

Prostate cancer still appears to be a very important malignant disease in Western societies. It can be said that prostate cancer morbidity rises with age in old men. The natural history of prostate cancer is rather simple. The majority of patients who are diagnosed with localized prostate cancer are treated with curative intent with either radiation or surgery. Patients in whom treatment with curative intent is unsuccessful and those who present with metastasis are candidates for androgen suppression. Skeletal-related events are defined by the existence of changes in antineoplastic therapy to treat bone pain, pathological bone fractures, radiation therapy to bone, spinal cord compression, and surgery to bone. Bone pathology correlates with cancer course and has been shown to influence survival. Pathobiology provides new insights into mechanisms responsible for bone metastases related to prostate cancer. Several studies have evaluated risk factors for bone loss and fractures in prostate cancer patients receiving androgen-deprivation therapy. The three risk factors that have been reliably identified in patients with locally advanced disease are older age, low body mass index and a long duration of androgen-deprivation therapy. In metastatic prostate cancer, the sole reliable risk factor is elevated levels of deoxy pyridinoline. Zoledronic acid has a crucial impact on combined therapy with hormones, then with chemotherapy, and additionally can be used in the prevention of osteoporosis/osteopenia related to cancer treatment.

**Key words:** prostate cancer, osteosclerotic bone metastasis, zoledronic acid, cancer bone loss.

## Pathophysiological basis for utility of zoledronic acid in patients with prostate cancer

*Podstawy patofizjologiczne stosowania kwasu zoledronowego u chorych na raka gruczołu krokowego*

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### Introduction

Prostate cancer still appears to be a very important malignant disease in Western societies. It can be said that prostate cancer morbidity rises with age in old men. In the Department of Oncology, I and my colleagues appreciate how the malignancy is important for the men who are our patients, and for us oncologists as well. Looking backward, we have published in past issues of the Journal two articles considering prostate cancer itself, and in links with malignant bone pathology related to this cancer, and one additional article on a bony problem seen in renal cancer patients [1-3]. Therefore, we want to focus on newer data that have been published since 2005 or to venture into the past if needed to make a better elucidation.

Epidemiological studies suggest a vital role of steroid hormones in prostate carcinogenesis, and because the prostate is a target for both androgens and oestrogens, both of them have been implicated in prostatic tumorigenesis. Several experimental and clinical observations are strong enough to be pointed out in prostate cancer: 1) Prostate cancer does not occur in eunuchs castrated early in life, 2) Testosterone levels decline with age, while plasma free oestrogen levels remain unchanged or increase during aging when prostate cancer develops, 3) American blacks, who have the highest incidence of prostate cancer in the world, exhibit elevations of both plasma-free testosterone and oestradiol levels, 4) Testosterone in cooperation with oestradiol induces prostatic hyperplasia and dysplasia in mice, and induces prostate cancer in rats [4-7].

The prostate cancer patient's age at diagnosis is rather blurred, and showing only older men as prostate cancer sufferers seems to be only half true. Evidence for this statement has come from Punglia et al. [8] study. In their elegant study, the authors investigated 6 691 men who underwent PSA-base screening for prostate cancer. Of these men, 705 (11%) underwent biopsy of the prostate. By taking into account the notion that a biopsy depends only on the PSA-test result and other observed variables, ROC (receiver-operating characteristics) were used. The ROC of the PSA tests adjusted for analysis compared with unadjusted for analysis were 0.86 vs. 0.69,  $p < 0.001$  for men less than 60 years old, and the same analysis for men aged over 60 gave results of 0.72 vs. 0.62,  $p = 0.008$ . If the threshold PSA value for performing biopsy was set at 4.1 ng/ml, 82% of cancers in younger men and 65% of cancers in older men would be missed. I have to hint at the screening problem of prostate cancer that there are no effective methods to reduce mortality due to this malignancy as the result of early detection after screening. Such highly sophisticated computer techniques as artificial neural networks (ANN) are being harnessed to obtain clinical

