

# Personalized medicine and treatment approaches in non-small-cell lung carcinoma

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**Abstract:** Chemotherapy has been the traditional backbone for the management of metastatic lung cancer. Multiple trials have shown the benefits of treatment with platinum doublets in lung cancer. This “one treatment fits all” approach was further refined by the introduction of targeted agents and discovery of subpopulations of patients who benefited from treatment with these agents. It has also become evident that certain histologic subtypes of non-small-cell lung cancer respond better to one cytotoxic chemotherapy versus others. This has led to the concept of using histology to guide therapy. With the introduction of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and the discovery of activating mutations in the EGFR gene, further personalization of treatment for subgroups of patients has become a reality. More recently, the presence of a fusion gene, echinoderm microtubule-associated protein-like 4 – anaplastic lymphoma kinase (EML4-ALK), was identified as the driver mutation in yet another subgroup of patients, and subsequent studies have led to approval of crizotinib in this group of patients. In this article, efforts in personalizing delivery of care based on the histological subtypes of lung cancer and the role of K-RAS and EGFR mutations, EML4/ALK translocation, and ERCC1 (excision repair cross-complementing 1) and EGFR expression in choosing appropriate treatments for patients with advanced lung cancer are discussed. This article also reviews the problem of resistance to EGFR tyrosine kinase inhibitors and the ongoing trials that target novel pathways and mechanisms that are implicated in resistance.

**Keywords:** NSCLC, EGFR, cancer treatment

Lung cancer is affecting an ever increasing number of patients, and is now one of the world's leading causes of cancer-related deaths. There have been many developments in the management of lung cancer, especially in non-small-cell lung cancer (NSCLC). These new treatments take into account the histology and molecular characteristics of the tumor as well as patient characteristics.

The management of NSCLC has historically relied on the use of cytotoxic chemotherapy, and responses have been modest. Advances in our understanding of the tumor biology, along with identification of specific molecular alterations, have allowed a more personalized approach for treatment of some patients with this disease.

In advanced NSCLC, use of platinum doublets has been the accepted standard of care. Studies such as ECOG 1594<sup>1</sup> and SWOG 9509<sup>2</sup> compared several platinum-based doublet regimens and found them to be equally effective, with minor differences in toxicity profile. None of these studies reported a particular advantage in any of the subgroups analyzed. These studies further highlighted the plateau that had been reached in treatment of NSCLC using cytotoxic agents, and indicated a need for change in our

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strategy to address this disease. In this article we review findings that we believe have had the most impact in management of this disease.

## Role of histology

The simplest way of personalizing delivery of care in NSCLC is to use histology. This area had not been fully explored in the past, largely because most of the available agents had equal efficacy in the various histologies. However that changed with the introduction of bevacizumab and pemetrexed.

## Bevacizumab

New blood vessel development is important for tumor growth, and the vascular endothelial growth factor (VEGF) group of proteins is important in the development of new blood vessels. The VEGF family of proteins includes VEGF-A, B, C, D, and E, and placental growth factor 1 and 2. The VEGF proteins, in conjunction with their receptors, act to increase vascular permeability, endothelial cell activation, proliferation, and migration, leading to angiogenesis and subsequent tumor growth and proliferation.<sup>3,4</sup> Bevacizumab is a humanized mouse monoclonal antibody against VEGF. It binds and inactivates VEGF-A without any effects on the other members of the VEGF family of proteins.<sup>5</sup> Its clinical efficacy in the treatment of multiple tumor types, including breast, colorectal, renal cell, and glioblastoma, has been established.<sup>6-13</sup>

In an attempt to improve the clinical efficacy of platinum doublets in lung cancer, combination treatments with bevacizumab were explored. In Phase I studies, bevacizumab was shown to be safe in combination with chemotherapy including carboplatin and paclitaxel.<sup>14</sup> In the pivotal ECOG 4599 trial, the combination of carboplatin/paclitaxel/bevacizumab for up to six cycles, followed by bevacizumab every 3 weeks, until progression, improved the median survival and median progression-free survival (PFS) as compared with carboplatin and paclitaxel alone. Patients with squamous histology were excluded because of the risk of life-threatening bleeding.<sup>15</sup>

