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THE DEGREE OF DOCTOR OF PHYLOSOPHY IN PHARMACOLOGY AND TOXICOLOGY

PAIN AND ANALGESIC USE ASSOCIATED WITH SREs IN PATIENTS WITH ADVANCED BREAST CANCER AND BONE METASTASES TREATED WITH BONE-MODIFYING AGENTS: A RETROSPECTIVE OBSERVATIONAL STUDY

Noemi Simeone

Director of the PhD Program
Prof. Silvana Gaetani

Supervisor
Prof. Patrizia Campolongo

Co-Supervisor
Prof. Augusto Caraceni

Index

1. Introduction

1.1 Breast Cancer

Breast cancer (BC) is a prevalent malignancy affecting women globally. Based on molecular and histological evidence, BC can be classified into three main subtypes: hormone receptor-positive BC (expressing estrogen receptor [ER+] or progesterone receptor [PR+]), human epidermal growth factor receptor 2-positive BC (HER2+), and triple-negative breast cancer (TNBC), which lacks ER, PR, and HER2 expression (ER−, PR−, HER2−). [1]

1.1.1 Pathology

1.1.1.1 Histological Classification

Epithelial malignancies are categorized into in situ carcinoma and invasive cancer. In in situ carcinoma, tumor cells do not invade the stromal tissue. This category is further classified into ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). In DCIS, neoplastic epithelial lesions remain confined to the mammary ducts, accounting for approximately 20% of all breast cancers. LCIS, on the other hand, is often multifocal and is bilateral in approximately 20-60% of cases. LCIS presents in two main forms: classic and pleomorphic. The pleomorphic type is characterized by larger, more atypical cells and carries a higher risk of progression to invasive carcinoma in both breasts. Invasive carcinoma includes predominantly infiltrating ductal carcinoma (IDC), which comprises approximately 75% of cases, and infiltrating lobular carcinoma (ILC), which accounts for around 10%. [5]

1.1.1.2 Molecular Classification

Breast cancer exhibits significant heterogeneity, with tumors that may appear similar in clinicopathological characteristics often showing diverse clinical behaviors and treatment responses. Gene expression profiling through microarray technology has thus become an essential tool for understanding molecular subtypes and guiding targeted therapies. Key biomarkers, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), have been widely used to classify breast cancer into subtypes—luminal A, luminal B, HER2-enriched, and basal-like/triple-negative—which influence both prognosis and therapeutic strategies. The identification of specific molecular pathways associated with these subtypes has led to the development of targeted therapies, such as endocrine therapies for ER-positive tumors, HER2 inhibitors for HER2-positive tumors, and, more recently, immune checkpoint inhibitors and PARP

inhibitors for certain triple-negative breast cancers. The integration of gene expression profiling into clinical practice supports a precision medicine approach, aiming to optimize treatment efficacy and improve patient outcomes.

Gene expression profiles generally correspond to the four immunophenotypic subgroups, though substantial heterogeneity exists within each subgroup.

These subgroups include:

- **Luminal A**: Characterized by positive hormone receptors, negative HER2, and low Ki67 proliferation index; tubular and classic lobular carcinomas are common in this group.
- **Luminal B**: Can be either HER2-positive or HER2-negative, with positive hormone receptors and a high Ki67 proliferation index.
- **HER2-positive (non-luminal)**: Defined by HER2 overexpression without hormone receptor expression.
- **Triple-negative**: Lacking expression of hormone receptors and HER2, and representing approximately 80% of the basal-like subtype. [6]

Other essential prognostic classifications include the TNM staging system, which assesses the extent of disease locally (T for tumor size and spread), in lymph nodes (N for nodal involvement), and in terms of metastasis (M for distant spread). Additionally, genomic prognostic testing offers a more refined, albeit costly, approach. The only tests with available data from randomized clinical trials (RCTs) are *MammaPrint* and *Oncotype DX*, both of which provide valuable insights into recurrence risk and guide treatment decisions. [7]

Important prognostic factors that aid in selecting the appropriate treatment include tumor size, axillary lymph node status, histological grade and type, proliferative activity (Ki67 index), vascular invasion, immunophenotype, patient age (with those under 35 having a poorer prognosis), and genomic and molecular classifiers. [8]

1.1.2 Clinical Presentation

Clinically, breast cancer rarely presents as generalized breast enlargement or diffuse thickening. Pain may be present, though it is seldom an isolated presenting symptom of breast cancer. In most cases, particularly in countries where mammographic screening is available, diagnosis occurs following the detection of a mass during routine physical examination or mammography. The presentation may be metastatic from the onset, with symptoms such as pathological fractures, abdominal pain, jaundice, or dyspnea. Clinical signs indicative of advanced breast cancer includes fixation of the mass to the chest wall or overlying skin, the presence of satellite nodules, or skin ulceration. Additionally,

assessment of the axillary, supraclavicular, and infraclavicular lymph nodes is crucial to evaluate potential lymphatic involvement. Two distinct clinical entities with unique presentations are Paget's disease of the nipple and inflammatory breast cancer. In Paget's disease of the nipple the tumor extends to the skin overlying the nipple and areola, presenting as an eczematous or psoriasiform skin lesion. Malignant cells, known as Paget cells, are present in the epidermis. This type of lesion may indicate an underlying in situ carcinoma or invasive carcinoma, necessitating prompt diagnosis and treatment. Inflammatory breast cancer presents with erythema, breast swelling, and generalized tenderness, often in the absence of a palpable mass. The skin may appear discolored or exhibit a thickened, peau d'orange texture, and nipple discharge is frequently observed. [9]

1.1.3 Diagnosis

A thorough physical examination, complete blood count, and full biochemical profile are necessary to determine an appropriate therapeutic approach and to identify or rule out comorbidities. A biopsy should be carried out to confirm histology and re-assess tumour biology (ER, PgR, HER2). In high-risk situations for metastasis (e.g., clinically positive axillary lymph nodes, tumor size ≥ 5 cm, and aggressive tumor biology), computed tomography (CT) of the chest and abdomen and bone scintigraphy are recommended, as well as for symptomatic patients with clinical or laboratory signs suggestive of metastatic disease. [18F]2-fluoro-2-deoxy-D-glucose (18F-FDG) positron emission tomography (PET)–CT may be used instead of CT and bone scans or when previous investigations are inconclusive. [10] Brain imaging should not be routinely carried out in all asymptomatic patients at initial MBC diagnosis or during disease monitoring. Symptomatic patients should always undergo brain imaging, preferably with magnetic resonance imaging (MRI).

1.1.4 Treatment of Metastatic Disease

The selection of treatment for breast cancer is inherently complex and varied, necessitating careful consideration of factors such as immunophenotype, prior treatments, expected toxicities, duration of disease-free interval, extent and location of metastases, physiological age, performance status, comorbidities, and menopausal status (particularly for endocrine therapy). Each patient’s treatment strategy should be based on a tailored risk–benefit evaluation that considers both patient- and disease-specific factors, comorbid conditions, and individual preferences. Treatment decisions should be made through a collaborative, patient-centered approach. Participation in clinical trials is recommended whenever available. In metastatic breast cancer, it is essential to evaluate whether the locoregional tumor is operable and whether a locoregional approach can be implemented alongside systemic therapy (including endocrine therapy, chemotherapy, and biologic agents) and follow-up

management. Systemic therapy is tailored based on the immunophenotypic and molecular characteristics of the tumor, the patient's menopausal status, the number and locations of metastases, the organs involved, previous treatments and their associated toxicities, disease-free interval, the need for close follow-up, and the patient's preferences. The treatment of metastases can improve overall survival and alleviate metastasis-related symptoms, thereby enhancing the patient's quality of life. Radiotherapy (RT) is frequently used in the treatment of isolated, symptomatic bone lesions or local cutaneous recurrences that are not amenable to surgical resection. Additionally, it is the most effective treatment for brain metastases. [11] Enhancing the patient's quality of life (QoL) is a central aspect of managing metastatic breast cancer, as these patients often experience a range of challenging symptoms. Common issues include pain (see section 1.4), constipation, dyspnea, nausea, and other symptoms that arise during the course of the disease, which may have a prolonged prognosis. Supportive care should always be part of the treatment plan and early introduction of expert palliative care may help to better control symptoms.

1.1.5 Systemic Therapy

1.1.5.1 Endocrine Therapy

Hormone therapy involves administering drugs that interfere with estrogen activity. Its mechanisms of action include: preventing tumor cells from responding to endogenous hormones through antiestrogen agents (such as Tamoxifen and Fulvestrant); inhibiting estrogen production by blocking aromatase, the enzyme responsible for converting androgens to estrogens (aromatase inhibitors); and reducing ovarian estrogen production using LHRH analogs. This strategy is effective only in hormone-responsive tumors (ER+ and/or PgR+).

Antiestrogens. The most commonly used antiestrogen is Tamoxifen, frequently administered in premenopausal women. Common side effects include hot flashes and sweating, weight gain, fluid retention, vaginal dryness, insomnia, musculoskeletal pain, and mood swings or depression. Regular follow-up is essential due to an increased risk of endometrial cancer, deep vein thrombosis, and stroke—rare but significant events. [12]

Aromatase Inhibitors. Aromatase inhibitors are drugs that reduce circulating estrogen levels by inhibiting aromatase enzymes in adipose tissue, which convert androgens to estrogens. They are preferred over Tamoxifen in postmenopausal women and are often administered as a first-line treatment in combination with cyclin-dependent kinase 4/6 (CDK4/6) inhibitors.

In later treatment lines, aromatase inhibitors are combined with mammalian target of rapamycin (mTOR) inhibitors. The most commonly used aromatase inhibitors—letrozole, anastrozole, and exemestane—are taken orally. Side effects are similar to those of Tamoxifen but have less impact on

mood changes. However, prolonged use increases the risk of osteoporosis, making regular bone density monitoring essential, along with the possible use of medications to counteract bone loss. There is no evidence of an increased risk of endometrial cancer, thrombosis, or stroke with aromatase inhibitors. [13]

LHRH Analogues. LHRH analogues inhibit ovarian estrogen production, resulting in ovarian ablation. They are indicated for use in premenopausal women and are often combined with another antiestrogen agent. Side effects are similar to menopausal symptoms, including hot flashes, sweating, decreased libido, headache, and mood swings. Commonly used LHRH analogues include triptorelin embonate, goserelin, and leuprolide acetate. [14]

1.1.5.2 Chemotherapy

Systemic pharmacological treatment involves the administration of drugs targeting cells with high replication rates, primarily aiming at tumor cells. This approach seeks to slow or halt disease progression and is designed to be as selective as possible for cancer cells while sparing healthy cells. It is particularly applied in ER-negative tumors or ER-positive tumors that are resistant to endocrine therapy, as well as in cases of visceral crisis.

Treatment selection is based on factors such as previously administered adjuvant drugs, cumulative doses reached, disease-free interval duration, and performance status. Among the most active agents are anthracyclines (e.g., doxorubicin, epirubicin, liposomal doxorubicin), taxanes (e.g., paclitaxel, docetaxel, nab-paclitaxel), antimetabolites (e.g., capecitabine), vinca alkaloids (e.g., vinorelbine), non-taxane microtubule inhibitors (e.g., eribulin), and platinum-based agents such as cisplatin (especially in BRCA-1 mutation cases) and carboplatin. Less commonly used drugs include cyclophosphamide, gemcitabine, 5-fluorouracil, methotrexate, mitoxantrone, and mitomycin C. The tissues most affected by chemotherapy are those with high cellular turnover, such as the skin and appendages, leading to alopecia; the bone marrow, resulting in anemia, thrombocytopenia, neutropenia, or leukopenia depending on the cell line affected; and the gastrointestinal tract, causing symptoms such as nausea, vomiting, diarrhea, loss of appetite, or oral stomatitis. Other potential side effects include asthenia and fatigue, arthralgia, myalgia, headache, renal or hepatic toxicity, peripheral neuropathy, and reduced fertility. Each chemotherapy regimen is associated with specific adverse effects (AEs). [15]

1.1.5.3 Biological Therapy

In recent years, monoclonal antibodies, tyrosine kinase inhibitors (TKIs), chemo-immuno conjugates, and CT-IO (chemo-immunotherapy) agents have been developed, demonstrating significant efficacy.

The first biologic drug was created to target tumors expressing HER-2 receptors. Among these "anti-HER-2" biologics, Trastuzumab (Herceptin) is the most widely used. These agents have a different toxicity profile than chemotherapy; for example, anti-HER2 drugs may cause headaches, flu-like symptoms, and allergic reactions. Trastuzumab is not recommended for patients with cardiac issues due to a potential risk of cardiotoxicity, especially with prolonged use. Another commonly used anti-HER-2 monoclonal antibody is Pertuzumab, which has a similar toxicity profile. Selective TKIs for HER-2 include Tucatinib, Lapatinib, and Neratinib. [16] e [2]. Two important new drugs are also available: trastuzumab deruxtecan (T-Dxd), which combines an anti-HER2 antibody with a novel topoisomerase inhibitor (a tetrapeptide-based linker with cytotoxic payload), and sacituzumab govitecan, an antibody directed against the trophoblast cell-surface antigen 2 (Trop-2), a metabolite of irinotecan and a topoisomerase I inhibitor. Trop-2 is a transmembrane calcium signal transducer highly expressed by breast cancer cells. [17] Among biologic agents used in the treatment of HER-2 negative metastatic tumors, Bevacizumab is commonly applied; it is a monoclonal antibody targeting vascular endothelial growth factor (VEGF), thereby inhibiting tumor angiogenesis. PARP inhibitors (poly-ADP-ribose polymerase inhibitors), enzymes involved in DNA repair processes and cell death, are also frequently used. [2]

CDK4/6 inhibitors are commonly used for metastatic ER+/HER2- breast cancers. Three drugs have been developed to selectively inhibit CDK4/6—enzymes involved in tumor proliferation—each with distinct potency and toxicity profiles: Palbociclib, Ribociclib, and Abemaciclib. [18]

1.1.5.4 Palliative Radiotherapy

Radiotherapy is a localized, non-invasive treatment that uses high-energy ionizing radiation, typically X-rays or gamma rays, to induce necrosis of tumor cells. To assess the response to therapy, an interval of 6-8 weeks is required following the completion of treatment. There are several applications of RT: Curative or Radical RT: Aims to completely eradicate the tumor.

