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REVIEW

Targeting the oncogenic Met receptor by antibodies and gene therapy

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THE ONCOGENIC MET RECEPTOR

In cancer cells, loss of growth control is the devastating phenotypic feature of genetic alterations. Even if a plethora of mutations almost invariably affects transformed cells, the vast majority of them are irrelevant or dispensable to the expression of the malignant phenotype ('passenger' mutations). On the contrary, specific lesions affecting genes directly involved in the control of cell growth, in few instances a single oncogene, have the role of 'drivers', sustaining the disease.[1] In this scenario, it is conceivable that a drug aimed at the product of a driver gene will fulfill the properties for the long-sought 'targeted' anti-cancer therapy.[2]

Receptors with tyrosine kinase activity are interesting candidates for targeted therapy, as they are often aberrantly activated in human cancers.[3] Among different receptors with tyrosine kinase activity, the hepatocyte growth factor (HGF) receptor, encoded by the Met oncogene, is a 'master gene' controlling the genetic program known as 'invasive growth'.[4] In some cancers, Met behaves as a driver, that is, it has been selected in the Darwinian tumor evolution for the long-term maintenance of the transformed phenotype: those cancers appear to be dependent on (or 'addicted' to) Met signaling for their growth and survival. Such constitutive signaling results from a transmissible genetic lesion, mutation or—more often—gene amplification. Described for the first time in hereditary and sporadic papillary renal tumors,[5] Met point mutations have been later found in a vast variety of solid cancers (Table 1) and a number of them have been functionally validated for their ability to trigger constitutive Met activation (Figure 1). Interestingly, the frequency of missense genetic alterations is enriched in metastatic lesions from head and neck squamous cell carcinomas[6] and in cancer of unknown primary origin.[7] Met gene amplification is spread among 2–4% percent of epithelial cancers (COSMIC database: www.sanger.ac.uk) and it is also one of the molecular mechanisms responsible for secondary resistance to anti-epithelial growth factor receptor (EGFR) therapy in non-small cell lung cancer (NSCLC) and colorectal cancers.[8–10] According to the concept of Met addiction, metastatic gastric and esophagogastric cancer patients, selected on the basis of Met gene amplification, benefit from anti-Met therapy.[11,12]

As well as in normal cells, Met stimulates cell growth, 'scattering', invasion, protection from apoptosis in transformed cells and angiogenesis in the tumor microenvironment, thereby acting as a powerful expedient for cancer survival and dissemination ('expedience').[13] Notably, Met behaves as a 'stress-response gene' and it is overexpressed as a transcriptional adaptation of cancer cells to unfavorable microenvironmental conditions, including hypoxia[14] and therapeutic ionizing radiations.[15] Both Met 'addiction' and 'expedience' are independent from the ligand HGF. However, superimposition of the ligand, released by paracrine or autocrine circuits, may exacerbate the phenotype. Within the hidden roots of the tumor, some cells get special benefit from Met expression. As an example, in a subset of glioblastomas, high Met expression sustains the stem-like and the invasive phenotype of cancer stem cells.[16] Furthermore, in metastatic colorectal cancers, the subpopulation of cancer-initiating cells takes advantage of Met signaling to blunt the therapeutic response to EGFR-targeting agents.[17]

Because of its dual role as a necessary oncogene for some tumor and as an adjuvant, prometastatic gene for others, Met is a validated target for therapeutic intervention.

HOW TO INHIBIT MET SIGNALING

To quench Met signaling, a number of drugs have been developed, including chemical kinase inhibitors and monoclonal antibodies (mAb) targeting either the ligand (HGF) or the receptor (Met).[18,19] Small molecule tyrosine kinase inhibitors translocate through the plasma membrane and interact with the receptor kinase domain, acting as ATP mimetics.[20] Both selective and non-selective inhibitors are under development. On one hand, selective drugs would be preferable, as, in principle, a narrow specificity window reduces off-target side effects. On the other hand, simultaneous inhibition of several intracellular signaling pathways may contribute to therapeutic efficacy. TKI activity can be hindered by the presence of point mutations affecting the tridimensional structure of the receptor kinase domain, not

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Table 1. Human cancers carrying Met gene missense mutations[a]

