Inhibition of Protein Kinase c-Src as a Therapeutic Approach for Cancer and Bone Metastases

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Abstract: c-Src is a proto-oncogene involved in the genesis of and invasion by many cancers. This non-receptor tyrosine kinase also plays a crucial role in bone homeostasis, since inhibition or deletion of c-Src impairs the function of osteoclasts, the bone resorbing cells. It is thus conceivable that c-Src could be a suitable target for the pharmacological treatment of cancers, skeletal metastases and diseases of bone loss, such as osteoporosis. The pyrrolo-pyrimidines CGP77675 and CGP76030 proved to be effective in preventing bone loss in animal models, while the effect of AZD0530, a dually active inhibitor of c-Src and Bcr-ABL, on bone resorption, has been tested in a Phase I clinical trials with promising results. As far as the metastatic bone disease is concerned, c-Src inhibitors could potentially have inhibitory effects both on osteoclasts and on tumour cells, and could disrupt the vicious circle established between these cell types in the bone microenvironment. In accord with this idea, CGP76030 is able to reduce the incidence of osteolytic lesions and of visceral metastases, and to suppress morbidity and lethality in a bone metastasis mouse model without obvious adverse effects. The purine-based c-Src inhibitor AP23451 and the dual c-Src/Abl inhibitors AP22408 and AP23236 proved efficacious in reducing bone metastases in preclinical studies. These results open a new avenue for the development of innovative therapies for the treatment of bone metastatic disease.

Key Words: c-Src inhibitor, cancer, bone metastasis.

STRUCTURE AND FUNCTION OF C-SRC

c-Src is a non-receptor tyrosine kinase that belongs to a family consisting of nine proteins: Src, Fyn, Yes, Lck, Lyn, Hck, Fgr, Blk and Yrk, the latter only in chicken, with their domain structures having considerable homology [1-3]. Src, Fyn and Yes are ubiquitously expressed, while the expression of other family members is more restricted, especially to cells of the haematopoietic lineage. c-Src is most abundant in platelets [4,5], neural tissue [6], and osteoclasts [7,8], where it is implicated in multiple pathways that regulate cell growth, migration, and survival [9]. Its activity increases in response to a number of signals, especially downstream of tyrosine kinase growth factor receptors, G-protein coupled receptors, integrin receptors for adhesion molecules, and cytokine receptors [10].

As showed in Fig. (**1**), the main structural and functional domains of c-Src are:

- a NH2-terminal membrane-association domain, named SH4, whose myristoylation allows binding of c-Src with the inner surface of the plasma membrane;
- a unique domain, in which the members of the c-Src family display the greatest sequence divergence;
- a SH3 domain, which mediates protein-protein interactions by binding to proline-rich sequences;
- a SH2 domain, involved in phosphotyrosine-mediated protein-protein interactions;
- a SH2-kinase linker, which is a loop functioning as a pseudo-SH3-binding site;
- a catalytic SH1 domain, the most conserved domain in all tyrosine kinases, which contains the ATP-binding pocket;
- a COOH-terminal tail that upon phosphorylation can bind c-Src SH2 domain.

Two major tyrosine phosphorylation sites are present in c-Src, which regulate its activity: $Tyr^{(416/419)}$ (416 in chicken, 418 in human) and $Tyr^{527/530}$ (527 in chicken, 530 9n human). The autophosphorylation of the former is necessary for the full c-Src kinase activity. In contrast, the $Tyr^{527/530}$, located in the COOH-terminal tail, is a negative regulatory site, whose phosphorylation blocks c-Src activity [1,2, 11].

c-Src is normally maintained in an inactive, or "closed" conformation Fig. (**1B**), where the SH2 domain is engaged with the phosphorylated $Tyr^{527/530}$, the SH3 domain binds the SH2-kinase linker sequence and the $Tyr^{416/419}$ is dephosphorylated. Dephosphorylation of $Tyr^{527/530}$ disrupts its intramolecular interaction with the SH2 domain and this open conformational state allows autophosphorylation of $Tyr^{416/419}$, resulting in c-Src activation. Phosphorylated $Tyr^{416/419}$ is displaced from the substrate-binding pocket, giving the kinase access to substrates, as shown in Fig. (**1B**). At the cellular level, binding of the SH2 domain of c-Src to tyrosine phosphorylated growth factor receptors displaces $Tyr^{527/530}$, allowing $Tyr^{416/419}$ phosphorylation and c-Src activation. Conversely, dissociation of c-Src from the growth factor receptor allows the intramolecular interactions to reform, thereby inactivating c-Src [1,2].

Two tyrosine kinases, named c-Src kinase (CSK) and CSK homologous kinase (CHK), are capable of phosphorylating $Tyr^{527/530}$, which results in c-Src inactivation.