## Pemetrexed

Pemetrexed is an antifolate that inhibits the enzyme thymidylate synthase and other folate dependent enzymes. A retrospective analysis of a Phase III trial that compared pemetrexed to docetaxel in patients with recurrent NSCLC indicated superior survival in patients with non-squamous subtypes of NSCLC in the pemetrexed-treated arm. A subsequent Phase III trial in patients with previously

untreated advanced NSCLC was then conducted, with a predetermined analysis for histology.<sup>16,17</sup> This study demonstrated that cisplatin/pemetrexed was non-inferior to the cisplatin/gemcitabine arm in the general patient population, with a median survival of 10.3 months in both arms. However, this predetermined subset analysis based on histology showed that patients with non-squamous histology, adenocarcinoma, large cell carcinoma, and others, tended to have a statistically significant improvement in survival. Survival was reported as 12.6 versus 10.9 months for adenocarcinoma, 10.4 versus 6.7 months for large cell carcinoma, and 11.8 versus 10.4 months for non-squamous overall. An analysis of the squamous subgroup showed that there was a statistically significant detriment to the use of pemetrexed-based regimen, with an overall survival of 9.4 versus 10.8 months favoring the gemcitabine-based regimen.

## Nab-paclitaxel

A recently reported study suggests possible superior response rates in patients with squamous cell histology when treated with nab-paclitaxel. In this Phase III study comparing carboplatin/paclitaxel versus carboplatin/nab-paclitaxel, the combination of nab-paclitaxel/carboplatin had a better response rate in patients with squamous cell carcinoma (41% versus 24%,  $P < 0.001$ ) in the nab-paclitaxel arm.<sup>18</sup> Confirmatory studies are needed with this agent.

## Summary

Use of histology as a means of personalizing chemotherapy in patients with advanced NSCLC is now possible. Although selection of chemotherapy based on histology alone is not the ultimate goal of personalized care, it does avoid unnecessary toxicity in a subset of patients with advanced disease. At this point, the available agents show preferential clinical benefit only in patients with non-squamous histology.

## Molecular targets in lung cancer, suppressing the identifiable oncogenic drivers

Identification of several driver mutations and a translocation in NSCLC tumors has led to development of two drugs that have had a major impact on the treatment of patients with such tumors. The main driver mutations in lung cancer are seen in several genes including epidermal growth factor receptor (EGFR), K-RAS, and MEK. The echinoderm microtubule-associated protein-like 4 – anaplastic lymphoma kinase (EML4-ALK) translocation, EGFR expression determined by immunohistochemistry (IHC), and excision repair

cross-complementing (ERCC) expression are additional determinants of response to various agents.

## K-RAS mutation

The K-RAS mutation was first described in human lung cancers in the 1980s, where it was found in tumor tissue but not in normal host tissues.<sup>19</sup> The Ras family belongs to the super-family of guanosine triphosphatases (GTPases) and is composed of several members. Different stimuli from cell surface, through activation of various proteins, can activate members of this family. Once activated, Ras protein stimulates the initiation of several signaling cascades. In the case of K-RAS, these include: Raf/MEK/ERK (promoting proliferation) and PI3K/Akt (inhibiting apoptosis). Stimulation of EGFR also activates K-RAS.

K-RAS mutations have been found in approximately 17% of all NSCLC, and are seen in 27%–34% of adenocarcinomas and non-squamous tumors, but are rarely seen in squamous cell carcinomas.<sup>20,21</sup> As a predictor of prognosis, data from several clinical trials indicate that having a K-RAS mutation may be associated with a poorer overall prognosis. A meta-analysis of studies looking into survival of patients with lung cancer and K-RAS mutations showed decreased overall survival for patients with this mutation, with a hazard ratio (HR) of 1.35 (95% confidence interval [CI] 1.16–1.56). In adenocarcinomas, the HR was 1.59 (95% CI 1.26–2.02).<sup>22</sup> A retrospective study based on tumor samples from the ECOG 3590 study, in which patients were randomized to either postoperative radiotherapy or chemoradiotherapy, showed no statistically significant difference in survival in wild-type versus mutant K-RAS tumors. However, a multivariate analysis looking at prognostic factors found that K-RAS mutational status was a weak prognostic factor (relative risk 0.641,  $P = 0.066$ ).<sup>21</sup>

