Current Topics

Target Therapy for Cancer: Anti-cancer Drugs Targeting Growth-Factor Signaling Molecules

Receptor Tyrosine Kinases and Targeted Cancer Therapeutics

Kenji Takeuchi* and Fumiaki Ito

Department of Biochemistry, Faculty of Pharmaceutical Sciences, Setsunan University; Hirakata, Osaka 573–0101, Japan.

Received June 22, 2011

The majority of growth factor receptors are composed of extracellular, transmembrane, and cytoplasmic tyrosine kinase (TK) domains. Receptor tyrosine kinase (RTK) activation regulates many key processes including cell growth and survival. However, dysregulation of RTK has been found in a wide range of cancers, and it has been shown to correlate with the development and progression of numerous cancers. Therefore, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Low-molecular-weight TK inhibitors (TKIs), such as imatinib, targeting tumors with mutant c-Kit, and gefitinib, targeting non-small cell lung cancer with mutant epidermal growth factor receptor (EGFR), have received marketing approval in Japan. MET, fibroblast growth factor receptor (FGFR), and insulin-like growth factor-I receptor (IGF-IR) are frequently genetically altered in advanced cancers. TKIs of these receptors have not yet appeared on the market, but many anticancer drug candidates are currently undergoing clinical trials. Most of these TKIs were designed to compete with ATP at the ATP-binding site within the TK domain. This review will focus on small-molecule TKIs targeting MET, FGFR, and IGF-IR and discuss the merits and demerits of two types of agents, i.e., those with only one or a few targets and those directed at multiple targets. Targeting agents specifically inhibiting the target kinase were previously searched for based on the hypothesis that a narrow target window might reduce unexpected side effects, but agents with multiple targets have been recently developed to overcome tumors resistant against a single-targeting agent.

Key words cancer; receptor tyrosine kinase; tyrosine kinase inhibitor; MET; fibroblast growth factor receptor; insulin-like growth factor-I receptor

1. INTRODUCTION

Growth factors and their receptors are the core components of signal transduction pathways. The majority of growth factor receptors are composed of extracellular, transmembrane, and cytoplasmic tyrosine kinase domains. Growth factor binding to the extracellular domain leads to the activation of the cytoplasmic tyrosine kinase. Receptor tyrosine kinase (RTK) activation regulates many key processes in cell growth, survival, organ morphogenesis, neovascularization, and tissue repair and regeneration. In normal cells, RTK activity is strictly regulated; but dysregulation or constitutive activation of RTK has been found in a wide range of cancers. The deregulated activation occurs by gain-of-function mutation, gene rearrangement, gene amplification, over-expression or abnormal autocrine, endocrine or paracrine stimulation of both receptor and ligand; and, in some cases, it has been shown to correlate with the development and progression of numerous human cancers. Since RTKs have been implicated in many aspects of the malignant phenotype, they are emerging as promising therapeutic targets.

Cancer therapy targeting RTKs will be successful only if the targeted RTK is a major regulator of cancer cell survival. Cancer cells contain multiple genetic and epigenetic abnormalities. Despite this complexity, their survival and/or proliferation can often be impaired by the inactivation of a single oncogene. This phenomenon, called “oncogene addiction,” provides a rationale for molecular targeted therapy. Convincing evidence for the concept of oncogene addiction comes from the increasing number of examples of the therapeutic efficacy of antibodies or small molecules that target a specific oncogene. Many targeting molecular cancer therapeutics have received marketing approval in Japan. Among them, antibodies or small molecules that target growth factor receptors are listed in Table 1. In breast cancer, over-expression of HER2 (ErbB2) occurs in approximately 25% of patients and is associated with shorter survival. The epidermal growth factor receptor (EGFR) is widely up-regulated in solid tumors and mediates many characteristics of malignant phenotype, including proliferation, protection from apoptosis, and tumor cell motility. These findings led to the development of antibodies that target HER2 and EGFR, and the validity of both growth factor receptors as therapeutic targets is illustrated by the successes of trastuzumab and cetuximab.

