

Src as a therapeutic target in men with prostate cancer and bone metastases

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While responsive to androgen ablation in its early stages, prostate cancer eventually becomes castration-resistant and metastasizes preferentially to bone. Once this happens, the disease carries considerable morbidity and is incurable. The process of bone metastasis involves a complex interplay between tumour and bone tissue. The eventual characteristic clinical presentation of disorganized osteoblastic bone lesions is preceded by a facilitatory osteoblastic phase; an osteoblastic component then continues to underlie the process. Increasing evidence has shown a ubiquitous role for Src (a proto-oncogene tyrosine-protein kinase) in multiple tumour

and bone-signalling processes involved in prostate tumour progression, driving proliferation, survival, migration and transition to androgen-independent growth. It is also intimately involved in positively regulating osteoclast physiology. As such, this molecule represents an attractive target for managing progressing prostate cancer. Encouraging results have been obtained in preclinical and clinical studies using Src inhibitors like AZD0530 and dasatinib. Both compounds reduced markers of bone resorption, in patients with cancer and those with advanced castration-resistant prostate cancer, respectively. Moreover, because Src is central to many mechanisms thought

to be responsible for the development of castration resistance, adding Src inhibitors to a treatment regimen might reverse this phenomenon. As a result, many Src inhibitors are in preclinical development. This review explores Src inhibition as a strategy for managing bone metastasis in prostate cancer, with a particular focus on targeting the critical osteoclastic response. Other emerging and novel approaches are also considered.

KEYWORDS

prostate cancer, dasatinib, Src kinases

INTRODUCTION

Prostate cancer has a high global incidence and mortality. It is the most common malignancy and the second most common cause of cancer death in North American males [1]. Advanced disease is most associated with prostate cancer-specific mortality and much of the morbidity of the disease. Prostate cancer metastasizes preferentially to bone, and is unique among solid tumours in that this is often the only clinically detectable site of metastasis [2]. Patients with metastatic disease experience intractable pain, pathological fractures and, in cases of spinal involvement, significant morbidity from nerve compression [2]. These events diminish patients' quality of life and incur an additional burden for treatment costs [3].

Prostate cancer progresses from diagnosis to death via one or more stages in a continuum, whereby patients might have increasing PSA levels both before and after androgen ablation, followed eventually, in either case, by metastatic spread [4]. Surgical and hormonal castration are effective only in early-stage, castration-responsive disease

[2]. However, once the cancer becomes castration-resistant it becomes incurable. Second-line hormonal therapies and other related strategies are only minimally effective; for those patients able to tolerate aggressive treatment, palliative chemotherapy remains the only subsequent option. The goal is to extend the time between development of castration resistance and progression requiring chemotherapy. As progression in prostate cancer is synonymous with bone-related morbidity, approaches for managing, or ideally preventing/delaying bone metastasis are key.

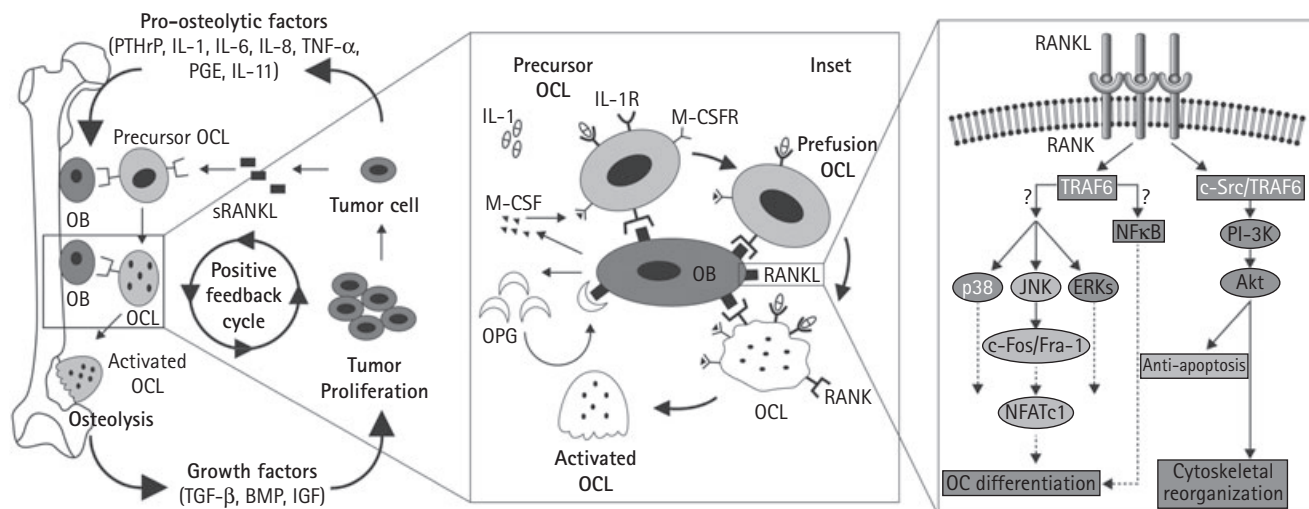
Bone spread in cancer is the result of a complex interaction between tumour cells and specialized bone cells termed osteoclasts and osteoblasts. Before osteoblast activation, osteoclasts break down normal bone tissue and initiate bone remodelling. In normal bone, resorption and formation are in equilibrium, but tumour invasion disrupts this balance, producing a net loss of normal bone tissue/net gain of abnormal highly disorganized bone tissue, both of which weaken the skeleton and predispose to fractures [5,6]. Furthermore, fractures have been correlated with poor outcomes and reduced survival [7].