Rak gruczołu krokowego jest nadal wielkim wyzwaniem społecznym w krajach Zachodu. Wiadomo, że zachorowanie na ten nowotwór złośliwy wzrasta z wiekiem mężczyźni. Przebieg choroby jest stosunkowo prosty. U większości chorych raka gruczołu krokowego rozpoznaje się w stadium zlokalizowanym i zostają oni poddani leczeniu operacyjnemu oraz radioterapii. W przypadku braku skuteczności takiego leczenia chorzy stają się kandydatami do hormonoterapii. Wydarzenia kliniczne związane z patologią kostną obejmują: konieczność zmiany terapii w celu zwiększenia działania przeciwbólowego, potrzebę stosowania radioterapii, wystąpienie złamania kompresyjnego kręgosłupa oraz wykonanie ortopedycznego zabiegu operacyjnego. Patologia w obrębie kości ma związek z przebiegiem choroby nowotworowej i wpływa na krótsze przeżycie. Patobiologia odkrywa nowe mechanizmy związane z powstawaniem przerzutów do kości w przebiegu raka gruczołu krokowego. Kilka badań klinicznych wykazało istnienie czynników ryzyka związanych z pojawieniem się przerzutów raka gruczołu krokowego do kości. Należą do nich: starszy wiek, niski współczynnik BMI oraz długotwałe leczenie hormonalne w przebiegu raka gruczołu krokowego. W przypadku stadium przerzutowego tego raka podstawowym czynnikiem prognostycznym jest zwiększone stężenie deoksyrydony. Kwas zoledronowy ma istotny wpływ na leczenie skojarzone raka gruczołu krokowego poprzez łączenie go z hormonoterapią, następnie z chemioterapią. Może być także stosowany jako leczenie zapobiegające osteoporozie/osteopenii towarzyszącej przebiegowi i leczeniu tego nowotworu.

**Słowa kluczowe:** rak gruczołu krokowego, osteoblastyczne przerzuty do kości, kwas zoledronowy, osteoporoza nowotworowa.

benefits. Unfortunately, such methods are currently under scrutiny and are far from clinical usage [9].

The natural history of prostate cancer is rather simple. The majority of patients who are diagnosed with localized prostate cancer are treated with curative intent with either radiation or surgery. Patients in whom treatment with curative intent is unsuccessful and those who present with metastasis are candidates for androgen suppression. The median duration of time-to-progression while on this front-line hormone therapy is 14 to 30 months [10]. This latter therapy is strengthened by the early works of Huggins and Hodges [11]. After the time of effective hormonal therapy, prostate cancer undergoes transformation into a more aggressive form readily biochemically (PSA decrease) responding to any hormonal modifications, but for a short time. From this point, a novel therapy needs to be sought to get clinical benefit. There are several therapeutic options to be used: antiandrogen withdrawal, secondary antiandrogens, corticosteroids and ketoconazole, compounds with oestrogenic properties, cytostatic drugs (docetaxel), and new therapies with small molecules [12]. At the time to make a proper decision on treatment, it is worth pointing out that quality of life in patients with prostate cancer has a prognostic role for morbidity and mortality in hormone-refractory disease [13].

### Molecular profiling of prostate cancer

A complex organism consists of both post-mitotic and renewable somatic tissues. Mitosis is a biological process having an impact on normal function at the cellular level. The same process, on the other hand, can begin tumorigenesis. Malignant tumours are able to originate from almost all types of normal tissues. Cancer is characterized by uncontrolled cell proliferation causing tumour growth. The biological characterization of a tumour reflects its progression. Some kind of regulatory alteration seems to have an impact on changed genomic pattern readily seen both at the gene and protein level. This multi-step process embraces the genome of a given cell, including mutant alleles of proto-oncogenes, tumour suppressor genes, regulators of the cell cycle machinery, and oncogenes responsible for coding adhesins [14].

Identification of genes that have a crucial impact on carcinogenesis and progression of cancer would be of the greatest clinical interest. DNA microarray is a molecular technology which has a seminal role on studying the biology of human cancers, better molecular level diagnostics, and creativity of novel drugs. This technology offers analysis of thousands of genes or oligonucleotides at the same time during one experiment. Glass slides with printed sequences are made automatically, and are available commercially as well. The entire processing is also highly automated. High throughput genetic analysis can detect genes that would be of clinical interest as prognostic or predictive factors. Some of the factors would be considered as a good target for new drugs which could be synthesized according to the rules of combinatorial chemistry [15, 16].

