Cell-autonomous and non-cell-autonomous mechanisms of HGF/MET-driven resistance to targeted therapies: from basic research to a clinical perspective.

CELL AUTONOMOUS AND NON-CELL AUTONOMOUS MECHANISMS OF HGF/MET–DRIVEN RESISTANCE TO TARGETED THERAPIES: FROM BASIC RESEARCH TO A CLINICAL PERSPECTIVE

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ABSTRACT

Targeted therapies have opened new perspectives in clinical oncology. However, clinicians have observed a lack of response in a relevant percentage of patients and frequent relapse in patients who initially respond. Therefore, a compelling challenge is to identify mechanisms underlying resistance and strategies to circumvent these hurdles.

A growing body of evidence indicates that MET, the tyrosine kinase receptor for hepatocyte growth factor (HGF), is frequently implicated in resistance to targeted therapies. In this review we highlight cell autonomous and non-cell autonomous mechanisms through which MET drives resistance, and we discuss some unsolved issues related to the selection of patients who could benefit from combined therapies.

STATEMENT OF SIGNIFICANCE

Resistance is, at present, the major limitation toof the efficacy of targeted therapies. Inappropriate MET activation is very frequently implicated in the onset of primary and secondary resistance to these therapies. Deciphering the role of the HGF/MET axis in resistance to different drugs could guide the design of new clinical trials based on combinatorial therapies and it might help to overcome, or possibly prevent, the onset of resistance.

INTRODUCTION

Targeted therapies by means of compounds that inhibit a specific target molecule give a new perspective in the treatment of cancer (1). In contrast to conventional

chemotherapy, which acts mainly on dividing cells, targeted drugs specifically act on subpopulations of cells directly involved in tumor progression in a more specific way.

The frequent alteration of receptor tyrosine kinases (RTK) in human malignancies led them to be considered targets for anti-neoplastic therapies; this resulted in the development of several inhibitors with strong clinical activity. The concept of "oncogene addiction", that is, the dependence of tumor cells on the constitutive activation of a single oncogene for their proliferation and survival, has added further rationale to the use of targeted therapies (2). In a fraction of patients with cancer, targeted therapies have shown excellent results, leading to dramatic tumor regression. However, a substantial percentage of patients selected to express the target of the drug do not benefit from treatment (primary resistance) and, almost invariably, initially responsive patients develop resistance to treatment and undergo tumor relapse (secondary resistance). Therefore, an important challenge associated with targeted therapies is to predict mechanisms that could cause resistance to treatment and to find ways to solve, or even prevent, this problem.

Recent studies aimed at investigating the molecular mechanisms responsible for primary and secondary resistance to targeted therapies mainly relied on *in vitro* experiments performed in tumor cells. These cells are highly heterogeneous and often display genomic instability. The therapeutic treatment can thus select preexisting clones with alterations in signaling pathways that are able to compensate for the drug-inhibited kinase. The use of *in vitro* models allowed the identification of a number of molecular mechanisms responsible for acquired resistance to tyrosine kinase inhibitors (TKI). Although most of these mechanisms have also been validated in patients, cellular models have clear limitations, such as the artificial growing conditions and the lack of epithelial-stromal interactions that typically occur *in vivo*.

Indeed, very recent data support the concept that the tumor microenvironment plays an important role in sustaining resistance to targeted therapies, for example, by producing ligands that - in a paracrine manner - activate signals able to compensate for the drug-inhibited pathways in tumor cells. Thus, an emerging concept is that resistance of tumor cells to targeted therapies can be due to cell autonomous and/or non-cell autonomous mechanisms. Our knowledge of the prevalence of either mechanism in different clinical situations is largely incomplete.

In this review we will discuss the role of the HGF/MET system in mediating resistance to anticancer kinase inhibitors, both in cell autonomous and in non-cell autonomous manners, and the translational implications of these findings.

The HGF/MET Pathway

The *MET* proto-oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF) (3, 4). Ligand binding induces MET activation, which drives a complex biological program defined as "invasive growth", resulting from the promotion of several biological activities such as cell proliferation, cell invasion and protection from apoptosis (Figure 1). MET-driven invasive growth is a physiological program that occurs during embryonic development and in adulthood during tissue regeneration. However, it has been shown that the inappropriate activation of this program contributes to several aspects of tumor progression. MET-activated signaling pathways are shared with many other RTKs and include the mitogen-activated protein kinase (MAPK) and PI3 Kinase-AKT pathways, STAT3, RAC1, and the NF-κB pathway (Figure 1). Many studies have focused on the specific role played by each of these pathways in the different MET-induced biological activities (for a review see (5)).

MET-mediated signaling results from pathways directly activated by this receptor, but it can also be modulated by the cross talk between MET and different membrane receptors, acting in complex interacting networks (Figure 2). *In vitro* data suggest that this cross-talk is not essential for cell survival but enables a better integration of the signals present in the extracellular environment. Under physiological conditions, these networks are probably redundant. However, it is likely that under pathological conditions these interacting receptors cooperate in promoting tumorigenesis and/or metastasis and in inducing resistance to targeted drugs. The first network of interaction involves MET and adhesive receptors such as CD44 and the α6β4 integrin. CD44, a transmembrane receptor for hyaluronic acid - a major component of the extracellular matrix - has been implicated in tumor progression and metastasis (6). The CD44v6 variant, necessary for the activation of some MET intracellular transducers, functions as an amplifying platform, linking the MET cytoplasmic tail to the actin cytoskeleton and sustaining activation of the MAPK cascade (7, 8). The α6β4 integrin, independent of its adhesive role, acts as a supplementary docking platform for amplification of PI-3 Kinase, MAPK, and SRC-dependent pathways (9). B family plexins, the receptors for semaphorins, can transactivate MET in the absence of HGF and promote pro-invasive signals (10). In addition, the interaction between MET and FAS, is important in the modulation of apoptosis (11). MET also interacts with other tyrosine kinase receptors, such as those belonging to the EGFR family. In fact, reciprocal transphosphorylation between these receptors has been shown in different systems and their ability to substitute for each other has been shown in tumor cells (12-14).

MET/HGF and cancer

In transformed tissues, gain of an invasive growth program is advantageous for cancer progression and metastasis. In fact, constitutive MET activation can contribute to several aspects of tumor progression since it induces neoplastic cells to disaggregate from the tumor mass, erode basement membranes, infiltrate stromal matrices, and eventually colonize new tissues to form metastases (for a review see (15)).

Data produced by many laboratories provide compelling evidence that HGF/MET signaling plays an important role in the development and malignant progression of tumors, particularly in tumor invasiveness and metastasis. Preclinical studies show that cells ectopically overexpressing MET or HGF are tumorigenic and metastatic in nude mice, while MET inhibition decreases these properties (15). Moreover, cancer cell lines exhibiting *MET* gene amplification are "addicted" to MET (which means that they are dependent on this receptor for their growth and survival) and MET inhibition results either in a block in proliferation or cell death (16-18).

As shown in Figure 3, deregulated MET activation in cancer can be due to different molecular alterations. Clinical data show that MET overexpression, in the absence of gene amplification, is the most frequent cause of constitutive MET activation in human tumors and often correlates with poor prognosis. Overexpression can be caused by several factors, such as hypoxia (19), activation of upstream oncogenes (20, 21), inactivation of tumor suppressor genes (22) or loss of microRNAs (23, 24). *MET* gene amplification, which drives increased expression and constitutive receptor activation, has been described in selected histotypes such as gastro-esophageal, colorectal, endometrial and lung carcinomas, glioblastomas and medulloblastomas (reviewed in (25)). Autocrine MET activation has been described in sarcoma,

glioblastoma, breast carcinoma (reviewed in (25)) and, very recently, in a high percentage of acute myelogenous leukemia (26). Finally, the unequivocal evidence linking *MET* and human cancer came from the identification of germline activating mutations in patients suffering from ehreditary papillary renal carcinomas (27, 28). Activating mutations in sporadically occurring tumors are relatively rare and have been mainly found in lung and kidney carcinomas and hepatoblastoma (reviewed in (25)). These mutations are located in the tyrosine kinase domain, the juxtamembrane portion, or in the extracellular Sema (semaphorin) domain. Although overexpression can render MET activation independent from HGF stimulation, in most cases the ligand is still required for full receptor activation. This is also true for MET receptor variants containing activating mutations that need HGF to fully activate their kinase activity (29).

In light of the functional role played by the HGF/MET axis in different human tumors, in the last decade several strategies have been designed to inhibit the activation of the MET receptor, and multiple agents are currently in clinical trials (for a summary, see Figure.4).

Activation of the HGF/MET axis in sustaining resistance to targeted therapies

Clinical practice, as well as experimental evidence, has clearly shown that biological therapies are effective only in a certain percentage of tumors expressing the appropriate target. Moreover, even responding tumors develop resistance to treatment quite rapidly. These observations prompted researchers to investigate the mechanisms responsible for primary and secondary resistance. The first studies were focused on the changes occurring in tumor cells, considered more likely to be responsible for the phenomenon due to their high genomic instability (Figure 5). However, many data

have unveiled a critical role for the tumor microenvironment in generating and sustaining resistance. The tumor stroma, in fact, produces growth factors and other molecules (such as extracellular matrix components) that can activate signaling pathways in tumor cells that are able to overcome the inhibitory effect of the drug (Figure 6).

