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**Receptor tyrosine kinases: characterisation, mechanism of action  
and therapeutic interests for bone cancers**

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## **Abstract**

Bone cancers are characterised by the development of tumour cells in bone sites, associated with a dysregulation of their environment. In the last two decades, numerous therapeutic strategies have been developed to target the cancer cells or tumour niche. As the crosstalk between these two entities is tightly controlled by the release of polypeptide mediators activating signaling pathways through several receptor tyrosine kinases (RTKs), RTK inhibitors have been designed. These inhibitors have shown exciting clinical impacts, such as imatinib mesylate which has become a reference treatment for chronic myeloid leukaemia and gastrointestinal tumours. The present review gives an overview of the main molecular and functional characteristics of RTKs, and focuses on the clinical applications that are envisaged and already assessed for the treatment of bone sarcomas and bone metastases.

## 1. Introduction

To be able to play their physiological role (intra- and inter-cellular signal transmission, adaptation to changes in the microenvironment), cells must be able to receive, integrate and respond to numerous extracellular messengers. These communications between cells and their environment are made possible through the attachment of molecules considered as messengers to their receptors, identified as effectors (cytokines, growth factors, etc). As proposed by Ehrlich in 1910, "to act, a substance must be fixed." These receptors are essentially located at the cell membrane, although there are also intra-cytoplasmic receptors such as steroid hormone that can be translocated into the nucleus to regulate expression of numerous genes. Membrane receptors possess: (i) an extracellular hydrophilic domain, often glycosylated, which recognises the ligand; (ii) a hydrophobic trans-membrane domain that makes embedding possible within the lipid bilayer of the plasma membrane; and (iii) an intra-cytoplasmic domain dedicated to signal transduction within the cell. The binding of a ligand to its receptor is specific, reversible and involves a large number of low-energy bonds (hydrogen, ionic, hydrophobic, Van der Waals). Thus, at equilibrium, the dissociation rate is equal to the rate of association. Among the receptors of cytokine/growth factors, six types of receptor have intrinsic enzymatic activity (kinase or phosphatase receptors, and guanylyl cyclase-coupled receptors) or not (the G protein-coupled receptors, the receptor-type "channel", and cytokine receptors).

The *guanylyl cyclase-coupled receptors* include natriuretic peptide, nitric oxide, carbon monoxide and enterotoxin receptors. The binding of the ligand to the extracellular domain of its receptor leads to intracellular activation of the guanylate cyclase domain of the receptor chain, and to synthesis of a cyclic GMP for activating the cAMP-dependent protein kinase environment [1]. The *G protein-coupled receptors* are characterised by seven transmembrane domains. The trimeric G proteins located on the cytoplasmic side of the cell membrane

transduce and amplify cell signalling through the production of cyclic AMP. The chemokine receptors are included in this family environment [2]. The *ion channel linked receptors* are ligand-dependent ion channels and their opening or closing activities are associated with the nature of the ligand. These receptors can be ionotropic or metabotropic. In the first case, the receptor is actually the pore, and opens following a conformational change made possible by the ligand binding. On the contrary, in the case of metabotropic receptors, ligand-stimulated receptors activate a ligand-independent channel through the intracellular effector environment [3]. *Cytokine receptors* can be divided into four groups: i) receptors with an immunoglobulin-like ectodomain (IL-1 $\alpha$ / $\beta$ , IL-18); ii) the trimeric members of the TNF receptor superfamily (which include, for instance, RANK, TRAIL receptors, TNF receptors- $\alpha$ / $\beta$ ); iii), class I-cytokine receptors (or haematopoietin receptors) environment [4] and iv) class II-cytokine receptors (or interferon and IL-10 receptors) [5]. Class I/II- cytokine receptors have oligomeric structures, where a specific  $\alpha$ -chain warrants specific ligand recognition, while one or two channels ( $\beta$ / $\gamma$ ) are used for signal transduction. For instance, the receptors of interleukins (IL) 2, 4, 7, 9 and 15 consist in a specific chain to the cytokine, and the shared IL-2  $\gamma$ -receptor chain, IL-2 and IL-34 also share a  $\beta$ -receptor chain environment [6]. Similarly, the IL-6 cytokine family (IL-6, IL-11, CNTF, OSM and LIF) shares the gp130 receptor chain environment [7]. Among the cytokine receptor families, some are characterised by intrinsic kinase activity and consequently by their ability for autophosphorylation. They form the receptor tyrosine kinase (RTK) family.

All of these receptors tightly control tissue homeostasis, and any dysregulation of these ligand-receptor systems (mutations, overexpression, etc) disturbs cell communication and leads to pathological situations. Bone formation and bone remodelling are then controlled by a large panel of cytokines and growth factors regulating the dialogue between osteoblasts, osteoclasts and their environment [8]. It has been recognised that cancer cells (bone sarcomas,

metastatic cells originating from carcinomas) dysregulate the balance between osteoblasts and osteoclasts, activate osteoclastogenesis and then stimulate bone resorption. Consequently, activated osteoclasts resorb the extracellular bone matrix and release numerous growth factors entrapped in the organic matrix, which stimulate in turn the proliferation of cancer cells. Based on these observations, numerous chemical drugs have been developed to specifically target the various receptor tyrosine kinases activated by mutations, or by the ligands present in the tumour microenvironment. The present review summarises the classification, structure and mechanism, and focuses on the targeting of action of the receptor tyrosine kinases. Their use in the treatment of bone cancers (bone sarcomas and bone metastases) is described and discussed.

## **2. The receptor tyrosine kinase (RTK) family**

### **2.1. Classification and structure of RTKs**

Protein kinases are key enzymes in the regulation of various cellular processes that catalyse the transfer of a phosphate group from ATP to a hydroxyl group of a serine or a threonine. Among the 90 identified genes encoding proteins with tyrosine kinase activity, 58 encode receptors divided into 20 subfamilies [9, 10] (Table I). Of these subfamilies, EGFR / ErbB (class I), the receptor for insulin (class II), for PDGF (Class III), for FGF (class IV), for VEGF (class V) and HGF (MET, Class VI) are strongly associated with oncological diseases. These RTKs are characterised by a single trans-membrane domain and a glycosylated N-terminal extracellular domain with a high number of disulfide bonds. This extracellular domain is involved in the dimerisation process of the receptors, and consequently in ligand recognition (Figure 1). The composition of these domains (immunoglobulin domains, rich in leucine, lysine and cysteine, fibronectin type III domain, etc.) depends on the classes of RTKs and then defines the specificity of the ligands. The RTKs are inserted into the cell membrane

thanks to an  $\alpha$ -helix trans-membrane domain composed of 20 amino acids. The trans-membrane domain plays a key role in the formation and stabilisation of the dimer of the receptor chains. In the lipid environment of the cell membrane, the  $\alpha$ -helix are non-covalently oligomerised [11] (Figure 1). This type of process makes it possible to pre-dimerise the RTKs in the cell membrane capable of interacting with the corresponding ligand [12].

The cytoplasmic domain harbours a specific domain with tyrosine kinase activity that is involved in the catalysis of the ATP-dependent phosphorylation of receptor chains. It includes two domains: a juxtamembrane region composed of 40 to 80 amino acids corresponding to the tyrosine kinase domain and a carboxy-terminal region. The tyrosine kinase domain is composed of 12 subdomains organised into two lobes, connected by the kinase insert domain (subdomain V) (Figure 1). The tyrosine kinase domain includes an activation loop, whose orientation (and phosphorylation) determines the active or inactive state of the kinase domain. The ATP required for kinase activity is housed between the two lobes. The small lobe (named lobe N, for N-terminal, subdomains I to IV), composed of  $\beta$ -sheets and one  $\alpha$  helice, binds, stabilises and orients the ATP previously complexed with  $Mg^{2+}$  ions. The large lobe (named C, for C-terminal, subdomains VI to IX) is mainly composed of  $\alpha$  helices, and plays a part in the chelation of ATP by  $Mg^{2+}$  ATP. It then binds the protein substrate containing the tyrosine target and catalyses the transfer of the phosphate group from the ATP to the receptor chains [13]. The size of the tyrosine kinase domain is relatively constant between the different RTKs. On the contrary, the size and content of the juxta- and C-terminal domains, vary considerably between the RTK families, conferring the specificity of intracellular signals. For instance, the intracellular domain of PDGFR $\beta$  has 552 amino acids, the intracellular domain of EGFR has 542 amino acids, while the FGFR1 shows 425 and TrkA only 356 amino acid residues. The number of tyrosine residues (phosphorylable or not) and their distribution vary significantly between the RTKs. Thus, 27 tyrosine residues are detected for the PDGFR $\beta$  (of which 19 can

be phosphorylated) and only 11 tyrosines can be detected in TrkA (with 6 phosphorylatable tyrosines) (Bradshaw *et al.* 2013). However, a pair of tyrosine residues phosphorylated after RTK activation is found in the activation loop and is required for the functionality of the receptor. The activation of these tyrosine residues stabilises the “open” conformation of the activation loop and both lobes, and also allows the ATP and peptidic substrate environment to bind [13]. An additional, third tyrosine amino acid (located in a close upstream domain) participates in the conformational change of the activation loop. All the mutations on these tyrosine residues result in inactivation of the receptor chains. EGFR is an exception in the RTK families and it has only one tyrosine residue at this position, which is not essential for receptor chain activation and function.

## **2.2. General mechanism of action**

It is admitted that the binding of a dimeric ligand to its receptor chains increases the proximity or/and stabilises the receptor chains that will be then auto-phosphorylated through their kinase domains (a process called trans-phosphorylation). This non-covalent dimerisation is associated with conformational changes that lead to the activation of the cytoplasmic kinase domains of the receptors. In most cases, one of the two receptor chains will trans-phosphorylate specific cytoplasmic tyrosines from the other monomeric chain environment [14]. In some cases, the constitutive form of the RTKs is a dimer such as insulin receptors. In addition, some ligands such as EGF are monomeric, and their binding to their receptor induces a conformational change that shifts the intra-molecular loop and exposes a binding domain in the receptor that results in its dimerisation environment [15]. In others, the dimerisation of the ligand is required to activate the receptor chain (*i.e.* the NGF - TrkA system environment [16]).