In the JBR.10 study, where the use of adjuvant chemotherapy with cisplatin/vinorelbine versus observation in patients with resected lung cancer was examined, survival of patients with tumors that expressed wild-type K-RAS was prolonged by adjuvant chemotherapy compared with observation (HR = 0.69; 95% CI 0.49–0.97;  $P = 0.03$ ). However, there was no apparent benefit from chemotherapy in patients with tumors that expressed K-RAS mutations (HR = 0.95; 95% CI 0.53–1.71;  $P = 0.87$ ).<sup>23</sup> In the SATURN trial, which investigated the use of erlotinib as maintenance in patients who had stable or nonprogressive disease after treatment with four cycles of a platinum doublet, presence of K-RAS mutation was associated with a poorer PFS.<sup>24</sup> Multiple other studies have also indicated that patients treated with

EGFR inhibitors such as erlotinib and gefitinib in the presence of K-RAS mutations have poorer response rates and survival.<sup>25–28</sup>

Targeting of K-RAS as a therapeutic target has been difficult. Several strategies have been employed. One approach with early promise relied on the use of drugs that belonged to the class of farnesyl transferase inhibitors, which prevent post-translational modification and farnesylation of a wide variety of proteins, including RAS. However, clinical trials of many of these compounds have failed to show significant benefits.<sup>29,30</sup> Another compound with early promise was farnesylthiosalicylic acid, or salirasib, which decreases the activity of activated RAS by competitively inhibiting the attachment of GTP-bound RAS to the plasma membrane. This compound has failed to show any benefit in Phase II trials.<sup>31</sup> Perhaps a more practical strategy is targeting pathways that are downstream or parallel to K-RAS, which may be easier to target. These include RAF, MEK, and PI3K/Akt, mTor, or c-MET.

Table 1 gives a listing of current clinic trials with different molecularly targeted agents aimed at K-RAS mutated tumors. Figure 1 shows a simplified schema for pathways that are important in lung cancer.

## EGFR mutations

The EGFR is a transmembrane receptor that belongs to the HER/erb family of receptor tyrosine kinases, which includes HER1, HER2, HER3, and HER4. When activated by its ligand, it undergoes homo- or heterodimerization with other family members, resulting in phosphorylation of the cytoplasmic domain and downstream signaling through multiple pathways, activating gene transcription, cell growth, and proliferation.<sup>32</sup> EGFR tyrosine kinase inhibitors (EGFR TKI), erlotinib and gefitinib, are well tolerated oral agents that have been tested in multiple trials.<sup>33–42</sup>

The most significant advancement in this area has been the discovery of activating mutations in the EGFR gene. This finding, confirmed independently by two groups, has had a significant impact in personalizing treatment of advanced NSCLC. These groups showed that the response to TKI therapy correlated with the presence of activating mutations present in the tyrosine kinase domain of the EGFR receptor. Exons 18 to 21 of the EGFR gene codes for the tyrosine kinase portion of the EGFR receptor, and mutations in any of these regions may confer either sensitivity or resistance to EGFR TKI directed therapy. The most common mutation is a deletion in exon 19. The second most common type of mutation is point mutations in exon 21, the most common

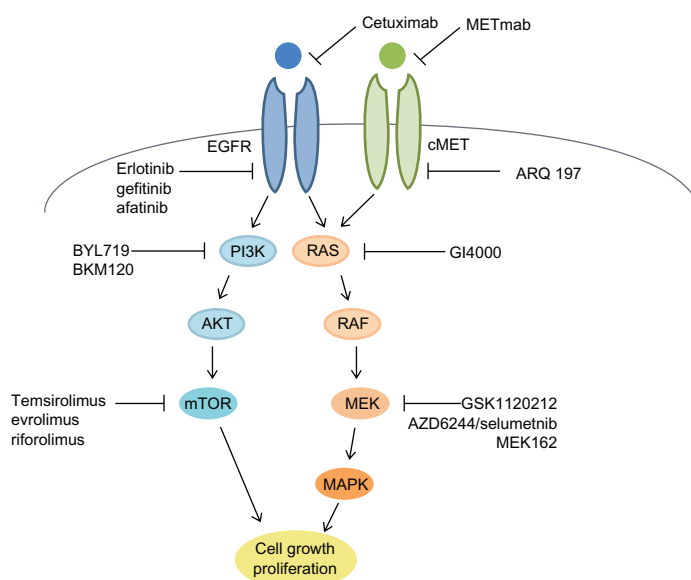
**Table 1** Proposed mechanisms of action of drugs in clinical trials addressing K-RAS mutated tumors

