Known and novel roles of the MET oncogene in cancer: A coherent approach to targeted therapy

Known and novel roles of the METoncogene in cancer: a coherent approach to targeted therapy

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Abstract

The MET oncogene encodes an unconventional receptor tyrosine kinase with pleiotropic functions: it initiates and sustains neoplastic transformation when genetically altered ('oncogene addiction') and fosters cancer cell survival and tumour dissemination when transcriptionally activated in the context of an adaptive response to adverse microenvironmental conditions ('oncogene expedience'). Moreover, MET is an intrinsic modulator of the self-renewal and clonogenic ability of cancer stem cells ('oncogene inherence'). Here, we provide the latest findings on MET function in cancer by focusing on newly identified genetic abnormalities in tumour cells and recently described non-mutational MET activities in stromal cells and cancer stem cells. We discuss how MET drives cancer clonal evolution and progression towards metastasis, both ab initio and under therapeutic pressure. We then elaborate on the use of MET inhibitors in the clinic with a critical appraisal of failures and successes. Ultimately, we advocate a rationale to improve the outcome of anti-MET therapies on the basis of thorough consideration of the entire spectrum of MET-mediated biological responses, which implicates adequate patient stratification, meaningful biomarkers and appropriate clinical end points.

Introduction

The MET receptor tyrosine kinase plays a well-defined role as a selectable oncogenic driver of tumour proliferation. Moreover, it is endowed with additional properties, only marginally related to cell proliferation, that enable tumour cells to survive, trigger the early steps of coagulation to organize fibrin 'nests' that support clonal expansion, retain their tumorigenic potential over time in the face of therapeutic pressures and immune attacks, and spread across the organism (reviewed in ref.1). This multifaceted biological outcome is sustained by a complex signalling apparatus, which includes physical interactions with scaffold (adaptor) proteins and cooperation with structurally related surface receptors (Box 1).

The proficiency of MET in counteracting adverse microenvironmental conditions is tightly associated with its ability to elicit a genetic programme known as invasive growth[2,3,4]. The invasive growth concept — which was originally coined to explain the ability of cancer cells to move and proliferate in response to growth factors — has been recently redefined to incorporate epithelial-to-mesenchymal transition (EMT), a reversible, plastic condition where two properties — cell stemness and dissemination — are believed to be concomitantly activated and interdependent[5]. Invasive growth implies the ability to sustain this complex phenotype across long-distance tissue migration and thus to survive a variety of stressful conditions[2.] Invasive growth and EMT are regulated by several extracellular signals and require specific transcription factors[5]. One of these signals is the MET ligand[6,7], which is best known as hepatocyte growth factor (HGF) after its initial identification in the serum of hepatectomized rodents[8] and was independently discovered as scatter factor[9] because of its ability to induce disassembly and dissemination of epithelial cell monolayers (reviewed in ref.10). The invasive growth and EMT programme is not an aberrant trait unique to metastatic tumour cells, but it is a physiological process required for embryonic development and the repair of injured tissues[11]. From this perspective, cancer invasive growth or EMT can be seen as the inappropriate activation of an inherent property of stem and progenitor cells rather than an acquired cancer hallmark[12].

How invasive growth is prompted at a certain stage of tumour progression is enigmatic. Genetic alterations causing metastasis are difficult to identify because they are selectable only if they lead to a growth advantage and amplification of the mutated cell subclone[13,14]. MET can play this role in a limited number of tumours, where underlying genetic lesions leading to kinase hyperactivation are selected along the natural history of the cancer and are required to maintain the transformed and metastatic phenotype ('oncogene addiction'). In this context, MET

targeting typically results in inhibition of tumour growth[15,16]. In mouse models, there is also evidence that silencing of genetically amplified MET is effective in preventing experimental metastases[17].

However, the role of MET in tumours in general, and in metastasis in particular, is mostly associated not with clonal selection but rather with the ability of MET to sustain cell adaptation to adverse environmental conditions, a function that we termed 'oncogene expedience' (ref.15). The underlying principle of expedience is MET activation by overexpression of the wild-type (WT) receptor, which can be attained through transcriptional upregulation by a variety of stimuli such as hypoxia[18], inflammatory cytokines, pro-angiogenic factors and mitogens — including HGF itself[19] — that are abundant in the reactive stroma of overt tumours[20,21]. In this context, activation of WT MET is a late event that increases the intrinsic malignant properties of already transformed cells by mainly conveying anti-apoptotic and pro-invasive signals. In terms of the implications for therapy, targeting overexpressed MET (in the absence of its structural alterations) is expected to impair cancer cell survival and progression towards metastasis, rather than impairing growth[22]. Expedience, being based on MET transcriptional upregulation, is not an inheritable mechanism, but it can contribute to selection, as MET is preferentially inducible in stem and progenitor cells, which physiologically express it[3]. These cells are often the tumour cell of origin and pass on their phenotypic traits to the tumour cell population, in particular to a subpopulation that retains the maximum tumorigenic potential, known as cancer stem cells (CSCs)[3]. We define 'oncogene inherence' as the innate expression and activity of WT MET in CSCs, a trait inherited in association with tumorigenic potential.

This Review analyses the contribution of MET addiction, expedience and inherence to cancer onset, progression and therapeutic response; in each of these different contexts, we try to envisage the expected outcome of MET inhibition. Finally, we build on the past failures of MET-targeted therapies to suggest how to redirect and evaluate MET targeting to achieve real benefits for patients.

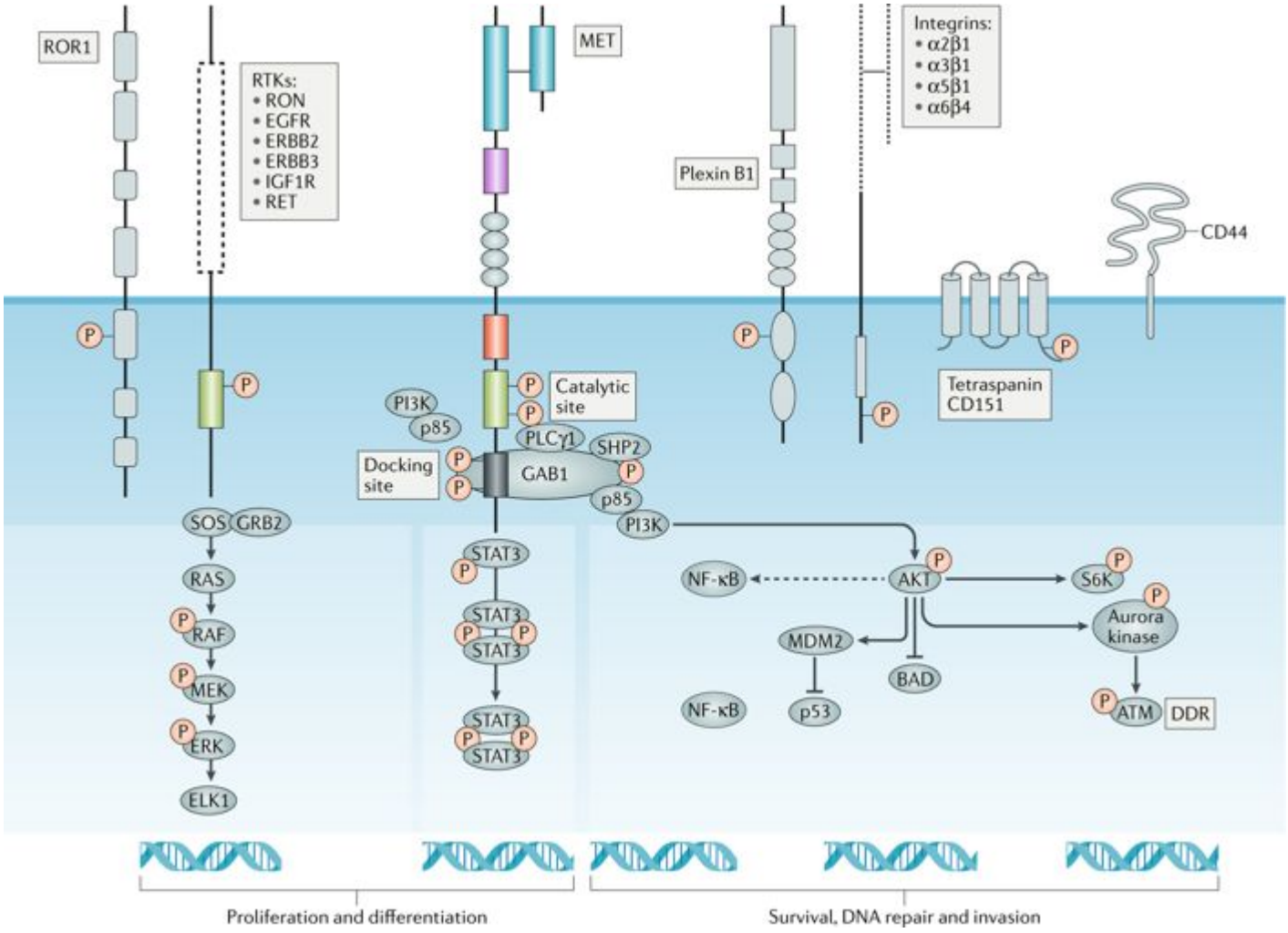
Box 1 | The MET signalling apparatus

After binding hepatocyte growth factor (HGF), MET is activated by dimerization and *trans*-phosphorylation of two juxtamembrane 'catalytic' tyrosines. The following step is phosphorylation (P) of two additional 'docking' tyrosines in the carboxy-terminal tail (see the figure), which are embedded in the sequence Y^{1349}VHVXXXY^{1356}VNV, tethering multiple SH2 domain-containing signal-relay molecules, such as PI3K, growth factor receptor-bound protein 2 (GRB2), phospholipase Cγ 1 (PLCγ1) and signal transducer and activator of transcription 3 (STAT3)[173,174]. MET also associates with GRB2-associated binding protein 1 (GAB1), a multi-adaptor protein that, following phosphorylation by MET, provides additional binding sites for GRB2, PI3K, SH2 domain-containing tyrosine phosphatase 2 (SHP2; also known as PTPN11), PLCγ1 and other signal transducers[175,176]. The complexity of the MET signalling network is further increased by the ability of MET to bind other surface receptors, including the MET homologue RON (also known as MST1R)[177], other tyrosine kinases[178], ROR1 (ref.[179]), CD44 (ref.[180]), plexin B1 (ref.[181]), integrins[182] and the tetraspanin CD151 (ref.[183]). All these membrane-spanning interaction partners qualitatively and/or quantitatively modulate MET signalling outputs for efficient execution of MET-dependent biological processes. MET stimulates proliferation by activating RAS and the distal MAPK cascade through the GRB2–son of sevenless (SOS) complex, which can directly bind the carboxy-terminal tail of MET or can interact indirectly through GAB1.

