

# Next-generation sequencing and molecular therapy

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## ABSTRACT

**Cancers contain a plethora of mutations, few of which are critical to maintaining a state of malignancy. With our ever-expanding understanding of the genomic complexity of cancer, potentially actionable biomarkers whose inhibition could cripple cancer growth are increasingly being elucidated. Modern cancer drug development has largely switched from cytotoxic agents to targeted therapies and immunotherapy, with noteworthy success in several cancer types including non-small-cell lung cancer (NSCLC), breast cancer and melanoma. Next-generation sequencing offers high-throughput, widescale genomic interrogation in a far more efficient and affordable manner than previous sequencing methods. This facilitates detection of potentially actionable mutations and fusions for individual patients and contributes to the identification of novel predictive and prognostic biomarkers in a population. Challenges in the technical aspects of biopsy and sequencing, interpretation, and development of targeted therapies against common genomic aberrations will need to be addressed for personalised medicine to become a reality for more patients with cancer.**

## Introduction

Healthy cells become cancerous when accumulated mutations involving key genes allow sustained proliferation with replicative immortality, avoidance of cell death and growth suppression signals, invasion, metastasis and immune evasion. With the advent of cancer genomics, the ability to characterise and treat cancers at the molecular level based on DNA, RNA and protein biomarkers has allowed classification of cancers into more precisely defined subpopulations. Examples of crucial driver mutations that have been successfully targeted include *BRAF* V600E in melanoma and *EGFR* in non-small cell lung cancer (NSCLC).<sup>1,2</sup> The increasing shift toward precision medicine over past 5 years has also seen first-wave approvals of anticancer drugs for 'tumour-agnostic' indications; that is, drugs that can be used to treat any kind of cancer, regardless of tissue of origin.<sup>3</sup> The move toward

more comprehensive genomic profiling using tissue and liquid biopsy next-generation sequencing (NGS) has the potential to identify greater treatment options for patients. As the cost and accessibility of this technology improve and the repertoire of targeted therapies continues to expand, we have the potential to truly move toward an era of personalised anticancer treatment as standard.

## Genomic alterations in cancers

Cancer-causing genomic alterations either enhance the function of pro-growth oncogenes, such as *BRAF*, or abrogate that of tumour suppressor genes, such as *TP53*. Inheritance of these mutations in the germline accounts for a minority of cancers; more commonly, they arise sporadically in somatic cells. The minimum number of such mutations required to facilitate carcinogenesis is estimated between two and eight,<sup>4</sup> but most cancers contain thousands

## Key points

Cancers are characterised by a wide variety of mutations, most of which are non-pathogenic. Identifying and targeting critical driver oncogenes can be a highly effective treatment strategy.

The advancement of DNA sequencing technologies to include next-generation sequencing (NGS) facilitates comprehensive profiling of the cancer genome to identify potential targetable alterations.

There is heterogeneity within the tumoral genome between deposits and over time. These may be captured through liquid biopsy, which is complementary to tissue biopsy.

Identical genomic alterations do not necessarily confer the same sensitivity to targeted therapy across different tumour types.

Systemic anticancer therapy is progressively moving toward precise molecular, in addition to histologic, characterisation of tumours as standard. Genomic profiling is increasingly directing treatment.

**KEYWORDS:** cancer genomics, targeted therapy, next-generation sequencing, liquid biopsy

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of genomic alterations, most of which are nonpathogenic. The four common types of alteration are point mutations, insertion/deletion, copy number variations (CNV) and rearrangements (also known as fusions or translocations). Epigenetic changes can also cause aberrant expression of crucial genes. The reliance of a cancer on an individual pathogenic mutation varies, from 'oncogene-addicted' *BRAF*-mutant melanomas to lung cancers containing a large number of smoking-induced mutations, the individual inhibition of which might be insufficient to cripple tumoural growth.

