Met is a tyrosine kinase that has hepatocyte growth factor as its ligand. Met plays a major role in cell growth, migration and morphological changes. Overexpression of hepatocyte growth factor and Met and mutations and amplification of MET have been noted in many forms of cancer and are reportedly correlated with cancer progression and a poor prognosis. Over the past few years, these molecules have attracted attention as targets of molecularly targeted therapies. This article describes the association relationship between hepatocyte growth factor/Met and cancer and it describes the latest findings regarding inhibitors to target hepatocyte growth factor/Met that are currently being developed.

Key words: HGF – Met – EGFR-TKI resistance – tyrosine kinase

ABNORMALITIES IN HGF/MET SIGNALING PATHWAYS IN CANCER

A transmembrane tyrosine kinase receptor, Met is a heterodimer consisting of a 45 kDa extracellular α-subunit and 145 kDa transmembrane β-subunit. Hepatocyte growth factor (HGF) is the only known ligand of the tyrosine kinase receptor Met. When the ligand HGF binds to Met’s Sema domain, Met dimerizes. In accordance with changes in its three-dimensional structure, tyrosine residues 1230, 1234 and 1235 in the tyrosine kinase domain are phosphorylated. Tyrosine residues 1349 and 1356 in the C-terminal region are also phosphorylated, and adapter proteins bind to these residues, activating Met. When Met is activated and adapter proteins bind to the tyrosine residues in the C-terminal region, activation of downstream signaling pathways such as PI3K/Akt, Ras/Rac/Rho and Ras/MAPK is facilitated. This signaling is known to induce cell growth, survival, and migration and angiogenesis (Fig. 1) (1,2).

Enhancement of abnormal HGF/Met signaling as a result of overexpression of HGF and Met and mutations and amplification of MET is associated with the progression of various forms of cancer. Overexpression of HGF has been noted in numerous forms of cancer, such as lung cancer (50%), breast cancer (91%), stomach cancer (87%), colon cancer (95%), cancer of the head and neck (45%) and liver cancer (33%) (3). Elevated levels of HGF in the blood are reportedly a factor for a poor prognosis for several forms of cancer (4). Moreover, overexpression of HGF in lung cancer is also known to be a factor for resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKIs). Overexpression of Met in tumor tissue has been noted in a range of cancers, such as lung cancer, stomach cancer, breast cancer, kidney cancer and colon cancer. Overexpression of Met is reportedly a factor for a poor prognosis. MET-activating mutations in Met’s tyrosine kinase domain have been noted in hereditary and sporadic renal cell carcinomas, pediatric liver cancer and squamous cell carcinoma of the head and neck. Other mutations in Met’s juxtamembrane region or in its Sema domain have been noted in cancers such as stomach cancer, breast cancer, pleural mesothelioma and small-cell lung cancer. MET amplification has been noted primarily in gastrointestinal cancers such as stomach cancer, esophageal cancer and colon cancer. In addition, Met inhibitors have become a key therapy to treat lung cancer over the past few years. MET amplification is reportedly involved in acquisition of resistance to EGFR-TKIs. An association between HGF/Met genetic abnormalities and cancer has been noted for
numerous forms of cancer. HGF/Met signaling is also reportedly involved in resistance to TKIs such as sunitinib and lapatinib as well as to EGFR-TKIs (5,6). HGF and Met are therapeutic targets that have attracted a great deal of attention over the past few years.