In the absence of the ligand, the activation loop self-regulates activation of the receptor because its “closed” conformation inhibits catalytic activity (*cis*-inhibition). Dimerisation of the RTK chains following ligand binding induces the rotation of the N- and C- lobes, as well as the major axis of the protein. The activation loop, which is masked by its tyrosine residues, the ATP binding site, moves to enable ATP binding and the autophosphorylation of tyrosine residues located on the opposite receptor chain. The *trans* phosphorylation of key tyrosine residues located in the activation loop stabilises the “open” conformation, and breaks the binding between these tyrosines and the binding sites to the protein substrates, making it possible to access the C lobe, then activating its kinase activity. In addition, other tyrosine residues are phosphorylated by protein kinases previously recruited on the phosphorylated tyrosines of the RTK environment [17]. Several molecular “brakes” in kinase activity have been developed to limit phosphorylation levels. These molecular domains are located in the activation loop, in the juxtamembrane domain (KIT, PDGFR) or in the C-terminal domain (*i.e.* Tie2). In the last two cases, these molecular repressions will be removed by *cis*-phosphorylation of the RTKs during the ligand binding-induced conformational changes [18]. Phosphorylation of the catalytic domain of the RTKs activates and increases the activity of the kinase domain, whereas the non-catalytic domains create various anchoring sites for cytoplasmic targets involved in intracellular signal transduction. These tyrosines are mostly located on the juxta-membrane and C-terminal domains, and at the insert kinase domain residues, allowing the binding, activation and phosphorylation of numerous cytoplasmic proteins that will then relay the signal towards various intracellular activation pathways. These proteins have SH2 or PTB domains that recognise tyrosine phosphorylated receptor chains, and have intrinsic enzymatic activity, such as Src or PLC $\gamma$ , or serve as adapter proteins for recruiting other enzymes, such as Grb2 linked to the MAPK activation pathway. The proteins recruited by their SH2 domains are named "adapter", while those that

bind directly to the receptor chains or to the Grb2 adaptative protein are called "anchoring proteins". Adaptive and anchoring proteins can bind to similar phosphorylated tyrosine residues or to several tyrosine residues from the same receptor chains. Thus, Gab1 binds to tyrosine<sup>1068</sup> and tyrosine<sup>1086</sup> of EGFR. Insulin and FGF receptors bind to a protein assembly that can be phosphorylated and used as adaptive proteins [19].

### **2.3. RTKs and activated signalling pathways**

RTKs are considered as protein platforms, or the starting point for many cellular signalling pathways by recruiting enzymatic effectors (PLC $\gamma$ , PI3K, Src, etc) either directly on to their intra-cytoplasmic domain, or indirectly through adapter proteins (Grb2, Shc, etc.), forming complexes capable of activating intracellular enzymes (Ras, etc.) (Figure 2). RTK downstream signalling pathways are mainly MAPK, PI3K, Src, and other signalling pathways involving PLC $\gamma$ , JAK / STAT, etc. While the early stages of signal transduction following the activation of RTKs is based mainly on tyrosine phosphorylation, signal propagation associates various phosphorylations on serine / threonine residues in the majority of cellular processes, as well as other processes such as ubiquitination, glycosylation or acetylation [20].

The **MAPK pathway** plays a part in controlling cell proliferation, cell death or differentiation, and migration, as well as promoting angiogenesis. The MAPK signalling cascade is divided into four major pathways used by RTKs and leading to ERK1/2 activation (Figure 2). After activation of the RTKs by their ligand, the adaptive protein Grb2 binds by its SH2 domains, the phosphorylated tyrosine residues of the receptor chains and the adaptive protein SOS by their SH3 domain, which is bound to the PIP2 membrane. This binding allows the activation of Ras, a small G protein, via SOS, a GEF protein exchanging the GDP for a GTP. In fact, Ras oscillates between its active and inactive state, thus acting as a "switch" for intracellular effector molecules. Once activated, Ras allows phosphorylated signal transduction through recruitment and phosphorylation of Raf kinases A, B or C (or MAP3K)

[21]. Activated Raf phosphorylates MEK1 and MEK2 (or MAP2K1/2) on serine<sup>218</sup>/serine<sup>222</sup> and serine<sup>222</sup>/serine<sup>226</sup> residues of their activation loop, and activated MEK1/2 itself catalyses the phosphorylation of Erk1 and Erk2 (or MAPK1/2) on their threonine<sup>202/185</sup> and tyrosine<sup>204/187</sup> residues. Phosphorylated Erk1/2 will be then translocated to the nucleus to activate transcription factors that will regulate the transcription of genes involved in the survival and growth of the cells, or activate cytosolic proteins, such as RSK1/2, which target cytoplasmic effectors or will finally be translocated into the nucleus to act as a transcription factor [22].

The targets of these transcription factors are transcriptional regulators such as STAT, Elk-1, CREB or H3 histone that activate transcription of early genes. Of these early genes, *c-Fos*, *c-Jun* or *c-Myc* stimulate the expression of other genes such as cyclin D1 or CDK6, which control progression in the G<sub>1</sub> phase and G<sub>1</sub>/S transition. When RTK activation, and therefore that of Erk1/2, is maintained, expression of the previous proteins is stabilised as c-Fos, which is phosphorylated on threonine residues by its RSK1/2 and Erk1/2, and forms the complex AP-1 with c-Jun, which also activates the transcription of target genes (Figure 2). The MAPK pathway also activates three additional pathways: p38, JNK and ERK5. In the first pathway, p38 $\alpha/\beta/\gamma/\delta$  are activated by a MAP2K such as MKK3 or MKK6, previously activated by a MAP3K such as TAK1, and consequently, p38 induces the transcription of various genes involved in cell proliferation, angiogenesis, inflammation and the production of immunomodulatory cytokines. In the JNK pathway, the TAK1-, MEKK1-, or MLK-MAP3Ks activate the MAP2K4 or MAP2K7, which activates JNK1, 2 or 3, for instance, and lead to the control of cell apoptosis or the development of the immune system [23]. In the ERK5 pathway, WNK1 activates MEKK2 and 3, which phosphorylates MEK5, leading to ERK5 activation. The translocation of ERK5 into the nucleus regulates cell proliferation and survival by activating the transcription of cyclin D1 for example, allowing G<sub>1</sub>/S transition in

the cell cycle in the same way as Erk1/2. ERK5 also has more specific substrates, such as the MEF2 transcription factor family, the pro-apoptotic protein BAD, connexin 43, etc. [24].

The **PI3K/Akt/mTOR pathway** controls cell cycle progression, the cell survival/cell apoptosis balance. Its activation facilitates cell proliferation and migration, the metabolism of glucose, etc. PI3K is a "lipid" kinase that phosphorylates membrane lipids via its catalytic p110 subunit ( $\alpha$ ,  $\beta$  or  $\delta$ ) once recruited by its two SH2 domains from the p85 regulatory subunit on activated RTKs. PIP2 then forms PIP3 (phosphatidylinositol 3,4,5-triphosphate) by transferring a phosphate group, and Akt (PKB, for Protein Kinase B) and PDK-1 then bind to the membrane, where the PDK-1 activated by PIP3 phosphorylates Akt (Figure 2). Activated Akt becomes an activation crossroad for many proteins, allowing cells to survive by inhibiting, ubiquitinating and degrading pro-apoptotic proteins such as BAD and p53, and by inducing the expression of anti-apoptotics such as Bcl-2 or Akt. In addition, Akt also induces cell proliferation by activating various cyclins and by inhibiting several cell cycle repressors such as p21 or p27. Akt also allows the transcription of pro-angiogenic genes such as VEGF and HIF-1 $\alpha$ , which are involved in numerous oncological processes. In addition, Akt inhibits the glucose metabolism by suppressing GSK3, and regulates the lipid metabolism through mTOR activation [25].

The role of the **Src pathway** in signal transmission within the cell was demonstrated for the first time in fibroblasts stimulated with PDGF [26]. Src, Fyn and Yes belong to the Src family, are activated by RTKs, and are associated with numerous other kinases such as Ras, PI3K, PLC $\gamma$  or FAKs. The members of the Src family therefore have redundant functions in the intracellular signalling pathways described below. Src family members are recruited on RTKs (EGFR, FGFR, IGFR, MCSF-R, HGFR, etc.) after their activation and transmit mitogen signals inducing DNA synthesis, cell survival, cytoskeleton rearrangements, cell adhesion and motility, but also control receptor turnover [27]. Src family members can bind

phosphorylated residues by their SH2 domains, resulting in kinase activity after conformational modifications. This activation is very complex and requires the recruitment of Ras and Ral GTPases. Several studies have shown that SFKs may regulate activation of RTKs directly by phosphorylating tyrosine residues such as tyrosine<sup>845</sup>, tyrosine<sup>1101</sup> and EGFR [28]. c-Src can be recruited within membrane complexes formed by integrins, and then phosphorylate these RTKs [29]. Furthermore, the Shp2 protein tyrosine phosphatase also plays a key role in this activation by blocking the activities of negative regulators (Csk for instance) [30].

PLC $\gamma$ , and JAK / STAT are additional signalling pathways associated with RTK activation. Various RTKs can bind through their phosphorylated tyrosine residue, the SH2 domains of STAT transcription factors, as demonstrated for MET and STAT3. The activation of these transcription factors results in their dimerisation and translocation into the nucleus to activate specific target genes [31].

### **2.3. Feedback loops controlling RTK activation**

RTK activities are tightly controlled by numerous positive or negative molecular feedback loops that prolong the auto-activation of the receptors and signal amplitude, by inducing the production of the ligand for instance. Such feedback loops are essential for stabilising the RTK system [32]. These controls include proteins already present within the cell that are mobilised on activation of RTKs and/or subjected to post-translational modifications for immediately regulating the signal induced (early negative feedback) (Figure 3). They also associate the synthesis of response elements (late negative feedback) such as IEGs early or DEGs late genes that regulate the activity of AP-1, c-Myc, p53 or the MAPKs. Thus, Erk1/2, a downstream protagonist of the MAPK pathway, directly inhibits (early negative feedback) the phosphorylation of the effector proteins by inhibiting the kinase activity of upstream

enzymes (RAF and MEK) [33]. In addition, the translocation of Erk1/2 into the core may also activate the expression of transcriptional repressors, such as phosphatases (*e.g.*: DSPs) to inhibit MAPK activity (negative feedback late) [34].

