



## Clinical cancer genomic profiling

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**Abstract** | Technological innovation and rapid reduction in sequencing costs have enabled the genomic profiling of hundreds of cancer-associated genes as a component of routine cancer care. Tumour genomic profiling can refine cancer subtype classification, identify which patients are most likely to benefit from systemic therapies and screen for germline variants that influence heritable cancer risk. Here, we discuss ongoing efforts to enhance the clinical utility of tumour genomic profiling by integrating tumour and germline analyses, characterizing allelic context and identifying mutational signatures that influence therapy response. We also discuss the potential clinical utility of more comprehensive whole-genome and whole-transcriptome sequencing and ultra-sensitive cell-free DNA profiling platforms, which allow for minimally invasive, serial analyses of tumour-derived DNA in blood.

### Precision oncology

The process of using molecular data from the analysis of a patient's tumour or healthy cells to inform treatment selection.

### Companion diagnostics

Within the context of precision oncology, companion diagnostics are medical devices designed to identify the subset of patients most likely to respond to and benefit from a targeted or other systemic or local therapy.

**Next-generation sequencing (NGS).** Massively parallel high-throughput sequencing methods designed to analyse DNA or RNA more rapidly and at higher resolution than older methods such as Sanger sequencing.

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Cancer is a genetic disease resulting from the accumulation of mutations in genes that regulate cell division, survival, invasion or other hallmarks of the transformed phenotype. Some cancers are indolent and remain latent and localized for years, whereas others rapidly invade nearby organs or metastasize to distant sites. Researchers have long sought to understand the biological basis of this variability of clinical outcomes by subclassifying cancers into increasingly small but more phenotypically uniform subtypes. Historically, tumour classification was based primarily on cell type or tissue of origin and morphological characteristics, in particular, histological appearance under light microscopy<sup>1</sup>. Greater insight into the molecular pathophysiology of cancer has prompted the adoption of molecular classification schemes that integrate genomic information with clinical characteristics to better predict an individual patient's risk of recurrence or cancer-specific death<sup>2</sup>. As the likelihood of response to cytotoxic<sup>3</sup>, immune<sup>4</sup> and targeted therapies<sup>5–7</sup> often varies as a function of molecular tumour subtype, accurate tumour classification is crucial to ensure optimal treatment selection.

For some cancer subtypes, a pathognomonic genomic alteration is both a driver of tumour initiation and a potential therapeutic vulnerability. For example, almost all chronic myelogenous leukaemias (CMLs) have a translocation (the Philadelphia chromosome) involving the *ABL1* tyrosine kinase gene on chromosome 9 and the breakpoint cluster region (*BCR*) on chromosome 22 (REFS<sup>8,9</sup>). The resulting *BCR-ABL* translocation is constitutively active, and drugs such as imatinib that selectively inhibit *ABL1* are highly effective in patients with CML<sup>10</sup>. The success of imatinib in patients with CML generated widespread hope — which some would later characterize as hype<sup>11</sup> — that adoption of clinical tumour genomic profiling would quickly enable

the development of more effective and less toxic personalized treatment strategies for all patients with cancer<sup>12</sup>. Although progress has been slower and more incremental than many predicted, lung cancer quickly emerged as a cancer type in which precision oncology has been transformative (BOX 1). A diversity of targetable molecular alterations, including *EGFR* and *BRAF* mutations and *ALK*, *ROS1* and *RET* fusions, have a central role in lung cancer pathogenesis. As tumours harbouring these molecular drivers are often indistinguishable under light microscopy<sup>13</sup>, clinical tumour genomic profiling is now broadly viewed as necessary by oncologists to ensure optimal therapy selection in patients with advanced lung cancer.

The expansion in the number of therapeutically actionable genes in lung and other cancer types exposed the limitations of single-analyte companion diagnostics, which were historically co-developed with new targeted therapies<sup>14–16</sup>. In the early days of precision oncology, most companion diagnostic tests could detect only a single mutation, such as *BRAF* p.Val600Glu (*BRAF*-V600E), or a single gene fusion, such as *EML4-ALK*. The proliferation of clinically validated and investigational drug targets, and more recently tumour-agnostic biomarkers of drug response, and the limited tumour tissue available for analysis for many patients with cancer prompted the development of multiplexed diagnostic assays (initially based on mass spectrometry or PCR technology) that could define the mutational status of several dozen cancer-associated genes in a single reaction<sup>17–19</sup>. The more recent development of next-generation sequencing (NGS)-based platforms has made feasible the concurrent analysis of hundreds of genes or even the entire genome using small quantities of tumour tissue collected by needle biopsy<sup>20–23</sup> or DNA shed from tumour cells into plasma, so-called cell-free DNA (cfDNA)<sup>24,25</sup>.

**Box 1 | Lung cancer as a model for precision oncology**

The epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors gefitinib and erlotinib were the first targeted therapies to receive US Food and Drug Administration (FDA) authorization for the treatment of patients with lung cancer<sup>187,188</sup>. EGFR inhibitors were initially tested in a tumour- and mutation-agnostic manner, and, while limited clinical activity was noted in most cancer types, rapid symptomatic relief and dramatic tumour regressions were observed in a minority (15–20%) of patients with lung cancer. Responses were more common in never-smokers and in Asian women, suggesting that underlying differences in disease pathogenesis were the likely basis for the variable responses to EGFR inhibitors among patients with lung cancer<sup>189,190</sup>. Retrospective studies later revealed that the vast majority of responders had somatic gain-of-function mutations in the EGFR gene that induce constitutive EGFR activation and oncogene dependence<sup>191–193</sup>.

As the preclinical development of EGFR tyrosine kinase inhibitors pre-dated large-scale sequencing initiatives such as The Cancer Genome Atlas (TCGA), which have since defined the genomic landscape of most cancer subtypes, it was not known before the initial clinical studies of gefitinib and erlotinib that activating mutations in EGFR were common in lung cancer. Indeed, EGFR inhibitors initially received FDA approval for the treatment of all patients with non-small-cell lung cancer (NSCLC) irrespective of EGFR mutational status, and for several years thereafter the clinical utility of EGFR mutational testing was an area of active debate and disagreement<sup>194</sup>. Given the limited treatment options available at the time for patients with metastatic lung cancer and the possibility that EGFR overexpression or ligand-driven EGFR kinase activation could also confer EGFR dependence and drug sensitivity, many clinicians advocated treatment of all patients with metastatic NSCLC with EGFR tyrosine kinase inhibitors irrespective of EGFR mutational status.

Over the past decade, several clinical and laboratory observations have led to the recommendation that clinical tumour genomic profiling is necessary for all patients with locally advanced and metastatic lung cancer to guide treatment selection. First, the subsequent identification and clinical validation of additional targetable oncogenic alterations in ALK<sup>195</sup>, ROS1 (REF. <sup>196</sup>), RET<sup>197</sup>, BRAF<sup>198</sup>, ERBB2 (REFS <sup>199,200</sup>) and MET<sup>201,202</sup> in a largely mutually exclusive pattern in patients with lung cancer suggested that these and other not yet druggable oncogenic alterations, such as KRAS mutations, delineated distinct subsets of patients with lung cancer whose prognosis, clinical characteristics and response to therapy were dictated in part by the presence or absence of these recurrent genomic alterations<sup>196,203</sup>. Second, clinical trials in patients with lung and colorectal cancer suggested that EGFR-targeted therapies were not only ineffective but could be harmful to patients with EGFR wild-type tumours owing to their toxicities or drug-induced acceleration of tumour growth<sup>85,188,204,205</sup>. The latter possibility, while initially deemed unlikely by some, was supported by concurrent studies indicating that RAF inhibitors could paradoxically activate MAPK pathway signalling in BRAF wild-type cells and accelerate the growth of occult skin and other BRAF wild-type cancers<sup>206–209</sup>. As the tumour type with the largest number of genes for which targeted therapies have been US Food and Drug Administration (FDA) approved, the need to identify multiple lung cancer-specific and tumour-agnostic biomarkers of drug response in large numbers of patients with lung cancer has prompted the development and clinical adoption of larger next-generation sequencing-based tumour and cell-free DNA sequencing panels.

In turn, the modest incremental cost of adding additional cancer genes to NGS-based diagnostic panels has made the development of drugs targeting increasingly smaller and molecularly defined subsets of patients with cancer logically and financially feasible. The efficient clinical development of inhibitors effective in cancers driven by rare genomic mutations required the concurrent development of novel clinical trial designs such as basket trials, a study design in which eligibility is based on mutational status instead of organ site<sup>26–28</sup>. With the subsequent validation in basket studies of tumour-agnostic biomarkers of drug response, such as microsatellite instability<sup>29</sup> and NTRK gene fusions<sup>30</sup>, many oncologists now believe that NGS-based tumour genomic profiling should be offered to all patients with cancer who are not candidates for curative-intent local or systemic therapy.

**Cell-free DNA**

(cfDNA). In the context of this article, circulating tumour DNA, that is, DNA fragments shed by the tumour into the blood.

**Basket trials**

A clinical trial design that prospectively accrues patients with a specific molecular alteration irrespective of tumour type.

In this Review, we discuss the current landscape of clinical actionability in precision oncology and the challenges of variant interpretation that have arisen as a result of the rapid and widespread adoption of larger panel NGS-based diagnostic platforms. The complementary use of clinical tumour NGS to assist with not only therapy selection but also cancer subtype diagnosis and the assessment of heritable cancer risk are summarized in FIG. 1. Many of the technical challenges posed by the use of clinical samples for NGS-based tumour genomic profiling have been discussed elsewhere<sup>31</sup>, thus we focus here on ongoing efforts to derive greater clinical value from tumour genomic profiling data through more robust evidence-based variant classification, integration of tumour and germline sequencing and the identification of allelic configurations and mutational signatures predictive of drug response. Finally, we review the potential future role for more comprehensive diagnostic platforms such as whole-genome and RNA sequencing and ultra-sensitive cfDNA profiling platforms that can noninvasively monitor tumour recurrence and identify adaptive changes that mediate drug resistance.

**DNA mutations as biomarkers of therapy response**

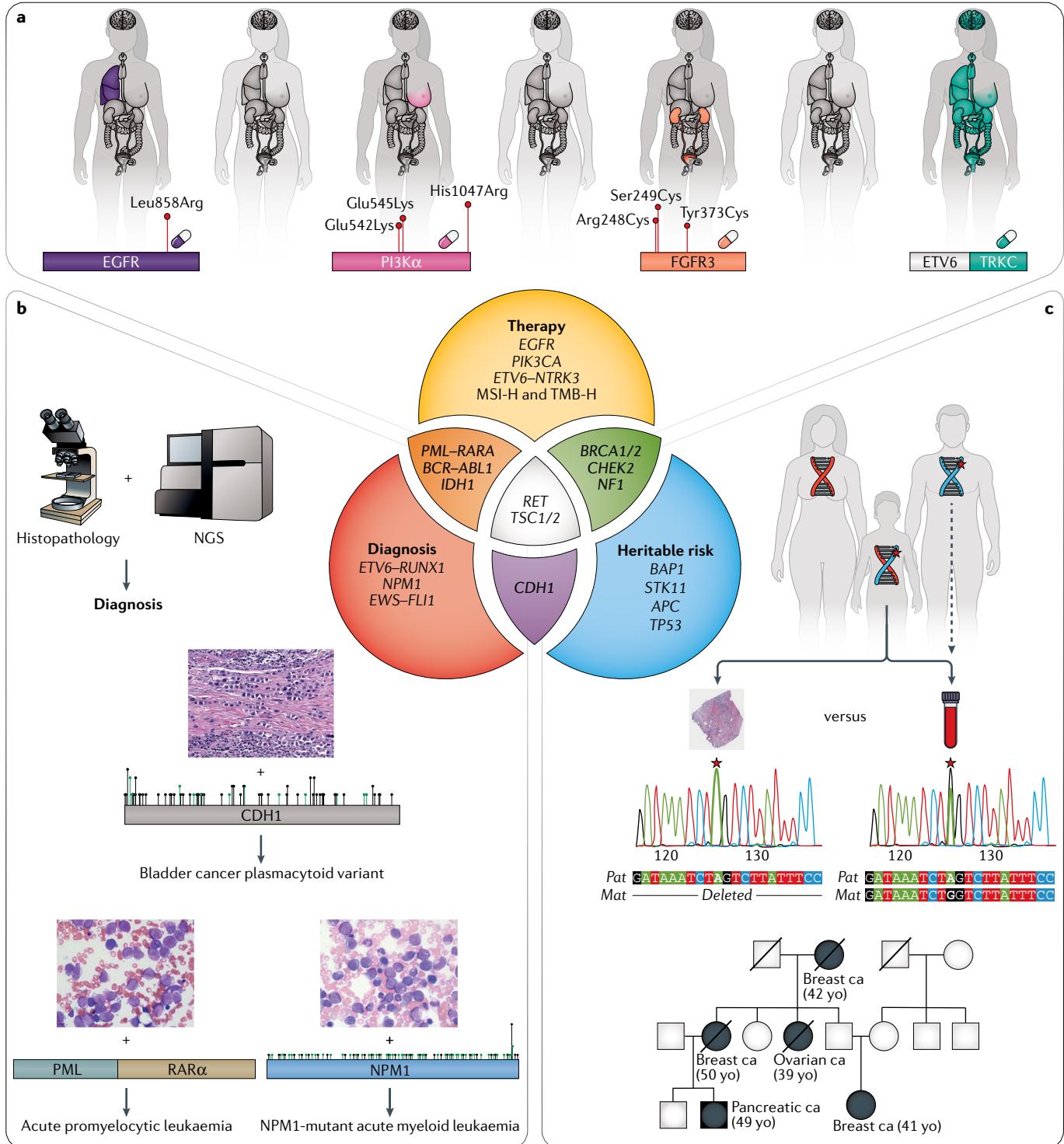
Not all mutations in the same gene have the same biological properties and the same clinical implications. Somatic mutations are subclassified into those that are oncogenic (drivers) versus those that are biologically inert (passengers)<sup>32,33</sup>. Among the drivers, a subset are predictive biomarkers of drug response or so-called clinically actionable mutations. The success of precision oncology strategies requires clinicians to distinguish clinically actionable molecular alterations from benign variants and understand how differences in the biological properties of individual mutant alleles influence treatment response and patient outcomes.

Currently, most mutations that are clinically actionable are the targets of small-molecule kinase inhibitors or antibodies that bind to cell surface receptors. Actionable mutations may also increase sensitivity to drugs that function through a synthetic lethal mechanism such as poly(ADP-ribose) polymerase (PARP) inhibitors, which are most effective in tumours with loss-of-function mutations in genes that mediate homologous recombination-based DNA repair such as *BRCA1* and *BRCA2* (REFS <sup>34,35</sup>). Gene mutations in DNA repair pathways have also been shown to be predictive of response to cytotoxic chemotherapies<sup>36,37</sup> and immunotherapy<sup>29,38</sup>.

**Challenges of variant interpretation.** A major hurdle to the broader adoption of precision oncology is the difficulty in distinguishing functional from benign variants, even in well-studied cancer genes. In the case of oncogenes, functional mutations typically enhance the enzymatic activity or induce oncoprotein signalling through dimerization or as a result of altered affinity for and activation of downstream effectors. As only a small subset of the potential mutations in an oncogene are capable of inducing activation via these mechanisms, population-based studies have been used to identify likely gain-of-function mutations based on their recurrence, so-called mutational hotspots<sup>39–42</sup>. Although mutational recurrence

within a population is typically a result of positive selection for a functional phenotype, non-functional recurrent variants ('passenger hotspots') can arise at

inherently mutable sites, for example, APOBEC3A-mediated deamination of cytosine within DNA short hairpin loops<sup>42,43</sup>.



