB) [28,59,61], mismatch gene repair defects resulting in high microsatellite instability (MSI-H) (and, thus, high TMB) [29,62], *PBRM1* alterations [63,64], and *PDL1* amplification [65]. Specifically, TMB has been shown to predict clinical benefit from checkpoint inhibitors [28]. In an analysis of 151 of 1638 patients who were treated with immunotherapeutic regimens and had TMB evaluation, high ( $\geq 20$  mutations/mb) TMB was independently associated with significant improvement in PFS and OS compared to low to intermediate TMB [28]. Other studies have however questioned the use of TMB as a biomarker [66,67].

Given its strong association with response to immunotherapy, MSI-H is an established biomarker for response to checkpoint inhibitors [68,69]. MSI-H tumors have high TMB, often accumulating  $> 1000$  non-synonymous genomic mutations, leading to tumor-specific proteins, known as neoantigens. Due to high clinical benefit rates, immunotherapeutic regimens have been approved by the FDA for the treatment of patients with advanced MSI-H colorectal cancer [70–72] or MSI-H tumors, irrespective of the organ of origin [73]. Finally, defects in DNA proofreading proteins polymerase  $\delta$  (*POLD1*) and polymerase  $\epsilon$  (*POLE*) lead to increased TMB and are associated with response to immunotherapy [59,74,75]. For instance, of 4 patients with non-small cell lung cancer with deleterious mutations in *POLD1* and *POLE* (whole-exome sequencing, [WES]), 3 patients with the highest TMB responded to pembrolizumab [59]. Defects in other DNA repair systems might also be associated with response to immunotherapy. The predictive role of homologous recombination deficiency (HRD) is being evaluated in various tumors, including breast and ovarian cancer. Early phase clinical trials demonstrating that these patients may benefit from the addition of immunotherapy to poly ADP-ribose polymerase (PARP) inhibitors, should be confirmed with additional studies [76,77].

Furthermore, *PBRM1* molecular alterations are evaluated as genomic biomarkers predicting checkpoint inhibitor responsiveness. Specifically, *PBRM1* alterations were evaluated in a study of 35 patients with metastatic renal cell cancer treated with anti-programmed death-1 (PD-1) regimens [63]. WES revealed loss-of-function (LOF) mutations in the *PBRM1* gene that predicted response to immunotherapy. Notably, the *PBRM1* gene encodes for a protein of the chromatin remodeling complex, possibly interfering with hypoxia, and immune signaling pathways [63].

Another biomarker that predicts benefit from immunotherapy is PD-L1 amplification [65]. In a retrospective analysis, this marker was identified in 0.7% (843 of 118,187) patients of various tumor types and it did not always correlate with PD-L1 expression. Six of 9 (66.7%) patients with PD-L1-amplified solid tumors had an objective response to checkpoint inhibitors, and their median PFS was 15.2 months [65]. PDL1 expression, assessed by immunohistochemistry on tumor cells or immune cells can be used as a response marker, albeit a suboptimal one [78]. Approximately 20% of FDA approvals of immunotherapeutic agents are based on companion PD-L1 diagnostic testing [79].

Genomic markers may also predict resistance—loss of JAK2 and beta 2 microglobulin mutations [80]—or hyper-progression (accelerated progression) after checkpoint blockade—*MDM2* amplification and *EGFR* alterations [81]. WES of tumor tissue from 4 patients with advanced melanoma whose disease was resistant to anti-PD1 therapy, demonstrated LOF mutations in genes involved in interferon-receptor signaling and in antigen presentation (JAK1/2,  $\beta$ 2-microglobulin) [80]. Importantly, PTEN loss is associated with resistance to immunotherapy in patients with melanoma, suggesting that targeting the PI3K/AKT/mTOR pathway may overcome resistance to immunotherapy [82]. In our opinion, it is plausible that when PI3K/AKT/mTOR pathway alterations or PTEN loss are the key drivers of the disease, immunotherapy may have limited, if any, antitumor activity. Similarly, STK11 mutations and  $\beta$ -catenin pathway alterations are reportedly associated with resistance to immunotherapy [83,84].

In summary, the available biomarkers are insufficient to adequately predict response to immunotherapy. Novel strategies may enhance our ability to identify biomarkers longitudinally, incorporating ctDNA analysis [85] or tumor tissue immune, genomic, transcriptomic, and proteomic analysis.

#### Adoptive cell therapy

Adoptive cell therapy (ACT) is an innovative personalized treatment approach that enhances a patient's immune system leading to specific tumor cell killing. Immune cells derived from a patient's blood or tissue are expanded *in vitro* and then reinfused into the patient. These immune cells may be reprogrammed to recognize tumor-specific antigens [60,86]. Types of ACT include tumor-infiltrating lymphocyte (TIL) therapy, chimeric antigen receptor (CAR) T-cell therapy, engineered T-cell receptor (TCR) therapy and natural killer (NK) cell therapy.

