[Frontiers in Bioscience, Landmark, 18, 454-473, January 1, 2013]

Biological significance and targeting of c-Met tyrosine kinase receptor in cancer

Liliane Goetsch[1], Veronique Caussanel[1], Nathalie Corvaia[1]

[1]Centre d'Immunologie Pierre Fabre, 5 avenue Napoleon III F-74164 Saint Julien en Genevois, France

TABLE OF CONTENTS

1. ABSTRACT

c-Met is a tyrosine kinase receptor largely described to be involved in cancer progression and metastasis. In such pathologic situation, many alterations of this receptor were noticed that include transcriptional overexpression, gene amplification, somatic or germline mutations and/or ligand dependent autocrine/paracrine loops. More recently it has also been suggested that c-Met would be involved in resistance to targeted therapies directed towards EGFR or angiogenesis. Major efforts from a large number of pharmaceutical companies are invested dedicated to evaluate the efficacy of either small molecule inhibitors or monoclonal antibodies directed against c-Met or its unique ligand HGF. A series of promising results from the first completed clinical trials indicated that compounds targeting c-Met have an acceptable toxicity profile and that efficacy was noticed in some treated patients. Non squamous NSCLC patients that express more often high levels of c-Met seemed to represent a most sensitive subset for and anti-c-Met/erlotinib therapy. Many Phase III trials are currently recruiting and a particular effort was performed in order to discover biomarkers associated with efficacy and patient selection. This review will provide an overview of the current knowledge on the c-Met axis for development of novel therapeutics in Oncology.

2. INTRODUCTION

Within the past decade, the receptor tyrosine kinase (RTK) c-Met has emerged as a potential target for cancer therapy. Activation of c-Met occurs via hepatocyte growth factor (HGF) binding and results in the activation of a series of signaling patways such as PI3K/Akt, Src, MAPK/Erk, STAT3 and FAK(1). c-Met is involved in tumor cell proliferation, migration, invasion, angiogenesis, survival and metastasis(2-5). c-Met pathway is frequently deregulated in a wide variety of human malignancies, including gastric, lung, colon, breast, bladder, head and neck thyroid, pancreatic and prostate cancers as well as hematological malignancies and central nervous system tumors(6-9). There are many aberrant situations related to c-Met status in cancer patients. It has been found to be transcriptionally up regulated in many tumors resulting in its significant over-expression (10). Gene amplification(11), germline(2,12) or somatic(8,13) mutations also leads to constitutive activation of c-Met. Finally HGF dependent autocrine loops are also described in gliomas(14). More recently c-Met has been described as potentially responsible for resistance to targeted therapies(15-17). Indeed there is accumulating evidence that acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors and angiogenic inhibitors

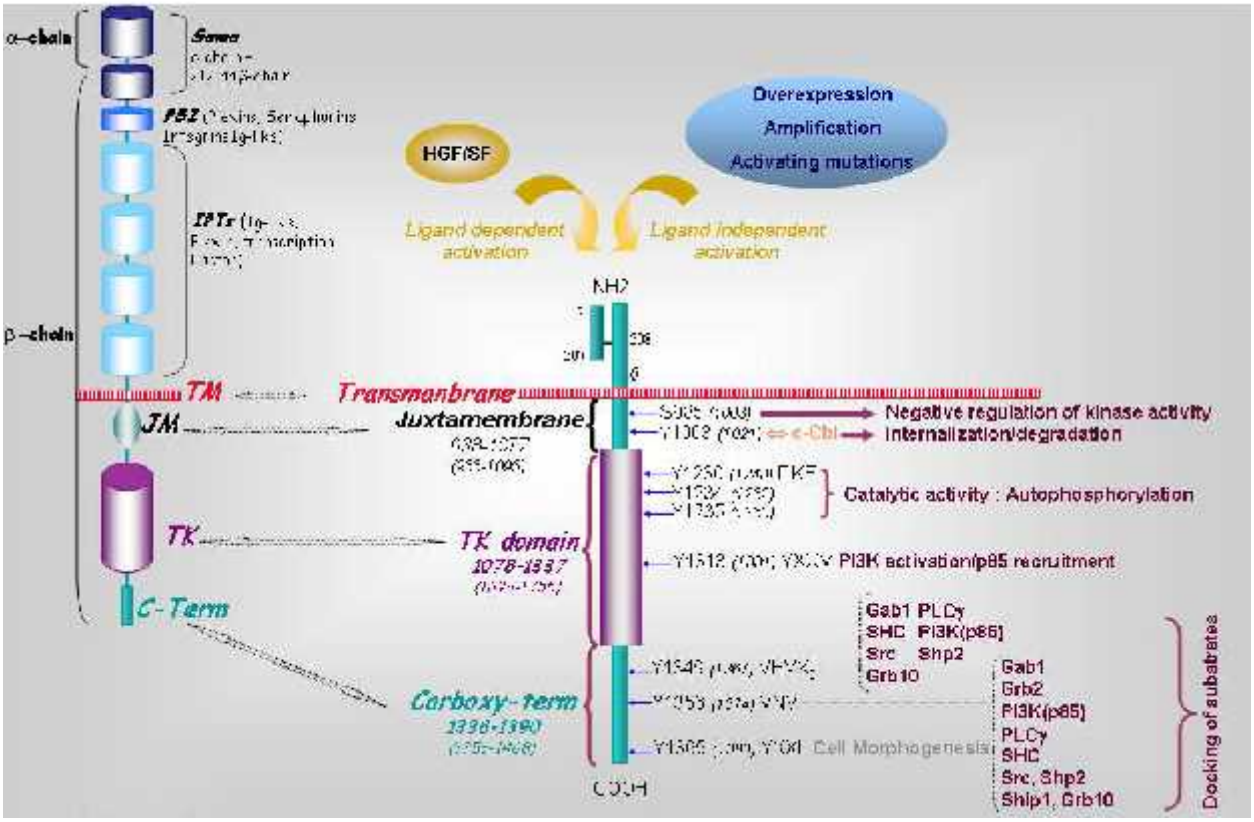

Figure 1. The c-Met receptor. C-Met is a transmembrane RTK with an extracellular domain consisting in 3 regions ; an N-terlinal region, a plexin semaphoring integrin domain (PSI) and a four unit immunoglobulin-like fold, plexins, transcription factors (IPT) domain. The intracellular C-terminal portion includes a juxtamembrane domain (JM), the TK domain and two tyrosines (Y1349 and Y135-) that form a unique-substrate docking site. In this figure, amino-acid residues are identified using the X54559 accession number. The corresponding J02958 accession numbers are written between brackets.

can be due, at least in part to increased activations of the c-Met pathway.

Inhibiting c-Met signaling is emerging as a promising strategy and several inhibitors are in various stages of clinical development. Promising results have been reported in lung, gastric, prostate and papillary renal cancer patients treated with these compounds. The main challenge facing the effective use of HGF/c-Met-targeted antagonists for cancer treatment remain the identification of the right subset of patients(18).

3. THE c-Met RECEPTOR IN CANCER DISEASES

The c-Met structure and functions

The c-Met receptor belongs to a sub-family of receptor tyrosine kinases (RTKs). It is formed by proteolytic processing of a 190 kDa glycosylated precursor in the post-golgi compartment and is composed of a 50 kDa extracellular α-chain disulfide-bounded to a 145 kDa membrane spanning β-chain. The extracellular portion of c-Met is composed of three domains. The N-terminal 500 residues are folded into a large semaphoring (SEMA) domain, which includes the whole α-chain and part of the β-subunit. The SEMA domain shares homologies with domains found in members of the semaphorin and plexin families of receptors. The PSI (also found in plexins, semaphorins and integrins) follows the SEMA domain, spans 50 residues and includes four disulfide bounds. It is though to function as a linking module that orients the extracellular fragment of c-Met for proper ligand binding(19). Further downstream are four immunoglobulin-plexin-transcription (IPT) domains which are relating to immunoglobulin-like domains. Intracellularly, the c-Met receptor contains a tyrosine kinase catalytic domain and a multifunctional docking site (20,21) (Figure 1). Several reports claim that the SEMA domain is the only ligand binding domain in c-Met(22). However, Basilico *et al.*(23) claim that IPT repeats 3 and 4, located closest to the juxtamembrane (JM) domain, also mediate high affinity ligand binding. c-Met has a single known ligand referred as hepatocyte growth factor (HGF) and also known as scatter factor (SF). This growth factor, mainly produced by mesenchymal cells, is widely distributed in the extracellular matrix of most tissues, where it is sequestrated in its inactive form by heparin like proteoglycans. HGF acts as a pleiotropic factor and cytokine, promoting many functions such as mitogenesis (24,25), motogenesis(26), invasion(27-29), morphogenetic differentiation(30-32) and angiogenesis(33,34). It is also involved in resistance to

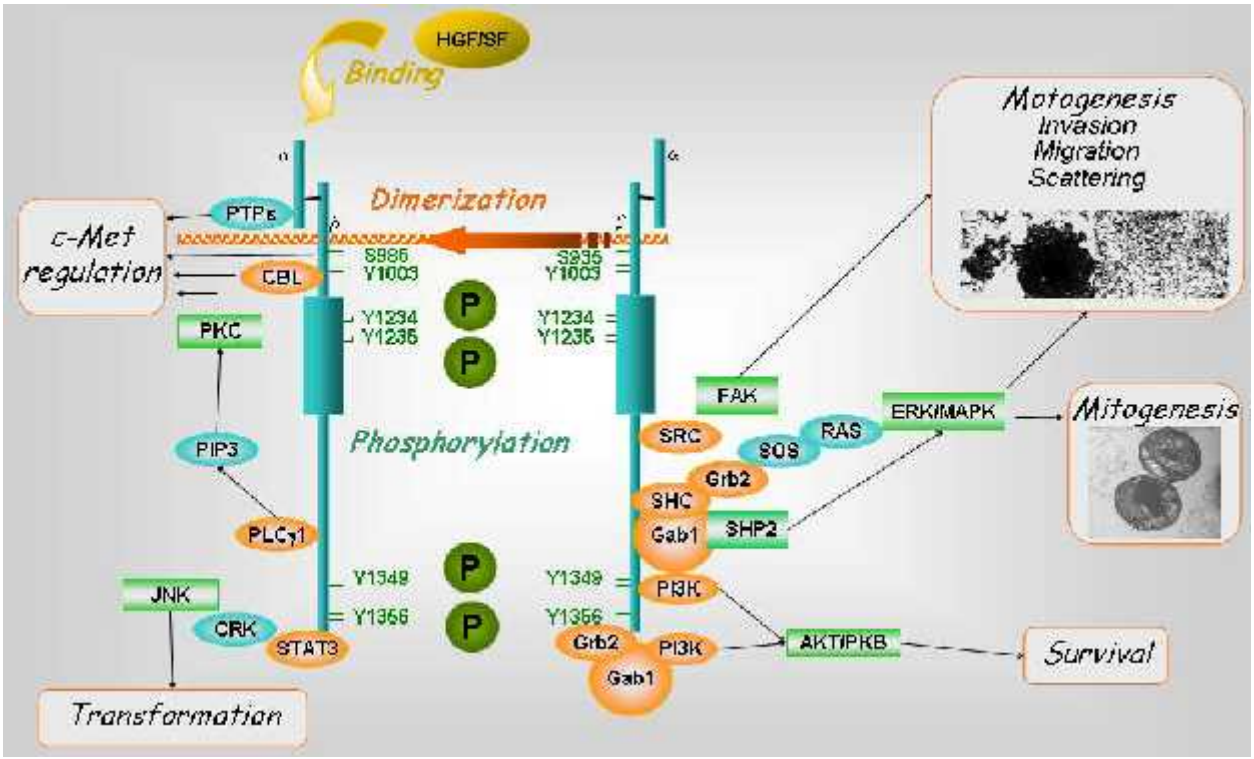

Figure 2. c-Met Functions. c-Met mediates multiple signaling cascades, ultimately resulting in transformation, mitogenesis, motogenesis, and survival.

apoptosis and is considered as an epithelial-mesenchymal transition promoter(35). Under physiological conditions, the first step of c-Met activation involves HGF binding, that results in receptor dimerization, triggering conformational changes that activates c-Met tyrosine kinase activity(7). Normally expressed by epithelial and endothelial cells, c-Met plays a role in embryological development(36-38), tissue regeneration(39) and development of various organs(36,37,40-44) . However c-Met is also described as an oncogene that plays a major role in tumorigenesis and cancer progression(2). It is expressed in a variety of human cancers(11) in which many HGF/c-M*et al*terations have been described that may confer a substantial growth advantage and invasive potential to cancer cells. Among them, transcriptional up-regulation of c-Met(10,45-52), gene amplification(11,53-56), somatic(8,13) or germline(2,12) mutations were described. Several situations are also observed where autocrine HGF/c-Met loops induce aberrant activation of c-Met(9,14,57,58). Receptor activation could occur either upon ligand stimulation or independently of the presence of HGF. In this latter case, cell signaling is driven by spontaneous dimerization, when c-Met is over-expressed on cancer cells(59,60).

3.1. The c-Met signaling pathways

The complex phenotype that results from c-Met signaling involves a series of molecular events which have been described in many recent reviews(61-63). Activation of c-Met leads to the autophosphorylation of specific tyrosine residues (Y1230/1234/1235) within the activating loop of the TK domain which in turn autophosphorylate of the carboxy-terminal bidentate substrate binding site Y1349/Y1356. These two tyrosines form a tandem SH2 recognition motive unique to c-Met (Y1349VHVX3Y1356VNV) which, stimulates multiple downstream signaling pathways(35) (Figure 2) via a series of effector proteins including Grb2, SHC, PI3K, SHIP-2, SRC and STAT3. In addition, unique to c-Met is its association with the adaptor protein Gab1 (64), a multi-adaptor protein that, once bound to and phosphorylated by c-Met, creates binding sites for more downstream adaptors. Gab1 can bind directly, through a unique 13-amino-acid c-Met binding site, and indirectly through c-Met-bound Grb2. Additional tyrosines could also contribute to c-Met signaling. When phosphorylated, Y1313 binds and activates PI3K probably promoting cell viability and motility(65). In addition Y1365 is involved in the regulation of cell morphogenesis(65).