By decreasing the amplitude of the signals generated and the stimulation of cellular activity, adapter proteins such as kinases, phosphatases and ubiquitin ligases located in the cytoplasm are the first early negative regulators of RTK activities [35]. The signal generated is then attenuated, based on the ubiquitination of RTKs by the E3 ubiquitin ligase c-CBL for instance, which leads to the endocytosis of the receptors and their degradation in the lysosomal compartment [36]. After activation by the ligand, the RTK is effectively clustered in clathrin-rich membrane regions and then internalised in clathrin-dependent endocytic vesicles to reduce the induced signal [37].

### **3. RTKs in oncology**

#### **3.1. RTK mutations and carcinogenesis**

RTKs are involved in numerous pathological disorders, especially in oncology. Around 30% of RTKs are mutated or overexpressed in various human cancers (MET, KIT, FLT3, etc.) [38]. Oncogenic mutations or gene duplications in the juxtamembrane region of KIT and FLT3 result in constitutive activation of these receptors in the absence of their ligand, and are consequently directly linked to the carcinogenesis process [39]. Duplications in the juxtamembrane region of FLT3 are responsible, for instance, for the constitutive activation of the receptor in 15-30% of cases of acute myeloid leukaemia [40] and in 65% of gastrointestinal stromal tumours (GISTs) [41]. Autocrine stimulation or overexpression of EGFR were also associated with many solid tumours. Thus, EGFR/ErbB-1 and ErbB-2 are overexpressed in lung [42], breast [43, 44] and prostate [45, 46] cancer, and their expression

is linked to marked aggressiveness and poor prognosis. Such observations have strengthened the therapeutic development of RTK inhibitors in the last three decades.

### **3.2. RTK inhibitors and bone cancers**

#### **3.2.1. RTK inhibitors target the bone tumour niche**

Primary malignant bone tumours (bone sarcomas) and bone metastases (from breast, prostate carcinomas, etc.) are characterised by their ability to dysregulate their micro-environment and especially the balance between bone apposition and bone resorption. Osteoblasts [8, 45-51] and osteoclasts [8, 52-54] express numerous RTKs and are then cellular targets of the corresponding ligands released in the cancer micro-environment. Based on these observations, the impact of RTK inhibitors has been assessed in bone remodelling. Recently, Bao *et al.*, using broad kinase inhibitor screening applied to the mouse MC3T3-E1 osteoprogenitor cell line, identified two families of inhibitor affecting cell survival differentially [55]. The first family included pro-osteoblastic drugs such as lapatinib (EGFR/HER2 inhibitor), erlotinib (EGFR inhibitor) and sunitinib (FLT3/PDGFR/VEGFR/CSF-1R inhibitor), which stimulated osteoblastic proliferation. In contrast, the second family grouped together seven kinase inhibitors (GSK1838705A, PF-04691502, masitinib targeting KIT or XL880 targeting MET and VEGFR), which inhibited osteoblast viability in a dose- and time-dependent manner. Nilotinib and CEP-751 may be added to the second family. Nilotinib potently inhibited osteoblast proliferation [56]. While nilotinib inhibits numerous RTKs (KIT, EPHA3, EPHA8, DDR1, DDR2, PDGFRB), its effects may be associated with the inhibition of PDGFR [65]. Pinski *et al.* demonstrated that proliferation induced apoptosis, but not quiescent human osteoblasts after treatment with CEP-751, a trk receptor tyrosine kinase inhibitor [57]. Similarly, inhibiting IGF1R also led to the inhibition of proliferation and induction of apoptosis of osteoblasts [58]. Nevertheless, these RTK inhibitors, due to their multiple

targeting, exert very complex effects and can exert dual activities on bone cells. Imatinib mesylate (Gleevec), which targets a broad range of tyrosine kinase proteins, including bcr/abl, c-kit, cFMS and the PDGFR among others, is able to inhibit osteoblast proliferation and also to activate their activities through the inhibition of PDGFR $\beta$  activity [59]. Gobin *et al.* confirmed recently this dual activity depending on the doses of inhibitor used. Low doses of imatinib mesylate increased the *in vitro* mineralisation process, and high doses of the drug markedly affected mineral deposits [60].

RTKs are also expressed by osteoclast precursors and mature osteoclasts, and numerous studies have shown that RTK inhibitors strongly affect osteoclastogenesis and bone resorption. Imatinib mesylate decreases osteoclastogenesis, and increases mature osteoclast apoptosis through the inhibition of cFMS signalling [61]. Sorafenib, an RET, and VEGFR inhibitors similarly target osteoclasts [62]. Dasatinib abolishes osteoclast formation *in vitro* by inhibiting cFMS activation, and increases osteoblast activities by repressing PDGFR signalling [63]. In addition, these authors demonstrated that the administration of dasatinib in animals resulted in dysregulated bone remodelling in favour of an increase in bone formation, which may be associated with the inhibition of osteoclast activity [63]. In 2012, Garcia-Gomez *et al.* confirmed the anabolic and anti-catabolic effects of dasatinib [64]. Overall, these works revealed that bone cells are potential targets for RTK inhibitors, and that using RTK inhibitors in an oncological bone context will have an impact on the bone tumour niche.

### **3.2.2. RTK inhibitors as therapeutic drugs for bone sarcomas**

Bone sarcomas derive from the mesoderm, and sarcoma cells originate from mesenchymal stem cells [65]. Osteosarcoma and Ewing's sarcomas are the two main types of bone sarcoma diagnosed in children and young adults. The peak of incidence for both tumours is at puberty, suggesting that there is a strong link with bone growth and the numerous growth factors,



hormones and cytokines released during this period. In this context, RTK inhibitors assessed on bone cells were also assessed in bone sarcomas (Table II) [66, 67]. Recently, Rettew *et al.* identified several RTKs by using a phosphoproteomic approach and demonstrated that Axl, EphB2, FGFR2, IGF-1R and Ret more specifically controlled the behaviour of human osteosarcoma cells *in vitro* from a functional point of view [68]. PDGFR was also identified as a therapeutic target in osteosarcoma, and selective inhibition of PDGFR activation led to apoptosis of osteosarcoma cells *in vitro* [69]. These data were confirmed by a phosphoreceptor tyrosine kinase array kit which identified seven receptors (PDGFR $\beta$ , Axl, RYK, EGFR, EphA2 and 10, IGF1R) as molecular targets for imatinib mesylate [60]. In this study, the authors showed that imatinib mesylate induced anti-proliferatives in pre-clinical models of osteosarcoma, and that of the seven modulated RTKs, PDGFR $\alpha$  appeared as the main target of the drug. Similar observations were made in Ewing's sarcoma [70]. Unfortunately, clinical investigations demonstrated only low or no efficacy in children with relapse bone sarcomas, even in patients selected for tumour expression of KIT or PDGFR $\alpha$  [71-73] (Table II). Dasatinib and Sunitinib were used in phase I clinical trials and defined the doses usable in a paediatric context [77, 79]. Although no objective responses were observed, 4 patients with sarcomas were in a stable condition [79]. Complementary investigations are needed to evaluate the therapeutic efficacy of dasatinib and sunitinib in sarcomas. Pazotinib, targeting VEGFR, PDGFR and c-KIT, and sorafenib, targeting RET and VEGFR, had interesting benefits in paediatric sarcomas [71, 54, 85] (Table II).

Protein assays have identified new RTKs with potential therapeutic benefits. Axl is thus expressed in most osteosarcomas [86] and a correlation was found between its expression and the clinical outcome [87, 88]. In addition, Fleuren *et al.* demonstrated that high Axl expression correlated with worse overall survival compared to Ewing's sarcoma patients with lower expression [89] similar to MET [90]. The MET inhibitor (PF-2341066) then appeared

efficient in a xenograft model of osteosarcoma [91]. EphA2 was the most abundant surface protein on cancer cells and may be involved in the pathogenesis of osteosarcoma by modulating bone remodelling and the communications between tumour cells and their environment [92-94]. Recently, Kuijjer *et al.* provided an *in vitro* rationale for using IGF1R inhibitors in osteosarcoma [95]. However, IGF1R mRNA expression, cell surface expression, copy number, and mutation status were not associated with tumour responsiveness to anti-IGF1R targeting [96]. EGFR are expressed by osteosarcoma cells, but gefitinib and BIBW2992 targeting the receptors were not effective on osteosarcoma cells, so the question of EGFR targeting remains open [97]. Similarly, HER-2 is expressed by osteosarcoma cells but its prognostic relevance is still controversial [98] and the results for the patients treated were limited [99]. A randomised study of patients with HER2-positive osteosarcoma would be of major interest for better understanding the role of HER-2 in the pathogenesis of bone sarcomas, and for evaluating their therapeutic value. EphA10 and RYK are two other RTKs expressed by osteosarcoma cells and represent other therapeutic opportunities [100, 101].

Overall, these data revealed the potential therapeutic interest for targeting RTKs in bone sarcomas. Clinical investigations must nevertheless be adapted to the expression/mutation/activation state of RTKs, which is the prerequisite for patient enrolment.

### **3.2.3. RTK inhibitors: therapeutic benefits for bone metastases**

As with bone sarcomas, bone metastatic cells, from breast or prostate carcinoma for instance, dysregulate local bone remodelling and the associated TRKs/growth factors, which in turn facilitate tumour development [102]. Consequently, numerous TRKs and their ligands have been associated with the pathogenesis of carcinomas and their capacity to form bone metastases. Many investigations at the pre-clinical and clinical levels have thus been developed in the last 10 years (Table III). Unfortunately, whilst most of the drugs developed had interesting anti-cancer effects on the primary tumours or/and the establishment of bone

metastases, the results of the clinical trials were often disappointing. Imatinib mesylate for instance, which is very efficient in soft tissue sarcomas, had no palliative or clinical activity in metastatic castration-resistant prostate cancer [105]. Combining it with bisphosphonates and docetaxel did not improve overall survival and brings into question the value of PDGFR inhibition with taxane chemotherapy in prostate cancer bone metastases [105-107]. Similarly, phase III clinical trials did not confirm the combination of dasatinib (which targets c-KIT, EPHA2, PDGFR) and docetaxel in chemotherapy-naïve patients with metastatic castration-resistant prostate cancer (Table III). Sunitinib initially appeared promising in metastatic castration-resistant prostate cancer [115], however the phase III clinical trial did not significantly prolong the overall survival of patients after failure of a docetaxel-based regimen [116]. Sorafenib was developed to target RET and VEGFR [120] and has a moderate activity as a second-line treatment for metastatic castration-resistant prostate cancer [122]. HGFR (c-MET) and its ligand HGF control numerous cellular signalling cascades that direct cell growth, proliferation, survival, and motility, and also regulate the epithelial-mesenchymal transition (EMT) with a strong impact on the development of metastases. Cabozantinib was specifically developed to inhibit the downstream signalling pathways transduced by c-MET and VEGFR [124-131]. Cabozantinib is currently approved by the U.S. Food and Drug Administration for the treatment of progressive, metastatic medullary thyroid cancer. The clinical evaluation demonstrated in phase II clinical trials that the use of this drug appeared clinically relevant in castration-resistant prostate cancer patients, as it improved bone scans and bone biomarkers, and reduced both soft tissue lesions and the number of circulating tumour cells [133-134]. The phase III COMET-II trials that cabozantinib has not fulfilled the promise reported in the phase II trials (Exelixis announcement: <http://www.exelixis.com/investors-media/press-releases>). Indeed, 50% of patients in the cabozantinib arm reported a pain response, compared to 17 percent of patients in the control

