Abstract

The iterative discovery in various malignancies during the past decades that a number of aberrant tumorigenic processes and signal transduction pathways are mediated by “druggable” protein kinases has led to a revolutionary change in drug development. In non-small cell lung cancer (NSCLC), the ErbB family of receptors (e.g., EGFR [epidermal growth factor receptor], HER2 [human epidermal growth factor receptor 2]), RAS (rat sarcoma gene), BRAF (v-raf murine sarcoma viral oncogene homolog B1), MAPK (mitogen-activated protein kinase) c-MET (c-mesenchymal-epithelial transition), FGFR (fibroblast growth factor receptor), DDR2 (discoidin domain receptor 2), PIK3CA (phosphatidylinositol-4,5-bisphosphate3-kinase, catalytic subunit alpha), PTEN (phosphatase and tensin homolog), AKT (protein kinase B), ALK (anaplastic lymphoma kinase), RET (rearranged during transfection), ROS1 (reactive oxygen species 1) and EPH (erythropoietin-producing hepatoma) are key targets of various agents currently in clinical development. These oncogenic targets exert their selective growth advantage through various intercommunicating pathways, such as through RAS/RAF/MEK, phosphoinositide 3-kinase/PI3K/AKT/mammalian target of rapamycin and SRC-signal transduction and transcription signaling. The recent clinical studies, EGFR tyrosine kinase inhibitors and crizotinib were considered as strongly effective targeted therapies in metastatic NSCLC. Currently, five molecular targeted agents were approved for treatment of advanced NSCLC: Gefitinib, erlotinib and afatinib for positive EGFR mutation, crizotinib for positive echinoderm microtubule-associated protein-like 4 (EML4)-ALK translocation and bevacizumab. Moreover, oncogenic mutant proteins are subject to regulation by protein trafficking pathways, specifically through the heat shock protein 90 system. Drug combinations affecting various nodes in these signaling and intracellular processes are predicted and demonstrated to be synergistic and advantageous in overcoming treatment resistance compared with monotherapy approaches. Understanding the role of the tumor microenvironment in the development and maintenance of the malignant phenotype provided additional therapeutic approaches as well. More recently, improved knowledge on tumor immunology has set the stage for promising immunotherapies in NSCLC. This review will focus on the rationale for the development of targeted therapies in NSCLC and the various strategies employed in preventing or overcoming the inevitable occurrence of treatment resistance.

Keywords: Drug resistance, heat shock protein 90 inhibitors, non-small cell lung cancer, programmed cell death-1 receptor inhibitors, protein kinase inhibitors
INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States and worldwide. The 2013 estimated new cases and deaths in the United States are 228,190 and 159,480 cases, respectively.[1] The 5-year survival rate is 5-10% for locally advanced/advanced stage non-small cell lung cancer (NSCLC) patients and it has remained essentially unchanged over the past decades before the advent of the targeted therapy era.[2] Despite the generally poor long-term outcomes of advanced stage disease, prolonged survival can be seen in some groups of patients. This is because NSCLC is a heterogeneous disease - its natural history is unique in every patient as tumor-related heterogeneity in terms of histological and molecular features affect treatment outcomes.[3] Both targeted and comprehensive genome-wide studies have demonstrated various recurrent genetic and epigenetic changes in lung cancer that confer oncogenic properties, many of which have differential frequencies according to histologic subtype.[4-10] Indeed, the changing landscape of lung cancer therapy was heralded by the discovery that the presence of activating kinase domain mutations in the epidermal growth factor receptor (EGFR) can identify a subset of patients who can greatly benefit from EGFR tyrosine kinase inhibitors (TKIs). This, along with the breakthroughs in imaging and genetic sequencing technologies, ushered in the era of precision medicine. The pertinent oncogenic pathways in lung cancer are summarized in Figure 1. This article delineates the rationale for the development of various targeted agents in NSCLC. Table 1 provides a brief summary comparing the genotypic differences by histologic subtype. Clinical issues of safety and toxicity will be described briefly where pertinent as this topic has recently been reviewed in greater detail elsewhere.[11]

SIGNALLING RECEPTORS

ErbB family of receptors

EGFR (also termed human epidermal growth factor receptor 1 [HER1] or ErbB 1) is a member of the ErbB family (consisting of 4 members: EGFR, HER2, HER3 and HER4) of cell surface receptor tyrosine kinase (RTK).[14] It is a 170-kDa RTK with an extracellular ligand-binding domain, a transmembrane region and an intracellular tyrosine kinase. The RTKs form homodimers and heterodimers after binding to specific ligands (except the orphan receptor HER2, which does not interact with any ligand directly), leading to autophosphorylation of tyrosine residues on the intracellular TK domain.[15] This interaction recruits a diverse set of signal transduction cascades including the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR), signal transduction and transcription (STAT) transcription and RAS/RAF/mitogen-activated protein kinase (MAPK) proliferation pathway [Figure 1].[14] In 2004, somatic mutations in the TK domain of EGFR, found most frequently in adenocarcinomas from patients in Asia who were never or former smokers, were strongly correlated with sensitivity to EGFR TKIs.[46] These mutations are mostly distributed in four exons (exon 18 to exon 21).[13,46] In-frame deletions of exon 19 (44%; E746-A750 deletion) and L858R substitutions in exon 21 (41%) are the most prevalent mutations associated with sensitivity to EGFR TKIs. The point mutations in exon 18 (G719C, G719S and G719A) and exon 20 (V765A and T783A) are less frequent; 5 and 1%, respectively.[16] More recently, an 18-bp insertion in exon 19, comprising about 1% of all EGFR mutations, has been reported to be correlated with sensitivity to EGFR TKIs.[47] Presence of the “classical” mutations in exons 18, 19 and 21 are the best predictive biomarker for the efficacy of EGFR TKIs such as erlotinib and gefitinib, with superior response rate (RR) and progression-free survival (PFS) compared with conventional chemotherapy or best supportive therapy in patients with tumors harboring EGFR TKI-sensitive mutations.[48] Until date, the EGFR TKI erlotinib (gefitinib is another TKI approved in other countries) is approved for first-line, second-or third-line and maintenance monotherapy for NSCLC based on highlighted Phase III trials in Table 2.[48-63] Recently, the Food and Drug Administration (FDA) approved afatinib (Gilotrif) for the first-line treatment of patients with metastatic NSCLC whose tumors have EGFR exon 19 deletions or exon 21 (L858R) substitution mutations based on the demonstration of improved PFS in a multi-center, international, open-label, Phase III trial [Table 2].[44] In comparison, cetuximab (Erbitux), an immunoglobulin G chimeric monoclonal antibody (mAb) against EGFR, which competitively inhibits ligand binding, had only been investigated in combination with chemotherapy in Phase III trials of molecularly unselected NSCLC [Table 2].[64] Fluorescent in-situ hybridization (FISH) assay to determine EGFR copy number and gene amplification had demonstrated potential promise as a predictive marker of response to cetuximab in a small study[65] and is thus being evaluated as a predictive biomarker of cetuximab in the ongoing Phase III study S0819 (NCT00946712). However, no biomarker has been found to consistently correlate with the benefit from cetuximab in the concluded Phase III clinical studies for NSCLC, including EGFR FISH or KRAS (Kirsten-rous avian sarcoma) mutation status, which is in contrast with experience in metastatic colon cancer.[66] Other mAbs against EGFR under investigation in trials for NSCLC include necitumumab, panitumumab, nimotuzumab, matuzumab and zalutumumab [Table 3].
Figure 1: Cell signaling pathways in lung cancer.
Depicted are the cellular signaling pathways involved in the proliferation, differentiation, growth, metastasis, resistance to apoptosis and angiogenesis in neoplasms, highlighting the targets amenable to therapeutic interventions in lung cancer therapy. Membrane-bound members of the ErbB family of receptors, MET, VEGFR and IGF-1R mediate mitogenic signals from extracellular ligands, such as EGF, HGF, VEGF and IGF, respectively. The Ras/Raf/MEK/ERK (mitogen-activated protein kinase, MAPK) and PI3K/AKT/mTOR pathways are major intracellular axes that regulate intracellular signaling traffic (AKT: Protein kinase B, AMPK-1: 5'-AMP-activated protein kinase catalytic subunit alpha-1, EGF: Epidermal growth factor, EGFR: Epidermal growth factor receptor, eif-4F: Eukaryotic initiation factor-4 complex, EML4-ALK: Echinoderm microtubule-associated protein-like 4 fused with the anaplastic lymphoma kinase, ERK: Extracellular signal-regulated kinases, 4E-BP1: 4E binding protein-1, GAP: GTPase; activating protein, GDP: Guanosine diphosphate, GEF: Guanine nucleotide exchange factors, GRB2: Growth factor receptor-bound protein 2, GTP: Guanosine triphosphate, HER: Human epidermal growth factor receptors, HGF: Hepatocyte growth factor, IGF: Insulin growth factors, IGF-1R: Insulin-like growth factor 1 receptor, IRS: Insulin receptor substrate, MEK: Mitogen-activated protein kinase, mTOR: Mammalian target of rapamycin, PDK1: 3-phosphoinositide – dependent protein kinase 1, PI3K: Phosphatidylinositide 3-kinase, PTEN: Phosphatase and tensin homolog, rpS6: Ribosomal protein S6, S6K1: 40S ribosomal protein S6 kinase, SHC: Src homology/collagen, SOS: Son of sevenless, TSC: Tuberous sclerosis; VEGFR: Vascular endothelial growth factor receptor).

Table 1: Molecular alterations in NSCLC

| Molecular alterations | Frequency in NSCLC % | Clinical relevance |
|-----------------------|----------------------|--------------------|
| AKT1 mutation[12]     | 1-1.5                | Predominantly found in squamous cell carcinoma. |
| BRAF mutation[13,14]   | 3-5                  | V600E mutation is the most common, found in smokers and nonsmokers alike. Non-V600E found predominantly in smokers. EGFR mutation may occur concomitantly in few cases. BRAF mutation may arise as the mechanism of acquired resistance to EGFR TKI. |
| Adenocarcinoma         | <1                   | Predominantly found in squamous cell carcinoma. Associated with sensitivity to multikinase inhibitors that inhibit DDR2 such as dasatinib, sorafenib and ponatinib. |
| Squamous cell carcinoma|                      |                    |
| DDR2 mutation[5]       | 3-4                  | Predominantly found in adenocarcinoma. Exon 19 deletion and exon 21 point mutation L858R constitute the majority of cases with sensitivity to EGFR TKIs. Recently reported as potential rare cause of acquired mechanism to ALK inhibitors. |
| EGFR mutation[15,16]   |                      | Predominantly found in adenocarcinomas and nonsmokers. Exon 19 deletion and exon 21 point mutation L858R constitute the majority of cases with sensitivity to EGFR TKIs. Recently reported as potential rare cause of acquired mechanism to ALK inhibitors. |
| Asian                  |                      |                    |
| Caucasian              | 30-50                |                    |
| EML4-ALK fusion gene[17,18] | 3-6              | Predominantly found in adenocarcinoma. Relatively more frequent in younger patients, men and non-smokers. |
| EPHA2 G391R mutation[19] | Up to 7           | Activating mutation in squamous cell carcinoma. Increases sensitivity to mTOR inhibitors in preclinical models. |
| FGFR1 amplification[20] |                      | Sensitive to pan-FGFR or selective FGFR1 inhibitors. Presence of activated MAPK signaling may result in resistance to FGFR1 inhibition alone. |
| Adenocarcinoma         | 3                    |                    |
| Squamous cell carcinoma| 21                   |                    |
| FGFR fusion gene[21]   |                      |                    |
| Squamous cell carcinoma| <1-2                | Gene fusions with FGFR1, 2 and 3 have been reported, with FGFR3-TACC3 fusion most frequently reported to date, reported in squamous cell carcinoma. Fusion proteins maybe sensitive to FGFR inhibitors, with FGFR3 fusions appearing to be more sensitive to FGFR inhibitors relative to FGFR3 activating point mutations in preclinical studies. |

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Table 1: Contd...

