cMET Exon 14 Skipping: From the Structure to the Clinic

Nele Van Der Steen, MSc,[a,b,c] Elisa Giovannetti, MD, PhD,[a,d] Patrick Pauwels, MD, PhD,[b,c] Godefridus J. Peters, MD, PhD,[a] David S. Hong, MD, PhD,[e] Federico Cappuzzo, MD, PhD,[f] Fred R. Hirsch, MD, PhD,[g] Christian Rolfo, MD, PhD[c,h,*]

[a]*Department of Medical Oncology, VU University Medical Center, Amsterdam, The Netherlands*
[b]*Department of Pathology, Antwerp University Hospital, Edegem, Antwerp, Belgium*
[c]*Center for Oncological Research, University of Antwerp, Wilrijk, Antwerp, Belgium*
[d]*Cancer Pharmacology Lab, Italian Association for Cancer Research Start-Up Unit, University of Pisa, Hospital of Cisanello, Pisa, Italy*
[e]*Investigational Cancer Therapeutics, Division of Cancer Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, Texas*
[f]*Department of Oncology, Tuscan Tumor Institute, Florence, Italy*
[g]*Division of Medical Oncology, University of Colorado, Aurora, Colorado*
[h]*Phase I Early Clinical Trials Unit, Oncology Department, Antwerp University Hospital, Antwerp, Belgium*

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ABSTRACT

The abnormal stimulation of the multiple signal transduction pathways downstream of the receptor tyrosine kinase mesenchymal-epithelial transition factor (cMET) promotes cellular transformation, tumor motility, and invasion. Therefore, cMET has been the focus of prognostic and therapeutic studies in different tumor types, including non–small cell lung cancer. In particular, several cMET inhibitors have been developed as innovative therapeutic candidates and are currently under investigation in clinical trials. However, one of the challenges in establishing effective targeted treatments against cMET remains the accurate identification of biomarkers for the selection of responsive subsets of patients. Recently, splice site mutations have been discovered in cMET that lead to the skipping of exon 14, impairing the breakdown of the receptor. Patients with NSCLC who are carrying this splice variant typically overexpress the cMET receptor and show a response to small molecule inhibitors of cMET. Here, we review the main differences at the structural level between the wild-type and the splice variants of cMET and their influence on cMET signaling. We clarify the reason why this variant responds to small molecule inhibitors and their prognostic/predictive role.

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Keywords: NSCLC; cMET; Exon 14 skipping; Targeted therapies

Introduction

The receptor tyrosine kinase mesenchymal-epithelial transition factor (cMET) is known as an oncogenic driver in non–small cell lung cancer (NSCLC), together with its ligand hepatocyte growth factor (HGF). However, the question of which biomarker to use for patient selection remains.[1] Earlier clinical trials used cMET expression as a biomarker,[2] but nowadays MNNG HOS Transforming gene (*cMET*) amplification is used. However, the best cutoff value still has to be determined. Besides amplification, several mutations in the kinase domain of the receptor have been described, although their impact on treatment is not yet clear.[3] Besides amplification, several

*Corresponding author.

Drs. Hirsch and Rolfo contributed equally to this article.

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Address for correspondence: Christian Rolfo, MD, PhD, Antwerp University Hospital, Oncology, Wilrijkstraat 10, Edegem, Antwerp 2650, Belgium. E-mail: christian.rolfo@uza.be

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mutations in the kinase domain of the receptor have been described, although their impact on treatment is not yet clear.[3] A splice variant of cMET has also been discovered,[4,5] and in recent years it has been shown that this confers sensitivity to cMET small molecule inhibitors.[6,7] This splice variant is not restricted to NSCLC tumors but has also been detected in gastrointestinal malignancies.[8] This review will focus on this splice site variant of cMET, explaining the structural and functional differences with the wild-type receptor and discussing some patient cases treated with cMET small molecule inhibitors, reviewing the literature up to January 4, 2016, as found in PubMed.

cMET Signaling

The cMET pathway plays an important role in embryogenesis, during which it is necessary for the migration of myogenic precursor cells,[9,10] the correct formation of lymph and blood vessels,[11,12] and the mitosis of hepatocytes.[13] In adults, cMET activation is mainly involved in wound healing, in which it stimulates cell migration and mitosis.[14] During cancer growth, cMET is involved in vasculogenesis,[15] migration, and invasion.[16] Moreover, the receptor is known to be of importance in resistance against epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs),[17] chemotherapy,[18,19] and radiation therapy.[20,21]

