Chapter

TARGETED THERAPIES IN NON-SMALL CELL LUNG CANCER

Susan Heavey, Ken O'Byrne and Kathy Gately*

Department of Clinical Medicine, Trinity College Dublin,
St. James's Hospital, Dublin, Ireland

ABSTRACT

The majority of non-small cell lung cancer (NSCLC) patients present with advanced disease and with a 5 year survival rate of <15% for these patients, treatment outcomes are considered extremely disappointing. Standard chemotherapy regimens provide some improvement to ~40% of patients. However, intrinsic and acquired chemoresistance are a significant problem and hinder sustained long term benefits of such treatments. Advances in proteomic and genomic profiling have increased our understanding of the aberrant molecular mechanisms that are driving an individual's tumour. The increased sensitivity of these technologies has enabled molecular profiling at the stage of initial biopsy and thus has paved the way for a more personalised approach to the treatment of cancer patients. Improvements in diagnostics together with a wave of new small molecule inhibitors and monoclonal antibodies that target "driver" mutations has revolutionised the treatment of cancer.

To date there are essentially three targeted agents approved for clinical use in NSCLC. The tyrosine kinase inhibitor (TKI) erlotinib, which targets the epidermal growth factor receptor (EGFR) TK domain, has proven to be

* kgately@stjames.ie

an effective treatment strategy in patients who harbour activating mutations in the *EGFR* TK domain. Bevacizumab a monoclonal antibody targeting the vascular endothelial growth factor (VEGF) can improve survival, response rates, and progression-free survival when used in combination with chemotherapy. *Crizotinib*, a small-molecule drug, inhibits the tyrosine kinase activity of the echinoderm microtubule-associated protein-like 4 anaplastic lymphoma kinase (EML4-ALK) fusion protein, resulting in decreased tumour cell growth, migration, and invasiveness in patients with locally advanced or metastatic NSCLC. Several other agents are in clinical testing that target a number of key signalling molecules including: KRAS, HER2, BRAF, MET, PI3K/PI3K-mTOR, MEK1, and IGF- 1R. Often several pathways are activated simultaneously and crosstalk between pathways allows tumour cells to escape the inhibition of a single targeted agent. This chapter will explore the clinical development of currently available targeted therapies for NSCLC as well as targeted agents currently in clinical trials and will examine the synergy between cytotoxic therapies.

INTRODUCTION

Only approximately 20-30% of newly diagnosed NSCLC patients present with early stage disease and are suitable candidates for resection. Traditionally decisions on therapeutic options were based on the histology of the tumour. The standard treatment for advanced NSCLC patients with a good performance status (0-1) is platinum-based chemotherapy and partial responses are achieved in 30-40% of patients [1]. Complete responses are very rare in advanced NSCLC and those that do respond initially will develop acquired resistance to treatment. With a 5 year survival rate of <15% for patients with advanced disease, treatment outcomes are considered disappointing. A randomised phase III trial demonstrated better results for pemetrexed than for gemcitabine in patients with non-squamous NSCLC [2]. However, conventional chemotherapy has reached a plateau of effectiveness in improving patient survival in all tumour histologies and a concerted effort to further classify tumours at the molecular level is critical.

Lung cancer is a heterogeneous disease and often several signalling pathways are driving the oncogenic behaviour of tumours. Several key "driver" genes are mutated in NSCLC including, epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), KRAS, human epidermal growth factor receptor 2 (HER2), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), MET, phosphatidylinositol 3-kinase catalytic α (PIK3CA), AKT and **mitogen-activated protein kinase kinase 1 (**MAP2K1) (Figure 1). In fact, these genes contribute to the pathogenesis of several cancer types. Other aberrations such as

variation in gene copy numbers, translocations between genes and single nucleotide polymorphisms (SNPs) can occur in a proportion of cells within a tumour. These aberrant genes are obvious targets for inhibition and thus the design of drugs that will selectively block these oncogenic varieties while sparing their normal counterparts seems like the ideal strategy to annihilate these rogue cells. A table summarising the frequency of mutations and availability of targeted therapies in NSCLC is shown below (Table 1). However, there is no guarantee that only the oncogenic variant is druggable, and like all targeted therapies the cancer cell will eventually activate alternative signalling pathways to evade these targeted inhibitors.

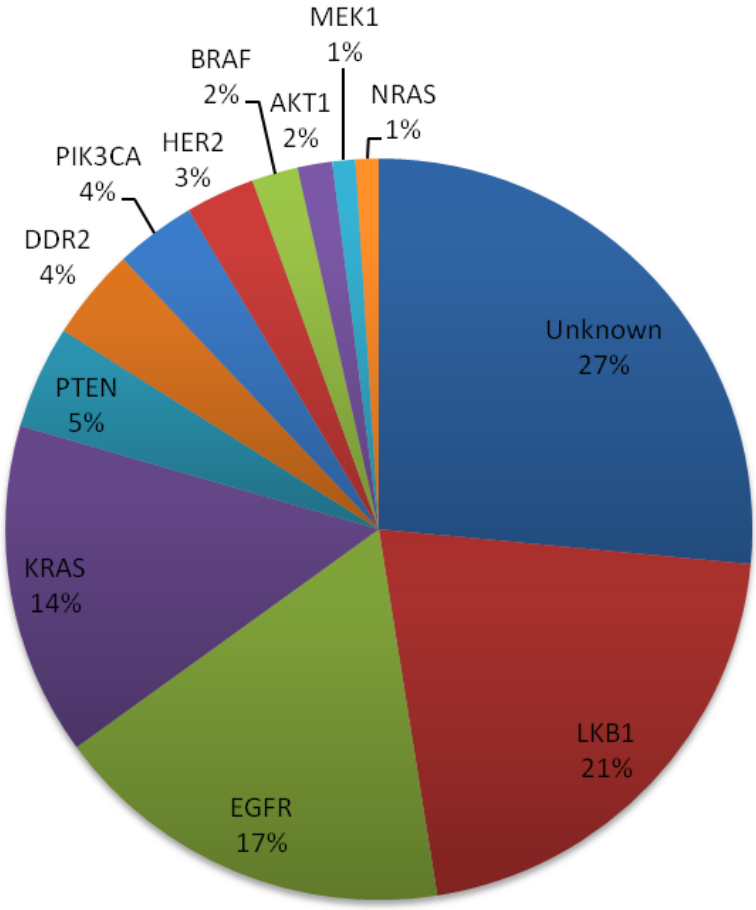

Figure 1. Mutation rates in Non-Small Cell Lung Cancer.