The downstream response to c-Met activation relies on signaling modulators common to many RTKs including the MAPK, PI3K, PLCγ1, STAT3 and FAK pathways. For the MAPK cascade, docking proteins such as SHC and Grb2 bind to the phosphotyrosine residues of the activated receptor. Grb2 binds then to the guanine nucleotide exchange factor SOS that becomes activated and promotes the removal of GDP from Ras. Ras can in turn bind GTP and becomes active leading to the indirect activation of Raf kinases which subsequently activate the MAPK effector kinase MEK, and finally ERK/MAPK that translocate to the nucleus and activate transcription factors

responsible for regulating a large number of genes involved in cell cycle progression, proliferation and motility. SHP2 can also link c-Met signaling to the MAPK cascade, as sequestration of SHP2 to Gab1 is responsible for extending the duration of MAPK phosphorylation. The PI3K pathway is also largely involved in c-Met signaling(66). The p85 subunit of PI3K can bind either directly to phosphorylated c-Met or indirectly through Gab1, which then signals through AKT/protein kinase B. This latter axis is primarily responsible for the cell survival response to c-Met signaling as Akt is able to suppress apoptosis through inactivation of the pro-apoptotic protein Bcl-2 antagonist of cell death BAD and to activate the E3 ubiquitin-protein ligase MDM2, which promotes the degradation of the pro-apoptotic protein p53. Transformation downstream to c-Met is mediated by the phosphorylation of JNK that occurs via binding to CRK. STAT3 has also been involved in transformation(67,68). The direct binding of STAT3 to c-Met results in STAT3 phosphorylation, homodimerization through the SH2 domains and translocation to the nucleus where they play the role of transcription factors to regulate a series of genes. This has been shown to result in tubulogenesis and invasion. However, other published data have found that, STAT3 is also required for c-Met-induced transformation with no effect on proliferation, invasion or branching morphogenesis. Finally, FAK activated through phosphorylation by SRC kinase family leads to cell migration and promotion of anchorage-independent growth.

Many mechanisms are involved in c-Met regulation. Phosphorylation of S985 located within the JM domain negatively regulates kinase activity(71). Another important regulatory site, also located in the JM domain of c-Met involves the Y1003 residue. When phosphorylated, Y1003 binds to c-Cbl, which ubiquitinates activated c-Met, inducing receptor internalization, trafficking to late endosomes and ultimate degradation(69,70). A negative regulation is also induced by binding of PLCγ to c-Met that leads to the activation of PKC that in turn inhibits c-Met activation(71). Independently of PKC activation, an intracellular increase of calcium can also result in a negative regulation of c-Met(72). c-Met is also the substrate for several protein tyrosine phosphatases (PTPs), including receptor PTPs such as DEP1 (also known as PTPRJ), LAR (also described as PTPRF)(73,74) or non-receptor PTPs among which PTP1B (also described as PTPN1) and TCPTP (also known as PTPN2)(75). These phosphatases inhibit c-Met signals by triggering dephosphorylation of either the catalytic domain of c-Met (PTP1B or TCPTP) or docking tyrosines (DEP1). More recently, a new mechanism regulating protein expression has been discovered that involved microRNAs, which are small non-protein-coding RNAs that function as negative gene regulators. Three microRNAs that negatively regulate c-Met (miR-34b, miR-34c and miR-199a) have already been identified(76).

4. HGF/c-Met AXIS ALTERATIONS IN CANCER DISEASES

4.1. c-Met over-expression

Over-expression of c-Met has been shown to be sufficient for transformation of normal cells both in *in vitro* and *in vivo* models (77-79). Over-expression can occur by transcriptional up-regulation that possibly results from upstream gene mutations. Various activated oncogenes (Ras, Ret, ETS) have been shown to induce c-Met over-expression(80). c-Met has been identified as a transcriptional target of β-catenin(81), PAX5(82) and MACC1(83). Some data indicated that changes in the tumor microenvironment are also involved(84). For example, hypoxia up regulates c-Met expression through the binding of HIF-1 to the c-Met promoter.

Over-expression could also result of *c-Met* gene amplification and many reports demonstrated that in such cases tumors were described as addicted to c-Met. Several cell lines from gastric(11,53,85,86), esophageal(11,87), liver metastasis of colorectal carcinomas(56) and NSCLCs(54,88) display an amplification of the *c-Met* gene. For such cells, it has been shown that c-Met inhibition results in a significant proliferative impairment and in a massive apoptotic cell death(89-91). This dramatic response is similar to the one observed in EGF-R-addicted cell lines upon treatment with EGFR inhibitors(92-94). An analysis of a panel of gastric cancer cell lines by using qPCR identified increased *c-Met* gene copy number in 29% of cases(85). In all amplified cell lines, FISH analysis showed the *c-Met* gene copies to be integrated within its specific chromosomal locus, consistent with so-called homogeneously stained regions (HSRs). An 8-fold cutoff of gene amplification, as measured by qPCR, has been selected to provide a clear distinction between gastric cancer cells with low-level aneuploidy (Amp-) versus those with high-level specific HSR-amplification of *c-Met* (Amp+). As expected, Amp+ cells displayed dramatic elevation in c-Met protein expression, compared to Amp- ones. Furthermore Amp+ cells also displayed high levels of baseline ligand-independent c-Met activation. To confirm the potential therapeutic relevance of these observations, Amp- and Amp+ cells were treated with PHA-665752. Five of 5 Amp+ cell lines appeared to be highly sensitive to this drug whereas no effect was observed in any of the 12 Amp- cell lines. These observations strongly demonstrated that *c-Met* amplification in gastric cancers may constitute an important molecular biomarker. Similar observations have been performed with NSCLC cell lines(88): (i) a subset of them carried *c-Met* amplification associated with c-Met over-expression, (ii) these cells are dependent on c-Met for their proliferation and survival. This property is opposed to what is observed for non-amplified NSCLC cell lines which have very low level of basal c-Met activation and that are not dependent on c-Met for growth. A parallel between preclinical studies and the clinical situation regarding *c-Met* amplification should be considered. c-Met has been reported to be over-expressed in many epithelial cancers, but gene amplification is most common in gastric cancers, where 10-20% of all primary tumors and up to 40% of the scirrhous histological subtype exhibited increased *c-Met* gene copy number(86,90,95). Using FFPE primary tumor tissues from 482 patients with gastric cancers to analyze their c-Met expression it has been demonstrated that patients with *c-Met* amplification and concomitant c-Met protein activation showed the worst outcome in subgroup analyses(96). Amplified *c-Met* gene

has been found as a frequent event (21%) in chemotherapy and EGFR-TKIs naïve NSCLC patients(97). In this latter case, *c-Met* gene amplification could be associated with *EGFR* amplification (43% of the *c-Met* gene amplified patients), but not with the presence of K-Ras mutation. In gastric cancer tissues c-Met expression correlated with advanced tumor stages(48,91,98-100). *c-Met* amplification has been reported to occur at a higher percentage in diffuse type gastric carcinomas compared to intestinal type cancers (101). Nakajima *et al.*(91) described *c-Met* amplification as an independent poor prognostic factor for gastric carcinoma. Amemiya *et al.* (99) found a significantly higher frequency of c-Met expression in stage IV gastric cancer with liver metastasis than in stage IV carcinoma without liver metastasis. Studies on patients with colon cancer also underlined that *c-Met* gene amplification was associated with advanced stage colorectal cancer (stage IV) and liver metastases(102), proposing the role of c-Met as a useful indicator of liver metastasis. Huang *et al*(98) described a correlation between depth of tumor invasion and c-Met expression but did not find an association with lymph node, liver or peritoneal metastasis. More recently, Kong *et al*(103) suggested that over-expression of c-Met was associated with shorter survival time and poor treatment response in glioblastoma. However, no data indicated whether the observed over-expression of c-Met resulted from a *c-Met* gene amplification. Altogether, these investigations point to the involvement of c-Met in tumor progression for a subset of patients with high grade and metastatic disease. In contrast with these data, Drebber *et al*(104), did not found any correlation between c-Met protein expression and tumor stage according to TNM classification, nor a higher rate of c-Met positive carcinomas in diffuse type carcinoma compared to intestinal type. However, and in agreement other published data(91), they found a statistically significant correlation between high c-Met expression and poor prognosis, confirming the role of c-Met as an independent prognostic factor.

Taken together, these data highly suggest the relevance of *c-Met* amplification for gastric, colon and NSCLC patient selection. However, recent studies emphasis on the importance of differentiating patients with *c-Met* amplification from the one harboring a polysomy of chromosome 7(105) in order to determine whether tumors from both subsets are likely to be c-Met driven. Indeed it is known that breast tumors with an increase *HER2* gene copy number as a result of polysomy 17 behave as HER2-negative tumors(106).

4.2. Phosphorylation of c-Met

Phosphorylation of c-Met (p-c-Met) was detected in cancer cells, but was rare in normal tissue. When assessing for functional activity of tumor tissues with p-c-Met staining, 44% of adenocarcinomas, 86% of large cell, 71% of squamous cell, 40% of carcinoids and 100% of SCLC demonstrated c-Met phosphorylation at the Y1003 c-Cbl binding site. Likewise, autophosphorylation at the Y1230/1234/1235 site was observed in 33% of adenocarcinomas, 57% of large cell and 50% of SCLC(107). In bladder cancer, although c-Met, pY1234/1235 c-Met and pY1349 c-Met were associated with pT stage, multivariate analysis identified pY1349 c-Met expression only as a significant factor for high pT stage. Expression of pY1349 c-Met was a significant predictor of metastasis and survival of patients with bladder cancer(108).

Moreover, Zucali *et al*(109) observed that pY1003 c-Met membrane staining was significantly correlated to primary gefitinib resistance and shorter time to progression in NSCLC. A multivariate analysis investigating EGFR-TKIs in NSCLC confirmed an increased risk of disease progression in the subset of patient whose over-expressed pY1003 and interestingly, all these patients presented typical EGFR-TKIs non responder characteristics. Among the patients with strong pY1003 c-Met over-expression, 3 out of 4 analyzed by FISH exhibited also high MET copy numbers and 7 out of 9 (78%) had also positive expression of pAkt. None of these patients harbored EGFR19 mutations or experienced some benefit from EGFR-TKIs when treated.

4.3. Mutated c-Met forms

More than 20 different somatic or germline mutations have been described so far for c-Met by sequencing DNA from patient samples(110). The major part of these mutations is missense mutations within different domains of the receptor. Extensive mutation analysis of the c-Met gene in sporadic human primary tumors and cell lines indicate that c-Met gene mutations are quite rare events in human primary carcinogenesis, expected for hereditary papillary renal carcinoma (HPRC)(2,111). However, they are frequently associated with tumor progression(112-114). Indeed, a significant number of c-Met mutations, mostly located in the tyrosine kinase domain, have been found in metastases of cell carcinomas(8). In HPRC, a genetic connection has been established between c-Met and cancer occurrence. Upon sequencing the c-Met gene from affected members of HPRC families and from patients with sporadic papillary carcinomas, 14 different mutations have been characterized, that result in amino acid substitutions in the receptor kinase domain(2,111). Eight of them are germline (g) mutations (M1149T(g), V1206L(g), V1238I(g), D1246N(g), Y1248C(g), V1110I(g), H1112Y(g), Y1248D(g)) and six are somatic (s) ones (L1213V(s), D1246H(s), Y1248H(s), M1268T(s), H1112L(s), H1124D(s)). Four of these mutations, are of particular interest: i) D1246N(g) and D1246H(s), located at positions homologous to a naturally-occurring mutation in the tyrosine kinase receptor c-Kit (D816V), ii) M1268T(s) corresponding to a position homologous to a mutation in the Ret proto-oncogene (M918T) and iii) V1110I(g) is a mutation homologous to the chicken proto-oncogene, c-erbB (V157I)(115). Mutated Kit alleles are found in patients with mastocytosis and acute myeloid leukaemia of M2 subtype(116,117) and missense mutations in Ret are associated with multiple endocrine neoplasia type 2B(118). This suggests that alteration of these residues is a critical event in deregulating tyrosine kinase receptors.

When transfected in NIH 3T3 cells, all of the above described mutants induced stronger autophosphorylation than wild type c-Met did(119). However, M1268T was a very strong activating mutation whereas others resulted only in moderate (D1246N, D1246H, Y1248H, Y1248C, Y1248D, H1112Y) to weak (V1238I, V1206L, M1149T, V1110I, H1112L, H1124D) activation. The level of intrinsic activation of c-Met phosphorylation was tightly correlated with a transformed phenotype of NIH 3T3 cells and their ability to form foci when cultured *in vitro*. Moreover cells expressing each of the mutant c-Met proteins were able to form tumors in athymic nude mice more readily than cells expressing wild-type c-Met. As with the foci formation assay, a strong correlation was observed between autophosphorylation activity and tumorogenesis, with M1268T activating mutation being the most tumorigenic one. Additional experiments with cells carrying the M1268T form of c-M*et al*so demonstrate that i) they were highly metastatic *in vivo*, and ii) able to induce scattering in MDCK cells, compared to the wild type c-Met cells(3). In recent studies, analysis of c-Met trafficking indicated that internalization of this active mutant was enhanced compared with wild type but also that recycling of the M1268T mutant was more efficient (a property also observed for the D1246N mutant)(120). After acute HGF stimulation, wild type receptor was degraded over several hours(121), but both activated mutants were largely refractory to degradation. This recycling could be part of the oncogenicity of these mutants.