**Fig. 1 | Clinical applications of tumour sequencing.** Tumour profiling can contribute to patient care by identifying mutations or structural variants that are predictive biomarkers of drug response (part a), assist with cancer subtype diagnosis (part b), or confer increased heritable cancer risk (part c). As highlighted in part a, predictive biomarkers of drug response can be either tumour subtype specific or tumour agnostic. Some somatic alterations, such as the BCR-ABL1 translocation, are both diagnostic for a cancer subtype and predictive biomarkers of drug response. A subset of

germline mutations, such as inactivating mutations in the tumour suppressor genes *BRCA1* and *BRCA2*, are both predictive of drug response and associated with increased heritable risk. Germline mutations of *CDH1* are associated with increased risk of developing hereditary diffuse gastric cancer, whereas somatic *CDH1* mutations are pathognomonic of the plasmacytoid variant subtype of bladder cancer. EGFR, epidermal growth factor receptor; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; TMB-H, tumour mutational burden-high; yo, years old.

**Mutational signatures**

Patterns of base pair substitutions or structural abnormalities that are often characteristic of exogenous or endogenous mutational processes (such as tobacco smoking or DNA repair pathway mutations).

**Somatic mutations**

Non-heritable mutations that may arise in any cell except germ cells (sperm or ova). Although somatic mutations have classically been defined as those found specifically in tumour but not in healthy cells, accumulation of somatic mutations is common in non-transformed, histologically normal-appearing cells as patients age.

**Drivers**

Mutations that enhance tumour cell fitness by providing a growth or survival advantage.

**Passengers**

Biologically inert mutations with no impact on tumour cell fitness.

**Clinically actionable mutations**

A subset of driver mutations that are predictive biomarkers of drug response or resistance.

**Synthetic lethal mechanism**

An interaction between two genes whereby loss of function of both (due to mutation, epigenetic silencing or drug inhibition) results in tumour cell death, whereas loss of function or inhibition of either individual gene does not.

**Mutational hotspots**

Mutations identified in a population of patients with cancer more frequently than expected by chance.

**Germline variants**

Heritable mutations that were present in germ cells and consequently found in all cells of the descendants.

For tumour suppressor genes, protein-truncating variants (frameshift, nonsense or splice site mutations) are often presumed to be oncogenic (classified as ‘likely oncogenic’) based on their ability to promote nonsense-mediated mRNA decay<sup>44</sup>. Oncogenic and likely oncogenic mutations are therefore typically distributed throughout the coding sequence of tumour suppressor genes, making computational inferences such as recurrence less useful for distinguishing between functional and benign variants.

Neomorphic mutations confer novel phenotypes that do not simply potentiate or impair wild-type protein function. Therefore, failure to observe a canonical phenotype in laboratory-based biological studies does not always signify that a mutation is a benign variant. For example, while a primary function of the *PIK3R1*-encoded p85α subunit of PI3 kinase is to regulate the p110α catalytic product of the *PIK3CA* locus, the mutant protein arising from a common recurrent *PIK3R1*-truncating mutation (p.Arg348\*) is unable to bind to p110α. Instead, it augments cell survival by acting as a scaffold for signalling intermediaries within the JNK pathway, resulting in increased JNK and MAPK pathway activity<sup>45,46</sup>.

Gain-of-function mutations in the same oncogene can also have distinct biological properties that result in differences in drug sensitivity. Mutant allele-specific drug sensitivities may be due to differences in drug binding affinity or, in the case of *BRAF*, differences in the ability of the mutant to promote *BRAF* dimer formation<sup>47</sup>. Further complicating the task of variant classification, mutations can have different phenotypes depending on tumour lineage or co-mutational context<sup>48</sup>. For example, laboratory studies indicate that the RAF inhibitor vemurafenib potently inhibits the *BRAF*-V600E mutant protein in cell-free assays<sup>49</sup>. However, the likelihood that a patient with a *BRAF*-V600E mutant tumour will respond to vemurafenib is strongly influenced by tumour type. Whereas the majority of patients with melanoma and histiocytosis respond to vemurafenib, RAF inhibitor monotherapy has limited clinical activity in patients with colorectal cancer<sup>5,50</sup> owing to intrinsic RAF inhibitor resistance mediated by EGFR-activated RAF dimer formation. This biological insight was the molecular basis for combination trials of the anti-EGFR antibody cetuximab and the RAF inhibitor encorafenib, which was FDA approved for patients with *BRAF*-V600E mutant colorectal cancer in April 2020 (REF.<sup>51</sup>). In sum, despite large-scale efforts over the past several decades to define the biological properties of cancer mutations as a guide to clinical interpretation, many if not most somatic mutations identified by clinical tumour genomic profiling are variants of unknown biological and clinical significance. This remains true even for extensively studied cancer genes such as *BRAF*, for which targeted inhibitors are approved for clinical use by the FDA.

The recognition that different mutations within the same gene often have different biological properties has made it challenging for point-of-care clinicians to interpret the large number of somatic and germline variants that emerge from clinical tumour genomic profiling<sup>52,53</sup>.

Of particular concern is that patients may be treated with the wrong therapy based on a failure by clinicians to recognize that certain mutations in an actionable cancer gene are either inert or intrinsically resistant to the drug chosen. For example, while many *EGFR* exon 20 mutations are oncogenic, they are typically insensitive to the FDA-approved EGFR inhibitors erlotinib and osimertinib<sup>54</sup>. The challenge of clinical variant interpretation has prompted the development of physician support tools such as OncoKB<sup>55</sup>, CIViC (Clinical Interpretation of Variants in Cancer)<sup>56</sup>, the Jackson Laboratory Clinical Knowledgebase (JAX-CKB)<sup>57</sup>, the Precision Medicine Knowledge Base (PMKB)<sup>58</sup>, the Cancer Genome Interpreter Cancer Biomarkers database (CGI)<sup>59</sup> and Personalized Cancer Therapy (PCT) resource<sup>60</sup>, which seek to catalogue the known biological properties and clinical implications of individual mutant alleles. As our understanding of the clinical implications of individual mutations is constantly evolving, and as new investigational drugs enter clinical testing and are either approved by regulatory agencies or fail to show efficacy in genetically defined populations, these variant interpretation tools need to be continuously updated to remain comprehensive and accurate through expert curation, crowdsourcing or a combination of the two.

**The current landscape of clinical actionability.** A long-standing criticism of the precision oncology field is that its proponents often overstate the clinical actionability of individual genes or genomic variants. Mutations that are clinically validated and FDA-recognized as predictive biomarkers of drug response are often grouped together as clinically actionable with mutations identified as the putative basis for outlier exceptional responses or variants that correlate with enhanced sensitivity in cell-based screens<sup>61,62</sup>. To better communicate the strength of evidence supporting the clinical actionability of individual mutant alleles, many variant knowledge bases stratify genomic alterations based on the level of clinical and/or biological data supporting their use as predictive biomarkers of drug response or resistance. Optimally, these databases should also incorporate information as to how lineage and co-mutational context influence the likelihood of clinical response.

As an example, in the OncoKB knowledge base, level 1 and 2 mutations are those variants established through clinical experience (preferably prospective randomized clinical trials) to be clinically validated predictive biomarkers of drug response to FDA-approved therapies in a specific cancer subtype (TABLE 1). Lower levels are assigned to mutations with compelling but not yet definitive clinical evidence (level 3) or only laboratory evidence (level 4) that the mutation is predictive of drug sensitivity<sup>55</sup>. As different mutations in the same gene can have different phenotypes, individual variants in cancer-associated genes can be assigned to different OncoKB levels. Furthermore, as tumour lineage often influences the likelihood of drug response, the same mutation can be assigned to different OncoKB levels in different cancer types.

In contrast to the germline setting, where interpretation of germline variant pathogenicity has been

Table 1 | OncoKB standard-care biomarkers

Genetic biomarker	Indication	Targeted therapy
<i>Level of evidence 1</i>		
BCR-ABL1 fusion	ALL, CML	Imatinib, dasatinib, bosutinib, nilotinib
ABL1 Thr315Ile		Ponatinib
ALK fusions	NSCLC	Crizotinib, ceritinib, alectinib
ALK fusions and oncogenic mutations	NSCLC	Brigatinib, lorlatinib
ATM, BARD1, BRIP1, CDK12, CHEK1/CHEK2, FANCL, PALB2, RAD51B/RAD51C/RAD51D, RAD54L loss-of-function mutations	Prostate cancer	Olaparib
BRCA1/BRCA2 loss-of-function mutations	Ovarian cancer, peritoneal serous carcinoma, prostate cancer	Olaparib, rucaparib, niraparib <sup>a</sup>
BRAF Val600Glu	Anaplastic thyroid cancer, NSCLC	Dabrafenib + trametinib
	Colorectal cancer	Encorafenib + cetuximab
BRAF Val600Glu/Lys	Melanoma	Dabrafenib + trametinib, encorafenib + binimetinib, vemurafenib + cobimetinib, vemurafenib + c obimetinib + atezolizumab
BRAF Val600	Erdheim–Chester disease	Vemurafenib
EGFR exon 19 deletions, EGFR Leu858Arg	NSCLC	Erlotinib, gefitinib, afatinib, dacomitinib, osimertinib
EGFR Gly719, Leu861Gln, Ser768Ile		Afatinib
EGFR Thr790Met		Osimertinib
ERBB2 amplification	Breast cancer	Trastuzumab ± chemotherapy ± pertuzumab/capecitabine + tucatinib, ado-trastuzumab emtansine, lapatinib + letrozole/capecitabine, neratinib ± capecitabine, fam-trastuzumab deruxtecan-nxki
	Oesophagogastric cancer	Trastuzumab, fam-trastuzumab deruxtecan-nxki
EZH2 Ala692Val, Tyr646Cys/Phe/Hys/Asn/Ser	Follicular lymphoma	Tazemetostat
FGFR2 fusions	Cholangiocarcinoma	Pemigatinib
FGFR2 fusions and FGFR3 fusions and hotspots (FGFR3 Gly370Cys, Arg248Cys, Ser249Cys, Tyr373Cys)	Bladder cancer	Erdafitinib
FLT3 internal tandem duplication and FLT3 oncogenic mutants, including Asp835 and Ile836	Acute myeloid leukaemia	Gilteritinib
IDH1/IDH2 oncogenic mutations	Bladder cancer	Ivosidenib, enasidenib
KIT exons 11, 9, 17, KIT Val654Ala and KIT Thr670Ile oncogenic mutations	Gastrointestinal stromal tumours	Imatinib, sunitinib, regorafenib, ripretinib
KRAS wild-type	Colorectal cancer	Cetuximab, panitumumab, regorafenib
MET exon 14 splice/exon-skipping mutations	NSCLC	Capmatinib
NF1 loss-of-function mutations	Neurofibroma	Selumetinib
MSI-H/TMB-H	All solid tumours	Pembrolizumab
MSI-H	Colorectal cancer	Ipilimumab + nivolumab, nivolumab
NTRK1/NTRK2/NTRK3 fusions	All solid tumours	Larotrectinib, entrectinib
COL1A1-PDGFB fusion	Dermatofibrosarcoma protuberans	Imatinib
FIP1L1-PDGFRα fusion	CEL-NOS	Imatinib
PDGFRα/PDGFRβ fusions	Myelodysplastic/myeloproliferative neoplasms	Imatinib

Table 1 (cont.) | OncoKB standard-care biomarkers

Genetic biomarker	Indication	Targeted therapy
<i>Level of evidence 1 (cont.)</i>		
PDGFR $\alpha$ Asp842Val, Asp842Tyr, Asp842_Hys845del, Asp842_Hys845insVal	Gastrointestinal stromal tumour	Avapritinib
PIK3CA oncogenic mutations	ER $^+$ /HER2 $^-$ breast cancer	Alpelisib + fulvestrant
RET fusions	NSCLC	Selpercatinib, pralsetinib
	Thyroid cancer	
RET oncogenic mutations	Medullary thyroid cancer	Selpercatinib, pralsetinib
ROS1 fusions	NSCLC	Crizotinib, entrectinib
SMARCB1 loss-of-function mutations	Epithelioid sarcoma	Tazemetostat
TSC1/TSC2 loss-of-function mutations	Subependymal giant cell astrocytomas	Everolimus
<i>Level of evidence 2</i>		
BCR-ABL1 fusion	ALL	Bosutinib, nilotinib
ABL1 Glu255Lys, Glu255Val, Phe317Cys, Phe317Ile, Phe317Leu, Phe317Val, Phe359Cys, Phe359Ile, Phe359Val, Thr315Ala, Tyr253His	ALL, CML	Bosutinib, dasatinib, nilotinib
ALK fusions	Inflammatory myofibroblastic tumour	Crizotinib, ceritinib
BRAF Val600Glu	Ganglioglioma, hairy cell leukaemia, pilocytic astrocytoma, pleomorphic xanthoastrocytoma	Cobimetinib + vemurafenib, trametinib + dabrafenib, vemurafenib
BRCA1/BRCA2 loss-of-function mutations	Breast cancer	Olaparib, talazoparib
CDK4 amplification	Dedifferentiated liposarcoma, well-differentiated liposarcoma	Palbociclib, abemaciclib
EGFR Ala763_Tyr764insPheGlnGluAla <sup>b</sup>	NSCLC	Erlotinib
ERBB2 amplification	Colorectal cancer	Pertuzumab + trastuzumab, lapatinib + trastuzumab
	Uterine serous carcinoma/uterine papillary serous carcinoma	Trastuzumab + carboplatin–taxol regimen
ERBB2 oncogenic mutations	NSCLC	Ado-trastuzumab emtansine
KIT exon 17 oncogenic mutations	Gastrointestinal stromal tumour	Sorafenib
KIT oncogenic mutations	Melanoma	Imatinib, sunitinib
MET exon 14 splice mutations, amplification	NSCLC	Crizotinib
MET amplification	Renal cell carcinoma	Cabozantinib
PDGFR $\alpha$ oncogenic mutations, PDGFR $\alpha$ Asp842Val	Gastrointestinal stromal tumour	Imatinib, dasatinib
RET fusions	NSCLC	Cabozantinib

ALL, B cell acute lymphoblastic leukaemia/lymphoma; CEL-NOS, chronic eosinophilic leukaemia, not otherwise specified; CML, chronic myelogenous leukaemia; EGFR, epidermal growth factor receptor; MSI-H, microsatellite instability-high; NSCLC, non-small-cell lung cancer; TMB-H, tumour mutational burden-high. <sup>a</sup>FDA approval of this drug is mutation-agnostic. <sup>b</sup>While most EGFR exon 20 insertions are resistant to FDA-approved EGFR inhibitors, Ala763\_Tyr764insPheGlnGluAla is sensitive to erlotinib.

standardized through a joint effort of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)<sup>63</sup>, to date there is no broad consensus among knowledge bases and regulators on how best to define clinical actionability of somatic variants. To address this issue, the AMP, the American Society for Clinical Oncology (ASCO)<sup>64</sup> and the European Society of Medical Oncology (ESMO) have

created working groups to develop guidelines on how best to classify the clinical and biological relevance of genomic variants identified by clinical tumour genomic profiling assays<sup>65–67</sup>. Additionally, cross-institutional consortia such as the Variant Interpretation Cancer Consortium (VICC)<sup>67</sup> and the somatic variant working group of ClinGen<sup>68</sup> have been formed to standardize best practices and define vocabulary that in the future could

**Resistance mutations**  
Mutations that increase tumour cell fitness under the selective pressure of a systemic therapy.