| Molecular alterations | Frequency in NSCLC % | Clinical relevance |
|-----------------------|----------------------|--------------------|
| HER2 exon 20 insertion mutation[^22,23] | 2-4 | Predominantly found in adenocarcinomas in nonsmokers. May rarely occur simultaneously with EGFR mutation. Associated with sensitivity to HER2-targeting agents. |
| HER2 amplification[^24,25,26] | Up to 23 | Occurs de novo or as the mechanism of acquired resistance to EGFR TKIs. |
| KRAS mutation[^27] | Asian: 5; Caucasian: 20-30 | Predominantly found in Adenocarcinoma and smokers. Occurs de novo or as the mechanism of acquired resistance to ALK or BRAF inhibitors. May contribute to resistance to PI3K inhibitors. |
| LKB1 mutation[^38] | Asian: 7-8; Caucasian: 30 | More common in adenocarcinomas than in squamous cell carcinomas. Concurrent mutation with KRAS may confer resistance to MEK inhibitors. |
| MEK1 mutation[^279] | <1 | Primary found in lung adenocarcinoma. Associated with sensitivity to MEK inhibitors. |
| MET mutation[^30,31] | <5 | Found in extracellular and juxta membrane domains in lung cancer. Kinase domain mutations have yet to be identified in NSCLC. Kinase domain mutations may arise as acquired resistance to MET kinase inhibitors. |
| MET amplification[^32,33] | 21 | Occurs de novo or as the mechanism of acquired resistance to EGFR TKIs. Associated with sensitivity to MET inhibitors. |
| NRAS mutation[^323] | <1 | Primary found in lung adenocarcinomas and smokers. Associated with sensitivity to MEK inhibitors. This region encodes for PDGFRa and KIT. Amplification alone does not predict for sensitivity to PDGFR/KIT inhibitors. |
| Amplification of chromosomal segment 4q12[^24] | Adenocarcinoma: 3-7; Squamous cell carcinoma: 8-10 | Occurs de novo or as the mechanism of acquired resistance to EGFR TKIs. Frequently occurs simultaneously with other mutations. Associated with sensitivity to PI3K inhibitors. |
| PIK3CA mutation[^35,36] | Adenocarcinoma: 0-2.5; Squamous cell carcinoma: 3-9 | Associated with sensitivity to PI3K inhibitors. May occur concomitantly with PIK3CA mutation. |
| PIK3CA amplification[^35,36] | Adenocarcinoma: 5-10; Squamous cell carcinoma: 37-43 | Associated with sensitivity to PI3K inhibitors. May occur concomitantly with PIK3CA mutation. |
| Loss of PTEN[^36,37,38] | Adenocarcinoma: 4; Squamous cell carcinoma: 21 | Associated with PI3K pathway activation and resistance to EGFR TKIs. Associated with sensitivity to PI3K inhibitors. |
| PTEN mutation[^39] | Adenocarcinoma: 1.7; Squamous cell carcinoma: 10.2 | Associated with PI3K pathway activation and resistance to EGFR TKIs. Associated with sensitivity to PI3K inhibitors. |
| RET fusion gene[^40,41] | 1-2 | Predominantly found in adenocarcinomas in nonsmokers. Associated with sensitivity to multikinase inhibitors that inhibit RET such as vandetanib and cabozantinib. |
| ROS1 fusion gene[^42] | 2 | More frequent in non-smokers, adenocarcinoma and younger patients. Associated with sensitivity to ALK inhibitors. |
| Trk (A, B, C) mutations[^43] | 3-5 | TrkB mutants lack transforming ability and thus of questionable role in patients selection for evaluation of Trk inhibitors. |

NSCLC: Non-small cell lung cancer; AKT: Protein kinase B; DDR2: Discoidin domain receptor 2; EGFR: Epidermal growth factor receptor; EML4: Echinoderm microtubule-associated protein-like 4; ALK: Anaplastic lymphoma kinase; HER2: Human epidermal growth factor receptor; MEK: Mitogen-activated protein kinase; PTEN: Phosphatase and tensin homolog; Trk: Tropomyosin-related kinase; TKI: Tyrosine kinase inhibitor; mTOR: Mammalian target of rapamycin; MAPK: Mitogen-activated protein kinase; RET: Rearranged during transfection; PIK3CA: Phosphoinositide-3-kinase, catalytic, alpha polypeptide; FGFR: Fibroblast growth factor receptor; MET: Mesenchymal-epithelial transition; BRAF: V-raf murine sarcoma viral oncogene homolog B1; Kras: Kirsten-rous avian sarcoma; LKB: Liver kinase; NRAS: Neuroblastoma RAS viral oncogene homolog; ROS1: Reactive oxygen species 1; PDGFR: Platelet-derived growth factor; KIT: The feline sarcoma viral oncogene v-kit.

Table 2: Summary of highlighted Phase III trials of EGFR inhibitors in advanced NSCLC

| Trials | N | Primary endpoint | Treatment | ORR (%) | P value | HR (95% CI) | Median PFS (mo) | HR (95% CI) | Median OS (mo) |
|--------|---|----------------|-----------|---------|---------|------------|----------------|------------|----------------|
| EGFR TKIs | | | | | | | | | |
| First line Trials | | | | | | | | | |
| IPASS[^46,47] | 1,217 | PFS | Gefitinib | 43 | (P=0.001) | 0.74 (0.63-0.85) | 5.7 mo | >0.001 | 18.8 mo |
| Asian, chemo-naïve, non/light smokers, adenocarcinoma | | | | | | | | | |

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| Trials                                      | N   | Primary endpoint | Treatment                      | ORR (%) | Median PFS (mo) | Median OS (mo) |
|---------------------------------------------|-----|------------------|--------------------------------|---------|----------------|----------------|
|                                             |     |                  | Paclitaxel/carboplatin         | 32.2    | 5.8 mo         | 17.4 mo        |
|                                             |     |                  | Subgroups                      |         |                |                |
|                                             |     |                  | Mutation+ve (benefit in gefitinib arm) | 9.5 versus 6.3 mo | 0.48 (0.36-0.64) | (P<0.001)  |
|                                             |     |                  |                                  |         |                |                |
|                                             |     |                  | Mutation –ve (benefit in chemo arm) | 1.5 versus 5.5 mo | 2.85 (2.05-3.98) | (P<0.001)  |
|                                             |     |                  |                                  |         |                |                |
| First-Signal[35]                           | 313 | OS               | Gefitinib                      | 53.5    | 6.1 mo         | 21.3 mo        |
| Asian, chemo-naive, non-smokers, adenocarcinoma |     |                  |                                  |         |                |                |
|                                             |     |                  | Subgroups                      |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 46.3    | 6.6 mo         | 23.3 mo        |
|                                             |     |                  |                                  |         |                |                |
| WJTOG3405[36]                              | 177 | PFS              | Gefitinib                      | 62.1    | 9.2 mo         | 30.9 mo        |
| Japanese, chemo-naive, EGFR mutation positive |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation –ve         | 6.1     | 0.48 (0.34-0.71) | (P<0.001)  |
| NEJ002[37]                                 | 200 | PFS              | Gefitinib                      | 73.7    | 10.8 mo        | 30.5 mo        |
| Japanese, chemo-naive, EGFR mutation positive |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation –ve         | 5.5     | 2.85 (2.05-3.98) | (P<0.001)  |
| OPTIMAL[38]                                 | 154 | PFS              | Gefitinib                      | 83%     | 13.1 mo        | Results not mature at time of publication |
| Chinese, chemo-naive, EGFR mutation positive |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 36%     | 4.6 mo         |                |
| EURTAC[39]                                 | 174 | PFS              | Gefitinib                      | 54.4    | 9.4 mo         | 22.9 mo        |
| Caucasian, chemo-naive, EGFR mutation positive |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 36%     | 4.6 mo         |                |
| LUX-lung 3[40]                             | 345 | PFS              | Gefitinib                      | 50.4    | 11.1 mo        | 16.6 mo        |
| chemo-naive, EGFR mutation positive        |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 50.4    | 11.1 mo        | 16.6 mo        |
| Maintenance                                |     |                  | Platinum-based regimen         | 10.5    | 5.2 mo         | 18.8 mo        |
| SATURN[41]                                 | 889 | PFS              | Erlotinib                      | 83%     | 13.1 mo        |                |
|                                             |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 36%     | 4.6 mo         |                |
| ATLAS[42]                                  | 768 | PFS              | Gefitinib                      | 54.4    | 9.4 mo         | 22.9 mo        |
|                                             |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib mutation+ve          | 36%     | 4.6 mo         |                |
| WJTOG0203[43]                              | 598 | OS               | Gefitinib                      | 50.4    | 11.1 mo        | 16.6 mo        |
| Superior OS in gefitinib arm in adenocarcinoma |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib                      | 50.4    | 11.1 mo        | 16.6 mo        |
| BeTa[44]                                   | 636 | OS               | Gefitinib                      | 13      | 3.4            | 9.3            |
|                                             |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib                      | 13      | 3.4            | 9.3            |
| 2nd and 3rd-line                          |     |                  |                                 |         |                |                |
| BR21[45]                                   | 731 | OS               | Gefitinib                      | 8.9     | 2.2 mo         | 6.7 mo         |
|                                             |     |                  |                                  |         |                |                |
|                                             |     |                  | Gefitinib                      | 8.9     | 2.2 mo         | 6.7 mo         |

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Table 2: Contd...

| Trials                     | N     | Primary endpoint | Treatment         | ORR (%) | Median PFS (mo) | Median OS (mo) |
|----------------------------|-------|------------------|-------------------|---------|----------------|---------------|
|                            |       |                  |                   | P value | HR (95% CI)    | HR (95% CI)   |
| ISEL[59] OS advantage      | 1,692 | OS               | Placebo           | <1      | 1.8 mo         | 4.7 mo        |
| in non-smokers and Asians  |       |                  | Gefitinib         | 8 (P<0.0001) | 3 mo* (P=0.0006) | 5.6 mo          | 0.89 (0.77-1.02) (P=0.087) |
| INTEREST[60] Non-inferiority trial | 1,466 | OS               | Placebo           | 1.3     | 2.6 mo         | 5.1 mo        |
|                            |       |                  | Gefitinib         | 9.1 (P=0.33) | 2.2 mo (P=0.47) | 7.6 mo          | 1.02 (0.9-0.15) |
| TAILOR[62] EGFR WT         | 218   | OS               | Docetaxel         | 7.6     | 2.7 mo         | 8.0 mo        |
| population                |       |                  | NA                | 0.7 (0.53-0.94) | (P=0.02) | NA |
| EGFR antibody             |       |                  |                    |         |                |               |
| FLEX[61]                   | 1,125 | OS               | Cetuximab+vinorelbine+cisplatin | 36 | 4.8 mo | 11.3 mo |
|                            |       |                  |                    | (P=0.01) |                 | 0.87 (0.76-0.99) (P=0.04) |
|                            |       |                  | Vinorelbine+cisplatin | 29 | 4.4 mo | 10.0 mo |
|                            |       |                  |                    | (P=0.0066) |                 | 0.902 (0.761-1.069) (P=0.01358) |
|                            |       |                  | Taxane+carboplatin | 17.2% | 4.24 mo | 8.38 mo |

NSCLC: Non-small cell lung cancer; EGFR: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; IPASS: Iressa Pan-Asia Study; WJTTOG: West Japan thoracic oncology group; NEJ: North-East Japan; PFS: Progression-free survival; ORR: Observed response rate; HR: Hazard ratio; CI: Confidence interval; OS: Overall survival; SATURN: Sequential Tarceva in Unresectable NSCLC; Bev: Bevacizumab; ISEL: Iressa survival evaluation in lung cancer; INTEREST: Iressa NSCLC trial evaluating response and survival versus taxotere; FLEX: First-line in Lung cancer with Erbitux; BMS: Bristol_Myers Squibb; WT: Wild-type

Table 3: Highlighted ongoing Phase I, II and III studies in NSCLC for novel ErbB inhibitors

| Targeted agents | Current phase of development | Targets | Designs |
|-----------------|------------------------------|---------|---------|
| TKIs            |                              |         |         |
| Lapatinib       | Phase II                     | EGFR, HER2 | Single arm in combination with pemetrexed |
|                 | NCT00528281                  | Reversible TKI | |
| Neratinib (HKI272) | Phase II                   | EGFR, HER2 | Single agent, 3 arms treatment based on EGFR mutation status |
|                 | NCT00266877                  | Irreversible TKI | |
| Afatinib (BIBW2992) | Phase I, II and III         | EGFR, HER2, HER4 | Single agent and combination with either chemotherapy, radiation or other targeted agents in stage IV or adjuvant setting Intermittent or pulse high-dose therapy |
|                 | NCT01553942                  | Irreversible TKI | |
|                 | NCT01746251                  |          |         |
|                 | NCT01647711                  |          |         |
|                 | NCT01853826                  |          |         |
| Icotinib (BPI-2009H) | Phase II and III            | EGFR | Single agent and combination with either chemotherapy, radiation or other targeted agents |
|                 | NCT01690390                  |          |         |
|                 | NCT01707329                  |          |         |
|                 | NCT01516983                  |          |         |
|                 | NCT01719536/(Convince)      |          |         |
| Dacomitinib (PF00299804) | Phase III                   | EGFR, HER2, HER4 | Single agent in comparison with gefitinib or erlotinib as treatment of advanced NSCLC |
|                 | NCT01774721/(Archer 1050)   | Irreversible TKI | |
|                 | NCT01360554/(ARCHER 1009)   |          |         |
| Poziotinib (HM781-36B) | Phase II                     | EGFR, HER2, HER4 and | Single arm, 1st line monotherapy in EGFR mutation lung adenocarcinoma |
|                 | NCT01819428                  | TEC family irreversible TKI | |
|                 | NCT01718847                  |          |         |
| CO-1686         | Phase III                    | EGFR T790M mutant | Single agent in previously treated mutant EGFR NSCLC |
|                 | NCT01526928                  |          |         |
| AP26113         | Phase III                    | Dual reversible ALK/ EGFR inhibitor | Single agent in NSCLC/other cancers with ALK gene rearrangement or mutated EGFR |
|                 | NCT01449461                  |          |         |

