

## c-Met: A Potential Target for Current Non-Small-Cell Lung Cancer Therapeutics

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### Abstract

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. In early stages of NSCLC tumor development (stage I/II), surgical resection is often performed; however, when the cancer becomes metastatic, chemotherapy is most commonly implemented. Due to the fact that traditional chemotherapies result in adverse and cytotoxic effects on healthy cells in addition to NSCLC cells, targeted therapeutics have been extensively developed over recent years to combat the disease. These targeted therapies include small molecule inhibitors and monoclonal antibodies (MAbs), some of which are used as first-line treatments for NSCLC patients. Several inhibitors against the mesenchymal-epithelial transition factor (c-Met), and its ligand hepatocyte growth factor (HGF), have shown promising results in NSCLC clinical trials. For example, crizotinib, a multi-kinase inhibitor has been approved by the FDA for the treatment of ALK positive NSCLC. c-Met is known to be overexpressed, mutated and gene amplified, specifically in NSCLC, and has also been implicated in the development of resistance against other small-molecule inhibitors (e.g. EGFR). Thus, this review will discuss the current developments and usages of c-Met inhibitors in NSCLC, and their potential for future therapeutic advancement.

**Keywords:** c-Met; NSCLC; Tyrosine kinase inhibitors; HGF; Monoclonal antibody

### Abbreviations:

HGF: Hepatocyte Growth Factor; PSI: Plexin-Semaphorin-Integrin; IPT: Immunoglobulin-like-Plexin Transcription; GRB2: Growth-factor Receptor Bound protein-2; GAB1: GRB2-Associated protein-1; SOS: Son of Sevenless; FAK: Focal Adhesion Kinase; SHP2: SRC homology 2 domain-containing phosphatase 2; PLCγ: Phospholipase gamma C; PI3K: Phosphatidylinositol 3-kinase; SHC: Src Homology 2 domain; STAT 3/5: Signal Transducer and Activator of Transcription 3/5

### Introduction

More than one-quarter of all cancer-related deaths in the United States are estimated to be due to lung cancer in 2014. Lung cancer is the leading cause of cancer deaths in men aged 40 and older and women aged 60 and older. Furthermore, lung cancer is one of the few cancers to show minimal improvement in cancer survival rates in the United States (measured from 1975 to 2009) [1]. Lung and bronchus cancer deaths (159,260 deaths) are estimated to have one of the highest malignancy mortalities in the United States this year [1].

c-Met is a receptor tyrosine kinase (RTK) encoded by a proto-oncogene located on chromosome 7q21-31 [2]. c-Met has a ligand binding domain located extracellularly, a transmembrane portion, and an intracellular tyrosine kinase domain [3]. Hepatocyte growth factor/scatter factor (HGF), which is secreted by mesenchymal cells, is the only known ligand that stimulates the c-Met receptor [3]. Binding of HGF stimulates c-Met, resulting in autophosphorylation of its tyrosine kinase domain, which causes downstream signaling and further

activation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)-Akt pathways [4].

Cancer cells, including NSCLC, express mutated versions of c-Met, which can result in increased activation and tumorigenesis [5]. Nearly 5% of all NSCLCs contain c-Met mutations, and c-Met amplification has been observed in approximately 20% of patients treated with EGFR inhibitors [6]. Lutterbach et al. has also shown that amplification of the MET gene causes overexpression of the c-Met receptor in NSCLC cell lines [7]. Activation of the c-Met receptor often results in synergistic effects with other RTKs located on the cell surface, resulting in further cancer cell proliferation, migration, and anti-apoptotic activity [8].

Inhibition of altered receptor tyrosine kinases, such as c-Met, has become a therapeutic strategy for patients with tumors that overexpress or contain mutated forms of these RTKs. For example, crizotinib, a small molecule inhibitor of c-Met, ALK, and ROS1, has also shown great potential in the treatment of ALK/c-Met positive NSCLC. Patients treated with crizotinib have experienced improvements in progression-free survival, quality of life, and lung cancer symptoms [9]. Patients treated with c-Met TKIs have exhibited resistance to therapy in some cases, warranting further studies in this direction. Recent studies from our laboratory indicate that activation of alternative signaling pathways, such as Wnt and mTOR, could be the cause of this resistance and combinatorial treatment with inhibitors against c-Met, Wnt, and mTOR could overcome this resistance mechanism [10].

