Cancer Letters 443 (2019) 189–202

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Oncogene addiction as a foundation of targeted cancer therapy: The paradigm of the MET receptor tyrosine kinase

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ARTICLE INFO

Keywords:
Oncogene addiction
Receptor tyrosine kinases
Targeted anticancer therapy
MET
DNA damage response
Immunotherapy

ABSTRACT

Following nearly two decades of its conception, the phenomenon of oncogene addiction still represents a key concept of how progresses in basic research can translate to unprecedented translational breakthroughs. Coined by Bernard Weinstein, this term refers to the phenomenon by which cancer cells can exhibit dependence on a single oncogenic protein or signaling pathway for sustaining proliferation and survival, despite the wide burden of genetic lesions characterizing their genomic background, revealing thus a promising Achilles' heel of cancer. Importantly, this concept aided the design and clinical implementation of molecularly targeted anticancer therapies, further supporting the paradigm shift witnessed in clinical oncology towards an individual-based, personalized era. In this review, we outline the path of discovery concerning the oncogene addiction concept and focus on the MET receptor tyrosine kinase as a model addicting oncoprotein to further explore potential and pitfalls stemming from the implementation of anticancer strategies targeting tumor dependencies beyond their blending with other therapeutic opportunities.

1. Introduction

The unprecedented efforts to extensively dissect the molecular landscapes underlying individual tumor subtypes support the notion that each cancer type is *per se* a mosaic of diverse disorders, driven potentially by different lesions. Indeed, the heterogeneous picture emerging from genomic, transcriptomic, metabolomic and proteomic studies aiming at unravelling cancer molecular hallmarks aided the conception of a new era in clinical oncology, governed by the notion that anticancer strategies could be tailored *ad hoc* to the individual patient according to underlying druggable vulnerabilities [1–4]. In this context, the formulation of the oncogene addiction concept by Bernard Weinstein represents a milestone for the subsequent design and clinical implementation of molecularly targeted anticancer strategies [5,6]. Coined nearly two decades ago, this term refers to the phenomenon by which cancer cells can exhibit dependence on a single oncogenic protein or signaling pathway for sustaining proliferation and survival, despite the wide burden of genetic lesions characterizing their genomic background, thus revealing a promising Achilles' heel of cancer [6–8]. Implicit in this hypothesis is the theory that the targeted inhibition of the addicting oncoprotein should exert profound effects on the hijacked cancer survival apparatus while sparing normal cells that do not display such dependency, which corresponds to the definition of any effective anticancer therapeutic strategy. In this scenario, a prominent role is played by receptor tyrosine kinases (RTKs), as their aberrant activation was found to fuel the malignant phenotype in a variety of cancer subtypes, thus representing a major class of proteins with addicting potential [9]. Remarkably, this observation appears to underlie the clinical implementation of several tyrosine kinase inhibitors (TKIs), including imatinib, crizotinib, trastuzumab, gefitinib, or erlotinib [10]. Beyond their well-established roles in driving tumorigenesis of specific subtypes of human cancer, accumulating data indicate a crosstalk between tyrosine kinases and the DNA damage response (DDR) machinery, which appears to translate in enhanced sensitivity to DNA-damaging agents (DDAs) upon inhibition of the addicting driving oncoprotein ([11–15]), revealing thus a potential case of a signaling circuit regulated by oncogene addiction in cancer cells. In this context, the MET RTK for hepatocyte growth factor (HGF) represents a model recapitulating both the classically associated functions of RTKs in cancer, as well as their involvement in rewiring the DDR machinery [16].

In this review, we outline the molecular foundation underlying the oncogene addiction concept and we discuss how the definition of this phenomenon aided the conception of targeted anticancer strategies, thus further sustaining the design and implementation of personalized approaches in clinical oncology. Furthermore, we employ the MET RTK as a model oncoprotein with addicting properties to further discuss the

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https://doi.org/10.1016/j.canlet.2018.12.001
Received 21 September 2018; Received in revised form 20 November 2018; Accepted 3 December 2018

role of RTK systems in cancer, beyond the potential and pitfalls stemming from the implementation of anticancer strategies targeting tumor dependencies. Finally, we explore the crosstalk between RTK systems and the DDR machinery and outline preliminary data concerning the implementation of immunotherapy in combination with TKIs to propose these as two possible therapeutic strategies that could counteract or at least delay the appearance of resistance mechanisms.

2. The roots of oncogene addiction

Preclinical studies supporting the phenomenon of oncogene addiction derive from both cell culture-based experiments as well as mouse tumor models. One of the first experimental observations in this respect can be traced back to a decade prior to the introduction of the term itself, when inhibition of MYC by antisense RNA was shown to associate with differentiation and inhibited proliferation of tumor-derived cellular models [17]. Similarly, inhibition of H-RAS oncoprotein was shown to induce apoptosis in mouse melanoma models and parallel observations were obtained by inhibiting K-RAS in mouse lung adenocarcinomas [18,19], implying the importance of RAS signaling in mediating the survival of these tumor models. Subsequent studies aimed at dissecting specific cancer dependencies revealed novel potential targets for intervention, the resulting landscape enriched by protein kinases, which were shown to represent key targets in a variety of recently developed anticancer therapeutic strategies, as further discussed below. Early preclinical evidence supporting the role of protein kinases in establishing oncogene addiction derives from studies by Druker and colleagues, who showed that IL-3-dependent, imatinib-resistant M07 human megakaryocytic cells and 32D murine myeloid cells reverted to an IL-3-independent, imatinib-sensitive phenotype following transfection with BCR-ABL [20]. Subsequently, Fan and colleagues extended these observations to immortalized rodent fibroblasts transformed with inhibitor-sensitized versions of v-erbB or v-src. Interestingly, inhibition of these oncogenes following transformation was accompanied by cell cycle arrest, decreased levels of D- and A-type cyclins and enhanced p27 levels, suggesting that mammalian cells can become dependent on aberrant oncogenic signaling [21]. An elegant series of studies performed on inducible transgenic mouse models provided additional insights into the phenomenon of oncogene addiction; for example, mouse models of leukemia [22] and breast cancer [23] have demonstrated the dependency of such tumors on BCR-ABL and erbB2, respectively.

Despite remarkable efforts have been invested aiming at identifying key proteins with addicting potential as well as to fill the gap in our understanding of the mechanistic underpinnings of oncogene addiction, the molecular basis accounting for this phenomenon is still not completely elucidated. In this respect, the genetic streamlining hypothesis postulates that cancer cells, due to selective pressure of the tumor environment and the tumorigenic process itself, undergo active deterioration of any cellular circuitry that is not providing advantage to cell viability or cellular fitness, thus rendering the entire cellular apparatus more susceptible to acute perturbations than the normal counterpart [24]. Similarly, the oncogenic shock model stems from experimental observations proposing that upon acute disruption of the addicting oncogene, pro-survival as well as pro-apoptotic signals previously emanating from the targeted oncoprotein decay at different rates, with the pro-survival ones (such as AKT, ERK and STAT) decaying more rapidly than the apoptotic ones (namely p38) [25]. The perturbed system thus enters a brief window of time during which pro-apoptotic signals exceed pro-survival ones, resulting in "oncogenic shock" and commitment to the apoptotic event. The aforementioned hypothesis has been demonstrated in cellular systems addicted to breakpoint cluster region-Abelson tyrosine kinase (BCR-ABL), SRC and epidermal growth factor receptor (EGFR), suggesting shared mechanisms involved in oncoprotein-driven apoptosis [26,27]. Finally, synthetic lethality relationships were suggested to occur within activated oncogenes in cancer cells [28,29]. Two genes are defined as synthetically lethal if inactivation of both genes results in cell death, whereas activation of either gene is compatible with cell survival. Implicit in this theory is that elimination of the oncogene would lead to cell death of cancer cells while sparing normal ones, where such a synthetic lethal interaction is not present. One remarkable example of how this model can be exploited in therapeutic settings is represented by synthetic lethal interactions described between BRCA1 and/or BRCA2 gene products, which play an imperative role in homologous recombination repair (HR) of double-strand breaks (DSBs), and poly [ADP-ribose)]-polymerase 1 (PARP1), an essential protein in base-excision repair (BER) of single-strand breaks (SSBs) [30,31]. Inhibition of PARP1 as a mean to inactivate BER was shown to lead to the deadly accumulation of DNA lesions in BRCA-deficient cells, as these are unable to repair DSBs originating from SSBs [32,33].