Seeking specific sets of genes that could form the signature of a specific metastatic propensity appears to be a good idea to build a step towards targeted therapy against particular behaviour of cancer. Experimental results exist for such a notion. In breast cancer Massague et al. [17] have identified a multigenic program mediating metastasis to bone. The authors used human breast cancer cell line subpopulations with elevated metastatic activity to detect genes that act cooperatively to cause osteolytic skeletal metastasis. Overexpression of this bone metastasis gene set induces existence of bone metastases. Clinical trials were carried out in 107 breast cancer patients by Smid et al. [18], who established a panel of 69 genes changed in the cells forming bone metastasis. Several ESTs (expressed sequence tags) were well-known factors (TFF1-trefoil factor, FGF1, FGF2, CD24, and EGF receptor-MAPK pathway). While using the comparative genomic hybridization method there is a possibility to detect alterations in the small number of

disseminated cells, even 10-20. This is a typical situation when the sample originates from bone marrow aspirates of prostate cancer patients [19].

Cancer associated genes can be silenced by hypermethylation of CpG islands within a promoter. There are differences in switching off genes in prostatic intraepithelial neoplasia, benign prostatic hyperplasia, and prostate cancer. Jeronimo et al. [20] have shown quantitative progressive increase of promoter methylation levels of several cancer-related genes associated with carcinogenesis of prostate cancer. Inactivation of p27 (inhibitor of the cell cycle progression) and the gene PTEN have been detected in most advanced prostate cancers. Lack of functions of both genes cooperates in prostate carcinogenesis. Moreover, p27 has a tumour suppressive function independent of its role in regulation of the cell proliferation in the prostate [21, 22]. Detection of several factors that have a real impact on carcinogenesis is the aim of many translational research activities. Some well-known factors, for instance HER-2, do not have clinical value, and therefore there is a great need to find novel factors, e.g. hepsin. The latter factor did not affect prostate cancer cell proliferation but caused disorganization of the basement membrane and promoted primary prostate cancer progression and metastasis to bone, liver, and lung [23, 24]. Validated clinical data and further analysis are capable of giving patients and physicians hope for better drugs.

A new direction in prostate cancer detection and treatment is associated with cancer stem cell biology. Stem cells are defined as cells that have the ability to perpetuate themselves through self-renewal and to generate mature cells of a particular tissue through differentiation. Cancer stem cells have been detected in many cancers including breast, brain, colon, head and neck, and prostate. The cells could have an impact on tumour growth and maintenance. It is still debatable whether target cancer stem cells may prove more effective than current cancer treatments [25, 26]. Xin et al. [27] have identified murine prostate cells (expressing Sca-1 marker) capable of regenerating tubular structures. Genetic changes within PTEN/Akt signalling in such cells leads to the initiation of tumorigenesis, and progression with subsequent increase in the number Sca-1 positive cells.

Molecular profiling is a fascinating and promising technology, but its incorporation into clinical decision making requires robust evidence. With the exception of breast cancer, there is little evidence concerning the role of molecular profiles in comparison with classic factors. From a technical viewpoint, there are some caveats to be solved because a single methodology has not offered full assessment of the expression of cancer cell transcriptome [28, 29].

### **Pathobiology of osteosclerotic metastasis of prostate cancer**

Skeletal-related events are defined by the existence of changes in antineoplastic therapy to treat bone pain, pathological bone fractures, radiation therapy to bone, spinal cord compression, and surgery to bone. Bone pathology