Given the oncogenic role of aberrant signaling from RTK, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Examples of successful therapeutic intervention by use of RTK inhibitors include imatinib, for treatment of gastrointestinal stromal tumors with mutant c-Kit, and gefitinib and erlotinib, for treatment of non-small cell lung cancers with mutant EGFR. Sunitinib inhibits multiple RTKs besides BCR-ABL and SRC. These multiple RTKs include kinases of the platelet-derived growth factor receptor (PDGFR) and vascular enrichments of evidence for the concept of oncogene addiction comes from the increasing number of examples of the therapeutic efficacy of antibodies or small molecules that target a specific oncogene. Many targeting molecular cancer therapeutics have received marketing approval in Japan. Among them, antibodies or small molecules that target growth factor receptors are listed in Table 1. In breast cancer, over-expression of HER2 (ErbB2) occurs in approximately 25% of patients and is associated with shorter survival. The epidermal growth factor receptor (EGFR) is widely up-regulated in solid tumors and mediates many characteristics of malignant phenotype, including proliferation, protection from apoptosis, and tumor cell motility. These findings led to the development of antibodies that target HER2 and EGFR, and the validity of both growth factor receptors as therapeutic targets is illustrated by the successes of trastuzumab and cetuximab.

Given the oncogenic role of aberrant signaling from RTK, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Examples of successful therapeutic intervention by use of RTK inhibitors include imatinib, for treatment of gastrointestinal stromal tumors with mutant c-Kit, and gefitinib and erlotinib, for treatment of non-small cell lung cancers with mutant EGFR. Sunitinib inhibits multiple RTKs besides BCR-ABL and SRC. These multiple RTKs include kinases of the platelet-derived growth factor receptor (PDGFR) and vascular enrichments of evidence for the concept of oncogene addiction comes from the increasing number of examples of the therapeutic efficacy of antibodies or small molecules that target a specific oncogene. Many targeting molecular cancer therapeutics have received marketing approval in Japan. Among them, antibodies or small molecules that target growth factor receptors are listed in Table 1. In breast cancer, over-expression of HER2 (ErbB2) occurs in approximately 25% of patients and is associated with shorter survival. The epidermal growth factor receptor (EGFR) is widely up-regulated in solid tumors and mediates many characteristics of malignant phenotype, including proliferation, protection from apoptosis, and tumor cell motility. These findings led to the development of antibodies that target HER2 and EGFR, and the validity of both growth factor receptors as therapeutic targets is illustrated by the successes of trastuzumab and cetuximab.

Given the oncogenic role of aberrant signaling from RTK, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Examples of successful therapeutic intervention by use of RTK inhibitors include imatinib, for treatment of gastrointestinal stromal tumors with mutant c-Kit, and gefitinib and erlotinib, for treatment of non-small cell lung cancers with mutant EGFR. Sunitinib inhibits multiple RTKs besides BCR-ABL and SRC. These multiple RTKs include kinases of the platelet-derived growth factor receptor (PDGFR) and vascular enrichments of evidence for the concept of oncogene addiction comes from the increasing number of examples of the therapeutic efficacy of antibodies or small molecules that target a specific oncogene. Many targeting molecular cancer therapeutics have received marketing approval in Japan. Among them, antibodies or small molecules that target growth factor receptors are listed in Table 1. In breast cancer, over-expression of HER2 (ErbB2) occurs in approximately 25% of patients and is associated with shorter survival. The epidermal growth factor receptor (EGFR) is widely up-regulated in solid tumors and mediates many characteristics of malignant phenotype, including proliferation, protection from apoptosis, and tumor cell motility. These findings led to the development of antibodies that target HER2 and EGFR, and the validity of both growth factor receptors as therapeutic targets is illustrated by the successes of trastuzumab and cetuximab.