3. Oncogene addiction from bench to bedside

Oncogene addiction appears to provide an exploitable therapeutic opportunity in which targeted inhibition of the addicting oncoprotein can have profound effects on the signaling balance of cancer cells. Given the key role of tyrosine kinases in driving tumorigenesis and in the maintenance of the malignant phenotype, it is not surprising that this class of proteins became a suitable target candidate in cancer therapy (Table 1).

The first indication of how oncogene addiction can be exploited in the clinic strongly links with the introduction of the BCR-ABL inhibitor imatinib in treating chronic myeloid leukemia (CML). The Philadelphia chromosome, which was found to correlate with the onset of CML ([34]), derives from a chromosomal translocation between the long arms of chromosomes 9 and 22, which in turn results in the production of a fusion transcript between the BCR and ABL genes [35,36]. This

Table 1
Examples of oncoproteins with addicting properties and their relevance in clinical practice.

Kinase	Kinase type	Main tumor types	Targeting drug	Main target modifications conferring addiction	Drug-resistance mutations	Secondary mutation-targeting drug
ABL	Cytoplasmic/nuclear tyrosine kinase	CML	Imatinib (Gleevec)	BCR-ABL fusion	T315I	Dasatinib (Sprycel)
ALK	RTK	NSCLC	Crizotinib (Xalkori)	EML4-ALK fusion	L1196M	Ceritinib (Zykadia)
B-RAF	Serine/threonine kinase	Melanoma, colorectal	Vemurafenib (Zelboraf)	V600E point mutation		
C-MET	RTK	Gastric, NSCLC, HNSCC, papillary RCC, others	EMD1214063 (Tepotinib) (phase II)	Gene amplification, point mutations (Y1230 C/H/D, Y1235D, M1268T)		
EGFR	RTK	NSCLC, colorectal, glioblastoma, HNSCC, others	Gefitinb (Iressa), Erlotinib (Tarceva)	Exon 18 – point mutations Exon 20 – insertions Exon 19 – deletions (LREA) Exon 21 – point mutations (L858R)	T790M	Osimertinib (Tagrisso)
HER2	RTK	Breast, ovarian, NSCLC, others	Trastuzumab (Herceptin)	Gene amplification, Exon 20 - insertions		

specific chimeric protein plays a key role in CML development and progression, thus emerging as one of the first targetable addictive oncoproteins in the clinic [37]. The development of the small molecule tyrosine kinase inhibitor STI571 (imatinib, gleevec (Novartis)) showed almost 100% complete responses in phase 1 clinical trials and these remarkable outcomes were confirmed in further clinical studies, leading to the fast-track FDA approval of the drug in 2001 [38,39].

Another example of how oncogene addiction can be exploited in clinical settings derives from the discovery that 25–30% of breast cancer patients display amplification of the gene encoding the receptor tyrosine-protein kinase erbB-2 (HER2), which correlates with poor prognosis [40]. The first agent targeting HER2 activity, trastuzumab (herceptin (Genentech)), showed responses in about 30% of HER2-overexpressing breast cancer patients [40].

Similar observations were reported following the introduction of the small molecule compounds gefitinib (iressa (AstraZeneca)) and erlotinib (tarceva (Roche)), both targeting epidermal growth factor receptor (EGFR) tyrosine kinase activity. Initial testings of these compounds in non-small cell lung cancer (NSCLC) patients were disappointing, with response rates of approximately 10% only [41]. However, this relatively small subpopulation of responsive patients displayed remarkable responses beyond common clinical and epidemiologic characteristics [42]. Subsequent genetic analysis of tumor subtypes present in these cohorts led to the discovery of EGFR-activating mutations as predictors of responses to EGFR inhibition [43,44]. Further studies showed 50–70% response rates (RR), increased progression-free survival (PFS) and overall survival (OS) rates in selected patients population displaying activating EGFR mutations, underlying the importance of patient stratification in personalized clinical settings involving the application of kinase inhibitors [45].

Beyond tyrosine kinases, mutations in the BRAF serine/threonine kinase were commonly found in various types of human malignancies [46]. Particularly, 74–90% of BRAF-mutated melanomas harbor the V600E ($BRAF^{V600E}$) mutated allele resulting from a substitution of valine with glutamic acid at codon 600 [46]. The small molecule inhibitor PLX4032 (vemurafenib (Plexxikon)) was shown to improve OS and PFS in melanoma patients carrying the BRAF V600E mutations in a phase III clinical trial [47]. Similar observations were obtained from an extended follow-up of this study, in which authors reported that vemurafenib increases median OS as well as median PFS as compared to treatment with the alkylating agent dacarbazine [48].

A more recent example of a clinical application of oncogene addiction resides in the introduction of the inhibition of anaplastic lymphoma kinase (ALK) in therapy of NSCLC patients displaying a fusion protein between echinoderm microtubule-associated protein like-4 (EML4) and ALK, which corresponds to 2–5% of NSCLC cases [49]. The EML4-ALK fusion protein associates with constitutively active ALK with tumorigenic potential [50]. Importantly, its inhibition by PF-02341066 (crizotinib (Pfizer)) produced striking results in a phase I clinical trial, with a 57% response rate in 82 ALK-rearranged patients ([51]), leading to the direct execution of a phase III trial in second-line and first-line settings, randomizing ALK-positive NSCLC patients with the final aim to compare efficacy of crizotinib *vs.* standard chemotherapy. In 2011, crizotinib got accelerated US FDA approval for the treatment of patients with advanced NSCLC carrying ALK rearrangements [52].

4. Oncogene addiction and the déjà-vu emergence of acquired resistance

The issue of acquired resistance to targeted anticancer therapies first emerged during a clinical trial testing the efficacy of imatinib in blast-crisis CML patients [41]. Subsequent analysis of patients who acquired insensitivity to imatinib despite initial remission revealed high rates of mutations in the BCR-ABL gene [41]. Particularly, the amino-acid substitution T315I, also referred to as “gatekeeper” mutation, was found to impede the insertion of the compound into the ATP-binding pocket of the kinase by steric hindrance while preserving kinase activity, thus resulting in drug insensitivity [41,53]. In addition, other mutations were found to prevent drug binding by affecting the conformational changes required for proper interaction between the compound and the kinase active site [54]. In this respect, novel compounds targeting the mutated form of the protein were developed to treat relapsing CML patients displaying resistance to imatinib, such as dasatinib (sprycel (Bristol-Myers Squibb)), nilotinib (tasigna (Novartis)) and ponatinib, the only inhibitor that can block the activity of the T315I BCR-ABL mutation [55–57].

The development of resistance to targeted therapies by the acquisition of secondary mutations that hinder drug binding to the target kinase catalytic site represents a recurrent theme in the landscape of targeted therapies and was documented for a variety of oncogene-addicted tumors, including EGFR in NSCLC [58]. In this context, the first resistance mechanism described relates to the acquisition of a secondary mutation in exon 20 of the *EGFR* gene, which results in a threonine to methionine substitution at residue 790 ([59]) and has been found to account for 50% of cancers displaying acquired resistance to EGFR TKIs [60]. Another resistance mechanism identified in NSCLC tumors resistant to gefitinib corresponds to amplification of the gene encoding the MET receptor [61].