Mechanism of action	Drug name	Clinical trial	Tumor characteristics
MEK inhibitor	Selumetinib/AZD6244	NCT01229150, NCT00890825, NCT01239290	k-RAS mutated NSCLC
	GSK1120212 MEK162	NCT01362296, NCT00687622 NCT01337765, NCT01449058, NCT01363232, NCT00959127	k-RAS mutated NSCLC Tumors with k-RAS, NRAS, and/or BRAF mutations
c-MET inhibitor	ARQ 197	NCT01395758	k-RAS mutated NSCLC
mTOR inhibitor	Retaspimycin HCl (IPI-504) + everolimus	NCT01427946	k-RAS mutated NSCLC
	Ridaforolimus	NCT00818675	k-RAS mutated NSCLC
mTor+ PI3K inhibitors	BEZ235	NCT01337765	Tumors with k-RAS, NRAS, and/or BRAF mutations
PI3K inhibitor	BYL719	NCT01449058	Tumors with k-RAS, NRAS, and/or BRAF mutations
	BKM120	NCT01363232	Tumors with k-RAS, NRAS, and/or BRAF mutations
HSP90 inhibitor	Retaspimycin HCl (IPI-504) + everolimus	NCT01427946	k-RAS mutated NSCLC
Virus killing Ras activated cells	Reovirus serotype 3-Dearing strain (REOLYSIN)	NCT00861627	k-RAS mutated NSCLC
Recombinant mutant Ras protein	GI-4000	NCT00655161	k-RAS mutated NSCLC

of these being L858R. Besides mutations in these exons, there can be activating mutations in exon 18 and exon 20, but these are much less common. Most mutations in exon 20 are associated with a resistance to TKI.<sup>43-45</sup>

Initial studies with EGFR TKIs enrolled all-comers without any knowledge of the mutational status of patients. BR.21 was a large, randomized, placebo-controlled, double-blind Phase III trial that evaluated the efficacy of erlotinib

versus placebo in patients with previously treated NSCLC. A reported median PFS of 2.2 months in the erlotinib group versus 1.8 months in the placebo arm led to the approval of this agent. The median overall survival was 6.7 and 4.7 months in the erlotinib and placebo arms, respectively. A subsequent subgroup analysis showed that the maximum benefit was seen in women ( $P = 0.006$ ), nonsmokers ( $P < 0.001$ ), Asians ( $P = 0.02$ ), and adenocarcinoma histology ( $P < 0.001$ ).<sup>33</sup>

**Figure 1** Simplified schema of molecular pathways involved in lung cancer with proposed mechanisms of action of established and newer agents.

We now know that these groups have a higher likelihood of harboring EGFR-activating mutations, and are therefore more likely to respond to TKI therapy.

Attempts at combining either erlotinib or gefitinib with chemotherapy have not been successful. These studies for the most part have enrolled patients without prior knowledge of their mutational status.<sup>46–49</sup>

The IPASS trial (Iressa Pan-Asia Study) was an open-label Phase III study that compared gefitinib 250 mg daily with carboplatin/paclitaxel every 3 weeks for up to six cycles, in previously untreated patients with NSCLC. The mutational status of the patients was not known at study entry but was determined if adequate tissue was available during the study. In the subgroup of patients with activating EGFR mutations, gefitinib had a superior PFS (HR for progression = 0.48; 95% CI 0.36–0.64;  $P < 0.001$ ). The objective response rate was 71.2% with gefitinib, versus 47.3% with carboplatin–paclitaxel, in the mutation-positive subgroup ( $P < 0.001$ ).<sup>50</sup> Maemondo et al conducted a Phase III trial that also compared gefitinib 250 mg to carboplatin/paclitaxel in patients with sensitive EGFR mutations. This study also demonstrated a significantly increased median PFS, 10.8 versus 5.4 months in the gefitinib and chemotherapy arms respectively (HR 0.30; 95% CI 0.22–0.41;  $P < 0.001$ ). The response rate, similar to the IPASS study, was also significantly higher (73.7% versus 30.7%) in the gefitinib arm ( $P < 0.001$ ).<sup>51</sup> Most of these studies were conducted in Asia and used gefitinib as the study agent. The OPTIMAL study, a Phase III study that compared erlotinib to carboplatin/