Intrinsic resistance or the development of acquired resistance to targeted therapies may be due to the multilevel complex cross-talk that occurs between the targets of the biological agent and several signal transduction pathways. Blocking only one of these pathways may not always be an effective treatment strategy as this approach may open the door for others to act as bypass mechanisms within tumour cells. The most promising approaches to the treatment of NSCLC, will be drugs with multiple targets or the use of a combination of targeted therapies.

Table 1. Frequency of mutations and availability of targeted therapies in NSCLC.

Gene	Alteration	Frequency in NSCLC
AKT1	Mutation	1-2%
ALK	Rearrangement	3–7%
BRAF	Mutation	1–3%
DDR2	Mutation	~4%
EGFR	Mutation	10–35%
FGFR1	Amplification	20%
HER2	Mutation	2–4%
KRAS	Mutation	8-21%
LKB1	Mutation	9-33%
MEK1	Mutation	1%
MET	Amplification	2–4%
NRAS	Mutation	1%
PIK3CA	Mutation	2-5%
PIK3CA	Amplification	12-20%
PTEN	Mutation	4–5%
PTEN	Loss	24-44%
RET	Rearrangement	1%
ROS1	Rearrangement	1%

Key:

Drugs approved in NSCLC
Drugs approved in NSCLC for ALK subtype & effective here
Drugs approved in other cancers
Drugs in clinical development

TARGETED THERAPY IN LUNG CANCER

A deeper understanding of the pathobiology of human malignancies has led to successful application of targeted therapeutic strategies in several cancers [3-6].

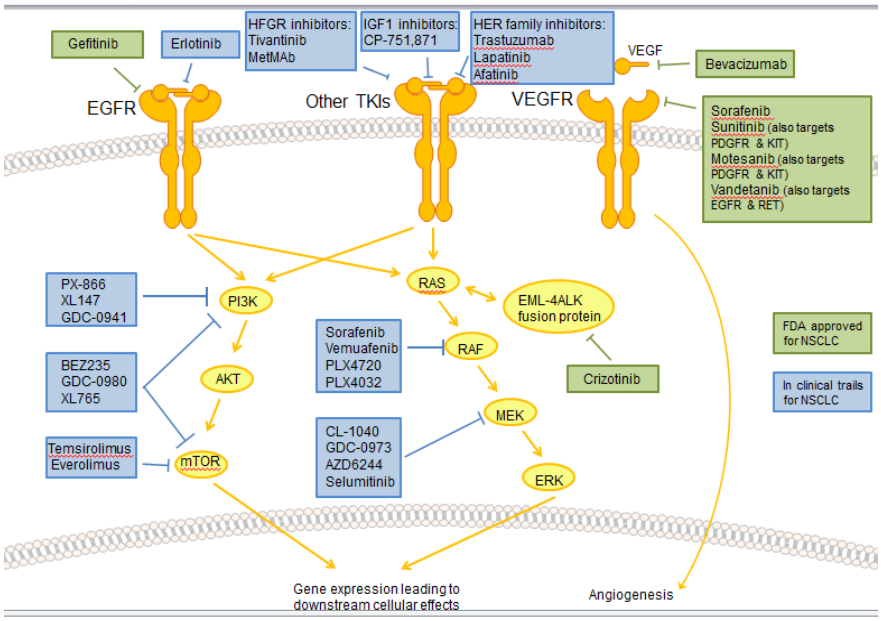

Figure 2. Targeted Therapies in NSCLC.

In NSCLC several targeted inhibitors are currently being evaluated in clinical trials and there are essentially three targeted agents approved for clinical use (Figure 2). The EGFR TKIs gefitinib and erlotinib which target the EGFR TK domain, have proven to be an effective treatment strategy in patients who harbour activating mutations in the *EGFR* TK domain. Bevacizumab a monoclonal antibody targeting VEGF can improve survival, response rates, and progression-free survival when used in combination with chemotherapy. *Crizotinib*, a small-molecule drug, inhibits the tyrosine kinase activity of the EML4-ALK fusion protein, resulting in decreased tumour cell growth, migration, and invasiveness in patients with locally advanced or metastatic NSCLC. With increasing studies showing the importance of matching the treatment to the mutation the Lung Cancer Mutation Consortium (LCMC) in collaboration with the National Lung Cancer Partnership was established to promote molecular tumour mutation testing for lung cancer patients. The LCMC aims to screen a large population of patients to create a unique national data set to determine the frequency of certain driver mutations and explore opportunities for clinical trial enrollment. Molecular profiling of 830 patients, indicated that 60% of tumors exhibit known driver mutations including 25% KRAS, 23% EGFR, and 6% ALK rearrangements [7]. These data remind us that in 40% of tumours there are as yet unknown lung

cancer drivers. Interestingly 95% of molecular lesions are mutually exclusive and the presence of one mutation in lieu of another can influence response to targeted therapy. Companion diagnostics that enable the monitoring of changes in patients' tumour genotypes throughout the course of their disease together with a combination approach to targeted therapy can potentially reduce toxicities and improve patient outcomes.

EGFR

The EGFR signalling pathway plays an essential role in the pathogenesis of NSCLC with EGFR protein expression seen in up to 85% of NSCLC patients. Initial studies failed to demonstrate the prognostic relevance of EGFR expression in NSCLC or to predict response to oral EGFR TKIs [8-12]. However, a recent study has shown that EGFR expression does predict response to EGFR-TKIs if an intracellular binding antibody is used for immunohistochemistry analysis [13]. Somatic mutations in the EGFR TK domain proved to be a better predictor of response to EGFR TKIs and were first described in 2004. Mutations are either in-frame deletions or amino acid substitutions clustered around the ATP-binding pocket of the TK domain (exons 18-21). The two most common mutations are in-frame deletions in exon 19 (del 19) or a substitution mutation in exon 21 (L858R) and these account for 85-90% of the drug-sensitive EGFR mutations seen in NSCLC. Two other sensitising mutations are substitutions in exons 18 (G719A/C) and 21 (L861Q) [14].

These mutations are more common in female, never smokers with adenocarcinomas, who are of Asian ethnicity [15, 16]. Cells harbouring these "driver" EGFR mutations are said to be in a state of "oncogenic addiction" and are highly dependent on the constitutively active EGFR signalling pathway [17]. A germline T790M EGFR mutation was reported in a family with multiple cases of NSCLC and was shown to confer a growth advantage to the cancer cells [18]. Both T790M positive cells and those having a "double mutant" (T790M and L858R mutations) have been shown to have enhanced kinase activity [19] and increased tyrosine phosphorylating activity, respectively [20]. The T790M mutation has also emerged as a secondary point mutation that is present in approximately half of lung cancer patients who develop resistance to EGFR TKIs. Pinter *et al*. have shown that there is little correlation between EGFR expression (IHC) and EGFR-activating mutations, increased EGFR gene copy number or response to EGFR TKI [9]. Although *EGFR* amplification can indicate response to EGFR TKIs, mutation status is the best predictor of response and is used

routinely to identify NSCLC tumours that are most likely to benefit from treatment [15, 21-25].