**EGFR-TKI RESISTANCE AND HGF/MET SIGNALING**

*EGFR*-activating mutations (exon 19 deletion or a point mutation in L858R in exon 21) are detected in 10–30% of non-small-cell lung cancers, and non-small-cell lung cancers with *EGFR*-activating mutations respond well to the EGFR-TKIs gefitinib and erlotinib (7). Nevertheless, many patients acquire drug resistance after 6 months to a year. Over the past few years, the mechanisms of resistance to EGFR-TKIs have been clarified. The first reported mechanism was a gatekeeper mutation (T790M) in exon 20 in *EGFR* (8). Met amplification is reportedly a primary mechanism for EGFR-TKI resistance; another is high levels of HGF expression (which the current authors noted) (9,10). As part of a joint study at 10 facilities in Japan, the current authors analyzed tumor specimens from patients with EGFR-TKI resistance. Analysis of specimens of 23 tumors from 23 patients with acquired resistance revealed the T790M mutation in 12/23 tumors (52%), Met amplification in 2/23 (9%) and high levels of HGF expression in 14/23 (61%). The increased incidence of activation of HGF/Met signaling may contribute to acquisition of resistance to EGFR-TKIs (11). High levels of HGF expression play three roles in the acquisition of EGFR-TKI resistance. The first is where Met is activated by HGF, and Met in turn activates Gab1/PI3K/Akt signaling; survival signaling occurs via these alternate pathways, inducing EGFR-TKI resistance. The second role that high HGF levels play is via stimulating growth of subpopulations of cells with Met amplification. A preclinical study cultured HCC827 lung cancer cells with *EGFR*-activating mutations in the presence of HGF and EGFR-TKIs. Growth of subpopulations of cells with Met amplification was stimulated, and these cells accounted for most of the cell growth (12). The third role that high HGF levels play is as a factor for resistance of T790M-mutant tumors to treatment with next-generation EGFR-TKIs such as irreversible EGFR-TKIs (CL-387, CL-785 and BIBW2992) or a mutant EGFR-selective TKI (WZ4002) (13,14). In a study involving lung cancer cell lines, the current authors found that HGF induced resistance to gefitinib and erlotinib as well as to next-generation EGFR-TKIs. EGFR-TKI resistance induced by HGF should be overcome by HGF/Met inhibitors. The current authors used E7050, a Met inhibitor, to determine whether EGFR-TKI resistance due to high levels of HGF expression could be overcome. E7050 is an ATP-competitive small molecular compound with an IC₅₀ of 23 nM in relation to Met in a cell-free system. Clinical trials involving several forms of cancer are currently underway. Gefitinib powerfully inhibits the growth of PC-9 and HCC827 lung cancer cells with *EGFR*-activating mutations, but addition of HGF causes those cells to become resistant. However, gefitinib resistance as a result of added HGF is overcome by combined use of E7050. Gefitinib-resistant clones were created by long-term exposure to HGF and gefitinib, and the growth was found to be inhibited by combined use of E7050. One model of resistance due to HGF produced by tumor stroma is a mouse model in which HGF-producing human MRC-5 fibroblasts were subcutaneously implanted along with PC-9 cells. Combined therapy with E7050 was found to overcome gefitinib resistance due to HGF produced by MRC-5 cells, and this therapy was found to have tumor-shrinking action (14). In the future, the effectiveness with which combination therapy with EGFR-TKIs and E7050 overcomes resistance due to HGF/Met should be verified in clinical trials while carefully assessing the safety of that therapy.

**CLINICAL DEVELOPMENT OF HGF/MET INHIBITORS**

Numerous HGF/Met inhibitors besides E7050 are being developed, and clinical trials are being conducted with a wide range of cancers (Table 1). Ficlatuzumab is an anti-HGF antibody. A Phase II trial involving Asians with little history of smoking and untreated Stage IIB/IV lung adenocarcinoma (188 patients) has been conducted. The trial compared gefitinib alone (94 patients) and gefitinib + ficlatuzumab (94 patients) (15). Differences in the response rate (the trial’s primary endpoint) were not noted, but the two treatments did not result in significant differences in progression-free survival (4.7 vs. 5.6 months). However, stratified analysis of biomarkers in patients with stroma that expressed high levels of HGF indicated that patients administered gefitinib + ficlatuzumab had a significantly longer overall survival compared with patients administered gefitinib alone (94 patients).
However, the trial had a small sample, and so further studies are needed to verify the effectiveness of anti-HGF antibodies. HGF’s involvement in lung cancer treated with EGFR-TKIs was ascertained only with regard to the resistance it induced in EGFR-mutant lung cancer cells. This is directly related to the trial design. In other words, a trial should be designed so that only patients with EGFR-mutant lung cancer are selected and so that anti-HGF antibodies are added to therapy with EGFR-TKIs. Otherwise, the trial cannot verify the effectiveness of those antibodies.

MetMAb (onartuzumab) is a human monovalent anti-Met monoclonal antibody. Many anti-Met antibodies have a drawback in that they bind with Met, facilitating dimerization. As a result, they act agonistically. Thus, MetMAb was created to inhibit the activation of Met by HGF; MetMAb is monovalent, and so it avoids dimerizing Met even if it binds to Met. Since MetMAb has action to inhibit ligand-induced Met activation, it may not have action to inhibit amplified Met. A Phase I trial was conducted with a patient who had metastatic gastric cancer that was refractory to chemotherapy and expression of both HGF and Met. A complete response as a result of treatment with MetMAb was noted for 2 years, suggesting that MetMAb is effective in treating cancer with abnormal HGF/Met (16). A Phase II trial was conducted with 137 patients with non-small-cell lung cancer that was refractory to chemotherapy that did not include EGFR-TKIs. Patients were assigned to one of two groups, a group receiving MetMAb + erlotinib or a group receiving a placebo + erlotinib. The trial verified the efficacy of adding MetMAb to erlotinib. There were no significant differences in the progression-free survival of the two groups of patients; patients who were also administered MetMAb had a progression-free survival of 2.2 months, while patients given a placebo had a progression-free survival of 2.6 months (hazard ratio: 1.09, \( P = 0.687 \)). However, levels of Met expression according to immunostaining were classified as high and low levels and then analyzed. Results revealed that the progression-free survival for patients with high levels of Met expression was 2.9 months for those who were also administered MetMAb and 1.5 months for those who were given a placebo. Patients who were also administered MetMAb had a progression-free survival that was about two times longer, and so significant improvement was noted (hazard ratio: 0.53, \( P = 0.04 \)). In addition, the overall survival for patients with high levels of Met expression was 12.6 months for those who were also administered MetMAb and 3.8 months for those who were given a placebo. Patients who were also administered MetMAb had an overall survival that was about three times longer (hazard ratio: 0.37, \( P = 0.002 \)). In the future, indicators of HGF/Met inhibitors must be verified further for combined therapy with EGFR-TKIs.