PI3K can be activated directly by MET and/or indirectly by RAS[184]. PI3K activation leads to the formation of phosphatidylinositol-3,4,5-triphosphate (PtdInsP3), which recruits the PH domain-containing serine/threonine kinase AKT to the plasma membrane. AKT is then able to inhibit apoptosis through inactivation of BCL-2-associated agonist of cell death (BAD) and activation of the E3 ubiquitin-ligase MDM2, which mediates degradation of the pro-apoptotic protein p53. Moreover, AKT positively regulates the transcriptional activity of the pro-survival factor nuclear factor-κB (NF-κB) by inducing phosphorylation and subsequent degradation of the inhibitor of κB (IκB). AKT also controls protein synthesis through phosphorylation of S6 kinase (S6K) and, as recently described[79], DNA repair through phosphorylation of Aurora kinase and the ensuing activation of the ataxia telangiectasia mutated (ATM) kinase, which plays a critical role in the maintenance of genomic stability. Another consequence of PI3K activity is increased cell migration through membrane compartmentalization of the guanine nucleotide exchange factor T

lymphoma invasion and metastasis-inducing protein 1 (TIAM1). This event leads to activation of RAC, a small GTPase involved in the directional motility of cells[185].

The docking of STAT3 to the carboxy-terminal tail of MET is followed by MET-dependent STAT3 tyrosine phosphorylation, which results in STAT3 dissociation from the receptor and homodimerization with other STAT3 molecules. STAT3 dimers translocate to the nucleus, where they transcriptionally regulate the expression of genes that are implicated in cell proliferation or differentiation[174].



DDR, DNA damage response; EGFR, epidermal growth factor receptor; IGF1R, insulin-like growth factor 1 receptor; p85, PI3K regulatory subunit; RTK, receptor tyrosine kinase.

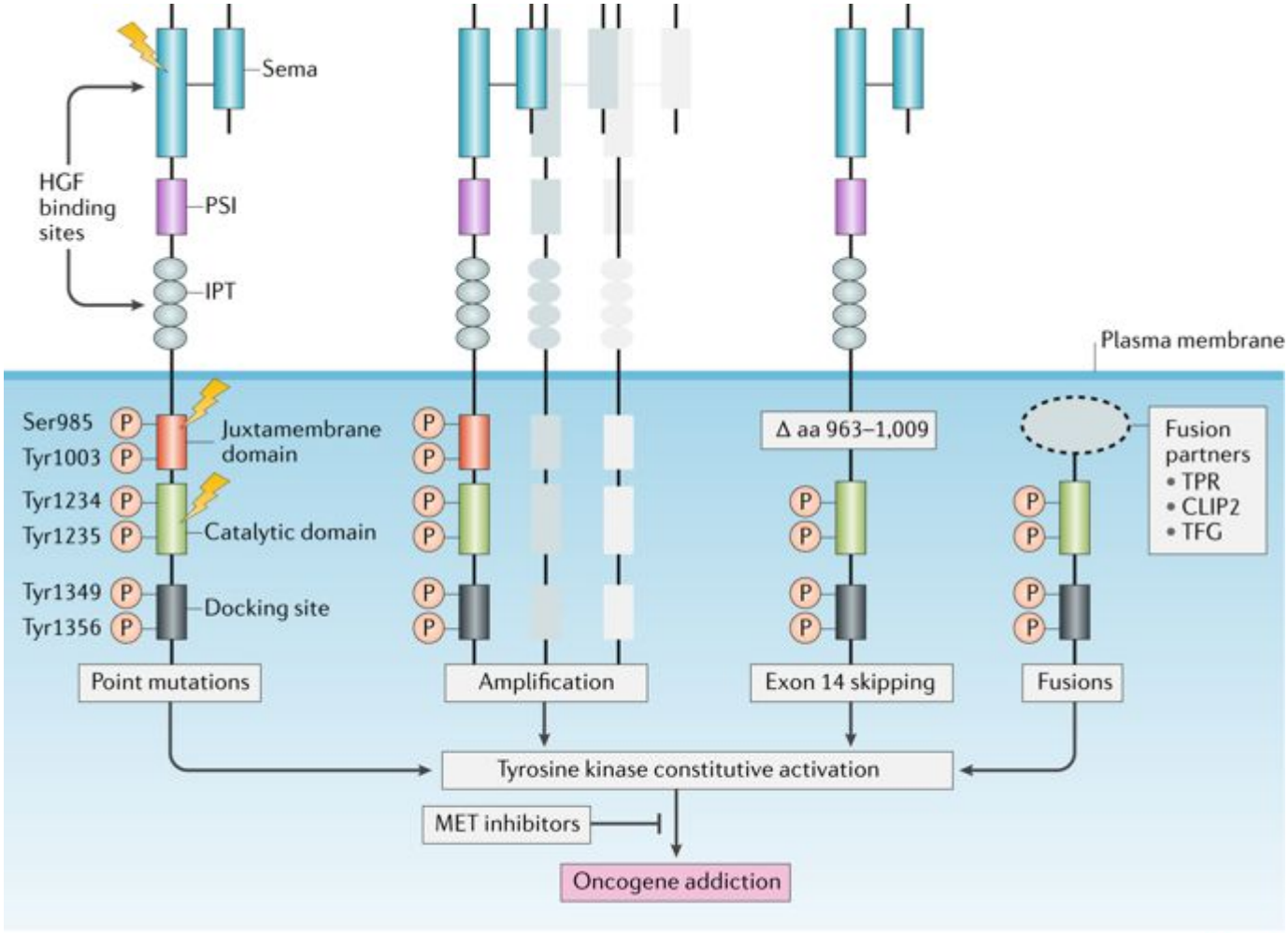
MET oncogene addiction

Established paradigms of MET genetic alterations

MET amplification in gastric cancer and MET-activating point mutations in renal cell carcinoma, either germline or sporadic, were the first MET genetic lesions identified in humans. Such alterations were later found to occur in most solid tumours with an overall frequency of 1–4% (Fig. 1; Supplementary Table 1) (reviewed in ref.15). Some cancer types appear to be more prone to acquiring MET genetic alterations, including cancer of unknown primary site (CUP), which often harbour MET mutations (Box 2), and melanomas, in which a remarkable frequency of MET amplification (12%; n = 183) has been identified by whole-genome analysis[23]. The MET protein structural changes or overexpression that arise from these genetic alterations result in constitutive activation of MET kinase, which becomes independent of, or hypersensitive to, ligand stimulation[24,25]. This bestows MET with persistent

signalling, a cell-autonomous mechanism of cell proliferation responsible for oncogene addiction. Targeting of constitutively active MET typically results in inhibition of tumour growth in experimental models and in patients[15,16] (Fig. 1).

Fig. 1: MET structural alterations and oncogene addiction.



The main structural domains and aminoacidic residues involved in MET functional regulation through phosphorylation (P) are indicated. The hepatocyte growth factor (HGF) binding site is complex. Structural analysis identified the semaphorin (sema) domain[200,201], and ligand-binding and functional studies revealed the presence of additional sites in the immunoglobulin-like, plexins, transcription factors (IPT) domains[202,203,204]. Point mutations (blizzard symbol; detailed in Supplementary Table 1) are concentrated in domains critical for ligand binding (sema) or receptor signalling (juxtamembrane and catalytic). Newly discovered MET gene alterations include mutations in exon 14 splicing sites (Supplementary Table 1), which cause exon skipping and deletion of the entire juxtamembrane amino acid sequence (Δ aa 963–1,009) as well as oncogenic fusions. Among the latter, the prototype is translocated promoter region (TPR)–MET205. This construct and the recently discovered CAP-Gly domain-containing linker protein 2 (CLIP2)–MET (1,235 amino acids) and TRK-fused gene (TFG)–MET (574 amino acids) constructs contain MET exons 15–21, that is, the entire sequence downstream of the juxtamembrane domain. This is fused at its amino terminus either with exons 1–12 from CLIP2 or with exons 1–4 from TFG. Other fusion proteins featuring the entire MET sequence fused at its amino terminus with various fragments of the protein-tyrosine phosphatase receptor type Z polypeptide 1 (PTPRZ1) protein57 are not shown. PSI, plexin–semaphorin–integrin domain.

In recent years, evidence of tumour genetic heterogeneity has highlighted that cancer cells often harbour oncogenic drivers of cell proliferation in a mutually exclusive fashion. A classic example is glioblastoma, where epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and MET are amplified in different

cells, which remain intermixed in some areas of the tumour, while, in other areas, they form more abundant homogeneous subclones[26]. This has several important consequences for therapeutic intervention: first, owing to the limited size of tissue samples usually available for tumour genetic analysis (sampling bias), the frequency of alterations of MET (or other oncogenes) can be underestimated across cohorts of patients and individual patients with MET alterations can be missed; and second, MET alterations can represent an acquired mechanism of resistance against inhibitors of other oncogenic drivers such as EGFR. This was originally observed in cases of EGFR-mutated non-small-cell lung cancers (NSCLCs; 15% of tumours; n = 27), where EGFR blockade eradicated EGFR-mutated subclones but positively selected for those harbouring MET amplification[27]. Amplification leads to kinase overexpression and constitutive activation, with the ensuing propagation of downstream survival signals that substitute for those of EGFR and impose resistance to EGFR inactivation (reviewed in ref.28). The MET-amplified subclones can pre-exist in the original tumour population because of genetic heterogeneity[27] but can also be induced de novo by treatment in a stochastic fashion owing to inherent genomic instability[29]. Selection of MET amplification can drive resistance not only in NSCLC but also in colorectal cancer treated with EGFR antibodies[30,31] or BRAF small molecule inhibitors[32]. Preclinical in vitro and in vivo studies have demonstrated that pharmacological targeting of MET in this specific genetic context reverts resistance and restores sensitivity to EGFR inhibition[30,32]. Vice versa, the use of MET inhibitors in experimental models[33,34] and in patients with gastro-oesophageal cancer[35] or NSCLC[36] has provided the first evidence for mechanisms of acquired resistance to such inhibitors. Among these mechanisms are RAS mutation[35], amplification of HER2 (also known as ERBB2)[35] and the MET^{D1228V} mutation[36].

Box 2 MET mutations in cancers of unknown primary: oncogenic drivers or metastasis promoters?

Cancer of unknown primary site (CUP) are disseminated tumours whose origin is undetermined to the best diagnostic standard: full body imaging cannot identify a site harbouring a presumptive primary mass, and exhaustive immunohistochemical analysis, although able to recognize evidence of epithelial differentiation in about 80% of cases, cannot detect markers that associate the tumour with a specific organ. Thus, the prominent biological features are an early metastatic activity, possibly arising together with cancer transformation itself, and abortive differentiation[186]. These tumours should offer an opportunity to understand the long-sought — but still largely mysterious — genetic determinants of metastasis and the relationship between persistence in undifferentiated states, which is distinctive of stem and/or progenitor cells, and the propensity to disseminate from original locations and grow in ectopic sites.

