ANNUAL REVIEWS

Annual Review of Biochemistry

Cancer Treatment in the Genomic Era

Gary J. Doherty,[1] Michele Petruzzelli,[1,2,*] Emma Beddowes,[1,3,*] Saif S. Ahmad,[1,2,3,*] Carlos Caldas,[1,3] and Richard J. Gilbertson[1,3]

[1]Department of Oncology, Addenbrooke's Hospital, Cambridge University Hospitals National Health Service (NHS) Foundation Trust, Cambridge CB2 0QQ, United Kingdom; email: gd231@cam.ac.uk, Richard.Gilbertson@cruk.cam.ac.uk

[2]Medical Research Council (MRC) Cancer Unit, University of Cambridge, Cambridge CB2 0XZ, United Kingdom

[3]Cancer Research United Kingdom Cambridge Institute, University of Cambridge, Cambridge CB2 0RE, United Kingdom

Annu. Rev. Biochem. 2019. 88:247–80

First published as a Review in Advance on March 22, 2019

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-062917-011840

*These authors contributed equally to this article

Keywords

cancer genomics, precision oncology, personalized cancer medicine, therapeutic actionability, cancer treatment

Abstract

The complexity of human cancer underlies its devastating clinical consequences. Drugs designed to target the genetic alterations that drive cancer have improved the outcome for many patients, but not the majority of them. Here, we review the genomic landscape of cancer, how genomic data can provide much more than a sum of its parts, and the approaches developed to identify and validate genomic alterations with potential therapeutic value. We highlight notable successes and pitfalls in predicting the value of potential therapeutic targets and discuss the use of multi-omic data to better understand cancer dependencies and drug sensitivity. We discuss how integrated approaches to collecting, curating, and sharing these large data sets might improve the identification and prioritization of cancer vulnerabilities as well as patient stratification within clinical trials. Finally, we outline how future approaches might improve the efficiency and speed of translating genomic data into clinically effective therapies and how the use of unbiased genome-wide information can identify novel predictive biomarkers that can be either simple or complex.

Contents

INTRODUCTION

Our understanding of the processes that distinguish malignant from normal tissue has improved dramatically in recent years. This progress has been brought about through major advances in cell, molecular, and cancer biology as well as drug development and preclinical cancer modeling. However, this new understanding has outstripped our ability to translate knowledge into effective treatments for cancer. Most new cancer medicines usually provide benefit for only a minority of patients, highlighting our incomplete understanding of the complexity of malignant diseases.

Cancers arising from the same organ continue to be subclassified using simple histological techniques. Such subclassification provides basic information to guide treatment and predict prognosis, but it has limited impact on individual treatment decisions, especially when cancers resist therapy (during or afterwards). These basic morphology features can be supplemented with immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH, a locus-specific structural genomic analysis) assays that extend understanding of disease biology and allow a degree of molecular subtyping: for example, enabling the development of molecularly directed treatment approaches for estrogen receptor (ER)–expressing breast cancers (1) and those with *HER2* gene amplification (2). These approaches have also been used to identify treatment-relevant biomarkers in other cancers (3).

The explosion of genomic investigation of human cancers, driven by technological advances and large-scale, international sequencing efforts, has unmasked the remarkable complexity of cancer (4–8). Identification of a genetic alteration, even in a gene known to drive cancer in preclinical models, does not necessarily mean that the malignant process remains, or ever was, dependent on the alteration (9, 10). Further, cancers of the same histological type often display massive intra- and intertumoral genomic variation (11–15). These complexities, and the sheer volume of data that can now be acquired for each tumor, make it difficult to understand how best to disrupt the biology of primary and relapsed cancers for therapeutic gain.

Next-generation sequencing has been adapted rapidly from a pure research tool to one used increasingly in clinical research and patient management. For example, this has involved the introduction of limited gene-panel and whole-exome sequencing, creating a wealth of individualized knowledge about patients' tumors (16). These approaches have direct positive implications for patient management—for example, facilitating the recruitment of patients to molecularly stratified clinical trials (17). However, it is widely agreed that converting the tsunami of genomic data that is now being acquired from patients with cancer into effective genomically guided therapy is an enormous task that will require extensive, multidisciplinary collaborations. It is clear that knowledge of a single, putatively targetable genomic alteration in a patient's tumor is usually not translatable into durable clinical responses (18). Insights into the heterogeneity of cancer provided by genomic analyses suggest that effective targeting of genetic alterations will require at least an understanding of whether a particular alteration is present in all, or only a few, tumor cells, whether the alteration has functional consequences for the cancer cell, and whether these functional consequences are relevant to cancer progression and/or maintenance. Here, we provide a genome-focused view to dealing with these issues: discussing current sequencing methodologies and how we can best interpret their findings to guide therapy. Currently, approved genomically guided cancer therapies can all be defined using targeted genetic approaches, and we explore the extra information that broad, unbiased genomics can provide and how this can help define novel simple (based on single genomic alterations) and more complex, actionable biomarkers. (See the sidebar titled Important Definitions for definitions of some complex concepts.)

IMPORTANT DEFINITIONS

Basket trial: A clinical trial that includes patients with distinct tumor types (histologies/primary disease sites) that share a specific biomarker, usually a specific genomic alteration, and treats them with a specific therapeutic strategy. Basket trials test the hypothesis that it is the specific biomarker that defines the therapeutic response, rather than the specific tumor type.

Putatively actionable genomic alteration: A DNA alteration in a patient's tumor that might predict clinical response to a matched drug. The degree of confidence in the prediction of clinical response (actionability) is proportional to the level of evidence that (*a*) the specific alteration is necessary for cancer maintenance/progression and (*b*) the matched drug directly acts to abrogate those pathway(s) that lead to cancer maintenance/progression in cancer cells as a result of the specific alteration.

Synthetic lethality: Cell death that arises from a combination of two or more deficiencies (genomically driven or drug induced) and the individual deficiencies alone do not result in cell death.

Umbrella trial: A clinical trial that includes patients with a specific tumor type and matches patients with specific cancer biomarkers, usually genomic alterations, to distinct trial arms, each with a therapeutic strategy predicted to target that biomarker.

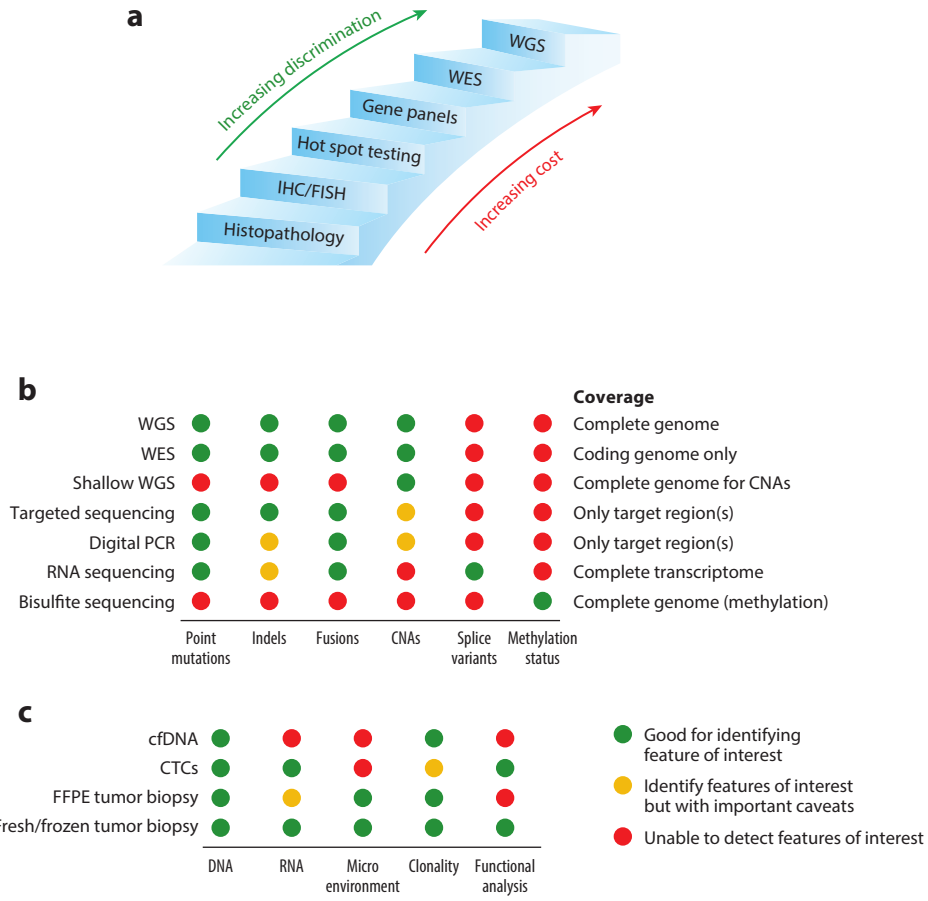

Figure 1

Approaches for studying genomic alterations in cancer. (*a*) Illustration of stepwise increases in information obtained through application of genomic technologies, demonstrating the trade-offs between discrimination of genomic alterations and cost. (*b*) Illustration of the advantages and limitations of genomic technologies. (*c*) Illustration of the advantages and limitations of tumor-derived substrates for genomic analyses. Abbreviations: cfDNA, cell-free DNA; CNAs, copy number alterations; CTCs, circulating tumor cells; FFPE, formalin-fixed and paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; indels, insertions and deletions; WES, whole-exome sequencing; WGS, whole-genome sequencing.

IDENTIFICATION OF CANCER-RELEVANT GENOMIC ALTERATIONS

Many techniques exist to identify alterations in the cancer genome, epigenome, transcriptome, and proteome (**Figure 1**). Each reveals distinct features of the cancer. As only limited amounts of tumor material are available from many patients, especially those with advanced cancer, care needs to be taken when prioritizing which genome-wide studies should be performed to identify clinically actionable biomarkers. Multiparametric techniques that can elucidate many features of the cancer at once on single slides [e.g., imaging mass cytometry (19)] may preserve material for deeper analyses, although these have not yet been clinically validated.

Technologies for identifying clinically relevant cancer alterations are shown in **Figure 1*a*,*b*** with associated advantages and limitations. Genome-wide approaches include whole-genome

sequencing (WGS), which provides the most complete information at the genomic level, covering exonic, intronic, and intergenic regions, including promoters and enhancers, as well as chromosomal rearrangements. Whole-exome sequencing (WES) is sufficient to find alterations that result in predicted alterations in proteins but does not cover intronic/intergenic DNA, and both WGS and WES usually have low coverage. Shallow WGS can provide information on copy number alterations (CNAs).

Gene panels provide information on a more focused set of known cancer-associated genes (typically up to 500). The relative affordability, high analytic validity, and ease of use with low sample inputs of these approaches have led to their frequent use for clinical trial stratification (20). Gene panels can also be used to detect CNAs and gene fusions for which breakpoints are known. Reverse transcriptase-polymerase chain reaction (RT-PCR)/microarray panels also provide important focused information, such as the Mammaprint® and Oncotype DX® panels that can subclassify risk in early breast cancer cases (21, 22). Hot spot panels are widely used to identify, with high sensitivity, patients with specific locus alterations who may benefit from currently approved drugs (e.g., activating *BRAF V600* mutations and BRAF inhibitors) or to provide information on intrinsic resistance to approved drugs [e.g., activating *KRAS* mutations and epidermal growth factor receptor (EGFR) inhibitors], but these panels are limited in the extent of target gene coverage (23). The choice of analytical approaches for an individual patient should be in favor of those most likely to show alterations of clinical relevance, which currently means some degree of genomic interrogation. The Foundation Medicine company has developed an industrial platform for targeted sequencing and identified that >75% of patients harbor mutations in at least 1 of 10 driver genes in their cancers; their platform has been widely used by clinicians for determining putatively actionable alterations, both from tumor specimen–derived DNA and from cell-free DNA (cfDNA)/circulating tumor DNA (ctDNA) (**https://www.foundationmedicine.com/**). Approaches in academic centers have usually focused on in-house gene panels, such as the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) panel, and both this and the FoundationOne CDx assay have been approved by the US Food and Drug Administration (FDA) as diagnostic tests.

The study of genomic alterations in cancer is also dependent on the nature of the clinical material. In this regard, technological advances have improved our ability to recover nucleic acids from formalin-fixed and paraffin-embedded (FFPE) tumor tissue—the most common substrate available for analysis in routine pathology laboratories—but it must be noted that degradation of macromolecules in long-term stored FFPE samples hampers analysis, and fresh samples are always preferred when feasible (**Figure 1*c***).

Genomic analysis is not confined solely to tumor biopsies. cfDNA/ctDNA or circulating tumor cells (CTCs) acquired from the blood—so-called liquid biopsies—also allow indirect genomic analysis of tumors, typically by digital PCR or tagged-amplicon deep sequencing (24, 25). These approaches have the advantages of being relatively noninvasive and enabling real-time, recurrent sampling of the patient's cancer genome. In this regard, ctDNA concentrations correlate with tumor burden (26) and can be used to monitor tumor relapse and treatment resistance (27). Digital PCR techniques can even detect ctDNA from localized cancers, supporting investigation of its utility in cancer screening (28). Techniques such as hybridization capture sequencing, which utilizes unique molecular tags that can discriminate low mutant allelic frequencies, improve the sensitivity of detection (27). ctDNA also holds promise to identify subclones of tumor cells likely to become dominant and may reconfirm the status of important predictive biomarkers after each treatment line, potentially avoiding the need for rebiopsy for treatment decisions. The sensitivity of genomic analysis of CTCs is limited by the reliability of their isolation from blood, but CTCs have the advantages of containing intact tumor genomes [unlike cfDNA, in which DNA fragments

WGS: whole-genome sequencing

WES: whole-exome sequencing

CNAs: copy number alterations

FFPE: formalin-fixed and paraffin-embedded

cfDNA: cell-free DNA

ctDNA: circulating tumor DNA

CTCs: circulating tumor cells

are 167 bp on average (29)] and allowing transcriptomic, proteomic, and functional analyses to be performed (30). However, it is unclear how CTCs differ from anchored cancer cells within the tumor microenvironment, and caution must be taken when interpreting data from these approaches.

RNAseq: RNA sequencing

Other approaches are required to provide information about gene expression. RNA sequencing (RNAseq) or transcriptomics can be unbiased, exploring the full transcriptome, or can be directed toward RNAs of interest. Whole-genome RNAseq data often require challenging deconvolution, particularly when the sample contains significant stromal infiltration, and benchmarking initiatives have helped create pipelines for data analysis (31). Single-cell RNAseq also allows deep profiling of the tumor microenvironment as well as intratumoral heterogeneity (32). Regardless of the approach, RNAseq can provide comprehensive information on gene expression from both mutant and wild-type alleles as well as splice variants and fusion transcripts. With regard to the latter, RNAseq is a more rapid and reliable means of detecting gene fusions than DNA sequencing and can be performed, albeit on selected gene targets, on FFPE material using NanoString technology (33, 34). In addition to mRNA profiling, RNAseq also provides information on expressed noncoding RNAs and microRNAs.