None of these mutations in the c-Met proto-oncogene were detected in other tested tumor types including metanephric adenomas, pancreatic and colon carcinomas, malignant melanomas, papillary thyroid carcinomas, Wilm's tumors, gliomas, bladder and prostate cancers(111). However, Park *et al.*(13) described 3 other missense mutations (T1191I(s), K1262R(s), M1268I(s)) in the kinase domain of c-Met that were exclusively found in childhood hepatocellular carcinomas (HCC). Other activating and oncogenic mutations of c-Met were also found in head and neck squamous carcinomas metastases (Y1253D(s))(8,112) or in colorectal metastases (N1118Y(s))(112). Additional mutations were observed within the semaphoring domain (E168D, L229F, S323G, N375S, M431V, N454(9,107)I (107,122). Seven percent of mesothelioma patients (3 of 43) were found to carry N375S, M431V and N454I mutations within this domain(9). Mutations were also described within the juxtamembrane domain (P1009S(g), T1010I(g), R988C, S1058P, N948S, alternative splice product skipping entire juxtamembrane domain)(12,107,123), and in the c-Cbl domain binding site of c-Met (Y1021F)(124). In this latter case, observed in NSCLC, the mutation leaded to a loss of Cbl E3-ligase binding which enhances c-Met protein stability resulting in a sustained c-Met activation(125).

5. CROSSTALK BETWEEN c-Met AND OTHER SIGNALING PATHWAYS

c-Met interacts with other membrane receptors and many different molecules acts as c-Met partners. Among them, interactions with plexin family members(126), integrin $\alpha 6\beta 4$, adhesive molecules such as CD44v6(127), tetraspanines(128,129), FAS but also other TKR were described. Relating to TKR a large number of reports have documented that c-Met was involved in mechanisms that can drive the escape from TKI therapies that occurred either by amplification of an oncogenic kinase gene or by substitution of the signaling functions of the targeted kinase by up-regulation of compensatory pathways. Some of these resistance mechanisms will be described thereafter.

5.1. Crosstalk BETWEEN c-Met and EGFR

An increasing number of preclinical data, including combination experiments, are in agreement with the major role of EGFR and c-Met crosstalks in resistance to EGFR therapies(15,54,130). Gefitinib and erlotinib inhibit the EGFR kinase and are approved to treat NSCLCs that have activating mutations such as i) deletions involving at least 12 nucleotides in exon 19 that eliminate a conserved LREA motif or ii) single point mutations in exon 21 (L858R) of the EGFR gene(92,93,131,132). While most of these EGFR mutant NSCLCs initially respond to EGFR inhibitors, the vast majority of these tumors finally become resistant to that therapy. In about 50% of the cases, resistance is due to the occurrence of a secondary mutation in EGFR described as T790M(133,134) or D761Y(134). To explore additional mechanisms of gefitinib resistance in NSCLC, Engelman *et al.*(15) isolated resistant clones of the gefitinib hypersensitive EGFR exon 19 mutant HCC827 cell line. The selected resistant cells maintained activation of Her3, PI3K and Akt in presence of Gefitinib. Performing genome wide copy number analyses and mRNA expression profiling of resistant versus parental cells, the authors found that resistant cells had amplified the *c-Met* oncogene. Treatment of resistant cells with either a TKI specific for c-Met or gefitinib alone did not inhibit cell viability nor affect Her3 and Akt phosphorylation. However, the combined treatment impaired significantly resistant cell growth and fully suppressed Her3 and Akt phosphorylation. Co-precipitation experiments strongly suggested that in gefitinib-resistant cells *c-Met*, that has been amplified, could trigger the activation of Her3, independent of EGFR kinase activity. Altogether these data suggest that resistant cells had escaped from drug-mediated EGFR inhibition by recruiting c-Met as a compensatory input to activate Her3, PI3K and Akt as survival pathways(135). The clinical relevance of *in vitro* results has been evaluated by analyzing *c-Met* copy status in tumors from 18 patients who had shown partial responses to gefitinib or erlotinib during initial treatment but who showed signs of tumor relapse while still receiving the treatment. In available paired tumor samples, 2 out of 8 specimens showed *c-Met* gene amplification in resistant but not in pre-therapy tumor samples indicating that therapeutic pressure may have induced this gene amplification. Including all samples, *c-Met* gene amplification was detected in 22% resistant lung cancers. Similar results were also observed by Bean *et al.* (54) who found a *c-Met* gene amplification in 9 of 43 (21%) patients with acquired resistance to gefitinib or erlotinib versus 2 of 62 (3%) in patients unexposed to EGFR kinase inhibitors. To determine whether *c-Met* amplification is also responsible for primary resistance to

gefitinib therapy in EGFR-positive NSCLC patients, a larger study was performed in colorectal cancer patients (136). In the selected cohort, *K-RAS*, *Her2* and *EGFR* gene status was determined, in order to evaluate the impact of c-Met without the confounding effect of other biological mechanisms potentially responsible for primary resistances. A c-Met FISH analysis was carried out on tumor biopsies from patients before exposure to EGFR-TKIs and no patient with amplified *c-Met* was identified while a large percentage of the studied patients displayed a primary resistance to gefitinib therapy.

More recently it has been described that high levels of HGF immunoreactivity were detected in lung adenocarcinoma from patients who showed intrinsic or acquired resistance to gefitinib without relation to T790M second mutation or *c-Met* amplification(137). Using Her3 siRNA, Yano *et al.*(137) demonstrated that HGF treatment completely restored PI3K/Akt signaling independently of Her3 in both PC-9 and HCC827 cell lines. Additional studies confirmed the role of HGF in mediating EGFR TKI resistance(138). Indeed Turke *et al.* demonstrated that HGF can independently rescue both PI3K/AKT and ERK signaling in presence of gefitinib but also that, unlike in c-Met-amplified resistant cancers, HGF-mediated resistance occurred through Gab1 and not ERBB3 signaling. Higher levels of HGF can be detected in tumor specimen from patients with NSCLC that are clinically resistant to gefitinib or erlotinib, compared with pretreatment tumors specimens. Notably, in some patients without evidence of EGFR T190M or *c-Met* amplification, HGF expression was greater in the resistant specimen than in pretreatment specimens, supporting the role of HGF alone in promoting drug resistance. A second role of HGF was described indicating that the c-Met ligand was able to accelerate the emergence of *c-Met* amplification in HCC827 cells both *in vitro* and *in vivo*. This process requires concomitant EGFR inhibition suggesting that this latter inhibition provides a unique proliferative advantage to a subset of cells with high c-Met expression, thus facilitating their rapid clonal expansion. These findings indicate that involvement of c-Met signaling is a unique resistance mechanism to kinase inhibitors that can occur through multiple independent mechanisms such as amplification or ligand mediated activation that can lead to rapid drug resistance when combined.

Taken together, these data support that the development of anti-c-Met therapeutic strategies should be focused on patients with acquired EGFR-TKI resistance. However, targeting c-Met might also be used as combinations with EGFR-targeted therapies to sensitize patients to gefitinib. Clinical trial combining ARQ197, a selective non-ATP competitive inhibitor of c-Met and erlotinib has been investigated in a randomized trial comparing the combination regimen to erlotinib monotherapy in $2^{nd}/3^{rd}$ line NSCLC(139).

5.2. Crosstalk between c-Met AND Her2

Her2 was identified as an important regulator of cancer cell proliferation. As a consequence, many strategies that aimed at reducing the presence or activity of the Her2 receptor have been developed with trastuzumab being the most successful(140). However, resistance to trastuzumab, both inherent and treatment acquired represents a significant barrier to the effective treatment of Her2-positive breast cancer. c-Met and its ligand, HGF, are frequently over-expressed in breast cancer and correlate with decreased relapse-free survival(141-146). Indeed, c-Met receptor over-expression is an independent predictor of poor prognosis in breast cancer(52,147,148). A recent immunohistochemical study of Her2+ breast tumors showed that a subset of patients (5 of 20) were strongly positive for c-Met receptor expression(149). This is of particular importance since c-Met and Her2 have been shown to synergize in promoting cellular invasion, suggesting that tumors expressing both receptors may be more aggressive(150). More recently, a physical interaction between Her2 and c-Met has been demonstrated by co-immunoprecipitation experiments(151). Shattuck *et al.*(17) showed that attenuation of c-Met activity using siRNA or the small molecule inhibitor of c-Met SU11274, led to sensitization to trastuzumab treatment. Moreover, c-Met activation protected cell from the growth inhibitory effect of trastuzumab by preventing trastuzumab-induced p27 induction. In addition, they demonstrated that c-Met is co-expressed along with Her2 in Her2-over-expressing breast cancer cells, as well as in Her2+ clinical breast cancer samples. Finally, gene expression analysis of trastuzumab and vinorelbine-resistant tumor samples showed that HGF and c-Met expression was significantly increased. The average increase in expression was ~1.5-fold for HGF and 2.0-fold for c-Met receptor in the resistant tumors as compared to the sensitive ones. These data suggested that c-Met receptor signaling plays a role in trastuzumab resistance. However, as patients were treated with a combined therapy, more define experiments are needed to confirm c-Met implication in trastuzumab resistance. Liu *et al.*(152) recently evaluated baseline tumor c-Met expression levels and clinical outcome to lapatinib, an EGFR/Her2 inhibitor, in 64 Her2+ advanced breast cancer patients enrolled in a trial with lapatinib monotherapy as first-line treatment. In this study, a trend towards an association between increased c-Met expression and decreased response was observed. Patients with high Her2 and low c-Met gene expression had the longest PFS compared to patients with low Her2 and high *c-Met* gene expression. Based on these data, a combination study of lapatinib and GSK1363089, a multi-kinase c-Met inhibitor, has been considered in Her2+ breast cancer patients with high *c-Met* gene expression(152).

6. ONGOING CLINICAL TRIALS: SAFETY, EFFICACY AND FIRST SELECTED BIOMARKERS

Several strategies targeting the HGF/c-Met axis have been explored including monoclonal antibodies against either c-Met or HGF, multikinase inhibitors and selective tyrosine kinase inhibitors. Some of these compounds are currently evaluated in clinical trials(153,154) and many recent reviews recapitulate these ongoing trials(155). In this review, we will particularly focus our topic on the two most advanced selective

Table 1. Ongoing clinical trials for the selective TKI ARQ 197 (Tivantinib)

Selective c-Met inhibitor : ARQ 197 (Tivantinib)
Type: not ATP-competitive, Company: ARQULE, High Status: Phase III
Clinical developpements (clinical trails.gov) : phase-indication-drug/combo-start date-trial number-status
Phase 2 - Non-small-cell Lung Cancer Advanced or Metastatic EGFR Mutation-positive - ARQ 197 + Erlotinib, May 2012; NCT01580735 - Not yet recruiting
Phase 2 - Metastatic Triple-Negative Breast Cancer - ARQ 197; April 2012; NCT01542996 - Not yet recruiting
Phase 2 - Recurrent or Metastatic Breast Cancer - ARQ 197; March 2012; NCT01575522 - Recruiting
Phase 2 - Metastatic Prostate Cancer - ARQ 197; January 2012; NCT01519414 - Recruiting
Phase 1 - Solid Tumors – ARQ 197 + omeprazole + s-warfarin + caffeine + vitamin K + digoxin + midazolam; December 2011; NCT01517399 - Recruiting
Phase 2 - Multiple Myeloma and Plasma Cell Neoplasm - ARQ 197; November 2011; NCT01447914 - Recruiting
Phase 1 - Advanced Solid Tumors – Pazopanib + ARQ 197; October 2011; NCT01468922 - Recruiting
Phase 3 - Non-small-cell Lung Cancer – Erlotinib +/- ARQ 197; July 2011; NCT01377376 - Recruiting
Phase 2 - Metastatic Non-Small Cell Lung Cancer - ARQ 197 + Erlotinib versus Pemetrexed, Docetaxel or Gemcitabine; July 2011; NCT01395758 - Recruiting
Phase 1 - Advanced/Recurrent Non-small-cell Lung Cancer - ARQ 197 + Erlotinib; December 2010; NCT01251796 - Active, not recruiting
Phase 3 - Non Squamous, Non-small-cell Lung Cancer - Erlotinib +/- ARQ 197; November 2010; NCT01244191 - Recruiting
Phase 1|Phase 2 - Advanced Solid Tumors - ARQ 197 as monotherapy or in combination with other drug(s)– (extension protocol for patients who have been treated in previous ARQ 197 studies); August 2010; NCT01178411 - Recruiting
Phase 1 - Solid Tumors - ARQ 197 formulations; July 2010; NCT01149720 - Completed
Phase 2 - Gastric Cancer - ARQ 197; June 2010; NCT01152645 - Active, not recruiting
Phase 1 - Non-small-cell Lung Cancer - ARQ 197 + Erlotinib; February 2010; NCT01069757 - Active, not recruiting
Phase 2 - Gastric Cancer - ARQ 197 Versus Investigator's Choice of Second-Line Chemotherapy; February 2010; NCT01070290 - **Withdrawn**
Phase 1|Phase 2 - Metastatic Colorectal Cancer - ARQ 197 + Cetuximab + Irinotecan|; January 2010; NCT01075048 - Active, not recruiting
Phase 2 - Non-CNS Germ Cell Tumors (Seminomas and Nonseminomas) - ARQ 197; January 2010; NCT01055067 - Active, not recruiting
Phase 2 - Unresectable Hepatocellular Carcinoma - ARQ 197; September 2009; NCT00988741 - Active, not recruiting
Phase 1 - Advanced Solid Tumors - ARQ 197 + sorafenib; September 2009; NCT00827177 - Active, not recruiting
Phase 1 - Advanced Solid Tumors - ARQ 197 + gemcitabine; March 2009; NCT00874042 - Completed
Phase 1 - Cirrhosis Hepatocellular Carcinoma - ARQ 197; January 2009; NCT00802555 - Active, not recruiting
Phase 2 - Non Small Cell Lung Cancer - ARQ 197 + Erlotinib; September 2008; NCT00777309 - Completed
Phase 1 - Healthy Volunteers - ARQ 197 Polymorphs; April 2008; NCT00658554 - Completed
Phase 1 - Healthy Volunteers - ARQ 197 Pharmacokinetic (PK) Profile; March 2008; NCT00651638 - Completed
Phase 1 - Advanced Solid Tumors - ARQ 197; February 2008; NCT00612703 - Completed
Phase 1 - Solid Tumors - ARQ 197; January 2008; NCT00609921 - Active, not recruiting
Phase 2 - Pancreatic Neoplasms - ARQ 197 Versus Gemcitabine; November 2007; NCT00558207 - Completed
Phase 2 - Renal Cell Carcinoma (RCC)|Alveolar Soft Part Sarcoma (ASPS)|Clear Cell Sarcoma (CCS) - ARQ 197; October 2007; NCT00557609 - Completed
Phase 1 - Cancer, Advanced Solid Tumors - ARQ 197; April 2007; NCT00612209 - Completed
Phase 1 - Metastatic Solid Tumors - ARQ 197; January 2006; NCT00302172 - Completed

ompounds under development in each therapeutic class of molecule in order to describe the common adverse events and the observed efficacy of therapies targeting c-Met.