Cancer	*No. mutated/No. analyzed*
Bladder carcinoma	3/156
Breast carcinoma	10/1935
Cancer of unknown primary origin	10/288
Childhood hepatocellular carcinoma	12/479
Cholangiocarcinoma	2/130
Colorectal carcinoma	75/1158
Endometrial carcinosarcoma	20/427
Epithelial ovarian carcinoma	12/918
Esophageal adenocarcinoma	4/280
Follicular and papillary thyroid carcinoma	4/807
Gastric cancer	5/427
Glioma	8/1059
Head and neck squamous cell carcinoma	63/653
Hereditary and sporadic papillary renal cell carcinoma	29/962
Leukemia	5/1382
Melanoma	6/1005
Mesothelioma	6/84
Neuroendocrine tumor	3/556
Osteosarcoma	1/170
Prostate carcinoma	3/497
Small cell and non-small cell lung cancer	95/3225
Uveal melanoma	1/170

[a]data from COSMIC database www.sanger.ac.uk.

allowing the drug to house properly into the ATP binding pocket. This has been described in cases of primary and secondary resistance to small molecule inhibitors of EGFR, Kit and BCR-Abl.[21–24] Moreover, on the long run, kinase inhibition could result in receptor stabilization and stifling of the therapeutic intervention. Phosphorylated receptors with tyrosine kinase activity are indeed subjected to physiological downregulation via the endocytic pathway[25] and thus, in the presence of a TKI, that maintains the receptor in an unphosphorylated status, downregulation is impaired. In the case of Met, endocytosis relies on the ubiquitin-ligase Cbl, recruited to the receptor both directly to the phosphorylated Y^{1021} (ref. 26) and indirectly—through the adaptor Grb2—to the phosphorylated Y^{1374} (ref. 27) in cells treated with specific tyrosine kinase inhibitors Met accumulates at the cell surface[28] (Lanzetti *et al.*, in preparation).

From a number of view points, the antibody approach shows various advantages. First of all, antibodies are more specific than small chemical tyrosine kinase inhibitors. Thanks to their target specificity and natural design, they are generally well tolerated. Antibodies can inhibit tumor cells through different mechanisms, elicited one by one or in combination. They can directly sequester the ligand or interfere with receptor functions through different (possibly synergic) mechanisms: (i) competing with the ligand for the binding site; (ii), blocking the homo- or heterodimerization required to unleash signal transduction; (iii) inducing receptor downregulation. Antibodies can stimulate an immune reaction against cancer cells via ADCC (antibody-dependent cellular cytotoxicity), CDC (complement-dependent cytotoxicity) and/or ADCP (antibody-dependent cellular phagocytosis). Ideally, antibodies can selectively recognize tumor-specific receptor conformations. Moreover, bi-specific engineered antibodies would simultaneously block multiple targets. Antibodies, binding the extracellular domain of the receptor, recognize their targets even affected by different intracellular mutations and are less prone to resistance generated by a secondary mutation. Finally, antibodies are insensitive to multidrug resistance, a phenotype that aggressive cancer cells acquire, thanks to amplification of a gene encoding, a pump that extrudes drugs from the cytoplasm.[29]

