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Review Article

Targeted therapies in development for non-small cell lung cancer

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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 lym phoma 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/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

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INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality in the United States and world-wide. 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]

SIGNALING 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).[44] 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.[45] 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].[44] 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).[15,46] 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].[61] 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].

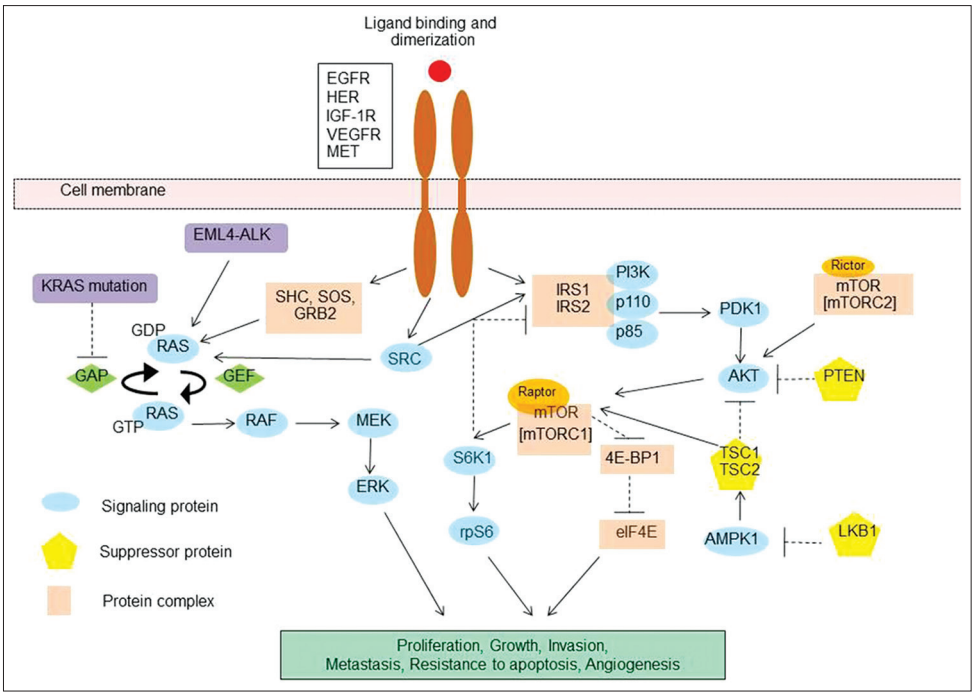

Figure 1: Cell signaling pathways in lung cancer.
Depicted are the cellular signaling pathways involved in the proliferation, differentiation, growth, metastasis, resistance to apoptosis and angiogenesis in neoplasms, highlighting the targets amenable to therapeutic interventions in lung cancer therapy. 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]		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	3-5	
Squamous cell carcinoma	<1	
DDR2 mutation[5]	3-4	Predominantly found in squamous cell carcinoma. Associated with sensitivity to multikinase inhibitors that inhibit DDR2 such as dasatinib, sorafenib and ponatinib.
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	30-50	
Caucasian	10-12	
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]		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.
Squamous cell carcinoma	<1-2	
Adenocarcinoma	<1	

