

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Skeletal metastases and impact of anticancer and bone-targeted agents in patients with castration-resistant prostate cancer

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1615145> since 2016-11-21T20:18:51Z

Published version:

DOI:10.1016/j.ctrv.2016.02.002

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in *CANCER TREATMENT REVIEWS*, 44, 2016, 10.1016/j.ctrv.2016.02.002.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.ctrv.2016.02.002

The publisher's version is available at:

<http://linkinghub.elsevier.com/retrieve/pii/S0305737216000177>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1615145>

Skeletal metastases and impact of anticancer and bone-targeted agents in patients with castration-resistant prostate cancer

Francesca Vignani*^a, Valentina Bertaglia*^a, Consuelo Buttigliero^a, Marcello Tucci^a, Giorgio V.

Scagliotti^a, Massimo Di Maio^a.

* These Authors equally contributed to the review

^a Division of Medical Oncology, Department of Oncology, University of Turin at San Luigi Gonzaga Hospital, Orbassano, Turin, Italy

Corresponding Author: Marcello Tucci, MD

Division of Medical Oncology, Department of Oncology, University of Turin, San Luigi Gonzaga Hospital, Regione Gonzole 10, 10043 Orbassano (Turin), Italy;

Tel. +390119026414;

Fax +390119015184;

e-mail: marcello.tucci@gmail.com

Skeletal metastases and impact of anticancer and bone-targeted agents in patients with castration-resistant prostate cancer

Skeletal metastases and impact of anticancer and bone-targeted agents in patients with castration-resistant prostate cancer

ABSTRACT

Incidence of bone metastases is very high in advanced prostate cancer patients. Bone metastases likely have a significant impact on functional status and quality of life, not only related to pain, but also to the relevant risk of skeletal-related events. A better understanding of mechanisms associated with bone metastatic disease secondary to prostate cancer and more specifically to the cross-talk between tumor cells and bone microenvironment in metastatic progression represented the background for the development of new effective bone-targeted therapies. Furthermore, a better knowledge of biological mechanisms driving disease progression led to significant advances in the treatment of castration-resistant prostate cancer, with the development and approval of new effective drugs. Aim of this review is to outline the physiopathology of bone metastases in prostate cancer and summarize the main results of clinical trials conducted with different drugs to control morbidity induced by skeletal metastases and bone disease progression. For each agent, therapeutic effect on bone metastases has been measured in terms of pain control and/or incidence of skeletal-related events, usually defined as a composite endpoint, including the need for local treatment (radiation therapy or surgery), spinal cord compression, pathological bone fractures. In details, data obtained with chemotherapy (mitoxantrone, docetaxel, cabazitaxel), new generation hormonal agents (abiraterone, enzalutamide), radium-223, bone-targeted agents (zoledronic acid, denosumab) and with several experimental agents (cabozantinib, dasatinib, anti-endothelin and other agents) in patients with castration-resistant prostate cancer are reviewed.

KEYWORDS

Castration resistant prostate cancer, bone metastasis, skeletal related event, new generation hormonal agents, chemotherapy, bone-targeted therapy

INTRODUCTION

In developed countries, prostate cancer is the most common type of cancer diagnosed among men, with more than 1,100,000 new cases worldwide every year [1]. In this cancer, bone represents a preferential site of metastases, and patients with advanced disease have a very high incidence of bone metastases [2]. Autopsy data from prostate cancer patients indicate an incidence of secondary bone lesions as high as 65%-75%, preceded only by multiple myeloma [3]. These bone metastases are typically osteosclerotic (i.e. with increased osteoblastic activity), and likely to produce a significant impact on patients' functional status and quality of life (QoL), not only related to pain, but also to the relevant risk of skeletal-related events (SREs) that can negatively impact physical well-being and activities of daily living [4,5]. According to Food and Drug Administration [6], skeletal related events (SREs) include pathologic bone fractures (both vertebral and non-vertebral), spinal cord compression, surgery to bone, radiotherapy to bone. To estimate the incidence of SREs in patients with prostate cancer and bone metastases the control arm of the trials testing bisphosphonates may be used as a reference value [7]. In a 15-month observation period, nearly half (44.2%) of those patients experienced at least one SRE.

SREs may have a relevant impact on survival of prostate cancer patients with bone metastases. In a landmark analysis of a randomized trial comparing zoledronic acid (ZA) versus placebo, patients without SREs in the first six months had significantly better 1-year survival rate compared to patients suffering from one or more SRE [8]. Furthermore, survival of patients with multiple events was worse than propensity-matched patients with only one SRE, although this difference was not statistically significant. A secondary analysis of randomized trials with ZA showed that, in patients with metastatic prostate cancer similarly to other tumor types, the incidence of pathological fractures is associated with a significantly increased risk of death [7]. In details, patients with pathological

fractures had a 29% increase in the risk of death at the unadjusted analysis (Hazard Ratio [HR] 1.29, 95% confidence interval [CI] 1.01-1.65), with comparable results observed for both vertebral and non-vertebral fractures. Adjusted analyses for prognostic covariates, including previous SRE occurrence and performance status, led to comparable results. As expected, although prostate cancer patients with metastatic spinal cord compression had a relatively better life expectancy compared to other tumors, this complication has a relevant impact on survival [9,10].

Patients with a SRE have a significantly worse QoL [5,8] and when assessed by validated instruments, such as the Functional Assessment of Cancer Therapy-General (FACT-G) and the Brief Pain Inventory (BPI), a clearly worse outcome was observed in patients with SREs compared to those without, with statistically significant differences in FACT-G total score, in functional well-being, physical well-being, emotional well-being and in BPI score [8]. When all types of SRE were considered as a whole (need for radiation, pathological fractures, other SRE) there was a statistically significant and clinically relevant decline in QoL in all domains [5]. Of course, treatment of SRE can improve QoL: radiation therapy can produce a significant reduction of pain [5], while treatment of spinal cord compression may improve performance status [10]. The occurrence of bone complications is also likely to be responsible for the increased direct and indirect costs of patients' management [11].

All SREs are associated with relevant health resource utilization, including both inpatient hospitalizations and outpatient or emergency department visits and procedures [12-14]. Furthermore, those studies trying to calculate the costs associated with SREs may have underestimated their global impact in terms of health resource utilization, due to the exclusion of patients with low performance status or life expectancy, and the exclusion of resource consumption associated with bone pain management [15].

Recently, the management of castration-resistant prostate cancer (CRPC) significantly changed, with approval of several new drugs [16]. This evolving therapeutic landscape was paired by a better knowledge of biological mechanisms driving disease progression. Nowadays, we know that AR signalling pathway has a significant activity also in CRPC and that the interplay between prostate cancer cells and bone microenvironment plays a crucial role in bone metastatic progression.

Aim of this review is to outline the physiopathology of bone metastases in prostate cancer and the contribution of each of these new agents in terms of control of morbidity induced by skeletal metastases and bone disease progression.

PHYSIOPATHOLOGY OF BONE METASTASES IN PROSTATE CANCER

Bone metastasis is a complex event due to the interaction among cancer cells, normal bone cells and bone microenvironment, leading to a severe disruption of physiological bone remodeling [17]. The latter is a dynamic process, critical to maintain skeletal integrity, responsible for replacement of old bone with a mechanically more competent bone. It occurs at specialized skeleton sites - called "bone remodeling units" - and is characterized by a functional sequence: osteoclast-mediated bone resorption followed by osteoblast-induced bone apposition [18,19].

In the early phase of bone remodeling, osteoclasts are attracted to bone surface, in which these cells excavate the Howship's lacuna, a resorption cavity. Following the cavity formation, osteoclasts produce several factors responsible for osteoblasts attraction to the sites of previous resorption. This sequence of events is called "coupling phenomenon". As described by Paget in 1889, tumor cells are "the seeds" which need a favorable "soil" in order to thrive at metastatic sites [20]. Skeletal microenvironment is an ideal "soil", due to presence of growth factors and cytokines stored in the bone matrix and released during cross-talk between bone-resident cells and cancer cells [21].