Overall, targeting the addicting oncoprotein in cancer cells can be bypassed by resistance mechanisms that seem to act either in a vertical or in a horizontal fashion: in the first scenario, acquired lesions at the level of the inhibited oncoprotein re-stabilize the previously inactivated signaling pathways (e.g. T790M mutation within the EGFR gene), in the latter, parallel signaling axes are activated, which have the potential to substitute for the inhibited protein, thus providing redundant survival signals (e.g. MET amplification upon EGFR targeting) (Fig. 1). Importantly, heterogeneity among the population of cancer cells may imply the presence of pre-exisiting insensitive subclones which may be selected by drug exposure, thus contributing to acquired resistance mechanisms as well [41]. In this scenario, we envision combinatorial strategies targeting several tumor vulnerabilities simultaneously as important tools to potentially counteract or delay the emergence of resistance mechanisms. In this review, we will focus our attention on two distinct strategies, which are discussed in sections 5.5 and 6.

Importantly, the notion of oncogenic shock has been employed as a model in order to explain the striking differences observed in the proportion of responses across different cancer subtypes to targeted inhibition of tyrosine kinases. Particularly, one hypothesis in this respect suggests that the key to predict targeted therapies efficacy resides in the timing of decay of pro-survival as well as pro-apoptotic signals upon disruption of oncogene activity, which may represent a specific feature of individual oncogenes [62]. Indeed, apoptosis could prevail in those systems governed by growth inhibitory signals slowly shutting down upon targeted disruption of oncogene activity. Conversely, resistance mechanisms may potentially occur in systems characterized by rapid elimination of pro-apoptotic factors emanating by the targeted oncoprotein, allowing escape from apoptosis and giving time for survival signaling pathways to restore [62]. Early evidence supporting the relevance of oncogenic shock in response to targeted therapies derives from a study conducted on mutant BRAF models [63]. This study has suggested BRAF-mediated activation of the SPRY family of RTKs inhibitory proteins, implying that targeted inhibition of BRAF activity results in mitogen-activated protein kinase (MAPK)-dependent survival signaling decay as well as in inhibition of SPRY expression, which in turn eliminates SPRY-mediated inhibition of RTKs activity in response to growth factors. Importantly, this feedback mechanism seems to translate in rapid activation of EGFR in BRAF-mutant colorectal cancer upon targeted inhibition of BRAF, leading to development of horizontal resistance [63,64]. The existence of paradoxical feedback pathways involving activation of signal transducer and activator of transcription 3 (STAT3) and their importance in tilting the balance towards survival upon acute disruption of oncogene activity has been described in

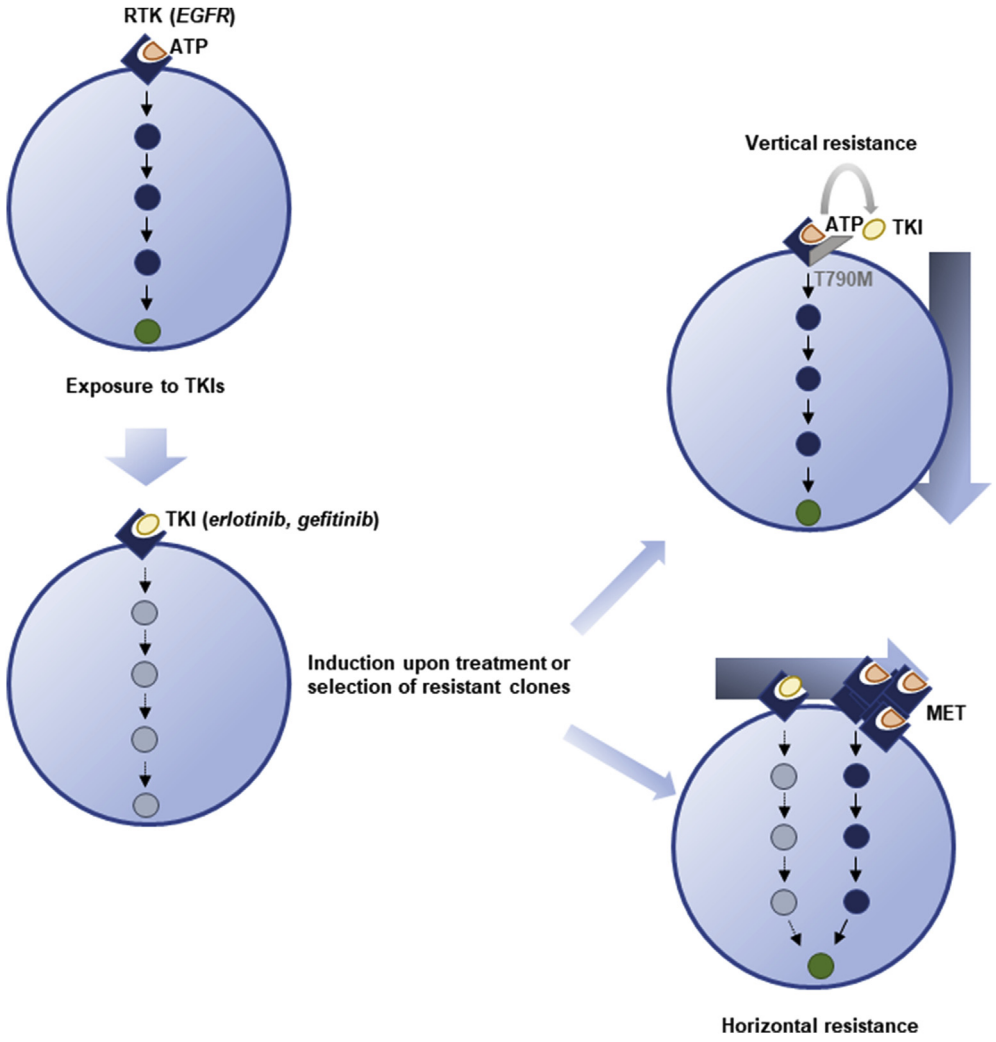

Fig. 1. Models of acquired resistance upon targeted inhibition of the addicting oncoprotein, which can be classified as either a horizontal or a vertical resistance. These events might be induced by treatment or due to selection of pre-existing resistant clones. Rectangles, receptor kinases. Dots, downstream effectors. Green, pro-survival factors. Blue, activated pathway. Grey, disrupted pathway.

several models for addiction, including EGFR, HER2, ALK and MET [65].

5. Addiction to RTKs: MET as a paradigm

The MET RTK for HGF, also known as scatter factor (SF), has acquired widespread attention in the field of cancer research since its discovery in 1984, where it was first identified as part of the nucleoporin translocated promoter region (TPR)-MET translocation product in a human osteosarcoma cellular model [66,67]. Beyond its role as oncogene in various human malignancies, the MET signaling pathway was found to play key roles in a plethora of physiological and developmental cellular processes [68]. Indeed, its expression was observed on epithelial cells of a variety of organs, including kidney, liver, muscle, pancreas, prostate and bone marrow [16]. Under physiological conditions, the interaction between MET and its ligand HGF, which is secreted by cells of mesenchymal origin, triggers the "invasive growth" (also known as "cell scattering") morphogenetic program ([68–70]), which plays an imperative role during embryogenesis for epithelial growth, morphogenesis and differentiation, thus representing a master developmental regulator [68]. Beyond its key role during organ development, MET and its ligand are essential during adulthood for organ protection and regeneration upon insult, recruitment in adult hematopoiesis and regulation of bone remodelling [71–76]. Examples of aforementioned scenarios include regenerating liver [77], skeletal muscle [78] and infarcted myocardium [79]. Furthermore, MET was shown to play an essential role in the regulation of the immune system [80].