gemcitabine, in patients with advanced NSCLC harboring activating EGFR mutations, also yielded a median PFS that was significantly superior in the erlotinib arm compared with the chemotherapy arm (13.1 versus 4.6 months; HR 0.16, 95% CI 0.10–0.26;  $P < 0.0001$ ).<sup>52</sup> Similar results were seen in the EURTAC trial, another Phase III study that compared erlotinib and platinum-based chemotherapy in patients with activating EGFR mutations.<sup>53</sup> In all of these studies, use of an EGFR TKI in the front-line setting showed a good response rate, in excess of 50%, and a statistically significant improvement in PFS. Table 2 summarizes Phase III data looking at the use of EGFR TKIs in the first-line setting in advanced NSCLC.

Together, these studies establish the role of EGFR TKIs in treatment of patients with EGFR-activating mutations at the time of diagnosis. It is imperative to obtain molecular markers on all patients with adenocarcinomas in an attempt to tailor treatment based on their tumor characteristics beyond histology.

### Detection methods

There are multiple methods that help identify various mutations in the EGFR gene. Once the tissue is deemed adequate, DNA is extracted for analysis; this step is crucial, as an unreliable process can lead to inaccurate results. Purified DNA is then amplified using different techniques before it is tested for mutations. Testing for mutations is accomplished using two basic methodologies. One approach is to screen the EGFR gene for all mutations both known and unknown;

**Table 2** Phase III studies with EGFR TKIs in the first-line setting in advanced NSCLC

Study	Population studied	Treatment arms	Results
IPASS <sup>50</sup>	Adenocarcinoma	Carboplatin/paclitaxel vs Gefitinib	Intention to treat group had a HR = 0.74; 95% CI, 0.65–0.85; $P < 0.001$ . EGFR mutated group had a HR = 0.48; 95% CI, 0.36–0.64; $P < 0.001$ . Response Rate was 71.2% vs 47.3%, ( $P < 0.001$ ) in the Gefitinib and chemotherapy groups respectively, within the EGFR mutated subgroup.
NEJSG 002 <sup>51</sup>	EGFR activating mutation positive	Carboplatin/paclitaxel vs gefitinib	Gefitinib group had a HR = 0.30; 95% CI, 0.22–0.41; $P < 0.001$ . Response rate was 73.7% vs 30.7%, ( $P < 0.001$ ) in the Gefitinib and chemotherapy arms respectively.
WJTOG3405 <sup>54</sup>	EGFR activating mutation positive	Cisplatin/docetaxel vs gefitinib	Gefitinib group had a HR = 0.489; 95% CI, 0.336–0.710; $P < 0.0001$ . Response rate was 62.1% vs 32.2% ( $P < 0.0001$ ) in the Gefitinib and chemotherapy arms respectively.
OPTIMAL (CTONG-0802) <sup>52</sup>	EGFR activating mutation positive	Gemcitabine/carboplatin vs erlotinib	Erlotinib group had a HR = 0.16; 95% CI, 0.10–0.26; $P < 0.0001$ . Response rate was 83% vs 36% ( $P < 0.0001$ ) in the Erlotinib and chemotherapy arms respectively.
EURTAC <sup>53</sup>	EGFR activating mutation positive	Platinum doublet vs erlotinib	Erlotinib group had a HR, 0.80; $P = 0.42$ . Response rate was 54.5% vs 10.5% ( $P < 0.0001$ ) in the Erlotinib and chemotherapy arms respectively.

this method usually needs a greater proportion of tumor cells in the sample. Examples of this approach are polymerase chain reaction (PCR) and direct sequencing, melting analysis, and pyrosequencing.<sup>55,56</sup> In general, these methods take additional time and need a greater percentage of tumor cells in the sample, and therefore would require a larger pathological specimen; however, novel mutations can be detected using this method. The second approach is to look for specific known mutations. This approach tends to be more sensitive and requires less tissue; however, the disadvantage of this method is that it can detect only known mutations. Examples of this approach are ARMS (amplification refractory mutation system), PNA clamp (peptide nucleic acid clamp), SNaPshot (a multiplexed PCR-based assay), ME PCR (mutation-enriched PCR), and PCR invader.<sup>57–60</sup> The choice of testing method may be controlled by institutional preferences; however, this should take into account the size and quality of the tumor sample and the likelihood of false negatives and false positives.<sup>61</sup>

### Resistance to EGFR TKIs

Inherent or primary resistance to EGFR TKIs can occur as a result of a mutation in the EGFR TK domain. The most common mutation is an insertion in exon 20, and rarely a mutation in exon 19.<sup>62</sup> All of these mutations are rare, and it is much more likely that there are additional mutations in downstream pathways such as K-RAS, or amplification in parallel pathways.