Depending on whether mechanisms favour a dominant osteoclastic or osteoblastic response, bone metastasis in cancer produces a primarily osteolytic or osteoblastic clinical presentation [2]; however, the underlying aetiology is complex and incompletely understood, and there might be both histological and phenotypic heterogeneity [8].

Interestingly, levels of urinary N-telopeptide (a marker of bone collagen breakdown) and serum bone-specific alkaline phosphatase (a marker of bone formation) were *both* higher in patients with metastatic cancer with a blastic disease presentation than in those with predominantly osteolytic lesions, suggesting a close link between bone resorption and deposition, with one process intimately driving the other [6]. The formation and activity of osteoclasts is controlled by osteoblasts [2,5]. Interference in regulatory cross-talk between osteoclasts and osteoblasts by tumour cells results in dysregulated signalling and abnormal bone formation, and facilitates further tumour spread [5].

Metastasis to bone in prostate cancer is outwardly an osteoblastic process, whereby

FIG. 1. Cellular and molecular interactions in bone metastases. The positive feedback cycle and osteolytic bone metastasis. Several factors secreted by the tumour cells and osteoclasts (PTHrP, IL-1, IL-8, IL-11, soluble RANKL, TNF- β , BMP, IGF, and TGF- β and PGE) underlie the osteolytic process in metastatic disease. Inset Box 1: These factors are stimulatory for tumour growth and increase tumour burden and, in turn, up-regulate osteolysis even further. Inset Box 2: RANKL-RANK trimerization causes the recruitment of adaptor molecules (TRAF6) and triggers c-Src signalling which up-regulates the PI-3K/Akt axis. This up-regulates both the osteoclast activity, apoptosis, activation of MAPKs and the NF κ B complex. Important downstream regulators of osteoclast formation also include c-Fos, Fra-1 and nuclear factor-activated T cells c1 (NFATc1). JNK, jun kinase; NF κ B, nuclear factor B; PI-3K, phosphatidylinositol 3 kinase; TRAF6, tumour necrosis factor receptor associated factor 6; BMP, bone morphogenetic protein; M-CSF, macrophage colony-stimulating factor; OB, osteoblast; OCL, osteoclast; PG, prostaglandin; PTHrP, parathyroid hormone-related peptide; Adapted from [12] and [13] with permission.



tumour cells induce osteoblast differentiation and activation [2]. Prostate tumour cells are also dependent on factors secreted by osteoblasts for their growth. While metastatic bone lesions in advanced prostate cancer are apparently osteoblastic, there is evidence that increased osteolytic activity also occurs in the background [8]. For example, men with advanced prostate cancer show elevated levels of bone resorption markers, reflecting up-regulated osteoclastic activity. As elevated bone markers correlate with poor outcomes, this points to relevance in the clinic [9].

Prostate tumour cells in the bone environment produce factors that directly or indirectly induce osteoclastogenesis, a process termed 'osteomimicry', while the resulting bone degradation releases growth factors from the bone matrix, which in turn stimulate tumour cell growth [8]. Moreover, metastatic prostate tumour cells also produce a range of survival factors identical to growth factors that are produced directly or indirectly by bone matrix components, and are involved in bone remodelling; these mitogens therefore also act as promoters of osteoblast activity [10].

Thus an osteoclastic reaction to micrometastatic invasion in early progression of prostate cancer is the essential first step

that allows 'seeding' of tumour cells in bone tissue and thereafter initiates changes in the tumour/bone microenvironment that favour osteoblastic deposition; osteolysis is then locally attenuated, although it remains a continuous, underlying factor in metastatic establishment and spread [10]. Consequently, a complex interplay of interdependency between tumour and bone tissues leads to escalating abnormal bone deposition in the form of osteoblastic lesions. Src is a key signalling molecule in normal and abnormal bone turnover, involved in both osteoclast and osteoblast function [11].

Strategies which inhibit osteoclast function could potentially reduce tumour-promoting growth factor release, as well as stabilizing bone tissue by reducing bone resorption, thus reducing morbidity and ameliorating further metastatic progression. Inhibiting osteoclast function might also interrupt the tumour-osteoclast-osteoblast cross-talk signalling axes, potentially reducing the imperative for excess bone deposition (Fig. 1) [12,13]. In this review I explore such novel approaches for managing bone metastasis in progressing prostate cancer, with a particular focus on Src signalling blockade. Concomitant or separate targeting of osteoblast activity and function is also discussed.

SRC FAMILY KINASE (SFK) SIGNALLING IN HEALTH AND DISEASE

Bone remodelling involves the resorption of bone by osteoclasts and formation of bone by osteoblasts; healthy bone requires continual recruitment and differentiation of both cell types in appropriate numbers. Src plays a pivotal role in this process by positively regulating osteoclasts and negatively regulating osteoblasts. Osteoclast-specific dysregulation of Src produced osteopetrosis in Src-deficient mice [14], while other studies confirmed an osteoblastic component of this phenomenon, whereby decreased Src expression also stimulated osteoblast differentiation and bone formation [15]. High levels of Src were found in mature osteoclasts [16], and Src activity was critical for osteoclast energy production and bone resorbing activity [15,17].