C-SRC AS PHARMACOLOGICAL TARGET IN DISEASES OF BONE LOSS

c-Src and Osteoclast Functions

Numerous studies have provided compelling evidence that c-Src plays a crucial role in osteoclast function. The interest in c-Src within the bone metabolism field was stirred by the work from Soriano and co-workers [12], which demonstrated that, despite the ubiquitous expression of c-Src, knockout mice showed only a bone phenotype, as they developed osteopetrosis, a disease characterized by excess bone mass due to the lack of osteoclast activity [13,14]. In particular, c-Src-deficient mice had a high number of osteoclasts, which failed to form a polarized ruffled membrane [15, 16] and presented a migration defect with a consequent inability to resorb bone. On the basis of these findings, c-Src is believed to have a unique role in osteoclasts, since other c-Src family members apparently cannot substitute for all c-Src functions in c-Src-deficient mice.

A key function of c-Src in osteoclasts is to promote the rapid assembly and disassembly of the podosomes, which are important

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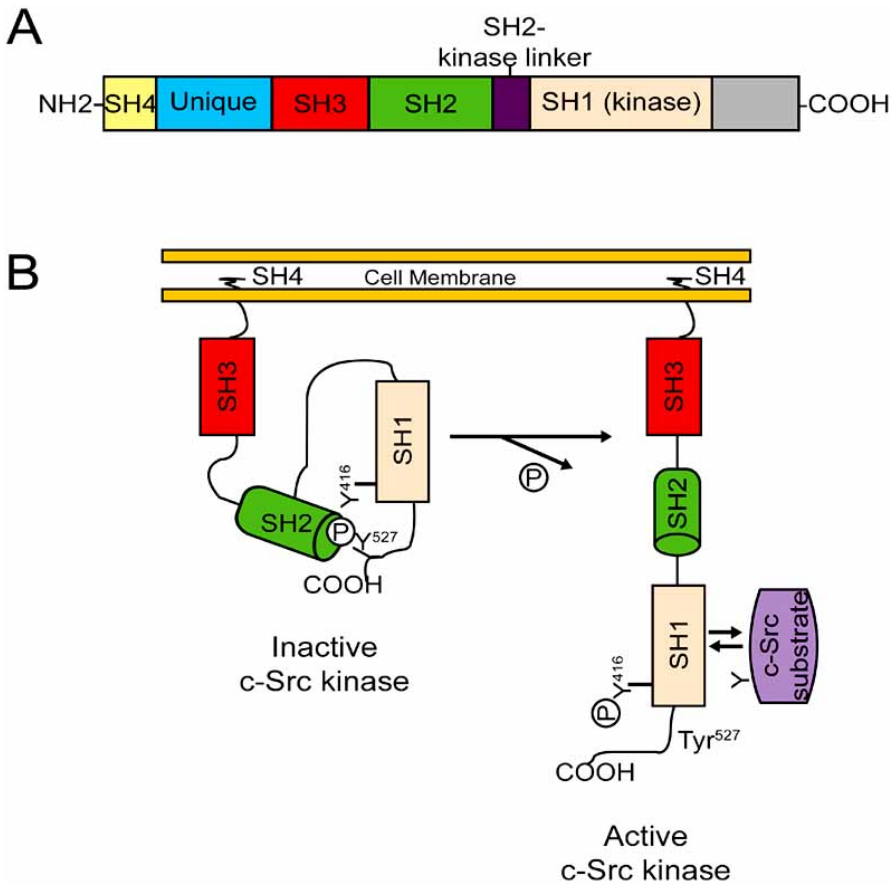

Fig. (1A). Schematic illustration of the primary structure of c-Src. The most important domains of c-Src are shown from the NH2- to the COOH-terminus. See text for details. (**B**) Schematic representation of c-Src activation. c-Src normally is maintained in an inactive or "closed" conformation, where Tyr^{527} is phosphorylated and binds to the SH2 domain, Tyr^{416} is dephosphorylated and the SH3 domain is engaged with the SH2 kinase linker. Dephosphorylation of Tyr^{527} leads to an "open" conformation, allowing autophosphorylation of Tyr^{416} and interaction of c-Src with substrates.

for osteoclast's resorption activity. Once recruited to the activated integrins, especially alphaVbeta3, by the adhesion tyrosine kinase Pyk2, c-Src binds to and phosphorylates Cbl and Cbl-b, homologous adapter proteins with ubiquitin ligase activity. The c-Cbl proteins, in turn, recruit and activate additional signalling effectors, which regulate cell polarity, cell attachment and motility [17, 18]. c-Src and the c-Cbl proteins are also involved in signalling cascades that are activated by several important receptors for osteoclast formation and function, such as RANK (receptor activator of nuclear factor kappa B) and the macrophage colony-stimulating factor receptor [18].