#### Penetrance

A measure of the proportion of individuals with a mutant allele in a defined population who manifest the associated phenotype. For germline variants associated with increased cancer predisposition, the penetrance is the proportion of patients who develop the associated cancer type.

guide FDA recognition of variant databases as physician support tools<sup>69</sup>.

To estimate the current fraction of patients with cancer for whom tumour genomic profiling would be predicted to directly influence therapy selection, we analysed a cohort of 52,069 solid tumour samples sequenced as of November 2020 at Memorial Sloan Kettering (MSK) Cancer Center using the MSK-IMPACT clinical NGS platform<sup>20</sup>. In this prospective dataset, 92% of tumours harboured at least one oncogenic mutation, and 34% harboured at least one mutation classified as a predictive biomarker of response to an FDA-approved or investigational drug based on compelling clinical data (OncokB, levels 1–3A)<sup>55</sup> (FIG. 2a,b).

Although there has been significant focus on the use of broad-panel NGS to identify investigational biomarkers of drug response, the increasingly widespread adoption of NGS-based clinical tumour profiling has been driven primarily by the need to identify somatic and germline mutations that are predictive biomarkers of response to FDA-approved therapies. Since 2017, there has been an approximately threefold increase from 9%<sup>20</sup> to 30% in the fraction of tumours for which a disease-matched, standard-care predictive biomarker of response to an FDA-approved therapy would have been identified by tumour NGS, defined as OncokB levels 1 and 2 (FIG. 2b). This increase in standard-care clinical actionability is partially the result of recent FDA approvals of first-in-class selective inhibitors of IDH2 (acute myeloid leukaemia (AML), 2017 (REFS<sup>70,71</sup>)), IDH1 (AML, 2018 (REF.<sup>72</sup>)), TRKA/TRKB/TRKC (tumour-agnostic, 2018 (REF.<sup>6</sup>)), PI3Kα (breast cancer, 2019 (REF.<sup>73</sup>)), FGFR3 (bladder cancer, 2019 (REF.<sup>74</sup>)), FGFR2 (cholangiocarcinoma, 2020 (REF.<sup>75</sup>)) and RET (lung and thyroid cancer, 2020 (REFS<sup>76,77</sup>)). The landscape of clinical actionability has also been expanded through FDA approvals of additional indications for PARP inhibitors for breast cancer (in 2018 (REF.<sup>78</sup>)), pancreatic cancer (in 2019 (REF.<sup>79</sup>)) and prostate cancer (in 2020 (REFS<sup>35,80</sup>)) and RAF and MEK inhibitors for lung cancer (in 2017 (REF.<sup>81</sup>)), Erdheim–Chester disease (in 2017 (REF.<sup>82</sup>)), anaplastic thyroid cancer (in 2018 (REF.<sup>83</sup>)) and colorectal cancer (in combination with the anti-EGFR antibody cetuximab, in 2018 (REF.<sup>50</sup>)).

Additionally, three classes of molecular alterations (*NTRK1/NTRK2/NTRK3* fusions, microsatellite instability-high/deficient mismatch repair (MSI-H/dMMR) and tumour mutational burden-high (TMB-H, defined as ≥10 mutations per megabase of DNA)) are now recognized by the FDA as tumour-agnostic biomarkers of drug response. As at least one of these tumour-agnostic predictive biomarkers has been reported in essentially all cancer subtypes, tumour molecular profiling is now viewed by most oncologists as a clinical necessity in all patients with metastatic cancer who require systemic therapy<sup>6,84</sup>.

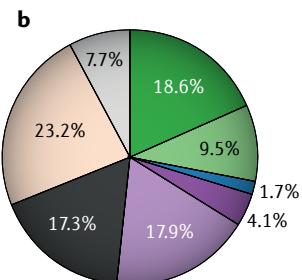
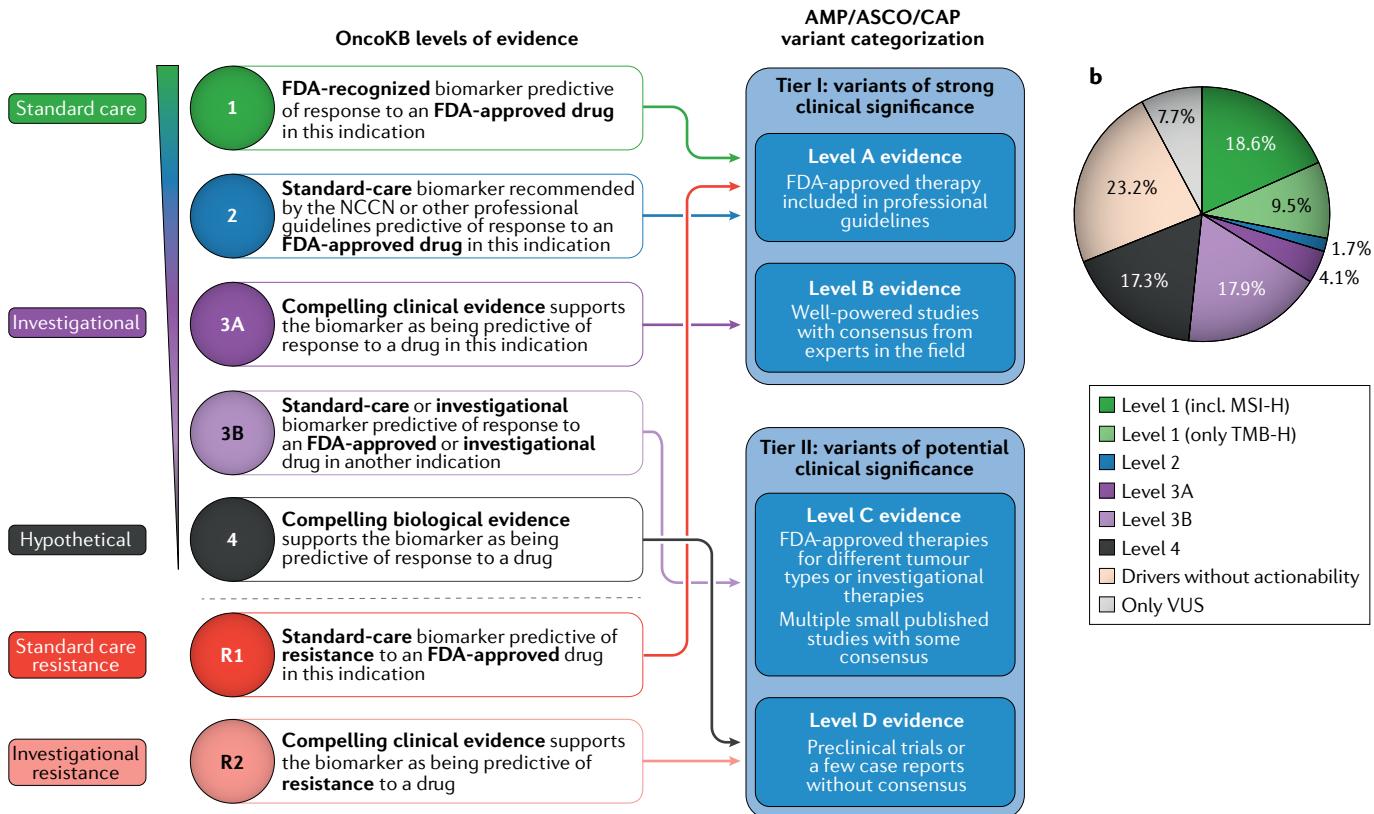
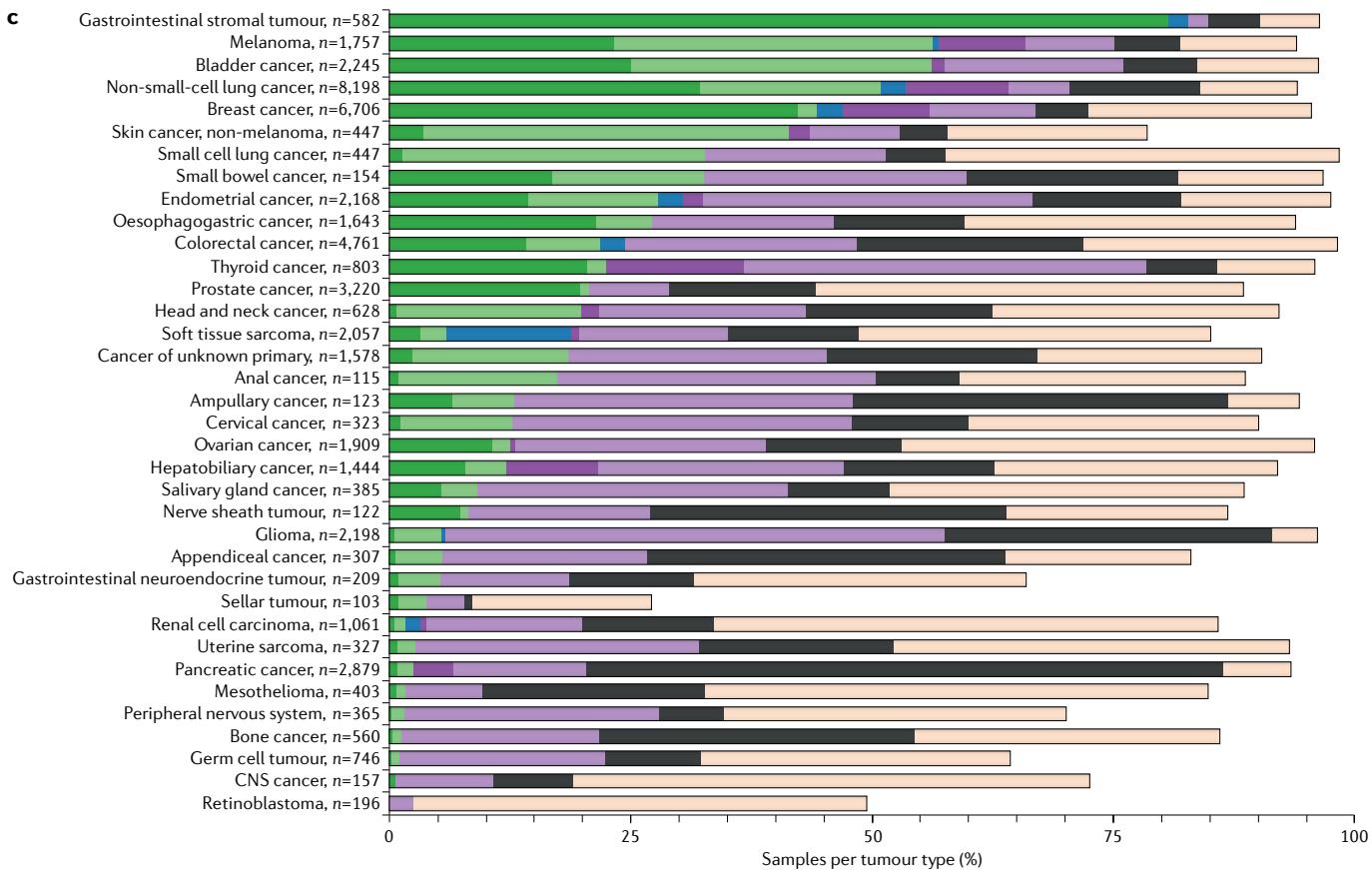
Although the recent FDA approvals of additional genotype-directed therapies represent clear progress, the likelihood of identifying a clinically actionable genomic alteration remains highly tumour-type dependent, ranging from >80% in gastrointestinal stromal tumours (GIST), and 64% in non-small-cell lung cancer, to <10%

in gliomas, pancreatic cancers and mesotheliomas (FIG. 2c). One could also argue that the recent increase in standard-care actionability overstates to some degree the benefits of tumour profiling by NGS, as many TMB-H tumours arise in patients with tumour types for which immune checkpoint blockade is clinically indicated irrespective of MSI or TMB status (FIG. 2c; BOX 2). However, tumour profiling with NGS can also benefit patients by identifying mutations predictive of drug resistance (so-called resistance mutations) such as *KRAS* and *BRAF* mutations, which are associated with intrinsic resistance to standard-care anti-EGFR antibodies in patients with colorectal cancer<sup>85,86</sup>. Clinical tumour genomic profiling can therefore benefit patients by allowing them to avoid potentially toxic therapies unlikely to result in clinical benefit based on their specific tumour molecular profile.

In the near term, the most substantial barrier to the broader success of precision oncology paradigms is not the limited number of genes included in current NGS-based panels but the large number of ‘undruggable’ oncogenic mutations identified by tumour genomic profiling. The prospective MSK-IMPACT experience indicates that undruggable mitogenic drivers are common in patients who lack clinically actionable alterations. Examples of oncogenic mutations that are not currently actionable include those in *NFI*, *PTEN*, *RB1*, *STK11* and *KEAP1* (REFS<sup>20,66</sup>). For some undruggable drivers, downstream effectors are potentially targetable (for example, the use of MEK inhibitors in *KRAS*-mutant tumours). Drugs targeting downstream effectors do not, however, typically demonstrate the levels of clinical efficacy of drugs that directly inhibit the mutated oncoprotein<sup>87,88</sup>. Recent promising results with covalent inhibitors of *KRAS* p.Gly12Cys<sup>89</sup> have generated hope that novel approaches to drug development including targeted protein degraders and synthetic lethal approaches will result in a continued expansion in the landscape of clinical actionability over the next several years.

#### Integration of somatic and germline profiling

**Heritable cancer risk and pharmacogenomics.** It has long been recognized that some families and ethnic groups are at greater risk of developing particular cancers. For example, individuals of Ashkenazi Jewish descent are at increased risk of developing early-onset breast and ovarian cancer<sup>90,91</sup>. By applying segregation analysis of incidence patterns to a cohort of 1,579 patients with breast cancer, Newman et al.<sup>92</sup> were the first to show that an autosomal dominant and highly penetrant susceptibility allele was the basis for familial clustering of early-onset breast cancer cases. Positional cloning subsequently localized the first candidate breast cancer susceptibility gene, designated *BRCA1*, to chromosome 17q21 (REFS<sup>93–95</sup>). Clinical testing for pathogenic germline variants in *BRCA1* and additional high and moderate penetrance cancer predisposition genes, including *BRCA2*, *CHEK2*, *PALB2*, *ATM*, *VHL*, *BAP1* and *MSH2*, among others, is now a component of the standard management of an expanding number of cancer types<sup>96</sup>. These tests use a DNA sample derived from healthy-appearing (‘normal’) tissue, typically blood, nail clippings or a buccal swab. As with tumour genomic

**a Mapping between the OncoKB levels of evidence and the AMP/ASCO/CAP consensus recommendation****c**

◀ Fig. 2 | Current landscape of clinical actionability. **a** | OncoKB levels of evidence. Individual mutations or structural alterations are annotated based on the level of evidence that the alteration is a predictive biomarker of drug response in a specific cancer subtype (left panel). Mapping of OncoKB levels of evidence to the Association of Molecular Pathology (AMP)–American Society of Clinical Oncology (ASCO)–College of American Pathologists (CAP) evidence-based variant categorization<sup>64</sup> (right panel). **b** | Clinical actionability of solid tumour samples prospectively analysed using the MSK-IMPACT clinical next-generation sequencing tumour profiling assay ( $n=52,069$ ). Fraction of samples across all solid tumour cancer types that harbour a mutation considered clinically actionable according to the OncoKB levels of evidence (pie chart). Tumours with level 1 alterations based only on the tumour-agnostic tumour mutational burden-high (TMB-H) approval of pembrolizumab are shown in lighter green (dark green, all other level 1 tumours; blue, level 2; dark purple, level 3A; light purple, level 3B; black, level 4; peach, non-actionable oncogenic drivers only; and light grey, tumours with variants of unknown significance (VUS) only). **c** | Clinical actionability of tumour samples as a function of common solid tumour types. Similar analysis to part **b** of solid tumours in the prospective MSK-IMPACT cohort but shown as a function of cancer subtype. All MSK-IMPACT sequencing results are made available through the AACR GENIE Consortium<sup>20,66</sup>. CNS, central nervous system; MSI-H, microsatellite instability-high.

profiling assays, single-gene germline tests have gradually been replaced by multigene NGS-based panels designed to identify pathogenic germline variants in a dozen or more heritable cancer-associated genes.