Given the oncogenic role of aberrant signaling from RTK, RTK has become an attractive therapeutic target. One way to effectively block signaling from RTK is inhibition of its catalytic activity with small-molecule inhibitors. Examples of successful therapeutic intervention by use of RTK inhibitors include imatinib, for treatment of gastrointestinal stromal tumors with mutant c-Kit, and gefitinib and erlotinib, for treatment of non-small cell lung cancers with mutant EGFR. Sunitinib inhibits multiple RTKs besides BCR-ABL and SRC. These multiple RTKs include kinases of the platelet-derived growth factor receptor (PDGFR) and vascular enrichments of evidence for the concept of oncogene addiction comes from the increasing number of examples of the therapeutic efficacy of antibodies or small molecules that target a specific oncogene. Many targeting molecular cancer therapeutics have received marketing approval in Japan. Among them, antibodies or small molecules that target growth factor receptors are listed in Table 1. In breast cancer, over-expression of HER2 (ErbB2) occurs in approximately 25% of patients and is associated with shorter survival. The epidermal growth factor receptor (EGFR) is widely up-regulated in solid tumors and mediates many characteristics of malignant phenotype, including proliferation, protection from apoptosis, and tumor cell motility. These findings led to the development of antibodies that target HER2 and EGFR, and the validity of both growth factor receptors as therapeutic targets is illustrated by the successes of trastuzumab and cetuximab.
2. MET

2.1. MET and Cancer  MET is a transmembrane protein composed of an extracellular α-chain disulfide-bonded to a membrane-spanning β-chain. The tyrosine kinase resides in the cytosolic portion of the β-chain. Hepatocyte growth factor/scatter factor (HGF/SF) binding activates MET via intracellular phosphorylation, thus initiating RAS/mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT signaling as well as several other pathways (Fig. 1). In vivo, HGF/MET signaling leads to increased cell growth, survival, motility, dispersion, migration, invasion/metastasis, angiogenesis, wound healing, and tissue regeneration.\(^{8,9}\) MET mutations have been reported to occur in papillary renal cell carcinoma, head and neck cancer, gastric cancer, colorectal cancer, ovarian cancer, and childhood hepatocellular carcinoma.\(^{10–14}\) MET amplification has been detected in patients with gastric, esophageal, colorectal cancers, and glioblastomas.\(^{15−18}\) Over-expression of MET has also been detected in cell lines established from Asian-prevalent head and neck cancer, nasopharyngeal cancer, pediatric tumors including osteogenic sarcoma and neuroblastoma, and hematopoietic malignancies including multiple myeloma and adult T-cell leukemia.\(^{19−23}\) Constitutive MET activation due to MET amplification and/or MET mutation has been found to be a driver of proliferation and survival. MET expression correlates with an aggressive phenotype and poor prognosis.\(^{24,25}\) Cells engineered to express high levels of wild or mutant MET display a proliferative, motogenic, and/or invasive phenotype, and grow as metastatic tumors in nude mice.\(^{26,27}\)

## Table 1. Example of Targeted Molecular Cancer Therapeutics Received Marketing Approval in Japan

<table>
<thead>
<tr>
<th>Drug type</th>
<th>Drug</th>
<th>Disease indication</th>
<th>Primary molecular target</th>
<th>Approval date in Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>Trastuzumab (Herceptin)</td>
<td>Breast cancer</td>
<td>HER2</td>
<td>April 4th, 2001</td>
</tr>
<tr>
<td></td>
<td>Bevacizumab (Avastin)</td>
<td>Metastatic colorectal carcinoma</td>
<td>VEGFR</td>
<td>February 22nd, 2007</td>
</tr>
<tr>
<td></td>
<td>Cetuximab (Erbitux)</td>
<td>EGFR-expressing metastatic colorectal cancer</td>
<td>EGFR</td>
<td>July 16th, 2008</td>
</tr>
<tr>
<td></td>
<td>Panitumumab (Vectibix)</td>
<td>Wild-type KRAS-expressing metastatic colorectal cancer</td>
<td>EGFR</td>
<td>April 16th, 2010</td>
</tr>
<tr>
<td>Small molecule</td>
<td>Imatinib (Gleevec)</td>
<td>CML, GIST</td>
<td>BCR-ABL, c-KIT, PDGFR</td>
<td>November 21st, 2001</td>
</tr>
<tr>
<td></td>
<td>Gefitinib (Iressa)</td>
<td>Metastatic non-small-cell lung cancer</td>
<td>EGFR</td>
<td>July 5th, 2002</td>
</tr>
<tr>
<td></td>
<td>Erlotinib (Tarceva)</td>
<td>Metastatic non-small-cell lung cancer</td>
<td>EGFR</td>
<td>October 19th, 2007</td>
</tr>
<tr>
<td></td>
<td>Sorafenib (Nexavar)</td>
<td>Renal cell cancer</td>
<td>VEGFR, c-RAF, PDGFR</td>
<td>January 25th, 2008</td>
</tr>
<tr>
<td></td>
<td>Sunitinib (Sutent)</td>
<td>Gleevec-resistant CML</td>
<td>BCR-ABL, SRC</td>
<td>April 18th, 2008</td>
</tr>
<tr>
<td></td>
<td>Nilotinib (Tasigna)</td>
<td>Gleevec-resistant CML</td>
<td>BCR-ABL</td>
<td>January 21st, 2009</td>
</tr>
<tr>
<td></td>
<td>Dasatinib (Sprycel)</td>
<td>Gleevec-resistant CML</td>
<td>BCR-ABL, SRC</td>
<td>January 21st, 2009</td>
</tr>
<tr>
<td></td>
<td>Lapatinib (Tykerb)</td>
<td>HER2-positive breast cancer</td>
<td>EGFR, HER2</td>
<td>April 22nd, 2009</td>
</tr>
</tbody>
</table>