Alongside nucleic acid–based approaches, epigenomic profiling can provide a global view of likely gene expression patterns, and proteomics (by mass spectrometry) allows interrogation of cancer-specific posttranslational modifications and can elucidate the activation status of proteins in pathways (35, 36). Epigenomic modifications are stable, allowing interrogation of DNA methylation status using cfDNA, but may change significantly after treatment (as is the case for transcriptional profiles), and caution should be taken when interpreting results from archival samples. These approaches have not yet achieved widespread clinical utility (see the section titled Other Paths to Novel Therapies).

WHAT MAKES AN IDEAL PREDICTIVE GENOMIC BIOMARKER?

Biomarkers, including those provided by genomic analyses, can be viewed generally as prognostic or predictive. Prognostic genomic biomarkers are those associated with a particular level of disease aggression, usually judged in the context of current conventional therapy. Predictive genomic biomarkers are those that identify tumors likely to respond to, or resist, a particular therapeutic agent or strategy. Predictive biomarkers can be positive [e.g., activating *EGFR* mutations, predicting responsiveness to EGFR-directed tyrosine kinase inhibitors (TKIs) in lung adenocarcinoma (37)] or negative [e.g., activating *KRAS* mutations, predicting resistance to EGFR-directed monoclonal antibodies in colorectal cancer (CRC) (38, 39)].

The ideal positive predictive biomarker is one that can be targeted directly and induces a sustained, clinical response in all patients whose tumors harbor the biomarker. This generally means that the genomic alteration should be a sine qua non clonal driver of the disease [either by gain-of-function (GOF) or by loss-of-function (LOF) consequences to proteins] and that coexisting cell signaling pathways and intratumoral heterogeneity are unable to provide pathways to treatment resistance. Although predictive genomic biomarkers are limited to the tumor cells themselves, the biomarker may still drive cancer owing to functional modulation of other cells (e.g., immune cells or other constituents of the tumor stroma). Biomarkers, although ideally single and independent of other variables for ease of clinical utility, may also be compound/complex and could in principle integrate information from multiple modalities of interrogation (e.g., genomic, transcriptomic, proteomic, metabolomic, and network approaches as well as tumor microenvironment, immune signatures, and even microbiomics). To increase the proportion of patients responding to genomically targeted treatments, to reduce the proportion of patients who do not respond yet are still exposed to treatment toxicities and burdens, and to develop treatments for the majority of patients

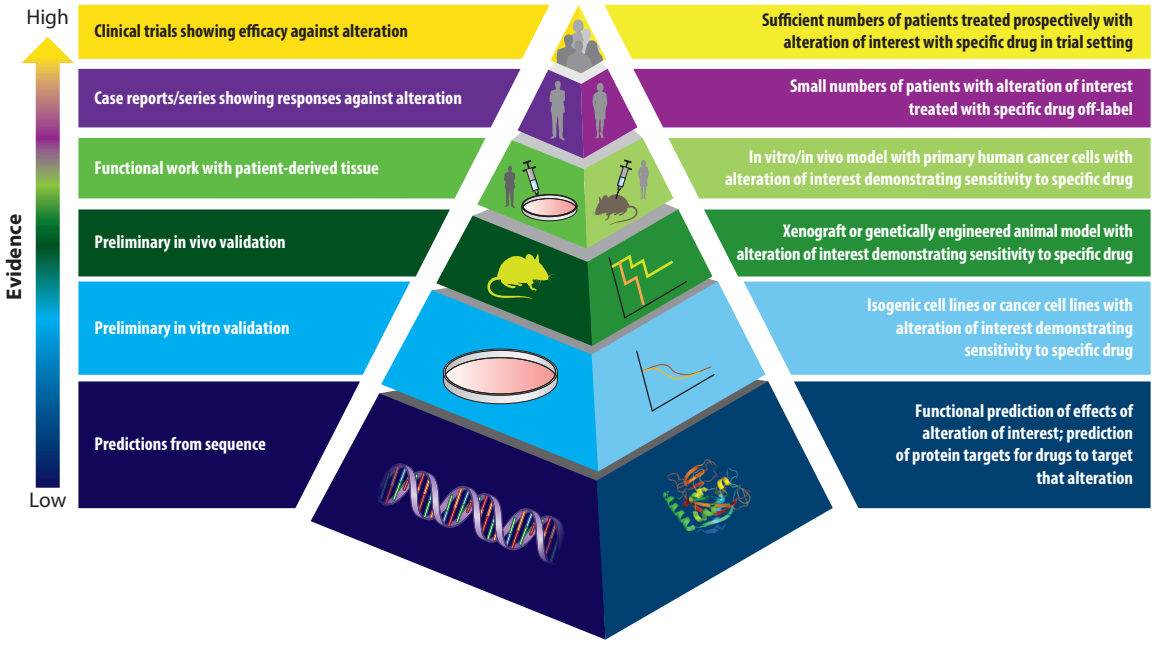

Figure 2

Hierarchy of evidence for determining putative clinical actionability. This illustration highlights key sources of evidence for the determination of putative clinical actionability. The lower levels of the pyramid depict lower levels of evidence. As one moves up the pyramid, higher levels of evidence are depicted and the probability that a specific drug will be clinically efficacious in targeting a specific genomic alteration is increased.

who do not have genomically targeted treatments available, we must embrace both the complexity and the uniqueness of individual cancers, which will mean novel approaches to determine putative clinical actionability of specific genomic alterations as well as increased reliance on complex, compound biomarkers.

HOW TO IDENTIFY AND VALIDATE CLINICALLY RELEVANT CANCER GENOMIC ALTERATIONS

When faced with a curated output of genetic data sets, the physician must be able to appropriately interpret this information to help decide if any genomic alteration should change the clinical management of their patient. Sequencing approaches have identified many classes of genomic alterations predicted to change protein function in cancer, each of which may, or may not, be biologically and clinically relevant. Silent alterations are referred to as passenger changes, whereas driver changes are those that are relevant to cancer cells and might be therapeutically actionable. Evidence that can inform the clinician about therapeutic actionability is shown in **Figure 2** and must be contextualized with information about confidence in alteration calling, clonality, and co-occurring genomic alterations.

Inferences from Sequence Data

A large number of cancer-relevant genomic alterations occur only rarely in cancer. These signals can therefore be indistinguishable from passenger mutations. The background mutation rate

and the mutation frequency (relative to background) are important variables for determining the power required to detect such rare but relevant genomic alterations (40). Often, many thousands of patient samples are required, posing a particular problem for rare cancers. Most genomic alterations found in cancer have no experimental data to support or refute their potential clinical actionability, which must therefore be inferred (with varying degrees of confidence) from a broad array of sources, despite the majority still likely representing passenger alterations.

LOF: loss-of-function

GOF: gain-of-function

The pipeline for identifying putatively actionable alterations necessarily involves filtering steps, including distinguishing sequencing errors from true alterations and somatic alterations from germline variants. As low-frequency variants are difficult to distinguish from background mutations, matched germline controls from the same patient make this process more reliable. There are many software tools available to assist variant calling [e.g., VarScan 2 (41)] and CNA calling [e.g., EXCAVATOR (42)].

Cancer-associated genomic alteration databases, including COSMIC (43) and ClinVar (44), allow determination of whether a specific genomic alteration is unique or recurrent. Because early stage and advanced cancers behave differently, and have distinct mutational spectra and clonality (45), we must strive to apply the right data set to each question. That said, recurrence of a specific alteration in that cancer type, or across cancer types, at a prevalence unlikely to be by chance alone implies a selection pressure for this alteration and increases the likelihood of relevance. Recurrent cancer-associated mutations (even if scattered across different regions of a single gene), hot spot regions of mutations (e.g., in a kinase domain), and differences in the ratio of synonymous versus nonsynonymous mutations can also implicate that gene as relevant but give no specific information regarding the phenotypic consequences of each individual alteration. In cases for which functional information regarding the protein is already known, LOF/GOF consequences can often be inferred—for example, when key enzymatic/DNA binding residues are disrupted, protein truncation results in loss of enzymatic domains or important regulatory domains, or focused locus amplification or deletion is found. In cases for which the structure of the protein is known, modeling can predict functional consequences as well as likely drug sensitivities through active site fitting if that protein is the drug target (46). For drugs targeting mutant proteins, it is particularly important to know if the binding site for the drug remains accessible to it in the mutant form. Functional inferences can also be made from knowledge of similar mutations occurring at the same conserved sites in paralogous sequences. One should also integrate the possible relevance of coexisting alterations (and known mutually exclusive/co-occurring alterations from large data sets), the predicted activation state of other proteins in the pathway(s), as well as biological plausibility and any relevant experimental/clinical data. Taken together, these data should allow attribution of likely significance (e.g., pathogenic/likely pathogenic/passenger/likely passenger/unclear), ideally with some indication of confidence despite often subjective conclusions. Several online databases have integrated preclinical and clinical data to suggest the potential significance of specific alterations [e.g., OncoKB (47), My Cancer Genome (48), Cancer Genome Interpreter (49), and the Personalized Cancer Therapy knowledge base (50)], but the methods by which they do so vary (e.g., from preclinical or clinical data, or by predicted protein function), sometimes leading to conflicting conclusions. All evidence must be aggregated and interpreted by the clinician or (ideally) a multidisciplinary Molecular Tumor Board (MTB) before clinical recommendations are made.

Analyses of large-scale data sets have helped widen the set of putative driver alterations. For example, a computational analysis of 9,423 tumor exomes from many different cancer types identified >3,000 putative missense driver alterations, including ~300 likely cancer driver genes, and experimental validations suggest 60–85% of these are true drivers (51). Fifty-seven percent of tumors had putatively therapeutically actionable alterations, although it is unclear from such interrogations if these are founder or progressor mutations, which has relevance with regard to the

cancers' maintained dependencies. Using a similar data set, an analysis of mutations, copy number, transcriptomics, gene fusions, and DNA methylation showed that 89% of tumors had at least one (likely) driving alteration in one of ten canonical pathways and 30% had multiple potentially targetable alterations, providing possible opportunities for sequential or combination therapeutic approaches (52). Validating drug-specific actionability for all of these in clinical models will prove time-consuming and challenging with current technologies, and ultra-high-throughput methods are required.

Matching Specific Genomic Alterations with Specific Drugs

Well over 80% of gastrointestinal stromal tumor patients can be matched to approved therapies on the basis of specific genomic alterations (53). However, ~80% of gliomas have putatively actionable genomic alterations, and no targeted therapies for these alterations have been approved, despite high-quality clinical attempts (54). Therefore, great caution must be taken when interpreting putative actionability. Actionability must be matched not just to a drug on the same pathway (as often suggested by actionability conclusions from large-scale analyses), or even a drug known to inhibit the protein of interest, but must be rationally and experimentally validated for a specific drug in an alteration-specific manner.

In recent years, functional characterization and druggability data have emerged first from clinical studies [see the basket approach for neratinib in **Supplemental Text 1** (9)]. In cases for which drugs are already approved for use in patients with specific alterations, actionability is usually unambiguous (although, again, often only the minority of patients can expect a response). Until we develop better methods of predicting actionability with a specific drug in a specific cancer context, only high-level evidence on drug-specific actionability should be used for patient management decisions, whereas lower-level evidence should be used mainly in the context of matching patients to biomarker-directed clinical trials (**Figure 2**). Strong preclinical evidence can sometimes justify off-label use of a specific drug in patients with no other treatment options, but patients must be carefully counseled about the risks associated with such drugs and the low probability of benefit.

Supplemental Material >

Preclinical Data

Genetically engineered mouse models have provided irrefutable evidence of the role of mutated genes in cancer development and maintenance and are important models for assessing drug sensitivity and resistance. However, modeling disease in living organisms is expensive, complex, and time-consuming, limiting its use for screening thousands of genomic alterations for therapeutic potential. Further, translation rates from murine models to approved therapies remain low in part because of the often poor design of preclinical studies and important differences between the murine and human diseases (55). It is more practical to determine the differential drug sensitivities of cultured cancer cells that harbor mutant or wild-type genomic alterations. Although tumor-derived cells are more representative of the mutational context of the disease, there are usually insufficient numbers available to adequately represent more than the common alterations. Thus, isogenic cell lines in which mutations are genetically engineered are useful to determine the effects of single genomic alterations, providing important preliminary information about actionability. Various high-throughput techniques are making in vitro mutational screening more rapid and comprehensive. For example, retroviral transduction recently identified four novel activating mutations in *HER2* and determined the drug actionability of both single and compound *HER2* mutations (56), and saturation mutagenesis enabled screening of 6,810 *ERK2* mutations for functional consequences and drug sensitivity (57). Such approaches can also incorporate

transcriptomics and proteomics to determine the impact of genetic alterations or drug treatment on signaling pathways. Discoveries made through these approaches can then be validated and studied further in vivo to determine differential drug sensitivities in a more tissue-relevant context.

TKI: tyrosine kinase inhibitor

Clinical Data

Ultimately, evidence of the clinical actionability of a genomic alteration must come from large, late-phase trials that include many patients with the alteration of interest. A thorough literature review is usually necessary to determine the denominator of patients with the specific alteration that have been treated with each specific targeted drug as well as the numbers that have responded and have not. Response durations and drug-related toxicities should also be taken into account. Case reports and case series are often useful, but these are particularly prone to publication bias in favor of positive outcomes and should be used mainly for hypothesis generation for prospective trials. One must also consider the relevance of other coreported genomic data, including comutations and CNAs, that may affect treatment response/resistance. Clinical data from other cancer types may be helpful, although great caution should be taken with inferring actionability in one cancer type from data obtained in another. As an example of this, in a basket trial in which patients with activating *BRAF V600* mutations were treated with vemurafenib, response rates were 48% in melanoma, 37% in non-small-cell lung cancer (NSCLC), and only 5% in CRC (10). In CRC patients, activation of EGFR by feedback loops is responsible for primary resistance, and combined approaches targeting both BRAF and EGFR in CRC appear promising (58).

THE LANDSCAPE OF GENOMIC ALTERATIONS IN CANCER AND ACTIONABLE TARGETS

Chromosomal Structural Variants and Gene Fusions

Chromosomal rearrangements play important roles in carcinogenesis, although the frequency of these alterations varies greatly between and within cancer subtypes (59). Chromosomal rearrangements are best detected in an unbiased manner by WGS, but specific alterations can be elucidated using targeted genetic approaches. The implications of large-scale genomic rearrangements are unclear and currently best appreciated in the context of changes in the function of specific genes, which include changes in gene promoters/enhancers/suppressors (modulating gene transcription) or formation of hybrid genes (with exonic regions derived from two distinct genes). Rearrangements can be balanced (through translocation, insertion, and inversion rearrangements) or unbalanced (through deletion events). Although >9,000 gene fusion events have been identified in cancer (60), most are likely to be nonpathogenic. Further understanding of the roles of nonexonic DNA sequences (and how these are altered in cancer cells) and of how genome structure and function correlate in cancer cells is greatly needed to translate our genomics-based knowledge of structural variants into novel therapeutic approaches.