Cell autonomous role of MET in sustaining resistance to RTK inhibitors

Non-small cell lung cancer (NSCLC)

The first clinical evidence of a role of MET in sustaining resistance to EGFR inhibitors came from two works studying patients affected by non-small cell lung cancer and treated with the reversible EGFR inhibitors gefitinib and erlotinib. Engelman and colleagues (30) started from the *in vitro* observation that lung cancer cells, originally sensitive to EGFR inhibitors, became resistant to long-term treatment with gefitinib as a consequence of *MET* gene amplification. They also demonstrated that sensitivity to EGFR inhibitors could be restored by simultaneous treatment with anti-MET and anti-EGFR drugs, thus proving the causative role of MET in sustaining acquired resistance. They observed that MET causes resistance to gefitinib by transphosphorylating HER-3 and driving HER3-dependent activation of the PI3K pathway. When they analyzed 18 NSCLC patients, all displaying acquired resistance to either erlotinib or gefitinib, they found that 22% of these patients displayed *MET* amplification. Notably, one patient bearing two independent metastases showed *MET* amplification only in one metastatic lesion, while the other displayed the EGFR T790M mutation, known to impair response to these drugs.

The work published by Bean and colleagues (31) confirmed these results. Indeed, using high-resolution genome-wide profiling of NSCLCs before and after treatment

with anti-EGFR drugs, they found *MET* amplification in 9 out of 43 patients (21%) with acquired resistance, compared with 2 out of 62 (3%) untreated patients. Interestingly, they observed that 40% of the samples with *MET* amplification also harbored the EGFR T790M mutation. *MET* amplification was also found in a NSCLC cell line, in which the same cells harbored (i) an EGFR mutation associated with drug sensitivity, (ii) an EGFR mutation associated with drug resistance and (iii) *MET* amplification, thus showing that all these genetic lesions can occur within the same cell population. Finally, this work also showed that treatment with a MET inhibitor was able to overcome acquired resistance to EGFR kinase inhibitors, even in cells harboring the T790M mutation. It has to be noted that more recent studies in re-biopsed specimens from larger cohorts revealed a lower frequency of *MET* amplification in resistant tumors compared with that reported by Engelman and Bean, ranging from 5 to 11% (32, 33). Given the results of these two studies, combination therapy with MET kinase inhibitors and anti-EGFR drugs should be considered for patients whose tumors have become resistant to gefitinib or erlotinib and harbor *MET* amplification.

One of the questions stemming from these studies is the origin of the cells displaying *MET* amplification: did the cell lines and the tumors harbor pre-existing *MET*-amplified clones and did the drug simply create a favorable environment capable of selecting them? Or, alternatively, did the drug induce a stress able to promote DNA breaks in fragile sites, such as the one in which the *MET* gene is located (34)? To answer these questions, Turke and colleagues (35) analyzed the HCC827 NSCLC cell line that, upon gefitinib treatment, became resistant via *MET* amplification. When they studied the parental HCC827 cells using high-throughput FISH, they identified 6 out of 4237 cells harboring a significant increase in *MET* copy number. On the

contrary, they did not find any *MET*-amplified subpopulation in two other NSCLC cell lines, namely H3255 and PC-9, which, upon acquisition of resistance to gefitinib, displayed the EGFR secondary resistance mutation T790M but not *MET* amplification. To further validate these observations in the clinical setting, the authors evaluated specimens obtained from 27 patients who developed acquired resistance to either gefitinib or erlotinib and found *MET* amplification in 4 of these samples. In all of these patients, the pretreatment specimens revealed rare (<1%) tumor cells with *MET* amplification. In contrast, only 1 out of 8 cases of resistance to anti-EGFR drugs resulting from EGFR secondary resistance mutations displayed rare *MET*-amplified tumor cells. Altogether, these data suggest that *MET*-amplified tumor cells pre-exist and that these cells are selected during the course of therapy in patients and NSCLC cell lines that are characterized by *MET* amplification as a mechanism of secondary resistance to EGFR inhibitors. However, it remains to be clarified if this is true only in NSCLC or if it is a common feature of other cancers.

Another interesting observation stemming from this work is that the presence of HGF in the environment, concomitant with EGFR inhibition, strongly favors the emergence of *MET*-amplified cells. *In vitro* experiments showed that the exposure of cells to HGF reduced the time required for the onset of resistance to EGFR inhibitors from several months to 14 days. Therefore, it is conceivable that, in the presence of EGFR inhibitors, HGF provides a proliferative advantage to cells with *MET* amplification and favors their clonal expansion. Thus, the combination of gene amplification and ligand-mediated activation represents a very powerful mechanism that renders lung cancer cells resistant to targeted therapies.

HER2-positive metastatic breast cancer

Several studies have shown that in HER2-overexpressing or amplified breast cancers the anti-HER2 humanized recombinant monoclonal antibody trastuzumab, in combination with chemotherapy, was significantly more effective than chemotherapy alone, both in the metastatic and the adjuvant settings (36, 37). In spite of the effectiveness of this treatment, only one third of patients who are eligible for the treatment respond; moreover, most of the patients who initially respond show disease progression within 1 year of treatment. Among the mechanisms of resistance to trastuzumab treatment (38-40), a role for MET has been reported by Shattuck and colleagues (41), who found that inhibition of MET sensitizes cells to trastuzumab-mediated growth inhibition, whereas MET activation protects cells against trastuzumab. Based on these results, Minuti et al. (42) recently evaluated the relationship between *MET* and *HGF* copy number and responsiveness to trastuzumab in 130 HER2-positive metastatic breast cancer patients using FISH. MET-positive cases (defined as ratio mean MET/mean CEP 7 > 2) had a significantly higher trastuzumab failure rate (44.4% vs 16%; P=0.001) and a significantly shorter time to progression (TTP; 5.7 vs 9.9 months; HR 1.74; P=0.006) compared with *MET*-negative cases. Similar results were obtained when *HGF* FISH status was evaluated. Interestingly, *MET* and *HGF*positivity were highly correlated (P<0.001), suggesting the presence of chromosome aneuploidy, rather than single amplification of either of the two genes. These results, together with the preclinical studies, suggest the possible effectiveness of combined anti-HER2 and anti-MET therapies in a subgroup of HER2-positive metastatic breast cancer patients.

Colon cancer

The EGFR-targeted monoclonal antibodies cetuximab and panitumumab are effective in a subset of metastatic colorectal tumors. The presence of KRAS mutations and deregulation of effectors of the EGFR signaling cascade (PIK3CA, PTEN, NRAS) or of EGFR modulators (HER2, EGFR ligands) are thought to impair primary response to EGFR blockade (43-45). Moreover, all responding patients develop resistance, which occurs through emergence of *KRAS* mutations in approximately 50% of the cases (46, 47) . A recent report has shown that amplification of the *MET* proto-oncogene can be responsible for *de novo* and acquired resistance to anti-EGFR therapy in colorectal cancer patients (48). Notably, in xenografts derived from *MET*-amplified tumors, treatment with MET kinase inhibitors overcame resistance to EGFR blockade. These results highlight the role of MET in mediating primary and secondary resistance to anti-EGFR therapies in colorectal cancer and offer novel opportunities to design clinical studies with combined EGFR/MET inhibitors.

Glioblastoma

Recent work performed by Jun and colleagues (49) revealed a role of MET in mediating resistance to gefitinib in a pre-clinical model of glioblastoma multiforme. Inhibition of EGFR resulted in a substantial change in global gene expression levels and, in particular, increased MET expression, which resulted in sustained prosurvival Akt signaling. Pharmacological inhibition of MET overcame the resistance to EGFR inhibition, further supporting the wide importance of MET/EGFR interaction in multiple tumor types.

A general problem: definition of MET *amplification*

Since these works imply a clinically relevant role for *MET* gene amplification in sustaining resistance to anti-EGFR therapies, a critical point is how to define *MET* amplification. Even though FISH seems to be the best way to identify *MET* gene copy number, standardized criteria to define *MET* FISH positive tumors have not yet been determined. The two most commonly used criteria are the University of Colorado Cancer Center Criteria (UICC) (50) and the Cappuzzo scoring system (51). Both of these scoring systems, however, evaluate aneuploidy and real *MET* amplification at the same time. From a biological point of view this can potentially confound the results, since chromosome 7, on which *MET* is located, also contains the genes for HGF and EGFR. Thus, chromosomal duplication increases not only the number of *MET* copies but also the copy number of its ligand and of EGFR, the therapeutic target. A recent work (52) has compared the Cappuzzo scoring system with the PathVysion system, which takes into account only real amplifications. The latter, in fact, considers a ratio between total *MET* copy number/Chr 7 copy number ≥ 2.0 as positive. The analysis performed on 138 lung adenocarcinoma patients revealed *MET* FISH positivity in 15% and 4% of cases according to the Cappuzzo scoring system and to PathVision, respectively. This means that in the great majority of cases, the increased *MET* copy number is not due to a real gene amplification but rather to chromosomal aneuploidy. Whether these two biological conditions are associated with a different clinical behavior should be assessed in future studies.