In pioneering experimental in vivo models, MET was found to be associated with metastatic ability, either as result of activating mutations[187] or autocrine signalling loops[188,189,190,191]. In human CUP biopsy samples, an increased frequency of MET mutations was unexpectedly found in the extracellular domain[192] (Fig. 1; Supplementary Table 1). Some of these mutations have been shown to confer transforming ability; however, the inferred molecular mechanism is not as obvious as in the case of intracellular mutations. Predicting the possible functional consequence of these mutations, which fall in the semaphorin (sema) domain[193], one can hypothesize that they affect residues critical for ligand–receptor interaction. Interestingly, mutations in the extracellular domain (in particular E168D, which is otherwise very rare in most cancer tissues) recur in brain metastasis not only from CUP[192] but also from metastatic lung cancer[194], raising the fascinating possibility that this mutation can confer a selective advantage for migrating to and thriving in the brain, a tissue producing abundant hepatocyte growth factor (HGF)[195].

Novel concepts of MET gene alterations

In demonstrating the oncogenic activity of MET mutants, emphasis was laid on the ability of mutations to upregulate kinase activity. This property was attributed to both amplified receptors and mutations in the catalytic domain (Fig. 1). Recently, renewed attention has been paid to MET genetic alterations that involve the loss of a mechanism of kinase downregulation, which is associated with the juxtamembrane region of the receptor. This region contains a serine residue (Ser985) that becomes phosphorylated by protein kinase C (PKC) and contributes to terminating the

kinase activity of MET[37,38] and a tyrosine residue (Tyr1003) that associates with the E3 ubiquitin-ligase CBL, which is required for MET internalization and degradation39,40. Mutation of Tyr1003 prevents receptor downregulation and results in oncogenic activation[39,41]. In lung cancer, increased tumorigenicity and cell motility were also associated with the expression of MET variants harbouring other point mutations in the juxtamembrane domain[42] (Fig. 1; Supplementary Table 1).

The juxtamembrane region, encoded by exon 14, can be alternatively spliced, generating MET variants detectable in normal tissues[43]. Recently, mutations in exon 14 splicing sites, which lead to 'exon skipping' and permanent deletion of the juxtamembrane region from the MET transcript, have been described in various solid tumours[44](Fig. 1; Supplementary Table 1). Exon 14 deletion was originally discovered in lung cancers, and tumour cells expressing this variant were shown to display canonical oncogenic ability[45,46]. Consistent with this, insertion of exon 14 into the oncogenic gene fusion translocated promoter region (TPR)–MET, which consists of the MET sequence downstream from the juxtamembrane domain fused to the dimerization motif of TPR, resulted in decreased oncogenic potential[47]. Recent extensive analyses reported a significant frequency of MET exon 14 deletions in NSCLCs[44,48,49,50,51,52]. This genetic alteration is mostly associated with lung adenocarcinomas (3%; n = 687)50, lung adenosquamous carcinomas (5%; n = 687)[50] and, remarkably, with the rare but highly aggressive and chemoresistant lung sarcomatoid tumours (32% in one study (n = 22)50 and 22% in another study (n = 36)[51]). Importantly, lung adenocarcinomas with MET exon 14 deletion displayed substantial clinical response to MET inhibition[44,52]. Owing to its relative frequency and it being an actionable genomic event, the MET exon 14 deletion can be considered a major oncogenic target in NSCLC, together with EGFR and anaplastic lymphoma kinase (ALK) genetic alterations[50]. Other than lung cancer, tumours displaying a significant frequency of MET exon 14 deletions are gastrointestinal carcinomas[53], gliomas, sarcomas and CUP[44].

For decades, the only known MET gene rearrangement in human tumours was TPR–MET, which mostly occurs in gastric cancer[54]. Recently, the advent of RNA sequencing (RNA-seq) analysis, applied to the comprehensive tumour collection (7,000 samples) of The Cancer Genome Atlas (TCGA), uncovered new fusion proteins encompassing the MET intracellular domain fused at its amino terminus with a dimerization motif that potentially give rise to oncogenic variants55. These fusions occurring at low frequencies, as those of other receptor tyrosine kinases, were found in lung adenocarcinoma (1 out of 513 patients), hepatocellular carcinoma (1 out of 194 patients), papillary renal carcinoma (2 out of 198 patients) and thyroid carcinoma (1 out of 498 patients)[55]. A new type of MET gene rearrangement (protein-tyrosine phosphatase receptor type Z polypeptide 1 (PTPRZ1)–MET), including the entire MET sequence fused at its amino terminus with a variable number of exons from the PTPRZ1 gene, encoding a tyrosine phosphatase, was found in brain tumours such as low-grade gliomas[55], secondary glioblastomas arising in adults from progression of lower-grade gliomas (15%; n = 40)[56] and paediatric glioblastomas[57]. In the latter, MET gene rearrangements occur at a remarkably high frequency (10%; n = 53) and, besides PTPRZ1–MET, generate other fusion proteins encompassing the MET intracellular domain downstream from the juxtamembrane region fused either with CAP-Gly domain-containing linker protein 2 (CLIP2) or TRK-fused gene (TFG)57 (Fig. 1). Such fusion proteins are oncogenic and actionable in experimental models and in patients[57]. Overall, gliomas display a relatively high frequency of METgenetic alterations: besides the aforementioned lesions, approximately 2% of glioblastomas (n = 251) exhibit MET amplification[58] and another 6% of patients with high-grade gliomas (grade III gliomas and glioblastomas; n = 102) have tumours with a deletion of exons 7 and 8 encoding the extracellular domains of MET, resulting in a constitutively active tyrosine kinase located in the cytosol[59].

MET as a survival expedient

The MET gene promoter contains binding sites for several transcription factors60, which, among others, include activator protein 1 (AP-1)61, SP1 (ref.62), PAX3 (ref.63), ETS1 (ref.64,65), Y-box-binding protein 1 (YB1; also known as YBX1)[66], hypoxia-inducible factor 1α (HIF1α)18 and nuclear factor-κB (NF-κB)[67]. All of these transcriptional regulators are induced or activated by manifold upstream cues, including aberrant oncogenic signalling, adverse environmental contexts and mutagenic agents. Importantly, many of the stimuli that induce MET transcription in cancer cells also induce HGF upregulation in the tumour stroma[67,68,69], generating a

feedforward stimulatory circuit that increases MET activation. As MET mostly conveys anti-apoptotic and migratory signals, its transcriptional upregulation is utilized by cancer cells as a safeguard mechanism to escape such intrinsic or extrinsic insults. We call this biological situation, whereby regulated overexpression of an oncogene product is leveraged to bypass selective barriers along the evolutionary trajectory of the tumour (including under therapy), oncogene expedience[15]. This scenario differs from oncogene addiction in that it is not sustained by genetically based activation of the oncogene — the oncoprotein activity is regulated by expression, not by mutation — and in that it drives tumour progression rather than cancer initiation.

MET expedience and response to anticancer therapies

One condition that induces MET transcription is low oxygen tension (hypoxia)[18], a frequent occurrence in tumours due to irregular vascularization and high interstitial pressure. By pruning or disrupting tumour blood vessels, anti-angiogenic therapy by antibody-mediated or small molecule-mediated blockade of vascular endothelial growth factor (VEGF)-dependent signals exacerbates tumour hypoxia, leading to MET overexpression, constitutive kinase activation and the ensuing onset of MET-dependent invasive growth[70] by cancer cells (Fig. 2a). This microenvironmental regulation of MET expression might explain why inhibitors of tumour angiogenesis, while effective on the growth of the primary tumour, precipitate local invasion and distant metastases in some preclinical models[71,72]. Accordingly, concurrent MET targeting mitigates tumour aggressiveness due to anti-VEGF therapy in pancreatic neuroendocrine tumours and pancreatic carcinoma[73]. Although the mechanistic connection between hypoxia, MET upregulation and cancer cell invasion is well established[15], data on the causal involvement of VEGF blockade in supporting cancer cell dissemination vary according to the pharmacological characteristics, dosage and schedule of the anti-angiogenic agents used[74,75], which makes results hard to generalize. It is also worth noting that the detrimental effects of anti-angiogenic agents have been documented in mouse models treated with monotherapies[71,72]. This is in contrast to clinical practice, in which anti-angiogenic therapies are not administered as single agents but are administered together with cytotoxic chemotherapy[76]. In patients, the combination of VEGF antibodies and standard chemotherapy has increased the progression-free survival (PFS) of many individuals but has had little effect on overall survival (OS)[77]. Whether this lack of ultimate benefit is due to MET-mediated evasive resistance or to other forms of drug adaptation remains to be determined. In addition, MET can contribute to the pro-invasive outcome of VEGF blockade through its direct association with VEGF receptor 2 (VEGFR2); in glioblastoma, VEGF inhibition impairs the recruitment of protein-tyrosine phosphatase 1B (PTP1B), a MET-inhibitory phosphatase, to the VEGFR2–MET complex, resulting in increased MET activation and MET-dependent local tumour invasion[78].

