Lung Cancer 103 (2017) 27–37

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Review

The race to target *MET* exon 14 skipping alterations in non-small cell lung cancer: The Why, the How, the Who, the Unknown, and the Inevitable

Thanyanan Reungwetwattana[a], Ying Liang[b], Viola Zhu[c,d], Sai-Hong Ignatius Ou[d,*]

[a] *Division of Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand*
[b] *Department of Medical Oncology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou 510060, China*
[c] *Long Beach Veterans Administration Hospital, Long Beach, CA 90822, USA*
[d] *Chao Family Comprehensive Cancer Center, Department of Medicine, Division of Hematology-Oncology, University of California Irvine School of Medicine, Orange County, CA 92868, USA*

ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
11 November 2016
Accepted 13 November 2016

Keywords:
MET exon 14 skipping
Lung cancer
Tyrosine kinase inhibitor
Targeted therapy
Clinical trial
Companion diagnostics

ABSTRACT

A number of small molecule tyrosine kinase inhibitors (TKIs) have now been approved for the treatment of non-small cell lung cancers (NSCLC), including those targeted against epidermal growth factor receptor, anaplastic lymphoma kinase, and ROS1. Despite a wealth of agents developed to target the receptor tyrosine kinase, MET, clinical outcomes have as yet been disappointing, leading to pessimism about the role of MET in the pathogenesis of NSCLC. However, in recent years, there has been a renewed interest in *MET* exon 14 alterations as potential drivers of lung cancer.

MET exon 14 alterations, which result in increased MET protein levels due to disrupted ubiquitin-mediated degradation, occur at a prevalence of around 3% in adenocarcinomas and around 2% in other lung neoplasms, making them attractive targets for the treatment of lung cancer. At least five MET-targeted TKIs, including crizotinib, cabozantinib, capmatinib, tepotinib, and glesatinib, are being investigated clinically for patients with *MET* exon 14 altered-NSCLC. A further two compounds have shown activity in preclinical models. In this article, we review the current clinical and preclinical data available for these TKIs, along with a number of other potential therapeutic options, including antibodies and immunotherapy. A number of questions remain unanswered regarding the future of MET TKIs, but unfortunately, the development of resistance to targeted therapies is inevitable. Resistance is expected to arise as a result of receptor tyrosine kinase mutation or from upregulation of MET ligand expression; potential strategies to overcome resistance are proposed.

Contents

Abbreviation: ALK, anaplastic lymphoma kinase; CNS, central nervous system; EGFR, epidermal growth factor receptor; FDA, food and drug administration; GCN, gene copy number; HGF, hepatocyte growth factor; IC_{50}, half inhibitory concentration; IHC, immunohistochemistry; mAb, monoclonal antibody; *MET*ex14, *MET* exon 14; *MET*ex14+ NSCLC, *MET*ex14 altered NSCLC; NSCLC, non-small cell lung cancer; ORR, overall response rate; PD-1, programmed cell death protein 1; RTK, receptor tyrosine kinase; SqCC, squamous cell carcinoma; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden; VEGFR, vascular endothelial growth factor receptor.

* Corresponding author at: Chao Family Comprehensive Cancer Center, Division of Hematology–Medical Oncology, Department of Medicine, University of California Irvine Medical Center, 101 City Drive, Bldg 56, RT 81, Rm 241, Orange County, CA 92868–3298, USA.
E-mail address: ignatius.ou@uci.edu (S.-H.I. Ou).

http://dx.doi.org/10.1016/j.lungcan.2016.11.011

1. Introduction (the Why)

Orally available small molecule tyrosine kinase inhibitors (TKIs) have now been approved for epidermal growth factor receptor (*EGFR*)-mutated, anaplastic lymphoma kinase (*ALK*)-rearranged, and *ROS1*-rearranged non-small cell lung cancers (NSCLC), altering the treatment landscape of NSCLC [1]. Alterations (point mutations, amplifications, protein overexpression, and fusions) in another receptor tyrosine kinase (RTK), the hepatocyte growth factor (HGF) receptor (MET), have been identified in NSCLC, and a plethora of MET-targeted agents (small molecular TKIs and antibodies against HGF or MET) have been investigated in this disease type [2]. Disappointingly, despite the wide spectrum of MET alterations in NSCLC, randomized trials with MET inhibitors have not resulted in clinical benefit [3–5]. These disappointing results have led to pessimism about the role of MET in the pathogenesis of NSCLC and the validity of MET as a targetable driver in NSCLC. This review will concentrate on the recent re-emergence of *MET* exon 14 (*MET*ex14) splicing alterations in NSCLC that has led to renewed optimism of *MET*ex14 alteration as a targetable mutation that may lead to the approval of MET-specific inhibitors in NSCLC.

1.1. *METex14 splicing mutations in NSCLC*

The MET signaling pathway has recently been reviewed in detail [6]. The timeline of events leading to the recognition of *MET*ex14 alteration as an important driver in lung cancer is summarized in Fig. 1. In 1994, an alternatively spliced MET RTK with deletion of the 47-amino acid juxtamembrane region of the MET receptor was reported [7], followed by the demonstration that mutation of a tyrosine residue at position 1001 in this juxtamembrane region led to a partial gain of function [8]. In 2001, Peschard and colleagues reported that mutation of tyrosine residue 1003 in the binding domain of the E3 ubiquitin-protein ligase, c-CBL, abolished c-CBL binding to MET, disrupting c-CBL-mediated degradation and leading to MET oncogenic activity [9]. Y1003 is located in the juxtamembrane region of MET and is encoded by exon 14 [6]. Subsequently, mutations in the *MET*ex14 splice sites were reported by Ma and colleagues in small cell lung cancer in 2003 and NSCLC in 2005 [10,11]. The significance of these splice site mutations was further characterized by Kong-Beltran and colleagues in 2006, when they identified both single nucleotide substitutions and small deletions in the 5′ and 3′ splice sites around *MET*ex14 in lung tumor samples, and demonstrated that these mutations resulted in *MET*ex14 skipping. The exon 14-spliced protein had abolished c-CBL E3 ligase binding, resulting in decreased ubiquitination, and leading to a relative increase in MET protein levels. Additionally, MET Y1003 mutation was shown to result in decreased ubiquitination and increased stability of the MET protein. Both *MET*ex14-spliced and MET Y1003-mutated proteins were transforming *in vitro* and in a xenograft model that was inhibited by an anti-MET antibody [12]. Since then, sporadic case series have reported the incidence of *MET*ex14 alterations in NSCLC to be around 2–4% [13–15]. It was not until 2015 that large scale molecular profiling of *MET*ex14 alterations in 38,028 tumor samples by Frampton and colleagues led to renewed focus on *MET*ex14 alterations in lung carcinomas [16]. Among the 221 tumor samples harboring *MET*ex14 alterations, 193 were in lung carcinomas, including 131 lung adenocarcinoma samples. No other common solid tumor malignancies harbored *MET*ex14 alterations to the same degree as lung neoplasms [16]. Furthermore, in 2015, an *in vitro* model using the CRISPR/Cas9 system in HEK293 cell lines demonstrated that *MET*ex14 deletion resulted in higher MET protein expression levels, enhanced MET phosphorylation, prolonged MET activation, and enhanced cellular growth, colony formation, and MET inhibitor sensitivity [17]. Contemporaneously, case reports and case series have reported that patients with *MET*ex14 altered NSCLC (*MET*ex14+ NSCLC) respond to MET TKIs [18–22].

Since late 2015, reports characterizing patients with *MET*ex14+ NSCLC have been published in rapid succession in the literature (Table 1) [16,23–31]. To date, it can be summarized that *MET*ex14 alterations are found in a relatively elderly population of patients with NSCLC, and are enriched in sarcomatoid histologies, with a prevalence ranging from 8 to 22% [25,31]. On average, *MET*ex14 alterations occurred at a prevalence of about 3% in lung adenocarcinoma, and notably, at a prevalence of slightly higher than 2% in squamous cell carcinoma (SqCC) [31]. Available data on the overlap between *MET*ex14 alterations, *MET* amplification, and *MET* point mutations are sparse, but concurrent *MET* amplification has been reported in 15–21% of *MET*ex14+ NSCLC [24,26,31], and MET Y1003X mutations account for around 2% of the *MET*ex14 alterations in NSCLC [31]. Based on 28 patients, Awad and colleagues showed that Stage IV *MET*ex14–mutated NSCLCs were significantly more likely to have concurrent *MET* genomic amplification and