correlates with cancer course and has been shown to influence survival. Retrospective analysis of 3 049 patients with multiple myeloma (n=513), breast cancer (n=1130), prostate cancer (n=640), and lung cancer or other solid tumours (n=766) gave a crucial insight into fracture incidence (multiple myeloma – 43%, breast cancer – 35%, prostate cancer – 19%, and lung cancer – 17%). Moreover, breast cancer patients who developed a pathological fracture had a significant 32% increased risk of death relative to patients without a fracture (HR=1.32,  $p<0.01$ ). On the other hand, patients with multiple myeloma and prostate cancer had a >20% increased risk of death [30]. So the results have highlighted the great need for monitoring bone lesions which respond to combined treatment anticancer drugs with bisphosphonates. Existence of bone metastases and high N-telopeptide of type I collagen (NTX) urinary levels have correlated with increased risk of skeletal-related events and death. The analysis of Lipton et al. [31] has embraced breast cancer (n=578), hormone-refractory prostate cancer (n=472), and non-small-cell lung cancer (n=291). The authors have shed light on zoledronic acid as a drug reducing the risks of skeletal complications and death. Normalization of urinary levels of NTX within 3 months of treatment has been used as a good prognostic marker.

Bone lesions associated with the course of cancer are infrequently silent, but they are usually responsible for severe pain which can be intractable. Very often bone cancer pain is a devastating manifestation of metastatic cancer. Mechanisms that drive bone cancer pain include tumour-directed osteoclast-mediated osteolysis, tumour-induced nerve injury, stimulation of endothelin A receptor, and host cell production of nerve growth factor. All the mechanisms appear to be a potential target of novel therapies [32]. Zoledronic acid seems to fulfil the criteria of an active drug against bone pain on metastasizing hormone-independent prostate cancer and can be used together with <sup>153</sup>Sm-ETMP [33].

Paget [34] coined “the soil and seed hypothesis”, stating that certain tumour cells (seeds) will colonize distant organs (soil) because of the presence of a favourable environment for their localization and growth. The metastatic cells are detached from a primary tumour, enter the circulation, and finally enter the bone microenvironment. The preferential skeletal localization of tumour cells is attributed to the biological and molecular characteristics of tumour cells as well as the bone environment [35].

Bone is considered as a unique environment for metastasis. Cancer metastases located in bone are usually multifocal and have a propensity for the haematopoietic marrow sites in the proximal long bones and axial skeleton, i.e. vertebrae, pelvis, ribs, and skull [36]. Sinusoids and sluggish blood flow in the metaphysis facilitate the interaction between endothelium and tumour cells. This step is crucial at colonization to initiate a metastasis lesion [37]. Bone metastases are classified as osteolytic, osteoblastic, or mixed. The classification is mainly based on radiographic appearance.

Bone metastasis is frequently manifested as osteolytic lesions on radiography with prior, sometimes even

**Table 1.** Pro-osteoblastic factors produced by tumour cells

Name of factor	Pathophysiological role	References
Wnt (wingless)	inhibits osteoclasts, stimulates osteoblasts	40
DKK-1 (dickkopf homolog 1)	inhibitor of Wnt pathway in osteoblasts	41
ET-1 (endothelin)	stimulates proliferation of osteoblasts, promotes mineralization, potentiates other factors on osteoblasts, inhibits osteoclast motility	42
OPG (osteoprotegerin)	inhibits osteoclasts through binding to RANKL (receptor activator of nuclear factor-kappaB ligand)	43
BMP (bone morphogenetic protein)	stimulates osteoblast proliferation and survival	44
IGFs (insulin-like growth factors)	stimulates osteoblast proliferation and survival	45
IL-6 (interleukin-6)	regulator of osteoblast functions	46
TGF-beta	stimulates osteoblast proliferation	47
uPA (urokinase)	stimulates osteoblast proliferation	48
PDGF – BB (platelet-derived growth factor-BB)	promotes angiogenesis and osteoblast activity	49
FGFs (fibroblast growth factors)	promote differentiation and proliferation of osteoblasts	50
PSA (prostate-specific antigen)	activates latent TGF-beta and inactivates parathyroid hormone-related peptide	51
VEGF (vascular endothelial growth factor)	promotes differentiation of osteoblasts	52

tormenting, pain. Numerous studies performed in animals have suggested the existence of a vicious cycle in pathogenesis of an osteolytic metastasis. Tumour cells secrete various soluble factors which promote osteoclast differentiation, proliferation, and activation causing increased osteolysis. Growth factors such as TGF-beta, insulin-like factors, FGFs, and BMP are stimulators of growth and survival of cancer cells. As the cooperation between tumour cells and bone cells is under progress, the tumour cells secrete more pro-osteolytic factors that force osteolysis and perpetuate the vicious cycle [38].