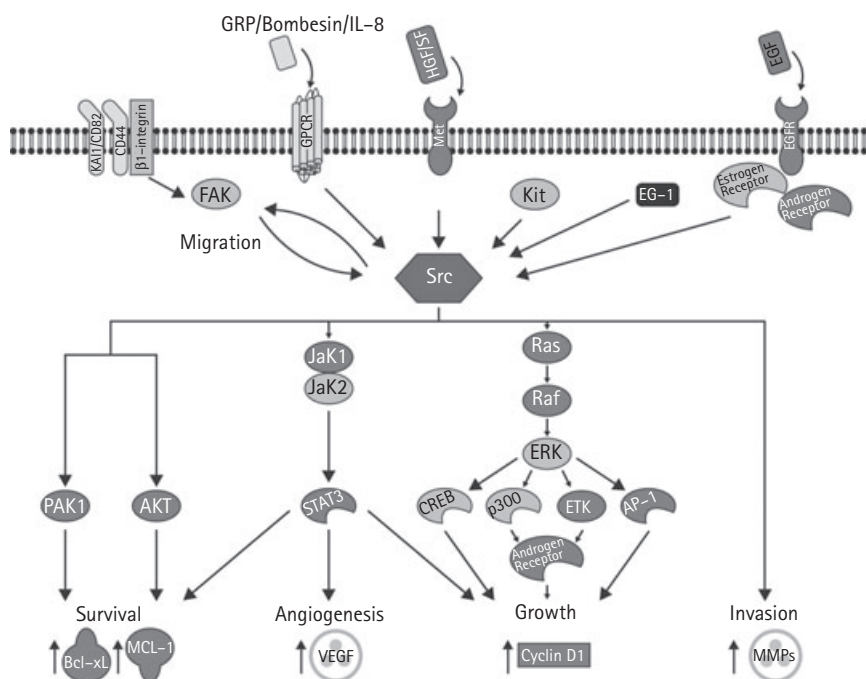
Src signalling is pivotal in osteoclast physiology. Src-deficient osteoclasts showed decreased migration [18] and impaired bone resorptive functioning *in vitro* [19]. Moreover, targeted disruption of Src in mice caused a defect in osteoclast-mediated bone resorption, leading to osteopetrosis [20]. Normal osteoclast function was restored by transgenic expression of Src in Src-knockout

mice; interestingly, even kinase-deficient mutants of *src* were effective, suggesting that Src recruits and/or activates downstream kinases [21]. Various *in vitro* studies using inhibitors of Src were consistent with its integral role in osteoclast function [22–25]. Lastly, suppression of Src also interfered with chloride-channel transport and vesicular acidification, processes required to solubilize bone mineral during bone resorption by osteoclasts [26].

For osteoblast function, there is extensive published evidence of a central role of Src; e.g. chemical or genetic inhibition of Src activation in osteoblasts significantly ablated the induction of connective tissue growth factor (CTGF), a functional anabolic mediator regulating osteoblast differentiation and function [27]. Extracellular receptor kinase (Erk) activation was simultaneously prevented, supporting a role for Src as an upstream mediator of Erk in regulating CTGF expression in osteoblasts. Yes-associated protein (YAP), a downstream target of SFKs, interacted with both Src and Yes to suppress the activation of Runx2, an osteoblast-related transcription factor required for osteoblast maturation [28]. Interference with the Src-YAP-Runx2 signalling pathway at any level inhibited YAP tyrosine phosphorylation, disrupted the Runx2-YAP interaction, and thus abrogated consequent downstream assembly of subnuclear regulatory complexes and resulting changes in osteoblast gene expression. Finally, Wnt proteins, lipid-modified signalling molecules influencing cell proliferation, differentiation and survival, prolonged the survival of both uncommitted osteoblast progenitors and differentiated osteoblasts in culture, an effect to which Src/Erk signalling was a significant contributor [29].

SFKs are intimately involved in prostate cancer progression. Src, androgen receptor and oestradiol receptor complex signalling triggered proliferation of prostate cancer cells *in vitro* [30]. Several studies have shown how SFKs might also contribute to castration-resistant prostate cancer by mediating signals from various factors, including growth factors, neuropeptides and chemokines, e.g. interleukin-8 (IL-8) [30–33]. Notably, there is evidence that Src might be involved in the initial transition from a castration-sensitive to castration-resistant state [31,34]. Finally, inhibition of Src signalling decreased proliferation, invasion

FIG. 2. Role of SRC in the bone metastases. Adapted with permission from [40].



and migration of prostate cancer cell lines *in vitro* [35–37].

Src has an important role in both tumour development and bone metabolism; therefore, Src signalling might be particularly important in patients with prostate cancer, in whom the skeleton is the preferential site for spread and disease advance. Bone metastases in prostate cancer comprise both osteolytic and osteoblastic components; the clinical presentation is usually predominantly osteoblastic, although this might partly reflect the diagnostic techniques used most often. Src is involved in many signalling pathways involved in neoplastic bone remodelling. In bone-derived metastatic castration-resistant prostate cancer PC-3 cells *in vitro*, Src was an integral component of a signalling complex regulating cell migration [38]. Src mediated CXCL12/CXCR4 transactivation of human epidermal growth factor receptor-2 (HER2) in lipid rafts of prostate cancer cells [39]. The CXCL12/CXCR4/HER2 signalling axis promotes bone matrix degradation, tumour cell invasion, growth of tumour in the bone microenvironment, and enhanced osteolysis.