Paired analysis of tumour and germline samples has long been standard for research-focused whole-exome sequencing (WES) and whole-genome sequencing (WGS) studies<sup>97</sup>. Filtering of germline variants using a patient-matched normal DNA sample allows for more accurate classification of mutations as somatic versus inherited germline variation. Tumour-only NGS-based sequencing panels — now widely used as companion diagnostic tests for therapy selection — rely on existing germline databases and computational algorithms to distinguish somatic from germline variants<sup>97</sup>. This can lead to misclassification of germline variants as somatic mutations, in particular for racial and ethnic minority patients, who are currently under-represented in germline variant databases<sup>98</sup>.

An equally important benefit of the incorporation of paired germline DNA analysis into tumour genomic profiling platforms is the opportunity to reanalyse the paired normal sample to identify pathogenic germline variants associated with increased heritable cancer risk. Paired tumour–germline genomic profiling has been shown to identify at least one pathogenic or likely pathogenic germline variant in a cancer predisposition gene in 8–18% of patients with cancer<sup>96,99,100</sup>. Of note, 25–50% of patients with cancer with pathogenic germline variants in population-based tumour genomic profiling cohorts would not have been referred for germline genetic analysis based on historical clinical guidelines<sup>96</sup>. On this basis, there is increasing support among oncologists for the testing of all patients with cancer for pathogenic germline variants to appropriately counsel patients as to their personal risk of a subsequent cancer and family members as to their risk of harbouring the same heritable cancer predisposition<sup>101,102</sup>.

Chemotherapy response and toxicity has also been linked to genetic differences in genes that have a role in the absorption or metabolism of cytotoxic and hormonal therapies<sup>103,104</sup>. As an example, genome-wide association studies (GWAS) of patients with breast cancer have

identified an association between germline variants in the genes encoding the cytochrome P450 enzymes CYP2D6 (REF.<sup>105</sup>) and CYP2C8 (REF.<sup>106</sup>) with altered tamoxifen metabolism and increased risk of paclitaxel-associated neuropathy, respectively. Similarly, germline polymorphisms in the *TYMS* gene, which encodes thymidylate synthetase, are associated with increased likelihood of response and toxicity to capecitabine and 5-fluorouracil, antimetabolites that competitively bind to and irreversibly inhibit thymidylate synthetase<sup>107</sup>. Although a lack of expert consensus regarding the clinical utility of these pharmacogenomic biomarkers has limited their clinical adoption, germline variants that influence drug metabolism or absorption could be easily incorporated into future tumour–germline paired NGS panels.

**Clonal haematopoiesis.** Clonal haematopoiesis, which is associated with increasing age and prior treatment with radiation or cytotoxic chemotherapies<sup>108</sup>, is a confounding factor for the accurate classification of somatic mutations. Clonal haematopoiesis-derived somatic mutations can be present in tumour-only sequencing data at variant allele frequencies above the thresholds typically used for somatic mutation calling. Therefore, in the absence of patient-matched germline DNA sequencing data (which is typically derived from blood), clonal haematopoiesis-derived somatic mutations can be misclassified as tumour somatic mutations<sup>109</sup>. As a subset of clonal haematopoiesis mutations are in clinically actionable cancer genes, tumour-only profiling may result in patients receiving inappropriate treatment owing to misclassification of clonal haematopoiesis-derived somatic variants as tumour-derived. In this context, incorrect treatment selection resulting from misinterpretation of clonal haematopoiesis-derived actionable mutations could include the inappropriate use of a drug (for example, a clonal haematopoiesis-derived *ATM* mutation in a patient with prostate cancer prompting the use of PARP inhibitors) or the inappropriate withholding of therapy (for example, a clonal haematopoiesis-derived *KRAS* mutation in a patient with metastatic colorectal cancer contraindicating the otherwise standard-care use of the anti-EGFR antibodies cetuximab and panitumumab). As mutational calling at lower allele frequencies is associated with a greater risk of misclassification of clonal haematopoiesis-derived mutations as tumour-derived, sequencing of paired cell-free (plasma) and white blood cell (buffy coat) samples will be of particular importance for cfDNA analyses<sup>110</sup>.

**Logistical challenges of integrated somatic–germline sequencing.** Although the historical focus of germline genetic testing was to identify variants associated with increased heritable cancer risk, the success of PARP inhibitors in patients with mutations in genes associated with homologous recombination repair has led to a convergence in the clinical need for tumour and germline genetic testing. PARP inhibitors are most effective in homologous recombination-deficient tumours, in particular, in patients with loss-of-function mutations in *BRCA1* and *BRCA2* (REF.<sup>34</sup>). As both somatic and germline *BRCA1* and *BRCA2* mutations can confer

**Whole-exome sequencing (WES).** Sequencing of all protein-coding regions (or exons) in the genome.

**Whole-genome sequencing (WGS).** Sequencing of the entire genome including non-coding sequences.

**Clonal haematopoiesis**  
The acquisition of somatic genomic alterations in haematopoietic stem and/or progenitor cells, resulting in clonal expansion.

## Box 2 | Tumour genomic profiling and immunotherapy sensitivity

Microsatellite instability-high/deficient mismatch repair (MSI-H/dMMR) and tumour mutational burden-high (TMB-H, defined as  $\geq 10$  mutations per megabase of DNA) are FDA-recognized predictive biomarkers of response to the anti-PD1 antibody pembrolizumab. Dramatic, durable responses have been observed in chemotherapy-refractory metastatic MSI-H/dMMR tumours in cancer types in which immune checkpoint inhibitors are not broadly active (for example, colorectal and prostate cancers)<sup>29</sup>. A failure to test all patients with metastatic disease for MSI-H/dMMR can therefore result in some patients not receiving effective, potentially curative, standard therapy. As next-generation sequencing (NGS)-based tumour sequencing assays can robustly detect microsatellite instability, the need to efficiently screen all patients with metastatic solid tumours for the MSI-H/dMMR phenotype to assess eligibility for immune checkpoint inhibitor therapy has been a rationale for the broader adoption of NGS-based tumour genomic profiling in patients with treatment-refractory solid tumours.

There is greater disagreement among oncologists as to the appropriateness of the tumour-agnostic approval of pembrolizumab for TMB-H tumours<sup>29,84,210</sup> as the label expansion was based largely on a single-arm study of 102 patients, in which a 29% objective response rate was observed in the TMB-H cohort<sup>211</sup>. Many of the responses were, however, durable, extending over 2 years, with many ongoing<sup>212,213</sup>. Despite its limitations as a predictive biomarker, the TMB-H label expansion provides a new and potentially transformative therapeutic option for patients with cancer types in which immune checkpoint inhibitors have yet to establish a role because response rates are low in biomarker-unselected patients (for example, prostate and pancreatic cancers<sup>214,215</sup>). Importantly, the TMB-H approval will also facilitate access to immune checkpoint inhibitor therapy for patients with rare cancers, for whom enrolment in adequately powered clinical trials is difficult. Future studies will be needed to determine whether the underlying mechanism of hypermutation influences the likelihood of immunotherapy response and to refine the optimal TMB-H cut-off, which in retrospective studies varies as a function of cancer type<sup>210</sup>.

Alterations in individual oncogenes have also been associated with greater or lesser likelihood of immune checkpoint inhibitor response. As an example, WNT pathway activation is associated with T cell exclusion and innate resistance to immune checkpoint blockade in preclinical models<sup>216,217</sup>, and clinical data suggest that WNT/β-catenin pathway mutations are a predictive biomarker of immune checkpoint inhibitor resistance in hepatocellular carcinoma<sup>218</sup>. In the phase III clinical trial testing the anti-PDL1 inhibitor atezolizumab in lung cancer, patients with ALK fusion-positive or EGFR-mutant tumours had a low likelihood of responding to immune checkpoint blockade, but this may simply reflect the low mutational burden of these molecularly defined lung cancer subtypes<sup>219,220</sup>. Host factors such as human leukocyte antigen (HLA) genotype and variations in the tumour microbiome may also influence sensitivity to immunotherapies and could potentially be assayed by NGS-based panels<sup>221,222</sup>. It has also been proposed that tumours with a viral aetiology are more likely to respond to immunotherapy<sup>223–225</sup>, and probes designed to capture viral DNA are being incorporated into newer NGS-based tumour genomic profiling assays.

Finally, whole-exome and whole-genome sequencing combined with machine learning approaches that can predict which mutated peptides bind with high-affinity to autologous HLA molecules have made possible the development of personalized cancer vaccines<sup>226,227</sup>. In such a scenario, tumour NGS functions not as a predictive biomarker of drug response but rather as the initial step in the development of personalized immunotherapies that target the neoantigens present exclusively in a patient's tumour but not healthy cells. As a more off-the-shelf alternative, T cell-based therapies are in development that target shared neoantigens that arise from recurrent mutations in commonly mutated oncogenes<sup>228</sup>. In sum, while previously viewed as a means to guide the selection of targeted therapies, tumour genomic profiling has quickly established an additional role in optimizing the use of immunotherapies in patients with cancer.

PARP inhibitor sensitivity, neither tumour-only nor germline-only profiling can identify all patients who are candidates for PARP inhibitor therapy. Additional examples of germline mutations that are predictive biomarkers of drug response include *ALK* mutations in children with neuroblastoma<sup>111</sup> and *RET* mutations in patients with medullary thyroid cancer<sup>112,113</sup>. Germline mutations in DNA repair pathways that result in elevated TMB have also been associated with increased likelihood of response to immunotherapy. For example, most tumours that arise in patients with Lynch syndrome are mismatch-repair deficient, which is a tumour-agnostic biomarker of response to the anti-PD1 antibody pembrolizumab<sup>114</sup>. Similarly, germline mutations in the *MBD4* gene, which encodes a DNA glycosylase, are associated with hypermutation and immunotherapy response in uveal melanoma<sup>115,116</sup>.

The need for broad-based germline genetic testing in patients with cancer as a prelude to treatment selection has raised several ethical and logistic hurdles. First, several states regulate germline genetic testing over concerns about the risk of discrimination (for example, with respect to insurance or employment) targeting patients with a heritable cancer susceptibility<sup>117–120</sup>. Privacy concerns linked to accessibility to germline genetic data are particularly acute, as results can have an impact not only on the index cancer patient but also on family members who may be germline mutation carriers and therefore

at higher risk of cancer<sup>121</sup>. The finding of a pathogenic germline variant can also result in distress and anxiety for patients and family members<sup>122</sup>. These concerns have led some experts to propose that counselling by specially trained nurses or physicians be required before germline genetic testing to ensure that patients fully understand the risks associated with an incidental finding of a pathogenic germline variant. The need to test an expanding fraction of patients with cancer for potentially actionable germline genetic alterations as a guide to therapy selection has upended the traditional pre-test counselling model, as there are currently insufficient genetic counsellors to advise all patients with cancer<sup>123,124</sup> (as opposed to the subset of patients referred to genetic counsellors on the basis of historical clinical guidelines). A requirement for referral for pre-test counselling could also markedly delay genetic testing and the reporting of results to treating physicians, which could prove harmful in patients with rapidly progressive metastatic disease who are candidates for PARP, ALK or RET inhibitors<sup>125</sup>. The burden of obtaining consent and advising patients about the risks of germline testing is therefore expected to fall increasingly on point-of-care medical, surgical and radiation oncologists, many of whom have limited training in cancer genomics, are less experienced in interpreting the clinical implications of variants of unknown significance and less likely to propose cascade testing of family members<sup>126,127</sup>.

Paired tumour–germline sequencing can add to assay costs owing to the need to obtain, extract and analyse DNA from a normal sample<sup>128</sup>. In our opinion, these labour and reagent costs are offset by the greater efficiency of variant interpretation in the setting of paired tumour and germline genetic sequencing. However, for many providers, the logistical hurdles and potential delays involved in coordinating collection and shipment of both tumour and healthy tissue samples have been a barrier to the broader adoption of paired tumour–germline sequencing<sup>129</sup>. Despite these logistical hurdles and the potential risks associated with an incidental finding of a pathogenic germline variant, we expect that the need to identify potentially actionable germline variants in an increasing number of cancer types will drive the broader adoption of paired tumour–germline genomic testing in patients with cancer.

### Alternatives to panel-based tumour NGS

**Whole-exome and whole-genome sequencing.** A primary limitation of targeted NGS panels is that they fail to detect mutations in genes not included in the assay design. In response to declining sequencing costs, panel sizes have steadily grown from several dozen to hundreds of cancer-associated genes<sup>20</sup>. Although clinical WES and WGS assays are offered by select academic laboratories and commercial providers<sup>130–133</sup>, the wider adoption of clinical WES and WGS has to date been limited for several reasons. One hurdle is that the tumour material available for many patients is of insufficient quantity, quality or purity for these broader sequencing platforms. In designing clinical NGS platforms, the limitations imposed by cost and sequencing capacity require the balancing of sequencing breadth and depth. Given this trade-off, the higher depth of coverage of targeted NGS assays provides an advantage over WES and WGS assays for maximizing detection of alterations in genes that are clinically validated biomarkers of drug response, in particular, in samples with poor DNA quality or significant stromal contamination. More widespread adoption of WGS will require further reductions in sequencing costs and technological improvements to enable the use of lower-quality, archival formalin-fixed, paraffin-embedded (FFPE) tumour tissue. Of note, although additional rare and private (that is, genetic alterations specific to one individual) oncogenic gene fusions, non-coding mutations and structural alterations that dysregulate gene expression will certainly be discovered in the coming years, the likelihood of identifying a clinically actionable mutation or structural variant by WES or WGS that would not have been detected by current large-panel NGS assays is low. Therefore, we and others predict that the adoption of clinical WGS will be driven more by the need to robustly characterize mutational signatures predictive of drug response (for example, structural signatures of homologous recombination deficiency) rather than by the need to identify incremental discrete oncogenic alterations<sup>134,135</sup>.