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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 TKI
KRAS mutation[27]		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.
Asian	5	
Caucasian	20-30	
LKB1 mutation[28]		More common in adenocarcinomas than in squamous cell carcinomas. Concurrent mutation with KRAS may confer resistance to MEK inhibitors.
Asian	7-8	
Caucasian	30	
MEK1 mutation[29]	<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[24,32]	21	Occurs *de novo* or as the mechanism of acquired resistance to EGFR TKIs. Associated with sensitivity to MET inhibitors.
NRAS mutation[33]	<1	Primary found in lung adenocarcinomas and smokers. Associated with sensitivity to MEK inhibitors.
Amplification of chromosomal segment 4q12[34]		This region encodes for PDGFRa and KIT. Amplification alone does not predict for sensitivity to PDGFR/KIT inhibitors.
Adenocarcinoma	3-7	
Squamous cell carcinoma	8-10	
PIK3CA mutation[35,36]		Occurs *de novo* or as the mechanism of acquired resistance to EGFR TKI. Frequently occurs simultaneously with other mutations. Associated with sensitivity to PI3K inhibitors.
Adenocarcinoma	0-2.5	
Squamous cell carcinoma	3-9	
PIK3CA amplification[35,36]		Associated with sensitivity to PI3K inhibitors. May occur concomitantly with PIK3CA mutation.
Adenocarcinoma	5-10	
Squamous cell carcinoma	37-43	
Loss of PTEN[36,37,38]		Associated with PI3K pathway activation and resistance to EGFR TKI. Associated with sensitivity to PI3K inhibitors.
Adenocarcinoma	4	
Squamous cell carcinoma	21	
PTEN mutation[39]		Associated with PI3K pathway activation and resistance to EGFR TKI. Associated with sensitivity to PI3K inhibitors.
Adenocarcinoma	1.7	
Squamous cell carcinoma	10.2	
RET fusion gene[40-42]	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[4]	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; EPH: Erythropoietin-producing hepatoma; HER: Human epidermal growth factor receptors; 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	Median PFS (mo) HR (95% CI) (*P* value)	Median OS (mo) HR (95% CI) (*P* value)
EGFR TKIs						
First line						
IPASS[48,49] Asian, chemo-naïve, non/light smokers, adenocarcinoma	1,217	PFS	Gefitinib	43 (*P*=0.001)	5.7 mo 0.74 (0.65-0.85) (*P*<0.001)	18.8 mo 0.90 (0.79-1.02) (*P*=0.109)

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Table 2: Contd...

Trials	*N*	Primary endpoint	Treatment	ORR (%) *P* value	Median PFS (mo) HR (95% CI) (*P* value)	Median OS (mo) HR (95% CI) (*P* value)
			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*<0.0001)	21 mo (0.76-1.33) (*P*=0.99)
			Mutation –ve (benefit in chemo arm)		1.5 versus 5.5 mo 2.85 (2.05-3.98) (*P*<0.0001)	12 mo 0.86 (0.86-1.63) (*P*=0.309)
First-Signal[50] Asian, chemo-naïve, non-smokers, adenocarcinoma	313	OS	Gefitinib	53.5 (*P*=0.15)	6.1 mo 0.81 (0.64-1.03) (*P*=0.044)	21.3 mo 1.03 (0.76-1.40) (*P*=0.43)
			Gemcitabine/cisplatin	46.3	6.6 mo	23.3 mo
		Subgroups	Gefitinib mutation+ve		7.9 mo 0.39 (0.21-0.71) (*P*=0.009)	
			Gefitinib mutation –ve		2.1 mo	
WJTOG3405[51] Japanese, chemo-naïve, EGFR mutation positive	177	PFS	Gefitinib	62.1 (*P*<0.0001)	9.2 mo 0.48 (0.34-0.71) (*P*<0.0001)	30.9 mo
			Docetaxel/cisplatin	32.2	6.3 mo	Not reached
NEJ002[52] Japanese, chemo-naïve, EGFR mutation positive	200	PFS	Gefitinib	73.7 (*P*<0.001)	10.8 mo 0.30 (0.22-0.41) (*P*<0.001)	30.5 mo (*P*=0.31)
			Paclitaxel/carboplatin	30.7	5.4 mo	23.6 mo
OPTIMAL[53] Chinese, chemo-naïve, EGFR mutation positive	154	PFS	Erlotinib	83% (*P*<0.0001)	13.1 mo 0.16 (0.10-0.26) (*P*<0.0001)	Results not mature at time of publication
			Gemcitabine/carboplatin	36%	4.6 mo	
EURTAC[54] Caucasian, chemo-naïve, EGFR mutation positive	174	PFS	Erlotinib	54.4 (*P*<0.0001)	9.4 mo 0.42 (*P*<0.0001)	22.9 mo 0.8 (*P*=0.42)
			Platinum-based regimen	10.5	5.2 mo	18.8 mo
LUX-lung 3[64] chemo-naïve, EGFR mutation positive	345	PFS	Afatinib	50.4 (*P*<0.001)	11.1 mo 0.58 (*P*=0.001)	16.6 mo 1.12 (*P*=0.6)
			Pemetrexed/cisplatin	19.1	6.9 mo	14.8
Maintenance						
SATURN[55]	889	PFS	Erlotinib	11.9 (*P*=0.0006)	12.3 weeks 0.71 (0.62-0.82) (*P*<0.0001)	12 mo 0.81 (0.70-0.95) (*P*=0.0088)
			Placebo	5.4	11.1 weeks	11 mo
ATLAS[56]	768	PFS	Bev+erlotinib	NA	4.76 mo 0.72 (0.59-0.88) (P=0.0012)	NA
			Bev+placebo		3.75 mo	
BeTa[63]	636	OS	Erlotinib+Bev	13	3.4 0.62 (0.52-0.75)	9.3 0.97 (0.80-1.18) (*P*=0.76)
			Erlotinib+placebo	6	1.5	9.2
WJTOG0203[57] Superior OS in gefitinib arm in adenocarcinoma	598	OS	Chemo 3 cycles+gefitinib until PD	34.2	4.6 mo 0.68 (0.57-0.80) (*P*<0.001)	13.7 mo 0.86 (0.72-1.03) (*P*=0.31)
			Chemo 6 cycles	29.3	4.3 mo	12.9 mo
2nd and 3rd-line						
BR21[58]	731	OS	Erlotinib	8.9 (*P*<0.001)	2.2 mo 0.61 (0.51-0.74) (*P*<0.0001)	6.7 mo 0.73 (0.58-0.85) (*P*<0.001)