Another important role of c-Src in osteoclasts is the regulation of vesicle transport and secretion of proteases. Moreover, c-Src is also involved in the organization of microtubules and actin polymers [19-21], and in cell survival [22]. Finally, a recent paper from Miyazaki and colleagues [23] showed a c-Src kinase activity also within mitochondria, which seems to be essential for the regulation of osteoclastic bone resorption.

c-Src as Pharmacological Target in Osteoclasts

Given the crucial role of c-Src in osteoclast physiology, it is conceivable that this molecule is a potential pharmacological target for the treatment of diseases of bone loss. One of the first compounds employed to inhibit c-Src activity was the antibiotic herbimycin A that, even while displaying an inhibitory effect *versus* other protein kinases, proved to be efficacious in inhibiting osteoclastic bone resorption *in vitro* and hypercalcemia *in vivo* [24]. By now, numerous other compounds have been synthesised and tested *in vitro* and *in vivo*, and for some of them clinical trials are in progress.

According to the mechanisms of action, c-Src inhibitors can be classified into two major groups [3]:

- Inhibitors of the tyrosine kinase activity [adenosine triphosphate (ATP) binding domain-mediated];
- Inhibitors of protein-protein (SH2-, SH3-, or substrate binding domain-mediated) interaction.

Between the two groups, the first seems to be the most promising in terms of potency, selectivity and therapeutic application. This group includes the following heterocyclic ATP analogs, as shown in Fig. (**2**):

- pyrazolo-[2,3-*d*]pyrimidines (PP1 and PP2);
- pyrrolo-[2,3-*d*]pyrimidines (CGP76030 and CGP77675);
- pyrido-[2,3-*d*]pyrimidines (PD166585, PD173955 and PD180970);
- quinoline carbonitriles (SKI606);
- olomucines (NVP-AAK980 and CGP79883).

Among the 5,7-diphenyl-pyrrolo [2,3-*d*]-pyrimidines synthesised, promising results have been obtained with two compounds, named CGP77675 and CGP76030 (Novartis Pharma, Basel, Switzerland) whose enzymatic selectivity is reported in Table **1**. These compounds proved to be potent and fairly selective c-Src inhibitors [25,26] relative to other non-receptor tyrosine kinases. Equipotent inhibition of c-Yes, a member of the c-Src family, is likely not important for bone, as c-Yes expression in osteoclasts is much lower than that of c-Src [7].

CGP77675 inhibited human c-Src in enzymatic assays and cellular Src in a Src-overexpressing cell line, with IC_{50} of 20 and 200

