Novel therapeutic targets on the horizon for lung cancer

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Lung cancer is a leading cause of cancer-related mortality worldwide, and is classically divided into two major histological subtypes: non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Although NSCLC and SCLC are considered distinct entities with different genomic landscapes, emerging evidence highlights a convergence in therapeutically relevant targets for both histologies. In adenocarcinomas with defined alterations such as *EGFR* mutations and *ALK* translocations, targeted therapies are now first-line standard of care. By contrast, many experimental and targeted agents remain largely unsuccessful for SCLC. Intense preclinical research and clinical trials are underway to exploit unique traits of lung cancer, such as oncogene dependency, DNA damage response, angiogenesis, and cellular plasticity arising from presence of cancer stem cell lineages. In addition, the promising clinical activity observed in NSCLC in response to immune checkpoint blockade has spurred great interest in the field of immunooncology, with the scope to develop a diverse repertoire of synergistic and personalised immunotherapeutics. In this Review, we discuss novel therapeutic agents for lung cancer that are in early-stage development, and how prospective clinical trials and drug development may be shaped by a deeper understanding of this heterogeneous disease.

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Introduction

Regardless of histological subtype, most patients with lung cancer present with advanced-stage disease, in which systemic treatment interventions are largely palliative.[1] Even in patients with early-stage resectable or locally advanced disease receiving definitive chemo-radiation, up to 90% of patients eventually relapse.[2] Although non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) bear a distinct clinical course, the classification has been largely constrained by management approaches in a prior era dominated by platinum-based chemotherapy, radiotherapy, or best supportive care.[3] The past decade has seen the introduction of second-generation and third-generation cytotoxics, as well as the use of anti-angiogenic therapies in combination with chemotherapy; together, these have marginally improved median overall survival of advanced NSCLC to 12 months (figure 1).[4–6] However, one of the most important therapeutic advances has been the identification of distinct molecular subsets amenable to targeted therapies, as well as the early success of immune checkpoint inhibitors.[7–10] These approaches show how therapeutic vulnerabilities can be identified on an individual patient basis, and have resulted in the integration of genomic and protein-based biomarker testing into clinical management algorithms to facilitate selection of optimal treatment.

Collective efforts by international sequencing consortiums, such as The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium, coupled with the pervasive use of next-generation sequencing technologies within hospitals and laboratories, have made it possible to stratify patients into actionable subgroups at diagnosis and following treatment failure. The initial clinical experience in screening for and targeting specific somatic alterations in advanced NSCLC has provided a unique opportunity to leverage future drug development efforts, overcoming the hurdles imposed by population differences in biomarker prevalence,[11] the varying incidence of tractable targets for specific phenotypes (eg, histological subtypes),[12] and detailed characterisation of the determinants of acquired resistance.[13]

Key traits in NSCLC and SCLC have been revealed through a greater appreciation of the genetic landscape, improved preclinical modelling of disease, and clinical insights from the vulnerabilities to targeted therapeutics (figure 2). The increased collaborative culture between clinicians, scientists, industry, and regulatory authorities has resulted in a new generation of science-driven translational clinical trials, alongside the rapid implementation and adoption of new technology (eg, point-of-care sequencing), thus accelerating the pace of drug development. Consequently, there is a rich pipeline of therapeutic candidates that need to be efficiently evaluated and prioritised in the context of early clinical trials. In this Review, we discuss some of the key developments and advances in the predominant subtypes of lung cancer, as well as how the design of prospective clinical trials and drug development can be informed by a deeper understanding of the biology of this disease.

What defines an ideal target?

The simplest definition of an ideal target is one that leads to the elimination of cancerous cells with a high therapeutic index and wide therapeutic window. This ability is dependent on the ratio between the dominance of the trait against the normal physiological function of the target, as well as the selectivity of a pharmacokinetically favourable compound. Drug efficacy (in vitro and in vivo) often forms the basis for clinical testing, with prerequisite demonstration of therapeutic effect in at least two or more cell line and xenograft models.[14] However, because not all therapeutic modalities can be readily or satisfactorily evaluated in vivo, consideration should be given to appraisal of the representativeness of the experimental model system. For example, preclinical modelling for treatments that use immune-mediated mechanisms for cell type-specific

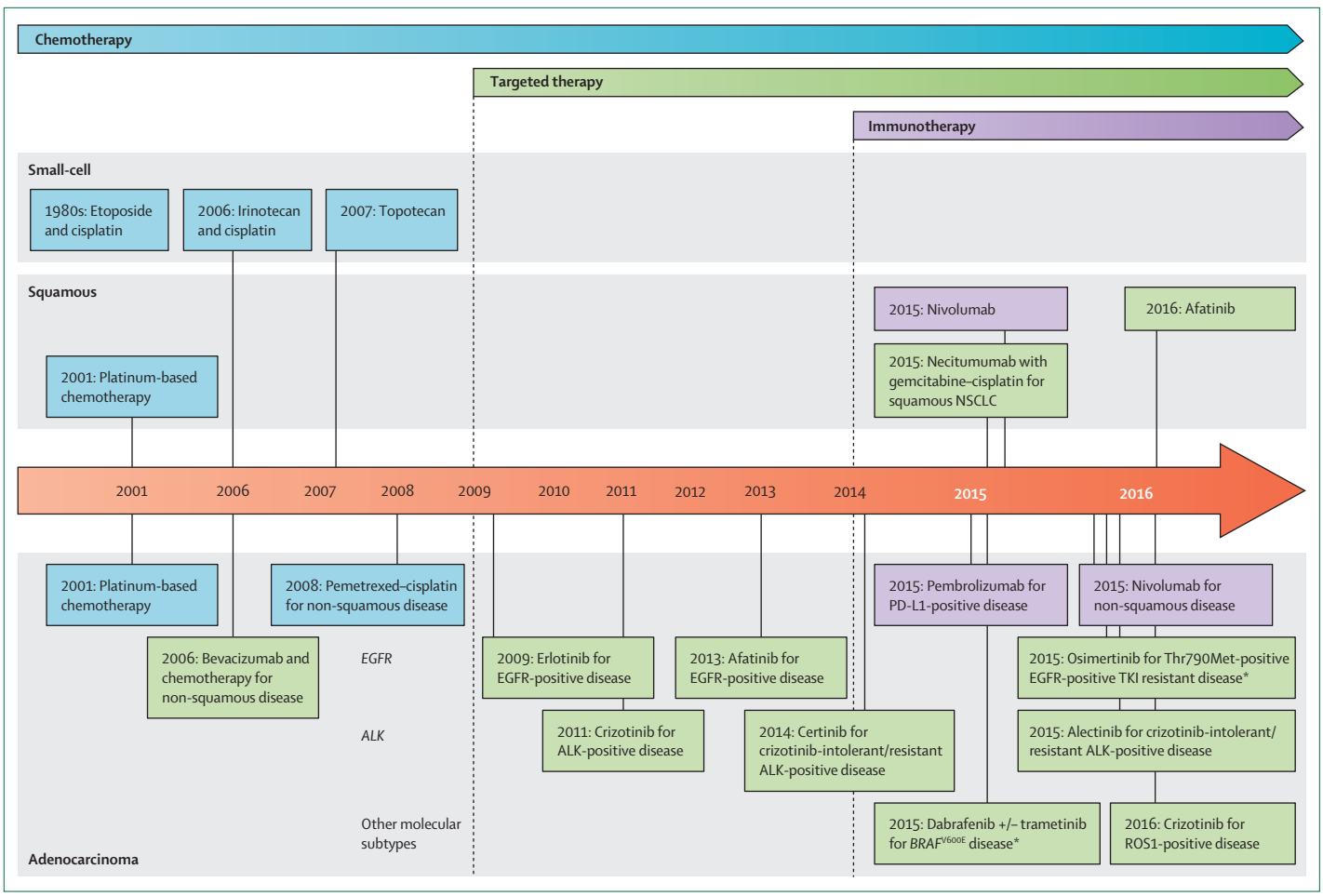

Figure 1: **Timeline of key therapeutic advances for advanced-stage disease in predominant histological subgroups of lung cancer**
NSCLC=non-small-cell lung cancer. TKI=tyrosine-kinase inhibitor. *US Food and Drug Administration (FDA)-designated breakthrough therapy.

elimination is particularly challenging because non-human host immunity might not be representative of human physiology. Nevertheless, studies in mice have been instrumental in the development of the mechanistic understanding and identification of immunological targets such as CTLA-4 and PD-1.[15] An integral part of this challenge is the development of a stable mouse model that can be used for the prediction of personalised immunotherapeutic outcomes. Immunocompetent humanised mouse model systems do not sufficiently recapitulate all the complex immunological parameters needed for the study of normal HLA-restricted antigen-specific T-cell responses.[16] Despite the limitations of xenograft models in the evaluation of certain therapeutic classes, patient-derived models and co-clinical xenograft trials have provided a more robust preclinical basis for entry into clinical testing.[17]

The presence of a selection biomarker specific to the putative mode of action of any novel therapeutic agent has emerged as an important requisite. With an increased ability to sequence the cancer genome at a large scale, any uncharacterised mutations can now be readily modelled with CRISPR technology, thus providing insights into their contributions toward disease progression and their value as predictive genomic biomarkers or drug development targets. Unbiased functional screens using an appropriate phenotypic readout are another powerful tool to rapidly uncover efficacious drugs or facilitate biomarker discovery.[18]

Most predictive biomarkers to date consist of single genomic or protein alterations, and have been implemented in conjunction with the first wave of successful targeted therapies in NSCLC. Importantly, emerging targets should have an accompanying plan for co-developing a companion diagnostic, and the performance of an analytically validated fit-for-purpose assay increasingly constitutes a cornerstone for success in early-phase trials. Once established, retrospective analyses of a specific biomarker should be performed on archival samples of different cancer types, providing molecular epidemiological data that can inform optimal trial design and enrolment strategies.