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Despite the dramatic responses to EGFR TKIs, most of the patients develop disease progression within one 1 year, usually because of secondary or acquired resistance. Treatment resistance, whether primary/de novo or secondary/acquired, is generally mediated by mechanisms that enable the persistence of aberrant mitogen-activated protein kinase (MAPK) pathway activation, such as the presence of T790M mutation (constituting 50–60% of acquired resistance) in exon 20 or most EGFR exon 20 insertions, which reduce binding affinity to the first-generation TKIs, amplification of HER2 or MEK1 amplifications, activating mutations in RAS or BRAF, MAPK-independent pathways on the other hand involve either acquired phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) mutations, amplification of mesenchymal-epithelial transition (MET) proto-oncogene, which provides a bypass avenue through transactivation of HER3/PI3K signaling or impairment of cell death mechanisms as seen with certain germline polymorphic variants of the proapoptotic molecule proapoptotic BCL-2-interacting mediator (BIM) (pro-apoptotic Bcl-2 family member). Other documented phenomena to explain treatment resistance are epithelial-to-mesenchymal transition mediated by either AXL kinase activation or activation of transforming growth factor-β pathway through downregulation of MED12, as well as phenotypic transformation to small cell histology.

De novo mutations in HER2 occur in 2–4% of NSCLC, up to approximately 10% in adenocarcinomas. Majority (>95%) of these represent small insertions in exon 20, largely (>80%) represented by a 12 basepair in-frame insertion causing a duplication of the amino acids YVMA that results in constitutive activation of HER2. These mutations appear to occur predominantly in women and never smokers and frequently are associated with either HER2 or EGFR copy number gains or gene amplifications in a Chinese lung adenocarcinoma cohort, though there was no such gender association reported by the North American cohort. Concurrent HER2 alterations by FISH also occurred at a much lower frequency in the North American group. In general, HER2 mutations are mutually exclusive with EGFR mutations though co-existence of both EGFR and HER2 mutations simultaneously had been described in the literature. In contrast to HER2 mutations, HER2 gene amplification or copy number gains as assessed by FISH had been reported in up to 23% of NSCLC cases in Western Hemisphere. Based on cumulative experience to date, it is anticipated that durable clinical benefit with ErbB-targeted therapies in NSCLC will most likely be best predicted by the presence of relevant activating mutations compared with mere presence of gene amplification or copy number changes.

Second-and third-generation EGFR TKIs are developed as part of the strategy to overcome treatment resistance to first-generation EGFR TKIs. Second-generation agents include the irreversible inhibitors of the ErbB family of receptors: Afatinib (also known as BIBW 2992, which targets EGFR, HER2, HER4), dacomitinib (also known as PF0299804, which targets EGFR, HER2, HER4) and neratinib (also known as HKI272, which targets EGFR...
Further development of neratinib or down regulation of EML4-ALK. The observed variability of response to T790M mutant include WZ4002, CO-1686 and Poziotinib (also known as HM781-36B), a new potent irreversible inhibitor of EGFR, HER2, HER4 and TEC family of kinases inhibitor (BTK, BLK and BMX) demonstrated preclinical efficacy against T790M mutant at 8-fold lower doses compared to afatinib. Early phase clinical trials of poziotinib and CO-1686 are still ongoing but data from preclinical modeling suggest that acquired resistance to these next-generation TKIs emerge by increased extracellular signal-regulated kinases (ERK) activation, such as through amplification of MEK1 or down regulation of negative regulators of ERK signaling, which may in turn be overcome by the use of MEK inhibitors. Akin to the clinically successful combinatorial strategy of BRAF and MEK inhibition used in BRAF-mutant melanoma, the combination of WZ4002 and a MEK inhibitor appears to be preclinically effective in treating drug-resistant tumors as well as in delaying the emergence of tertiary drug-resistant clones. Another new agent, which started its first-in-man Phase I clinical testing in early 2013 is AZD9291, which is being developed for patients with acquired resistance to EGFR TKI, including T790M. The multitargeted EGFR/HER2/vascular endothelial growth factor receptor/EphB4 inhibitor XL547 and the dual reversible anaplastic lymphoma kinase (ALK)/EGFR inhibitor AP26113 also demonstrated preclinical activity against EGFR T790M mutant tumors. However, in the Phase II study of XL647 (KD019) in patients with acquired resistance to EGFR TKI, only 3% observed response rate (ORR) was observed, with disease progression as the best tumor response seen in 67% of the cases with documented T790M. Early phase data of AP26113 suggested a preliminary hint of clinical activity, though relatively modest, in patients with resistance to other EGFR TKIs.

Clinical antitumor responses to EGFR TKIs in cases with EGFR exon 20 insertion, such as A763_Y764insFQEA, have been reported. The observed variability of response to EGFR TKIs with EGFR exon 20 insertions is thought to be related to functional differences arising from the heterogeneity in the location of the insertion, whereby insertions in the more distal region from 773 to 775 would be predicted to have the most significant drug-binding effect compared with insertions involving amino acids more proximally at 764-770. Exon 20 mutations in HER2 in contrast tend to be more homogeneous and located in the most proximal region between codons 775 and 881. Clinical responses to HER2-targeted antibody and second-generation EGFR TKI therapies (specifically afatinib) have been documented for NSCLC with HER2 mutations.

Third-generation EGFR inhibitors designed to inhibit the EGFR T790M mutant include WZ4002, CO-1686 and AZD9291. Poziotinib (also known as HM781-36B), a new potent irreversible inhibitor of EGFR, HER2, HER4 and TEC family of kinases inhibitor (BTK, BLK and BMX) demonstrated preclinical efficacy against T790M mutant at 8-fold lower doses compared to afatinib. Early phase clinical trials of poziotinib and CO-1686 are still ongoing but data from preclinical modeling suggest that acquired resistance to these next-generation TKIs emerge by increased extracellular signal-regulated kinases (ERK) activation, such as through amplification of MEK1 or down regulation of negative regulators of ERK signaling, which may in turn be overcome by the use of MEK inhibitors. Akin to the clinically successful combinatorial strategy of BRAF and MEK inhibition used in BRAF-mutant melanoma, the combination of WZ4002 and a MEK inhibitor appears to be preclinically effective in treating drug-resistant tumors as well as in delaying the emergence of tertiary drug-resistant clones. Another new agent, which started its first-in-man Phase I clinical testing in early 2013 is AZD9291, which is being developed for patients with acquired resistance to EGFR TKI, including T790M. The multitargeted EGFR/HER2/vascular endothelial growth factor receptor/EphB4 inhibitor XL547 and the dual reversible anaplastic lymphoma kinase (ALK)/EGFR inhibitor AP26113 also demonstrated preclinical activity against EGFR T790M mutant tumors. However, in the Phase II study of XL647 (KD019) in patients with acquired resistance to EGFR TKI, only 3% observed response rate (ORR) was observed, with disease progression as the best tumor response seen in 67% of the cases with documented T790M. Early phase data of AP26113 suggested a preliminary hint of clinical activity, though relatively modest, in patients with resistance to other EGFR TKIs.

A different approach in addressing EGFR TKI resistance involves the use of combination regimens. Therefore, the combination of erlotinib with cetuximab, the combination of erlotinib with MM-121, a fully human mAb that targets HER3, in patients with acquired resistance to EGFR TKI did not show sufficient clinical activity for further investigation in this population. The combination of afatinib and cetuximab was the first to report very promising overall RR of 36%, including ORR of 29% in the T790M cases. Other combination regimens, such as with c-MET inhibitors, heat shock protein 90 (HSP90), PI3K/mTOR inhibitors will be discussed subsequently under each respective pathway.

**ALK and leukocyte tyrosine kinase receptors**

The echinoderm microtubule-associated protein-like 4 (EML4)-ALK fusion gene (EML4 fused with the ALK) is one of the newer molecular targets elucidated in NSCLC. The ALK is a member of the insulin superfamily of RTKs normally expressed only in the CNS, small intestine and testis. The ALK translocation (t(2;5)[p23;q35]) was originally found in a subset of anaplastic large cell lymphomas in 1994. The translocation of 2 genes in the short arm of chromosome 2, between the C-terminal kinase domain
of ALK and the N-terminal portion of the EML4, was discovered in a NSCLC patient in Japan in 2007.\(^\text{[105]}\) This translocation causes aberrant activation of downstream oncogenic signaling pathways such as the RAS/RAF/MEK, PI3K/AKT/mTOR and the Janus kinase (JAK)/STAT signaling pathway, leading to cell proliferation, invasion and inhibition of apoptosis [Figure 1].\(^\text{[117]}\) EML4-ALK translocation is found in 3–6% of all cases of NSCLC.\(^\text{[105,106]}\) It is more frequent in younger patients, men and never-smokers/light smokers with NSCLC.\(^\text{[105,106]}\) EML4-ALK translocation is mutually exclusive with EGFR or KRAS mutations in the ALK inhibitor-naïve population.\(^\text{[105]}\) There are several EML4-ALK translocation variants in lung cancer, in addition to other ALK fusion partners (e.g., kinesin family member 5B [KIF5B], KLC, TFG).\(^\text{[107]}\) Crizotinib (PF-02341066), an oral dual ALK/MET inhibitor, is currently the only FDA-approved agent for advanced ALK-positive NSCLC. This was based on the high ORR of approximately 60% seen in early phase studies which recruited a heavily pre-treated population, wherein treatment response to conventional cytotoxic chemotherapy is otherwise generally < 10% in this setting.\(^\text{[108]}\) More recent data from ongoing Phase III studies confirm superior PFS in patients who received crizotinib as second-line therapy compared with chemotherapy (hazard ratio [HR] 0.49, \(P < 0.0001\)).\(^\text{[109]}\) The gold standard assay for detection of EML4-ALK is FISH. Other assays in evaluation involve real-time polymerase chain reaction, next-generation sequencing and immunohistochemical approaches.\(^\text{[110]}\) Each diagnostic platform has advantages and disadvantages and standardization efforts are currently ongoing. Recently, Yi et al. proposed to test ALK positivity by a combination of immunohistochemical and FISH techniques in NSCLC, similar to algorithmic HER-2 testing in breast cancer.\(^\text{[111]}\) This method might be a cost-effective and accurate screening modality, but further validation is warranted.

Despite the remarkable initial responses, acquired resistance to crizotinib develops within a year.\(^\text{[112]}\) Various mechanisms of acquired resistance have been documented, several of which may co-exist simultaneously. Multiple secondary mutations have already been identified in patients treated with crizotinib.\(^\text{[112,113]}\) Homologous to the gatekeeper EGFR T790M mutation is the L1196M substitution which, unlike EGFR T790M, does not appear to confer a growth disadvantage to cells.\(^\text{[113]}\) Other secondary mutations such as G1269A, C1156Y, L1152R and 1151Tins may affect affinity of the mutant ALK for either adenosine triphosphate (ATP) or drug and these differences have ramifications on the development of next-generation ALK inhibitors, which have varied mutation-specific efficacy amongst different agents. Other resistance mechanisms implicated include amplification of ALK gene, aberrant activation of other kinases such as amplification of KIT or direct MAPK pathway activation as represented by either KRAS mutation, upregulation of EGFR or detection of an activating EGFR mutation not seen in the initial tumor tissue.\(^\text{[113-117]}\) Another potential approach that maybe effective is dual inhibition of PI3K and MEK pathway, which demonstrated significant activity in an ALK-translocated NSCLC cell line.\(^\text{[118]}\)

Several second-generation agents against crizotinib-resistant EML4-ALK-positive cancers are being developed. HSP90 inhibitors also show preclinical and clinical activity in ALK-rearranged NSCLC and may have broader activity across different ALK mutations.\(^\text{[114,119-121]}\) The reversible dual ALK/EGFR inhibitor AP26113 is a more potent ALK inhibitor than crizotinib and demonstrates preclinical activity against various secondary mutations resistant to crizotinib, including L1196 and G1269A.\(^\text{[122]}\) In an ongoing Phase I dose-escalation study, it demonstrated activity in up to two-thirds of crizotinib-resistant ALK-positive patients. LDK378 is a selective ALK inhibitor with weak c-MET activity which showed substantial clinical activity, with an ORR of 81% at doses > 400 mg in ALK-positive NSCLC patients previously treated with crizotinib.\(^\text{[123]}\) Both agents demonstrated tumor responses against crizotinib-resistant brain metastases.