6.1. ARQ 197 Tyrosine kinase inhibitor

The main problem in tyrosine kinase development is the selectivity of the compound and regarding the inhibition of the c-Met the capacity to block both ligand-dependent and ligand independent activation of the receptor.

The most advanced selective TKI, ARQ197 (also described as tivantinib), is a maleimide derivative developed by Arqule. ARQ197 is an orally non ATP-competitive inhibitor of c-Met with a high selectivity assessed against 230 other kinases. ARQ197 inhibits HGF-stimulated and constitutive c-Met phosphorylation in multiple human cancer cell lines(156). It demonstrated anti-cancer activities in breast, prostate, colon, and pancreatic xenograft models(157,158) as well as a reduction of pulmonary metastasis in the HT29 colon carcinoma model(159). Combination studies have evidenced a synergy between ARQ197 and sorefanib a multikinase inhibitor RAF, c-KIT, FLT-3, VEGFR-2, VEGFR-3 et PDGFR-β(160).

ARQ197 is currently being evaluated in 17 active or recruiting clinical trials (Table 1): 8 phases I, 7 phases II and 2 phase III, on cancer patients with non squamous non-small cell lung cancer, colorectal cancer, hepatocellular carcinoma (HCC), pancreatic and other advanced solid malignancies either alone or in combination with another treatment: erlotinib, sorafenib, pazopanib, irinotecan/cetuximab or gemcitabine. Final results of an open-label dose escalation phase I in advanced solid tumors were recently reported(161) and demonstrated a favorable safety profile for ARQ197, with grade 1-2 fatigue nausea and vomiting as common drug related adverse events, up to the dose of 360 mg bid. A dose limiting toxicity (DLT) of grade 3 febrile neutropenia was observed in two patients in the 400 mg twice daily cohort. One of these patient also experienced two other grade 3 DTLs relating to mucosal inflammation and palmar-plantar erythrodysesthesia. Data from this study recommended the use of a 360 mg twice daily schedule for phase II studies. In this study, pre- and postoperative paired tumor biopsies were performed and p-c-Met and FAK were evaluated by immunohistochemistry (IHC) as pharmacodynamic (PD) markers. Post therapy, intratumoral c-Met, p-c-Met and p-FAK decreased and an increase of apoptosis was shown by a TUNEL assay.

Circulating endothelial cells (CEC) were also evaluated and it has been shown that CEC declined in 61% of patient suggesting an anti-angiogenic effect of ARQ197(162). The best treatment response in this phase I was stable disease (SD) for over 4 month in 14 patients (27%), with minor regressions in gastric and Merkel cell carcinomas. One patient with T276A MET mutation experienced SD for 20 weeks and had a marked improvement in symptoms. Another phase I dose–escalation study in combination with sorafenib in advanced solid tumors was performed. The recommended selected dose for phase II was ARQ197 360 mg twice daily plus sorafenib 400 mg twice daily. The most commonly reported adverse effects of any grade were fatigue, diarrhea, anorexia and rash. A best response of SD for 7-32 weeks was demonstrated(163). Taken together these results showed that the tested combination was safe and may have therapeutic potential.

As mentioned above, recent data suggested that c-Met could be involved in resistance to EGFR targeted therapies. Regarding to these observations(20,137,139), a phase I trial, combining ARQ197 and Erlotinib, has been completed that determined the recommended doses for phase II. A schedule of 360 mg bid and 150 mg daily in a 28 cycle for ARQ197 and erlotinib respectively (139) has been selected for the ongoing phase II versus erlotinib plus placebo conducted in patients (N=167) with inoperable, locally advanced non-small cell lung cancer (NSCLC)(164). Progression-free survival (PFS) was prolonged with the combined treatment compared to erlotinib plus placebo (16.1 vs 9.7 weeks). This improvement of PFS was paralleled with a similar improvement in median overall survival (36.6 vs 29.4 weeks). Patients with non squamous histology benefited most, with a 9.2-week improvement in median PFS (18.9 vs 9.7 weeks) and a 13.7-week improvement in median overall survival (43.1 vs 29.4 weeks). Subgroup analysis showed benefit of the combination in patients with *c-Met* fluorescent *in situ* hybridization (FISH) gene copy number greater than 4, EGFR wild-type status and KRAS mutation status. There was no worsening of outcome in patients with low *c-Met* gene copy number(165). Interestingly, this study also demonstrated a potential effect of the combination on metastatic dissemination and once again patients with non squamous histology experienced the most significant effect with median time to metastatic disease being increased from 3.6 to 11.0 months(166). An exploratory biomarker IHC analysis confirmed that non squamous NSCLC tumors are more often positive for c-MET expression than squamous tumors(167). Two ongoing, randomized, phase III trials (MARQUEE™ and ATTENTION™) are enrolling only non squamous NSCLC patients, which should enrich the population de facto with c-MET+ patients.

6.2. MetMab monovalent antibody targeting c-Met

Most strategies targeting c-Met with antibodies resulted in the generation of molecules bearing high intrinsic agonist activities probably linked to cross-linking and then activation of the receptor(1,168,169).To overcome this issue, Genentech developed, MetMab (OA5D5 also described as onartuzumab), a recombinant humanized aglycosylated monovalent (one armed) monoclonal antibody, produced in *E coli*, that has full antagonist HGF activity whereas the bivalent counterpart antibody 5D5 behaves as an agonist compound to c-Met(169). MetMab is able to displace HGF and has significant antitumor activities in autocrine or paracrine tumors. However, no activity was observed with this antibody on autophosphorylated cell lines(170). Many clinical trials are ongoing for MetMab evaluation (Table 2). Based on preclinical data, a dose escalating phase I from 10, 20, 30 mg/kg every 3 weeks was performed(171). MetMab appears to be safe and generally well tolerated. It had a terminal half- life of ~10 days and a clearance of ~7.5 mg/kg/day that is approximately 2 times faster than a traditional bivalent antibody. Change in serum HGF and in circulating IL8 levels were evaluated as efficacy markers by ELISA. Depending on patient, no change or decrease in both HGF and IL8 were observed. A confirmed complete response (CR) was observed in one patient (20 mg/kg) with a diagnostic profile suggestive of “autocrine” biology: no evidence of c-Met mutation, no c-Met amplification (fluorescent *in situ* hybridization) and intratumoral c-Met and HGF protein expression observed by IHC. This patient is the only one demonstrating a sustain decrease in HGF following MetMab treatment. This latter result suggests that the serum HGF level could be a useful biomarker of response to MetMab. However this hypothesis has to be confirmed with a significant number of patients. Based on results obtained in phase I and in preclinical combination studies of MetMab with erlotinib or with an anti-VEGF Mab(172) in xenograft experiments(172), combination trials have been designed. A phase I study of MetMab in combination with Bevacizumab in patients with advanced solid malignancies was completed that includes three parts : a 3+3 dose escalation ranging from 1 to 30 mg/kg (N=21); an expansion protocol at 15 mg/Kg every 3 weeks (N=13); and a combination of MetMab at 10 and 15 mg/kg plus bevacizumab at 15 mg/kg every 3 weeks (N=9) (173). The most frequently adverse events observed was fatigue, peripheral edema and hypoalbuminemia. No grade 3-5 treatment-related adverse events were reported with the combination. A CR was observed in one patient with gastric carcinoma after 4 cycles of single-agent MetMab. A phase II trial of MetMab in combination with bevacizumab plus paclitaxel in patients with triple negative breast cancer is currently ongoing. A phase II study evaluating MetMab in combinaison wih erlotinib in patients with advanced NSCLC has been completed(174). This study included 128 patients with all histologies following at least one chemotherapy containing regimen for stage IIIb/IV disease. Patients were scored by IHC for the expression of c-Met. In patients with high c-Met (Scores 2+ or 3+), the combination of MetMab Plus erlotinib resulted in significant improvement of both PFS and OS resulting in a near three fold decrease in the risk of death.

7. FUTURE PERSPECTIVES

Numerous alterations of the c-Met oncogene are described in a wide range of cancers, including over-expression of the receptor and/or its ligand(10,14), amplification of the *c-Met* gene(11) and presence of

Table 2. Ongoing clinical trials for the MetMab monovalent antibody targeting c-Met (Onartuzumab)

Anti-c-Met Monovalent Antibody : Onartuzumab, MetMab (OA5D5)
Produit: Humanized One armed, Company: GENENTECH, High Status Phase III
Clinical developpements (clinical trails.gov): phase-indication-drug/combo-start date-trial number-status
Phase 2 - Metastatic HER2-Negative Gastroesophageal Cancer – Metmab + mFOLFOX6, August 2012 ; NCT01590719 - Not yet recruiting
Phase 2 - Non-Small Cell Lung Cancer – MetMab + bevacizumab + paclitaxel + platinum OR MetMAb + pemetrexed + platinum, February 2012; NCT01496742 - Not yet recruiting
Phase 2 - Non-Small Cell Lung Cancer – MetMAb + Paclitaxel + Platinum, February 2012; NCT01519804 - Not yet recruiting
Phase 3 - metastatic Non-Small Met Diagnostic Positive Cell Lung Cancer - MetMAb+ Erlotinib; January 2012; NCT01456325 - Recruiting
Phase 2 - Metastatic Colorectal Cancer – MetMAb + FOLFOX + Bevacizumab as First-Line ; September 2011; NCT01418222 - Recruiting
Phase 2 – Metastatic, Triple Negative Breast Cancer -Metmab +/Or Bevacizumab + Paclitaxel ; March 2011; NCT01186991 - Recruiting
Phase 2 - Non-Small Cell Lung Cancer – MetMAb + Erlotinib; April 2009; NCT00854308 - Active, not recruiting
Phase 1 - Locally Advanced or Metastatic Solid Tumors- MetMAb alone or MetMAb + bevacizumab ; August 2007; NCT01068977 - Completed

activating mutations(2,8,12,13). Each of these may result in cell transformation and malignant progression(2-5). Abundant literature suggests that c-Met might be a valuable prognostic marker, predicting progression, aggressiveness and poor outcome in many cancer types, including thyroid(46), breast(52,147), gastric(48,91,98-100), colorectal(56,102), glioma and rhabdomyosarcoma(58).

Could some of these alterations, linked to tumor progression and poor patient outcome, be selected as stratification markers? The main challenge in translating the preclinical studies into effective clinical therapies is the accurate identification of patients that could benefit from the tested therapy. In that perspective, many pharmaceutical companies started trials in patients carrying tumors in which c-Met amplification or mutations have been reported such as gastric or papillary renal carcinomas (www.clinicaltrials.gov). Some preliminary results with selective compounds targeting c-Met described patients with stable disease(175), partial regression(175,176) or complete response(171). However, a better knowledge of the target and its network is critical in order to improve the frequency of responsive patients by identifying genetically defined selected subset of patients. Many data are available on oncogenic addiction, a proposed mechanism by which a tumor cell becomes largely dependent on a single activated oncogene(177,178). It has also been suggested that oncogene addiction leads to activation of both survival and apoptotic pathways, but in viable tumor cells, the apoptotic signal is outweighed by the pro-survival (179). Inhibition of this dominant oncogenic signal can result in a dramatic modification of the balance between mediators of survival and apoptotic signal that results in cell death (179). The strong impairment of cancer cell proliferation and the massive apoptosis observed in cell lines amplified for *c-Met* when treated with a targeted c-Met therapy could be a marker of such addiction in gastric and NSCL patients. However the key point with this approach will be to estimate the *c-Met* copy number that will switch tumor behavior towards addiction for c-Met. This criterion should probably not be considered as an isolated factor but in the context of the whole tumor cell including also data on the status of other RTK such as EGFR, Her2, VEGFR or adaptor signaling protein such as PTEN or K-RAS. c-Met has been shown to physically interact with other RTK and to be over-expressed, amplified and/or activated *in situ*ations where its partners are mutated or become resistant to therapies. As discussed by Pillay *et al.*(180), the plasticity of the oncogenic network may have significant implications for targeted therapies. Indeed their work suggested that some tumor cells can harbor a hierarchy of activated oncogenes, possibly arising through their ongoing evolution. Thus even targeting the dominant oncogene in some solid tumors may be relatively ineffective as its inhibition could be rapidly overcome by the promotion of other activated oncogenes to a dominant position within the cell. This hypothesis is fully exemplified by situations where *c-Met* is amplified when cells become resistant to EGFR or Her2 therapies(15,151,152). With regard to these observations, a strategy to determine the most likely responsive subset of patients for testing a targeted therapy to c-Met should be to include patients characterized for a panel of RTKs to perform combined or sequential therapies. Moreover, amplification of the *c-Met* gene only represents a subset of situations where c-Met is over-expressed. In a significant number of patients, over-expression of c-Met results from a deregulation of transcriptional regulation. Even if there is no clear rationale linking such over-expression of c-Met to a particular susceptibility to targeted therapies, the level of c-Met phosphorylation and the nature of activated tyrosine residues (108,109,181) could be analyzed in order to identify responsive subset of patients. The presence of major mutations such as K-RAS or PTEN should be taken into account and for TKI, mutations that occur in the TK-domain should be carefully monitored to either exclude patients with the ones responsible for resistance or to identify those that could sensitize patients to therapy.