- Preoperative or Neoadjuvant RT: Administered before surgery to reduce tumor size, facilitating surgical removal.
- Postoperative or Adjuvant RT: Reduces the risk of recurrence following surgery.
- Intraoperative RT (IORT): Applied during surgery.
- Palliative RT: Aims to slow tumor growth and relieve symptoms, particularly pain, thus improving quality of life (QoL).
- Ablative RT: Used for small tumors with high doses through stereotactic techniques.

- Total Body Irradiation (TBI): Used for specific cancers such as leukemia or lymphoma. [19]

Palliative RT is particularly valuable for metastatic sites in bone or soft tissue, as it can slow tumor progression and alleviate associated symptoms. [20]

Despite significant advancements in the precision of ionizing radiation beams, RT is not without adverse effects, as some healthy cells are inevitably exposed. This can result in acute, subacute, and late-onset side effects.

In palliative RT, common adverse effects include breast tension (especially in cases of carcinomatous mastitis) secondary to edema, often accompanied by sharp, generally tolerable, and short-lived pain; skin changes such as erythema, itching, burning, and excoriations; increased breast firmness, edema, and fibrosis; systemic side effects such as asthenia and fatigue; dysphagia (notably with RT to the spine), which usually resolves quickly with appropriate medical therapy and discontinuation of RT; and initial exacerbation of bone pain (for RT to bone metastases). [21]

1.2 Skeletal Metastases

The treatment of bone metastases is a primary focus of this study, specifically comparing Zoledronic Acid (ZA) and Denosumab (Dmab) in terms of their effectiveness in reducing skeletal-related events (SREs) and in pain palliation. Skeletal metastasis refers to the secondary localization of cancer within bone tissue. Bone metastases are indicative of advanced-stage disease and, while treatable, are not curable. Due to the high associated symptom burden, they are a leading cause of morbidity and mortality in cancer patients. [22] Bone is the third most common site of metastasis after the liver and lungs, and all cancer types have the potential to metastasize to bone. More than half of skeletal lesions are caused by prostate and breast cancer, whereas bone metastases are less frequently observed in cancers of the gastrointestinal tract.

Approximately 80% of bone lesions are located in the axial skeleton, with fewer instances in the appendicular skeleton. This is partly due to the fact that appendicular skeletal segments are often only partially included in anatomical or functional imaging studies and are frequently identified based on clinical symptoms. [22]

The primary SREs include fractures, spinal cord compression (SCC), and hypercalcemia (see section 1.3).

1.2.1 Pathogenic Mechanisms

A key step in bone metastasis remains the attachment of osteoclasts to the bone surface. The binding of RANKL to RANK activates osteoclast precursors, promoting their differentiation into mature

osteoclasts. Osteoprotegerin (OPG) prevents the RANK-RANKL interaction by binding to RANKL, thereby promoting bone formation and reducing bone resorption (see Fig. 1). Tumor cells release PTHrP, IL-6, TNF-alpha, and other factors that stimulate osteoblasts to release RANKL, thereby enhancing osteoclastic activity. Additionally, substances are secreted that suppress OPG production, further promoting the RANKL-RANK interaction. The complexity of the pathological mechanisms involved underlies the phenotypic variability of skeletal metastases, which may present as predominantly osteoblastic, as seen in prostate cancer, or more osteolytic, as in breast or lung cancer. [23]

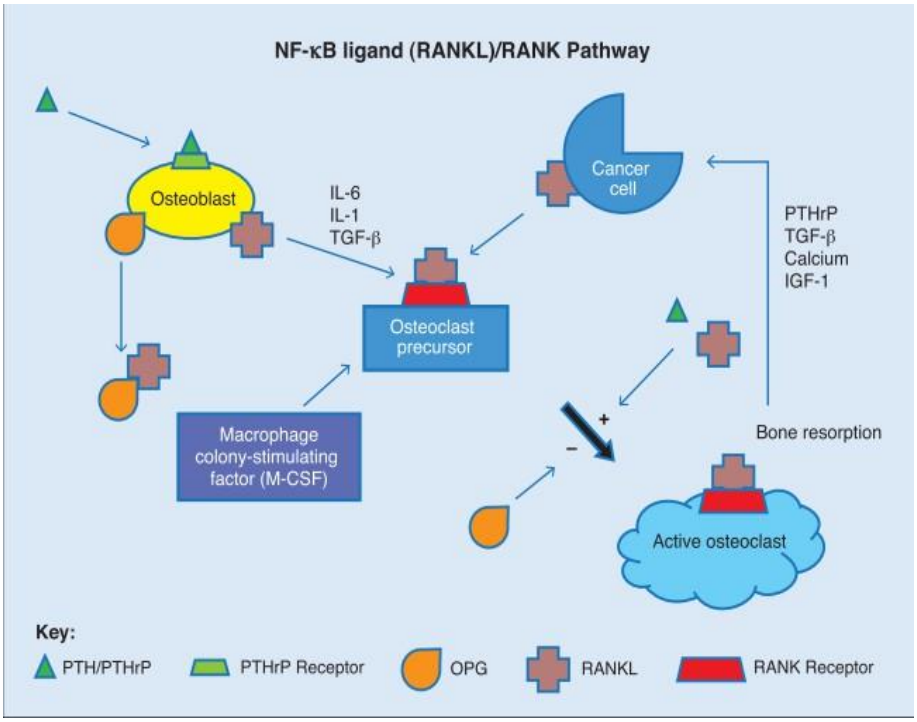

Fig. 1. Osteoblasts are activated by PTHrP, leading to increased production of RANKL, which interacts with RANK receptors on hematopoietic precursors of osteoclasts, resulting in the formation of mature, active osteoclasts. Mature osteoclasts resorb bone, releasing minerals and growth factors stored within the bone, including PTHrP. OPG can inhibit this process by binding to RANKL.

1.2.2 Clinical Presentation

Skeletal metastases are rarely asymptomatic. The primary presenting symptom of skeletal involvement is pain, which may have a complex pathogenesis involving various mechanisms. Pain is associated with the local release of cytokines and biochemical mediators from tumor cells, irritation of the periosteum, and stimulation of intraosseous and periosteal nerve structures. Mechanical mechanisms may also contribute due to the mass effect of tumor tissue within the bone and compression of surrounding structures. [24]

Pain is usually localized and may be exacerbated by the occurrence of SREs such as pathological fractures (the most frequent events), SCC, and, less commonly, hypercalcemia (which may present with abdominal pain). It is important to note that the development of acute or progressively worsening back pain, especially when associated with radiographic abnormalities and/or neurological symptoms, should always prompt an evaluation to rule out SCC.

1.2.3 Diagnosis

The diagnosis of skeletal metastases is based on suggestive clinical findings and appropriate imaging. In a smaller number of cases, skeletal metastases may be detected incidentally during radiological or nuclear medicine assessments that evaluate bone status, particularly in cancers with locally advanced extension or a risk of local or distant recurrence. Diagnostic evaluation should be completed with assessments of calcium levels, alkaline phosphatase, parathyroid hormone (PTH), and vitamin D. Conventional radiography is a rapid, cost-effective, and accessible option for evaluating bone metastases, offering high specificity but low sensitivity, as it cannot detect subcentimeter or marrow-trabecular lesions. Scintigraphy, on the other hand, has high sensitivity and lower specificity, capable of detecting osteoblastic reactions indicative of bone damage. However, MRI is superior to these methods, particularly for assessing the presence of vertebral metastases. Bone scintigraphy is indicated in cases with clinical symptoms, elevated calcium or alkaline phosphatase levels, or when recommended by specific diagnostic protocols. Further evaluation with CT or MRI is advised to assess potential fractures or evaluate fracture risk. FDG-PET is increasingly used and is superior to bone scintigraphy for identifying secondary skeletal lesions. The low resolution of PET is partially addressed by image fusion with CT or MRI in combined PET-CT or PET-MRI exams. However, the high cost and limited availability of this equipment restrict its use when the sole objective is the detection of bone lesions. [25]

1.2.4 Skeletal Complications

1.2.4.1 Pathological Fractures

1.2.4.1.1 Pathophysiology

Skeletal metastases disrupt the balance of bone remodeling, favoring increased resorption, which significantly reduces the bone's load-bearing capacity. [26] The accumulation of microfractures can lead to full pathological fractures, which are more common in lytic metastases, particularly in the vertebrae and ribs. Significant damage occurs in both trabecular and cortical bone, with cortical bone damage playing a more critical role in the pathogenesis of fractures. [27]

1.2.4.1.2 Clinical Presentation and Diagnosis

Generally, the patient presents with pain, particularly exacerbated by movement, along with deformity, localized swelling, and loss of function. In this context, patients may experience fractures even during routine activities, such as turning in bed or walking upstairs; therefore, considerable caution is required when undertaking movements. Asymptomatic skeletal metastasis may also be present, which could lead to bone fractures following even minimal exertion. [27]

In patients reporting cervical or dorsal pain, common differential diagnoses include osteoporotic collapses or degenerative diseases. Vertebral fractures can lead to severe neuropathic pain due to nerve injuries, potentially resulting in neurological syndromes such as Horner's syndrome in cases of metastasis at the C7-T1 levels, or spinal cord compressions resulting from vertebral collapses. However, the most debilitating disabilities are associated with long bone fractures or epidural extension of tumors within the spine. High-risk patients include the elderly, those with osteoporosis, and individuals undergoing long-term steroid treatment. There are available scoring systems based on the characteristics of the lesion (lytic, blastic, or mixed), the location, the extent of cortical involvement, and the presence of pain, which can provide insight into the risk of fracture. To diagnose a pathological fracture, a radiograph is typically sufficient. [28]

1.2.5 Treatment

The best treatment is to prevent the occurrence of fractures, and for this purpose, bone resorption inhibitors are crucial, as they reduce the incidence of pathological fractures, prevent other skeletal-related events (SREs), and even enhance repair in the event of a fracture. A surgical synthesis of the fracture is possible; however, in weight-bearing bones, orthopedic fixation is generally preferred. This approach typically results in immediate pain relief. Radiation therapy is indicated for localized pain that poorly responds to medication, in an area corresponding to imaging studies that confirm bone metastases. Up to 80% of patients report pain relief after RT. A physiotherapy consultation is

also essential for the prescription of precautionary advice and tools to stabilize the affected area. [29]

1.2.5.1 Hypercalcemia

1.2.5.1.1 Pathophysiology

The normal range for serum calcium is between 8.5 and 10.3 mg/dL (or 2.12 to 2.57 mM), with approximately half of the serum calcium existing in a free ionized form (Ca++) and the other half bound to proteins (primarily albumin) or complexed with anions. Changes in albumin levels can affect the total concentration of ionized calcium, which is active on cellular membranes. Each change of 1 mg/dL in albumin is associated with an approximate change in calcium of 0.8 mg/dL; this correction is important in patients with advanced cancer, given their low protein levels. If left untreated, hypercalcemia can become life-threatening. [30]

In the differential diagnosis, the following conditions must be taken into account: paraneoplastic hypercalcemia due to the release of PTHrP, hypercalcemia associated with bone metastases, primary hyperparathyroidism, vitamin D toxicity, hyperthyroidism, and sarcoidosis. [31]

Therefore, in cancer patients, hypercalcemia may be directly caused by the lytic lesions of skeletal metastases or by the paraneoplastic secretion of PTHrP, which plays a significant role in most cases of paraneoplastic hypercalcemia. Furthermore, circulating levels of PTHrP are elevated in up to two-thirds of patients with skeletal metastases.

Tubular reabsorption of calcium is increased due to dehydration and the effects of PTHrP on the renal tubules. Local production of PTHrP and other osteolytic factors by tumor cells in the bone stimulates bone resorption by osteoclasts: tumor-derived cytokines alter the balance between osteoprotegerin, whose production is decreased, and RANKL, which is increased. The net result is an enhancement of osteoclast proliferation and activity. [32]

1.2.5.2 Clinical Presentation and Diagnosis

Hypercalcemia is not always clinically evident, as the symptoms are nonspecific. Manifestations may be neurological (irritability, depression, lethargy, sedation, delirium, stupor, and even coma), gastrointestinal (nausea, vomiting, constipation, anorexia), renal (polyuria, dehydration, decreased glomerular filtration rate, renal insufficiency), and cardiovascular (bradycardia, arrhythmia, shortened QT interval, prolonged PR interval, and T wave enlargement). [30]

1.2.5.2.1 Treatment

The most common therapies include parenteral rehydration with intravenous (IV) or subcutaneous (SC) fluid administration using saline solutions at a rate of 1-3 L per day or more, and the

administration of bisphosphonates (BPs): clodronate (1500 mg IV), pamidronate (90 mg IV), ibandronate (4 mg IV), and zoledronic acid (4 mg IV). Generally, the preferred pharmacological treatment is the administration of IV BPs, in conjunction with hydration using 1-3 liters of saline solution over a 24-hour period. Zoledronic acid has been shown to be the most effective for acute treatment.

Corticosteroids such as dexamethasone (6-16 mg per day) or prednisone (40-100 mg per day) are recommended only for steroid-responsive tumors (such as multiple myeloma and lymphoma) or when symptoms can be improved with steroids. Calcitonin (4-8 IU/kg subcutaneously every 6-12 hours) is a natural anti-osteoclastic hormone, with its main advantages being a rapid onset of action and negligible toxicity; it is typically administered subcutaneously or intramuscularly. [30]

It is essential to remember to calculate the corrected calcium (Calcium Correction for Hypoalbuminemia); the formula is as follows:

Calcium Correction for Hypoalbuminemia

The adjustment formula is as follows:

SI Units (mmol/L) Ca (ionized) = Measured calcium level + [0.02 (40 –serum albumin)]

Conventional Units (mg/dL) Ca (ionized) = Measured calcium level + [0.8 (4 – serum albumin)] [30]

Do not hydrate the patient prior to the administration of BPs, use diuretics without adequate hydration, administer phosphates, and wait an appropriate amount of time before the subsequent reevaluation of serum calcium levels.