5.1. The HGF-MET axis: structure and modes of activation

HGF is synthesized as a single-chain precursor that is subsequently proteolytically cleaved to produce α- and β-chain heterodimers. The α-chain contains a hairpin loop (HL) domain and four kringle (K) domains, while the β domain is composed by a serine protease homology domain (SPH) [81]. HGF can bind to MET through two distinct binding sites that display different affinity to the receptor. Specifically, the α-chain contains the high-affinity site and binds to the IPT3 and IPT4 domain, whereas the low-affinity site is located within the β-chain and interacts with the SEMA domain of the target receptor [82,83].

MET on the other hand is a 190 kDa heterodimeric protein composed by a 145 kDa transmembrane β subunit linked to a 45 kDa extracellular α subunit by disulfide bond [81] (Fig. 2). The extracellular β subunit is composed by a semaphorin (SEMA) domain, which completely encompasses the α subunit, followed by an integrin-rich domain (PSI) and four immunoglobulin-like domains (IPTs), these latter linking the extracellular portion of the receptor to the transmembrane helix. The intracellular portion is composed by the juxtamembrane domain,

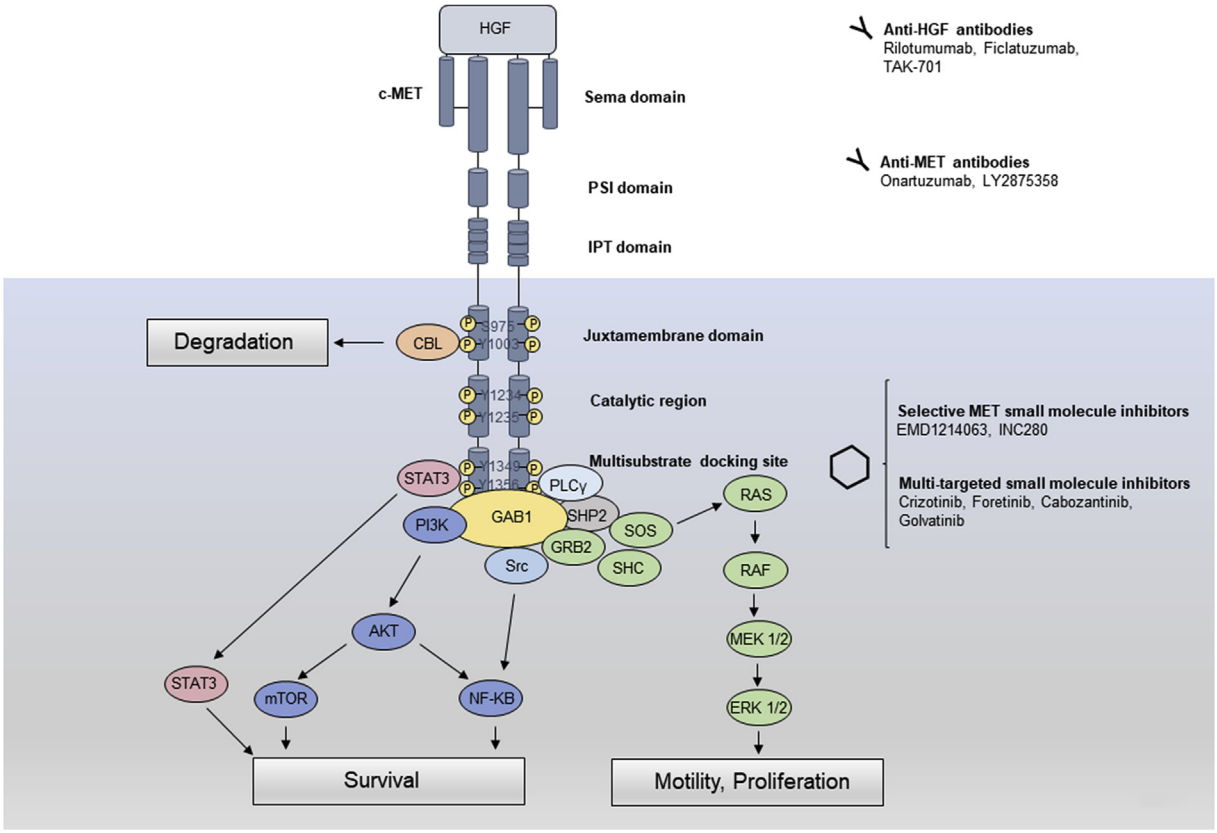

Fig. 2. Schematic representation of MET and the downstream signaling pathways, with emphasis on classes of MET/HGF axis inhibitors and their binding sites.

the catalytic region and the C-terminal docking site. Each of these portions are involved in orchestrating specific functions emanating from the kinase: the juxtamembrane domain contains two key phosphorylation sites, Ser975 and Tyr1003, involved in inhibition of protein kinase activity, while the catalytic portion harbors Tyr1234 and Tyr1235, which positively modulate MET activity upon trans-phosphorylation. Finally, phosphorylation at Tyr1349 and Tyr1356 at the docking site triggers recruitment of adaptor and effector proteins following MET activation, resulting in signal propagation at the cellular level [84,85]. These include the growth factor receptor-bound protein 2 (GRB2), SRC homology 2 domain-containing (SHC), phosphatidylinositol-3 kinase (PI3K), phospholipase C γ (PLCγ), tyrosine phosphatase SHP2, SRC homology 2-containing inositol 5-phosphatase 1 (SHIP1), STAT3 and the docking protein GRB2-associated binding protein (GAB1) [86–89]. The recruitment of adaptor proteins triggers the activation of signaling networks which modulate a variety of MET-mediated cellular functions, including migration, proliferation and cell survival [85]. One such pathway is represented by the MAPK pathway initiated by the interaction between the adaptor GRB2, SHC and the guanine nucleotide exchanger son-of-Sevenless (SOS) [90]. Specifically, SOS can activate the small GTPase RAS by promoting the exchange of guanine diphosphate (GDP) to guanine triphosphate (GTP), which in the active GTP-bound conformation can interact with the serine/threonine kinase RAF. Active RAF phosphorylates MEK1/2, which in turn triggers activation of ERK1/2, a key regulator of transcriptional factors that modulate the expression of genes involved in cell motility and proliferation. Indeed, the importance of this signaling pathway in invasive growth was well established [91–95].

Another major signaling branch emanating from active MET and responsible for downstream signal transduction is represented by the PI3K pathway, which is initiated upon direct interaction of PI3K with GAB1. Phosphatidylinositol-3,4,5-trisphosphate is then generated through phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP2) by PI3K, which in turn interacts with AKT and pyruvate dehydrogenase kinase 1 (PDK1). PDK1 subsequently phosphorylates AKT and this event triggers activation of the mTOR complex (mTORC1) ([96]), leading to cell proliferation and migration [95].

Although MET signaling is primarily mediated by the aforementioned pathways, other signal transducers have been shown to participate in MET-mediated regulation of cellular functions. One such example is the signal transducer STAT3, which was found to bind to the active receptor via SH2 domains subsequently undergoing phosphorylation, homodimerization and translocation to the nucleus, where active dimers act as transcriptional regulators of genes involved in cell proliferation, differentiation and formation of branched tubules [86,97].

Among MET signal transduction effectors, the NF-KB pathway was described as a key regulator of cell proliferation and resistance against apoptosis. Specifically, MET-driven activation of NF-kB is mediated by PI3K/AKT and SRC signaling and results in NF-KB nuclear translocation, where it regulates the transcription of anti-apoptotic and mitogenic genes [98,99].