What is the clinically relevant number of *MET* copies in order to confer resistance to targeted therapies? This question has been addressed by Suda et al. (53), who found that the *MET* gene copy number was augmented in proportion to erlotinib resistance in cells treated with increasing concentration of the drug. By using FISH analysis they observed that when *MET* gene copy number had increased by more than four-fold

(that is at least 8 gene copies), the cells were able to proliferate in the presence of micromolar concentrations of the drug, a clinically achievable dose. If these data hold true, the threshold suggested by the UICC and the Cappuzzo scoring systems might be too low to identify situations in which the level of *MET* amplification is high enough to have a strong clinical impact. However, it is essential to consider that since HGF is available *in vivo*, lower amplification levels can lead to ligand-dependent activation of the MET signaling pathway that is sufficient to drive resistance. At the moment, this question is still open and preclinical and clinical studies are needed to clarify this critical point.

Non-cell autonomous role of MET in sustaining resistance to RTK inhibitors

Cell autonomous or "intrinsic" mechanisms of resistance (such as activation of alternative signaling pathways, onset of secondary mutations in drug targets, amplification of the target gene, activation of efflux pumps) have been deeply investigated and therapeutic strategies and new drugs have been generated to overcome these molecular alterations. On the contrary, non-cell autonomous mechanisms of resistance to therapy have only recently been investigated. In fact, the concept that the tumor behaves as an organ, in which the interaction between cancer cells and the peritumoral environment is critical to sustain its survival, is relatively new. The first focus was on the role of angiogenesis and resulted in many studies aimed at blocking tumor growth by interfering with its vascularization (reviewed in (54). However, it soon became clear that other cells of the microenvironment, such as cancer-associated fibroblasts and inflammatory cells, can also modulate the biological properties of tumor cells, impacting on their growth and invasion potential (55). More recently it has been shown that the microenvironment can also contribute to both

primary and secondary resistance to anticancer therapies. Stromal cells, in fact, secrete factors able to activateredundant signaling programs in cancer cells that render them resistant to targeted treatments. One of the first reports to show this, from Williams et al. (56), found that in an animal model of BCR-ABL+ chronic myelogenous leukemia, neoplastic cells were sensitive to the ABL specific inhibitor imatinib only *in vitro* but not *in vivo*, due to the secretion of host cytokines.

Several recent papers have highlighted the notion that HGF secreted by cells of the microenvironment can play a critical role in primary and secondary resistance to several target therapies in the context of different tumors.

Using a panel of kinase-addicted human cancer cell lines, Wilson and colleagues (57), showed that most of these cells could be rescued from drug sensitivity by exposure to growth factors usually secreted by microenvironmental cells. HGF, FGF (fibroblast growth factor) and NRG1 (neuregulin 1) were the most broadly active ligands and could induce either a “partial rescue” or a “complete rescue” (that is a shift of the IC50 curve of more than ten-fold or a complete suppression of drug response) in the tested cell lines. The two downstream pathways commonly engaged by these ligand were the PI3K-AKT and MAPK pathways, but only HGF was able to simultaneously rescue both of these pathways. In the cell lines examined, HGF promoted resistance to several targeted therapies, including not only those against tyrosine kinases (such as ALK and members of the EGFR family), but also therapies against serine/threonine kinases (for example RAF).

An important question is the following: how does the stimulation with HGF exert its role in promoting resistance? Does it simply activate downstream signals or, while doing so, does it also select cells able to better respond to HGF? A strong evidence in favor of the latter hypothesis was provided in the work of Turke et al.

(35), who showed that in EGFR-mutant NSCLCs, HGF treatment promoted the emergence of cell subpopulations displaying *MET* amplification prior to exposure to the anti-EGFR drug gefitinib. Indeed, *MET*-amplified cells do not have any growth advantage in the absence of gefitinib, which targets EGFR-addicted cells, but they are enriched by the selective pressure of the drug during ligand exposure. Similar results were also obtained by Straussman et al. (58), who showed that HGF treatment could rapidly promote resistance to lapatinib (a HER2 inhibitor) in *HER2*-amplified breast cancer cells, likely by driving selection of a subpopulation of *MET*-amplified cells.

HGF is an angiogenic factor (59) that is able to activate MET in endothelial cells, in part by inducing VEGF production and decreasing thrombospondin-1 expression (60). Although anti-angiogenic therapies have shown efficacy and survival benefits in clinical trials, the majority of patients develop resistance during treatment and undergo progression. Several studies have suggested that factors such as FGF2 or platelet derived growth factor, produced by either cancer or stromal cells, can contribute to the onset of resistance (57, 61). Shojaei and colleagues (62) have shown that HGF production by the tumor stroma can also contribute to resistance to treatment with sunitinib, a multikinase inhibitor targeting the VEGF pathway. Indeed, they found that resistant tumors displayed a greater concentration of HGF and a high MET expression level in endothelial cells. They also showed that combinatorial treatment with sunitinib and an anti-MET TKI was more effective than sunitinib alone. Moreover, systemic injection of HGF conferred resistance to sunitinib treatment by sustaining tumor angiogenesis. Altogether, these data suggest that cotargeting of VEGF/HGF mediated pathways could be a strategy to circumvent resistance to anti-angiogenic therapies.

Melanoma

The role of HGF in mediating resistance to RAF inhibition in melanomas was studied by Straussman et al. (58), who showed that HGF secretion by stromal cells resulted in the activation of MET and reactivation of PI3K-AKT and MAPK pathways and in primary resistance to RAF inhibition. Moreover, they found a significant correlation between HGF expression by stromal cells and primary resistance to RAF inhibition. Importantly, dual inhibition of RAF and either HGF or MET reversed drug resistance. Interestingly, none of the other 22 RTK ligands tested were able to rescue BRAF-mutated melanoma cells from the BRAF inhibitor. In accordance with what was described by Wilson et al., (52) among all the evaluated ligands, only HGF was able to simultaneously and efficiently activate both the PI3K-AKT and MAPK pathways. These data suggest that a combined anti RAF and anti-MET/HGF therapy might represent a useful option in patients affected by RAF-mutated tumors, such as melanomas, colon cancer and glioblastomas.

Non-small cell lung cancer

The first paper showing that HGF can induce gefitinib resistance in lung adenocarcinomas with EGFR activating mutations was that of Yano et al. (63), who found that HGF acts by restoring PI3K-AKT activation via phosphorylation of MET (but not of EGFR or HER3); moreover, they showed that strong immunoreactivity for HGF was detected in patients displaying primary or secondary resistance to gefitinib. The same authors subsequently found that high HGF immunohistochemical reactivity was present in 29% of non-responding patients and that HGF expression was significantly higher in tumors with acquired resistance than in sensitive ones (64). Plasma levels of HGF were evaluated by Tanaka et al. (65), who observed that

administration of EGFR kinase inhibitors significantly increased plasma HGF levels 15 days after treatment. Moreover, high levels of plasma HGF before treatment were found in patients with primary resistance to anti-EGFR drugs. Finally, Yamada et al. (66) recently showed that, in lung cancer, HGF can induce resistance not only to small TKIs but also to Cetuximab, an anti-EGFR monoclonal antibody, and that in preclinical *in vivo* models this is due to the secretion of HGF by stromal fibroblasts.

Data published by the same authors indicate that a similar HGF-mediated mechanism can induce resistance to ALK inhibitors in lung cancer cells addicted to an activated form of the tyrosine kinase ALK (67). ALK fusion proteins are present in 3-7% of unselected NSCLCs; treatment of these patients with ALK inhibitors showed a response rate of 54%, with a 91% disease control rate (partial response + stable disease (68)). However, almost all the responding patients developed resistance to treatment and underwent relapse. Resistance was due to several mechanisms such as *ALK* gene amplification, secondary mutations, or coactivation of other RTKs like EGFR and HER2 (69). Yamada et al. showed that paracrine activation by either EGFR or MET ligands from the microenvironment can trigger resistance to ALK inhibitors. Similar results were also reported *in vitro* by Harbinski et al. (61). These data suggest that the dual MET/ALK inhibitor crizotinib may be more effective than more specific ALK inhibitors in treating NSCLCs expressing ALK fusion proteins.

Breast, gastric and colorectal cancer

Triple negative breast cancers (that is, breast cancers negative for the expression of estrogen receptor, progesterone receptor and HER2) display EGFR overexpression in 54% of cases, but they are not responsive to EGFR-targeting drugs. Mueller et al. (70) showed that HGF treatment of breast cancer cells that are sensitive

to EGFR TKIs conferred resistance to treatment. Interestingly, EGFR silencing abrogated HGF-induced cell survival, indicating a critical role for EGFR/MET cross-talk. These data support the hypothesis that poor efficacy in the clinical treatment of triple negative breast cancers overexpressing EGFR may be due to crosstalk between EGFR and MET. Thus, simultaneous targeting of both the receptors could be an effective therapeutic strategy.

More than 15% of gastric cancers display amplification of HER2. *In vitro* studies showed that lapatinib, a dual TKI targeting HER2 and EGFR, strongly decreased the viability of gastric cancer cells with amplified *HER2* (71). However, HGF-mediated MET activation rescued cells form lapatinib-induced inhibition and reactivated downstream pathways. Finally, Liska et al. (72) showed that in colorectal cancer cells, HGF-induced MET activation could release cells from cetuximab-mediated EGFR inhibition, suggesting that inhibition of the MET/HGF pathway may also improve the response to EGFR inhibitors in colorectal cancer.