Most of the reagents and methods are available to evaluate abnormal c-Met situations. FISH analysis may be used to monitor gene amplification. Mutations of either c-Met or protein adaptors related to c-Met could be evaluated by DNA sequencing performed on biopsies or blood samples from patients. IHC methods have been described to determine subcellular distribution of c-Met and its phosphorylation status. Circulation HGF measurement could be achieved by ELISA and a suitable electroluminescent assay has been published to quantify circulation c-Met resulting from the shedding process. Some of these parameters have been, or are currently, evaluated in ongoing clinical trials. However much more patients have to be included in clinical trials and more parameters must be studied during trials to establish correlations between one, or a set of biomarkers, and a response to the tested therapy. Regarding to EGFR

situation, clinical trials started more than 10 years ago and the critical importance of K-RAS has only been discovered recently while many compounds have been approved.

In conclusion, i) the diversity of abnormal situations involving c-Met, ii) the complex network between c-Met and many cell surface components including other RTK and, iii) the number of c-Met mutations identified, point out to the need of a careful monitoring of patients included in clinical trials. In addition to determining the level of c-Met over-expression/amplification, the activation status, potential c-Met mutations, but also expression of c-Met partners, treatment resistance and impairment of signaling pathway such as K-RAS and PTEN might be taken into account to increase the probability of identifying the most relevant subset of responsive patients.

8. ACKNOWLEDGMENT

We acknowledge Claire Catry for helping in manuscript preparation.

9. REFERENCES

1 Comoglio, P.M., Giordano S.,& Trusolino L.: Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nat Rev Drug Discov.* 7, 504-516 (2008)

2 Schmidt, L., Duh F.M., Chen F., Kishida T., Glenn G., Choyke P., Scherer S.W., Zhuang Z., Lubensky I., Dean M., Allikmets R., Chidambaram A., Bergerheim U.R., Feltis J.T., Casadevall C., Zamarron A., Bernues M., Richard S., Lips C.J., Walther M.M., Tsui L.C., Geil L., Orcutt M.L., Stackhouse T., Lipan J., Slife L., Brauch H., Decker J., Niehans G., Hughson M.D., Moch H., Storkel S., Lerman M.I., Linehan W.M.,& Zbar B.: Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat.Genet.* 16, 68-73 (1997)

3 Gallego, M.I., Bierie B.,& Hennighausen L.: Targeted expression of HGF/SF in mouse mammary epithelium leads to metastatic adenosquamous carcinomas through the activation of multiple signal transduction pathways. *Oncogene*. 22, 8498-8508 (2003)

4 Jeffers, M., Fiscella M., Webb C.P., Anver M., Koochekpour S.,& Vande Woude G.F.: The mutationally activated Met receptor mediates motility and metastasis. *Proc Natl Acad Sci U S A.* 95, 14417-14422 (1998)

5 Takayama, H., Larochelle W.J., Sharp R., Otsuka T., Kriebel P., Anver M., Aaronson S.A.,& Merlino G.: Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor. *Proc Natl Acad Sci U S A.* 94, 701-706 (1997)

6 Liu, X., Yao W., Newton R.C.,& Scherle P.A.: Targeting the c-MET signaling pathway for cancer therapy. *Expert.Opin.Investig.Drugs.* 17, 997-1011 (2008)

7 Birchmeier, C., Birchmeier W., Gherardi E.,& Vande Woude G.F.: Met, metastasis, motility and more. *Nat Rev Mol Cell Biol.* 4, 915-925 (2003)

8 Di Renzo, M.F., Olivero M., Martone T., Maffe A., Maggiora P., Stefani A.D., Valente G., Giordano S., Cortesina G.,& Comoglio P.M.: Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. *Oncogene*. 19, 1547-1555 (2000)

9 Ferracini, R., Di Renzo M.F., Scotlandi K., Baldini N., Olivero M., Lollini P., Cremona O., Campanacci M.,& Comoglio P.M.: The Met/HGF receptor is over-expressed in human osteosarcomas and is activated by either a paracrine or an autocrine circuit. *Oncogene*. 10, 739-749 (1995)

10 Di Renzo, M.F., Olivero M., Ferro S., Prat M., Bongarzone I., Pilotti S., Belfiore A., Costantino A., Vigneri R., Pierotti M.A.,& .: Overexpression of the c-MET/HGF receptor gene in human thyroid carcinomas. *Oncogene*. 7, 2549-2553 (1992)

11 Houldsworth, J., Cordon-Cardo C., Ladanyi M., Kelsen D.P.,& Chaganti R.S.: Gene amplification in gastric and esophageal adenocarcinomas. *Cancer Res*. 50, 6417-6422 (1990)

12 Lee, J.H., Han S.U., Cho H., Jennings B., Gerrard B., Dean M., Schmidt L., Zbar B.,& Vande Woude G.F.: A novel germ line juxtamembrane Met mutation in human gastric cancer. *Oncogene*. 19, 4947-4953 (2000)

13 Park, W.S., Dong S.M., Kim S.Y., Na E.Y., Shin M.S., Pi J.H., Kim B.J., Bae J.H., Hong Y.K., Lee K.S., Lee S.H., Yoo N.J., Jang J.J., Pack S., Zhuang Z., Schmidt L., Zbar B.,& Lee J.Y.: Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res*. 59, 307-310 (1999)

14 Koochekpour, S., Jeffers M., Rulong S., Taylor G., Klineberg E., Hudson E.A., Resau J.H.,& Vande Woude G.F.: Met and hepatocyte growth factor/scatter factor expression in human gliomas. *Cancer Res*. 57, 5391-5398 (1997)

15 Engelman, J.A., Zejnullahu K., Mitsudomi T., Song Y., Hyland C., Park J.O., Lindeman N., Gale C.M., Zhao X., Christensen J., Kosaka T., Holmes A.J., Rogers A.M., Cappuzzo F., Mok T., Lee C., Johnson B.E., Cantley L.C.,& Janne P.A.: MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*. 316, 1039-1043 (2007)

16 Huang, P.H., Mukasa A., Bonavia R., Flynn R.A., Brewer Z.E., Cavenee W.K., Furnari F.B.,& White F.M.: Quantitative analysis of EGFRvIII cellular signaling

networks reveals a combinatorial therapeutic strategy for glioblastoma. *Proc Natl Acad Sci U S A.* 104, 12867-12872 (2007)

17 Shattuck, D.L., Miller J.K., Carraway K.L., III,& Sweeney C.: Met receptor contributes to trastuzumab resistance of Her2-overexpressing breast cancer cells. *Cancer Res.* 68, 1471-1477 (2008)

18 Goetsch, L.,Caussanel V.: Selection criteria for c-Met-targeted therapies: emerging evidence for biomarkers. *Biomark.Med.* 4, 149-170 (2010)

19 Kozlov, G., Perreault A., Schrag J.D., Park M., Cygler M., Gehring K.,& Ekiel I.: Insights into function of PSI domains from structure of the Met receptor PSI domain. *Biochem Biophys Res Commun.* 321, 234-240 (2004)

20 Giordano, S., Ponzetto C., Di Renzo M.F., Cooper C.S.,& Comoglio P.M.: Tyrosine kinase receptor indistinguishable from the c-met protein. *Nature.* 339, 155-156 (1989)

21 Park, M., Dean M., Kaul K., Braun M.J., Gonda M.A.,& Vande W.G.: Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. *Proc Natl Acad Sci U S A.* 84, 6379-6383 (1987)

22 Gherardi, E., Youles M.E., Miguel R.N., Blundell T.L., Iamele L., Gough J., Bandyopadhyay A., Hartmann G.,& Butler P.J.: Functional map and domain structure of MET, the product of the c-met protooncogene and receptor for hepatocyte growth factor/scatter factor. *Proc Natl Acad Sci U S A.* 100, 12039-12044 (2003)

23 Basilico, C., Arnesano A., Galluzzo M., Comoglio P.M.,& Michieli P.: A high affinity hepatocyte growth factor-binding site in the immunoglobulin-like region of Met. *J Biol Chem.* 283, 21267-21277 (2008)

24 Nakamura, T., Teramoto H.,& Ichihara A.: Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. *Proc Natl Acad Sci U S A.* 83, 6489-6493 (1986)

25 Rubin, J.S., Chan A.M., Bottaro D.P., Burgess W.H., Taylor W.G., Cech A.C., Hirschfield D.W., Wong J., Miki T., Finch P.W.,& .: A broad-spectrum human lung fibroblast-derived mitogen is a variant of hepatocyte growth factor. *Proc Natl Acad Sci U S A.* 88, 415-419 (1991)

26 Stoker, M., Gherardi E., Perryman M.,& Gray J.: Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature.* 327, 239-242 (1987)

27 Weidner, K.M., Behrens J., Vandekerckhove J.,& Birchmeier W.: Scatter factor: molecular characteristics and effect on the invasiveness of epithelial cells. *J Cell Biol.* 111, 2097-2108 (1990)

28 Rong, S., Segal S., Anver M., Resau J.H.,& Vande Woude G.F.: Invasiveness and metastasis of NIH 3T3 cells induced by Met-hepatocyte growth factor/scatter factor autocrine stimulation. *Proc Natl Acad Sci U S A.* 91, 4731-4735 (1994)

29 Jeffers, M., Rong S.,& Vande Woude G.F.: Enhanced tumorigenicity and invasion-metastasis by hepatocyte growth factor/scatter factor-met signalling in human cells concomitant with induction of the urokinase proteolysis network. *Mol.Cell Biol.* 16, 1115-1125 (1996)

30 Montesano, R., Matsumoto K., Nakamura T.,& Orci L.: Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell.* 67, 901-908 (1991)

31 Brinkmann, V., Foroutan H., Sachs M., Weidner K.M.,& Birchmeier W.: Hepatocyte growth factor/scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells. *J Cell Biol.* 131, 1573-1586 (1995)

32 Tsarfaty, I., Resau J.H., Rulong S., Keydar I., Faletto D.L.,& Vande Woude G.F.: The met proto-oncogene receptor and lumen formation. *Science.* 257, 1258-1261 (1992)

33 Bussolino, F., Di Renzo M.F., Ziche M., Bocchietto E., Olivero M., Naldini L., Gaudino G., Tamagnone L., Coffer A.,& Comoglio P.M.: Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. *J Cell Biol.* 119, 629-641 (1992)

34 Grant, D.S., Kleinman H.K., Goldberg I.D., Bhargava M.M., Nickoloff B.J., Kinsella J.L., Polverini P.,& Rosen E.M.: Scatter factor induces blood vessel formation *in vivo*. *Proc Natl.Acad.Sci.U.S.A.* 90, 1937-1941 (1993)

35 Mazzone, M.,Comoglio P.M.: The Met pathway: master switch and drug target in cancer progression. *FASEB J.* 20, 1611-1621 (2006)

36 Bladt, F., Riethmacher D., Isenmann S., Aguzzi A.,& Birchmeier C.: Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature.* 376, 768-771 (1995)

37 Schmidt, C., Bladt F., Goedecke S., Brinkmann V., Zschiesche W., Sharpe M., Gherardi E.,& Birchmeier C.: Scatter factor/hepatocyte growth factor is essential for liver development. *Nature.* 373, 699-702 (1995)

38 Uehara, Y., Minowa O., Mori C., Shiota K., Kuno J., Noda T.,& Kitamura N.: Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature.* 373, 702-705 (1995)

39 Nusrat, A., Parkos C.A., Bacarra A.E., Godowski P.J., Delp-Archer C., Rosen E.M.,& Madara J.L.: Hepatocyte growth factor/scatter factor effects on epithelia. Regulation of intercellular junctions in transformed and nontransformed cell lines, basolateral polarization of c-met receptor in transformed and natural intestinal epithelia, and

induction of rapid wound repair in a transformed model epithelium. *J Clin Invest*. 93, 2056-2065 (1994)

40 Santos, O.F., Barros E.J., Yang X.M., Matsumoto K., Nakamura T., Park M.,& Nigam S.K.: Involvement of hepatocyte growth factor in kidney development. *Dev Biol*. 163, 525-529 (1994)

41 Woolf, A.S., Kolatsi-Joannou M., Hardman P., Andermarcher E., Moorby C., Fine L.G., Jat P.S., Noble M.D.,& Gherardi E.: Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. *J Cell Biol*. 128, 171-184 (1995)

42 Yang, Y., Spitzer E., Meyer D., Sachs M., Niemann C., Hartmann G., Weidner K.M., Birchmeier C.,& Birchmeier W.: Sequential requirement of hepatocyte growth factor and neuregulin in the morphogenesis and differentiation of the mammary gland. *J Cell Biol*. 131, 215-226 (1995)

43 Soriano, J.V., Pepper M.S., Nakamura T., Orci L.,& Montesano R.: Hepatocyte growth factor stimulates extensive development of branching duct-like structures by cloned mammary gland epithelial cells. *J Cell Sci*. 108 (Pt 2), 413-430 (1995)

44 Streit, A., Stern C.D., Thery C., Ireland G.W., Aparicio S., Sharpe M.J.,& Gherardi E.: A role for HGF/SF in neural induction and its expression in Hensen's node during gastrulation. *Development*. 121, 813-824 (1995)