1.2.5.3 Spinal Cord Compression

1.2.5.3.1 Pathophysiology

Spinal cord compression (SCC) is an oncological emergency that requires high doses of corticosteroids, RT, and/or surgical decompression and stabilization of the spine to prevent permanent neurological damage. Most cases of SCC in patients with metastatic cancer are caused by metastasis of the vertebral body that invades the epidural space and compresses the spinal cord or cauda equina anteriorly. Extensions of the vertebral body mass may also occur, along with epidural abscesses, hemorrhages, or disk herniations. Rarely, there is invasion of the vertebral foramen.

1.2.5.3.2 Clinical Presentation and Diagnosis

Early diagnosis is crucial, as symptoms may precede neurological emergencies by several weeks. Pain is the initial symptom in approximately 90% of SCC cases. However, due to its nonspecific nature, a diagnosis is often made only in the presence of neurological symptoms and signs. Localized pain is frequently mechanical in nature and worsens with movement. However, if pain intensifies while in the supine position, this should prompt suspicion of SCC in patients with known bone metastases that present an increased risk. Radicular pain arises when vertebral metastases compress a nerve root, resulting in unilateral or bilateral radiation based on the SCC location. In such instances, neurological signs may manifest as diminished deep tendon reflexes, weakness, and sensory changes. [33] Involvement of the spinal cord is characterized by symptoms consistent with myelopathy, including paresthesias or sensory loss, as well as motor deficits in the caudally innervated territories, which increase in relation to the degree of SCC. Generally, there is flaccid paresis, but signs of pyramidal involvement may also be observed, such as the presence of clonus and hyperreflexia. Importantly, sphincter control may be affected, leading to urinary retention. The signs and symptoms of myelopathy depend on the location of the compressions. For example, a lesion at the T12-L1 level may present with conus medullaris syndrome, which, in addition to sensory and motor manifestations, also includes early loss of sphincter control. If epidural compression occurs at levels below L1, it will result in compression of the cauda equina roots, leading to neurological signs of peripheral and asymmetric involvement, along with potential sphincter dysfunction. The appearance of a Lhermitte's sign (a sensation of electric shocks during passive flexion of the neck) may also indicate SCC; this sign is more commonly seen in cases of SCC affecting the posterior cord of the spinal cord in the cervical or upper thoracic regions. The diagnostic technique is MRI with contrast agent, which is highly sensitive for assessing neoplastic vertebral invasion, as well as involvement of the central nervous system and peripheral nerves. However, if MRI is not available, a CT scan with contrast agent may also be utilized. [34]

1.2.5.3.3 Treatment

Treatment includes corticosteroids, RT, and neurosurgical intervention. Steroids can reduce edema, alleviate pain, preserve neurological function, and improve functional outcomes after definitive treatment. In cases of SCC with severe or rapidly progressing neurological deficits, or with MRI evidence of a severe lesion or myelographic block (high-grade lesions), high-dose dexamethasone is recommended. For low-grade lesions, a lower-dose regimen may be utilized. The choice between RT and surgery is multidisciplinary and depends on a variety of clinical characteristics. [33] Surgical intervention is indicated in patients with recent onset of symptoms (less than twenty-four hours), progressive paraplegia, and urinary retention. Additionally, more than three vertebral segments must

be involved, and the patient should have a life expectancy of several weeks. If symptoms have been present for more than twenty-four hours, RT is indicated, as surgical decompression rarely leads to recovery of motor or bladder function. [35]

1.2.6 Treatment

To reduce the psychosocial distress associated with the disease and, especially, the fragmentation in the approach to patients with bone metastases, it is essential to create organizational models based on the multidisciplinary nature of diagnostic, therapeutic, and rehabilitative interventions. The involved professionals include palliative care specialists, oncologists, radiation therapists, orthopedic and/or neurosurgeons, and radiologists, and may be complemented by nuclear medicine physicians, interventional radiologists, and other healthcare professionals.

The therapeutic approach for bone metastases includes RT, surgery, administration of radiopharmaceuticals, BPs, or Dmab (see sections 1.3.1 and 1.3.2), as well as specific anticancer therapy.

Indications for RT for bone metastases include pain, risk of pathological fractures, and neurological complications arising from SCC, either as a standalone treatment or in combination with surgery. Recent data have demonstrated the feasibility and effectiveness of treating bone pain through percutaneous image-guided radiofrequency ablation of bone metastases (RFA). Additionally, RT plays an important role in the treatment of skeletal metastases in cases of oligometastatic bone disease, using a stereotactic ablative radiation approach, not only in terms of symptom control and prevention of pathological fracture risk but also in terms of tumor regression. [36] [37]

Surgery is indicated for long bone fractures, hip joint involvement, or for decompression in cases of spinal cord involvement. It may also be performed in high-risk situations for pathological fractures. The main factor to consider regarding a surgical approach for skeletal metastases is the prognosis of the patients: the intervention should be limited to cases where a rapid recovery is anticipated and when fractures could lead to severe complications impacting survival. Elaborate patient assessment systems have been developed to guide the selection for surgical interventions, including the Tokuhashi score, which takes into account performance status, the number of bone lesions, the number of vertebral lesions, the presence and resectability of visceral disease, the site of the primary tumor, and the presence of paralysis. Examples of techniques used include intramedullary nailing (for long bone lesions aimed at palliation of bone pain and restoration of acceptable functionality), as well as percutaneous vertebroplasty and kyphoplasty. Surgical intervention for long bone lesions should be followed by radiation treatment to inhibit further tumor growth. Additionally, radiopharmaceuticals are available for the treatment of skeletal metastases (phosphorus-32,

strontium-89, and samarium-153). The delivered dose varies depending on the size of the tumor and the properties of the isotope. In current clinical practice, the most commonly used radiopharmaceutical in patients previously treated for prostate cancer without visceral metastases is radium-223, an α-emitter that has demonstrated an increase in survival compared to placebo. [36] [37] Included in the treatment options for managing bone metastases are specific anticancer therapies, which exert an effect on skeletal metastases by targeting all sites of disease. [36]

1.3 Bone-targeting strategies

The following section will discuss bone resorption inhibitors, specifically BPs and Dmab, which play a crucial role in reducing the incidence of SREs. This chapter is particularly important for the objectives of our study.

1.3.1 Biphosphonates

Bisphosphonates inhibit osteoclast activity by binding to the hydroxyapatite crystals within the bone matrix, resulting in high local concentrations in resorption lacunae. These drugs are subsequently internalized by osteoclasts, leading to their apoptosis. Presently, BPs are the most widely prescribed and effective bone-modifying agents for the treatment of conditions associated with increased bone resorption, including osteoporosis, Paget's disease, and tumor-related osteolysis.

1.3.1.1 Mechanism of action and Pharmacodynamics

Bisphosphonates are synthetic chemical analogs of inorganic pyrophosphate (PPi) and are characterized by a central phosphorus-carbon-phosphorus (P-C-P) structure with two variable side chains covalently attached to the central carbon. [38] BPs exhibit pharmacological similarities to PPi while also displaying notable biochemical differences, particularly in their binding mechanisms to bone and their effects on bone resorption. In contrast to pyrophosphate, BPs contain a carbon atom that connects the phosphate groups, rendering the molecule resistant to biological degradation and enhancing its chemical stability and resistance to enzymatic or acidic hydrolysis. The phosphorus-carbon-phosphorus (P-C-P) structure contributes to the strong affinity of BPs for hydroxyapatite binding. Additionally, the various substituents on the BPs confer distinct levels of stability and resistance to resorption.

Pyrophosphate is a byproduct of many synthetic reactions in the body and can inhibit calcification by binding to hydroxyapatite crystals. Bisphosphonates are preferentially incorporated into sites of active bone remodeling, a characteristic that accounts for their significant utility in diseases associated with accelerated bone turnover. The remarkable selectivity of BPs for bone tissue, as opposed to other

tissues, underlies their efficacy and safety. Other cell types that internalize BPs include osteoblasts, macrophages, epithelial and endothelial cells, monocytes, and certain neoplastic cells, such as those found in prostate cancer and multiple myeloma. [39]. Bisphosphonates exert their effects on osteoclasts through two primary molecular mechanisms, allowing classification into two main groups based on their mechanism of action. First-generation, nitrogen-free BPs, such as clodronate and etidronate, act as pyrophosphate analogs and are metabolized by osteoclasts into ATP analogs. The accumulation of these ATP analogs within osteoclasts inhibits their function and induces apoptosis, likely due to inhibition of ATP-dependent enzymes.

In contrast, nitrogen-containing second- and third-generation BPs (N-BPs), such as pamidronate and alendronate (second generation) and ibandronate and zoledronic acid (third generation), target specific metabolic pathways. They interfere with the biosynthetic pathway for cholesterol and other sterols, essential for the post-translational modification of small GTPases, including Ras, Rab, Rho, and Rac—key proteins involved in cellular signaling. [39]

Bisphosphonates preferentially localize to sites of active bone remodeling and act directly on mature osteoclasts, decreasing their bone resorption activity, reducing H+ and Ca++ extraction, and inducing osteoclast apoptosis. [40]

1.3.1.2 Adverse Events

Clinically, BPs are generally well tolerated, with no significant differences in toxicity profiles observed across various tumor types in multiple studies. As BPs are primarily cleared by the kidneys, their administration should be tailored according to the patient's creatinine clearance levels. Renal impairment associated with BPs use is defined as an increase in serum creatinine of more than 0.5 mg/dL in patients with a normal baseline creatinine (< 1.4 mg/dL), an increase of more than 1.0 mg/dL in patients with elevated baseline creatinine (> 1.4 mg/dL), or a doubling of serum creatinine relative to baseline. Based on this definition, the incidence of renal impairment in patients treated with BPs is estimated at 12%. [41]

Additionally, as serum calcium levels decrease, hypocalcemia (and secondary hyperparathyroidism) may occur, which in turn can lead to muscle cramps and dry skin. The most common adverse events (AEs) include the onset of flu-like symptoms (such as fever, arthralgia, myalgia, and weakness), anemia, and symptoms involving the upper gastrointestinal tract (e.g., nausea, vomiting, epigastric pain, and dyspepsia) due to mucosal irritation.

Multifocal bone pain and/or myalgia are commonly observed, typically occurring within 24 hours and lasting up to 3 days. The intensity of pain is variable and can be severe. This condition is self-limiting but may require analgesic therapy. [42] Ocular inflammation may occur in rare cases,

manifesting as nonspecific conjunctivitis, uveitis, iritis, episcleritis, or scleritis, with incidence rates remaining very low, between 0.05% and 1%. Nonspecific conjunctivitis is the most common ocular side effect, typically resolving spontaneously or with a brief course of corticosteroids. More severe ocular adverse effects, such as uveitis and scleritis, necessitate discontinuation of the treatment. These events are generally mild to moderate and self-limiting. [43]

A unique side effect of BPs is the risk of developing ONJ. ONJ was previously recognized as a complication following RT for head and neck cancer or among workers exposed to metabolically active white phosphorus. The incidence of ONJ in osteoporotic patients treated with daily oral ibandronate or 5 mg of intravenous zoledronic acid (ZA) twice a year remains relatively low, whereas an increased incidence has been observed with routine intravenous administration of BPs, particularly with monthly 4 mg doses of ZA in metastatic patients.

According to the American Association of Oral and Maxillofacial Surgeons, the criteria for defining medication-related osteonecrosis of the jaw (MRONJ) include: current or previous treatment with BPs, exposed bone in the maxillofacial region persisting for more than eight weeks, and no history of RT to the jaw. It is important not to confuse ONJ with other common clinical conditions, such as alveolar osteitis, sinusitis, gingivitis, periodontitis, dental caries, periapical pathology, and temporomandibular joint (TMJ) disorders. [44]

Several studies have highlighted the importance of the route of administration, cumulative dose, and potency of BPs in the development of ONJ. Intravenous administration results in higher drug exposure and therefore an increased risk compared to the oral route.

A cohort study [44] suggests that the administration of Dmab is more frequently associated with the risk of developing ONJ compared to bisphosphonates (BP) [incidence of 28.3 per 10,000 patient-years with Dmab vs. 4.5 with BP, with HR= 6.3 (95% CI, $p < 0.001$)]. However, as also highlighted by the review by Anastasilakis et al., the overall risk of ONJ remains significantly lower than the benefits across all patient categories. [45]

Dentoalveolar surgery is a significant risk factor (RF); before initiating treatment, patients undergo dental clearance, followed by a six-monthly follow-up after starting therapy. Other RFs include: local risk factors (presence of chronic periodontal disease, poor oral hygiene, oral infections, dental caries, tooth extractions, ill-fitting removable prostheses, implants, trauma, fractures, or oral surgical procedures during treatment with BPs or Dmab); drug-related factors (type of bisphosphonate, cumulative dose, and duration of treatment; duration of Dmab therapy); concomitant systemic conditions (diabetes, peripheral vascular disease, anemia, rheumatoid arthritis, etc.); and smoking or antiplatelet therapy. [2] [46]

For patients initiating intravenous BP therapy, the primary objective is to minimize the risk of ONJ development. While a small percentage of patients may develop ONJ spontaneously, the majority

encounter this complication following dentoalveolar surgical procedures. The decision to discontinue BP therapy should be a collaborative discussion among the prescriber, treating oncologist, oral and maxillofacial surgeon, dentist, and patient. Discontinuation in patients with ONJ has been associated with a gradual improvement in clinical disease. Stopping oral BP therapy for 6–12 months may lead to spontaneous sequestration or resolution following surgical curettage, which varies in effectiveness for eradicating necrotic bone. Ozone therapy also has a role in the treatment of mild-to-moderate ONJ.

1.3.1.3 Use of Biphosphonates in the treatment of Skeletal Metastases

Zoledronic acid (ZA), a third-generation intravenous BP, has demonstrated significant benefits for patients with bone metastases. It effectively reduces SREs by approximately 31%. In clinical studies, ZA has proven more effective than pamidronate in both inhibiting bone resorption and treating malignant hypercalcemia. [47]

Zoledronic acid is safe and convenient to administer in an outpatient setting, with a shorter infusion time than other BPs. The recommended dose is 4 mg, administered intravenously over a 15-minute infusion every 4 weeks, although recent data also support extended intervals of up to 12 weeks (Italian Medicines Agency, AIFA). Patients should be adequately hydrated, and oral vitamin D3 supplementation is recommended. Based on the most robust evidence in the literature, the Italian Association of Medical Oncology (AIOM) suggests a treatment duration of 2 years, with potential continuation based on the individual risk of SREs and toxicity.