Clinical translation

The idea of targeting the HGF/MET axis as a strategy to avoid or circumvent resistance to molecular therapies seems very attractive in light of the preclinical data collected in the last few years. Recent works have shown that only a small number of oncogenic tyrosine kinases display a compensatory ability when the “driver” kinase is inhibited. Among the tested kinases, MET seems to be one of the most effective, since it simultaneously activates the RAS/MAPK and the PI3K/AKT pathways with high efficiency. As previously described, preclinical experiments have shown that combinatorial treatments with a drug blocking the driver kinase plus a MET/HGF inhibitor delay the onset of secondary resistance or even overcome resistance to the

targeting treatment. Notably, clinical data also support the value of combining a MET/HGF inhibitor with already approved targeted therapies in order to delay patient relapse. In particular, a recent phase II clinical trial with MetMAb (Onartuzumab, a one-armed antibody designed to compete with HGF by binding the extracellular portion of MET) (73), performed in patients with advanced NSCLC has shown the clinical efficacy of the combined therapy. This Phase II study showed that patients whose tumors had high MET levels experienced a 2-fold increase in progression-free survival in response to METMAb plus erlotinib compared to erlotinib alone. Moreover, the combination of METMAb plus erlotinib tripled the overall survival compared with erlotinib alone in patients harboring tumors with high MET expression (74). Overall, although this trial was not designed to investigate the role of MET in acquired EGFR TKI resistance, these results suggest that the combined anti EGFR/MET treatment can delay the onset of resistance. This is likely the consequence of preventing the outgrowth of clones displaying MET activation, which are favored in the presence of anti-EGFR drugs.

A clinically relevant point is how to select patients who could benefit from the combined treatment. There are currently no available validated biomarkers that can be used to predict the molecular mechanisms that will sustain resistance in different patients. Therefore, at the moment, preventing resistance is more a dream than a close reality, since the presence of cell autonomous and non-cell autonomous mechanisms leaves many possibilities open. In addition, the clinical perspective is different in the case of patients with primary (innate) or secondary (acquired) resistance.

With regard to primary resistance, preclinical models identified local increases in HGF /MET activation as possible reasons for resistance to target therapies. Relying on these data, it would be important to stratify patients on the basis of HGF/MET

status in the tumor, in order to select patients who could benefit from the treatment with a single agent and patients who would need a combined therapy with MET inhibitors. The main problem related to this stratification is that, at present, no technique to quantify HGF or MET activation in patient-derived samples has been standardized and routinely used. In particular: 1) there are no anti-HGF antibodies of sufficient quality for routine use in a pathological setting. This issue could be overcome by the use of recently developed *in situ* hybridization techniques (75). However, because of the high costs and the technical difficulties, this approach will likely be applicable only on a large-scale in the future; 2) the phosphorylation of tyrosine receptors such as MET is rapidly lost in cells and tissues at room temperature. Since adequate preservation of the surgical resection/biopsy is not routinely done, immunohistochemical analysis with antiphospho-MET antibodies or phosphoproteomic approaches would rarely be informative. Moreover, the lack of standard in anti-MET antibodies for IHC and in the scoring system to evaluate MET activation by IHC is a key limitation. These technical problems in evaluating MET activation in patient samples may also suggest that the involvement of the HGF/MET axis in primary and secondary resistance has probably been underestimated; and 3) intratumoral heterogeneity and multiple layers of mechanisms of pathway activation represent additional problems.

With regard to secondary resistance, molecular analysis of the relapsed tissues is becoming mandatory, since many clinical studies have shown that the expression profile as well as the genomic status of the resistant tumors/metastases can be very different from that of the primary neoplasm. The identification of *MET* gene amplification in resistant samples will demand the use of a combined anti-EGFR/anti-

MET therapy. This kind of analysis (evaluation of *MET* gene amplification upon relapse, for example by FISH) is, at present, not routinely performed in all centers, but it will probably become mandatory in the near future. In addition, it would be of great clinical impact to monitor the possible onset of *MET* gene amplification in the tumor during the treatment targeting the "driver" kinase (e.g. anti-EGFR drugs in lung cancer). It would thus be possible to foresee the development of resistance before the radiological appearance of the relapsed tumor and to start a combined therapy with MET inhibitors in advance of relapse. At the moment, the only known possibility to repeatedly monitor -to some extent- a tumor or a metastasis in a patient is by analyzing tumor-free DNA in the circulating blood (the recent concept of "liquid biopsy"). This strategy has been successfully used to identify the onset of acquired resistance to anti-EGFR antibodies in colorectal cancer due to KRAS mutations (47). However, since MET alterations implicated in the acquisition of resistance are quantitative and not qualitative (i.e. increased gene copy number and not mutations), their identification in the blood appears more difficult.

To evaluate the efficacy of combined treatments in counteracting both primary and secondary resistance, it is now possible to use mouse models that mimic the complexity of a specific patient's cancer. In practical terms, immunocompromised mice can be directly engrafted with tumor surgical specimens/biopsies to obtain "xenopatients" that, even upon serial passages, retain the histological and genomic features of their original tumors (76). Once the "signature" of a tumor is identified, different drugs or combinations can be tested in these "avatars", to select the most efficient combination for the patient. Other than being feasible in relatively few cancer centers, the main problem to evaluate HGF or MET as therapeutic targets through this approach is the observation -often reported in the literature- of the low

cross-reactivity between human MET and murine HGF (77). The decreased ability of the mouse ligand to activate human MET could lead to the underestimation of the role played by the stromal murine HGF on tumor cells expressing human MET.

CONCLUSIONS

Recently, we have gained a better understanding of the molecular mechanisms underlying primary and secondary resistance to targeted therapies. According to Vogelstein (46), "only a limited number of genes are likely to be able to exert a resistance phenotype", and recent studies have shown that the HGF/MET axis is one such pathway. Drugs that target this tyrosine kinase are now available and offer the opportunity to treat patients to overcome resistance, but the problem of identifying patients who could benefit most from these treatments still remains. Personalized approaches based on genomic and functional profiles will be critical to select these patients and to evaluate the complex behavior of their tumors over time.

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REFERENCES

1. Gschwind A, Fischer OM, Ullrich A. The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat Rev Cancer. 2004;4:361-70.

2. Weinstein IB, Joe AK. Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. Nat Clin Pract Oncol. 2006;3:448-57.

3. Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM. Tyrosine kinase receptor indistinguishable from the c-met protein. Nature. 1989;339:155-6.

4. Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science. 1991;251:802-4.

5. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol. 2010;11:834-48.

6. Misra S, Heldin P, Hascall VC, Karamanos NK, Skandalis SS, Markwald RR, et al. Hyaluronan-CD44 interactions as potential targets for cancer therapy. FEBS J. 2011;278:1429-43.

7. Orian-Rousseau V, Chen L, Sleeman JP, Herrlich P, Ponta H. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev. 2002;16:3074-86.

8. Orian-Rousseau V, Morrison H, Matzke A, Kastilan T, Pace G, Herrlich P, et al. Hepatocyte growth factor-induced Ras activation requires ERM proteins linked to both CD44v6 and F-actin. Mol Biol Cell. 2007;18:76-83.

9. Trusolino L, Bertotti A, Comoglio PM. A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. Cell. 2001;107:643-54.

10. Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, et al. The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat Cell Biol. 2002;4:720-4.

11. Wang X, DeFrances MC, Dai Y, Pediaditakis P, Johnson C, Bell A, et al. A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. Mol Cell. 2002;9:411-21.

12. Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem. 2000;275:8806-11.

13. Bergström JD, Westermark B, Heldin NE. Epidermal growth factor receptor signaling activates met in human anaplastic thyroid carcinoma cells. Exp Cell Res. 2000;259:293-9.

14. Fischer OM, Giordano S, Comoglio PM, Ullrich A. Reactive oxygen species mediate Met receptor transactivation by G protein-coupled receptors and the epidermal growth factor receptor in human carcinoma cells. J Biol Chem. 2004;279:28970-8.

15. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol. 2003;4:915-25.

16. McDermott U, Sharma SV, Dowell L, Greninger P, Montagut C, Lamb J, et al. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. Proc Natl Acad Sci U S A. 2007;104:19936-41.

17. Smolen GA, Sordella R, Muir B, Mohapatra G, Barmettler A, Archibald H, et al. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. Proc Natl Acad Sci U S A. 2006;103:2316-21.

18. Corso S, Migliore C, Ghiso E, De Rosa G, Comoglio PM, Giordano S. Silencing the MET oncogene leads to regression of experimental tumors and metastases. Oncogene. 2008;27:684-93.

19. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell. 2003;3:347-61.

20. Ivan M, Bond JA, Prat M, Comoglio PM, Wynford-Thomas D. Activated ras and ret oncogenes induce over-expression of c-met (hepatocyte growth factor receptor) in human thyroid epithelial cells. Oncogene. 1997;14:2417-23.

21. Gambarotta G, Boccaccio C, Giordano S, Andŏ M, Stella MC, Comoglio PM. Ets up-regulates MET transcription. Oncogene. 1996;13:1911-7.

22. Hwang CI, Matoso A, Corney DC, Flesken-Nikitin A, Körner S, Wang W, et al. Wild-type p53 controls cell motility and invasion by dual regulation of MET expression. Proc Natl Acad Sci U S A. 2011;108:14240-5.