TARGETING EGFR

Cetuximab (Erbitux) is a chimeric monoclonal G1 (IgG1) antibody that binds to EGFR with high affinity and blocks ligand binding, inducing receptor internalisation and degradation resulting in inhibition of EGFR expression. It is licensed to treat both metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck (SCCHN). However, after initial positive results in Phase II trials in patients with advanced NSCLC, two Phase III trials evaluating Cetuximab in addition to first-line chemotherapy showed only a small benefit in overall survival (OS). Interestingly, a subgroup analysis of patients with high EGFR expression by IHC from the FLEX Phase III trial demonstrated a greater survival benefit [26]. If this data can be validated prospectively than cetuximab may be approved as a standard treatment in the future.

The EGFR TKIs gefitinib (Iressa) and erlotinib (Tarceva) have been licensed to treat advanced or metastatic NSCLC. Activating mutations in the EGFR tyrosine kinase domain have been shown to demonstrate different sensitivities to EGFR TKIs, with exon 19 deletions being more likely to respond than those of exon 21 [27-31]. Conversely, some substitution mutations in exon 20 (T790M) and exon 21 (T854A) are known to confer resistance to some EGFR TKIs [32-34] Although mutated tumours initially respond to treatment with the EGFR TKIs erlotinib and gefitinib, almost all will eventually develop acquired resistance to these drugs [32, 35]. A mutation at T790M has been shown to confer resistance in 50% of cases [36-38]. It was initially thought that the substitution of the larger methionine residue may cause steric hindrance to the binding of the drugs [32, 39] A structurally similar reversible TKI is, however, able to overcome the T790M mutation [40]. The T790M mutation may result in increased affinity of EGFR for ATP compared with erlotinib or gefitinib and this is the primary mechanism by which the mutation confers drug resistance [41] Thus, it should be possible to overcome the resistance by developing TKIs that have a higher affinity for the T790M kinase [33, 42, 43]. New generation EGFR TKIs, that bind irreversibly to the EGFR-TK, forming covalent cross-links with EGFR, such as afatinib (BIBW 2992), have been shown to be active against tumours resistant to reversible EGFR TKIs [32, 42-46] and offer an alternative therapy strategy. New-generation TKIs may have a longer duration of action than reversible agents. In order to change the therapeutic agent at the optimal time to prevent tumour progression, it would be

useful to know when the T790M mutation has developed. A recent publication found T790M in up to 38% of patients not previously treated with a TKI [47]. The existence of a T790M mutation in a few cancer cells at diagnosis confers a shorter time to tumour progression [48]. Such cells are subsequently 'cloned out' during the patient's treatment with EGFR TKIs indicating that these patients may benefit from treatment with new-generation EGFR TKIs from the beginning. Alternatively screening for the emergence of the T790M mutation during treatment may allow early identification of acquired resistance to TKIs and treatment to be tailored as necessary. In addition to these specific EGFR mutations, other factors such as amplification or activation of the insulin-like growth factor receptor [49] and MET amplification [50] have also been shown to confer resistance to EGFR TKIs. These nuances of the EGFR discussed above demonstrate that both preselection of patients most likely to respond to EGFR-targeted therapy and screening during therapy are crucial to determine the appropriate treatment regimen.

VEGF AND VEGFR

The vascular endothelial growth factor (VEGF) ligand is released from endothelial cells in response to tissue injury or hypoxia, and leads to the formation of new blood vessels. It has been implicated in tumorigenesis and metastasis and is an important pro-angiogenic mediator. There are five members of the VEGF family (VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF) with VEGF-A the most clinically active. There are 3 VEGF receptor (VEGFR) tyrosine kinases (VEGFR-1, VEGFR-2 and VEGFR-3) with VEGFR-2 involved in most angiogenic responses. Overexpression of VEGF promotes vascular permeability, which enhances tumor nutrient exchange, extravasation of macromolecular proteins and the influx of tumor cells [51]. Several studies have identified VEGF overexpression as a poor prognostic indicator in NSCLC and SCLC [52, 53].

TARGETING VEGFR WITH BEVACIZUMAB

The anti-tumor effects of VEGF pathway inhibition have been validated in preclinical models [54, 55], and subsequently led to the development of VEGF antagonists such as the monoclonal antibody, bevacizumab (Avastin). The Phase III ECOG 4599 trial established the efficacy of bevacizumab by randomizing

metastatic, non-squamous lung cancer patients to carboplatin, paclitaxel and bevacizumab versus chemotherapy alone [56]. The addition of bevacizumab to chemotherapy resulted in better median survival (12.3 vs 10.3 months, hazard ratio (HR) 0.79) and a higher response rate (RR; 35 vs 15%, $p < 0.001$) when compared with chemotherapy alone. Based on this study, bevacizumab gained FDA and EMEA approval in 2006 as first-line therapy in combination with carboplatin and paclitaxel for advanced nonsquamous NSCLC.

TARGETING VEGFR WITH TKIS

After the success of bevacizumab a number of compounds targeting angiogenesis have since gone into preclinical and clinical testing. Several of these newer agents are multitarget tyrosine kinase inhibitors (MTKIs) which target several pathways concomitantly. Sorafenib inhibits the kinase activity of C-RAF, B-RAF and targets platelet-derived growth factor receptor family (PDGFR-β and stem cell factor receptor (KIT)) as well as VEGFR2/3. Sorafenib inhibits MEK and ERK phosphorylation in various cancer cell lines and tumor xenografts [57]. Sunitinib is a multitargeted inhibitor of VEGFR, PDGFR, KIT and FLT3 (FMS-like tyrosine kinase 3). Both MTKIs were approved by the FDA to treat metastatic renal cell carcinoma and sunitinib has been approved for gastrointestinal stromal tumours. However these drugs have not produced as promising results as in the non-specific biomarker setting. These drugs are also associated with having higher toxicities.

In a large Phase III placebo-controlled study investigating the efficacy of sorafenib with carboplatin and pacliltaxel (CP), the addition of sorafenib to chemotherapy-naïve patients with advanced NSCLC failed to show clinical benefit [58]. In a setting when the biomarker status of a patient is known, such as in the case of the BATTLE trial, sorafenib has shown varying results [59]. Sorafenib was found to have activity against tumors in patients with wild-type K-RAS, whereas patients who had EGFR mutations fared worse on sorafenib. Such results illustrate the importance of biomarkers in predicting the sensitivity or resistance of patients to targeted therapy. BATTLE-2 is a follow-up trial seeking to pursue the interesting data found in BATTLE-1 regarding KRAS mutations and the positive results of sorafenib. Thus, only two arms from the original BATTLE-1 trial (erlotinib and sorafenib) were continued in BATTLE-2.