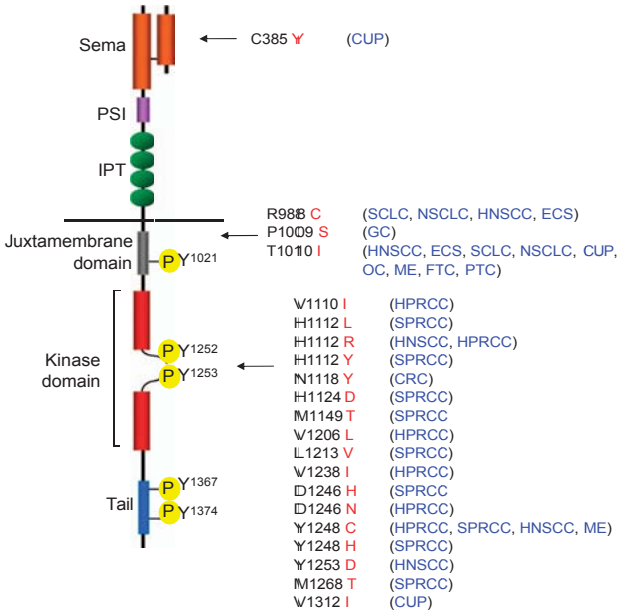

Figure 1. Missense mutations activating Met. Met receptor is a transmembrane protein with an extracellular region, including Sema domain (orange boxes), PSI (violet box) and IPTs (green circles), a transmembrane region and an intracellular region, including the juxtamembrane domain (gray box), the kinase domain (red boxes) and the tail (blue box). P-signed yellow circles indicated critical phosphorylated tyrosine residues; Y^{1021} negatively regulates Met signaling, Y^{1252} and Y^{1253} are the major phosphorylation sites, positively regulating the receptor kinase activity, and Y^{1367} and Y^{1374} are the docking sites for intracellular transducers. In black are reported wild-type amino acids (one letter code plus a number indicating their position, according to the sequence with accession number J02958) and in red the corresponding mutated amino acids found in human tumor samples. Arrows indicate Met domains where mutations are located. The missense mutations reported here have been functionally validated. In blue are indicated the tumors where the genetic modifications have been scored. CUP, cancer of unknown primary origin; ECS, endometrial carcinosarcoma; EOC, epithelial ovarian carcinoma; FTC, follicular thyroid carcinoma; GC, gastric carcinoma; HNSCC, head and neck squamous cell carcinoma; HPRCC, hereditary papillary renal cell carcinoma; ME, melanoma; PTC, papillary thyroid carcinoma; SPRCC, sporadic papillary renal cell carcinoma.

On the other hand, the major antibody disadvantages include their possible agonist activity and the requirement of a good level of antigen expression at the cell surface. The therapeutic effect can also be affected by poor antibody penetration into the tumor. Moreover, antibodies are delivered by complex protocols to obtain a long-lasting level in the circulation. Finally prolonged protein infusion may give rise to side effects, including host-neutralizing immune response.

ANTIBODIES AGAINST HGF (LIGAND)

A mix of five different antibodies (called Amix), recognizing distinct epitopes on the HGF α-chain, is required to obtain neutralization of the ligand–receptor interaction.[30] The combination of heparin with three out of five antibodies from this mixture exert a superior inhibitory effect.[31] Amgen developed five different 'fully human' anti-HGF antibodies using XenoMouse technology.[32] One of them, AMG102/Rilotumumab binds preferentially to the mature biologically active form of HGF, interacting with the amino-terminal portion of the β-chain[33] (Figure 2a) and