Gene fusions can result in proteins with aberrant function (GOF or LOF). They can result in deregulated transcription; for example, in follicular lymphoma the t(14;18)(q32;q21) translocation places *BCL2* under control of the immunoglobulin heavy locus (*IGH*) regulatory regions, leading to *BCL2* overexpression (61, 62). Fusions can also result in dysregulated enzymatic function such as the *BCR-ABL1* balanced gene translocation in chronic myelogenous leukemia (CML) (63). This fusion gene is the target for imatinib (a TKI with multiple targets), which was the first genomically directed therapy for malignancy (64). Imatinib has transformed dramatically the life expectancy of CML patients—most now die *with* CML, rather than *because* of it (65). Activating gene fusions

are much rarer in solid cancers but are enriched in certain cancer types and in pediatric patients (66), and some are strong oncogenic drivers and targets for particularly successful therapies with impressive durations of response (67–69).

Gene fusions can be detected using WES/WGS as well as by gene panels and FISH when the alteration is known; however, RNAseq is the gold standard approach for identifying gene fusions (70). Even though more frequently recurring fusion events are more likely to be pathogenic and drive cancer, a large number of rarer fusions are also likely to do so. Although each may be found only in a small number of cancers, taken together, these rare fusions probably comprise the bulk of putatively actionable gene fusions. To highlight therapeutic actionability of gene fusions and the usually incremental nature of precision oncology, we focus on one highly successful example of gene fusion targeting in solid cancers—using anaplastic lymphoma kinase (ALK) inhibitors to treat *ALK* fusion-positive NSCLC (see **Supplemental Text 2**).

FISH: fluorescence in situ hybridization

FDA: US Food and Drug Administration

Supplemental Material >

Given the successes of targeting more common gene fusions such as *ALK* in NSCLC, attention is now being paid to less common fusion gene drivers. Rare, activating gene fusions involving the neurotrophic tyrosine receptor kinase (NTRK) family of receptor tyrosine kinases (RTKs), which play important roles in neuronal development, have been reported in a variety of solid cancers (at 0.2–3% prevalence). Such fusions result in overexpression or constitutive activity of NTRK tyrosine kinases (TKs). Entrectinib potently inhibits the tropomyosin receptor kinase (TRK) products of NTRK1–3, as well as the ROS1 and ALK TKs (68). In a combined analysis of results from the ALKA-372-001 and STARTRK-1 trials ($n = 119$ in total), 60 patients had *NTRK/ROS1/ALK* genetic rearrangements, 25 of whom were naive to treatments targeting the rearrangement of interest, had therapeutic exposures consistent with the eventual randomized phase II dose, and were evaluable for response (71). The overall response rates (ORRs) for *NTRK*-rearranged cancers, *ROS1*-rearranged cancers, and *ALK*-rearranged cancers were 100% ($n = 4$), 86% ($n = 14$), and 57% ($n = 7$), respectively, with promising signals of response durability. The intracranial ORR was 63% ($n = 8$), and one patient with extensive intracranial disease had a complete intracranial response that lasted for at least 15 months. These results are being followed up in the STARTRK-2 basket study.

Larotrectinib is a potent, brain-penetrant pan-TRK inhibitor that was tested in a phase I/II basket study in children and a phase II basket study in adults (69). Of the 55 patients treated, the ORR was 75%, with a disease control rate of at least 88% and responses observed across diverse tumor types and ages. Seventy-one percent of responses were ongoing at 1 year, when the median progression-free survival (PFS) had not yet been reached, and these results led to breakthrough therapy designation by the FDA. These results underline the importance of finding activating gene fusions, given the profound clinical benefit that inhibiting these can provide.

Loss-of-Function Gene Alterations

These alterations inactivate or negatively affect the function of the protein product and encompass a wide spectrum of genomic alterations, from missense mutations through indels to chromosomal structural variants. For cases in which these alterations impact cancer progression or maintenance, the relevant genes are commonly known as tumor suppressors. Unlike GOF alterations (see below), LOF alterations do not present immediately attractive targets for therapeutics, but synthetic lethality approaches can be applied to identify and exploit vulnerabilities exposed by the LOF.

Synthetic lethal interactions describe the relationship between two genes whereby inactivation of either gene alone permits viability but combined inactivation results in cell death (72). Exploiting this with therapeutics should result in limited toxicity to normal cells (as they do not already harbor a deficiency). Poly(ADP-ribose) polymerase 1 and 2 (PARP1 and PARP2) are important

sensors of DNA damage that bind to single-strand breaks and other types of DNA damage and act in DNA damage response (DDR) signal transduction through posttranslational modification of substrate proteins by poly(ADP-ribose) chains (72). *BRCA1/2* mutant cancers are defective in homologous recombination (HR), which is required for error-free double-strand DNA break (DSB) repair (73), and thus rely on PARP activity for DNA repair (74, 75). Synthetic lethality from PARP inhibition in BRCA1/2-deficient cancers is thought, in part, to be related to trapping of PARP, which usually removes itself from single-stranded DNA (ssDNA) through autoPARylation (76). Trapped PARP abrogates ssDNA break repair and provides the substrate for conversion into DSBs. BRCA1/2-deficient cells lacking in HR are therefore unable to repair these lesions efficiently, relying on error-prone repair mechanisms such as nonhomologous end joining (77). BRCA1/2-deficient cancers are sensitive to PARP inhibition (74, 75), for example, by olaparib, rucaparib, and niraparib, which are all approved for the treatment of ovarian cancer. Although responses have been higher in *BRCA1/2* mutant cases, a significant population of *BRCA1/2* wild-type cases responds to these therapies (78). These other routes to achieving synthetic lethality with PARP inhibitors are beginning to be understood—many *BRCA1/2* wild-type cancers are phenocopies of *BRCA1/2* mutants with regard to HR defects and have similar vulnerabilities (79), highlighting the importance of genomics-based diagnostic approaches. As resistance remains inevitable with PARP inhibition, other approaches have identified other synthetic lethal targets, such as Polymerase θ (POL θ) (80, 81) and RAD52 (82), and the success of PARP inhibitors has led to intensive study of other vulnerabilities across the spectrum of DNA damage repair.

Synthetic lethality can even occur between two components of the same complex, for example, between BAF47 and BRG1 (both are parts of the SWI–SNF complex, which promotes mobilization of nucleosomes along DNA, thereby regulating gene expression and facilitating DNA repair). Members of the SWI–SNF complex are mutated in ∼20% of cancers (83). *BAF47* is commonly mutated in malignant rhabdoid tumors in young patients (84), and inhibition of EZH2 (the enzymatic portion of the polycomb repressor complex, which leads to *CDKN2A* epigenetic silencing when BAF47 is deficient) leads to *CDKN2A* re-expression and is under clinical investigation for this disease (72, 85). Although previous approaches employing epigenomic targeting have been disappointing, they have mainly targeted enzymes that switch global histone posttranslational modifications, and it is likely that more focused synthetic lethality approaches will lead to a new wave of epigenomically targeted therapies. Similar synthetic lethality approaches are being investigated in tumors with defects in metabolic pathways, such as succinate dehydrogenase tumors, which are dependent on pyruvate carboxylase activity (86).

Gain-of-Function Gene Alterations

Cancer-relevant GOF alterations affect either the amount or the structure of the resultant proteins, leading to acquisition of novel properties or deregulated activity. Point mutations are the most common reasons for these changes, and many of these affect the RTK/RAS/RAF/MEK/ERK and related PI3K/AKT/mTOR signaling pathways (52). GOF alterations can also occur through indels as well as gene amplifications (hypermorphs in the setting of a wild-type allele) or structural variants. GOF mutations may be pathogenic heterozygous with a wild-type allele, which can also affect the functional consequences of the mutation [as occurs with *KRAS* (87, 88)]. Often, however, functional inactivation of the other allele occurs, usually through mutation or deletion [loss of heterozygosity (LOH)]. GOF alterations are most simply targeted by inhibition of the mutant protein or downstream effectors.

Activating *BRAF* missense mutations are common in melanoma [>40% of cases, most involving V600 (with 90% of these being V600E)], and these can be targeted directly using the BRAF

mutant inhibitors vemurafenib and dabrafenib [with monotherapy response rates for advanced V600E cases being 48% and 50%, respectively (89, 90)]. Targeting MEK1/2 (just downstream of BRAF) with trametinib (a MEK1/2 inhibitor) alone led to a lower ORR of 22% (91). As pathway/MEK activation was identified as an important resistance mechanism to BRAF inhibitor monotherapy, combination treatment with BRAF and MEK inhibitors was then explored. The addition of trametinib to dabrafenib improved response rates from 51% to 64% and the 1-year overall survival from 65% to 72% ($p = 0.005$) and also abrogated the paradoxical activation of KRAS that occurs with BRAF-targeted monotherapy and leads to an increased risk of cutaneous squamous cell carcinoma (1% in the combination arm versus 18% in the dabrafenib-only arm) (92). Comparable results were achieved with the addition of cobimetinib (another MEK1/2 inhibitor) to vemurafenib (93).

Even within a single gene, multiple mechanisms may produce GOF consequences (e.g., activation mechanisms for rarer *BRAF* mutants that are not druggable using dabrafenib/vemurafenib). Distinct mutations in codons 12/13/61 in KRAS all impair GTPase-activating protein (GAP)–assisted guanosine diphosphate (GDP) hydrolysis but do so by different structural mechanisms, and the relative prevalence of each of these mutations varies significantly among KRAS-driven cancers, probably as a result of the mutational spectra produced by certain mutagens and selection for specific properties of the altered protein in specific cell types (94). Further, *KRAS* mutant zygosity has important biological and therapeutic consequences (95). Many approaches have been applied to directly target KRAS in *KRAS* mutant cancers, given the high prevalence of such mutations in diverse tumor types (e.g., >98% of pancreatic adenocarcinomas and ~30% of lung adenocarcinomas), yet no drugs have been approved specifically for *KRAS* mutant cancers, reflecting both our incomplete understanding of the signaling pathways in which KRAS resides and the absence of a binding pocket on KRAS that is amenable to traditional drug screening (94) [although many different approaches have been tested, including targeting KRAS G12C specifically with a covalent inhibitor that can induce *Kras* G12C tumor regression in murine models (96)]. Activating *KRAS* mutations are generally considered to confer signaling independence from upstream RTKs of the ERBB family [which includes the epidermal growth factor receptor (EGFR), HER2, ERBB3, and ERBB4] and others, leading to constitutive pro-proliferative and pro-survival signaling. Consistent with this textbook model, *KRAS* mutant lung cancers and CRCs are insensitive to EGFR inhibitors, and activating *EGFR* and *KRAS* mutations are mutually exclusive in cancers. However, these *KRAS* mutant cancers are not clinically sensitive to MEK1/2 inhibition alone (97). Further, *KRAS* mutant lung cancer cell lines remain dependent on IGFR activity for PI3K activation (98). In lung cancer and CRC cell lines, overexpression of ERBB3 (which heterodimerizes with other ERBB family members to form active signaling units) is induced by MEK inhibition downstream of activated KRAS, leading to the identification of ERBB and MEK inhibitors as synthetic lethal in combination (99). Recently it was shown in a murine model that *Kras* G12D-driven lung tumors remain dependent on signaling through the ERBB family (100). The pan-ERBB TKI neratinib markedly suppressed *Kras* G12D-driven lung tumors, and although ineffective alone in extending survival of mice with established tumors, neratinib provided added benefit to MEK1/2 inhibition with trametinib (100). Such results again highlight the caution that should be taken when applying our current understanding of signaling pathways to clinical contexts—however rational a drug target might seem (e.g., MEK1/2 inhibition in *KRAS* mutant cancers), it should be experimentally validated before actionability with a specific drug is tested clinically.

Impressive clinical benefit has been achieved by directly targeting mutant RTKs. Activating *EGFR* mutations occur in up to 20% of lung adenocarcinomas, with significant geographical variance—they are most common in Asian women who have never smoked tobacco (101). The most recurrent mutations in *EGFR* promote constitutive EGFR activity and can be targeted with

Supplemental Material >

drugs that inhibit EGFR TK activity. The clinical development of this important target has mirrored the piecemeal approach for ALK inhibitors (described in **Supplemental Text 2**), and several generations of drugs with specific properties that allow them to target different subsets of mutations, or efficiently cross the blood-brain barrier (which can hinder reaching therapeutically relevant concentrations of systemic drugs in the central nervous system), have been developed (37, 102). A common resistance mutation found in patients that progress on first- and second-generation EGFR TKIs (such as gefitinib, erlotinib, and afatinib) is the gatekeeper mutation *T790M* (found in the ATP binding pocket of EGFR), which is responsible for drug resistance in ~60% of cases and increases the affinity of EGFR for ATP (103). Emergence of the *T790M* mutation can be detected by rebiopsy or by analysis of cfDNA (the latter method being widely used for this application). Unlike first- and second-generation EGFR TKIs, osimertinib binds covalently to EGFR via C797, with an increased affinity for mutant over wild-type EGFR proteins (including T790M) (104). In the FLAURA trial, in treatment-naive NSCLC patients with *EGFR* exon 19 deletions or *L858R* mutations, the response rate was 80% for osimertinib, similar to gefitinib/erlotinib (standard-of-care arm), but with a median duration of response of 17.2 months (versus 8.5 months with standard of care), leading to its approval in the first-line setting (105). The impact of each EGFR-targeted TKI on less common *EGFR* mutations is less clear [although case reports and series have provided some insights (106)], as are their utilities in other cancers harboring activating *EGFR* mutations.

Zygosity and Copy Number Alterations

Changes in zygosity can lead to loss of wild-type protein production as well as increased production of the mutant protein, with obvious implications for oncogenes and tumor suppressor genes. Any LOH must therefore be examined to interpret the likely functional outcome. CNAs are focal amplifications or deletions of a restricted region of a chromosome that may lead to changes in gene expression and should ideally be reported alongside genomic alterations. The underlying mechanisms of these alterations are not fully understood, but it is thought that they arise from a combination of DNA strand breaks alongside impaired cell-cycle checkpoint functionality/DNA repair mechanisms or though replication of extrachromosomal DNA from an excised DNA fragment (present in ~50% of cancers) (107). Absolute copy numbers of mutant and wild-type alleles appear to be the most important variables, given their effects on mutant/wild-type protein production. CNAs, although best determined by WGS/WES, can also be determined using gene panels and cfDNA analysis. Large-scale integrated analyses of genomic and transcriptomic data, such as the METABRIC study of ~2,000 breast cancers, highlighted CNAs as the most important variables determining gene expression, particularly with *cis*-acting CNAs (with regard to mutant alleles), and revealed new driver targets driven by CNAs (108, 109). This study also identified a good prognosis group of cancers with very low levels of CNAs. CNAs can also be readily determined using shallow WGS, which can identify both regional CNAs and genome-wide signatures that can be used to subclassify cancers, as has been shown for ovarian cancer (110). This is a powerful, but inexpensive, tool that could easily be incorporated into routine genomic interrogation.