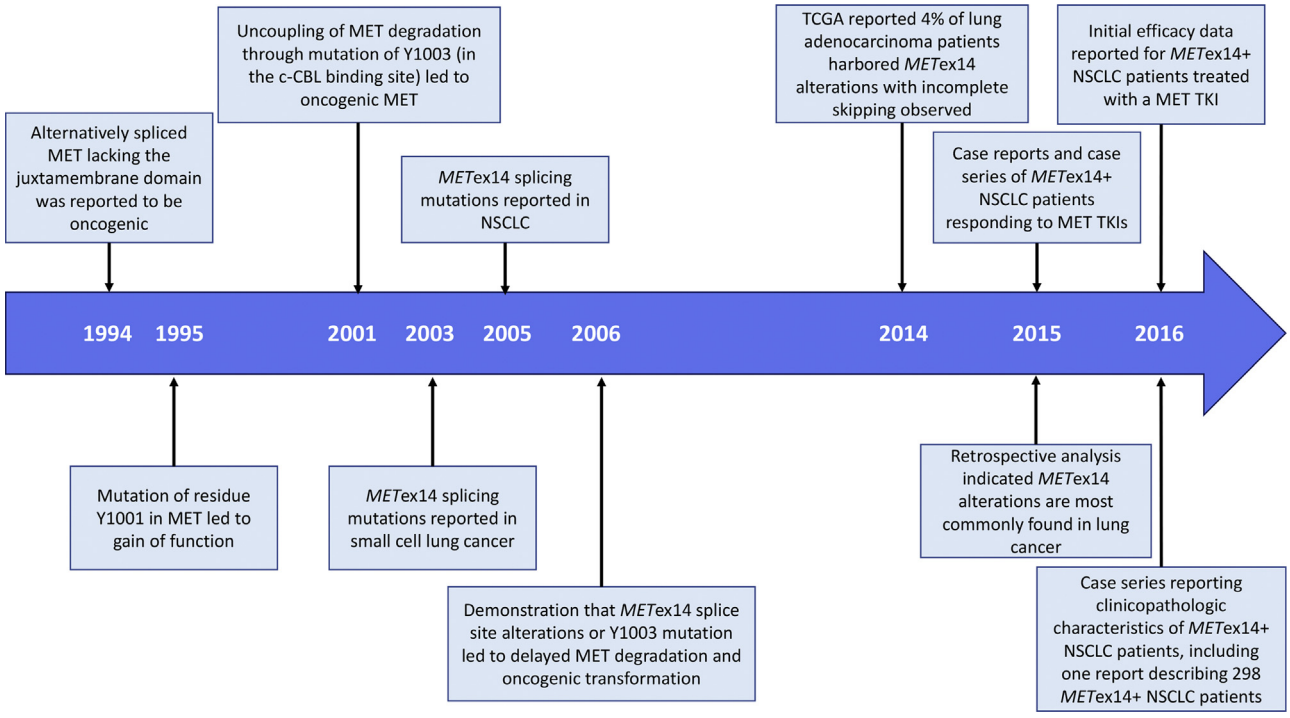

Fig. 1. Timeline of the emergence of *MET* exon14 skipping alterations in NSCLC.
*MET*ex14: *MET* exon 14; NSCLC: non-small cell lung cancer; TCGA: The Cancer Genome Atlas; TKI: tyrosine kinase inhibitor.

strong MET immunohistochemical expression than stage IA to IIIB *MET*ex14–mutated NSCLCs [24]. However, a much larger series of 298 *MET*ex14+ patients did not show the correlation between *MET* amplification and advanced stage [31].

2. Clinical trial design considerations of a MET TKI trial targeting *MET*ex14+ NSCLC (the How)

There are several MET TKIs in development that target *MET*ex14+ NSCLC, and completing accrual as soon as possible is of paramount importance given the relatively low incidence of *MET*ex14+ NSCLC. Eligibility criteria for MET TKI trials in *MET*ex14+ NSCLC should thus be tailored to allow speedy accrual. From the report of Schrock and colleagues, *MET*ex14 alterations occur in approximately 2% of patients with SqCC [31], and these patients have been shown to respond to MET TKIs [16,27,31]. Therefore, it is advised to include both major histologies (adenocarcinoma and SqCC) in addition to sarcomatoid lung cancer.

It is generally accepted that in NSCLC with a validated targetable driver mutation, highly effective targeted therapy should work regardless of prior lines of chemotherapy. For example, a phase 2 study of crizotinib in ROS1 inhibitor-naïve *ROS1+* NSCLC patients revealed no difference in the overall response rate (ORR) whether patients were treatment naïve or had received more than three prior chemotherapy regimens [32]. Thus, a clinical trial of MET TKIs in *MET*ex14+ NSCLC patients should allow any prior lines of therapy (chemotherapy or immunotherapy). Indeed, the recently presented phase 2 trial of crizotinib in *MET*ex14+ NSCLC patients allowed both treatment-naïve and chemotherapy-refractory patients [33].

Finally, a sensitive, reliable, and rapid molecular test that can be easily performed in diagnostics laboratories should be incorporated into MET TKI trials, for the purpose of getting a compendium of diagnostics for *MET*ex14 alterations approved. As shown in Table 1, most of the methods used to detect *MET*ex14 alterations, including MET Y1003 mutation, are based on DNA sequencing. Thus, the actual results of *MET*ex14 skipping are inferred from the substitutions and indels observed at the splice donor and acceptor sites. As demonstrated by The Cancer Genome Atlas (TCGA) analysis of adenocarcinoma, some of the *MET*ex14 alterations detected at the DNA level resulted in incomplete (80%) *MET*ex14 skipping [14]. Thus, confirmation of actual *MET*ex14 skipping by differential *MET* exon expression [15,21], quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) [24], or direct RNA sequencing [15,27] should be part of the test for *MET*ex14 alterations. Additionally, both DNA and RNA sequencing methods will be needed to detect Y1003, the "functional analog" of *MET*ex14 skipping alterations, which accounts for approximately 2% of all *MET*ex14 alterations [31].

3. MET TKI in *MET*ex14 skipping alterations in NSCLC (the Who)

Multiple MET inhibitors, including both small molecule TKIs and monoclonal antibodies (mAbs) against MET or its ligand, HGF, have been in clinical development since the early 2000 s [34]. TKIs can generally be divided into three types (I, II, and III) [35,36]. The binding of ATP to the MET kinase domain has been succinctly reviewed by Gherardi and colleagues [35]. The apo-MET kinase adopts a unique autoinhibitory conformation with the activation loop locked into the ATP triphosphate binding site *via* a salt bridge between D1228 and K1110. Type I MET-inhibitors are ATP-competitive, and bind to this MET unique autoinhibitory conformation with characteristic interaction (π-stacking) with Y1230 in the MET activation loop. Type I inhibitors can be further divided into two types: type Ia and type Ib. The potency of type Ia inhibitors comes from interaction with Y1230, the hinge, and the solvent front glycine residue G1163 (analogous to the same position as ALK G1202 and ROS1 G2032), whereas type Ib series have stronger interactions with Y1230 and the hinge, but normally no interaction with G1163. Type Ib inhibitors are highly specific for MET with fewer off target effects as compared with type Ia inhibitors.

Table 1
Studies reporting the clinicopathologic characteristics of *MET* exon 14 skipping alterations.

Study	Histology studied	Region	Diagnostic method	Concurrent *MET* amplification	Concurrent genomic alterations reported	Prevalence
Okuda et al, 2008 [76]	All NSCLC histologies	Nagoya, Japan	Sanger sequencing	No	Not reported	1.7% (3/178)
Onozato et al, 2009 [13]	All NSCLC histologies (211 adenocarcinoma)	Nagoya, Japan	RT-PCR followed by genomic sequencing	No	Not reported	3.3% (7/211) adenocarcinoma
Seo et al, 2012 [14]	Adenocarcinoma	Seoul, Republic of Korea	RNA sequencing (transcriptome sequencing)	No	Not reported	3.4% (3/87)
TCGA, 2014 [15]	Adenocarcinoma	USA	WES	Not tested	Not reported	4.3% (10/230)
Frampton et al, 2015 [16]	All histologies	USA	Hybrid capture NGS	Not reported	*MDM2* amplification; *CDK4* amplification	3% (131/4402) adenocarcinoma; 2.3% (62/2669) other lung histologies
Park et al, 2015 [23]	Adenocarcinoma	Seoul, Republic of Korea	RT-PCR followed by genomic DNA sequencing	No	Not reported	2.9% (2/70)
Awad et al, 2016 [24]	All NSCLC histologies	Boston, USA	NGS, confirmation by qRT-PCR	21%	46% *MDM2* amplification	3% (28/933) non-squamous NSCLC; 0% (0/132) squamous cell carcinoma
Liu et al, 2016 [25]	Pulmonary sarcomatoid carcinoma	New York, USA	WES, RT-PCR followed by confirmation by Sanger sequencing	Not tested	Not reported	22.2% (8/36)
Tong et al, 2016 [26]	All NSCLC histologies	Hong Kong, China	PCR-Sanger sequencing	33.3%	Not reported	2.6% (10/392) adenocarcinoma; 4.8% (1/21) adenosquamous cell; 31.8% (7/22) pulmonary sarcomatoid carcinoma; 0% (0/180) squamous carcinoma; 0% (0/45) large cell carcinoma
Heist et al, 2016 [27]	All histologies	Boston, USA	Anchored multiplex RNA sequencing, confirmed by DNA sequencing	1/16 with borderline *MET* amplification	Not reported	19% (10/54) of the enriched cohort (wild-type *EGFR*, *KRAS*, *BRAF*, *ERBB2*, *ALK*, and *ROS1*); 5.6% (5/89) of clinical samples
Saito et al, 2016 [28]	Adenocarcinoma	Tokyo, Japan	RNA sequencing*	No	Not reported	2.8% (9/319)
Zheng et al, 2016 [29]	All NSCLC histologies	Shanghai, China	qRT-PCR	Not tested	Not reported	1.6% (21/1305) adenocarcinoma; 4.2% (2/48) adenosquamous; 0% (0/417) squamous cell carcinoma
Liu et al, 2016 [30]	All NSCLC histologies	Guangdong, China	NGS, Sanger sequencing	No	Not reported	0.9% (10/1101) adenocarcinoma; 5% (1/20) squamous cell; 0.7% (1/136) adenosquamous cell carcinoma
Schrock et al, 2016 [31]	All histologies	USA	Hybrid capture NGS	14.8%	34.6% *MDM2* amplification; 21.1% *CDK4* amplification; 6.4% *EGFR* amplification	2.8% (205/7140) adenocarcinoma; 2.1% (25/1206) squamous cell carcinoma; 8.2% (8/98) adenosquamous carcinoma; 7.7% (8/107) sarcomatoid carcinoma; 3.0% (49/1659) NOS
Halmos et al, 2016 [99]	All NSCLC	USA	Hybrid capture based comprehensive genomic profiling	Not reported	Not reported	11.5% (16/139) lung sarcomatoid carcinoma; 2.8% other NSCLC