Osteoblasts are the key players involved in forming the woven bone noted in an osteosclerotic metastasis. Cancer cells produce many biologically active factors such as cytokines, transcription factors, and growth factors. They are responsible for activation of osteoblasts. As far as the osteoblastic pathway is concerned, activated osteoblasts secrete numerous growth factors during the formation of osteosclerotic bone (TGF-beta, BMP, VEGF). The secreted factors stimulate more pro-osteoblastic factors, which in turn amplify the formation of woven bone and drive the vicious cycle [39]. Table 1 shows some of the most important factors that play a pivotal role in formation of osteoblastic metastasis.

Endothelin appears to be a physiological regulator of bone remodelling. On the other hand, this cytokine plays a key role in forming osteoblastic metastasis in prostate cancer dissemination. Endothelin 1 (ET-1) is a potent vasoconstrictor that binds to ETa and ETb receptors. ET-1 is produced by bone cells and stimulates mitogenesis of osteoblasts which express both ETa and ETb receptors. Moreover, ET-1 can decrease osteoclast activity and motility. If so ET-1 should be an important factor playing a pivotal

role in pathogenesis of osteoblastic metastasis in prostate cancer and other malignant diseases with bone osteoblastic lesions. Experimental data have shown that ET-1 stimulates osteoblastic bone metastasis formation by the mechanism affecting receptor ETa. This molecular pathway seems to be a potential target for clinical therapy [53, 54].

Atrasentan (ETa receptor antagonist) has shown efficacy in the treatment of osteoblastic lesions in preclinical models of prostate cancer bone metastasis and in phase I prostate cancer patients. However, subsequent results of clinical trials of phase II or III have not turned out to be clinically relevant [55, 56]. And again, novel experimental studies dealing with combination of docetaxel with atrasentan as a treatment of bone metastasis prostate cancer have shown activity of the composed therapy, but this notion needs to be confirmed in controlled clinical trials [57].

Overweight and obesity are well-known factors responsible for higher risks of cancer morbidity. A large number of available studies do not support an association between body mass and incidence of prostate cancer. But additionally, some studies support evidence that obesity is associated with an increase in risks of advanced prostate cancer or death from that malignancy [58].

Leptin is one of the major cytokines produced by adipocytes and controls body weight homeostasis by food intake and energy expenditure [59]. The physiological role of leptin is not limited only to metabolism of the fat tissue but has extended to bone remodelling since the beginning of the 21<sup>st</sup> century. Leptin-deficient ob/ob mice are obese and develop multiple pathologies associated with metabolic syndromes, and additionally, skeletal abnormalities are clearly seen. Genetic correction of that defect is sufficiently efficacious [60]. So leptin has been defined as a powerful

inhibitor of bone formation *in vivo*. This anti-osteogenic function involves leptin binding to its receptors on ventromedial hypothalamic neurons, the autonomous nervous system and beta-adrenergic receptors on osteoblasts. Leptin does not have its receptors on osteoblasts. Clinically, leptin is associated with bone loss, and therefore can be defined as a determinant of bone formation; in other words, leptin has an anti-osteogenic function [61, 62]. So another therapeutic method is pending for investigation to establish a potential role of leptin in patients with skeletal pathology, not only malignant. The latter notion can be supported by experimental results that have shown a strong interaction between cell-adipocyte and prostate cancer cells through leptin crosstalk. Leptin may be a potent target for therapy due to its pro-invasive activity on prostate cancer cells, and stimulates proliferation of androgen-independent prostate cancer cells [63, 64].