LTK is a RTK, which shares a high degree of homology (nearly 80% identical) with ALK and is expressed in hematopoietic cells, brain and placenta.\(^\text{[104,124]}\) Though its function is not well understood, it is thought to promote growth and survival through activation of RAS/MAPK and PI3K/AKT signaling pathways.\(^\text{[125]}\) Mutations in LTK at residues F568 and R669, which correspond to the activating mutations F1174 L and R1275Q in ALK, demonstrated transforming potential, with anchorage-independent growth of the mutant-expressing cells inhibited by crizotinib or a pan-JAK inhibitor. Recurrent somatically acquired LTK mutations, including the R669 variant, have been described in approximately 1.5% of NSCLC.\(^\text{[126]}\) Although there are no clinical outcomes data yet, it is anticipated that these mutations may potentially be responsive to ALK inhibitors.

**Erythropoietin-producing hepatoma family of receptors**

The EPH receptor family of EphA and EphB receptors generates an unusual bidirectional signaling whereby kinase-induced forward signaling occurs in the receptor-expressing cell, whereas reverse signaling through the Src family kinases occurs in the membrane-bound ephrin ligand-expressing cell.\(^\text{[127]}\) This bidirectional signaling, as well as the balance of signaling through its catalytically deficient
forms (e.g., EphB6), accounts for the context-dependent oncogenic or tumor-suppressor functions described.\[128\] Mutations in almost all of the Eph receptors have been reported in NSCLC, most frequently for EphA5, EphA3, EphB1, EphA7 and EphB6, ranging between 2% and 5% in lung adenocarcinomas.\[9\] Of note, these mutations are not mutually exclusive with each other, e.g., 58% of samples with EphA3 mutation also have at least another Eph receptor mutation.\[129\] Although wild-type EphA2 kinase activity is independent of ligand-binding, binding to its ligand ephrinA1 negatively regulates growth and migration\[130\] and thus agonistic antibodies have been developed to stimulate tumor suppressor activity. Whether this strategy can overcome the oncogenic effect of the recurrent somatic G391R mutation in the first fibronectin Type III domain of EphA2 reported in 7% of squamous cell lung carcinomas (SQCLC) is unknown. This mutation promotes cell survival and metastasis through activation of p130Cas\[131\] which appears to be responsive to mTOR inhibition.\[19\] In comparison, several kinase domain mutations in EPHA3 result in diminished phosphorylation of the EPHA3 receptor, which functionally attenuates the tumor-suppressive effects of wild-type EPHA3 through regulation of AKT activity.\[131\] Various multikinase inhibitors currently in clinical use potently target Eph receptors, such as dasatinib and nilotinib. XL647 (also known as PRIM-001, KD019), a reversible ATP-competitive inhibitor of various kinases (EGFR, VEGFR2, FLT4, c-kit) in the nanomolar range including EphB4, is currently being compared in a Phase III study to erlotinib in NSCLC as second-or third-line therapy. As the Eph/ephrin system is poorly understood and drugs are in early development, there are currently no genotypically-defined clinical studies specific for this pathway.

**Insulin-like growth factor receptor**

The IGF-1R is an emerging target for cancer treatment because it is overexpressed in many cancers, including NSCLC. IGF-1R is activated by the binding of IGF ligands, IGF-1 or IGF-2, to the extracellular domain of IGF-1R.\[132\] IGF-1R signaling involves the activation of various intracellular signaling pathways as shown in Figure 1.\[133\] A predictive biomarker of response to IGF-1R has not been established yet. Despite highly promising Phase II trial data in NSCLC for figitumumab, a human mAb against IGF-1R, the lack of OS benefit and concern for increased toxicities, including treatment-related fatalities, in the two Phase III studies (first-line in combination with chemotherapy; combination with erlotinib in previously treated patients) dampened the enthusiasm for the development of this class of drugs for lung cancer.\[134\] The Phase I/II study of erlotinib in combination with a different mAb, cixutumumab, was also not developed further clinically as the combination was toxic and efficacy was low in unselected patients.\[135\] Two randomized Phase II studies incorporating cixutumumab in combination with first-line chemotherapy for patients with non-squamous NSCLC are ongoing (NCT00955305, NCT01263782).

Unfortunately, small-molecule IGF-1R TKIs faced obstacles in their clinical development due to the experience encountered with the mAbs as briefly described above. Owing to the significant homology between IGF-1R and insulin receptor (InsR) TK domains, these drugs generally inhibit both IGF-1R and InsR signaling and are associated with on-target metabolic derangements. Although this may be viewed as a disadvantage, hyperglycemia from IGF-1R TKIs is not life-threatening and is clinically manageable.\[133\] Moreover, inhibition of the InsR signaling may in fact prevent the bypass effect of drug-induced hyperinsulinemia and overcome the increased sensitivity to the growth stimulatory effects of insulin induced by IGF-1R blockade, which is thought to underlie the failure of IGF-1R mAbs in the clinical trials aforementioned.\[137\] This is a similar rationale for combining IGF-1R mAb with inhibitors of mTOR, which is downstream of insulin signaling, as supported by preclinical models showing that resistance to IGF-1R therapy arises through induced activation of AKT and mTOR.\[138\] A Phase I study of linsitinib (a dual TKI of IGF-1R and InsR) in combination with erlotinib demonstrated that 2 out of 4 partial responses (PR) were in patients who had NSCLC.\[139\] This combination is being evaluated in ongoing Phase II trials [Table 4]. AXL1717 (Axelar AB) is a TKI of the IGF-1R that does not inhibit the closely related InsR. A Phase I study of AXL1717 showed 4 out of 6 patients with SQCLC who had an objective response with tumor necrosis on positron emission tomography scan and prolonged disease control.\[20\] A randomized Phase II study comparing it to docetaxel as second or third-line therapy in NSCLC is ongoing (NCT01561456).

**Fibroblast growth factor receptor**

At the turn of the new millennium, identification of therapeutic targets for SQCLC had lagged behind adenocarcinoma of the lung. This impasse was first surmounted by genomic analyses showing the presence of focal fibroblast growth factor receptor FGFR1 amplification sensitive to treatment with small molecule FGFR TKIs in approximately 20% of SQCLC.\[140\] FGFR1 is a member of FGFR family of 4 highly conserved RTKs whose activation leads to downstream signaling through PI3K/AKT and RAS/RAF/MEK/MAPK pathways [Figure 1].\[141\] Mutations are seen across the four FGFR members, but individually occur less frequently. More recently, FGFR gene fusions were also described in NSCLC, namely BAG4-FGFR1, FGFR2-CIT, FGFR2-KIAA1997 and FGFR3-TACC3.\[1\] These fusion products can exhibit
Table 4: Highlighted ongoing clinical studies of select novel targeted therapies in advanced NSCLC

| Targeted agents | Current phase of development | Design |
|-----------------|-----------------------------|--------|
| **BRAF inhibitors** | | |
| Dabrafenib (GSK2118436) | NCT01336634 Phase II | Single agent in advanced NSCLC with BRAF mutations |
| Vemurafenib (PLX4032) | NCRR 396 Phase II | Single agent in patients with BRAFV600 mutation-positive cancers (excluding melanoma and papillary thyroid cancers) |
| LGX818 | NCT01543698 Phase I | Combination with sorafenib for patients with BRAF mutations refractory to standard therapy |
| ROS212054 (PLX3603) | NCT01143753 Phase II | Combined with MEK162 in patients with BRAFV600-dependent advanced solid tumors |
| RAF 265 (dual BRAF/VEGFR2 inhibitor) | NCT01352273 Phase I | Combined with MEK162 in patients with advanced solid tumors harboring RAS or BRAFV600E mutations |
| **MEK inhibitors** | | |
| Trametinib (GS1120212) | NCT01192165 Phase I | Trametinib in combination with docetaxel; erlotinib; pemetrexed; pemetrexed+cisplatin; or nab-paclitaxel in advanced solid tumors |
| | NCT01155453 Phase I | Open-label study of BKM120 in combination with trametinib in patients with advanced solid tumors |
| | NCT01362296 Phase I | Trametinib compared with docetaxel in patients with targeted mutations (KRAS, NRAS, BRAF, MEK1) in advanced NSCLC (2nd line treatment) |
| Selumetinib (AZD6244) | NCT01586624 Phase I | Vandetanib in combination with selumetinib NSCLC (expansion cohort) |
| | NCT01229150 Phase II | Combination with erlotinib in KRAS wild type and KRAS mutant advanced NSCLC |
| | NCT01750281 Phase II | Combination with docetaxel, compared with placebo in advanced NSCLC patients as a 2nd line treatment |
| | NCT00890825 Phase II | Combination with docetaxel, compared with docetaxel alone, in 2nd line patients with KRAS mutation NSCLC |
| | NCT01248247 Phase II | Battle-2 program |
| GDC-0973 | NCT00996892 Phase I | Combined with GDC-0941 (pan-PI3K inhibitor) in advanced solid tumors |
| | NCT01562275 Phase I | Combined with GDC-0068 (AKT inhibitor) in advanced solid tumors |
| **ERK inhibitors** | | |
| BVDS23 | NCT01781429 Phase I/I | Single agent in advanced solid tumors |
| MK-8353 (SCH900353) | NCT01358331 Phase I | Single agent in advanced solid tumors |
| **ALK inhibitors** | | |
| Crizotinib (PF-02341066) | NCT01441128 Phase I | Stage IIIB/IV NSCLC. Crizotinib+PF0299804 (pan-ERBB inhibitor) |
| | NCT01121575 Phase I | Stage IIIB/IV NSCLC. Crizotinib+dacomitinib (PF00299804) versus PF0299804 alone until progression |
| | NCT00932893 Phase III (PROFILE 1007) | Advanced NSCLC with ALK gene fusion progressed after platinum-based chemotherapy. Crizotinib versus docetaxel or pemetrexed |
| | NCT01154140 Phase III (PROFILE 1014) | Previously untreated non-squamous advanced NSCLC with ALK gene fusion |
| LDK378 | NCT01685060 Phase II | Single-arm study in patients with ALK-activated NSCLC previously treated with chemotherapy and crizotinib |
| | NCT01685138 Phase II | Single-arm study in crizotinib naïve patients with ALK-activated NSCLC |
| ASP3026 | NCT01401504 Phase I | Patients with advanced solid tumors |
| AP26113 | NCT01449461 Phase II | This agent also has EGFR inhibitory activity. The expansion cohort will focus on ALK and EGFR mutant NSCLC |
| X396 | NCT01625234 Phase I | Patients with advanced solid tumors |

Contd...
| Targeted agents | Current phase of development | Design |
|----------------|-----------------------------|--------|
| CH5424802      | NCT01588028 Phase I and Phase II | ALK-rearranged NSCLC |
| **Multikinase inhibitors** | | |
| Sorafenib (BAY43-9006) | NCT00870532 Phase I NCT00609804 Phase II NCT00863746 Phase III | Sorafenib and erlotinib or sorafenib alone in patients progressing on erlotinib after 2-3 previous treatment |
| Dovitinib (TKI258) | NCT01676714 Phase II NCT01831726 Phase II | Single agent for tumor with mutations or translocations of FGFR, PDGFR, VEGF, cKIT, FLT3, CSFR1, Trk and RET |
| Ponatinib (AP24534) | NCT Phase I/II NCT01813734 Phase II | Single agent in advanced squamous cell lung cancers with FGFR kinase alterations |
| Nintedanib (BIBF1120) | NCT01346540 Phase I NCT00693992 Phase II NCT00863746 Phase III | Sorafenib and erlotinib or sorafenib alone in patients progressing on erlotinib after 2-3 previous treatment |
| Sunitinib (SU11248) | NCT00698815 Phase II NCT00693992 Phase II NCT00863746 Phase III | Sorafenib versus placebo in relapsed advanced non-squamous NSCLC |
| Pazopanib (GW786034) | NCT01027598 Phase II | Single agent for tumor with mutations or translocations of FGFR, PDGFR, VEGF, cKIT, FLT3, CSFR1, Trk and RET |
| Cediranib (AZD2171) | NCT006993992 Phase III | Cediranib (20 mg) versus placebo in patients receiving paclitaxel/carboplatin |
| Vargatef (BIBF1120) | NCT00805194 Phase III NCT00806819 Phase III | Oral BIBF 1120 plus docetaxel versus placebo plus docetaxel in patients after failure of 1st line chemotherapy |
| Vandetanib (ZD6474) | NCT01823068 Phase II NCT01582191 Phase I | Single agent in NSCLC patients with RET rearrangement |
| Cabozantinib (XL184) | NCT01639508 Phase II | Patients with KIF5B/RET positive advanced NSCLC |
| XL647 (PRIM-001, KD019) | NCT01487174 Phase III | Patients with KIF5B/RET positive advanced NSCLC |
| Dasatinib (BMS-354825) | NCT00787267 Phase II NCT01514864 Phase II | Dasatinib in previously treated NSCLC |
| **c-MET/hepatocyte growth factor pathway inhibitors** | | |
| EMD1214063 | NCT01014936 Phase I | Single agent under 2 different regimens in advanced solid tumors |
| INC280 | NCT01324479 Phase I NCT01610336 Phase II | Combination with gefitinib in patients with EGFR mutated, c-MET-amplified NSCLC progressed after EGFR Inhibitor treatment |
| Crizotinib (PF-02341066) | NCT00585195 (A8081001) Phase I | Advanced solid malignancies that are known to be sensitive to PF-02341066 inhibition, e.g. ALK, MET and ROS |
| Cabozantinib (XL184) | NCT01639508 Phase II | Patients with KIF5B/RET positive advanced NSCLC |
| Tivantinib (ARQ197) | NCT01377376, NCT01244191 Phase II | Dasatinib in pretreated NSCLC patients with harboring a DDR2 mutation or an inactivating BRAF mutation |