arm receiving mitoxantrone/ prednisone. This difference in pain response between the arms was not statistically significant. Tivantinib, another c-MET inhibitor, has shown promising therapeutic value in pre-clinical models [136-137]. Erlotinib has moderate clinical effect as a single-agent in chemotherapy-naïve castration-resistant cancer [142] and its combination with docetaxel did not show any added therapeutic value [143]. Genitinib, lapatinib and vandetanib alone or in combination with other drugs failed to show significant therapeutic activity compared to the conventional drugs in breast and prostate cancers (Table III). Dovotinib is a recently developed multi-RTK inhibitor (FGFR, VEGFR) that has shown interesting pre-clinical activity in metastatic castration-resistant prostate cancer: anti-angiogenic activity, anti-tumour activity and clinical activity in 34 patients with bone metastases [159 ]. However, its combination with histone deacetylase inhibitor did not show any additional value [160]. Clinical trials are required to confirm its therapeutic value.

Although numerous RTK inhibitors initially appeared to be of great interest, based on pre-clinical assessments, most of them have not fulfilled the promise hoped in phase I/II studies. The absence of significant results with their use can be explained by the multiplicity of their targets and the complexity of the mechanisms involved. Indeed, these drugs will affect not only the tumor cells but also its environment. Thus, the Cabozantinib, like dovotinib for instance for which the clinical activity needs to be confirmed, affects the coupling between cancer cells and the bone tumour niche [159, 161, 162]. The bone tumour microenvironment (in bone sarcoma and bone metastases) is then described as a sanctuary that controls at least in part the tumour growth and contributes to the drug resistance acquisition [163, 164]. By modulating the tumour microenvironment, RTK could have a positive and/or a negative impact on the tumour development.

#### **4. Conclusion**

In the last 15 years, there have been high expectations in oncology of therapies with RTK inhibitors. Imatinib mesylate was the first to show spectacular clinical success in chronic myeloid leukaemia patients, and has become the first line of treatment. Gastro-intestinal stromal tumour (GIST) is the second success for the use of an RTK inhibitor, and imatinib mesylate is the standard of care in patients who are at high risk for GIST recurrence following resection [165]. Unfortunately, patients develop resistance and relapse due to protein point mutations and/or the introduction of molecular feedback loops. Many other RTK inhibitors have shown disappointing results in clinical applications after encouraging pre-clinical results. In all cases, the efficacy of RTK inhibitors is linked with their ability to disrupt the crosstalk between tumour cells and their environment. A better understanding of both intracellular signal modulating by these RTK inhibitors, and the feedback loops developed during the establishment of resistance, will increase the chances of success for these drugs. In addition, adapted investigational approaches will be needed to define the expression profile of the RTK genuinely activated/mutated/expressed in patients before their inclusion in clinical trials.

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## REFERENCES

- [1] Garbers D L, Koesling D, Schultz G. Guanylyl cyclase receptors. » *Molecular Biology of the Cell* 1994;5: 1□5.
- [2] Bhattacharya M, Babwah AV, Ferguson SSG. Small GTP-Binding Protein-Coupled Receptors. *Biochem Soc Trans* 2004;32:1040□44.
- [3] Li S, Wong AHC, Liu F. Ligand-gated ion channel interacting proteins and their role in neuroprotection. *Frontiers Cell Neurosci* 2014;8:125.
- [4] Liongue C, Ward AC. Evolution of Class I Cytokine Receptors. *BMC Evol Biol* 2007; 7:120.
- [5] Langer JA., Cutrone EC, Kotenko S. The Class II Cytokine Receptor (CRF2) Family: Overview and Patterns of Receptor–ligand Interactions. *Cytokine Growth Factor Rev* 2004;15:33□48.
- [6] Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Curr Opin Immunol* 2011;23:598-604.
- [7] Blanchard F, Duplomb L, Baud'huin M, Brounais B. The dual role of IL-6-type cytokines on bone remodeling and bone tumors. *Cytokine Growth Factor Rev* 2009;20:19-28.
- [8]. Heymann D Ed. Bone Cancer, Primary bone cancers and bone metastases, 2<sup>nd</sup> Ed, San Diego, USA, Academic Press ; 2014.
- [9] Robins DR, Wu YM, Lin SF. The Protein Tyrosine Kinase Family of the Human Genome. *Oncogene* 2000;19:5548□57.
- [10] Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature*. 2001;411:355-65.
- [11] Arkin IT. Structural aspects of oligomerization taking place between the transmembrane  $\alpha$ -helices of bitopic membrane proteins. *Biochim Biophys Acta* 2002;1565:347-63.
- [12] Moriki T, Maruyama H, Maruyama IN. Activation of Preformed EGF Receptor Dimers by Ligand-Induced Rotation of the Transmembrane Domain. *J Mol Biol* 2001;311:1011□26.
- [13] Hubbard SR, Till JH. Protein Tyrosine Kinase Structure and Function. *Ann Rev Biochem* 2000;69:373□98.
- [14] Lemmon MA, Schlessinger J. Cell Signaling by Receptor Tyrosine Kinases. *Cell* 2010;141:1117□34.
- [15] Ogiso H, Ishitani R, Nureki O, Fukai S, Yamanaka M, Kim JH, et al. Crystal Structure of the Complex of Human Epidermal Growth Factor and Receptor Extracellular Domains. *Cell* 2002;110:775□87.

- [16] Bradshaw RA, Chalkley RJ, Biarc J, Burlingame AL. Receptor tyrosine kinase signaling mechanisms: Devolving TrkA responses with phosphoproteomics. *Adv Biol Regul* 2013;53: 87–96.
- [17] Hubbard SR. Autoinhibitory Mechanisms in Receptor Tyrosine Kinases. *Frontiers in Bioscience* 2002;7:330–40.
- [18] Hubbard SR, Miller WT. Receptor tyrosine kinases: mechanisms of activation and signaling. *Curr Opin Cell Biol* 2007;19:117–23.
- [19] Liu Y, Rohrschneider LR. The Gift of Gab. *FEBS Lett* 2002;515:1–7.
- [20] Choudhary C, Mann M. Decoding Signalling Networks by Mass Spectrometry-Based Proteomics. *Nature Rev Mol Cell Biol* 2010;11:427–39.
- [21] Cseh B, Doma E, Baccarini M. “RAF” neighborhood: Protein–protein interaction in the Raf/Mek/Erk pathway. *FEBS Lett* 2014;588: 2398–406.
- [22] Roskoski Jr R. ERK1/2 MAP kinases: Structure, function, and regulation. *Pharmacol Res* 2012;66:105–43.
- [23] Raman M, Chen W, Cobb MH. Differential Regulation and Properties of MAPKs. *Oncogene* 2007;26: 3100–112.
- [24] Cargnello M, et Roux PP. Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. *Microbiol Mol Biol Rev* 2011;75:50–83.
- [25] Song G, Ouyang G, Bao S. The Activation of Akt/PKB Signaling Pathway and Cell Survival. *J Cell Mol Med* 2005;9:59–71.
- [26] Ralston R, Bishop JM. The Product of the Protooncogene c-Src Is Modified during the Cellular Response to Platelet-Derived Growth Factor. *Proc Natl Acad Sci USA* 1985;82: 7845–49.
- [27] Bromann PA, Korkaya H, Courtneidge SA. The Interplay between Src Family Kinases and Receptor Tyrosine Kinases. *Oncogene* 2004;23:7957–68.
- [28] Biscardi JS, Maa MC, Tice DA, Cox ME, Leu TH, Parsons SJ. C-Src-Mediated Phosphorylation of the Epidermal Growth Factor Receptor on Tyr845 and Tyr1101 Is Associated with Modulation of Receptor Function. *J Biol Chem* 1999;274: 8335–43.
- [29] Moro L, Dolce L, Cabodi S, Bergatto E, Erba EB, Smerigli M, et al. Integrin-Induced Epidermal Growth Factor (EGF) Receptor Activation Requires c-Src and p130Cas and Leads to Phosphorylation of Specific EGF Receptor Tyrosines. *J Biol Chem* 2002;277: 9405–9414.
- [30] Goi T, Shipitsin M, Lu Z, Foster DA, Klinz SG, Feig LA. An EGF receptor/Ral-GTPase Signaling Cascade Regulates c-Src Activity and Substrate Specificity. *EMBO J* 2000;19:623–30.