In the metastatic cascade, the first step is the homing of tumor cells to skeletal tissue [21,22]. This process is not a casual event, but is due to the production by bone microenvironment of the same chemotactic factors responsible for the migration of hematopoietic stem cells into the bone marrow. These cells are localized at a specific site, the hematopoietic stem cell niche, where they may remain quiescent or divide and then differentiate. An important chemotactic factor is the stromal-derived factor-1 (SDF-1), also called CXCL12. This cytokine, mainly produced by osteoblasts, interacts with the CXCR4 receptor on hematopoietic stem cells, inducing their homing to the bone marrow [21-24]. The pathway SDF-1/CXCR4 is also able to modulate the attraction of prostatic tumor cells to bone. Some preclinical studies showed a significant expression of CXCR4 on the

surface of prostate cancer cells [25]. The induction of SDF-1 expression from bone marrow endothelial cells favors prostatic cancer migration and adhesion to extracellular bone matrix [22,26]. Therefore, prostatic tumor cells are able to compete with hematopoietic stem cells for the place in the bone marrow niche; this complex process determines the formation of so-called “onco-niche”, in which cancer cell may remain in a state of dormancy or may start to colonize and invade (**Figure A**) [17,21,22].

During the metastatic colonization of the bone, prostate cancer cells interfere with the physiological bone remodeling due to the release of paracrine factors physiologically involved in the regulation of both osteoclastic and osteoblastic activity (**Figure B**). The early, crucial phase of this process is the abnormal increase of osteoclast-mediated bone resorption, due to several growth factors and cytokines, as transforming growth factor β 1 (TGF β 1), parathyroid-hormone-related peptide and interleukin 6 [27]. These factors lead to the activation of the receptor activator nuclear kappa B (RANK) / RANK ligand (RANKL) pathway, which plays a central role in bone resorption regulation. RANKL, produced by osteoblasts, binds its receptor RANK on osteoclasts surface, favoring osteoclast maturation, survival and activity [17,21]. Increased osteolysis is crucial for the seeding of prostate cancer cells, and is also associated with the release from the bone matrix of several growth and survival factors, responsible for tumor progression [27]. In the subsequent phase of skeletal colonization there is an excessive bone apposition, which becomes dominant compared to bone resorption. This is due to growth factors including basic fibroblast growth factor, bone morphogenic proteins, endothelin-1 (ET-1), tumour growth factor β 1 and insulin-like growth factor 1, that are released by cancer cells and from bone matrix and stimulate both osteoblasts activity and tumor proliferation. Prostatic cancer cells may also contribute to bone apposition by gaining the same functional activities of osteoblasts (“osteomimicry”) [27].

The complex interaction between bone microenvironment and tumor cells leads to the so-called “vicious cycle”, that induces cancer progression [17].

Prostate cancer patients with bone metastases frequently have SREs due to increased osteolysis in typically osteoblastic bone lesions [17,28]. Increased osteoclastic activity is not only confined to metastatic sites, but it may be considered a more generalized event [17,28]. This is caused by secondary hyperparathyroidism, due to the so-called “bone hunger syndrome”, a metabolic derangement in which calcium entrapment in skeletal tissue, due to increased osteoblastic activity, leads to hyperparathyroidism in response to serum calcium deficiency [29]. Compensatory increase of parathyroid hormone secretion is responsible of osteoclasts activation at distant sites.

Furthermore, an additional cause of bone resorption is represented by iatrogenic osteoporosis, induced by androgen deprivation treatment [28].

Skeletal related events: different definitions.

In older trials, therapeutic effect on bone metastases was measured in terms of pain, decrease in biochemical markers of bone turnover, serial imaging assessment showing healing of bone lesions [30]. In recent trials, SREs have been defined as a composite endpoint, mostly including the need for local treatment (radiotherapy or surgery), spinal cord compression and pathological bone fractures [31-36]. Radiotherapy may include treatment of uncontrolled pain, treatment or prevention of imminent pathologic fractures, treatment or prevention of spinal cord compression. Surgery may include procedures to stabilize pathologic fractures or spinal cord compression, but also procedures aimed to prevent these SREs. Some trials consider only skeletal symptomatic events (SSE), other trials include also asymptomatic bone fractures. Only in some trials the use of radioisotopes is explicitly included among the radiation therapy procedures. **Table A** summarizes the definition of SREs in selected randomized trials conducted in patients with metastatic prostate cancer, using SREs as primary or secondary endpoint.

IMPACT OF ANTI-CANCER TREATMENTS

Chemotherapy

Mitoxantrone -

At the beginning of this century, mitoxantrone plus prednisone was commonly used in CRPC patients for its palliative role, despite the negative outcome of randomized trials that did not show a significant improvement in overall survival (OS) [37,38]. In one trial CRPC patients with pain received mitoxantrone plus prednisone or prednisone alone (**Table B**) [37]. Most of the enrolled patients (96%) had bone metastases. The primary endpoint was palliative response, defined as pain decrease without an increase in analgesics use. Palliative response rate was 29% with mitoxantrone plus prednisone and 12% with prednisone alone ($p=0.01$). Decrease in analgesics use without an increase in pain, one of the secondary endpoints, was comparable in the two arms. Later, another trial compared hydrocortisone alone vs. hydrocortisone plus mitoxantrone (**Table B**) [38]. Although there was no significant OS benefit, which was the primary endpoint, frequency and severity of pain were significantly better with mitoxantrone. Unfortunately, none of the trials included a description of SREs.

Docetaxel -

Before the TAX-327 [39] and the SWOG 99-16 study [40], that demonstrated the efficacy of docetaxel, no OS benefit had been shown with chemotherapy in CRPC patients. Both those two trials had OS as primary endpoint, while the impact on pain was among secondary endpoints (**Table B**). TAX-327 study compared two docetaxel schedules (every-3-week or weekly) plus prednisone versus mitoxantrone plus prednisone, and showed a significant OS benefit with every-3-week docetaxel [39,41]. Most patients (91%) had bone metastases and 45% had baseline pain. A reduction in pain was more frequently documented with every-3-week docetaxel than with mitoxantrone [39]. Pain response was

associated with OS outcome: median survival was 18.6 months among patients who achieved a pain response versus 12.5 months in patients who did not obtain pain response. However, improvement in median OS with every-3-week docetaxel was 3.9 months among men without significant baseline pain, and 2.4 months among those with baseline pain, suggesting that OS benefit associated with docetaxel is not limited to symptomatic patients obtaining pain response [42,43]. In the SWOG 99-16 trial, patients were randomized to docetaxel plus estramustine versus mitoxantrone plus prednisone [40]. Patients in docetaxel-estramustine arm had a significant OS improvement, although pain relief was similar in the two arms. In both these randomized trials, no specific SREs description was available.

Although not referred to CRPC but conducted in the “earlier” setting of hormone-naïve prostate cancer patients, in the STAMPEDE trial, the addition of docetaxel to androgen deprivation treatment (ADT) produced not only a relevant OS benefit (HR 0.78; p=0.006), but also a significant reduction in the time to first reported SSE (HR 0.60; p= 0.13 x 10⁻⁵) (Table B) [44].

Cabazitaxel -

In preclinical and clinical models, cabazitaxel showed significant efficacy in docetaxel-resistant and refractory prostate carcinomas [45,46]. In the randomized phase III TROPIC trial, comparing cabazitaxel plus prednisone versus mitoxantrone plus prednisone in patients with metastatic CRPC after docetaxel failure, cabazitaxel was associated with a significant prolongation of OS [47]. More than 80% of patients had bone metastases, and about 45% had baseline pain. Secondary endpoints included pain response and time to pain progression, and cabazitaxel showed similar pain improvement compared to mitoxantrone (Table B) [48]. In an expanded access program conducted in United Kingdom, 31%-57% of patients treated with cabazitaxel reported “no pain or discomfort”

during treatment at various cycles, compared to 22% at baseline [49]. No specific description of the impact on SREs of cabazitaxel is available.