Beyond its key role as a transducer of MET signaling pathways, the scaffold protein GRB2 was found to be directly involved in MET degradation by recruiting the E3 ubiquitin ligases c-CBL and CBL-B to Tyr1003 within the receptor juxtamembrane domain, which in turn leads to protein ubiquitination and subsequent recruitment to multivesicular bodies, thus ensuring termination of MET functions upon its degradation [100].

5.2. *The other side of the coin: role of MET RTK in cancer*

As illustrated by previous prominent examples, RTKs were demonstrated to play imperative roles in driving tumorigenesis and in the maintenance of the malignant phenotype of specific cancer subtypes, thus representing a major class of proteins with addicting potential [9].

Similarly, aberrant regulation of the MET/HGF axis with subsequent alteration of downstream signaling pathways was found to contribute to tumorigenesis, cancer progression and invasiveness [101]. Importantly, the aforementioned "invasive growth" program, beyond the established relevance in physiological functions, can be exploited by cancer cells for migration and dissemination processes [102]. Indeed, the role of aberrant MET activation in driving tumor invasiveness and metastatic growth was well established [102]. Moreover, MET and HGF were found to modulate key regulatory signaling events involved in cell proliferation, angiogenesis and cancer stem cell regulation, thus their aberrant activation representing a major driver of oncogenesis [84]. In this context, aberrant MET signaling can result from a variety of different mechanisms, including transcriptional deregulation, gene translocation or amplification, activating point mutations, establishment of HGF/MET autocrine loops, autocrine or paracrine activation or crosstalk with other receptors and downstream signaling players [103]. Notably, MET overexpression and genetic mutations in the MET gene were reported in various human malignancies, including hereditary papillary renal cell carcinoma (RCC), sporadic papillary RCC, gastric, head and neck cancers, NSCLC, breast and ovarian carcinomas as well as in childhood hepatocellular carcinoma (HCC) and were found to correlate with poor clinical outcome [102,104–108]. MET overexpression results either from alterations in transcriptional regulation or gene amplification and was observed in various human malignancies; furthermore it was found to associate with poor prognosis and metastatic dissemination [95]. Examples include NSCLC, HCC and colorectal cancer. In the latter, MET overexpression was observed in 50% of primary tumors *vs.* 70% of metastatic ones, underlying the key role of MET in driving cancer cell invasiveness [101,104–106,108–110].

MET overexpression by transcriptional regulation can result from dysregulation of the transcription factors ETS and SP1 or by alterations of transcriptional repressors such as the micro RNAs miR-1, miR-34 and miR-449a [111–113]. A key mediator of this event under hypoxic conditions is hypoxia-inducible factor α (HIF-1α) [112]. The second mechanism triggering MET overexpression relies on MET gene amplification, which was observed in a variety of human cancers including gastric and esophageal carcinoma, glioblastoma, medulloblastoma and NSCLC [84]. In the latter, *MET* amplification was found to correlate with poor outcome ([114–116]), whereas in gastric cancer MET overexpression is predictive for lower OS rate [101,117–120]. Similarly, in colorectal cancer elevated *MET* copy number associates with progression to metastatic stages of the disease [110]. Importantly, *MET* amplification was found to correlate with poor prognosis in lung adenocarcinoma and has emerged as an important resistance mechanism in the landscape of targeted therapies against the tyrosine kinase receptor EGFR [121–125]. Of note, elevated *MET* gene copy number was found to correlate with enhanced protein expression, suggesting ligand-independent, constitutive activity of the receptor [126].

Beyond MET overexpression, aberrant MET expression can result from point mutations within the *MET* gene, which were reported in numerous tumors, including melanoma, lung, breast, ovarian and head and neck cancers [109]. Such mutations can result in decreased affinity of the receptor for its ligand, such as the N375S mutation localized within the SEMA domain, which was detected in a subset of lung cancer patients ([127]), or they can trigger constitutive receptor activation by affecting Tyr1003, the binding site of the c-CBL. Indeed, mutated Tyr1003 was detected in NSCLC and melanoma tumors [106,128]. In this respect, a variety of mutations localized within the MET juxtamembrane domain has been reported in lung cancer, HNSCC, gastric cancer and melanoma patients [106,109,129–132]. Finally, a third class of mutations can alter MET kinase activity as these are localized within the catalytic domain of the receptor (examples include Y1230 C/H/D, Y1235D, M1268T) and trigger constitutive, ligand-independent activity of the kinase by stabilizing MET in an active conformational state. These mutations were first identified in papillary RCC [104] and were subsequently reported in childhood HCC and HNSCC, where a point mutation in the MET catalytic domain (Y1253D) was found to correlate with decreased metastasis-free survival [133].

An additional mechanism triggering aberrant MET activity resides in HGF overexpression. Indeed, MET and its ligand were found to be co-expressed in cancer cells, thus generating autocrine or paracrine receptor activation loops [134]. Specifically, HGF overexpression was detected in a variety of different tumors, including NSCLC, HNSCC, gastric carcinoma, clear cell RCC (ccRCC), breast cancer, acute myeloid leukemia (AML), osteosarcoma, melanoma and glioma and was associated with poor clinical outcome and an aggressive tumor phenotype [134,135]. Notably, overexpression of HGF and its receptor were shown to trigger metastatic events in rat models [136].

As aforementioned, MET was shown to interact with other RTK systems, including members of the EGFR family ([137]), and these events may trigger aberrant MET activity in human cancers. In this context, activation of MET and of the key downstream effectors AKT and ERK1/2 were found to undergo synergistic modulation by HGF and EGF in NSCLC cellular models displaying active MET and EGFR receptors, and EGF alone was found to induce MET activation [138]. Notably, proliferation and motility were also regulated synergistically by the combination of the two growth factors, suggesting a crosstalk between the two RTKs [61,138,139].

5.3. The potential of exploiting MET dependency in cancer therapy

The key role of the MET/HGF axis in progression and invasiveness of a wide variety of human malignancies suggests that targeted strategies inhibiting MET signaling could result in significant reduction in survival and acquisition of invasive traits of cancer cells displaying dependency on MET for sustaining growth and proliferation. However, any efficient targeted-based anticancer therapy requires patient stratification in order to evaluate *a priori* which patient population could benefit from the administration of a specific compound. In this respect, the identification of predictive biomarkers for drug efficacy represents an imperative step towards effective precision medicine-based approaches. Furthermore, these should include evaluation of combinatorial strategies efficacy, which could act synergistically on reducing cancer cell proliferation [140].

The design of compounds inhibiting the initiation of the signaling cascade propagating by active MET relies on strategies designed to specifically target one of the serial processes regulating MET activation. Particularly, these can be classified as HGF/MET antagonists or antibodies against HGF of MET that abrogate interactions of HGF with its ligand, small-molecule MET inhibitors or inhibitors of downstream effectors of MET signaling circuitries. Several novel compounds targeting either MET or HGF showed positive responses in preclinical studies and are currently under clinical evaluation [140] (Table 2).

5.3.1. Targeting MET by small molecule inhibitors

Small molecule inhibitors are low molecular weight compounds that display high affinity for the receptor catalytic site, thus preventing receptor transactivation and propagation of the signal to downstream effectors. In this respect, small molecule inhibitors can be sub-divided in class I inhibitors, which correspond to ATP-competitive inhibitors, whereas another class of ATP-competitive small molecules, defined as class II inhibitors, differs from the previous one in the mode of interaction with the substrate, as it engages several residues within the catalytic domain upon conformational changes [140–143]. Finally, non-ATP-competitive binding can occur with the inactive conformation of the receptor, leading to structural stabilization and subsequent inhibition of receptor activity [143]. Several small molecules inhibitors selective for MET RTK were developed and are currently undergoing clinical evaluation. Examples include EMD1214063/tepotinib (Merck Serono), a highly selective type II ATP-competitive MET inhibitor which was shown to exert antitumoral activity in preclinical studies as

Table 2
Overview of prominent MET/HGF inhibitors and antibodies under clinical evaluation.