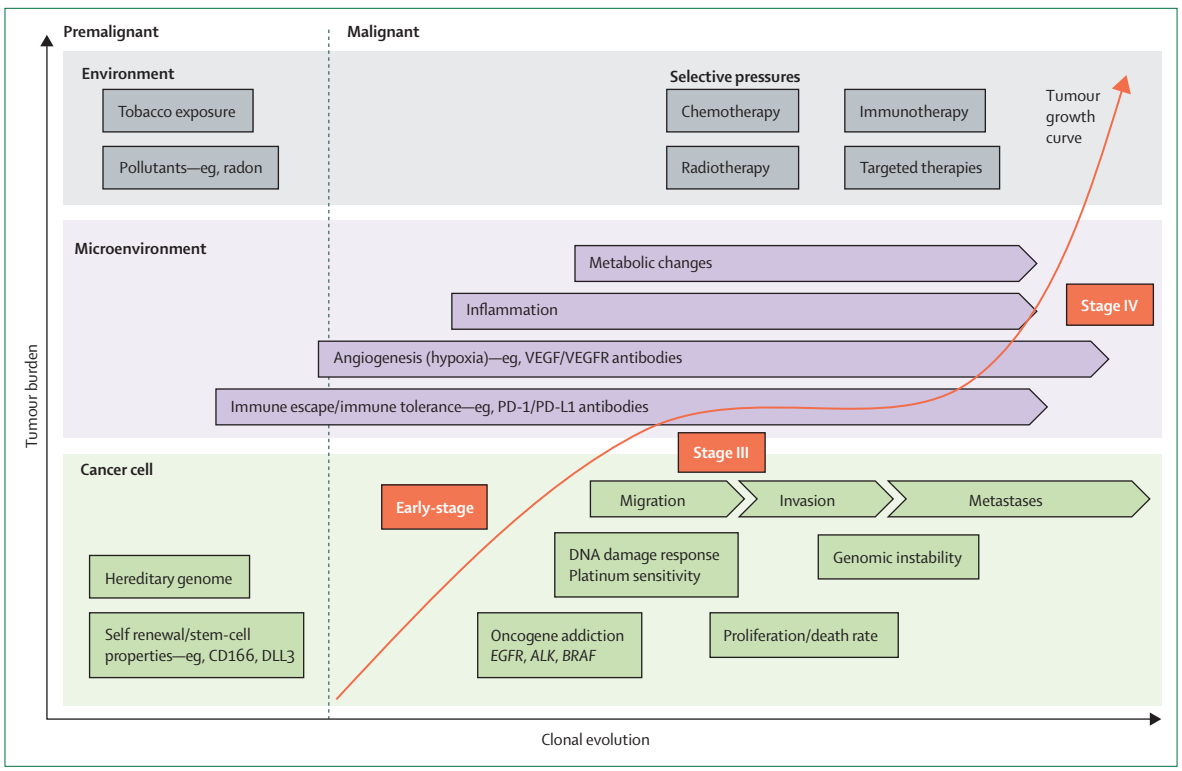

Figure 2: **Key therapeutically relevant hallmarks of lung cancer**
The process of tumorigenesis occurs through acquisition of mutational alterations induced by environmental insults like smoking, pollutants, metabolic changes, targeted therapies, and chemotherapy, which exert selective pressures on the cell. Clonal evolution of the tumour is additionally driven by key traits of lung cancer (oncogene-induced growth, angiogenesis, stem-cell-like properties, DNA damage response defects). Eventual metastases will result from increasing tumour burden with dynamic fluxes in dominant traits with disease progression.

Because predominant cancer traits can evolve over time, fresh tissue biopsies are required in many contemporary trials. Therefore, with a diverse repertoire of mechanism-based interventions, due consideration should be given to the identification of specific windows of opportunity for the evaluation of activity of novel agents, which in turn need to be measured through clinical or science-based endpoints suited to the mode of action.

Identification of genomic targets

Many therapeutically tractable genomic lesions comprise of gain-of-function alterations that result in structural modifications and subsequent pathway activation. These alterations include somatic point mutations in kinase domains, chromosomal rearrangements, or focal amplification of a genomic region. Regardless of the mechanism of activation, considerable enthusiasm exists for the development of targeted therapies, even for rare molecular subsets (table). An alternative approach exploits synthetically lethal interactions between a therapeutic agent and one or more separate genomic alterations.[38] With this approach, each individual alteration is non-lethal in isolation (and could facilitate tumour development—eg, in loss of a tumour suppressor gene such as *BRCA1*). However, co-occurrence of a second hit, either through a genomic event or exposure to a drug, results in cell death.

Comprehensive large-scale profiling of lung cancers has systematically identified substantially altered genes (table). Many alterations do not represent tractable targets, and the sensitivity for the identification of driver genes is a function of the frequency of alterations and number of background mutations. Therefore, cancer types with a high mutational burden, such as smoking-related lung cancers, require thousands of samples to be sequenced before sufficient sensitivity can be reached for the identification of low-frequency driver genes.[39] In addition, the increasing number of multidimensional datasets has facilitated comparisons across different cancer types. In a TCGA multiplatform analysis,[40] 3527 specimens from 12 cancer types were reclassified into 11 major tumour histology agnostic subtypes. For example, a subset of squamous-cell lung carcinoma clustered together with other aerodigestive and bladder cancers of epithelial origin,[40] providing a rationale to define druggable subsets across cancer types.

Targeting of oncogenic drivers: clinical insights

The application of targeted therapies in two of the most common genomic drivers in NSCLC—*EGFR* mutations and *ALK* rearrangements—has been instructive. In both molecular subgroups, the proportion of patients who achieved a response was as high as 70% in pivotal phase 3 trials,[7,8] setting a benchmark for clinically effective

	Frequency (%)			Approved drugs and selected agents in development
	Adenocarcinoma[19-22]	Squamous-cell carcinoma[23]	Small-cell carcinoma[24]	
TP53	35·1–61·4%	81%	93·6%	AZD 1775 (NCT02593019, NCT02513563)
EGFR	14·3–39·6%	0%	0%	Erlotinib, gefitinib, afatinib (FDA-approved), osimertinib (Thr790Met) (FDA-approved, NCT02151981)
KRAS	14·9–32·6%	0%	0%	NA
MEK1 (*MAP2K1*)[25]	<1%	<1%	0%	MEK 162/binimetinib (NCT01859026), VS-6063/defactinib (NCT01951690), trametinib (NCT02642042, NCT02258607), AZD6244/selumetinib (NCT02583542), cobimetinib (NCT02457793)
RB1	3·3–4·3%	7%	80%	Palbociclib (NCT01291017)
ALK (fusion)[26]	3–13%	NA	NA	Crizotinib, ceritinib (FDA-approved), alectinib (NCT01801111), X-396 (NCT01625234), brigatinib (NCT02737501), PF-06463922 (NCT01970865)
MYC[26]	31%	Rare	16%	MLN8237/alisertib (NCT02038647), OTX105/MK-8628 (NCT02259114), BMS-986158 (NCT02419417)
FGFR1 (amp)[27,28]	1%	20%	5·6%	BGJ 398 (NCT01004224), TKI258/dovitinib (NCT01676714), nintedanib (NCT01948141), BAY1163877 (NCT01976741), GSK3052230 (NCT01868022), AZD4547 (NCT02154490)
RET[26]	1–2%	NA	NA	Cabozantinib (NCT01639508), vandetanib (NCT01823068), lenvantinib (NCT01877083), AP24534/ponatinib (NCT01935336)
MET[29]				
Amplification (de novo)	1–4%	0%	0%	XL184/cabozantinib (NCT01639508, NCT02132598)
Amplification (EGFR TKI-resistant)	10–20%	0%	0%	INC280/capmatinib (NCT02414139, NCT01911507)
Exon 14 skipping[30]	3–4%	0%	0%	MSC2156119J/tepotinib (NCT01982955), AZD6094/volitinib (NCT02374645, NCT02143466), MGCD265 (NCT02544633), crizotinib (NCT00585195)
PTEN	2·2%	8%	10%	Buparlisib (NCT01297491)
PIK3CA	..	..	..	Buparlisib (NCT01297491)
Mutation	4·4–6·9%	16%	0%	BYL719/alpelisib (NCT02276027)
Amplification[31-34]	2–9%	30–40%	5%	GDC-0032/taselisib (NCT01862081), AZD8186 (NCT01884285), IPI-549 (NCT02637531), LY3023414 (NCT02443337), AZD2014 (NCT02403895, NCT02664935)
BRAF[35]	1–2% (1% *BRAF*^{V600E})	0%	0%	Dabrafenib (NCT01336634), LGX818 (NCT02109653), vemurafenib (NCT02314481)
ROS1	2%	0%	0%	Crizotinib (FDA-approved), PF-06463922 (NCT01970865), AP26113 (NCT01449461)
NTRK1[36,37]	0·1–3·3%	0%	0%	LOXO-101 (NCT02576431), PLX7486 (NCT01804530), entrectinib (NCT02568267, NCT02097810)
HER2	..	..	..	Afatinib (NCT02369484, NCT02597946)
Mutation	2–4%	0%	0%	Pyrotinib (NCT02535507)
Amplification[26]	5–10%	0%	0%	AP32788 (NCT02716116)
DDR2[26]	0%	3·8%	0%	NA
IGFR1[26]	0%	0%	95%	BI 836845 (IGF/IGFR pathway inhibitor) (NCT02191891)

FDA=US Food and Drug Administration.

Table: **Frequencies of gene alterations across lung cancer subtypes, and targeted agents**

targeted therapies. Perhaps the most striking feature is the seemingly universal emergence of drug resistance, either through a secondary mutation that negatively affects drug binding, or by invoking alternative bypass pathways. For example, in acquired resistance to first-generation EGFR tyrosine-kinase inhibitors (TKIs), sustained EGFR signalling can be achieved through clonal expansion of cells harbouring a Thr790Met mutation, activating bypass pathways (eg, *MET* amplification), or transformation into other histological types.[41] Data for ALK TKI resistance further highlight the possibility of differing resistance landscapes emerging from first-generation and second-generation ALK inhibitors. While the Leu1196Met mutation in the ALK kinase domain is a common resistance mechanism to crizotinib, and can be overcome by second-generation ALK inhibitors like alectinib and ceritinib, resistance to second-generation agents can still subsequently ensue with a Gly1202Arg mutation.[42] Thus, the pharmacological activity of novel agents and their associated off-target effects impose varying degrees of selective pressures that dictate how a tumour might navigate the drug resistance or cancer cell fitness landscape.

Another important observation is the ability for more potent or mechanism-specific TKIs to elicit significant antitumour responses. For instance, the development of a wild-type-sparing Thr790Met mutation-specific EGFR TKI demonstrated an overall response of 59% on the basis of two single-arm studies (AURA extension [NCT01802632] and AURA2 [NCT02094261]), resulting in

the accelerated approval of osimertinib by the US Food and Drug Administration (FDA) in November, 2015. However, similar to first-generation EGFR TKIs, resistance mechanisms specific to osimertinib can arise—eg, a novel Cys797Ser *EGFR* mutation, which affects the covalent binding site of third-generation TKIs.[43] Whether the use of later-generation inhibitors in the first-line setting will achieve more durable disease control compared with first-generation or second-generation EGFR TKIs or induce alternative resistance mechanisms remains unclear. A key priority is to define the optimal sequence and combinations of available targeted therapies to circumvent drug resistance. Several first-line trials are examining the role of a second-generation ALK inhibitor alectinib against crizotinib (NCT02075840), and third-generation Thr790Met inhibitors against erlotinib or gefitinib (NCT02296125).