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Table 2: Contd...

Trials	*N*	Primary endpoint	Treatment	ORR (%) *P* value	Median PFS (mo) HR (95% CI) (*P* value)	Median OS (mo) HR (95% CI) (*P* value)
			Placebo	<1	1.8 mo	4.7 mo
ISEL[59] OS advantage in non-smokers and Asians	1,692	OS	Gefitinib	8 (*P*<0.0001)	3 mo* (*P*=0.0006)	5.6 mo 0.89 (0.77-1.02) (*P*=0.087)
			Placebo	1.3	2.6 mo	5.1 mo
INTEREST[60] Non-inferiority trial	1,466	OS	Gefitinib	9.1 (*P*=0.33)	2.2 mo 1.04 (0.93-1.18) (*P*=0.47)	7.6 mo 1.02 (0.9-0.15)
			Docetaxel	7.6	2.7 mo	8 mo
TAILOR[62] EGFR WT population	218	OS	Docetaxel	NA	0.7 (0.53-0.94) (*P*=0.02)	NA
			Erlotinib		Docetaxel is superior over erlotinib	
EGFR antibody						
FLEX[61]	1,125	OS	Cetuximab+vinorelbine+cisplatin	36 (*P*=0.01)	4.8 mo	11.3 mo 0.87 (0.76-0.99) (*P*=0.04)
			Vinorelbine+cisplatin	29	4.8 mo	10 mo
BMS099[66]	676	PFS	Cetuximab+taxane*+carboplatin	25.7% (*P*=0.0066)	4.4 mo 0.902 (0.761-1.069) (*P*=0.2358)	9.69 mo 0.89 (0.754-1.051) (*P*=0.1685)
			Taxane*+carboplatin	17.2%	4.24 mo	8.38 mo

NSLCC: Non-small cell lung cancer; EGFR: Epidermal growth factor receptor; TKI: Tyrosine kinase inhibitor; IPASS: Iressa Pan-Asia Study; WJTOG: 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 NCT00528281	EGFR, HER2 Reversible TKI	Single arm in combination with pemetrexed
Neratinib (HKI272)	Phase II NCT00266877	EGFR, HER2 Irreversible TKI	Single agent, 3 arms treatment based on EGFR mutation status
Afatinib (BIBW2992)	Phase I, II and III NCT01553942 NCT01746251 NCT01647711 NCT01853826	EGFR, HER2, HER4 Irreversible TKI	Single agent and combination with either chemotherapy, radiation or other targeted agents in stage IV or adjuvant setting Intermittent or pulse high-dose therapy
Icotinib (BPI-2009H)	Phase II and III NCT01690390 NCT01707329 NCT01516983 NCT01719536/(Convince)	EGFR	Single agent and combination with either chemotherapy, radiation or other targeted agents
Dacomitinib (PF00299804)	Phase III NCT01774721/(Archer 1050) NCT01360554/(ARCHER 1009)	EGFR, HER2, HER4 Irreversible TKI	Single agent in comparison with gefitinib or erlotinib as treatment of advanced NSCLC
Poziotinib (HM781-36B)	Phase II NCT01819428 NCT01718847	EGFR, HER2, HER4 and TEC family irreversible TKI	Single arm, 1st line monotherapy in EGFR mutation lung adenocarcinoma Single arm, 2nd line monotherapy in lung adenocarcinoma acquired resistance to prior EGFR TKIs
CO-1686	Phase I/II NCT01526928	EGFR T790M mutant	Single agent in previously treated mutant EGFR NSCLC
AP26113	Phase I/II NCT01449461	Dual reversible ALK/ EGFR inhibitor	Single agent in NSCLC/other cancers with ALK gene rearrangement or mutated EGFR

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Table 3: Contd...