Compound name	Properties	Producer	Target	Evaluation in the clinic
Tepotinib	Type II ATP-competitive inhibitor	Merck Serono	MET	Phase II [140]
Capmatinib	Type I ATP-competitive inhibitor	Novartis	MET	Phase I [79,135]
Crizotinib	Type I ATP-competitive inhibitor	Pfizer	EML4-ALK, MET	Approved [141]
Foretinib	Type II ATP-competitive inhibitor	GSK/Exelixis	MET, AXL, VEGFR, RON	Phase I [142]
Cabozantinib	Type II ATP-competitive inhibitor	Exelixis	MET, VEGFR2, RET, KIT, FLT3	Approved [79]
Golvatinib	ATP-competitive inhibitor	Eisai	MET, VEFGR2	Phase II [79]
Onartuzumab	Humanized monoclonal antibody	Genentech	MET	Phase III [143]
LY-2875358	Humanized bivalent antibody	Eli Lilly	MET	Phase I [79]
Rilotumumab	Human monoclonal antibody	Amgen	HGF	Phase III [79]
Ficlatuzumab	Humanized monoclonal antibody	Aveo	HGF	Phase II [79]
TAK-701	Humanized monoclonal antibody	Takeda Pharmaceuticals Co	HGF	Phase I [79,135]

well as in phase II trial of advanced solid tumors [144,145] or INC280/capmatinib (Novartis), a type I ATP-competitive inhibitor which displayed favorable pharmacokinetic profile beside manageable side effects in a phase I trial of advanced, refractory tumors [84,140]. Beyond selective tyrosine kinase inhibitors (TKIs), several multi-targeted inhibitors targeting also MET were designed. One such example is crizotinib (Pfizer), a type I ATP-competitive inhibitor which was approved for NSCLC patients harbouring the EML4-ALK translocation in 2011 [146]. Similarly, the multikinase type II ATP-competitive inhibitor foretinib (GSK/Exelixis) was shown to target MET, AXL, vascular endothelial growth factor receptor (VEGFR) and the recepteur d'origine nantais (RON) and to display clinical efficacy and required safety standards in patients with metastatic or unresectable tumors [147]. Other examples include the type II ATP-competitive inhibitor cabozantinib/cabometyx/cometriq (Exelixis), which targets MET, VEGFR2, RET, KIT and FLT3 ([84]) and was approved for treatment of patients with progressive, metastatic thyroid carcinoma (MTC) as well as renal cell carcinoma and the ATP-competitive inhibitor golvatinib (Eisai), which is currently in phase II clinical evaluation for solid tumors and displayed affinity for MET as well as for VEFGR2 ([84]).

5.3.2. Antibodies-based strategies

As aforementioned, binding of HGF to its receptor can be inhibited by antibodies that target either MET or HGF itself. One example of the first scenario is the humanized monovalent monoclonal antibody onartuzumab (Genentech), which is currently being evaluated in phase III trials for patients with metastatic HER2-negative, MET-positive gastroesophageal cancer [148]. Notably, the same agent was administered in combination with erlotinib in advanced NSCLC patients with MET-positive tumors and the combinatorial strategy showed promising preliminary results [149]. Similarly, LY-2875358 (Eli Lilly), a humanized bivalent anti-MET antibody whose inhibitory effect on MET signaling is exerted by blocking HGF binding to the receptor, subsequently leading to MET internalization and degradation, is currently being evaluated in a phase I study in combination with erlotinib [84].

Besides these MET-directed targeted strategies, several antibodies recognizing its ligand have been designed and their safety and efficacy is currently being evaluated. One such example is the human monoclonal antibody rilotumumab (Amgen), which is currently being administered to a cohort of patients with advanced MET-positive gastric or gastroesophageal junction adenocarcinoma in a phase III study in combination with the chemotherapeutic agents epirubicin, cisplatin and capecitabine [84]. Ficlatuzumab (Aveo), a humanized monoclonal HGF-directed antibody, showed positive results in a phase II study focused on adenocarcinoma patients [84]. Finally, TAK-701 (Takeda Pharmaceuticals Co), a humanized monoclonal anti-HGF antibody, showed manageable toxicity in a phase I study [84,140].

5.4. The pitfalls of exploiting MET dependency in cancer therapy

As aforementioned, resistance to targeted therapies seems to occur either in a horizontal or in a vertical fashion. In this respect, targeting MET was shown to be bypassed by the development or selection of lesions localized within the MET kinase domain (*vertical*) as well as by upregulation of alternative parallel pathways driven by other RTKs such as EGFR and fibroblast growth factor receptors (FGFRs), which can substitute for the inhibited protein and provide redundant survival signals (*horizontal*) [84]. Remarkably, it was previously reported that exposure of the gastric MET-addicted cancer cell line GTL-16 to increasing doses of the MET small molecule inhibitors PHA-665752 or JNJ38877605 drives acquired resistance to MET inhibition by amplification and subsequent overexpression of K-RAS oncoprotein [150]. Moreover, overexpression of a MET variant characterized by the amino acid substitution Y1230H within the activation loop in the MET-positive gastric carcinoma cellular model SNU-638 was shown to trigger resistance to MET inhibition exerted by PHA-665752 and crizotinib [151]. The authors of the aforementioned study showed occurrence of an alternative mechanism for resistance to MET-targeted therapies, involving overexpression of TGFα and subsequent activation of EGFR. Another mechanism of resistance to MET inhibition previously identified in AML cells involves increased HGF expression, which restores MET activity, thus compensating for MET signaling decay exerted by the multi-substrate inhibitor crizotinib [134].

5.5. The DDR and RTK systems: points of intersection with therapeutic potential

Genome integrity is constantly challenged by a diverse array of DNA lesions that can result from exposure to a multitude of environmental agents such as ionizing and UV radiations, as well as from endogenous products of cellular metabolic reactions including reactive oxygen species (ROS). To counteract the harmful effects of these lesions, the DDR machinery has evolved as a complex network of signaling events which sense, recognize and repair damaged DNA, orchestrating a wide variety of cellular responses that may trigger cell cycle arrest or cell death [152]. Indeed, both widely used anticancer strategies – radiotherapy and chemotherapy – exploit the effects of severe DNA damage to induce cytotoxicity and subsequent death of highly proliferative cells. On the other hand, genomic instability can trigger tumorigenesis, thus revealing the importance of DNA repair mechanisms, both as key events driving the tumorigenic processes as well as exploitable anticancer targets [153,154]. Accumulating data seem to suggest a crosstalk between the DDR machinery and several RTK systems, which translates in enhanced DNA-damaging agents (DDAs)-induced toxicity upon targeted inhibition of RTKs. In this respect, the MET RTK represents a paradigm of a RTK system modulating the cellular response to DNA damage. Early observations pointing to a potential intersection between MET and the DDR machinery emerged in 1998, when it was reported by Fan et al. that HGF can protect breast cancer cells from apoptosis induced by a variety of DNA-damaging agents through stabilization of the anti-apoptotic protein BCL-X_L [155]. Mechanistically this phenomenon was shown to be regulated by the adaptor protein GAB1, which triggers

BCL-X_L stabilization via activation of the PI3K pathway. Additionally, HGF was reported to increase ionizing radiation (IR)- and adriamycin-induced DSBs repair [155–157]. Further studies aiming at dissecting the MET-DDR crosstalk demonstrated increased expression of the receptor and its ligand HGF upon induction of DNA damage [158–160].