New generation hormonal agents

Abiraterone -

Abiraterone acetate (AA) is a potent, selective and irreversible inhibitor of CYP17, a critical enzyme in androgens synthesis [50]. The randomized trial COU-AA-301 compared AA plus prednisone vs. placebo plus prednisone in patients with metastatic CRPC (mCRPC) progressing after chemotherapy [51]. AA plus prednisone demonstrated a significant survival benefit [51,52]. At baseline, about 90% of patients in both arms had bone metastases, with similar pain scores. Incidence of SREs was 29% with AA and 33% with placebo; time to first SRE was significantly longer with AA (median 25.0 vs. 20.3 months; HR 0.615; $p=0.0001$) [35] (**Table B**). The most common SRE (expressed as rate per 100 patients-years of exposure) was bone radiation (24% with AA vs. 46.1% with placebo); others included pathologic fracture (6.0% vs. 4.0%), bone surgery (1.7% vs. 1.0%), and spinal cord compression (7.3% vs. 14.0%). In patients with clinically significant pain at baseline, AA produced significantly more palliation (45.0% vs. 28.8%; $p=0.0005$) and faster palliation of pain intensity (median time to palliation 5.6 vs. 13.7 months; $p=0.0018$) [35]. Iuliani and al. investigated AA activity on bone microenvironment in an *in vitro* model and in a clinical prospective cohort of 49 mCRPC patients, in which serum markers of bone turnover (ALP and CTX) were measured at baseline and every 3 months during treatment with AA [53]. AA was associated with a statistically significant inhibition of osteoclast differentiation and with osteoblasts differentiation. During treatment, patients had a progressive CTX reduction along with an increase of ALP values. In conclusion, this study demonstrated a direct bone anabolic and anti-resorptive effect of AA.

The randomized trial COU-AA-302 evaluated AA with prednisone compared to placebo plus prednisone in asymptomatic or mildly symptomatic mCRPC docetaxel-naive patients (**Table B**) [54]. Co-primary endpoints included radiographic progression-free survival (rPFS) and OS. The proportion of patients with bone disease only (51% and 49% in

experimental and control arm, respectively), and that of patients with more than 10 bone lesions (49% and 47%, respectively), were similar in the two arms. AA improved both OS and rPFS. Furthermore, secondary endpoints, such as time to symptomatic deterioration, time to pain progression and PSA PFS were significantly improved. Treatment with AA was associated with a significant improvement in time to opiate use (median not reached vs. 23.7 months; $p=0.001$), in time to increase in pain (median 26.7 vs. 18.4 months, $p=0.049$), and in time to progression of pain interference (median 10.3 vs. 7.4 months; $p=0.005$). Unfortunately, no data are available about the impact of treatments on SREs occurrence. A *post hoc* analysis evaluated the safety and efficacy of AA with concomitant bone targeted therapies (BTT) [55]. Overall, 34% of patients in experimental arm and 31% in control arm received concomitant BTT. Superiority of AA was confirmed both with and without BTT. Furthermore, although the interpretation of these results is limited by their *post hoc* nature, concomitant BTT prolonged time to opioid use (HR 0.80; $p=0.036$), time to performance status deterioration (HR 0.75; $p<0.001$) and was associated with better OS (HR 0.75; $p=0.01$). In a retrospective study of mCRPC patients treated with AA, out of 123 patients with baseline pain, 29% reported an improvement during treatment, 32% no change and 28% a worsening [56].

Enzalutamide -

Enzalutamide is an AR antagonist, more potent than first-generation drugs [57]. Similarly to abiraterone, enzalutamide is approved for the treatment of both patients with mCRPC progressing after chemotherapy and chemotherapy-naive patients. The AFFIRM phase III trial randomized men with mCRPC progressing after chemotherapy to enzalutamide versus placebo (**Table B**) [58]. At baseline, proportion of patients with bone lesions (about 92%), proportion of patients with more than 20 lesions (38%), and intensity of pain were similar between arms. Enzalutamide demonstrated a significant improvement in OS which

was the primary end point of the study, and its superiority was confirmed in all secondary endpoints. In details, median time to first SRE was 16.7 months with enzalutamide versus 13.3 months with placebo (HR 0.69; $p < 0.001$) (**Table B**) [34]. Approximately half of patients were receiving a bisphosphonate at baseline. Time to first SRE was significantly improved by enzalutamide in patients not receiving bisphosphonate (HR 0.614; $p = 0.0005$) and not significantly in patients who were receiving bisphosphonate (HR 0.762; $p = 0.553$), although the study was not designed and powered to test this interaction. Enzalutamide provided consistent benefits in several pain measures, including pain severity, pain interference and pain palliation. Pain palliation was achieved in 45% of patients with enzalutamide versus 7% with placebo ($p = 0.0079$).

The phase III PREVAIL study compared enzalutamide versus placebo in asymptomatic or mildly symptomatic patients with chemotherapy-naïve mCRPC (**Table B**), having OS and rPFS as co-primary endpoints [59]. Both were significantly improved with enzalutamide. At baseline, number of bone lesions and pain intensity were similar between arms. Although median time to first SRE was similar in the two arms, the risk of first SRE was significantly decreased with enzalutamide (HR 0.72; 95%CI 0.61-0.84; $p < 0.001$) (**Table B**) [60]. Median time to pain progression was 5.7 months with enzalutamide versus 5.6 months with placebo (HR 0.62, 95%CI 0.53-0.74; $p < 0.0001$). At week 13, progression of pain was significantly less common with enzalutamide (29%) than with placebo (42%, $p < 0.0001$).

Radium-223 -

Radium-223 dichloride is a α particle-emitting agent [61] and, as a calcium mimetic, is taken up into areas of high bone turnover, such as bone metastases [62]. Once radium-223 binds bone, α particles induce double-stranded DNA breaks, causing a local cytotoxic effect [63]. To date, it is the only radionuclide that showed OS benefit in CRPC. The phase III trial ALSYMPCA randomized mCRPC patients with bone metastases and without visceral metastases to receive either radium-223 or placebo in addition to the best standard of care (**Table C**) [64], having OS as the primary endpoint. Time to first SSE and time to increase in alkaline phosphatase (ALP) were among secondary endpoints. At baseline, number of bone lesions and pain intensity were similar between the arms. OS was significantly prolonged by radium-223 and time to first SSE was also improved (median 15.6 vs. 9.8 months; HR 0.66, 95%CI 0.52-0.83; $p < 0.001$). The use of external beam radiation therapy to treat bone pain and the risk of spinal cord compression were significantly reduced, while radium-223 did not significantly reduce the risk of symptomatic pathological bone fracture and the need for tumor-related surgery. Decrease in ALP $\geq 30\%$ occurred in 47% with radium-223 vs. 3% with placebo ($p < 0.001$) [36]. Radium-223 provided a delay in biochemical (ALP) progression (median 7.4 vs. 3.8 months). In the ALSYMPCA study, 55% of patients required opioids at baseline [65]. Data about pain response were not collected, however in patients without opioids at baseline the proportion of patients who received opioids during study was 36% with radium-223 versus 50% with placebo, and radium-223 significantly delayed time to opioids use (HR 0.62; 95%CI 0.46–0.85). At baseline, 41% of patients were treated with BTT, and radium-223 increased OS regardless of bisphosphonate use. Delay in SSEs with radium-223 was reported both in patients not treated with BTT (although not statistically significant: median 11.8 vs. 8.4 months; HR 0.77; $p = 0.07$) and in patients treated with bisphosphonates (median 19.6 vs. 10.2 months; HR 0.49; $p = 0.00048$). In 2015, a systematic review evaluated the efficacy of

radiopharmaceuticals (89-strontium-chloride, 153-samarium-EDTMP, 186-rhenium-HEDP, 188-rhenium-HEDP and 223-radium-chloride) for palliation of bone pain from prostate cancer [66]. Pain response rates greater than 50%–60% were observed with all radionuclides. However, this review did not identify which radionuclide provides the best level and duration of pain relief, and OS results are not easily interpreted, because most studies were underpowered.