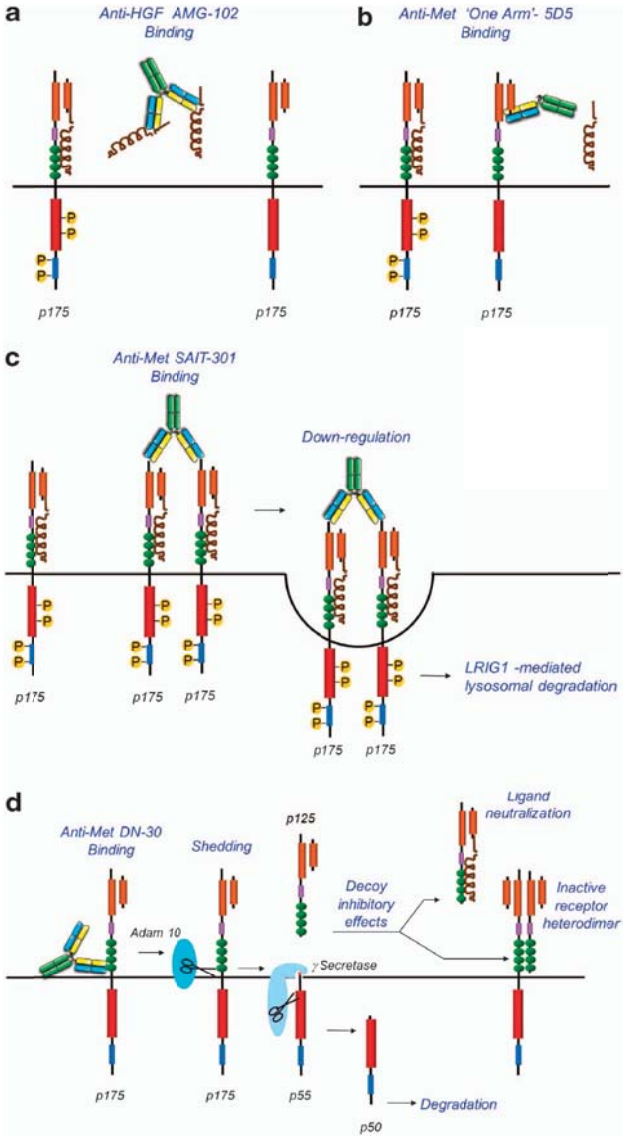

Figure 2. Mechanisms underlying antibody-mediated Met inhibition. Met (p175), interacting with its ligand HGF(drawn in brown), is activated by phosphorylation on a number of tyrosine residues (P-signed yellow circles). (a) AMG-102 antibody binds to HGF beta chain. Antibody-sequestered ligands are displaced from Met. (b) One Arm-5D5 binds to Met SEMA domain, competing with HGF for Met binding. (c) SAIT301 interacts with Met and induces receptor downregulation followed by LRIG1 protein-mediated Met degradation into the lysosomes. (d) DN30 antibody binds to Met IPT domain, enhancing Met cleavage by the metalloprotease ADAM-10. The receptor amino-terminal fragment (p125 Met), is released in the extracellular environment where it acts as 'decoy', sequestering HGF and generating inactive heterodimers with the residual full-length transmembrane Met receptors. The membrane-linked carboxy-terminal fragment (p55 Met) becomes substrate of a second transmembrane protease, γ-secretase, that releases a cytoplasmic p50 Met fragment addressed to degradation pathways. Met domain color codes: orange, Sema; violet, PSI; green, IPT; red, kinase; blue: carboxy-terminal tail. Antibody domain color codes: green, Fc; yellow, heavy chain; blue, light chain.

synergizes with chemotherapy[34] and radiotherapy.[35] A blocking murine anti-HGF antibody, L2G7, inhibits various biological activities induced by HGF in experimental brain tumors.[36] The humanized version of L2G7, called TAK-701, abrogates the acquired resistance to EGFR-targeted therapy experimentally induced *in vitro* by HGF overexpression,[37] but failed to inhibit the growth of xenografts obtained from childhood tumors carrying concomitant HGF expression and Met phosphorylation.[38] AV299/Ficlatuzumab, a humanized antibody developed by Aveo Pharmaceuticals, provided a survival benefit when tested in an orthotopic mouse glioblastoma model, exerting its therapeutic potential both alone and in conjunction with the standard-of-care chemotherapy (temozolomide).[39] Other anti-HGF molecules have been described more recently: an antibody generated by rabbit immunization with the HGF–cMet complex (SFN68)[40] and two anti-HGF nanobodies (1E2 and 6E10).[41] The characterization of these molecules was done in preclinical models that employed tumor cells featuring an HGF/Met autocrine loop, either natural or by transfection. In this contest, artificially obtained HGF expression models can be considered somehow tautological. In the vast majority of human cancers, Met activation is due to receptor overexpression, secondary to gene amplification ('addiction') or transcriptional activation ('expedience') and less frequently to gene mutations. In both instances, activation of the oncogenic receptor is ligand independent and thus antibodies against HGF are not expected—in principle—to elicit any therapeutic response. Nonetheless, HGF supports tumor growth and metastasis dissemination acting not only on tumor cells, but also on tumor microenvironment, favouring angiogenesis[42] and sustaining 'cancer-associated-fibroblasts'[43] and 'tumor-associated-macrophages'[44,45] that are known to endorse tumor growth.

Rilotumumab and Ficlatuzumab are in clinical trials. Rilotumumab has been tested as monotherapy in patients carrying recurrent glioblastomas,[46] metastatic renal carcinomas[47] or ovarian cancers[48] and in combination with chemotherapy in prostate cancers[49] or with antiangiogenic agents in advanced solid tumors.[50] Ficlatuzumab was tested both as monotherapy and in association with EGFR inhibitors in NSCLCs.[51] Until now, the results of these clinical studies are somehow puzzling, reporting cases of sporadic responses without reaching the primary endpoints.