The binding of HGF to cMET causes dimerization of the receptor (Fig. 1). This activates the autophosphorylation activity of the receptor, which leads to the phosphorylation of Y1234 and Y1235 and ultimately to the phosphorylation of the docking sites Y1349 and Y1356.[22] Next, the adaptor proteins growth factor receptor bound protein 2, growth factor receptor bound protein–associated binding protein 1, and Src homology 2 domain containing bind to the docking sites of the receptor, starting downstream signaling.[23–25] This signaling leads to the activation of the Ras,[26] phosphoinositide 3–kinase (PI3K)–protein kinase B,[27] signal transducer and activator of transcription 3,[28] and nuclear factor kappa light-chain enhancer of activated B cells[29,30] signaling cascades. However, upon activation of cMET Y1003, which is a docking site for the E3-ligase Casitas B-lineage lymphoma (c-Cbl), is also phosphorylated.[31] c-Cbl ubiquitylates cMET, thus marking it for breakdown. Thereafter, the receptor is internalized and is either recycled back to the membrane or degraded in the lysosomes.[32]

cMET Structure

The *cMET* gene is situated on chromosome 7 and is initially translated as a precursor protein. After cleavage by furin, this precursor protein is split into the 50-kDa

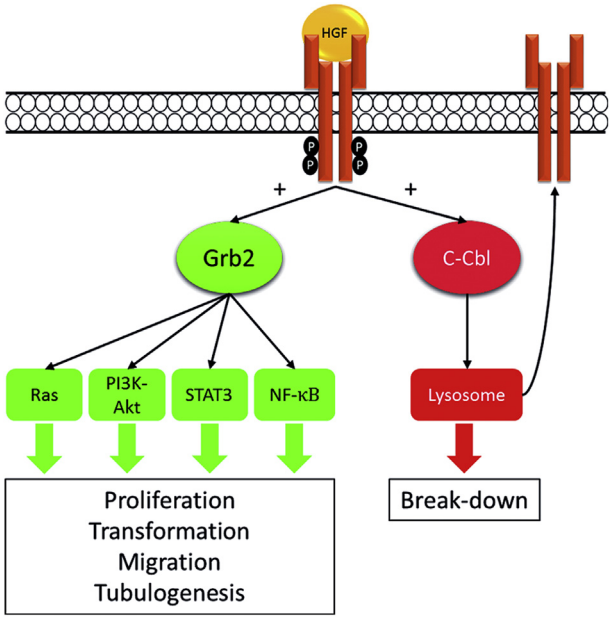

Figure 1. Schematic of the mesenchymal-epithelial transition (cMET) pathway. Upon binding of hepatocyte growth factor, the cMET receptor dimerizes and autophosphorylates. This leads to the binding of adaptor proteins. These adaptor proteins recruit and activate several signaling pathways such as Ras, phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt), signal transducer and activator of transcription 3 (STAT3), and nuclear factor kappa light-chain enhancer of activated B cells (NF-κB). These signalization cascades ultimately lead to cell proliferation, transformation, migration, and tubulogenesis. The E3-ligase c-Cbl also binds the receptor, leading to ubiquitylation and the internalization of cMET into the lysosomes. Next the receptor is either broken down or recycled back to the cell membrane.

α-chain and a 140-kDa β-chain, which are linked by disulfide bonds to form the mature protein[33] (Fig. 2). The α-chain is situated entirely extracellularly, whereas the β-chain is situated extracellularly and intracellularly. The N-terminus of the receptor contains the semaphorin domain, which has the structure of a seven-bladed β-propellor.[34] This semaphorin domain contains the ligand-binding domain and is also necessary for receptor dimerization.[35] A plexin-semaphorin-integrin domain connects the sema domain with a stalk of four immunoglobulin-plexin-transcription domains. This plexin-semaphorin-integrin domain is needed for the correct positioning of the ligand binding domain.[36] Next, the transmembrane domain is spanning the cellular membrane and is connected to the juxtamembrane domain, which contains the c-Cbl binding site, including Y1003. The tyrosine kinase domain consists of two lobes that form the adenosine triphosphate (ATP)-binding pocket. During inactivity of the receptor, entrance to this pocket is blocked by the activation loop. Upon phosphorylation of Y1230, Y1234, and Y1235, conformational changes take place that discard the