Bone-targeted agents

Zoledronic acid and other bisphosphonates–

Bisphosphonates reduce excessive bone turnover while preserving bone structure and mineralization. In early 1990s, several trials were initiated to investigate the use of bisphosphonates in prostate cancer: PR04 trial investigated the efficacy of sodium clodronate in locally advanced PC with negative bone scan, while PR05 investigated the same compound in bone metastatic hormone sensitive patient [67, 68]. Both trials resulted negative in terms of bone metastases- free survival and symptomatic bone PFS advantage, respectively. Mature data about OS, that was secondary endpoint, were published later: these data showed a benefit in OS only in PR05 patients, not in PR04 patients [69].

The first agent approved for the management of bone metastases in CRPC patients was zoledronic acid (ZA), a third-generation bisphosphonate. A phase III trial compared ZA versus placebo, demonstrating a significant reduction in the incidence of at least one SRE during the 24-month study period (**Table C**) [31,70]. Proportion of patients with at least one SRE was 49% with placebo and 38% with ZA ($p=0.028$). Furthermore, ZA significantly prolonged time to first SRE (HR 0.67; $p=0.009$), and time to first and subsequent SRE (HR 0.64, $p= 0.002$). The annual SRE incidence was 0.77 with ZA versus 1.47 with placebo ($p=0.005$) [71]. Pain scores and use of analgesics favored ZA. There were no differences either in disease progression or in OS [31,70]. In this study, 70% of patients treated with ZA had normalization within 1 month of the urinary levels of N-telopeptide (NTX), a markers of bone resorption. The normalization of NTX levels within 3 months correlated with a 59% reduction in the risk of death ($p<0.0001$) [71].

The TRAPEZE trial investigated the efficacy of addition of ZA and/or strontium-89 to docetaxel in CRPC patients [72]. Patients were randomized to receive docetaxel plus prednisolone: alone; with ZA; with a single dose of Sr89 after cycle 6 or both. Sr89

improved clinical progression free survival (CPFS), but not OS. ZA did not improve CPFS or OS but did significantly improve median SRE-free interval, mostly post-progression, suggesting a role as post-chemotherapy maintenance therapy. (**Table C**)

Of note, several trials have tested the role of zoledronic acid in patients with “earlier” phase of disease. The CALGB90202 study randomized castration-sensitive prostate cancer patients to ZA or placebo, with the aim of detecting a reduction in the risk of first SRE (~~Table C~~) [73]. Unfortunately, the primary endpoint was not met. Early treatment with ZA was not associated with a decreased SRE risk, compared with treatment initiation after progression to castration-resistant disease. Similarly, in the abovementioned STAMPEDE trial (~~Table B~~), the addition of ZA to ADT in hormone-naïve patients did not translate into a significant benefit in time to first SSE, both in the entire population and in the subgroup of patients with bone metastases [44]. On the contrary, the arm testing the addition of both docetaxel and ZA to ADT produced a significant benefit, but similar to the benefit obtained with docetaxel alone.

The ZEUS study investigated the efficacy of ZA for the prevention of bone metastasis in high-risk non-metastatic prostate cancer patients receiving ADT [74]: there was no difference in the occurrence of bone metastasis. After a median follow-up of 4.8 years, the proportion of bone metastasis was 14.7% with ZA and 13.2% in control group ($p=0.65$).

In conclusion, data about a *post hoc* analysis of RADAR trial, conducted in patients with locally advanced prostate cancer, must be mentioned but regarded cautiously [75]. RADAR trial investigated whether 18 months of androgen suppression (intermediate-term androgen suppression, ITAS) plus radiotherapy with or without 18 months of ZA is more effective than 6 months of neoadjuvant androgen suppression (short-term androgen suppression, STAS) plus radiotherapy with or without ZA. Secondary endpoint data and *post hoc* analyses showed that ITAS plus ZA reduce PSA progression and decrease need for secondary therapeutic intervention, in patients with Gleason 8-10 tumors. However,

neither prostate cancer-specific mortality nor all-cause mortality differed between control and experimental groups.

Considering this negative evidence in castration-sensitive and high-risk non metastatic prostate cancer patients, CRPC is the only setting of disease with proven efficacy of ZA in the management of bone metastases.

Denosumab -

Denosumab is a fully human monoclonal antibody against RANKL, and prevents the activation of its receptor, RANK, thus inhibiting osteoclast formation, function and survival, decreasing bone resorption and increasing bone mass and strength [32]. In a phase III trial that compared denosumab versus ZA in patients with bone metastatic CRPC, denosumab produced a 3.6 months significant improvement in median time to first SRE [32] (**Table C**). Furthermore, denosumab significantly delayed time to first and subsequent SREs (rate ratio 0.82, $p=0.008$). The two groups had a similar OS and time-to-disease progression. At week 13, median decrease in concentration of urinary N-telopeptide adjusted for creatinine (uNTX/Cr) and serum bone ALP were significantly greater with denosumab [32]. An exploratory analysis showed that, compared with ZA, denosumab significantly reduced also the risk of first SSE (HR 0.78, $p=0.005$) and first and subsequent SSEs (rate ratio 0.78, $p=0.004$) [76].

Of note, similarly to ZA, denosumab has subsequently been also tested in non-metastatic patients to evaluate its efficacy in delaying time to bone metastases. In a phase III, placebo- controlled trial in non-metastatic CRPC patients at high risk for bone metastasis, denosumab generated a 4.2- month improvement in median bone metastasis-free survival (BMFS, HR 0.85, $p=0.028$), in contrast with above mentioned ZEUS trial results that, however, were obtained in hormone sensitive patients [77]. Denosumab also produced a 33% reduction in the risk of symptomatic bone metastasis. However, there was no impact

on time to overall prostate cancer progression or OS (Table C) [77]. The relationship between both PSA value and PSA doubling time (PSADT) at baseline with BMFS was explored [78]. In the placebo group, patients with PSADT < 8 months had a shorter BMFS. Denosumab consistently increased BMFS among men with PSADT \leq 10 months (HR 0.84; $p=0.042$), \leq 6 months (HR 0.77; $p=0.006$) and \leq 4 months (HR 0.71; $p=0.004$) [78]. Based on these results, beyond its efficacy in metastatic CRPC, denosumab has also shown a role in prolonging BMFS in high-risk non metastatic patients.

New drugs

Several new drugs have been recently or are currently being tested in prostate cancer patients. Here we summarize the results reported in studies investigating cabozantinib, dasatinib, anti-endothelin drugs, cathepsin K inhibitors and aflibercept, with specific details about bone disease control, although all these drugs did not show any improvement of survival benefit in phase III studies.

Cabozantinib -

Cabozantinib is an oral tyrosine kinase inhibitor that blocks MET, vascular endothelial growth factor receptor 2 (VEGFR-2) as well as other tyrosine kinases including RET, KIT, AXL and FLT3 [79]. MET is overexpressed in bone metastases from solid tumours, such as prostate cancer, and is involved in proliferation, differentiation and migration of osteoblasts and osteoclasts [80]. In a phase II randomized discontinuation trial, cabozantinib produced a relevant PFS prolongation compared with placebo [81]. Of note, cabozantinib showed a partial or complete resolution of bone lesions in 56% and 19% of patients and 64% of patients who received analgesics experienced an improvement in pain intensity, while 46% stopped or reduced narcotics. Similarly, in a non-randomized phase II trial, cabozantinib produced pain palliation and pain relief in 42% and 57% of patients respectively [82]. Disappointingly, two phase III randomized trials produced negative results (**Table C**) [83,84]. In the COMET-1 trial, that compared cabozantinib versus prednisone in men with progressive mCRPC pre-treated with docetaxel, abiraterone and/or enzalutamide, cabozantinib improved PFS and bone scan response, but no OS improvement was observed [83]. In the COMET-2 trial, cabozantinib was compared versus mitoxantrone in men with progressive mCRPC, and the primary endpoint of pain palliation was not met [84].