Descriptions of transformation of adenocarcinoma to squamous-cell and small-cell histologies after development of TKI resistance provide insights into the potential for plasticity of cancer stem cells. The morphological differences between adenocarcinoma, squamous-cell carcinoma, and small-cell carcinoma were previously attributed to specific cell-of-origins, in which NSCLCs are thought to have originated from bronchioloalveolar stem cells or alveolar type II cells, whereas SCLCs may have originated from neuroendocrine cells.[44] Recent data have suggested a key role for loss of Rb in small-cell transformation,[45] highlighting how histology can be dictated by genomic events. This finding is supported by conditional mouse models in which inactivation of *TP53* and *RB1* using adenoviral vectors targeting Cre recombinase in different lung epithelial cells show preferential formation of SCLC from neuroendocrine cells.[46] Therefore, under sufficient selective pressures, the emergence of new genetic or epigenetic events can result in late phenotypic changes, suggesting the presence of a multipotent stem cell-of-origin within the stem cell niches, even in established tumours. These findings are consistent with the ability of specific genetic lesions to influence the fate of a common cell-of-origin. For instance, *KRAS* and *TP53* mutations result in the development of lung adenocarcinoma, whereas the disruption of *RB1* and *TP53* promotes SCLC formation.[47]

Targeting of low-frequency driver alterations in the clinic has been illuminating. $BRAF^{V600E}$ (ie, Val600GLu) mutations and *NTRK* and *ROS1* rearrangements are infrequent alterations (approximately 1% across adenocarcinoma [table]) for which the quality of clinical responses to target-specific TKIs has varied. Equally brisk and durable responses have been observed when drugging low-frequency drivers (as compared with a common driver such as *EGFR* mutations), highlighting the fact that oncogenic potential does not necessarily correlate with the population prevalence of an alteration. By contrast, genomic alterations that can appear to be genuine drivers in preclinical models (eg, *PIK3CA* mutations) might not necessarily translate into actionable targets in the clinic due to the genomic context in which they are found. *PIK3CA* mutations are often found as co-occuring driver alterations, or occur as a subclonal event.[48,49] Thus, the context in which alterations are acquired—ie, determined by environmental exposure, individual risk factors (hereditary genome), cell-of-origin (epigenetic factors), and the genetic context of early (clonal) versus late (subclonal) alterations—ultimately dictates the therapeutic vulnerability of the target and drug response.[7,8,50,51] In support of this notion, no significant clinical activity was observed in the BASALT-1 trial (NCT01297491),[50] in which patients with NSCLC selected for *PIK3CA* mutations and PTEN loss were treated with the pan-class I PI3K inhibitor buparlisib. This trial, which terminated early due to lack of efficacy, showed overall response of 3·2% (two of 63 patients), and 12-week progression-free survival of 20·0% for the squamous group and 23·3% for the adenocarcinoma group.

Emerging targets in other oncogenic drivers

KRAS mutations are one of the most common mutations in NSCLC (adenocarcinomas) and while previously thought to be non-druggable, several promising strategies are now in development. Targeting of downstream pathways with MEK inhibitors (eg, selumetinib and trametinib) has yielded encouraging results, with the combination of selumetinib with docetaxel achieving response rates of 36% (16 of 44 patients) versus 0% with docetaxel alone;[52] a phase 3 trial of docetaxel with selumetinib or placebo in *KRAS*-mutant NSCLC is ongoing (NCT01933932). More recently, novel approaches to target GTP-bound RAS have been reported, specifically in the context of *KRAS* Gly12Cys mutations. These direct and indirect strategies include the exploitation of a novel allosteric site in a binding pocket of the mutant cysteine residue, and the discovery of allele-specific inhibitors that block the nucleotide exchange mechanism, trapping KRAS in the inactive state.[53,54]

The clinical experience in targeting oncogenic drivers in squamous-cell and small-cell carcinoma is more limited. In part, the predominant smoking-related aetiology for these histological subtypes and consequential high mutational burden restricts the identification of recurrent substantially mutated genes. Nevertheless, some tractable targets are observed across major histological subtypes. For example, activation of the MET/HGF pathway has been described in adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma, and can be activated through diverse mechanisms, including MET amplification and exon 14 skipping mutations. MET amplification (defined as ≥6 copies by FISH) has been reported in 2–4% of de-novo untreated NSCLC and in up to 20% of EGFR TKI-resistant NSCLC,[29,55–58] whereas MET exon 14 skipping mutations are present in 3–4% of lung

adenocarcinoma cases.[30] Published data remain limited, however, with only small case series revealing meaningful clinical responses to MET inhibitors.[21,30,59–61]

Similar to MET or HGF signalling, diverse mechanisms of activating the FGFR pathway have been reported. *FGFR1* amplification is one of the commonly adopted selection biomarkers, and in early-stage squamous-cell lung carcinoma has been found to be associated with poor survival and smoking.[62,63] Prevalence of *FGFR1* amplification in squamous-cell lung carcinoma varies in different studies, from 9·7% to 21·1% by single-nucleotide polymorphism (SNP) arrays and 22·2% by FISH,[27,64] whereas the prevalence in adenocarcinoma has been found to be low at 3%.[64,65] Although encouraging clinical activity has been observed in ongoing clinical trials of highly selective FGFR 1–3 inhibitors, such as BGJ398, AZD4547, and PD173074, the development of FGFR inhibitors has faced many challenges, including inconsistencies in molecular screening strategies for FGFR activation (mutations, copy number, translocations, gene expression), definitions of appropriate amplification cutoffs, and management of adverse events related to on-target effects (eg, hyperphosphataemia, paronychia, and haemoptysis). In SCLC, although *FGFR1* amplification has been reported in more than 20% of cases,[66] molecular prescreening has been complicated by a lack of tissue specimens and an insufficient number of patients fit for early-phase trials.

MYC family alterations are also an emerging target, with a prevalence of up to 20% in SCLC.[67] Preclinical studies have revealed SCLC to be particularly susceptible to inhibition of aurora kinases, with activation of MYC family genes being a key determinant.[68,69] In a phase 1 trial,[70] alisertib, a highly specific aurora kinase A inhibitor, demonstrated a response of up to 21% in platinum-refractory SCLC; this finding prompted a randomised phase 2 trial comparing paclitaxel with or without alisertib in second-line SCLC (NCT02038647).

Targeting of tumour suppressors

TP53 is the most commonly altered gene across all lung cancer subtypes, but remains an elusive target. P53 function can be impaired through overexpression of regulatory proteins or inactivating mutations, compromising its critical role in determining cell fate through DNA repair, cell cycle arrest, apoptosis, and senescence. Two broad therapeutic strategies have been used: activation of wild-type P53, most commonly through small molecules that bind to MDM2 by mimicking key residues of P53 (eg, nutilin), and restoration of normal function to mutant P53 (eg, PRIMA-1^{Met} [APR-246]).[71] The latter is achieved through covalently modifying cysteine residues and misfolded P53 proteins (specifically in the context of Arg273His and Arg175His P53 mutations), thereby affecting DNA binding. Preclinical studies have demonstrated significant antitumour effects through induction of apoptosis in *TP53*-mutant murine SCLC models,[71] and in a clinical phase 1 trial[72]—which was conducted primarily in haematological malignancies and prostate cancer—no major adverse events other than fatigue and giddiness were observed.

Given substantial crosstalk between P53 and DNA repair pathways, combinatorial approaches have also been examined in the context of TP53-deficient NSCLC. Stress-activated P38 mitogen-activated protein kinase pathway (MK2) is a crucial component of DNA damage response, and P53 mutant and MK2 deficiency were synthetically lethal in a mouse NSCLC model treated with cisplatin.[73] In a genome-wide short hairpin RNA screen exploiting reactivation of intact functional P53 with nutilin, genetic or pharmacological inhibition of ATM or MET kinase was able to convert the cellular response from cell cycle arrest to apoptosis,[74] underscoring the potential for synthetically lethal strategies in P53-mutant lung cancers. No P53-directed therapies have been approved, which is probably a reflection of the complex biology and diverse roles of P53 in cellular homoeostasis.

Targeting of epigenetic mechanisms

Epigenetic mechanisms of gene regulation generally entail covalent modifications of DNA and histone proteins, such as methylation or acetylation. The ability for transcriptional machinery to access specific DNA loci, or the recruitment of epigenetic regulators, results in different chromatin states influencing gene expression changes.[38] Mutations in epigenetic regulators occur frequently: mutations in *SMARCA4/BRG1* and *ARID1A*, both members of the SWI/SNF complex, are found in 8% and 10% of adenocarcinomas, respectively;[26] mutations in *MLL2,* a histone methyltransferase, is found in 19% of squamous-cell carcinomas; and mutations in *CREBBP/CBP* and *EP300* histone acetyltransferase co-activators are found in 18% of SCLC cases.[26] Unlike somatic alterations in driver genes, one of the attractions in manipulation of epigenetic mechanisms is the potential for reversibility, making them ideal candidates for anticancer drugs.

Histone deacetylases and DNA methyltransferase inhibitors are the most advanced compounds explored in the clinic, although results of single-agent studies (regardless of class) and combinations with chemotherapy have been disappointing. Combinations with targeted therapies are ongoing; of note is a phase 1b trial[75] of vorinostat in combination with gefitinib, chosen specifically to overcome primary resistance to *BIM* deletion polymorphism. Preclinical studies confirmed a dose-dependent increase in expression of the BH3 domain, resulting in marked apoptosis and regression in EGFR-mutant BIM-deleted NSCLC xenograft models.[76] Overall, one of the major challenges has been the lack of selectivity of epigenetic targeting agents, and an incomplete understanding of mechanisms

for synergy in most combinations. For this reason, predictive biomarkers for specific epigenetic inhibitors need to be better defined than they are at present.

Another class of epigenetic targeting agents are directed against chromatin readers. Chromatin readers have specialised domains that bind to covalent modification of nucleosomes and, when mutated, result in the inability to decipher the epigenetic landscape.[38] Bromodomain and extra-terminal (BET) domain proteins, including BRD2, BRD3, BRD4, and BRDT, function as transcriptional co-activators and facilitate target gene transcription.[77] BET bromodomain inhibitors have been found to modulate epigenetic signalling in a cell context-dependent manner with diverse downstream consequences. For example, sensitivity of lung adenocarcinoma cell lines to the BET inhibitor JQ1 may be mediated by the suppression of the oncogenic transcription factor FOSL1, rather than through the MYC pathway.[78] By contrast, SCLC demonstrates higher sensitivity to JQ1, particularly in *MYC*-amplified models.[79] Downregulation of MYC-dependent transcription has also been observed in *KRAS*-mutant *LKB1* wild-type in vitro and in mouse models, highlighting the importance of genomic context even in an epigenetic targeting agent.[80] Several BET inhibitors are currently in early-phase clinical trials, such as OTX015 in advanced solid tumours including NSCLC (NCT02259114), and BMS-986158 in some advanced solid tumours including SCLC (NCT02419417).

Therapeutically relevant hallmarks of lung cancer

The inability to cure oncogene-driven lung cancer despite substantial cytoreduction with highly effective targeted therapies underscores the major limitation of targeting single genetic lesions. The emerging genomic complexity of treatment-naive and treatment-resistant lung cancers has further revealed vast potential for tumour adapation, as well as the diverse phenotypes that can emerge through acquiring multiple somatic alterations.[50,81–83] Furthermore, many genomic alterations commonly co-occur, and pose a striking challenge when genomic-guided therapeutics are to be assigned. Elucidation of cancer phenotypes, or hallmarks, is a complementary approach to targeting of lung cancer.

Exploitation of DNA damage repair

DNA damage response is of interest in lung cancer because of the strong aetiological link with smoking and response to platinum-based chemotherapy. Furthermore, the differences in time to the development of lung cancer in lifelong heavy smokers, and the absence of smoking-related mutational signatures in some patients despite chronic tobacco exposure, suggest individual differences in efficiency of DNA repair.[83,84] DNA damage response is a complex multistep process involving multiple DNA repair proteins, coordination of cell cycle checkpoints, and effectors of cellular fates.[85] This complexity has led to multiple studies examining the clinical relevance of key DNA repair proteins such as *ERCC1*,[86] and even randomised controlled trials stratifying patients on the basis of *RRM1* and *ERCC1* gene expression levels to four different chemotherapy arms.[87] Unfortunately, the interpretation of the results from these studies has been challenging because of the absence of rigorous biomarker data for patient selection, as well as inadequacies in the clinical trials that included suboptimal comparative controls and non-targeted chemotherapy regimens.[88,89] To this end, unbiased high-throughput drug screens have recently identified PARP-1/2 inhibitors as synthetically lethal partners in an isogeneic ERCC1-deficient NSCLC line.[90] In another study that compared reverse-phase protein array profiles between SCLC and NSCLC, PARP1 and E2F1 co-activator targets (eg, EZH2) were significantly increased, corresponding to an increased sensitivity to the PARP inhibitor olaparib in vitro, as well as in combination with etoposide and cisplatin.[91] Multiple trials are ongoing exploring both monotherapy and PARP inhibitors, such as talazoparib; in a phase 1 trial,[92] Response Evaluation Criteria In Solid Tumors (RECIST) confirmed responses in two (18%) of 11 patients with refractory SCLC. Other PARP inhibitors, such as olaparib, are being explored in combination with gefitinib in EGFR-mutant NSCLC,[93] and the addition of veliparib to paclitaxel and carboplatin against chemotherapy alone has suggested improved activity, although non-significantly, particularly in advanced squamous lung cancers.[94]

A major challenge has been the identification of patients with impaired DNA damage response who are therapeutically vulnerable to inhibitors of the DNA repair pathway through synthetic lethality. Genomic sequencing studies can only provide circumstantial evidence to infer the quality of an individual's DNA damage response (eg, mutational burden or mutations in genes involved in DNA repair); similarly, single gene or protein biomarkers provide an incomplete picture. A functional DNA damage response assay would ideally be required to accurately stratify patients.