23. Migliore C, Martin V, Leoni VP, Restivo A, Atzori L, Petrelli A, et al. MiR-1 downregulation cooperates with MACC1 in promoting MET overexpression in human colon cancer. Clin Cancer Res. 2012;18:737-47.

24. Taulli R, Bersani F, Foglizzo V, Linari A, Vigna E, Ladanyi M, et al. The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. J Clin Invest. 2009;119:2366-78.

25. Peters S, Adjei AA. MET: a promising anticancer therapeutic target. Nat Rev Clin Oncol. 2012;9:314-26.

26. Kentsis A, Reed C, Rice KL, Sanda T, Rodig SJ, Tholouli E, et al. Autocrine activation of the MET receptor tyrosine kinase in acute myeloid leukemia. Nat Med. 2012;18:1118-22.

27. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 1997;16:68-73.

28. Schmidt L, Junker K, Nakaigawa N, Kinjerski T, Weirich G, Miller M, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene. 1999;18:2343-50.

29. Michieli P, Basilico C, Pennacchietti S, Maffè A, Tamagnone L, Giordano S, et al. Mutant Met-mediated transformation is ligand-dependent and can be inhibited by HGF antagonists. Oncogene. 1999;18:5221-31.

30. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007;316:1039-43.

31. Bean J, Brennan C, Shih JY, Riely G, Viale A, Wang L, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A. 2007;104:20932-7.

32. Arcila ME, Oxnard GR, Nafa K, Riely GJ, Solomon SB, Zakowski MF, et al. Rebiopsy of lung cancer patients with acquired resistance to EGFR inhibitors and enhanced detection of the T790M mutation using a locked nucleic acid-based assay. Clin Cancer Res. 2011;17:1169-80.

33. Yu HA, Arcila ME, Rekhtman N, Sima CS, Zakowski MF, Pao W, et al. Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with EGFR-Mutant Lung Cancers. Clin Cancer Res. 2013.

34. Miller CT, Lin L, Casper AM, Lim J, Thomas DG, Orringer MB, et al. Genomic amplification of MET with boundaries within fragile site FRA7G and upregulation of MET pathways in esophageal adenocarcinoma. Oncogene. 2006;25:409-18.

35. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010;17:77-88.

36. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344:783-92.

37. Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011;365:1273-83.

38. Valabrega G, Montemurro F, Sarotto I, Petrelli A, Rubini P, Tacchetti C, et al. TGFalpha expression impairs Trastuzumab-induced HER2 downregulation. Oncogene. 2005;24:3002-10.

39. Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst. 2007;99:628-38.

40. Berns K, Horlings HM, Hennessy BT, Madiredjo M, Hijmans EM, Beelen K, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell. 2007;12:395-402.

41. Shattuck DL, Miller JK, Carraway KL, Sweeney C. Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. Cancer Res. 2008;68:1471-7.

42. Minuti G, Cappuzzo F, Duchnowska R, Jassem J, Fabi A, O'Brien T, et al. Increased MET and HGF gene copy numbers are associated with trastuzumab failure in HER2-positive metastatic breast cancer. Br J Cancer. 2012;107:793-9.

43. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol. 2010;11:753-62.

44. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discovery. 2011;1:508-23.

45. Yonesaka K, Zejnullahu K, Okamoto I, Satoh T, Cappuzzo F, Souglakos J, et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Science translational medicine. 2011;3:99ra86.

46. Diaz LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486:537-40.

47. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012;486:532-6.

48. Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, et al. Amplification of the *MET* receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discovery; in press.

49. Jun HJ, Acquaviva J, Chi D, Lessard J, Zhu H, Woolfenden S, et al. Acquired MET expression confers resistance to EGFR inhibition in a mouse model of glioblastoma multiforme. Oncogene. 2012;31:3039-50.

50. Go H, Jeon YK, Park HJ, Sung SW, Seo JW, Chung DH. High MET gene copy number leads to shorter survival in patients with non-small cell lung cancer. J Thorac Oncol. 2010;5:305-13.

51. Cappuzzo F, Marchetti A, Skokan M, Rossi E, Gajapathy S, Felicioni L, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. J Clin Oncol. 2009;27:1667-74.

52. Tanaka A, Sueoka-Aragane N, Nakamura T, Takeda Y, Mitsuoka M, Yamasaki F, et al. Co-existence of positive MET FISH status with EGFR mutations signifies poor prognosis in lung adenocarcinoma patients. Lung Cancer. 2012;75:89-94.

53. Suda K, Murakami I, Katayama T, Tomizawa K, Osada H, Sekido Y, et al. Reciprocal and complementary role of MET amplification and EGFR T790M mutation in acquired resistance to kinase inhibitors in lung cancer. Clin Cancer Res. 2010;16:5489-98.

54. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer. 2002;2:727-39.

55. Meads MB, Gatenby RA, Dalton WS. Environment-mediated drug resistance: a major contributor to minimal residual disease. Nat Rev Cancer. 2009;9:665-74.

56. Williams RT, den Besten W, Sherr CJ. Cytokine-dependent imatinib resistance in mouse BCR-ABL+, Arf-null lymphoblastic leukemia. Genes Dev. 2007;21:2283-7.

57. Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. Nature. 2012;487:505-9.

58. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature. 2012;487:500-4.

59. Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol. 1992;119:629-41.

60. Zhang YW, Su Y, Volpert OV, Vande Woude GF. Hepatocyte growth factor/scatter factor mediates angiogenesis through positive VEGF and negative thrombospondin 1 regulation. Proc Natl Acad Sci U S A. 2003;100:12718-23.

61. Harbinski F, Craig VJ, Sanghavi S, Jeffery D, Liu L, Sheppard KA, et al. Rescue screens with secreted proteins reveal compensatory potential of receptor tyrosine kinases in driving cancer growth. Cancer Discov. 2012;2:948-59.

62. Shojaei F, Lee JH, Simmons BH, Wong A, Esparza CO, Plumlee PA, et al. HGF/c-Met acts as an alternative angiogenic pathway in sunitinib-resistant tumors. Cancer Res. 2010;70:10090-100.

63. Yano S, Wang W, Li Q, Matsumoto K, Sakurama H, Nakamura T, et al. Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. Cancer Res. 2008;68:9479-87.

64. Yano S, Yamada T, Takeuchi S, Tachibana K, Minami Y, Yatabe Y, et al. Hepatocyte growth factor expression in EGFR mutant lung cancer with intrinsic and acquired resistance to tyrosine kinase inhibitors in a Japanese cohort. J Thorac Oncol. 2011;6:2011-7.

65. Tanaka H, Kimura T, Kudoh S, Mitsuoka S, Watanabe T, Suzumura T, et al. Reaction of plasma hepatocyte growth factor levels in non-small cell lung cancer patients treated with EGFR-TKIs. Int J Cancer. 2011;129:1410-6.

66. Yamada T, Takeuchi S, Kita K, Bando H, Nakamura T, Matsumoto K, et al. Hepatocyte growth factor induces resistance to anti-epidermal growth factor receptor antibody in lung cancer. J Thorac Oncol. 2012;7:272-80.

67. Yamada T, Takeuchi S, Nakade J, Kita K, Nakagawa T, Nanjo S, et al. Paracrine receptor activation by microenvironment triggers bypass survival signals and ALK inhibitor resistance in EML4-ALK lung cancer cells. Clin Cancer Res. 2012;18:3592-602.

68. L C, D K, GJ R, PA J, FH B, DR C, et al. Initial phase II results with crizotinib in advanced *ALK*-positive non-small cell lung cancer (NSCLC): PROFILE 1005. Print this page. 2011 ASCO Annual Meeting; 2011.

69. Sasaki T, Koivunen J, Ogino A, Yanagita M, Nikiforow S, Zheng W, et al. A novel ALK secondary mutation and EGFR signaling cause resistance to ALK kinase inhibitors. Cancer Res. 2011;71:6051-60.

70. Mueller KL, Madden JM, Zoratti GL, Kuperwasser C, List K, Boerner JL. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 2012;14:R104.

71. Chen CT, Kim H, Liska D, Gao S, Christensen JG, Weiser MR. MET activation mediates resistance to lapatinib inhibition of HER2-amplified gastric cancer cells. Mol Cancer Ther. 2012;11:660-9.

72. Liska D, Chen CT, Bachleitner-Hofmann T, Christensen JG, Weiser MR. HGF rescues colorectal cancer cells from EGFR inhibition via MET activation. Clin Cancer Res. 2011;17:472-82.

73. Martens T, Schmidt NO, Eckerich C, Fillbrandt R, Merchant M, Schwall R, et al. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. Clin Cancer Res. 2006;12:6144-52.

74. ESMO, MIlan 2010, 510P.

75. Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn. 2012;14:22-9.

76. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1:508-23.

77. Ikebuchi F, Oka K, Mizuno S, Fukuta K, Hayata D, Ohnishi H, et al. Dissociation of c-Met phosphotyrosine sites in human cells in response to mouse hepatocyte growth factor but not human hepatocyte growth factor: the possible roles of different amino acids in different species. Cell Biochem Funct. 2012.

FIGURE LEGENDS

Figure 1. MET-induced signaling pathways and biological activities.