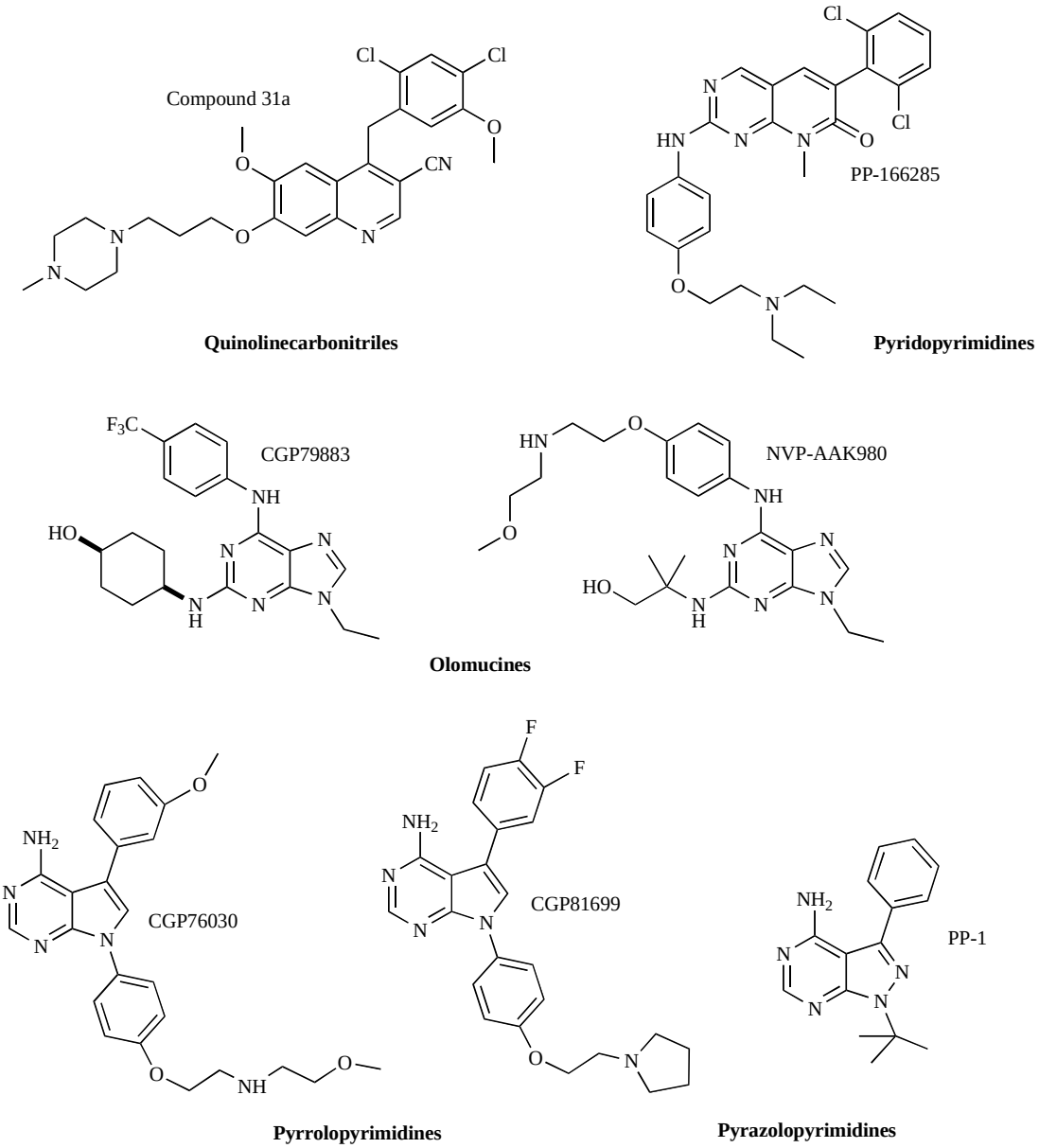

Fig. (2). Chemical structure of the principal c-Src inhibitors discussed in the text.

nM, respectively [25]. In rat foetal long bone cultures, CGP77675 inhibited parathyroid hormone-induced bone resorption with an IC_{50} of 0.8 micromol/L [25]. *In vivo*, it dose-dependently reduced the hypercalcaemia induced in mice by interleukin-1beta and partly prevented bone loss and microarchitectural changes in young ovariectomised rats [25].

Similar effects were observed for the CGP76030 compound that was previously administered to hypercalcaemic animals and animals treated with the bone loss inducer retinoic acid [27]. Moreover, a drop in osteoclast number associated with clear evidence of cell damage, suggestive of apoptosis, was found in these animals. Further *in vitro* study showed that treatment with CGP77675 or CGP76030 disrupted osteoclast actin rings and impaired adhesion and bone resorption in a dose-dependent manner [27]. Despite the *in vivo* results of Soriano and colleagues [12] that indicated no change in osteoclast number, inhibition of osteoclastogenesis was observed *in vitro* [27]. This could be explained either by lack of induction of compensation mechanisms after acute administration of the inhibitors, which may instead prevent changes in osteoclast numbers in Src-null animals or by differences between *in vitro* cultures and more complex *in vivo* mechanisms. Finally, both inhibitors induced apoptosis in mature osteoclasts by a mechanism driven by late ERK1/2 activation [27].

Recent *in vivo* studies showed the ability of CGP76030 to fully prevent hypercalcemia and bone loss in retinoid-treated male thyroparathyroidectomised (TPTX) rats at 100 mg/kg [3]. In ovariectomised (OVX) female rats, measurements of serum calcium and urine hydroxyproline showed a significant prevention of bone loss at 10-20 mg/kg in both young and old animals. In addition, OVX-induced disruption of trabecular bone microarchitecture was partially prevented by treatment with 50 mg/kg CGP76030 administered orally twice a day [3].

As far as the purine-derived olomucine class is concerned, the compound NVP-AAK980 was tested only in short-term retinoid-treated rat model, where it was active at 30 and 100 mg/kg, with the latter dose showing a full protection against hypercalcemia [3].

Among the compounds being tested in clinical trials, AZD0530 should be mentioned, an orally-active, dually-specific, small molecular weight inhibitor of c-Src and BCR-Abl that has been developed by Astra Zeneca [28]. The efficacy of AZD0530 on bone resorption has been demonstrated in two Phase I clinical trials: one single ascending dose and one multiple ascending dose (60-250 mg) study in healthy male volunteers [29].

C-SRC AS PHARMACOLOGICAL TARGET IN CANCER

Several studies demonstrated that c-Src, the first proto-oncogene to be described, plays an important role in the genesis and progression of human cancers, including carcinomas of the breast, colon, prostate, lung and ovary, and in myeloproliferative disorders [1,30, 31]. The role of c-Src in tumorigenesis is based on its support of cell proliferation and migration. c-Src activity increases during cancer progression, with the highest expression and/or activity in metastases [32,33]. In colorectal carcinoma c-Src activity can be predictive of poor prognosis [34].