Contd...
### Table 4: Contd...

| Targeted agents | Current phase of development | Design |
|-----------------|------------------------------|--------|
| **Targeted agents** | | | |
| | | | |
| Foretinib (GSK1363089) | NCT01068587 | Phase II | Tivantinib/erlotinib in relapsed NSCLC with EGFR mutant or EGFR unknown. Previously known as XL880 |
| | NCT01773018 | Phase I | Single agent in advanced solid tumors |
| | | | |
| **ABT700** | NCT01472016 | Phase I | Single agent or in combination with oxaliplatin and capecitabine in advanced solid tumors |
| | | | |
| **Onartuzumab (OAM4558g)** | NCT01456325 | Phase III | Onartuzumab/erlotinib in pretreated advanced NSCLC patients with Met positive |
| | NCT01519804 | Phase II | Paclitaxel/cisplatin or Carboplatin/onartuzumab as first-line treatment for stage IIIb/IV squamous cell type |
| | NCT01496742 | Phase II | Onartuzumab versus placebo in combination with either Bev/platinum/paclitaxel or pemetrexed/platinum in untreated stage IIIb/IV non-squamous NSCLC |
| | | | |
| **Rilotumumab** | NCT01233687 | Phase I/II | Rilotumumab and erlotinib in previously treated NSCLC |
| | | | |
| **Ficlatuzumab** | NCT01039948 | Phase I/II | Combination with gefitinib in Asian NSCLC patients |
| | | | |
| **mTOR inhibitors** | | | |
| | | | |
| Sirolimus | NCT00923273 | Phase II | Combination with pemetrexed in relapsed NSCLC |
| | | | |
| Everolimus | NCT01700400 | Phase I | Everolimus/pemetrexed/carboplatin/bevacizumab (bev) in combination for stage IV non-squamous NSCLC |
| | NCT00406276 | Phase II | Everolimus plus docetaxel in patients with metastatic or recurrent NSCLC |
| | NCT00457119 | Phase II | Combination of everolimus/carboplatin/paclitaxel/bev in NSCLC as a first-line treatment |
| | NCT00406276 | Phase II | Everolimus plus docetaxel in patients with metastatic or recurrent NSCLC |
| | NCT01317615 | Phase IV | Combination of everolimus/paclitaxel/carboplatin in patients with advanced large cell with neuroendocrine differentiation lung cancer |
| | | | |
| Temsirolimus | NCT00079235 | Phase II | Temsirolimus in patients with advanced NSCLC |
| | NCT00921310 | Phase III | Combination of temsirolimus and pemetrexed in recurrent NSCLC |
| | | | |
| **mTORC1/2 inhibitors** | | | |
| | | | |
| CC223 | NCT01545947 | Phase I | Open-label study of CC-223 in combination with erlotinib or azacitidine in advanced NSCLC |
| | MLN0128 | NCT01351350 | Phase I | Open-label study of MLN0128 in combination with paclitaxel, with/without trastuzumab, in advanced cancer |
| | PX866 | NCT01204099 | Phase III | PX866 and docetaxel in patients with NSCLC and head and neck cancer |
| | Buparlisib (BKM120) | NCT01363232 | Phase I | Combination of BKM120/MEK162 in advanced solid tumors |
| | NCT01723800 | Phase I | Combination of BKM120/carboplatin/pemetrexed in advanced non-squamous NSCLC |
| | NCT01487265 | Phase III | Erlotinib and BKM120 in patients with advanced NSCLC previously sensitive to erlotinib |
| | NCT01570296 | Phase I | Gefitinib in combination with BKM120 in advanced NSCLC with tumor harbour molecular alterations of PI3K pathway and known to overexpress EGFR |
| | | | |
| GDC0941 | NCT00974584 | Phase I | Combination with either paclitaxel/carboplatin (with or without bev) or pemetrexed/cisplatin/bev in advanced NSCLC |
| | NCT01493843 | Phase II | Carboplatin/paclitaxel and carboplatin/paclitaxel/bev with and without GDC-0941 in advanced NSCLC |

Contd...
### Table 4: Contd...

| Targeted agents          | Current phase of development | Design                                                                                                                                 |
|--------------------------|------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| **AKT inhibitors**       |                              |                                                                                                                                          |
| MK2206                   | NCT01147211                  | MK-2206 combined with gefitinib in NSCLC population enriched with EGFR mutation                                                        |
|                          | NCT01294306                  | Combination of MK2206/erlotinib in advanced NSCLC patients pretreated with erlotinib                                                  |
| GDC0068                  | NCT01362374                  | Combined with either docetaxel or fluoropyrimidine plus oxaliplatin in advanced solid tumors                                          |
| AZD5363                  | NCT01226316                  | Single agent in solid tumors bearing either AKT1 or PIK3CA mutation                                                                   |
| **Insulin-like growth factor pathway** |                          |                                                                                                                                          |
| Cixutumumab (IMC-A12)    | NCT00955305                  | Paclitaxel, carboplatin, bevacizumab with or without cixutumumab in non-squamous histology                                              |
|                          | NCT01263782                  | Biomarker-integrated study in advanced NSCLC as front line setting                                                                     |
| Dalotuzumab (MK0646)     | NCT00799240                  | Combined with pemetrexed/cisplatin in non-squamous type                                                                               |
| Ganitumab (AMG479)       | NCT01122199                  | Combined with RAD001 in advanced solid tumors                                                                                          |
|                          | NCT01708161                  | Combined with BYL719 in patients with PIK3CA mutated or amplified advanced solid tumors                                               |
| BIIB022                  | NCT00970580                  | Combination with paclitaxel/carboplatin advanced NSCLC                                                                               |
| Linstitinib (OSI906)     | NCT01211077                  | Erlotinib in combination linstitinib in chemonaive patients with EGFR mutation                                                        |
|                          | NCT01186861                  | Maintenance linstitinib plus erlotinib in patients with non-progression Following after 1st line chemotherapy                         |
| AXL1717                  | NCT01561456                  | AXL1717 compared to docetaxel in previously treated patients in advanced NSCLC                                                       |
| **Heat shock protein 90 (HSP90) inhibitors** |                          |                                                                                                                                          |
| Ganetespib (STA9090)     | NCT01562015                  | Single arm ganetespib in subjects ALK-positive NSCLC                                                                                    |
|                          | NCT01579994                  | Crizotinib and ganetespib in ALK positive lung cancers                                                                                |
|                          | NCT01348126                  | Combination with docetaxel versus docetaxel alone advanced NSCLC                                                                       |
| Retaspimycin (IPIS04)    | NCT01427946                  | Combination with everolimus in KRAS mutant NSCLC                                                                                      |
|                          | Phase I/I                    |                                                                                                                                          |
| AU1Y922                  | NCT01259089                  | Adenocarcinoma with acquired resistance to EGFR TKIs                                                                                |
|                          | NCT01752400                  | ALK-rearranged advanced NSCLC with acquired resistance to prior ALK TKIs                                                               |
|                          | NCT01646125                  | AU1Y922 versus pemetrexed or docetaxel in EGFR mutations and progressed on prior EGFR TKIs                                               |
| AT13387                  | NCT01712217                  | Alone and in combination with crizotinib NSCLC                                                                                         |
|                          | Phase I/I                    |                                                                                                                                          |
| DS-2248                  | NCT01288430                  | Single agent in advanced solid tumors                                                                                                  |
| **Selective FGFR inhibitors** |                          |                                                                                                                                          |
| JNJ-42756493             | NCT01703481                  | Single agent, with expansion cohort in KRAS wild-type tumors with FGFR 1, 2 or 4 gene amplifications                                      |
| BGJ398                   | NCT01004224                  | Single agent in advanced solid tumors with FGFR1 or FGFR2 amplification or FGFR3 mutation                                             |
| AZD4547                  | NCT01795768                  | Patients with FGFR1 or FGFR2 amplified in Squamous cell lung cancer, gastric, esophageal and breast cancer                              |
|                          | NCT01824901                  | Docetaxel with or without AZD4547 in recurrent FGFR1-amplified squamous NSCLC                                                        |

Contd...
Table 4: Contd...

| Targeted agents | Current phase of development | Design |
|-----------------|------------------------------|--------|
| PLX7486         | NCT01804530 Phase I          | Single agent and in combination with gemcitabine and nab-paclitaxel in advanced solid tumors |
| Anti-PD-1       |                              |        |
| Nivolumab (BMS-936558) | NCT01454102 Phase I          | Nivolumab in combination with gemcitabine/cisplatin, pemetrexed/cisplatin, carboplatin/paclitaxel, bevacizumab maintenance, erlotinib, ipilimumab or as nonotherapy in 1st line or in switch maintenance in advanced NSCLC |
|                 | NCT01721759 Phase II         | Single arm in advanced squamous cell lung cancer who have received at least 2 prior regimens |
|                 | NCT01673867 Phase III        | Open-label randomized trial of nivoluma versus docetaxel in previously treated advanced non-squamous cell lung cancer |
|                 | NCT01642004 Phase III        | Open-label randomized trial of nivoluma versus docetaxel in previously treated advanced squamous cell lung cancer |
| Lambrolizumab (MK3475) | NCT01295827 Phase I          | Single agent MK-3475 in patients with advanced or carcinoma, melanoma and NSCLC |
| Anti-PDL-1      |                              |        |
| MEDI4736        | NCT01693562 Phase I          | In advanced solid tumors |
| MDX1105-01 (BMS-936559) | NCT00729664 Phase I          | Administered every 14 days in advanced solid tumors |

| NSCLC: Non-small cell lung cancer; TKI: Tyrosine kinase inhibitor; VEGFR: Vascular endothelial growth factor receptor; ALK: Anaplastic lymphoma kinase; Bev: Bevacizumab; KIF5B: Kinesin family member 5B; MET: Mesenchymal-epithelial transition factor; DDR2: Discoidin domain receptor 2; EGFR: Epidermal growth factor receptor; FLT3: FMS-like tyrosine kinase 3; Trk: Tropomyosin-related kinase; ERK: Extracellular signal-regulated kinases; AKT: Protein kinase B; RET: Rearranged during transfection; FGFR: Fibroblast growth factor receptor; MEK: Mitogen-activated protein kinase; PIK3CA: Phosphoinositide-3-kinase, catalytic, alpha polypeptide; BRAF: V-raf murine sarcoma viral oncogene homolog B1; RAS: no need for expansion; KRAS: Kirsten-rous avian sarcoma; ROS: Reactive oxygen species 1; NCT: National clinical trial; PDGFR: Platelet-derived growth factor receptor; CSF1R: Colony stimulating factor receptor-1 |

oligomerization capability, resulting in FGFR TK activation sensitive to FGFR TKIs.\[21\] FGFR3-TACC3 fusion was the most frequently reported to date, found in approximately 1.8% of SQCLC screened.\[142\] Owing to the high degree of homology between VEGFR2, PDGFR (platelet-derived growth factor receptor) and FGFR TK domain, various oral multikinase inhibitors currently in clinical use or early phase development demonstrate ability to inhibit FGFR1 in nanomolar concentrations (e.g. nintedanib, briivanib, dovitinib, sorafenib, pazopanib, ponatinib, etc.). Ponatinib has pan-FGFR activity and is thus being explored in a Phase II/III for advanced SQCLC with FGFR kinase alterations (NCT01761747). Dovitinib is also being evaluated in a modular Phase II study in tumors with activated pathways that maybe inhibited by dovitinib, such as mutations or translocations of FGFR, PDGFR, VEGF, cKIT, FLT3, CSF1R, tropomyosin-related kinase (Trk) and rearranged during transfection (RET) (NCT01831726). More selective FGFR inhibitors in early phase clinical testing include JNJ-42756493, BGJ398 and AZD4547. An Eastern Cooperative Oncology Group randomized Phase II study of docetaxel with or without AZD4547 in patients with FGFR1-amplified SQCLC is being planned (NCT01820364).