Secondary or acquired resistance occurs after a period of response to EGFR-targeted therapy. The most common mutation is the T790M mutation, a point mutation in exon 20 resulting in substitution of a threonine residue with methionine, seen in about 50% of tumors that relapse after exposure to EGFR TKIs. Some studies have suggested that this mutation is already present in some tumor cells even before exposure to the drug, and that treatment leads to selection of the mutated clone.<sup>62,63</sup> Another acquired mutation has recently been reported in exon 21. This change leads to substitution of alanine for threonine at position 854 (T854A) and interferes with the inhibition of tyrosine phosphorylation by erlotinib.<sup>64</sup>

To address the issue of resistance, second generation EGFR TKIs have been developed. These agents are irreversible inhibitors and covalently bind to Cys-797 of the EGFR ATP binding domain, and they seem to overcome resistance introduced by the T790M mutation that is seen with gefitinib and erlotinib. The frontrunners in the development of second generation irreversible TKIs are BIBW 2992 (afatinib) and PF00299804.

Afatinib inhibits both EGFR and HER2 and has been found to be very active in NSCLC with EGFR mutations.<sup>65</sup> Interestingly, it has also been found to be effective in treating patients with de novo T790M mutations.<sup>66</sup> In a Phase IIb/III double-blind placebo-controlled trial of afatinib in patients who had failed 1–2 lines of chemotherapy and either erlotinib or gefitinib plus best supportive care compared with placebo with best supportive care, afatinib showed a statistically significant PFS and response rates compared with placebo.<sup>67</sup> In a single-arm Phase II study in patients who progressed after erlotinib or gefitinib, afatinib showed significant benefits in terms of disease control rate and a median PFS of 4.4 months. This study was conducted in a highly enriched population of patients with previously known EGFR mutations.<sup>68</sup> More recently, a combination of afatinib and cetuximab, allowing for a complete EGFR blockade, has shown clinical benefit with a 36% partial response (PR) rate overall and a 29% PR rate in confirmed T790M mutations.<sup>69</sup>

PF00299804 is a pan HER inhibitor, targeting EGFR, HER2, and HER4. In preliminary studies, it has been shown to be effective in patients with EGFR mutations in the first-line setting.<sup>70</sup> In a randomized Phase II study comparing it with erlotinib in patients with NSCLC following progression on chemotherapy, an overall PFS advantage was achieved.<sup>71,72</sup> A phase I/II study of this agent in Asian patients who were refractory to chemotherapy and erlotinib or gefitinib, showed antitumor activity without significant toxicity.<sup>73</sup> Further studies with this agent are pending.

Another important determinant of resistance to EGFR therapy is c-MET expression. MET amplification can lead to secondary or primary resistance in patients with EGFR TKI sensitive mutations. MET amplification has been seen in approximately 20%–22% of lung cancer tissue samples that had become resistant to erlotinib or gefitinib, compared with only about 3% of untreated patients. This resistance is thought to be secondary to HER3-mediated activation of the PI3K-AKT pathway.<sup>74,75</sup>

MetMab (Genentech, Inc, South San Francisco, CA) is a monovalent antibody that targets c-MET and prevents its activation by hepatocyte growth factor. Patients with recurrent advanced NSCLC were randomly assigned to treatment with erlotinib versus erlotinib and MetMab in a randomized Phase II trial. High expression of c-MET (defined as a majority of tumor cells with  $\geq 50\%$  MET expression by IHC) was associated with statistically superior PFS (1.5 versus 3.0 months,  $P = 0.01$ ), and overall survival (4.6 months versus 12.6 months,  $P = 0.002$ ) in favor of the MetMab arm.