1.3.2 Denosumab

Denosumab is a human monoclonal antibody directed against the receptor activator of nuclear factor Kappa-B ligand (RANKL), studied as a treatment for postmenopausal osteoporosis and bone lysis in rheumatoid arthritis or metastatic tumors. [48]

1.3.2.1 Mechanism of action and Method of Administration

Denosumab directly inhibits the RANK/RANKL signaling pathway, which is essential for osteoclast activation and survival. By sequestering RANKL and preventing its binding to the receptor on osteoclast surfaces, Dmab acts as a highly effective inhibitor of bone resorption. Unlike BPs, Dmab does not accumulate in the bone matrix, and delays or interruptions in dosing are associated with a rebound effect on bone turnover, leading to rapid loss of bone mineral density and an increased risk of vertebral fractures. [48]

For patients with a sustained high risk of fractures due to prior osteoporotic fractures (e.g., femoral,

vertebral, or peripheral fractures with a lumbar or femoral T-score <−2.5) and/or comorbidities or notable risk factors for skeletal fragility (such as chronic glucocorticoid therapy, aromatase inhibitor therapy, diabetes mellitus, inflammatory diseases, or patient frailty), extending Dmab therapy up to a maximum of 10 years is recommended.

Safety and efficacy data for Dmab support its use for up to 10 consecutive years of treatment. An initial reassessment of the individual patient's fracture risk is recommended after 5 years of therapy to evaluate the risk/benefit balance regarding treatment continuation or discontinuation. Pending results from longer-term clinical studies, exceeding this treatment duration may be considered on an individual basis in specific clinical conditions, such as in patients with renal insufficiency. [49]

The recommended dosage in patients with skeletal metastases is one subcutaneous injection of 120 mg every 28 days. For further information on reducing the risk of skeletal-related events (SREs) and the duration of treatment in patients with bone metastases, see Section 2.1.

1.3.2.2 Adverse Events

Due to the risk of severe hypocalcemia during Dmab therapy, baseline calcium levels should be monitored. Additionally, after the initial injections, patients should receive daily supplementation of vitamin D and calcium. Compared to BPs, Dmab has negligible renal toxicity and lower infusion-related toxicity (e.g., skin rash). Other reported adverse effects include arthralgia and increased susceptibility to infections of the urinary and respiratory tracts, as well as a risk of diverticulitis. The only contraindications for the drug are hypocalcemia or hypersensitivity to the active substance or excipients. The occurrence of an adverse effect requires drug discontinuation and subsequent reassessment for potential resumption. [50]

As with BPs, it is necessary to monitor for the risk of ONJ during Dmab therapy. A recent retrospective observational study was conducted on patients with metastatic bone cancer who received at least one dose of Dmab and had a follow-up visit. The objective was to assess the real-life incidence of Dmab-related ONJ.

The study reports that 56 patients developed ONJ, with cumulative incidence rates of 5.7% (95% CI: 4.2%–7.8%) at 24 months and 9.8% (95% CI: 7.6%–12.7%) at 48 months. The incidence was significantly higher in the middle-aged group ($56 < \text{age} \leq 73$) in both univariate and multivariate analyses ($p = 0.029$ and 0.0106, respectively). Multivariate Cox analysis revealed that dental procedures (Hazard Ratio [HR] = 3.67; $p = 0.0001$), tooth extractions (HR = 23.40; $p < 0.0001$), and prior bisphosphonate administration (HR = 2.62; $p = 0.0024$) were significantly associated with an increased risk of ONJ.

The study concludes that these findings confirm a clinically relevant incidence of Dmab-induced ONJ and identify dental treatments, particularly extractions, administered during or prior to Dmab therapy,

as a significant risk factor. [51] Important limitations of the study included the absence of a control group and the unknown impact of any prior BP therapy. Additionally, due to the retrospective nature of the study, information was lacking regarding ONJ risk factors, as well as the administration intervals of chemotherapy, hormone therapy, and corticosteroids. Nonetheless, the sample size is substantial, and this study is among the few that analyze real-world data.

1.4 Pain

The International Association for the Study of Pain (IASP) has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." Pain assessment is complex due to its subjective sensory and emotional nature, lacking objective diagnostic tests and relying instead on subjective evaluation. The entire pain experience is subjective, necessitating a multidimensional approach for accurate measurement. A model to better understand this phenomenon was proposed by John Loeser, who identified four primary concepts: nociception, pain, suffering, and pain behavior. Another important concept, suggested by Melzack and Casey, posits three major sensory and psychological dimensions of pain: sensory-discriminative, motivational-affective, and cognitive-evaluative. This underscores the complexity of pain definition and assessment, which is predominantly subjective.
The main categories of pain classification are:

- Nociceptive pain, caused by the activation of nociceptors following a noxious stimulus to non-nervous tissues, which may be visceral or somatic.
- Neuropathic pain, resulting from injury or disease within the somatosensory nervous system and characterized by sensory disturbances on examination, including both positive (hypersensitivity) and negative (hyposensitivity) symptoms.

Diagnosing neuropathic pain is challenging. Guidelines offer support through four primary criteria:

- a relevant neurological history of injury or disease;
- a significant neuroanatomical distribution of the pain;
- pain associated with sensory disturbances in the same significant neuroanatomical distribution;
- diagnostic tests confirming injury or disease in the somatosensory system.

If the first two criteria are present, a diagnosis of neuropathic pain is possible; if the third criterion is also positive, the diagnosis is probable; and if all four criteria are met, the diagnosis is definitive. [52]

However, for the purposes of this study, it is useful to focus on cancer-related pain. In this type of pain, both neuropathic and nociceptive components are often present simultaneously, resulting in mixed pain.

1.4.1 Cancer Pain

Pain affects approximately 40% of patients with advanced cancer, with about 85% of cases attributed to the tumor itself and the remainder to antitumor therapy or non-cancer-related causes. A meta-analysis of 143 studies shows a high prevalence of cancer-related pain (50-94%) at the end of life due to progressive oncological diseases, compared to other non-oncological chronic diseases such as COPD (21-57%), heart failure (14-78%), or renal failure (11-83%). Additionally, in malignancies, pain often coexists with other symptoms that occur less frequently in non-oncological chronic diseases: psychophysical fatigue, anorexia and cachexia, dyspnea, depression, and death-related anxiety. [53]

Pain is the predominant symptom in cancer diseases, making a thorough and accurate assessment essential. For this purpose, evaluating the clinical characteristics of the pain becomes important, as it can guide its etiology and, consequently, the therapeutic approach.

1.4.1.1 Pain Intensity

While the concept of intensity is essential for quantifying pain, it does not capture all the components involved. Given that pain encompasses multiple dimensions (sensory, emotional, cognitive, and social), adopting multidimensional models has become necessary, despite the historical reliance on unidimensional scales for assessment.

Among unidimensional scales, which assess only one aspect of pain (most often intensity), are the following:

- **Visual Analogue Scales (VAS)**: These consist of horizontal or vertical 10 cm lines with endpoints defined as "no pain" and "worst possible pain." The patient places a mark on the line at the point that reflects their perceived pain intensity. These scales are rarely used in clinical practice because the scoring system is complex and time-consuming, and they are often less comprehensible to patients than numeric and verbal scales, particularly for elderly individuals or those with cognitive impairment.
- **Numeric Scales** (es. Numerical Rating Scale, NRS): These scales consist of a series of numbers, typically ranging from 0 to 10, where 0 represents 'no pain' and 10 represents the 'worst possible pain'. Pain scores between 7 and 10 are

considered severe, between 5 and 6 moderate, and between 4 and 1 mild. Due to their simplicity and ease of understanding, they are the most commonly used in clinical practice.

- **Verbal Scales** (es. Verbal Rating Scale, VRS): consist of a series of adjectives used to describe pain intensity, each assigned a numerical value. While they are easy to understand, the difference in meaning between adjectives is often not equal, making the analysis of the obtained values more challenging. Both VAS and NRS have been shown to produce comparable results in the assessment of acute and chronic pain intensity. The choice between these two commonly used pain assessment tools often depends on the practicality of administration. The accurate measurement of pain intensity is essential for evaluating the efficacy of therapeutic interventions. Multidimensional scales, which are tailored to the individual patient and clinical context, are more complex. The most commonly used are the McGill Pain Questionnaire (MPQ) and the Brief Pain Inventory (BPI):
- McGill Pain Questionnaire (MPQ): the scale employs a multidimensional approach to pain assessment, using 78 descriptors categorized into 20 subclasses. It evaluates sensory, affective, and evaluative aspects of pain. Patients indicate the location and distribution of pain on a body map and select words that best describe their experience. The resulting scores provide a detailed profile of the pain, including the Pain Rating Index, Number of Words Chosen, and Present Pain Intensity; [54]
- Brief Pain Inventory (BPI): the scale provides a comprehensive assessment of the pain experience, evaluating not only pain intensity but also its impact on daily functioning and emotional state. It quantifies worst, average, and least pain in the past 24 hours and current pain levels. Furthermore, it assesses the degree to which pain interferes with general activities, work, mood, social interactions, and sleep. [55]

Assessing pain intensity is essential for determining the efficacy of therapeutic interventions. [56]

1.4.1.2 Anatomical Location and Distribution

The anatomical location and distribution of pain is another critical factor. Pain may be localized to a specific area (focal), multiple areas (multifocal), or be diffuse throughout the body. It is common to have pain in more than one location, particularly in the case of metastatic disease.

1.4.1.3 Quality

The quality of pain is another crucial factor in elucidating its underlying pathophysiology. A burning quality, often accompanied by tingling and electric shocks, is suggestive of neuropathic pain, whereas diffuse, cramp-like, and colicky pain is more commonly associated with visceral pain and nociceptive mechanisms.

1.4.1.4 Temporal Variations in cancer pain

Finally, temporal fluctuations in pain intensity should be considered. Pain can be categorized as acute or chronic (persisting for at least three months), and can be either continuous or intermittent. Cancer patients frequently experience Breakthrough Pain (BTP), characterized by:

- A transient exacerbation of underlying pain, often precipitated by a specific event;
- A relatively well-controlled baseline pain state;
- The requirement for alterations in the therapeutic regimen or the addition of supplemental analgesics.

Breakthrough pain substantially diminishes patients' quality of life, highlighting the importance of accurate diagnosis and effective treatment. [57] [58]. Based on common pain characteristics and underlying causes, various pain syndromes have been identified. In oncology patients, the most prevalent syndromes include bone pain, visceral pain, soft tissue injury pain, neuropathic pain, and those induced by cancer treatments.

1.4.2 Taxonomy of Chronic Pain

To address the need for standardized pain management, the IASP introduced a chronic pain taxonomy. This taxonomy is comprised of five axes that provide a framework for characterizing the complex nature of chronic pain. The first axis is the anatomical site, referring to the predominant location of the pain. The second axis is constituted by the systems from which the pain originates, for example, the respiratory and cardiovascular systems, the connective and musculoskeletal systems, the cutaneous, subcutaneous, and glandular annex systems, the nervous system (from whose pathologies neuropathic pain originates), and the psychological system (from whose dysfunction somatoform pain can arise). The third axis classifies the temporal characteristics of pain, including its episodic or continuous nature. The fourth describes its intensity and the fifth identifies the etiology: genetic, traumatic, infectious, neoplastic, metabolic, degenerative and mechanical, dysfunctional, and psychological. [59]

The ICD-10, the International Statistical Classification of Diseases and Related Health Problems, is another internationally recognized classification system. [60]

1.4.3 Semiology of Pain

Clinical semiotics involves systematic collection and interpretation of signs and symptoms to arrive at a diagnosis. It includes:

- physical examination using the four classic techniques;
- functional assessment of organ systems; and
- specialized diagnostic procedures.

Pain, a symptom often accompanied by diagnostic signs, can provide insights into the underlying pathophysiology, tissue involvement, and contributing factors, guiding the selection of appropriate therapeutic interventions.

The pathophysiology of chronic pain involves a process of sensitization, leading to a lowered pain threshold. This sensitization can occur at peripheral, spinal, or central levels, including nociceptors, spinal cord neurons, and the brain. Peripheral sensitization is triggered by the release of inflammatory mediators, such as prostaglandins and bradykinin, at the site of injury. These substances lower the pain threshold of nociceptors and alter sodium channel function, leading to increased excitability. Consequently, previously non-painful stimuli can elicit pain (allodynia), and in severe cases, spontaneous pain may occur. [61]

Spinal sensitization is characterized by altered transmission between the first and second order neurons in the dorsal horn of the spinal cord. This transmission can be modulated by endogenous substances (endorphins, serotonin and norepinephrine reuptake inhibitors, endocannabinoids, etc.) or by pharmacological agents (such as paracetamol, opioids, tricyclic antidepressants, etc.). Persistent peripheral pathogenic mechanisms can induce hyper excitability of spinal neurons, characterized by high-frequency and high-intensity impulses. Both specific and wide dynamic range neurons, located deeper in the dorsal horn, are involved. Specific neurons, with a lowered stimulation threshold, activate rapidly and increase their firing rate, thus enhancing pain perception. Wide dynamic range neurons lose their ability to discriminate between tactile and pain sensations, leading to a condition where a tactile stimulus from the same spinal segment can elicit a painful sensation of different intensity. Patients experience more pain than that generated by peripheral neurons alone and perceive it in a larger area compared to the simple lesion site, even experiencing pain with mere pressure or touch of healthy skin (secondary allodynia). In peripheral neuropathic pain, there is hyperexcitability of the ectopic site, which develops following injury to a peripheral nerve and its fibers. Abnormal

impulses originating from the ectopic site generate sensations such as constrictive pain, burning, paresthesias, or electric shock sensations. This phenomenon is caused by anomalous activation of sodium channels, which underlie the transmission of nerve impulses, and can be spontaneous or, more frequently, secondary to various external stimuli such as mechanical stimuli (e.g., entrapment), inflammation, or ischemia. Following injury to the somatosensory system, complex changes occur at the level of the dorsal root ganglion and spinal cord synapses, leading to the development of ectopic foci. These changes include increased excitability of sensory neurons, enhanced neurotransmitter release, and reduced inhibitory modulation. These mechanisms contribute to the generation of spontaneous and ectopic discharges, underlying neuropathic pain. To comprehensively assess pain, it is essential to evaluate: pain intensity, functional impact, patient-reported outcomes, pain quality, temporal patterns, provoking and relieving factors, associated symptoms, and physical examination findings. [61]

1.4.4 An Overview of Cancer Pain

The WHO guidelines have been widely adopted as the benchmark for effective cancer pain management, providing a robust evidence-based approach.