Two different types of anticancer therapeutic agents, antibody and small molecule, that target growth factor receptors have received marketing approval in Japan. The bcr/abl rearrangement causes production of an abnormal tyrosine kinase molecule (BCR-ABL). SRC is a non-receptor protein tyrosine kinase that plays a multitude of roles in cell signaling. CML, Chronic myelogenous leukemia; GIST, Gastrointestinal stromal tumor.

doethelial growth factor receptor (VEGFR), both of which play a role in both tumor angiogenesis and tumor cell proliferation.\(^{59}\) Although the molecular mechanism of sensitivity to RTK inhibitors has not yet fully been elucidated, the induction of apoptosis has been considered as the major mechanism for tyrosine kinase inhibitor (TKI)-mediated anticancer effects; and a variety of Bcl-2 family members serve as the determinants that control apoptosis.\(^{61}\)

In addition to the aforementioned RTKs, the receptor for hepatocyte growth factor (MET), fibroblast growth factor receptor (FGFR), and insulin-like growth factor-I receptor (IGF-I-IR) are frequently genetically altered or otherwise dysregulated in advanced cancers, suggesting them to be attractive therapeutic targets.\(^{71}\) TKIs of these receptors have not yet been approved for marketing; however, many anticancer drug candidates are currently undergoing clinical trials. This review will focus on small-molecule TKIs targeting MET, FGFR, and IGF-I-IR.

2.2. MET-Targeted Therapy  The HGF/MET signaling pathway is dysregulated in many human cancers and is an attractive candidate for targeted cancer therapy. A staurosporine analogue, K252a is a broad-spectrum kinase inhibitor derived from a group of natural alkaloids. It was the first small molecule found to be active as a MET inhibitor, supporting the concept of development of MET TKIs.\(^{28}\) Subsequently, PHA665752, chemically known as (R)-5-((2,6-dichlorobenzyl)sulfonyl)-3-((3,5-dimethyl-4-(2-(pyrrolidin-1-ylmethyl)pyrrolidine-1-carbonyl)-1H-pyrrol-2-yl)methyl)ene)indolin-2-one, was identified as a more selective MET inhibitor.\(^{29}\) PHA665752 potently inhibits MET enzyme activity with a \(K_{i}\) of 4 nM and an \(IC_{50}\) of 9 nM. It is \(>50\times\) selective over a large panel of other tyrosine and serine-threonine kinases. In cells, PHA665752 inhibits MET phosphorylation and MET-dependent motility, invasion, and proliferation, and
modulates known MET signal transducers including RAS/MAPK and PI3K/AKT. Its cytoreductive activity was also demonstrated in a gastric carcinoma xenograft model. These in vitro and in vivo studies support the therapeutic potential of targeting MET in cancers where MET plays a role in tumor growth or metastasis. However, PHA665752 is not a viable clinical agent due to its poor pharmacokinetic properties, resulting in low oral bioavailability. Based on the co-crystal structure of PHA665752 with the MET kinase domain, orally available TKI inhibitor PF2341066, which is chemically known as \((\text{R})-3-(1-(2,6\text{-dichloro-3-fluorophenyl})\text{-ethoxy})-5-(1-(\text{piperidin-4-yl})\text{-1H-pyrazol-4-yl})\text{pyridin-2-amine}\), was designed. PF2341066 as well as PHA665752 competitively antagonize occupancy of the ATP-binding site, preventing TK activation and downstream signaling. Recently, PF2341066 was shown to inhibit the anaplastic lymphoma kinase (ALK) pathway, suggesting that this TKI could be effective against non-small cell lung cancers harboring ALK gene alterations. AM7, \((5-(3\text{-fluoro-4-}((6\text{-methyl-oxo})-7-((3\text{-4-morpholinyl}propyl)oxy)-4\text{-quinolinyl})oxy)-3\text{-methyl-2-(phenylmethyl)-4\text{-(3H})-pyrimidinone})\), is the first MET inhibitor to be described that inhibits MET mutants previously shown to be resistant to other molecules. AM7 has a different MET binding modality compared with other published MET TKIs including SU11274.