- [31] Livio T, Berlotti A, Comoglio PM. MET signaling: principles and functions in development, organ regeneration and cancer. *Nature Rev Mol Cell Biol* 2010;11:834-848.
- [32] Freeman M. Feedback control of intercellular signaling in development. *Nature* 2000 ;408:313-319.
- [33] Amit I, Citri A, Shay T, Lu Y, Katz M, Zhang F, Tarcic G, Siwak D, Lahad J, Jacob-Hirsch J, Amariglio N, Vaisman N, Segal E, Rechavi G, Alon U, Mills GB, Domany E, Yarden Y. A module of negative feedback regulators defines growth factor signaling. *Nature Genet* 2007 ;39 :503-512.
- [34] Santos SD, Verveer PJ, Bastiaens PI. Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. *Nat Cell Biol* 2007;9:324-30.
- [35] Dikic I, Giordano S. Negative receptor signalling. *Curr Opin Cell Biol* 2003;15:128-35.
- [36] Marmor MD, Yarden Y. Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. *Oncogene* 2004 ;23:2057-70.
- [37] Jiang X, Huang F, Marusyk A, Sorkin A. Grb2 regulates internalization of EGF receptors through clathrin-coated pits. *Mol Biol Cell* 2003 ;14:858-70.
- [38] Lu Z, Jiang G, -Jensen P, Hunter T. Epidermal growth factor-induced tumor cell invasion and metastasis initiated by dephosphorylation and downregulation of focal adhesion kinase. *Mol Cell Biol*. 2001;21:4016-31.
- [39] Petti LM, Irusta PM, DiMaio D. Oncogenic activation of the PDGF beta receptor by the transmembrane domain of p185neu\*. *Oncogene* 1998 ;16:843-51.
- [40] Meshinchi S, Appelbaum FR. Structural and functional alterations of FLT3 in acute myeloid leukemia. *Clin Cancer Res* 2009 ;15:4263-9.
- [41] Antonescu CR. The GIST paradigm: lessons for other kinase-driven cancers. *J Pathol* 2011;223:251-61.
- [42] Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129-39.
- [43] Bose R, Kavuri SM, Searleman AC, Shen W, Shen D, Koboldt DC, Monsey J, Goel N, Aronson AB, Li S, Ma CX, Ding L, Mardis ER, Ellis MJ. Activating HER2 mutations in HER2 gene amplification negative breast cancer. *Cancer Discov* 2013;3:224-37.
- [44] Nakajima H, Ishikawa Y, Furuya M, Sano T, Ohno Y, Horiguchi J, Oyama T. Protein expression, gene amplification, and mutational analysis of EGFR in triple-negative breast cancer. *Breast Cancer* 2014;21:66-74.
- [45] Peraldo-Neia C, Migliardi G, Mello-Grand M, Montemurro F, Segir R, Pignochino Y, Cavalloni G, Torchio B, Mosso L, Chiorino G, Aglietta M. Epidermal Growth Factor



Receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in primary prostate cancer. *BMC Cancer*. 2011;11:31.

[46] Fu M, Zhang W, Shan L, Song J, Shang D, Ying J, Zhao J. Mutation status of somatic EGFR and KRAS genes in Chinese patients with prostate cancer (PCa). *Virchows Arch* 2014; 464:575-81.

[47] Marie PJ. Signaling pathways affecting skeletal health. *Curr Osteoporos Rep* 2012; 10:190-8.

[48] Marie PJ. Fibroblast growth factor signaling controlling bone formation: an update. *Gene* 2012; 498:1-4

[49] Dai J, Rabie AB. VEGF: an essential mediator of both angiogenesis and endochondral ossification. *J Dent Res* 2007;86:937-50.

[50] Al-Kharobi H, El-Gendy R, Devine DA, Beattie J. The role of the insulin-like growth factor (IGF) axis in osteogenic and odontogenic differentiation. *Cell Mol Life Sci* 2014; 71:1469-76

[51] Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *Bonekey Rep* 2014; 3:481.

[52] Heymann D, Guicheux J, Gouin F, Passuti N, Daculsi G. Cytokines, growth factors and osteoclasts. *Cytokine* 1998; 10:155-68.

[53] Clarkin CE, Gerstenfeld LC. VEGF and bone cell signalling: an essential vessel for communication? *Cell Biochem Funct* 2013; 31:1-11

[54] Crane JL, Cao X. Function of matrix IGF-1 in coupling bone resorption and formation. *J Mol Med (Berl)* 2014; 92:107-15.

[55] Bao NR, Lu M, Bin FW, Chang ZY, Meng J, Zhou LW, Guo T, Zhao JN. Systematic screen with kinases inhibitors reveals kinases play distinct roles in growth of osteoprogenitor cells. *Int J Clin Exp Pathol* 2013; 6:2082-91.

[56] O'Sullivan S, Lin JM, Watson M, Callon K, Tong PC, Naot D, Horne A, Aati O, Porteous F, Gamble G, Cornish J, Browett P, Grey A. The skeletal effects of the tyrosine kinase inhibitor nilotinib. *Bone* 2011; 49:281–289.

[57] Pinski J, Weeraratna A, Uzgaré AR, Arnold JT, Denmeade SR, Isaacs JT. Trk receptor inhibition induces apoptosis of proliferating but not quiescent human osteoblasts. *Cancer Res* 2002; 62:986-9.

[58] Duan Z, Choy E, Harmon D, Yang C, Ryu K, Schwab J, Mankin H, Hornicek FJ. Insulin-like growth factor-I receptor tyrosine kinase inhibitor cyclo lignan picropodophyllin inhibits proliferation and induces apoptosis in multidrug resistant osteosarcoma cell lines. *Mol Cancer Ther* 2009; 8:2122–2130.

[59] Vandyke K, Fitter S, Dewar AL, Hughes TP, Zannettino AC. Dysregulation of bone

remodeling by imatinib mesylate. *Blood* 2010; 115(4):766-74.

[60] Gobin B, Moriceau G, Ory B, Charrier C, Brion R, Blanchard F, Redini F, Heymann D. Imatinib mesylate exerts anti-proliferative effects on osteosarcoma cells and inhibits the tumour growth in immunocompetent murine models. *PLoS One* 2014; 9:e90795.

[61] El Hajj Dib I, Gallet M, Mentaverri R, Sévenet N, Brazier M, Kamel S. Imatinib mesylate (Gleevec) enhances mature osteoclast apoptosis and suppresses osteoclast bone resorbing activity. *Eur J Pharmacol* 2006; 551:27-33.

[62] Rimondi E, Secchiero P, Melloni E, Grill V, Zauli G. Sorafenib inhibits in vitro osteoclastogenesis by down-modulating Mcl-1. *Invest New Drugs* 2013; 31:780-6.

[63] Vandyke K, Dewar AL, Diamond P, Fitter S, Schultz CG, Sims NA, Zannettino AC. The tyrosine kinase inhibitor dasatinib dysregulates bone remodeling through inhibition of osteoclasts in vivo. *J Bone Miner Res* 2010; 5:1759-70.

[64] Garcia-Gomez A, Ocio EM, Crusoe E, Santamaria C, Hernández-Campo P, Blanco JF, Sanchez-Guijo FM, Hernandez-Iglesias T, Brinon JG, Fisac-Herrero RM, Lee FY, Pandiella A, San Miguel JF, Garayoa M. Dasatinib as a bone-modifying agent: anabolic and anti-resorptive effects. *PLoS One* 2012; 7:e34914.

[65] Heymann D, Redini F. Bone sarcomas : pathogenesis and new therapeutic approaches. *IBMS BoneKey* 2011 ; 8 :402-414.

[66] Heymann D, Redini F. Targeted therapies for bone sarcomas. *Bonekey Rep* 2013; 2:378.

[67] Gaspar N, Di Giannatale A, Geoerger B, Redini F, Corradini N, Enz-Werle N, Tirode F, Marec-Berard P, Gentet JC, Laurence V, Piperno-Neumann S, Oberlin O, Brugieres L. Bone sarcomas: from biology to targeted therapies. *Sarcoma* 2012 ; 301975.

[68] Rettew AN, Getty PJ, Greenfield EM. Receptor tyrosine kinases in osteosarcoma: not just the usual suspects. *Adv Exp Med Biol* 2014;804:47-66.

[69] McGary EC, Weber K, Mills L, Doucet M, Lewis V, Lev DC, Fidler IJ, Bar-Eli M. Inhibition of platelet-derived growth factor-mediated proliferation of osteosarcoma cells by the novel tyrosine kinase inhibitor STI571. *Clin Cancer Res* 2002; 8:3584-91.

[70] Ikeda AK, Judelson DR, Federman N, Glaser KB, Landaw EM, Denny CT, Sakamoto KM. ABT-869 inhibits the proliferation of Ewing Sarcoma cells and suppresses platelet-derived growth factor receptor beta and c-KIT signaling pathways. *Mol Cancer Ther* 2010; 9:653-60.

[71] Chugh R, Wathen JK, Maki RG, Benjamin RS, Patel SR, Meyers PA, Priebat DA, Reinke DK, Thomas DG, Keohan ML, Samuels BL, Baker LH. Phase II multicenter trial of imatinib in 10 histologic subtypes of sarcoma using a bayesian hierarchical statistical model. *J Clin Oncol* 2009;27:3148–3153.

[72] Chao J, Budd GT, Chu P, Frankel P, Garcia D, Junqueira M, Loera S, Somlo G, Sato J, Chow WA. Phase II clinical trial of imatinib mesylate in therapy of KIT and/or PDGFRalpha-

expressing Ewing sarcoma family of tumours and desmoplastic small round cell tumours. *Anticancer Res* 2010;30:547–552.

[73] Bond M, Bernstein ML, Pappo A, Schultz KR, Krailo M, Blaney SM, Adamson PC. A phase II study of imatinib mesylate in children with refractory or relapsed solid tumours: a Children's Oncology Group study. *Pediatr Blood Cancer* 2008;50:254–258.

[74] Gonzalez I, Andreu EJ, Panizo A, Inoges S, Fontalba A, Fernandez-Luna JL, Gaboli M, Sierrasesumaga L, Martín-Algarra S, Pardo J, Prosper F, de Alava E. Imatinib inhibits proliferation of Ewing tumour cells mediated by the stem cell factor/KIT receptor pathway, and sensitizes cells to vincristine and doxorubicin-induced apoptosis. *Clin Cancer Res* 2004;10:751–761.

[75] Timeus F, Crescenzo N, Fandi A, Doria A, Foglia L, Cordero di Montezemolo L. In vitro antiproliferative and antimigratory activity of dasatinib in neuroblastoma and Ewing sarcoma cell lines. *Oncol Rep* 2008;19:353–359.

[76] Hingorani P, Zhang W, Gorlick R, Kolb EA. Inhibition of Src phosphorylation alters metastatic potential of osteosarcoma in vitro but not in vivo. *Clin Cancer Res* 2009;15:3416–3422.

[77] Aplenc R, Blaney SM, Strauss LC, Balis FM, Shusterman S, Ingle AM et al. Pediatric phase I trial and pharmacokinetic study of dasatinib: a report from the children's oncology group phase I consortium. *J Clin Oncol* 2011;29:839–844.

[78] Maris JM, Courtright J, Houghton PJ, Morton CL, Kolb EA, Lock R, Tajbakhsh M, Reynolds CP, Keir ST, Wu J, Smith MA. Initial testing (stage 1) of sunitinib by the pediatric preclinical testing program. *Pediatr Blood Cancer* 2008;51:42–48.