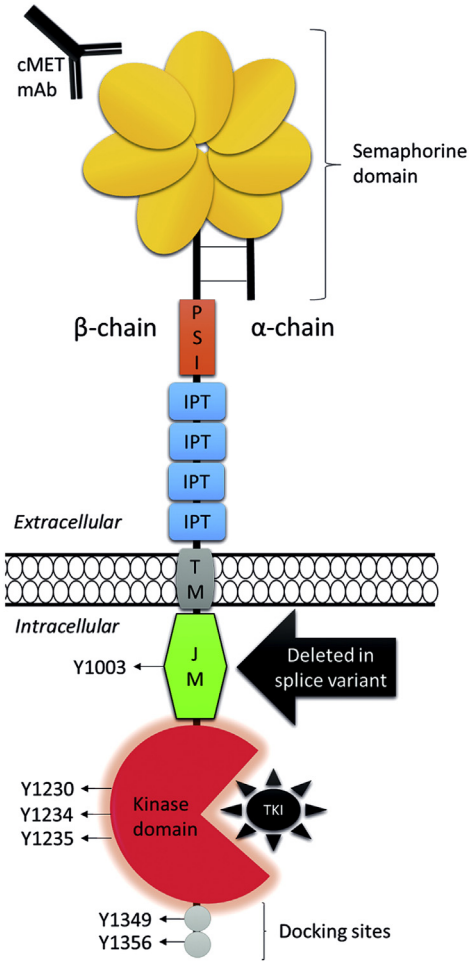

Figure 2. Structure of mesenchymal-epithelial transition (cMET). This figure represents the structure of the cMET receptor from the N-terminus to the C-terminus, with the semaphorin domain, plexin-semaphorin-integrin (PSI) domain, immunoglobulin-plexin-transcription (IPT) domain, transmembrane domain (TM), juxtamembrane domain (JM), kinase domain, and docking sites shown. When exon 14 skipping occurs, the juxtamembrane domain is deleted from the receptor. cMET monoclonal antibodies (mAbs) target the extracellular semaphorin domain, containing the ligand-binding site. cMET-tyrosine kinase inhibitors (TKIs) target the ATP-binding pocket of the kinase domain. Important tyrosines are situated on the receptor.

activation loop from the ATP-binding site.[37] Finally, the N-terminus of the receptor contains the docking sites (Y1349 and Y1356) for the adaptor proteins that are required for signaling.[38]

The Splicing Mechanism

In eukaryotes, most genes consist of exons separated by larger intronic sequences (Fig. 3). Once a gene gets translated to pre-mRNA, these introns need to be spliced out to obtain the mature mRNA, which is translated to protein in a next step. There are four important conserved cis-acting sequences necessary for the spliceosome to function: the 5′ splice site (SS) GU, the branching site A, the polypyrimidine tract, and the 3′ SS AG. The assembly of the spliceosome is started by the formation of complex A by the binding of U1 small nuclear ribonucleoprotein at the 5′SS and the binding of U2 small nuclear ribonucleoprotein to both the branch site and the 3′SS. Here the polypyrimidine tract is necessary for a correct identification and assembly of complex A.[39,40] Through the binding of U4, U5, and U6, complex B is formed, after which U1 and U4 are excluded again.[41] Finally, the association of cell division cycle 5 like (CDC5L) results in the active spliceosome. The splicing reaction consists of two transesterifications. In a first step, the 2′-OH group of the branching site adenosine attacks the 5′SS, which results in the formation of a lariat. After the repositioning of the spliceosome[42] the resulting free –OH group of the 5′SS will attack the 3′SS, resulting in the ligation of both exons and the exclusion of the intron in the form of a lariat, as also reviewed by Smith et al.[43]

Normal splicing is regulated by several factors. First, there are cis-acting enhancer and silencer sequences that can be either intronic or exonic. The enhancer sequences are binding sites of Ser-Arg–rich proteins, which promote splicing. The silencer sequences, on the other hand, function as binding sites for heterogeneous nuclear ribonucleoproteins that act as splicing inhibitors. The combined effects of these proteins contribute to the splicing efficiency and fidelity.[44] Next, the formation of secondary structures can shorten the relative distance between both splice sites, thus facilitating splicing.