**RNA sequencing.** Capture-based DNA sequencing panels are not optimal for the detection of oncogenic gene fusions. Although gene fusions can be detected

by capture-based NGS sequencing panels through the inclusion of probes targeting intronic regions, the large size of some introns and the variable location of fusion breakpoints make the tiling of all relevant intronic regions prohibitively expensive. RNA sequencing (RNA-seq) is far better suited for fusion detection and can also provide information on gene expression that cannot be derived from DNA-based tumour profiling. Unfortunately, the generation of high-quality whole-transcriptome RNA-seq data from the limited and often low-quality archival tumour samples available for most patients with cancer has proved challenging. There are also computational challenges with RNA-seq data associated with batch and sample quality-associated artefacts<sup>136</sup>. As an alternative, RNA-based gene panels have been developed that use anchored multiplex PCR technology that can robustly detect gene fusions using small amounts of FFPE-derived RNA<sup>137</sup>. The small number of genes covered by current RNA-based fusion panels will likely restrict their use in the near future to the validation of putative gene fusions detected by DNA-based tumour profiling or the analysis of patient samples in which the pre-test suspicion of a gene fusion is high (for example, sarcomas and lung cancers in which DNA testing failed to detect an oncogenic driver). As an alternative, exome-capture RNA-seq has shown promise for the detection of fusions in archival FFPE tumour samples<sup>138</sup>. Finally, diagnostic assays capable of characterizing global DNA methylation patterns are likely to prove useful in refining tumour subtype classification and for identifying the likely primary site in patients with cancers of unknown primary<sup>139</sup>. The latter may be particularly relevant for cfDNA-based screening platforms as discussed in the next section. Novel diagnostic platforms that can identify epigenetic modifications or changes in protein expression or activation that are predictive of treatment response are also in development.

**Cell-free DNA.** In contrast to broader tumour sequencing platforms such as WES, narrower but ultrasensitive gene panels capable of detecting small quantities of circulating tumour-derived DNA in plasma have been quick to show evidence of clinical utility. Tumour biopsy samples are often of insufficient quantity and quality for tumour genomic profiling, and analysis of a single primary or metastatic tumour site may fail to capture spatial heterogeneity or treatment-associated clonal evolution. Profiling of plasma cfDNA can overcome many of these limitations by enabling minimally invasive analysis of tumour-derived DNA that can be serially repeated as patients progress from localized to metastatic disease or develop resistance to systemic therapies. Tumour-derived cfDNA can also be present in cerebrospinal fluid<sup>140</sup>, pleural fluid<sup>141</sup>, ascitic fluid<sup>142</sup> or in urine<sup>143</sup>. Beyond its use to identify actionable mutations, cfDNA analysis has shown promise as a platform to screen for occult cancers in high-risk populations, for the detection of minimal residual disease in patients treated with curative intent, and as a means to quantify treatment response in patients with metastatic disease<sup>144–149</sup>.

The challenge of cfDNA analysis is that the quantity of circulating tumour DNA is typically low, and it

#### Capture-based DNA sequencing

A method for selectively sequencing targeted regions of the genome using baits that hybridize with specific regions of DNA.

**Clonal mutations**

Mutations present in all of a patient's cancer cells.

can be difficult to distinguish between tumour-derived somatic mutations, somatic mutations arising from clonal haematopoiesis and artefacts induced by DNA oxidation, PCR amplification errors or through the process of DNA sequencing<sup>110</sup>. DNA barcoding-based error suppression methods have recently been developed to filter out mutations resulting from PCR or sequencing artefacts<sup>150</sup> (FIG. 3). These methods rely on a 20–40-fold greater depth of sequencing coverage than needed for tumour-based NGS panels, and therefore cfDNA assays typically analyse significantly fewer genes. The limited sequencing breadth of current cfDNA panels and the low fraction of tumour-derived DNA in plasma also make some genomic features such as deletions and mutational signatures more challenging to detect in blood. Negative cfDNA results in individual patients must be interpreted with caution as some tumours do not shed sufficient DNA to allow for mutation detection using even the most sensitive of assays. Tumour and cfDNA sequencing are therefore likely to be complementary strategies for identifying potentially actionable genomic alterations in patients in need of systemic therapy.

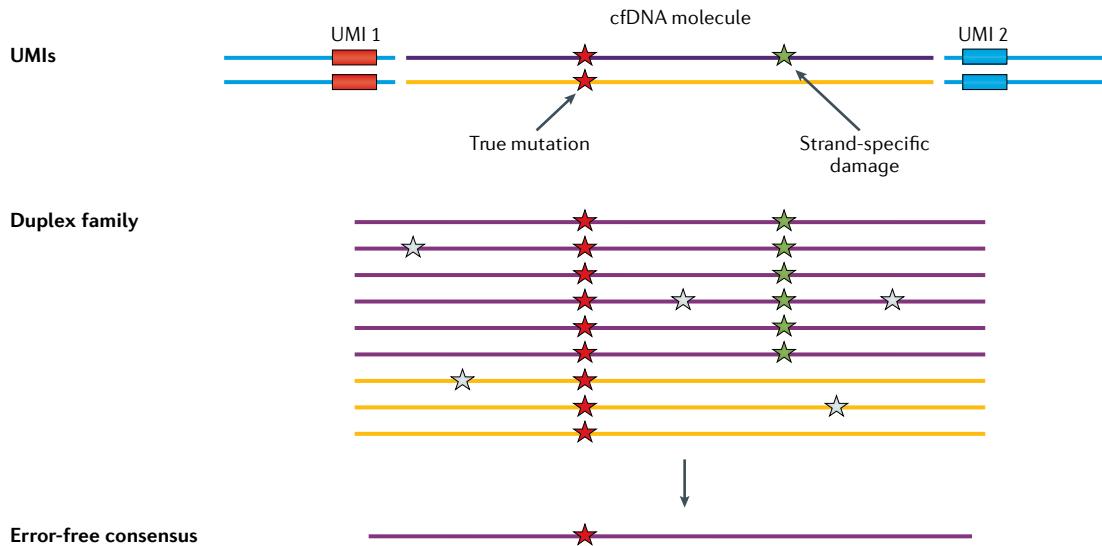
Despite their limitations, NGS-based multigene cfDNA assays are rapidly being adopted in lung cancer, as oncologists need to screen for multiple targetable genomic alterations in a short time frame, and cfDNA results can on average be delivered more quickly<sup>151</sup>. In particular, for patients who had a diagnostic tumour biopsy or surgery at another institution, results from cfDNA testing can be available weeks or even months faster than tumour testing given the logistical delays associated with requesting and processing tumour tissue. cfDNA sequencing is also likely to be particularly useful for identifying targetable genomic alterations in tumours with a predominant pattern of osseous spread, such as breast<sup>152</sup> and prostate<sup>153</sup>.

cancers, given the challenges of obtaining high-quality genomic material from bone biopsies.

In the minimal residual disease setting, where the fraction of tumour-derived cfDNA is expected to be very low, personalized cfDNA assays designed to detect multiple clonal mutations identified by tumour sequencing (some of which may be non-functional variants) appear to be more sensitive than NGS-based panels, which are better suited to the detection of a limited number of actionable alterations in patients with greater tumour burden or metastatic disease<sup>154–156</sup>. Finally, there are millions of CpG sites across the human genome, and DNA methylation patterns differ as a function of cancer subtype<sup>157</sup>. Therefore, for screening applications, cfDNA assays designed to detect aberrant patterns of DNA methylation may be more sensitive than NGS-based cfDNA panels, while also providing information as to the likely primary site of disease to guide subsequent imaging or diagnostic endoscopies<sup>110</sup>.

**Allelic configuration and mutational signatures**

**Mutational clonality.** New somatic alterations arise stochastically as a result of tumour cell-intrinsic deficiencies in DNA replication, repair or chromosomal segregation, or as a result of ongoing exposure to mutagens such as cigarette smoke or drugs, including cytotoxic chemotherapies, which induce DNA damage. Cancer genomes are also constantly evolving, with new mutations and structural variants arising as tumours invade, metastasize or adapt to therapy-induced selective pressures. Ongoing mutation and clonal selection during the course of a patient's disease therefore result in complex patterns of intra-lesional and lesion-to-lesion genomic heterogeneity that may diminish the effectiveness of precision oncology approaches. Current tumour sequencing



**Fig. 3 | Detection of low-frequency mutations in tumour cell-free DNA.** As the fraction of cell-free DNA (cfDNA) derived from tumour cells is often very low, tumour-specific somatic mutations are typically present in blood at very low allele frequencies. For mutations present at low allele frequencies (<1%), ultra-deep sequencing and error correction are needed to distinguish PCR and sequencing errors from tumour-derived somatic variants in blood. Error suppression can be achieved through the use of unique molecular indices (UMIs) with dual index barcodes, which allow for the filtering of sequencing artefacts. Red stars represent true somatic mutations, whereas green stars are strand-specific damage and white stars sequencing errors. Image courtesy of M. F. Berger, Memorial Sloan Kettering Cancer Center, New York, USA.

**Subclonal mutations**  
Mutations present in only a fraction of a patient's cancer cells.

**Cancer cell fraction**  
The estimated fraction of cancer cells that harbour a specific mutation.

**Variant allele frequency**  
The fraction of mutant versus total sequencing reads at the mutation locus.

**Allelic configuration**  
The number of mutant and wild-type alleles, which because of copy number gain or loss can be less than or greater than two.

**Knudsen's two-hit hypothesis**  
The hypothesis, proposed by Alfred Knudsen in 1971, that for tumour suppressor genes that are recessive in nature, in order for a phenotype to manifest, both alleles must be inactivated (biallelic inactivation). This may be achieved through multiple mechanisms including deletions (either chromosomal or subchromosomal), epigenetic silencing or mutation.

**Loss of heterozygosity**  
A common form of allelic imbalance in which a heterozygous somatic alteration becomes homozygous following loss of the wild-type allele.

**Clonal fitness**  
The relative growth, survival or metastatic potential advantage of a cancer cell clone over other cancer cells within the tumour or non-cancer cells. The term fitness here derives its origins from the concept of natural selection in evolutionary biology.

**Integer copy number**  
The copy number of a gene or localized DNA segment represented as an integer value. For missense mutations, the number of mutated and wild-type alleles in the cell.

reports are mutation-centric in that mutations and oncogenic structural variants such as amplification of oncogenes, deletions encompassing tumour suppressor genes and in-frame oncogenic translocations are reported as independent and unrelated events (FIG. 4a). Future NGS reports will seek to better place mutations in context by reporting their clonality and potential functional interaction with co-occurring driver mutations (FIG. 4b).

Some driver mutations arise early in tumour development and are clonal<sup>158</sup>. Others arise later during metastatic progression or as mediators of resistance to systemic therapies and are subclonal. For kinase inhibitors, antitumour activity is presumed to be greatest in patients in whom the targeted mutation is clonal, as targeting subclonal mutations would be expected to result in rapid selection for cancer cells lacking the drug-sensitizing mutation. More broadly, cancers that have a high degree of tumour heterogeneity may be less sensitive to a variety of systemic therapies including cytotoxic chemotherapies and immunotherapies, as the existence of a larger number of genetically distinct subclones before treatment initiation increases the odds that a therapy-resistant clone already exists pre-treatment<sup>159,160</sup>.

Computational algorithms have been developed that leverage the high depth of sequencing coverage generated by NGS-based tumour profiling to convert variant allele frequencies into cancer cell fractions<sup>161,162</sup>, a numerical estimation of the fraction of cancer cells harbouring a particular mutation. For single-nucleotide variants (SNVs), cancer cell fraction can be inferred from NGS data using estimates of variant allele frequency, tumour purity and local copy number. The accuracy of cancer cell fraction estimates can, however, be affected by tissue quality, sequencing depth and the breadth of the sequencing panel<sup>163</sup>. The clinical utility of cancer cell fraction for guiding treatment decisions remains poorly defined, and estimates of mutational clonality are not routinely included in clinical tumour profiling reports. A major concern over the use of clonality inferences is that the results may not provide an accurate reflection of the patient's current disease burden. For example, a drug-sensitizing mutation that is subclonal in the surgically resected primary tumour site may be clonal in all metastatic sites<sup>7</sup>. Mutational clonality can also vary between metastatic sites<sup>164</sup>. Therefore, the use of clonal inferences derived from a single tumour biopsy sample may give an incorrect assessment of the clonality of an actionable mutation in the patient's overall disease burden. Subclonal drug-sensitizing mutations have also been shown to arise in parallel with sensitizing mutations in other genes through the process of convergent evolution<sup>165</sup>. Therefore, although retrospective studies suggest that pre-existing tumour heterogeneity and mutational clonality often influence drug response<sup>7,166</sup>, how best to incorporate this knowledge into clinical practice guidelines requires further study.

**Allelic configuration.** The methods used to infer the cancer cell fraction of somatic mutations from NGS-based tumour profiling data can provide information as to the allelic configuration of individual mutations or global

structural variant signatures that may influence treatment response (FIG. 4b,c). Many tumour suppressor genes have a phenotype only in the setting of biallelic inactivation of both gene copies (the Knudsen's two-hit hypothesis). Therefore, the ability to assess for loss of heterozygosity resulting from deletion of the non-mutated wild-type allele may provide clinicians with insight as to whether targeting a particular tumour suppressor gene mutation is likely to be effective<sup>167</sup>. Biallelic inactivation of tumour suppressor genes can also result from dysregulation of epigenetic modifiers such as promoter methylation<sup>168</sup>, and therefore the clinical utility of analysing tumours for only DNA-mediated loss of heterozygosity events without the concurrent ability to detect epigenetically driven gene silencing remains an area of substantial controversy.