Upper panel: Hepatocyte growth factor (HGF) promotes dimerization and activation of MET at the plasma membrane. The phopsphorylation of cytoplasmic tyrosines creates binding sites for several SH2-containing intracellular tranducers such as Growth factor receptor-bound protein 2 (Grb2), Grb2-Associated Binding protein 1 (GAB1), phospholipase Cγ (PLCγ), Phosphoinositide 3-Kinase (PI3K), and Signal Transducer and Activator of Transcription 3 (STAT3). Signals originated by the RAS-MAPK, PI3K-AKT and STAT3 pathways reach the nucleus and modulate gene transcription and DNA replication. RAC1-dependent signals control cytoskeletal modifications. *Lower panels*: Invasive growth is a complex biological program which results from the combination of different biological activities (shown in the cartoon) such as proliferation, protection from apoptosis, motility and differentiation. BM = basement membrane.

Figure 2. MET interaction with other membrane receptors.

The strength and duration of MET-induced signals are regulated by a network of co-receptors (such as adhesive receptors, death receptors, B plexins and other tyrosine kinase receptors) that physically associate with MET. The roles taken on by these interactions are reported below the corresponding receptors.

Figure 3. MET alterations in human tumors.

Deregulated MET activation in several human tumors can be due to different molecular alterations. (A) The most common mechanism is MET overexpression in the absence of gene amplification, due to hypoxia, activation of upstream oncogenes, inactivation of tumor suppressor genes or loss of microRNAs. (B) *MET* gene amplification drives receptor overexpression and constitutive activation. (C) Activating germline mutations have been identified in patients affected by hereditary papillary renal carcinomas; somatic mutations, located either in the intracellular or in the extracellular domain, have been found in the indicted cancer types (D) Autocrine MET activation is generally due to ectopic MET expression in cells producing HGF.

Figure 4. HGF/MET inhibitors in active clinical trials.

Anti-MET inhibitors fall in two main categories: monoclonal antibodies directed against MET or HGF (upper panels) and small kinase inhibitors (lower panel). The table shows a list of the active clinical trials targeting either HGF or MET.

Figure 5. Cell autonomous mechanisms of resistance to target therapies involving the HGF/MET axis.

(A) *MET* gene amplification results in overexpression of the receptor and HER3 transphosphorylation in NSCLC cells in which EGFR is inhibited by specific TKIs. (B) Chromosome 7 duplication results in increased copy number of both *HGF* and *MET* and, thus, in increased MET-dependent signal transduction. This results in resistance to trastuzumab in breast cancer cells. (C) Upon EGFR inhibition, a substantial increase of MET expression is responsible for sustained pro-survival AKT signaling, leading to resistance to EGFR TKIs in glioblastoma. (D) *MET* gene

amplification, causing receptor overexpression and activation of downstream pathways, induces resistance to cetuximab and panitumumab in colorectal cancer.

Figure 6. Non cell-autonomous mechanisms of resistance to target therapies involving the HGF/MET axis.

(A) HGF secretion by stromal cells results in MET activation and stimulation of PI3K-AKT and MAPK pathways in tumor cells presenting BRAF activating mutations or constitutive RTK activation. This leads to resistance to inhibitors targeting BRAF or growth factor receptors, respectively. (B) HGF production by stromal cells can contribute to induce resistance to treatment with sunitinib by activating MET-dependent pathways in endothelial cells that compensate for VEGFR inhibition.

Figure 1

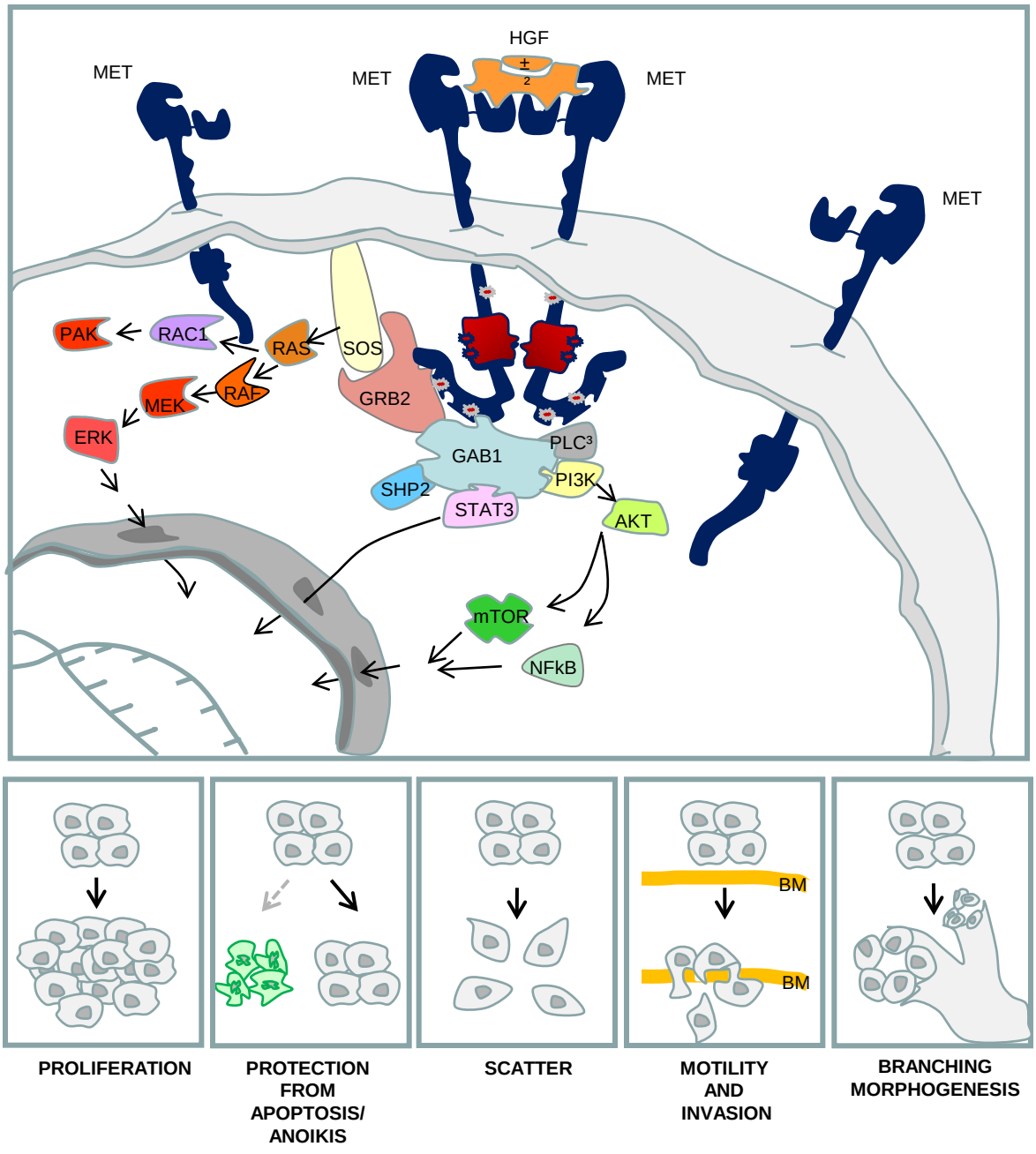

HGF
MET
MET
MET
MET
PAK
RAC1
RAS
SOS
MEK
RAF
ERK
GRB2
GAB1
PLC³
PI3K
SHP2
STAT3
AKT
mTOR
NFkB
BM
BM
PROLIFERATION
PROTECTION FROM APOPTOSIS/ ANOIKIS
SCATTER
MOTILITY AND INVASION
BRANCHING MORPHOGENESIS