3. FGFR

3.1. FGFR and Cancer

The FGFR family consists of four members—FGFR1, FGFR2, FGFR3, and FGFR4. FGFRs are glycoproteins composed of extracellular immunoglobulin (Ig)-like domains, a hydrophobic transmembrane region, and a cytoplasmic part containing the tyrosine kinase domain. They transduce signals from their high-affinity ligands (FGFs) to RAS/MAPK and PI3K/AKT pathways through FGFR substrate 2, and also to the diacylglycerol/protein kinase C pathway through phospholipase \(C_\gamma\). While FGFR signaling is clearly important in physiological processes such as cell proliferation, motility, and survival, the aberrant regulation of FGFRs and FGFRs is associated with cancer.
Gene amplification and over-expression of FGFRs have been observed in a variety of human cancers: FGFR1 over-expression in uterine carcinoma and luminal B-type breast cancers, and FGFR2 over-expression in breast cancer and gastric cancer.\(^4\text{-}^9\) FGFR1 amplification may be a major contributor to poor prognosis in luminal-type breast cancers. Missense mutations of FGFRs have also been observed in many human cancers: FGFR2 mutations in breast cancer, gastric cancer, lung cancer, ovarian cancer, and endometrial cancer, and FGFR3 mutations in uterine carcinoma. FGFR2 missense mutations found as a cluster around the hinge region and the third Ig-like domain induce oncogenic FGFR2 activation due to the altered ligand-receptor specificity, thus creating an FGF autocrine loop.\(^5\text{-}^6\) FGFR2 activation due to the acquisition of ligand independency.\(^5\text{-}^8\) Missense mutations found as a cluster around the hinge region and the third Ig-like domain induce oncogenic FGFR2 activation due to the altered ligand-receptor specificity, thus creating an FGF autocrine loop.\(^5\) FGFR2 missense mutations within the kinase domain are observed in uterine cancer. These mutations induce oncogenic FGFR2 activation due to the acquisition of ligand independency.\(^5\text{-}^6\)

FGFR3 mutations are the most common genetic alteration in uterine carcinoma and result in constitutive activation of the receptor.\(^5\text{-}^7\) Therefore, FGFR3 as well as FGFR1 may be valid therapeutic targets in uterine carcinoma. Collectively, FGFR1, FGFR2, and FGFR3 can be promising proteins for specific and targeted therapeutic approaches.

3.2. FGFR-Targeted Therapy FGFR TKIs that are more effective and have fewer side effects than older chemotherapeutic drugs have recently been developed. PD173074, a synthetic compound of the pyrido[2,3-d]pyrimidine class, is such a TKI. PD173074 was initially found as a nanomolar-effective TKI of FGFR1; and it also inhibits the autophosphorylation of FGFR3 with an IC\(_{50}\) value of approximately 5 nM and exerts a remarkable inhibitory effect in uterine carcinomas and multiple myelomas expressing a mutated FGFR3; and it inhibits the proliferation and survival of endometrial cancer cells with activating FGFR2 mutations.\(^5\text{-}^8\) PD173074 also blocks the mitogenesis of breast cancer cells through G1-arrest mediated by down-regulation of cyclin D1 and cyclin D2, indicating that FGFR signaling is linked to the cell-cycle machinery via D-type cyclins.\(^5\text{-}^8\) Finally, PD173074, which also acts as a submicromolar inhibitor of VEGF2R, blocks angiogenesis induced by either FGF or VEGF with no apparent toxicity.\(^5\text{-}^8\)

