# Bone and joint disorders: screening and early clinical drug development

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CHAPTER 1

# Introduction

The skeleton offers support, shape and protection to organs and surrounding soft tissues. With the joints, it provides a framework for muscles and nerves to maintain posture and control movement. Beside this mechanical function, it has other important roles, particularly in calcium-phosphate homeostasis and hematopoiesis.[1,2] As hematopoiesis is not a primary subject of this thesis, it will not be discussed in this introduction.

# Bone

## CONSTRUCTION AND METABOLISM

Skeletal formation in humans is initiated in the first 2-3 months of the fetal development. Two types of osseous formation can be distinguished: (1) endochondral ossification, development by differentiation of mesenchyme into cartilage that is gradually replaced by bone leading to long bones that compile limbs and spine, and (2) intramembranous ossification, in which mesenchyme condenses to a thick membrane that slowly mineralizes resulting in flat bones such as the skull, mandible and clavicles.

Throughout life, bone is modeled by osteoclasts, governed by hormonal- and mechanical influences (stress and strain). Bone formation during life is supplied by osteoblasts in the periosteum.[1–3]

The main chemical component of bone is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) that, as the other components of bone, is subject to capture or release by the continuous processes of bone formation and breakdown. As such, bone is a highly metabolic active organ: approximately 10-15% of bone is renewed each year in adults.[2] This turn-over enables the bone to contribute to the calcium- and phosphate homeostasis.

The calcium – parathyroid hormone (PTH) – vitamin D axis plays the pivotal role in physiological bone- and calcium/phosphate homeostasis: the blood calcium concentration exerts negative feedback to PTH production and secretion. PTH increases renal calcium reabsorption and osteoclastic bone resorption; as such, hypocalcemia is compensated from sources available within the body.[4] In addition, the active metabolite of vitamin D, calcitriol, induces calcium absorption from the gut.[5] Thus, calcium- and/or vitamin D deficiency may lead to hyperparathyroidism and osteoporosis.[4,5]

Another role in bone homeostasis relates to gonadal hormones: estrogens and androgens. The importance of estrogens in bone homeostasis is demonstrated by their role in the etiology of post-menopausal osteoporosis. Androgens (predominantly testosterone), also have an important role in bone homeostasis. They contribute to the achievement of a higher peak bone mass in males than in females, in adolescence.[6–9] Physiologically, serum concentrations of sex hormones change with age in both men and women.[10] The most abrupt change occurs in women, in the menopause transition. At menopause a relatively abrupt decrease of total estradiol and increase of follicle stimulating hormone (FSH) occurs.[10] In men, androsterone-precursor dehydroepiandrosterone sulfate levels (and thereby testosterone) gradually decrease- and FSH increases with age.[11] As a result of these hormonal changes, bone mineral density (BMD) decreases with age in both sexes.[12]

## OSTEOPOROSIS

Osteoporosis is defined by the world health organization as a BMD T-score < -2.5, i.e. measured BMD more than 2.5 standard deviations below the average of a reference population of young Caucasian women.[13] The current gold standard for measurement of BMD is dual energy X-ray absorptiometry (DXA).[13] Low BMD increases the risk of nontraumatic or low-energetic fractures and associated morbidity and mortality.[14,15]

The complications of low BMD may be avoided by implementation of screening to enable early diagnosis and treatment. A strategy for early detection could include standardized screening of patients at risk of developing osteoporosis. Subsequently, osteoporosis and associated fragility fractures may be prevented by timely adjustments in bone-deteriorating therapy, hormone replacement, osteopenia- and osteoporosis treatment with bisphosphonates and calcium regulating compounds.[16–18]

Conditions which influence BMD include endocrine- and metabolic diseases such as adrenal insufficiency, Cushing syndrome, hyperparathyroidism, hypogonadism and type I diabetes mellitus.[19] Furthermore, changes in gonadal hormones may be induced by pharmacotherapy, e.g. glucocorticoids, estrogen modulators and androgen deprivation therapy.[19]

Urogenital malignancies may also harm BMD. In case of testicular germ cell tumors, a negative effect has not been unequivocally demonstrated yet. These tumors may affect the BMD through the simultaneous presence of a hypogonadal state, as testicular dysgenesis, and associated Leydig cell dysfunction is more prevalent among these patients. In addition, testicular germ cell tumors are treated by orchiectomy, often combined with chemotherapy. Both treatment modalities influence the gonadal state and chemotherapy has an independent negative effect on BMD.[20,21]

Prostate cancer treatment often involves multiple years of androgen deprivation therapy, which subsequently leads to enhanced risk of low BMD.[22] This risk of low BMD is recognized in this population and screening and treatment is advised by several guidelines.[23,24]