Exposure to carcinogens, congenital or acquired dysfunction of DNA repair proteins, age and other factors can result in discrete, predictable patterns of widespread mutation within the cancer genome, a genomic 'signature', which could have prognostic and predictive properties. For example, sporadic or inherited deficiency in DNA mismatch repair (MMR) proteins compromises the ability to adequately repair DNA damage, manifesting as an accumulation of mutations in 'microsatellite' regions of the genome. This translates into a high burden of mutated, neoantigenic proteins potentially recognisable by the immune system. Such microsatellite instability-high (MSI-H) tumours have demonstrated enhanced responsiveness to immune checkpoint inhibitors.<sup>5,6</sup> The Cancer Genome Atlas (TCGA) has comprehensively profiled over 20,000 cancers and matched normal samples across 33 tumour types,<sup>7</sup> elucidating hitherto unrecognised distinct genomic subgroups in several cancers, with implications for response rates to existing and emerging therapies.

### Genomic profiling strategies

Sanger sequencing has been the conventional standard for detecting DNA mutations at predetermined loci, but the method can only analyse one sequence at a time and is inefficient for wide-scale genomic interrogation. NGS has increased the throughput of DNA sequencing by a factor of several hundred thousand.<sup>8</sup> Bases are mapped temporally rather than spatially, whereby each string of replicated DNA contributes to the qualification of hundreds of consecutive bases, allowing large numbers of DNA sequences to be analysed in parallel across a microarray.<sup>9</sup> The pioneering Human Genome Project used Sanger sequencing to characterise a near-complete human genome for the first time, taking multiple laboratories 13 years.<sup>10</sup> With modern NGS, a large panel of target genes, the entire coding region of the genome (whole-exome sequencing, WES) or even the entire genome (whole-genome sequencing, WGS) can be sequenced within days to weeks at a cost point of a few hundred pounds for a large DNA panel. Transcriptome or RNA sequencing analyse only the coding regions of the genome and are better suited to detecting gene rearrangement; however, RNA is inherently less stable than DNA.

### Targeted therapies

Targeted therapy has continued to revolutionise the treatment of both solid tumours and haematologic malignancies since the approval of imatinib for chronic myeloid leukaemia in 2001, after it was shown to dramatically improve outcomes through inhibition of the characteristic BCR-ABL fusion gene product of this cancer.<sup>11</sup> A plethora of research in, and drug development against, potentially targetable oncogenes has ensued, and the list of actionable mutations continues to expand (Table 1), with

NSCLC at the helm. Given that suppression of an oncogene is simpler than restoration of a lost tumour suppressor gene, most therapies use the former strategy. Targeted drugs in the form of monoclonal antibodies (mAbs) against growth factor receptors and small-molecule kinase inhibitors of intracellular signalling enzymes have yielded successful results, although the magnitude of benefit varies with different targets and tumour types, from response rates in excess of 80% for first-line osimertinib in NSCLC with *EGFR* exon 19 deletion or L858R point mutation<sup>2</sup> to 28% with mobocertinib for cases with the rarer and more resistant *EGFR* exon 20 insertion.<sup>12</sup>

Dual blockade of the HER2 oncoprotein with trastuzumab and pertuzumab together with chemotherapy yields pathological complete response rates in excess of 50% in modern neoadjuvant trials in breast cancers demonstrating HER2 amplification or overexpression.<sup>13,14</sup> Targeted therapies do not necessarily show equal agnostic efficacy against different tumour types; these drugs are somewhat less effective in gastroesophageal cancers with the same alteration.<sup>15–18</sup> Along with these mAbs, several HER2-targeting tyrosine kinase inhibitors have been developed, as well as the antibody–drug conjugates trastuzumab-emtansine and trastuzumab-deruxtecan. The latter demonstrated impressive activity in HER2-positive colorectal, NSCLC, gastroesophageal and breast cancers,<sup>19–21</sup> and is effective in tumours with lower or less consistent HER2 expression than required for response to trastuzumab.<sup>22</sup>

Approximately 40% of cutaneous melanoma cases are driven by a point mutation in the *BRAF* oncogene, against which dual targeting of *BRAF* and the downstream signalling protein MEK yields exceptionally reliable and rapid responses.<sup>1</sup> Recently, tebentafusp, a novel bispecific drug incorporating targeted and immunotherapy approaches, was the first to show tumour-specific survival benefit in uveal melanoma, reducing the risk of death by nearly a half compared with physician's therapy of choice.<sup>23</sup> Tebentafusp comprises a T cell receptor targeted against melanoma marker gp100, fused to the  $\zeta$  domain of the CD3 co-stimulator to engage and activate endogenous T cells.