In conclusion, Src is an important convergence point for signal transduction and regulation in many signalling pathways

in prostate cancer, involved in tumour cell proliferation, survival, migration and crucially, transition to androgen-independent growth (Fig. 2) [40]. Notably, targeting the micrometastatic (osteoclastic) stage of progressive prostate cancer, or even the transition phase between micrometastasis and macrometastatic establishment, while diagnostically challenging, might prevent, inhibit or delay disease progression [10]. Ultimately, as a first step between palliation and cure, successful management of the pathology and symptoms of bone metastatic prostate cancer could transform it into a chronic, rather than a fatal, disease. Src inhibitors are a promising avenue towards achieving this goal.

SRC SIGNALLING AS A THERAPEUTIC TARGET FOR METASTATIC PROSTATE CANCER

Agents in clinical development for castration-resistant prostate cancer include AZD0530 and dasatinib. AZD0530 is an orally active Src/Abl inhibitor in early clinical development. In preclinical testing, AZD0530 dose-dependently inhibited Src, proliferation and migration in a range of prostate cancer cell lines, including cells derived from bone metastatic castration-resistant tumours [41]. AZD0530 was a potent inhibitor of

osteoclast-mediated bone resorption in studies using isolated rabbit bone slices and organ cultures of neonatal mouse calvariae [42], and inhibited the formation and activity of human osteoclasts *in vitro* [43]. In mice bearing orthotopic tumours from castration-independent prostate tumour cell line clones, AZD0530 completely inhibited metastasis [44]. AZD0530 also retarded osteolytic lesions in a mouse model of castration-resistant bone metastatic prostate cancer [44,45].

AZD0530 showed pharmacokinetics consistent with once-daily dosing, and mainly mild adverse effects, in a Phase I study in healthy subjects [46]. The minimum plasma concentration remained above the 50% inhibitory concentration for Src. In healthy men from the same study population, AZD0530 lowered serum and urine levels of bone resorption markers, suggesting inhibition of osteoclastic activity via suppression of Src [47]. In a Phase I study in patients with cancer, AZD0530 inhibited phosphorylation of Src substrates and dose-dependently significantly reduced levels of markers of osteoclastic bone resorption [48]. This agent is currently being evaluated as monotherapy in a Phase II trial in patients with castration-resistant prostate cancer (ClinicalTrials.gov identifier: NCT00513071).

Dasatinib (Sprycel®, Bristol-Myers Squibb, New York, USA) is a small-molecule tyrosine kinase inhibitor with activity against several receptor and nonreceptor tyrosine kinases, including SFKs, platelet-derived growth factor receptor (PDGFR), c-kit, Bcr-Abl and ephrins [49]. Although dasatinib is currently approved for treating patients with all phases of chronic myelogenous leukaemia and Philadelphia chromosome-positive acute lymphoblastic leukaemia after unsuccessful previous therapy, its spectrum of antikinase activities has led to further evaluation in various solid tumours, including prostate cancer.

Preliminary results from a Phase II study in patients with advanced castration-resistant prostate cancer support a favourable effect of dasatinib on bone metastases. Of 27 patients with bone scans at 12 weeks, 16 were stable and one was improved. Two of five patients with two or more bone scans at 24 weeks had stable disease. Of the 37 patients with evaluable urinary N-telopeptide levels, including those who continued on bisphosphonate therapy, 21 (57%) had a $\geq 35\%$ decrease from baseline [50].

Dasatinib has well recorded safety and tolerability data in patients with cancer. The anti-metastatic and anti-osteoclastic effects that dasatinib has shown in preclinical studies could translate into clinical benefits for patients with advanced prostate cancer that has metastasized, or would likely metastasize, to bone. Early data from the above Phase II trial seem to support this premise. Reversal or amelioration of metastatic progression might extend the period before a patient requires chemotherapy; this could extend patient survival, as well as providing an acceptable option for older, frailer patients unable to tolerate aggressive treatment.

Preclinical proof-of-concept studies showed that dasatinib blocked the kinase activities of the SFKs Lyn and Src, and inhibited related downstream signalling, in both androgen-dependent and -independent human prostate cancer cells, at low nanomolar concentrations [35]. These effects correlated with inhibition of cell adhesion, migration and invasion in *in-vitro* model systems, supporting a potential effect of the drug on metastasis. Further evidence was obtained from a study in which dasatinib inhibited growth and lymph node metastasis of prostate cancer in an orthotopic nude mouse model [51]. Dasatinib also showed inhibitory effects on osteoclast-mediated bone resorption in an animal model [52]. Furthermore, dasatinib inhibited migration and invasion, and induced apoptosis in bone sarcoma cell cultures, confirming its ability to affect metastatic tumour tissue/bone-derived tissue [53].

In addition to its anti-Src effects, PDGFR-blocking effects might also contribute to the clinical efficacy of dasatinib. The PDGFR inhibitor ST1571 reduced lymph and bone metastasis of experimental prostate cancer in mice, suggesting a beneficial effect of inhibiting the PDGF signalling axis [54]. Combining the PDGFR inhibitor with paclitaxel produced clinically significant effects. The multikinase inhibitory effects of dasatinib might be beneficial in patients with the heterogeneous advanced phase of prostate cancer, with or without conventional chemotherapy.