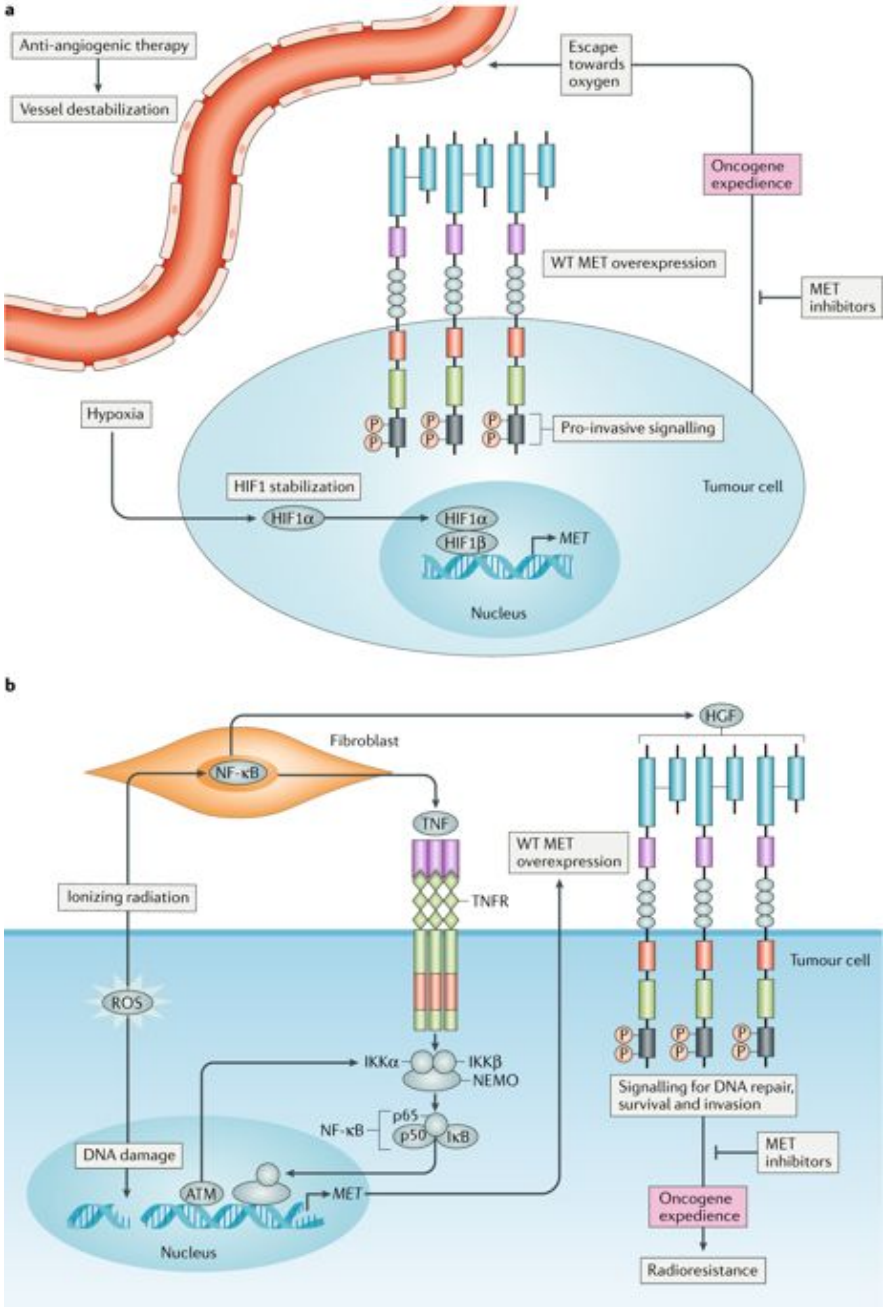


Fig. 2: MET oncogene expedience.

a | Anti-angiogenic therapy promotes tumour invasion via MET transcriptional upregulation. Anti-angiogenic agents cause tumour vessel destabilization and decreased tumour oxygenation. Hypoxia prevents proteasomal degradation of hypoxia-inducible factor 1α (HIF1α), which, together with its transcription partner HIF1β, upregulates MET expression. This is followed by activation of pro-invasive signalling that fosters cancer cell escape towards oxygen via intravasation and dissemination. Concomitant administration of MET inhibitors can prevent pro-metastatic activities often fostered by anti-angiogenic agents. b | Radiotherapy promotes MET transcriptional upregulation and radioresistance. Ionizing radiation in the therapeutic range (5–10 Gy) induces DNA damage detected by protein complexes including the ataxia telangiectasia mutated (ATM) kinase, which orchestrates the DNA damage response and, among other effectors, activates transcription factor nuclear factor-κB (NF-κB)[67] via the inhibitor of κB (IκB) kinase (IKK) complex. NF-κB, in turn, upregulates transcription of a panel of genes including MET, which becomes overexpressed at the cell surface, is hypersensitive to hepatocyte growth factor (HGF) and/or is activated in the absence of the ligand67. In irradiated cancer tissues, NF-κB signalling and the ensuing MET upregulation are further enhanced by tumour necrosis factor (TNF)[69], HGF[67] and, possibly, other factors206 secreted by tumour-associated fibroblasts. NEMO, NF-κB essential modulator; P, phosphorylation; ROS, reactive oxygen species; TNFR, TNF receptor; WT, wild-type.

MET is also transcriptionally induced by therapeutic doses of ionizing radiation through a signalling pathway that involves the ataxia telangiectasia mutated (ATM) kinase, which detects DNA double-strand breaks, and the transcription factor NF-κB[67]. MET upregulation orchestrates an adaptive response to radiation-induced damage by concomitantly promoting tumour cell invasion, escape from apoptosis and AKT-mediated and ATM-mediated activation of DNA repair mechanisms[79] (Fig. 2b). As a result, MET inhibition in glioblastoma mouse models increases the therapeutic efficacy of radiotherapy[79] (see below for more details). Besides ATM, another enzyme critically implicated in double-strand DNA repair is poly(ADP-ribose) polymerase 1 (PARP1). In breast cancer, MET has been shown to phosphorylate PARP1 (ref.80). This event increases PARP1 enzymatic activity and reduces its binding affinity to small molecule inhibitors, thereby impairing responsiveness to PARP pharmacological blockade80. Accordingly, the combination of MET and PARP inhibitors has synergistic activity in suppressing mammary tumour growth in vitro and in xenograft models[80].

The crosstalk between stromal HGF and MET-expressing cancer cells

The tumour microenvironment (TME) is composed of stromal cells that sustain cancer growth and progression through paracrine and autocrine signals[81]. Tumour-associated stromal cells include components of haematopoietic origin, which leave the circulation and home to tumours in response to chemoattractants, and resident cells, which pre-exist in the tissue in which the tumour develops. Cells that infiltrate tumours after extravasation from blood vessels comprise myeloid cells and lymphocytes. Among myeloid cells, mononuclear phagocytes derived from circulating monocytes (macrophages and dendritic cells (DCs)) and polymorphonuclear cells called neutrophils are particularly prominent in the TME. Tissue-resident cells are components of normal connective tissues and change their properties and morphology under the influence of cancer and myeloid cells; they include vascular cells (endothelial cells and pericytes), fibroblasts and resident leukocytes (macrophages, mast cells and DCs). HGF is typically secreted by cancer-associated fibroblasts (CAFs) and acts in a paracrine fashion on MET expressed by adjacent epithelial cancer cells[1,20]. HGF is also produced by neutrophils[82] and macrophages[83].

Activation of WT MET leads to drug resistance not only as a consequence of receptor overexpression but also following paracrine secretion of HGF by cells of the tumour-reactive stroma[84,85,86] (Fig. 3). Independent experimental approaches, including co-cultures of cancer and stromal cell lines[84] and drug screens of cancer cell lines in the presence of exogenously administered[85] or ectopically transfected[86]growth factors, all identified HGF as a prevalent survival factor that exerts protective activity against targeted therapies in several tumour contexts, in particular, in EGFR-mutated and ALK-translocated NSCLC and in BRAF-mutated melanoma[84,85,86,87]. Intriguingly, HGF also reduces sensitivity to MET small molecule inhibitors in MET-amplified cancer cell lines and patient-derived xenografts (PDXs), and preliminary evidence indicates that an HGF-neutralizing antibody can restore full responsiveness to MET blockade by kinase inhibitors[88]. At the clinical level, high expression of stromal HGF, as detected in biopsy specimens[84], or elevated HGF plasma concentrations[85] seem to correlate with weaker responses to BRAF inhibitors in patients with BRAF-mutated melanoma. However, it is difficult to establish whether the association between high HGF levels and poor outcome can be convincingly ascribed to HGF-induced drug resistance or, rather, to the inherent aggressiveness of HGF-overexpressing tumours, which could negatively affect prognosis irrespective of therapy.

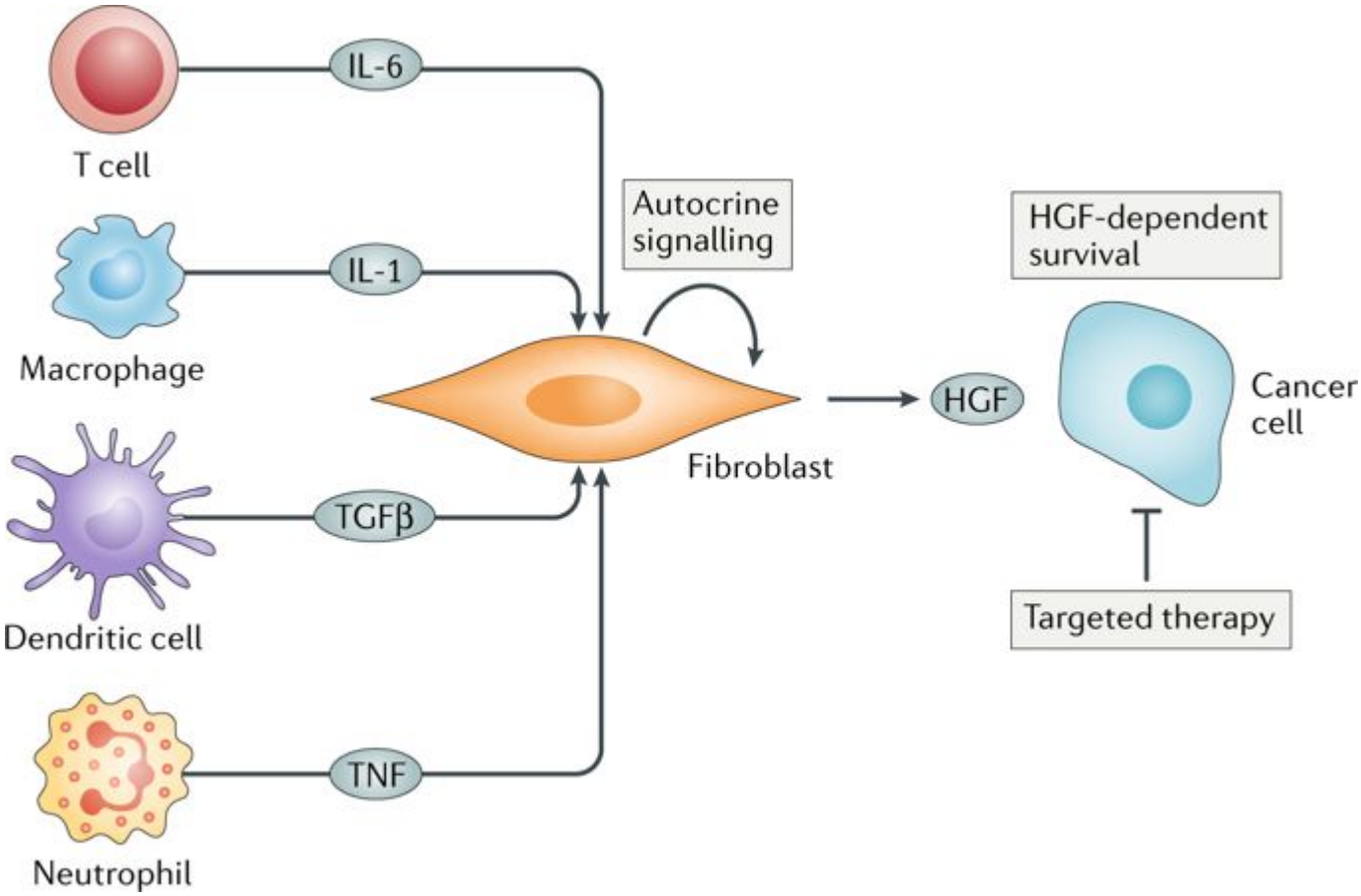


Fig. 3: Stromal HGF protects cancer cells from targeted therapy.

