Understanding the impact of bone metastases in non-small cell lung cancer

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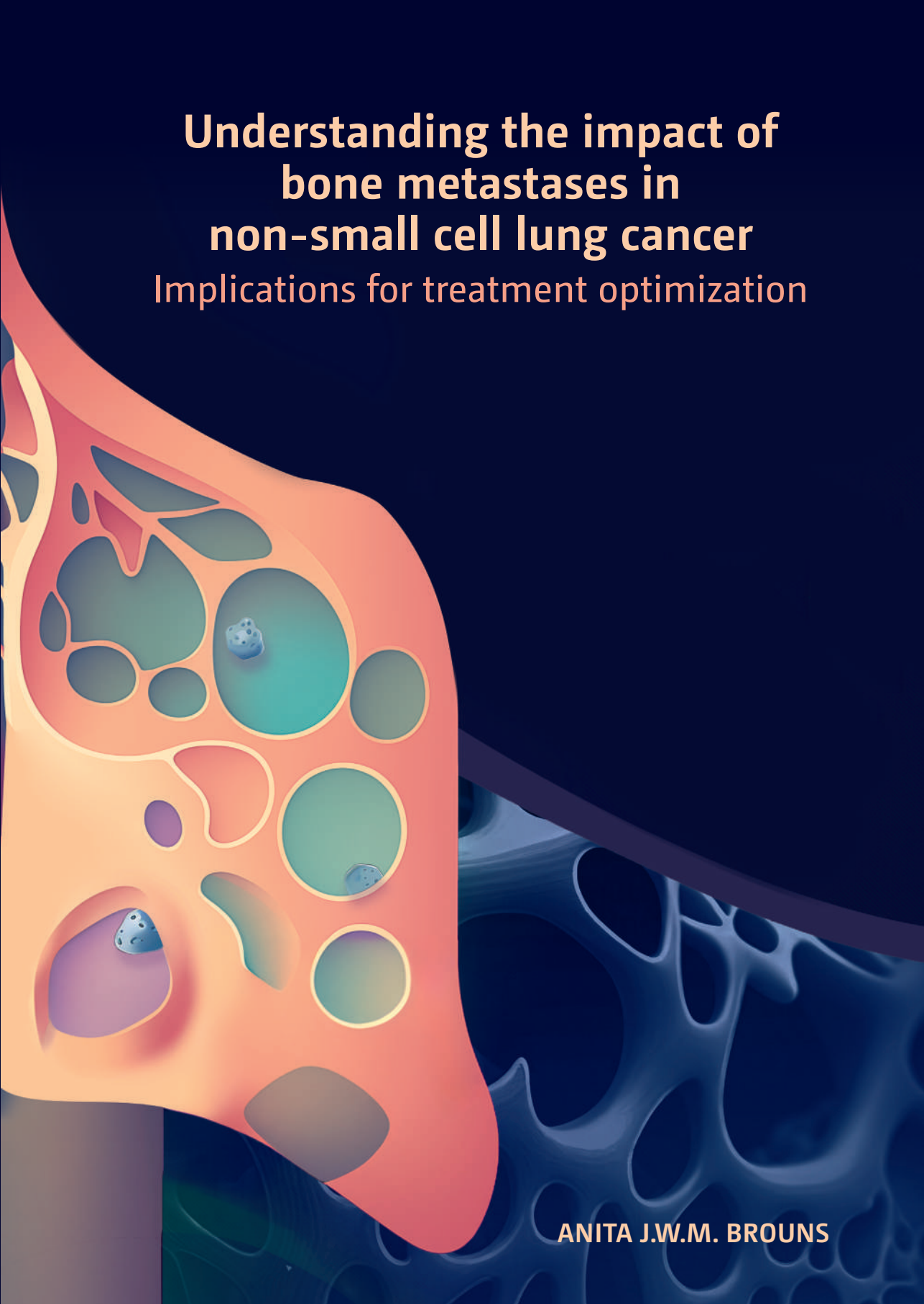
Implications for treatment optimization



ANITA J.W.M. BROUNS

Understanding the impact of bone metastases in non-small cell lung cancer: implications for treatment optimization

Anita J.W.M. Brouns

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Understanding the impact of bone metastases in non-small cell lung cancer: implications for treatment optimization

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Promotor
Prof. dr. Anne-Marie C. Dingemans, Erasmus MC.

Copromotoren
Dr. Lizza E.L. Hendriks,
Dr. Gerben P. Bootsma.