SRC INHIBITION FOR RESTORING CASTRATION SENSITIVITY

The androgen receptor (AR) signalling axis is a survival-factor pathway, with androgens

being the primary, but not the only, survival factors for prostate cancer cells [10]. After therapeutic androgen ablation, even when levels are at or below castrate values, numerous other, less potent, survival factors can compensate for the absence of androgenic ligands, by activating or reinforcing AR transcription in a ligand-independent manner [10]. Cross-talk between steroid receptors such as AR and growth factors needed for tumour survival and progression occurs at many levels, contributing to castration resistance.

Central to many such processes is the signalling mediator Src; e.g. under short-term hormonal deprivation, AR regulated HER phosphorylation and signalling in a Src-dependent manner [30]. The neuropeptides bombesin and neurotensin induced androgen-independent growth in androgen-dependent prostate cells via indirect AR activation; Src was a key signalling molecule in the downstream pathway that was responsible [31]. In another study, IL-8 conferred androgen-independent growth and migration in similar cells; again, AR activation was mediated by signalling involving Src [32]. Transition from androgen dependence to androgen independence was associated with constitutive activation of a signalling pathway with Src at its apex [34]. In summary, Src is involved in anti-apoptotic pathways driven by AR activation via various mediators in prostate cancer, and as such is a promising target for overcoming castration resistance [41].

Because of this, there is prolific development of Src inhibitors. Agents with preclinical anti-Src activity relevant to bone metastasis in prostate cancer include: PD173955, CGP76030, CGP77675, UCS15A, AP22161, AP22408, AP23451 and AP23588 [11,55]. PD173955, a SFK family selective inhibitor, had significant antiproliferative activity in prostate cancer cells *in vitro* [56]. Two Src inhibitors, CGP76030 and CGP77675, reduced the *in vitro* proliferation, adherence, spread, and chemotactic migration of an androgen-independent prostate carcinoma cell line derived from bone metastasis [36]. The nonkinase Src signal transduction inhibitor UCS15A dose-dependently inhibited *in-vitro* bone resorption activity of mouse osteoclast-like multinucleated cells, and inhibited bone resorption in mouse calvaria organ cultures [57]. The Src SH2 domain-selective binding compound AP22161 inhibited rabbit

osteoclast-mediated resorption of dentine in cellular assays [23]. Finally, an osteoclast-selective Src SH2-binding inhibitor, AP22408, showed bone-targeting properties, and was antiresorptive in a parathyroid hormone-induced rat model of bone resorption [58].

OTHER SIGNAL-BLOCKING STRATEGIES IN BONE METASTATIC PROSTATE CANCER

Detailed consideration of the range of established and investigational strategies for treating bone metastasis in malignancy is outside the scope of this review, although these have been amply reviewed elsewhere [59,60]. However, of note in terms of an established class of bone-preserving agents are the bisphosphonates. These agents bind to bone surfaces, where they act as a mechanical barrier to osteoclast-mediated resorption; they also inhibit recruitment of osteoclast precursors, prevent migration of osteoclasts toward bone, and inhibit the production of various mediators involved in bone remodelling [61]. When taken up by osteoclasts, bisphosphonates interfere with various signal-transduction pathways and enzymes, resulting in osteoclast apoptosis and inhibition of osteolysis [55].

Recently, there have been reports of direct antitumour effects of bisphosphonates that might contribute to their inhibitory effects on bone metastases [8,62–64]. These are thought to comprise one or more signal-disruption mechanisms producing antiproliferative, anti-invasive and anti-angiogenic actions. One of the newer bisphosphonates, zoledronic acid, reduced the incidence of skeletal-related events in men with castration-resistant advanced prostate cancer [61,62,65]. In fact, zoledronic acid is the only bisphosphonate that has shown significant clinical benefit in this type of tumour [62]. Although zoledronic acid has a generally acceptable safety profile, treatment is by intravenous infusion, and monitoring of serum creatinine is required [61,66].

Other strategies to minimize or reverse the osteolytic component of bone metastasis in prostate cancer include Fc-OPG, a stabilized construct of osteoprotegerin (OPG); the latter molecule is a critical regulator of osteoclastogenesis, and preclinical studies have reported promising effects of both Fc-coupled OPG, or induced tumour OPG overexpression, on prostate cancer

establishment in bone [8]. While OPG inhibits osteoclast differentiation by interfering with the receptor activator of nuclear factor B (RANK)/RANK ligand (RANKL) interaction, it is also a survival factor for prostate cancer cells. Other approaches for blocking the RANK/RANKL interaction include soluble RANKL-Fc, and AMG-162 (a monoclonal antibody specific for human RANKL) [8,67].

Finally, strategies to ameliorate or reverse the osteoblastic response in prostate cancer progression to bone include targeted inhibition of the endothelin receptor signalling axis, as endothelin-1 is a significant osteoblast stimulatory factor [8]. Moreover, on ligand binding and activation, the endothelin-A receptor triggers a parallel activation of several signal-transducing pathways influencing cell growth and proliferation affecting both tumour and host bone responses [68]. Atrasentan (ABT-627) is an endothelin-A receptor antagonist that has shown some promising results in clinical trials. It delayed the progression of castration-resistant prostate cancer in some men, accompanied by attenuated blood PSA levels and bone formation markers. Men with prostate cancer and radiological evidence of bone metastases had a 19% delay in time to disease progression after atrasentan treatment [8,69,70].