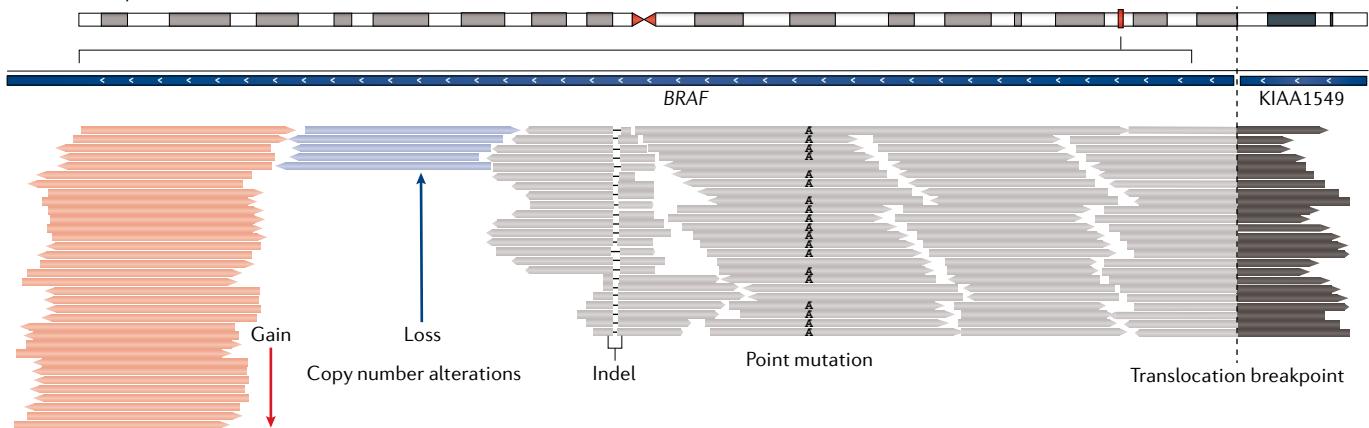
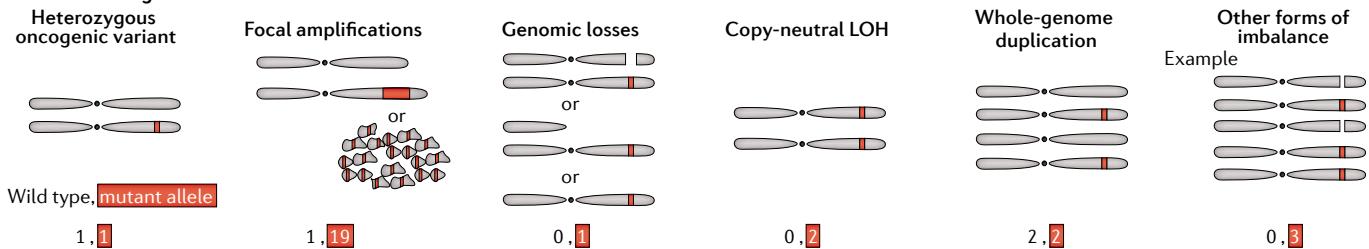
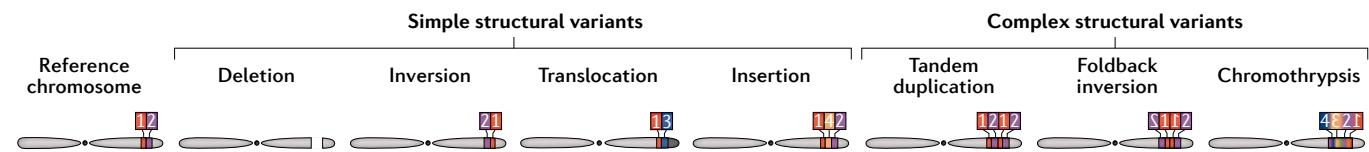
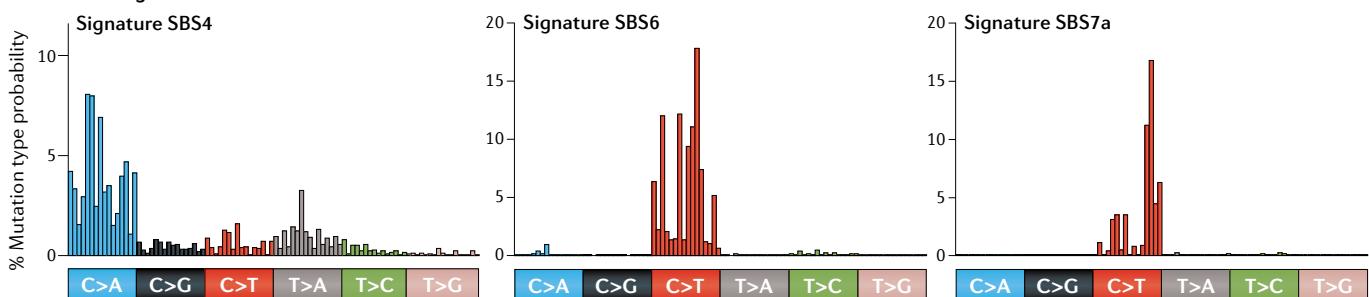
Although less recognized, the allelic configuration of gain-of-function mutations can also influence oncogene dependence and drug sensitivity. For example, 12–15% of *PIK3CA*-mutated breast cancers harbour not one but two mutations in the same *PIK3CA* allele<sup>169</sup>. These compound *PIK3CA* mutations activate PI3 kinase signalling to a greater degree than the individual *PIK3CA* mutations and are predictive of greater sensitivity to PI3Ka inhibitors<sup>169</sup>. Allelic imbalance resulting in gain of additional copies of mutated oncogenes or loss of the wild-type allele can also enhance clonal fitness or modulate sensitivity to targeted therapies<sup>167</sup>. For example, loss of the wild-type *BRAF* allele in *BRAF*-V600E-mutant tumours may confer greater sensitivity to vemurafenib, a selective RAF inhibitor<sup>167</sup>. A more precise estimation of integer copy number from tumour NGS data could also help clinicians interpret the significance of amplifications involving targetable oncogenes, as higher levels of gene amplification may be associated with greater oncogene dependence and drug sensitivity<sup>161</sup>.

**Co-mutational context.** Most tumour sequencing reports annotate point mutations, copy number alterations and structural variants such as translocations independently of each other. Co-occurring mutations that confer drug resistance or enhance drug sensitivity are rarely highlighted as such. For example, mutations in *PTEN* and *NF1* have been shown to influence RAF inhibitor response in *BRAF*-mutated melanoma<sup>170–173</sup>. Conversely, tumours with two or more alterations that activate the same pathways, for example, concurrent *TSC1* and *NF2* alterations, both of which activate mTORC1, exhibit greater drug sensitivity than tumours with either alteration alone<sup>61</sup>. Enhancements to clinical reporting detailing epistatic relationships between two or more co-occurring mutations and highlighting whether two mutations in the same gene are present in *cis* or in *trans* may in the future help guide treatment selection or refine prognostication.

**Mutational signatures.** Extending analyses beyond consideration of individual genetic alterations to patterns of single, doublet and clustered base pair substitutions, small insertions or deletions and larger structural alterations is likely to provide additional insight into the biological and clinical significance of mutations

**a Allele level**

Reference sequence

**b Allelic configuration****c Structural variants****d Mutation signatures****Fig. 4 | Expanding the clinical utility of tumour genomic profiling.**

**a** Current clinical tumour genomic reports are mutation centric with mutations, small insertions/deletions (indels), gene amplifications, deletions and translocations reported as discrete events. **b** Future next-generation sequencing (NGS) reports will seek to report additional genomic features such as allelic context (the number of mutant and wild-type copies of an actionable cancer gene estimated to be present in the cancer cell), which may influence drug response or patient prognosis. **c** Broader sequencing platforms such as whole-genome sequencing can detect the presence of complex structural variants such as tandem duplications, foldback inversions or chromothripsis (thousands of clustered chromosomal rearrangements in a single genomic region). **d** Global patterns of complex

genomic variants and single base substitution signatures may provide insight into the likely cause of a patient's cancer or may be more predictive of response to poly(ADP-ribose) polymerase (PARP) inhibitors or immunotherapy than single gene mutational status alone. For example, single base substitution (SBS) signature SBS4 is associated with tobacco smoking and likely arises owing to DNA damage by mutagens such as benzo[a]pyrene present in tobacco smoke<sup>176</sup>, whereas SBS7 is common in skin cancers arising in sun-exposed areas and is believed to be the result of ultraviolet light-mediated mutagenesis. Mutational signature SBS6 is associated with defective DNA mismatch repair and is commonly found in tumours with microsatellite instability, which is predictive of response to the immune checkpoint inhibitor pembrolizumab.

in potentially actionable cancer genes<sup>174</sup>. By combining the six possible SNV classes together with their trinucleotide contexts, all SNVs can be classified into 1 of 96 possible combinations. Analysis of patterns of these SNV substitutions has led to the identification of 81 distinct SNV-based mutational signatures, many of which are linked to specific defects in DNA repair, or drug or toxin exposure<sup>175,176</sup>. The presence or absence of a mutational signature can aid in the clinical interpretation of potentially actionable mutations. For example, not all tumours with mutations in the Lynch syndrome-associated genes *MLH1*, *MSH2*, *MSH6* and *PMS2* have evidence of dMMR, but those that do often have a distinctive pattern of single base substitutions (FIG. 4d). Integration of mutational signature analysis with an analysis of microsatellite regions using MSIsensor<sup>177</sup> or other bioinformatic tools<sup>178,179</sup> can help define the biological and clinical significance of mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2* by determining whether there is phenotypic evidence of mismatch repair deficiency<sup>180</sup>. Such phenotypic characterization of microsatellite instability is likely a more robust predictor of immunotherapy sensitivity than simply the presence or absence of a mutation in a Lynch syndrome-associated gene<sup>180</sup>.

The ability to robustly characterize mutational signatures may prove to be the most clinically significant incremental benefit of WGS over targeted panel sequencing assays. Structural alterations that have been associated with a poor prognosis or predictive of drug response include focal tandem duplications resulting from *CDK12* loss-of-function mutations<sup>181</sup>, foldback inversions and interstitial deletions that may be characteristic of differential aberrant DNA repair processes in ovarian cancer<sup>182</sup>, and reciprocal loss of heterozygosity, which is commonly observed in germ cell tumours and may be associated with cisplatin resistance<sup>183</sup> (FIG. 4c). WGS-scale assays can also robustly characterize single and doublet base substitutions and small insertion and deletion signatures that may be more predictive of immunotherapy or PARP inhibitor response than the mutational status of individual cancer genes<sup>181,182,184</sup>.

### Balancing regulation and innovation

Although the quality assurance goals of governmental regulation of tumour genomic profiling assays is laudable, the paradigm of companion diagnostic tests as currently implemented does not best facilitate the iterative improvements needed to promote rapid innovation<sup>185,186</sup>. Of particular concern is the possibility that drug reimbursement and drug access may in the future require the use of a specific companion diagnostic test. While reimbursement for NGS-based tumour genomic profiling in patients with cancer has been largely justified to date by the need to screen for predictive biomarkers of drug response, these profiling assays are multipurpose diagnostic platforms that can also help ensure the correct tumour subtype classification, inform prognosis and determine whether a cancer arose as a result of a heritable cancer predisposition. Multigene NGS panels also enable the concurrent reporting of established and investigational biomarkers, facilitating the development

of novel therapies directed at new drug targets. Viewed in this way, NGS-based tumour profiling has the potential to reduce costs and improve patient outcomes by replacing and improving upon more limited PCR, fluorescence in situ hybridization, immunohistochemistry and small-panel NGS-based analytics.

Improvements to tumour NGS assays, such as the addition of newly identified cancer-associated genes, are likely to be incremental and facilitated by declining sequencing costs. These newer assay versions should not need to demonstrate clinical utility. Instead, regulators should focus on ensuring that the assays used for patient care are accurate, reproducible and performed by qualified personnel. Such a nimble regulatory structure would ensure quality while maximizing the potential for innovation in this rapidly evolving field. A flexible and adaptive approach to the regulation of clinical tumour profiling assays would also allow individual hospital-based and commercial laboratories to quickly adopt novel methods of DNA extraction, library preparation, sequencing or bioinformatic analysis, presuming such changes do not negatively impact the sensitivity or specificity of detecting established biomarkers. In sum, regulatory agencies should seek to encourage the development of more cost-effective assays that are tissue-efficient and capable of detecting all gene alterations and mutational signatures required to guide the care of patients with cancer, recognizing that no single assay developed to date is optimal for all applications in all cancer types.

### Conclusion and perspectives

The continued decline in sequencing costs and the identification of new genomic biomarkers predictive of drug response has driven the rapid adoption of multigene NGS-based tumour genomic profiling panels as a component of routine cancer care. In addition to the identification of predictive biomarkers of therapy response, tumour genomic profiling can identify somatic and germline mutations that refine or confirm a patient's cancer subtype diagnosis and provide clinicians with insight into heritable cancer risk and the likelihood of cancer recurrence and death. The future adoption of broader sequencing panels or WGS methods should enable more accurate assessments of mutational clonality, allelic context and the identification of mutational and structural signatures that are predictive of drug response. The emergence of NGS-based platforms capable of detecting and analysing tumour-derived DNA in plasma will also allow the development of diagnostic assays to screen high-risk patients for occult cancers, assess for minimal residual disease following curative-intent therapy, monitor and better quantify treatment response and assess for clonal evolution under the selection pressure of therapy as a prelude to the development of rational combination strategies that prevent or delay drug resistance.

Data-sharing initiatives that allow for integration of tumour genomic profiling data with detailed clinical annotation and treatment response data, such as AACR GENIE<sup>6</sup>, will be crucial in assessing the clinical utility of tumour genomic profiling, in particular, in rare cancer

types. As not all mutations in the same gene have the same biological effect or clinical significance, improvements to variant knowledge bases and clinical reporting will be needed to help communicate to point-of-care clinicians the therapeutic, diagnostic and prognostic relevance of individual mutations, epistatic interactions between co-occurring mutations and genome-wide

mutational signatures. A significant expansion in the fraction of patients with cancer who benefit from a precision medicine approach will also require the development of new therapies effective against tumours driven by currently undruggable oncogenic drivers.