In contrast, aberrant splicing can be caused by secondary structures occluding either the 3′SS, 5′SS, or both, leading to alternative splicing of the pre-mRNA by impairing the binding of spliceosome proteins.[45] They also influence the efficiency of splicing by occluding silencer or enhancer sequences or promote alternative splicing by favoring decoy splice sites.[46] Also, mutations affecting one of the four conserved sequences prevent correct splicing of the exon, thus favoring the selection of decoy splice sites. This can lead to the skipping of exons or the inclusion of introns. Furthermore, mutations in the enhancer or silencer sequences affecting the binding of Ser-Arg–rich proteins or heterogeneous nuclear ribonucleoproteins can also lead to aberrant splicing, which in turn can play a role in cancer growth.[47]

cMET Splice Variant

In 1994 Lee et al. described for the first time a shorter variant of the cMET receptor in mice. This variant had an in-frame deletion of 47 amino acids in the juxtamembrane domain.[4] When this variant was transiently expressed in mouse NIH3T3 fibroblasts, these cells were able to form colonies in soft agar and became

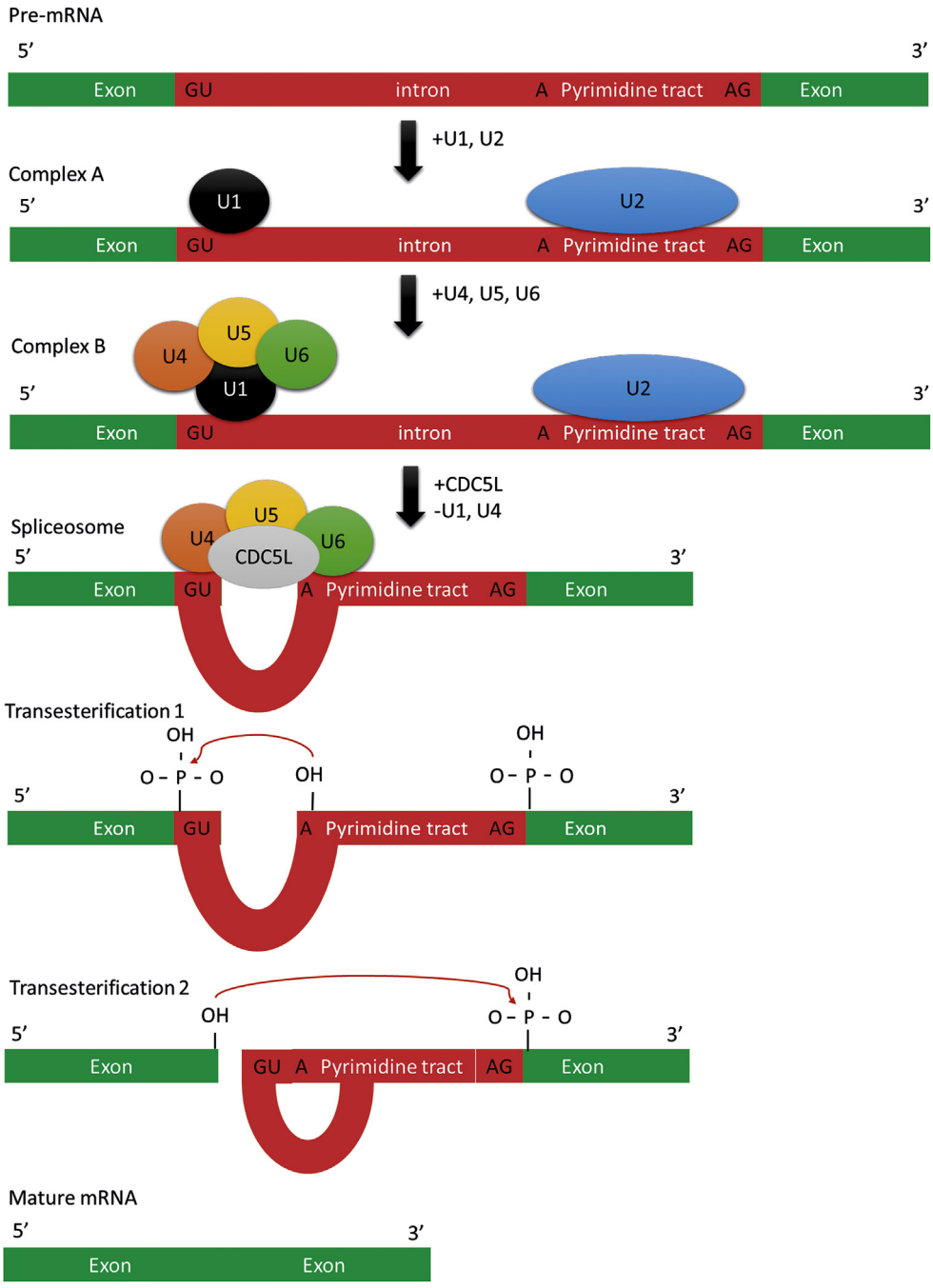

Figure 3. mRNA splicing. This schematic represents the different steps in mRNA splicing, with the important nucleotides, proteins, and chemical reactions shown. Splicing starts with the formation of complex A by the binding of U1 small nuclear ribonucleoprotein at the 5′SS and the binding of U2 small nuclear ribonucleoprotein to both the branch site and the 3′SS. Through the binding of U4, U5, and U6 complex B is formed, after which U1 and U4 are excluded. The association of cell division cycle 5 like results in the active spliceosome. The splicing reaction itself consists of two transesterifications. In a first step the 2′-OH group of the branching site adenosine attacks the 5′SS, which results in the formation of a lariat. The resulting free -OH group of the 5′SS will attack the 3′SS, resulting in the ligation of both exons and the exclusion of the intron in the form of a lariat.

tumorigenic in vivo. This shorter cMET variant showed no significant difference in phosphorylation compared with the wild-type receptor but has a delayed ubiquitinylation.[48] Moreover the p85 subunit of PI3K shows a stronger association with the splice variant of cMET, thus affecting downstream signaling.[49] In particular PI3K is important for cMET-driven migration, tubulogenesis, and apoptosis resistance.[25] The deleted part of the receptor also contains the Y1003, which is the binding site of c-Cbl. The levels of phosphorylated c-Cbl