### c-Met Structure and Function

c-Met is a member of the RTK family, which is considered the second largest family of membrane receptors [11]. c-Met is a disulfide-

linked heterodimer that spans the width of the cell membrane [4,12] and is comprised of a 50- kDa  $\alpha$ -chain and a 140- kDa  $\beta$ -chain [13]. The extracellular region of c-Met is composed of a seven-bladed  $\beta$ -propeller semaphorin (sema) domain, a PSI domain and four immunoglobulin-like repeat domains. c-Met is stimulated by its ligand, HGF, the only known naturally occurring stimulating factor for this receptor. HGF was originally identified as a growth factor for hepatocytes and a motogenic factor for fibroblasts [13]. HGF has six domains, including one N-terminal domain, four kringle domains, and a C-terminal domain [13]. Binding of the HGF  $\beta$ -domain to the sema domain of c-Met triggers c-Met dimerization and tyrosine kinase activation [12-14].

The intracellular region of the c-Met receptor is composed of a juxtamembrane (JM) domain, a tyrosine kinase domain (TKD), and a carboxyterminal tail, also known as the bidentate or multi-substrate docking site [12]. The tyrosine kinase region is responsible for initiating the downstream signaling pathways through trans-autophosphorylation of the tyrosine residues Y1234 and Y1235 that are located on the TKD. The residues Y1349 and Y1356, which are located on the carboxyterminal tail, are then trans-phosphorylated and act as a multiple substrate docking station for downstream signaling transducers [15]. Moreover, the bidentate docking site is essential and exclusive to the subfamily of Met receptors, and allows for direct and indirect interactions with proteins, such as GRB2-associated binder (GAB1), growth factor receptor bound protein-2 (GRB2), and PI3K [16]. c-Met signaling is normally terminated by the recruitment of c-CBL, which is an E3 ubiquitin ligase. c-CBL binds to the Y1003 region of the JM domain and triggers the internalization of the c-Met receptor via clathrin-coated vesicles [13].

The activation of the c-Met RTK leads to the subsequent activation, binding, and phosphorylation of adaptor proteins GAB1 and GRB2, which are involved in directing c-Met signaling to regulate cell shape and motility, and to facilitate the oncogenic transforming activity of c-Met, respectively [17-19]. Additionally, mutations in the GRB2-binding site result in a complete loss of c-Met biological functioning, which includes GAB1-dependent activity [19,20]. Once formed, the phosphorylated c-Met adaptor protein complex leads to downstream signaling, primarily through the PI3K/Akt, MAPK, and activators of transcription (STAT) pathways [4,15]. Specifically, the PI3K/Akt pathway is important in cell survival and the MAPK pathway is important for cell proliferation. Other downstream signaling proteins include Focal Adhesion Kinase (FAK) and paxillin. Paxillin has been found to be highly expressed, amplified and to correlate with c-Met expression in lung cancer [21]. Paxillin has also been found to play a key role in focal adhesion, and may regulate FAK activity and other protein interactions [20]. FAK also acts as a protein recruiter and helps mediate functions such as cell growth, survival, and migration [20,22]. Additional functions of these pathways include angiogenesis, metabolism, protein synthesis, transcription, apoptosis, differentiation, organism development, and cell cycle regulation and progression [13,19,23,24].