However, in c-MET low expressing patients, PFS was better in the erlotinib treatment arm (9.2 months versus 5.5 months,  $P = 0.021$ ).<sup>76</sup>

Other MET inhibitors have also been tested in NSCLC. A recent Phase II trial looked at combining erlotinib with tivantinib (ARQ197) in patients who have received at least one line of therapy that did not include a TKI. The study did not meet its primary endpoint of superior PFS, but subset analysis showed that it had benefit in K-RAS mutated patients. A Phase III trial, MARQUEE (Met inhibitor ARQ 197 plus erlotinib versus erlotinib plus placebo in NSCLC) is underway to further clarify the role of ARQ 197 in this population.<sup>77</sup>

## Resistance to chemotherapy

Chemotherapeutic agents were designed with the rationale that rapidly dividing cells are more sensitive to DNA damage. A number of DNA repair genes have been studied and are under investigation as potential targets to improve the therapeutic index of existing cytotoxic agents. Aberrant activation of DNA repair in response to chemotherapy-induced DNA damage is a major mechanism of drug resistance. As an example, the ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia Rad3-related) kinases have been identified as important triggers of chemotherapy resistance. These proteins are required for DNA repair in response to DNA damage and are required for cell cycle arrest. ATM and ATR regulate a series of proteins that prevent initiation of DNA replication.<sup>78</sup> Therefore, targeting specific signaling component of the DNA damage response can increase efficacy of chemotherapy. This approach is being actively tested using several agents.<sup>79</sup>

In lung cancer, one of the best studied pathways is the excision repair cross-complementation group 1 (ERCC-1). ERCC-1 is needed for DNA damage repair following exposure to platinum compounds like cisplatin and carboplatin. A low level of expression of ERCC-1 suggests that tumors may not be able to repair platinum-induced damage. A retrospective analysis of ERCC-1 expression in resected specimens of patients enrolled in the International Adjuvant Lung Cancer Trial (IALT) study showed that ERCC-1 negative patients had a better overall survival in the chemotherapy arm, with a median overall survival of 56 months compared with 42 months in the control arm. In ERCC-1 positive patients there was no apparent benefit to adjuvant chemotherapy.<sup>80</sup> Randomized studies in which patients are assigned to various treatments based on the level of ERCC-1 expression are ongoing.

## EML4-ALK translocation

The EML4-ALK gene rearrangement is a relatively new finding in lung cancer and was first reported in 2007. In this translocation, a deletion and translocation in chromosome 2p brings the EML4 gene in conjunction with ALK receptor tyrosine kinase, making a fusion protein in which the tyrosine kinase activity is permanently turned on.<sup>81</sup> EML4-ALK rearrangement is detected in approximately 5% of patients with adenocarcinomas. Like EGFR mutations, EML4-ALK positive tumors are usually seen in nonsmokers or very light smokers, and tend to be adenocarcinomas. Crizotinib, a C-MET inhibitor, was found to have significant activity in patients with tumors that expressed EML4/ALK rearrangement in a Phase I trial.<sup>82</sup> Based on the results of this Phase I study, an enriched population of ALK-positive patients were added to the study. The overall response rate was 57%, with 27% of the patients having stable disease. The disease control rate over a period of 8 weeks was 87%.<sup>83</sup> PFS in 119 patients in this Phase I study was 10 months (95% CI 8–15 months), and the median overall survival had not been reached.<sup>84</sup> Two subsequent Phase II studies looked at the use of crizotinib in ALK + NSCLC. The first Phase II study involved 117 patients with ALK + advanced NSCLC. Two complete responses (CRs) and 69 PRs for a response rate of 61% (95% CI 52%–70%), with the median response duration of 48.1 weeks, have been reported. In the second Phase II single-arm study of 136 ALK-positive patients, there was 1 CR and 67 PRs with a response rate of 50% (95% CI 42%–59%), and a median response duration of 41.9 weeks. Based on these two studies, the Food and Drug Administration granted approval for the use of crizotinib in ALK + NSCLC.<sup>85</sup> Clinical trials with other ALK inhibitors are now ongoing and are summarized in Table 3.