ALK: anaplastic lymphoma kinase; CDK4: cyclin-dependent kinase 4; EGFR: epidermal growth factor receptor; MDM2: Mouse double minute 2 homolog; NGS: next-generation sequencing; NOS: not otherwise specified; NSCLC: non-small cell lung cancer; qRT-PCR, quantitative RT-PCR; RT-PCR: real-time polymerase chain reaction; WES, whole-exome sequencing.

* Based on Sunami et al., 2016 [100].

Type II inhibitors are also ATP-competitive, but bind to the ATP adenine binding site and extend to the hydrophobic back pocket. As such, type II inhibitors generally distort the apo-MET autoinhibitory conformation and bind to an induced conformation with a certain penalty; potency depends on the activation state of the MET protein. Normally, there is no interaction with the solvent front residue G1163; thus, type II inhibitors could potentially rescue solvent front mutations. However, as type II inhibitors occupy the induced back

Table 2
Tyrosine kinase inhibitors targeting *MET* exon 14 skipping alterations.

Compound	Company	Targets	Type of inhibitor	Enzyme IC_{50}, nM	Cellular IC_{50} (cell line), nM	Clinicaltrials.gov NCT number/EuDraCT number
Crizotinib [39,83]	Pfizer	MET, ALK, ROS1	Type Ia	<1.0	8; 8.6 ± 2 (A549)	NCT00585195 (PROFILE-1001) NCT02465060 (NCI-MATCH) NCT02499614 (METROS) NCT02664935 (Matrix)
Capmatinib [47]	Novartis	MET	Type Ib	0.13	0.4 (H596);* 0.7 (A549)	NCT02750215 NCT01324479
Tepotinib [51]	Merck	MET	Type Ib	3	9 (EBC-1)	NCT02864992/2015-005696-24
Savolitinib [54,57]	AstraZeneca/Hutchison China Meditech	MET	Type Ib	5	4 (H1993)	NCT02897479
AMG337 [58]	Amgen/Nanbio	MET	Type Ib	1		No current clinical trials; *in vitro* activity against *MET*ex14+ gastric cell line (Hs746T)
Cabozantinib [61]	Elexis	MET, VEGFR2, RET, KIT, TIE-2, AXL	Type II	1.3	7.8 (PC3)	NCT01639508
Glesatinib [67]	Mirati Therapeutics	MET, VEGFR, RON, TIE-2	Type II	1	20 (MKN45)	NCT02544633
Merestinib [70]	Lilly	MET, TIE-1, AXL, ROS1, DDR1/2, FLT3, MERTK, RON, MKNK1/2	Type II	4.7	35 (H460); 52 (S114)	NCT02920996

ALK: anaplastic lymphoma kinase; DDR1/2: discoidin domain receptor tyrosine kinase 1/2; EGFR: epidermal growth factor receptor; FLT-3: FMS-like tyrosine kinase-3; IC_{50}: half inhibitory concentration; MERTK: MER receptor tyrosine kinase; MKNK1/2: MAP kinase-interacting serine/threonine-protein kinase 1/2; NSCLC: non-small cell lung cancer; VEGFR: vascular endothelial growth factor receptor.
* H596 is a NSCLC cell line that harbors *MET*ex14 deletions.

pocket with high energy, clinically, it is easy to develop resistance from a free energy point if MET is activated by HGF [37]. Type III inhibitors bind to allosteric sites distinct from the ATP binding site [35]; currently there are no type III inhibitors being tested in clinical trials for oncology. Type I and II MET TKIs in development in *MET*ex14+ NSCLC are shown in Table 2.

3.1. Type Ia inhibitors

3.1.1. Crizotinib (Xalkori, PF-02341066)

Crizotinib (PF-02341066, Xalkori; developed by Pfizer) is an orally available, ATP-competitive, type I inhibitor, with potent inhibitory activity against MET (half inhibitory concentration [IC_{50}], 8–11 nM), ALK (IC_{50}, 24–60 nM), and ROS1 (IC_{50}, 55 nM) [38–40]. In MET-dependent cancer cell lines, crizotinib inhibits autophosphorylation of MET, which in turn leads to inhibition of signal transduction and cell proliferation, and induction of apoptosis [38,41].

In August 2011, crizotinib was approved for the treatment of *ALK*-rearranged NSCLC [42], and in March 2016, was approved for treatment of *ROS1*-rearranged NSCLC by the US Food and Drug Administration (FDA) [43]. Crizotinib has also demonstrated clinical activity in NSCLC with *MET* amplification, especially among patients with high *MET* amplification, proving it is a bona fide MET inhibitor [40,44]. Preliminary results were recently presented from the PROFILE-1001 study in which 21 treatment-naïve or chemotherapy-refractory *MET*ex14+ NSCLC patients were enrolled, 18 of which were evaluable at the time of presentation. Within a relatively short 5.3 months of median follow-up time, the ORR was 44% [33]. Additionally, crizotinib is the TKI for the *MET*ex14 arms of two large phase 2 basket trials; the NCI-MATCH trial (NCT02465060) in the US and the National Lung Matrix trial (NCT02664935) in the UK, for patients with *MET*ex14+ solid malignancies and lung cancer respectively [45,46]. A phase 2 trial, the METROS study, is ongoing in pretreated patients with metastatic NSCLC with *MET* or *ROS* aberrations (Table 2) [45]. With the safety data of crizotinib firmly established, it is likely that crizotinib will be the first MET TKI to receive FDA approval for *MET*ex14+ NSCLC in the near future.

3.2. Type Ib inhibitors

3.2.1. Capmatinib (INC280)

Capmatinib (INC280; Novartis) is also an oral, ATP-competitive, type Ib MET inhibitor. It has extremely potent inhibitory activity against MET, with a cellular kinase IC_{50} of 0.13 nM, and a cell-based IC_{50} of 0.4–0.7 nM (Table 1) [47]. Capmatinib inhibits signaling pathways and cell proliferation, induces apoptosis in MET-dependent cell lines, and demonstrates single-agent antitumor activity in MET-driven mouse xenograft models [47]. The efficacy and safety of capmatinib as a single agent were investigated in a phase 1 study in patients with advanced solid tumors, including a cohort of *EGFR*-wild-type *MET*+ (mutated, amplified, or rearranged) NSCLC patients (N = 55). The drug was well tolerated, with no grade 3/4 adverse events occurring in over 10% of patients, and preliminary activity was observed in NSCLC patients with high *MET* gene copy number (GCN) ≥6 or MET overexpression as measured by immunohistochemistry (IHC 3 +), with an ORR of 47% and 24%, respectively [48]. Four *MET*ex14+ NSCLC patients were enrolled and all achieved significant reductions in tumor volume (>45%) [22,48]. A phase 2 trial of capmatinib that includes a study arm investigating its clinical efficacy in *MET*ex14+ NSCLC patients is currently ongoing (GEOMETRY *mono*-1) [45]. There is also an investigator-initiated phase 2 study of capmatinib in patients with *MET*ex14+ NSCLC who have received a prior MET inhibitor (Table 2) [45]. Furthermore, capmatinib has been investigated in combination with gefitinib in patients with *EGFR*-mutated NSCLC, and acquired *MET* amplification as a resistance mechanism to EGFR inhibitors; the ORR was 50% in patients with *MET* GCN ≥6 [49]. A phase 2 study (GEOME-

TRY duo-1) is ongoing for this clinical setting, in combination with erlotinib [50].