Similar to other TKIs, an anticipated mechanism of acquired resistance to FGFR inhibitors, particularly with FGFR-selective inhibitors, is the emergence of a secondary gatekeeper mutation, which has been modeled recently as the V555M alteration in FGFR3.\[143\] Co-existence of other activated signaling pathways, like MAPK pathway, may also underlie intrinsic resistance to FGFR1 inhibition.\[143,144\] Conversely, activated FGFR pathway can mediate resistance to other targeted therapies such as EGFR, HER, MET, BRAF and angiogenesis inhibitors.\[145-149\] This provides rationale for combination therapy, which is anticipated to result in improved efficacy and in delayed emergence of treatment resistance. Combination trials are either underway (NCT01820364) or in early stages of planning.

**Discoidin domain receptor 2**

DDR2 (located on 1q23) is a RTK, which binds collagen and has been shown to promote cell migration, proliferation and survival.\[43\] Conventional Sanger sequencing performed in a discovery-validation study of 290 SQCLC tumors identified the presence of DDR2 gene mutations in 3.2% of primary squamous cell carcinoma (SCC) tumor samples.\[15\] No alterations in DDR2 gene copy number or protein expression was found. Functional characterization revealed that these mutations are oncogenic and that DDR2-driven transformation is sensitive to treatment by dasatinib, an oral multikinase inhibitor dual-specific Src and Abl kinase. Other FDA-approved kinase inhibitors with DDR2-inhibitor activity include imatinib, nilotinib, ponatinib, sorafenib and pazopanib. Several studies of dasatinib in NSCLC were terminated due to either slow accrual or toxicity concerns.
A Phase II trial with dasatinib in previously treated patients with advanced NSCLC regardless of molecular profile is ongoing (NCT00787267). Another Phase II study recruiting SQCLC patients with DDR2 mutation is evaluating dasatinib as first-or subsequent-line therapy in this patient population (NCT01514864).

**PDGFRα and KIT**

Recurrent amplification of chromosomal segment 4q12 was identified in 3–7% of lung adenocarcinomas and 8–10% of SQCLC. Preclinical models in NSCLC cell lines with focal high level-amplitude gains in this chromosomal segment implicate the potential oncogenic role of PDGFRα and KIT. However, amplification alone is not sufficient to predict treatment sensitivity to specific kinase inhibitors as only one out of six cell lines with 4q12 amplification were found to be sensitive to treatment with imatinib or sunitinib, multikinase inhibitors which include KIT and PDGFRα in their spectrum of drug targets. Various nonsynonymous mutations in PDGFRα and KIT are reported in approximately 4–2% of NSCLC, respectively. There is an ongoing Phase II study in NSCLC, SCLC and thymic malignancies that allocate several targeted therapies according to the tumor’s molecular profile (NCT01306045). Patients with PDGFRα mutation or gene amplification or KIT mutation are assigned treatment using sunitinib in this study. It is anticipated that this enrichment strategy will yield better tumor RRs and corresponding clinical benefit compared to the more modest result of approximately 2–10% RR seen in older NSCLC trials in the molecularly unselected population.

**SIGNALING PATHWAYS**

**RAS/RAF/MEK/ERK pathway**

The RAS family of proteins are oncogenes discovered in animals through a cancer-causing retrovirus and encoded by 3 genes; H-RAS, K-RAS and N-RAS. All 3 of these genes are commonly mutated in human cancers, leading to constitutively activated proteins locked in the guanosine triphosphate (GTP)-bound “on” state. RAS genes encode G proteins downstream of RTKs such as EGFR [Figure 1]. Activated RAS/RAF/MEK/ERK pathway regulates cell growth, differentiation and apoptosis by interacting with multiple effectors. In 15–25% of patients with NSCLC, KRAS mutations are present and 97% of KRAS mutant cases are exon 2 (codons 12 or 13) mutations. In contrast to EGFR mutations, KRAS mutations are found in 20–30% of white patients but in only 5% of East Asian patients with lung adenocarcinomas. KRAS mutations are predominantly found in adenocarcinomas of smokers and in general are mutually exclusive with EGFR and HER2 mutations. Testing for the presence of KRAS mutations as a predictive biomarker of response to chemotherapy or EGFR therapies in NSCLC is controversial and to date does not preclude the use of EGFR TKI though the clinical benefit is likely to be marginal compared with patients with wild-type KRAS NSCLC.

NRAS mutations, in comparison, are present in <1% of lung cancers, primarily in adenocarcinomas. A distinct subset of tumors with inactivating mutations in the tumor suppressor gene NF1, which manifest the hyperactivated RAS phenotype in the absence of mutations in RAS itself, is found in approximately 7% of lung adenocarcinomas. NF1 encodes neurofibromin 1, a GTPase-activating protein that negatively regulates RAS signaling. Mutations in BRAF, a member of the RAF family of serine/threonine kinases, have been reported in 1–4.9% of NSCLC, predominantly in adenocarcinomas. The V600E mutation is the most common found in NSCLC (56.8%) and is associated with micropapillary features, female gender and poor OS. Unlike EGFR and HER2 mutations, BRAF mutations tend to be found commonly among smokers. Furthermore, unlike EGFR and HER2 mutations, several non-V600 BRAF mutations found in NSCLC are inactivating (e.g., D594G, G466V, Y472). Even though, downstream MEK and ERK activation are, comparatively much less compared with BRAF V600E mutants, these phosphorylation events are paradoxically still at or above those observed with wild-type in BRAF kinase-impaired/kinase-inactivating mutants, presumably due to transactivation of CRAF. Interestingly, kinase-impaired BRAF mutations in NSCLC appear to be sensitive to dasatinib, providing rationale for an ongoing Phase II study in NSCLC patients with inactivating BRAF mutations (NCT01514864). Finally, somatic activating mutation in exon 2 of MEK1, a dual-specificity serine/threonine and tyrosine kinase, is seen in approximately 1% of lung adenocarcinomas.

Because the development of RAS inhibitors have been largely unsuccessful to date (e.g., farnesyltransferase inhibitors), various investigations thus have focused on the modulation of downstream proteins or protein trafficking pathways. Preclinical models have shown that MEK inhibitors can induce significant tumor regressions in KRAS- or BRAF-induced lung tumors. Splice site mutations that lead to higher expression of the active RAC1b isoform, a G-protein that promotes KRAS-induced lung tumorigenesis, may be potentially associated with sensitivity to MEK inhibition. However, the kinase-impaired D594G or G469E BRAF mutations are known to be highly resistant to MEK inhibitors in melanoma cell lines. In addition, resistance to MEK inhibition is thought to arise potentially through emergence of secondary mutations in MEK, either in conjunction with...
with or independently of the emergence of KRAS or BRAF amplification in cells harboring KRAS mutation or BRAF V600E mutation, respectively. Co-inhibition of BRAF may overcome resistance to MEK inhibition alone in the BRAF-mutant cases, thus providing rationale for combination regimens in this particular setting.\textsuperscript{[168,169]} Regardless of the mechanism involved, ERK inhibition can block proliferation of MEK inhibitor-resistant tumors. In addition, dual MEK-ERK inhibition shows additive/synergistic effect and can delay emergence of and potentially overcome, acquired MEK inhibitor resistance.\textsuperscript{[166]}

The heterogeneity in response to MEK inhibition in KRAS mutant lung cancers is attributed to the presence of activated AKT or STAT3 pathway, thus providing rationale for combination regimens with the corresponding inhibitors.\textsuperscript{[170]} Moreover, the combination of a MEK inhibitor with either a PI3K inhibitor or BCL-XL inhibitor results in marked synergistic tumor regression in mice bearing KRAS-mutant lung cancers.\textsuperscript{[171,172]} Similarly, the addition of selumetinib, a MEK inhibitor, markedly improved the response of KRAS mutant tumors to docetaxel.\textsuperscript{[172]} However, the concurrent loss of LKB1 (also known as STK11), a tumor suppressor gene, abrogated the synergistic effect of the combination of docetaxel with selumetinib in a KRAS mutant lung tumor model, likely through activated AKT and SRC pathways.\textsuperscript{[173]} This is very relevant as concurrent KRAS and LKB1 mutation is observed in 4–10% of NSCLC. Although there are no published reports of MEK inhibition in NF1-deficient lung cancer models specifically at this time, lessons can be extrapolated from other tumor models. MEK inhibition can suppress the growth of NF1-deficient acute myeloid leukemias\textsuperscript{[174]} and myeloproliferative disorders\textsuperscript{[175]} but is only effective against a subset of NF-1 deficient GBM (glioblastoma multiforme).\textsuperscript{[176]} In NF1-deficient GBM resistant to MEK inhibition alone, combination therapy with a dual PI3K/mTOR inhibitor overcomes this treatment resistance.\textsuperscript{[176]} On a similar theme, HER3 activation that results in recruitment of PI3K/AKT signaling was observed to underlie the resistance to dual BRAF and MEK inhibition in melanoma.\textsuperscript{[177]}

Several potent and selective MEK inhibitors such as selumetinib (AZD6244) and trametinib (GSK1120212) are in clinical testing for NSCLC. Other MEK inhibitors, such as GDC-0973 (XL518), refametinib (BAY 86-9766, RDEA119), pimasertib (AS703026/MSC1936369B), MEK162, WX-554, etc., are in early clinical development. Table 4 highlights several clinical trials, either ongoing or soon to be activated, of various targeted therapies in development. A randomized, placebo-controlled Phase II study of selumetinib plus docetaxel in KRAS-mutant NSCLC has shown promising efficacy, albeit with a higher number of adverse events than docetaxel alone in previously treated advanced KRAS-mutant NSCLC.\textsuperscript{[178]} A phase I trial of selumetinib in combination with the dual EGFR/VEGFR inhibitor vandetanib is ongoing, with planned expansion cohort in NSCLC. There are multiple studies underway evaluating the combination of a MEK inhibitor with inhibitors of the PI3K/AKT/mTOR pathway, which will be discussed later. Various ERK inhibitors such as BVD-523 and MK-8353 (SCH 900353) are in early clinical testing. The combination of PI3K inhibitor and the ERK inhibitor shows synergistic antiproliferative activity in preclinical models.\textsuperscript{[179]} Similarly, the dual PI3K/ERK inhibitor AEZS-132 demonstrated significant activity in several xenograft models.\textsuperscript{[180]}

Tumor responses in NSCLC with BRAF V600E mutation had been reported with vemurafenib (currently approved for use in advanced stage melanoma) and dabrafenib, both potent inhibitors of wild-type BRAF, BRAF V600E and C-RAF.\textsuperscript{[181–183]} Other BRAF inhibitors in development include ARQ736, RO5212054 (PLX3603), LGX818 and RAF265 (dual BRAF/VEGFR2 inhibitor). An open-label, Phase II study of vemurafenib (NCRN396:VE BASKET study) is ongoing for BRAF V600 mutation-positive solid tumors (excluding melanoma and papillary thyroid cancer). A cautionary note with BRAF inhibitors in general is that in cells with mutant RAS or wild-type RAS/RAF, paradoxical ERK pathway activation by RAF inhibitors has been well-described by various investigators due to RAF dimerization, leading to CRAF activation.\textsuperscript{[184–188]} This is thought to explain the occurrence of cutaneous SCC/keratoacanthomas in some patients who were treated with BRAF inhibitors as monotherapy. Newer generation agents are thus being developed that can overcome this paradoxical activation, so-called “paradox breakers,” such as PLX PB-3, which do not activate the MAPK pathway in cells with activated RAS or EGFR and do not upregulate EGFR ligands.\textsuperscript{[186]} Alternatively, combination of BRAF inhibitor and MEK inhibitor can overcome this limitation and this approach has shown clinical efficacy and better safety profile as predicted by the preclinical models.

Knowledge on acquired resistance to BRAF inhibitors is largely derived from melanoma studies. In contrast to many TKI inhibitors, acquisition of secondary gatekeeper mutations in the RAF kinase has yet to be reported in clinical cases of acquired resistance. Mechanisms involved either reactivation of MAPK pathway (e.g. V600E BRAF amplification; alternate splicing of BRAF; CRAF overexpression; upregulation of COT kinase, FGFR or PDGFRβ; activating mutations in NRAS or MEK1)\textsuperscript{[187–192]} or alternate MAPK-independent pathway signaling, e.g., activating PI3K/mTOR/AKT pathway

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through PIK3CA mutations. An interesting observation reported recently is that vemurafenib-resistant melanomas acquire dependence on vemurafenib for their continued proliferation. Drug cessation in fact results in regression of drug-resistant tumors, thus providing a rationale for investigating an intermittent schedule of drug administration to delay the onset of acquired resistance. In the case of NSCLC, acquired resistance to dabrafenib through the emergence of KRAS mutation has been recently described in a case report.