ANTIBODIES AGAINST MET (RECEPTOR)

R13 and R28 are two Met antibodies that must be combined to interfere with HGF binding and to elicit ADCC.[52] It has been hypothesized that the first interacts with Met, facilitating the binding of the second, locking the receptor in an inactive conformation. A panel of other antibodies was generated using the Met SEMA domain and alive Met-expressing cell lines as immunogens.[53] These mAbs, whose mechanism of action is poorly characterized, seem to interfere with the cell surface receptor recycling. SAIT301, a humanized antibody, has the peculiar ability to promote Met degradation by the LRIG1-mediated lysosomal pathway[54] (Figure 2c). SAIT301 inhibits the growth, in clonogenic assays *in vitro*, of cells derived from Cetuximab-resistant Met-overexpressing lung tumors. A humanized nanobody has been generated by fusing a building block targeting Met with a second one binding human serum albumin, for half-life extension. Inhibitory properties have been detected *in vitro*, on myeloma cells harboring an HGF-Met autocrine loop.[55] Two humanized antibodies, LY2875358 (Ely Lilly) and H224G11/ABT700 (Pierre Fabre–Abbott), are currently in early clinical trials. LY2875358 induces internalization and degradation of Met *in vitro* and marked antitumor activity in Met-amplified NSCLC xenografts *in vivo*.[56] Currently, it is being tested in phase I/II alone, in patients with advanced solid tumors, or in combination with Erlotinib in NSCLC.[57] H224G11/ABT700 blocks HGF binding, inhibits Met dimerization, phosphorylation and induces Met intracellular downregulation *in vitro*; moreover, it triggers a significant ADCC activity.[58] At present, this antibody is being evaluated alone, or plus Docetaxel, or plus FOLFIRI/Cetuximab, or plus Erlotinib, in

advanced solid tumors displaying Met gene amplification or Met receptor overexpression.

The more advanced antibody scouting clinical applications is 5D5, screened by Genentech on the basis of its ability to compete with HGF for Met binding. Due to its bivalent structure, it induces Met dimerization, behaving as a full agonist.[59] By molecular engineering ('knob into hole' modification of the CH3 domain),[60] a monovalent antibody devoid of agonistic activity was then generated, the so called One Arm-5D5 (OA-5D5). This molecule, 'humanized' and 'affinity maturated' (MetMab/Onartuzumab)[61] (Figure 2b), acts as an inhibitor in preclinical models of HGF-dependent tumors such as glioblastomas[61,62] and pancreatic carcinomas.[63] Phase I studies demonstrated Met specificity and tolerability.[64] Interestingly, a patient with chemorefractory metastatic gastric cancer, treated with Onartuzumab in the Phase I trial, showed complete remission, lasting 2 years, unfortunately followed by a refractory multiple carcinomatosis.[11] This case represents the first durable complete response obtained with a monoclonal antibody targeting Met. Phase II trials are currently ongoing (see www.clinicaltrials.gov), combining Onartuzumab with chemotherapy and/or targeting agents, in lung cancers, triple-negative metastatic breast cancers, metastatic colon cancers,[65] gastroesophageal cancers and glioblastomas. The final results of phase II trial conducted on patients with refractory NSCLC in combination with an EGFR inhibitor, showed improvement in progression-free and overall survival in tumors with high Met expression (Met 'diagnostic positive'). A 'paradox' response was observed in tumors defined as 'Met diagnostic negative' that have a worse clinical outcome than controls.[66] The mechanistic explanation of the paradox is still waiting. Phase III trials on NSCLC (plus Erlotinib) and gastroesophageal cancers (plus mFOLFOX6) are ongoing. 5D5 has also been engineered into a bispecific '*ad hoc*' antibody, to simultaneously target Met and EGFR.[67]

THE ANTI-MET DN30 SERIES

The monoclonal antibody DN30 is a mouse IgG2A engaging the extracellular moiety of the human Met receptor.[68] It binds, with subnanomolar affinity, to the IPT domain[69] promoting '*shedding*'.[70] Shedding is a physiologic cellular mechanism of protein degradation working to maintain the homeostasis of the cell surface.[71] It is a tightly regulated negative feedback mechanism to terminate receptor signaling. Met shedding takes place in two steps (Figure 2d): first, a surface metalloprotease, ADAM-10, cleaves the extracellular domain of Met, recognizing a specific sequence immediately upstream the transmembrane moiety;[72] then, the membrane-linked Met C-terminal fragment becomes substrate of a second transmembrane protease, γ-secretase, that detaches the intracellular kinase domain from the membrane and rapidly addresses it toward the proteasome.[73] An alternative lysosomal-dependent second route of Met degradation has also been described.[74] The DN30-induced shedding most likely relies on an antibody-induced Met conformational change, that results in exposition of the motif targeted by ADAM-10. As a consequence of these proteolytic events, the net number of Met receptors exposed at the cell surface is reduced and, concomitantly, free Met extracellular domains are released. The free domains harbor functional HGF binding sites, thus sequester the ligand (HGF) from the environment, acting as '*decoys*'. Moreover, the free domains form inactive heterodimers with the full-length transmembrane Met receptors that survived cleavage[75] (Figure 2d). The biological response(s) to DN30 are remarkable: inhibition of 'anchorage independent' growth and invasion *in vitro* and impairment of tumor growth and metastasis dissemination *in vivo*.[70] Due to its mechanism of action, based on the straightforward elimination of Met from the surface (Figure 3), independently of receptor activation (phosphorylated vs unphosphorylated state), DN30 has a strong advantage over other Met antibodies, as it is effective in the full spectrum of Met activation mechanisms, whether HGF-dependent or independent (that is, induced by mutations or overexpression).