Autocrine and paracrine signalling of stromal cells leads to abundant production of hepatocyte growth factor (HGF) in the tumour microenvironment. HGF is transcriptionally induced in cancer-associated fibroblasts and macrophages by inflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF)[68,69], as well as pro-invasive and pro-angiogenic growth factors such as transforming growth factor-β (TGFβ)11. IL-1, IL-6 and TGFβ are primarily secreted by fibroblasts[21] and macrophages[207]. IL-6 is also produced by T cells[208], and TGFβ is also produced by dendritic cells[209]. The primary source of TNF is neutrophils[210]. Stromal HGF conveys survival signals to cancer cells, rendering them less susceptible to therapy-induced apoptosis. This protective activity has been primarily documented in anaplastic lymphoma kinase (ALK)-translocated non-small-cell lung cancer and BRAF-mutant melanoma[84,85,86].

Similar to MET expression in cancer cells, HGF can also be transcriptionally induced in stromal cells by microenvironmental cues such as pro-angiogenic growth factors and inflammatory cytokines[20,68,89]. Moreover, the tumour stroma is replete with proteases capable of converting single-chain, inactive pro-HGF into a two-chain moiety capable of MET activation[90]. This massive bioavailability of active ligand in the interstitial compartment, coupled with increased expression of the active receptor in cancer cells, could be a general means by which tumours can restrain the effectiveness of targeted therapies (Fig. 3). Such an adaptive mechanism is expected to be particularly prominent in tumours that display hypoxic and/or inflammatory features.

The crosstalk between HGF and MET in the tumour stroma

Crosstalk between HGF-producing and MET-expressing cells occurs not only between stromal and epithelial components in TMEs but also among stromal cells, in particular, tumour-associated macrophages (TAMs) and neutrophils. Under basal conditions, these myeloid cells have barely detectable levels of MET[91]. However, MET expression can be induced (or increased) by inflammatory stimuli, leading to autocrine feedforward loops with

functional consequences[92]. For example, differentiation of monocytes into macrophages is accompanied by METtranscriptional upregulation and induction of cell-autonomous pro-HGF convertase activity[83]. In situations of chronic inflammation, such as renal fibrosis[93] and multiple sclerosis[94], this HGF–MET autocrine signalling plays a key role in attenuating inflammation and supporting tissue repair[95], thus shifting macrophage polarization from an immunologically active phenotype (M1) to a trophic, growth-stimulating state (M2). In tumours, this functional skewing results in tumour-promoting activities such as the creation of an immunosuppressive microenvironment, increased angiogenesis and increased secretion of survival factors and matrix metalloproteinases (MMPs)[96]. Moreover, HGF stimulates TAMs, CAFs and cancer cells to produce stromal cell-derived factor 1 (SDF1; also known as CXCL12)[97,98,99], a chemokine that boosts the recruitment of circulating leukocytes, including monocytes, into the tumour stroma[100]. These waves of infiltrating macrophages, in addition to resident TAMs, further increase the local abundance of HGF.

Ligand-dependent activation of MET in DCs potentiates their migration into the draining lymph nodes in inflamed skin[101]. However, increased DC representation in lymphatic tissues could blunt, rather than exacerbate, T cell-mediated immune responses: in a mouse model of allergic airway inflammation, hydrodynamic delivery of an HGF-expressing vector reduced disease severity by impairing the antigen-presenting ability of lung DCs and the ensuing activation of T cells[102]. Mature DCs that engage T cells in a non-inflammatory setting promote peripheral tolerance through activation of regulatory T cells or induction of anergy in responder cells[103]. Congruent with the observation that it might exert immunosuppressive functions, HGF has been demonstrated to promote tolerogenic DCs in models of organ allograft rejection[104] and experimental autoimmunity[105,106]. Mechanistically, the tolerogenic properties of HGF could rely on SRC-dependent and PI3K-dependent inhibition of NF-κB, a key transcription factor for the induction of inflammatory molecules[107,108]. Several tumour-derived cytokines contribute to the generation of tolerogenic DCs[109]. The possibility that deregulated HGF production or activation within the TME stimulates tumour growth by fostering DC tolerogenic potential is reasonable and deserves investigation.

In the tumour stroma, the pro-tumorigenic function of MET in TAMs could be counteracted by neutrophils, in which MET signalling appears to prompt tumoricidal activities[110]. Endothelial cell-derived and cancer cell-derived tumour necrosis factor (TNF) upregulates MET expression in neutrophils; this induction facilitates neutrophil transmigration across the tumour-activated endothelium and is permissive for HGF-dependent production of nitric oxide synthase (NOS) and the ensuing release of nitric oxide, which kills cancer cells[110]. These findings suggest that the therapeutic benefit of MET blockade in cancer cells can be counterbalanced by the pro-tumorigenic outcomes evoked by MET inhibition in neutrophils[110]. This antitumoural function of MET in neutrophils may be confined to incipient tumours; indeed, production of cytotoxic nitric oxide was shown to decay at later stages of tumour development, and in overt tumours, depletion of neutrophils inhibited, rather than favoured, tumour growth[111]. Notably, recent evidence indicates that neutrophils acquire immunosuppressive properties once recruited to T cell-inflamed TMEs[112]; accordingly, by hampering the reactive mobilization of immunosuppressive neutrophils into tumours, MET inhibitors have been shown to increase the efficacy of cancer immunotherapies in mouse cancer models[112].

MET expression in myeloid cells can also be adoptively acquired from tumour-derived exosomes[113]. In particular, a MET cargo delivered from melanoma-derived exosomes was found to reprogramme bone marrow-derived cells (BMDCs) towards a pro-vasculogenic and pro-metastatic phenotype, which favoured cancer cell seeding and survival at secondary sites[114].

MET oncogene inherence

MET overexpression in tumours can result from not only upregulated transcription in single cells but also relative expansion of cells that express MET over those that do not. In tumours, an expansion of cells with stem and/or progenitor cell features, at the expense of differentiated cells, is observed, which reverses the ratio between stem and/or progenitor cells and differentiated cells that is typical of normal tissues[3]. As previously discussed, in normal tissues, MET is expressed in stem and/or progenitor cells rather than in cells with differentiated features; therefore, its widespread expression in some tumours can be a marker of expansion of cells with stem and/or progenitor

features, the so-called CSCs[3]. In these cells, which often derive from direct transformation of normal stem and/or progenitor cells[115], MET can foster stem-like functions essential for tumour initiation, propagation, regeneration and dissemination, irrespective of its oncogenic activation (addiction) or pro-survival activity (expedience). We define the ability of MET to promote the CSC phenotype by sustaining properties inherent in the stem and/or progenitor cell of origin as a paradigm of 'inherence' (Fig. 4).

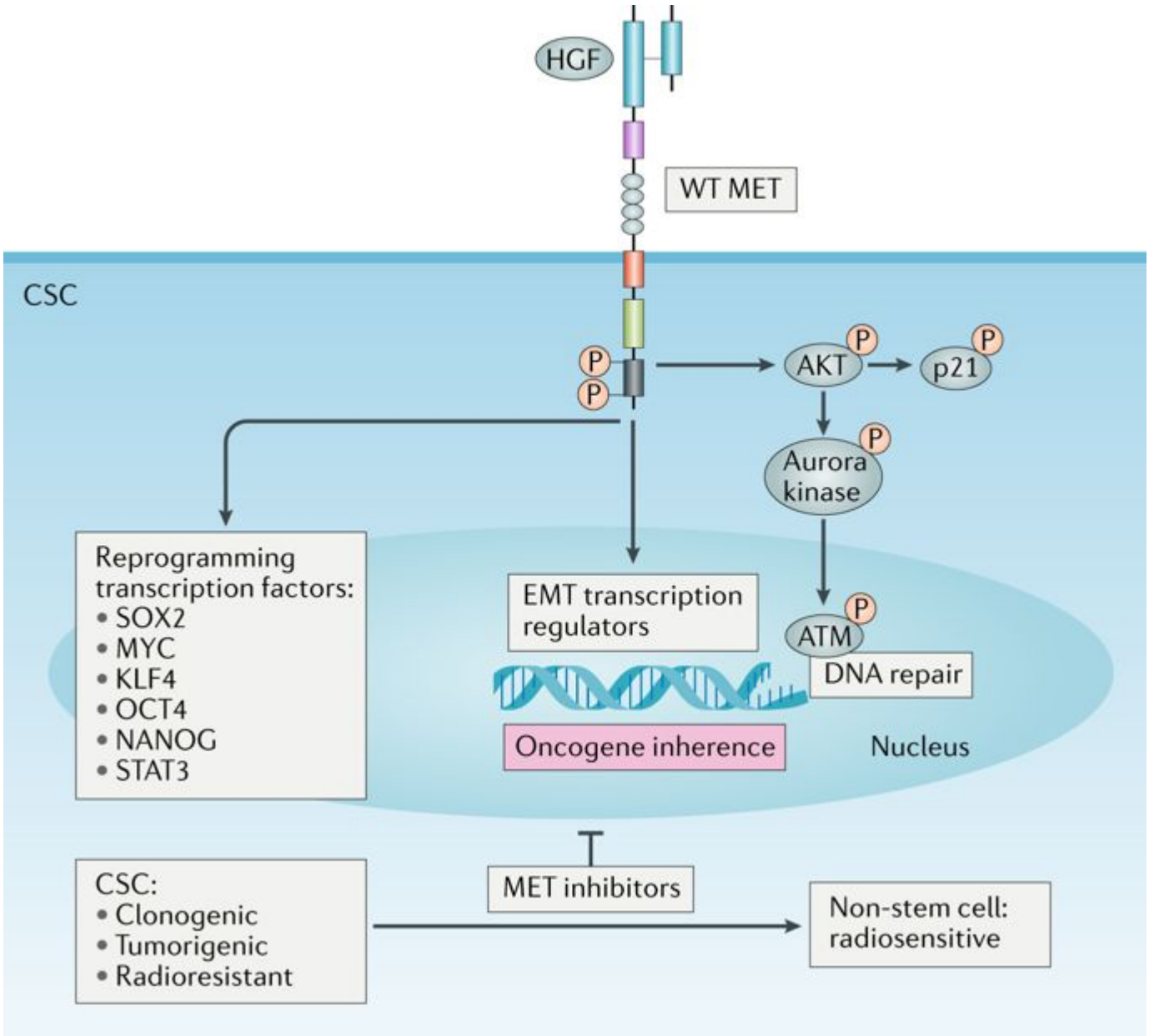


Fig. 4: The MET oncogene in cancer stem cells: a paradigm of inherence

Wild-type (WT) MET signalling drives genetic programmes essential for the cancer stem cell (CSC) phenotype, which are sustained by reprogramming transcription factors and transcriptional regulators of epithelial-to-mesenchymal transition (EMT). In addition, MET fosters radioresistance by initiating signalling pathways leading to increased DNA repair and survival, a property inherent in glioblastoma stem cells and other CSCs. MET inhibition blocks these pathways, turning the radioresistance of CSCs into radiosensitivity. ATM, ataxia telangiectasia mutated; HGF, hepatocyte growth factor; KLF4, Krüppel-like factor 4; OCT4, octamer-binding protein 4 (also known as POU5F1); P, phosphorylation; SOX2, SRY-box 2; STAT3, signal transducer and activator of transcription 3.