3.2.2. Tepotinib (MSC2156119J, EMD 1214063)

Tepotinib (EMD1214063, MSC2156119J; Merck) is another oral, ATP-competitive, and highly selective MET inhibitor [51]. Preclinical antitumor activity was observed in a number of murine xenograft models of human tumors, including in the ligand-independent, *MET*-amplified EBC-1 NSCLC model. Antitumor activity was also seen in other non-lung tumor models, regardless of whether MET activation was HGF dependent or independent [51].

The efficacy and safety of tepotinib was assessed in a phase 1 single-agent study in patients with solid tumors, including NSCLC. Tepotinib was well tolerated and showed antitumor activity, especially in patients with overexpressed or amplified MET [52]. A phase 2 trial investigating the activity of tepotinib in *MET*ex14+ NSCLC patients has been initiated (Table 2) [45,53]

3.2.3. Savolitinib (AZD6094, volitinib, HMPL-504)

Savolitinib (AZD6094, volitinib, HMPL-504; AstraZeneca) is an orally available inhibitor, with potent, selective activity against MET [54]. In preclinical studies, savolitinib inhibited phosphorylation of MET and downstream signaling, and had antitumor activity against a number of xenograft models, including those of *EGFR*- and *KRAS*-wild-type NSCLC [55]. The safety and efficacy of savolitinib as a single agent was investigated in a phase 1 study in patients with solid tumors, including NSCLC. The drug was well tolerated, and preliminary antitumor activity was observed [56]. Savolitinib has also demonstrated inhibitory activity against *MET*ex14 alterations and MET Y1003 mutations in cell lines with acquired resistance to savolitinib mediated by MYC overexpression [57]. A phase 2 trial of savolitinib in patients with *MET*ex14+ positive pulmonary sarcomatoid carcinoma has recently been initiated in China (Table 2) [45].

3.2.4. AMG-337

AMG337 (Amgen, Nanbio) is another highly selective, orally available ATP-competitive MET inhibitor [58]. In a phase 1/2 trial of esophageal, gastro-esophageal junction, and gastric carcinoma, AMG337 demonstrated an impressive ORR of 62% (8/13) in a subset of patients that harbored high *MET* amplification, indicating that it is a bonda fide oral MET inhibitor [59]. AMG337 has also been shown *in vitro* to inhibit migration, invasion, and anchorage-independent growth in gastric cell lines harboring *MET*ex14 deletion that have been stimulated by purified HGF [60]. Currently there are no clinical trials investigating the activity of AMG337 against *MET*ex14+ NSCLC [45,53].

3.3. Type II inhibitors

3.3.1. Cabozantinib (Cometriq, XL184)

Cabozantinib (XL184, Cometriq; Elexis) is an oral, type II MET inhibitor, effective against MET. Cabozantinib is multi-targeted, and exhibits activity against other kinases, including vascular endothelial growth factor receptor 2 (VEGFR2), KIT, RET, and AXL. Preclinical studies have demonstrated inhibition of both MET and VEGFR2 phosphorylation, and dose-dependent inhibition of tumor growth in a variety of mouse models, including lung [61].

Cabozantinib was approved for medullary thyroid cancer in 2012 [62]. Cabozantinib demonstrated modest single-agent activity in a phase 2 trial in patients with metastatic NSCLC (ORR 10%) [63]. In patients with *EGFR*-mutated NSCLC with resistance to EGFR TKIs, clinical activity was observed following treatment with cabozantinib and erlotinib; however, it should be noted that *MET* amplification was not detected in the study population [64]. A recent publication described a patient with lung adenocarcinoma with EGFR+/*MET* amplification who developed a new mutation in MET (D1228V) after progression on savolitinib and osimertinib, and subsequently responded to treatment with cabozantinib and erlotinib [65]. One case series reported a *MET*ex14+ NSCLC patient with concurrent *MET* amplification who achieved a complete response to cabozantinib [21]. Currently there is an investigator-initiated, single institution phase 2 study focusing on patients with *MET*-mutated NSCLC, among other actionable driver mutations, in NSCLC (Table 2) [45].

3.3.2. Glesatinib (MGCD265)

Glesatinib (MGCD265; Mirati Therapeutics) is a type II oral inhibitor, with activity against MET, VEGFR1/2/3, RON, and TIE-2. Preclinical antitumor activity was demonstrated in NSCLC xenograft models, including in an EGFR TKI-resistant model, when combined with erlotinib [66]. Subsequent studies in a gastric cancer xenograft model revealed that, in addition to the typically reported cellular activities, glesatinib in combination with erlotinib disrupted the glycolysis pathway, suggesting a novel mechanism of action for this drug [67].

In an ongoing phase 1 study in patients with *MET*+ or *AXL*-rearranged advanced solid tumors, glesatinib demonstrated preliminary single-agent activity, with all three patients with *MET* dysregulated NSCLC (two with *MET*ex14 alterations and one with increased GCN) showing significant tumor regression at the first assessment [68]. A phase 2 study is currently recruiting patients with *MET*-dysregulated (mutated or amplified) advanced or metastatic NSCLC (Table 2) [69].

3.3.3. Merestinib (LY2801653)

Merestinib (Lilly, Inc) is a multi-targeted TKI that can inhibit MET, RON, AXL, MER receptor tyrosine kinase (MERTK), TIE-2, TIE-1, ROS1, and discoidin domain receptor tyrosine kinase 1 (DDR1) [70]. The *in vitro* IC_{50} of merestinib against MET is 4.7 nM and the cell-based IC_{50} is 35–52 nM, depending on the cell lines utilized [70]. Treatment with merestinib inhibited the constitutive activation of MET signaling and resulted in inhibition of NCI-H441 cell proliferation, anchorage-independent growth, migration, and invasion. Additionally, merestinib inhibited orthopedic H441 primary tumor growth or metastasis and resulted in longer survival of mice bearing the H441 orthopedic transplant compared with vehicle injection [71]. In the H1993 NSCLC cell line, which harbors *MET* amplification and also overexpression of RON, merestinib was superior to crizotinib in terms of cellular growth inhibitory activity (9.28 nM versus 45.4 nM, respectively) [72].

A phase 2 study has recently been initiated that includes a cohort of patients with *MET*ex14-mutated NSCLC (Table 2) [45].

4. Clinicopathologic characteristics of *MET*ex14+ NSCLC patients (the Unknown)

Although there have been many reports on the clinicopathologic characteristics of *MET*ex14+ NSCLC patients (Table 1), much remains to be discovered. Firstly, the prevalence of brain metastasis at the time of diagnosis of *MET*ex14+ NSCLC patients is unknown. Even more importantly, the prevalence of central nervous system (CNS) progression from MET TKIs is unknown. If the natural history of *MET*ex14+ NSCLC disease mirrors that of *ALK*+ NSCLC [73], then progression in the CNS will be a significant therapeutic challenge to MET-targeted therapy, and the ability of MET TKIs to penetrate the CNS will be of paramount importance. Preliminary CNS activity of cabozantinib in a *MET*ex14+ NSCLC patient has been observed [74]. Secondly, while it is generally established that *MET* amplification is a poor prognostic factor in NSCLC [73,75–77], the prognostic significance of *MET*ex14 alterations in NSCLC is

unknown. Thirdly, Schrock and colleagues have demonstrated that tumors with *MET*ex14+ alterations with concurrent *MET* amplification harbored a significantly higher total mutational burden (TMB) [31]. It remains to be determined whether concurrent *MET* amplification modulates the response to MET TKIs, as this molecular alteration could affect the efficacy of MET TKIs if it is not properly accounted for. Fourthly, Schrock and colleagues also reported a high incidence of concurrent *MDM2* amplification [31]. *MDM2* encodes an E3 ubiquitin-protein ligase, and whether *MDM2* amplification is a response to the absence of an E3 ubiquitin-protein ligase binding site in the *MET*ex14 altered protein, or whether it has another role in *MET*ex14+ NSCLC, such as resistance to TKIs, remains to be investigated [78].

5. Potential strategies to overcome resistance to MET TKIs (the Inevitable)

Resistance to targeted therapy with a single agent TKI invariably occurs (Fig. 2). The tight binding of the MET TKIs to the ATP-pocket of MET RTK is facilitated by several hydrophobic interactions at several specific amino acids. *In vitro* mutagenesis assays with MET TKIs in MET-dependent tumors have identified several predominant resistance mutations against type I inhibitors (Y1230, D1228) [79,80] and several minor resistance mutations (F1200, V1155) [80]. Indeed, acquired crizotinib resistance mutations, MET D1228N and MET Y1230C, have recently been described in patients with *MET*ex14+ NSCLC refractory to crizotinib [81,82]. Residue D1228 is important to stabilize the orientation of the activation loop in the inactive autoinhibitory conformation of MET, and D1228N mutations have been shown to switch the turnover number (K_{cat} [s^{-1}]) of crizotinib from 0.27 ± 0.05 (inactivated MET [unphosphorylated]) to 11.3 ± 3.0 (activated [phosphorylated]) [83].