#### TILs

ACT of TILs is based on the use of T-cells that have infiltrated a patient's tumor. Autologous cells are being harvested and administered to patients after their expansion and activation. This approach has shown promising results in metastatic melanoma [87–90], nasopharyngeal, and cervical carcinoma [91,92]. In three sequential clinical trials in patients with metastatic melanoma who had failed standard therapy, the use of autologous TILs was associated with objective response rates of 49%, 52%, and 72%, respectively; durable CRs were reported in 22% (20 of 93) of patients; and clinical benefit was observed irrespectively of prior therapy [87]. Ongoing clinical trials assess the role of TIL therapy in various solid tumors (NCT03645928, NCT03935893, NCT03108495, NCT03083873).

#### CAR T-cells

CAR T-cells are a type of adoptive T-cell therapy in which autologous T-lymphocytes are genetically engineered to recognize the antigens expressed on malignant cells [93]. Adoptive T-cell therapy has resulted in remarkably high rates of durable CR in hematologic malignancies, including in patients with refractory disease. Therefore, the FDA has approved CAR T-cells for the treatment of pediatric patients and young adults with relapsed/refractory B-cell precursor acute lymphoblastic leukemia (Kymriah™, <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-tisagenlecleucel-adults-relapsed-or-refractory-large-b-cell-lymphoma>) and adult patients with relapsed/refractory diffuse large B-cell lymphoma (Yescarta™, <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/yescarta-axicabtagene-ciloleucel>). CAR T-cells are currently being evaluated in solid tumors [94,95].

#### TCR therapy

This approach uses T-cell receptor (TCR) engineered T-cells, and involves retroviruses that enable integration of new TCR transgene targeting antigens, which are expressed at high levels on different cancers into the genome of T-cells [96]. TCR therapy has been assessed in hematologic and solid malignancies [97–101]. Current trials evaluate treatment-associated toxicity, binding affinity to tumor antigens and efficacy in carefully selected patients with increased tumor burden.

#### NK cell therapy

Natural killer (NK) cells are cytotoxic lymphocytes that play a critical role in innate immunity. NK cells do not cause graft-versus-host disease, which makes them promising candidates for cancer treatment. Treatment of relapsed/refractory acute myeloid leukemia with haplo-identical NK cells and recombinant human interleukin-15 induced CR in 32% of patients [102]. Clinical trials are currently evaluating CAR-NK cells in hematologic (NCT03056339, NCT00995137) and solid (NCT03656705, NCT03383978) malignancies.

#### Personalized vaccines (vaccinomics)

The accumulation of somatic mutations in cancer can generate cancer-specific neo-epitopes. Autologous T-cells often identify these neo-epitopes as foreign bodies, which makes them ideal cancer vaccine targets. Every cancer has its own unique mutations, but a small number of neo-antigens are shared between cancers. Theoretically, technological advances will soon result in rapid mapping of mutations within a genome, rational selection of vaccine targets such as neo-epitopes, and on-demand production of vaccines tailored to a patient's individual tumor. Alternatively, off-the-shelf vaccines for tumors with shared epitopes might also be exploitable.

Several personalized vaccines are currently being evaluated in clinical trials [103,104]. For example, investigators used computational prediction of neo-epitopes to design personalized RNA mutanome vaccines for patients with metastatic melanoma [103]. Two of the five patients treated had objective responses to the vaccine alone, while a third patient had a CR to treatment with the vaccine combined with PD-1 blockade [103]. In another study of vaccine-induced polyfunctional CD4+ and CD8+ T-cells targeting unique neoantigens in patients with melanoma [104], four of six vaccinated patients had no recurrence at 25 months after vaccination [104].

Sipuleucel-T, the first FDA-approved therapeutic cancer vaccine, is produced via *ex vivo* activation of autologous peripheral-blood mononuclear cells by a recombinant fusion protein comprised of prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor [105]. Sipuleucel-T is used to treat metastatic castration-resistant prostate cancer on the basis of results of a randomized, double-blind, placebo-controlled phase III trial in which patients who received Sipuleucel-T had longer survival than those who received placebo (25.8 months vs. 21.7 months, respectively;  $p = 0.03$ ) [105].

#### Challenges and solutions for the optimal implementation of precision medicine

Genomic studies have unveiled the reality of tumors—they are tremendously heterogeneous and complex, and optimized therapy often does not result from classical clinical research and practice models.