[79] Dubois SG, Shusterman S, Ingle AM, Ahern CH, Reid JM, Wu B, Baruchel S, Glade-Bender J, Ivy P, Grier HE, Adamson PC, Blaney SM. Phase I and pharmacokinetic study of sunitinib in pediatric patients with refractory solid tumours: a children's oncology group study. *Clin Cancer Res* 2011;17:5113–5122.

[80] Keir ST, Morton CL, Wu J, Kurmasheva RT, Houghton PJ, Smith MA. Initial testing of the multitargeted kinase inhibitor pazopanib by the pediatric preclinical testing program. *Pediatr Blood Cancer* 2012;59:586–588.

[81] Glade Bender JL, Lee A, Reid JM, Baruchel S, Roberts T, Voss SD, Wu B, Ahern CH, Ingle AM, Harris P, Weigel BJ, Blaney SM. Phase I Pharmacokinetic and Pharmacodynamic Study of Pazopanib in Children With Soft Tissue Sarcoma and Other Refractory Solid Tumors: A Children's Oncology Group Phase I Consortium Report. *J Clin Oncol* 2013; 31: 3034–3043.

[82] Kumar S, Mokhtari RB, Sheikh R, Wu B, Zhang L, Xu P, Man S, Oliveira ID, Yeger H, Kerbel RS, Baruchel S. Metronomic oral topotecan with pazopanib is an active antiangiogenic regimen in mouse models of aggressive pediatric solid tumour. *Clin Cancer Res* 2011;17:5656–5667.

[83] Pignochino Y, Grignani G, Cavalloni G, Motta M, Tapparo M, Bruno S, Bottos A,

Gammaitoni L, Migliardi G, Camussi G, Alberghini M, Torchio B, Ferrari S, Bussolino F, Fagioli F, Picci P, Aglietta M. Sorafenib blocks tumour growth, angiogenesis and metastatic potential in preclinical models of osteosarcoma through a mechanism potentially involving the inhibition of ERK1/2, MCL-1 and ezrin pathways. *Mol Cancer* 2009;8:118.

[84] Navid F, Baker SD, McCarville MB, Stewart CF, Billups CA, Wu J, Davidoff AM, Spunt SL, Furman WL, McGregor LM, Hu S, Panetta JC, Turner D, Fofana D, Reddick WE, Leung W, Santana VM. Phase I and Clinical Pharmacology Study of Bevacizumab, Sorafenib, and Low-dose Cyclophosphamide in Children and Young Adults with Refractory/Recurrent Solid Tumors. *Clin Cancer Res* 2013; 19: 236–246.

[85] Grignani G, Palmerini E, Dileo P, Asaftei SD, D'Ambrosio L, Pignochino Y, Mercuri M, Picci P, Fagioli F, Casali PG, Ferrari S, Aglietta M. A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study. *Ann Oncol* 2012;23:508–516.

[86] Han J, Tian R, Yong B, Luo C, Tan P, Shen J, Peng T. Gas6/Axl mediates tumor cell apoptosis, migration and invasion and predicts the clinical outcome of osteosarcoma patients. *Biochem Biophys Res Commun* 2013; 435:493-500.

[87] Rettew AN, Getty PJ, Greenfield EM. Receptor tyrosine kinases in osteosarcoma: not just the usual suspects. *Adv Exp Med Biol* 2014;804:47-66.

[88] Zhang Y, Tang YJ, Man Y, Pan F, Li ZH, Jia LS. Knockdown of AXL receptor tyrosine kinase in osteosarcoma cells leads to decreased proliferation and increased apoptosis. *Int J Immunopathol Pharmacol* 2013; 26:179–188

[89] Fleuren ED, Hillebrandt-Roeffen MH, Flucke UE, Te Loo DM, Boerman OC, van der Graaf WT, Versleijen-Jonkers YM. The role of AXL and the in vitro activity of the receptor tyrosine kinase inhibitor BGB324 in Ewing sarcoma. *Oncotarget*, in press.

[90] Fleuren ED, Roeffen MH, Leenders WP, Flucke UE, Vlenterie M, Schreuder HW, Boerman OC, van der Graaf WT, Versleijen-Jonkers YM. Expression and clinical relevance of MET and ALK in Ewing sarcomas. *Int J Cancer* 2013;133:427-36.

91] Sampson ER, Martin BA, Morris AE, Xie C, Schwarz EM, O'Keefe RJ, Rosier RN. The orally bioavailable met inhibitor PF-2341066 inhibits osteosarcoma growth and osteolysis/matrix production in a xenograft model. *J Bone Miner Res* 2011;26:1283-94.

[92] Fritsche-Guenther R, Noske A, Ungethüm U, Kuban RJ, Schlag PM, Tunn PU, Karle J, Krenn V, Dietel M, Sers C. De novo expression of EphA2 in osteosarcoma modulates activation of the mitogenic signalling pathway. *Histopathol* 2010;57: 836–850

[93] Mstuo K, Otaki N. Bone cell interactions through Eph/ephrin: bone modeling, remodeling and associated diseases. *Cell Adh Migr* 2012 ;6: 148–156

[94] Posthumadeboer J, Piersma SR, Pham TV, van Egmond PW, Knol JC, Cleton-Jansen AM, van Geer MA, van Beusechem VW, Kaspers GJ, van Royen BJ, Jiménez CR, Helder MN. Surface proteomic analysis of osteosarcoma identifies EPHA2 as receptor for targeted

drug delivery. *Br J Cancer* 2013;109: 2142–2154.

[95] Kuijjer ML, Peterse EF, van den Akker BE, Briaire-de Bruijn IH, Serra M, Meza-Zepeda LA, Myklebost O, Hassan AB, Hogendoorn PC, Cleton-Jansen AM. IR/IGF1R signaling as potential target for treatment of high-grade osteosarcoma. *BMC Cancer* 2013 ; 13: 245.

[96] Cao Y, Roth M, Piperdi S, Montoya K, Sowers R, Rao P, Geller D, Houghton P, Kolb EA, Gill J, Gorlick R. Insulin-like growth factor 1 receptor and response to anti-IGF1R antibody therapy in osteosarcoma. *PLoS One* 2014;9:e106249.

[97] Lee JA, Ko Y, Kim DH, Lim JS, Kong CB, Cho WH, Jeon DG, Lee SY, Koh JS. Epidermal growth factor receptor: is it a feasible target for the treatment of osteosarcoma? *Cancer Res Treat* 2012 ;44: 202–209

[98] Gill J, Geller D, Gorlick R. HER-2 involvement in osteosarcoma. *Adv Exp Med Biol* 2014;804:161-77.

[99] Ebb D, Meyers P, Grier H, Bernstein M, Gorlick R, Lipshultz SE, Krailo M, Devidas M, Barkauskas DA, Siegal GP, Ferguson WS, Letson GD, Marcus K, Goorin A, Beardsley P, Marina N. Phase II trial of trastuzumab in combination with cytotoxic chemotherapy for treatment of metastatic osteosarcoma with human epidermal growth factor receptor 2 overexpression: a report from the children's oncology group. *J Clin Oncol* 2012;30:2545-51.

[100] Macheda ML, Stacker SA. Importance of Wnt signaling in the tumour stroma microenvironment. *Curr Cancer Drug Targets* 2008 ;8: 454–465.

[101] Truitt L, Freywald A. Dancing with the dead: Eph receptors and their kinase-null partners. *Biochem Cell Biol* 2011 ;89: 115–129

[102] Clézardin P. Pathophysiology of bone metastases and new molecular targets involved in bone remodelling. *Bull Cancer* 2013;100:1083-91.

[103] Uehara H, Kim SJ, Karashima T, Shepherd DL, Fan D, Tsan R, Killion JJ, Logothetis C, Mathew P, Fidler IJ. Effects of blocking platelet-derived growth factor-receptor signaling in a mouse model of experimental prostate cancer bone metastases. *J Natl Cancer Inst* 2003;95:458-70.

[104] Kim SJ, Uehara H, Yazici S, Busby JE, Nakamura T, He J, Maya M, Logothetis C, Mathew P, Wang X, Do KA, Fan D, Fidler IJ. Targeting platelet-derived growth factor receptor on endothelial cells of multidrug-resistant prostate cancer. *J Natl Cancer Inst* 2006;98:783-93.

[105] Tiffany NM, Wersinger EM, Garzotto M, Beer TM. Imatinib mesylate and zoledronic acid in androgen-independent prostate cancer. *Urology* 2004;63:934-9.

[106] Mathew P, Thall PF, Jones D, Perez C, Bucana C, Troncoso P, Kim SJ, Fidler IJ, Logothetis C. Platelet-derived growth factor receptor inhibitor imatinib mesylate and docetaxel: a modular phase I trial in androgen-independent prostate cancer. *J Clin Oncol* 2004;22:3323-9.