BEOORDELINGSCOMMISSIE

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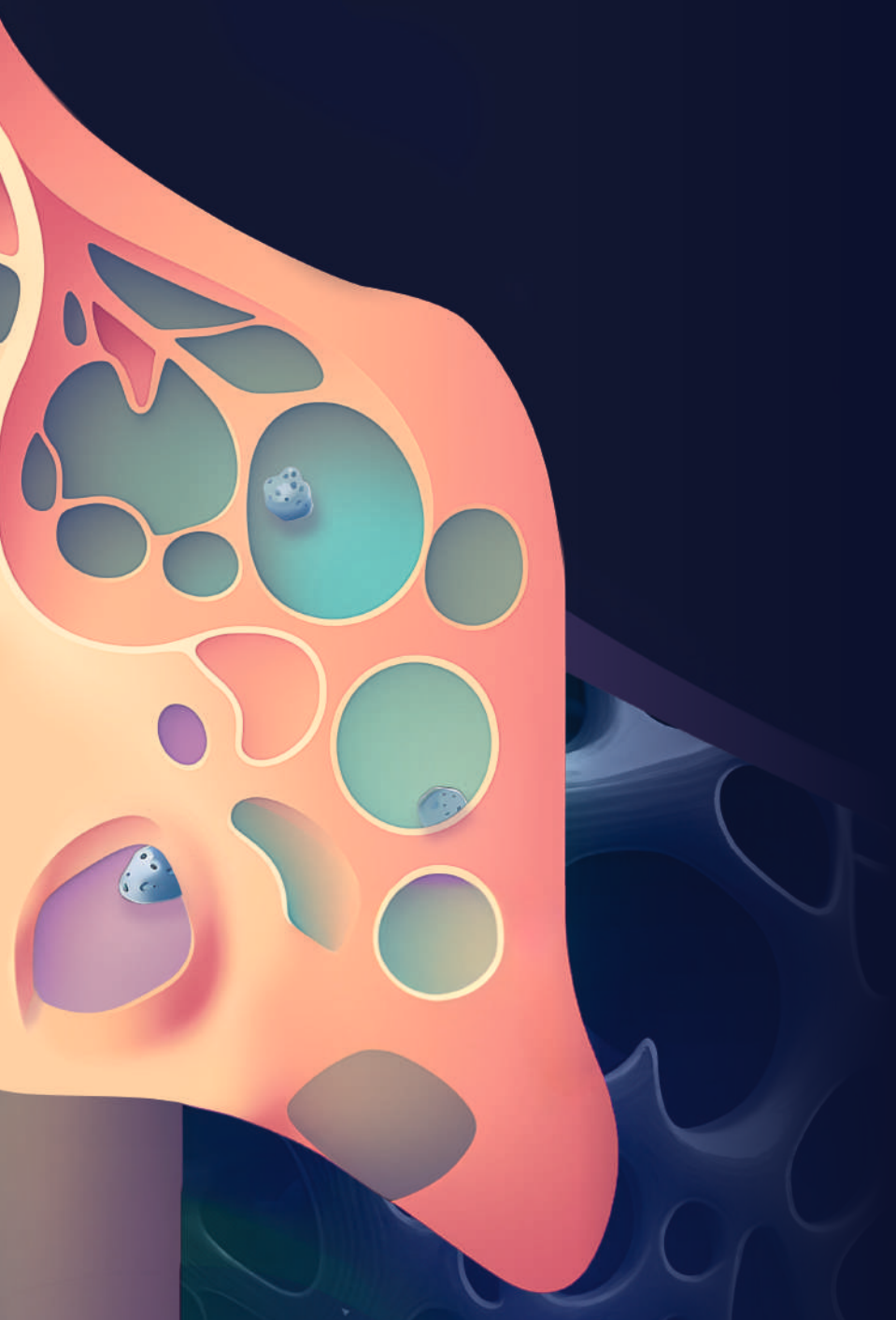
CHAPTER 1



General introduction and outline of this thesis

1.1 EPIDEMIOLOGY OF LUNG CANCER

Lung cancer is one of the most frequently diagnosed cancers worldwide and contributed to 12% of all new cancer cases in 2022 (1). Lung cancer is classified into non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), which accounts for 85% and 15%, respectively (2). NSCLC is subdivided into different subtypes: non-squamous cell carcinomas are the most common, followed by squamous cell carcinoma (SCC) and other rarer subtypes (2). In the Netherlands 13,910 patients were diagnosed with lung cancer in 2020, half of them already had metastatic disease at initial diagnosis (3).

1.2 DIAGNOSIS OF LUNG CANCER

The diagnostic imaging work-up of a suspected lung cancer requires at least a computer tomography scan (CT scan) of the chest and upper abdomen (4). Although bone metastases can be diagnosed on CT, 2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computed tomography (FDG-PET-CT) scan and bone scintigraphy are more sensitive (5). An FDG-PET-CT scan and often also dedicated brain imaging are necessary to rule out distant metastases if a patient has a potentially curable lung cancer.