1.4.4.1 WHO Reccomendations

According to WHO recommendations, medications should be administered at fixed intervals (by the clock), with rescue medication available for breakthrough pain episodes. The oral route (by mouth) is preferred due to its less invasive nature and greater patient acceptance. Doses should be titrated to the individual patient's characteristics. The goal is to identify the minimum effective dose to minimize adverse effects. Effective chronic pain management involves a stepwise approach, starting with less potent analgesics and progressing to stronger ones if pain is not adequately controlled. Adjuvant medications should be added as needed. Specifically, pharmacological strategies for treating cancer pain target the underlying pathophysiological mechanisms: peripheral sensitization, propagation of action potentials, spinal transmission, and descending inhibitory pathways. The first guideline on cancer pain, published in 1986 by the World Health Organization (WHO) and titled 'Cancer Pain Relief', introduced a landmark three-step analgesic ladder (Figure 2) that remains a cornerstone of cancer pain management. This strategy, updated in 1996, has served as the foundation for subsequent guidelines. [62]

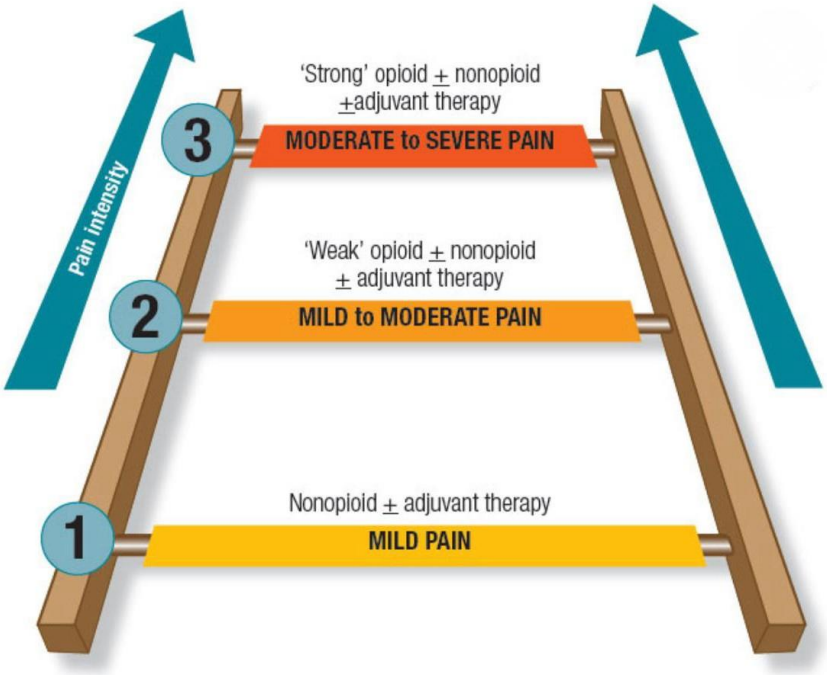

Fig. 2 WHO three-step analgesic scale: first level non-opioid; second level weak opiates; third level strong opiates. At each level it is possible to associate adjuvant therapy.

The first step employs non-steroidal anti-inflammatory drugs (NSAIDs)/acetaminophen for mild pain. Weak opioids, with or without NSAIDs/acetaminophen, are used for mild to moderate pain. Strong opioids, with or without adjuvants, are reserved for moderate to severe pain. Adjuvants, such as certain anticonvulsants, antidepressants, and corticosteroids, can be used at any step to enhance analgesia. [58] [63]

1.4.4.2 Non-Opioid Drugs

Non-opioid analgesics, like paracetamol and NSAIDs, have a ceiling effect on analgesia. Further dose increases beyond this point will not improve pain control and may lead to adverse effects. Progression to the next step of the WHO analgesic ladder is often necessary. However, the combination of NSAIDs and opioids can provide superior analgesia, enhance patient satisfaction, and reduce opioid requirements without compromising safety. NSAIDs are effective for acute inflammatory pain but can cause serious adverse effects, especially with long-term use. By inhibiting both COX-1 and COX-2, NSAIDs can induce gastrointestinal ulcers, renal impairment, and an increased risk of cardiovascular events.

1.4.4.3 Opioid Drugs

Codeine, often combined with non-opioids, is a commonly used weak opioid. Tramadol and tapentadol are other weak opioids available in various formulations. Strong opioids include morphine, oxycodone, hydromorphone, fentanyl, and buprenorphine. A common approach to morphine administration involves a regular dosing schedule every four hours with rescue doses as needed. The regular dose is highly variable, ranging from 5-10 mg to over 250 mg, and there is no ceiling effect. Oxycodone is approximately 1.5 times stronger than morphine, while hydromorphone is up to 5 times stronger, and fentanyl up to 100 times stronger. Fentanyl, the most commonly used lipophilic opioid, is also well-accepted by patients due to its option for transdermal systemic administration. [63]

The main adverse effects of opioid drugs, which act on specific receptors in the spinal cord and brainstem, as well as in the gastrointestinal tract and systemically, include drowsiness and mental confusion, especially in elderly patients, along with symptoms such as constipation, nausea, and vomiting. Drowsiness and mental confusion may occur in 10-20% of cases during the first days of therapy but typically resolve within 3-5 days, particularly when treatment is initiated at low doses.

In recent years, the non-medical use of opioids, combined with specific healthcare policies and media pressure, has drawn attention—especially in the United States—to the life-threatening toxicities of opioids (acute respiratory failure in overdoses). However, these drugs remain central to cancer pain management, and among patients with pain due to advanced, incurable cancer, the risks of abuse or misuse are limited and do not compromise their safety and effectiveness. [64] e [65]

Finally, an important phenomenon that may occur is tolerance, in which patients require higher doses to achieve adequate pain control, as well as withdrawal symptoms.

1.4.4.4 Adjuvant Drugs

Adjuvant medications for pain management include anticonvulsants, antidepressants, and corticosteroids, each targeting specific aspects of neuropathic pain. Anticonvulsants, such as gabapentin and pregabalin, are effective in managing postherpetic neuralgia, painful diabetic neuropathy, and spinal cord injury pain, though their role in oncology-induced neuropathy remains less conclusive. Pregabalin's anxiolytic properties make it a suitable choice for patients with concurrent anxiety symptoms. [66]

Tricyclic antidepressants, particularly amitriptyline, have shown efficacy across multiple chronic pain syndromes, including migraine, postherpetic neuralgia, painful diabetic neuropathy, central post-stroke pain, and cancer-related neuropathic pain. Imipramine also provides relief for painful diabetic neuropathy and idiopathic chest pain. Among newer agents, serotonin-norepinephrine reuptake inhibitors (SNRIs), such as duloxetine, have emerged as promising adjuvants with proven benefits in neuropathic pain, demonstrating a favorable tolerability profile compared to tricyclic antidepressants.

2. Rationale of the Study

2.1 Background

Bone metastases (BM) are common in patients with metastatic breast cancer (MBC), affecting up to 80% of this population. These metastases trigger increased osteoclast activity, leading to local bone destruction and a variety of skeletal complications, including severe bone pain, hypercalcemia of malignancy, pathological fractures, SCC, the need for RT to alleviate pain or prevent fractures, surgical interventions to manage or prevent pathological fractures. These significant adverse outcomes are known as SREs, and arise in up to 64% of patients with MBC. [67] [68] SREs can impair both patient's quality of life (QoL) and life expectancy, increasing health care costs. [69]. Major randomized controlled trials (RCTs) evaluating the efficacy of antiresorptive agents (BPs and Dmab) have shown a reduction in the incidence of SREs and a delay in the onset of both initial and subsequent SREs in cancer patients. [70], [71], [72], [73], [74], [75]

In their study Alison et al. concluded that Dmab is superior to ZA in delaying [HR, 0.82; 95% CI, 0.71–0.95; P= .01 for superiority] or reducing the incidence of SREs (time to first SRE rate ratio, 0.77; 95% CI, 0.66–0.89; P= .001). Dmab demonstrated good tolerability, required no renal function monitoring, and provided the advantage of convenient administration via subcutaneous injection. A higher incidence of renal AEs and hypersensitivity/idiosyncratic reactions was observed with ZA, while hypocalcemia was more frequent with Dmab. ONJ was rare for both drugs (2.0% with Dmab; 1.4% with ZA; P = .39) [70] These findings led to the approval of BPs and Dmab in Italy for patients with skeletal metastases, regardless of underlying pathology or related symptoms. As recommended by the American Society of Clinical Oncology (ASCO) and by the European School of Medical Oncology (ESMO) as well, BMAs should be used in all patients with MBC with evidence of BM, regardless of whether or not they exhibit symptoms. BMAs should normally continue indefinitely, until clinical deterioration or intolerable toxicity occurs. [76]

Pain is another significant aspect of skeletal metastases, which may arise from the direct impact of the metastases or a SREs. [77] A 2002 Cochrane review examined the effectiveness of BPs in providing pain relief associated with bone metastases. [78] The authors conclude that while there was some evidence suggesting that BP provided pain relief, the heterogeneity in methods used to assess pain and endpoints (proportion of pain-free patients, reduction in unspecified major pain, mean and/or median pain scores, median analgesic consumption, at least 20% reduction from baseline pain score) precluded the ability to make a strong recommendation. A 2011 Cochrane review on pain relief also confirmed the weakness of the evidence in this area. Additionally, none of the studies reviewed considered BP administration beyond two years. RCTs have shown that both Dmab and ZA are

effective in pain management. progression Specifically, Dmab has been shown to delay pain onset or progression. [79], [80]. The 2013 study by Charles et al. [80] indicates that Dmab is more effective than ZA in preventing the onset of pain, though both drugs demonstrate similar analgesic effects. Additionally, the study notes that fewer patients treated with Dmab required strong opioids and experienced a longer time to pain progression (Dmab 8.5 months vs. ZA 7.4 months; P = 0.08). Among patients with no or mild baseline pain, Dmab was associated with a 4-month delay in progression to moderate/severe pain compared to ZA (9.7 months vs. 5.8 months; P = 0.002). [81] The efficacy of bone-modifying agents in patients with breast cancer bone metastases has been demonstrated. However, heterogeneity in pain assessment tools and treatment protocols hinders meta-analysis. [82], [83] Consequently, there is limited evidence supporting the use of these drugs for pain control in this patient population.

A 2017 systematic review represents the most recent update on the topic of pain and bone-modifying agents. The researchers included 43 studies (8,595 and 7,590 patients treated with BPs and Dmab, respectively). Among the 28 placebo-controlled studies, 22 found no significant analgesic benefit from BPs. Furthermore, no studies evaluating Dmab directly assessed pain relief. Therefore, the evidence supporting an analgesic role for bone-modifying agents remains limited. [84]

In conclusion, the evidence supporting the use of anti-resorptive agents for pain management remains limited and is often derived from post-hoc analyses of RCTs. Despite their statistical strength, RCTs are frequently conducted on select patient populations and over relatively short periods. As a result, findings generated under these idealized conditions may lack generalizability to patients in routine clinical practice. Additionally, small sample sizes often restrict the ability to detect efficacy differences in specific subgroups, rare AEs, or long-term effects. Therefore, the generalizability of RCT results is frequently limited, underscoring the importance of complementing RCT data with real-world evidence.

2.2 Aim of the Study

The objective of this study is to assess the impact of Dmab and ZA on bone pain management and the reduction of SREs in an unselected cohort of breast cancer patients with skeletal metastases who have received at least two years of anti-resorptive therapy.

3. Methods

3.1 Study Design

A retrospective, single-center observational study was conducted to assess patients with breast

cancer-related bone metastases who received a minimum of 24 monthly infusions of either ZA or Dmab at the Palliative Care Unit of the IRCCS Foundation National Cancer Institute of Milan, Italy, from January 2008 to January 2023. The study was approved by the Territorial Ethics Committee of Lombardia 4 (study identification number: INT 139/23). Due to the retrospective nature of the study and the unavailability or death of patients, obtaining informed consent was not feasible.

3.2 Inclusion and Exclusion Criteria

Patients were deemed eligible if they met the following criteria:

- age of 18 years or older
- confirmed diagnosis of breast cancer
- radiographic or biopsy-proven evidence of at least one bone metastasis
- completion of at least 24 monthly administrations of ZA or Dmab.

3.3 Data Collection

The list of patients who received 24 doses of ZA or Dmab was obtained from electronic hospital databases. Patients follow-up consisted of five subsequent visits, respectively 3, 6, 12, 18 and 24 months after baseline evaluation. The following baseline data for ZA or dmab were retrieved from electronic medical records: age at the start of treatment, date of cancer diagnosis, histotype, status of oestrogen (ER) and progesterone receptor (PgR), and human epidermal growth factor receptor-2 (HER-2), presence of metastases other than bone at initial diagnosis, history of skeletal morbidities prior to study entry, and baseline Eastern Cooperative Oncology Group (ECOG) performance status, date and number of bone metastases at diagnosis, previous therapy with others BMA and, in this case, the date of the last administration, date and type of previous bone complications, ongoing antineoplastic therapy (i.e. chemotherapy, hormone therapy, biological therapy), week average pain intensity measured through a 0-10 numerical rating scale (NRS) where 0 indicates “No pain” and 10 “The worst possible pain” [85], type of analgesic according to the World Health Organization (WHO) analgesic ladder and opioids consumption doses were expressed as mgs of oral morphine equivalent (OME), following the European Association for Palliative Care (EAPC) recommendation.