Targeted agents	Current phase of development	Targets	Designs
AZD9291	Phase I NCT01802632	Pan-HER inhibitor	Single agent in NSCLC patients with acquired resistance to EGFR TKI
MAb			
MM-121 (SAR256212)	Phase I, II NCT00994123 NCT01436565	Fully human HER3 mAb	Combination with erlotinib or PI3K inhibitor
Panitumumab (ABX-EGF mAb)	Phase II NCT01038037	EGFR Human IgG2 mAb	Combination with chemotherapy
Necitumumab (IMC-11F8)	Phase II, III NCT01763788 NCT00981058/(SQUIRE) NCT00982111/(INSPIRE)	EGFR Human IgG2 mAb	Combination with platinum-doublet chemotherapy
Pertuzumab (rhuMAb2C4)	Phase II NCT00063154 NCT00855894	HER dimerization inhibitor Humanized murine mAb	Single agent Single arm in combination with erlonib
Nimotuzumab (h-R3)	Phase II NCT01498562 NCT01393080	EGFR Humanized mAb	Combination with gefitinib Combination with liposomal paclitaxel and carboplatin
Matuzumab (EMD72000)	Phase II NCT00111839	EGFR Humanized mAb	Combination with pemetrexed

TKI: Tyrosine kinase inhibitor; NSCLC: Non-small cell lung cancer; EGFR: Epidermal growth factor receptor; HER: Human epidermal growth factor receptors; mAb: Monoclonal antibody; IgG: Immunoglobulin G; ALK: Anaplastic lymphoma kinase; EGF: Epidermal growth factor;

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.[67] 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[68] 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*.[69-71] MAPK-independent pathways on the other hand involve either acquired phosphoinositide-3-kinase, catalytic, alpha polypeptide (*PIK3CA*) mutations,[71] amplification of mesenchymal-epithelial transition (*MET*) proto-oncogene, which provides a bypass avenue through transactivation of HER3/PI3K signaling,[24,72] or by impairment of cell death mechanisms as seen with certain germline polymorphic variants of the proapoptotic molecule pro-apoptotic BCL-2-interacting mediator (BIM) (pro-apoptotic Bcl-2 family member).[73] 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*[74-76] as well as phenotypic transformation to small cell histology.[22]

De novo mutations in *HER2* occur in 2-4% of NSCLC, up to approximately 10% in adenocarcinomas.[23,77] 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*.[78,79] 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,[80] 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.[78] In general, *HER2* mutations are mutually exclusive with *EGFR* mutations[80] though co-existence of both *EGFR* and *HER2* mutations simultaneously had been described in the literature.[25] 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.[26,81] 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

and HER2). These agents have been or are being evaluated in NSCLC-specific clinical trials [Table 3]. Afatinib and dacomitinib, which are the furthest in clinical development, have demonstrated superior PFS compared with either chemotherapy or erlotinib, but no differences in overall survival (OS) in the reported randomized Phase II/III studies to date.[64,82-84] Further development of neratinib in NSCLC is unlikely given its low clinical activity due to dosing limitations arising from diarrhea-related toxicities.[85] Pelitinib (EKB-569) and canertinib (CI-1033) have also been discontinued from further clinical development. Based on the clinical data of achievable plasma concentrations of continuous daily dosing of neratinib, dacomitinib and afatinib, it is thought that primary resistance to these agents will still be encountered for the *EGFR* T790M mutation as well as exon 20 insertions[86] and clinical trial results thus far with the second-generation EGFR TKIs had generally supported this prediction with few exceptions (see below). Thus, an alternate schedule of drug administration, such as intermittent or pulse high-dose therapy using afatinib to determine its activity against T790M (NCT01647711), is under investigation. Intermittent high-dose schedule to attain higher central nervous system (CNS) penetration has demonstrated some efficacy in treating disease progression confined to the CNS wherein, the pathophysiology is different from acquired resistance in extracranial sites of malignancy.[87]