Another MTKI that has been investigated in NSCLC is Motesanib which targets VEGFR 1 - 3, PDGFR and KIT. A Phase II three arm study comparing motesanib and CP with single agent motesanib and single agent bevacizumab

showed comparable results between the motesanib and bevacizumab arms in advanced NSCLC patients but with higher toxicity in the motesanib arms [60]. A larger Phase III placebo-controlled study with motesanib and CP was temporarily halted due to a high incidence of hemoptysis in patients with squamous cell histology, but reopened later for non-squamous NSCLC patients with results available in 2013 [61].

Other therapies that target and antagonize circulating VEGF are currently undergoing investigations [62]. VEGF and EGFR are now shown to have interconnected downstream pathways, potentiating the effectiveness of their dual inhibition in cancer therapy. Vandetanib is a small molecule that inhibits VEGFR, EGFR as well as RET and additional kinases, and may be beneficial in treating patients with solid tumors [63]. The effectiveness of dual EGFR/VEGF inhibition to single EGFR blockade was evaluated in a two-part crossover, randomized Phase II trial [64]. Vandetanib (300 mg) was tested against gefitinib (250 mg) and following progression on the randomized primary treatment, patients were crossed over to the opposing treatment arm. Median PFS was 11.0 weeks for vandetanib and 8.1 weeks for gefitinib (HR = 0.69) with no difference found in OS. DC for more than 8 weeks was observed in 45% of patients in the vandetanib arm compared with only 34% patients taking gefitinib.

The efficacy of vandetanib was evaluated in four randomized phase III clinical trials in NSCLC in combination with docetaxel (ZODIAC) [65], pemetrexed (ZEAL) (66] or as a single agent (ZEST and ZEPHYR) [67, 68]. Both combination trials with vandetanib resulted in the improvement of lung cancer symptom control. However, ZODIAC was the only trial to meet its primary endpoint of progression free survival (PFS) while no study showed advantage in overall survival (OS). As of October 2009, there are no longer planned developments for the use of vandetanib in treating advanced NSCLC in the USA and the EU. The decision was made based on the lack of benefit to OS and the insufficiency of using PFS as the primary end point for approval.

EML4-ALK

EML4-ALK is an aberrant fusion gene that is the result of a chromosomal rearrangement between the N-terminal half of the echinoderm microtubule-associated proteinlike 4 (EML4) gene and intracellular kinase domain of the anaplastic lymphoma kinase (ALK) gene. This leads to constitutive, ligand-independent activation of the *ALK* kinase, resulting in aberrant activation of downstream oncogenic signalling pathways including PI3K and MAPK, as well

as STAT3 dependent pathways that promote cell proliferation, stromal invasion, and apoptotic inhibition. Other reported ALK fusions in NSCLC include TFG [69] and kinesin family member 5B (KIF5B) [70, 71] are less frequently observed. EML4-ALK was initially discovered in anaplastic large-cell lymphoma [72] however, in 2007, Soda *et al*. described ALK activation in a subset of NSCLC that exhibited this fusion between EML4 and ALK in the short arm of chromosome 2p [73]. This study detected the EML4–ALK fusion transcript in 5 of 75 Japanese (6.7%) NSCLC patients however further studies revealed that it occurs in approximately in 2-7% of patients with NSCLC adenocarcinomas. ALK rearrangements are more common in never smokers or light smokers and in those with adenocarcinoma especially the acinar histology in East Asia or the signet-ring or cribriform morphology in the West. This variant is always positive for TTF-1 [74, 75]. In general, EML4–ALK and EGFR and KRAS mutations are mutual exclusive [76] however counterexamples have also been reported [77]. Patients with ALK rearrangements are not thought to benefit from EGFR TKIs.

TARGETING ALK

Although the incidence of EML4–ALK is low in NSCLC its importance is significant due to its relatively specific and well-tolerated ALK inhibitor, crizotinib (Xalkori) [78, 79]. Crizotinib is a small molecule TKI originally developed as a mesenchymal epithelial transition growth factor (c-Met) inhibitor [80] but was discovered to be a potent ALK inhibitor. It binds to ALK blocking the driver kinase activity and inhibiting tumour growth. This inhibitor was first described in 2007 and in less than 3 years an early-phase clinical trial yielded impressive responses in patients with advanced lung cancer containing *ALK* rearrangements (Kwak *et al*.) 82 patients, most of whom were previously treated, were enrolled in the study and received crizotinib (250 mg) twice daily in 28-day cycles [81]. At a mean treatment duration of 6.4 months, the overall response rate was 57% (47 of 82 patients, with 46 confirmed partial responses and 1 confirmed complete response); 27 patients (33%) had stable disease. A total of 63 of 82 patients (77%) were continuing to receive crizotinib at the time of data cut-off, and the estimated probability of 6-month progression-free survival was 72%, with no median for the study reached. The drug resulted in grade 1 or 2 (mild) gastrointestinal side effects. The benefit of testing for ALK rearrangements was demonstrated in phase I and phase II trials of the ALK inhibitor crizotinib. Patients with the EML4-ALK fusion, nearly all of whom had progressed despite

at least 1 prior line of therapy, showed response rates of approximately 50% to 60% crizotinib. Response duration was 42-48 weeks [78, 79].

Data obtained from clinical trials has shown that in patients who had received prior treatments that either failed or worked only for a brief period of time, crizotinib offered a 72% chance the tumour would shrink or remain stable for at least six months. As with the EGFR inhibitors, however, tumours tend to adapt to target therapies, and eventually render them ineffective. The FDA approved crizotinib along with a companion diagnostic to evaluate ALK rearrangements on FISH for advanced stage non-small cell lung cancer which has been determined to be ALK positive.

In patients who received crizotinib as second-line therapy, the 1-year overall survival rate was 70% and the 2-year overall survival rate was 55%. By contrast, ALK -positive matched controls had a 1-year survival of 44% and a 2-year survival of 12%, whereas ALK -negative controls had a 1-year survival of 47% and a 2-year survival of 32%. These data suggest that the presence of the ALK gene fusion itself does not confer a poorer outcome but that the use of crizotinib in ALK-positive patients can improve outcome [82].

Immunohistochemistry, may become a standard-of-care, high concordance with FISH having been established for IHC 3+ or IHC 0 [83]. Intermediate IHC scores may, however, still require FISH. Several different antibodies are in development [76]. Already, a crizotinib resistance mechanism has been identified, a "gatekeeper" mutation L1196M [84-86]. Interestingly the ROS gene is also an "off-target" of crizotinib. Activation of ROS can be found in about 1.7% of NSCLC and crizotinib appears to have marked activity in these cases [87]. However, there is a case report of a patient with NSCLC harboring MET amplification who responded to this agent [88].