### Challenges

Although tremendous advances have been made over the past few decades, the long path ahead to precision medicine-based oncology as standard is fraught with challenges relating to accessibility, tissue procurement and interpretation, and furthering development of novel targeted therapies. Performing NGS requires a competent laboratory as well as various specialists trained in obtaining and preparing tissue and interpreting results, generally through a Molecular Tumour Board. Access to these highly specialised services is variable outside of major metropolitan areas. The time required to return results is often measured in short weeks, which might be unsuitable for patients presenting in visceral crisis requiring urgent commencement of directed therapy. In most cases, testing requires an invasive biopsy containing sufficient DNA. Although the cost of comprehensive genomic profiling has fallen, the cumulative financial burden of testing all patients routinely must be balanced against the expected yield of actionable results. Hierarchical testing, whereby rarer mutations are only tested subsequent to a more common, mutually exclusive mutation being ruled out, is cost-efficient but this serial rather than parallel approach creates issues around availability of tissue and can add significantly to turnaround time.

**Table 1. Selected targetable biomarkers in solid tumours**

Biomarker	Alteration	Targeted therapies	Drug type	Tumour types with licensed indication
<i>ALK</i>	Rearrangement	Alectinib, brigatinib, lorlatinib, crizotinib	SM	NSCLC
<i>BRAF</i> V600E	Point mutation	Dabrafenib + trametinib (MEK inhibitor), vemurafenib + cobimetinib, encorafenib + binimetinib	SM	Melanoma, NSCLC, tumour agnostic (dabrafenib + trametinib)
<i>EGFR</i>	Deletion, point mutation	Erlotinib, gefitinib, afatinib, osimertinib	SM	NSCLC
<i>FGFR2</i>	Rearrangement	Pemigatinib, infigratinib <sup>b</sup>	SM	BTC
<i>FGFR3</i>	Rearrangement	Erdafitinib	SM	Urothelial <sup>b</sup>
FR $\alpha$	Overexpression <sup>a</sup>	Mirvetuximab soravtansine	ADC	Ovarian
<i>HER2/ERBB2</i>	Amplification, overexpression	Trastuzumab, pertuzumab Tucatinib, neratinib, lapatinib Trastuzumab deruxtecan	mAb SM ADC	Breast, oesophagogastric (trastuzumab only) Breast Breast, gastric/GOJ <sup>b</sup> , NSCLC <sup>b</sup>
HRD	Numerous alterations lead to this phenotype e.g. <i>BRCA1</i> loss, <i>ATM</i> loss	PARP inhibitors e.g. olaparib, niraparib, rucaparib, talazoparib	SM	Ovarian, breast, prostate
<i>IDH1</i>	Point mutation	Ivosidenib	SM	BTC <sup>b</sup>
<i>KIT</i>	Point mutation, deletion	Imatinib, sunitinib, avapritinib, regorafenib	SM	GIST
<i>KRAS</i> G12C	Point mutation	Sotorasib, adagrasib <sup>b</sup>	SM	NSCLC
<i>MET</i>	Amplification, skip mutation	Capmatinib <sup>b</sup> , tepotinib	SM	NSCLC
MSI-H	Point mutation, deletion, epigenetic	Immune checkpoint inhibitors eg nivolumab, pembrolizumab, ipilimumab,	mAb	Tumour-agnostic
Nectin-4	Overexpression <sup>a</sup>	Enfortumab vedotin	ADC	Urothelial
<i>NTRK</i>	Rearrangement	Entrectinib, larotrectinib	SM	Agnostic
<i>PDGFRA</i>	Point mutation	Avapritinib	SM	GIST
<i>PIK3CA</i>	Point mutation	Alpelisib	SM	Breast
<i>RET</i>	Rearrangement	Selpercatinib, pralsetinib	SM	Thyroid, NSCLC
<i>ROS1</i>	Rearrangement	Crizotinib, entrectinib	SM	NSCLC
TF	Overexpression <sup>a</sup>	Tisotumab vedotin	ADC	Cervical <sup>b</sup>
Trop-2	Overexpression <sup>a</sup>	Sacituzumab govitecan	ADC	Breast, urothelial <sup>b</sup>
<i>VHL</i>	Point mutation, insertion/deletion, rearrangement, epigenetic	Belzutifan (blocks downstream HIF2 $\alpha$ )	SM	RCC in Von Hippel Lindau disease