5.1. Switching from type I to type II inhibitors or vice versa for acquired resistance mutations

Given that type I inhibitors require stacking with Y1230 to bind to MET, mutations in Y1230 abolish the binding of type I MET TKIs. For example, the IC_{50} of AMG337 against wild-type MET is 1 nM, but the IC_{50s} against Y1230H and D1228H are 1077 nM and >4000 nM, respectively [58]. Switching from a type I MET TKI to type II MET TKI may overcome this group of resistance mutations; for example, the IC_{50s} of merestinib against wild-type Y1230C and D1228N MET are 42 nM, 54 nM, and 111 nM, respectively [70]. Clinical trials that allow switching from type I inhibitors (the predominant TKIs in clinical trial) to type II inhibitors will have to be conducted to thoroughly test this hypothesis, but data from one case study is supportive: a patient with lung adenocarcinoma and a D1228H point mutation responded to cabozantinib after progression on savolitinib [65]. Additionally, the ability to detect the acquired resistance mechanism will be important to ensure the success of these "non-cross resistant" trials.

5.2. "Total" MET signaling axis blockade

An important difference between RTK-rearranged NSCLC and *MET*ex14+ NSCLC is that the ligand binding domain of *MET*ex14+ NSCLC is intact, and ligand-mediated activation of the MET receptor protein is ongoing. Therefore, one potential mechanism of resistance is the upregulation of HGF, which can potentially increase the percentage of MET receptors in the active (phosphorylated) conformation, increasing the Michaelis constant (K_m, the concentration of substrate that permits the enzyme to achieve half the V_{max}, the maximum rate of the reaction) of ATP inhibitors, and essentially abolishing the binding of type II inhibitors to MET [37]. Thus, to overcome or delay the emergence of resistance to MET TKIs, it may be advisable to combine MET TKIs with antibodies against HGF, or antibodies that block ligand binding to or dimerization of the MET receptor. This is similar to the design rationale of the 4th generation EGFR TKIs [84].

5.2.1. Combination with antibodies against MET

5.2.1.1. Emibetuzumab (LY2875358). Emibetuzumab (LY2875358; Lilly) is a humanized, bivalent, IgG4 mAb, designed to block both ligand-dependent and ligand-independent MET signaling (binding affinity 0.8 pM) [85]. It induces internalization and degradation of MET, inhibiting proliferation of tumor cells with *MET* amplification and providing antitumor activity in xenograft models of NSCLC [85]. Preliminary, but limited, single-agent clinical activity was observed in a phase I trial in patients with MET+ (IHC ≥2+) solid tumors, including NSCLC [86].

5.2.1.2. ABT-700. ABT-700 (hz224G11; AbbVie) is a humanized, IgG1 mAb with nanomolar affinity for human MET. It exhibited preclinical activity in cell lines, inhibiting HGF binding and subsequent MET phosphorylation, and also induced apoptosis in gastric cancer xenografts [87]. The safety and preliminary efficacy of ABT-700 is currently being investigated in a phase 1 trial in patients with MET dysregulated solid tumors [45].

5.2.2. Combination with antibodies against HGF

5.2.2.1. Ficlatuzumab. Ficlatuzumab (AV-299; Aveo) is a humanized IgG1 mAb, capable of binding HGF with high affinity and inhibiting activity at sub-nanomolar concentrations [88]. In a phase 2 study in Asian patients with NSCLC, ficlatuzumab in combination with gefitinib showed preliminary efficacy in the *EGFR*-mutated, low-MET population [89]. Clinical development is ongoing, including a proof-of-concept study (FOCAL) of ficlatuzumab in combination with erlotinib in patients with *EGFR*-mutant NSCLC selected using BDX004, a serum-based proteomic companion diagnostic test [45,90].

5.2.3. Combination with antibody–drug conjugates

5.2.3.1. ABBV-399. Antibody–drug conjugates represent a novel class of drugs that utilize targeted antibodies to deliver cytotoxic agents directly to tumor cells. ABBV-399 (AbbVie) is a first-in-class conjugate of a MET Ab, ABT-700, and an antimicrotubule agent, monomethyl auristatin E. Preliminary analysis from a phase 1 study in patients (N = 45) with metastatic solid tumors that included an expansion cohort of 10 patients with MET+ (IHC ≥2+) NSCLC reported promising antitumor activity in these MET+ patients (30% response) [91]. Given that the major sequela of *MET*ex14 deletion is persistence of the MET protein, this molecular subgroup of NSCLC is a good target for ABBV-399.

5.3. Combination with immunotherapy

Immunotherapy utilizing anti-programmed cell death protein 1 (anti-PD-1) antibodies has been approved in the US for platinum-refractory NSCLC based on significant overall survival benefit over single-agent docetaxel [92–94]. Rivzi and colleagues have reported that mutational load is closely related to response to pembrolizumab [95]. Tumor mutational load can serve as a surrogate marker for the reservoir of neoantigens, with higher mutation load corresponding to higher reservoir of neoantigens. The average TMB in *MET*ex14+ NSCLC patients was 6.9 mutations/Mb (range 0–197.9), which is slightly lower than the overall average of 10.7 mutations/Mb for all lung cancer cases [31], but higher than the average TMB for *EGFR*-mutated (mean, 4.5) and *ALK+* NSCLC (mean, 2.8) patients [96]. Gainor and colleagues reported that for *EGFR*-mutated and *ALK+* NSCLC patients, the incidence of co-expression of PD-L1 is low, as is response to anti-PD-L1/PD-1

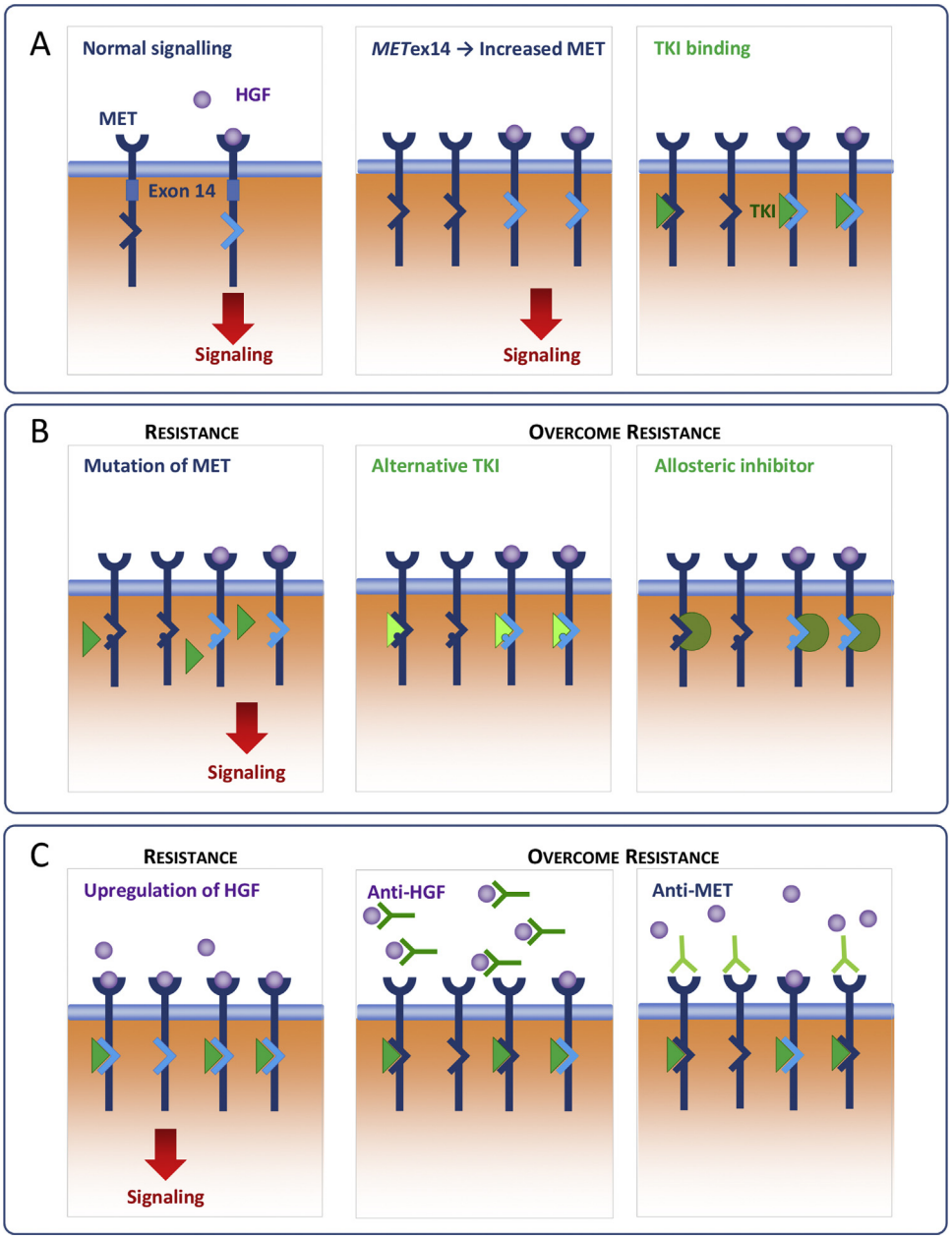

Fig. 2. Mechanisms of resistance to single-agent MET TKIs in the ligand-dependent setting, and potential strategies to overcome this resistance. (A) HGF-mediated MET signaling can be blocked by TKI binding. (B) TKI resistance can result from mutation of *MET*; this can be overcome by use of a different inhibitor. (C) TKI resistance can result from upregulation of HGF; this can be overcome by use of anti-HGF or anti-MET monoclonal antibodies.
HGF: hepatocyte growth factor; TKI: tyrosine kinase inhibitor.

therapy [97]. While the expression level of PD-L1 among patients with *MET*ex14+ NSCLC is currently unknown, a higher average TMB among *MET*ex14+ NSCLC patients than *EGFR*+ or *ALK*+ NSCLC patients, especially among the *MET* amplified subgroup, would suggest that the combination of MET TKIs and immunotherapy may be successful.