Targeting of the cell cycle and checkpoint kinases

Cell cycle checkpoint inhibitors, such as CDK4/6 inhibitors, have been explored in lung cancer. In a phase 2 trial[95] of palbociclib (PD0332991) in previously treated patients with advanced NSCLC and inactivated CDKN2A, stable disease was achieved in eight (50%) of 16 evaluable patients. In a phase 1 study[96] comprising of patients with NSCLC, another CDK4/6 inhibitor, abemaciclib (LY2835219), showed a partial response in one (2%) of 49 patients and overall disease control in 25 (51%) patients. In addition, there was a trend towards improved activity in *KRAS*-mutant cases (disease control in 14 [54%] of 26 patients) compared with KRAS-wild-type cases (disease control in seven [37%] of 19 patients).[96] Palbociclib is currently being evaluated in a phase 1/2

trial in combination with the MEK inhibitor, PD0325901, in *KRAS*-mutant patients with NSCLC (NCT02022982), as well as in the biomarker-targeted LUNG-MAP study incorporating a basket-trial design for recurrent squamous-cell lung carcinoma (NCT02154490).

Cell cycle proteins are inextricably linked to DNA damage response, and several inhibitors of checkpoint kinases are being tested in preclinical and clinical trials. Chk1, Wee1, and ATR are key regulators of the G2 checkpoint in the cell cycle, and also regulate CDK activity during S-phase; inhibition results in G2 checkpoint abrogation and contributes to cytotoxic effects of DNA damage. Additional factors such as P53 deficiency, MYC overexpression, *RAS* mutations, and reduced level of the repair protein ERCC1 might enhance efficacy and lead to sensitisation to these inhibitors.[97] Ongoing trials with Chk1 inhibitors include a phase 1 study of single agent LY2606368 in patients with advanced cancers including NSCLC (NCT01115790). Despite Chk1 inhibitors displaying antitumour activity on their own, larger gains could be sought by synergising them with conventional chemotherapy and radiotherapy. Preclinical analyses and a phase 1 trial of the Chk1 inhibitor LY2603618 in combination with pemetrexed and cisplatin have reported a partial response in two (14%) of 14 patients with NSCLC,[98] and a phase 1/2 study in patients with metastatic NSCLC (NCT01139775) is underway.

Other examples of targeting of a checkpoint kinase include the combination of the ATR inhibitor VX-970 with cisplatin and the Chk1 inhibitor AZD7762 with gemcitabine.[99] Several clinical trials are testing the Wee1 inhibitor AZD1775 combined with chemotherapy in the setting of NSCLC (NCT02087241), as well as SCLC with *MYC* amplification or *CDKNA* alterations in P53 mutant context (NCT02688907). Cell cycle-associated kinases such as the polo-like kinases (PLK1-3) and aurora kinases (A, B, and C) are also promising targets for lung cancer therapy. PLK-1 is regarded as the dominant kinase in the family, and overexpression of PLK-1 has been noted in many malignancies, including NSCLC. BI2536 and BI6727 are two PLK-1 inhibitors that are being studied as a single agent or in combination with chemotherapy (eg, pemetrexed). However, the impact of using these inhibitors will depend on the cell cycle state within each cell and varies both across and within individual tumours, restricting the predictive potential of biomarker analysis on bulk tissue from single biopsies.[100]

Targeting of cancer stem-like cells

The observed small-cell transformation in the drug-resistant state lends weight to the hypothesis that rare, cancer stem cell-like fractions exhibit self-renewing capacity and have the potential to differentiate into diverse cancer cell populations.[101] However, the establishment of the presence of this dynamic tumour subpopulation has been challenging, bearing in mind that cancer stem cells are functionally defined. Stem cell markers such as CD133, CD166, CD44, and ALDH1 and signalling pathways such as Hedgehog, Notch, and Wnt pathways have been examined mostly in bulk tumour samples. One important clinical implication of cellular heterogeneity is the diverse resistance mechanisms displayed by specific subsets of tumour cells to therapy. Mounting evidence suggests that therapy-resistant cells are enriched for cancer stem cell-like properties, thus favouring relapse into a more aggressive disease and metastasis.[102–104]

One approach has involved the development of strategies to specifically target cancer stem cell pools. This targeting might involve the ablation or induced differentiation of cancer stem cells to halt tumour growth or promote their sensitivity to front-line therapies. Biotech companies such as Boston Biomedical (Cambridge, MA, USA), Verastem (Needham, MA, USA), and OncoMed (Redwood City, CA, USA) have devoted drug discovery pipelines that focus on cancer stem cell therapeutics: examples include napabucasin (BBI608), defactinib (VS-6063), and demcizumab (a δ-like ligand [DLL4] antibody).[105] Several clinical trials are ongoing to understand the impact of putative cancer stem cell-specific agents in altering disease outcome, such as the phase 1/2 study of napabucasin in combination with immune checkpoint inhibitors in adult patients with advanced cancers (NCT02467361), and another phase 2 trial testing defactinib in patients with *KRAS*-mutant NSCLC (NCT01951690). Demcizumab, which targets a ligand of the Notch receptor, is currently in a phase 1/2 trial for patients with untreated extensive-stage SCLC (NCT01859741). In a recent phase 1 clinical trial of rovalpituzumab—a drug conjugate comprising a DLL3 antibody, a stable linker, and an active cytotoxic payload—a subgroup of DLL3-positive patients with relapsed SCLC showed a partial response in ten (34%) of 29 patients, and stable disease in nine patients (31%).[106] With DLL3 expression observed in up to 70% of SCLCs,[107] this promising approach is under assessment in a phase 2 setting. Therefore, development of cancer stem cell therapeutics represents an area of promising potential.

An alternative approach is targeting of signalling pathways associated with cancer stem cell-like cell fractions.[108–110] Although several driver pathways, such as Wnt and Hedgehog, have been suggested to promote lung cancer stem cell function, evidence for the role of Notch signalling appears to be the strongest.[111–113] Expression analyses have revealed the Notch pathway to be activated in ALDH-positive or CD133-positive cancer stem cells, and pharmacological treatment with a γ-secretase inhibitor has been found to disrupt cancer stem cell activity and confers sensitivity to chemotherapy.[111,114] RO4929097 is a selective γ-secretase small-molecule inhibitor against Notch signalling with antitumour activity shown in NSCLC xenograft models,[115] and was evaluated in patients with advanced NSCLC who

had completed front-line chemotherapy; this phase 2 trial (NCT01193868) was later terminated due to the limited efficacy observed across different cancer types, and cessation of drug production. Another phase 1 trial studying the Notch inhibitor BMS-906024 in patients with advanced solid tumours (NCT01292655) is underway. Nonetheless, the role of cancer stem cell-signalling pathway signatures derived from bulk tumours in informing cancer stem cell content and helping in tailoring novel therapeutics has not been evaluated. Whether the pharmacological inhibition of these pathways disrupts bona-fide cancer stem cells in vivo, and whether this translates to a durable clinical response, remains uncertain.

Combinational therapies using classes of drugs that act on distinct traits provide an attractive strategy to improve treatment efficacy. Cancer stem cell-targeted agents have been combined with chemotherapy, thus disrupting heterogeneous carcinoma cell types; such an approach has been explored in a phase 1b study of the anti-cancer stem cell agent demcizumab in combination with pemetrexed and carboplatin in patients with first-line non-squamous NSCLC (NCT01189968). Results showed encouraging early clinical activity with tolerable toxicities and complete responses in one (3%) of 40 patients and partial responses in 19 (47%) patients, according to RECIST criteria. In light of this, a randomised phase 2 trial (DENALI) of demcizumab with carboplatin and pemetrexed in first-line non-squamous NSCLC has been initiated (NCT02259582). In parallel, the use of epigenetic drugs—which typically disrupt histone or DNA modifications—to treat cancers is gaining momentum. The epigenetic landscape of cancer stem cells is distinct from the corresponding bulk, differentiated cancer cells, and these differences result in the global reprogramming of gene expression patterns.[116–118] For instance, the histone demethylase LSD1, an epigenetic regulator, can support the maintenance of lung cancer stem cells; its inhibition promotes cell differentiation, reducing tumour growth in SCLC.[119,120] These results, and other emerging evidence, underscore the need to evaluate epigenetic regulators as clinically viable molecular targets in cancer stem cells.

Targeting of tumour metabolism

To date, the majority of studies have been directed at elucidation of the metabolic alterations of neoplastic cells and how they differ from normal tissues. These findings are largely centred upon the reactivation of the Warburg effect, or cancer cell addiction to serine, glutamine, and glycine.[121,122] These seminal observations form important starting points for understanding of broad metabolic variations between highly divergent cell types. Nonetheless, some studies have begun to highlight differences in metabolic alterations between heterogeneous cell types. The ability to identify a therapeutic window during which the metabolic properties of cancer cells might be exploited is crucial to such targeting.

The metabolic enzyme GLDC is increased specifically in lung cancer stem cells and is responsible for their proliferation.[110] GLDC catalyses the conversion of glycine to methyl-tetrahydrofolate, which is one of the key steps in the serine-glycine pathway that feeds into the one-carbon metabolism cycle. Other enzymes of the serine-glycine pathway, which include SHMT2 and PHGDH, are also elevated in a specific subpopulation of cells within other cancer types, sparking interest in the development of targeted inhibitors against these metabolic enzymes.[123,124] Drugs targeting GLDC, PHGDH (anti-serine biosynthesis), and SHMT2 (anti-glycine biosynthesis) are in preclinical studies.[125]

Harnessing the immune system

The ability to induce tumour responses across adenocarcinoma, squamous-cell carcinoma, and SCLC with single-agent immune checkpoint inhibitors (PD-1 or PD-L1 antibodies) provides incontrovertible evidence for the ability of the immune system to eradicate cancer cells.[126] The key promise of cancer immunotherapy has been the unprecedented appearance of durable responses in patients with advanced incurable NSCLC. Although this outcome already occurs in a minority of patients, combinatorial approaches are expected to achieve durable responses in the remaining patients. Immunotherapeutic strategies can be divided into two broad categories: host-targeting strategies and tumour-directed strategies (figure 3).