CNAs have generally been more challenging to target clinically than mutant proteins, owing to the fact that wild-type proteins in nontumor cells must also be inhibited if the altered gene is also the drug target. *HER2* amplification leads to sensitivity to multiple HER2-targeted therapies in breast cancer and gastroesophageal cancer (111). *FGFR2* and *MET* are also frequently amplified in solid cancers, and it appears that high-level clonal amplification dictates sensitivity to inhibitors targeting amplified genes (112). Amplified genes whose protein products are presented on the plasma membrane are attractive targets for antibody–drug conjugates. These deliver a cytotoxic

payload preferentially to cells with the highest target protein levels (113), such as the strategy adopted for ado-trastuzumab emtansine (which is approved for advanced HER2-overexpressing breast cancer) (114). It is highly likely that we will see a significant increase in the number of CNA-targeted therapies approved in the coming years.

MMR: mismatch repair

MSI: microsatellite instability

Germline Variants

Although the importance of germline variants has been appreciated for decades in familial cancer syndromes, it is increasingly appreciated that significant cancer-predisposing germline variants are more common than previously thought [e.g., ~12% of prostate and ~16% of renal cancer patients have germline genomic alterations in important cancer-associated genes (115, 116)]. It is unclear if germline-associated and identical somatic variants can be targeted by similar approaches, but clinical responses to PARP inhibitors in *BRCA1/2* mutant cancers suggest that they can be (117), although differences in clonality and toxicity may exist. Germline testing alongside somatic testing in the same patient improves the detection of somatic mutations as well as provides important information about risk for other family members.

Genetic Instability and Tumor Mutational Burden

Changes in chromosomal number or structure as a result of chromosomal instability (CIN) are observed in most cancer types and are generally associated with a poor prognosis (118). CIN is thought to be an early event and a driving force behind the acquisition of further mutations and the development of intratumoral heterogeneity (119, 120). Chromosomally unstable tumors include features such as large-scale amplifications and deletions, LOH, translocations, and inversions. Large aberrant genomic amplifications and deletions can be caused by DNA replication stress, via the slowing or collapsing of replication forks (121). Replication stress can be caused by a number of factors including oncogene activation and endogenous factors (e.g., oxidative stress) (122, 123). Mutations in DNA repair genes (e.g., *BRCA1/2*) are also important drivers of CIN. Whole-chromosome aneuploidy is also a common feature of cancer, being generally attributed to chromosome mis-segregation. Whether aneuploidy is a cause or consequence of CIN is a subject of debate, and it is thought that aneuploidy can be both tumor promoting and suppressing, depending on the context, though overall it appears to contribute to increased tumor fitness and promote genomic evolution (124–126).

Mismatch repair (MMR) fixes a type of DNA damage that occurs when DNA polymerase slips, which would otherwise result in frameshift mutations. Microsatellite instability (MSI) can occur secondary to germline or somatic mutations in, or hypermethylation of, MMR genes (e.g., *MSH2* or *MLH1*) (127). In CRC, MSI is known to lead to tumor development and is usually associated with a better prognosis compared with microsatellite-stable tumors. A less common form of genomic instability is acquired through LOF alterations in genes involved in base excision repair (BER) or nucleotide excision repair (NER) pathways, leading to important consequences for defense against mutagens. Patients with xeroderma pigmentosum have germline mutations in NER genes and a dramatic predisposition to the development of skin cancer due to an impaired ability to repair ultraviolet light–induced DNA damage. Genetically unstable tumors can accumulate large numbers of CNAs and small-scale mutations, although the frequency of CNAs generally appears to be inversely proportional to mutation frequency, leading to the concepts of M (mutation) and C (CNA) classes of cancer (7).

Independent of functional aberrations, genomic alterations in cancer that affect amino acid sequences can provide information about possible neoantigen production and immunogenicity

ICPI: immune checkpoint inhibitor

[whereby presentation of mutant peptides on MHC class I proteins can be detected by cytotoxic T lymphocytes (CTLs), leading to tumor cell death]. Antigen presentation is restricted by human leukocyte antigen (HLA) haplotypes and depends on the presence of CTLs with cognate T cell receptors (TCRs) and the balance of stimulatory and inhibitory signals from the tumor cell or microenvironment. Tumor-infiltrating lymphocytes (TILs) are emerging as important biomarkers for the potential immunogenicity of cancers, for example, in triple-negative breast cancers for which TIL infiltration is predictive of prognosis (128). Their presence presumably reflects either ongoing effective antitumoral immunity or an immune response that lacks the final steps to convert production and homing of tumor-specific TILs into tumor cell killing. Programmed death-ligand 1 (PD-L1), which binds the T cell inhibitory checkpoint receptor PD-1 (negatively regulating T cell activity), is an important positive predictive biomarker for response of cancers to the anti-PD-1/PD-L1 immune checkpoint inhibitor (ICPI) monoclonal antibodies (129). Agents targeting a raft of other stimulatory and inhibitory immune cell checkpoints are currently in clinical development, and an anti-CTLA4 antibody (ipilimumab) was the first to be approved. Anti-CTLA4 and anti-PD-1/PD-L1 antibodies are more potent in combination in melanoma, with manageable toxicity (130, 131). Because elucidation of all these immune variables is impractical for individual patients, tumor mutational burden (TMB) has emerged as a surrogate biomarker for the number of neoantigens and has been shown to be predictive of response to ICPIs (132). TMB is higher in cancers exposed to potent carcinogens, such as tobacco-exposed lung cancers and melanomas, and TMB-high tumors are more likely to respond to ICPIs. MMR deficiency also produces high neoantigen burden, and pembrolizumab [an anti-PD-1 ICPI with efficacy against MMR-deficient cancers (133)] has been granted accelerated approval by the FDA for these (or other MSI-high) tumors regardless of their initial origin or histology.

Broad Genomic Classifications

Alongside identification of individual predictive biomarkers as described above, analyses of large-scale sequencing data have used mutational clustering to allow more specific taxonomic classifications of cancers. For example, analysis of ~2,000 breast cancers in the METABRIC cohort has revealed 10 integrative clusters, with distinct features and prognoses (134). Histopathological subclassifications are inferior to genomic taxonomic classification in survival prediction, and genomic taxonomies cannot be reliably inferred using traditional clinicopathological methods alone (135). Genomic taxonomies will allow the usually broad histopathological eligibility for clinical trials to be narrowed to help search for response signals in certain subclasses and may explain some of the differential responses observed in clinical trials with nongenomically matched agents. By elimination of much of the noise from the presence of other subtypes, they will also allow further identification of subclass-specific drivers. Unbiased genomic interrogation yields insights beyond those that can be discerned from focused studies—it is only by looking at broad patterns of genomic alterations that we can reliably identify biological subtypes of cancers with distinct cells of origin and initiator and progressor genomic alterations and hone our search for novel therapeutic approaches.

Clonality and Heterogeneity

Genomics approaches have shown that solid cancers usually comprise multiple subclones, promoted by genetic instability. The presence of subclones is likely to be responsible for much of the intrinsic and acquired resistance observed with genomically targeted agents (136). After truncal (often initiating) genomic alterations, branched evolution results in tumor cell subclones,

potentially with differing fitness and sensitivities to specific therapies (136). Heterogeneity is a dynamic property, changing as certain fitter subclones win out, either in cancers progressing in the absence of treatment or as a result of subclonal death or skewed signaling networks in response to sustained therapy. Diligent sequencing of many parts of the same tumor and different metastatic sites has led to significant insights into intratumoral and intertumoral heterogeneity. For example, sequencing of multiple sites of single renal cell carcinoma tumors demonstrated that intratumoral heterogeneity is common and approximately two-thirds of mutations are not detectable in all sequenced regions (12). Biopsies underestimate tumor clonality (being from a limited region of a single disease site), but measuring allelic frequencies of mutations even in these small samples remains important. Sampling of various metastatic sites, or analysis of cfDNA, which is safer and more practical (137), allows a more global assessment of cancer clonality.

Deterministic evolutionary outcomes have also been shown in renal cell carcinoma, for which seven evolutionary subtypes were identified in a study of 1,206 primary regions from 101 patients (13), and genetic diversity and chromosomal complexity have emerged as important predictors of outcome. The degrees of intratumoral and intertumoral heterogeneity vary significantly between tumor types, from high intrinsic levels of subclones in hepatocellular carcinomas to lower levels seen in melanoma (11). Significant heterogeneity is also seen between primary tumors and metastatic sites, unsurprising given their distinct microenvironments, now evidenced by multiple studies in multiple cancer types. For example, in breast cancer, eight genes were shown to be more frequently mutated in metastatic sites, and mTOR pathway gene mutations and APOBEC mutational signatures were more frequently observed in metastatic HER2-negative, hormone receptor–positive tumors than in primary tumors (8). Branched evolution and subclonality have important implications for the application of genomically targeted agents in individual patients, and we must move away from the concept of binary alterations (presence or absence of the alteration) to incorporate measures of clonality. For example, ~15% of PI3K/AKT pathway alterations across cancers are subclonal (139), and although *PTEN* loss appears clonal in triple-negative breast cancer (140), it is often subclonal in prostate cancer (141) (reviewed in 142). These important observations are likely the cause of some of the significant intrinsic resistance associated with genomically targeted therapies matched to known strong driver alterations.

IMPLICATIONS OF GENOMIC DATA FOR CLINICAL TRIALS

From January 1, 2006, to June 1, 2018, 34 targeted drugs or combinations of targeted drugs (excluding hormonal therapies alone) were approved by the FDA for first-line treatment of advanced cancers (or after radiation therapy alone). Of these, 16 have been approved coupled with genomic biomarkers (including HER2 positivity, which is routinely detected by in situ hybridization or FISH but reflects a clearly defined genomic alteration); 17 have been approved coupled with nongenomic biomarkers ($n = 4$) or without a necessary biomarker ($n = 13$); and 1 has indications for which this requirement varies (pembrolizumab). Indications for monotherapies and combinations are shown in **Tables 1** and **2**, alongside any biomarkers required for their use. For those drugs coupled with genomic biomarkers, response rates by cancer type and by targeted genomic alteration are shown in **Figure 3*a***. Response rates are generally higher when coupled to a genomic biomarker (**Figure 3*b***; $p < 0.0001$) than when coupled with a nongenomic or no biomarker. Of the 33 individual targeted agents that have been approved, 20 are kinase inhibitors, with the most common targets being EGFR, ALK, BRAF, MEK, and VEGFR; 5 are anti-PD-1/PD-L1 antibodies; and the rest inhibit SMO, CDKs, or mTOR.

Although approved genomically targeted drugs have markedly changed the outcomes for subsets of patients, the small number of targets for approved drugs has led some to question the

Table 1 FDA-approved, genomically matched targeted therapies in the first-line setting with targets, coapprovals, indications, and response rates

Genomic biomarker	Indication	Drug or combination	Major drug target(s)	Response rate
Activating *BRAF* mutation	Melanoma	Binimetinib and encorafenib	BRAF and MEK1/2	63%
Activating *BRAF* mutation	Melanoma	Cobimetinib and vemurafenib	BRAF and MEK1/2	70%
Activating *BRAF* mutation	Melanoma	Dabrafenib	BRAF	52%
Activating *BRAF* mutation	Melanoma	Dabrafenib and trametinib	BRAF and MEK1/2	66%
Activating *BRAF* mutation	Anaplastic thyroid carcinoma	Dabrafenib and trametinib	BRAF and MEK1/2	61%
Activating *BRAF* mutation	NSCLC	Dabrafenib and trametinib	BRAF and MEK1/2	63%
Activating *BRAF* mutation	Melanoma	Trametinib	MEK1/2	22%
Activating *BRAF* mutation	Melanoma	Vemurafenib	BRAF	48%
Activating *EGFR* mutation	NSCLC	Afatinib	EGFR	66%
Activating *EGFR* mutation	NSCLC	Erlotinib	EGFR	65%
Activating *EGFR* mutation	NSCLC	Gefitinib	EGFR	70%
Activating *EGFR* mutation	NSCLC	Osimertinib	EGFR	77%
ALK translocation	NSCLC	Alectinib	ALK	83%
ALK translocation	NSCLC	Brigatinib	ALK	48%
ALK translocation	NSCLC	Ceritinib	ALK	73%
ALK translocation	NSCLC	Crizotinib	ALK	65%
c-KIT mutation	GIST	Imatinib	Multiple TKs	53%
HER2 amplification	Breast cancer	Lapatinib and letrozole	EGFR/HER2 and hormonal therapy	28%
MMR deficiency/MSI high	Any tumor origin	Pembrolizumab	PD-1	40%
ROS1 translocation	NSCLC	Crizotinib	ROS1	70%

This table shows all the fully FDA-approved drugs or combinations (between January 1, 2006, and June 1, 2018) against advanced tumors that can be used in the first-line setting (or after radiation alone) and are dependent on the detection of a specific genomic alteration in tumors, alongside response rates used in the creation of **Figure 3**. For consistency, response rates given here were taken from the individual drug labels at **https://www.fda.gov**.
Abbreviations: FDA, US Food and Drug Administration; GIST, gastrointestinal stromal tumor; MMR, mismatch repair; MSI, microsatellite instability; NSCLC, non-small-cell lung cancer; TK, tyrosine kinase.

broad utility of genomically targeted cancer therapeutics. Arguments against following this path have been strengthened by some disappointing outcomes from approaches to match patients to drugs on the basis of putatively actionable genomic alterations. The SHIVA trial was a randomized phase II trial including patients with any genomically profiled metastatic tumor (refractory to standard-of-care treatment) who received 1 of 10 matched regimens or the physician's treatment of choice (143). This was a negative study, presumably as a result of heavy reliance on treating patients with *PI3K/AKT/mTOR* alterations with the weak mTOR inhibitor everolimus. The MOSCATO-01 study used a PFS1/2 outcome measure (to determine if genomically matched treatment could result in greater PFS than the last therapy) and demonstrated that 33% of patients had improved outcomes with genomically matched treatment [using a >30% increase in PFS as a positive outcome (occurred in 63/193 patients) (144)]. The MD Anderson IMPACT study of 3,743 patients (including 1,307 patients with at least one genomic alteration) demonstrated an improvement in 3-year overall survival from 7% (with nonmatched treatments) to 15% with genomically matched treatments (145). SAFIR 02, the ASCO TAPUR study, and NCI-MATCH (see later in this section) are ongoing and will report further general outcomes for these approaches. Positive outcomes are expected, as outcomes from large numbers of phase I and II trials have shown that