Dasatinib -

SRC, a non-receptor protein tyrosine kinase, is a key signalling molecule in tumorigenesis and bone metabolism [85]. SRC signalling has a central role in tumour growth, invasion, metastasis, and is a mediator of osteoclast activity and function, involved in pathogenesis of prostate carcinoma bone metastases [86]. Dasatinib is a potent oral inhibitor of several tyrosine kinases including SRC, SFKs members and BCR-ABL [87]. In a phase I/II trial, dasatinib was evaluated in combination with docetaxel in chemotherapy-naïve or docetaxel pre-treated mCRPC patients [88]. Fourteen patients (30%) had disappearance of at least one bone lesion and 19 patients (41%) had stable bone scans. Most of the patients had decrease in urinary NTX and BALP (87% and 76%, respectively). In a phase II trial, conducted in mCRPC chemotherapy-naïve patients [89], dasatinib showed again a significant reduction of urinary NTX and ALP. In the randomized phase III READY trial, dasatinib plus docetaxel was compared to docetaxel plus placebo in mCRPC chemotherapy-naïve patients (**Table C**) [90], with OS as primary endpoint and SREs and pain palliation as secondary end points. Dasatinib failed to improve OS, while median time to first SRE was 31.1 months with placebo and not reached with dasatinib (HR 0.81, $p=0.08$). Reduction in pain intensity was not significantly different between arms.

Anti-endothelin -

Endothelins (ET-1, ET-2 and ET-3) are a family of small peptides with multiple roles including regulation of the vasomotor tone, nociception, hormone production and cellular proliferation [91]. ET-1 stimulates osteoblast activity and plays a key role in promoting prostate cancer growth and metastasis [92]. The activity of ET-1 is mediated by endothelin A receptor (ET-A) [93]. In preclinical models, endothelin receptor antagonists showed inhibition of the development and progression of metastases [94].

Atrasentan is a potent, oral, selective ET-A antagonist that inhibits the osteoblast-dependent formation of new bone induced by metastatic cancer cells [95]. In a phase II, placebo-controlled trial, atrasentan was tested in hormone refractory metastatic prostate cancer (HRPC) patients [96]. The primary endpoint was the rate of pain relief after 12 weeks, that was not met. However, atrasentan 10 mg produced a statistically significant improvement in BPI, particularly the benefit was demonstrated in pain interference with relations with other people ($p=0.031$) and in the worst pain in the last 24 hours ($p=0.03$). In another phase II trial in asymptomatic HRPC patients [94], markers of bone deposition and resorption were significantly reduced with atrasentan compared to placebo. A phase III randomized, placebo-controlled trial evaluating atrasentan in non-metastatic HRPC (**Table C**) did not meet the primary endpoint of delaying time to disease progression and did not show a significant improvement in time to first skeletal lesion, although atrasentan lengthened PSA doubling time ($p=0.031$) and slowed BALP increase ($p<0.001$) [97].

Zibotentan is an oral, selective ET-A antagonist, competing with ET-1 for receptor binding and therefore indirectly increasing pro-apoptotic signalling. Three trials (ENTHUSE) evaluated zibotentan in CRPC patients (**Table C**) [98-100]. Disappointingly, in these trials there was no significant improvement either in OS, the primary endpoint, or in secondary endpoints, including time to pain progression and pain response.

Cathepsin K inhibitors

Cathepsin K is a cysteine protease, expressed in osteoclasts and various type of cancers [101]. It plays a key role in osteoclast-mediated bone resorption and promotes tumor cells invasion [102]. Cathepsin K inhibitors have been studied for post-menopausal osteoporosis and bone metastatic disease [103]. Odanacatib, a cathepsin K inhibitor, has been evaluated in a randomized, double blind trial in order to assess the efficacy and safety in reducing markers of bone resorption in bone metastatic breast cancer patients

[104]. Forty-three patients were randomized to oral odanacatib 5 mg daily for 4 weeks or intravenous ZA 4 mg given once at study initiation. The study showed that odanacatib reduced uNTx similarly to ZA after 4 weeks of treatment [104]. Two phase III clinical trials were planned in order to evaluate its efficacy and safety in prolonging time to first bone metastasis in CRPC patients (NCT00691899) and in reducing risk of bone metastases in women with breast cancer (NCT00692458). Unfortunately, these studies were closed before starting accrual [105]. Further clinical trials are needed in order to obtain more clinical informations.

Aflibercept

Aflibercept is an anti-angiogenic agent with high affinity to the isoform VEGF-A, it also binds VEGF-B and platelet-derived growth factors PIGF1 and PIGF2 [106]. A recent phase III, randomized, double-blind placebo-controlled trial (VENICE) has evaluated docetaxel plus aflibercept vs docetaxel plus placebo in 1224 mCRPC patients [107]. The primary endpoint was OS; secondary endpoints included PFS, PSA-PFS, time to first SRE and pain-PFS. Aflibercept has not met its primary endpoint (22.1 months vs 21.1 months; $p=0.38$). There were not differences in terms of secondary end-points, in particular median time to first SRE was 15.3 months in aflibercept group vs 15.0 months in placebo group ($p=0.31$).

CONCLUSIONS

Recently, a better understanding of mechanisms associated with bone metastatic disease in prostate cancer and, more specifically, the crucial role of cross-talk between tumor cells and bone micro-environment in metastatic progression provided the basis for the development of new effective bone-targeted therapies.

There is no question that prostate cancer cells have a strong bone tropism, and their dissemination into the bone alters the equilibrium between osteoclasts and osteoblasts. Although bone lesions secondary to prostate carcinoma are mainly characterized by aberrant osteoblast activation, osteolysis is common and is responsible of increased incidence of SREs that are dramatic clinical events, able to decrease QoL, autonomy and survival of CRPC patients.

Abnormal osteoclast activity is the rationale for the administration of potent osteolysis inhibitors, such as zoledronic acid and denosumab. These agents reduce the burden of bone metastatic disease, although this benefit does not translate in an improvement in survival.

Recently, a new treatment opportunity for patients with prostate cancer and bone metastases is represented by radium-223. Notably, this α -emitter, when used in men with CRPC and bone metastases, not only showed efficacy in preventing symptomatic skeletal events, but it was the first bone-targeted therapy associated with a significant OS improvement.

Additionally, in the last five years, highly effective new systemic agents have significantly changed the treatment landscape of CRPC patients, improving their life expectancy [12]. Some of these therapies also documented efficacy in delaying SRE and improving bone pain. Trials testing the concomitant administration of radium-223 with abiraterone (NCT02043678), enzalutamide (NCT02194842) and docetaxel (NCT01106352) are ongoing. Results of these studies will help to better understand how to combine systemic

new agents with bone-targeted therapies, in order to effectively interfere with the “seed” and with the “soil” at the same time.

Figure legends

Figure A. Mechanisms of shift from hematopoietic stem cell niche to “onco-niche”.

A) Hematopoietic stem cell into the bone marrow is localized in the hematopoietic stem cell niche in connection with osteoblasts through SDF-1/CXCR4 pathway. Prostatic tumor cells are able to compete with hematopoietic stem cell for the place in the bone marrow using SDF-1/CXCR4 axis, favoring the formation of “onco-niche”.

B) In the “onco-niche”, cancer cell may remain in a state of dormancy or may start to colonize and invade bone.

Figure B. Pathogenesis of “vicious cycle” that underpin osteoblastic bone metastases from prostate carcinoma.

A) In the early phase of metastatic colonization osteolysis predominates due to production of transforming growth factor β 1 (TGF β 1), parathyroid-hormone-related peptide (PTHrP) and interleukin 6 (IL-6). These factors activate the receptor activator nuclear kappa B (RANK)/RANK ligand (RANKL) pathway, which is responsible of bone resorption stimulation.

B) The increase of osteolysis causes the release from bone matrix of growth factors and cytokines responsible for neoplastic proliferation.