3.4 SRE Study Definition

As reported in the literature, the need for orthopedic surgery or bone RT performed prophylactically prior to a SRE has often been categorized as a SRE. However, the primary aim of these therapies is to prevent the occurrence of actual skeletal complications. Thus, in this study, for the purposes of data collection and analysis, RT and orthopedic surgery events were considered as competing factors alongside anti-resorptive drugs in delaying SREs and bone pain.

In conclusion, the study defined a skeletal-related event (SRE) as follows:

- Pathological fracture (vertebral or non-vertebral) as evidenced by radiographic imaging (CT, MRI, PET, or standard X-ray);
- Spinal cord compression as demonstrated by CT or MRI;
- Hypercalcemia as defined by CTCAE v5 laboratory criteria. [86]

The incidence of RT and bone surgery for pain management and fracture prevention or treatment was also recorded.

3.5 Comparison Interventions

The two patient groups compared received the following treatments:

1. Dmab 120 mg, one subcutaneous injection every 28 days for 24 administrations. No dose adjustment was required with Dmab.
2. ZA 4 mg (or dose adjusted for glomerular filtration rate per prescribing information), administered as a 15-minute IV infusion every 28 days

3.6 Objectives and Endpoints

3.6.1 Primary End Point

- Effect of ZA and Dmab on bone pain.

3.6.2 Secondary End points

- Effect of ZA and Dmab on the onset of the first SRE;
- Effect of ZA and Dmab on the need for RT and/or orthopedic surgery.

3.6.3 Endpoints of the Primary Objective

To measure the effect of bone-modifying agents on pain, the study evaluates:

- the type of analgesic used according to the WHO analgesic ladder (non-opioids, weak opioids, and strong opioids) during the observation period; [88]
- the average pain intensity over the past week at each evaluation point, measured using the NRS scale. [89] Pain data were collected at baseline and subsequently at 3, 6, 12, 18, and 24 months;
- the trend in opioid dosage, expressed in mg of oral morphine equivalents (OME), over the observation period.

3.6.4 Endpoints of the Secondary Objective

- Incidence of SREs in the study populations.
- Incidence of RT and/or orthopedic surgery.

3.6.5 Statistical Analysis

Demographic characteristics were summarized through median (first and third quartile) for numeric features, absolute and relative frequency for categorical ones stratifying by the received treatment. The first skeletal event was defined as the first event occurred between the following: pathological fracture, SCC, and hypercalcemia; the corresponding time was defined as the difference between the date of event occurrence and the date of star treatment with Dmab and ZA. A similar assessment was conducted for the need for orthopedic RT or orthopedic surgery. As noted, these treatments may prevent skeletal-related events, qualifying as competing events. Time to the last injection was

censored for patients without SREs or who received RT or bone surgery before 24 injections. The analgesic effect of Dmab or ZA was evaluated using three Bayesian mixed-effects longitudinal models with the WHO pain scale, NRS, and morphine dosage as outcomes. Given the different nature of these measures, the effect of Dmab on pain was inferred by jointly interpreting the results of these models. In more detail, the fixed effects included:

- a categorical time variable with 5 levels to model differences in the response variable at various evaluation times, namely 3, 6, 12, 18, and 24 months, relative to baseline;
- prior BPs use and treatment type (Dmab vs. ZA);
- three time-dependent variables that could vary at each evaluation: occurrence of SRE, type of oncology therapy, and bone surgery or RT (noting, however, that therapies may change between two different time points).

Following evaluation of all model parameters as potential causal effects using predicted log-pointwise density, only the value of the dependent variable was retained as a patient-specific causal effect to account for heterogeneity in repeated measures within the same patient. Coefficient distributions were derived by sampling from the posterior distribution of the specified models via a Markov Chain Monte Carlo (MCMC) method. The coefficient estimates were represented by the mean of the posterior distribution samples. Given that the WHO scale is an ordinal variable, a cumulative longitudinal Bayesian mixed-effects model was specified accordingly. For OME, a log1p transformation was applied as the response variable in the Gaussian longitudinal Bayesian mixed-effects model to address zero inflation in the variable's distribution, with conditional model effects subsequently back-transformed to the original scale. A Gaussian mixed-effects model was also specified for the NRS as the response variable. The warm-up period was set to 5,000, with a thinning interval of 2. Trace plots were utilized to evaluate MCMC convergence. To clarify the effects of the two drugs, the conditional effects of each model were isolated.

To assess the effect of Dmab in delaying the onset of the first SRE compared to ZA, the Aalen-Johansen estimator (a variant of the Kaplan-Meier estimator) was used to construct cumulative incidence curves. Gray's test was applied for the statistical comparison of incidence curves. A competing risk analysis was then conducted using a multivariable Fine-Gray model to better quantify the impact of Dmab in delaying the onset of the first skeletal event. Additionally, a multivariable Fine-Gray model was also used to quantify the association between Dmab and competing events. The models included the following covariates as adjustment factors: age, time from metastatic diagnosis and initiation of palliative care, presence of a baseline SRE, WHO scale (used to consider pain from

an objective perspective), NRS scale (used to account for pain from a subjective perspective), prior BP use, and baseline oncology therapy. As the WHO scale and morphine dose were highly correlated, it was not appropriate to include both as covariates due to multicollinearity. In frequentist analyses (cumulative incidence curves and Fine-Gray models), interval estimates are presented as 95% confidence intervals, while in Bayesian frameworks, 95% credible intervals were constructed. Statistical analyses were conducted using R Studio version 4.3.2.

4. Results

4.1 Patients Characteristics

A total of 364 BC patients with bone metastases treated with BMAs (ZA:194 pts, Dmab: 170 pts) for at least 24 administrations were identified. We retrospectively reviewed the medical charts of 832 patients from the hospital database, with 463 ZA group and 396 to the Dmab group. Based on predefined inclusion criteria, only 43.5% of these cases were included in the final analysis. Male patients (n=4) were excluded due to their minimal representation, which was insufficient for a robust assessment of potential gender-based differences. Including this limited subset could have introduced bias and affected the reliability of our findings, as it would not provide a statistically meaningful comparison between genders. Therefore, a total of 364 BC patients with bone metastases treated with BMAs (ZA:194 pts, Dmab: 170 pts) for at least 24 administrations were identified. Patients' characteristics stratifying by treatment are reported as median (IQR) or absolute and relative frequencies as appropriate are reported in Table 1.

Characteristic	**Zoledronic Acid**, N =194 N %	**Denosumab**, N = 170 N %	**p-value***2*
Median Age , years (Q1-Q3)	62.2 (52.7, 70.9)	62.2 (50.5, 72.0)	0.85
ECOG PS			0.063
0	119.0 (61.3%)	118.0 (69.4%)	
1	69.0 (35.6%)	43.0 (25.3%)	
2	6.0 (3.1%)	9.0 (5.3%)	
Hystotype			0.47
Invasive Ductal Carcinoma	144.0 (76.6%)	111.0 (71.2%)	
Invasive Lobular Carcinoma	29.0 (15.4%)	26.0 (16.7%)	
Invasive Ductal Carcinoma and Others	1.0 (0.5%)	3.0 (1.9%)	
Invasive Ductal Carcinoma and Lobular Carcinoma	14.0 (7.4%)	16.0 (10.3%)	
Unknown	6	14	
Positive Hormone receptor PR status	164.0 (85.9%)	123.0 (82.0%)	0.33
Unknown	3	20	
Positive Hormone receptor ER status	177.0 (92.7%)	143.0 (93.5%)	0.77
Unknown	3	17	
Positive HER2 status	37.0 (20.1%)	36.0 (29.8%)	0.053
Unknown	10	49	
Median Time from cancer breast diagnosis and initial bone metastases, months (Q1-Q3)	77.1 (25.2, 148.6)	67.6 (16.3, 154.4)	0.76
Basal Skeletal Event	39.0 (20.1%)	47.0 (27.6%)	0.091
Median Time from bone metastasis diagnosis and start of ZA/Dmab Treatment, months (Q1-Q3)	4.0 (2.2, 15.1)	2.2 (1.3, 4.3)	<0.001
Number of bone Metastases to initial diagnoses			0.005
One	164.0 (84.5%)	123.0 (72.4%)	
Multiple	30.0 (15.5%)	47.0 (27.6%)	
Previous Bisphophonate administration	27.0 (13.9%)	12.0 (7.1%)	0.035
Time from last bisphosphonate and new one	17.3 (9.7, 27.9)	1.1 (1.0, 2.6)	0.002
(Missing)	169	158	
Metastasis			0.23
Only bone	120.0 (61.9%)	88.0 (51.8%)	
Thoracic (Lung	37.0 (19.1%)	48.0 (28.2%)	
Abdomen-Pelvi (liver	13.0 (6.7%)	14.0 (8.2%)	
Thoracic, Head and Neck (brain	20.0 (10.3%)	14.0 (8.2%)	
Head and Neck, Abdomen-Pelvi	1.0 (0.5%)	1.0 (0.6%)	
Thoracic, Head and Neck, Abdomen-Pelvi	3.0 (1.5%)	5.0 (2.9%)	
Therapy ongoing			<0.001
None	41.0 (21.1%)	8.0 (4.7%)	
Hormonotherapy	55.0 (28.4%)	53.0 (31.2%)	
Chemotherapy	37.0 (19.1%)	22.0 (12.9%)	
Radiotherapy and others*	50.0 (25.8%)	43.0 (25.3%)	
Chemo and/or Biological	4.0 (2.1%)	8.0 (4.7%)	
Hormono and/or Biological	7.0 (3.6%)	34.0 (20.0%)	

Table 1. Baseline Demographics Characteristics n 364
* *Radiotherapy interrupted within 1 month*
1 Median (IQR) or Frequency (%)
2 Wilcoxon rank sum test; Pearson's Chi-squared test; Wilcoxon rank sum exact test; Fisher's exact test

Patient's characteristics were generally balanced for age, ECOG, PR/ER status but some statistically significant difference was observed. Patients treated with Dmab differ significantly from those

treated with ZA in terms of oncological treatments (chemotherapy vs hormonal and biological therapies) and median time between the diagnosis of bone metastasis and the initiation of bone-modifying therapy.

4.2 Results of the Primary Objective

The analysis reveals that Dmab has a superior analgesic effect compared to ZA. The three pain assessment methods used, along with their variations over the observation period, are presented in Table 2 in terms of mean values and absolute or relative frequencies. Table 2 also includes data on oncological therapies and any SREs/need for RT or orthopedic surgery, relative to the study groups.

Characteristic	**0, N = 194**	**3, N = 194**	**6, N = 194**	**12, N = 194**	**18, N = 194**	**24, N = 194**	**p-value §**
			Zoledronic Acid				
Dosage meq Morphine (mg)	0.0 (0.0, 15.0)	0.0 (0.0, 15.0)	0.0 (0.0, 15.0)	7.8 (0.0, 22.0)	0.0 (0.0, 18.8)	8.0 (0.0, 22.0)	0.004
WHO ladder							<0.001
0	121.0 (62.4%)	64.0 (33.0%)	58.0 (29.9%)	59.0 (30.4%)	62.0 (32.0%)	56.0 (28.9%)	
1	10.0 (5.2%)	45.0 (23.2%)	49.0 (25.3%)	37.0 (19.1%)	38.0 (19.6%)	37.0 (19.1%)	
2	32.0 (16.5%)	53.0 (27.3%)	53.0 (27.3%)	57.0 (29.4%)	56.0 (28.9%)	64.0 (33.0%)	
3	31.0 (16.0%)	32.0 (16.5%)	34.0 (17.5%)	41.0 (21.1%)	38.0 (19.6%)	37.0 (19.1%)	
NRS pain (0-10)	2.0 (0.0, 4.0)	1.0 (0.0, 3.0)	1.0 (0.0, 2.0)	1.0 (0.0, 3.0)	1.0 (0.0, 3.0)	2.0 (0.0, 3.0)	<0.001
Therapy							<0.001
None	41.0 (21.1%)	22.0 (11.3%)	20.0 (10.3%)	16.0 (8.2%)	8.0 (4.1%)	14.0 (7.2%)	
Chemotherapy	37.0 (19.1%)	55.0 (28.4%)	48.0 (24.7%)	39.0 (20.1%)	45.0 (23.2%)	41.0 (21.1%)	
Hormonotherapy	55.0 (28.4%)	98.0 (50.5%)	102.0 (52.6%)	116.0 (59.8%)	117.0 (60.3%)	112.0 (57.7%)	
Chemo and/or Biological	4.0 (2.1%)	12.0 (6.2%)	14.0 (7.2%)	12.0 (6.2%)	12.0 (6.2%)	10.0 (5.2%)	
Hormono and/or Biological	7.0 (3.6%)	7.0 (3.6%)	10.0 (5.2%)	11.0 (5.7%)	12.0 (6.2%)	17.0 (8.8%)	
Pathologic Fracture	33.0 (17.0%)	7.0 (3.6%)	15.0 (7.7%)	16.0 (8.2%)	9.0 (4.6%)	17.0 (8.8%)	<0.001
~~Spinal Cord Compression~~	5.0 (2.6%)	0.0 (0.0%)	2.0 (1.0%)	0.0 (0.0%)	1.0 (0.5%)	1.0 (0.5%)	0.062
Hypercalcemia	6.0 (3.1%)	3.0 (1.5%)	9.0 (4.6%)	13.0 (6.7%)	13.0 (6.7%)	19.0 (9.8%)	0.006
Radiotherapy	50.0 (25.8%)	33.0 (17.0%)	20.0 (10.3%)	25.0 (12.9%)	26.0 (13.4%)	19.0 (9.8%)	<0.001
Surgery	7.0 (3.6%)	2.0 (1.0%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0%)	<0.001