It has been shown that due to its bivalent nature, DN30 elicits a partial agonistic activity on Met, promoting some biological responses:[68,76] in fact, it can stimulate scattering and wound healing, but it does not induce proliferation, invasion or branching morphogenesis. The native DN30 mAb can be thus considered a 'Janus' molecule, endowed with both antagonistic and agonistic properties. The two activities have been disassociated by the simple conversion of the bivalent antibody into the monovalent Fab fragment.[77] DN30 Fab binds Met with high affinity (subnanomolar) and induces Met shedding, while it is unable to stimulate Met phosphorylation, thus behaving as a pure antagonist, and resulting in a potent inhibitor. DN30 Fab abrogates Met downstream signaling, producing a strong impairment of Met-mediated biological responses.[77] *In vitro,* it inhibits the growth of Met-addicted tumor cells, inducing G_0 growth arrest and apoptosis, and it reduces anchorage-independent growth in a wide panel of Met-expressing cells. *In vivo*, upon intratumoral administration, it blunts the growth of tumors featuring Met addiction. The DN30 Fab is a very attractive therapeutic tool, but its short half-life in plasma, due to renal clearance, limits its clinical applications. To extend the half-life, DN30 Fab has been conjugated with Poly-Etilen-Glycol,[77] improving its efficiency *in vivo*.

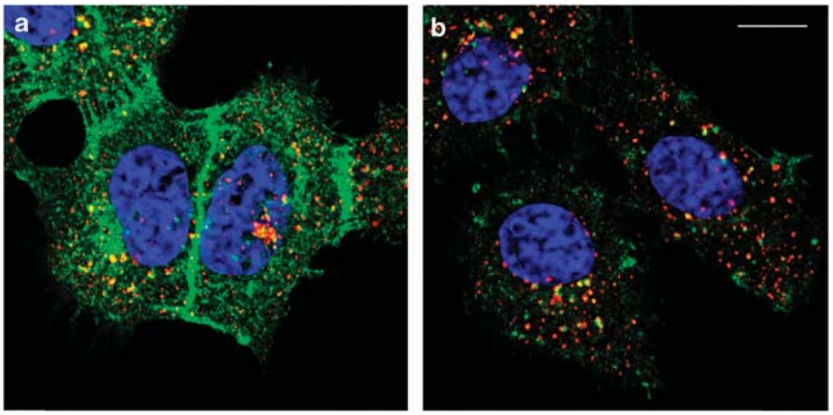

Figure 3. The monovalent DN30 Fab wipes out the Met receptor from the surface of NSCL cancer cells. H1993 cells were incubated for 1 h at 4 °C and shifted at 37 °C for 15 min in the absence (a) or in the presence (b) of DN30 Fab, 1 μM. Cells were then stained to reveal Met (in green) and EEA1, a marker of the early endosomal compartment (in red). Bar is 10 μm.

Table 2. Antibodies against HGF (ligand) or Met (receptor)