**MET/hepatocyte growth factor pathway**

MET factor receptor or HGF receptor triggers key intracellular signaling cascades, such as Src, STAT3, PI3K/AKT/mTOR and RAS/RAF/MAPK, upon binding to its ligand HGF [Figure 1]. MET kinase is implicated in cancer cell proliferation, invasion, migration and angiogenesis. Dysregulation of the HGF/MET signaling pathway can occur through HGF or MET overexpression, MET gene amplification and mutations. MET amplification occurs in 1-5% of unselected early-stage NSCLC cases, which have been associated with poor prognosis. Engelman et al. reported that 22% of lung cancers with acquired resistance to EGFR TKIs had MET amplification, driving HER3-dependent activation of PI3K.

Several strategies to antagonize MET signaling are currently under investigation, such as anti-HGF mAb (ficlatuzumab, rilotumumab and TAK701), Anti-MET mAb (onartuzumab) and TKIs (both selective and non-selective TKIs). Accrual to the randomized Phase III trial evaluating the combination of the selective non-ATP dependent c-MET TKI tivantinib (ARQ 197) and erlotinib compared to erlotinib combined with the placebo as second-line therapy in advanced EGFR TKI-naïve NSCLC patients was halted in October 2012 after a planned interim analysis revealed lack of PFS improvement in the overall population and that the primary endpoint of OS improvement will not be met. In comparison, the randomized, placebo-controlled Phase III study of onartuzumab (MetMab) in combination with erlotinib versus erlotinib in NSCLC patients with Met-positive tumor as determined by immunohistochemistry (IHC) is ongoing. The rationale for the study was based on promising PFS and OS data with the onartuzumab/erlotinib combination compared to erlotinib/placebo in the preceding randomized Phase II study in patients with Met-positive tumors.

Cabozantinib and crizotinib are both oral TKIs that include c-met in their spectrum of activity. There is a case report of durable tumor response seen with crizotinib in a NSCLC patient with de novo MET amplification, but without ALK translocation attesting to additional clinical settings for the use of crizotinib in NSCLC. The combination of crizotinib with the pan-ErbB inhibitor dacomitinib is being evaluated in an ongoing Phase I study (NCT01121575). Similarly, the c-MET and AXL inhibitory profile of cabozantinib provides rationale for its combination with EGFR TKI to overcome and delay treatment resistance. A Phase I/II study of cabozantinib with erlotinib reported partial tumor response seen in a patient with MET amplification. A PR with cabozantinib monotherapy was also recently reported in a patient with ALK fusion-positive tumor, implying either the co-existence of activated c-met signaling in the tumor or that cabozantinib has potential ALK inhibitory activity as well. Although the current understanding of mechanisms of acquired resistance to c-MET inhibitors is limited, preclinical cellular models of acquired resistance show that wild-type KRAS amplification and overexpression provides a mechanism of escape from MET dependence, thus overcoming the inhibitory effect of a c-met TKI.

Another mechanism documented to date is the emergence of secondary mutations in the MET kinase domain, some of which are identical to known activating mutations in the MET kinase domain, such as Y1230C and D1228N, found in patients with papillary renal cell carcinoma.

**PI3K/AKT/mTOR pathway**

The PI3K/AKT/mTOR signaling pathway was first identified in the 1990s. It is activated early in lung carcinogenesis by multiple signaling nodes such as RAS, EGFR, IGF-1R and c-MET [Figure 1]. It plays a role in cell growth, cell proliferation, angiogenesis and anti-apoptosis/cell survival, which mediates treatment resistance against systemic chemotherapy and radiation. The main downstream signaling hub is mediated by mTOR in response to growth factor stimuli and leads to the modulation of the eukaryotic initiation factor 4E binding protein-1 and the 40S ribosomal protein S6 kinase involved in the regulation of translation and protein synthesis [Figure 1]. The tumor suppressor gene phosphatase and tensin homolog (PTEN) inhibits the PI3K/AKT signaling pathway by dephosphorylating PIP3 to inactivate AKT. Loss or inactivating mutations of PTEN results in hyperactivation of the PI3K pathway, similar to what is achieved by somatic activating “gain of function” mutations in the PIK3CA gene itself. This explains the observation that these two events are mutually exclusive in most human tumors. Furthermore, loss of PTEN with subsequent pAKT overexpression is associated with poor prognosis.

Loss of PTEN as assessed by absent PTEN protein expression determined by IHC is found in 24-44% of NSCLC and up to 75% if cases with weak PTEN expression were included. Epigenetic silencing may partially explain PTEN loss in some cases as mutations or homozygous deletions of PTEN gene are rare in NSCLC.
Mutation and amplification of PIK3CA, the gene that encodes the catalytic p110 subunit α isomorf of PI3K principally involved in regulating cell proliferation and growth, is found in up to 10% and up to 45% of patients with NSCLC respectively and is associated with increased PI3K activity and AKT expression. Preclinical as well as early clinical studies have shown that the presence of PIK3CA mutations in cancer cells confer treatment sensitivity to single-agent PI3K pathway inhibitors. However, PIK3CA mutations can frequently occur simultaneously with other oncogenic drivers such as activating mutations in the MAPK pathway, particularly with lung adenocarcinomas where this is estimated to occur in 49-86% of cases. Treatment resistance to PI3K inhibitor monotherapy is anticipated and documented in preclinical models with this dual activated PI3K/MAPK genotypic profile. However, this compound pathway activation may not necessarily predict lack of clinical benefit when PI3K inhibitors are used in combination with cytotoxic agents or even as monotherapy in some settings such as in gynecologic malignancies.

Lastly, the oncogenic E17K mutation in AKT1, the isoform principally involved in regulating cellular processes such as apoptosis, is rare in NSCLC and primarily found in SQCLC with prevalence of approximately 1-1.5%. This mutation is associated with increased membrane localization and autophosphorylation of AKT. This mutation also appears to be generally mutually exclusive with PIK3CA mutations, again suggesting functional redundancy in the evolution of the hyperactivated PI3K pathway phenotype.

Various PI3K inhibitors, which include isoform-specific, pan-class I or dual PI3K/mTORC inhibitors, are in clinical development. Despite the broad similarities of p110α with other protein kinases, mutagenesis studies to anticipate mechanisms of acquired resistance suggest that appearance of secondary mutations, unlike the case with TKIs, appear unlikely to be the cause of resistance to PI3K inhibitors since most mutations led to loss of enzymatic activity. This was also true for analogous gatekeeper mutations, which render the kinase either catalytically inactive or with minimal function. Instead, preclinical models suggest that overexpression of IGF-1R can mediate resistance, whereas targeted functional inhibition or knockdown of IGF-1R expression can reverse PI3K resistance. A number of ATP-competitive pan-AKT inhibitors are in early clinical development (GSK2110183, GSK 2141795, GDC-0068, AZD5363). These agents induce hyperphosphorylation of AKT, a phenomenon termed “inhibitor hijacking of kinase activation,” resulting in AKT membrane localization though in a nonfunctional state while downstream signaling is inhibited. Non-ATP competitive allosteric inhibitors, such as MK2206, are thought to have less off-target effects and do not induce hyperphosphorylation of AKT at the threonine 308 and serine 473 residues, thus with no theoretical concerns of untoward effects during drug dissociation from the catalytically active hyperphosphorylated AKT. Lastly, dual mTORC1 and 2 inhibitors were developed to overcome the limitation of paradoxical feedback activation of AKT with first-generation rapalogs which inhibit mTORC1 (downstream to AKT), but not mTORC2 (upstream to AKT).

Owing to the highly interconnected relationship between the PI3K/AKT/mTOR and RAF/RAF/MEK/ERK pathways along with preclinical evidence showing superior outcomes with dual pathway-blockade particularly in tumors with compound mutations, several clinical trials have been launched exploring such combination strategies. Early phase studies suggest that dual inhibition may potentially exhibit improved antitumor efficacy compared with single-pathway inhibition, although potentially at the cost of increased toxicities such as skin rash, mucositis and transaminase elevations for some combinations of MEK inhibitors (selumetinib or trametinib) with either AKT inhibitor (MK2206 or GSK2141765) or the mTOR inhibitor everolimus. In comparison, the combination of the MEK inhibitor GDC-0973 with the pan-PI3K inhibitor GDC-0941 can be safely combined to date. Similarly, the combination of trametinib with the PI3K inhibitor BKM120 appears to be tolerable as well. Although dose escalation is still ongoing for both studies, objective tumor responses have already been seen in patients with RAS/RAF mutant tumors (melanoma, pancreatic cancer, gynecologic malignancies). Because increased toxicity from this combination is anticipated with continuous treatment, alternative dosing schedules (i.e. interrupted dosing of one or both agents) were tested in various preclinical models, which exhibited similar cytotoxicity to continuous dual inhibition, thus providing rationale for intermittent dosing, which was explored in the Phase I study of GDC-0973 and GDC-0941. Indeed, early clinical data suggest that higher doses can be tolerated with intermittent dosing compared with the continuous schedule. A number of Phase I studies evaluating continuous or intermittent dosing schedules of other MEK and/or PI3K inhibitors are ongoing, with expansion cohorts planned to include patients with NSCLC. Several Phase Ib/II studies of EGFR TKI (erlotinib, gefitinib, vandetanib) in combination with inhibitors of the PI3K/AKT/mTOR pathway, such as BKM120, MK2206 (AKT inhibitor) or everolimus are either ongoing or underway, some of which incorporate enrichment strategies for patients with tumors that harbor the activated PI3K pathway signature (NCT01570296, NCT01487265, NCT01582191). Monotherapy trials using mTOR inhibitors,
either as first or subsequent lines of therapy, showed minimal activity in the molecularly unselected population.\textsuperscript{221,222} The combination of gefitinib with everolimus similarly showed minimal activity in the molecularly unselected population. However, it was interesting to note that objective tumor responses were seen in two patients with the rare \textit{KRAS} G12F mutation. However, overall RR in the \textit{KRAS} mutant patients was low at 13%.\textsuperscript{223} The combination of the irreversible EGFR TKI pelitinib (development discontinued) with temsirolimus was associated with moderate toxicities, with maximally tolerated dose of pelitinib at less than half of its monotherapy dose.\textsuperscript{224} Lastly, the Phase I study of erlotinib in combination with the dual mTORC1/2 inhibitor XL765 has completed its dose-escalation, though updated results have not yet been reported.\textsuperscript{225}

**HSP90 pathway**

HSP90 is a molecular chaperone involved in the posttranslational folding, stability, activation and maturation of over 200 client proteins, including oncogenic proteins such as EGFR, HER2, MET, BRAF, ALK, ROS1, RET, etc., and their mutant forms, essential to signal transduction and cell cycle.\textsuperscript{226} It is also in turn regulated by several genetic and epigenetic mechanisms. Protein trafficking through HSP90 chaperone is not a system by itself, but it is a part of the ubiquitin proteasome system.\textsuperscript{226,227} Inhibition of HSP90 by antagonists, first established with geldanamycin and its derivatives such as 17-AAG (tanespimycin), abrogates its chaperone function and targets client proteins for proteasomal degradation. Due to the unique mechanism of action, inhibition of HSP90 has a broad therapeutic application, which includes potential activity in settings of acquired resistance to various targeted agents. However, early clinical development of HSP90 inhibitors was beleaguered by drug formulation and hepatotoxicity issues (thought to be related to the nucleophile reactions arising from the quinone component in the geldanamycin chemotype), in addition to tepid antitumor activity in the clinic.\textsuperscript{227} Adding to the complexity, it is recognized that HSP90 inhibitors can paradoxically promote AKT and ERK activation, one mechanism of which is by transient activation of their client protein kinase.\textsuperscript{228} Nonetheless, encouraging clinical responses in NSCLC patients with ALK rearrangements have been documented in various early phase studies across several HSP90 inhibitors.\textsuperscript{121,229} The recent study by Socinski et al. showed that ganetespib monotherapy had manageable side effect profile as well as clinical activity in heavily pretreated patients with advanced NSCLCs, particularly in patients with tumors harboring ALK gene rearrangement.\textsuperscript{229} PFS rates at 16 weeks were 13.3%, 5.9-9.7% in patient with positive EGFR mutation, KRAS mutation and non-EGFR/non-KRAS mutation, respectively. Four patients out of 98 patients (4%) achieved PR; all had disease that harbored ALK gene rearrangement.\textsuperscript{229} HSP90 inhibitors in development currently include the water-soluble 17-AAG hydroquinone retaspimycin (IP1504), the non-quinone PU-H71 and the nongeldanamycin agents ganetespib (STA9090), AUY922, AT13387, DS-2248 and XL888. Clinical trials evaluating the combination of HSP90 inhibitors with EGFR, BRAF, ALK or PI3K inhibitors are either ongoing or underway (NCT01259089, NCT01657591, NCT01772797, NCT01613950, NCT01712217).