Another key role of c-Src is the regulation of specific angiogenic factors that promote tumour progression. Recent studies demonstrated that this tyrosine kinase regulates both constitutive and growth factor-induced VEGF and IL-8 expression [35,36]. Increased c-Src activity in tumour cells stimulated VEGF expression, resulting in increasing activation of VEGF receptors on endothelial cells. VEGF receptor activation in turn leads to association of c-Src with these receptors, thus increasing its activity in endothelial cells as well [31].

Prostate Cancer

c-Src inhibitors CGP76030 and CGP77675 proved to be efficacious in reducing *in vitro* migration, invasion and adhesion of the human prostate cancer cell line PC3 [37]. Other c-Src inhibitors belonging to pyrazolo[3,4-d]pyrimidines class, named S135 and S140, inhibited PC3 cell proliferation, adhesion and EGF-mediated migratory ability [38].

Breast Cancer

It has been documented that c-Src kinase activity is 4-fold to 20-fold greater in human mammary carcinomas than in normal tissues [39]. In addition, c-Src plays a critical role in ErbB2-mediated breast cancer invasion and metastases [1]. It has been observed that maximally active c-Src was over-expressed in a subset of tamoxifen-resistant breast cancer variants, thus suggesting that inhibitors of c-Src activity may delay or prevent progression and metastasis of estrogen receptor-positive tumours [40].

Colon Cancer

In this type of cancer, the activation and function of c-Src have been extensively studied and various c-Src inhibitor compounds have been tested. In particular, a recent study demonstrated that PP2 reduced urokinase-plasminogen activator receptor (uPAR) transcription in human colon cancer cells, with a consequent reduction of their invasiveness [41]. Moreover, treatment of human colon tumour cells with pyrido[2,3-d] pyrrolo pyrimidine compounds inhibited their growth in culture as well as colony formation in soft agar [42].

Finally, both CGP76030 and the olomoucine NVP-AAK980 inhibited proliferation and viability in colon cancer cells, with similar potency with respect to the clinically used compound 5-fluorouracil [3]. *In vivo*, both c-Src inhibitors were, however, inactive in the HT-29 xenograft models [3].

Pancreatic Cancer

In an orthotopic implantation model performed using the L3.6pl human pancreatic tumour cells, where c-Src expression was down-regulated by siRNA, Trevino and colleagues [35] showed a significant reduction of the incidence of metastases, suggesting that c-Src activity is critical to tumour progression. Moreover, in animals bearing established wild-type tumour and treated with the Src/Abl-selective inhibitor BMS-354825 (dasatinib, Bristol Myers Squibb), tumour and the incidence of metastases were reduced as compared to controls [35].

c-Src inhibition by the AZM475271 compound (Astra Zeneca) alone or in combination with gemcitabine, inhibited growth and metastases of orthotopically implanted human pancreatic carcinoma cells. Moreover, this treatment significantly reduced tumour cell proliferation and tumour microvessel density and increased apoptosis *in vivo* [43].

Other c-Src Inhibitors in Cancer Treatment

From the pyrido-pyrimidine class, PD180970 is the most recently reported compound. In CD34-positive leukaemia cells from patients with imatinib (Novartis)-resistant chronic myeloid leukemia (CML), this compound induced apoptosis [44].

Dasatinib (BMS354825) is a dually-specific c-Src and Abl kinase inhibitor that can bind BCR-Abl in both the active and inactive conformations [45]. Recent studies demonstrated that this compound was 300-fold more potent than imatinib against the wild-type Bcr-Abl, with little or no inhibitory effect against normal haematopoietic progenitors [46,47]. Dasatinib demonstrated *in vitro* and *in vivo* activity against 14 of 15 BCR-Abl mutants that are resistants to imatinib. Moreover, promising results have been obtained from a Phase I study performed in Philadelphia chromosome-positive CML patients after failure or intolerance to imatinib, as reported by Talpaz and colleagues [48].

Among dual c-Src/Abl kinase inhibitors, the AP23464 compound proved to be efficacious in inhibiting proliferation of human CML cell lines and BCR-Abl-transduced Ba/F3 cells [1]. In preclinical studies, AP23464 was more potent than PP1 in causing growth inhibition and c-Src kinase inhibition in the human myeloid cell line MV4-11 [1].

Finally, the compound SKI-606, a c-Src/Abl kinase dual inhibitor, was efficacious in several *in vitro* and *in vivo* CML models of resistance to imatinib [49].

C-SRC AND BONE METASTASES

Bone metastases are a major complication of many types of cancers, occurring in up to 70 percent of patients with advanced breast and prostate carcinomas [50]. Once skeletal metastases have developed, the chances of survival dramatically drop. In particular, it has been estimated that only 20 percent of breast cancer patients are still alive five years after the diagnosis of bone metastases [51]. Moreover, the quality of life is also seriously compromised, as skeletal metastases can cause severe pain, pathologic fractures, nerve-compression syndromes and hypercalcaemia.