### **How zoledronic acid takes action on prostate cancer cells, bone cells, and cells of the microenvironment**

Bisphosphonates are synthetic analogues of pyrophosphate molecule. They have a greater stability than natural molecules and are resistant to enzymatic hydrolysis by osteoclasts. Nitrogen-containing bisphosphonates such as pamidronate, alendronate, ibandronate, and zoledronic acid interfere with a key enzyme acting in the mevalonate pathway, i.e. farnesyl-diphosphate synthase. Zoledronic acid is characterized by containing two nitrogen atoms, and therefore is the most active compound in inhibiting farnesyl-diphosphate synthase. This leads to reduction of the levels of farnesyl and geranylgeranyl diphosphate, which are required for the prenylation of small GTPases, e.g. Ras and Rho [65-67]. On the other hand, there is a wide range of mechanisms that are involved in destroying prostate cancer, and they are different from the well-known, e.g. independent action from expression of Ras protein of zoledronic acid against prostate cancer cells [68].

Direct activity of zoledronic acid against prostate cancer cell lines has been presented in a number of experimental investigations. There are also known many mechanisms in which zoledronic acid is involved. It reduces cell adhesion to the extracellular matrix and bone, tumour cell migration, tumour cell invasion, and proliferation of prostate cancer cells [69, 70].

Having shown zoledronic acid as a nitrogen-containing compound with high biologic activity, it is worth pointing out the possibility of its utility in clinics as part of combined therapy. Experimental data have defined a certain role of zoledronic acid in combination with doxorubicin in breast and prostate cancer cells. Namely, this sequence consisting of zoledronic acid (not other bisphosphonates) and doxorubicin can offer a higher rate of apoptosis in both investigated cancers [71].

A combination therapy may offer better results as could come from mixing various drugs having synergistic activity, of course, with acceptable toxicity profile. Several experimental investigations have supported the notion. mTOR activity is increased in advanced prostate cancer as

the result of well-defined mutations of PTEN. And everolimus is a new orally available mTOR inhibitor with potential activity against prostate cancer. Early *in vivo* results carried out on animals have shown inhibition of growth of prostate cancer after sole treatment with everolimus, and in combination with docetaxel and zoledronic acid. All drugs had augmented inhibitory activity on prostate cancer growth *in vivo* [72]. Everolimus may be useful as a radiosensitizer for PTEN mutated prostate cancer cells to improve tumour cell kill [73].

Bone cells are sensitive to the action of zoledronic acid. Several studies have suggested that bisphosphonates promote apoptosis of osteoclasts. Apoptosis is characterized by various cellular changes, including DNA fragmentation, mitochondrial swelling, and chromatin condensation. There are significant differences in the response of the hormone-sensitive and hormone-resistant prostate cancer cells. The latter cells, p53 efficient, are characterized by high levels of apoptosis and accumulation in the S phase after 24 hours, whereas hormone-sensitive prostate cells exhibits a slow rise in apoptotic cells with a strong G1 phase arrest. So it is possible to hypothesize that zoledronic acid induces apoptosis by distinct mechanisms depending on the p53 status of the target cells [74]. On the other hand, new experimental data have suggested that though zoledronic acid is capable of keeping inhibited bone resorption and bone loss associated with mixed osteoblastic/osteolytic bone metastases, it does not reduce the incidence of prostate cancer bone metastases [75].

Since the time when Dr Folkman isolated the first tumour factor responsible for angiogenesis, this process has been investigated with unprecedented impetus [76]. Angiogenesis appears to be an important prognostic factor for patients with androgen-independent prostate cancer. The most powerful plasma marker of angiogenesis, VEGF, may have clinical significance in patients with hormone-refractory prostate cancer. Plasma levels of VEGF median 83 pg/ml (range 4-885 pg/ml) correlated with survival [77]. Zoledronic acid and other bisphosphonates inhibit angiogenesis in experimental systems using testosterone-stimulated vascular regrowth in the ventral prostate. Other circulating angiogenic factors, with their modulation representing anti-angiogenic activity, are changed long-term by zoledronic acid [78, 79].