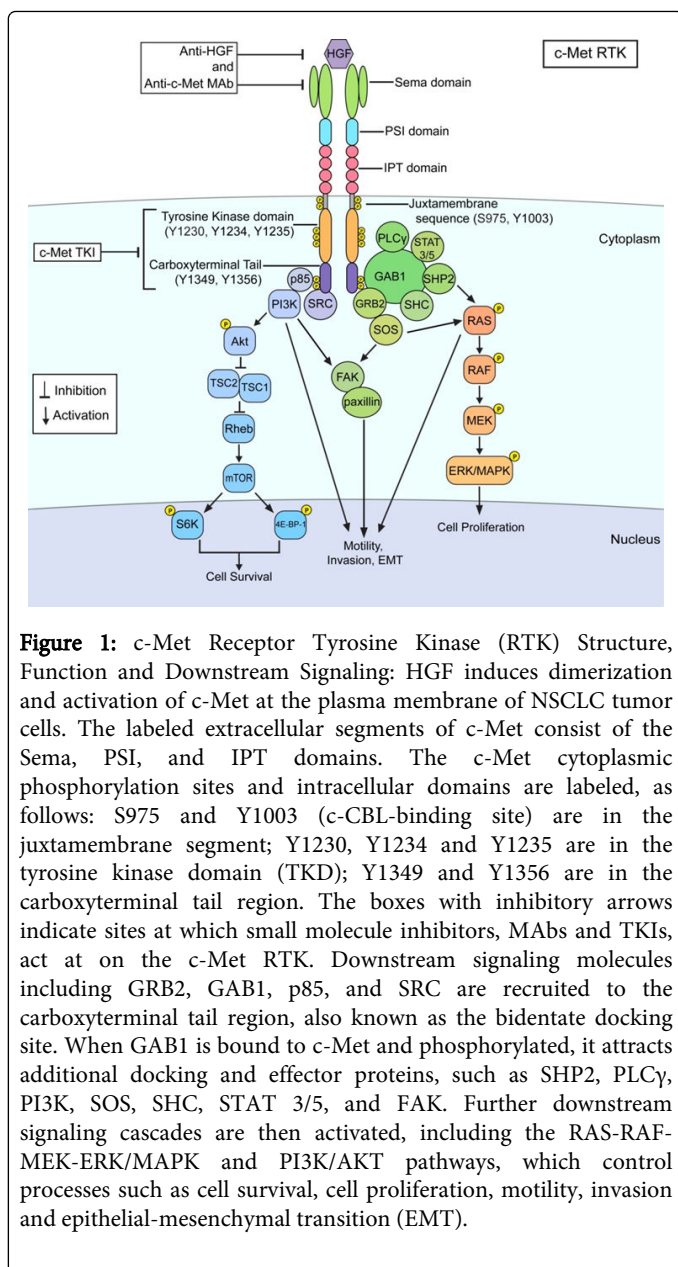
### c-Met Dysregulation in NSCLC

c-Met dysregulation is often associated with tumor growth, invasion and metastasis [4,12,25]. Uncontrolled c-Met signaling can occur through overexpression of c-Met/HGF, MET amplification, activating mutations, and autocrine signaling, resulting in an increase in malignancy of NSCLC [12]. Certain tumor cells express both c-Met and its ligand, HGF, establishing potential for an autocrine loop that

allows secreted HGF to bind to c-Met and cause constitutive activation of the receptor and its downstream signaling pathways. This constitutive activation of c-Met enhances tumor growth and invasive behavior, often leading to malignant progression of NSCLC tumors and also correlating with poor prognosis [6].

Mutations of the MET gene on exons 2 and 14 have been linked to the development of approximately 5% of all NSCLC cases [6]. In previous studies, novel MET mutations have been recorded in the extracellular sema domain and the JM domain in NSCLC cell lines [26]. Specifically, there is a somatic intronic mutation that causes an exon 14 JM domain deletion, which results in the loss of c-CBL E3-ligase binding and increased tumorigenicity [27]. Mutations in the MET gene (T1010I and R988C) and alternatively spliced skipping variants of the JM domain are linked to an increase in the rate of phosphorylation of a signal transducer and adaptor protein, paxillin [28], which is also overexpressed in NSCLC [13]. In one study that included 141 Asian, 76 Caucasian, and 66 African American lung cancer patients, the type and frequency of MET mutations present in each ethnicity differed amongst each group. A significant amount of East Asian patients were found to have the N375S MET mutation. This mutation is associated with the development of squamous-cell carcinoma and resistance to c-Met inhibition and was more prevalent amongst male smokers [6]. In a second study of 188 adenocarcinoma patients, three somatic MET mutations were detected, Arg988del and Tyr1021Asn in exon 13 which encodes the JM domain, and Gly1260Cys in exon 18, which encodes the kinase domain [6].