45 Ruco, L.P., Ranalli T., Marzullo A., Bianco P., Prat M., Comoglio P.M.,& Baroni C.D.: Expression of Met protein in thyroid tumours. *J.Pathol*. 180, 266-270 (1996)

46 Chen, B.K., Ohtsuki Y., Furihata M., Takeuchi T., Iwata J., Liang S.B.,& Sonobe H.: Overexpression of c-Met protein in human thyroid tumors correlated with lymph node metastasis and clinicopathologic stage. *Pathol.Res.Pract*. 195, 427-433 (1999)

47 Di Renzo, M.F., Olivero M., Katsaros D., Crepaldi T., Gaglia P., Zola P., Sismondi P.,& Comoglio P.M.: Overexpression of the Met/HGF receptor in ovarian cancer. *Int J Cancer*. 58, 658-662 (1994)

48 Di Renzo, M.F., Poulsom R., Olivero M., Comoglio P.M.,& Lemoine N.R.: Expression of the Met/hepatocyte growth factor receptor in human pancreatic cancer. *Cancer Res*. 55, 1129-1138 (1995)

49 Humphrey, P.A., Zhu X., Zarnegar R., Swanson P.E., Ratliff T.L., Vollmer R.T.,& Day M.L.: Hepatocyte growth factor and its receptor (c-MET) in prostatic carcinoma. *Am.J.Pathol*. 147, 386-396 (1995)

50 Natali, P.G., Prat M., Nicotra M.R., Bigotti A., Olivero M., Comoglio P.M.,& Di Renzo M.F.: Overexpression of the met/HGF receptor in renal cell carcinomas. *Int J Cancer*. 69, 212-217 (1996)

51 Ueki, T., Fujimoto J., Suzuki T., Yamamoto H.,& Okamoto E.: Expression of hepatocyte growth factor and its receptor, the c-met proto-oncogene, in hepatocellular carcinoma. *Hepatology*. 25, 619-623 (1997)

52 Camp, R.L., Rimm E.B.,& Rimm D.L.: Met expression is associated with poor outcome in patients with axillary lymph node negative breast carcinoma. *Cancer*. 86, 2259-2265 (1999)

53 Kuniyasu, H., Yasui W., Kitadai Y., Yokozaki H., Ito H.,& Tahara E.: Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem Biophys Res Commun*. 189, 227-232 (1992)

54 Bean, J., Brennan C., Shih J.Y., Riely G., Viale A., Wang L., Chitale D., Motoi N., Szoke J., Broderick S., Balak M., Chang W.C., Yu C.J., Gazdar A., Pass H., Rusch V., Gerald W., Huang S.F., Yang P.C., Miller V., Ladanyi M., Yang C.H.,& Pao W.: 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*. 104, 20932-20937 (2007)

55 Tong, C.Y., Hui A.B., Yin X.L., Pang J.C., Zhu X.L., Poon W.S.,& Ng H.K.: Detection of oncogene amplifications in medulloblastomas by comparative genomic hybridization and array-based comparative genomic hybridization. *J.Neurosurg*. 100, 187-193 (2004)

56 Di Renzo, M.F., Olivero M., Giacomini A., Porte H., Chastre E., Mirossay L., Nordlinger B., Bretti S., Bottardi S., Giordano S.,& .: Overexpression and amplification of the met/HGF receptor gene during the progression of colorectal cancer. *Clin Cancer Res*. 1, 147-154 (1995)

57 Tuck, A.B., Park M., Sterns E.E., Boag A.,& Elliott B.E.: Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. *Am.J.Pathol*. 148, 225-232 (1996)

58 Ferracini, R., Olivero M., Di Renzo M.F., Martano M., De Giovanni C., Nanni P., Basso G., Scotlandi K., Lollini P.L.,& Comoglio P.M.: Retrogenic expression of the MET proto-oncogene correlates with the invasive phenotype of human rhabdomyosarcomas. *Oncogene*. 12, 1697-1705 (1996)

59 Rodrigues, G.A.,Park M.: Dimerization mediated through a leucine zipper activates the oncogenic potential of the met receptor tyrosine kinase. *Mol.Cell Biol*. 13, 6711-6722 (1993)

60 Zhen, Z., Giordano S., Longati P., Medico E., Campiglio M.,& Comoglio P.M.: Structural and

functional domains critical for constitutive activation of the HGF-receptor (Met) *Oncogene*. 9, 1691-1697 (1994)

61 Trusolino, L., Bertotti A.,& Comoglio P.M.: MET signalling: principles and functions in development, organ regeneration and cancer. *Nat.Rev.Mol.Cell Biol.* 11, 834-848 (2010)

62 Organ, S.L.,Tsao M.S.: An overview of the c-MET signaling pathway. *Ther.Adv.Med.Oncol.* 3, S7-S19 (2011)

63 Gherardi, E., Birchmeier W., Birchmeier C.,& Vande W.G.: Targeting MET in cancer: rationale and progress. *Nat.Rev.Cancer*. 12, 89-103 (2012)

64 Weidner, K.M., Di Cesare S., Sachs M., Brinkmann V., Behrens J.,& Birchmeier W.: Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature*. 384, 173-176 (1996)

65 Maulik, G., Madhiwala P., Brooks S., Ma P.C., Kijima T., Tibaldi E.V., Schaefer E., Parmar K.,& Salgia R.: Activated c-Met signals through PI3K with dramatic effects on cytoskeletal functions in small cell lung cancer. *J.Cell Mol.Med.* 6, 539-553 (2002)

66 Ponzetto, C., Bardelli A., Zhen Z., Maina F., dalla Z.P., Giordano S., Graziani A., Panayotou G.,& Comoglio P.M.: A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell*. 77, 261-271 (1994)

67 Boccaccio, C., Ando M., Tamagnone L., Bardelli A., Michieli P., Battistini C.,& Comoglio P.M.: Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature*. 391, 285-288 (1998)

68 Zhang, Y.W., Wang L.M., Jove R.,& Vande Woude G.F.: Requirement of Stat3 signaling for HGF/SF-Met mediated tumorigenesis. *Oncogene*. 21, 217-226 (2002)

69 Petrelli, A., Gilestro G.F., Lanzardo S., Comoglio P.M., Migone N.,& Giordano S.: The endophilin-CIN85-Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature*. 416, 187-190 (2002)

70 Abella, J.V., Peschard P., Naujokas M.A., Lin T., Saucier C., Urbe S.,& Park M.: Met/Hepatocyte growth factor receptor ubiquitination suppresses transformation and is required for Hrs phosphorylation. *Mol.Cell Biol.* 25, 9632-9645 (2005)

71 Gandino, L., Longati P., Medico E., Prat M.,& Comoglio P.M.: Phosphorylation of serine 985 negatively regulates the hepatocyte growth factor receptor kinase. *J Biol Chem*. 269, 1815-1820 (1994)

72 Gandino, L., Munaron L., Naldini L., Ferracini R., Magni M.,& Comoglio P.M.: Intracellular calcium regulates the tyrosine kinase receptor encoded by the MET oncogene. *J Biol Chem*. 266, 16098-16104 (1991)

73 Palka, H.L., Park M.,& Tonks N.K.: Hepatocyte growth factor receptor tyrosine kinase met is a substrate of the receptor protein-tyrosine phosphatase DEP-1. *J Biol Chem*. 278, 5728-5735 (2003)

74 Machide, M., Hashigasako A., Matsumoto K.,& Nakamura T.: Contact inhibition of hepatocyte growth regulated by functional association of the c-Met/hepatocyte growth factor receptor and LAR protein-tyrosine phosphatase. *J Biol Chem*. 281, 8765-8772 (2006)

75 Sangwan, V., Paliouras G.N., Abella J.V., Dube N., Monast A., Tremblay M.L.,& Park M.: Regulation of the Met receptor-tyrosine kinase by the protein-tyrosine phosphatase 1B and T-cell phosphatase. *J.Biol.Chem.* 283, 34374-34383 (2008)

76 Migliore, C., Petrelli A., Ghiso E., Corso S., Capparuccia L., Eramo A., Comoglio P.M.,& Giordano S.: MicroRNAs impair MET-mediated invasive growth. *Cancer Res.* 68, 10128-10136 (2008)

77 Patane, S., Avnet S., Coltella N., Costa B., Sponza S., Olivero M., Vigna E., Naldini L., Baldini N., Ferracini R., Corso S., Giordano S., Comoglio P.M.,& Di Renzo M.F.: MET overexpression turns human primary osteoblasts into osteosarcomas. *Cancer Res*. 66, 4750-4757 (2006)

78 Wang, R., Ferrell L.D., Faouzi S., Maher J.J.,& Bishop J.M.: Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. *J Cell Biol*. 153, 1023-1034 (2001)

79 Yu, Y.,Merlino G.: Constitutive c-Met signaling through a nonautocrine mechanism promotes metastasis in a transgenic transplantation model. *Cancer Res*. 62, 2951-2956 (2002)

80 Ivan, M., Bond J.A., Prat M., Comoglio P.M.,& Wynford-Thomas D.: Activated ras and ret oncogenes induce over-expression of c-met (hepatocyte growth factor receptor) in human thyroid epithelial cells. *Oncogene*. 14, 2417-2423 (1997)

81 Patil, M.A., Lee S.A., Macias E., Lam E.T., Xu C., Jones K.D., Ho C., Rodriguez-Puebla M.,& Chen X.: Role of cyclin D1 as a mediator of c-Met- and beta-catenin-induced hepatocarcinogenesis. *Cancer Res.* 69, 253-261 (2009)

82 Kanteti, R., Nallasura V., Loganathan S., Tretiakova M., Kroll T., Krishnaswamy S., Faoro L., Cagle P., Husain A.N., Vokes E.E., Lang D.,& Salgia R.: PAX5 is expressed in small-cell lung cancer and positively regulates c-Met transcription. *Lab Invest.* 89, 301-314 (2009)

83 Stein, U., Walther W., Arlt F., Schwabe H., Smith J., Fichtner I., Birchmeier W.,& Schlag P.M.: MACC1, a newly identified key regulator of HGF-MET signaling, predicts colon cancer metastasis. *Nat.Med.* 15, 59-67 (2009)

84 Pennacchietti, S., Michieli P., Galluzzo M., Mazzone M., Giordano S.,& Comoglio P.M.: Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell*. 3, 347-361 (2003)

85 Smolen, G.A., Sordella R., Muir B., Mohapatra G., Barmettler A., Archibald H., Kim W.J., Okimoto R.A., Bell D.W., Sgroi D.C., Christensen J.G., Settleman J.,& Haber D.A.: 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*. 103, 2316-2321 (2006)

86 Nessling, M., Solinas-Toldo S., Wilgenbus K.K., Borchard F.,& Lichter P.: Mapping of chromosomal imbalances in gastric adenocarcinoma revealed amplified protooncogenes MYCN, MET, WNT2, and ERBB2. *Genes Chromosomes.Cancer*. 23, 307-316 (1998)

87 Miller, C.T., Lin L., Casper A.M., Lim J., Thomas D.G., Orringer M.B., Chang A.C., Chambers A.F., Giordano T.J., Glover T.W.,& Beer D.G.: Genomic amplification of MET with boundaries within fragile site FRA7G and upregulation of MET pathways in esophageal adenocarcinoma. *Oncogene*. 25, 409-418 (2006)

88 Lutterbach, B., Zeng Q., Davis L.J., Hatch H., Hang G., Kohl N.E., Gibbs J.B.,& Pan B.S.: Lung cancer cell lines harboring MET gene amplification are dependent on Met for growth and survival. *Cancer Res.* 67, 2081-2088 (2007)

89 Burgess, T., Coxon A., Meyer S., Sun J., Rex K., Tsuruda T., Chen Q., Ho S.Y., Li L., Kaufman S., McDorman K., Cattley R.C., Sun J., Elliott G., Zhang K., Feng X., Jia X.C., Green L., Radinsky R.,& Kendall R.: Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. *Cancer Res.* 66, 1721-1729 (2006)

90 Sakakura, C., Mori T., Sakabe T., Ariyama Y., Shinomiya T., Date K., Hagiwara A., Yamaguchi T., Takahashi T., Nakamura Y., Abe T.,& Inazawa J.: Gains, losses, and amplifications of genomic materials in primary gastric cancers analyzed by comparative genomic hybridization. *Genes Chromosomes.Cancer*. 24, 299-305 (1999)

91 Nakajima, M., Sawada H., Yamada Y., Watanabe A., Tatsumi M., Yamashita J., Matsuda M., Sakaguchi T., Hirao T.,& Nakano H.: The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer*. 85, 1894-1902 (1999)

92 Lynch, T.J., Bell D.W., Sordella R., Gurubhagavatula S., Okimoto R.A., Brannigan B.W., Harris P.L., Haserlat S.M., Supko J.G., Haluska F.G., Louis D.N., Christiani D.C., Settleman J.,& Haber D.A.: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N.Engl.J.Med.* 350, 2129-2139 (2004)

93 Paez, J.G., Janne P.A., Lee J.C., Tracy S., Greulich H., Gabriel S., Herman P., Kaye F.J., Lindeman N., Boggon T.J., Naoki K., Sasaki H., Fujii Y., Eck M.J., Sellers W.R., Johnson B.E.,& Meyerson M.: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 304, 1497-1500 (2004)

94 Pao, W., Miller V., Zakowski M., Doherty J., Politi K., Sarkaria I., Singh B., Heelan R., Rusch V., Fulton L., Mardis E., Kupfer D., Wilson R., Kris M.,& Varmus H.: EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A*. 101, 13306-13311 (2004)

95 Kuniyasu, H., Yasui W., Kitadai Y., Yokozaki H., Ito H.,& Tahara E.: Frequent amplification of the c-met gene in scirrhous type stomach cancer. *Biochem.Biophys.Res.Commun.* 189, 227-232 (1992)