Table 2 FDA-approved, nongenomically matched targeted therapies in the first-line setting with targets, coapprovals, indications, and response rates

Biomarker required	Indication	Drug or combination	Major drug target(s)	Response rate
None	Merkel cell carcinoma	Avelumab	PD-L1	33%
None	Renal cell carcinoma	Cabozantinib	MET/AXL/VEGFR	33%
None	Medullary thyroid cancer	Cabozantinib	MET/AXL/VEGFR	28%
None	Neuroendocrine tumor	Everolimus	mTOR	2%
None	Pancreatic neuroendocrine tumor	Everolimus	mTOR	5%
None	Melanoma	Ipilimumab	CTLA4	11%
None	Melanoma	Ipilimumab and nivolumab	CTLA4 and PD-1	60%
None	Renal cell carcinoma	Ipilimumab and nivolumab	CTLA4 and PD-1	42%
None	Thyroid cancer	Lenvatinib	VEGFR	65%
None	Melanoma	Nivolumab	PD-1	40%
None	Renal cell carcinoma	Pazopanib	VEGFR/c-KIT/FGFR/PDGFR	30%
None	Melanoma	Pembrolizumab	PD-1	34%
None	Basal cell carcinoma	Sonidegib	Smoothened	58%
None	Thyroid cancer	Sorafenib	VEGFR/PDGFR/RAF	12%
None	Hepatocellular carcinoma	Sorafenib	VEGFR/PDGFR/RAF	2%
None	Pancreatic neuroendocrine tumor	Sunitinib	VEGFR/PDGFR	9%
None	Renal cell carcinoma	Sunitinib	VEGFR/PDGFR	28%
None	Renal cell carcinoma	Temsirolimus	mTOR	9%
None	Basal cell carcinoma	Vismodegib	Smoothened	30%
ER positivity	Breast cancer	Abemaciclib and aromatase inhibitor	CDK4/6 and hormonal therapy	59%
ER positivity	Breast cancer	Palbociclib and letrozole	CDK4/6 and hormonal therapy	55%
ER positivity	Breast cancer	Ribociclib and aromatase inhibitor	CDK4/6 and hormonal therapy	53%
PD-L1 positivity	Urothelial carcinoma	Atezolizumab	PD-L1	23%

This table shows all the fully FDA-approved drugs or combinations (between January 1, 2006, and June 1, 2018) against advanced tumors that can be used in the first-line setting (or after radiation alone) and are not dependent on the detection of a specific genomic alteration in tumors, alongside response rates used in the creation of **Figure 3**. For consistency, response rates given here were taken from the individual drug labels at **https://www.fda.gov**.
Abbreviations: ER, estrogen receptor; FDA, US Food and Drug Administration.

genomic biomarkers significantly outperform other biomarkers in terms of response rates with matched therapies.

To determine the true actionability of specific genomic alterations, a sufficient number of patients with a particular alteration must be treated with a specific drug, giving the study sufficient power to infer clinically meaningful efficacy. Previously, studies have often relied on screening patients for single (often rare) alterations and matching them to a specific drug in a single trial, leading to low rates of trial participation. Currently, only 2–5% of patients that are genomically profiled are matched to therapies in clinical trials, as being matched depends on local trial availability and strict trial-specific eligibility criteria, although enrollment rates in clinical trials are improving. To speed up the drug development process, and to widen the number of therapies

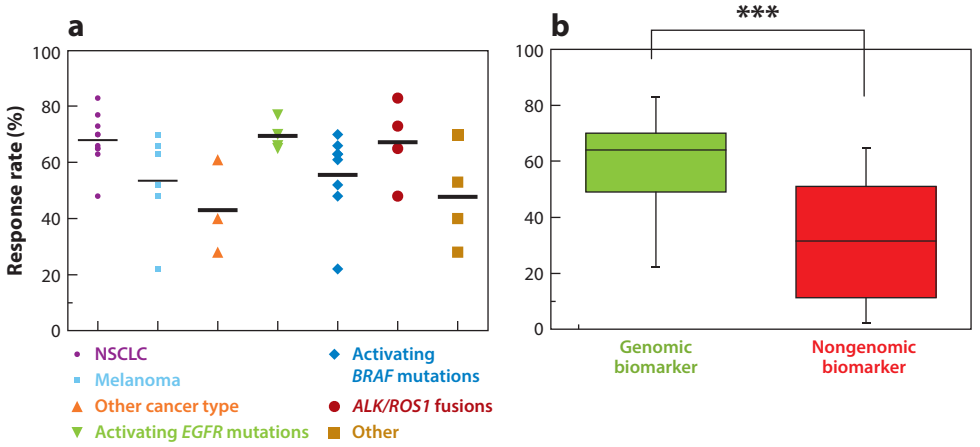

Figure 3

Graphs showing response rates to FDA-approved targeted therapies for advanced tumors in the first-line setting or after radiation therapy alone (from January 1, 2006, to June 1, 2018). (*a*) Individual response rates are shown for FDA-approved genomic biomarker–directed drugs separated by cancer indication and by genomic alteration target. The indications and drug(s) from which the response rates were generated for this figure are highlighted in **Table 1**. (*b*) Response rates are shown for FDA-approved, genomically targeted therapies based on a genomic biomarker and for those that are not based on a genomic biomarker or do not require a biomarker. The indications and drug(s) from which the response rates were generated for this figure are highlighted in **Tables 1** and **2**. A two-sided Mann-Whitney test was performed that shows significantly higher response rates for genomically matched therapies ($p < 0.0001$). Abbreviations: FDA, US Food and Drug Administration; NSCLC, non-small-cell lung cancer.

individual patients may be eligible to receive in clinical trial contexts, two main trial approaches have been developed. Umbrella trials are disease specific, include patients with a single type of cancer, and use genomic profiling to match patients with specific alterations (or pathway alterations) to trial arms, in which the patient receives a specific drug or combination with putative utility against that alteration. Examples of large umbrella trials include iSpy2, Lung-MAP, ALCHEMIST, MATRIX, and BATTLE-2. Basket trials include genomically profiled patients with one of many permitted cancer types. Patients with a specific alteration are treated with a drug or drugs targeting the alteration, and drug efficacy is assessed with respect to the specific alteration and not with regard to the cancer type. This approach is particularly useful for determining the efficacy of drugs targeting rarer alterations, for which cancer-specific trials are challenging owing to low patient numbers. Examples of large basket trials with multiple arms include NCI-MATCH, MyPathway, Basket of Baskets, and TAPUR. NCI-MATCH is the most ambitious, hosting >30 treatment arms and with matching primarily done on the basis of genomic selection (146). It is important that all clinical trials report the maximal amount of information to the community about the successes and failures in specific tumor types of specific drugs targeted to specific alterations. In **Supplemental Text 1**, we focus on a basket trial of neratinib targeted against HER2 mutations to highlight the power of basket approaches and the importance of full outcome reporting (9). Cell-of-origin analyses have highlighted that certain cancers arising from different organs may be more similar to each other than to other cancers from their originating tissue (6), with important potential consequences for patient management and lending support to basket trial approaches. However, the trial results discussed in **Supplemental Text 1** (9) and the vemurafenib basket trial described above (10) highlight tissue-specific caveats with regard to pan-cancer actionability.

Supplemental Material >

Sustainable trial platforms with adaptive protocols and dynamic trial arms (accruing new arms for novel alterations and matched drugs), as well as expansion cohorts to allow efficacy to be demonstrated in subsets for which preliminary responses have been promising, can dramatically speed up the drug discovery process. To identify rare alterations, it is necessary to genomically profile large numbers of patients, with the knowledge that usually only a minority will be successfully matched to a trial arm. This approach necessitates large national or international collaborations to find enough patients to provide statistical power to determine preliminary efficacy for genomically matched drugs and unfortunately with the associated inequity that results from these trials still being concentrated in large academic centers. Most large-scale trial approaches have been concentrated in North America. The Basket of Baskets trial is an ambitious collaboration between the seven comprehensive cancer centers that comprise Cancer Core Europe (147), which has broken down many complex legal and regulatory barriers to achieve a sustainable trial platform that will include many basket studies to match as many patients as possible to therapies. These studies will open synchronously across all sites. The success of these approaches is reliant upon the discovery of novel genomic biomarkers and matched drugs, but these trials can also be adapted to incorporate biomarkers from other sources as well as compound biomarkers.

The FDA breakthrough therapy designation was launched to speed up the regulatory process and improve access to therapies that have promising efficacy and safety data, and it recognizes the difficulties inherent to performing large phase III trials for rare cancers and for rare genomic alterations (148). It also appreciates the difficulties in performing superiority trials in which the novel therapy is predicted to significantly outperform standard-of-care treatments (and matching to a standard-of-care arm thereby presents significant ethical issues). This designation has resulted in important alterations to trial designs, particularly through significant expansion of promising phase II study cohorts.

OTHER FUTURE DIRECTIONS AND REQUIREMENTS

To identify novel biomarkers from genomics-based approaches and translate these to clinical utility, several concurrent approaches are required. We must interrogate the data that we already have in novel ways, improve preclinical models to investigate and validate putatively actionable biomarkers, interrogate data from multiple omics-based approaches, increase the recruitment to biomarker-matched clinical trials, and incorporate complex and compound biomarkers into clinical trial design. We must also alter our patient management to learn as much as possible from every patient and expand and develop comprehensive databases with matched omics and clinical data.

Comprehensive Analysis and Sharing of Patient-Level Data

As discussed above, large-scale approaches have furthered our knowledge of cancer genomics, identified novel putatively actionable targets, provided important insights into cancer progression and clonality, and highlighted similarities and differences between cancer types and subtypes. Collaborative approaches and pipelines between preclinical and clinical investigators across academia and industry are now required to identify, prioritize, and validate the most promising targets from these approaches, feeding findings directly into clinical trials. Such collaboration will require significant investment from academia, government, and industry and coordinated international cooperation (149).

Genomics is only one part of the cancer puzzle and can only make general predictions about the phenotypic consequences of particular alterations, although network approaches have helped

to refine these predictions. In a high-throughput manner, tumor cell transcriptomics provides information that is closer to phenotype by highlighting likely protein levels and changes in signaling pathways in cancer cells (150). Proteomics, though currently more expensive and more difficult to interpret than nucleic acid profiling, is one step closer, allowing interrogation of actual protein expression and posttranscriptional modifications (36). These approaches provide little insight into the tumor microenvironment, and single-cell transcriptomics, imaging mass cytometry, and immunological approaches must also be integrated alongside epigenomics, metabolomics, radiomics, and microbiomics. Given the complexity of cancer, it is not surprising that such complex approaches are required—traditional approaches have focused on the low-hanging fruit presented by strong, directly targetable drivers, and only integrative approaches that identify emergent properties that are not easily discernable from the sum of the parts will likely deliver broad patient benefit. Some of these benefits will be discernable from genomics data alone through artificial intelligence (AI) approaches, which must now be applied to genomic data sets.

To help deliver on this, data curation and sharing must be comprehensive and useful for both the research community (for hypothesis generation and interrogation) and the clinical community (to be able to act on identified alterations in individual patients). In **Supplemental Text 3**, we highlight what information these databases should ideally include to optimize utility for both communities, alongside practical, cost, and ethical considerations. We also discuss ongoing transformative approaches to data sharing and their implications for clinical centers.

Supplemental Material >

Completing the Bench-to-Bedside Loop to Improve and Personalize Validation of Putatively Actionable Targets

Even without integrating other omics approaches, large-scale genomic data sets have highlighted many more putatively actionable alterations than can ever feasibly be tested in clinical trials. To ensure that evidence of actionability before clinical testing is of the highest quality, we must improve the throughput and translatability of preclinical models. As described above, high-throughput systems for testing the consequences and druggability of large numbers of genomic alterations in isogenic cell lines have been developed, but these systems currently consider consequences to cells in only two dimensions, devoid of elements of the tumor microenvironment. This leads to conclusions that certain drugs are not useful, when they might be in vivo, and many positive results in two dimensions are not borne out when tested in more complex models (151). High-throughput in vitro screening in three dimensions goes some way to addressing these issues and can prioritize hypotheses for xenograft experiments in murine models, in which stromal elements are co-opted and humanization of murine immunity may also help improve translatability to cancer patients. However, these approaches do not address the highly individual nature of patients' cancers.

Figure 4 highlights some attractive ways to complete the bench-to-bedside loop at the level of individual patients. Expansion of patient-derived CTCs allows individualized drug screening in vitro (152), either prioritizing drugs against putatively actionable alterations or using an unbiased approach. Drug panels should prioritize drugs for which safe, pharmacodynamically validated doses have already been established in humans but should ideally also include other molecules to generate and test new hypotheses. CTCs or biopsy specimens can also be grown into organoids, which can recapitulate the genetic, histological, and molecular features of the tumor of origin, are amenable to drug screening, and have been shown to predict clinical drug response (153, 154). Hits can then be further validated through murine xenograft experiments, although it is unclear if CTCs are truly representative of cells in tumors themselves and how precisely they represent intratumoral/intertumoral heterogeneity. CTCs also do not allow examination of the context of the patient's tumor microenvironment.

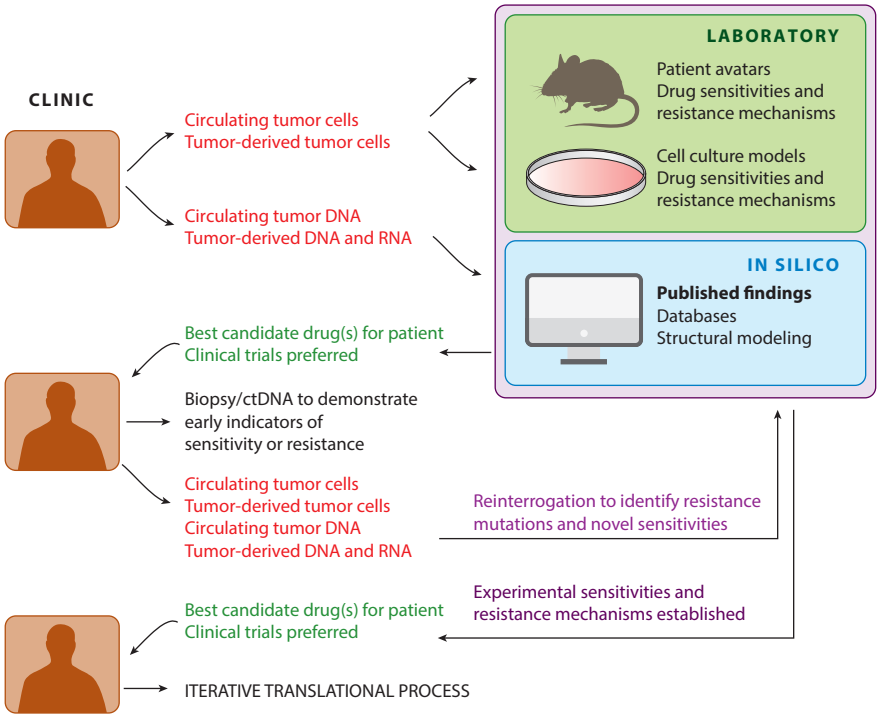

Figure 4

Novel approaches to determining and assessing actionability at the level of a single patient: completing the bench-to-bedside loop. This illustration shows a possible iterative process whereby a patient's derived material is used for functional and bioinformatic analysis alongside that patient's treatment journey. This analysis informs the choices of drugs targeted against specific genomic alterations, taking into account experimental sensitivities and resistances. Abbreviation: ctDNA, circulating tumor DNA.