6. Conclusions

Only two years ago, amplification of *MET* in NSCLC was postulated to be the target that would lead to the eventual regulatory approval of MET TKIs [98]. As the incidence of true *de novo MET* amplification remains low (0.8%) and the incidence of *MET* amplification as a resistance mechanism to EGFR TKIs is only 5%, the road to regulatory approval of MET TKIs seemed uncertain [98]. There has since been renewed interest in *MET*ex14 alterations in NSCLC, with the prevalence closer to 3%, and reports of responses to MET TKIs. Dedicated clinical trials with MET TKIs in *MET*ex14+ NSCLC are underway, which are likely to lead to the eventual approvals of MET TKIs.

Conflicts of interest

Thanyanan Reungwetwattana has served on advisory boards for AstraZeneca, Boehringer Ingelheim, Novartis, Pfizer, MSD, Bristol-Myers Squibb, and Roche; speaker bureaus for AstraZeneca, Roche, Boehringer Ingelheim, Novartis, Pfizer, and Sanofi; and provided clinical research support for AstraZeneca, Roche, and

Novartis. Ying Liang has no conflict of interest. Viola Zhu has served on advisory boards for AstraZeneca and Bayer. Sai-Hong Ignatius Ou has served on advisory boards for Roche, ARIAD, AstraZeneca, Novartis, and Boehringer Ingelheim; speaker bureaus for Roche/Genentech, AstraZeneca, Novartis, and Boehringer Ingelheim; and his institution has received clinical research support from Pfizer, Roche/Genentech, Daiichi Sankyo, AstraZeneca, Clovis, Novartis, and ARIAD.

Acknowledgments

This work was supported by Novartis Pharmaceuticals Corporation. Editorial assistance was provided by Laura Hilditch PhD from Articulate Science, and was funded by Novartis Pharmaceuticals Corporation. We would like to thank J. Jean Cui PhD, lead designer of crizotinib, for her insightful suggestions and review of the manuscript.

References

[1] A.S. Tsao, G.V. Scagliotti, P.A. Bunn Jr., et al., Scientific advances in lung cancer 2015, J. Thorac. Oncol. 11 (2016) 613–638.
[2] A.A. Sadiq, R. Salgia, MET as a possible target for non-small-cell lung cancer, J. Clin. Oncol. 31 (2013) 1089–1096.
[3] G. Scagliotti, J. von Pawel, S. Novello, et al., Phase III multinational, randomized, double-blind, placebo-controlled study of tivantinib (ARQ 197) plus erlotinib versus erlotinib alone in previously treated patients with locally advanced or metastatic nonsquamous non-small-cell lung cancer, J. Clin. Oncol. 33 (2015) 2667–2674.
[4] H. Yoshioka, K. Azuma, N. Yamamoto, et al., A randomized, double-blind, placebo-controlled, phase III trial of erlotinib with or without a c-Met inhibitor tivantinib (ARQ 197) in Asian patients with previously treated stage IIIB/IV nonsquamous nonsmall-cell lung cancer harboring wild-type epidermal growth factor receptor (ATTENTION study), Ann. Oncol. 26 (2015) 2066–2072.
[5] D.R. Spigel, M.J. Edelman, K. O'Byrne, et al., Onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV NSCLC: Results from the pivotal phase III randomized, multicenter, placebo-controlled METLung (OAM4971 g) global trial, J. Clin. Oncol. 32 (5s) (2014) 8000.
[6] N. Van Der Steen, E. Giovannetti, P. Pauwels, et al., cMET exon 14 skipping: from the structure to the clinic, J. Thorac. Oncol. 11 (2016) 1423–1432.
[7] C.C. Lee, K.M. Yamada, Identification of a novel type of alternative splicing of a tyrosine kinase receptor: juxtamembrane deletion of the c-Met protein kinase C serine phosphorylation regulatory site, J. Biol. Chem. 269 (1994) 19457–19461.
[8] K.M. Weidner, M. Sachs, D. Riethmacher, W. Birchmeier, Mutation of juxtamembrane tyrosine residue 1001 suppresses loss-of-function mutations of the met receptor in epithelial cells, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 2597–2601.
[9] P. Peschard, T.M. Fournier, L. Lamorte, et al., Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein, Mol. Cell 8 (2001) 995–1004.
[10] P.C. Ma, T. Kijima, G. Maulik, et al., c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions, Cancer Res. 63 (2003) 6272–6281.
[11] P.C. Ma, R. Jagadeeswaran, S. Jagadeesh, et al., Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer, Cancer Res. 65 (2005) 1479–1488.
[12] M. Kong-Beltran, S. Seshagiri, J. Zha, et al., Somatic mutations lead to an oncogenic deletion of met in lung cancer, Cancer Res. 66 (2006) 283–289.
[13] R. Onozato, T. Kosaka, H. Kuwano, Y. Sekido, Y. Yatabe, T. Mitsudomi, Activation of MET by gene amplification or by splice mutations deleting the juxtamembrane domain in primary resected lung cancers, J. Thorac. Oncol. 4 (2009) 5–11.
[14] J.S. Seo, Y.S. Ju, W.C. Lee, et al., The transcriptional landscape and mutational profile of lung adenocarcinoma, Genome Res. 22 (2012) 2109–2119.
[15] Cancer Genome Atlas Research Network, Comprehensive molecular profiling of lung adenocarcinoma, Nature 511 (2014) 543–550.
[16] G.M. Frampton, S.M. Ali, M. Rosenzweig, et al., Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors, Cancer Discov. 5 (2015) 850–859.
[17] Y. Togashi, H. Mizuuchi, S. Tomida, et al., MET gene exon 14 deletion created using the CRISPR/Cas9 system enhances cellular growth and sensitivity to a MET inhibitor, Lung Cancer 90 (2015) 590–597.
[18] S.N. Waqar, D. Morgensztern, J. Sehn, MET mutation associated with responsiveness to crizotinib, J. Thorac. Oncol. 10 (2015) e29–31.
[19] M.A. Mendenhall, J.W. Goldman, MET-Mutated NSCLC with major response to crizotinib, J. Thorac. Oncol. 10 (2015) e33–4.
[20] R.W. Jenkins, G.R. Oxnard, S. Elkin, E.K. Sullivan, J.L. Carter, D.A. Barbie, Response to crizotinib in a patient with lung adenocarcinoma harboring a MET splice site mutation, Clin. Lung Cancer 16 (2015) e101–4.
[21] P.K. Paik, A. Drilon, P.D. Fan, et al., Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping, Cancer Discov. 5 (2015) 842–849.
[22] C. Lee, D. Usenko, G.M. Frampton, C. McMahon, S.M. Ali, J. Weiss, MET 14 deletion in sarcomatoid non-small-cell lung cancer detected by next-generation sequencing and successfully treated with a MET inhibitor, J. Thorac. Oncol. 10 (2015) e113–4.
[23] S. Park, J. Koh, D.W. Kim, et al., MET amplification, protein expression, and mutations in pulmonary adenocarcinoma, Lung Cancer 90 (2015) 381–387.
[24] M.M. Awad, G.R. Oxnard, D.M. Jackman, et al., MET exon 14 mutations in non-small-cell lung cancer are associated with advanced age and stage-dependent MET genomic amplification and c-Met overexpression, J. Clin. Oncol. 34 (2016) 721–730.
[25] X. Liu, Y. Jia, M.B. Stoopler, et al., Next-generation sequencing of pulmonary sarcomatoid carcinoma reveals high frequency of actionable MET gene mutations, J. Clin. Oncol. 34 (2016) 794–802.
[26] J.H. Tong, S.F. Yeung, A.W. Chan, et al., MET amplification and exon 14 splice site mutation define unique molecular subgroups of non-small cell lung carcinoma with poor prognosis, Clin. Cancer Res. 22 (2016) 3048–3056.
[27] R.S. Heist, H.S. Shim, S. Gingipally, et al., MET exon 14 skipping in non-small cell lung cancer, Oncologist 21 (2016) 481–486.
[28] M. Saito, K. Shiraishi, H. Kunitoh, S. Takenoshita, J. Yokota, T. Kohno, Gene aberrations for precision medicine against lung adenocarcinoma, Cancer Sci. 107 (2016) 713–720.
[29] D. Zheng, R. Wang, T. Ye, et al., MET exon 14 skipping defines a unique molecular class of non-small cell lung cancer, Oncotarget (2016) (Epub ahead of print).
[30] S.Y. Liu, L.Y. Gou, A.N. Li, et al., The unique characteristics of MET exon 14 mutation in chinese patients with NSCLC, J. Thorac. Oncol. 11 (2016) 1503–1510.
[31] A.B. Schrock, G.M. Frampton, J. Suh, et al., Characterization of 298 lung cancer patients harboring MET exon 14 skipping (METex14) alterations, J. Thorac. Oncol. 11 (2016) 1493–1502.
[32] K. Goto, J. Yang, D. Kim, S. Lu, T. Seto, I. Yang, Phase II study of crizotinib in East Asian patients with ROS1-positive advanced non-small cell lung cancer, J. Clin. Oncol. 34 (Suppl. (34)) (2016) 9022.
[33] A.E. Drilon, D.R. Camidge, S.I. Ou, et al., Efficacy and safety of crizotinib in patients (pts) with advanced *MET* exon 14-altered non-small cell lung cancer (NSCLC), J. Clin. Oncol. 34 (2016) 108.
[34] A. Backes, B. Zech, B. Felber, B. Klebl, G. Muller, Small-molecule inhibitors binding to protein kinases: part I: exceptions from the traditional pharmacophore approach of type I inhibition, Expert Opin Drug Discov 3 (2008) 1409–1425.
[35] E. Gherardi, W. Birchmeier, C. Birchmeier, G. Vande Woude, Targeting MET in cancer: rationale and progress, Nat. Rev. Cancer 12 (2012) 89–103.
[36] A. Backes, B. Zech, B. Felber, B. Klebl, G. Muller, Small-molecule inhibitors binding to protein kinase: part II: the novel pharmacophore approach of type II and type III inhibition, Expert Opin Drug Discov 3 (2008) 1427–1449.
[37] S. Pennacchietti, M. Cazzanti, A. Bertotti, et al., Microenvironment-derived HGF overcomes genetically determined sensitivity to anti-MET drugs, Cancer Res. 74 (2014) 6598–6609.
[38] H.Y. Zou, Q. Li, J.H. Lee, et al., An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms, Cancer Res. 67 (2007) 4408–4417.
[39] J.J. Cui, M. Tran-Dube, H. Shen, et al., Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK), J. Med. Chem. 54 (2011) 6342–6363.
[40] D.R. Camidge, S.I. Ou, G. Shapiro, et al., Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC), J. Clin. Oncol. 32 (5s) (2014) 8001.
[41] J. Tanizaki, I. Okamoto, K. Okamoto, et al., MET tyrosine kinase inhibitor crizotinib (PF-02341066) shows differential antitumor effects in non-small cell lung cancer according to MET alterations, J. Thorac. Oncol. 6 (2011) 1624–1631.
[42] S.M. Malik, V.E. Maher, K.E. Bijwaard, U.S. et al, Food and drug administration approval: crizotinib for treatment of advanced or metastatic non-small cell lung cancer that is anaplastic lymphoma kinase positive, Clin. Cancer Res. 20 (2014) 2029–2034.
[43] D. Kazandjian, G.M. Blumenthal, L. Luo, et al., Benefit-Risk summary of crizotinib for the treatment of patients with ROS1 alteration-positive, metastatic non-small cell lung cancer, Oncologist 21 (2016) 974–980.
[44] S.H. Ou, E.L. Kwak, C. Siwak-Tapp, et al., Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification, J. Thorac. Oncol. 6 (2011) 942–946.
[45] ClinicalTrials.gov. Available from: https://clinicaltrials.gov/. Accessed October 2016.
[46] M. Di Maio, P. Bironzo, G.V. Scagliotti, MET exon 14 mutation: another actionable genomic variation in patients with advanced NSCLC, Transl. Cancer Res. 5 (2016) S101–5.