96 Lee, J., Seo J.W., Jun H.J., Ki C.S., Park S.H., Park Y.S., Lim H.Y., Choi M.G., Bae J.M., Sohn T.S., Noh J.H., Kim S., Jang H.L., Kim J.Y., Kim K.M., Kang W.K.,& Park J.O.: Impact of MET amplification on gastric cancer: Possible roles as a novel prognostic marker and a potential therapeutic target. *Oncol.Rep.* (2011)

97 Beau-Faller, M., Ruppert A.M., Voegeli A.C., Neuville A., Meyer N., Guerin E., Legrain M., Mennecier B., Wihlm J.M., Massard G., Quoix E., Oudet P.,& Gaub M.P.: MET gene copy number in non-small cell lung cancer: molecular analysis in a targeted tyrosine kinase inhibitor naive cohort. *J.Thorac.Oncol.* 3, 331-339 (2008)

98 Huang, T.J., Wang J.Y., Lin S.R., Lian S.T.,& Hsieh J.S.: Overexpression of the c-met protooncogene in human gastric carcinoma--correlation to clinical features. *Acta Oncol.* 40, 638-643 (2001)

99 Amemiya, H., Kono K., Itakura J., Tang R.F., Takahashi A., An F.Q., Kamei S., Iizuka H., Fujii H.,& Matsumoto Y.: c-Met expression in gastric cancer with liver metastasis. *Oncology*. 63, 286-296 (2002)

100 Prat, M., Narsimhan R.P., Crepaldi T., Nicotra M.R., Natali P.G.,& Comoglio P.M.: The receptor encoded by the human c-MET oncogene is expressed in hepatocytes, epithelial cells and solid tumors. *Int J Cancer*. 49, 323-328 (1991)

101 Wu, M.S., Shun C.T., Wang H.P., Sheu J.C., Lee W.J., Wang T.H.,& Lin J.T.: Genetic alterations in gastric cancer: relation to histological subtypes, tumor stage, and Helicobacter pylori infection. *Gastroenterology*. 112, 1457-1465 (1997)

102 Zeng, Z.S., Weiser M.R., Kuntz E., Chen C.T., Khan S.A., Forslund A., Nash G.M., Gimbel M., Yamaguchi Y., Culliford A.T., D'Alessio M., Barany F.,& Paty P.B.: c-Met gene amplification is associated with advanced stage colorectal cancer and liver metastases. *Cancer Lett.* 265, 258-269 (2008)

103 Kong, D.S., Song S.Y., Kim D.H., Joo K.M., Yoo J.S., Koh J.S., Dong S.M., Suh Y.L., Lee J.I., Park K., Kim J.H.,& Nam D.H.: Prognostic significance of c-Met expression in glioblastomas. *Cancer*. 115, 140-148 (2009)

104 Drebber, U., Baldus S.E., Nolden B., Grass G., Bollschweiler E., Dienes H.P., Holscher A.H.,& Monig S.P.: The overexpression of c-met as a prognostic indicator for gastric carcinoma compared to p53 and p21 nuclear accumulation. *Oncol.Rep.* 19, 1477-1483 (2008)

105 Janjigian, Y.Y., Tang L.H., Coit D.G., Kelsen D.P., Francone T.D., Weiser M.R., Jhanwar S.C.,& Shah M.A.: MET expression and amplification in patients with localized gastric cancer. *Cancer Epidemiol.Biomarkers Prev.* 20, 1021-1027 (2011)

106 Vanden, B., I, Van Loo P., Drijkoningen M., Neven P., Smeets A., Christiaens M.R., Paridaens R.,& Wolf-Peeters C.: Polysomy 17 in breast cancer: clinicopathologic significance and impact on HER-2 testing. *J.Clin.Oncol.* 26, 4869-4874 (2008)

107 Ma, P.C., Jagadeeswaran R., Jagadeesh S., Tretiakova M.S., Nallasura V., Fox E.A., Hansen M., Schaefer E., Naoki K., Lader A., Richards W., Sugarbaker D., Husain A.N., Christensen J.G.,& Salgia R.: Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res*. 65, 1479-1488 (2005)

108 Miyata, Y., Sagara Y., Kanda S., Hayashi T.,& Kanetake H.: Phosphorylated hepatocyte growth factor receptor/c-Met is associated with tumor growth and prognosis in patients with bladder cancer: correlation with matrix metalloproteinase-2 and -7 and E-cadherin. *Hum.Pathol.* 40, 496-504 (2009)

109 Zucali, P.A., Ruiz M.G., Giovannetti E., Destro A., Varella-Garcia M., Floor K., Ceresoli G.L., Rodriguez J.A., Garassino I., Comoglio P., Roncalli M., Santoro A.,& Giaccone G.: Role of cMET expression in non-small-cell lung cancer patients treated with EGFR tyrosine kinase inhibitors. *Ann.Oncol.* 19, 1605-1612 (2008)

110 Danilkovitch-Miagkova, A.,Zbar B.: Dysregulation of Met receptor tyrosine kinase activity in invasive tumors. *J Clin Invest.* 109, 863-867 (2002)

111 Schmidt, L., Junker K., Nakaigawa N., Kinjerski T., Weirich G., Miller M., Lubensky I., Neumann H.P., Brauch H., Decker J., Vocke C., Brown J.A., Jenkins R., Richard S., Bergerheim U., Gerrard B., Dean M., Linehan W.M.,& Zbar B.: Novel mutations of the MET proto-oncogene in papillary renal carcinomas. *Oncogene*. 18, 2343-2350 (1999)

112 Lorenzato, A., Olivero M., Patane S., Rosso E., Oliaro A., Comoglio P.M.,& Di Renzo M.F.: Novel somatic mutations of the MET oncogene in human carcinoma metastases activating cell motility and invasion. *Cancer Res.* 62, 7025-7030 (2002)

113 Park, W.S., Oh R.R., Kim Y.S., Park J.Y., Shin M.S., Lee H.K., Lee S.H., Yoo N.J.,& Lee J.Y.: Absence of mutations in the kinase domain of the Met gene and frequent expression of Met and HGF/SF protein in primary gastric carcinomas. *APMIS*. 108, 195-200 (2000)

114 Tanyi, J., Tory K., Rigo J., Jr., Nagy B.,& Papp Z.: Evaluation of the tyrosine kinase domain of the Met proto-oncogene in sporadic ovarian carcinomas*. *Pathol.Oncol.Res.* 5, 187-191 (1999)

115 Shu, H.K., Pelley R.J.,& Kung H.J.: Tissue-specific transformation by epidermal growth factor receptor: a single point mutation within the ATP-binding pocket of the erbB product increases its intrinsic kinase activity and activates its sarcomagenic potential. *Proc Natl Acad Sci U S A.* 87, 9103-9107 (1990)

116 Piao, X.,Bernstein A.: A point mutation in the catalytic domain of c-kit induces growth factor independence, tumorigenicity, and differentiation of mast cells. *Blood*. 87, 3117-3123 (1996)

117 Beghini, A., Larizza L., Cairoli R.,& Morra E.: c-kit activating mutations and mast cell proliferation in human leukemia. *Blood*. 92, 701-702 (1998)

118 Hofstra, R.M., Landsvater R.M., Ceccherini I., Stulp R.P., Stelwagen T., Luo Y., Pasini B., Hoppener J.W., van Amstel H.K., Romeo G.,& .: A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature*. 367, 375-376 (1994)

119 Jeffers, M., Schmidt L., Nakaigawa N., Webb C.P., Weirich G., Kishida T., Zbar B.,& Vande Woude G.F.: Activating mutations for the met tyrosine kinase receptor in human cancer. *Proc Natl Acad Sci U S A.* 94, 11445-11450 (1997)

120 Joffre, C., Barrow R., Menard L., Calleja V., Hart I.R.,& Kermorgant S.: A direct role for Met endocytosis in tumorigenesis. *Nat.Cell Biol.* 13, 827-837 (2011)

121 Hammond, D.E., Urbe S., Vande Woude G.F.,& Clague M.J.: Down-regulation of MET, the receptor for hepatocyte growth factor. *Oncogene.* 20, 2761-2770 (2001)

122 Jagadeeswaran, R., Ma P.C., Seiwert T.Y., Jagadeeswaran S., Zumba O., Nallasura V., Ahmed S., Filiberti R., Paganuzzi M., Puntoni R., Kratzke R.A., Gordon G.J., Sugarbaker D.J., Bueno R., Janamanchi V., Bindokas V.P., Kindler H.L.,& Salgia R.: Functional analysis of c-Met/hepatocyte growth factor pathway in malignant pleural mesothelioma. *Cancer Res.* 66, 352-361 (2006)

123 Ma, P.C., Kijima T., Maulik G., Fox E.A., Sattler M., Griffin J.D., Johnson B.E.,& Salgia R.: c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. *Cancer Res*. 63, 6272-6281 (2003)

124 Peschard, P., Fournier T.M., Lamorte L., Naujokas M.A., Band H., Langdon W.Y.,& Park M.: Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. *Mol Cell*. 8, 995-1004 (2001)

125 Kong-Beltran, M., Seshagiri S., Zha J., Zhu W., Bhawe K., Mendoza N., Holcomb T., Pujara K., Stinson J., Fu L., Severin C., Rangell L., Schwall R., Amler L., Wickramasinghe D.,& Yauch R.: Somatic mutations lead to an oncogenic deletion of met in lung cancer. *Cancer Res*. 66, 283-289 (2006)

126 Conrotto, P., Corso S., Gamberini S., Comoglio P.M.,& Giordano S.: Interplay between scatter factor receptors and B plexins controls invasive growth. *Oncogene*. 23, 5131-5137 (2004)

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

128 Sridhar, S.C.,Miranti C.K.: Tetraspanin KAI1/CD82 suppresses invasion by inhibiting integrin-dependent crosstalk with c-Met receptor and Src kinases. *Oncogene*. 25, 2367-2378 (2006)

129 Franco, M., Muratori C., Corso S., Tenaglia E., Bertotti A., Capparuccia L., Trusolino L., Comoglio P.M.,& Tamagnone L.: The tetraspanin CD151 is required for Met-dependent signaling and tumor cell growth. *J.Biol.Chem.* 285, 38756-38764 (2010)

130 Tang, Z., Du R., Jiang S., Wu C., Barkauskas D.S., Richey J., Molter J., Lam M., Flask C., Gerson S., Dowlati A., Liu L., Lee Z., Halmos B., Wang Y., Kern J.A.,& Ma P.C.: Dual MET-EGFR combinatorial inhibition against T790M-EGFR-mediated erlotinib-resistant lung cancer. *Br J Cancer*. 99, 911-922 (2008)

131 Druker, B.J., Sawyers C.L., Kantarjian H., Resta D.J., Reese S.F., Ford J.M., Capdeville R.,& Talpaz M.: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N.Engl.J.Med.* 344, 1038-1042 (2001)

132 Demetri, G.D., von Mehren M., Blanke C.D., Van den Abbeele A.D., Eisenberg B., Roberts P.J., Heinrich M.C., Tuveson D.A., Singer S., Janicek M., Fletcher J.A., Silverman S.G., Silberman S.L., Capdeville R., Kiese B., Peng B., Dimitrijevic S., Druker B.J., Corless C., Fletcher C.D.,& Joensuu H.: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N.Engl.J.Med.* 347, 472-480 (2002)

133 Kosaka, T., Yatabe Y., Endoh H., Yoshida K., Hida T., Tsuboi M., Tada H., Kuwano H.,& Mitsudomi T.: Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. *Clin Cancer Res*. 12, 5764-5769 (2006)

134 Balak, M.N., Gong Y., Riely G.J., Somwar R., Li A.R., Zakowski M.F., Chiang A., Yang G., Ouerfelli O., Kris M.G., Ladanyi M., Miller V.A.,& Pao W.: Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. *Clin Cancer Res*. 12, 6494-6501 (2006)

135 Engelman, J.A.,Janne P.A.: Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin.Cancer Res.* 14, 2895-2899 (2008)

136 Cappuzzo, F., Finocchiaro G., Rossi E., Janne P.A., Carnaghi C., Calandri C., Bencardino K., Ligorio C., Ciardiello F., Pressiani T., Destro A., Roncalli M., Crino L., Franklin W.A., Santoro A.,& Varella-Garcia M.: EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients. *Ann.Oncol.* 19, 717-723 (2008)

137 Yano, S., Wang W., Li Q., Matsumoto K., Sakurama H., Nakamura T., Ogino H., Kakiuchi S., Hanibuchi M., Nishioka Y., Uehara H., Mitsudomi T., Yatabe Y., Nakamura T.,& Sone S.: Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations. *Cancer Res*. 68, 9479-9487 (2008)

138 Turke, A.B., Zejnullahu K., Wu Y.L., Song Y., Dias-Santagata D., Lifshits E., Toschi L., Rogers A., Mok T., Sequist L., Lindeman N.I., Murphy C., Akhavanfard S., Yeap B.Y., Xiao Y., Capelletti M., Iafrate A.J., Lee C., Christensen J.G., Engelman J.A.,& Janne P.A.: Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell.* 17, 77-88 (2010)

139 Laux, I., Goldman J., Just R., Brady K., Li J., Schwartz B., Savage R., Garmey E.,& Rosen L.: Phase I dose escalation trial (ARQ 197-111) evaluating combination of selective c-Met inhibitor ARQ 197 and erlotinib. *J Clin Oncol*. 27, 3549 (2009)

140 Hudis, C.A.: Trastuzumab--mechanism of action and use in clinical practice. *N.Engl.J.Med.* 357, 39-51 (2007)

141 Edakuni, G., Sasatomi E., Satoh T., Tokunaga O.,& Miyazaki K.: Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. *Pathol.Int.* 51, 172-178 (2001)