ADC = antibody–drug conjugate; BTC = biliary tract cancer; CRC = colorectal cancer; FR $\alpha$  = folate receptor alpha; GIST = gastrointestinal stromal tumour; HCC = hepatocellular carcinoma; HNSCC = head/neck squamous cell carcinoma; HRD = homologous recombination repair deficiency (genomic signature); mAb = monoclonal antibody; MSI-H = microsatellite instability-high (genomic signature); MMR = mismatch repair; NSCLC = non-small cell lung cancer; SM = small-molecule inhibitor; TF = tissue factor.

<sup>a</sup>Targeted therapy approved regardless of biomarker status. Biomarker not routinely tested.

<sup>b</sup>Approved by US Food and Drug Administration.

The genome of a cancer evolves over the course of the disease, particularly in response to the selection pressure of anticancer therapy. As such, there is both spatial and temporal heterogeneity in the complement of mutations seen in a patient's cancer. A

biopsy from a single site at a single point in time might not be representative of the disease as a whole. Liquid biopsy, a blood test for circulating tumour DNA (ctDNA), is likely to become a vital tool facilitating sampling of DNA shed from widespread

metastatic sites simultaneously, and allowing easy serial re-collection on disease progression to detect acquired resistance mutations without repeated invasive tissue biopsies.

When true tumoural mutations are detected, pathogenic drivers must be distinguished from incidental passenger mutations. The understanding of the pathological, prognostic and predictive roles of each biomarker is convoluted and dynamic, and the presence of a known driver mutation does not guarantee the availability of a targeted therapy. Some of the most common cancer-causing alterations, including *KRAS* and *TP53*, have been notoriously difficult to drug. Although remarkable success has been realised with several of the aforementioned targeted strategies, many more putative biomarkers fail to predict therapeutic activity in early-phase trials,<sup>24,25</sup> outlining the need for further biomarker characterisation and drug development.

### Future directions

As cancers are increasingly divided into molecularly defined, homogenous subgroups, individual populations shrink and recruiting to large phase III trials exclusive to patients with rarer mutations can be difficult. In such populations, in which a novel targeted agent has shown impressive response rates in early-phase trials, a new paradigm of drug approval based on phase I or II trial data has emerged. In 2011, the US Food and Drug Administration (FDA) approved the anaplastic lymphoma kinase (ALK) inhibitor crizotinib for NSCLC with ALK rearrangement after a single-arm phase I study of 82 such patients demonstrated a response rate of 57%, superior to that seen with conventional platinum-doublet chemotherapy.<sup>26</sup> Issues around regulatory approval using small numbers of patients in a study without appropriate controls are challenging and there is likely to be increasing reliance on real-world evidence to provide data for regulatory and funding authorities.

The increasing availability of NGS and enthusiasm for developing new targeted therapies have seen the emergence of multi-arm, biomarker-directed basket and umbrella trials seeking to detect efficacy signals in multiple biomarkers in parallel, such as the National Lung Matrix and FOCUS-4 trials in NSCLC and colorectal cancer, respectively. Testing rates will escalate in coming years as NHS England strives to facilitate access to NGS as routine for patients with cancer and rare conditions, having established a centralised network of seven Genomic Laboratory Hubs.<sup>27</sup> We are not yet at the stage where most patients with cancer will return actionable results, but the vast amounts of biomarker data collected will contribute to the identification of further prognostic and predictive markers for ongoing drug development, potentially heralding an era of personalised medicine in oncology. ■

### Declaration of interests

C Morton has received honoraria from MSD. D Sarker reports personal fees for honoraria/advisory boards/consulting from MSD, Bayer, Eisai, AstraZeneca, Surface Oncology, Sirtex Medical, Roche, and AAA; nonfinancial support from MiNA Therapeutics and Medivir; and grants from UCB and Inspirata. P Ross reports personal fees for honoraria/advisory boards/consulting from Amgen, AstraZeneca, Eisai, Merck, Sirtex Medical, Boston Scientific, Roche, Servier. Non-financial support from HMP Education and Oncosil and grants from Sanofi and Sirtex medical.

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