[47] X. Liu, Q. Wang, G. Yang, et al., A novel kinase inhibitor, INCB28060, blocks c-MET-dependent signaling, neoplastic activities, and cross-talk with EGFR and HER-3, Clin. Cancer Res. 17 (2011) 7127–7138.
[48] M.H. Schuler, R. Berardi, W. Lim, et al., Phase (Ph) I study of the safety and efficacy of the cMET inhibitor capmatinib (INC280) in patients (pts) with advanced cMET+ non-small cell lung cancer (NSCLC), J. Clin. Oncol. 34 (2016) 9067 (s).
[49] Y. Wu, D. Kim, E. Felip, et al., Phase (Ph) II safety and efficacy results of a single-arm ph ib/II study of capmatinib (INC280) + gefitinib in patients (pts) with EGFR-mutated (mut), cMET-positive (cMET+) non-small cell lung cancer (NSCLC), J. Clin. Oncol. 34 (2016) 9020 (s).
[50] E.F. Smit, H. Kopp, D. Kim, et al., GEOMETRY duo-1: A phase (Ph) Ib/II, multicenter trial of oral cMET inhibitor capmatinib (INC280) ± erlotinib vs platinum + pemetrexed in adult patients (pts) with epidermal growth factor receptor (EGFR)-mutated, cMET-amplified, locally advanced/metastatic non-small cell lung cancer (NSCLC) with acquired resistance to prior EGFR tyrosine kinase inhibitor (TKI) therapy, J. Clin. Oncol. 34 (2016) (s) TPS9109.
[51] F. Bladt, B. Faden, M. Friese-Hamim, et al., EMD 1214063 and EMD 1204831 constitute a new class of potent and highly selective c-Met inhibitors, Clin. Cancer Res. 19 (2013) 2941–2951.
[52] G.S. Falchook, R. Kurzrock, H.M. Amin, et al., Efficacy, safety, biomarkers, and phase II dose modeling in a phase I trial of the oral selective c-Met inhibitor tepotinib (MSC2156119J), J. Clin. Oncol. 33 (2015) 2591 (s).
[53] EU Clinical Trials register. Available from: https://www.clinicaltrialsregister.eu/. Accessed October 2016.
[54] H. Jia, G. Dai, J. Weng, et al., Discovery of (S)-1-(1-(Imidazo[1,2-a]pyridin-6-yl)ethyl)-6-(1-methyl-1H-pyrazol-4-yl)-1H-[1,2,3]triazolo[4,5-b]pyrazine (volitinib) as a highly potent and selective mesenchymal-epithelial transition factor (c-Met) inhibitor in clinical development for treatment of cancer, J. Med. Chem. 57 (2014) 7577–7589.
[55] C. D'Cruz, M. Frigault, A. Adam, et al., Targeting MET in preclinical models to support the clinical development of Volitinib in NSCLC, Cancer Res. 74 (2014) 3114.
[56] H.K. Gan, J. Lickliter, M. Millward, et al., First-in-human phase I study of a selective c-Met inhibitor volitinib (HMP504/AZD6094) in patients with advanced solid tumors, J. Clin. Oncol. 32 (5s) (2014) 11111.
[57] E. Barry, R. Henry, A. Borodovsky, E. Maloney, B. Ladd, M. Frigault, Activity of svaolitinib against MET ex14 mutations and resistance to METi through decoupling from MYC expression, AACR Meet. Abstr. (2016) 1150.
[58] P.E. Hughes, K. Rex, S. Caenepeel, et al., In vitro and in vivo activity of AMG 337, a potent and selective MET kinase inhibitor, in MET-dependent cancer models, Mol. Cancer Ther. 15 (2016) 1568–1579.
[59] E. Kwak, P. LoRusso, O. Hamid, F. Janku, M. Kittaneh, D. Catenacci, Clinical activity of AMG337, an oral MET kinase inhibitor, in adult patients (pts) with MET-amplified gastroesophageal junction (GEJ), gastric (G), or esophageal (E) cancer, J. Clin. Oncol. 33 (Suppl) (2015) 1.
[60] F. Cecchi, S. Rabizadeh, P. Weingarten, C. Tsai, L. Zhou, T. Hembrough, MET activation via exon 14 skipping mutations (METex14del): gastrointestinal prevalence and sensitivity to MET inhibitor AMG337, Ann. Oncol. 27 (Suppl. 2ii11) (2016) P–038.
[61] F.M. Yakes, J. Chen, J. Tan, et al., Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth, Mol. Cancer Ther. 10 (2011) 2298–2308.
[62] R. Elisei, M.J. Schlumberger, S.P. Muller, et al., Cabozantinib in progressive medullary thyroid cancer, J. Clin. Oncol. 31 (2013) 3639–3646.
[63] B.A. Hellerstedt, G. Edelman, N.J. Vogelzang, et al., Activity of cabozantinib (XL184) in metastatic NSCLC: Results from a phase II randomized discontinuation trial (RDT), J. Clin. Oncol. 30 (2012) 7514 (s).
[64] K.L. Reckamp, P.C. Mack, N. Ruel, et al., Biomarker analysis of a phase II trial of cabozantinib and erlotinib in patients (pts) with EGFR-mutant NSCLC with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) resistance: a California Cancer consortium phase II trial (NCI 9303), J. Clin. Oncol. 33 (15s) (2015) 8087.
[65] M. Bahcall, T. Sim, C.P. Paweletz, et al., Acquired MET D1228V mutation and resistance to MET inhibition in lung cancer, Cancer Discov. (2016).
[66] J.M. Besterman, M. Fournel, I. Dupont, et al., Potent preclinical antitumor activity of MGCD265, an oral Met/VEGFR kinase inhibitor in phase II clinical development, in combination with taxanes or erlotinib, J. Clin. Oncol. 28 (2010) e13595 (s).
[67] C. Bonfils, N. Beaulieu, M. Fournel, H. Ste-Croix, J.M. Besterman, C.R. Maroun, The combination of MGCD265, a Met/VEGFR inhibitor in clinical development, and erlotinib potently inhibits tumor growth by altering multiple pathways including glycolysis, Cancer Res. 72 (2012) 1790.
[68] C.K. Kollmannsberger, S. Sharma, G. Shapiro, et al., Phase I study of receptor tyrosine kinase (RTK) inhibitor, MGCD265, in patients (pts) with advanced solid tumors, J. Clin. Oncol. 33 (2015) 2589 (s).
[69] I.I. Rybkin, E.A. Kio, A. Masood, et al., Amethyst NSCLC trial: phase 2, parallel-arm study of receptor tyrosine kinase (RTK) inhibitor, MGCD265, in patients (pts) with advanced or metastatic non-small cell lung cancer (NSCLC) with activating genetic alterations in mesenchymal-epithelial transition factor (*MET*), J. Clin. Oncol. 34 (2016) (s):TPS9099.
[70] S.B. Yan, V.L. Peek, R. Ajamie, et al., LY2801653 is an orally bioavailable multi-kinase inhibitor with potent activity against MET, MST1R, and other oncoproteins, and displays anti-tumor activities in mouse xenograft models, Invest. New Drugs 31 (2013) 833–844.