Patient avatars are created by xenografting tumor-derived cells from a patient (including stromal components) into murine models to allow in vivo testing of drugs in real-time alongside that patient's clinical journey (155, 156). Although expensive and space- and time-consuming (requiring large animal colonies and with a lag period that limits their immediate clinical utility for patients who need to start treatment), avatars offer the possibility of treating tumor cells in a more relevant context. Heterogeneity is maintained at least in early passages, including intratumoral clonality, which is also maintained in short-term cultures of xenograft-derived tumor cells that allow for high-throughput drug screening in vitro (157). However, human stromal components are gradually replaced by murine stroma, and the contributions of immunity are necessarily lost or diluted—although partially humanized immune systems can help (158), these do not precisely model the patient's unique immune system. In addition to providing important information about sensitivity/resistance to specific drugs, these models can allow for the determination of likely resistance mechanisms, and findings can be relayed to the treating clinician to inform treatment choices. It is unclear how these approaches to patient management will be handled by regulatory authorities, nor is it clear how treatment with off-label drugs in this setting might be approved.

Other Paths to Novel Therapies

The individual patient-centered approaches described above should proceed alongside more basic science approaches for determining novel drug targets. For example, to build on the success of

synthetic lethality, approaches using isogenic cell lines, knockout screens, or ISLE [identification of clinically relevant synthetic lethality; this method predicts the most clinically useful synthetic lethal interactions from laboratory screening results (159)] can help prioritize putative synthetic lethal interactions and have successfully predicted response. Examination of pairs of genes that are never comutated in the same tumor may also aid prioritization, and approaches to examine the whole genome for synthetic lethal interactions are underway (160, 161).

Dysregulated transcriptional networks are being explored as potential therapeutic targets. These impart important phenotypic differences to cancer cells and have led to the concept of transcriptional addiction. For example, most human cancers amplify or deregulate the transcription factor MYC, driving anabolic processes and metabolic adaptation. Although MYC is not currently directly targetable in humans, inhibition of MYC function in murine models leads to widespread tumoral cell death and is remarkably well tolerated (162). Promising approaches for targeting transcriptional addiction include targeting the BET bromodomain coactivator fusions that occur in many cancer types with BET inhibitors (163). Transcriptomics can also identify clinically relevant immune signatures and help investigate resistance mechanisms, for example, the highly recurrent signatures that converge on c-MET, YAP1, and LEF1 in treated melanoma patients (164). Gene signatures, from microarray/RT-PCR approaches [e.g., Mammaprint® (21) and Oncotype DX® (22)], have helped identify which early breast cancer patients are cured by surgery and endocrine therapy alone and which require cytotoxic chemotherapy, reducing unnecessary treatment burdens and toxicities for thousands of women. Such approaches underline the importance of refining biomarkers to produce clinically feasible, useful, and validated tools.

Proteomic analysis, as described above, is a powerful tool for determining true protein characteristics and can identify novel clusters of drivers that are not necessarily discernable from transcriptomics. Efforts to reduce the expense and improve the speed, sensitivity, and analytic validity of proteomics will help unveil its true power. Epigenomics [using chromatin immunoprecipitation sequencing (ChIP-seq) or methylation sequencing (methyl-seq)] has uncovered important cancer-associated changes in genome-wide transcriptional and chromatin regulation (which is often dysregulated through mutation of chromatin modulators), revealing possible novel treatment approaches. Although broad-brush targeting of epigenetic modifications [e.g., with histone deacetylase (HDAC) and DNA methyltransferase inhibitors] has yielded disappointing results, insights into genome reorganization and synthetic lethal approaches have identified specific candidate targets. Epigenomics allows cellular phenotypes to be explored and potentially targeted, and future predictive biomarkers may include epigenomically elucidated categories such as stemness and epithelial-to-mesenchyme transitions, employing drugs to reverse these states [e.g., the use of all-trans retinoic acid (ATRA) in neuroblastoma]. As described above, EZH2 has emerged as a promising target (85), as has mutant IDH1/2 (which results in widespread epigenetic changes) (165). Currently, epigenomic biomarkers are used to identify patients with hypermethylated MMR genes [leading to MMR deficiency and opening up treatment possibilities with ICPIs (133)] or methylated *MGMT* [leading to increased sensitivity to alkylating agents (e.g., temozolomide in glioblastoma) (166)].

Nearly a century after the discovery of the Warburg effect (167), high-sensitivity mass spectrometry–based metabolomics has emerged as an exciting prospect for highlighting differences in small molecules in cancer cells (and can be applied directly to tumor specimens) and has highlighted therapeutic vulnerabilities given the particular reliance of cancer cells on certain metabolic pathways (e.g., to make amino acids, fats, or the nucleic acids that are necessary for anabolic cell growth; reviewed in 168, 169). Integration of multi-omic analyses for 92 breast cancer patients has highlighted some of these, such as fatty acid dependencies for luminal B cancers and alterations in glucose/glutamine metabolism in HER2-positive and basal cancers (170). Common

mutations in metabolic genes are driving alterations in certain cancers (e.g., *IDH1/2*, fumarate hydratase, and succinate dehydrogenase), leading to attempts to target these using synthetic lethality approaches (171).

Network and systems biology approaches are required to integrate multi-omic data (particularly given the highly nonlinear interplay between cancer cells and their microenvironment) to provide novel targets for therapy (172, 173). Although such approaches have not yet been applied to the clinical trial setting (where reductionist methods continue to reign supreme), they have elucidated mechanisms underlying observed network changes that are attractive targets for therapy and are likely to be much more predictive of drug sensitivities than hypotheses based on single genomic alterations. Network approaches include identification of proteins that interact extensively with altered cancer genes (aberration hubs), and these approaches can find novel targets, such as the identification of spleen TK as a key proliferation factor in renal cell carcinoma (174). It is important that we incorporate machine learning and AI into our exploration of multi-omic data sets, to find novel features that will predict drug sensitivities in ways that are currently unimaginable and reveal broad pan-cancer targets.

Given promising durable clinical responses to ICPIs, much research is now focusing on refining biomarkers of response to these drugs and finding novel ways to exploit anticancer immunity. Methods include neoantigen prediction and validation, TCR sequencing, and the exploration of diverse intratumoral and systemic immune cell populations. A large-scale approach akin to The Cancer Genome Atlas (TCGA) is required to systematically probe the immunome for specific cancer types, including single-cell RNAseq of tumor-infiltrating cells. Beyond allowing neoantigen creation, genomic alterations may also provide insights, at least in some patients—for example, a small subset of patients harbor *PDL1* gene amplifications (including 10.5% of patients with hepatocellular cholangiocarcinomas) (175, 176). Of nine patients (with diverse cancers) harboring *PDL1* amplification that were treated with ICPIs, six had objective responses, including a patient with glioblastoma, which is usually refractory to ICPIs. Genomic alterations can also predict which patients are unlikely to benefit from ICPIs, such as melanoma patients with *JAK2* or β2-microglobulin deletions (177). Network approaches may help refine this further.

To tackle heterogeneity, reliable identification and targeting of truncal (as opposed to subclonal) genomic alterations should help tackle intrinsic subclonal resistance, and such approaches can also help identify truncal neoantigens to inform immune-directed and cell-based therapeutic approaches. Intermittent drug treatments may exploit changes in the relative fitness of subclones that occur between drug-naive and treated states and help tackle some consequences of heterogeneity.

The combinatorial drug space is challenging to investigate, although drug combinations will usually be required to ensure extensive tumor cell killing and to prevent resistance mechanisms from appearing (to allow durable responses). Combinations can include targets from different pathways or several targets from the same pathway to counteract regulatory feedback mechanisms. Aside from the methods described above, identification of synthetic rescues (178), in which inhibition of one target is compensated for by activity of a rescuing protein or pathway, may help inform combinatorial approaches.

Radiogenomics and Radiotherapy

Radiogenomics incorporates radiomic data (in which digitalized images are mined for certain features and matched to clinical data) and genomic data and is a promising approach to determine differences between primary tumors and individual metastatic sites (and provide early readouts

DDR: DNA damage response

of treatment response), particularly when combined with novel imaging approaches (e.g., the use of pathway-specific imaging probes). This review has focused on druggability with regard to systemic treatments, yet RT is a key modality in the management of >40% of cancer patients cured of their disease and is highly effective in symptom control (179). Given that RT works by inducing direct or indirect DNA damage (and potentially immunogenic cell death leading to investigation of ICPI–RT combination approaches), its use must be reconsidered in light of genomic data to improve outcomes. Cancer cells are more likely to have DDR deficiencies (119), leading to the development of approaches utilizing RT to increase DNA damage to unassailable levels in these cells (e.g., in ATM-deficient cancers). A multimodality approach through combined use of DDR inhibitors (e.g., ATM/ATR/PARP inhibitors) and targeted RT may increase radiosensitivity. For example, the brain-penetrant ATM inhibitor AZD1390 shows promising radiosensitizing effects in *TP53* mutant glioma models (180) and is in early phase clinical development alongside RT (NCT03423628). Efforts have been made to develop predictors of radiosensitivity—for example, the pan-cancer radiosensitivity index, which is based on gene expression data for 10 genes in 48 cell lines and has been validated in multiple cancers (although predominantly retrospectively) (181). Further, a novel gene-expression radiosensitivity signature has been developed for breast cancer, and in an independent data set, this signature outperformed all traditional clinicopathological features for predicting local recurrence (182). Numerous other signatures have also been developed across numerous cancer subtypes. However, definitive and internationally recognized approaches for predicting radiosensitivity have not been agreed upon (reviewed in 183). Notably, only one radiosensitizing drug–RT combination has been approved in the last 12 years [cetuximab–RT in head and neck cancer (184)], and whether it is truly radiosensitizing remains unclear. To improve on this, integrated data sharing as described above is required to allow investigation of radiosensitivity and radioresistance signatures at an appropriate scale.

CONCLUSIONS

Genomic sequencing of human tumors has led to a dramatic improvement in our understanding of cancer, yet many challenges remain in converting these important data into approaches for developing effective anticancer therapies. To improve translatability of genomic findings, large-scale international efforts are required to identify and validate drug-specific vulnerabilities that can be prioritized for clinical testing in well-designed umbrella and basket clinical trials. These approaches will require close collaboration between basic scientists, bioinformaticians, clinical scientists, clinicians, industry, and funding bodies. Future approaches must effectively integrate clinical data with multi-omic data sets, using systems biology and AI methods to identify novel cancer vulnerabilities. These approaches require significant investment in data collection, curation, and sharing. In the meantime, many genomic tools are available for clinical use to match patients to approved therapies, or to fuel recruitment to genomically matched clinical trials, and although the success to date of genomically driven drug development has been disappointing, the clinical benefit afforded by approved agents underlines persistence with this approach. The cart (genomic data) has overtaken the horse (clinical actionability), leading to frequent clinical dilemmas whereby genomic alterations with putative therapeutic actionability lead to imprudent use of off-label drugs. We urge clinicians to approach patient-specific management decisions with caution and with detailed interrogation of evidence that supports or refutes use of a specific drug against a specific alteration. We urge researchers to get the horse back before the cart by diligent characterization and prioritization of putatively actionable alterations—and diligent assessment of the extra information that genomics (versus targeted genetic sequencing) provides—to produce novel genomics-based predictive biomarkers.

DISCLOSURE STATEMENT

C.C. has received research grants from Genentech, Roche, Servier, and Astra Zeneca, all administered by the University of Cambridge, and is a board member of the following organizations: Astra Zeneca–Innovative Medicines and Early Development External Science Panel, European Association for Cancer Research, Organisation of European Cancer Institutes, European Academy of Cancer Sciences, and Cancer Core Europe.