Figure 2

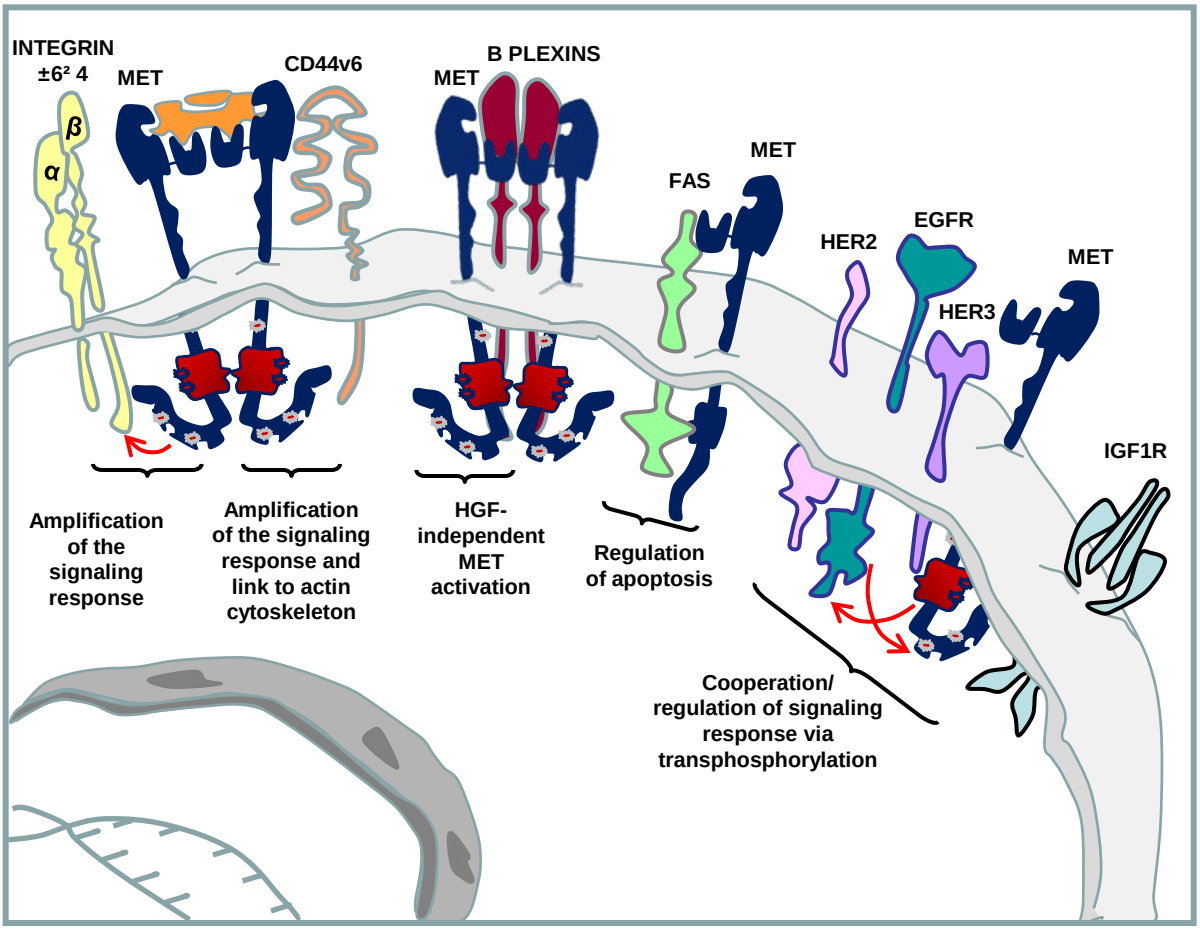
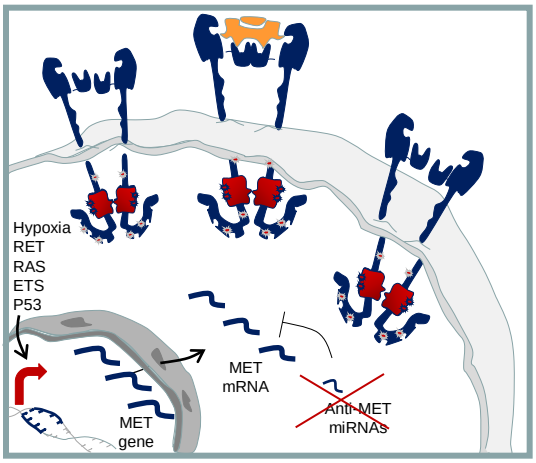

INTEGRIN
±6² 4
β
α
MET
CD44v6
B PLEXINS
MET
FAS
MET
HER2
EGFR
HER3
MET
IGF1R
Amplification of the signaling response
Amplification of the signaling response and link to actin cytoskeleton
HGF-independent MET activation
Regulation of apoptosis
Cooperation/ regulation of signaling response via transphosphorylation

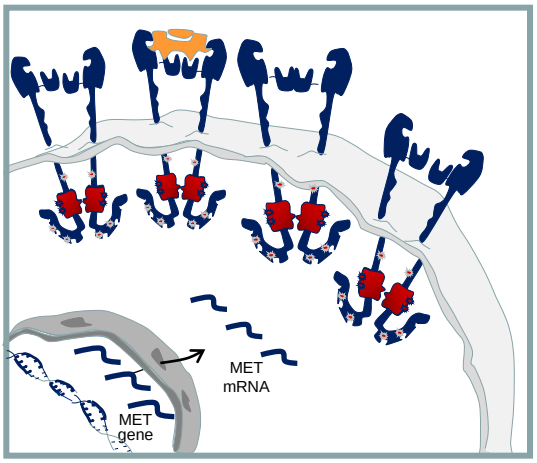
Figure 3

A MET overexpression



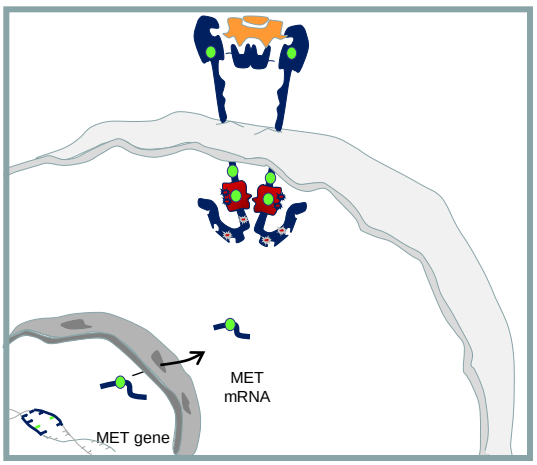
- Renal cancer
- Gastic cancer
- Hepatocellular cancer
- Thyroid cancer
- Ovarian cancer
- Colorectal cancer
- Breast cancer
- Oral squamous cell cancer
- Pancreatic cancer
- Prostatic cancer

B MET gene amplification



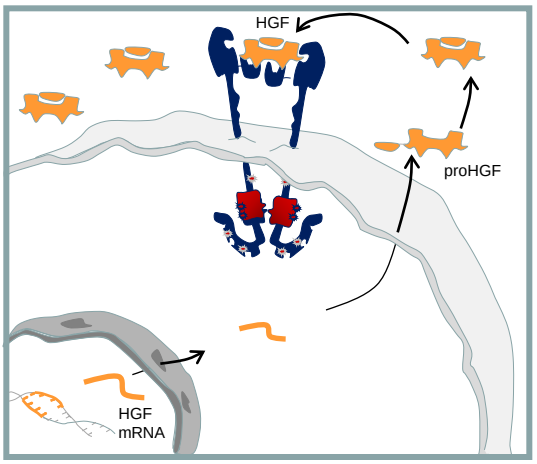
- Gastric cancer
- Esophageal cancer
- Colorectal cancer
- Non-Small Cell Lung cancer
- Glioblastoma
- Medulloblastoma
- Endometrial cancer

C MET germline/somatic mutations



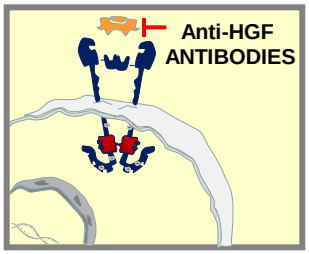
- Papillary renal cell cancer (germline and somatic)
- Gastic cancer (germline)
- Childhood hepatocellular cancer (somatic)
- Lymphnode metastases of head and neck squamous cell cancer (somatic)
- Glioma (somatic)
- Lymphomas (somatic)
- Melanoma (somatic)
- Mesothelioma (somatic)
- Non-Small Cell lung cancer (somatic)
- Small Cell lung cancer (somatic)
- Ovary cancer (somatic)
- Tyroid cancer (somatic)

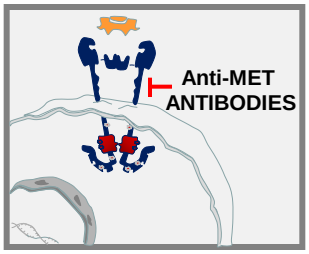
D HGF autocrine production

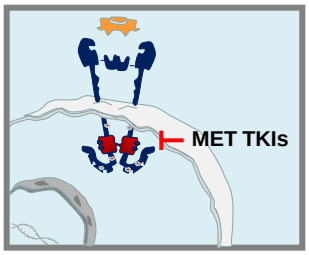


- Soft-tissue Sarcoma
- Osteosarcoma
- Acute myelogenous leukemia
- Glioblastoma
- Breast cancer

Figure 4







COMPOUND	COMPANY	FEATURES	CLINICAL DEVELOPMENT
FiclatuzuMAb (AV-299)	AVEO	MAb	Phase II: NSCLC Phase I: solid tumors, limphoma, multiple mieloma
RilotumuMAb (AMG-102)	Amgen	MAb	Phase II: colorectal, prostate, renal cell and gynaecological cancer, mesothelioma, glioma,SCLC and NSCLC Phase III: gastro-esophageal cancer
TAK-701	Millenium	MAb	Phase I: solid tumors (completed)
OnartuzuMAb	Genentech Roche	MAb	Phase II: NSCLC, breast and colorectal cancer. Phase III: NSCLC
LY-2875358	Eli Lilly	MAb	Phase I: solid tumors, lymphoma
Tivantinib	ArQule	Specific anti-MET TKI (non-ATP competitor)	Phase II: NSCLC, gastric, colorectal, renal cell, head and neck , prostate cancer Phase III: NSCLC, Hepatocelular cancer
EMD 1214063 **EMD 1204831**	EMD Serono	Specific anti-MET TKI (ATP competitor)	Phase I: solid tumors
INCB28060	Novartis/ Incyte	Specific anti-MET TKI (ATP competitor)	Phase I: solid tumors
Foretinib	Glaxo SmithKline	Non-Specific anti-MET TKI (ATP competitor): MET, VEGFR2, AXL, PDGFR, KIT, FLT3,TIE2	Phase II: NSCLC, breast, gastric, papillary renal and head and neck cancer.
Crizotinib	Pfizer	Non-Specific anti-MET TKI (ATP competitor): MET,ROS1, ALK, AXL,RON,TIE2	Phase III: ALK-altered NSCLC Phase I and II:lung cancer, anaplastic large cell lymphoma and others
Cabozantinib	Exelixis	Non-Specific anti-MET TKI (ATP competitor): MET, VEGFR2, RET, KIT, FLT3,TIE2	Phase III: medullary thyroid cancer, prostate cancer Phase II: NSCLC, brain, prostate , breast cancer and other solid tumors
AMG 208	Amgen	Non-Specific anti-MET TKI (ATP competitor): MET, RON	Phase I: solid tumors, lymphoma
MGCD-265	Methylgene	Non-Specific anti-MET TKI (ATP competitor): MET, RON, VEGFR1, TIE2 VEGFR2, VEGFR3,	Phase II: NSCLC
E7050	Eisai	Non-Specific anti-MET TKI (ATP competitor): MET, VEGFR2	Phase II: Hepatocellular cancer, glioblastoma, melanoma, gastric cancer, head and neck cancer