Several high-level FGFR gene amplifications have been identified in breast cancer.\(^5\text{-}^8\) Amplification of FGFR1 is associated with early relapse and poor survival, specifically in estrogen receptor-positive breast cancer.\(^5\text{-}^8\) Amplification of FGFR1 is uncommon in HER2-amplified tumors, suggesting that amplification of FGFR1 and HER2 may be mutually exclusive ways of activating similar downstream pathways.\(^5\text{-}^8\) Agents that target HER2 improve the prognosis of patients with HER2-amplified breast cancers. However, patients who initially respond to such a targeted therapy eventually develop resistance to the treatment. In HER2 TKI-resistant breast cancer cells (UACC812/LR), the FGFR2 gene is highly amplified, accompanied by over-expression of FGFR2 and reduced expression of HER2. The IC\(_{50}\) of PD173074 is 10000 times lower in UACC812/LR than in the parent cells. Azuma and colleagues suggest that a switch of addiction from the HER2 to the FGFR2 pathway enables cancer cells to become resistant to HER2-targeted therapy.\(^5\text{-}^8\) While the last decade has seen advances in the treatment of hormone-receptor and HER2-positive breast cancers, outcomes for women with estrogen receptor-, progesterone receptor-, and HER2-negative- or triple negative breast cancers (TNBCs) have a relatively poor prognosis and cannot be effectively treated with current targeted therapies. FGFR2 amplification was identified in TNBC cell lines, and cell lines with FGFR2 amplification are highly sensitive to PD173074.\(^5\text{-}^8\)

Angiogenesis, which is regulated by growth factors, in particular, FGF and VEGF, is an important process for tumor growth. Brivanib, (R)-1-(4-(4-fluoro-2-methyl-1H-indol-5-oloxo)-5-methylpyrrolo[2,1-F][1,2,4]triazin-6-olxy)-propan-2-ol, is a selective dual TKI of FGF and VEGF signaling. Brivanib alamine, the orally administrated t-alanine ester prodrug, has strong antiangiogenic effects, as well as potent direct effects, on tumor cells across a range of tumor types, including liver and colon.\(^6\text{-}^8\) Sorafenib, 4-(4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)phenoxy)-N-methylpicolinamide 4-methylbenzenesulfonate, a kinase inhibitor of VEGFR, PDGFR, c-Kit, and Raf, has been approved for the treatment of hepatocellular carcinoma. Because brivanib targets both the FGF and VEGF signaling pathways, it may gain an advantage over sorafenib for the treatment of hepatocellular carcinoma.

4. IGF-IR

4.1. IGF-IR and Cancer A member of the IGF family, the IGF-IR is a ubiquitously expressed type 1 transmembrane heterotetrameric receptor composed of two extracellular ligand-binding \(a\)-subunits (M, 130000 each) and two \(b\)-subunits (M, 90000 each). Each \(b\)-subunit contains an extracellular, a transmembrane, an intracellular tyrosine kinase, and a C-terminal domain.\(^7\text{-}^8\) On binding of specific ligands (IGF-I and IGF-II), IGF-IR undergoes autophosphorylation of the tyrosine residues in the tyrosine kinase domain of its \(b\)-subunit, leading to subsequent tyrosine phosphorylation of two major substrates, i.e., insulin receptor substrate 1 (IRS1) and SHC, resulting in the activation of the PI3K/AKT and RAS/MAPK pathways (Fig. 1).\(^7\text{-}^8\) These activated pathways regulate the function and expression of proteins involved in cell survival and proliferation.\(^7\text{-}^8\) Under normal physiological conditions, the IGF system is tightly regulated, allowing homeostatic growth. In tumor cells, these same molecules are activated, either by mutation, chromosomal translocation, abnormal stimulation (autocrine, endocrine or paracrine), or loss of genomic imprinting. In humans, the IGF-II gene is normally maternally imprinted.\(^7\text{-}^8\) However, bi-allelic expression (loss of imprinting; LOI) of the IGF-II gene has been reported in colorectal carcinomas, Wilms’ tumor, juvenile nasopharyngeal angiofibromas, and childhood acute lymphoblastic leukemia.\(^7\text{-}^8\) In cells that express both parental IGF-II alleles, the increase in IGF-II production may be the major mechanism promoting cancer development. IGF-IR gene amplification has been reported in malignant melanoma, primary breast cancers, and pancreatic adenocarcinoma.\(^7\text{-}^8\) High concentrations of IGF-I have been documented in several common cancers, including prostate cancer and premenopausal breast cancer.\(^7\text{-}^8\) Therefore, the IGF-IR can be a promising protein for specific and targeted therapeutic approaches.