### BONE TUMORS- AND METASTASES

Bone lesions, benign and malignant, are another cause of skeletal fragility. Primary bone tumors are relatively rare,[25] but the skeleton is a preferred location for metastases from several primary solid organ tumors such as prostate- breast- lung- and renal cancer.[26–28] According to the seed and soil theory, malignant cells can colonize another organ only if the microenvironment is conductive to their implantation.[29]

A preparatory process of the 'soil' (pre-metastatic site) finds place before cells from the primary tumor can settle. It implies the induction of an inflammatory state, that makes circulating tumor cells admissible for settlement, growth in the tissue and thereby development of metastases. The induction of such an inflammatory state occurs via signaling through exosomes excreted by a primary tumor. Integrins expressed on the surface of the exosomes condition tropism to organs located at a distance (i.e. the preferred metastatic sites). There, they are internalized and initiate the inflammatory state by activation of chemokines.[30,31] Circulating tumor cells are directed by chemokine gradients and – in case of bone metastases – cytokine receptor activator of nuclear factor kappa-B ligand (RANKL), which makes the prepared inflammatory sites ideal targets for settling.[26,31,32]

As chemokines are tissue specific, the overlap between expression of chemokine subtypes in the targeted tissues and chemokine subtype receptors on circulating tumor cells is decisive for the preferred sites for metastases of a certain tumor type. Finally, a complex interplay of matrix proteins, lysyl oxidases, proteases and micro-RNA's enables further tumor cell invasion- and survival of the intruding cells at the metastatic site.[30] In case of bone, the growth of metastases causes pain, local weakening of the bone, and thereby fracture risk.

Metastatic sites have tissue-specific properties that most other tissues do not have (e.g. state of inflammation, high metabolism, modified vascular structure). By the time a solid tumor measures 2-3 mm, angiogenesis is induced.[33] Tumor neovasculature differs from that of normal tissue, presenting dilated, leaky and irregular of shape.[33] These malformations allow extravasation of large particles (macromolecules, nanoparticles, lipidic particles) into the tissue. Once absorbed into the tissue, the particles are retained longer than in other tissue due to poor lymph drainage. This phenomenon is the 'enhanced permeability and retention effect'.[34]

Anti-cancer treatments may take advantage of this effect to enable tumor targeting. If a relatively large proportion of the administered (often toxic) dose is available at the target sites, that may result in a better response, whilst limiting systemic side effects. A such, targeting may result in a longer survival and preservation of quality of life.

The preferred methods for diagnosis and follow-up of bone metastases in case of a known underlying malignancy, are skeletal scintigraphy and (positron emission tomography)-computed tomography (PET)-CT) scan.[35,36] Fluodeoxyglucose (FDG) PET imaging utilizes the high metabolism in the tumor sites, to visualize tumor sites. The tumor takes up most (radioactive) glucose, which can be detected and converted to an image by the PET-scanner.

## Joint

Synovial joints are essential for mobility. In addition to the osseous epiphyses, joints consist of cartilage, synovium, synovial fluid and in some cases menisci and ligaments. Inflammatory joint disease is the most common cause of joint disorder induced movement impairment, primarily due to the prevalence of osteoarthritis (OA).[37,38] Many joint pathologies are left undiscussed here, as those are not subject of this thesis.

### OSTEOARTHRITIS

Although highly prevalent, the exact etiology of OA remains largely unknown. It was long thought that OA was caused by 'wear and tear' of the cartilage, but current insights add other important factors. OA is now known as a multifactorial and highly heterogeneous disease, risk factors for which include: a history of traumatic joint injury, obesity, aging, biomechanical factors, and hereditary factors.[39,40]

Multiple joint tissues are involved in osteoarthritis: cartilage was long thought to play the primary role, as it is non-vascularized and the supply of nutrients and oxygen to the chondrocytes is restricted, and repair is hindered, all resulting in cartilage degeneration. Although the cartilage may be damaged in an OA-affected joint, it is an aneural tissue, and pain only

appears if innervated tissues are involved.[41] Synovium and subchondral bone are now also recognized to be involved in osteoarthritis from an early stage on.[41–43] Inflamed synovium produces catabolic and pro-inflammatory mediators, which can alter the balance within the cartilage matrix and thereby advance OA.[44]