- [107] Mathew P, Thall PF, Bucana CD, Oh WK, Morris MJ, Jones DM, Johnson MM, Wen S, Pagliaro LC, Tannir NM, Tu SM, Meluch AA, Smith L, Cohen L, Kim SJ, Troncoso P, Fidler IJ, Logothetis CJ. Platelet-derived growth factor receptor inhibition and chemotherapy for castration-resistant prostate cancer with bone metastases. *Clin Cancer Res* 2007;13:5816-24.
- [108] Liu Y, Karaca M, Zhang Z, Gioeli D, Earp HS, Whang YE. Dasatinib inhibits site-specific tyrosine phosphorylation of androgen receptor by Ack1 and Src kinases. *Oncogene*. 2010;29:3208-16.
- [109] Araujo JC, Poblenz A, Corn P, Parikh NU, Starbuck MW, Thompson JT, Lee F, Logothetis CJ, Darnay BG. Dasatinib inhibits both osteoclast activation and prostate cancer PC-3-cell-induced osteoclast formation. *Cancer Biol Ther* 2009;8:2153-9.
- [109] Koreckij T, Nguyen H, Brown LG, Yu EY, Vessella RL, Corey E. Dasatinib inhibits the growth of prostate cancer in bone and provides additional protection from osteolysis. *Br J Cancer* 2009;101:263-8.
- [110] Takahashi S, Miyazaki M, Okamoto I, Ito Y, Ueda K, Seriu T, Nakagawa K, Hatake K. Phase I study of dasatinib (BMS-354825) in Japanese patients with solid tumors. *Cancer Sci* 2011;102:2058-64.
- [111] Yu EY, Massard C, Gross ME, Carducci MA, Culine S, Hudes G, Posadas EM, Sternberg CN, Wilding G, Trudel GC, Paliwal P, Fizazi K. Once-daily dasatinib: expansion of phase II study evaluating safety and efficacy of dasatinib in patients with metastatic castration-resistant prostate cancer. *Urology* 2011;77:1166-71.
- [112] Yu EY, Wilding G, Posadas E, Gross M, Culine S, Massard C, Morris MJ, Hudes G, Calabrò F, Cheng S, Trudel GC, Paliwal P, Sternberg CN. Phase II study of dasatinib in patients with metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2009;15:7421-8.
- [113] Araujo JC, Trudel GC, Saad F, Armstrong AJ, Yu EY, Bellmunt J, Wilding G, McCaffrey J, Serrano SV, Matveev VB, Efsthathiou E, Oudard S, Morris MJ, Sizer B, Goebell PJ, Heidenreich A, de Bono JS, Begbie S, Hong JH, Richardet E, Gallardo E, Paliwal P, Durham S, Cheng S, Logothetis CJ. Docetaxel and dasatinib or placebo in men with metastatic castration-resistant prostate cancer (READY): a randomised, double-blind phase 3 trial. *Lancet Oncol* 2013;14:1307-16.
- [114] Zwolak P, Jasinski P, Terai K, Gallus NJ, Ericson ME, Clohisy DR, Dudek AZ. Addition of receptor tyrosine kinase inhibitor to radiation increases tumour control in an orthotopic murine model of breast cancer metastasis in bone. *Eur J Cancer* 2008;44:2506-17.
- [115] Sonpavde G, Periman PO, Bernold D, Weckstein D, Fleming MT, Galsky MD, Berry WR, Zhan F, Boehm KA, Asmar L, Hutson TE. Sunitinib malate for metastatic castration-resistant prostate cancer following docetaxel-based chemotherapy. *Ann Oncol* 2010;21:319-24.
- [116] Michaelson MD, Oudard S, Ou YC, Sengeløv L, Saad F, Houede N, Ostler P, Stenzl A, Daugaard G, Jones R, Laestadius F, Ullén A, Bahl A, Castellano D, Gschwend J, Maurina T,

Chow Maneval E, Wang SL, Lechuga MJ, Paolini J, Chen I. Randomized, placebo-controlled, phase III trial of sunitinib plus prednisone versus prednisone alone in progressive, metastatic, castration-resistant prostate cancer. *J Clin Oncol* 2014;32:76-82.

[117] Bachelot T, Garcia-Saenz JA, Verma S, Gutierrez M, Pivot X, Kozloff MF, Prady C, Huang X, Khosravan R, Wang Z, Cesari R, Tassell V, Kern KA, Blay JY, Lluch A. Sunitinib in combination with trastuzumab for the treatment of advanced breast cancer: activity and safety results from a phase II study. *BMC Cancer* 2014;14:166.

[118] Beuselinck B, Oudard S, Rixe O, Wolter P, Blesius A, Ayllon J, Elaidi R, Schöffski P, Barrascout E, Morel A, Escudier B, Lang H, Zucman-Rossi J, Medioni J. Negative impact of bone metastasis on outcome in clear-cell renal cell carcinoma treated with sunitinib. *Ann Oncol* 2011;22:794-800.

[119] Keizman D, Ish-Shalom M, Pili R, Hammers H, Eisenberger MA, Sinibaldi V, Boursi B, Maimon N, Gottfried M, Hayat H, Peer A, Kovel S, Sella A, Berger R, Carducci MA. Bisphosphonates combined with sunitinib may improve the response rate, progression free survival and overall survival of patients with bone metastases from renal cell carcinoma. *Eur J Cancer* 2012;48:1031-7.

[120] Merz M, Komljenovic D, Zwick S, Semmler W, Bäuerle T. Sorafenib tosylate and paclitaxel induce anti-angiogenic, anti-tumour and anti-resorptive effects in experimental breast cancer bone metastases. *Eur J Cancer* 2011;47:277-86.

[121] Dahut WL, Scripture C, Posadas E, Jain L, Gulley JL, Arlen PM, Wright JJ, Yu Y, Cao L, Steinberg SM, Aragon-Ching JB, Venitz J, Jones E, Chen CC, Figg WD. A phase II clinical trial of sorafenib in androgen-independent prostate cancer. *Clin Cancer Res* 2008;14:209-14.

[122] Aragon-Ching JB, Jain L, Gulley JL, Arlen PM, Wright JJ, Steinberg SM, Draper D, Venitz J, Jones E, Chen CC, Figg WD, Dahut WL. Final analysis of a phase II trial using sorafenib for metastatic castration-resistant prostate cancer. *BJU Int* 2009;103:1636-40..

[123] Sciarra A, Autran Gomez AM, Gentilucci A, Parente U, Salciccia S, Gentile V, Di Silverio F. Adjuvant therapy with sorafenib in bone metastases bilateral renal carcinoma: a case report. *Eur Urol*. 2007;52:597-9.

[124] Humphrey PA, Zhu X, Zarnegar R, Swanson PE, Ratliff TL, Vollmer RT, Day ML. Hepatocyte growth factor and its receptor (c-MET) in prostatic carcinoma. *Am J Pathol* 1995;147:386-96.

[125] Pisters LL, Troncoso P, Zhau HE, Li W, von Eschenbach AC, Chung LW. c-met proto-oncogene expression in benign and malignant human prostate tissues. *J Urol* 1995;154:293-8.

[126] Nagy J, Curry GW, Hillan KJ, McKay IC, Mallon E, Purushotham AD, George WD. Hepatocyte growth factor/scatter factor expression and c-met in primary breast cancer. *Surg Oncol* 1996;5:15-21.

[127] Tuck AB, Park M, Sterns EE, Boag A, Elliott BE. Coexpression of hepatocyte growth factor and receptor (Met) in human breast carcinoma. *Am J Pathol* 1996;148:225-32.

- [128] Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4(12):915-25.
- [129] Ponzo MG, Lesurf R, Petkiewicz S, O'Malley FP, Pinnaduwaage D, Andrulis IL, Bull SB, Chughtai N, Zuo D, Souleimanova M, Germain D, Omeroglu A, Cardiff RD, Hallett M, Park M. Met induces mammary tumors with diverse histologies and is associated with poor outcome and human basal breast cancer. *Proc Natl Acad Sci U S A*. 2009;106:12903-8.
- [130] Previdi S, Scolari F, Chilà R, Ricci F, Abbadessa G, Brogginì M. Combination of the c-Met inhibitor tivantinib and zoledronic acid prevents tumor bone engraftment and inhibits progression of established bone metastases in a breast xenograft model. *PLoS One* 2013;8:e79101.
- [131] Graham TJ, Box G, Tunariu N, Crespo M, Spinks TJ, Miranda S, Attard G, de Bono J, Eccles SA, Davies FE, Robinson SP. Preclinical evaluation of imaging biomarkers for prostate cancer bone metastasis and response to cabozantinib. *J Natl Cancer Inst*, in press.
- [132] Lee RJ, Saylor PJ, Michaelson MD, Rothenberg SM, Smas ME, Miyamoto DT, Gurski CA, Xie W, Maheswaran S, Haber DA, Goldin JG, Smith MR. A dose-ranging study of cabozantinib in men with castration-resistant prostate cancer and bone metastases. *Clin Cancer Res* 2013;19:3088-94.
- [133] Smith MR, Sweeney CJ, Corn Smith MR, Sweeney CJ, Corn PG, Rathkopf DE, Smith DC, Hussain M, George DJ, Higano CS, Harzstark AL, Sartor AO, Vogelzang NJ, Gordon MS, de Bono JS, Haas NB, Logothetis CJ, Elfiky A, Scheffold C, Laird AD, Schimmoller F, Basch EM, Scher HI. Cabozantinib in chemotherapy-pretreated metastatic castration-resistant prostate cancer: results of a phase II nonrandomized expansion study. *J Clin Oncol* 2014;32:3391-9.
- [134] Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, Vogelzang NJ, Small EJ, Harzstark AL, Gordon MS, Vaishampayan UN, Haas NB, Spira AI, Lara PN Jr, Lin CC, Srinivas S, Sella A, Schöffski P, Scheffold C, Weitzman AL, Hussain M. Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial. *J Clin Oncol* 2013;31:412-9.
- [135] Basch E, Autio KA, Smith MR, Bennett AV, Weitzman AL, Scheffold C, Sweeney C, Rathkopf DE, Smith DC, George DJ, Higano CS, Harzstark AL, Sartor AO, Gordon MS, Vogelzang NJ, de Bono JS, Haas NB, Corn PG, Schimmoller F, Scher HI. Effects of Cabozantinib on Pain and Narcotic Use in Patients with Castration-resistant Prostate Cancer: Results from a Phase 2 Nonrandomized Expansion Cohort. *Eur Urol* in press.
- [136] Previdi S, Scolari F, Chilà R, Ricci F, Abbadessa G, Brogginì M. Combination of the c-Met inhibitor tivantinib and zoledronic acid prevents tumor bone engraftment and inhibits progression of established bone metastases in a breast xenograft model. *PLoS One* 2013;8:e79101.
- [137] Previdi S, Abbadessa G, Dalò F, France DS, Brogginì M. Breast cancer-derived bone metastasis can be effectively reduced through specific c-MET inhibitor tivantinib (ARQ 197) and shRNA c-MET knockdown. *Mol Cancer Ther* 2012;11:214-23.