Targeting of checkpoint inhibition, and beyond

Two PD-1 antibodies—nivolumab and pembrolizumab—were approved for use in the second-line setting for NSCLC in 2015,[10,127,128] and are currently under assessment in the first-line setting (NCT02041533, NCT02477826, NCT02142738, NCT02220894). Further combinations across checkpoint inhibitors are beginning to show promise. Ipilimumab and tremelimumab are CTLA-4-targeting antibodies undergoing clinical development in lung cancer in combination with PD-1 and PD-L1 antibodies; following impressive phase 1b results,[129] a nivolumab–ipilimumab combination, and a durvalumab–tremelimumab combination are being tested in phase 3 trials versus standard-of-care chemotherapy as first-line treatment for both PD-L1-positive and PD-L1-negative patients with NSCLC (CheckMate 227, NCT02477826; MYSTIC, NCT02453282; NEPTUNE, NCT02542293).

Antibodies that target other checkpoints are also being tested in clinical trials for solid cancers, although not specific to lung cancer. Two anti-LAG-3, BMS-986016 and LAG525, are being tested in phase 1 clinical trials alone and in combination with anti-PD-1 drugs (nivolumab [NCT01968109] and PDR001 [NCT02460224], respectively). MBG453, an anti-TIM3 antibody, is being studied as a single agent and in combination with

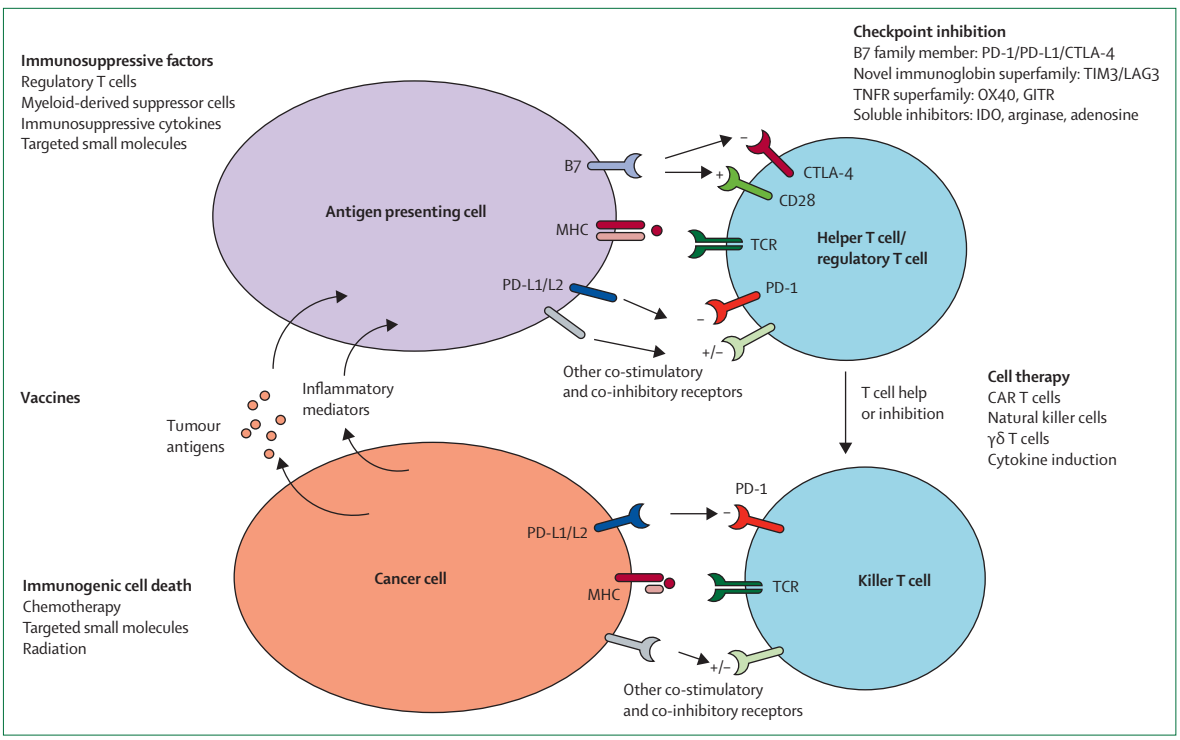

Figure 3: **Strategies to augment immune response to cancers**

PDR001 (NCT02608268). These antibodies are thought to target T-cell-specific immune checkpoints. An anti-KIR antibody that targets an NK-cell checkpoint is being tested with nivolumab in advanced solid tumours (NCT01714739).

In addition to immune checkpoint inhibitors, agonistic targeting of co-stimulatory molecules has also been explored. Co-stimulatory receptors relevant to T cells include CD28, CD27, 4-1BB, GITR, and OX40.[130] Several agonistic antibodies targeting these receptors are currently in development, and include the anti-GITR antibodies TRX518 and MEDI1873—both currently in phase 1 (NCT01239134, NCT02583165)—and the anti-OX40 antibody MEDI6383—currently in phase 1 (NCT02221960)—given alone or in combination with durvalumab, an anti-PD-1 antibody.

Therapeutic cancer vaccines are designed to elicit an immune response against shared or tumour-specific antigens, several of which have moved into late-stage trials. For example, GV1001, which targets the human telomerase reverse transcriptase subunit of telomerase—which is highly expressed in nearly all cancers but restricted in normal tissues—is being tested in a phase 3 study (NCT01579188) for patients with inoperable stage III NSCLC. Tergenpumatucel-L is a therapeutic vaccine consisting of human lung cancer cells genetically modified to include a mouse gene to which the immune system responds strongly, and is being tested in a phase 2/3 trial (NCT01774578) for patients with stage III or IV NSCLC. TG4010, which targets the MUC1 antigen, is being tested in a phase 2/3 study (NCT01383148) for patients with stage IV NSCLC. The phase 2b component of the trial met its primary endpoint, with significant improvement observed in PFS (hazard ratio 0·74, 95% CI 0·55–0·98, p=0·019), and the study entering the phase 3 component.[131] DRibble (DPV-001), a vaccine made from nine cancer antigens plus Toll-like receptor (TLR) adjuvants, is being tested in a phase 2 trial for patients with stage III NSCLC (NCT01909752). After successfully completing an earlier RNActive (CureVac GmbH; Tubingen, Germany) technology-based first-generation mRNA-based vaccine (CV9201) directed towards five tumour-associated antigens (MAGE-C1, MAGE-C2, NY-ESO-1, surviving, and 5T4), CureVac out-licensed a second-generation mRNA vaccine encoding for six overexpressed antigens to Boehringer Ingelheim in September, 2014.[132] This vaccine is being studied in the setting of patients with stage IV NSCLC, in combination with local radiation, or after partial response or stable disease in patients with chemotherapy-treated squamous-cell subtype, or patients with EGFR TKI-treated non-squamous subtypes that harbour activating *EGFR* mutations (NCT01915524).

Enhancement of tumour-directed immune responses

Cancer cell death can be immunogenic or non-immunogenic. Immunogenic cell death results in release of proinflammatory factors and recruitment of immune cells. Endoplasmic reticulum stress and autophagy result in calreticulin exposure in the outer

leaflet of pre-apoptotic cancer cells that additionally secrete ATP, which release nuclear protein HMGB1 as membranes become permeabilised during necrosis. Calreticulin, ATP, and HMGB1 bind to CD91, P2RX7, and TLR4, respectively, facilitating the recruitment of dendritic cells in the tumour bed, the engulfment of tumour antigens by dendritic cells, and optimal antigen presentation to T cells.[133] Radiation is commonly used in lung cancer and is known to cause calreticulin exposure, ATP release, and HMGB1 release. The concept of immunogenic cell death could underpin the rationale for strategies that combine standard treatments of chemotherapy, small-molecule inhibitors, and radiation therapy with immunotherapy. Studies to characterise the capacity of these treatments to cause immunogenic cell death specifically in lung cancer are needed for rational combinations of standard available cancer treatments to be harnessed synergistically with immunotherapeutic strategies.[134]

Several cell therapies are in development. A phase 2 trial of T cells genetically engineered to recognise NY-ESO-1, given alongside dendritic cells pulsed with NY-ESO-1 antigen as a vaccine, is being tested in patients with advanced or refractory malignancies, including lung cancer (NCT01697527). A phase 2 trial of tumour-infiltrating lymphocytes in patients with NSCLC following chemotherapy is open for enrolment (NCT02133196). Early-phase clinical trial testing is underway in cancers including those of lung, with various T cells engineered to target NY-ESO-1 (NCT01967823) as well as in combination with ipilimumab (NCT02070406), VEGFR2 (NCT01218867), MAGE-A3 (NCT02111850), mesothelin (NCT01583686, NCT02414269), and WT1-expressing NSCLC and mesothelioma (NCT02408016). Cytokine-induced killer cells represent a heterogeneous population of immune cells that have been expanded from peripheral blood mononuclear cells using cytokines. These cells have shown in-vitro killing in a variety of cancers.[135] γδ T cells potentially have both antigen-presenting capability as well as tumour cytolytic capacity, and could be a unique immune cell to harness for anticancer therapy.[136] Natural killer cells play a part in immune surveillance and have cytotoxic activity against cancer, and might also represent another population of immune cells that can harnessed for anticancer therapy.[137] Agents that specifically target immunosuppressive factors in the tumour microenvironment, including but not limited to, regulatory T cells, myeloid-derived suppressor cells, and immunouppressive cytokines, need to be explored in lung cancer.

The role of chimeric monoclonal antibodies has garnered renewed interest, including cetuximab, which can directly inhibit on-target EGFR signalling, as well as potentially elicit off-target antibody-dependent cell cytotoxicity and complement activation. Studies to harness complement activation therapeutically might improve the usefulness of existing therapeutic antibodies and also allow us to harness the utility of intrinsic autoantibodies against cancer. Numerous monocolonal antibodies are in clinical development, including bavituximab (SUNRISE, NCT01999673), patritumab (NCT02134015), rilotumumab (NCT02154490, NCT01233687), and IMMU-132, an antibody-drug conjugate of humanised monoclonal antibodies binding to Trop 2, conjugated to SN-38 (NCT01631552).

Can tumour adaptation be targeted?

The development of a drug-resistant state after treatment with EGFR TKIs through emergent genomic alterations such as Thr790Met underscores the effect of clonal evolution. Existing theories on the rise of the resistance mechanisms can often be divided into two schools of thought: pre-adaptive (pre-existent) or post-adaptive (directed adaption as a response to directed selection).[138] Similar to the setting in bacteriology, many of the genetic mutations (eg, antibiotic resistance) are pre-existing rather than being a response to selection pressure (pioneered by the work from Luria and Delbrück).[138,139] This situation is probably also true for tumour populations. When the number of cells is larger than is the inverse of the mutational rate, every position of the genome is mutated at least once,[140,141] suggesting that mutations might already be prevalent across a tumour, but below the detection threshold of current sequencing technology and sampling methods.