Skeletal metastases can be osteolytic or osteoblastic in nature, but this distinction is not so obvious because patients can have mixed lesions with both features. Moreover, secondary formation of bone can occur in response to bone destruction. Most patients with breast cancer develop preferentially osteolytic lesions [52], characterised by intense bone resorption activity leading to focal bone fragility. In contrast, the metastases in prostate cancer are predominantly osteosclerotic [53] and are characterised by new bone tissue with the features of primary "woven" bone formed by deregulated osteoblasts. Although the metastatic bone disease is characterised by high morbidity, its chronic nature makes the prognosis more favourable than visceral lesions, opening up an avenue for its persistent remission by medications.

Osteolytic Bone Metastases and the Vicious Circle

In osteolytic metastases, the destruction of bone is mediated by the osteoclasts rather than by the tumour cells [50,54]. However, tumour cells that colonise bone produce factors that directly or

indirectly induce the formation of osteoclasts. It is well known that the destruction of bone matrix by osteoclasts results, in turn, in the release of tumour-seeking factors therein stored, such as TGFbeta (transforming growth factor beta), IGFs (insulin-like growth factors), FGFs (fibroblast growth factors), PDGF (platelet-derived growth factor) and BMPs (bone morphogenetic proteins) that further stimulate cancer expansion [50,51,54] Fig. (**3**). This mutual enhancement between tumour cells and the bone microenvironment results in a vicious circle that progressively increases both bone destruction and the tumour burden Fig. (**3**).

Several molecules, released by tumour cells at the site of tumour bone colonisation, contribute to foster the vicious circle. The PTHrP (paraphyroid hormone-related peptide) is produced by tumour cells and it induces the expression of RANKL in the bone marrow stromal cells, as depicted in Fig. (**3**). Breast cancer cells also produce M-CSF (macrophage colony stimulating factor), PGE2 (Prostaglandin E2) and a number of pro-inflammatory cytokines such as IL-1 (interleukin-1), IL-6, TNFalpha (tumour necrosis factor alpha), GM-CSF (granulocyte macrophage-colony stimulating factor), which stimulate osteoclast formation and enhance their bone-resorption activity [50,54,55].

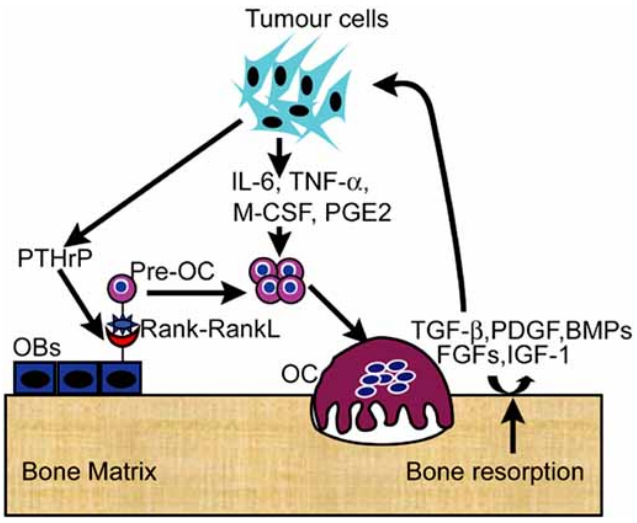

Fig. (3). Schematic representation of the vicious circle. Tumour cells intruding the bone marrow produce factors such as PTHrP, IL-6, TNFα, M-CSF, PGE2 which stimulate osteoclastogenesis either directly or stimulating RANKL expression in osteoblasts (PTHrP). Increased osteoclast formation leads to an increase of bone resorption, which in turn allows the release from the bone matrix of growth factors (BMPs, PDGF, TGFbeta, IGF-I, FGFs) that enhance tumour growth. (OBs=Osteoblasts; pre-OC=pre-osteoclast; OC=Osteoclast).

Thus, there is clearly a close relationship between bone destruction and tumour growth. However, definitive evidence that pharmacologically decreasing bone destruction reduces tumour burden in patients with bone metastases has not been clearly demonstrated. It may be necessary to break the vicious circle by blocking activated bone resorption, as well as tumour growth.

Current Treatments

To date the most efficacious therapy for treatment of patients with bone metastases are nitrogen-containing bisphosphonates, introduced into the clinical practice during the last decades [56,57]. These compounds bind to the bone surface, where they are taken-up by osteoclasts during the bone resorption process. Inside of osteoclast, bisphosphonates interfere with specific signal transduction pathways (i.e. mevalonate pathway) and enzymes (farnesyl pyrophosphate synthase), resulting in osteoclast apoptosis and inhibition of osteolysis. It has been demonstrated that treatment with zoledronic acid (Novartis) results in delayed skeletal events in patients with bone metastases from breast cancer, prostate cancer and multiple myeloma [58]. However, experimental studies in animals, as well as clinical experience in humans, have demonstrated that bisphosphonates do not significantly affect tumour cells, therefore tumour burden is not reduced and survival is not improved [59,60]. Patients benefit from a better quality of life mainly due to the improvement of pain and from reduced risk of fractures. Recent clinical evidence has demonstrated a close relationship between bisphosphonate treatment and the onset of the osteonecrosis of the jaw, a rare but severe pathology with as yet unclear mechanism [61].