Tivantinib (ARQ197) is a small molecular compound that is being developed as a selective non-adenosine triphosphate (ATP)-competitive Met inhibitor (17). Recently, tivantinib was found to have microtubule-disrupting activity similar to that of vincristine (16). A Phase III clinical trial (MARQUEE Trial) was conducted to verify the effectiveness of adding tivantinib to erlotinib in patients with advanced non-squamous non-small-cell lung cancer that was resistant to platinum-based agents, but the trial was halted.

Crizotinib (PF-2341066, brand name: Xalkori™) is an anaplastic lymphoma kinase (ALK) inhibitor. Crizotinib was approved for non-small-cell lung cancer that tested positive for the EML4-ALK fusion gene in the USA in 2011, and the drug was similarly approved in Japan in March 2012. In addition to its inhibition of ALK, crizotinib has ROS1- and Met-inhibiting activity. The drug is currently being developed with a focus on its Met-inhibiting activity. Patients with non-small-cell lung cancer with MET amplification that tested negative for the EML4-ALK fusion gene reportedly responded to crizotinib (18). In addition, improvement in clinical

### Table 1. HGF/Met inhibitors in clinical trials

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
<th>Tumor types in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rilotumumab</td>
<td>AMG102</td>
<td>HGF</td>
</tr>
<tr>
<td>Ficlatuzumab</td>
<td>AV-299</td>
<td>HGF</td>
</tr>
<tr>
<td>Onartuzumab</td>
<td>MetMAb</td>
<td>Lung, colon, breast</td>
</tr>
<tr>
<td>Small molecule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crizotinib (Xalkori™)</td>
<td>PF-2341066</td>
<td>Met, ALK, ROS1</td>
</tr>
<tr>
<td>Tivantinib</td>
<td>ARQ197</td>
<td>Lung, lymphoma</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>XL184</td>
<td>Met, VEGFR2, Ret, Flt-3, Kit, Tie2</td>
</tr>
<tr>
<td>Foretinib</td>
<td>XL880</td>
<td>Lung, breast, liver, renal, stomach, head and neck</td>
</tr>
<tr>
<td>Golvatinib</td>
<td>E7050</td>
<td>Met, VEGFR2</td>
</tr>
<tr>
<td>MGCD265</td>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>BMS-777607</td>
<td>Met, Ron</td>
<td>Solid tumors</td>
</tr>
<tr>
<td>AMG208</td>
<td>Met</td>
<td>Solid tumors</td>
</tr>
</tbody>
</table>

HGF, hepatocyte growth factor.
symptoms and tumor-shrinking action has been noted as a result of crizotinib administration in esophagogastric adenocarcinomas and glioblastoma multiforme with MET amplification (19,20). Thus, MET amplification may be a predictive biomarker for efficacy of Met inhibitors such as crizotinib.

CONCLUSION

Over 20 years have passed since Met and HGF were first discovered. Numerous studies have reported that abnormal HGF/Met signaling in cancer is related to disease progression, and Met and HGF have attracted attention as therapeutic targets. The efficacy and safety of numerous HGF/Met inhibitors are now being verified in clinical trials. Biomarkers and new molecularly targeted drugs are likely to be developed based on preclinical and clinical evidence regarding which inhibitors are efficacious in treating certain cancers with abnormal HGF/Met signaling.

Funding

This study was supported by Grants-in-Aid for Cancer Research (S.Y., 21390256), Scientific Research on Innovative Areas ‘Integrative Research on Cancer Microenvironment Network’ (S.Y., 22112010A01) and Grant-in-Aid for Project for Development of Innovative Research on Cancer Therapeutics (P-Direct) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

Conflict of interest statement

Takayuki Nakagawa is an employee of Eisai Co., Ltd for oncology research. Seiji Yano received honoraria from Chugai Pharmaceutical Co., Ltd and AstraZeneca. Seiji Yano received research funding from Chugai Pharmaceutical Co., Ltd, Kyowa Hakko Kirin Co., Ltd and Eisai Co., Ltd.

References