Clinical antitumor responses to EGFR TKIs in cases with *EGFR* exon 20 insertion, such as A763_Y764insFQEA, have been reported.[88-90] 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.[88] Exon 20 mutations in *HER2* in contrast tend to be more homogeneous and located in the most proximal region between codons 775 and 881.[78] Clinical responses to HER2-targeted antibody and second-generation EGFR TKI therapies (specifically afatinib) have been documented for NSCLC with *HER2* mutations.[25,91,92]

Third-generation EGFR inhibitors designed to inhibit the *EGFR* T790M mutant include WZ4002, CO-1686 and AZD9291.[93,94] 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.[95] 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.[69] 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.[96,97] 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.[98] Early phase data of AP26113 suggested a preliminary hint of clinical activity, though relatively modest, in patients with resistance to other EGFR TKIs.[99]

A different approach in addressing EGFR TKI resistance involves the use of combination regimens. Therefore, the combination of erlotinib with cetuximab.[100] and 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,[101] 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.[102] 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.[103] The *ALK* translocation (t[2;5][p23;q35]) was originally found in a subset of anaplastic large cell lymphomas in 1994.[104] 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.[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].[17] *EML4-ALK* translocation is found in 3-6% of all cases of NSCLC.[18,106] It is more frequent in younger patients, men and never-smokers/light smokers with NSCLC.[18,106] *EML4-ALK* translocation is mutually exclusive with *EGFR* or *KRAS* mutations in the ALK inhibitor-naïve population.[106] 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*).[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.[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, $P < 0.0001$).[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[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.[6] 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 $p130^{Cas}$ 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.[134,135] 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.[136] 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-KIAA1967* and *FGFR3-TACC3*.[21] 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)	NCRN 396 Phase II	Single agent in patients with BRAFV600 mutation-positive cancers (excluding melanoma and papillary thyroid cancers)
	NCT01531361 Phase I	Combination with sorafenib for patients with BRAF mutations relapsed/ refractory to standard therapy
LGX818	NCT01543698 Phase I/II	Combined with MEK162 in patients with BRAFV600-dependent advanced solid tumors
RO5212054 (PLX3603)	NCT01143753 Phase I	Single agent in patients with BRAFV600-mutated 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		
Trametenib (GSK1120212)	NCT01192165 Phase I	Trametenib in combination with docetaxel; erlotinib; pemetrexed; pemetrexed+carboplatin; pemetrexed+cisplatin; or nab-paclitaxel in advanced solid tumors
	NCT01155453 Phase I	Open-label study of BKM120 in combination with trametenib 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 (2^{nd} 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 2^{nd} line treatment
	NCT00890825 Phase II	Combination with docetaxel, compared with docetaxel alone, in 2^{nd} 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		
BVD523	NCT01781429 Phase I/II	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 PF00299804 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 I/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