Standard radiography is commonly used to confirm OA but is inadequate to detect early OA-related changes in the joints, and its correlation with clinical symptoms in early OA is poor.[45–48] Synovitis and subchondral bone activity cause early clinical symptoms such as joint swelling and pain but are not identifiable on standard radiographs. Synovitis can be identified by ultrasound and magnetic resonance imaging. Subchondral bone activity/turnover is apparent on magnetic resonance images as subchondral bone lesions.[41,44]

### CURRENT TREATMENTS

Current treatments for OA are restricted to symptom relief by minimization of pain and optimization of joint function. Various non-pharmacological and pharmacological interventions are available, all with only modest effects. Therefore, a combination of therapeutic approaches is commonly used. The choice of interventions is based on individual factors such as affected joints, disease extensiveness (mono-, oligo-, or poly-arthritis), and severity of symptoms, in addition to the presence of concurrent signs and symptoms such as muscle weakness, comorbidities, obesity, functional impairment, and depression.[49–52] Initially, non-pharmacological interventions such as exercise, weight loss, education and self-management programs are strongly recommended for all types of OA.[50] The next therapeutic step consists of pharmacological interventions, among which are: analgesic treatment, including oral and topical non-steroidal anti-inflammatory drugs (NSAIDS; selective and non-selective COX-2), paracetamol, tramadol, duloxetine, chondroitin, intra-articular steroid administration and topical capsaicin.[50]

The approved pharmacological interventions for OA treatment target symptoms and have no meaningful disease modifying effect. As a result, the condition worsens over time and in some cases leads to arthroplasty. Total hip- or knee replacement results in pain reduction in the majority of patients and their cost-effectiveness is well established.[53,54] Unfortunately, these surgical interventions for OA are commonly preceded by a long trajectory of pain and functional limitation. Also, the interventions are not successful for all patients and there is a risk of complications during the post-surgical trajectory, e.g. thromboembolic events or infection.[55–57]

It is plausible to assume that the increasing knowledge of the pathophysiology of OA will result in the identification of novel pathways/targets that can be exploited to develop disease modifying OA drugs (DMOADs). The involved tissues (synovium, subchondral bone, and cartilage) may each be targeted by potential DMOADs.

Cartilage regeneration may still give an effect; cartilage degeneration remains a key factor in OA and -especially in traumatic OA-, it influences joint loading and may enhance further joint degeneration. The growth of cartilage is a challenge, as the tissue is so poorly vascularized. Approaches to achieve this growth could be stimulation of cartilage stem progenitor cells, or the introduction of stem cells into the joint. So far, no pharmacotherapeutics have been registered in this class.

As inflamed synovium expresses mediators which further stimulate degenerative changes in OA,[41,42] muting synovitis may be a successful treatment strategy. So far, systemic- and local therapies with a general anti-inflammatory mechanism (corticosteroids) and disease modifying antirheumatic drugs, were unsuccessful.[58,59] At least two explanations for which may be applicable: the mechanism of action does not have the desired effect, or the exposure in the synovium was not sufficient to be effective.

Several attempts have been done to target the subchondral bone with therapies registered for other indications, but so far without success.[16,60]

When successful, DMOADs would lead to quality-of-life improvement.[61] This will, however, be a *bumpy road* as shown by the failed attempts to develop DMOADs so far. It is important to identify the reasons for these failures and in particular address possibly erroneous assumptions in animal-to-human translation, side effects, structural symptom discordance, incorrect structural endpoints.[62–66]

## Epidemiology

The personal- and societal burden caused by disorders affecting the bone and joints is significant – grouped musculoskeletal disorders rank as the most expensive category in healthcare expenses in the USA, with annual costs of $380.9 billion.[67]

Societal costs of osteoporosis fractures are $17.9 billion per annum in the USA.[68] Approximately 25% of osteoporosis-related healthcare cost, is on ac-

count of men.[69] Although long neglected, increasing attention is being paid to osteoporosis in men, and the Endocrine society guideline recommends screening of men at risk.[70]

Prostate cancer patients, particularly those undergoing androgen deprivation therapy, are known to be at risk for fractures, due to their state of pharmacologically induced hypogonadism.[71] Prostate cancer mostly occurs in men >65 years of age and is the second most diagnosed type of cancer in males worldwide, with 1.5 million newly diagnosed- and 0.4 million deaths per year.[25,72] Screening and treatment of prostate cancer patients for osteoporosis could prevent skeletal related events (fragility fractures), and thereby reduce personal- and societal burden. Although screening for osteoporosis in patients using ADT is widely recommended,[73–76] it is scarcely implemented.[77–79] The application of DXA in all these patients is costly and time consuming; a more efficient approach of screening may be available and preferable.[25,80]