Figure 5

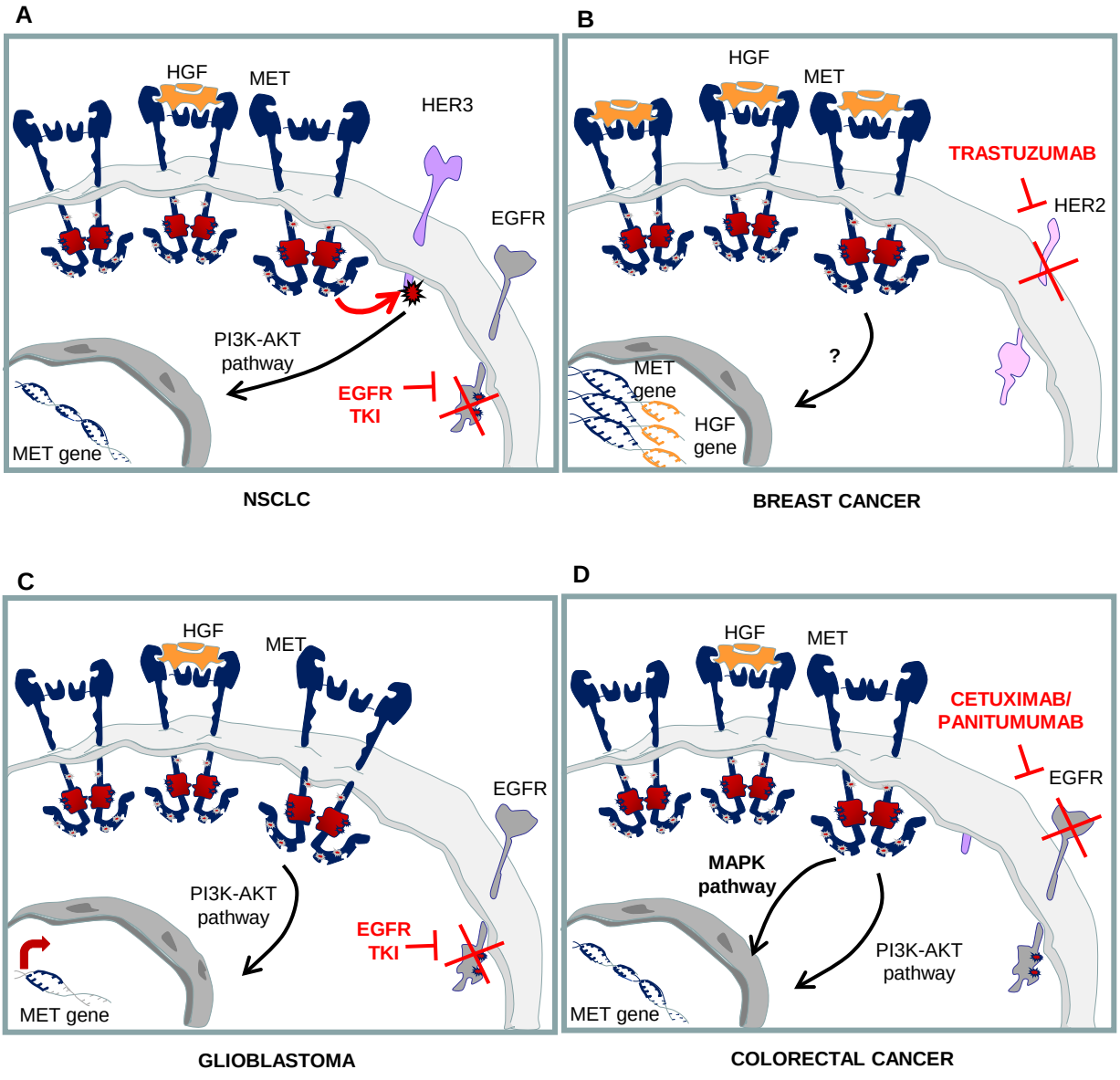
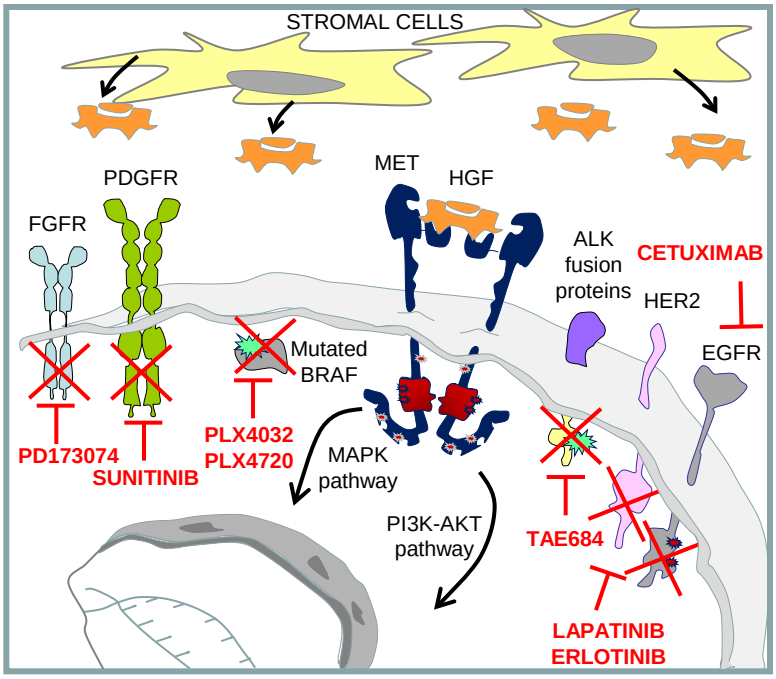

A
HGF
MET
HER3
EGFR
PI3K-AKT
pathway
EGFR
TKI
MET gene
NSCLC
B
HGF
MET
TRASTUZUMAB
HER2
?
MET
gene
HGF
gene
BREAST CANCER
C
HGF
MET
EGFR
PI3K-AKT
pathway
EGFR
TKI
MET gene
GLIOBLASTOMA
D
HGF
MET
CETUXIMAB/
PANITUMUMAB
EGFR
MAPK
pathway
PI3K-AKT
pathway
MET gene
COLORECTAL CANCER

Figure 6

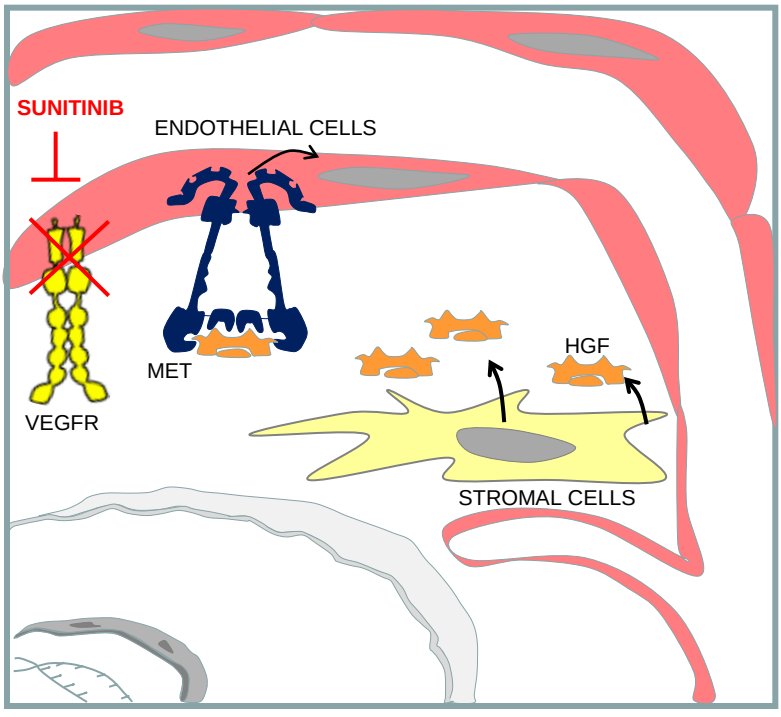
A



CANCER CELL LINES with constitutive EGFR activation, constitutive HER2 activation, amplified FGFR, amplified PDGFR, ALK translocation/mutation

COLON, GASTRIC, BREAST CANCER, NSCLC

B



TUMORS RESISTANT TO ANTI-ANGIOGENIC THERAPIES