4.2. IGF-IR-Targeted Therapy The orally available compound NVP-AEW541, a pyrrolo[2,3-d]pyrimidine deriv-
ative, is a small-molecule inhibitor of IGF-IR kinase.\textsuperscript{52} It is highly selective against IGF-IR, compared with the insulin receptor (IR) and other tyrosine kinases. The anti-neoplastic efficacy of NVP-AEW541 has recently been shown in many \textit{in vitro} and \textit{in vivo} studies.\textsuperscript{83} In these studies, cancer cells were treated with NVP-AEW541 alone or in combination with anti-tumor drugs such as gemcitabine. Picropodophyllin (PPP), chemically (5R,5aR,3aS,9R)-5-hydroxy-9-(3,4,5-trimethoxyphenyl)-5a,6,8a,9-tetrahydro-5H-[2]benzofuro[5,6-f] [1,3]benzodioxol-8-one, is a member of the cyclolignan family and a new selective inhibitor of IGF-IR.\textsuperscript{84} PPP inhibits tyrosine phosphorylation of Y1136 in the activation loop of the IGF-IR kinase domain.\textsuperscript{85} Interestingly, PPP does not interfere with the IGF-IR tyrosine kinase at the level of the ATP-binding site, suggesting that it may inhibit IGF-IR autophosphorylation at the substrate level.\textsuperscript{85} This is consistent with the fact that the ATP-binding sites of IGF-IR and IR kinases are identical. This agent has been shown to induce tumor regression and inhibition of metastasis in several models of human cancer.\textsuperscript{84—87} PPP does not affect the tumor growth of established xenografts composed of IGF-IR-negative cells, whereas IGF-IR-positive xenografts are fully responsive. Recent studies showed that oral PPP is well tolerated \textit{in vivo} and inhibits IGF-IR expression and growth of melanomas.\textsuperscript{88} It thus appears that PPP not only inhibits the activity of IGF-IR but also causes the down-regulation of the receptor.

Many tumors show altered expression of the IGF-IR and its ligands. Further, tumors are now known to express IRs, particularly the insulin receptor isoform A (IR-A). In cells that express IGF-IR and IR, IGF-IR/IR hybrids are formed by random association and can be activated by a high level of insulin. Although the classic insulin receptor isoform B (exon 11 +) only binds insulin, IR-A (exon 11 −) binds IGF-II in addition to insulin. It is plausible that the binding of IGF-II to IR-A may be implicated in development of tumors. Thus, IR is also a key component of the IGF signaling pathway, and inhibition of both IGF-IR and IR may be necessary for inhibiting IGF-mediated proliferation. BMS-554417, (S)-3-4-(2-(4-(2-(3-chlorophenyl)-2-hydroxyethylamino)-2-oxo-1,2-dihydropyridin-3-yl)-7-methyl-1H-benzo[d]imidazol-5-yl)piperazin-1-yl)propanenitrile, is the first dual-kinase inhibitor of the IGF-IR and IR to show antiproliferative activity against multiple cell types. Both NVP-AEW541 and PPP were designed for selectivity against the IGF-IR and not the IR due to the potential metabolic consequences of inhibiting IRs. IGF-IR TKIs with low selectivity are predicted to influence glucose tolerance by direct inhibition of the IR kinase.\textsuperscript{89} An increase in serum glucose has been reported in the BMS-554417-treated mice.