## Detection methods

Different methods have been used to test for EML4-ALK rearrangements. These include fluorescent in situ hybridization (FISH) probes, IHC for ALK expression and reverse transcriptase (RT)-PCR. The current gold standard for EML4-ALK testing is FISH. IHC was found to be concordant with FISH in the highly positive patients, but the concordance was not high in samples that stained weakly or moderately.<sup>86,87</sup> RT-PCR can help determine the partner gene and potential variants depending on the breakpoints; however, it will only detect specific breakpoints and may miss those that are not being looked for. Currently, the approved test in the United States is the FISH-based test.<sup>88</sup>

**Table 3** Current ALK inhibitors in clinical trials in various tumor types

LDK378	NCT01283516	Phase I	Tumors with ALK
IPI-504 HSP 90 inhibitor	NCT01228435	Phase II	ALK + lung cancer
AP26113 (ALK/EGFR inhibitor)	NCT01449461	Phase I/II	ALK + or EGFR + lung Ca
PF-02341066 (c-Met Alk inhibitor)	NCT00585195	Phase I	ALK + lymphoma
PF-02341066 (c-Met Alk inhibitor)	NCT00932893	Phase III	ALK + lung cancer second line

## Role of EGFR expression

EGFR expression, unlike EGFR activating mutations, is seen in about 62% of NSCLC patients, with maximum expression seen in squamous cell carcinoma (82%) and bronchoalveolar carcinomas (80%). In a univariate analysis, the presence of EGFR overexpression or increased copy number did not affect patients' prognosis, but there was a trend towards a less favorable prognosis.<sup>89</sup>

In the Phase III FLEX trial, patients with EGFR-expressing tumors were randomly assigned to receive cisplatin/vinorelbine or cisplatin/vinorelbine plus cetuximab. The median PFS was 11.3 versus 10.1 months in the chemotherapy plus cetuximab arm and chemotherapy alone arm respectively (HR = 0.871, 95% CI 0.762–0.996,  $P = 0.044$ ).<sup>90</sup> EGFR expression was defined as having any expression by IHC. The BMS 099 trial investigated the use of a carboplatin/paclitaxel combination with or without cetuximab in patients who were not selected for EGFR expression. This study failed to show any improvement in PFS or overall survival.<sup>91</sup> A retrospective analysis of the FLEX data, based on the level of EGFR expression in tumors as defined by the H score method, was recently published. In this analysis an H score  $\geq 200$  defined a high expressing tumor. Based on this definition, overall survival was better in the chemotherapy plus cetuximab arm in high versus low expressing tumors, with a median overall survival of 12.0 months compared with 9.6 months in the chemotherapy alone group.<sup>92</sup> This analysis suggests that better patient selection based on the level of EGFR expression, as defined by H score, might select a patient population that would benefit from Cetuximab treatment. This observation needs to be confirmed in a randomized prospective trial.

## Discussion

There has been a great deal of progress in the management of NSCLC, from selection of initial chemotherapy in the adjuvant setting based on histology and molecular characteristics to multi-targeted agents in the refractory, relapsed, and metastatic settings. Molecular features of the tumor are now guiding therapy, and there has been an explosion of targeted therapies based on a better understanding of driver mutations

and pathways of resistance. The coming years will see the use of next-generation TKIs like afatinib in addition to erlotinib, gefitinib, and crizotinib. Studies like the International Tailored Chemotherapy Adjuvant (ITACA) and Tailored Post-Surgical Therapy in Early Stage NSCLC (TASTE) trials will also help to further refine adjuvant chemotherapy based on predicted tumor response to chemotherapy, making it more personalized and individualized.

It is important to emphasize several points in this setting. The clinically useful drivers of personalized medicine in this disease that are validated either by larger Phase III trials or have regulatory approval fall into two categories; those driven by histology (bevacizumab and pemetrexed) and those driven by molecular markers (erlotinib and crizotinib). In the case of bevacizumab, the personalization of delivery of care is due to a toxicity factor in one histology, and in the case of pemetrexed, it is by preferential activity in nonsquamous histologies. Although there is much interest in and there is availability of testing for markers such as ERCC-1, there are no prospective Phase III trials or regulatory approval for the use of this test in the clinical decision-making process. Given the enormous interest in and availability of molecular testing, much of which is for markers that are not validated, caution should be used in interpreting the results and basing clinical decision making on untested markers.

We have a much better understanding of some of the driver mutations that impact patient outcome in lung cancer. In the coming years our ability to target pathways of interest as opposed to individual genes will open new possibilities for treatment. Use of system biology and studies using synthetic lethal interactions continue to identify close associations between genes that operate in related or redundant pathways. These investigations could ultimately lead to rationally designed clinical trials with combination therapies with multiple targeted agents. Management of toxicities and cost will then be challenges that need to be addressed. The future, however, is promising.

## Disclosure

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