				Denosumab			
Dosage meq Morphine	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.89
WHO ladder							0.67
0	100.0 (58.8%)	107.0 (62.9%)	115.0 (67.6%)	118.0 (69.4%)	115.0 (67.6%)	114.0 (67.1%)	
1	28.0 (16.5%)	23.0 (13.5%)	18.0 (10.6%)	22.0 (12.9%)	21.0 (12.4%)	21.0 (12.4%)	
2	22.0 (12.9%)	21.0 (12.4%)	16.0 (9.4%)	10.0 (5.9%)	12.0 (7.1%)	14.0 (8.2%)	
3	20.0 (11.8%)	19.0 (11.2%)	21.0 (12.4%)	20.0 (11.8%)	22.0 (12.9%)	21.0 (12.4%)	
NRS pain	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0.0 (0.0, 2.0)	0.52
Therapy							<0.001
None	10.0 (5.9%)	3.0 (1.8%)	6.0 (3.5%)	11.0 (6.5%)	12.0 (7.1%)	13.0 (7.6%)	
Chemotherapy	22.0 (12.9%)	31.0 (18.2%)	28.0 (16.5%)	27.0 (15.9%)	34.0 (20.0%)	48.0 (28.2%)	
Hormonotherapy	53.0 (31.2%)	66.0 (38.8%)	66.0 (38.8%)	69.0 (40.6%)	68.0 (40.0%)	55.0 (32.4%)	
Chemo and/or Biological	8.0 (4.7%)	14.0 (8.2%)	10.0 (5.9%)	4.0 (2.4%)	7.0 (4.1%)	7.0 (4.1%)	
Hormono and/or Biological	34.0 (20.0%)	56.0 (32.9%)	60.0 (35.3%)	59.0 (34.7%)	49.0 (28.8%)	47.0 (27.6%)	
Pathologic Fracture	41.0 (24.1%)	6.0 (3.5%)	10.0 (5.9%)	11.0 (6.5%)	13.0 (7.6%)	13.0 (7.6%)	<0.001
Spinal Cord Compression	8.0 (4.7%)	0.0 (0.0%)	1.0 (0.6%)	0.0 (0.0%)	1.0 (0.6%)	2.0 (1.2%)	0.001
Hypercalcemia	3.0 (1.8%)	3.0 (1.8%)	8.0 (4.7%)	7.0 (4.1%)	11.0 (6.5%)	21.0 (12.4%)	<0.001
Radiotherapy	43.0 (25.3%)	36.0 (21.2%)	7.0 (4.1%)	13.0 (7.6%)	18.0 (10.6%)	15.0 (8.8%)	<0.001
Surgery	9.0 (5.3%)	0.0 (0.0%)	2.0 (1.2%)	0.0 (0.0%)	2.0 (1.2%)	1.0 (0.6%)	<0.001

Table 2. Relative and absolute frequencies of analgesic use (according to the WHO scale), average pain score over the past week (according to the NRS), average dose of mg OME taken, frequencies of SREs or need for RT/orthopedic surgery, and adopted oncologic therapy.

§ Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test

To graphically represent the results of the primary objective, Figure 3 shows the outcomes of the multivariable longitudinal Bayesian mixed-effects models. In detail:

- Figure 3A reports the estimated Odds Ratio (OR) and the 95% Credible Intervals of the Cumulative Logit model build on the WHO analgesic scale. Dmab shows an OR of about 0.10 (0.042, 0.25 CI 95%), indicating about a 90% reduction in the likelihood of progression to the next step on the WHO scale compared to ZA;
- Figure 3B shows the conditional effects derived from the treatment model on the WHO scale. Patients treated with Dmab have a higher probability of being in level 0 of the WHO scale and a lower probability of being in the highest level compared to ZA;
- Figure 3C shows estimates of the coefficients of the Gaussian mixed effects model built for the NRS of pain. Dmab reduced NRS by an average of 0.38

points (-0.65, -0.11 95% CI) compared to ZA. It should be noted that the NRS ranges from 0 to 10, but the distribution of NRS in the study population is characterized;

- Figure 3D shows the conditional effects derived from the model of ZA/Dmab treatment on NRS score. Mean NRS score for the Dmab group was 1.82 (1.52, 2.10 95% CI) compared to 2.20 (1.91, 2.48 95% CI) in the ZA group;
- Figure 3E shows the estimates of the coefficients of the Gaussian mixed effects model built for the log1p transformation of the dosage. Dmab treatment showed stronger analgesic effect.

In details, Dmab group assumed an average of 0.42 meq (0.039, 1.09 95% CI) compared to 3.71 meq (1.69, 8.91 95% CI) in the ZA (Figure 3F). The mg OME has a distribution with 80% of values below 15 mg; this data should be related to the difference of 0.42 vs 3.71 mg in dose between the two groups.

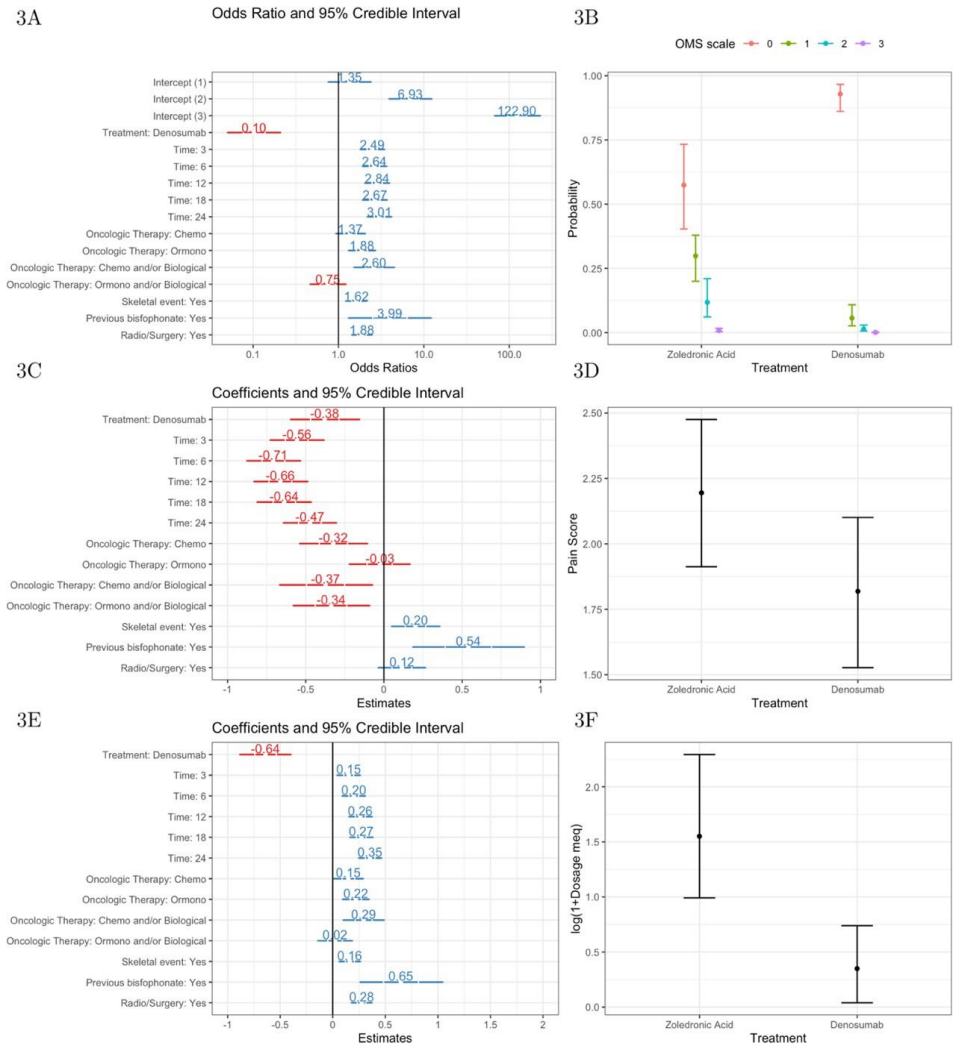

Fig. 3. Coefficients Estimates (95%) on the left and conditional effects on the right for each of the three Bayesian mixed effects longitudinal models.

Table 3 provides a numerical summary of what is shown in Figure 3.

WHO analgesic ladder				
Treatment	**OMS**	**Probability**	**CI 2.5%**	**CI 97.5%**
Zoledronic Acid	0	0.57	0.40	0.73
Denosumab	0	0.93	0.86	0.97
Zoledronic Acid	1	0.30	0.20	0.38
Denosumab	1	0.06	0.03	0.11
Zoledronic Acid	2	0.12	0.06	0.21
Denosumab	2	0.01	0.01	0.03
Zoledronic Acid	3	0.01	0.00	0.02
Denosumab	3	0.00	0.00	0.00
NRS (0-10)				
Treatment		Mean	**CI 2.5%**	**CI 97.5%**
Zoledronic Acid		2.20	1.91	2.48
Denosumab		1.82	1.53	2.10
OME (mg)				
Treatment		Mean	**CI 2.5%**	**CI 97.5%**
Zoledronic Acid		1.55	0.99	2.29
Denosumab		0.35	0.04	0.74

Table 3. Conditional Effects of the three Models and 95% CI, stratified for Treatment

At different observation times (0, 3, 6, 12, 18, and 24 months), in the group treated with Dmab, there is an increase in the percentage of patients not using analgesics (step 0 of the WHO scale) and a decrease in the percentage corresponding to steps 1 and 2 of the WHO scale. Conversely, in the ZA group, there is a decrease in the relative percentage of non-users and an increase in patients using weak or moderate-to-strong opioids. Examining the mean pain intensity at each evaluation point reveals consistent results: the NRS score is lower in the Dmab group compared to patients treated with ZA. Furthermore, patients treated with Dmab demonstrate a lower median mg OME dose

compared to those treated with ZA, with this difference evident at each evaluation time point.

Figure 4 illustrates pain control across all observation times, assessed through NRS scores and opioid dosage expressed in OME. Accordingly, the enhanced analgesic control associated with Dmab is sustained throughout each evaluation point. (Figure 4).

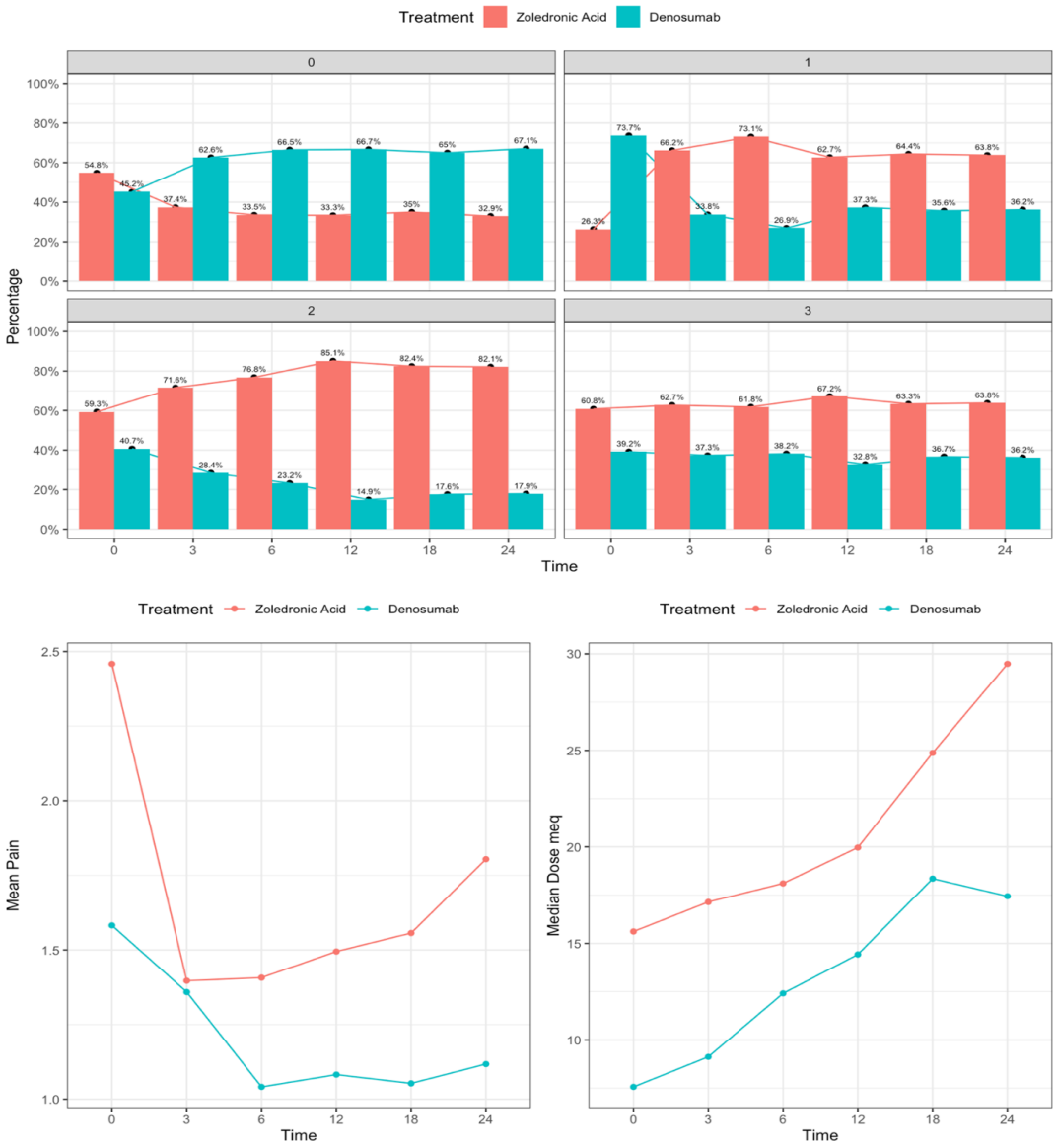

Fig. 1: Trend of WHO ladder, Mean NRS score, Median meq dosage over time.

4.3 Results of the Secondary Objectives

The cumulative incidence of the first skeletal event is 28.9% (24.2-33.7%; 95% CI, Figure 5).