- [138] Yin JJ, Zhang L, Munasinghe J, Linnoila RI, Kelly K. Ceranib/AZD2171 inhibits bone and brain metastasis in a preclinical model of advanced prostate cancer. *Cancer Res* 2010 ; 70 :8662-8673.
- [139] Ryan CJ, Stadler WM, Roth B, Hutcheon D, Conry S, Puchalski T, Morris C, Small EJ. Phase I dose escalation and pharmacokinetic study of AZD2171, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinase, in patients with hormone refractory prostate cancer (HRPC). *Invest New Drugs* 2007;25(5):445-51.
- [140] Bachelier R, Confavreux CB, Peyruchaud O, Croset M, Goehrig D, van der Pluijm G, Clézardin P. Combination of anti-angiogenic therapies reduces osteolysis and tumor burden in experimental breast cancer bone metastasis. *Int J Cancer* 2014;135:1319-29.
- [141] Furugaki K, Moriya Y, Iwai T, Yorozu K, Yanagisawa M, Kondoh K, Fujimoto-Ohuchi K, Mori K. Erlotinib inhibits osteolytic bone invasion of human non-small-cell lung cancer cell line NCI-H292. *Clin Exp Metastasis* 2011;28:649-59.
- [142] Nabhan C, Lestingi TM, Galvez A, Tolzien K, Kelby SK, Tsarwhas D, Newman S, Bitran JD. Erlotinib has moderate single-agent activity in chemotherapy-naïve castration-resistant prostate cancer: final results of a phase II trial. *Urology* 2009;74:665-71.
- [143] Gross M, Higano C, Pantuck A, Castellanos O, Green E, Nguyen K, Agus DB. A phase II trial of docetaxel and erlotinib as first-line therapy for elderly patients with androgen-independent prostate cancer. *BMC Cancer* 2007;7:142.
- [144] D'Alessio A, De Luca A, Maiello MR, Lamura L, Rachiglio AM, Napolitano M, Gallo M, Normanno N. Effects of the combined blockade of EGFR and ErbB-2 on signal transduction and regulation of cell cycle regulatory proteins in breast cancer cells. *Breast Cancer Res Treat* 2010;123:387-96.
- [145] Borghese C, Cattaruzza L, Pivetta E, Normanno N, De Luca A, Mazzucato M, Celegato M, Colombatti A, Aldinucci D. Gefitinib inhibits the cross-talk between mesenchymal stem cells and prostate cancer cells leading to tumor cell proliferation and inhibition of docetaxel activity. *J Cell Biochem* 2013;114:1135-44.
- [146] Sgambato A, Camerini A, Faraglia B, Ardito R, Bianchino G, Spada D, Boninsegna A, Valentini V, Cittadini A. Targeted inhibition of the epidermal growth factor receptor-tyrosine kinase by ZD1839 ('Iressa') induces cell-cycle arrest and inhibits proliferation in prostate cancer cells. *J Cell Physiol* 2004;201:97-105.
- [147] Pezaro C, Rosenthal MA, Gurney H, Davis ID, Underhill C, Boyer MJ, Kotasek D, Solomon B, Toner GC. An open-label, single-arm phase two trial of gefitinib in patients with advanced or metastatic castration-resistant prostate cancer. *Am J Clin Oncol* 2009;32:338-41.
- [148] Boccardo F, Rubagotti A, Conti G, Battaglia M, Cruciani G, Manganelli A, Ricci S, Lapini A. Prednisone plus gefitinib versus prednisone plus placebo in the treatment of hormone-refractory prostate cancer: a randomized phase II trial. *Oncology* 2008;74:223-8.
- [149] Salzberg M, Rochlitz C, Morant R, Thalmann G, Pedrazzini A, Roggero E,

Schönenberger A, Knuth A, Borner M. An open-label, noncomparative phase II trial to evaluate the efficacy and safety of docetaxel in combination with gefitinib in patients with hormone-refractory metastatic prostate cancer. *Onkologie* 2007;30:355-60.

[150] Somlo G, Martel CL, Lau SK, Frankel P, Ruel C, Gu L, Hurria A, Chung C, Luu T, Morgan R Jr, Leong L, Koczywas M, McNamara M, Russell CA, Kane SE. A phase I/II prospective, single arm trial of gefitinib, trastuzumab, and docetaxel in patients with stage IV HER-2 positive metastatic breast cancer. *Breast Cancer Res Treat* 2012;131:899-906.

[151] Carlson RW, O'Neill A, Vidaurre T, Gomez HL, Badve SS, Sledge GW. A randomized trial of combination anastrozole plus gefitinib and of combination fulvestrant plus gefitinib in the treatment of postmenopausal women with hormone receptor positive metastatic breast cancer. *Breast Cancer Res Treat* 2012;133:1049-56.

[152] Osborne CK, Neven P, Dirix LY, Mackey JR, Robert J, Underhill C, Schiff R, Gutierrez C, Migliaccio I, Anagnostou VK, Rimm DL, Magill P, Sellers M. Gefitinib or placebo in combination with tamoxifen in patients with hormone receptor-positive metastatic breast cancer: a randomized phase II study. *Clin Cancer Res*. 201 ;17:1147-59.

[153] Cristofanilli M, Valero V, Mangalik A, Royce M, Rabinowitz I, Arena FP, Kroener JF, Curcio E, Watkins C, Bacus S, Cora EM, Anderson E, Magill PJ. Phase II, randomized trial to compare anastrozole combined with gefitinib or placebo in postmenopausal women with hormone receptor-positive metastatic breast cancer. *Clin Cancer Res* 2010;16:1904-14.

[154] Whang YE, Armstrong AJ, Rathmell WK, Godley PA, Kim WY, Pruthi RS, Wallen EM, Crane JM, Moore DT, Grigson G, Morris K, Watkins CP, George DJ. A phase II study of lapatinib, a dual EGFR and HER-2 tyrosine kinase inhibitor, in patients with castration-resistant prostate cancer. *Urol Oncol* 2013;31:82-6.

[155] Pircher M, Mlineritsch B, Fridrik MA, Dittrich C, Lang A, Petru E, Weltermann A, Thaler J, Hufnagl C, Gampenrieder SP, Rinnerthaler G, Ressler S, Ulmer H, Greil R. Lapatinib-plus-Pegylated Liposomal Doxorubicin in Advanced HER2-positive Breast Cancer Following Trastuzumab: A Phase II Trial. *Anticancer Res* 2015;35:517-21.

[156] Sridhar SS, Hotte SJ, Chin JL, Hudes GR, Gregg R, Trachtenberg J, Wang L, Tran-Thanh D, Pham NA, Tsao MS, Hedley D, Dancey JE, Moore MJ. A multicenter phase II clinical trial of lapatinib (GW572016) in hormonally untreated advanced prostate cancer. *Am J Clin Oncol* 2010;33:609-13.

[157] Azad AA, Beardsley EK, Hotte SJ, Ellard SL, Klotz L, Chin J, Kollmannsberger C, Mukherjee SD, Chi KN. A randomized phase II efficacy and safety study of vandetanib (ZD6474) in combination with bicalutamide versus bicalutamide alone in patients with chemotherapy naïve castration-resistant prostate cancer. *Invest New Drugs* 2014;32:746-52.

[158] Clemons MJ, Cochrane B, Pond GR, Califaretti N, Chia SK, Dent RA, Song X, Robidoux A, Parpia S, Warr D, Rayson D, Pritchard KI, Levine MN. Randomised, phase II, placebo-controlled, trial of fulvestrant plus vandetanib in postmenopausal women with bone only or bone predominant, hormone-receptor-positive metastatic breast cancer (MBC): the OCOG ZAMBONEY study. *Breast Cancer Res Treat* 2014;146:153-62.

- [159] Wan X, Corn PG, Yang J, Palanisamy N, Starbuck MW, Efstathiou E, Tapia EM, Zurita AJ, Aparicio A, Ravoory MK, Vazquez ES, Robinson DR, Wu YM, Cao X, Iyer MK, McKeehan W, Kundra V, Wang F, Troncso P, Chinnaiyan AM, Logothetis CJ, Navone NM. Prostate cancer cell-stromal cell crosstalk via FGFR1 mediates antitumor activity of dovitinib in bone metastases. *Sci Transl Med* 2014;6:252ra122.
- [160] Vallo S, Mani J, Stastny M, Makarević J, Juengel E, Tsaur I, Bartsch G, Haferkamp A, Blaheta RA. The prostate cancer blocking potential of the histone deacetylase inhibitor LBH589 is not enhanced by the multi receptor tyrosine kinase inhibitor TKI258. *Invest New Drugs* 2013;31:265-72.
- [161] Nguyen HM, Ruppender N, Zhang X, Brown LG, Gross TS, Morrissey C, Gulati R, Vessella RL, Schimmoller F, Aftab DT, Corey E. Cabozantinib inhibits growth of androgen-sensitive and castration-resistant prostate cancer and affects bone remodeling. *PLoS One* 2013;8:e78881.
- [162] Lee RJ, Smith MR. Cabozantinib and prostate cancer: inhibiting seed and disrupting soil? *Clin Cancer Res* 2014;20:525-7.
- [163] Meads MB, Hazlehurst LA, Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. *Clin Cancer Res* 2008 ; 14: 2519–26.
- [164] David E, Blanchard F, Heymann MF, De Pinieux G, Gouin F, Rédini F, Heymann D. The Bone Niche of Chondrosarcoma: A Sanctuary for Drug Resistance, Tumour Growth and also a Source of New Therapeutic Targets. *Sarcoma* 2011; 2011:932451.
- [165] Trent JC, Subramanian MP. Managing GIST in the imatinib era: optimization of adjuvant therapy. *Expert Rev Anticancer Ther* 2014;14:1445-59.

## Figure Legends

**Figure 1: General organisation of the molecular domains that make up the RTKs.** RTKs are characterised by the dimerisation of two receptor chains with an N-terminal (N) extracellular domain (ECM), and a C-terminal (C) intracellular domain (ICD). The extracellular domain is implicated in the recognition of the dimeric ligands and the formation of the receptor chain dimerisation process. The extracellular domain is associated with ligand recognition and is composed of various domains depending on the RTK class. The transmembrane-domain is composed of an  $\alpha$ -helix chain which contributes to the stabilisation of the dimeric receptor chains. The binding of a dimeric ligand (in red) to the extracellular domains of the receptor chains strengthens the stabilisation of the receptor chains, which are auto-phosphorylated through their tyrosine kinase domains and then transduced in specific downstream signalling pathways.

**Figure 2: Main signalling pathways activated by the ligand-induced RTK auto-phosphorylations.** The phosphorylation cascades initiated by the RTK phosphorylations lead to the activation of numerous transcription factors which consequently control the regulation of many physiological processes.

**Figure 3: The negative feedback loops regulating RTK activation.** The window of time required for inducing mRNA and protein synthesis after RTK activation is between 15 and 90 minutes. These mechanisms are tightly regulated by negative feedback loops. Indeed, the phosphorylation cascade induced by RTK activation leads to the activation of numerous transcription factors and simultaneously of their repressors. The translocation of the various transcription factors can also induce the expression of transcriptional repressors or

phosphates, which in turn can repress the corresponding transcription factors and/or the upstream kinase activities. + : activation; - : repression.