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- Sobin, L. H. The international histological classification of tumours. *Bull. World Health Organ.* **59**, 813–819 (1981).
- Hoadley, K. A. et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* **158**, 929–944 (2014).
- Bouwman, P. & Jonkers, J. The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. *Nat. Rev. Cancer* **12**, 587–598 (2012).
- Yarchoan, M., Hopkins, A. & Jaffee, E. M. Tumor mutational burden and response rate to PD-1 inhibition. *N. Engl. J. Med.* **377**, 2500–2501 (2017).
- Hyman, D. M. et al. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N. Engl. J. Med.* **373**, 726–736 (2015).
- Drlon, A. et al. Efficacy of larotrectinib in TRK fusion-positive cancers in adults and children. *N. Engl. J. Med.* **378**, 731–739 (2018).
- Hyman, D. M. et al. AKT inhibition in solid tumors with AKT1 mutations. *J. Clin. Oncol.* **35**, 2251–2259 (2017).
- Hungerford, D. A. & Nowell, P. C. A minute chromosome in human chronic granulocytic leukemia. *Science* **132**, 1497–1499 (1960).
- de Klein, A. et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature* **300**, 765–767 (1982).
- Kantarjian, H. et al. Improved survival in chronic myeloid leukemia since the introduction of imatinib therapy: a single-institution historical experience. *Blood* **119**, 1981–1987 (2012).
- Prasad, V. Perspective: the precision-oncology illusion. *Nature* **537**, S63 (2016).
- Hyman, D. M., Taylor, B. S. & Baselga, J. Implementing genome-driven oncology. *Cell* **168**, 584–599 (2017).
- Pan, Y. et al. ALK, ROS1 and RET fusions in 1139 lung adenocarcinomas: a comprehensive study of common and fusion pattern-specific clinicopathologic, histologic and cytologic features. *Lung Cancer* **84**, 121–126 (2014).
- Mansfield, E. A. FDA perspective on companion diagnostics: an evolving paradigm. *Clin. Cancer Res.* **20**, 1453–1457 (2014).
- Kris, M. G. et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* **311**, 1998–2006 (2014).
- Jordan, E. J. et al. Prospective comprehensive molecular characterization of lung adenocarcinomas for efficient patient matching to approved and emerging therapies. *Cancer Discov.* **7**, 596–609 (2017).
- Su, Z. et al. A platform for rapid detection of multiple oncogenic mutations with relevance to targeted therapy in non-small-cell lung cancer. *J. Mol. Diagn.* **13**, 74–84 (2011).
- MacConaill, L. E. et al. Prospective enterprise-level molecular genotyping of a cohort of cancer patients. *J. Mol. Diagn.* **16**, 660–672 (2014).
- Li, T., Kung, H.-J., Mack, P. C. & Candara, D. R. Genotyping and genomic profiling of non-small-cell lung cancer: implications for current and future therapies. *J. Clin. Oncol.* **31**, 1039–1049 (2013).
- Zehir, A. et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat. Med.* **23**, 703–713 (2017).
- Sholl, L. M. et al. Institutional implementation of clinical tumor profiling on an unselected cancer population. *JCI Insight* **1**, e87062 (2016).
- Meric-Bernstam, F. et al. Feasibility of large-scale genomic testing to facilitate enrollment onto genetically matched clinical trials. *J. Clin. Oncol.* **33**, 2753–2762 (2015).
- Stockley, T. L. et al. Molecular profiling of advanced solid tumors and patient outcomes with genotype-matched clinical trials: the Princess Margaret IMPACT/COMPACT trial. *Genome Med.* **8**, 109 (2016).
- Odegaard, J. I. et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clin. Cancer Res.* **24**, 3539–3549 (2018).
- Brannon, A. R. et al. Enhanced specificity of high sensitivity somatic variant profiling in cell-free DNA via paired normal sequencing: design, validation, and clinical experience of the MSK-ACCESS liquid biopsy assay. Preprint at *bioRxiv* <https://doi.org/10.21203/rs.3.rs-120695/v1> (2020).
- Tao, J. J., Schram, A. M. & Hyman, D. M. Basket studies: redefining clinical trials in the era of genome-driven oncology. *Annu. Rev. Med.* **69**, 319–331 (2018).
- Redig, A. J. & Jänne, P. A. Basket trials and the evolution of clinical trial design in an era of genomic medicine. *J. Clin. Oncol.* **33**, 975–977 (2015).
- Woodcock, J. & LaVange, L. M. Master protocols to study multiple therapies, multiple diseases, or both. *N. Engl. J. Med.* **377**, 62–70 (2017).
- Le, D. T. et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
- Drlon, A. E. et al. A phase II basket study of the oral TRK inhibitor LOXO-101 in adult subjects with NTRK fusion-positive tumors. *J. Clin. Oncol.* **34**, TPS2599–TPS2599 (2016).
- Berger, M. F. & Mardis, E. R. The emerging clinical relevance of genomics in cancer medicine. *Nat. Rev. Clin. Oncol.* **15**, 353–365 (2018).
- Vogelstein, B. et al. Cancer genome landscapes. *Science* **339**, 1546–1558 (2013).
- Bailey, M. H. et al. Comprehensive characterization of cancer driver genes and mutations. *Cell* **173**, 371–385.e18 (2018).
- Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).
- Mateo, J. et al. DNA-repair defects and olaparib in metastatic prostate cancer. *N. Engl. J. Med.* **373**, 1697–1708 (2015).
- Van Allen, E. M. et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. *Cancer Discov.* **4**, 1140–1153 (2014).
- Li, Q. et al. ERCC2 helicase domain mutations confer nucleotide excision repair deficiency and drive cisplatin sensitivity in muscle-invasive bladder cancer. *Clin. Cancer Res.* **25**, 977–988 (2019).
- Kelderman, S., Schumacher, T. N. & Kvistborg, P. Mismatch repair-deficient cancers are targets for anti-PD-1 therapy. *Cancer Cell* **28**, 11–13 (2015).
- Chang, M. T. et al. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. *Nat. Biotechnol.* **34**, 155–163 (2016).
- Chang, M. T. et al. Accelerating discovery of functional mutant alleles in cancer. *Cancer Discov.* **8**, 174–183 (2018).
- Hanrahan, A. J. et al. Leveraging systematic functional analysis to benchmark an *in silico* framework distinguishes driver from passenger MEK mutants in cancer. *Cancer Res.* **80**, 4233–301.e14 (2020).
- Hess, J. M. et al. Passenger hotspot mutations in cancer. *Cancer Cell* **36**, 288–301.e14 (2019).
- Buisson, R. et al. Passenger hotspot mutations in cancer driven by APOBEC3A and mesoscale genomic features. *Science* **364**, eaaw2872 (2019).
- Holbrook, J. A., Neu-Yilik, G., Hentze, M. W. & Kulozik, A. E. Nonsense-mediated decay approaches the clinic. *Nat. Genet.* **36**, 801–808 (2004).
- Cheung, L. W. T. et al. High frequency of PIK3R1 and PIK3R2 mutations in endometrial cancer elucidates a novel mechanism for regulation of PTEN protein stability. *Cancer Discov.* **1**, 170–185 (2011).
- Cheung, L. W. T. et al. Naturally occurring neomorphic PIK3R1 mutations activate the MAPK pathway, dictating therapeutic response to MAPK pathway inhibitors. *Cancer Cell* **26**, 479–494 (2014).
- Yao, Z. et al. BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. *Cancer Cell* **28**, 370–383 (2015).
- Zabransky, D. J. et al. HER2 missense mutations have distinct effects on oncogenic signaling and migration. *Proc. Natl. Acad. Sci. USA* **112**, E6205–E6214 (2015).
- Poulkakos, P. I., Zhang, C., Bollag, G., Shokat, K. M. & Rosen, N. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* **464**, 427–430 (2010).
- Kopetz, S. et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. *N. Engl. J. Med.* **381**, 1632–1643 (2019).
- Corcoran, R. B. et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAFV600E-mutant colorectal cancer. *Cancer Discov.* **8**, 428–443 (2018).
- Gray, S. W., Hicks-Corron, K., Cronin, A., Rollins, B. J. & Weeks, J. C. Physicians' attitudes about multiplex tumor genomic testing. *J. Clin. Oncol.* **32**, 1317–1323 (2014).
- Schram, A. M. et al. Oncologist use and perception of large panel next-generation tumor sequencing. *Ann. Oncol.* **28**, 2298–2304 (2017).
- Wu, J.-Y. et al. Lung cancer with epidermal growth factor receptor exon 20 mutations is associated with poor gefitinib treatment response. *Clin. Cancer Res.* **14**, 4877–4882 (2008).
- Chakravarty, D. et al. OncoKB: a precision oncology knowledge base. *JCO Precis. Oncol.* <https://doi.org/10.1200/PO.17.00011> (2017).
- Griffith, M. et al. CIVIC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat. Genet.* **49**, 170–174 (2017).
- Patterson, S. E. et al. The clinical trial landscape in oncology and connectivity of somatic mutational profiles to targeted therapies. *Hum. Genomics* **10**, 4 (2016).
- Huang, L. et al. The cancer precision medicine knowledge base for structured clinical-grade mutations and interpretations. *J. Am. Med. Inform. Assoc.* **24**, 513–519 (2017).
- Tamborero, D. et al. Cancer genome interpreter annotates the biological and clinical relevance of tumor alterations. *Genome Med.* **10**, 25 (2018).
- Dumbrava, E. I. & Meric-Bernstam, F. Personalized cancer therapy-leveraging a knowledge base for clinical decision-making. *Cold Spring Harb. Mol. Case Stud.* **4**, a001578 (2018).
- Iyer, G. et al. Genome sequencing identifies a basis for everolimus sensitivity. *Science* **338**, 221 (2012).
- Ross, J. S. et al. Comprehensive genomic profiling of carcinoma of unknown primary site: new routes to targeted therapies. *JAMA Oncol.* **1**, 40–49 (2015).
- Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
- Li, M. M. et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J. Mol. Diagn.* **19**, 4–23 (2017).
- Mateo, J. et al. A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO scale for clinical actionability of molecular targets (ESCAT). *Ann. Oncol.* **29**, 1895–1902 (2018).
- AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discov.* **7**, 818–831 (2017).
- Wagner, A. H. et al. A harmonized meta-knowledgebase of clinical interpretations of somatic genomic variants in cancer. *Nat. Genet.* **52**, 448–457 (2020).

68. Ritter, D. I. et al. Somatic cancer variant curation and harmonization through consensus minimum variant level data. *Genome Med.* **8**, 117 (2016).
69. Center for Devices & Radiological Health. FDA recognition of public human genetic variant databases. *FDA* <https://www.fda.gov/medical-devices/precision-medicine/fda-recognition-public-human-genetic-variant-databases> (2019).
70. Stein, E. M. et al. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. *Blood* **130**, 722–731 (2017).
71. Pollyea, D. A. et al. Enasidenib, an inhibitor of mutant IDH2 proteins, induces durable remissions in older patients with newly diagnosed acute myeloid leukemia. *Leukemia* **33**, 2575–2584 (2019).
72. DiNardo, C. D. et al. Durable remission with ivosidenib in IDH1-mutated relapsed or refractory AML. *N. Engl. J. Med.* **378**, 2386–2398 (2018).
73. Andrè, F. et al. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. *N. Engl. J. Med.* **380**, 1929–1940 (2019).
74. Loriot, Y. et al. Erdafitinib in locally advanced or metastatic urothelial carcinoma. *N. Engl. J. Med.* **381**, 338–348 (2019).
75. Abou-Alfa, G. K. et al. Pemigatinib for previously treated, locally advanced or metastatic cholangiocarcinoma: a multicentre, open-label, phase 2 study. *Lancet Oncol.* **21**, 671–684 (2020).
76. Drilon, A. et al. PL02.08 registrational results of LIBRETTO-001: a phase 1/2 trial of LOXO-292 in patients with RET fusion-positive lung cancers. *J. Thorac. Oncol.* **14**, S6–S7 (2019).
77. Wirth, L. et al. LBA93 - registrational results of LOXO-292 in patients with RET-altered thyroid cancers. *Ann. Oncol.* **30**, v933 (2019).
78. Litton, J. K. et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N. Engl. J. Med.* **379**, 753–763 (2018).
79. Golan, T. et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *N. Engl. J. Med.* **381**, 317–327 (2019).
80. Mateo, J. et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol.* **21**, 162–174 (2020).
81. Planchard, D. et al. Dabrafenib plus trametinib in patients with previously untreated BRAFV600E-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol.* **18**, 1307–1316 (2017).
82. Diamond, E. L. et al. Vemurafenib for BRAF V600-mutant erdheim-chester disease and langerhans cell histiocytosis: analysis of data from the histology-independent, phase 2, open-label VE-BASKET study. *JAMA Oncol.* **4**, 384–388 (2018).
83. Subbiah, V. et al. Dabrafenib and trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer. *J. Clin. Oncol.* **36**, 7–13 (2018).
84. Goodman, A. M. et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol. Cancer Ther.* **16**, 2598–2608 (2017).
85. Amado, R. G. et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **26**, 1626–1634 (2008).
86. De Roock, W. et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* **11**, 753–762 (2010).
87. Chung, V. et al. Effect of selumetinib and MK-2206 vs oxaliplatin and fluorouracil in patients with metastatic pancreatic cancer after prior therapy: SWOG S1115 study randomized clinical trial. *JAMA Oncol.* **3**, 516–522 (2017).
88. Jänne, P. A. et al. Selumetinib plus docetaxel compared with docetaxel alone and progression-free survival in patients with KRAS-mutant advanced non-small cell lung cancer: the SELECT-1 randomized clinical trial. *JAMA* **317**, 1844–1853 (2017).
89. Canon, J. et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* **575**, 217–223 (2019).
90. Tonin, P. et al. BRCA1 mutations in Ashkenazi Jewish women. *Am. J. Hum. Genet.* **57**, 189 (1995).
91. Abellioch, D. et al. The founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women. *Am. J. Hum. Genet.* **60**, 505–514 (1997).
92. Newman, B., Austin, M. A., Lee, M. & King, M. C. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. *Proc. Natl. Acad. Sci. USA* **85**, 3044–3048 (1988).
93. Hall, J. M. et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science* **250**, 1684–1689 (1990).
94. King, M.-C. The race' to clone BRCA1. *Science* **343**, 1462–1465 (2014).
95. Miki, Y. et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66–71 (1994).
96. Mandelker, D. et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA* **318**, 825–835 (2017).
97. Garofalo, A. et al. The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. *Genome Med.* **8**, 79 (2016).
98. Jones, S. et al. Personalized genomic analyses for cancer mutation discovery and interpretation. *Sci. Transl. Med.* **7**, 283ra53 (2015).
99. Zhang, J. et al. Germline mutations in predisposition genes in pediatric cancer. *N. Engl. J. Med.* **373**, 2336–2346 (2015).
100. Schrader, K. A. et al. Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncol.* **2**, 104–111 (2016).
101. Domchek, S. M. Germline genetic testing for breast cancer: Which patients? What genes? *Genet. Med.* **22**, 698–700 (2020).
102. Konstantinopoulos, P. A. et al. Germline and somatic tumor testing in epithelial ovarian cancer: ASCO guideline. *J. Clin. Oncol.* **38**, 1222–1245 (2020).
103. McLeod, H. L. Cancer pharmacogenomics: early promise, but concerted effort needed. *Science* **339**, 1563–1566 (2013).
104. Wang, L., McLeod, H. L. & Weinshilboum, R. M. Genomics and drug response. *N. Engl. J. Med.* **364**, 1144–1153 (2011).
105. Irvin, W. J. Jr. et al. Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced CYP2D6 metabolism: a multicenter study. *J. Clin. Oncol.* **29**, 3232–3239 (2011).
106. Hertz, D. L. et al. CYP2C8\* 3 predicts benefit/risk profile in breast cancer patients receiving neoadjuvant paclitaxel. *Breast Cancer Res. Treat.* **134**, 401–410 (2012).
107. Pullarkat, S. T. et al. Thymidylate synthase gene polymorphism determines response and toxicity of 5-FU chemotherapy. *Pharmacogenomics J.* **1**, 65–70 (2001).
108. Coombs, C. C. et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell* **21**, 374–382.e4 (2017).
109. Ptashkin, R. N. et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors. *JAMA Oncol.* **4**, 1589–1593 (2018).
110. Razavi, P. et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat. Med.* **25**, 1928–1937 (2019).
111. Carpenter, E. L. & Mossé, Y. P. Targeting ALK in neuroblastoma — preclinical and clinical advancements. *Nat. Rev. Clin. Oncol.* **9**, 391–399 (2012).
112. Wirth, L. J. et al. 1922P Exploratory patient-reported outcomes among patients with RET-mutant medullary thyroid cancer in LIBRETTO-001: a phase I/II trial of selpercatinib (LOXO-292). *Ann. Oncol.* **31** (Suppl. 4), S1089 (2020).
113. Wells, S. A. Jr. et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J. Clin. Oncol.* **30**, 134–141 (2012).
114. Yurgelun, M. B. & Hampel, H. Recent advances in Lynch syndrome: diagnosis, treatment, and cancer prevention. *Am. Soc. Clin. Oncol. Educ. Book* **38**, 101–109 (2018).
115. Rodrigues, M. et al. Outlier response to anti-PD1 in uveal melanoma reveals germline MBD4 mutations in hypermutated tumors. *Nat. Commun.* **9**, 1866 (2018).
116. Johansson, P. A. et al. Correction to: Prolonged stable disease in a uveal melanoma patient with germline MBD4 nonsense mutation treated with pembrolizumab and ipilimumab. *Immunogenetics* **71**, 511 (2019).
117. Schneid, T. in *Discrimination Law Issues for the Safety Professional* (ed. Schneid, T.) 161–194 (CRC Press, 2011).
118. National Human Genome Research Institute. Genetic Information Nondiscrimination Act (GINA) of 2008. *NIH* <https://www.genome.gov/24519851/genetic-information-nondiscrimination-act-of-2008> (2008).
119. Gniady, J. A. Regulating direct-to-consumer genetic testing: protecting the consumer without quashing a medical revolution. *Fordham Law Rev.* **76**, 2429–2475 (2008).
120. Ferreira-Gonzalez, A. et al. US system of oversight for genetic testing: a report from the Secretary's Advisory Committee on Genetics, Health and Society. *Per. Med.* **5**, 521–528 (2008).
121. Lolkeima, M. P. et al. Ethical, legal, and counseling challenges surrounding the return of genetic results in oncology. *J. Clin. Oncol.* **31**, 1842–1848 (2013).
122. Li, M. M. et al. Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet. Med.* **22**, 1142–1148 (2020).
123. Kurian, A. W. et al. Genetic testing and counseling among patients with newly diagnosed breast cancer. *JAMA* **317**, 531–534 (2017).
124. McNamara, D. Shortage of genetic counselors in face of growing need. *Medscape* <https://www.medscape.com/viewarticle/877135> (2017).
125. Eisen, A. et al. Genetic assessment wait time indicators in the high risk ontario breast screening program. *Mol. Genet. Genomic Med.* **6**, 213–223 (2018).
126. Culver, J. O., Hull, J. L., Dunne, D. F. & Burke, W. Oncologists' opinions on genetic testing for breast and ovarian cancer. *Genet. Med.* **3**, 120–125 (2001).
127. Teng, I. & Spigelman, A. Attitudes and knowledge of medical practitioners to hereditary cancer clinics and cancer genetic testing. *Fam. Cancer* **13**, 311–324 (2014).
128. Teer, J. K. et al. Evaluating somatic tumor mutation detection without matched normal samples. *Hum. Genomics* **11**, 22 (2017).
129. Damodaran, S., Berger, M. F. & Roychowdhury, S. Clinical tumor sequencing: opportunities and challenges for precision cancer medicine. *Am. Soc. Clin. Oncol. Educ. Book* **35**, e175–e182 (2015).
130. Robinson, D. R. et al. Integrative clinical genomics of metastatic cancer. *Nature* **548**, 297–303 (2017).
131. Van Allen, E. M. et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. *Nat. Med.* **20**, 682–688 (2014).
132. Van Allen, E. M. et al. A comparative assessment of clinical whole exome and transcriptome profiling across sequencing centers: implications for precision cancer medicine. *Oncotarget* **7**, 52888–52899 (2016).
133. Robbe, P. et al. Clinical whole-genome sequencing from routine formalin-fixed, paraffin-embedded specimens: pilot study for the 100,000 Genomes Project. *Genet. Med.* **20**, 1196–1205 (2018).
134. Staaf, J. et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. *Nat. Med.* **25**, 1526–1533 (2019).
135. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. *Nature* **578**, 82–93 (2020).
136. Conesa, A. et al. A survey of best practices for RNA-seq data analysis. *Genome Biol.* **17**, 13 (2016).
137. Zheng, Z. et al. Anchored multiplex PCR for targeted next-generation sequencing. *Nat. Med.* **20**, 1479–1484 (2014).
138. Cieslik, M. et al. The use of exome capture RNA-seq for highly degraded RNA with application to clinical cancer sequencing. *Genome Res.* **25**, 1372–1381 (2015).
139. Capper, D. et al. DNA methylation-based classification of central nervous system tumours. *Nature* **555**, 469–474 (2018).
140. Miller, A. M. et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. *Nature* **565**, 654–658 (2019).
141. Goto, K. et al. Epidermal growth factor receptor mutation status in circulating free DNA in serum: from IPASS, a phase III study of gefitinib or carboplatin/paclitaxel in non-small cell lung cancer. *J. Thorac. Oncol.* **7**, 115–121 (2012).
142. Husain, H. et al. Cell-free DNA from ascites and pleural effusions: molecular insights into genomic aberrations and disease biology. *Mol. Cancer Ther.* **16**, 948–955 (2017).