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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	Metronomic oral vinorelbine in combination with sorafenib
	NCT00609804 Phase II	Sorafenib and erlotinib or sorafenib alone in patients progressing on erlotinib
	NCT00863746 Phase III	Sorafenib versus placebo in relapsed advanced non-squamous NSCLC) after 2-3 previous treatment
Dovitinib (TKI258)	NCT01676714 Phase II	Single agent in advanced lung cancer and colorectal cancer
	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 II/III	Single agents in advanced squamous cell lung cancers with FGFR kinase alterations
	NCT01813734 Phase II	Single agent in advanced NSCLC harboring RET translocations
Nintedanib (BIBF1120)	NCT01346540 Phase I/II	Continuous BIBF1120 added to standard gemcitabine/cisplatin in 1st line squamous cell lung
Sunitinib (SU11248)	NCT00698815 Phase II	Pemetrexed or sunitinib or pemetrexed/sunitinib in 2nd line treatment
	NCT00693992 Phase III	As maintenance therapy in non-progressing patients after 4 cycles of platinum-based combination chemotherapy
	NCT01829217 Phase II	Adenocarcinoma lung cancers in in ever smokers (EGFR/KRAS/ALK wild type) or adenocarcinomas that have a mutation in the RET gene
Pazopanib (GW786034)	NCT01027598 Phase II	Erlotinib and pazopanib or erlotinib and placebo in patients with previously treated NSCLC
Cediranib (AZD2171)	NCT00795340 Phase III	Cediranib (20 mg) versus placebo in patients receiving paclitaxel/carboplatin
Vargatef (BIBF1120)	NCT00805194 Phase III	Oral BIBF 1120 plus docetaxel versus placebo plus docetaxel in patients after failure of 1st line chemotherapy
	NCT00806819 Phase III	BIBF 1120 plus pemetrexed versus pemetrexed/placebo in patients after failure of 1st line chemotherapy
Vandetanib (ZD6474)	NCT01823068 Phase II	Single agent in NSCLC patients with RET rearrangement
	NCT01582191 Phase I	Combination with everolimus
Cabozantinib (XL184)	NCT01639508 Phase II	Patients with KIF5B/RET positive advanced NSCLC
XL647 (PRIM-001, KD019)	NCT01487174 Phase III	KD019 versus erlotinib in pretreated, EGFR TKI-naive NSCLC patients
Dasatinib (BMS-354825)	NCT00787267 Phase II	Dasatinib in previously treated advanced NSCLC
	NCT01514864 Phase II	Dasatinib in pretreated NSCLC patients with harboring a DDR2 mutation or an inactivating BRAF mutation
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	Single agent in patients with c-MET dependent advanced solid tumors
	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-03241066 inhibition, e.g. ALK, MET and ROS
Cabozantinib (XL184)	NCT01639508 Phase II	Patients with KIF5B/RET positive advanced NSCLC
	NCT01708954 Phase II	Erlotinib, cabozantinib, or erlotinib/cabozantinib as 2nd or 3rd line therapy in EGFR wild-type NSCLC
Tivantinib (ARQ197)	NCT01377376, NCT01244191	Tivantinib/erlotinib versus placebo/erlotinib in previously treated with advanced non-squamous NSCLC (no EGFR mutation)

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Targeted agents	Current phase of development	Design
	phase III	
	NCT01580735 Phase II	Single-arm tivantinib/erlotinib in EGFR mutation-positive NSCLC
	NCT01395758 Phase II	Tivantinib/erlotinib versus single agent chemotherapy in previously treated KRAS mutation positive NSCLC
Foretinib (GSK1363089)	NCT01068587 Phase I/II	Foretinib/erlotinib in relapsed NSCLC with *EGFR* mutant or *EGFR* unknown. Previously known as XL880
Volitinib (HMPL504)	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 Ib/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 I/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 I/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 I/II	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
PI3K inhibitors		
PX866	NCT01204099 Phase I/II	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 I/II	Erlotinib and BKM120 in pataients 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

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Targeted agents	Current phase of development	Design
AKT inhibitors		
MK2206	NCT01147211 Phase I	MK-2206 combined with gefitinib in NSCLC population enriched with EGFR mutation
	NCT01294306 Phase II	Combination of MK2206/erlotinib in advanced NSCLC patients pretreated with erlotinib
GDC0068	NCT01362374 Phase I	Combined with either docetaxel or fluoropyrimidine plus oxaliplatin in advanced solid tumors
AZD5363	NCT01226316 Phase I	Single agent in solid tumors bearing either AKT1 or PIK3CA mutation
Insulin-like growth factor pathway		
Cixutumumab (IMC-A12)	NCT00955305 Phase II	Paclitaxel, carboplatin, bevacizumab with or without cixutumumab in non-squamous histology
	NCT01263782 Phase II	Biomarker-integrated study in advanced NSCLC as front line setting
Dalotuzumab (MK0646)	NCT00799240 Phase II	Combined with pemetrexed/cisplatin in non-squamous type
Ganitumab (AMG479)	NCT01122199 Phase I	Combined with RAD001 in advanced solid tumors
	NCT01708161 Phase I/II	Combined with BYL719 in patients with PIK3CA mutated or amplified advanced solid tumors
BIIB022	NCT00970580 Phase I	Combination with paclitaxel/carboplatin advanced NSCLC
Linsitinib (OSI906)	NCT01221077 Phase II	Erlotinib in combination linsitinib in chemonaive patients with EGFR mutation
	NCT01186861 Phase II	Maintenance linsitinib plus erlotinib in patients with non-progression Following after 1st line chemotherapy
AXL1717	NCT01561456 Phase II	AXL1717 compared to docetaxel in previously treated patients in advanced NSCLC
Heat shock protein 90 (HSP90) inhibitors		
Ganetespib (STA9090)	NCT01562015 Phase II	Single arm ganetespib in subjects ALK-positive NSCLC
	NCT01579994 Phase I/II	Crizotinib and ganetespib in ALK positive lung cancers
	NCT01348126 Phase II/III	Combination with docetaxel versus docetaxel alone advanced NSCLC
Retaspimycin (IPI504)	NCT01427946 Phase I/II	Combination with everolimus in KRAS mutant NSCLC
AUY922	NCT01259089 Phase I/II	Adenocarcinoma with acquired resistance to EGFR TKIs
	NCT01752400 Phase II	ALK-rearranged advanced NSCLC with acquired resistance to prior ALK TKIs
	NCT01646125 Phase II	AUY922 versus pemetrexed or docetaxel in *EGFR* mutations and progressed on prior EGFR TKIs
AT13387	NCT01712217 Phase I/II	Alone and in combination with crizotinib NSCLC
DS-2248	NCT01288430 Phase I	Single agent in advanced solid tumors
Selective FGFR inhibitors		
JNJ-42756493	NCT01703481 Phase I	Single agent, with expansion cohort in KRAS wild-type tumors with FGFR 1, 2 or 4 gene amplifications
BGJ398	NCT01004224 Phase I	Single agent in advanced solid tumors with FGFR1 or FGFR2 amplification or FGFR3 mutation
AZD4547	NCT01795768 Phase II	Patients with FGFR1 or FGFR2 amplified in Squamous cell lung cancer, gastric, esophageal and breast cancer
	NCT01824901 Phase I/II	Docetaxel with or without AZD4547 in recurrent FGFRI-amplified squamous NSCLC
Tropomyosin-related kinase inhibitor		