C) In the next phase of skeletal colonization bone neoapposition become dominant due to growth factors released by cancer cells and from bone matrix, such as basic fibroblast growth factor (bFGF), bone morphogenic proteins (BMPs), endothelin-1 (ET-1), tumour growth factor b1 (TGFb1) and insulin-like growth factor 1 (IGF-1), able to stimulate osteoblasts activity.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015;65:87-108.
2. Costa L, Badia X, Chow E, Lipton A, Wardley A. Impact of skeletal complications on patients' quality of life, mobility, and functional independence. *Support Care Cancer* 2008;16:879-89.
3. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev* 2001;27:165-76.
4. Broder MS, Gutierrez B, Cherepanov D, Linhares Y. Burden of skeletal-related events in prostate cancer: unmet need in pain improvement. *Support Care Cancer* 2015;23:237-47.
5. Weinfurt KP, Li Y, Castel LD, Saad F, Timbie JW, Glendenning GA et al. The significance of skeletal-related events for the health-related quality of life of patients with metastatic prostate cancer. *Ann Oncol*. 2005;16:579-84.
6. Clinical Trials Endpoints for the Approval of Cancer Drugs and Biologics, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER) 2007.
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. DePuy V, Anstrom KJ, Castel LD, Schulman KA, Weinfurt KP, Saad F. Effects of skeletal morbidities on longitudinal patient-reported outcomes and survival in patients with metastatic prostate cancer. *Support Care Cancer* 2007;15:869-76.
9. Aass N, Fossa SD. Pre- and post-treatment daily life function in patients with hormone resistant prostate carcinoma treated with radiotherapy for spinal cord compression. *Radiother Oncol* 2005;74:259-65.
10. Rades D, Stalpers LJ, Veninga T, Rudat V, Schulte R, Hoskin PJ. Evaluation of functional outcome and local control after radiotherapy for metastatic spinal cord compression in patients with prostate cancer. *J Urol*. 2007;175:552-6.
11. Krupski TL, Foley KA, Baser O, Long S, Macarios D, Litwin MS. Health care cost associated with prostate cancer, androgen deprivation therapy and bone complications. *J Urol* 2007;178:1423-8.
12. Bahl A, Hoefeler H, Duran I, Hechmati G, Garzon-Rodriguez C, Ashcroft J, et al. Health resource utilization associated with skeletal-related events in patients with advanced prostate cancer: a European subgroup analysis from an observational, multinational study. *J Clin Med* 2014; 3(3):883-96.
13. Hagiwara M, Delea TE, Saviile MW, Chung K. Healthcare utilization and costs associated with skeletal-related events in prostate cancer patients with bone metastases. *Prostate Cancer Prostatic Dis* 2013; 16:23–27.
14. Lage MJ, Barber BL, Harrison DJ, Jun S. The cost of treating skeletal-related events in patients with prostate cancer. *Am J Manag Care* 2008; 14:317–322.
15. Hechmati G, Cure S, Gouepo A, Hoefeler H, Lorusso V, Luftner D, et al. Cost of skeletal-related events in European patients with solid tumours and bone metastases: Data from a prospective multinational observational study. *J Med Econ* 2013; 16:691–700.
16. Tucci M, Scagliotti GV, Vignani F. Metastatic castration-resistant prostate cancer: time for innovation. *Future Oncol* 2015;11:91-106.

17. Guise T. Examining the metastatic niche: targeting the microenvironment. *Semin. Oncol* 2010;37(suppl2):S2-S14
18. Parfitt AM. The physiology and clinical significance of bone histomorphometry: Techniques and Interpretations. CRC Press 1983;143.
19. Autio KA, Morris MJ. Targeting bone physiology for the treatment of metastatic prostate cancer. *Clin Adv Hematol Oncol* 2013;11:134–43.
20. Paget S. The distribution of secondary growths in cancer of the breast. *Cancer Metastasis Rev* 1989;8:98–101.
21. Pedersen EA, Shiozawa Y, Pienta KJ, Taichman RS. The prostate cancer bone marrow niche: more than just 'fertile soil'. *Asian J Androl* 2012;14:423–7.
22. Weillbaecher K, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nat Rev Cancer* 2011;11:411–25.
23. Yu C, Shiozawa Y, Taichman RS, McCauley LK, Pienta K, Keller E. Prostate Cancer and Parasitism of the Bone Hematopoietic Stem Cell Niche. *Crit Rev Eukaryot Gene Expr* 2012;22:131–48.
24. Camacho DF, Pienta KJ. A multi-targeted approach to treating bone metastases. *Cancer Metastasis Rev* 2014;33:545–53.
25. Sun YX, Wang J, Shelburne CE, Lopatin DE, Chinnaiyan AM, Rubin MA et al. Expression of CXCR4 and CXCL12 (SDF-1) in human prostate cancers (PCa) in vivo. *J Cell Biochem* 2003;89:462–73.
26. Sun YX, Fang M, Wang J, Cooper CR, Pienta KJ, Taichman RS. Expression and activation of $\alpha\beta 3$ integrins by SDF-1/CXC12 increases the aggressiveness of prostate cancer cells. *Prostate* 2007;67:61–73.
27. Msaouel P, Pissimissis N, Halapas A, Koutsilieris M. Mechanisms of bone metastasis in prostate cancer: clinical implications. *Best Pract Res Clin Endocrinol Metab* 2008;22:341-55.
28. Tucci M, Mosca A, Lamanna G, Porpiglia F, Terzolo M, Vana F et al. Prognostic significance of disordered calcium metabolism in hormone-refractory prostate cancer patients with metastatic bone disease. *Prostate Cancer and Prostatic Diseases* 2009;12:94–9.
29. Rico H, Uson A, Hernandez ER, Prados P, Paramo P, Cabranes JA. Hyperparathyroidism in metastases of prostate carcinoma. A biochemical, hormonal and histomorphometric study. *Eur Urol* 1990;17:35-9.
30. Lipton A, Glover D, Harvey H, Grabelsky S, Zelenakas K, Macerata R, et al. Pamidronate in the treatment of bone metastases: results of 2 dose-ranging trials in patients with breast or prostate cancer. *Ann Oncol* 1994;5:31-5.
31. Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, et al. A randomized, placebo-controlled trial of zoledronic acid in patients with hormone-refractory metastatic prostate carcinoma. *J Natl Cancer Inst* 2002;94:1458-68.
32. Fizazi K, Carducci M, Smith M, Damião R, Brown J, Karsh L, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: a randomised, double-blind study. *Lancet* 2011;377:813-22.
33. Smith MR, Coleman RE, Klotz L, Pittman K, Milecki P, Ng S, et al. Denosumab for the prevention of skeletal complications in metastatic castration-resistant prostate cancer: comparison of skeletal-related events and symptomatic skeletal events. *Ann Oncol* 2015;26:368-74.
34. Fizazi K, Scher HI, Miller K, Basch E, Sternberg CN, Cella D, et al. Effect of enzalutamide on time to first skeletal-related event, pain, and quality of life in