In the clinics, MET and BCL-X_L expression was found to inversely correlate with complete remission of primary squamous cell cancers of the oropharynx trait following radiation therapy, and MET expression was shown to decrease survival in similar therapeutic settings [161,162]. These observations seem to translate in enhanced radiosensitivity upon MET inhibition. A first study confirming this hypothesis demonstrated that MET inhibition increases the response of human glioma xenografts to IR exposure [163]. Similarly, it was subsequently reported that MET inhibition exerts radiosensitivity in several glioblastoma cell lines by impairing DSBs repair and increasing apoptosis. Importantly, these findings were confirmed *in vivo* where MET inhibition combined with IR resulted in enhancement of tumor-growth-delay and in increased survival [164]. Similar observations were obtained in other models including NSCLC, prostate and papillary thyroid cancer [165,166].

Mechanistically, MET inhibition was shown to induce increase of γH2AX levels, a marker indicative for unresolved DNA damage, and to impair the activation of key components of the DDR machinery such as ATR, CHK1 and CDC25b, resulting in reduced survival of MET-overexpressing cellular models upon combinational treatment with IR or adriamycin [167]. Interestingly, MET inhibition was shown to inhibit HR by impairing RAD51 nuclear translocation upon DNA damage; notably MET inhibition was also accompanied by disruption of the RAD51-BRCA2 complex, which is of key importance for HR-mediated repair of DSBs [168,169]. Taken together, these observations support the involvement of the MET system in modulating the cellular response to IR and open a window to novel combinatorial anticancer approaches.

Of note, MET is not the only RTK system that was found to impact the cellular response to DNA damage. As aforementioned, EGFR was shown to interact with key players of the DDR machinery. In this respect, an early observation pointed to a direct interaction between EGFR and DNA-PKcs [170]. Subsequent studies demonstrated a key role of this interaction in DNA DSBs repair. Interestingly, EGFR inhibition exerted by the anti-EGFR antibody cetuximab or the small molecule inhibitor gefitinib was shown to be accompanied by disruption of EGFR-DNA-PKcs interactions, thus impairing DNA DSBs repair [171,172]. Additional evidence supporting the involvement of EGFR in modulating key components of the DDR machinery derives from a study reporting that EGFR inhibition by the small molecule inhibitor erlotinib impairs HR efficiency by translocation of BRCA1 in the cytosol, thus resulting in radiosensitivity [173]. The clinical relevance of these observations started to emerge upon a study conducted by Bonner et al. which showed that treatment of advanced head and neck cancer with radiotherapy in combination with the anti-EGFR antibody cetuximab is beneficial compared to the single agents administered alone both for improved locoregional control as well as in terms of reduced mortality [174].

Similarly, other RTKs were demonstrated to interact with key players of the DDR machinery, suggesting thus that the administration of TKIs in combinatorial settings with standard radiotherapy may provide a successful strategy to induce tumor radiosensitivity. In this context, IGF1R inhibition was shown to act synergistically with DDAs by enhancing antitumor activity and to exert radiosensitivity by inhibiting both NHEJ- and HR-mediated repair of DSBs [175,176]. Similar conclusions were drawn for other receptors including fibroblast growth factor receptor 3 (FGFR3), VEGFR and platelet-derived growth factor receptor (PDGFR) and the role of their inhibition on influencing cellular response to IR established [177,178]. Overall, these studies seem to reveal the RTK-DDR crosstalk as a druggable vulnerability of addicted cancer cells, which could be exploited therapeutically by the conception of combinatorial strategies implying TKIs in combination with DDAs. Importantly, these may provide higher clinical benefit than single agent-based therapies and potentially delay development of resistance mechanisms.

6. Oncogene addiction and immunotherapy

Highlighted as Breakthrough of the Year by *Science* in 2013, cancer immunotherapy represents a key paradigm shift witnessed over the last decade concerning cancer management and treatment. This has been supported by revolutionary clinical observations demonstrating that checkpoint blockade therapy can trigger T lymphocytes to eradicate cancer cells by recognizing tumor-specific neoantigens [179]. Indeed, immune checkpoint inhibitors, including those targeting programmed cell death-1 (PD-1) or the ligand PD-L1 demonstrate clinical benefit with enhanced OS compared to standard chemotherapy ([180–184]), which led to the approval of several of these compounds for clinical practice. One such compound is the PD-1 inhibitor pembrolizumab, which displayed higher PFS in combination with chemotherapy as compared to platinum-based chemotherapy alone in NSCLC patients [185]. Similarly, the PD-1 inhibitor nivolumab was approved in the second-line setting for the treatment of NSCLC ([180,181]), whereas the PD-L1 inhibitor atezolizumab showed improved OS compared to docetaxel in NSCLC patients in the phase II POPLAR trial and in the phase III OAK trial [183,186].

Importantly, observations deriving from these studies do not seem to support the use of immunotherapy for patients harboring EGFR-activating mutations or ALK rearrangements, although no conclusion can be drawn so far due to the limited number of patients analyzed carrying these specific mutations [187].

Of note, a recent meta-analysis showed that immune checkpoint inhibitors significantly improved OS compared to standard chemotherapy based on docetaxel in NSCLC EGFR wild-type patients, but not in the EGFR-mutant subgroup [188]. Similarly, a recent study reported an overall response rate lower than 5% in ALK-rearranged or EGFR-mutant NSCLC patients upon treatment with immune checkpoint inhibitors [189]. Importantly, Rizvi and colleagues previously demonstrated that in NSCLC patients treated with the anti-PD-1 antibody pembrolizumab, higher mutational burden was associated with improved ORR, durable clinical benefit and PFS ([190]), underlying the importance of the tumor genomic landscape to determine responsiveness to immunotherapy. Remarkably, oncogene-addicted tumors were shown to display lower mutational burden ([191]), which could partially explain the decreased efficacy of immunotherapy in these specific tumors.

Importantly, a strong correlation was found between high PD-L1 expression and EGFR mutations in NSCLC patients, and PD-L1 expression was shown to decrease in EGFR-mutant cell lines upon exposure to EGFR TKIs ([192,193]), suggesting that EGFR reshapes the tumor microenvironment to trigger immune escape in EGFR-addicted models. In this scenario, combinatorial strategies involving TKIs with immune checkpoint inhibitors may provide higher clinical benefit in oncogene-addicted tumors as compared to immunotherapy alone. Similarly, higher PD-L1 expression was observed in ALK-rearranged NSCLC cell lines, which decreased upon treatment with the ALK TKI alectinib, suggesting a possible crosstalk between the addicted oncoprotein and the tumor microenvironment which could be therapeutically exploited [194,195]. Notably, a phase I trial testing the combination of the EGFR TKI erlotinib and the anti-PD-1 antibody nivolumab in NSCLC patients harboring EGFR mutations displayed an ORR of 19% and PFS rate of 47% at 24 weeks [196]. Similarly, the combination of the third generation TKI osimertinib targeting the EGFR T790M mutant form and the immune checkpoint inhibitor durvalumab was tested in a phase Ib study, which showed ORR of 67% in T790M-positive and 21% in T790M-negative patients who had undergone previous treatment with EGFR TKIs, compared to 70% ORR in EGFR TKI-naïve patients [197]. However, 38% of the patients analyzed developed interstitial lung