Pemetrexed may have exceptional activity in ALK-rearranged NSCLC [89], with a response (in monotherapy or in combination with a platin) of 42% and a PFS of 9 months. Other publications have appeared in support [90, 91]) but more recently, those findings have been questioned. The ongoing profile studies should be informative.

KRAS

RAS oncogenes were first identified as the transforming factor in the Harvey and Kirsten strains of a mouse sarcoma virus [92]. KRAS is a member of a family of intracellular GTP-binding switch proteins called the GTPase superfamily. KRAS alternates between an active "on" state with a bound GTP and an inactive

"off" state with a bound GDP. It plays a key role in signal transduction downstream of transmembrane receptor tyrosine kinases, e.g. EGFR. After binding of growth factors such as the epidermal growth factor (EGF) and transforming growth factor α (TGFα) to the EGFR, GDP bound KRAS is then recruited by adaptor molecules. KRAS activation is accelerated by a protein called guanine nucleotide–exchange factor (GEF), which binds to the KRAS-GDP complex, causing dissociation of the bound GDP. GTP binds spontaneously to "empty" KRAS molecules, with release of GEF, activating downstream signalling predominantly via the RAF/MEK/ERK downstream signal transduction pathway (the classical MAPK pathway). Several other pathways may be stimulated by KRAS, including PI3K/AKT [93, 94]. Deactivation of KRAS, requires the assistance of a GTPase-activating protein (GAP), which binds to RAS-GTP and accelerates its intrinsic GTPase activity by a hundredfold.

Homologous *KRAS* mutations have been identified in several human cancers. These missense mutations impair GTP hydrolysis and thus promote formation of constitutively activated GTP-bound KRAS. In NSCLC, 97% of *KRAS* mutations occur in exon 2, codon 12 or 13 [95] and are more common in "smoking adenocarcinoma" patients (30%–40%) hence the hypothesis that there is a direct relationship to tobacco exposure. The mutations are G-to-T or G-to-C transversions (pyrimidine swapped for purine); however, in never-smokers with adenocarcinoma, "transition" mutations (G to A (purine for purine)) have occasionally been found (approximately 15%) [96]. Mutation subtype may alter downstream signal activation, with potential implications [97] for prognosis. *KRAS* mutations are rarer in never smokers and less common in East Asian vs. US/European patients [98]. In general, *KRAS* mutations are found in tumors wild type for *EGFR* or *ALK* defining a distinct molecular subset of the disease. KRAS mutations are prognostic for poor survival, independent of therapy and patients fail to benefit from adjuvant chemotherapy and seem to be resistant to EGFR-TKIs [99, 100].

TARGETING KRAS

Unlike other inactivating mutations which "switch off" signalling, *KRAS* mutations results in its persistent signal activation. For this reason KRAS has been difficult to target directly; as it requires reactivation, not inactivation, to switch the signalling off.

Potentially KRAS itself could be targeted by inhibiting: KRAS protein expression, membrane localization through posttranslational modification or

trafficking, blocking its interaction with GEFs, or enhancing KRAS/GAP interactions [101]. Although extensive studies have been undertaken to block prenylation of the KRAS C-terminal membrane anchoring by farnesyltransferase inhibiton, unfortunately to date, no agent has been developed that can modulate any of the above processes with success. Efforts to target downstream effector pathways including BRAF, MEK, and PI3K either singularly or in combination have been more successful and are described below.

Targeted inhibition of the molecular chaperone Hsp90 by investigational drug ganetespib, a synthetic second-generation Hsp90 inhibitor, slowed the growth of NSCLC cells which were *KRAS* mutation positive. The drug was even more active when combined with traditional lung cancer treatments and other investigational targeted therapies, according to preclinical study data [102].

The indirect value of KRAS in determining sensitivity to other targeted agents or to pemetrexed remains controversial. Recent data in NSCLC has shown that *KRAS* mutations may sensitize tumours to antifolates such as pemetrexed [105], possibly by upregulation of mir-181c, a micro RNA that can downregulate *KRAS*. As clinical evidence emerges, it is apparent that both KRAS mutation and amplification status should be considered for patient stratification prior to antifolate treatment.

HER2

HER2 is a member of the EGFR family of tyrosine kinase receptors that drive and regulate cell proliferation [103]. HER2 is overexpressed in 20% of NSCLC patients resulting in poor patient prognosis and survival [104]. Increased gene amplification has been documented in 2% to 23% of cases depending on the study [105, 106] and is more frequent (30%) in tumors with bronchioloalveolar carcinoma histology [107]. Mutations in the tyrosine kinase domain of HER2 were first identified by the Cancer Genome Project [108]. Occurring in 2 to 4% of cases somatic mutations are predominantly insertion/duplications between amino acids 774 and 779, or a missense mutation at 755 and result in the continuous activation of the HER2 receptor pathway [109]. EGFR and HER2 mutations are located in the C-helix region of the kinase domain and interestingly, HER2 in-frame insertions are found in a similar position to the deletion mutations observed in EGFR in NSCLC. Similar to EGFR and ALK, mutations occur more frequently in women, never-smokers and those with an adenocarcinoma sub-type. Studies have shown HER2 mutations, in exon 20 of the tyrosine kinase domain, are found

in 3 to 10% of lung adenocarcinomas [110, 111]. HER2, EGFR, and KRAS mutations seem to be mutually exclusive although a small proportion of patients may have coexisting HER2 and EGFR mutations [112]. At present, due to the relatively low mutation rate in the HER2 tyrosine kinase domain, routine patient screening and the availability of clinical trials has been limited.

TARGETING HER2

Early trials with the anti-HER2 monoclonal antibody (Trastuzumab) combined with chemotherapy in lung cancer patients with HER2 overexpression did not show a benefit for patients like the exceptional results seen in breast cancer [113]. Patients with amplified HER2 do not seem to benefit from anti-HER2 monoclonal antibodies (trastuzumab) or HER2 TKIs (lapatinib) [105-107, 114, 115]. Although patients with HER2 mutations are resistant to EGFR TKIs irrespective of their EGFR mutation status, they do benefit from HER2 targeted therapy. Patients who have previously shown resistance to chemotherapy and/or EGFR inhibitors benefit from trastuzumab plus chemotherapy [116]. The dual tyrosine kinase inhibitors lapatinib and afatanib, which target both EGFR and HER2, have shown evidence of activity in lung cancer patients with HER2 mutations [117]. Similarly, the pan-HER TKI PF00299804 inhibited cell growth in HER2- amplified and HER2-mutated NSCLC cell lines resistant to gefitinib [118].