In recent years, the inherent role of MET as a functional marker of normal and CSCs has been informed by findings in normal liver[116,117], mammary gland[118] and nervous tissues[119], gut epithelium[120] and tumours of the breast[118], colon[121], pancreas[122] and brain[79,123,124,125,126]. A further, although more indirect, example of inherence involves acute myeloid leukaemia (AML) blasts, which are cells whose phenotype reflects an early differentiation block downstream from the leukaemic stem cell. These cells strongly depend on WT MET activation by autocrine HGF and can be effectively targeted by MET inhibitors[127].

MET as a stem cell marker in the liver

Seminal studies showed that HGF, later identified as the MET ligand, was the most potent mitogen for cultured adult rodent hepatocytes[128], which, unlike most terminally differentiated cells in other tissues, retain the unique ability to mediate tissue regeneration[129]. Consistently, genetic ablation of Hgf in the mouse prevented liver development[130]. Later, MET was used in combination with other surface markers to isolate multipotent stem cells from the developing liver[131]. In the adult, MET is expressed by so-called oval cells, bipotent stem and/or progenitor cells residing in bile ducts that are capable of differentiating into either hepatocytes or biliary epithelium after reactivation in cases of extensive liver injury[129,132]. MET activity was found to be essential for mouse oval cell function during liver regeneration[116]. MET cooperates with EGFR in sustaining oval cell self-renewal and promotes commitment towards the hepatocyte lineage via AKT and signal transducer and activator of transcription 3 (STAT3) activation, while EGFR promotes biliary differentiation through NOTCH1 (ref.117). Interestingly, several murine models of chemical carcinogenesis indicate that oval cells expand in precancerous lesions, thereby generating the cell subpopulation that eventually progress into hepatocellular carcinomas and cholangiocarcinomas[132].

MET as a stem cell marker in the mammary gland and breast cancer

High levels of MET expression were measured in luminal progenitors, descendants of multipotent mammary stem cells committed towards ductal and secretory cell differentiation[118,133]. HGF stimulation was shown to expand and redirect these progenitors towards the stem cell state by increasing their clonogenic activity in vitro and their ability to reconstitute the mammary gland in vivo[118]. Interestingly, increased levels of MET expression and stem-like features are typical of basal-like breast cancer[134,135], which is thought to originate from luminal progenitors[136]. Association of MET expression with the cell of origin of breast cancer — which likely undergoes transformation into a CSC — is especially interesting in view of the well-known notion that in the mammary epithelium, activation of the EMT programme is a key mechanism of conversion into the stem cell state[137,138] (Fig. 4). Thus, breast cancer offers an example of MET inherence, where MET is a functional marker of the cell of origin, and its expression and signalling may be necessary to complement transformation by other oncogenes. Nonetheless, mutated MET may be in some instances a driver, as mouse models have shown that targeting the mutated oncogene to the mammary epithelium induces tumours with basal-like features[134].

MET as a stem cell marker in colorectal cancer

In colorectal cancers, CSCs were isolated in vitro as ‘colospheres’ (refs[121,139,140]). Here, MET is often expressed at high levels[121] in the absence of mutations or amplification, reflecting the expansion of a stem and/or progenitor population transformed by accumulation of different genetic lesions such as adenomatous polyposis coli (APC) loss and RAS mutations[3]. In this tumour type, the inherent role of MET signalling is supported by several observations. In colon adenocarcinoma tissues, HGF secreted by stromal myofibroblasts has been shown to activate the WNT self-renewal pathway and to replace EGF in sustaining CSC long-term propagation[141]. In mouse models of colorectal cancer, MET inhibition has been shown to increase the efficacy of the EGFR antibodies approved for treatment of metastatic tumours that lack activating mutations of the RAS pathway[121]. Interestingly, colorectal cancers with an intact RAS pathway often do not harbour obvious oncogenic drivers31 and yield CSCs that tightly depend on EGF, fibroblast growth factor (FGF) and HGF for their proliferation and survival, attesting to the importance of these growth factors as inherent regulators of CSC properties[142].

These findings are corroborated by a recent analysis of MET contribution to gut epithelium homeostasis, regeneration and adenoma formation in mouse models[120]. It was found that leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)-expressing intestinal stem cells form fully differentiation-competent mouse organoids in the presence of HGF as efficiently as they do in the presence of EGF[120], a factor so far considered essential for stem cell functions[143]. Redundancy between the two growth factors was evident in epithelial homeostasis in vivo, but lack of MET after genetic inactivation could not be fully compensated by EGFR in intestinal regeneration after irradiation[120]. Moreover, MET genetic disruption significantly impaired intestinal adenoma formation in mice with APC inactivation[120]. Interestingly, in intestinal stem cells, MET activity required co-expression of an isoform of CD44, CD44v4, which is a known target of the WNT pathway[144] and a marker of normal stem cells and CSCs in several tissues[145]. In earlier studies, another isoform of CD44, CD44v6, was recognized as a crucial requirement for MET signalling[146] and for invasion and metastasis of human colorectal CSCs after transplantation into the mouse[147]. This evidence suggests that the functional cooperation between MET and CD44 plays a key role in CSC dissemination across the organism, an essential prerequisite to formation of metastases[12].

MET as a stem cell marker in brain tumours

Barely detectable in differentiated nervous tissue, MET is expressed in neural stem cells[119], the prominent candidate for the cell of origin in gliomas, which are the most frequent primary brain tumours[148]. MET expression and function in gliomas are one of the best paradigms of inherence (Fig. 4). Early lineage-tracing experiments in genetically modified mice revealed endogenous MET expression and activity in stem-like cells of brain tumours[126]. Now, MET has been exploited as a cell surface marker to prospectively isolate stem-like cells from human glioblastomas, the most aggressive and frequent form of glioma[125]. Notably, expression of the WT MET gene is preferentially associated with the so-called ‘mesenchymal’ glioblastoma subtype, which owes its name to a gene expression profile reminiscent of stem and mesenchymal cells[149]. Consistently, MET is preferentially expressed in CSCs derived in culture as neurospheres from this subtype, whereas it is rarely expressed in the classical subtype, which is characterized by EGFR amplification and a strong dependence on EGFR signalling[123]. Interestingly, neurospheres, despite culture conditions favouring self-renewal, undergo a limited pseudodifferentiation process, which leads to the emergence of a progeny that tends to exhaust its clonogenic and tumorigenic potential. Retention of clonogenicity and tumorigenicity correlates with high levels of MET expression, whereas pseudodifferentiation and termination of CSC properties correlate with loss of MET expression[123]. In particular, MET can promote retention of glioblastoma CSC properties by activation of reprogramming transcription factors such as MYC[123,124] (Fig. 4). The role of HGF in glioblastoma presents striking similarities with that of transforming growth factor-β (TGFβ) in breast cancer: both growth factors support EMT and, concomitantly, sustain the stem cell state[123,137]. Consistently, TGFβ can also be essential for supporting glioblastoma CSCs by sustaining expression of master regulators of the stem cell state such as inhibitor of DNA binding (ID) transcriptional regulators[150].

MET at the intersection between expedience and inherence

Besides inherent MET expression and function, glioblastoma CSCs display inherent radioresistance. This is associated with the ability to activate the DNA damage response (DDR) owing to constitutive activation of checkpoint kinase 2 (CHK2) and the ATM kinase[151]. In glioblastoma CSCs, MET activity sustains the DDR via a signalling pathway involving a phosphorylation cascade mediated by AKT, ATM and Aurora kinase[79]. Concomitantly, the cell cycle inhibitor p21 is retained in the cytoplasm, where it is known to deploy anti-apoptotic functions[79] (Fig. 4). In preclinical glioblastoma models, MET inhibition was shown to radiosensitize CSCs, abating clonogenicity in vitro and tumorigenic potential in vivo[79]. Therefore, in terms of a therapeutic implication, targeting WT MET may overcome the intrinsic radioresistance of glioblastoma CSCs and help to prevent the so far inevitable recurrence of glioblastomas. It has been claimed that EMT confers radio-chemoresistance[12]. More likely, the above findings suggest that EMT and the mechanistic pathway of radio-chemoresistance are concomitantly regulated by the same upstream drivers, among which the MET oncogene is prominent (Fig. 4).

Targeting MET in the clinic

Several MET-targeting agents, including HGF and MET antibodies, as well as small molecule kinase inhibitors, are currently in early or advanced stages of clinical testing (Table 1; Supplementary Table 2).

Table 1 Recent and ongoing clinical trials with MET-targeting agents

Drug	Number of trials (phase)[a]	Cancer types	Principal outcome	Notes
HGF antibodies				
AMG 102 (rilotumumab)	1 (I), 5 (I/II), 7 (II), 2 (II/III) and 2 (III)	Mixed cancer[b], gastric cancer, glioblastoma, lung cancer, mesothelioma and prostate cancer	A phase III study combining rilotumumab with chemotherapy was terminated early on the basis of an increased number of deaths in the rilotumumab arm versus the placebo[162]	Humanized IgG2 monoclonal antibody
AV-299 (ficlatuzumab)	5 (I), 1 (I/II) and 1 (II)	AML, head and neck cancer, liver cancer and NSCLC	The phase II trial was prematurely terminated owing to a high rate of patient discontinuation[c]	Humanized IgG1 monoclonal antibody[211]
MET antibodies				
MetMab (onartuzumab)	5 (I), 1 (I/II), 7 (II) and 5 (III)	Mixed cancer, breast cancer, colorectal cancer, glioblastoma, $HER2^-$ and MET^+ gastric cancer, HCC and MET^+ NSCLC	One NSCLC phase III trial (NCT01456325) failed owing to inadequate patient selection based on MET expression identified by IHC[155]. Three of the four phase III studies have been completed with results pending, while one is currently active	One-armed monoclonal antibody
LY2875358 (emibetuzumab)	2 (I), 1 (I/II) and 3 (II)	Mixed cancer, gastric cancer and NSCLC	Results pending; recruitment of patients is based on MET expression as identified by IHC[212]	Bivalent monoclonal antibody
ARGX-111	1 (I)	Mixed cancer, gastric cancer, glioblastoma, liver cancer and renal cancer	Results pending	Bivalent monoclonal antibody endowed with the property of activating ADCC[213]