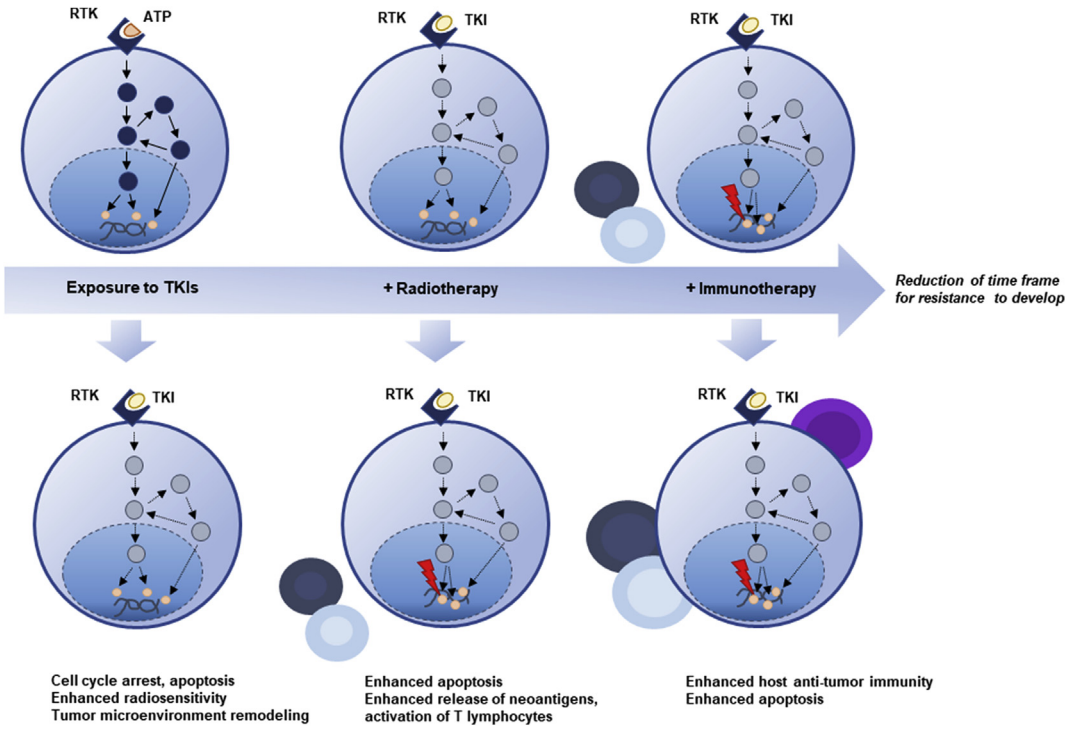

Fig. 3. Proposed model of triple combination strategy aiming at maximizing synergistic modulation of individual anticancer agents in order to delay the emergence of resistance mechanisms. Rectangles, receptor kinases. Dots, downstream effectors. Blue, activated pathway. Grey, disrupted pathway. Orange, players of DDR machinery. Blue, light blue and purple spheres, components of the immune system.

disease due to the combinatorial regime. Another phase Ib study combining the anti-PDL-1 antibody atezolizumab with erlotinib showed a promising ORR of 75%, although adverse effects occurred in 18% of the patients treated [198].

A similar scenario seems to emerge from clinical studies aiming at evaluating the efficacy of immunotherapy combined with targeted anticancer agents in melanoma patients. Of note, preclinical trials demonstrated that inhibition of the MAPK pathway may result in beneficial enhancing host anti-tumor immunity by increasing the melanocytic antigen expression beyond improving T-cell function and tumor infiltration ([199–202]). Thus, the combination of BRAF inhibitors with immunotherapy appears to provide a novel promising therapeutic strategy for this specific tumor subtype. In this respect, a phase I study analyzing the combination of the anti-PD-L1 antibody MEDI4736 with the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib showed a response rate (RR) of 69% accompanied by a disease control rate (DCR) of 79% in BRAF-mutant patients subjected to the three drugs, although severe adverse effects were reported [203]. In another phase 1b study, the combination of the anti-PD-L1 antibody atezolizumab with the BRAF inhibitor vemurafenib and the MEK inhibitor cobimetinib conducted on 29 BRAF-mutant advanced melanoma patients showed a RR of 83% and progression of the disease observed only in 3% of the patients, although high toxicity was reported [204]. The corresponding phase III study is currently ongoing ([205]), together with other trials aiming at evaluating the combination of targeted compounds with immunotherapy in BRAF-mutant melanoma patients [206,207].

Although several trials are ongoing, no data are currently available concerning the benefit of immunotherapy, alone or in combination with TKIs in other oncogene-addicted tumors. However, accumulating preclinical and correlative observations seem to suggest that activated oncoproteins with addicting properties may remodel the surrounding tumor microenvironment and thus affect the response to immunotherapy. In this context, MET activity levels were found to correlate with high PD-L1 levels in renal cell carcinoma [208]. Furthermore, MET amplification was associated with enhanced PD-L1 levels in erlotinib-resistant NSCLC cells [209]. Recently, Saigi and colleagues demonstrated that MET promotes PD-L1 expression in MET-addicted cells and that MET activation is accompanied by the expression of negative immunoresponse regulators as well as other key factors required for the establishment of immunosuppression, thus suggesting that MET may contribute to the ability of tumor cells to escape immunosurveillance by driving the establishment of an immunosuppressive environment [210].

Taken together, preliminary observations seem to point to a cross-talk between the addicting oncoprotein and the host immune system, accompanied by promising clinical results testing combination strategies involving immunotherapy with targeted anticancer compounds in EGFR- and BRAF-mutant tumors. It is thus imperative to acquire further knowledge concerning safety and efficacy of such combinatorial strategies beyond evaluating their potential as a novel tool to delay the emergence of resistance mechanisms following exposure to TKIs.

Notably, several strategies were reported to potentially enhance the immunogenicity of oncogene-addicted tumors and thus increase the efficacy of immunotherapy. One such approach resides in combining radiotherapy with immunotherapy, as radiotherapy was demonstrated to trigger the release of neoantigens through tumor cell death and activate specific T lymphocytes [211] to generate in some cases a powerful systemic eradication of metastatic lesions through abscopal effects [212]. Accordingly, we could envisage the benefit of triple combination regimes of radiotherapy with TKIs, which may not only result in enhancement of TKIs efficacy, but have also the host immune system to respond more effectively to immunotherapy which could be administered subsequently, as an example of therapeutic strategy aiming at maximizing synergistic modulation of the individual compounds in order to reduce the time frame for development of resistance mechanisms to prevail (Fig. 3).

7. Summary and outlook

Oncogene addiction refers to the phenomenon by which tumor cells develop a dependency on a driver oncogenic product that assumes the role of buttressing and fueling the malignant phenotype, thus setting the basis for the conception of anticancer therapies targeting single oncoproteins in defined populations of cancer patients. Despite being accompanied by an unprecedented translational impact, the clinical implementation of molecularly targeted anticancer strategies revealed the emergence of common patterns for resistance across most tumor subtypes, thus greatly limiting the benefits of oncoprotein-targeted therapies. These common themes for resistance should be deeply understood in order to be intercepted or at least anticipated. In this respect, combinations of distinct therapies may provide higher clinical benefit and delay the onset of resistance mechanisms. An example reported in this review resides in the administration of targeted anticancer agents with radiation therapy and/or immunotherapy. Furthermore, technical advances applied to the detection of resistant clones could aid faster remodelling of individual therapies in order to dismantle reservoirs of resistance mechanisms. Clonal heterogeneity and the capability of cancer cells to quickly adapt to the surrounding environment signify intrinsic hallmarks of human malignancies. We envisage that the key step towards the development of more successful anticancer strategies will probably be represented by the increasing capability to understand, and thus predict, cancer evolution in response to therapy in order to accompany it to the desired destination.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This work was supported by the Swiss National Science Foundation (grant no. 31003A-156816 to Y.Z.).

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