In contrast to the highly prevalent prostate cancer, testicular carcinoma is globally diagnosed in approximately 75 000 men per year and has an estimated mortality of ~10 000 per year.[72,81] Although not as prevalent, the mean age at diagnosis of testicular cancer is generally much lower than that of prostate cancer patients. In fact, testicular cancer is the most common type of cancer in men aged 18-40 years.[81] Curative rates for testicular cancer are high, thereby, these young men have longstanding consequences of late effects of testicular cancer, such as hypogonadism- cardiovascular disease or osteoporosis. The effects of testicular cancer to bone health are not as well-established as those of prostate cancer; screening for osteoporosis is not recommended in the European agency for urology (EAU) guideline for testicular cancer,[82] while the Endocrine society recommends screening of hypogonadal patients,[83] which testicular cancer survivors frequently are.[84]

OA is one of the largest contributors to healthcare cost due to musculoskeletal disorders.[67] Incidence of OA increases with age; globally 10% of men and 18% of women aged over 60 years have symptomatic OA.[37,38] OA leads to annual healthcare expenses of approximately $80.0 billion in the USA.[67] As such, it is a cause of long-term pain and disability in older adults, causing loss of work productivity and significant healthcare- and social support costs. Development of a DMOAD has the potential to relieve some of these costs.



## In conclusion

Musculoskeletal disorders have an enormous personal- and societal impact. Diagnosis and treatment of these disorders are most efficient if targeted screening, accurate diagnosis, and targeted treatment are available. To enable targeted screening, the population at risk must be well-defined and categorized if required. Subsequently, screening- and diagnostic methods must have good, or excellent predictive value and finally, treatment must target the disease, thus spare healthy tissues and processes and thereby avoid adverse events.

The aim of this thesis is to gain new insights about the diagnostic process- and treatment of pathological conditions of the bone and joints, namely male urological cancer-induced bone loss and OA.

## This thesis

This thesis consists of two sections; In Section I, screening, diagnosis and treatment of the consequences of male urological malignancies and their therapies to bone health are investigated (**Chapters 2-4**). Section II focuses on inflammatory arthritis and the early clinical development of compounds targeting inflammatory joint disease (**Chapters 5-7**).

### SECTION I: BONE IN MALE UROLOGICAL MALIGNANCIES

In this thesis, the vulnerability of the skeleton is studied in the context of two male urological malignancies: prostate cancer and testicular cancer. In both cancers a role is assigned to (treatment induced) hypogonadism, which could cause a low BMD, and secondary effects from anti-cancer therapy (androgen deprivation therapy, chemotherapy, corticosteroids). In prostate cancer osseous metastasis further contribute to detrimental skeletal effects. In this thesis, it was aimed to investigate options of strategic screening and early diagnosis of osteoporosis and targeted treatment of osseous metastases.

**Chapter 2** of describes a literature review of the effects of testicular cancer and its treatments on BMD, as measured in dual-energy X-ray absorptiometry (DXA), and outcomes of hormone levels and bone turnover markers that were reported in the investigated populations. Prostate cancer patients are at risk of having poor bone health due to years of androgen deprivation therapy. In addition to DXA, quantitative ultrasound (QUS) is another,

more accessible, method to evaluate BMD and bone structure, which can be applied directly in the clinic. However, it is not as frequently applied nor as well-established as DXA. A study for the accuracy and potential of QUS as a pre-screening tool in prostate cancer patients, is presented in **Chapter 3. Chapter 4** reports the results of a first-in-human clinical study in which patients with metastatic castration resistant prostate cancer with osseous metastases were included. The liposomal compound that was administered in this study, was designed to target osseous metastatic sites. The aims of the study were to investigate safety and tolerability and to explore efficacy.

## SECTION II: OSTEOARTHRITIS THERAPIES

The current pipeline of treatment advances in OA is described in a clinical trial database review in **Chapter 5**. One of the categories in which clinical trials are ongoing, is cartilage metabolism. **Chapter 6** describes the first-in-human study of a chondro-stimulating compound (LRX712). The compound was administered to patients with knee osteoarthritis. **Chapter 7** describes the outcomes of a first-in-human study with ART-IO2. This gene-therapy product aims to give inflammation-driven expression of an anti-inflammatory protein in the synovium, to inhibit the low-grade inflammatory state in the joint, and in the synovium in particular. In this study ART-IO2 was introduced into a target hand joint of patients with inflammatory hand arthritis. The main objectives of the studies in **Chapters 6 and 7**, were to establish safety and tolerability of the compounds, and to explore efficacy.

A summary, general discussion and conclusions of the thesis are described in **Chapter 8**, and a summary conclusion in Dutch is given in **Chapter 9**.



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