CONCLUSIONS

Advanced prostate cancer is associated with bone metastases which carry significant morbidity and impair quality of life. Once prostate cancer has become castration-resistant and has metastasized, therapeutic options are limited and the outlook is bleak. Src is a key regulator of bone metabolism in both health and disease, and is associated with increased metastatic potential. As such, Src provides an ideal therapeutic target in patients at risk of bone metastases, and represents an alternative to more aggressive forms of treatment, potentially delaying the need for chemotherapy.

There are extensive reports confirming the importance of Src in normal and abnormal bone function, notably its critical role in the bone resorptive function of osteoclasts, a key process which both facilitates tumour invasion and spread, and triggers disorganized osteoblastic bone deposition.

Preclinical studies have shown that inhibition of Src signalling restores the balance in bone turnover. SFK inhibitors like AZD0530 and dasatinib could potentially have clinical activity in patients with metastatic bone disease, perhaps alongside one or more of hormonal therapies, continuing/intensified androgen ablation, chemotherapy, and conventional treatments for treating osteoblastic lesions; or even in as-yet to be developed combination regimens or treatment sequences involving novel and traditional therapies with various mechanisms of action.

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REFERENCES

- 1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71–96
- 2 Logothetis CJ, Lin SH. Osteoblasts in prostate cancer metastasis to bone. *Nat Rev Cancer* 2005; **5**: 21–8
- 3 Lage MJ, Barber BL, Harrison DJ *et al.* The cost of treating skeletal-related events in patients with prostate cancer. *Am J Manag Care* 2008; **14**: 317–22
- 4 Scher HI, Halabi S, Tannock I *et al.* Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the prostate cancer clinical trials working group. *J Clin Oncol* 2008; **26**: 1148–59
- 5 Chirgwin JM, Mohammad KS, Guise TA. Tumor–bone cellular interactions in skeletal metastases. *J Musculoskelet Neuronal Interact* 2004; **4**: 308–18
- 6 Demers LM, Costa L, Lipton A. Biochemical markers and skeletal metastases. *Cancer* 2000; **88** (Suppl. 12): 2919–26
- 7 Saad F, Lipton A, Cook R, Chen YM, Smith M, Coleman R. Pathologic fractures correlate with reduced survival in patients with malignant bone disease. *Cancer* 2007; **110**: 1860–7