are similar in cells transfected with either wild-type cMET or the splice variant, but there is a decreased binding of c-Cbl to the splice variant. This leads to impaired breakdown of the receptor and gives an explanation for the higher expression of the splice variant compared with wild-type cMET.[50] Besides a splice variant of cMET, a fusion protein of Tpr-cMET is also missing the cMET juxtamembrane domain. When this domain is inserted into the fusion protein, the fusion protein loses its oncogenic property, illustrating the important regulatory function of this domain.[51]

The H596 NSCLC cell line carries a 3′ SS mutation, leading to the exon 14 skipping. When this cell line was treated with MetMAb in vitro, the cMET signal and proliferation of the cells was impaired in a dose-dependent manner.[50] Similarly, the concentration that inhibits 50% of HEK293 cells for crizotinib decreased fivefold after induction of the exon 14 deletion of cMET.[52] These responses to both small molecule inhibitors and anti-cMET monoclonal antibodies can be explained when looking at the changes in the receptor. The splice variant indeed shows a 47 amino acid deletion in the juxtamembrane of the receptor, situated intracellularly just below the cell membrane. Antibodies have been shown to bind the extracellular part of the receptor. Because this deletion does not influence their epitopes, they will still be able to bind the receptor and exert their inhibitory function. Small molecule inhibitors, on the other hand, bind intracellularly. Most inhibitors have been developed to interfere with the ATP-binding domain, which is situated more to the C-terminal part of the receptor than the juxtamembrane domain. Again, no conformational changes of these sites are evoked by the deletion, thus conferring the binding and inhibition of these molecules. The oncogenicity of the splice variant is mainly determined by the impaired breakdown (and thus longer signaling) of the receptor and the stronger association of the p85 PI3K subunit, provoking stronger signaling through this pathway. Given that this splice variant also needs HGF and ATP binding to be activated, both types of inhibitors will be able to block signaling through this splice variant.