Precision medicine studies (Tables 1 and 2) demonstrate the major challenges in designing trials for this new paradigm. First, the rate of matching patients to drugs in these trials ranges from 5% to 49% and is mostly in the 15% to 20% range. Failure to match patients is attributed to (i) enrollment of individuals with end-stage disease, who deteriorate or die early; (ii) use of small gene panels that yield limited actionable alterations; (iii) delays in receiving and interpreting genomic results;

and (iv) difficulty accessing targeted therapy drugs and/or limited drug availability. Some solutions provided by trials with higher matching rates, e.g., I-PREDICT<sup>12</sup> (matching rate, 49%), include: (i) use of clinical trial navigators and medication acquisition specialists; (ii) application of a large NGS panel with > 200 genes; (iii) creation of just-in-time electronic molecular tumor boards immediately upon physician request; and (iv) exploitation of biomarkers to match patients to chemotherapy, hormonal therapy, and immunotherapy (in addition to gene-targeted agents). The majority of these trials [2,3,12,24] have shown improvement in clinical outcomes when treatments are matched to drugs compared to when they are not. Importantly, malignancies have complicated molecular biology, and use of personalized combinations of drugs that address a higher percentage of the aberrations present in an individual cancer is associated with better outcomes than more limited matching [6,7,12,13].

Other major hurdles encountered in the implementation of precision medicine include the following: (i) Potential differences in response to matched therapy depending on histology and/or genomic co-alterations. In contrast to molecular abnormalities that predict tumor agnostic response to treatment (e.g., NTRK fusions, MSI-H) [27,71,73], selected genomic biomarkers are predictive in specific tumor histologies [106,107]. (ii) The heterogeneity, complexity, and constant evolution of genomic landscapes. Due to significant heterogeneity between primary tumor and metastatic sites, molecular profiling of tumor tissue obtained from a single lesion may not always be representative of the systemic disease [108,109]. Additionally, under the pressure of targeted treatments, tumor molecular profile constantly evolves, with emerging resistant clones and new molecular alterations driving disease progression [110,111]. (iii) The need to screen large numbers of patients in order to find specific/rare genomic defects (for instance, NTRK fusions) [27,106,107]. (iv) Incomplete biologic/molecular profiles with which to select therapy; suboptimal technology and resources to understand completely the drivers of cancer in individual patients; (v) Considerable delays in the activation of clinical trials; (vi) differences in the metabolism and adverse effects of study drugs in various ethnic groups; (vii) lack of agreement between assays from different diagnostic companies/laboratories; and (viii) most importantly, lack of access to drugs for patients with limited resources as well as excessive eligibility criteria that rule out large swaths of patients with real-world comorbidities. Approximately 3–5% of patients with cancer are enrolled on clinical trials and accrual is limited by overly restrictive eligibility criteria and limited access to drugs [112]. ASCO, the Friends of Cancer Research, and the FDA recommended to broaden eligibility criteria to allow more patients to participate in clinical trials and gain benefit from novel investigational therapies [113]; and consequently participants will be representative of the actual patient population, increasing generalizability of the results. Patient enrollment could be enhanced by national and worldwide collaborations, as shown in multi-institutional trials [114,115]. Finally, the Clinical Trials Transformation Initiative (CTTI), has been developed to examine the challenges and propose solutions to improve trial recruitment [116].

Several initiatives might help overcome the challenges introduced by our emerging understanding of cancer biology: (i) molecular profiling (tissue, blood) should be used at the time of diagnosis and during the course of the disease, the latter to monitor response and resistance; (ii) completion of molecular profiling should be expedited; and (iii) bioinformatic analysis should be optimized to include the key drivers of carcinogenesis.

With the current excitement about the promise of immunotherapy, a large proportion of patients are assigned to immunotherapy trials without undergoing molecular profiling or immune marker identification. Although a significant minority of these patients will experience a clinical benefit and prolonged survival, the majority will have disease progression and/or significant adverse events. Therefore, the incorporation of biomarkers into the selection of patients for immunotherapy needs to be optimized.

Finally, the immense potential of real-world data needs to be addressed. Validation of database information can be performed by comparing outcomes of clinical trials that led to approval with those in the database; if outcomes are similar, real-world data can then be used to rapidly predict new applications for medicines.