- 8 Vessella RL, Corey E. Targeting factors involved in bone remodeling as treatment strategies in prostate cancer bone metastasis. *Clin Cancer Res* 2006; **12** (Suppl. 20): 6285s–6290s
- 9 Smith MR, Cook RJ, Coleman R *et al.* Predictors of skeletal complications in men with hormone-refractory metastatic prostate cancer. *Urology* 2007; **70**: 315–9
- 10 Msaouel P, Pissimissis N, Halapas A *et al.* Mechanisms of bone metastasis in prostate cancer: clinical implications. *Best Pract Res Clin Endocrinol Metab* 2008; **22**: 341–55
- 11 Fizazi K. The role of Src in prostate cancer. *Ann Oncol* 2007; **18**: 1765–73
- 12 Virk MS, Lieberman JR. Tumor metastasis to bone. *Arthritis Res Ther* 2007; **9** (Suppl. 1): S5
- 13 Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. *Nat Rev Genet* 2003; **4**: 638–49
- 14 Lowe C, Yoneda T, Boyce BF *et al.* Osteopetrosis in Src-deficient mice is due to an autonomous defect of osteoclasts. *Proc Natl Acad Sci USA* 1993; **90**: 4485–9
- 15 Marzia M, Sims NA, Voit S *et al.* Decreased c-Src expression enhances osteoblast differentiation and bone formation. *J Cell Biol* 2000; **151**: 311–20
- 16 Horne WC, Neff L, Chatterjee D *et al.* Osteoclasts express high levels of pp60c-src in association with intracellular membranes. *J Cell Biol* 1992; **119**: 1003–13
- 17 Miyazaki T, Neff L, Tanaka S *et al.* Regulation of cytochrome c oxidase activity by c-Src in osteoclasts. *J Cell Biol* 2003; **160**: 709–18
- 18 Sanjay A, Houghton A, Neff L *et al.* Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha (v) beta (3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J Cell Biol* 2001; **152**: 181–95
- 19 Boyce BF, Yoneda T, Lowe C *et al.* Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. *J Clin Invest* 1992; **90**: 1622–7
- 20 Soriano P, Montgomery C, Geske R *et al.* Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* 1991; **64**: 693–702
- 21 Schwartzberg PL, Xing L, Hoffmann O *et al.* Rescue of osteoclast function by transgenic expression of kinase-deficient Src in src-/- mutant mice. *Genes Dev* 1997; **11**: 2835–44
- 22 Hall TJ, Schaeublin M, Missbach M. Evidence that c-src is involved in the process of osteoclastic bone resorption. *Biochem Biophys Res Commun* 1994; **199**: 1237–44
- 23 Violette SM, Shakespeare WC, Bartlett C *et al.* A Src SH2 selective binding compound inhibits osteoclast-mediated resorption. *Chem Biol* 2000; **7**: 225–35
- 24 Violette SM, Guan W, Bartlett C *et al.* Bone-targeted Src SH2 inhibitors block Src cellular activity and osteoclast-mediated resorption. *Bone* 2001; **28**: 54–64
- 25 Yoneda T, Lowe C, Lee CH *et al.* Herbimycin A, a pp60c-src tyrosine kinase inhibitor, inhibits osteoclastic bone resorption in vitro and hypercalcemia in vivo. *J Clin Invest* 1993; **91**: 2791–5
- 26 Edwards JC, Cohen C, Xu W *et al.* c-Src control of chloride channel support for osteoclast HCl transport and bone resorption. *J Biol Chem* 2006; **281**: 28011–22
- 27 Arnott JA, Zhang X, Sanjay A *et al.* Molecular requirements for induction of CTGF expression by TGF-beta1 in primary osteoblasts. *Bone* 2008; **42**: 871–85
- 28 Zaidi SK, Sullivan AJ, Medina R *et al.* Tyrosine phosphorylation controls Runx2-mediated subnuclear targeting of YAP to repress transcription. *EMBO J* 2004; **23**: 790–9
- 29 Almeida M, Han L, Bellido T *et al.* Wnt proteins prevent apoptosis of both uncommitted osteoblast progenitors and differentiated osteoblasts by beta-catenin-dependent and - independent signaling cascades involving Src/ERK and phosphatidylinositol 3-kinase/AKT. *J Biol Chem* 2005; **280**: 41342–51
- 30 Migliaccio A, Di Domenico M, Castoria G *et al.* Steroid receptor regulation of epidermal growth factor signaling through Src in breast and prostate cancer cells: steroid antagonist action. *Cancer Res* 2005; **65**: 10585–93
- 31 Lee LF, Guan J, Qiu Y *et al.* Neuropeptide-induced androgen independence in prostate cancer cells: roles of nonreceptor tyrosine kinases Etk/Bmx Src, and focal adhesion kinase. *Mol Cell Biol* 2001; **21**: 8385–97
- 32 Lee LF, Louie MC, Desai SJ *et al.* Interleukin-8 confers androgen-independent growth and migration of LNCaP: differential effects of tyrosine kinases Src and FAK. *Oncogene* 2004; **23**: 2197–205
- 33 Pandini G, Mineo R, Frasca F *et al.* Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. *Cancer Res* 2005; **65**: 1849–57
- 34 Unni E, Sun S, Nan B *et al.* Changes in androgen receptor nongenotropic signaling correlate with transition of LNCaP cells to androgen independence. *Cancer Res* 2004; **64**: 7156–68
- 35 Nam S, Kim D, Cheng JQ *et al.* Action of the Src family kinase inhibitor, dasatinib (BMS-354825), on human prostate cancer cells. *Cancer Res* 2005; **65**: 9185–9
- 36 Recchia I, Rucci N, Festuccia C *et al.* Pyrrolopyrimidine c-Src inhibitors reduce growth, adhesion, motility and invasion of prostate cancer cells in vitro. *Eur J Cancer* 2003; **39**: 1927–35
- 37 Slack JK, Adams RB, Rovin JD *et al.* Alterations in the focal adhesion kinase/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells. *Oncogene* 2001; **20**: 1152–63
- 38 Sahu SN, Nunez S, Bai G *et al.* Interaction of Pyk2 and PTP-PEST with leupaxin in prostate cancer cells. *Am J Physiol Cell Physiol* 2007; **292**: C2288–96
- 39 Chinni SR, Yamamoto H, Dong Z *et al.* CXCL12/CXCR4 transactivates HER2 in lipid rafts of prostate cancer cells and promotes growth of metastatic deposits in bone. *Mol Cancer Res* 2008; **6**: 446–57
- 40 Chang YM, Kung HJ, Evans CP. Nonreceptor tyrosine kinases in prostate cancer. *Neoplasia* 2007; **9**: 90–100
- 41 Chang YM, Bai L, Yang J *et al.* AZD0530 is a novel Src inhibitor with anti-proliferation and anti-migration properties in prostate cancer. *Proc Am Assoc Cancer Res* 2007; (Suppl.): Abstract LB–24
- 42 Mullender MG, Everts V, Green TP *et al.* Inhibition of osteoclastic bone resorption by the novel, potent, and selective c-Src/Abl kinase inhibitor, AZD0530. *Proc Am Assoc Cancer Res* 2005; **46** (Suppl.): Abstract 2923
- 43 Klein-Nulend J, van Duin MA, Green TP, Everts V, de Vries TJ. The dual specific Src/Abl kinase inhibitor AZD0530 inhibits the formation and activity of human osteoclasts. *J Clin Oncol* 2007; **25** (Suppl.): 3602
- 44 Evans CP, Lara PN, Kung H *et al.* Activity of the Src-kinase inhibitor AZD0530 in androgen-independent prostate cancer