Antibody	*Specifications*	*Target*	*Mechanism of action*	*Developmental stage*
Amix	Mixture of five murine mAb	HGF, α-chain	Ligand displacement	Preclinical
AMG102/ Rilotumumab	Human mAb	HGF, β-chain	Ligand displacement	Clinical phase II
L2G7, TAK-701	Murine, humanized mAb	HGF	Ligand displacement	Clinical phase I
SFN68	Rabbit/human chimeric mAb	HGF in complex with Met	Ligand displacement	Preclinical
1E2+6E10	Bispecific nanobodies	HGF and serum albumin	Ligand displacement	Preclinical
AV299/ Ficlatuzumab	Humanized mAb	HGF	Ligand displacement	Clinical phase II
R13+R28	Human recombinant mAbs	Met	Ligand competition+ADCC	Preclinical
LMH panel	Murine mAbs	Met precursor and/or mature form (Sema domain)	Interference with cell surface receptor recycling	Preclinical
SAIT301	Humanized mAb	Met	Receptor downregulation	Preclinical
Anti c-met nanobody	Bispecific nanobody	Met and serum albumine	Ligand competition	Preclinical
LY2875358	Humanized mAb	Met	Ligand competition, receptor internalization	Clinical phase II
H224G11/ ABT 700	Humanized mAb	Met	Ligand competition, receptor downregulation, ADCC	Clinical phase I/Ib
OA-5D5/ Onartuzumab	Monovalent humanized Fab+Fc	Met Sema domain	Ligand competition	Clinical phase II/III
MvDN30	Monovalent chimeric Fab	Met IPT domain	Receptor shedding	Preclinical

DN30 FAB GENE THERAPY: AN INNOVATIVE ROUTE FOR MET TARGETING

Conventional immunotherapy requires complex procedures to produce and purify the antibody and the hassle of frequent repeated administrations. Gene therapy, that is, transfer of genes encoding the therapeutic antibody, may bypass such limitations, allowing direct, continuous and sustained production of the therapeutic molecule by the host. Far from being a standard option for cancer therapy, it is a promising alternative to more toxic—or still absent—conventional treatments.[78] Gene therapy would be the preferred choice in the case of Fabs, such as DN30 Fab, to bypass the intrinsic limitation due to their extremely short half-life. Among the spectrum of vectors offered by the state-of-the-art genetic engineering, lentiviral vectors (LV) have the unique property to integrate the gene cargo into non-proliferating cells,[79] such as cancer stem cells where the Met oncogene plays a crucial role.[16,17] Concerns about LV biosafety have been overcome, as demonstrated by the Food and Drug Administration approval of clinical protocols employing LVs.[80,81] LVs are suitable both for local and systemic delivery. Local administration, such as intratumor infusion, offers some advantages, as (i) toxicity due to 'off-target' effects is minimized by the limited spread of the vector and (ii) the response is amplified, thanks to the secretion of the transgenic Fab into the surrounding tumor tissue ('bystander' effect). On the other hand, intratumor administration has some drawbacks, as a 'suicide' negative selection of vector-infected Fab synthesizing cancer cells takes place. This problem is circumvented by systemic administration of the vector, as the therapeutic protein is produced in a distant organ (notably liver and spleen), by cells shielded from suicide effects. To date, DN30 Fab gene therapy has been challenged in two preclinical models of Met-driven tumors, representative of unmet clinical needs: a glioblastoma multiforme, sustained by HGF-Met autocrine loop, and a Met-addicted NSCLC, presenting Met gene amplification. DN30 Fab gene therapy—both local and systemic—has given promising results, comparable or superior to those obtained by conventional administration of the purified antibody.[69,82] This approach, far from being an 'out of the box' clinical protocol, gives a strong proof of concept, encouraging further studies to explore gene therapy alone and in combination with molecular targeted or standard drugs.

CONCLUSION

The results obtained from ongoing clinical trials of HGF or Met antibodies are encouraging, once again validating the notion of the crucial role played by this oncogenic receptor in driving/ supporting the transformed phenotype of selected cases. It would be therefore highly relevant to define the actionable anti-Met therapeutic opportunities by molecular diagnosis.[83] In this respect, specific Met antibodies will be a valuable diagnostic tool for immunohistochemistry, as well as for use with innovative, non-invasive technologies such as imaging by quantitative PET and SPECT-Scan.[84] Between the current strategies to inhibit the oncogenic Met pathway, namely to target the ligand or the receptor (see Table 2), the receptor options seem to be more rationale, as it will hit cancer cells driven by both ligand-dependent and ligand-independent mechanisms of activation. While antibodies targeting the receptor binding site work through a mechanism functionally superimposable to that of ligand antibodies, those physically removing Met from the cell surface will have 'an extra oomph'.

CONFLICT OF INTEREST

PMC and EV are authors of the international patent WO2007090807 ('Anti-met monoclonal antibody, fragments and vectors thereof …') owned by Metheresis Translational Research SA (Switzerland); The University of Torino received financial support from Metheresis and PMC is a consultant. The company did not interfere at all in the preparation and in the submission of the review article.

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