Prostate development is a result of an epithelial-mesenchymal interaction which regulates complexity, at a functional sphere of the gland. The complex reactions embrace paracrine influences of androgens and oestrogens. Hormonal induction of prostate cancer is thought to require signalling by androgen and oestrogen receptors which are expressed in the prostate. There are many pathological pathways involved in carcinogenesis of the prostate. It has been believed that in the beginning of a prostate cancer hormonal interactions play a pivotal role with subsequent gradual independence of the hormones leading directly to hormone-refractory prostate cancer. So the micro-environment has a stable role from the development of a prostate through benign tumour formation up to the appearance of prostate cancer. One of the latest directions

of investigation of the microenvironment in the course of prostate cancer seems to be elucidation of the role of T lymphocyte infiltrations, but mostly gamma/delta type.

T lymphocytes recognize specific ligands by clonally distributed T-cell receptors (TCR). A TCR is composed of an alpha chain and a beta chain. In the case of a minor population of T lymphocytes their TCR is characterized by gamma/delta chains. Conventional alpha/beta T lymphocytes are specific for antigenic peptides presented by gene products of the major histocompatibility complex. Gamma/delta T lymphocytes directly recognize proteins and even non-proteinaceous phospholigands. The presented explanation has suggested so far that gamma/delta T lymphocytes and alpha/beta T lymphocytes recognize antigens in a fundamentally different way. The residence of gamma/delta T lymphocytes in epithelial tissues and their rapid mobilization in response to various stimuli guarantee their scrutiny in the nearest future by the tumour immunologist. T lymphocytes with gamma/delta receptor account for 2-5% of CD3+ T cells in the peripheral blood but constitute a major T-cell subset in other anatomic locations, the most abundant being in the intestine and skin [80, 81].

Among gamma/delta T lymphocytes, the human peripheral specific fraction of these T cells recognizes low molecular mass nonpeptide ligands. Such phosphoantigens comprise isoprenoid pathway metabolites originating from bacteria, protozoa, and host cells. On the other hand, pharmacological agents such as bisphosphonates (zoledronic acid) are responsible for accumulation of mentioned metabolites which sensitize tumour cells to a specific fraction of gamma/delta T cells. Because zoledronic acid is widely used in clinics, actually, there is another potential activity of the drug. Dieli et al. [82] have performed a pilot study focused on potential synergistic activity of zoledronic acid and IL-2 through activation of gamma/delta T lymphocytes in hormone-refractory prostate cancer. The authors have shown that 18 mortally ill patients with hormone-refractory prostate cancer could obtain a biochemical response to combined treatment with zoledronic acid and IL-2 (the most active therapy in comparison with zoledronic acid used alone or IL-2). The observation was also confirmed in breast cancer patients who were treated with zoledronic acid only [83].

#### **Clinical trial results of zoledronic acid in preventing cancer treatment-induced bone loss (CTIBL) in patients with prostate cancer**

Zoledronic acid is an interesting drug, having a nitrogen-containing domain with highly potent activity in bone. It is 100-fold more potent than pamidronate in preclinical models of osteoclast-mediated bone resorption. Zoledronic acid has a unique usage by infusion lasting only 15 minutes, which makes this drug very convenient for patients in comparison with other intravenous bisphosphonates. Zoledronic acid received broad regulatory approval for the treatment of bone metastases secondary to all solid tumour types and bone lesions from multiple myeloma based on the results of three large, randomized, phase III clinical trials enrolling more than 3,000 patients. More information on this

clinically relevant drug in patients with cancer-related bone lesions is available elsewhere [1, 84-86].

Several studies have evaluated risk factors for bone loss and fractures in prostate cancer patients receiving androgen-deprivation therapy. The three risk factors that have been reliably identified in patients with locally advanced disease are older age, low body mass index and a long duration of androgen-deprivation therapy. In metastatic prostate cancer, the sole reliable risk factor is elevated levels of deoxy pyridinoline. Other factors such as reducing alcohol intake, less smoking, participating in regular weight-bearing exercise and taking daily calcium and vitamin D supplements may be responsible for helping reduce bone loss in men and women at risk of osteoporosis [87].