Unlike other genes that code for RTKs, the MET gene is most often amplified in cancers, rather than mutated [29]. The MET gene is thought to be more often amplified due to its position on chromosome 7, within FRA7G, a known chromosomal common fragile site. Hellman et al. proposed that this fragile site is predisposed to breakage during conditions of "replication stress", during which selective pressure for gene amplification is applied and disappears once the oncogene obtains sufficient amplification [29,30]. By using quantitative real-time polymerase chain reaction assay for MET amplification, multiple studies have reported primary MET amplification to be in a wide range (2%–21%) of NSCLC lung adenocarcinomas, particularly in TKI-naive treatment groups. However, the Lung Cancer Mutation Consortium has also reported that only 4.1% of adenocarcinoma have high degrees MET amplification, determined by using fluorescence in situ hybridization (FISH) assay [31]. Furthermore, MET gene amplification has been suggested as a cause of EGFR TKI resistance in NSCLC. Specifically, several groups have reported MET gene amplification to be in approximately 20% of EGFR mutation-positive NSCLC patients with acquired resistance to EGFR TKIs [15,32]. However, MET gene amplification was not detected in the majority of these patients prior to EGFR TKI treatment [15,32], suggesting that MET amplification occurs in tumor cells previously treated with inhibitors against other RTKs. Another study observed ERBB3 transphosphorylation as a result of MET amplification and overexpression in NSCLC cells treated with gefitinib, which ultimately led to resistance against EGFR family inhibitors [11] (Figure 1).



## Agents targeting c-Met

A variety of molecules that target c-Met and control its overexpression in NSCLC patients have been discovered. These molecules can be generally classified as selective c-Met inhibitors, non-selective c-Met inhibitors and anti-Met antibodies. Selective inhibitors target the c-Met receptor and inactivate it by blocking the ATP binding site, whereas non-selective inhibitors interfere with the activity of multiple kinase receptors. On the other hand, monoclonal antibodies can control c-Met overexpression, either by binding to HGF or to the extracellular domain of the c-Met RTK.

Tivantinib, one of the most studied c-Met inhibitors, is a non-competitive ATP inhibitor that inhibits c-Met phosphorylation and its downstream signaling pathways [33]. Tivantinib has also been observed to induce apoptosis in c-Met activated cells by the ubiquitin/

proteasome pathway in vitro [34]. In addition to its activity against c-Met, recent studies have indicated that tivantinib may exhibit anti-cancer inhibitory effects due to disruption of microtubule formation [35]. There are many clinical trials that are completed or in progress involving tivantinib as a single drug or in combination with various TKIs or MABs. In a recent phase II trial, 167 patients with advanced NSCLC were treated with either 150 mg of erlotinib plus 360 mg of tivantinib (ET) or with 150 mg of erlotinib plus placebo (EP). The results showed a median progression free survival (PFS) of 3.8 months in patients treated with erlotinib plus tivantinib (ET arm) and 2.3 months in patients treated with erlotinib plus placebo (EP arm) [36]. A phase III trial has been planned on the basis of the data obtained from the above mentioned trial in NSCLC patients, in order to compute overall survival (OS) rates (NCT01377376). Other selective c-Met inhibitors include PF04217903, an ATP competitive small inhibitor, [37] AMG337 a proto-oncogene that disrupts c-Met signal transduction [38], and INCB028060 a small molecule which blocks c-Met phosphorylation [39].

Crizotinib is a non-selective c-Met inhibitor that has been approved by the FDA for treatment of patients with metastatic Anaplastic Lymphoma Kinase (ALK) positive NSCLC [40]. Crizotinib is a small multi-kinase inhibitor that competes with ATP by binding to the ATP binding pocket of c-Met and ALK [9]. A recent phase III trial enrolled 350 patients from 21 countries with advanced NSCLC and ALK gene rearrangements to evaluate the PFS of patients after treatment with 250mg crizotinib bi-daily. The study showed that PFS was twice as long when compared to patients treated with traditional cytotoxic chemotherapy. The PFS was recorded to be 7.7 months for crizotinib treated patients, and 3 months for patients treated with chemotherapy. There was no change in OS for either of the treatments, but tumor response was observed in 65% of crizotinib treated patients versus only 20% of patients treated with traditional chemotherapy [41].