## Conclusions and future perspectives

Remarkable biotechnological advances are transforming cancer care. Tumor and cell-free DNA profiling using NGS, as well as proteomic and RNA analysis, and a better understanding of immune mechanisms are optimizing cancer treatment selection. A major challenge in the therapeutic management of patients with advanced metastatic cancer is the complexity of tumor biology. This complexity is attributed to highly variable patterns of genetic and epigenetic diversity and clonal architecture associated with spatial expansion, proliferative self-renewal, migration, and invasion. The complexity is amplified by the dynamic, Darwinian evolutionary character of cancer cells, which undergo sequential searches for mechanisms to escape environmental constraints. Such cellular evolution involves the interplay of advantageous “driver” lesions, neutral or “passenger/hitchhiker” abnormalities, molecular changes in the tumor cells that increase the rate of other genomic anomalies, and modifications to the microenvironment and immune machinery that alter the fitness effects of other variables [117]. Strategies to address tumor complexity include targeting self-renewing cancer stem cells to overcome their plasticity and adaptability, impacting the microenvironment, and turning cancer into a chronic disease (using cytostatic drugs to suppress cell division and new mutations). The complicated nature of tumor biology is also the result of interactions between the tumor, host, and local ecosystem, including HLA type, genetic polymorphisms, microbiome, immune cell repertoire, and tumor microenvironment [118]. New strategies, some of which now have a proven track record, include gene-directed therapies and a host of immune-targeted approaches (e.g., checkpoint blockade, CAR T-cells, personalized vaccinomics) [118,119].

An overarching theme is that optimized therapy may require the utilization of combinations of drugs and/or strategies that attack the tumor from multiple angles. It is time to recognize the possibility that advanced computer implementation could generate real-world data that expand our understanding of cancer, rapidly identify new treatments, and create personalized drugs or immune therapies.

## Authors' contributions

All authors wrote and approved the paper.

## Funding

NIH/NCI, award number P30 CA016672.

## Declaration of Competing Interest

Dr. Apostolia-Maria Tsimberidou has the following financial relationships to disclose: Research Funding (Institution): Immatics, Parker Institute for Cancer Immunotherapy, Tempus, OBI Pharma, EMD Serono, Baxalta, ONYX, Bayer, Boston Biomedical, Placon Therapeutics, Karus Therapeutics, and Tvardi Therapeutics. Consulting or Advisory Role: Covance, Genentech, and Tempus.

Dr. Elena Fountzilias has the following financial relationships to disclose: Travel grant from Merck and K.A.M Oncology/Hematology; stock ownership Deciphera Pharmaceuticals, Inc.

Dr. Mina Nikanjam has the following financial relationships to disclose: Research Funding (Institution): Regeneron, Bristol Myers Squibb, Immunocore, Idera, and Merck.

Dr. Razelle Kurzrock has the following financial relationships to

disclose: Research Funding (Institution): Incyte, Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, Konica Minolta, Grifols, Biologic Dynamics, and Guardant. Consulting role: X-Biotech, Loxo, and Actuate Therapeutics. Speaker fees: Roche. Ownership interest: IDbyDNA and Curematch, Inc.