Drug	Number of trials (phase)[a]	Cancer types	Principal outcome	Notes
SAIT301	1 (I)	Mixed cancer	Results pending	Bivalent monoclonal antibody targeting the MET α-chain and exploiting CBL-independent degradation of MET to circumvent the detrimental effects induced by the agonist activities of other bivalent antibodies[214,215]
Multitarget tyrosine kinase inhibitors (small molecules)				
PF02341066 (crizotinib)	38 (I), 5 (I/II), 37 (II), 13 (III), 3 (IV) and case reports	Breast cancer, renal clear cell cancer, glioblastoma, inflammatory myofibroblastic tumours, lymphoma, papillary renal cancers, MET$^+$ gastric adenocarcinoma, MET$^+$ or RON$^+$metastatic urothelial cancer and NSCLC	Substantial antitumour activity in patients with oesophagogastric, lung and glioblastoma tumours and *MET*amplification and/or exon 14 deletion[52,154,169,216,217,218,219]	• Targets: ALK, ROS1 and MET • Approved for the treatment of NSCLC with EML4–ALK in 2011 and NSCLC with CD74–ROS1 in 2016
XL184 (cabozantinib)	19 (I), 3 (I/II), 37 (II), 6 (III), 2 (IV) and case reports	Breast cancer, glioblastoma, HCC, kidney cancer, medullary thyroid cancer, melanoma, NSCLC, ovarian cancer and prostate cancer	Complete response was reported for a patient with *MET* exon 14 deletion[52]. However, the majority of trials failed to show any benefit, likely because patients were not selected for *MET*alterations	• Targets: MET, RET and others. • Approved for treatment of medullary thyroid cancer
GSK1363089 (foretinib)	4 (I), 2 (I/II) and	Mixed cancer, breast cancer, gastric	Foretinib showed no activity in unselected patients with previously	• Targets: MET, RON, AXL, TIE2 and

Drug	Number of trials (phase)[a]	Cancer types	Principal outcome	Notes
	5 (II)	cancer, head and neck cancer, liver cancer, NSCLC and papillary renal cancer	treated metastatic gastric cancer[220]	VEGFR2 • In 2014, product development was terminated, and no other clinical trials have been started
MGCD265 (glesatinib)	5 (I) and 2 (II)	Mixed cancer and NSCLC	Results pending: phase II trial NCT02544633 is the only one that includes *MET* genetic alterations as a biomarker	Targets: MET and AXL
MP470 (amuvatinib)	2 (1) and 1 (II)	Mixed cancer, gastric cancer, glioblastoma, pancreatic cancer and SCLC	Results pending: patients are not selected for *MET* alterations	Targets: MET, RET, FLT3 and PDGFRA
E7050 (golvatinib)	4 (I) and 4 (I/II)	Mixed cancer, gastric cancer, head and neck cancer and HCC	Results pending: patients are not selected for *MET* alterations	Targets: MET and VEGFR2
Specific MET tyrosine kinase inhibitors (small molecules)				
ARQ197 (tivantinib)	21 (I), 4 (I/II), 17 (II) and 4 (III)	Mixed cancer, colorectal cancer, HCC, liver cancer, mesothelioma, NSCLC, stomach cancer and SCLC	Phase II and III trials failed despite reported weak overall survival benefit in patients with high MET expression[156,221,222] One phase III trial, recruiting patients with MET$^+$ HCC (NCT02029157), has remained open since 2013	Tivantinib is a questionable MET inhibitor; the effects observed are likely explained by the taxane-like cytotoxic activity[157,158]
INCB28060 (also known as INC280 and capmatinib)	9 (I), 5 (I/II), 11 (II) and 1 (IV)	Mixed cancer, colorectal cancer, glioblastoma, head and neck cancer, HCC, NSCLC and papillary renal cancer	In phase I and II trials, significant responses were reported in patients with high *MET* amplification and/or *MET* exon 14 deletion[44,223,224]	One phase IV rollover trial (NCT03040973) to assess long-term follow-up of MET-dependent tumours started in May 2017
AZD6094 (also known as	6 (I), 2 (I/II), 3	Mixed cancer, colorectal cancer,	Results pending	NA

Drug	Number of trials (phase)[a]	Cancer types	Principal outcome	Notes
HMPL-504, HMP-504, savolitinib or volitinib)	(II) and 1 (III)	gastric cancer, kidney cancer, NSCLC and papillary renal cancer		
AMG337	1 (I), 2 (I/II) and 2 (II)	Mixed cancer, renal clear cell cancer, oesophageal cancer and stomach cancer	Results pending: one phase II trial (NCT03147976) selecting patients with tumours overexpressing MET has been started. The phase II trial NCT02016534 including *MET*-amplified tumours was terminated owing to safety concerns	NA
MSC2156119J (tepotinib)	2 (I) and 2 (I/II)	Mixed cancer, lung cancer and NSCLC	Results pending: latest phase II trial (NCT02864992) will study tumours with *MET* exon 14 deletion that did not respond to chemotherapy	NA
OMO-1 (also known as JNJ-38877618)	1(I)	Mixed cancer, lung cancer and NSCLC	Results pending	Placebo-like adverse event profile observed up to the highest dose tested; favourable pharmacokinetic profile after oral dosing

1. For details, see Supplementary Table 2 and US National Library of Medicine. ADCC, antibody-dependent cell-mediated cytotoxicity; ALK, anaplastic lymphoma kinase; AML, acute myeloid leukaemia; EML4, echinoderm microtubule-associated protein-like 4; FLT3, FMS-like tyrosine kinase 3; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; IgG, immunoglobulin G; IHC, immunohistochemistry; NA, not applicable; NSCLC, non-small-cell lung cancer; PDGFRA, platelet-derived growth factor receptor-α; VEGFR2, vascular endothelial growth factor receptor 2. [a]As of October 2017. [b]Unselected, advanced cancer of various origin. [c]Report from Aveo Pharmaceuticals Inc.

Studies in immortalized cancer cell lines and PDXs have shown that only tumours harbouring MET genetic lesions (mostly amplification) respond to MET blockade with cell cycle arrest and/or apoptosis in vitro[152] and complete inhibition of tumour growth (and even tumour shrinkage) in vivo[30]. By contrast, targeting the HGF–MET axis in tumours with WT MET has little or no effect on cancer cell growth nor does it influence the cytotoxic effect of chemotherapy[153]; instead, pharmacological inactivation of WT MET impairs cell migration, survival from apoptotic insults and metastasis[22]. This preclinical evidence should inform the rational application of MET inhibitors in the clinic: only patients with tumours (both primary lesions and established metastases) displaying amplification or

mutational activation of the MET gene are likely to experience objective responses (tumour regressions) following treatment with MET-targeted therapies. In line with this assumption, a number of case reports have documented responsiveness to anti-MET therapy in patients with MET-amplified gastro-oesophageal cancer[154] and patients with NSCLC exhibiting high-level MET amplification[36] or MET exon-14-skipping variants[44,52].

The notion that only MET genetic lesions predict overt response to MET inhibition might explain the failure of two large, randomized phase III trials in patients with advanced NSCLC. These studies evaluated onartuzumab155 (a MET monoclonal antibody that impedes HGF binding) or tivantinib[156] (a questionable MET inhibitor that shows MET-independent microtubule-disrupting activity[157,158]) (Table 1) in combination with the EGFR inhibitor erlotinib, which is effective in NSCLCs harbouring EGFR-activating mutations[159] but has poor activity in WT EGFR-expressing tumours[160]. In both trials, no assessment of EGFR and MET genetic status or evaluation of MET activity (which can be gauged by the use of antibodies against the tyrosine-phosphorylated protein) were considered for upfront patient recruitment, and the only inclusion criterion (in the onartuzumab trial) was strong immunohistochemical staining for total MET protein in tumour cells. The lack of molecular stratification likely diluted individual responses in patients with genetically susceptible tumours. In fact, a post hoc exploratory analysis revealed longer OS in the subgroup of patients with tumours displaying high (more than four) MET copy number gain treated with tivantinib[156]. This correlation between high MET copy number and response to MET inhibition was not found in patients treated with onartuzumab[155]. However, this antibody acts by disrupting ligand–receptor binding and does not affect the intrinsic kinase activity of MET[161]. Such a mode of action might explain why onartuzumab was not active against MET-amplified tumours, which typically rely on constitutive, ligand-independent MET signalling for their proliferation and survival[152]. At the same time, the ability of onartuzumab to block the interaction between HGF and MET could be exploited to inhibit HGF-dependent invasion and survival in tumours without METamplification, with potentially positive effects on limiting tumour metastatic dissemination.

The fact that MET-targeting compounds fail to induce growth arrest in tumours with WT MET does not imply that MET blockade in this setting is futile. As discussed above, MET inhibitors might prove beneficial when given in combination with targeted therapies to intercept HGF-dependent survival cues[84,85,86] or together with ionizing radiation as a means to increase the effects of radiotherapy[79]. However, neutralization of HGF-dependent survival cues might be one explanation for why HGF antibodies result in paradoxical detrimental outcomes when compared with chemotherapy alone, as recently documented in a clinical trial that was discontinued prematurely after an independent data monitoring committee found a higher number of patient deaths in the HGF antibody group than in the control group[162] (Table 1; Supplementary Table 2). MET inhibitors could also prove useful owing to their ability to block MET functions in cells of the TME. Because MET signalling is pro-tumorigenic in macrophages, its neutralization could synergize with agents that induce macrophage depletion, such as antibodies[163] and small molecules[164] targeting colony-stimulating factor 1 receptor (CSF1R; also known as MCSFR or CD115). In addition, as MET activation supports the tolerogenic properties of DCs, MET inhibitors might cooperate with immune checkpoint inhibitors, for example, programmed cell death protein 1 (PD1) antibodies, to restore an immunostimulatory microenvironment and unleash the tumoricidal activity of cytotoxic T cells. A clinical trial (NCT02323126)[165] combining a MET-specific small molecule kinase inhibitor (INC280) with a PD1 monoclonal antibody in NSCLC is currently ongoing.

Clinical challenges and outlook

Established preclinical evidence and initial findings in patients are shaping the landscape of precision oncology with MET inhibitors. An increased understanding of the biological functions and appreciation of practical hurdles, including unexpected rebound effects observed after small molecule inhibitor discontinuation (Box 3), can now enable a more informed appraisal of the full potential of anti-MET therapies as they have done with other targeting agents.