142 Jin, L., Fuchs A., Schnitt S.J., Yao Y., Joseph A., Lamszus K., Park M., Goldberg I.D.,& Rosen E.M.: Expression of scatter factor and c-met receptor in benign and malignant breast tissue. *Cancer*. 79, 749-760 (1997)

143 Kang, J.Y., Dolled-Filhart M., Ocal I.T., Singh B., Lin C.Y., Dickson R.B., Rimm D.L.,& Camp R.L.: Tissue microarray analysis of hepatocyte growth factor/Met

pathway components reveals a role for Met, matriptase, and hepatocyte growth factor activator inhibitor 1 in the progression of node-negative breast cancer. *Cancer Res*. 63, 1101-1105 (2003)

144 Nagy, J., Curry G.W., Hillan K.J., McKay I.C., Mallon E., Purushotham A.D.,& George W.D.: Hepatocyte growth factor/scatter factor expression and c-met in primary breast cancer. *Surg.Oncol.* 5, 15-21 (1996)

145 Yamashita, J., Ogawa M., Yamashita S., Nomura K., Kuramoto M., Saishoji T.,& Shin S.: Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. *Cancer Res*. 54, 1630-1633 (1994)

146 Yao, Y., Jin L., Fuchs A., Joseph A., Hastings H.M., Goldberg I.D.,& Rosen E.M.: Scatter factor protein levels in human breast cancers: clinicopathological and biological correlations. *Am.J.Pathol.* 149, 1707-1717 (1996)

147 Ghoussoub, R.A., Dillon D.A., D'Aquila T., Rimm E.B., Fearon E.R.,& Rimm D.L.: Expression of c-met is a strong independent prognostic factor in breast carcinoma. *Cancer*. 82, 1513-1520 (1998)

148 Lee, W.Y., Chen H.H., Chow N.H., Su W.C., Lin P.W.,& Guo H.R.: Prognostic significance of co-expression of RON and MET receptors in node-negative breast cancer patients. *Clin Cancer Res*. 11, 2222-2228 (2005)

149 Lindemann, K., Resau J., Nahrig J., Kort E., Leeser B., Annecke K., Welk A., Schafer J., Vande Woude G.F., Lengyel E.,& Harbeck N.: Differential expression of c-Met, its ligand HGF/SF and HER2/neu in DCIS and adjacent normal breast tissue. *Histopathology*. 51, 54-62 (2007)

150 Khoury, H., Naujokas M.A., Zuo D., Sangwan V., Frigault M.M., Petkiewicz S., Dankort D.L., Muller W.J.,& Park M.: HGF converts ErbB2/Neu epithelial morphogenesis to cell invasion. *Mol.Biol.Cell*. 16, 550-561 (2005)

151 Agarwal, S., Zerillo C., Kolmakova J., Christensen J.G., Harris L.N., Rimm D.L., Digiovanna M.P.,& Stern D.F.: Association of constitutively activated hepatocyte growth factor receptor (Met) with resistance to a dual EGFR/Her2 inhibitor in non-small-cell lung cancer cells. *Br J Cancer*. 100, 941-949 (2009)

152 Liu, Y., Liu L., Shi H., Greger J.G., Jr., Jackson K.D., Marty-Ethgen P., Gilmer T.M.,& Martin A.: cMET expression in HER2+ MBC patients with first-line lapatinib (L) treatment. *ASCO Meeting Abstracts*. 27, 1073 (2009)

153 Migliore, C.,Giordano S.: Molecular cancer therapy: can our expectation be MET? *Eur.J.Cancer*. 44, 641-651 (2008)

154 Eder, J.P., Vande Woude G.F., Boerner S.A.,& LoRusso P.M.: Novel therapeutic inhibitors of the c-Met signaling pathway in cancer. *Clin Cancer Res*. 15, 2207-2214 (2009)

155 Cecchi, F., Rabe D.C.,& Bottaro D.P.: Targeting the HGF/Met signaling pathway in cancer therapy. *Expert.Opin.Ther.Targets.* (2012)

156 Jeay, S., Munshi N., Hill J., Moussa M., Ashwell M., Leggett D.,& Li C.: # 2369 ARQ 197, a highly selective small molecule inhibitor of c-Met, with selective antitumor properties in a broad spectrum of human cancer cells. *AACR 98th Annual Meeting*. Los Angeles, CA, USA, 14 april - 18 april (2007)

157 Li, Y., Chen D., Zhou W., Li W., Sun J., Hyatt E., Jiang Z., Makhija S., Leggett D.S.,& Li C.J.: Broad spectrum anti-cancer activity of ARQ 197, a highly selective oral c-Met Inhibitor, in multiple xenograft models. *AACR Meeting Abstracts.* 2007, 2216 (2007)

158 Anderson, K., Li C., Moreau J., Chan T.,& Rosenblatt M.: ARQ 197, a small molecule inhibitor of c-met, prevents bone metastasis in a humanized mouse model of breast cancer. *AACR Meeting Abstracts*. 2007, B184 (2007)

159 Li, Y., Zhou W., Chen D., Li W., Jiang Z., Chen T.,& Li C.J.: Anti-metastatic activity of ARQ 197, a highly selective oral small molecule inhibitor of c-Met, in experimental metastatic models of colon cancer. *AACR Meeting Abstracts*. 2007, 2191 (2007)

160 Chen, C.R., Szwaya J., Rojnuckarin A., Uppalapati U., Huang L., Nakuci E., Cornell-Kennon S., Brown J., McSweeney D., Bruseo C., Chan T.,& France D.: Abstract #820: Combination studies of tyrosine kinase inhibitors (TKIs): Assessment of potential cytotoxic synergy of ARQ 197 with sorafenib or sunitinib. *AACR Meeting Abstracts*. 2009, 820 (2009)

161 Yap, T.A., Olmos D., Brunetto A.T., Tunariu N., Barriuso J., Riisnaes R., Pope L., Clark J., Futreal A., Germuska M., Collins D., deSouza N.M., Leach M.O., Savage R.E., Waghorne C., Chai F., Garmey E., Schwartz B., Kaye S.B.,& de Bono J.S.: Phase I trial of a selective c-MET inhibitor ARQ 197 incorporating proof of mechanism pharmacodynamic studies. *J.Clin.Oncol.* 29, 1271-1279 (2011)

162 Adjei, A.A., Schwartz B.,& Garmey E.: Early clinical development of ARQ 197, a selective, non-ATP-competitive inhibitor targeting MET tyrosine kinase for the treatment of advanced cancers. *Oncologist.* 16, 788-799 (2011)

163 Adjei, A.A., Sosman J.A., Martell R.E., Dy G.K., Goff L.W., Ma W.W., Horn L., Fetterly G.J., Michael S.A., Means J.A., Chai F., Lamar M., Strauss G.M., Chiang W., Jarboe J., Schwartz B.E.,& Puzanov I.: Efficacy in selected tumor types in a phase I study of the c-MET inhibitor ARQ 197 in combination with sorafenib. *ASCO Meeting Abstracts*. 29, 3034 (2011)

164 Schiller, J.H., Akerley W.L., Brugger W., Ferrari D., Garmey E.G., Gerber D.E., Orlov S.V., Ramlau R., von Pawel J.,& Sequist L.V.: Results from ARQ 197-209: A global randomized placebo-controlled phase II clinical trial of erlotinib plus ARQ 197 versus erlotinib plus placebo in previously treated EGFR inhibitor-naive patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) *ASCO Meeting Abstracts*. 28, LBA7502 (2010)

165 Sequist, L.V., von Pawel J., Garmey E.G., Akerley W.L., Brugger W., Ferrari D., Chen Y., Costa D.B., Gerber D.E., Orlov S., Ramlau R., Arthur S., Gorbachevsky I., Schwartz B.,& Schiller J.H.: Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. *J.Clin.Oncol.* %20;29, 3307-3315 (2011)

166 Brugger, W., Sequist L.V., Akerley W.L., Ferraris D., Garmey E., Gerber D.E., Orlov S., Ramlau R., von Pawel J.,& Schiller J.H.: Final results from ARQ 197-209: a global randomized placebo-controlled phase 2 clinical trial of erlotinib plus ARQ 197 versus erlotinib plus placebo in previously treated EGFR-inhibitor naïve patients with advanced non-small cell lung cancer (NSCLC) (EMSO 3630) *Ann.Oncol.* 21, viii122-viii161 (2010)

167 Rodig, S., Sequist L.V., Schiller J.H., Chen Y., Halim A., Waghorne C., Zahir H.,& Schwartz B.: An exploratory biomarker analysis evaluating the effect of the c-MET inhibitor tivantinib (ARQ 197) and erlotinib in NSCLC patients in a randomized, double-blinded phase 2 study. *AACR 102nd Annual Meeting* (2012)

168 Prat, M., Crepaldi T., Pennacchietti S., Bussolino F.,& Comoglio P.M.: Agonistic monoclonal antibodies against the Met receptor dissect the biological responses to HGF. *J Cell Sci*. 111 (Pt 2), 237-247 (1998)

169 Schwall, R.H., Adams C.W., Zheng Z., Romero M., Mai E., Moffat B., Reilly D., Lim A., Duenas E., Dodge K., Simmons L., Huang A.,& Yansura D.G.: #1424 Inhibition of cMet activation by a one-armed antibody. *Proc Amer Assoc Cancer Res*. 2004, 327-32b (2004)

170 Martens, T., Schmidt N.O., Eckerich C., Fillbrandt R., Merchant M., Schwall R., Westphal M.,& Lamszus K.: A novel one-armed anti-c-Met antibody inhibits glioblastoma growth *in vivo*. *Clin Cancer Res*. 12, 6144-6152 (2006)

171 Salgia, R., Peterson A., Eppler S., Yu W., Polite B., Geary D., Wesolowski E., LaRosiliere M., Ratain M.,& Sovak M.: 411 POSTER A phase I, open-label, dose-escalation study of the safety and pharmacology of MetMAb, a monovalent antagonist antibody to the receptor c-Met, administered IV in patients with locally advanced or metastatic solid tumors. *Eur.J.Cancer*. Suppl. 6, 129-129 (2008)

172 Mark, M., Zhang Y., Su Y., Romero M.S., Severin C., Zheng Z., Mendoza N., Kaufman D.W., Vande Woude G.F.,& Filvaroff E.: # 556 POSTER MetMAb significantly enhances anti-tumor activity of anti-VEGF and/or erlotinib in several animal tumor models. *Eur.J.Cancer*. 6, 175-175 (2008)

173 Moss, R.A., Bothos J.G., Patel P.H., Peterson A.C., Eppler S.,& Bai S.: Final results from the phase I study of MetMAb, a monovalent antagonist antibody to the receptor Met, dosed as single agent and in combination with bevacizumab in patients with advanced solid malignancies. *AACR Annual Meeting, April 2–6, 2011, Orlando, Florida: abstract 4717* (2011)

174 Spigel, D.R., Ervin T.J., Ramlau R., Daniel D.B., Goldschmidt J.H., Blumenschein G.R., Krzakowski M.J., Robinet G., Clement-Duchene C., Barlesi F., Govindan R., Patel T., Orlov S.V., Wertheim M.S., Zha J., Pandita A., Yu W., Yauch R.L., Patel P.H.,& Peterson A.C.: Final efficacy results from OAM4558g, a randomized phase II study evaluating MetMAb or placebo in combination with erlotinib in advanced NSCLC. *ASCO Meeting Abstracts*. 29, 7505 (2011)

175 Mekhail, T., Rich T., Rosen L., Chai F., Semic-Suka Z., Savage R.E.,& Senzer N.: #3548 Final results: A dose escalation phase I study of ARQ 197, a selective c-Met inhibitor, in patients with metastatic solid tumors. *J Clin Oncol - 2009 ASCO Annual Meeting Proceedings*. 27 (2009)

176 Reardon, D.A., Cloughsey T.F., Raizer J.J., Laterra J., Schiff D., Yang X., Loh E.,& Wen P.Y.: # 2051 Phase II study of AMG 102, a fully human neutralizing antibody against hepatocyte growth factor/scatter factor, in patients with recurrent glioblastoma multiforme. *J Clin Oncol - 2008 ASCO Annual Meeting Proceedings*. 26 (2008)

177 Weinstein, I.B.,Joe A.: Oncogene addiction. *Cancer Res*. 68, 3077-3080 (2008)

178 Weinstein, I.B.,Joe A.K.: Mechanisms of disease: Oncogene addiction--a rationale for molecular targeting in cancer therapy. *Nat.Clin.Pract.Oncol.* 3, 448-457 (2006)

179 Sharma, S.V., Fischbach M.A., Haber D.A.,& Settleman J.: "Oncogenic shock": explaining oncogene addiction through differential signal attenuation. *Clin Cancer Res*. 12, 4392s-4395s (2006)

180 Pillay, V., Allaf L., Wilding A.L., Donoghue J.F., Court NW, Greenall S.A., Scott A.M.,& Johns T.G.: The plasticity of oncogene addiction: implications for targeted therapies directed to receptor tyrosine kinases. *Neoplasia*. 11, 448-458 (2009)

181 Canadas, I., Arumi M., Lema L., Martinez A., Grande E., Bellosillo B., Rojo F., Rovira A., Albanell J.,& Arriola E.: MET in small cell lung carcinoma (SCLC): Effects of a MET inhibitor in SCLC cell lines and prognostic role of MET status in patients. *ASCO Meeting Abstracts*. 27, e14617 (2009)

Key Words: c-Met, Targeted Cancer Therapy, Signaling, Clinical Trials, Biomarker, Review

Send correspondence to: Lxxxx Goetsch1, Centre d'Immunologie Pierre Fabre, 5 avenue Napoleon III F-74164 Saint Julien en Genevois, France, Tel: 33450353522, Fax: 33450353590, E-mail: liliane.goetsch@pierre-fabre.com