At present, patients who are identified as HER2-mutant are treated with first-line chemotherapy, with HER2 specific trials designated as second-line or greater therapy. The Lung Cancer Mutation Consortium is offering tumor testing for HER2 mutations with the aim of promoting, within all participating medical institutions, clinical trials of this uncommon mutation.

BRAF

B-RAF is a serine/threonine-protein kinase that plays a role in linking RAS GTPases with proteins of the MAPK family which are involved in cell division and differentiation [119]. Three members of RAF kinase family have been identified and include: A-RAF, B-RAF and c-RAF. B-RAF mutations are among the most common in cancer in general, and were first described in melanomas [120]. They are present in only 1% to 3% of lung cancers and are most commonly

found in adenocarcinomas and in former or current smokers. In melanoma, where the most promising results with BRAF inhibitors have been seen, about 80% of mutations affect the Val600 residue (exon 15) within the kinase domain.

Lung tumours also harbour non-Val600Glu mutations including the Leu596Val mutation in the kinase domain and the Gly468Ala mutation in the G loop of the activation domain, making testing more complicated [57, 120, 121]. The Lung Cancer Mutation Consortium is testing for multiple BRAF mutations, with direction to clinical trials being offered through the consortium.

TARGETING BRAF

Sorafenib (BAY43-9006, Nexavar) was the first RAF kinase inhibitor to enter clinical testing. This compound was initially developed as a selective inhibitor of RAF, however studies revealed other targets included VEGF receptor 2 and 3, PDGFR, FLT-3, c-KIT, and FGFR-1 [122]. The anti-tumor activity of sorafenib is actually thought to be anti-angiogenic rather than through RAF inhibition in patients with advanced cancer. Second-generation RAF inhibitors with greater selectivity for BRAF are in development and include PLX4032 (vemurafenib) and its close analogue PLX4720 (Plexxikon, Berkeley, CA, USA). Both are small molecule inhibitors selective for B-RAF and have shown excellent results in the treatment of melanoma patients with the Val600Glu mutation [123]. PLX4032 potently inhibits MAPK pathway activity in cells expressing ^{V600E}BRAF, however paradoxically it induces activation of ERK in BRAF wild-type tumor and normal cells [132, 133]. In BRAF wild-type tumor and normal cells, low concentrations of PLX4032 induces ERK signaling by transactivating non-drug bound RAF protomers in a process that is RAS-dependent. At higher concentrations, PLX4032 binds to both protomers within a dimer and inhibits all RAF activation [124]. Novel RAF inhibitors that potently suppress ERK activation in BRAF mutant cells but lack the paradoxical activation of ERK noted with PLX4032 in normal cells are also in development on the basis of the presumption that such agents would exhibit a broader therapeutic index [125].

C-MET

The *MET* gene is located on chromosome 7q21-q31 and encodes hepatocyte growth factor receptor (HGFR) [126]. Its paracrine ligand, hepatocyte growth

factor (HGF), is produced by stromal cells. Met signals via RAS, PI3K/AKT, and STAT, affecting mitosis, survival, angiogenesis, migration, invasion as well as mesenchymal–epithelial transversion. Upregulation in cancer cells results in "invasive growth" [127]. In NSCLC, *MET* amplification occurs in squamous-cell carcinoma and adenocarcinoma but HGFR overexpression maybe more common. Mutations in *MET* are rare with one study identifying only 3 mutations in a cohort of 188 lung adenocarcinomas; two in exon 13 encoding the juxtamembrane domain (Arg988del and Tyr1021Asn) and one in exon 18 encoding the kinase domain (Gly1260Cys) [128].

Two Japanese studies also identified an intronic splice variant leading to exon 14 deletions in 2–3% of NSCLC [129, 130]. Upregulation of MET may depend on prior exposure to therapy and may mediate resistance to it. Several studies indicate that MET amplification is responsible for ±20% of resistance to EGFR TKIs through a mechanism termed kinase switch [131–133], prompting the development of Met-inhibitory strategies.

TARGETING C-MET

Interestingly, crizotinib (PF-02341066 (Pfizer) described above as an approved targeted therapy for ALK-rearranged metastatic NSCLC, was originally developed as a c-MET inhibitor. A recent report [88] of a MET-amplified NSCLC patient with normal ALK treated with crizotinib showed the patient had a rapid, durable response to the dual inhibitor. As seen with other cancers crizotinib has a role in treating NSCLC patients with ALK rearrangements or MET amplification [134, 135].

Tivantinib (ARQ 197) is a small molecule inhibitor of c-Met with potential antineoplastic activity. A randomized, double-blind study (MARQUEE) evaluating erlotinib plus tivantinib versus erlotinib plus placebo in previously treated patients with locally advanced or metastatic, non-squamous, non-small cell lung cancer (NSCLC) was recently halted after interim analysis showed it was not expected to reach its primary endpoint of improvement in OS. This trial was based on a randomized phase II study (erlotinib ± tivantinib) in which non-squamous and KRAS M+ patients had benefited most with PFS (4.4 vs 2.3 months, P = 0.12) and OS (10.1 vs 6.9 months, P = 0.18) [136]. Tivantinib continues to be studied as a monotherapy and as a part of combinations to treat many cancers including NSCLC.

MetMAb (Hoffmann–La Roche), an anti-Met monoclonal antibody, achieved significant PFS and OS benefit in a randomized phase II trial (OAM4558g),

comparing MetMAb plus erlotinib (ME) to placebo plus erlotinib (PE) in 2nd/3rd line NSCLC. Benefit was not restricted to EGFR M+ or MET FISH + but was also seen in MET FISH-/IHC + [137]). Therefore MET expression may be more reliable than amplification in predicting MetMAb benefit. The effect in low expressors of Met appeared actually harmful, highlighting the importance of a companion diagnostic as MetMAb proceeds into phase III. Several phase I trials are in progress with inhibitors targeted towards specific and multiple kinases that are also active against c-Met. Multikinase inhibitor of c-Met and VEGFR-2 foretinib (GSK1363089 or XL880), and cabozantinib XL184 (Exelixis) demonstrate strong affinity for the hepatocyte growth factor receptor (Met) and vascular endothelial growth factor receptor 2 (VEGFR2).

Rilotumumab (AMG 102) (Amgen) is a fully humanised IgG2 monoclonal antibody that binds to and neutralises HGF, which prevents its binding to c-MET. A phase II trial of this drug in combination with chemotherapy is underway. MET appears to be the next major biomarker in metastatic NSCLC, with several inhibitors fast approaching the clinic.