## References

- Von Hoff DD, Stephenson Jr. JJ, Rosen P, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. *J Clin Oncol* 2010;28:4877–83.
- Tsimberidou AM, Iskander NG, Hong DS, et al. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. *Clin Cancer Res* 2012;18:6373–83.
- Tsimberidou AM, Wen S, Hong DS, et al. Personalized medicine for patients with advanced cancer in the phase I program at MD Anderson: validation and landmark analyses. *Clin Cancer Res* 2014;20:4827–36.
- Tsimberidou AM, Hong DS, Ye Y, et al. Initiative for Molecular Profiling and Advanced Cancer Therapy (IMPACT): An MD Anderson Precision Medicine Study. *JCO Precis. Oncol* 2017;2017..
- Le Tourneau C, Delord JP, Goncalves A, et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol* 2015;16:1324–34.
- Schwaederle M, Parker BA, Schwab RB, et al. Precision Oncology: The UC San Diego Moores Cancer Center PREDICT Experience. *Mol Cancer Ther* 2016;15:743–52.
- Wheler JJ, Janku F, Naing A, et al. Cancer Therapy Directed by Comprehensive Genomic Profiling: A Single Center Study. *Cancer Res* 2016;76:3690–701.
- Stockley TL, Oza AM, Berman HK, et al. Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret IMPACT/COMPACT trial. *Genome Med* 2016;8:109.
- Massard C, Michiels S, Ferte C, et al. High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial. *Cancer Discov* 2017;7:586–95.
- Hainsworth JD, Meric-Bernstam F, Swanton C, et al. Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study. *J Clin Oncol* 2018;36:536–42.
- Tredan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfILER trial. *Ann Oncol* 2019;30:757–65.
- Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med* 2019;25:744–50.
- Rodon J, Soria JC, Berger R, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. *Nat Med* 2019;25:751–8.
- Kim ES, Herbst RS, Wistuba II, et al. The BATTLE trial: personalizing therapy for lung cancer. *Cancer Discov* 2011;1:44–53.
- Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* 2014;311:1998–2006.
- Aisner DL, Sholl LM, Berry LD, et al. The Impact of Smoking and TP53 Mutations in Lung Adenocarcinoma Patients with Targetable Mutations-The Lung Cancer Mutation Consortium (LCMC2). *Clin Cancer Res* 2018;24:1038–47.
- Papadimitrakopoulou V, Lee JJ, Wistuba II, et al. The BATTLE-2 Study: A Biomarker-Integrated Targeted Therapy Study in Previously Treated Patients With Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* 2016;34:3638–47.
- Esserman LJ, Berry DA, DeMichele A, et al. Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL-CALGB 150007/150012, ACRIN 6657. *J Clin Oncol* 2012;30:3242–9.
- Andre F, Bachelot T, Commo F, et al. Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: a multicentre, prospective trial (SAFIR01/UNICANCER). *Lancet Oncol* 2014;15:267–74.
- Park JW, Liu MC, Yee D, et al. Adaptive Randomization of Neratinib in Early Breast Cancer. *N Engl J Med* 2016;375:11–22.
- Rugo HS, Olopade OI, DeMichele A, et al. Adaptive Randomization of Veliparib-Carboplatin Treatment in Breast Cancer. *N Engl J Med* 2016;375:23–34.
- Wheler J, Lee JJ, Kurzrock R. Unique molecular landscapes in cancer: implications for individualized, curated drug combinations. *Cancer Res* 2014;74:7181–4.
- Kurzrock R, Giles FJ. Precision oncology for patients with advanced cancer: the challenges of malignant snowflakes. *Cell Cycle* 2015;14:2219–21.
- Schwaederle M, Zhao M, Lee JJ, et al. Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. *JAMA Oncol* 2016;2:1452–9.
- Rosario SR, Long MD, Affronti HC, Rowsam AM, Eng KH, Smiraglia DJ. Pan-cancer analysis of transcriptional metabolic dysregulation using The Cancer Genome Atlas. *Nat Commun* 2018;9:5330.
- Long GV, Stroyakovskiy D, Gogas H, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med* 2014;371:1877–88.
- Drilon A, Laetsch TW, Kummar S, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med* 2018;378:731–9.
- Goodman AM, Kato S, Bazhenova L, et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther* 2017;16:2598–608.
- Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015;372:2509–20.
- Subbiah V, Kurzrock R. The Marriage Between Genomics and Immunotherapy: Mismatch Meets Its Match. *Oncologist* 2019;24:1–3.
- Kurzrock R, Shtalrid M, Romero P, et al. A novel c-abl protein product in Philadelphia-positive acute lymphoblastic leukaemia. *Nature* 1987;325:631–5.
- Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. *N Engl J Med* 1988;319:990–8.
- Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science* 2001;291:1304–51.
- Herbst RS, Gandara DR, Hirsch FR, et al. Lung Master Protocol (Lung-MAP)-A Biomarker-Driven Protocol for Accelerating Development of Therapies for Squamous Cell Lung Cancer: SWOG S1400. *Clin Cancer Res* 2015;21:1514–24.
- Pabla S, Conroy JM, Nesline MK, et al. Proliferative potential and resistance to immune checkpoint blockade in lung cancer patients. *J Immunother Cancer* 2019;7:27.
- Jennings LJ, Arcila ME, Corless C, et al. Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn* 2017;19:341–65.
- Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19:4–23.
- Genomic Profiling Tests Cleared by FDA. 2017. (Accessed 7/12/2019, at <https://www.cancer.gov/news-events/cancer-currents-blog/2017/genomic-profiling-tests-cancer>).
- Salami SS, Singhal U, Spratt DE, et al. Circulating Tumor Cells as a Predictor of Treatment Response in Clinically Localized Prostate Cancer. *JCO Precision Oncol* 2019;1–9.
- Abd Elmageed ZY, Yang Y, Thomas R, et al. Neoplastic reprogramming of patient-derived adipose stem cells by prostate cancer cell-associated exosomes. *Stem Cells* 2014;32:983–97.
- Chabon JJ, Simmons AD, Lovejoy AF, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. *Nat Commun* 2016;7:11815.
- Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018;15:81–94.
- Merker JD, Oxnard GR, Compton C, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *Arch Pathol Lab Med* 2018.
- Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004;351:781–91.
- Hiltermann TJ, Pore MM, van den Berg A, et al. Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. *Ann Oncol* 2012;23:2937–42.
- Hofman V, Ilie MI, Long E, et al. Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the Cell Search Assay and the isolation by size of epithelial tumor cell method. *Int J Cancer* 2011;129:1651–60.
- Tammaing M, de Wit S, Hiltermann TJ, et al. Circulating tumor cells in advanced non-small cell lung cancer patients are associated with worse tumor response to checkpoint inhibitors. *J Immunother Cancer* 2019;7:173.
- Rossi E, Fabbri F. CTCs 2020: Great Expectations or Unreasonable Dreams. *Cells* 2019;8.
- Heller G, McCormack R, Kheoh T, et al. Circulating Tumor Cell Number as a Response Measure of Prolonged Survival for Metastatic Castration-Resistant Prostate Cancer: A Comparison With Prostate-Specific Antigen Across Five Randomized Phase III Clinical Trials. *J Clin Oncol: Off J Am Soc Clin Oncol* 2018;36:572–80.
- Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med* 2018;379:111–21.
- Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
- Buffa FM, Camps C, Winchester L, et al. microRNA-associated progression pathways and potential therapeutic targets identified by integrated mRNA and microRNA expression profiling in breast cancer. *Cancer Res* 2011;71:5635–45.
- Jacobsen A, Silber J, Harinath G, Huse JT, Schultz N, Sander C. Analysis of microRNA-target interactions across diverse cancer types. *Nat Struct Mol Biol* 2013;20:1325–32.
- Michuda J, Igartua C, Taxter T, Bell JS, Pelosoff R, White K. Transcriptome-based cancer type prediction for tumors of unknown origin. *J Clin Oncol* 2019;37:3081–9.
- Bridgewater J, van Laar R, Floore A, Van'T Veer L. Gene expression profiling may improve diagnosis in patients with carcinoma of unknown primary. *Br J Cancer* 2008;98:1425–30.
- Tohill RW, Shi F, Paiman L, et al. Development and validation of a gene expression tumour classifier for cancer of unknown primary. *Pathology* 2015;47:7–12.
- Weidenbusch B, Richter GHS, Kesper MS, et al. Transcriptome based individualized therapy of refractory pediatric sarcomas: feasibility, tolerability and efficacy. *Oncotarget* 2018;9:20747–60.
- Worst BC, van Tilburg CM, Balasubramanian GP, et al. Next-generation personalised medicine for high-risk paediatric cancer patients - The INFORM pilot study. *Eur J Cancer* 2016;65:91–101.
- Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer.