New Potential Therapies for Treatment of Bone Metastases

It has been well established that in addition to its role in osteoclasts, c-Src has also important functions in malignant cells in which it regulates cell division, growth factor signalling, migration and invasion. Indeed, c-Src activity is up-regulated in many cancers. Thus, in patients with metastatic bone disease, c-Src inhibitors could potentially have negative effects not only on osteoclasts, but also on tumour cells and on their interactions with osteoclasts.

Among the c-Src inhibitors whose effect on osteoclasts and cancer cells has been tested both *in vitro* and *in vivo*, the following compounds have provided promising results.

CGP76030. As already mentioned, this compound, belonging to the 5,7-diphenyl-pyrrolo[2,3*d*] pyrimidine, successfully inhibited osteoclast bone resorption both *in vitro* and *in vivo* [3,27]. A recent report also demonstrated the ability of CGP76030 to reduce the incidence of bone metastases in an *in vivo* animal model [62]. This *in vivo* model employs the injection of human tumour cells in the arterial circulation through the left ventricle of the heart in four-week-old nude mice (Balb-c nu/nu). As a consequence, mice develop metastases in bones, preferentially in the hind limbs [63,64]. Fig. (**4**) shows the hind limb of a mouse after intracardiac injection with vehicle (PBS) Fig. (**4A**), or with the human breast cancer cells MDA-MB-231, Fig. (**4B**). Four weeks after injection it is possible to appreciate the presence of osteolytic lesions by X-ray, Fig. (**4B**), left panel, and to detect by histological staining with hematoxylin/eosin tumour cells that have invaded the bone marrow, Fig. (**4B**), middle panel. Histological sections stained for the osteoclast specific marker TRAcP (tartrate resistant acid phosphatase) clearly show high numbers of these cells lining the bone surface, Fig. (**4B**), right panel.

Treatment of mice inoculated with the MDA-MB-231 cells with CGP76030 (100 mg/kg/day p.o.) decreased the morbidity, Fig. (**4C**) and lethality, Fig. (**4D**) compared to vehicle-treated mice [61]. Moreover, CGP76030 was also able to reduce the incidence of bone, Fig. (**4E**), as well as of visceral metastases, Fig. (**4F**). Interestingly, the effect of CGP76030 seemed not to be restricted to the inhibition of osteoclast bone resorption [25,27], but it acted also on metastases developed in other organs. Worth noting is that this treatment did not induce any obvious detrimental effect in animals.

These results were consistent with data showing that intracardiac injection of nude mice with MDA-MB-231 cells stably transfected with a c-Src kinase-dead dominant-negative construct induced less morbidity, lethality and incidence of bone and visceral metastases relative to mice injected with parental MDA-MB-231 cells [62,65]. Finally, treatment of MDA-MB-231 cells with CGP76030 significantly reduced *in vitro* cell proliferation, migration and invasion in a concentration-dependent manner.

AP23451(ARIAD) is a purine-based c-Src tyrosine kinase inhibitor which contains a bisphosphonate group that conferrs bone-targeting properties [66]. *In vitro*, this compound inhibited osteoclast formation and stimulated osteoclast apoptosis, while *in vivo* studies showed its ability to dose-dependently prevent parathyroid hormone-induced bone resorption and hypercalcaemia, and ovariec-