- (AIPC): pre-clinical rationale for a phase II trial. *J Clin Oncol* 2006; **24** (Suppl.): 14542
- 45 Evans CP, Bai L, Kung H, Yang JC. Effect of the specific Src kinase inhibitor AZD0530 on osteolytic lesion in prostate cancer. *ASCO Meeting Genitourinary Cancers Symposium. J Clin Oncol* 2008; Abstract 170
 - 46 Lockton JA, Smethurst D, Macpherson M *et al*. Phase I ascending single and multiple dose studies to assess the safety, tolerability and pharmacokinetics of AZD0530, a highly selective, dual-specific Src-Abl inhibitor. *J Clin Oncol* 2005; **23** (Suppl.): 3125
 - 47 Eastell R, Hannon RA, Gallagher N *et al*. The effect of AZD0530, a highly selective, orally available Src/Abl kinase inhibitor, on biomarkers of bone resorption in healthy males. *J Clin Oncol* 2005; **23** (Suppl.): 3041
 - 48 Tabernero J, Cervantes A, Hoekman K *et al*. Phase I study of AZD0530, an oral potent inhibitor of Src kinase: first demonstration of inhibition of Src activity in human cancers. *J Clin Oncol* 2007; **25** (Suppl.): 3520
 - 49 Lombardo LJ, Lee FY, Chen P *et al*. Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)-piperazin-1-yl)-2-methylpyrimidin-4-ylamino) thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem* 2004; **47**: 6658-61
 - 50 Yu EY, Wilding G, Posadas E *et al*. Dasatinib in patients with hormone-refractory progressive prostate cancer: a phase II study. *J Clin Oncol* 2008; **26** (Suppl.): Abstract 5156
 - 51 Park SI, Zhang J, Phillips KA *et al*. Targeting SRC family kinases inhibits growth and lymph node metastases of prostate cancer in an orthotopic nude mouse model. *Cancer Res* 2008; **68**: 3323-33
 - 52 Luo FR, Camuso A, McGlinchey K *et al*. Evaluation of anti-osteoclastic activity of the novel, oral multi-targeted kinase inhibitor Dasatinib (BMS-354825). AACR-NCI-EORTC. *Int Conf Mol Targets Cancer Ther* 2005; (Suppl.): Abstract B178
 - 53 Shor AC, Keschman EA, Lee FY *et al*. Dasatinib inhibits migration and invasion in diverse human sarcoma cell lines and induces apoptosis in bone sarcoma cells dependent on SRC kinase for survival. *Cancer Res* 2007; **67**: 2800-8
 - 54 Uehara H, Kim SJ, Karashima T *et al*. 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
 - 55 Rucci N, Susa M, Teti A. Inhibition of protein kinase c-Src as a therapeutic approach for cancer and bone metastases. *Anticancer Agents Med Chem* 2008; **8**: 342-9
 - 56 Moasser MM, Srethapakdi M, Sachar KS *et al*. Inhibition of Src kinases by a selective tyrosine kinase inhibitor causes mitotic arrest. *Cancer Res* 1999; **59**: 6145-52
 - 57 Sharma SV, Oneyama C, Yamashita Y *et al*. UCS15A, a non-kinase inhibitor of Src signal transduction. *Oncogene* 2001; **20**: 2068-79
 - 58 Shakespeare W, Yang M, Bohacek R *et al*. Structure-based design of an osteoclast-selective, nonpeptide Src homology 2 inhibitor with in vivo antiresorptive activity. *Proc Natl Acad Sci USA* 2000; **97**: 9373-8
 - 59 Lipton A. Future treatment of bone metastases. *Clin Cancer Res* 2006; **12** (Suppl. 20): 6305s-6308s
 - 60 Susa M, Glatt M, Teti A. Tumor bone diseases: molecular mechanisms and opportunities for novel treatments. *Curr Med Chem Anticancer Agents* 2001; **1**: 313-29
 - 61 Pienta KJ, Smith DC. Advances in prostate cancer chemotherapy: a new era begins. *CA Cancer J Clin* 2005; **55**: 300-18
 - 62 Caraglia M, Santini D, Marra M *et al*. Emerging anti-cancer molecular mechanisms of aminobisphosphonates. *Endocr Relat Cancer* 2006; **13**: 7-26
 - 63 Oades GM, Coxon J, Colston KW. The potential role of bisphosphonates in prostate cancer. *Prostate Cancer Prostatic Dis* 2002; **5**: 264-72
 - 64 Saad F. New research findings on zoledronic acid: survival, pain, and anti-tumour effects. *Cancer Treat Rev* 2008; **34**: 183-92
 - 65 Saad F, Gleason DM, Murray R *et al*. Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer. *J Natl Cancer Inst* 2004; **96**: 879-82
 - 66 Saad F, Higano CS, Sartor O *et al*. The role of bisphosphonates in the treatment of prostate cancer: recommendations from an expert panel. *Clin Genitourin Cancer* 2006; **4**: 257-62
 - 67 Saad F, Markus R, Goessl C *et al*. Targeting the receptor activator of nuclear factor-kappaB (RANK) ligand in prostate cancer bone metastases. *BJU Int* 2008; **101**: 1071-5
 - 68 Carducci MA, Jimeno A. Targeting bone metastasis in prostate cancer with endothelin receptor antagonists. *Clin Cancer Res* 2006; **12** (Suppl. 20): 6296s-6300s
 - 69 Carducci MA, Saad F, Abrahamsson PA *et al*. A phase 3 randomized controlled trial of the efficacy and safety of atrasentan in men with metastatic hormone-refractory prostate cancer. *Cancer* 2007; **110**: 1959-66
 - 70 Petrylak DP. New paradigms for advanced prostate cancer. *Rev Urol* 2007; **9** (Suppl. 2): S3-12

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Abbreviations: AR, androgen receptor; IL, interleukin; ERK, extracellular receptor kinase; SFK, Src family kinase; CTGF, connective tissue growth factor; YAP, Yes-associated protein; HER, human epidermal growth factor receptor; PDGF(R), platelet-derived growth factor (receptor).