- Science 2015;348:124–8.
- [60] Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* 2015;348:62–8.
- [61] Jhaveri KL, Wang XV, Makker V, et al. Ado-trastuzumab emtansine (T-DM1) in patients with HER2-amplified tumors excluding breast and gastric/gastroesophageal junction (GEJ) adenocarcinomas: results from the NCI-MATCH trial (EAY131) subprotocol Q. *Ann Oncol* 2019;30:1821–30.
- [62] Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med* 2018;378:2093–104.
- [63] Miao D, Margolis CA, Gao W, et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. *Science* 2018;359:801–6.
- [64] Otto G. PBRM1 loss promotes tumour response to immunotherapy. *Nat Rev Clin Oncol* 2018;15:134–5.
- [65] Goodman AM, Piccioni D, Kato S, et al. Prevalence of PDL1 Amplification and Preliminary Response to Immune Checkpoint Blockade in Solid Tumors. *JAMA Oncol* 2018;4:1237–44.
- [66] Langer C, Gadgeel S, Borghaei H, et al. OA04.05 KEYNOTE- 021: TMB and Outcomes for Carboplatin and Pemetrexed With or Without Pembrolizumab for Nonsquamous NSCLC. *J Thoracic Oncol* 2019;14:S216.
- [67] Garassino M, Rodriguez-Abreu D, Gadgeel S, et al. OA04.06 Evaluation of TMB in KEYNOTE- 189: Pembrolizumab Plus Chemotherapy vs Placebo Plus Chemotherapy for Nonsquamous NSCLC. *J Thoracic Oncol* 2019;14:S216–7.
- [68] Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid Tumors. *Clin Cancer Res* 2019;25:3753–8.
- [69] Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018;24:1449–58.
- [70] Overman MJ, McDermott R, Leach JL, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017;18:1182–91.
- [71] Le DT, Kim TW, Cutsem EV, et al. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability–High/Mismatch Repair-Deficient Metastatic Colorectal Cancer: KEYNOTE-164. *J Clin Oncol* 2020;38:11–9.
- [72] Overman MJ, Lonardi S, Wong KYM, et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. *J Clin Oncol: Off J Am Soc Clin Oncol* 2018;36:773–9.
- [73] Marabelle A, Le DT, Ascierto PA, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol* 2020;38:1–10.
- [74] Gong J, Wang C, Lee PP, Chu P, Fakih M. Response to PD-1 Blockade in Microsatellite Stable Metastatic Colorectal Cancer Harboring a POLE Mutation. *J Natl Comprehensive Cancer Netw: NCCN* 2017;15:142–7.
- [75] van Gool IC, Eggink FA, Freeman-Mills L, et al. POLE Proofreading Mutations Elicit an Antitumor Immune Response in Endometrial Cancer. *Clin Cancer Res* 2015;21:3347–55.
- [76] Domchek S, Postel-Vinay S, Im S, et al. *Ann Oncol* 2019;30(suppl\_5). v475–v532 101093/annonc/mdz253.
- [77] Konstantinopoulos PA, Waggoner S, Vidal GA, et al. Single-Arm Phases 1 and 2 Trial of Niraparib in Combination With Pembrolizumab in Patients With Recurrent Platinum-Resistant Ovarian Carcinoma. *JAMA Oncol* 2019;5:1141–9.
- [78] Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. *Mol Cancer Ther* 2015;14:847–56.
- [79] Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. *J Immunother Cancer* 2019;7:278.
- [80] Zaretsky JM, Garcia-Diaz A, Shin DS, et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med* 2016;375:819–29.
- [81] Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after Immunotherapy: Analysis of Genomic Alterations Associated with Accelerated Growth Rate. *Clin Cancer Res* 2017;23:4242–50.
- [82] Peng W, Chen JQ, Liu C, et al. Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discov* 2016;6:202–16.
- [83] Koyama S, Akbay EA, Li YY, et al. STK11/LKB1 Deficiency Promotes Neutrophil Recruitment and Proinflammatory Cytokine Production to Suppress T-cell Activity in the Lung Tumor Microenvironment. *Cancer Res* 2016;76:999–1008.
- [84] Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic  $\beta$ -catenin signalling prevents anti-tumour immunity. *Nature* 2015;523:231–5.
- [85] Said R, Guilbert N, Oxnard GR, Tsimberidou AM. Circulating tumor DNA analysis in the era of precision oncology. *Oncotarget* 2020;11:188–211.
- [86] Schumacher TNM. T-cell-receptor gene therapy. *Nat Rev Immunol* 2002;2:512–9.
- [87] Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2011;17:4550–7.
- [88] Besser MJ, Shapira-Frommer R, Itzhaki O, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2013;19:4792–800.
- [89] Andersen R, Donia M, Ellebaek E, et al. Long-Lasting Complete Responses in Patients with Metastatic Melanoma after Adoptive Cell Therapy with Tumor-Infiltrating Lymphocytes and an Attenuated IL2 Regimen. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2016;22:3734–45.
- [90] Forget M-A, Haymaker C, Hess KR, et al. Prospective Analysis of Adoptive TIL Therapy in Patients with Metastatic Melanoma: Response, Impact of Anti-CTLA4, and Biomarkers to Predict Clinical Outcome. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2018;24:4416–28.