The cMET splice variant has been detected in NSCLC[50] and other types of cancer, such as gastric carcinoma[53] and gastrointestinal carcinomas.[8] Several splice site mutations in the cMET gene that ultimately lead to the deletion of exon 14 at the protein level have been described in cancer cells. Onozato et al.[54] found point mutations at the 5′SS or deletions of the 3′SS, the branch site, or the polypyrimidine tract. A missense mutation at Y1003 also ablates the c-Cbl binding site, resulting in the same effect as the skipping of exon 14.[55] Moreover, these somatic mutations were heterozygous at the DNA level, whereas at the protein level the truncated form is much more prevalent than the wild-type receptor.[54] Of note, several reports of patient cases showed that cMET exon 14 skipping is not mutually exclusive with cMET amplification.[6,7]

cMET Pathway-Directed Therapies

There are two classes of agents with clinical activity against receptor tyrosine kinases: antibodies, which target the extracellular domain of the receptor, and small molecule TKIs, which target the intracellular part by competing with ATP, thus inhibiting the autophosphorylation of the receptor and preventing downstream signaling.

Because of the highly conserved tyrosine kinase domain, selectivity is mainly determined by amino acids outside of the ATP binding pocket and their association with the unique side chains of each inhibitor. These structural differences among each receptor or receptor family are indeed taken into account during the design of the inhibitor to obtain selective or nonselective inhibition.[56,57]

Nowadays, several cMET small molecule TKIs are in clinical trials and even more are being developed. Examples of cMET TKIs include crizotinib, capmatinib, and cabozantinib, whereas onartuzumab and ARGX-111 are examples of cMET-targeting antibodies.

Crizotinib was originally developed as a cMET inhibitor,[58,59] but it has also shown activity against anaplastic lymphoma receptor tyrosine kinase (ALK) and ROS proto-oncogene 1, receptor tyrosine kinase (ROS1) rearrangements.[60] During a first in-human trial, PROFILE 1001, some exceptional responders had an ALK rearrangement, after which the attention shifted to this group of patients. In recent years however, some patients who showed cMET amplification (and no ALK rearrangement) were treated with crizotinib and showed remarkable responses, not only in NSCLC[61,62] but also in glioblastoma[63] and gastric[64] and esophageal carcinoma.[65] At the moment, several clinical trials are recruiting for treatment with crizotinib. The CREATE study (EORTC 90101) is recruiting patients with rare cMET-amplified tumors.[66] The METROS trial (NCT02499614) is recruiting patients with both cMET amplification and ROS1 rearrangement, and the AcSé trial (NCT02034981) is recruiting patients with cMET amplification, ALK rearrangement, and ROS1 rearrangement.

Capmatinib had a potent antitumor activity in cMET-dependent cell lines both in vitro and in vivo, but it also showed the cross-talk inhibition of EGFR and human epidermal growth factor receptor 3 activation.[67] This compound is still in phase I/II clinical trials investigating dose escalation (NCT01324479 and NCT02414139), as well as drug combinations with the EGFR TKIs erlotinib, gefitinib, or EGF816 (NCT02468661, NCT01610336 and

NCT02335944) or the anti–programmed cell death protein 1 monoclonal antibody nivolumab (NCT02323126).

Cabozantinib is a nonselective inhibitor that inhibits cMET, as well as vascular epidermal receptor growth factor, ret proto-oncogene, ROS1, AXL receptor tyrosine kinase (AXL), cKIT, and fms related tyrosine kinase 3. It also inhibits tubule formation. The combined effects are possibly responsible for inhibition of migration, invasion, and proliferation and for induction of both tumor and endothelial cell death.[68] In a few cases, cabozantinib was successfully used for the treatment of ret proto-oncogene rearrangement NSCLC.[69,70] Several clinical trials in NSCLC are ongoing, either with cabozantinib as monotherapy (NCT02132598 and NCT01639508) or in combination with erlotinib in patients who showed progressive disease after EGFR TKI treatment (NCT00596648).

MGCD265 is a small molecule inhibitor targeting both cMET and AXL kinase.[71] Currently a single drug phase I/II dose escalation study is being performed and selecting patients with solid tumors harboring cMET or AXL aberrations (NCT02544633).