5. PROSPECTS OF NEW TARGETED CANCER THERAPEUTICS

Cancer therapies using agents aimed at a single target have thus far been successful when the target controls the majority, if not all, of the critical signaling pathways for cell survival. There have been spectacular successes led by imatinib, gefitinib, and erlotinib. These successes verify the concept that a major clinical benefit can be gained from targeting a single target involved in malignancy. However, even though blocking the activity of a single target has been shown to prevent tumor progression in the short-term, eventual progression of cancer in the presence of this inhibitor has been observed in clinical studies. Therefore, resistance to treatment remains the major challenge to targeted cancer therapy.

Tumor cells can become resistant to TKIs due to new mutations in the tyrosine kinase such as seen in the secondary resistance mutations of mutated EGFR. They also acquire resistant to TKIs due to switching addictions from the targeted RTK to another RTK. Many studies have indicated the existence of crosstalk between various levels of different signal transduction cascades and redundant signaling pathways. Compensatory RTK signaling enables cancer cells to become resistant to antitumor agents that selectively target a single RTK. For example, IGF-IR expression has been associated with resistance to targeting agents, such as inhibitors of EGFR or HER2, in several experimental models; and co-targeting the IGF-IR together with the EGFR or HER2 can achieve better therapeutic effects in several types of cancer models.\textsuperscript{90—92}

MET expression has also been associated with resistance to targeting agents. \textit{MET} gene amplification is detected in 22% of lung cancer specimens from patients who had acquired resistance to gefitinib and erlotinib. Treatment with MET TKI restored gefitinib sensitivity in a lung cancer cell line that had acquired gefitinib resistance through \textit{MET} amplification.\textsuperscript{93} The treatment of NSCLC with MET TKIs in combination with erlotinib is now underway with promising results so far.\textsuperscript{94}

There are two types of targeting agents, those with a relatively narrow (only one or a few targets) and those with a broad one (multiple targets). Agents specifically inhibiting a given target kinase were previously searched for based on the hypothesis that a narrow target window might reduce unexpected side effects. However, agents with multiple targets have been recently developed to overcome the intrinsic or acquired resistance of tumors to a single targeting agent.\textsuperscript{95,96} The vast majority of human malignancies are very complex, and thus inhibiting a single target alone is likely to be therapeutically ineffective. A combination of different agents or single multiple targeted inhibitor of several pathways provides an opportunity to assess the potential benefit of simultaneous inhibition of the multiple targets. Such combinations could lower the effective dose of each agent but retain comparable or enhanced activity, thus reducing the likelihood of drug toxicity.\textsuperscript{97—99}

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and by funding from the Fugaku Trust for Medical Research.

REFERENCES

22) Hecht M., Papoutsi M., Tran H. D., Wilting J., Schweigerer L., 
21) Umeki K., Shiota G., Kawasaki H., 
16) Smolen G. A., Sordella R., Muir B., Mohapatra G., Barmettler A., 
15) Di Renzo M. F., Olivero M., Martone T., Maffe A., Maggiora P., 
14) Schmidt L., Duh F. M., Chen F., Kishida T., Glenn G., Choyke P., 
10) Choi Y. L., Tsukasaki K., O'Neill M. C., Yamada Y., Onimaru Y., 
9) Peruzzi B., Bottaro D. P., 
8) Ma P. C., Maulik G., Christensen J. G., Salgia R., 
7) Christensen J. G., Burrows J., Salgia R., 
6) Mendel D. B., Laird A. D., Xin X., Louie S. G., Christensen J. G., Li 
5) Archibald H., Kim W. J., Okimoto R. A., Bell D. W., Sgroi D. C., 
4) B. J., Bae J. H., Hong Y. K., Lee K. S., Lee S. H., Yoo N. J., Jang J. J., 
3) Aburatani H., Kamihira S., Nakamura T., Tomonaga M., 
2) Takada S., Kamihira S., Nakamura T., Tomonaga M., 
1) Morotti A., Mila S., Accornero P., Tagliabue E., Poncetto Z., Oncogene, 
27) Christensen J. G., Schreck R., Burrows J., Kuruganti P., Chan E., Le P., 