Characterisation of tumour heterogeneity through multisector sequencing often reveals a branched pattern of tumour evolution. Spatially sampled tumour populations are related by a common trunk (shared mutations across sectors) and subsequently differentiated with respect to each other by branch mutations (mutations found exclusive to a subset of sectors). Theoretically, targeting of truncal mutations can lead to effective therapies.[48,142] However, application of systems-based approaches such as ecological theory and game theory to the acquisition of drug resistance predicts that targeting of subclonal mutations can also provide important avenues for cancer treatment.[143–145] The TRACERx study,[146] together with the DARWIN trial (NCT02314481), is aiming to relate the clonal (or subclonal) dominance of targetable mutations to progression-free survival intervals in the setting of resectable and subsequently recurrent NSCLC. Although additional insights into the evolution of clonal heterogeneity can be gained through this approach, further efforts in the rationalisation of combinations to subvert drug resistance are ongoing. These efforts could either be directed at co-exisiting alterations, or by the inhibition of potential factors that promote heterogeneity (eg, APOBEC family of enzymes or chemokines such as interleukin 11), thereby forestalling clonal diversity and expansion.[147,148]

Drug development approaches and future perspectives

With the wide range of new therapeutic targets on the horizon for lung cancer, the foremost challenge remains how to expedite novel agents and combinations through the drug development pipeline, while balancing the competing priorities of patient safety and benefit, efficiency of study conduct, and scientific rigour in measuring treatment efficacy. Drug approvals are traditionally based on improvement of overall survival against standard-of-care therapies in the setting of phase 3 clinical trials—a relatively crude metric for evaluation of the usefulness of novel agents. Biomarker-directed clinical trials in defined molecular subsets (eg, EGFR and ALK) illustrate this shortcoming, in which the ethical obligation for crossover designs expose the problem of adoption of conventional overall survival endpoints.[149] The advent of immune-related response criteria is a further example of the limitations of RECIST response criteria and progression-free survival.[150] Furthermore, the burgeoning pipeline of compounds targeting a diverse range of cancer traits underscores the futility in persisting with existing drug development approaches.

New approval pathways such as the FDA breakthrough designation and the inception of trials with contemporary umbrella designs such as BATTLE-2 (NCT01248247), Lung-MAP in squamous-cell carcinoma (NCT02154490), and the UK National Lung Matrix trial (NCT02664935) require signficant commitment from academia, industry, and regulatory partners. Many of these biomarker-driven clinical trials require acquisition of fresh biopsy samples with the intention of providing a contemporaneous snapshot of one region of a tumour to study a panel of biomarkers for treatment allocation. By virtue of scale, these impressive efforts highlight the value of academic cooperative groups driving biomarker-driven translational clinical trials with multiple pharmaceutical partners, overcoming the challenge of trial feasibility in rare patient subsets. However, such single-marker drug studies still entail RECIST-defined endpoints and are conducted in advanced pre-treated NSCLC, and do not fully account for the fact that some novel agents might have a mechanism-based therapeutic niche.

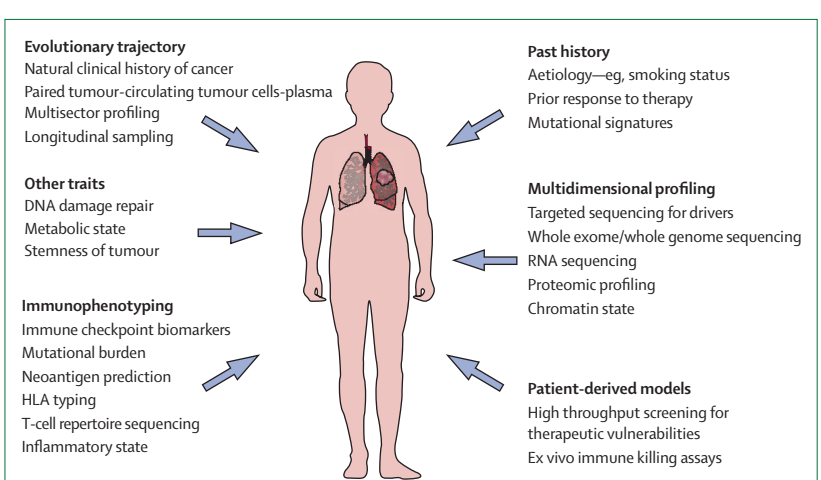

Figure 4: **Future of precision oncology for lung cancer**

In the event that there are insufficient data to support a selection biomarker, trials should incorporate an a priori plan for an adaptive design. In such early biomarker discovery trials, enrolment of a broad range of cancers where there is a good rationale for evaluating the target will be important, alongside development of an enrichment strategy based on a clinical phenotype for efficient enrolment of patient cohorts. Paired biopsy samples might provide information on pharmacodynamic effects such as the pathway (eg, target modulation) and cellular changes (eg, apoptosis) after drug exposure—as well as determine the mechanisms of resistance—to identify fit-for-purpose biomarkers.[151] Genomic and transcriptomic interrogation of responders could yield candidate biomarkers and should occur in near real-time, permiting adjustments in the target population with emerging experience.

This principle similarly applies in the enhancement of precision of a selection biomarker. Genomics studies alone can yield multiple candidate alterations that are potentially tractable targets. In such a scenario, additional multidimensional profiling of downstream transcriptomic or proteomic profiles could help determine dominant pathways.[89] Such analyses should be complemented by expedient in-vitro and in-vivo functional evaluation in either a patient-specific model or a patient-derived tumour xenograft repository to examine rational drug combinations. Although this approach is feasible with certain drug classes (eg, targeted therapeutics against signal transduction pathways), other combinations, such as immunotherapy-targeted regimens, will continue to pose challenges in terms of optimisation of efficacy and safety. Indeed, the high response rates often observed in targeted therapies in pathway-addicted tumours and the potential for long-term disease control with immune checkpoint inhibitors make a compelling case for combination of these agents. However, whether the therapeutic implications of current biomarkers (eg, PD-L1 expression) are similar in the context of different oncogenic drivers is less clear, in part attributed to the differences in demographics and relationship to smoking. Furthermore, the effect of targeted therapies on the immune response warrants further investigation, with recent data highlighting how trametinib (a MEK inhibitor) can enhance efficacy when optimally combined in a concurrent or phased sequential manner with immune checkpoint inhibitors.[152]

Dynamic changes in mutational frequencies through longitudinal or spatial sampling (eg, tissue or plasma, or both) over time can also aid the depiction of intratumoral heterogeneity. Thus, circulating-free DNA from plasma might have the benefit of depicting aggregate molecular portraits in a sequential manner, although major

Search strategy and selection criteria

We searched PubMed for articles published in English between Oct 1, 2015, and March 31, 2016, with the following terms: "targeted drug", "targeted therapy", "phase 1", "drug development", "emerging targets", "novel therapeutics", "early clinical trials", and "preclinical trials". We also searched abstracts on early clinical trials at major medical oncology conferences, including the American Association for Cancer Research, the American Society of Clinical Oncology, the European Society for Medical Oncology, and the Molecular Targets Meeting and World Conference on Lung Cancer.

limitations include the inability to resolve genomic context as well as low signal-to-noise ratio in discovering clinically relevant alterations.[153] Further clinical and technical validation is required to establish the appropriate sensitivity thresholds for circulating-free DNA as a predictive biomarker.[154] Furthermore, beyond genomic alterations, predominant phenotypic traits should be inferred for individual cancers through transcriptomic or functional studies in patient-derived models. Finally, a past medical and social history such as smoking status and response to prior therapies can provide the clinical context for hypothesis-driven drug development efforts (figure 4).

Conclusion

Depiction of the life history and important traits of individual cancers will become increasingly feasible—including aetiology, ancestry, genomic composition (driver and passenger events), chromatin states, and transcriptional or metabolic profiles—all of which might be taken into account when stratifying patients to relevant therapies. The ability to precisely define patient subsets to apply highly specific and individualised treatment options underscores the deficiencies of randomised studies due to inherent inadequacies of any control cohort. Novel methods for the evaluation of treatment efficacies are needed, and continued dialogue with regulatory authorities is crucial to accelerate patient access to innovative cancer medicines. Although identification of druggable genomic alterations appears to be reaching a plateau, a vast genetic and epigenetic landscape remains in the non-coding genome, chromatin regulation, and microenvironment that could yield novel therapeutic targets and predictive biomarkers for existing therapies. We envisage that the expanding armamentarium of current and next-generation therapeutics, coupled with ability to characterise genomic alterations, key traits, and evolutionary trajectories, will enable more nuanced cocktail-based interventions tailored to individual tumours.

Contributors

Conception and design of review by DSWT; and figures and illustrations by DSWT, W-LT, and EWN. All authors contributed equally to literature search, data collection, and assembly, as well as the manuscript writing.

Declaration of interests

DSWT reports personal fees from Pfizer, Bayer, BI, Merck, and Novartis, and grants from Bayer, GlaxoSmithKline, and Novartis, outside the submitted work. AMH reports funding for research collaboration from GlaxoSmithKline, outside the submitted work, and also has a patent PCT/SG2011/000437 pending. All other authors declare no competing interests.

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References

1 Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; **64:** 9–29.
2 Crino L, Weder W, van Meerbeeck J, Felip E. Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010; **21** (suppl 5)**:** v103–15.
3 Hyde L, Yee J, Wilson R, Patno ME. Cell type and the natural history of lung cancer *JAMA* 1965; **193:** 52–54.
4 Schiller JH, Harrington D, Belani CP, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002; **346:** 92–98.
5 Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008; **26:** 3543–51.
6 Sandler A, Gray R, Perry MC, et al. Paclitaxel–carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; **355:** 2542–50.
7 Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; **361:** 947–57.
8 Solomon BJ, Mok T, Kim D-W, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; **371:** 2167–77.
9 Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015; **372:** 2018–28.
10 Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. *N Engl J Med* 2015; **373:** 1627–39.
11 Tan DS, Mok TS, Rebbeck TR. Cancer Genomics: Diversity and disparity across ethnicity and geography. *J Clin Oncol* 2016; **34:** 91–101.
12 Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005; **97:** 339–46.
13 Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med* 2013; **19:** 1389–400.
14 Marx V. Models: stretching the skills of cell lines and mice. *Nat Methods* 2014; **11:** 617–20.
15 Budhu S, Wolchok J, Merghoub T. The importance of animal models in tumor immunity and immunotherapy. *Curr Opin Genet Dev* 2014; **24:** 46–51.
16 Sanmamed MF, Chester C, Melero I, Kohrt H. Defining the optimal murine models to investigate immune checkpoint blockers and their combination with other immunotherapies. *Ann Oncol* 2016; published online Feb 23. DOI: 10.1093/annonc/mdw041.
17 Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. *Cancer Res* 2013; **73:** 5315–19.
18 Walters WP, Namchuk M. Designing screens: how to make your hits a hit. *Nat Rev Drug Discov* 2003; **2:** 259–66.

19 Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature* 2008; **455:** 1069–75.

20 Imielinski M, Berger AH, Hammerman PS, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. *Cell* 2012; **150:** 1107–20.

21 The Cancer Genome Atlas Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; **489:** 519–25.

22 The Cancer Genome Atlas Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 2014; **511:** 543–50.

23 Wu K, Zhang X, Li F, et al. Frequent alterations in cytoskeleton remodelling genes in primary and metastatic lung adenocarcinomas. *Nat Commun* 2015; published online Dec 9. DOI:10.1038/ncomms10131.

24 George J, Lim JS, Jang SJ, et al. Comprehensive genomic profiles of small cell lung cancer. *Nature* 2015; **524:** 47–53.

25 Arcila ME, Drilon A, Sylvester BE, et al. MAP2K1 (MEK1) mutations define a distinct subset of lung adenocarcinoma associated with smoking. *Clin Cancer Res* 2015; **21:** 1935–43.

26 Shtivelman E, Hensing T, Simon GR, et al. Molecular pathways and therapeutic targets in lung cancer. *Oncotarget* 2014; **5:** 1392–433.

27 Weiss J, Sos ML, Seidel D, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. *Sci Transl Med* 2010; **2:** 62ra93.