143. Chang, H. W. et al. Urinary cell-free DNA as a potential tumor marker for bladder cancer. *Int. J. Biol. Markers* **22**, 287–294 (2007).
144. Diehl, F. et al. Circulating mutant DNA to assess tumor dynamics. *Nat. Med.* **14**, 985–990 (2008).
145. Bettegowda, C. et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci. Transl. Med.* **6**, 224ra24 (2014).
146. Lecomte, T. et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. *Int. J. Cancer* **100**, 542–548 (2002).
147. Murtaza, M. et al. Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* **497**, 108–112 (2013).
148. Dawson, S.-J. et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N. Engl. J. Med.* **368**, 1199–1209 (2013).
149. Garcia-Murillas, I. et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci. Transl. Med.* **7**, 302ra153 (2015).
150. Kinde, I., Wu, J., Papadopoulos, N., Kinzler, K. W. & Vogelstein, B. Detection and quantification of rare mutations with massively parallel sequencing. *Proc. Natl Acad. Sci. USA* **108**, 9530–9535 (2011).
151. Oxnard, G. R. et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. *J. Clin. Oncol.* **34**, 3375–3382 (2016).
152. Shaw, J. A. et al. Mutation analysis of cell-free DNA and single circulating tumor cells in metastatic breast cancer patients with high circulating tumor cell counts. *Clin. Cancer Res.* **23**, 88–96 (2017).
153. Goodall, J. et al. Circulating cell-free DNA to guide prostate cancer treatment with PARP inhibition. *Cancer Discov.* **7**, 1006–1017 (2017).
154. Reinert, T. et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol.* **5**, 1124–1131 (2019).
155. Christensen, E. et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultradeep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J. Clin. Oncol.* **37**, 1547–1557 (2019).
156. Coombes, R. C. et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. *Clin. Cancer Res.* **25**, 4255–4263 (2019).
157. Hao, X. et al. DNA methylation markers for diagnosis and prognosis of common cancers. *Proc. Natl Acad. Sci. USA* **114**, 7414–7419 (2017).
158. Nowell, P. C. The clonal evolution of tumor cell populations. *Science* **194**, 23–28 (1976).
159. McGranahan, N. et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463–1469 (2016).
160. Landau, D. A. et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell* **152**, 714–726 (2013).
161. Carter, S. L. et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat. Biotechnol.* **30**, 413–421 (2012).
162. Shen, R. & Seshan, V. E. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res.* **44**, e131 (2016).
163. Tarabichi, M. et al. A practical guide to cancer subclonal reconstruction from DNA sequencing. *Nat. Methods* **18**, 144–155 (2021).
164. Gerlinger, M. et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
165. Voss, M. H. et al. Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy. *Clin. Cancer Res.* **20**, 1955–1964 (2014).
166. Hyman, D. M. et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. *Nature* **554**, 189–194 (2018).
167. Bielski, C. M. et al. Widespread selection for oncogenic mutant allele imbalance in cancer. *Cancer Cell* **34**, 852–862.e4 (2018).
168. Jones, P. A. & Baylin, S. B. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* **3**, 415–428 (2002).
169. Vasan, N. et al. Double PIK3CA mutations in cis increase oncogenicity and sensitivity to PI3K $\alpha$  inhibitors. *Science* **366**, 714–723 (2019).
170. Paraiso, K. H. T. et al. PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. *Cancer Res.* **71**, 2750–2760 (2011).
171. Xing, F. et al. Concurrent loss of the PTEN and RB1 tumor suppressors attenuates RAF dependence in melanomas harboring (V600E)BRAF. *Oncogene* **31**, 446–457 (2012).
172. Whittaker, S. R. et al. A genome-scale RNA interference screen implicates NF1 loss in resistance to RAF inhibition. *Cancer Discov.* **3**, 350–362 (2013).
173. Nissan, M. H. et al. Loss of NF1 in cutaneous melanoma is associated with RAS activation and MEK dependence. *Cancer Res.* **74**, 2340–2350 (2014).
174. Alexandrov, L. B. & Stratton, M. R. Mutational signatures: the patterns of somatic mutations hidden in cancer genomes. *Curr. Opin. Genet. Dev.* **24**, 52–60 (2014).
175. Alexandrov, L. B. et al. Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
176. Alexandrov, L. B. et al. The repertoire of mutational signatures in human cancer. *Nature* **578**, 94–101 (2020).
177. Niu, B. et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* **30**, 1015–1016 (2014).
178. Escudie, F. et al. MIAMs: microsatellite instability detection on NGS amplicons data. *Bioinformatics* <https://doi.org/10.1093/bioinformatics/btz797> (2019).
179. Huang, M. N. et al. MSIseq: software for assessing microsatellite instability from catalogs of somatic mutations. *Sci. Rep.* **5**, 13521 (2015).
180. Abida, W. et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. *JAMA Oncol.* **5**, 471–478 (2019).
181. Wu, Y.-M. et al. Inactivation of CDK12 delineates a distinct immunogenic class of advanced prostate cancer. *Cell* **173**, 1770–1782.e14 (2018).
182. Wang, Y. K. et al. Genomic consequences of aberrant DNA repair mechanisms stratify ovarian cancer histotypes. *Nat. Genet.* **49**, 856–865 (2017).
183. Taylor-Weiner, A. et al. Genomic evolution and chemoresistance in germ-cell tumours. *Nature* **540**, 114–118 (2016).
184. Angus, L. et al. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. *Nat. Genet.* **51**, 1450–1458 (2019).
185. Salgado, R. et al. Addressing the dichotomy between individual and societal approaches to personalised medicine in oncology. *Eur. J. Cancer* **114**, 128–136 (2019).
186. Salgado, R. et al. How current assay approval policies are leading to unintended imprecision medicine. *Lancet Oncol.* **21**, 1399–1401 (2020).
187. Rosell, R. et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* **13**, 239–246 (2012).
188. Mok, T. S. et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **361**, 947–957 (2009).
189. Fukuoka, M. et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J. Clin. Oncol.* **21**, 2237–2246 (2003).
190. Kris, M. G. et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA* **290**, 2149–2158 (2003).
191. Paez, J. G. et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
192. Lynch, T. J. et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**, 2129–2139 (2004).
193. Pao, W. et al. EGF receptor gene mutations are common in lung cancers from ‘never smokers’ and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl Acad. Sci. USA* **101**, 13306–13311 (2004).
194. Hann, C. L. & Brahmer, J. R. Who should receive epidermal growth factor receptor inhibitors for non-small cell lung cancer and when?. *Curr. Treat. Options Oncol.* **8**, 28–37 (2007).
195. Soda, M. et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **448**, 561–566 (2007).
196. Davies, K. D. et al. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin. Cancer Res.* **18**, 4570–4579 (2012).
197. Takeuchi, K. et al. RET, ROS1 and ALK fusions in lung cancer. *Nat. Med.* **18**, 378–381 (2012).
198. Gautschi, O. et al. Targeted therapy for patients with BRAF-mutant lung cancer: results from the European EURA cohort. *J. Thorac. Oncol.* **10**, 1451–1457 (2015).
199. Stephens, P. et al. Lung cancer: intragenic ERBB2 kinase mutations in tumours. *Nature* **431**, 525–526 (2004).
200. Shimamura, T. et al. Non-small-cell lung cancer and Ba/F3 transformed cells harboring the ERBB2 G776insV\_G/C mutation are sensitive to the dual-specific epidermal growth factor receptor and ERBB2 inhibitor HKI-272. *Cancer Res.* **66**, 6487–6491 (2006).
201. The Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* **511**, 543–550 (2014).
202. Paik, P. K. et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Discov.* **8**, 842–849 (2015).
203. Govindan, R. et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* **150**, 1121–1134 (2012).
204. Douillard, J.-Y. et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J. Clin. Oncol.* **28**, 4697–4705 (2010).
205. Bokemeyer, C. et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann. Oncol.* **22**, 1535–1546 (2011).
206. Joseph, E. W. et al. The RAF inhibitor PLX4032 inhibits ERK signalling and tumor cell proliferation in a V600E BRAF-selective manner. *Proc. Natl Acad. Sci. USA* **107**, 14903–14908 (2010).
207. Su, F. et al. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N. Engl. J. Med.* **366**, 207–215 (2012).
208. Callahan, M. K. et al. Progression of RAS-mutant leukemia during RAF inhibitor treatment. *N. Engl. J. Med.* **367**, 2316–2321 (2012).
209. Sanchez-Laorden, B. et al. BRAF inhibitors induce metastasis in RAS mutant or inhibitor-resistant melanoma cells by reactivating MEK and ERK signaling. *Sci. Signal.* **7**, ra30 (2014).
210. Samstein, R. M. et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat. Genet.* **51**, 202–206 (2019).
211. Marabelle, A. et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol.* **21**, 1353–1365 (2020).
212. Hellmann, M. D. et al. Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer. *Cancer Cell* **33**, 853–861.e4 (2018).
213. Marabelle, A. et al. 1192O - Association of tumour mutational burden with outcomes in patients with select advanced solid tumours treated with pembrolizumab in KEYNOTE-158. *Ann. Oncol.* **30**, v477–v478 (2019).
214. Graff, J. N. et al. A phase II single-arm study of pembrolizumab with enzalutamide in men with metastatic castration-resistant prostate cancer progressing on enzalutamide alone. *J. Immunother. Cancer* **8**, e000642 (2020).
215. O'Reilly, E. M. et al. Durvalumab with or without tremelimumab for patients with metastatic pancreatic ductal adenocarcinoma: a phase 2 randomized clinical trial. *JAMA Oncol.* **5**, 1431–1438 (2019).
216. Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic  $\beta$ -catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231–235 (2015).
217. Xiao, Q. et al. DKK2 imparts tumor immunity evasion through  $\beta$ -catenin-independent suppression of cytotoxic immune-cell activation. *Nat. Med.* **24**, 262–270 (2018).
218. Hardling, J. J. et al. Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin. Cancer Res.* **25**, 2116–2126 (2019).

219. Peters, S. et al. Phase II trial of atezolizumab as first-line or subsequent therapy for patients with programmed death-ligand 1-selected advanced non–small-cell lung cancer (BIRCH). *J. Clin. Oncol.* **35**, 2781–2789 (2017).
220. Haratani, K. et al. Tumor immune microenvironment and nivolumab efficacy in EGFR mutation-positive non-small-cell lung cancer based on T790M status after disease progression during EGFR-TKI treatment. *Ann. Oncol.* **28**, 1532–1539 (2017).
221. Chowell, D. et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science* **359**, 582–587 (2018).
222. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2018).
223. D'Angelo, S. P. et al. Avelumab in patients with previously treated metastatic Merkel cell carcinoma: long-term data and biomarker analyses from the single-arm phase 2 JAVELIN Merkel 200 trial. *J. Immunother. Cancer* **8**, e000674 (2020).
224. Janjigian, Y. Y. et al. Genetic predictors of response to systemic therapy in esophagogastric cancer. *Cancer Discov.* **8**, 49–58 (2018).
225. Kim, S. T. et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat. Med.* **24**, 1449–1458 (2018).
226. Ott, P. A. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **547**, 217–221 (2017).
227. Keskin, D. B. et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* **565**, 234–239 (2019).
228. Wang, R. F. & Rosenberg, S. A. Human tumor antigens for cancer vaccine development. *Immunol. Rev.* **170**, 85–100 (1999).

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#### Author contributions

The authors contributed equally to all aspects of the article.

#### Competing interests

D.B.S. serves on the Scientific Advisory Board for Loxo Oncology at Eli Lilly, Pfizer, Scorpion Therapeutics, BridgeBio and Vividion Therapeutics, owned stock at Loxo Oncology at Eli Lilly and Scorpion Therapeutics, and received honoraria from Illumina and Eli Lilly. D.C. declares no competing interests.

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