Androgen-deprivation therapy is also responsible for induction of bone loss at a significant level, and therefore is a clinical concern in patients with hormone-sensitive prostate cancer receiving long lasting androgen-deprivation therapy (ADT). ADT decreases circulating levels of oestrogen and testosterone, both of which maintain bone mass through suppression of bone resorption and promotion of bone formation [88]. ADT accelerates bone loss beyond levels seen with aging (1-2% annually) [89]. This fact is of special concern because patients with prostate cancer typically have low bone mineral density (BMD) even before starting ADT due to age, prostate cancer, and other health complications. In patients with advanced prostate cancer for more than 2 years before initiation of ADT (n=174) 42% were osteoporotic in comparison with 27% (n=106) of age-matched controls (p=0.22) [90].

Retrospective analysis performed on prostate cancer patients (n=4 494) has suggested that ADT increases the risk of osteopenia/osteoporosis (30%) and pathological and non-pathological fractures (16% and 42%) [91]. SEER (Surveillance, Epidemiology, and End Results program) data from 1992-1997 (n=50 613) have been analyzed. 19.4% of patients with prostate cancer receiving ADT for more than 5 years had a fracture compared with 12.6% of those did not have such therapy [92]. In another retrospective study concerning patients with prostate cancer receiving ADT circa 27% of them had osteoporosis and 51% had osteopenia of the hip or the lumbar spine [93]. So prevention of bone metastasis appears to be an interesting therapeutic opportunity for better results of control of malignant disease prior to dissemination to the skeleton. There are many ongoing clinical trials such as NSABP B34 (clodronate vs. placebo), S0307 (clodronate vs. ibandronate vs. placebo). All the studies are focused on breast cancer. Patients with high-risk prostate cancer and non-small-cell lung cancer will receive zoledronic acid in the prevention of skeletal metastases in a clinical trial called ZEUS [94].

Hormone therapy of prostate cancer is responsible for hypogonadism. Gonadotropin-releasing hormone agonists (GnRH-A) are the current treatment for metastatic prostate cancer and recurrent non-metastatic disease. GnRH-A increase bone turnover in men with prostate cancer. Biochemical markers of osteoclast and osteoblast activity increase gradually after treatment with this class of drugs. The plateau of the markers is reached after about 6 months.

On the other hand GnRH-A are responsible for increased parathyroid hormone-mediated osteoclast activation, suggesting that changes in skeletal sensitivity to that hormone play an important role in the pathogenesis of hypogonadal bone loss [95].

Zoledronic acid and pamidronate, given intravenously every 3 months, have prevented androgen-deprivation induced bone loss in the hip and the lumbar spine in men with non-metastatic prostate cancer receiving GnRH-A. In contrast with pamidronate, zoledronic acid increased bone mineral density. In the lumbar spine bone mineral density increased by 5.6% in men medicated with zoledronic acid and decreased by 2.2% in the placebo group ( $p < 0.001$ ) [96]. The results were confirmed in a randomized controlled clinical trial considering 40 men with non-metastatic prostate cancer on GnRH-A therapy receiving zoledronic acid at the dose of 4 mg given intravenously per annum vs. placebo. This annual therapy by one infusion of zoledronic acid increased the mean BMD of the lumbar spine by 4% compared with a decrease of 3.1% in men receiving placebo ( $p < 0.001$ ) [97].

## Conclusion

Bone is frequently affected in many malignant diseases but in prostate cancer it needs special attention. Bone pathology is of clinical value due to poor prognosis for cancer patients with afflicted skeleton. In some neoplasms such as multiple myeloma, breast cancer, prostate cancer, and lung cancer a proper therapy is especially highly needed for better results. So almost for ten years, there has been observed progress in studying malignant bone problems. Another challenge appears to be resolved, namely, finding better control of pain, which significantly impairs quality of life in prostate cancer patients.

Bisphosphonates are well-accepted drugs in the management of secondary bone neoplasms. Nitrogen-containing bisphosphonates, among them zoledronic acid, offer multidirectional action in prostate cancer patients. The drugs have direct activity against prostate cancer cells. Additionally, zoledronic acid is a powerful regulator of bone remodelling in patients with prostate cancer receiving androgen deprivation therapy. Combining treatment of prostate cancer patients with zoledronic acid and other anticancer drugs appears to be a new path that needs to be followed in order to get better results.

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