Other common non-selective c-Met inhibitors include cabozantinib, foretinib, MGCD265, and E7050. Cabozantinib is a potent multi-kinase inhibitor of c-Met, VEGFR2, RET, KIT, AXL and FLT3 [42] that has been recently approved by the FDA for the treatment of patients with progressive metastatic medullary thyroid cancer (MTC) [43]. Foretinib is an ATP competitive inhibitor of c-Met and VEGFR-2 receptors [44], and is currently in phase II clinical trials in combination with erlotinib hydrochloride to stop tumor growth in NSCLC patients (NCT01068587). E7050 is also an ATP competitive dual-kinase inhibitor of c-Met and VEGFR, [45] and is a potential candidate for future NSCLC clinical trials. MGCD265, a multi kinase inhibitor of c-Met, VEGF, RON, along with Tie2 [46], is in early phases of clinical trials to study possible synergistic effects with erlotinib and/or docetaxel (NCT00975767).

Onartuzumab, commonly known as MetMab, is a fully humanized monovalent monoclonal anti-c-Met antibody. It is based on a human IgG1k framework sequence and binds to the extracellular domain of the c-Met RTK, inhibiting HGF ligand binding and hence receptor activation. In a recent phase II study, patients with recurrent NSCLC were randomly given a combination of onartuzumab and erlotinib or placebo and erlotinib. The co-primary endpoint was PFS in the MET positive population, as well as in the intent to treat (ITT) population. Although the ITT population showed no improvement in PFS or OS, the MET positive population showed improved PFS and OS when treated with onartuzumab along with erlotinib [47].

Ficlatuzumab (AV-299) is a high affinity IgG1 monoclonal antibody against hepatocyte growth factor. A phase I trial for patients with

unresectable NSCLC was carried out by administering ficlatuzumab (10 and 20 mg/kg every 2 weeks) in combination with gefitinib (250 mg daily), intravenously. 20 mg/kg ficlatuzumab + 250 mg gefitinib was the recommended dose, as no dose limiting toxicity was observed (NCT01039948). Rilotumumab (AMG 102) is another anti-HGF human monoclonal antibody that selectively binds to the active  $\beta$ -chain of the human HGF and inhibits it. A phase I/II trial is recruiting patients with untreated NSCLC to determine the efficacy of rilotumumab in combination with erlotinib (NCT01233687). TAK701 is also a humanized monoclonal antibody that binds to HGF and inhibits c-Met activation, and has shown promise in overcoming EGFR TKI resistance in pre-clinical NSCLC studies [48].

A recent study reported that gemcitabine, a ribonucleotide reductase, can inhibit the micrometastasis of NSCLC. Gemcitabine is known as a nucleoside analog, which suppresses tumor growth by affecting DNA replication and repair mechanisms, hence inducing apoptosis [49]. Gemcitabine targets tumor cells that are Epithelial Cell Adhesion Molecule associated antigen (EpCAM)-positive by inhibiting the HGF/c-Met pathway through a mechanism that is presently unclear [50]. In another recent study, it has also been shown that the knock-down of 6-phosphogluconate dehydrogenase, an enzyme in the oxidative pentose phosphate pathway, can downregulate the phosphorylation of c-Met [51].

## Conclusion

Many RTKs have been examined as targets for the treatment of NSCLC. The c-Met RTK has been an especially attractive target due to its known functionality in processes such as cell survival, tissue migration, and wound healing. In cancer, c-Met is often dysregulated by overexpression, gene amplification or mutation, and has been shown to contribute to sustained proliferation, invasion, metastasis, and angiogenesis of cancer cells. Specifically in NSCLC, c-Met has been found to be overexpressed, amplified and mutated, all of which are associated with poor prognosis and increased malignancy of NSCLC [52-54]. Thus, various therapies and drugs targeting c-Met are currently being tested to improve the progression free survival and overall survival of NSCLC patients. Although certain phase III clinical trials have failed to meet their endpoints, c-Met inhibitors have the potential to benefit specific subsets of NSCLC patients on a clinical basis. Therefore, it is extremely important to develop diagnostic testing, and to identify predictive biomarkers, to better determine the benefit of anti-c-Met/HGF therapy. Combinatorial therapies have also proved to be more effective in NSCLC clinical trials when compared to monotherapies, due to the development of resistance. Overall, further investigation is necessary to move c-Met inhibitors to the final stages of clinical development, in which they have potential to improve the status of NSCLC patients.

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