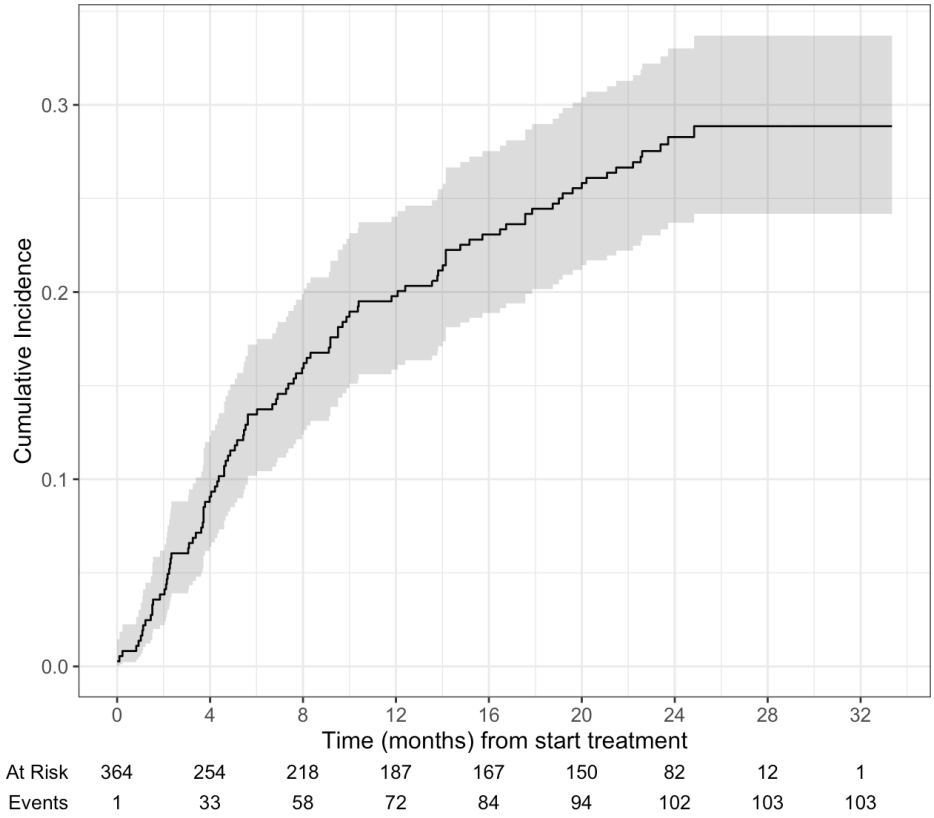

Fig. 5. Cumulative Incidence curve of the first skeletal event and the need for RT or bone surgery with 95% CI, stratified by treatment

Pathological fractures are the most frequent SREs (n=33 in ZA and 28 in Dmab), followed by hypercalcemia (n=23 in ZA, with 2 patients at grade 2 according to CTCAE, and 24 in Dmab), while SCC occurs in only 1 patient treated with ZA. Patients treated with Dmab have a comparable incidence to those treated with ZA regarding the first SRE or need for RT/orthopedic surgery. Table 4 shows the cumulative incidence at 12 and 24 months for the first SRE or need for RT/orthopedic surgery (competitive events). (Figure 6 e Table 4).

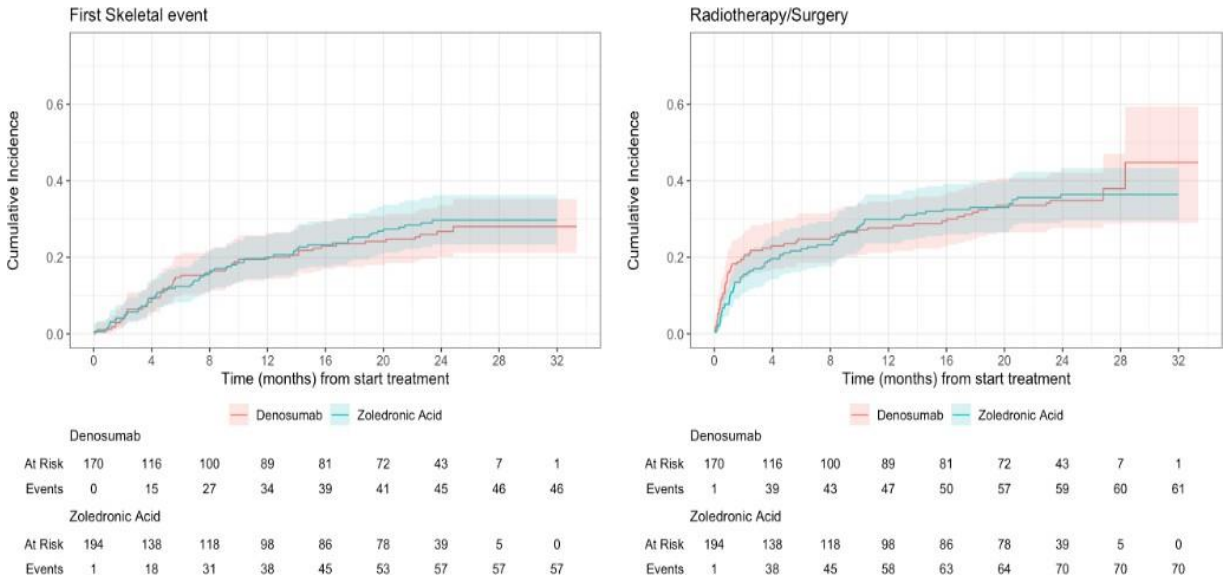

Fig. 6. Trend of WHO ladder, Mean NRS score, Median meq dosage over time

Variable	**12-months CI**	**24-months CI**	**p-value§**
First skeletal Event			
Treatment			0.6
Zoledronic Acid	20% (14%, 25%)	30% (23%, 36%)	
Denosumab	20% (14%, 26%)	27% (20%, 43%)	
Radiotherapy/Surgery			
Treatment			>0.9
Zoledronic Acid	30% (24%, 36%)	36% (30%, 43%)	
Denosumab	28% (21%, 35%)	35% (28%, 42%)	

Table 4: Cumulative Incidence at 12 and 24 months of the first skeletal event and radiotherapy or bone surgery

§ Gray's Test

Even in the multivariable Fine-Gray model constructed for the first SRE and the competing event, ZA and Dmab show no statistically significant difference (p = 0.8 and p = 0.2 for SRE and RT/surgery, respectively), as shown in Tables 5 and 6.

Variable	HR	95% CI	p-value
Age	0.98	0.96, 1.00	0.11
Basal Skeletal event			
No (Reference)	—	—	0.006
Yes	1.91	1.21, 3.01	
Treatment			
Zoledronic Acid (Reference)	—	—	0.8
Denosumab	0.95	0.61, 1.48	
Time from metastatic diagnosis	1.00	1.00, 1.00	0.10
Previous bisphosphonates			
No (Reference)	—	—	0.060
Yes	1.78	0.98, 3.25	
WHO ladder			
0 (Reference)	—	—	
1	0.88	0.45, 1.73	0.7
2	1.04	0.53, 2.03	0.9
3	0.65	0.31, 1.36	0.3
NRS pain (0-10)	0.99	0.88, 1.10	0.8
Oncologic Therapy			
None (Reference)	—	—	
Chemotherapy	0.72	0.34, 1.51	0.4
Hormonotherapy	0.65	0.35, 1.21	0.2
Chemo and/or Biological	0.65	0.21, 2.01	0.5
Hormono and/or Biological	0.71	0.30, 1.66	0.4
Radiotherapy	0.88	0.48, 1.60	0.7

Table 5. Results of the Fine-Gray model performed on the first SRE.

Characteristic	HR	95% CI	p-value
Age	1.00	0.99, 1.02	0.6
Basal Skeletal event			
No	—	—	
Yes	1.21	0.80, 1.82	0.4
Treatment			
Zoledronic Acid	—	—	
Denosumab	1.29	0.88, 1.89	0.2
Time from metastatic diagnosis	1.00	1.00, 1.00	0.3
Previous bisphosphonates			
No	—	—	
Yes	0.76	0.41, 1.41	0.4
WHO ladder			
0 (Reference)	—	—	
1	1.27	0.67, 2.40	0.5
2	1.59	0.91, 2.78	0.11
3	2.30	1.32, 4.03	0.004
NRS pain (0-10)	1.07	0.98, 1.17	0.12
Oncologic Therapy			
None (Reference)	—	—	
Chemotherapy	0.56	0.30, 1.60	0.074
Hormonotherapy	0.79	0.46, 1.36	0.4
Chemo and/or Biological	0.34	0.09, 1.32	0.12
Hormono and/or Biological	0.47	0.22, 1.00	0.050
Radiotherapy and others	0.31	0.17, 0.56	<0.001

Table 6. Results of the Fine-Gray model applied to the competing event (RT/surgery).

5. Discussion

SREs are regarded as a critical outcome in the treatment of skeletal metastases from breast cancer with bone-targeting agents, significantly impacting both patients and the healthcare system. However, focusing exclusively on SREs in discussing these therapies may be misleading, as bone pain can profoundly diminish the QoL in these patients. [77]. Healthcare system costs are linked to the progression of the patient's overall condition and the management of complications. Bone-targeting agents have demonstrated efficacy in delaying the onset of SREs and are approved in Italy for all patients with bone metastases, regardless of symptom presence or previous SREs. However, the effect of bone-targeting agents on bone pain is a less frequently discussed topic. Some RCTs and meta-analyses have demonstrated the efficacy of ZA in reducing bone pain when compared to placebo. In the meta-analysis by Kohno et al., ZA showed a beneficial effect on bone pain at 3, 12, and 24 months, as measured by the BPI. An analgesic effect has also been observed with Dmab; notably, it delays the onset of moderate-to-severe pain by 1.8 months compared to ZA. Furthermore, fewer patients on Dmab tend to escalate from weak to strong opioids or experience a QoL decline due to pain. [77]

More specifically, in a study on bone pain in breast cancer patients with skeletal metastases, Dmab delayed the progression to moderate-to-severe pain by 295 days compared to 176 days with ZA, although this difference was not statistically significant (HR 0.78; 95% CI 0.67-0.92; p-value = 0.142). [79]

This study was prompted by clinical observations of notable analgesic benefits of Dmab in patients with skeletal metastases from breast cancer. However, despite the studies referenced, there remains a lack of real-world data supporting the analgesic effect of this drug over extended periods of up to 24 months.

This is particularly relevant in therapeutic decision-making for common malignancies such as breast cancer with bone pain due to metastases. Therefore, the primary aim of this study was to assess the analgesic effect of these antiresorptive drugs in this specific population after at least 24 months of treatment.

As shown in Table 1, the populations in the ZA and Dmab groups are balanced for major demographic and clinical variables. An imbalance in the oncologic treatments used and the median time between the diagnosis of bone metastasis and the start of bone-targeting therapy may be due to the fact that Dmab has only been approved in Italy since 2013. Consequently, the effects of the emergence of biological therapies in oncology (e.g., CDK-4/6 inhibitors in first-line therapy combined with hormone therapy and new-generation anti-HER2 agents for breast cancer) and years of increased awareness of the use of bone-targeting agents are more evident in this patient group.

Defining outcomes is challenging due to the complexity of pain as a symptom. To provide a more

precise characterization, three parameters were assessed: the type of analgesic used according to the WHO analgesic scale, average pain intensity over the past week measured by NRS, and opioid dosage expressed in mg OME. The findings suggest that Dmab offers superior analgesic effects compared to ZA. Specifically, Dmab was associated with a lower frequency of escalation to higher steps on the WHO scale, a decreased likelihood of using higher-level analgesics, and an increased likelihood of not requiring pain medication

This trend is also confirmed in the assessment of pain using the NRS scale, with values averaging 0.38 points lower than those in the ZA group. Lastly, the median dose of mg OME was also lower in the Dmab group, with 0.42 mg compared to 3.71 mg in patients treated with ZA.

While the differences in NRS intensity and mean mg OME dose between the Dmab and ZA groups may appear modest, they should be interpreted in the context of 80% of patients having an NRS < 3 and an average opioid dose < 15 mg OME. Thus, the relative impact of Dmab compared to ZA in reducing pain intensity and subsequent opioid needs is significant.

This effect can be attributed to the inhibition of the RANK/RANK-L pathway, allowing the drug to block osteoclast activity and reduce the production of cytokines and inflammatory molecules. These molecules impact sensory nerve endings directly or indirectly by promoting inflammation in the surrounding microenvironment. [90], [91] These findings could suggest a direct analgesic effect of Dmab, leading to reduced pain intensity and lower opioid requirements. However, this hypothesis is not consistently supported, as some studies, including Charles et al. (2013) [81], did not observe a significant analgesic effect of Dmab.

The data on secondary objectives indicate no statistically significant difference between ZA and Dmab in delaying or preventing SREs. Figure 6 shows that the incidences are comparable between the two groups in terms of SRE occurrence as well as the need for RT or orthopedic surgery. This finding contrasts with previous results from earlier RCTs.

For instance, Alison et al. [70] concluded that Dmab was superior to ZA in delaying (HR= 0.82) or reducing SREs. However, this may be due to the fact that, in our study, RT and orthopedic surgery were considered competitive events rather than SREs. Additionally, studies in the literature were conducted under ideal and controlled conditions.

While ZA and Dmab show comparable efficacy in reducing SREs, Dmab provides certain advantages regarding administration and tolerability. It can be administered subcutaneously rather than intravenously and does not require dose adjustment for patients with renal insufficiency—a common condition in heavily treated patients with advanced cancer. ONJ remains an important but rare side effect of both drugs [51], necessitating preventive dental check-ups and follow-up at least every six months.

Despite the inherent limitations of a retrospective study, the sample size and broad inclusion/exclusion criteria allowed for a meaningful evaluation of the two drugs in a real-world context. A further strength of this study is its consideration of the impact of RT and orthopedic surgery on pain, classifying them as competing events rather than SREs. This approach underscores the specific effects of bone-targeting agents on bone pain and SREs.

6. Limitations of the Study

The main limitations of this study include:

- Monocentric design: Conducted at a single referral oncology center, which may limit the generalizability of the results.
- Retrospective nature: Exposing the study to recall and information biases.
- Uncontrolled setting: Increasing susceptibility to errors, data dispersion or incompleteness, and additional biases, such as selection bias.

Thus, while real-world studies are essential, it is important to recognize that data quality is, by definition, lower than that of RCTs.

7. Conclusions

In breast cancer patients with skeletal metastases, Dmab appears to offer superior long-term analgesic control and preventive benefits compared to ZA. Furthermore, both drugs demonstrate a similar effectiveness in preventing SREs.

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