PI3K

Phosphatidylinositol 3-kinases (PI3Ks) were discovered by Lewis Cantley and colleagues, who first published on their association with the polyoma middle T protein in 1985 [138]. PI3Ks have since been shown to be signal transducer enzymes that have the ability to phosphorylate the 3 position hydroxyl group of the inositol ring of phosphatidylinositol and phosphoinositides. The signals that PI3K family members help to potentiate induce the cell to grow, differentiate, proliferate, and increase survival, motility and intracellular trafficking. As such, these enzymes are strongly implicated in the development of the malignant phenotype. The PI3K/AKT/mTOR pathway has been implicated in lung tumourigenesis, with mutations, amplifications and epigenetic alterations observed at various points in the cascade [139]. The catalytic subunit of PI3K, p110, occurs in multiple isoforms, with the p110α isoform being encoded by PIK3CA. PIK3CA is frequently amplified or mutated in NSCLC, and is associated with increased AKT activity. PIK3CA aberrations are more frequently observed in squamous cell carcinomas (mutations: 2-7%; amplifications: 33-70%) than adenocarcinomas (mutations: 2%; amplifications: 6-19%) [140]. PIK3CA promoter methylation has also been observed in NSCLC, implying an alternative mechanism underlying inactivation of tumor-associated genes in lung carcinogenesis [139]. Further alterations in PI3K pathway genes such as PTEN

(mutation: 4-5%, loss 24-44%), LKB1 (mutation: 9-33%) and AKT1 (mutation: 1-2%) are all found in NSCLC. Alterations in these genes lead the PI3K pathway to become constitutively activated. Activated mTOR (p-mTOR) has been shown to be present in up to 90% of adenocarcinomas, 60% of large cell carcinomas and 40% of squamous cell carcinomas cases.We and others have shown that activation of this pathway in NSCLC leads to a more aggressive, drug-resistant disease which correlates to poor prognosis for patients [141].

TARGETING PI3K

The PI3K pathway represents an attractive target for therapeutic intervention and recently we have seen the development of several inhibitors targeting strategic points in the signalling cascade. Analogues of mTOR inhibitor Rapamycin (or 'Rapalogues') were first investigated as a method of inhibiting PI3K pathway signalling. The mTOR protein kinase nucleates two distinct multiprotein complexes, mTOR complex1 and 2 (mTORC1 and mTORC2), and these Rapalogues (including Everolimus and Temsirolimus) only inhibit mTORC1. Disappointing results from clinical trials indicated that this treatment strategy was not ideal, and as such 'second generation inhibitors' of mTOR, which inhibit the adenosine triphosphate site of the mTOR kinase domain, and crucially are able to block both mTORC1 and mTORC2 complexes, were developed [142]. Several second generation mTOR inhibitors also exert effects on PI3K, and as such several of these 'dual PI3K/mTOR inhibitors' such as BEZ235, GDC-0980 and XL765 are currently in clinical trials, with promising *in vitro* and *in vivo* data indicating that this dual inhibition strategy may be superior to targeting mTORC1 or PI3K alone. Pan PI3K inhibitors such as GDC-0941, XL147 and PX-866, and isoform-specific PI3K inhibitors such as P110α inhibitor BYL719, p110β inhibitor GSK2636771 and p110δ inhibitor GS1101 have also been developed and are currently under investigation. AKT, NfκB and other PI3K pathway protein inhibitors have also been investigated in the laboratory and clinical setting for effectiveness in NSCLC, with more recent strategies involving a combined treatment approach, as discussed later in this chapter. NSCLC represents a cancer which could benefit greatly from use of PI3K pathway targeted inhibitors such as these, due to the limited effectiveness of current standard treatments as well as the crucial role that the PI3K pathway has been shown to play in the progress, maintenance and inherent/acquired chemoresistance of NSCLC tumours.

MEK

The most common oncogene in human cancer is RAS, with 20% of all tumours harbouring an activating mutation in one of the RAS genes. As discussed earlier in this chapter, this gene plays a particularly important role in NSCLC, with 35% of NSCLC tumours having undergone a RAS activating mutation [143]. It is generally accepted that the RAS protein superfamily plays an important role in lung carcinogenesis and the maintenance of the malignant phenotype, with KRAS and BRAF being frequently mutated in NSCLC. It has been reported that one or other of KRAS and BRAF is mutated in 50% of NSCLC cases [144]. While strategies of targeting KRAS and BRAF have already been discussed, here, the relevance of KRAS downstream signalling effectors to NSCLC treatment will be examined.

Once activated through the binding of an extracellular mitogen to a membrane-bound ligand, RAS exchanges its GDP for a GTP, allowing it to activate signalling proteins which ultimately exert effects on cell proliferation, cell survival and other pro-carcinogenic phenotypes. The first identified and most extensively investigated effector of RAS is RAF, a serine/threonine kinase which phosphorylates MEK1 and MEK2, which in turn activate ERK1 and ERK2 by phosphorylation. Once activated, ERK can translocate to the nucleus and activate transcription factors such as ETS family members that can transcribe genes involved in the promotion of cell cycle progression.

Glu56Pro, Lys57Asn and GLN56Pro mutations occur in the non-kinase portion of MEK1. Somatic mutations including these have been identified in approximately 1% of NSCLC, predominantly adenocarcinoma [145].

TARGETING MEK

With the RAS-RAF-MEK signalling cascade offering a cancer cell ample opportunity to grow, divide and survive, there has been significant interest in developing methods of 'switching off' the pathway in cancers including NSCLC. A number of inhibitors of RAF have been investigated in both the laboratory and clinical settings, and are discussed earlier in this chapter.

The only known substrate for MEK is ERK, and since activation of MEK and ERK can be independent of RAS activation, inhibition of MEK has become a major focus of research over the last number of years. Marks et al. demonstrated in 2008 that cells harbouring MEK1 Lys57Asn and Gln56Pro mutations (which

are both gain-of-function mutations) can be sensitive to MEK inhibitor AZD6244 [145]. Results from a Phase II, open-label, randomized study to assess the efficacy and safety of AZD6244 versus pemetrexed in patients with NSCLC who had failed prior chemotherapeutic regimens indicated that while AZD6244 showed clinical activity as a second- or third- line treatment for patients with advanced NSCLC, it did not offer any advantage over standard treatment with pemetrexed [146]. A phase II study of the oral MEK inhibitor, CI-1040, in patients with cancers including advanced NSCLC concluded that it demonstrated insufficient antitumor activity to warrant further development [147]. Other inhibitors of MEK currently under investigation include PD0325901 [148], selumitinib [149] and GDC-0973 [150].

With numerous points of cross-talk between the RAS-RAF-MEK pathway and other pathways including the PI3K/AKT/mTOR pathway, cancer cells have the ability to overcome MEK targeted inhibition by utilising alternative signalling routes, which could explain the disappointing results that have been observed thus far with clinical MEK inhibition. As such recent thinking suggests a more promising role for these inhibitors in combination with other pathway inhibitors, as discussed below.