LITERATURE CITED

1. Brufsky AM. 2017. Long-term management of patients with hormone receptor-positive metastatic breast cancer: concepts for sequential and combination endocrine-based therapies. *Cancer Treat. Rev.* 59:22–32
2. Loibl S, Gianni L. 2017. HER2-positive breast cancer. *Lancet* 389(10087):2415–29
3. Dietel M. 2016. Molecular pathology: a requirement for precision medicine in cancer. *Oncol. Res. Treat.* 39(12):804–10
4. Hutter C, Zenklusen JC. 2018. The Cancer Genome Atlas: creating lasting value beyond its data. *Cell* 173(2):283–85
5. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, et al. 2018. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 173(2):321–37.e10
6. Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, et al. 2018. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* 173(2):291–304.e6
7. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. 2013. Emerging landscape of oncogenic signatures across human cancers. *Nat. Genet.* 45(10):1127–33
8. Lefebvre C, Bachelot T, Filleron T, Pedrero M, Campone M, et al. 2016. Mutational profile of metastatic breast cancers: a retrospective analysis. *PLOS Med.* 13(12):e1002201
9. Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, et al. 2018. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. *Nature* 554(7691):189–94
10. Hyman DM, Puzanov I, Subbiah V, Faris JE, Chau I, et al. 2015. Vemurafenib in multiple nonmelanoma cancers with *BRAF* V600 mutations. *N. Engl. J. Med.* 373(8):726–36
11. McGranahan N, Swanton C. 2017. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell* 168(4):613–28
12. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, et al. 2012. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* 366(10):883–92
13. Turajlic S, Xu H, Litchfield K, Rowan A, Horswell S, et al. 2018. Deterministic evolutionary trajectories influence primary tumor growth: TRACERx Renal. *Cell* 173(3):595–610.e11
14. Burrell RA, Swanton C. 2016. Re-evaluating clonal dominance in cancer evolution. *Trends Cancer* 2(5):263–76
15. Aparicio S, Caldas C. 2013. The implications of clonal genome evolution for cancer medicine. *N. Engl. J. Med.* 368(9):842–51
16. Offit K. 2016. The future of clinical cancer genomics. *Semin. Oncol.* 43(5):615–22
17. Hyman DM, Solit DB, Arcila ME, Cheng DT, Sabbatini P, et al. 2015. Precision medicine at Memorial Sloan Kettering Cancer Center: clinical next-generation sequencing enabling next-generation targeted therapy trials. *Drug Discov. Today* 20(12):1422–28
18. Hyman DM, Taylor BS, Baselga J. 2017. Implementing genome-driven oncology. *Cell* 168(4):584–99
19. Giesen C, Wang HAO, Schapiro D, Zivanovic N, Jacobs A, et al. 2014. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat. Methods* 11(4):417–22
20. Cheng ML, Berger MF, Hyman DM, Solit DB. 2018. Clinical tumour sequencing for precision oncology: time for a universal strategy. *Nat. Rev. Cancer* 18:527–28
21. Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, et al. 2016. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. *N. Engl. J. Med.* 375(8):717–29

22. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, et al. 2018. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. *N. Engl. J. Med.* 379(2):111–21
23. Lyle S, Powers W, Xu J, Huettner CS, Russell M, Protopopov A. 2017. Comparison of a large NGS panel to hot-spot testing and small panels for the ability to accurately stratify advanced colorectal cancer patients to appropriate treatment pathways. *J. Clin. Oncol.* 34:510
24. Perakis S, Speicher MR. 2017. Emerging concepts in liquid biopsies. *BMC Med.* 15(1):75
25. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, et al. 2017. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat. Rev. Cancer* 17(4):223–38
26. Dawson S-J, Tsui DWY, Murtaza M, Biggs H, Rueda OM, et al. 2013. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N. Engl. J. Med.* 368(13):1199–209
27. Murtaza M, Dawson S-J, Tsui DWY, Gale D, Forshew T, et al. 2013. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497(7447):108–12
28. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, et al. 2018. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 359(6378):926–30
29. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. 2016. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. *Cell* 164(1–2):57–68
30. Thiele J-A, Bethel K, Králíčková M, Kuhn P. 2017. Circulating tumor cells: fluid surrogates of solid tumors. *Annu. Rev. Pathol. Mech. Dis.* 12:419–47
31. Everaert C, Luypaert M, Maag JLV, Cheng QX, Dinger ME, et al. 2017. Benchmarking of RNA-sequencing analysis workflows using whole-transcriptome RT-qPCR expression data. *Sci. Rep.* 7(1):1559
32. Baslan T, Hicks J. 2017. Unravelling biology and shifting paradigms in cancer with single-cell sequencing. *Nat. Rev. Cancer* 17(9):557–69
33. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, et al. 2008. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat. Biotechnol.* 26(3):317–25
34. Veldman-Jones MH, Brant R, Rooney C, Geh C, Emery H, et al. 2015. Evaluating robustness and sensitivity of the NanoString technologies nCounter platform to enable multiplexed gene expression analysis of clinical samples. *Cancer Res.* 75(13):2587–93
35. Nebbioso A, Tambaro FP, Dell'Aversana C, Altucci L. 2018. Cancer epigenetics: moving forward. *PLOS Genet.* 14(6):e1007362
36. Belczacka I, Latosinska A, Metzger J, Marx D, Vlahou A, et al. 2018. Proteomics biomarkers for solid tumors: current status and future prospects. *Mass Spectrom. Rev.* 38:49–78
37. da Cunha Santos G, Shepherd FA, Tsao MS. 2011. EGFR mutations and lung cancer. *Annu. Rev. Pathol. Mech. Dis.* 6:49–69
38. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, et al. 2008. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* 26(10):1626–34
39. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, et al. 2008. *K-ras* mutations and benefit from cetuximab in advanced colorectal cancer. *N. Engl. J. Med.* 359(17):1757–65
40. Hofree M, Carter H, Kreisberg JF, Bandyopadhyay S, Mischel PS, et al. 2016. Challenges in identifying cancer genes by analysis of exome sequencing data. *Nat. Commun.* 7:12096
41. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, et al. 2012. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 22(3):568–76
42. Magi A, Tattini L, Cifola I, D'Aurizio R, Benelli M, et al. 2013. EXCAVATOR: detecting copy number variants from whole-exome sequencing data. *Genome Biol.* 14(10):R120
43. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, et al. 2017. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res.* 45(D1):D777–83
44. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, et al. 2018. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* 46(D1):D1062–67
45. Turajlic S, Xu H, Litchfield K, Rowan A, Chambers T, et al. 2018. Tracking cancer evolution reveals constrained routes to metastases: TRACERx Renal. *Cell* 173(3):581–94.e12
46. Robichaux JP, Elamin YY, Tan Z, Carter BW, Zhang S, et al. 2018. Mechanisms and clinical activity of an EGFR and HER2 exon 20-selective kinase inhibitor in non-small cell lung cancer. *Nat. Med.* 24(5):638–46

47. Chakravarty D, Gao J, Phillips S, Kundra R, Zhang H, et al. 2017. OncoKB: a precision oncology knowledge base. *JCO Precis. Oncol.* **https://doi.org/10.1200/PO.17.00011**
48. Taylor AD, Micheel CM, Anderson IA, Levy MA, Lovly CM. 2016. The path(way) less traveled: a pathway-oriented approach to providing information about precision cancer medicine on My Cancer Genome. *Transl. Oncol.* 9(2):163–65
49. Tamborero D, Rubio-Perez C, Deu-Pons J, Schroeder MP, Vivancos A, et al. 2018. Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations. *Genome Med*. 10(1):25
50. Dumbrava EI, Meric-Bernstam F. 2018. Personalized cancer therapy—leveraging a knowledge base for clinical decision-making. *Cold Spring Harb. Mol. Case Stud.* 4(2):a001578
51. Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, et al. 2018. Comprehensive characterization of cancer driver genes and mutations. *Cell* 173(2):371–85.e18
52. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, et al. 2018. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell* 173(2):321–37.e10
53. Niinuma T, Suzuki H, Sugai T. 2018. Molecular characterization and pathogenesis of gastrointestinal stromal tumor. *Transl. Gastroenterol. Hepatol.* 3:2
54. Staedtke V, Dzaye OD, Holdhoff M. 2016. Actionable molecular biomarkers in primary brain tumors. *Trends Cancer* 2(7):338–49
55. Mak IW, Evaniew N, Ghert M. 2014. Lost in translation: animal models and clinical trials in cancer treatment. *Am. J. Transl. Res.* 6(2):114–18
56. Nagano M, Kohsaka S, Ueno T, Kojima S, Saka K, et al. 2018. High-throughput functional evaluation of variants of unknown significance in ERBB2. *Clin. Cancer Res.* 24. **https://doi.org/10.1158/1078-0432.CCR-18-0991**
57. Brenan L, Andreev A, Cohen O, Pantel S, Kamburov A, et al. 2016. Phenotypic characterization of a comprehensive set of *MAPK1*/ERK2 missense mutants. *Cell Rep*. 17(4):1171–83
58. Strickler JH, Wu C, Bekaii-Saab T. 2017. Targeting BRAF in metastatic colorectal cancer: maximizing molecular approaches. *Cancer Treat. Rev.* 60:109–19
59. Hasty P, Montagna C. 2014. Chromosomal rearrangements in cancer: detection and potential causal mechanisms. *Mol. Cell. Oncol.* 1(1):e29904
60. Mertens F, Johansson B, Fioretos T, Mitelman F. 2015. The emerging complexity of gene fusions in cancer. *Nat. Rev. Cancer* 15(6):371–81
61. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. 1985. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* 228(4706):1440–43
62. Kridel R, Sehn LH, Gascoyne RD. 2012. Pathogenesis of follicular lymphoma. *J. Clin. Investig.* 122(10):3424–31
63. Ren R. 2005. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat. Rev. Cancer* 5(3):172–83
64. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, et al. 2003. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. *N. Engl. J. Med.* 348(11):994–1004
65. Viganò I, Di Giacomo N, Bozzani S, Antolini L, Piazza R, Gambacorti Passerini C. 2014. First-line treatment of 102 chronic myeloid leukemia patients with imatinib: a long-term single institution analysis. *Am. J. Hematol.* 89(10):E184–87
66. Dupain C, Harttrampf AC, Urbinati G, Geoerger B, Massaad-Massade L. 2017. Relevance of fusion genes in pediatric cancers: toward precision medicine. *Mol. Ther. Nucleic Acids* 6:315–26
67. Peters S, Camidge DR, Shaw AT, Gadgeel S, Ahn JS, et al. 2017. Alectinib versus crizotinib in untreated *ALK*-positive non-small-cell lung cancer. *N. Engl. J. Med.* 377(9):829–38
68. Ardini E, Menichincheri M, Banfi P, Bosotti R, De Ponti C, et al. 2016. Entrectinib, a pan-TRK, ROS1, and ALK inhibitor with activity in multiple molecularly defined cancer indications. *Mol. Cancer Ther.* 15(4):628–39
69. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, et al. 2018. Efficacy of larotrectinib in *TRK* fusion–positive cancers in adults and children. *N. Engl. J. Med.* 378(8):731–39

70. Stransky N, Cerami E, Schalm S, Kim JL, Lengauer C. 2014. The landscape of kinase fusions in cancer. *Nat. Commun.* 5(1):4846
71. Drilon A, Siena S, Ou S-HI, Patel M, Ahn MJ, et al. 2017. Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTRK-1). *Cancer Discov.* 7(4):400–9
72. Ashworth A, Lord CJ. 2018. Synthetic lethal therapies for cancer: What's next after PARP inhibitors? *Nat. Rev. Clin. Oncol.* 15:564–76
73. Venkitaraman AR. 2014. Cancer suppression by the chromosome custodians, BRCA1 and BRCA2. *Science* 343(6178):1470–75
74. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, et al. 2005. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 434(7035):913–17
75. Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, et al. 2005. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 434(7035):917–21
76. Ström CE, Johansson F, Uhlén M, Szigyarto CA-K, Erixon K, Helleday T. 2011. Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand intermediate. *Nucleic Acids Res.* 39(8):3166–75
77. Chang HHY, Pannunzio NR, Adachi N, Lieber MR. 2017. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* 18(8):495–506
78. Mirza MR, Pignata S, Ledermann JA. 2018. Latest clinical evidence and further development of PARP inhibitors in ovarian cancer. *Ann. Oncol.* 29(6):1366–76
79. Lee J-M, Ledermann JA, Kohn EC. 2014. PARP inhibitors for BRCA1/2 mutation-associated and BRCA-like malignancies. *Ann. Oncol.* 25(1):32–40
80. Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzerini-Denchi E, Sfeir A. 2015. Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. *Nature* 518(7538):254–57
81. Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, et al. 2015. Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. *Nature* 518(7538):258–62
82. Feng Z, Scott SP, Bussen W, Sharma GG, Guo G, et al. 2011. Rad52 inactivation is synthetically lethal with BRCA2 deficiency. *PNAS* 108(2):686–91
83. Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, et al. 2013. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat. Genet.* 45(6):592–601
84. Biegel JA, Zhou JY, Rorke LB, Stenstrom C, Wainwright LM, Fogelgren B. 1999. Germ-line and acquired mutations of *INI1* in atypical teratoid and rhabdoid tumors. *Cancer Res.* 59(1):74–79
85. Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, et al. 2015. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat. Med.* 21(12):1491–96
86. Cardaci S, Zheng L, MacKay G, van den Broek NJF, MacKenzie ED, et al. 2015. Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. *Nat. Cell Biol.* 17(10):1317–26
87. Westcott PMK, Halliwill KD, To MD, Rashid M, Rust AG, et al. 2015. The mutational landscapes of genetic and chemical models of *Kras*-driven lung cancer. *Nature* 517(7535):489–92
88. Doherty GJ, Kerr EM, Martins CP. 2017. *KRAS* allelic imbalance: strengths and weaknesses in numbers. *Trends Mol. Med.* 23(5):377–78
89. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, et al. 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* 364(26):2507–16
90. Hauschild A, Grob J-J, Demidov LV, Jouary T, Gutzmer R, et al. 2012. Dabrafenib in *BRAF*-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 380(9839):358–65
91. Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, et al. 2012. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med.* 367(2):107–14
92. Robert C, Karaszewska B, Schachter J, Rutkowski P, Mackiewicz A, et al. 2015. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N. Engl. J. Med.* 372(1):30–39

93. Larkin J, Ascierto PA, Dréno B, Atkinson V, Liszkay G, et al. 2014. Combined vemurafenib and cobimetinib in *BRAF*-mutated melanoma. *N. Engl. J. Med.* 371(20):1867–76
94. McCormick F. 2018. Targeting KRAS directly. *Annu. Rev. Cancer Biol.* 2:81–90
95. Kerr EM, Gaude E, Turrell FK, Frezza C, Martins CP. 2016. Mutant *Kras* copy number defines metabolic reprogramming and therapeutic susceptibilities. *Nature* 531(7592):110–13
96. Janes MR, Zhang J, Li L-S, Hansen R, Peters U, et al. 2018. Targeting KRAS mutant cancers with a covalent G12C-specific inhibitor. *Cell* 172(3):578–89.e17
97. Jänne PA, van den Heuvel MM, Barlesi F, Cobo M, Mazieres J, et al. 2017. Selumetinib plus docetaxel compared with docetaxel alone and progression-free survival in patients with *KRAS*-mutant advanced non-small cell lung cancer. *JAMA* 317(18):1844–53
98. Molina-Arcas M, Hancock DC, Sheridan C, Kumar MS, Downward J. 2013. Coordinate direct input of both KRAS and IGF1 receptor to activation of PI3 kinase in *KRAS*-mutant lung cancer. *Cancer Discov.* 3(5):548–63
99. Sun C, Hobor S, Bertotti A, Zecchin D, Huang S, et al. 2014. Intrinsic resistance to MEK inhibition in *KRAS* mutant lung and colon cancer through transcriptional induction of *ERBB3*. *Cell Rep.* 7(1):86–93
100. Kruspig B, Monteverde T, Neidler S, Hock A, Kerr E, et al. 2018. The ERBB network facilitates KRAS-driven lung tumorigenesis. *Sci. Transl. Med.* 10(446):eaao2565
101. Zhang Y-L, Yuan J-Q, Wang K-F, Fu X-H, Han X-R, et al. 2016. The prevalence of *EGFR* mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* 7(48):78985–93
102. Ahluwalia MS, Becker K, Levy BP. 2018. Epidermal growth factor receptor tyrosine kinase inhibitors for central nervous system metastases from non-small cell lung cancer. *Oncologist* 23(10):1199–209
103. Ko B, Paucar D, Halmos B. 2017. EGFR T790M: revealing the secrets of a gatekeeper. *Lung Cancer* 8:147–59
104. Cross DAE, Ashton SE, Ghiorghiu S, Eberlein C, Nebhan CA, et al. 2014. AZD9291, an irreversible EGFR TKI, overcomes T790M-mediated resistance to EGFR inhibitors in lung cancer. *Cancer Discov.* 4(9):1046–61
105. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, et al. 2018. Osimertinib in untreated *EGFR*-mutated advanced non-small-cell lung cancer. *N. Engl. J. Med.* 378(2):113–25
106. O'Kane GM, Bradbury PA, Feld R, Leighl NB, Liu G, et al. 2017. Uncommon *EGFR* mutations in advanced non-small cell lung cancer. *Lung Cancer* 109:137–44
107. Albertson DG. 2006. Gene amplification in cancer. *Trends Genet.* 22(8):447–55
108. Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, et al. 2012. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 486(7403):346–52
109. Pereira B, Chin S-F, Rueda OM, Vollan H-KM, Provenzano E, et al. 2016. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat. Commun.* 7:11479
110. Macintyre G, Goranova TE, De Silva D, Ennis D, Piskorz AM, et al. 2018. Copy number signatures and mutational processes in ovarian carcinoma. *Nat. Genet.* 50:1262–70
111. Parakh S, Gan HK, Parslow AC, Burvenich IJG, Burgess AW, Scott AM. 2017. Evolution of anti-HER2 therapies for cancer treatment. *Cancer Treat. Rev.* 59:1–21
112. Pearson A, Smyth E, Babina IS, Herrera-Abreu MT, Tarazona N, et al. 2016. High-level clonal FGFR amplification and response to FGFR inhibition in a translational clinical trial. *Cancer Discov.* 6(8):838–51
113. Beck A, Goetsch L, Dumontet C, Corvaïa N. 2017. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat. Rev. Drug Discov.* 16(5):315–37
114. Verma S, Miles D, Gianni L, Krop IE, Welslau M, et al. 2012. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N. Engl. J. Med.* 367:1783–91
115. Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W, et al. 2016. Inherited DNA-repair gene mutations in men with metastatic prostate cancer. *N. Engl. J. Med.* 375(5):443–53
116. Carlo MI, Mukherjee S, Mandelker D, Vijai J, Kemel Y, et al. 2018. Prevalence of germline mutations in cancer susceptibility genes in patients with advanced renal cell carcinoma. *JAMA Oncol.* 4:1228–35

117. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, et al. 2017. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 18(1):75–87
118. Sansregret L, Vanhaesebroeck B, Swanton C. 2018. Determinants and clinical implications of chromosomal instability in cancer. *Nat. Rev. Clin. Oncol.* 15(3):139–50
119. Lee J-K, Choi Y-L, Kwon M, Park PJ. 2016. Mechanisms and consequences of cancer genome instability: lessons from genome sequencing studies. *Annu. Rev. Pathol. Mech. Dis.* 11:283–312
120. Pikor L, Thu K, Vucic E, Lam W. 2013. The detection and implication of genome instability in cancer. *Cancer Metastasis Rev*. 32(3–4):341–52
121. Negrini S, Gorgoulis VG, Halazonetis TD. 2010. Genomic instability—an evolving hallmark of cancer. *Nat. Rev. Mol. Cell Biol.* 11(3):220–28
122. Fragkos M, Naim V. 2017. Rescue from replication stress during mitosis. *Cell Cycle* 16(7):613–33
123. Técher H, Koundrioukoff S, Nicolas A, Debatisse M. 2017. The impact of replication stress on replication dynamics and DNA damage in vertebrate cells. *Nat. Rev. Genet.* 18(9):535–50
124. Gordon DJ, Resio B, Pellman D. 2012. Causes and consequences of aneuploidy in cancer. *Nat. Rev. Genet.* 13(3):189–203
125. Sheltzer JM, Ko JH, Replogle JM, Habibe Burgos NC, Chung ES, et al. 2017. Single-chromosome gains commonly function as tumor suppressors. *Cancer Cell* 31(2):240–55
126. Santaguida S, Richardson A, Iyer DR, M'Saad O, Zasadil L, et al. 2017. Chromosome mis-segregation generates cell-cycle-arrested cells with complex karyotypes that are eliminated by the immune system. *Dev. Cell* 41(6):638–51.e5
127. Boland CR, Goel A. 2010. Microsatellite instability in colorectal cancer. *Gastroenterology* 138(6):2073–87.e3
128. Dieci MV, Mathieu MC, Guarneri V, Conte P, Delaloge S, et al. 2015. Prognostic and predictive value of tumor-infiltrating lymphocytes in two phase III randomized adjuvant breast cancer trials. *Ann. Oncol.* 26(8):1698–704
129. Maleki Vareki S, Garrigós C, Duran I. 2017. Biomarkers of response to PD-1/PD-L1 inhibition. *Crit. Rev. Oncol. Hematol.* 116:116–24
130. Pardoll DM. 2012. The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* 12(4):252–64
131. Sharma P, Allison JP. 2015. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 161(2):205–14
132. Goodman AM, Kato S, Bazhenova L, Patel SP, Frampton GM, et al. 2017. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol. Cancer Ther.* 16(11):2598–608
133. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, et al. 2015. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* 372(26):2509–20
134. Dawson S-J, Rueda OM, Aparicio S, Caldas C. 2013. A new genome-driven integrated classification of breast cancer and its implications. *EMBO J.* 32(5):617–28
135. Mukherjee A, Russell R, Chin S-F, Liu B, Rueda OM, et al. 2018. Associations between genomic stratification of breast cancer and centrally reviewed tumour pathology in the METABRIC cohort. *npj Breast Cancer* 4(1):5
136. Greaves M. 2015. Evolutionary determinants of cancer. *Cancer Discov*. 5(8):806–20
137. Murtaza M, Dawson S-J, Pogrebniak K, Rueda OM, Provenzano E, et al. 2015. Multifocal clonal evolution characterized using circulating tumour DNA in a case of metastatic breast cancer. *Nat. Commun.* 6(1):8760
138. Deleted in proof
139. McGranahan N, Favero F, de Bruin EC, Birkbak NJ, Szallasi Z, Swanton C. 2015. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci. Transl. Med.* 7(283):283ra54
140. Shah SP, Roth A, Goya R, Oloumi A, Ha G, et al. 2012. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 486(7403):395–99

141. Baca SC, Prandi D, Lawrence MS, Mosquera JM, Romanel A, et al. 2013. Punctuated evolution of prostate cancer genomes. *Cell* 153(3):666–77
142. Collins DC, Sundar R, Lim JSJ, Yap TA. 2017. Towards precision medicine in the clinic: from biomarker discovery to novel therapeutics. *Trends Pharmacol. Sci.* 38(1):25–40
143. Le Tourneau C, Delord J-P, Gonçalves A, Gavoille C, Dubot C, et al. 2015. 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*. 16(13):1324–34
144. Massard C, Michiels S, Ferté C, Le Deley M-C, Lacroix L, et al. 2017. High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 Trial. *Cancer Discov*. 7:586–95
145. Tsimberidou AM, Hong DS, Wheler JW, Falchook GS, Naing A, et al. 2018. Precision medicine: clinical outcomes including long-term survival according to the pathway targeted and treatment period—The IMPACT study. *J. Clin. Oncol.* 36. **https://doi.org/10.1200/JCO.2018.36.18_suppl.LBA2553**
146. Do K, O'Sullivan Coyne G, Chen AP. 2015. An overview of the NCI precision medicine trials—NCI MATCH and MPACT. *Chin. Clin. Oncol.* 4(3):31
147. Eggermont AMM, Caldas C, Ringborg U, Medema R, Tabernero J, Wiestler O. 2014. Cancer Core Europe: a consortium to address the cancer care–cancer research continuum challenge. *Eur. J. Cancer* 50(16):2745–46
148. Corrigan-Curay J, McKee AE, Stein P. 2018. Breakthrough-therapy designation—an FDA perspective. *N. Engl. J. Med.* 378(15):1457–58
149. Siu LL, Lawler M, Haussler D, Knoppers BM, Lewin J, et al. 2016. Facilitating a culture of responsible and effective sharing of cancer genome data. *Nat. Med.* 22(5):464–71
150. Raghavachari N, Garcia-Reyero N. 2018. Overview of gene expression analysis: transcriptomics. In *Methods in Molecular Biology*, Vol. 1783: *Gene Expression Analysis*, ed. N Raghavachari, N Garcia-Reyero, pp. 1–6. New York: Humana Press
151. Langhans SA. 2018. Three-dimensional in vitro cell culture models in drug discovery and drug repositioning. *Front. Pharmacol.* 9:6
152. Maheswaran S, Haber DA. 2015. Ex vivo culture of CTCs: an emerging resource to guide cancer therapy. *Cancer Res*. 75(12):2411–15
153. Weeber F, Ooft SN, Dijkstra KK, Voest EE. 2017. Tumor organoids as a pre-clinical cancer model for drug discovery. *Cell Chem. Biol.* 24(9):1092–100
154. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, et al. 2018. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 359(6378):920–26
155. Malaney P, Nicosia SV, Davé V. 2014. One mouse, one patient paradigm: new avatars of personalized cancer therapy. *Cancer Lett*. 344(1):1–12
156. Meehan TF, Conte N, Goldstein T, Inghirami G, Murakami MA, et al. 2017. PDX-MI: minimal information for patient-derived tumor xenograft models. *Cancer Res*. 77(21):e62–66
157. Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, et al. 2016. A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. *Cell* 167(1):260–74.e22
158. Morton JJ, Bird G, Keysar SB, Astling DP, Lyons TR, et al. 2016. XactMice: humanizing mouse bone marrow enables microenvironment reconstitution in a patient-derived xenograft model of head and neck cancer. *Oncogene* 35(3):290–300
159. Lee JS, Das A, Jerby-Arnon L, Arafeh R, Auslander N, et al. 2018. Harnessing synthetic lethality to predict the response to cancer treatment. *Nat. Commun.* 9(1):2546
160. Toledo CM, Ding Y, Hoellerbauer P, Davis RJ, Basom R, et al. 2015. Genome-wide CRISPR-Cas9 screens reveal loss of redundancy between PKMYT1 and WEE1 in glioblastoma stem-like cells. *Cell Rep*. 13(11):2425–39
161. Ashworth A, Lord CJ. 2018. Synthetic lethal therapies for cancer: what's next after PARP inhibitors? *Nat. Rev. Clin. Oncol.* 15:564–76
162. Jain M, Arvanitis C, Chu K, Dewey W, Leonhardt E, et al. 2002. Sustained loss of a neoplastic phenotype by brief inactivation of *MYC*. *Science* 297(5578):102–4

163. Jung M, Gelato KA, Fernández-Montalván A, Siegel S, Haendler B. 2015. Targeting BET bromodomains for cancer treatment. *Epigenomics* 7(3):487–501
164. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, et al. 2016. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 165(1):35–44
165. Molenaar RJ, Maciejewski JP, Wilmink JW, van Noorden CJF. 2018. Wild-type and mutated IDH1/2 enzymes and therapy responses. *Oncogene* 37(15):1949–60
166. Thon N, Kreth S, Kreth F-W. 2013. Personalized treatment strategies in glioblastoma: *MGMT* promoter methylation status. *Onco Targets Ther*. 6:1363–72
167. Warburg O. 1925. The metabolism of carcinoma cells. *J. Cancer Res.* 9(1):148–63
168. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. 2017. Cancer metabolism: a therapeutic perspective. *Nat. Rev. Clin. Oncol.* 14(1):11–31
169. Yu L, Li K, Zhang X. 2017. Next-generation metabolomics in lung cancer diagnosis, treatment and precision medicine: mini review. *Oncotarget* 8(70):115774–86
170. Cappelletti V, Iorio E, Miodini P, Silvestri M, Dugo M, Daidone MG. 2017. Metabolic footprints and molecular subtypes in breast cancer. *Dis. Markers* 2017:7687851
171. Zecchini V, Frezza C. 2017. Metabolic synthetic lethality in cancer therapy. *Biochim. Biophys. Acta Bioenerg.* 1858(8):723–31
172. Yugi K, Kubota H, Hatano A, Kuroda S. 2016. Trans-omics: how to reconstruct biochemical networks across multiple 'omic' layers. *Trends Biotechnol.* 34(4):276–90
173. Archer TC, Fertig EJ, Gosline SJC, Hafner M, Hughes SK, et al. 2016. Systems approaches to cancer biology. *Cancer Res.* 76(23):6774–77
174. Karimzadeh M, Jandaghi P, Papadakis AI, Trainor S, Rung J, et al. 2018. Aberration hubs in protein interaction networks highlight actionable targets in cancer. *Oncotarget* 9(38):25166–80
175. Goodman AM, Piccioni D, Kato S, Boichard A, Wang H-Y, et al. 2018. Prevalence of *PDL1* amplification and preliminary response to immune checkpoint blockade in solid tumors. *JAMA Oncol.* 4:1237–44
176. Ali HR, Glont S-E, Blows FM, Provenzano E, Dawson S-J, et al. 2015. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. *Ann. Oncol.* 26(7):1488–93
177. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, et al. 2016. Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N. Engl. J. Med.* 375(9):819–29
178. Motter AE, Gulbahce N, Almaas E, Barabási A-L. 2008. Predicting synthetic rescues in metabolic networks. *Mol. Syst. Biol.* 4:168
179. Oudard S, Rixe O, Beuselinck B, Linassier C, Banu E, et al. 2011. A phase II study of the cancer vaccine TG4010 alone and in combination with cytokines in patients with metastatic renal clear-cell carcinoma: clinical and immunological findings. *Cancer Immunol. Immunother.* 60(2):261–71
180. Durant ST, Zheng L, Wang Y, Chen K, Zhang L, et al. 2018. The brain-penetrant clinical ATM inhibitor AZD1390 radiosensitizes and improves survival of preclinical brain tumor models. *Sci. Adv.* 4(6):eaat1719
181. Eschrich SA, Fulp WJ, Pawitan Y, Foekens JA, Smid M, et al. 2012. Validation of a radiosensitivity molecular signature in breast cancer. *Clin. Cancer Res.* 18(18):5134–43
182. Zhao SG, Shilkrut M, Speers C, Liu M, Wilder-Romans K, et al. 2015. Development and validation of a novel platform-independent metastasis signature in human breast cancer. *PLOS ONE* 10(5):e0126631
183. Williams NL, Dan T, Zaorsky NG, Garg S, Den RB. 2017. The role of genomic techniques in predicting response to radiation therapy. *Oncology* 31(7):562–70
184. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, et al. 2006. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N. Engl. J. Med.* 354(6):567–78

Annual Review of Biochemistry

Volume 88, 2019

Contents

Errata

An online log of corrections to *Annual Review of Biochemistry* articles may be found at http://www.annualreviews.org/errata/biochem