Onartuzumab is a humanized monoclonal antibody that binds the HGF-binding domain of cMET. It has been investigated in phase I, II, and III trials in NSCLC (NCT01519804, NCT01496742, and NCT01456325),[72] but the recent failure of the phase III testing onartuzumab in combination with erlotinib has hampered its further development and approval.[2]

ARGX-111 is another example of a monoclonal antibody against cMET. It has an enhanced antibody-dependent cellular cytotoxicity, engaging the natural killer cells of the immune system to kill the cancer cells. This antibody is currently in phase I clinical trials.[73]

Treatment of Patients with cMET Exon 14 Skipping

Patients presenting with cMET exon 14 skipping respond to cMET small molecule inhibitors (Table 1).[74–77] Although most data are from isolated cases, they are of utmost importance to understand the potential of anti-cMET treatment in selected patients. First, these splice site mutations arise in men and women alike and appear independent of smoking habits. Cases have been described in all subtypes of NSCLC: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. A wide range of mutations provoke aberrant splicing. In one case, even two independent splice site mutations could be detected in the same tumor. Responses were achieved with treatment of crizotinib, capmatinib, and cabozantinib, ranging from stable disease to complete response, with most patients showing a response of approximately −50%. In two cases disease progression occurred after 5 to 11 months of cMET TKI therapy, suggesting that either resistance mutations[78] can arise or other driver pathways may be activated.[79] Unfortunately, there are not yet any data on these resistance mechanisms.

The question whether patients with cMET exon 14 skipping respond to cMET targeting antibodies remains to be answered, as no reports have described this situation to date. However, the fact that these antibodies bind the extracellular domain of the receptor strongly suggests that these treatments might be effective as well. In addition, treatment of the cMET exon 14–skipping cell line H596 with MetMAb led to a reduction in cMET signaling and cell proliferation, strengthening this prediction.[50]

Future Strategies for cMET Exon 14-Skipping NSCLC

Given that most patients receiving cMET inhibitors ultimately show progression, further knowledge about the resistance mechanisms is needed. There are several preclinical investigations suggesting the influence of other driver pathways (e.g., Kirsten rat sarcoma viral oncogene homolog,[80] Wnt and mammalian target of rapamycin,[81] fibroblast growth factor receptor 2,[82] and RAF and B-Raf proto-oncogene, serine/threonine kinase).[83] Preclinical studies should be conducted to see whether combination therapy against two pathways can overcome this resistance. Moreover, tumor tissue of progressive patients should be analyzed to determine the molecular mechanisms of resistance in patients of different tumor types and the relative importance of the different resistance mechanisms. Deletions in cyclin-dependent kinase inhibitor 2A and cyclin-dependent kinase inhibitor 2B in progressive lesions have been observed,[6] but the influence of these deletions on the response to cMET inhibitors is yet to be determined. Given the fact that both the wild-type receptor and the splice variant are sensitive to cMET inhibitors, it is possible that the resistance mechanisms are also similar, but this hypothesis needs to be proved.

Concluding Remarks

A new challenge arises for the routine detection of this cMET exon 14 skipping. Given the relative small deletion, it remains a question whether antibodies can be developed with enough specificity against this splice variant. No such antibody exists today. One of the solutions can be next-generation sequencing because many different mutations can indeed cause the aberrant splicing of exon 14 at the DNA level, making mRNA sequencing much more straightforward. However, the use of formalin-fixed paraffin-embedded samples in pathology, accompanied by the often poor quality of RNA, raise technical difficulties that should be overcome to implement the detection of this splice variant in clinical routine.