- [91] Comoli P, Pedrazzoli P, Maccario R, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. *J Clin Oncol: Off J Am Soc Clin Oncol* 2005;23:8942–9.
- [92] Stevanović S, Draper LM, Langhan MM, et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J Clin Oncol: Off J Am Soc Clin Oncol* 2015;33:1543–50.
- [93] Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014;371:1507–17.
- [94] ACTolog in Patients With Solid Cancers (ACTolog). 2016. (Accessed 6/24/2019, at <https://clinicaltrials.gov/ct2/show/NCT02876510?cond=actolog&rank=1>).
- [95] Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after Chimeric antigen receptor T-cell therapy. *N Engl J Med* 2016;375:2561–9.
- [96] Govers C, Sebestyén Z, Coccorsis M, Willemsen RA, Debets R. T cell receptor gene therapy: strategies for optimizing transgenic TCR pairing. *Trends Mol Med* 2010;16:77–87.
- [97] Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* 2009;114:535–46.
- [98] Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science (New York, NY)* 2006;314:126–9.
- [99] Chodon T, Comin-Anduix B, Chmielowski B, et al. Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2014;20:2457–65.
- [100] Kageyama S, Ikeda H, Miyahara Y, et al. Adoptive Transfer of MAGE-A4 T-cell Receptor Gene-Transduced Lymphocytes in Patients with Recurrent Esophageal Cancer. *Clin Cancer Res: Off J Am Assoc Cancer Res* 2015;21:2268–77.
- [101] Parkhurst MR, Yang JC, Langan RC, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther* 2011;19:620–6.
- [102] Cooley S, He F, Bachanova V, et al. First-in-human trial of rhIL-15 and haplo-identical natural killer cell therapy for advanced acute myeloid leukemia. *Blood Adv* 2019;3:1970–80.
- [103] Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 2017;547:222–6.
- [104] Ott PA, Hu Z, Keskin DB, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* 2017;547:217–21.
- [105] Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010;363:411–22.
- [106] Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med* 2015;373:726–36.
- [107] Ross JS, Ali SM, Fasan O, et al. ALK fusions in a wide variety of tumor types respond to anti-ALK targeted therapy. *Oncologist* 2017;22:1444–50.
- [108] Lovly CM, Salama AKS, Salgia R. Tumor Heterogeneity and Therapeutic Resistance. *Am Soc Clin Oncol Educ Book* 2016:e585–93.
- [109] Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *N Engl J Med* 2012;366:883–92.
- [110] Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2005;352:786–92.
- [111] Napolitano A, Vincenzi B. Secondary KIT mutations: the GIST of drug resistance and sensitivity. *Br J Cancer* 2019;120:577–8.
- [112] Murthy VH, Krumholz HM, Gross CP. Participation in cancer clinical trials: race-, sex-, and age-based disparities. *JAMA* 2004;291:2720–6.
- [113] Kim ES, Bruinooge SS, Roberts S, et al. Broadening Eligibility Criteria to Make Clinical Trials More Representative: American Society of Clinical Oncology and Friends of Cancer Research Joint Research Statement. *J Clin Oncol* 2017;35:3737–44.
- [114] Unger JM, Cook E, Tai E, Bleyer A. The Role of Clinical Trial Participation in Cancer Research: Barriers, Evidence, and Strategies. *Am Soc Clin Oncol Educ Book* 2016;35:185–98.
- [115] Trimble EL, Abrams JS, Meyer RM, et al. Improving cancer outcomes through international collaboration in academic cancer treatment trials. *J Clin Oncol* 2009;27:5109–14.
- [116] Huang GD, Bull J, Johnston McKee K, Mahon E, Harper B, Roberts JN. Clinical trials recruitment planning: A proposed framework from the Clinical Trials Transformation Initiative. *Contemporary Clin Trials* 2018;66:74–9.
- [117] Greaves M, Maley CC. Clonal evolution in cancer. *Nature* 2012;481:306–13.
- [118] Sahin U, Tureci O. Personalized vaccines for cancer immunotherapy. *Science* 2018;359:1355–60.
- [119] van Rooij N, van Buuren MM, Philips D, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin Oncol* 2013;31:e439–42.
- [120] Lee J, Kim ST, Kim K, et al. Tumor Genomic Profiling Guides Patients with Metastatic Gastric Cancer to Targeted Treatment: The VIKTORY Umbrella Trial. *Cancer Discov* 2019;9:1388–405.
- [121] Folprecht G, Aust DE, Roth A, et al. Improving access to molecularly defined clinical trials for patients with colorectal cancer: The EORTC SPECTAColor platform. *J Clin Oncol* 2015;33: 575–575.
- [122] Gerber DE, Oxnard GR, Mandrekars SJ, et al. ALCHEMIST: a clinical trial platform to bring genomic discovery and molecularly targeted therapies to early-stage lung cancer. *J Clin Oncol* 2015;33: TPS7583-TPS.