PI3K AND MEK CO-TARGETED INHIBITION

RAS has the ability to activate both the PI3K and MEK pathways, resulting in pro-carcinogenic phenotypes being expressed by a cell in response to an extracellular stimulus such as the binding of a growth factor to a receptor. Once one of these two pathways is blocked by a targeted therapy, it is both possible and plausible that the cell can overcome the treatment by signalling through the other pathway at any of a number of points of convergence, i.e. by using a 'bypass track'. This is one increasingly discussed explanation for the high frequency of resistance observed when patients are treated with cell signalling protein targeted inhibitors. As such, a promising strategy going forward is to utilize a combination approach to therapy for NSCLC patients, where both the PI3K and MEK pathways are inhibited. Research published in 2009 identified a robust increase in apoptosis and tumour shrinkage upon combined blockade of both of these pathways [151], with further *in vitro* and *in vivo* work showing promise for this strategy [152-154].

Recent *in vitro* data demonstrates that PI3K/mTOR inhibitor GDC-0941 synergizes with the MEK inhibitor U0126 in NSCLC cells [155]. A phase I trial is currently recruiting patients with solid tumours for combination treatment with

PI3K pathway inhibitor BKM120 and MAPK pathway inhibitor MEK162. Another Phase I trial is currently recruiting patients with locally advanced or metastatic solid tumours for co-targeted inhibition treatment with MEK inhibitor GDC-0973 and PI3K inhibitor GDC-0941. This approach may become more common in lung cancer treatment, as an estimated 70% of lung tumours display RAS-RAF-MEK pathway activation [144].

IGF1-R

The insulin-like growth factor receptor-1 (IGF-1R) is a transmembrane tyrosine kinase and is structurally homologous to the Insulin Receptor (IR). IGF-1R overexpression has been identified in several tumour types and protects cells from apoptosis-inducing agents, including hypoxia and anti-cancer drugs. The IGF-1R suppresses apoptosis primarily through the phosphoinositide 3-kinase (PI3K) pathway [156] and has been shown to activate other receptors involved in cancer including EGFR, VEGFR, ERα and AR [157]. Although several studies have shown that IGF-1R is not a prognostic marker in NSCLC [158-160]. Merrick et al showed that high IGF-1R expression is associated with ADC histology and a poorer survival in 191 resected NSCLC patients [161]. We have shown that high levels of both IGF-1R and EGFR correlates to poor survival in NSCLC patients (unpublished data) and high levels of IGF-1R correlates to a poor prognosis in SCLC [162]. Activation of the IGF-1R signalling pathway is also involved in the development of resistance to EGFR TKIs [163].

TARGETING IGF1R

Several different approaches are being investigated for targeting the IGF-1R, including small-molecule kinase inhibitors and IGF-1R antibodies. Initial results of a randomized phase II study showed that combining an anti-IGF-1R monoclonal antibody (figitumumab) with a platinum doublet (paclitaxel–carboplatin) in NSCLC patients resulted in a higher response rate and trends for superior progression-free survival and overall survival. The best results were observed in squamous cell patients [164]. However, a subsequent phase III study failed to validate these results although in a subset of patients with high IGF serum levels, the addition of figitumumab appeared to offer benefit over carboplatin–paclitaxel [165]. Studies have shown mTOR inhibition can activate

AKT through IGF-1R resulting in a synergistic interaction with IGF-1R inhibitors [166]. To this end, a phase I trial with cixutumumab in combination with temsirolimus was undertaken and resulted in stable disease in 47% of patients, although only 1 patient in this trial had NSCLC [167]. These clinical trials have indicated that high circulating levels of IGF-1 may be a potential biomarker that could identify patients who may benefit from IGF-1R inhibitors. Currently, the monoclonal antibody IMC-A12 is being tested in combination with a platinum doublet in stage IV NSCLC and in extensive stage SCLC. Several trials are ongoing that are testing the inhibition of both the EGFR and IGF-1R pathways. In one study, cetuximab is combined with cixutumumab and patients are randomized to gemcitabine–cisplatin or carboplatin–cetuximab with or without cixutumumab. Two randomized trials with the IGF-1R tyrosine kinase inhibitor (OSI-906) combined with erlotinib are ongoing. The first study, chemotherapy naïve patients with EGFR mutations are randomized to erlotinib combined with OSI-906 or to erlotinib and placebo. In the second study, patients who are eligible for erlotinib maintenance are assigned to the EGFR TKI plus OSI-906 *versus* placebo. Importantly the collection of of blood and tumor specimens for molecular marker testing is mandatory in these trials, which is crucial in determining markers of response to treatment.

THE FUTURE OF TARGETED THERAPY

The implementation of treatment protocols tailored to the molecular profile of an individual's tumour represents an important paradigm shift in how we treat cancer patients. This personalised approach will hopefully lead to substantial therapeutic improvements and increased patient survival. The success of EGFR and ALK targeted therapies have taught us that a greater emphasis should be placed on the collection of tumor specimens in "real-time" during clinical trials as in the case of the BATTLE trial. Further analyses of these tumor and blood specimens may identify unique patient subgroups that are sensitive to targeted treatments. A recently published study by Tsimberidou and colleagues describes a personalised medicine program undertaken, at the MD Anderson cancer center, in the context of early clinical trials, which involved using targeted agents matched with tumor molecular aberrations [168]. Of 1,144 patients analyzed, 460 (40.2%) had 1 or more aberration. Patients with one aberration treated with a matched therapy (n =175), compared with treatment without matching (n = 116) were associated with a higher overall response rate (27% vs. 5%; $P < 0.0001$), longer time-to-treatment failure (TTF; median, 5.2 vs. 2.2 months; $P < 0.0001$), and

longer survival (median, 13.4 vs. 9.0 months; P = 0.017). These data, although not randomized and patients had diverse tumor types and a median of 5 prior therapies, are extremely encouraging and represent a promising future for targeted therapies.

CONCLUSION

A deeper understanding of the aberrant molecular mechanisms of tumours has led to a shift from a "one size fits all" therapeutic approach to a more personalised targeted approach defined by each tumour's molecular characteristics. Patient screening using companion diagnostics is key to ensuring those with a compatible molecular profile receive an appropriate targeted agent thus improving overall survival and decreasing toxicity. However, in NSCLC, the acquisition of sufficient biopsy material remains a stubborn obstacle to the identification of a patient's molecular signature. Therefore the development of more sensitive technologies, that can generate a comprehensive genetic profile of tumor specimens in a time- and cost-effective manner, is crucial to the success of targeted therapies in NSCLC.

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