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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

NSLCC: 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: Endothelial 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; CSFR1: Colony stimulating factor receptor1

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, brivanib, 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, CSFR1, 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 (NCT01824901).

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.[5] 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.[34] Preclinical models in NSCLC cell lines with focal high level-amplitude gains in this chromosomal segment implicate the potential oncogenic role of *PDGFR* 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.[34] Various nonsynonymous mutations in *PDGFR* and *KIT* are reported in approximately 4-2% of NSCLC, respectively.[150] 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 *PDGFR* 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.[151,152]

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].[153] Activated RAS/RAF/MEK/ERK pathway regulates cell growth, differentiation and apoptosis by interacting with multiple effectors.[27] 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.[154] 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.[27] *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.[155]

NRAS mutations, in comparison, are present in <1% of lung cancers, primarily in adenocarcinomas.[13] 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.[6] *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.[14] 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.[156] Furthermore, unlike *EGFR* and *HER2* mutations, several non-V600 *BRAF* mutations found in NSCLC are inactivating (e.g., *D594G, G466V, Y472*).[29,156,157] 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.[157,158] 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).[157] 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.[159]

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.[160] Preclinical models have shown that MEK inhibitors can induce significant tumor regressions in KRAS - or BRAF-induced lung tumors.[161-163] Splice site mutations that lead to higher expression of the active RAC1b isoform, a G-protein that promotes KRAS-induced lung tumorigenesis,[164] may be potentially associated with sensitivity to MEK inhibition.[165] However, the kinase-impaired D594G or G469E *BRAF* mutations are known to be highly resistant to MEK inhibitors in melanoma cell lines.[29] In addition, resistance to MEK inhibition is thought to arise potentially through emergence of secondary mutations in *MEK*,[166,167] either in conjunction

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.[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.[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.[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.[171,28] Similarly, the addition of selumetinib, a MEK inhibitor, markedly improved the response of *KRAS* mutant tumors to docetaxel.[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.[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[174] and myeloproliferative disorders[175] but is only effective against a subset of NF-1 deficient GBM (glioblastoma multiforme).[176] In NF1-deficient GBM resistant to MEK inhibition alone, combination therapy with a dual PI3K/mTOR inhibitor overcomes this treatment resistance.[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.[177]

Several potent and selective MEK inhibitors such as selumetinib (AZD6244) and trametenib (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.[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.[179] Similarly, the dual PI3K/ERK inhibitor AEZS-132 demonstrated significant activity in several xenograft models.[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.[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.[158,184,185] 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.[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 are 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*)[187-192] or alternate MAPK-independent pathway signaling, e.g., activating PI3K/mTOR/AKT pathway

through *PIK3CA* mutations.[193] 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.[32] In the case of NSCLC, acquired resistance to dabrafenib through the emergence of *KRAS* mutation has been recently described in a case report.[183]