28 Schultheis AM, Bos M, Schmitz K, et al. Fibroblast growth factor receptor 1 (FGFR1) amplification is a potential therapeutic target in small-cell lung cancer. *Mod Pathol* 2014; **27:** 214–21.

29 Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci USA* 2007; **104:** 20932–37.

30 Frampton GM, Ali SM, Rosenzweig M, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov* 2015; **5:** 850–59.

31 Kawano O, Sasaki H, Okuda K, et al. PIK3CA gene amplification in Japanese non-small cell lung cancer. *Lung Cancer* 2007; **58:** 159–60.

32 Ji M, Guan H, Gao C, Shi B, Hou P. Highly frequent promoter methylation and PIK3CA amplification in non-small cell lung cancer (NSCLC). *BMC Cancer* 2011; **11:** 147.

33 Okudela K, Suzuki M, Kageyama S, et al. PIK3CA mutation and amplification in human lung cancer. *Pathol Int* 2007; **57:** 664–71.

34 Yamamoto H, Shigematsu H, Nomura M, et al. PIK3CA mutations and copy number gains in human lung cancers. *Cancer Res* 2008; **68:** 6913–21.

35 Luk PP, Yu B, Ng CC, et al. BRAF mutations in non-small cell lung cancer. *Transl Lung Cancer Res* 2015; **4:** 142–48.

36 Vaishnavi A, Capelletti M, Le AT, et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. *Nat Med* 2013; **19:** 1469–72.

37 Farago AF, Le LP, Zheng Z, et al. Durable clinical response to entrectinib in NTRK1-rearranged non-small cell lung cancer. *J Thorac Oncol* 2015; **10:** 1670–74.

38 McLornan DP, List A, Mufti GJ. Applying synthetic lethality for the selective targeting of cancer. *N Engl J Med* 2014; **371:** 1725–35.

39 Lawrence MS, Stojanov P, Mermel CH, et al. Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* 2014; **505:** 495–501.

40 Hoadley KA, Yau C, Wolf DM, et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell* 2014; **158:** 929–44.

41 Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. *Nat Rev Clin Oncol* 2014; **11:** 473–81.

42 Katayama R, Shaw AT, Khan TM, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med* 2012; **4:** 120ra17.

43 Thress KS, Paweletz CP, Felip E, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nat Med* 2015; **21:** 560–62.

44 Kim CFB, Jackson EL, Woolfenden AE, et al. Identification of bronchioalveolar stem cells in normal lung and lung cancer. *Cell* 2005; **121:** 823–35.

45 Niederst MJ, Sequist LV, Poirier JT, et al. RB loss in resistant *EGFR* mutant lung adenocarcinomas that transform to small-cell lung cancer. *Nat Commun* 2015; **6:** 6377.

46 Sutherland KD, Proost N, Brouns I, Adriaensen D, Song J-Y, Berns A. Cell of origin of small cell lung cancer: inactivation of Trp53 and Rb1 in distinct cell types of adult mouse lung. *Cancer Cell* 2011; **19:** 754–64.

47 Asselin-Labat M-L, Filby CE. Adult lung stem cells and their contribution to lung tumourigenesis. *Open Biol* 2012; **2:** 120094.

48 Chaft JE, Arcila ME, Paik PK, et al. Coexistence of PIK3CA and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol Cancer Ther* 2012; **11:** 485–91.

49 McGranahan N, Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* 2015; **27:** 15–26.

50 Vansteenkiste JF, Canon J-L, De Braud F, et al. Safety and efficacy of buparlisib (BKM120) in patients with PI3K pathway-activated non-small cell lung cancer: results from the phase II BASALT-1 study. *J Thorac Oncol* 2015; **10:** 1319–27.

51 Tan DS NR, Takano A, et al. Multiregion whole exome and transcriptome sequencing defines the genomic spectrum of EGFR+ NSCLC and reveals novel mechanisms of TKI resistance. The World Conference on Lung Cancer; Denver, Colorado; Sept 6–Sept 9, 2015. Abstr 3637.

52 Jänne PA, Shaw AT, Pereira JR, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. *Lancet Oncol* 2013; **14:** 38–47.

53 Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013; **503:** 548–51.

54 Lito P, Solomon M, Li L-S, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science* 2016; **351:** 604–08.

55 Cappuzzo F, Janne PA, Skokan M, et al. MET increased gene copy number and primary resistance to gefitinib therapy in non-small-cell lung cancer patients. *Ann Oncol* 2009; **20:** 298–304.

56 Chen HJ, Mok TS, Chen ZH, et al. Clinicopathologic and molecular features of epidermal growth factor receptor T790M mutation and c-MET amplification in tyrosine kinase inhibitor-resistant Chinese non-small cell lung cancer. *Pathol Oncol Res* 2009; **15:** 651–58.

57 Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 2007; **316:** 1039–43.

58 Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011; **3:** 75ra26.

59 Camidge DR, Shapiro G, Otterson GA, et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2014; **32** (suppl): abstr 8001.

60 Jenkins RW, Oxnard GR, Elkin S, Sullivan EK, Carter JL, Barbie DA. Response to crizotinib in a patient with lung adenocarcinoma harboring a MET splice site mutation. *Clin Lung Cancer* 2015; **16:** e101–04.

61 Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Discov* 2015; **5:** 842–49.

62 Weeden CE, Solomon B, Asselin-Labat ML. FGFR1 inhibition in lung squamous cell carcinoma: questions and controversies. *Cell Death Dis* 2015; **1:** 15049.

63 Kim HR, Kim DJ, Kang DR, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer. *J Clin Oncol* 2013; **31:** 731–37.

64 Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. *PLoS One* 2011; **6:** e20351.

65 Seo AN, Jin Y, Lee HJ, et al. FGFR1 amplification is associated with poor prognosis and smoking in non-small-cell lung cancer. *Virchows Arch* 2014; **465:** 547–58.

66 Thomas A, Lee J-H, Abdullaev Z, et al. Characterization of fibroblast growth factor receptor 1 in small-cell lung cancer. *J Thorac Oncol* 2014; **9:** 567–71.

67 Semenova EA, Nagel R, Berns A. Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes Dev* 2015; **29:** 1447–62.

68 Hook KE, Garza SJ, Lira ME, et al. An integrated genomic approach to identify predictive biomarkers of response to the aurora kinase inhibitor PF-03814735. *Mol Cancer Ther* 2012; **11:** 710–19.

69 Li J, Fang B, Kinose F, et al. Target identification in small cell lung cancer via integrated phenotypic screening and activity-based protein profiling. *Mol Cancer Ther* 2016; **15:** 334–42.

70 Melichar B, Adenis A, Lockhart AC, et al. Safety and activity of alisertib, an investigational aurora kinase A inhibitor, in patients with breast cancer, small-cell lung cancer, non-small-cell lung cancer, head and neck squamous-cell carcinoma, and gastro-oesophageal adenocarcinoma: a five-arm phase 2 study. *Lancet Oncol* 2015; **16:** 395–405.

71 Zandi R, Selivanova G, Christensen CL, Gerds TA, Willumsen BM, Poulsen HS. PRIMA-1Met/APR-246 induces apoptosis and tumor growth delay in small cell lung cancer expressing mutant p53. *Clin Cancer Res* 2011; **17:** 2830–41.

72 Lehmann S, Bykov VJ, Ali D, et al. Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. *J Clin Oncol* 2012; **30:** 3633–39.

73 Morandell S, Reinhardt HC, Cannell IG, et al. A reversible gene-targeting strategy identifies synthetic lethal interactions between MK2 and p53 in the DNA damage response in vivo. *Cell Rep* 2013; **5:** 868–77.

74 Sullivan KD, Padilla-Just N, Henry RE, et al. ATM and MET kinases are synthetic lethal with nongenotoxic activation of p53. *Nat Chem Biol* 2012; **8:** 646–54.

75 Han J-Y, Lee SH, Lee GK, et al. Phase I/II study of gefitinib (Iressa®) and vorinostat (IVORI) in previously treated patients with advanced non-small cell lung cancer. *Cancer Chemother Pharmacol* 2015; **75:** 475–83.

76 Nakagawa T, Takeuchi S, Yamada T, et al. EGFR-TKI resistance due to BIM polymorphism can be circumvented in combination with HDAC inhibition. *Cancer Res* 2013; **73:** 2428–34.

77 Barbieri I, Cannizzaro E, Dawson MA. Bromodomains as therapeutic targets in cancer. *Brief Funct Genomics* 2013; **12:** 219–30.

78 Lockwood WW, Zejnullahu K, Bradner JE, Varmus H. Sensitivity of human lung adenocarcinoma cell lines to targeted inhibition of BET epigenetic signaling proteins. *Proc Natl Acad Sci USA* 2012; **109:** 19408–13.

79 Kaur G, Reinhart RA, Monks A, Evans D, Morris J, Teicher BA. Bromodomain and hedgehog pathway targets in small cell lung cancer. *Cancer Res* 2014; **74** (suppl 19)**:** abstr 5508.

80 Shimamura T, Chen Z, Soucheray M, et al. Efficacy of BET bromodomain inhibition in Kras-mutant non–small cell lung cancer. *Clin Cancer Res* 2013; **19:** 6183–92.

81 Govindan R, Ding L, Griffith M, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell* 2012; **150:** 1121–34.

82 Zhang J, Liu Y, Li L, et al. Genome sequencing reveals the multicentric nature of multiple synchronous lung adenocarcinomas. *Cancer Res* 2015; **75** (suppl 15)**:** abstr 2982.

83 de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science* 2014; **346:** 251–56.

84 Zhang L, Wang X-F, Ma Y-S, et al. Quantitative assessment of the influence of TP63 gene polymorphisms and lung cancer risk: evidence based on 93,751 subjects. *PLoS One* 2014; **9:** e87004.

85 Roos WP, Thomas AD, Kaina B. DNA damage and the balance between survival and death in cancer biology. *Nat Rev Cancer* 2016; **16:** 20–33.

86 Olaussen KA, Dunant A, Fouret P, et al. DNA repair by ERCC1 in non–small-cell lung cancer and cisplatin-based adjuvant chemotherapy. *N Engl J Med* 2006; **355:** 983–91.

87 Bepler G, Williams C, Schell MJ, et al. Randomized international phase III trial of ERCC1 and RRM1 expression–based chemotherapy versus gemcitabine/carboplatin in advanced non–small-cell lung cancer. *J Clin Oncol* 2013; **31:** 2404–12.

88 Tan DS, Ng QS, Tan IB, Lim ST, Lim WT. Truth about ERCC1 in lung cancer. *J Clin Oncol* 2010; **28:** e162.

89 Tan DS-W, Gerlinger M, Teh B-T, Swanton C. Anti-cancer drug resistance: understanding the mechanisms through the use of integrative genomics and functional RNA interference. *Eur J Cancer* 2010; **46:** 2166–77.

90 Postel-Vinay S, Bajrami I, Friboulet L, et al. A high-throughput screen identifies PARP1/2 inhibitors as a potential therapy for ERCC1-deficient non-small cell lung cancer. *Oncogene* 2013; **32:** 5377–87.

91 Byers LA, Wang J, Nilsson MB, et al. Proteomic profiling identifies dysregulated pathways in small cell lung cancer and novel therapeutic targets including PARP1. *Cancer Discov* 2012; **2:** 798–811.