**ADDITIONAL TARGETS**

Other recurrent mutations in several other kinase genes documented in large-scale genome sequencing projects, whose biological functions are largely uncharacterized, but may have therapeutic potential in NSCLC, are discussed below.

**ROS1 rearrangement**

ROS1 rearrangement, a newly discovered driver mutation in NSCLC, was discovered by Rikova \textit{et al} in 2007.\textsuperscript{230} The estimated incidence is approximately 2% of lung adenocarcinoma (both Asian and non-Asian populations) and the patient characteristics are similar to \textit{ALK} and \textit{EGFR}-mutation positive patients (non-smokers, adenocarcinoma histology).\textsuperscript{14} Histologic examination in a small series of ROS1-rearranged NSCLC identified focal presence of either solid growth with signet-ring cells or cribriform architecture with abundant extracellular mucus in more than half of the cases, which phenotypically resemble ALK-rearranged NSCLC.\textsuperscript{231} ROS1 is one of the RTKs, which consist of an extracellular ligand-binding domain, a short transmembrane domain and intracellular TK domain.\textsuperscript{232} Wild-type ROS1 is located on chromosome 6. It has been previously reported that there is a 49% amino acid homology between human ROS and ALK within the kinase domain and 77% identity at the ATP-binding site.\textsuperscript{233,234} This led to the hypothesis that ALK inhibitors could act as ROS1 inhibitors which was subsequently confirmed in various preclinical studies.\textsuperscript{234,235} These rearrangements are also client proteins of the HSP pathway and thus similarly sensitive to treatment with HSP90 inhibitors.\textsuperscript{129} Aberrant ROS1 kinase activity leads to downstream activation of PI3K/ATK/mTOR, RAS/RAF/MEK/MAPK, vav 3 guanine nucleotide exchange factor 1 (VAV3) and Src-homology 2 domain-containing phosphatase (SHP)-1 and -2 pathways.\textsuperscript{236} Currently, 12 ROS1 fusion variants in NSCLC have been identified.\textsuperscript{237} ROS1 fusions represent a unique molecular subset of NSCLC with no overlap with other oncogene drivers. Highly promising clinical activity of crizotinib with ORR of 50-60% in this patient population has been recently reported.\textsuperscript{234}
Other ALK inhibitors in development [Table 4] have varied activity against ROS1 in their spectrum of inhibition.

**RET rearrangement**

RET is a RTK involved in cell proliferation, neuronal navigation, cell migration and cell differentiation through signaling through a ligand/coreceptor/RET multiprotein complex that activates various downstream pathways such as the RAS/RAF/MEK/ERK, PI3K/AKT and STAT pathways.142 Germline and somatic mutations in RET cause the multiple endocrine neoplasia type 2 syndrome and sporadic medullary thyroid cancer.40,41 Recently, a novel fusion gene between either KIF5B or coiled-coil domain containing 6 (CCDC6) and RET protooncogene (pericentric inversion in chromosome 10), was identified in lung adenocarcinoma.142,239 Takeuchi et al. screened for ALK and ROS1 gene rearrangement in 1528 patients with surgically removed tissues and discovered KIF5B-RET and CCDC6-RET fusion genes in 14 adenocarcinomas, mutually exclusive with EGFR and KRAS mutations.239 Wang et al. also reported finding RET fusions with KIF5B, CCDC6, or nuclear receptor coactivator 4 in 13 out of 936 surgically resected NSCLC patients.239 These studies estimate that RET fusions occur in approximately 1-2% of lung adenocarcinomas.142,239,239 Tumors with RET fusion gene tend to be more poorly differentiated tumors in never smokers and are associated with tumor size ≤ 3 cm, but with N2 lymph node involvement.239 Nonetheless, there appears to be no prognostic implication as there is no significant difference in recurrence-free survival and OS between RET-positive and RET-negative patients.

Vandetanib and cabozantinib (XL184) are small molecule inhibitors of multiple kinases including VEGFR2 and RET currently approved for treatment of metastatic medullary thyroid cancer. Other FDA-approved agents that demonstrate in vitro inhibition of RET include ponatinib (AP24534), axitinib, sunitinib and sorafenib.240 Several Phase III studies of vandetanib, sunitinib and sorafenib had been conducted in previously treated, genotypically unselected NSCLC patients as monotherapy or in combination regimens. No OS benefit was seen compared with the control arms in all studies to date.241,242 Nevertheless, anecdotal evidence of clinical benefit had been reported with vandetanib and cabozantinib in RET-positive NSCLC.243,244 A Phase II study specific for patients with KIF5B-RET positive advanced NSCLC using cabozantinib (potent inhibitor of c-MET, VEGFR2, c-KIT, Flt 1/3/4, Tie2, AXL and RET) is currently ongoing (NCT01639508). Mechanisms of acquired resistance are yet to be elucidated, but it is interesting to note that the gatekeeper mutation (RET V804 L/M) resistant to vandetanib retains high affinity to sunitinib in preclinical models.245 Similarly, ponatinib, an oral multikinase inhibitor, which is approved for use in chronic myeloid leukemia patients and demonstrated clinical efficacy against the ABL gatekeeper mutation T351I, has potent activity against RET kinase including the oncogenic RET V804M mutant resistant to vandetanib.246 Phase II studies are being planned to evaluate vandetanib, sunitinib, as well as ponatinib monotherapy in NSCLC harboring RET translocations (NCT01823068, NCT01829217, NCT01813734).

**JAK/STAT**

The Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) signaling pathway is implicated in numerous cellular processes such as hematopoesis and immunoregulation.247 Discovery of a recurrent constitutively activating JAK2 mutation V617F in myeloproliferative disorders consequently led to the eventual testing and approval of ruxolitinib, an oral JAK1 and JAK2 inhibitor, in the treatment of myelofibrosis. Multiple other agents, such as pacritinib, SAR30203, CYT387, etc., are in development in hematologic malignancies. Mutations in all members of the JAK family of kinases (JAK1, JAK2, JAK3 and TYK2) are seen in 1.5-2% of NSCLC.150

**FMS-like tyrosine kinase 3**

FLT3 is a member of the Type III RTK which includes KIT and PDGFR.248 This accounts for the close homology and consequently the overlapping spectrum of activity of various multitkine inhibitors such as sunitinib, sorafenib, nintedanib, dovitinib, etc., Activating mutations were first identified in hematologic malignancies, which subsequently were found to have a negative prognostic effect, spurring the development of more selective FLT3 inhibitors such as crenolanib, quizartinib, AC220, etc.249 Various FLT3 mutations have been reported in approximately 2% of NSCLC.150

**Trk family**

Neurotrophins essential to the survival and function of neurons mediate their effects through one or more of the Trk family of RTKs (TrkA, TrkB and TrkC). Mutations in all three members have been reported in NSCLC, at a frequency of approximately 3-4% each.180 Oncogenic TrkA and TrkC activity has been reported in some thyroid and colon cancers.250,251 However, functional characterization of some of the TrkB mutants revealed lack of transforming ability and thus of questionable role in patient selection for evaluation of Trk inhibitors such as the pan-Trk inhibitor PLX7486.252 Other Trk inhibitors in development include the dual cyclin-dependent kinase/TrkA inhibitor PHA-848125AC and the TrkC inhibitor AZD7451.
Evolving Area of Targeted Approach in Immune Checkpoint Proteins

Tumor cells have the uncanny ability to evade the immune response and several approaches are being developed to boost antitumor responses of T-cells and restore their ability to detect and attack cancer cells. A better understanding of the intricate balance between T-cell co-stimulatory and inhibitory signals under physiological conditions and during aberrant immune evasion/resistance led to the recent development of mAbs blocking the cytotoxic lymphocyte-associated antigen 4 (CTLA4) and the programmed cell death protein 1 (PD-1)-mediated T-cell events. CTLA4 is expressed exclusively on T-cells where it regulates the early stages of T-cell activation by actively delivering inhibitory signals to the T-cell. It also mediates signaling-independent T-cell inhibition by sequestration of ligands to counteract the activity of the T-cell co-stimulatory receptor, CD28. In contrast to the PD-1 pathways, there is no tumor specificity to the expression of the CTLA4 ligands. PD-1 is an immune checkpoint receptor expressed by activated T-cells and it mediates immunosuppression upon binding to PD-1 ligands (PD-L1 [B7-H1] and PD-L2 [B7-DC]), which are expressed by tumor cells, stromal cells, or both. The major role of PD1 is to limit the autoimmune and the inflammatory response in peripheral tissues by restricting T-cell activity. Blockade of the interaction between PD-1 and PD-L1 potentiates immune responses in vitro and in vivo antigrowth activity. In NSCLC tissue, PDL-1 positive cells are substantially increased when compared with adjacent lung parenchyma and PDL-1 expression on lung cancer cells also correlates with poor prognosis and decreased OS.

Ipilimumab, a fully human mAb against CTLA4, is already approved for use in advanced stages of melanoma. In NSCLC, a randomized Phase II trial of carboplatin with paclitaxel with or without ipilimumab showed a statistically significant improvement in immune-related PFS among patients who received the phased ipilimumab administration combination compared to those who received chemotherapy alone (5.7 vs. 4.6 mos, HR 0.72, P = 0.05), with subset analysis suggesting a trend toward greater clinical benefit among patients with SQCLC. This prompted the design and activation of a Phase III trial of this combination as first-line therapy in patients with squamous cell histology (NCT01285609). Two different strategies were pursued in Phase I studies evaluating the feasibility of PD-1 pathway blockade: Topalian et al. investigated a mAb directed at PD-1 (nivolumab, BMS-936558) and Brahmer et al. used mAb targeting PD-L1 (BMS-936559). Over 200 patients were enrolled in each trial with large cohorts of NSCLC patients included (122/296 and 75/205 NSCLC patients for anti-PD-1 and anti-PD-L1, respectively). Both these trials demonstrated remarkable sustained tumor regressions in the heavily pre-treated advanced NSCLC patients, with RR of 18% for anti-PD-1 and 10% for anti-PD-L1. The Grade 3 and 4 drug-related adverse event rates were low, at 14-9% for anti-PD-1 and anti-PD-L1, respectively. This is a distinct advantage compared with the adverse event rates associated with ipilimumab. Histology-specific Phase III studies comparing nivolumab with docetaxel as second-line therapy for patients with squamous (NCT01642004) or nonsquamous NSCLC (NCT01673867) are ongoing. MAbS that can block other inhibitory receptors, such as anti-killer cell immunoglobulin-like receptors (KIRs), are also in early clinical development. Identification and validation of predictive biomarkers, such as tumor expression of PD-1, for these therapies are intense areas of investigation.

Conclusion

The discovery of EGFR mutations and EML4-ALK rearrangement revolutionized the first-line treatment of NSCLC by targeted agents (erlotinib, gefitinib and crizotinib) and triggered the paradigm shift in developing genomically-driven clinical trials. Success in this approach has been confirmed largely in the population with metastatic disease and there are multiple studies ongoing or underway to further explore this in the adjuvant, neoadjuvant and post-radiation consolidation settings in NSCLC. Understanding the molecular drivers of NSCLC can assist in the optimal selection of therapy because distinct molecular subtypes may have overlapping clinical features but yet have heterogeneous outcomes to treatment. The development of novel targeted therapies represent an important and revolutionary change in oncology, but a common and inevitable theme is the specter of treatment resistance and thus investigations on the mechanisms of de novo and acquired resistance go hand-in-hand with drug development. In addition, the lack of significant activity of these targeted agents in the genotypically unselected patients underscores the need for a different approach in trial design during early phase clinical testing. With next-generation sequencing technologies discovering more genomic alterations and potential “druggable” targets, it is imperative to have a better understanding of the functional implications of these changes in order to establish the therapeutic relevance of purported drug targets and to facilitate the validation of biomarkers to be used in the identification of patients who will have the greatest likelihood of deriving benefit from target-specific therapies. Similarly, a better understanding of the therapeutic spectrum of available drugs will enable successful drug repurposing. All these efforts will ensure a more successful route from the initial steps of drug discovery to the final coveted phase of drug development.
widespread clinical use, thereby maximizing the probability of treatment success while minimizing the risks of exposure to adverse drug reactions and ineffective therapies.

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