- [123] Aftimos PG, Antunes De Melo e Oliveira AM, Hilbers F, et al. 1520First report of AURORA, the breast international group (BIG) molecular screening initiative for metastatic breast cancer (MBC) patients (pts). *Ann Oncol* 2019;30.
- [124] Slosberg ED, Kang BP, Peguero J, et al. Signature program: a platform of basket trials. *Oncotarget* 2018;9:21383–95.
- [125] Joshi SS, Maron SB, Lomnicki S, et al. Personalized antibodies for gastroesophageal adenocarcinoma (PANGEA): A phase II precision medicine trial (NCT02213289). *J Clin Oncol* 2018;36:TPS198-TPS.
- [126] Krop IE, Jegede O, Grilley-Olson JE, et al. Results from molecular analysis for therapy choice (MATCH) arm I: Taselisib for PIK3CA-mutated tumors. *J Clin Oncol* 2018;36. 101-101.
- [127] Jhaveri KL, Wang XV, Makker V, et al. Ado-trastuzumab emtansine in patients with HER2-amplified tumors excluding breast and gastric/gastroesophageal junction adenocarcinomas: results from the NCI-MATCH trial (EAY131) sub-protocol Q. *Ann Oncol* 2019 Nov 1;30(11):1821–30.
- [128] Chae YK, Vaklavas C, Cheng HH, et al. Molecular analysis for therapy choice (MATCH) arm W: Phase II study of AZD4547 in patients with tumors with aberrations in the FGFR pathway. *J Clin Oncol* 2018;36. 2503-2503.
- [129] Azad N, Overman M, Gray R, et al. Nivolumab is effective in mismatch repair-deficient noncolorectal cancers: results from Arm Z1D-A subprotocol of the NCI-MATCH (EAY131) study. *J Clin Oncol* 2020 Jan 20;38(3):214–22.
- [130] Mangat PK, Halabi S, Bruinooge SS, et al. Rationale and Design of the Targeted Agent and Profiling Utilization Registry (TAPUR) Study. *JCO Precis. Oncol* 2018;2018..