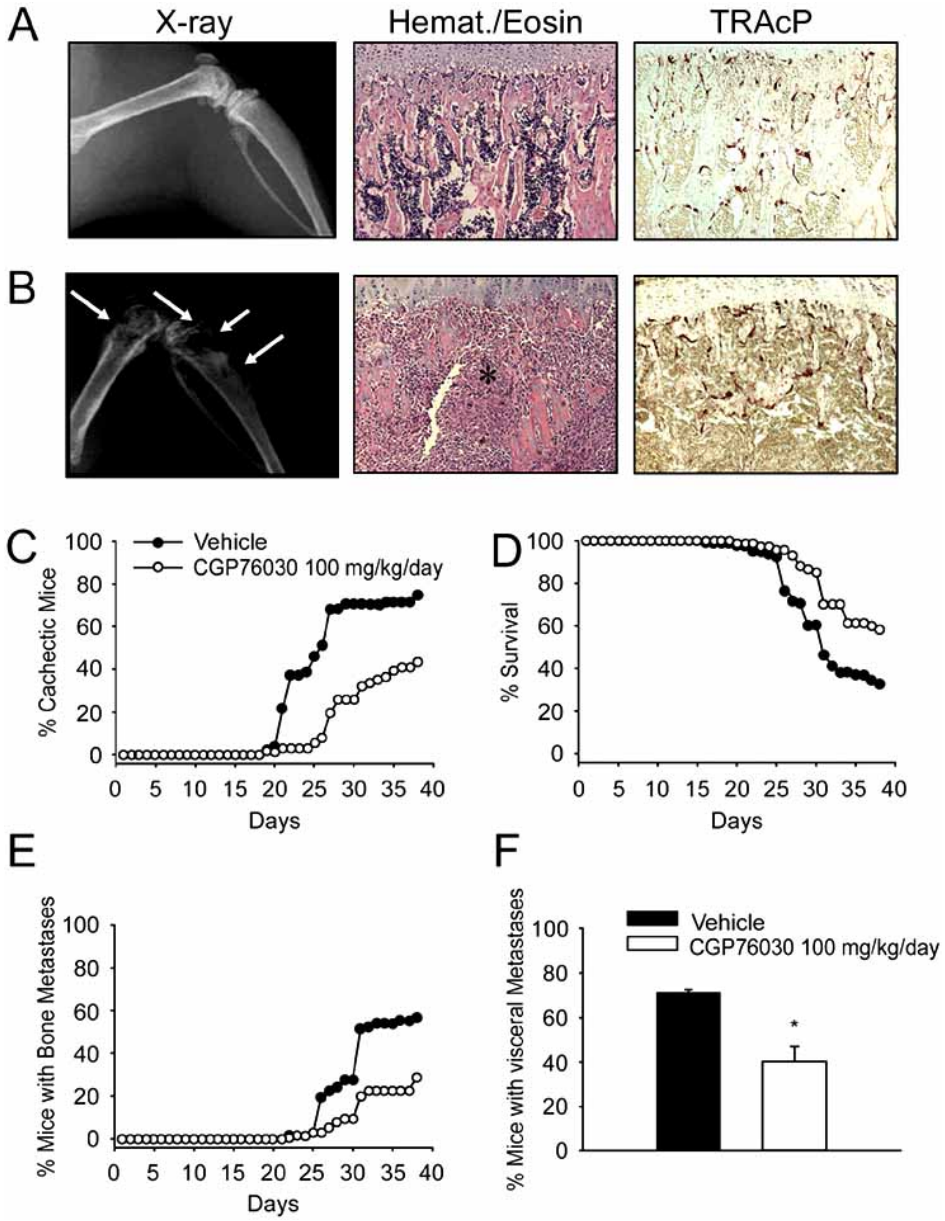

Fig. (4A-B). Hind limb of a BALB/c-nu/nu mouse inoculated in the left ventricle with vehicle alone (PBS) (**A**) or with 100,000/100 microL of MDA-MB-231 cells (**B**). Left panels: x-ray analysis (arrow, osteolytic lesions). Middle panels: haematoxylin/eosin staining (*, tumour mass). Right panels: histochemical staining for tartrate-resistant acid phosphatase activity (TRAcP). (**C-F**) *In vivo* treatment with the c-Src inhibitor CGP76030. As described in (**B**), from the day after injection of tumour cells, animals were treated with CGP76030 (100 mg/kg/day) or with vehicle alone. Mice were then monitored daily for body weight (cachexia) (**C**) and survival (**D**) and were weekly subjected to x-ray analysis to evaluate osteolytic lesions (**E**). After 38 days, animals were sacrificed and subjected to anatomical dissection to evaluate visceral metastases (**F**). Similar results were observed in 4 independent experiments. (**F**) *p=0.043 (N. of mice/group=8). A similar experiment has already been published by us in [62].

tomy-induced bone loss [67]. Interestingly, treatment of nude mice, which have been inoculated with the MDA-MB-231 cells *via* intracardiac injection, with this compound reduced metastasis-induced osteolysis [68] and resulted in smaller tumours developed in the bone marrow cavities [69].

AP22408 and **AP23236** (ARIAD) belong to the dual c-Src/Abl kinase inhibitor class, which blocks c-Src noncatalytic SH2 domain or catalytic activity in osteoclasts. These compounds are bone-targeted and are both active in the preclinical studies of osteoporosis and osteolytic bone metastases [1,70].

CONCLUSIONS

Proto-oncogene c-Src plays a role in the development and progression of many human cancers and in osteoclast function. Accordingly, c-Src inhibitors target both tumour cells and osteoclasts, thus disrupting their mutual interactions in bone metastases. Pharmacological treatment with c-Src inhibitors is promising for the treatment of bone metastasis complications, which could lead to a better quality of life and improved survival of patients. Pilot clinical studies support the feasibility of this treatment since c-Src inhibitors were found not to cause significant adverse effects in male volunteers [29].

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