[71] W. Wu, C. Bi, K.M. Credille, et al., Inhibition of tumor growth and metastasis in non-small cell lung cancer by LY2801653, an inhibitor of several oncokinases, including MET, Clin. Cancer Res. 19 (2013) 5699–5710.
[72] I. Kawada, R. Hasina, Q. Arif, et al., Dramatic antitumor effects of the dual MET/RON small-molecule inhibitor LY2801653 in non-small cell lung cancer, Cancer Res. 74 (2014) 884–895.
[73] D.B. Costa, A.T. Shaw, S.H. Ou, et al., Clinical experience with crizotinib in patients with advanced ALK-rearranged non-small-cell lung cancer and brain metastases, J. Clin. Oncol. 33 (2015) 1881–1888.
[74] S.J. Klempner, A. Borghei, B. Hakimian, S.M. Ali, S.H. Ignatius Ou, Brief report: intracranial activity of cabozantinib in MET exon14 positive NSCLC with brain metastases, J. Thorac. Oncol. (2016).
[75] S. Park, Y.L. Choi, C.O. Sung, et al., High MET copy number and MET overexpression: poor outcome in non-small cell lung cancer patients, Histol. Histopathol. 27 (2012) 197–207.
[76] K. Okuda, H. Sasaki, H. Yukiue, M. Yano, Y. Fujii, Met gene copy number predicts the prognosis for completely resected non-small cell lung cancer, Cancer Sci. 99 (2008) 2280–2285.
[77] F. Cappuzzo, A. Marchetti, M. Skokan, et al., Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients, J. Clin. Oncol. 27 (2009) 1667–1674.
[78] J.D. Oliner, A.Y. Saiki, S. Caenepeel, The role of MDM2 amplification and overexpression in tumorigenesis, Cold Spring Harb. Perspect. Med. 6 (2016), http://dx.doi.org/10.1101/cshperspect.a026336.
[79] J. Qi, M.A. McTigue, A. Rogers, et al., Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors, Cancer Res. 71 (2011) 1081–1091.
[80] R. Tiedt, E. Degenkolbe, P. Furet, et al., A drug resistance screen using a selective MET inhibitor reveals a spectrum of mutations that partially overlap with activating mutations found in cancer patients, Cancer Res. 71 (2011) 5255–5264.
[81] R.S. Heist, L.V. Sequist, D. Borger, et al., Acquired resistance to crizotinib in non-small cell lung cancer with MET exon 14 skipping, J. Thorac. Oncol. 11 (2016) 1242–1245.
[82] S.H. Ignatius Ou, L. Young, A.B. Schrock, et al., Emergence of pre-existing MET Y1230C mutation as a resistance mechanism to crizotinib in NSCLC with MET exon 14 skipping, J. Thorac. Oncol. (2016) (in press).
[83] S.L. Timofeevski, M.A. McTigue, K. Ryan, et al., Enzymatic characterization of c-Met receptor tyrosine kinase oncogenic mutants and kinetic studies with aminopyridine and triazolopyrazine inhibitors, Biochemistry 48 (2009) 5339–5349.
[84] Y. Jia, C.H. Yun, E. Park, et al., Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors, Nature 534 (2016) 129–132.
[85] L. Liu, W. Zeng, M.A. Wortinger, et al., LY2875358, a neutralizing and internalizing anti-MET bivalent antibody, inhibits HGF-dependent and HGF-independent MET activation and tumor growth, Clin. Cancer Res. 20 (2014) 6059–6070.
[86] M.S. Banck, R. Chugh, R.B. Natale, et al., Phase 1 results of emibetuzumab (LY2875358), a bivalent MET antibody, in patients with advanced castration-resistant prostate cancer, and MET positive renal cell carcinoma, non-small cell lung cancer, and hepatocellular carcinoma, Mol. Cancer Ther. 14 (2015) A55.
[87] A. Gonzalez, M. Broussas, C. Beau-Larvor, et al., A novel antagonist anti-cMet antibody with antitumor activities targeting both ligand-dependent and −independent c-Met receptors, Int. J. Cancer 139 (2016) 1851–1863.
[88] K. Meetze, K. Connolly, A. Boudrow, et al., Preclinical efficacy and pharmacodynamics of SCH 900105 (AV-299), an anti-HGF antibody, Cancer Res. 69 (2009) 1246.
[89] T.S.K. Mok, K. Park, S.L. Geater, et al., A randomized phase (PH) 2 study with exploratory biomarker analysis of ficlatuzumab (F) a humanized hepatocye growth factor (HGF) inhibitory mAb in combination with gefitinib (G) versus G in asian patients (PTS) with lung adenocarcionma (LA), Ann. Oncol. 23 (2012) (s9):1198P.
[90] N. Dupuis, J. Chang, M. Steers, Z. Weng, J. Gyuris, G. Pestano, Feasibility of serum proteomic companion diagnostic (CDx) test development on the microflex LT platform, Cancer Res. 75 (2015) 2011.
[91] J.H. Strickler, J.J. Nemunaitis, C.D. Weekes, et al., Phase 1, open-label, dose-escalation and expansion study of ABBV-399, an antibody drug conjugate (ADC) targeting c-Met, in patients (pts) with advanced solid tumors, J. Clin. Oncol. 34 (2016) 2510 (s).
[92] J. Brahmer, K.L. Reckamp, P. Baas, et al., Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer, N. Engl. J. Med. 373 (2015) 123–135.
[93] H. Borghaei, L. Paz-Ares, L. Horn, et al., Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer, N. Engl. J. Med. 373 (2015) 1627–1639.
[94] R.S. Herbst, P. Baas, D.W. Kim, et al., Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial, Lancet 387 (2016) 1540–1550.
[95] N.A. Rizvi, M.D. Hellmann, A. Snyder, et al., Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer, Science 348 (2015) 124–128.

[96] D. Spigel, A. Schrock, D. Fabrizio, et al., Total mutation burden (TMB) in lung cancer (LC) and relationship with response to PD-1/PD-L1 targeted therapies, J. Clin. Oncol. 34 (Suppl) (2016) 9017.
[97] J.F. Gainor, A.T. Shaw, L.V. Sequist, et al., EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-Small cell lung cancer (NSCLC): a retrospective analysis, Clin. Cancer Res. 22 (2016) 4585–4593.
[98] K. Garber, MET inhibitors start on road to recovery, Nat. Rev. Drug Discov. 13 (2014) 563–565.
[99] B. Halmos, G.M. Frampton, J. Suh, et al., Lung sarcomatoid carcinoma (LSC) harbors targetable genomic alterations and high mutational burden as observed by comprehensive genomic profiling (CGP), Ann. Oncol. 27 (2016) 1212PD.
[100] K. Sunami, K. Furuta, K. Tsuta, et al., Multiplex diagnosis of oncogenic fusion and MET exon skipping by molecular counting using formalin-fixed paraffin embedded lung adenocarcinoma tissues, J. Thorac. Oncol. 11 (2016) 203–212.