92 Wainberg ZA, Rafii S, Ramanathan RK, et al. Safety and antitumor activity of the PARP inhibitor BMN673 in a phase 1 trial recruiting metastatic small-cell lung cancer (SCLC) and germline BRCA-mutation carrier cancer patients. *Proc Am Soc Clin Oncol* 2014; **32** (suppl)**:** abstr 7522.

93 Massuti B, Campelo RG, Abreu DR, et al. Open, phase II randomized trial of gefitinib alone versus olaparib (AZD2281) plus gefitinib in advanced non-small cell lung cancer (NSCLC) patients (P) with epidermal growth factor receptor (EGFR) mutations: Spanish Lung Cancer Group trial (NCT=1513174/GECP-GOAL). *Proc Am Soc Clin Oncol* 2014; **32** (suppl)**:** abstr TPS8127.

94 Ramalingam SS, Blais N, Mazières J, et al. A randomized, double-blind, phase 2 trial of veliparib (ABT-888) with carboplatin and paclitaxel in previously untreated metastatic or advanced non-small cell lung cancer. *Ann Oncol* 2014; **25** (suppl 4)**:** iv426–iv470.

95 Gopalan PK, Pinder MC, Chiappori A, Ivey AM, Villegas AG, Kaye FJ. A phase II clinical trial of the CDK 4/6 inhibitor palbociclib (PD 0332991) in previously treated, advanced non-small cell lung cancer (NSCLC) patients with inactivated CDKN2A. *Proc Am Soc Clin Oncol* 2014; **32** (suppl)**:** abstr 8077.

96 Goldman JW, Gandhi L, Patnaik A, et al. Clinical activity of LY2835219, a novel cell cycle inhibitor selective for CDK4 and CDK6, in patients with non-small cell lung cancer. *Proc Am Soc Clin Oncol* 2014; **32** (suppl)**:** abstr 8026.

97 Syljuasen RG, Hasvold G, Hauge S, Helland A. Targeting lung cancer through inhibition of checkpoint kinases. *Front Genet* 2015; **6:** 70.

98 Calvo E, Chen VJ, Marshall M, et al. Preclinical analyses and phase I evaluation of LY2603618 administered in combination with pemetrexed and cisplatin in patients with advanced cancer. *Invest New Drugs* 2014; **32:** 955–68.

99 Hall AB, Newsome D, Wang Y, et al. Potentiation of tumor responses to DNA damaging therapy by the selective ATR inhibitor VX-970. *Oncotarget* 2014; **5:** 5674–85.

100 Camidge DR. Cell cycle-associated kinases as targets for therapy in lung cancer. *J Thorac Oncol* 2010; **5** (suppl 6)**:** S461–62.

101 Sourisseau T, Hassan KA, Wistuba I, et al. Lung cancer stem cell: fancy conceptual model of tumor biology or cornerstone of a forthcoming therapeutic breakthrough? *J Thorac Oncol* 2014; **9:** 7–17.

102 Tam WL, Lu H, Buikhuisen J, et al. Protein kinase C α is a central signaling node and therapeutic target for breast cancer stem cells. *Cancer cell* 2013; **24:** 347–64.

103 Ailles LE, Weissman IL. Cancer stem cells in solid tumors. *Curr Opin Biotechnol* 2007; **18:** 460–66.

104 Tam WL, Ng HH. Sox2: masterminding the root of cancer. *Cancer Cell* 2014; **26:** 3–5.

105 Kaiser J. The cancer stem cell gamble. *Science* 2015; **347:** 226–29.

106 Pietanza C. Safety, activity, and response durability assessment of single agent rovalpituzumab tesirine, a delta-like protein3 (DLL3)-targeted anibody drug conjugate (ADC), in small cell lung cancer (SCLC). European Cancer Congress; Vienna, Austria; Sept 26–Sept 29, 2015. Abstr LBA7.

107 Saunders LR, Bankovich AJ, Anderson WC, et al. A DLL3-targeted antibody-drug conjugate eradicates high-grade pulmonary neuroendocrine tumor-initiating cells in vivo. *Sci Transl Med* 2015; **7:** 302ra136.

108 Bertolini G, Roz L, Perego P, et al. Highly tumorigenic lung cancer CD133+ cells display stem-like features and are spared by cisplatin treatment. *Proc Natl Acad Sci USA* 2009; **106:** 16281–86.

109 Jiang F, Qiu Q, Khanna A, et al. Aldehyde dehydrogenase 1 is a tumor stem cell-associated marker in lung cancer. *Mol Cancer Res* 2009; **7:** 330–38.

110 Zhang WC, Shyh-Chang N, Yang H, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. *Cell* 2012; **148:** 259–72.

111 Liu Y-P, Yang C-J, Huang M-S, et al. Cisplatin selects for multidrug-resistant CD133+ cells in lung adenocarcinoma by activating Notch signaling. *Cancer Res* 2013; **73:** 406–16.

112 Zhang X, Lou Y, Wang H, et al. Wnt signaling regulates the stemness of lung cancer stem cells and its inhibitors exert anticancer effect on lung cancer SPC-A1 cells. *Med Oncol* 2015; **32:** 1–8.

113 Hassan KA, Wang L, Korkaya H, et al. Notch pathway activity identifies cells with cancer stem cell–like properties and correlates with worse survival in lung adenocarcinoma. *Clin Cancer Res* 2013; **19:** 1972–80.

114 Sullivan JP, Spinola M, Dodge M, et al. Aldehyde dehydrogenase activity selects for lung adenocarcinoma stem cells dependent on notch signaling. *Cancer Res* 2010; **70:** 9937–48.

115 Luistro L, He W, Smith M, et al. Preclinical profile of a potent γ-secretase inhibitor targeting notch signaling with in vivo efficacy and pharmacodynamic properties. *Cancer Res* 2009; **69:** 7672–80.

116 Chang C-J, Yang J-Y, Xia W, et al. EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-β-catenin signaling. *Cancer Cell* 2011; **19:** 86–100.

117 Abdouh M, Facchino S, Chatoo W, Balasingam V, Ferreira J, Bernier G. BMI1 sustains human glioblastoma multiforme stem cell renewal. *J Neurosci* 2009; **29:** 8884–96.

118 Trowbridge JJ, Sinha AU, Zhu N, Li M, Armstrong SA, Orkin SH. Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. *Genes Dev* 2012; **26:** 344–49.

119 Mohammad HP, Smitheman KN, Kamat CD, et al. A DNA hypomethylation signature predicts antitumor activity of LSD1 inhibitors in SCLC. *Cancer Cell* 2015; **28:** 57–69.

120 Stewart CA, Byers LA. Altering the course of small cell lung cancer: targeting cancer stem cells via LSD1 inhibition. *Cancer Cell* 2015; **28:** 4–6.

121 Chaneton B, Hillmann P, Zheng L, et al. Serine is a natural ligand and allosteric activator of pyruvate kinase M2. *Nature* 2012; **491:** 458–62.

122 Son J, Lyssiotis CA, Ying H, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 2013; **496:** 101–05.

123 Kim D, Fiske BP, Birsoy K, et al. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* 2015; **520:** 363–67.

124 Possemato R, Marks KM, Shaul YD, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011; **476:** 346–50.

125 DeBerardinis RJ. Serine metabolism: some tumors take the road less traveled. *Cell Metab* 2011; **14:** 285–86.

126 Drake CG, Lipson EJ, Brahmer JR. Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat Rev Clin Oncol* 2014; **11:** 24–37.

127 Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non–small-cell lung cancer. *N Engl J Med* 2015; **373:** 123–35.

128 Herbst RS, Baas P, Kim D-W, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2015, published online Dec 19. DOI:10.1016/S0140-6736(15)01281-7.

129 Antonia S, Goldberg SB, Balmanoukian A, et al. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* 2016; **17:** 299–308.

130 Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol* 2013; **13:** 227–42.

131 Quoix E, Lena H, Losonczy G, et al. TG4010 immunotherapy and first-line chemotherapy for advanced non-small-cell lung cancer (TIME): results from the phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial. *Lancet Oncol* 2016; **17:** 212–223.

132 Boehringer Ingelheim. Boehringer Ingelheim and CureVac announce collaboration to develop next generation lung cancer immunotherapy. Sept 18, 2014. https://www.boehringer-ingelheim.com/press-release/boehringer-ingelheim-and-curevac-collaboration (accessed July 6, 2016).

133 Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol* 2013; **31:** 51–72.

134 Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov* 2015; **14:** 561–84.

135 Sangiolo D. Cytokine induced killer cells as promising immunotherapy for solid tumors. *J Cancer* 2011; **2:** 363–68.

136 Silva-Santos B, Serre K, Norell H. gammadelta T cells in cancer. *Nat Rev Immunol* 2015; **15:** 683–91.

137 Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer* 2015; **16:** 7–19.

138 Luria SE, Delbrück M. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 1943; **28:** 491.

139 Newcombe HB. Origin of bacterial variants. *Nature* 1949; **164:** 150.

140 Bozic I, Antal T, Ohtsuki H, et al. Accumulation of driver and passenger mutations during tumor progression. *Proc Natl Acad Sci USA* 2010; **107:** 18545–50.

141 Frank SA. Dynamics of cancer: incidence, inheritance, and evolution. Princeton: Princeton University Press, 2007.

142 Fisher R, Pusztai L, Swanton C. Cancer heterogeneity: implications for targeted therapeutics. *Br J Cancer* 2013; **108:** 479–85.

143 Crespi B, Summers K. Evolutionary biology of cancer. *Trends Ecol Evol* 2005; **20:** 545–52.

144 Merlo LM, Pepper JW, Reid BJ, Maley CC. Cancer as an evolutionary and ecological process. *Nat Rev Cancer* 2006; **6:** 924–35.

145 Pienta KJ, McGregor N, Axelrod R, Axelrod DE. Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments. *Transl Oncol* 2008; **1:** 158–64.

146 Jamal-Hanjani M, Hackshaw A, Ngai Y, et al. Tracking genomic cancer evolution for precision medicine: the lung TRACERx study. *PLoS Biol* 2014; **12:** e1001906.

147 Marusyk A, Tabassum DP, Altrock PM, Almendro V, Michor F, Polyak K. Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. *Nature* 2014; **514:** 54–58.

148 Swanton C, McGranahan N, Starrett GJ, Harris RS. APOBEC enzymes: mutagenic fuel for cancer evolution and heterogeneity. *Cancer Discov* 2015; **5:** 704–12.

149 Neal JW, Gainor JF, Shaw AT. Developing biomarker-specific end points in lung cancer clinical trials. *Nat Rev Clin Oncol* 2015; **12:** 135–46.

150 Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumours: immune-related response criteria. *Clin Cancer Res* 2009; **15:** 7412–20.

151 Tan DS, Thomas GV, Garrett MD, et al. Biomarker-driven early clinical trials in oncology: a paradigm shift in drug development. *Cancer J* 2009; **15:** 406–20.

152 Liu L, Mayes PA, Eastman S, et al. The BRAF and MEK inhibitors dabrafenib and trametinib: effects on immune function and in combination with immunomodulatory antibodies targeting PD-1, PD-L1, and CTLA-4. *Clin Cancer Res* 2015; **21:** 1639–51.

153 Remon J, Gorham J, Besse B, Sculier J-P. Circulating free DNA, new dynamic marker in nonsmall cell lung cancer patients? *Eur Respir J* 2015; **46:** 1548–50.

154 Tsui DW, Berger MF. Profiling non-small cell lung cancer: from tumor to blood. *Clin Cancer Res* 2016; **22:** 790–92.