MET/hepatocyte growth factor pathway

MET factor receptor or HGF receptor triggers key intracellular signaling cascades, such as Src, STAT3, PIK3/AKT/mTOR and RAS/RAF/MAPK, upon binding to its ligand HGF [Figure 1].[194] 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.[30] *MET* amplification occurs in 1-5% of unselected early-stage NSCLC cases, which have been associated with poor prognosis.[30] Engelman *et al*. reported that 22% of lung cancers with acquired resistance to EGFR TKIs had *MET* amplification, driving HER3-dependent activation of PI3K.[72]

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.[195] 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.[196]

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.[197] The combination of crizotinib with the pan-ErbB inhibitor dacomitinib is being evaluated in an ongoing Phase I study (NCT01121575).[198] 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.[108] A PR with cabozantinib monotherapy was also recently reported in a patient with *ALK* fusion-positive tumor,[199] 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.[200] 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.[31]

PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR signaling pathway was first identified in the 1990s.[201] 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].[202] 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.[203] Furthermore, loss of PTEN with subsequent pAKT overexpression is associated with poor prognosis.[204] 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.[37,38] Epigenetic silencing may partially explain PTEN loss in some cases as mutations or homozygous deletions of PTEN gene are rare in NSCLC.[39]

Mutation and amplification of PIK3CA, the gene that encodes the catalytic p110 subunit α isoform of PI3K principally involved in regulating cell proliferation and growth, is found in up to10% and up to 45% of patients with NSCLC respectively and is associated with increased PI3K activity and AKT expression.[35,36] 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.[205] 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.[205-207] Treatment resistance to PI3K inhibitor monotherapy is anticipated and documented in preclinical models with this dual activated PI3K/MAPK genotypic profile.[28,208] 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.[12,205,209] 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%.[210] This mutation is associated with increased membrane localization and autophosphorylation of AKT1.[211] 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.[212]

Various PI3K inhibitors, which include isoform-specific, pan-class I or dual PI3K/mTORC inhibitors, are in clinical development. Despite the broad similarities of p110 α 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.[213] 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.[214] 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.[215] 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).[216]

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.[217] In comparison, the combination of the MEK inhibitor GDC-0973 with the pan-PI3K inhibitor GDC-0941[218] can be safely combined to date. Similarly, the combination of trametinib with the PI3K inhibitor BKM120 appears to be tolerable as well.[219] 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 malignances). 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.[118,220] Indeed, early clinical data suggest that higher doses can be tolerated with intermittent dosing compared with the continuous schedule.[218] 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.[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 *KRAS G12F* mutation. However, overall RR in the *KRAS* mutant patients was low at 13%.[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.[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.[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.[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.[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.[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.[228] Nonetheless, encouraging clinical responses in NSCLC patients with *ALK* rearrangements have been documented in various early phase studies across several HSP90 inhibitors.[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.[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.[229] HSP90 inhibitors in development currently include the water water-soluble 17-AAG hydroquinone retaspimycin (IPI504), 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 *et al* in 2007.[230] The estimated incidence is approximately 2% of lung adenocarcinoma (both Asian and non-Asian populations) and the patient characteristics are similar to *ALK* and *EGFR*-mutation positive patients (non-smokers, adenocarcinoma histology).[4] 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.[231] ROS1 is one of the RTKs, which consist of an extracellular ligand-binding domain, a short transmembrane domain and intracellular TK domain.[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.[233,234] This led to the hypothesis that ALK inhibitors could act as ROS1 inhibitors which was subsequently confirmed in various preclinical studies.[234,235] These rearrangements are also client proteins of the HSP pathway and thus similarly sensitive to treatment with HSP90 inhibitors.[120] Aberrant ROS1 kinase activity leads to downstream activation of PI3K/AKT/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.[236] Currently, 12 *ROS1* fusion variants in NSCLC have been identified.[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.[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.[42,238] 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.[238] 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.[42,238,239] 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 hematopoiesis 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 multikinase 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.[150] 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 anticancer 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.[253] 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.[254-256] The major role of PD1 is to limit the autoimmunity and the inflammatory response in peripheral tissues by restricting T-cell activity.[253] Blockade of the interaction between PD-1 and PD-L1 potentiates immune responses *in vitro* and *in vivo* antitumor activity.[257,258] 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.[259] 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.[260] 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).[261,262] 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 genotypically-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

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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