- men with castration-resistant prostate cancer: results from the randomised, phase 3 AFFIRM trial. *Lancet Oncol* 2014;15:1147-56.
35. Logothetis CJ, Basch E, Molina A, Fizazi K, North SA, Chi KN, et al. Effect of abiraterone acetate and prednisone compared with placebo and prednisone on pain control and skeletal-related events in patients with metastatic castration-resistant prostate cancer: exploratory analysis of data from the COU-AA-301 randomised trial. *Lancet Oncol* 2012;13:1210-7
 36. Sartor O, Coleman R, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, et al. Effect of radium-223 dichloride on symptomatic skeletal events in patients with castration-resistant prostate cancer and bone metastases: results from a phase 3, double-blind, randomised trial. *Lancet Oncol* 2014;15:738-46.
 37. Tannock IF, Osoba D, Stockler MR, Ernst DS, Neville AJ, Moore MJ, et al. Chemotherapy with mitoxantrone plus prednisone or prednisone alone for symptomatic hormone-resistant prostate cancer: a Canadian randomized trial with palliative end points. *J Clin Oncol* 1996;14:1756-64.
 38. Kantoff PW, Halabi S, Conaway M, Picus J, Kirshner J, Hars V, et al. Hydrocortisone with or without mitoxantrone in men with hormone-refractory prostate cancer: results of the cancer and leukemia group B 9182 study. *J Clin Oncol* 1999;17:2506-13.
 39. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *N Engl J Med* 2004;351:1502-12.
 40. Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, et al. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. *N Engl J Med* 2004;351:1513-20.
 41. Berthold DR, Pond GR, Soban F, de Wit R, Eisenberger M, Tannock IF. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer: updated survival in the TAX 327 study. *J Clin Oncol* 2008;26:242-5.
 42. Armstrong AJ, Garrett-Mayer E, Ou Yang YC, Carducci MA, Tannock I, de Wit R, et al. Prostate-specific antigen and pain surrogacy analysis in metastatic hormone-refractory prostate cancer. *J Clin Oncol* 2007;25:3965-70.
 43. Berthold DR, Pond GR, Roessner M, de Wit R, Eisenberger M, Tannock AI. Treatment of hormone-refractory prostate cancer with docetaxel or mitoxantrone: relationships between prostate-specific antigen, pain, and quality of life response and survival in the TAX-327 study. *Clin Cancer Res* 2008;14:2763-7.
 44. James ND, Sydes MR, Clarke NW, Mason MD, Dearnaley DP, Spears MR, et al. STAMPEDE investigators. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. *Lancet* 2015; [Epub ahead of print]
 45. Mita AC, Denis LJ, Rowinsky EK, Debono JS, Goetz AD, Ochoa L, et al. Phase I and pharmacokinetic study of XRP6258 (RPR 116258A), a novel taxane, administered as a 1-hour infusion every 3 weeks in patients with advanced solid tumors. *Clin Cancer Res* 2009;15:723-30.
 46. Bouchet BP, Galmarini CM. Cabazitaxel, a new taxane with favorable properties. *Drugs Today (Barc)* 2010;46:735-42.
 47. De Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant

- prostate cancer progressing after docetaxel treatment: a randomised open-label trial. *Lancet* 2010;376:1147-54.
48. Bahl A, Oudard S, Tombal B, Ozgüroglu M, Hansen S, Kocak I, et al. Impact of cabazitaxel on 2-year survival and palliation of tumour-related pain in men with metastatic castration-resistant prostate cancer treated in the TROPIC trial. *Ann Oncol* 2013;24:2402-8.
 49. Bahl A, Masson S, Malik Z, Birtle AJ, Sundar S, Jones RJ, et al. Final quality of life and safety data for patients with metastatic castration-resistant prostate cancer treated with cabazitaxel in the UK Early Access Programme (EAP) (NCT01254279) *BJU Int* 2015 Jan 30.
 50. Logothetis CJ, Efstathiou E, Manuguid F, Kirkpatrick P. Abiraterone acetate *Nat Rev Drug Discov* 2011;10:573-4.
 51. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med*. 2011;364:1995-2005.
 52. Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study *Lancet Oncol* 2012;13:983-92.
 53. Iuliani M, Pantano F, Buttigliero C, Fioramonti M, Bertaglia V, Vincenzi B, et al. Biological and clinical effects of abiraterone on anti-resorptive and anabolic activity in bone microenvironment. *Oncotarget* 2015;6:12520-8.
 54. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med* 2013;368:138-48.
 55. Saad F, Shore N, Van Poppel H, Rathkopf DE, Smith MR, de Bono JS, et al. Impact of bone-targeted therapies in chemotherapy-naïve metastatic castration-resistant prostate cancer patients treated with Abiraterone Acetate: post hoc analysis of study COU-AA-302. *Eur Urol* 2015;68:570-7.
 56. Caffo O, De Giorgi U, Fratino L, Lo Re G, Basso U, D'Angelo A, et al. Safety and clinical outcomes of patients treated with abiraterone acetate after docetaxel: results of the Italian Named Patient Programme. *BJU Int* 2014;115:764-71.
 57. Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science* 2009;324:787–90.
 58. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med* 2012;367:1187-97.
 59. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. *N Engl J Med* 2014;371:424-33.
 60. Loriot Y, Miller K, Sternberg CN, Fizazi K, De Bono JS, Chowdhury S, et al. Effect of enzalutamide on health-related quality of life, pain, and skeletal-related events in asymptomatic and minimally symptomatic, chemotherapy-naive patients with metastatic castration-resistant prostate cancer (PREVAIL): results from a randomised, phase 3 trial. *Lancet Oncol* 2015;16:509-21.
 61. Bruland OS, Nilsson S, Fisher DR, Larsen RH. High-linear energy transfer irradiation targeted to skeletal metastases by the alpha-emitter ²²³Ra: adjuvant or alternative to conventional modalities? *Clin Cancer Res* 2006;12:6250–7.

62. Henriksen G, Fisher DR, Roeske JC, Bruland OS, Larsen RH. Targeting of osseous sites with alpha-emitting ²²³Ra: comparison with the beta-emitter ⁸⁹Sr in mice. *J Nucl Med* 2003;44:252–9.
63. Lewington VJ. Bone-seeking radionuclides for therapy. *J Nucl Med* 2005;46(suppl 1):38S–47S.
64. Parker C, Nilsson S, Heinrich D, Helle SI, O'Sullivan JM, Fosså SD, et al. Alpha emitter radium-223 and survival in metastatic prostate cancer. *N Engl J Med* 2013; 369:213–23.
65. Nilsson S, Sartor AO, Buland OS, Fang F, Aksnes AK, Parker C, et al. Pain analysis from the phase III randomized ALSYMPCA study with radium-223 (Ra-223) in patients with castration-resistant prostate cancer (CRPC) with bone metastases. *J Clin Oncol* 2013;31(suppl 6).
66. Jong JM, Oprea-Lager DE, Hoof L, de Klerk JM, Bloemendal HJ, Verheul HM, et al. Radiopharmaceuticals for Palliation of Bone Pain in Patients with Castration-resistant Prostate Cancer Metastatic to Bone: A Systematic Review. *Eur Urol.* 2015 Sep 18.
67. Mason MD, Sydes MR, Glaholm J, Langlely RE, Huddart RA, Sokal M et al.; Medical Research Council PR04 Collaborators. Oral sodium clodronate for nonmetastatic prostate cancer--results of a randomized double-blind placebo-controlled trial: Medical Research Council PR04 (ISRCTN61384873). *J Natl Cancer Inst.* 2007;99(10):765-76.
68. Dearnaley DP, Sydes MR, Mason MD, Stott M, Powell CS, Robinson AC et al.; Medical Research Council Pr05 Collaborators. A double-blind, placebo-controlled, randomized trial of oral sodium clodronate for metastatic prostate cancer (MRC PR05 Trial). *J Natl Cancer Inst.* 2003 ;95(17):1300-11.
69. Dearnaley DP, Mason MD, Parmar MK, Sanders K, Sydes MR. Adjuvant therapy with oral sodium clodronate in locally advanced and metastatic prostate cancer: long-term overall survival results from the MRC PR04 and PR05 randomised controlled trials. *Lancet Oncol.* 2009;10(9):872-6.
70. Saad F, Gleason DM, Murray R, Tchekmedyian S, Venner P, Lacombe L, 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.
71. Lipton A, Cook R, Saad F, Major P, Garnero P, Terpos E, et al. Normalization of bone markers is associated with improved survival in patients with bone metastases from solid tumors and elevated bone resorption receiving zoledronic acid. *Cancer* 2008;113:193-201.
72. James ND, Pirrie S, Barton D, Brown JE, Billingham L, Collins SI et al. Clinical outcomes in patients with castrate-refractory prostate cancer (CRPC) metastatic to bone randomized in the factorial TRAPEZE trial to docetaxel (D) with strontium-89 (Sr89), zoledronic acid (ZA), neither, or both Abstract Number: LBA5000, presented at ASCO 2013 Annual Meeting
73. Smith MR, Halabi S, Ryan CJ, Hussain A, Vogelzang N, Stadler W, et al. Randomized controlled trial of early zoledronic acid in men with castration-sensitive prostate cancer and bone metastases: results of CALGB 90202 (alliance). *J Clin Oncol* 2014;32:1143-50.
74. Wirth M, Tammela T, Cicalese V, Gomez Veiga F, Delaere K, Miller K, et al. Prevention of bone metastases in patients with high-risk non metastatic prostate cancer treated with zoledronic acid: efficacy and safety results of the Zometa European Study (ZEUS). *Eur Urol* 2015;67:482-91.