It is now becoming increasingly clear that tumours are composed of genetically heterogeneous clones, some of which prosper while others regress depending on their ability to face microenvironmental selection

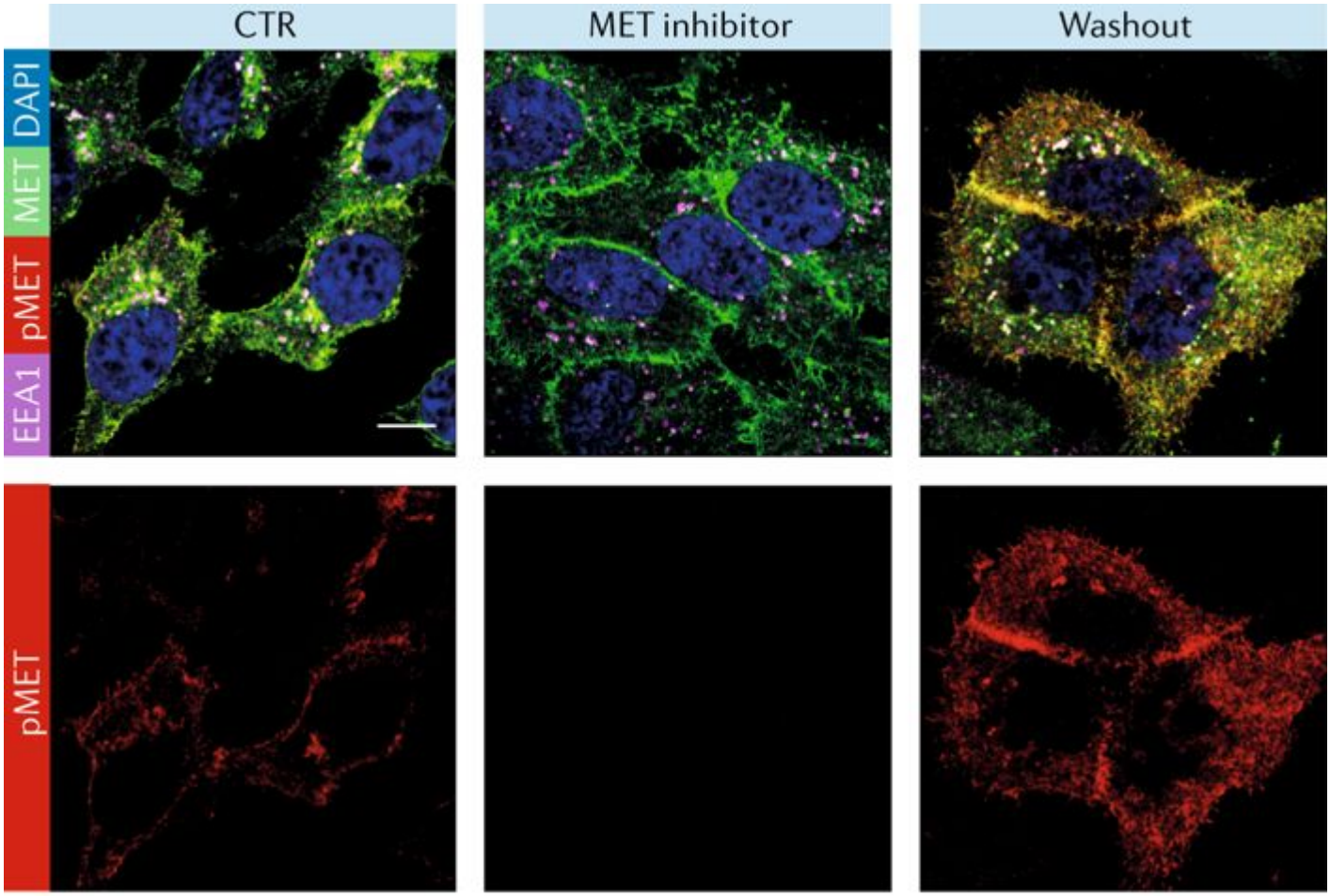
pressures166. MET genetic alterations may dominate the majority of tumour cells and dictate drug sensitivity; however, minor subclones harbouring other mutations that confer resistance to MET blockade may coexist and be positively selected under drug pressure. Resistant subclones need to be promptly detected to limit their emergence with appropriate and timely therapeutic interventions. Tracking incipient subclones harbouring resistance has now been made easier by technological platforms that enable genomic interrogation of liquid biopsies, which include factors such as circulating tumour DNA and circulating tumour cells[167,168]. Not only do these tools enable longitudinal evaluation of tumour genetic evolution in a non-invasive manner, they are also thought to overcome the spatial sampling limitation of solid tissue biopsy (although further work is needed to fully recognize their resolution power), thus offering a more informative picture of the tumour mutational burden as a whole.

Another issue that needs careful implementation is the categorization of response biomarkers. Detection of oncogenic mutations at a high allelic frequency is a reliable indication that such mutations sustain the transformed phenotype, making the mutant genes potentially strong candidates for targeted therapy. But this 'digital' output hardly applies to continuous variables with a normal distribution, in particular, transcript and protein expression in tissues, and to other 'analog' biomarkers scored by graded progressions, including gene copy number gains. For instance, evidence is emerging whereby the efficacy of MET inhibitors increases along with the extent of MET gene amplification[169]. To increase the applicability of biomarker analysis, it will be important to set dichotomous thresholds that distinguish between MET-positive versus MET-negative samples and HGF-high versus HGF-low tumours and to choose the appropriate methodology to accomplish this. For example, gene amplification can be measured by quantitative techniques (such as next-generation sequencing and array comparative genomic hybridization) on bulk tumour extracts or by qualitative techniques on tumour sections (such as in situ hybridization). While quantitative outputs are more reproducible, they may be biased by heavy stromal contamination in the tumour lysates, which dilutes cancer cell-specific signals; conversely, the merit of tissue visualization afforded by in situ hybridization is countered by the subjective nature of gene copy number assessment, which is typically conducted 'by eye' by the pathologist. One opportunity to address these hurdles is by capitalizing on large patient data sets obtained from different sources and with different technical approaches. By comparing diagnostic and therapeutic results and by analysing how and to what extent independent measurement strategies introduce deviations from the expected outcomes, it will be possible to extract reliable predictive correlations as a prelude to the development of standardized companion diagnostic tests. Such efforts will be improved by the availability of novel quantitative technologies to evaluate biomarkers, such as next-generation hybridization systems for in situ mRNA detection[170] and analysis of protein and/or phosphoprotein levels in tumour-derived circulating exosomes[171]. Ultimately, the establishment of standard, reliable consensus cut-offs for MET mutational and activation status will facilitate the introduction of robust algorithms for effective patient stratification and clinical decision making.

Box 3 | The flare effect: an overlooked problem

In clinical practice, discontinuation of treatment with drugs inhibiting tyrosine kinases (such as erlotinib and crizotinib) has been found to be associated with a substantial risk of rebound cancer growth or accelerated disease progression. This adverse effect, often overlooked, is known as 'disease-flare' (refs196,197). To provide a mechanistic explanation for this paradoxical phenomenon, detailed preclinical studies have been so far undertaken only for the MET receptor. The rationale is based on the knowledge that phosphorylation is involved not only in activation but also in downregulation of kinase receptors. In the case of MET, treatment with ATP-competitive small molecules severely impairs endocytosis, causing a local increase in the number of receptors at the cell surface, which likely favour receptor dimerization and ligand-independent autophosphorylation[198]. If MET inhibition is cytostatic and not cytotoxic, withdrawal of the inhibitor is followed by rapid receptor rephosphorylation. Tyrosine phosphorylation is not only increased with respect to the steady-state but also prolonged in duration owing to downmodulation of a negative feedback loop mediated by protein-tyrosine phosphatase 1B (PTP1B)[199], which requires receptor internalization. Thus, a rebound flare effect of receptor activity may take place, pushing quiescent cancer cells back into the cell cycle. Notably, treatment with a MET therapeutic antibody that induces 'shedding'

(proteolytic cleavage of the receptor at the cell surface) substantially prevents this effect, providing a rationale to combine, or alternate, MET-targeted drugs with different mechanisms of action[198].



H1993 lung cancer cells were treated overnight with 500 nM of the MET inhibitor JNJ-605 and then subsequently released from this inhibition for 24 h (washout)[198]. Controls (CTR) are untreated cells. Confocal images in the upper row show total MET (green), the early endosomal marker early endosome antigen 1 (EEA1; magenta) and nuclear staining (4',6-diamidino-2-phenylindole (DAPI); blue), and images in the bottom row show phosphorylated MET (pMET; red). Scale bar is 10 μm. Figure adapted from Pupo, E. et al. Rebound effects caused by withdrawal of MET kinase inhibitor are quenched by a MET therapeutic antibody. Cancer Res. 76, 5019–5029 (2016), with permission from AACR (ref.198).

Conclusions

The different roles of MET in human tumours underscore the importance of this kinase as a therapeutic target, but they also highlight the need for evidence-based translation of biological knowledge into clinical applications. The lessons learnt from the failure of clinical trials, in which the presence of MET genetic aberrations was not taken into consideration for positive selection of patients, and the successful outcome of initial studies, in which patient stratification based on tumour genetics has instead been implemented, are a testimony to how functional preclinical insights should inform clinical practice. Indeed, it was already clear from studies in cell lines and animal models that substantial reduction of cancer cell viability in vitro and overt tumour shrinkage in vivo occur only in those tumour settings in which stable and heritable genetic alterations of MET sustain oncogene addiction. In hindsight, this conclusion should have been drawn earlier: the finding that EGFR tyrosine kinase inhibitors work better than conventional chemotherapy in NSCLC harbouring EGFR-activating mutations[159], while chemotherapy is more effective than targeted treatment in WT EGFR-expressing tumours[160], is a clear indication of the strength of genetic assessment to identify oncogene-addicted tumours and in this way enrich for responding patients.

Preclinical models have clearly shown that inhibiting WT MET activity, albeit not productive in regressing tumours, is sufficient to reduce cell survival, local invasion and metastasis to distant sites[22,172]. These results can be explained by the role of MET as an anti-apoptotic and pro-invasive expedient and by its inherent activity as a clonogenic driver in CSCs. A sensible translation of these findings into the clinic would be in adjuvant therapy after tumour resection with curative intent — an ideal setting to eradicate the persistence and dissemination of subclinical tumour foci — with PFS and OS as clinical end points to assess treatment efficacy. While the rationale is sound, it has not been considered in some of the previous clinical trials. To specifically evaluate the advantage of MET inhibition, randomization of patients into cohorts who receive only standard-of-care therapy and cohorts who also receive the MET inhibitor is required, which calls for the accrual of an adequate number of individuals, using PFS and OS as end points. These trial designs in the adjuvant setting are usually implemented only after evidence of tumour regressions in the metastatic disease, a time when there is sufficient ground to embark on arduous but potentially high-gain projects. Thus, a first-in-human trial to assess the efficacy of MET inhibitors in preventing or delaying relapse after removal of the tumour bulk would be possible only upon a drastic change of mindset in pharmaceutical industries and regulatory authorities.

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Competing interests

P.M.C. is co-founder and scientific adviser of Octimet Oncology NV and Metis Precision Medicine B-Corp. C.B. and L.T. declare no competing interests.

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