Table 1. Overview cMET Exon 14 Skipping Patients Who Received Anti-cMET Therapy: An Overview of the Characteristics of the Patients Described Thus Far Who Presented with cMET Exon 14 Skipping and Were Treated with cMET Small Molecule Inhibitors

Age	Sex	Smoker	Cancer Type	Previous Treatments	cMET ex14 Splice Mutation	Other Genetic Information	cMET Inhibitor	Response	Ref
84	Female	Never	Stage III histiocytic sarcoma	None	c2888-5_2944del62	*TP53* pR175H *ZMYM3* c3008-1G>A	Crizotinib	-60% progression after 11 mo	7
82	Female	25 PY	Stage IV large cell lung cancer	Resection	c3028G>C	*TP53* pN30fs*14	Capmatinib	-53%	7
66	Female	4 PY	Stage I squamous carcinoma lung	Resection Gemcitabine + carboplatin Palliative radiotherapy Paclitaxel + carboplatin CHK1 inhibitor	c3028+1G>T	NA	Capmatinib	-61%	7
80	Female	Never	Stage Ia lung adenocarcinoma	Docetaxel Pemetrexed Radiotherapy	c3028G>C	*cMET* amplification	Cabozantinib	Stable disease	6
78	Male	Yes	Stage IV adenocarcinoma lung	Carboplatin + pemetrexed + bevacizumab Pemetrexed + bevacizumab Albumin-bound paclitaxel	c3024_3028delAGAAGGT ATATT	*CDKN2A* deletion *CDKN2B* deletion	Crizotinib	-30%	6
65	Male	Yes	Stage IV adenocarcinoma lung	Cisplatin + pemetrexed + bevacizumab Pemetrexed + bevacizumab Gemcitabine	c3028+1G>T	*EGFR* WT *ALK* WT	Crizotinib	-31%	6
90	Female	Never	Metastatic adenocarcinoma lung	Pemetrexed Gemcitabine	c3028G>T	*CDK4* amplification *MDM2* amplification	Crizotinib	-47%	6
64	Female	Never	Metastatic poorly differentiated adenocarcinoma	Chemotherapy (not specified)	c3028G>A	*EGFR*, *KRAS*, *BRAF*, *ALK*, *ROS1* WT cMET amplification	Crizotinib	Ongoing response at 8 mo	74
71	Male	15 PY	Metastatic lung adenocarcinoma	Radiotherapy (3000 cGy) Carboplatin + pemetrexed	c3082G>C	No *cMET* amplification	Crizotinib	Ongoing response at 6 mo	75
86	Male	Never	Metastatic lung adenocarcinoma	Radiotherapy Pemetrexed	c2887-18_2887-7del12	NA	Crizotinib	Response, but discontinued because of pneumonitis	76
61	Male	Never	Sarcomatoïd NSCLC	Radiotherapy Carboplatin + paclitaxel + bevacizumab	c2888-5_2890TTAAGATC>A c3028+2T>G c3280C>T	NA	Crizotinib	Partial response Progression after 5 mo	77

cMet, mesenchymal-epithelial transition factor; Ref, reference; *cMet*, MMNG HOS Transforming gene; *TP53*, tumor protein p53 gene; *ZMYM3*, zinc finger MYM-containing 3 gene; PY, pack-years; NA, not applicable; *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; *CDKN2B*, cyclin-dependent kinase inhibitor 2B gene; *EGFR*, epidermal growth factor receptor gene; WT, wild type; *ALK*, anaplastic lymphoma receptor tyrosine kinase gene; *CDK4*, cyclin-dependent kinase 4 gene; *MDM2*, MDM2 proto-oncogene, E3 ubiquitin protein ligase gene; *KRAS*, Kirsten rat sarcoma viral oncogene homolog gene; *ROS1*, ROS proto-oncogene 1, receptor tyrosine kinase gene; NSCLS, non–small cell lung cancer.

With the development of targeted therapies, optimal biomarkers to select the patients who would benefit most are warranted. Known examples include sensitizing mutations in EGFR for treatment with erlotinib/gefitinib,[84] and ALK rearrangement for treatment with crizotinib.[85] The selection of patients for treatment with cMET inhibitors still remains a challenge. Today, the most widely used biomarker is cMET, which has a prevalence of approximately 3% in NSCLC.[86] In recent years the splice variant of cMET that misses exon 14 has arisen as a new biomarker for therapy with cMET inhibitors, identifying a new patient population. cMET TKIs will inhibit the activity of this splice variant because the kinase domain remains unchanged and monoclonal antibodies against cMET can also still bind the unchanged extracellular part of the receptor. Patients presenting with cMET exon 14 skipping are eligible for therapy with cMET inhibitors, thus opening up a new population of approximately 4% in NSCLC.[55]

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