75. Denham JW, Joseph D, Lamb DS, Spry NA, Duchesne G, Matthews J et al. Short-term androgen suppression and radiotherapy versus intermediate-term androgen suppression and radiotherapy, with or without zoledronic acid, in men with locally advanced prostate cancer (TROG 03.04 RADAR): an open-label, randomised, phase 3 factorial trial. *Lancet Oncol*. 2014;15(10):1076-89.
76. Smith MR, Coleman RE, Klotz L, Pittman K, Milecki P, Ng S, et al. Denosumab for the prevention of skeletal complications in metastatic castration-resistant prostate cancer: comparison of skeletal-related events and symptomatic skeletal events. *Ann Oncol*. 2015;26:368-74.
77. Smith MR, Saad F, Coleman R, Shore N, Fizazi K, Tombal B, et al. Denosumab and bone-metastasis-free survival in men with castration-resistant prostate cancer: results of a phase 3, randomised, placebo-controlled trial. *Lancet* 2012;379:39-46.
78. Smith MR, Saad F, Oudard S, Shore N, Fizazi K, Sieber P, et al. Denosumab and bone metastasis-free survival in men with non metastatic castration-resistant prostate cancer: exploratory analyses by baseline prostate-specific antigen doubling time. *J Clin Oncol* 2013;31:3800-6.
79. Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther* 2011;10:2298–308.
80. Knudsen B, Gmyrek G, Inra J, Scherr DS, Vaughan ED, Nanus DM, et al. High expression of the Met receptor in prostate cancer metastasis to bone. *Urology* 2002;60:1113-7.
81. Smith DC, Smith MR, Sweeney C, Elfiky AA, Logothetis C, Corn PG, et al. Cabozantinib in patients with advanced prostate cancer: results of a phase II randomized discontinuation trial. *J Clin Oncol* 2013;3:412–9.
82. Basch E, Autio KA, Smith MR, Bennett AV, Weitzman AL, Scheffold C et al. 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* 2015;67:310-8.
83. Smith RM, De Bono JS, Sternberg C, Le Moulec S, Oudard S, De Giorgi U, et al. Final analysis of COMET-1: Cabozantinib versus prednisone in metastatic castration-resistant prostate cancer (mCRPC) patients previously treated with docetaxel and abiraterone and/or enzalutamide. *J Clin Oncol* 2015;33(suppl 7);abstr 139.
84. Basch EM, Scholz MC, De Bono JS, Vogelzang NJ, DeSouza PL, Marx GM, et al. Final analysis of COMET-2: Cabozantinib versus mitoxantrone/prednisone in metastatic castration-resistant prostate cancer (mCRPC) patients with moderate to severe pain who were previously treated with docetaxel and abiraterone and/or enzalutamide. *J Clin Oncol* 2015; 33(suppl 7);abstr 141.
85. Brunton VG, Frame MC. Src and focal adhesion kinase as therapeutic targets in cancer. *Curr Opin Pharmacol* 2008;8:427–32.
86. Saad F. Src as a therapeutic target in men with prostate cancer and bone metastases. *BJU Int* 2009;103:434–40.
87. Saad F, Lipton A. SRC kinase inhibition: targeting bone metastases and tumor growth in prostate and breast cancer. *Cancer Treatment Reviews*. 2010; 36:177–84.

88. Araujo JC, Mathew P, Armstrong AJ, Braud EL, Posadas E, Lonberg M et al. Dasatinib combined with docetaxel for castration-resistant prostate cancer: results from a phase 1-2 study. *Cancer*. 2012;118:63–71.
89. Yu EY, Wilding G, Posadas E, Gross M, Culine S, Massard C et al. Phase II study of dasatinib in patients with metastatic castration-resistant prostate cancer. *Clin Cancer Res*. 2009;15:7421-8.
90. Araujo JC, Trudel GC, Saad F, Armstrong AJ, Yu EY, Bellmunt J et al. 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.
91. Yanagisawa M, Inoue A, Ishikawa T, Kasuya Y, Kimura S, Kumagaye S ,et al. Primary structure, synthesis, and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. *Proc Natl Acad Sci USA* 1988;85:6964-7.
92. Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA , et al. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. *Nat Med* 1995;1:944–9.
93. Nelson JB, Chan-Tack K, Hedican SP, Magnuson SR, Opgenorth TJ, Bova GS, et al. Endothelin-1 production and decreased endothelin B receptor expression in advanced prostate cancer. *Cancer Res* 1996;56:663-8.
94. Nelson JB, Nabulsi AA, Vogelzang NJ, Breul J, Zonnenberg BA, Daliani DD, et al. Suppression of prostate cancer induced bone remodeling by the endothelin receptor A antagonist atrasentan. *J Urol* 2003;169:1143–9.
95. Nelson JB. Endothelin inhibition: novel therapy for prostate cancer. *J Urol*. 2003;170:S65-7.
96. Nelson JB. Endothelin receptor antagonists. *World J Urol* 2005;23:19-27.
97. Nelson JB, Love W, Chin JL, Saad F, Schulman CC, Sleep DJ, et al. Phase 3, randomized, controlled trial of atrasentan in patients with non metastatic, hormone-refractory prostate cancer. *Cancer* 2008;113:2478-87.
98. Fizazi K, Higano CS, Nelson JB, Gleave M, Miller K, Morris T, et al. Phase III, randomized, placebo-controlled study of docetaxel in combination with zibotentan in patients with metastatic castration-resistant prostate cancer. *J Clin Oncol*. 2013;31:1740-7.
99. Miller K, Moul JW, Gleave M, Fizazi K, Nelson JB, Morris T, et al. Phase III, randomized, placebo-controlled study of once-daily oral zibotentan (ZD4054) in patients with non-metastatic castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis* 2013;16:187-92.
100. Nelson JB, Fizazi K, Miller K, Higano C, Moul JW, Akaza H, et al. Phase 3, randomized, placebo-controlled study of zibotentan (ZD4054) in patients with castration-resistant prostate cancer metastatic to bone. *Cancer* 2012;118:5709-18.
101. Vasiljeva O, Reinheckel T, Peters C, Turk D, Turk V, Turk B. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr Pharm Des*. 2007;13:387–403.
102. Lecaille F, Kaleta J, Bromme D. Human and parasitic papain-like cysteine proteases: their role in physiology and pathology and recent developments in inhibitor design. *Chem Rev*. 2002;102:4459–48
103. Verbovšek U, Van Noorden CJ, Lah TT. Complexity of cancer protease biology: Cathepsin K expression and function in cancer progression. *Semin Cancer Biol*. 2015; 35:71-84.

104. Jensen AB, Wynne C, Ramirez G, He W, Song Y, Berd Y et al. The cathepsin K inhibitor odanacatib suppresses bone resorption in women with breast cancer and established bone metastases: results of a 4-week, double-blind, randomized, controlled trial. *Clin Breast Cancer*. 2010;10:452-58.
105. Deal C. Future therapeutic targets in osteoporosis. *Curr Opin Rheumatol* 2009;21:380–85
106. Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M et al. VEGF-trap: a VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci*. 2002;99:11393–98.
107. Tannock IF, Fizazi K, Ivanov S, Karlsson CT, Fléchon A, Skoneczna I et al. Aflibercept versus placebo in combination with docetaxel and prednisone for treatment of men with metastatic castration-resistant prostate cancer (VENICE): a phase 3, double-blind randomised trial. *Lancet Oncol*. 2013;14:760-8