The diagnosis of (lung) cancer is based on pathological examination of a biopsy from the primary tumor or a metastasis. Patients with newly diagnosed advanced non-squamous NSCLC should have broad molecular testing of their tumor as presence of targetable oncogenic drivers influences the treatment choice, with targeted therapies available for multiple oncogenic drivers (6, 7). Multiplex testing by next generation sequencing (NGS) is recommended for molecular subtyping, as less tumor cells are needed (2). An activating mutation in Kirsten rat sarcoma viral oncogene (*KRAS+*) is the most common oncogenic driver in NSCLC and is found in 25-30% of the patients with non-squamous NSCLC (2, 8). The epidermal growth factor receptor mutation (*EGFR+*), present in 14-16.6% of the Caucasian population, is the most common targetable oncogenic driver (9, 10). There are many less frequent oncogenic drivers and

for more and more of these drivers, targetable therapies are becoming available (Figure 1). Next to molecular subtyping of the tumor, determining of programmed death ligand 1 (PD-L1) protein expression on the tumor is necessary, especially for those without a targetable driver. PDL-1 protein expression has emerged as a biomarker for prediction of a tumor's response to immunotherapy and guides the decision for the optimal immunotherapy strategy (monotherapy or combination therapy) for an individual patient (11).

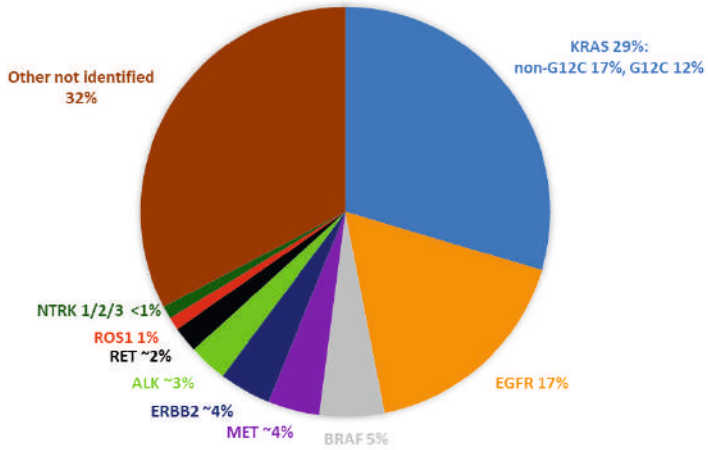


Figure 1: The frequencies of common oncogenic driver mutations in NSCLC. Based on a cohort of 4065 patients with advanced NSCLC by Singal and colleagues (12).
Abbreviations: KRAS: Kirsten rat sarcoma; EGFR: Epidermal Growth Factor Receptor; MET: mesenchymal epithelial transition; ERBB2: erythroblastic oncogene B2; ALK: anaplastic lymphoma kinase; RET: rearranged during transfection; ROS1: reactive oxygen species 1.

Staging of lung cancer is currently based on the 8th edition of the tumor, node and metastases (TNM) classification (13). Stages range from stage IA1, which represents a primary tumor ≤1cm without metastases, to stage IVB, which represents multiple (extrathoracic) metastases (14). The aim of the TNM classification is to classify patients with NSCLC into groups with the same overall survival (OS) and prognosis: stage IA1 has an excellent prognosis, whereas survival is rather poor in stage IVB (Table 1). In general, OS of patients with advanced NSCLC is increasing due to the introduction of targeted therapies as well as immunotherapy, resulting in long-term disease control for subgroups of patients (15). For example, five-year OS for those without an oncogenic driver, treated with mono-immunotherapy in case of a PD-L1 score of ≥50%, is 32% and five-year OS of patients with an activating *EGFR* mutation or ALK rearrangement, treated with targeted therapy is 35% and 62.5%, respectively (16-18). For the future, we expect a further increase in the five-year OS for *EGFR* mutated patients, as the Flaura trial (first line osimertinib in *EGFR* mutated advanced NSCLC) and the NEJ009 trial (combination gefitinib with carboplatinum/pemetrexed versus gefitinib alone) report median OS of 38.6 months and 50.9 months, respectively (19, 20). Five-year overall survival data of these studies are not mature. The introduction of combination chemotherapy and immunotherapy for metastatic NSCLC patients without an oncogenic driver leads to improved survival too: five-year survival rates of 18.4% for squamous NSCLC treated with carboplatin/paclitaxel or nab-paclitaxel and pembrolizumab (versus 9.7% while treated with chemotherapy only) and 19.4% for non-squamous NSCLC treated with pemetrexed/platinum and pembrolizumab (versus 11.3% while treated with chemotherapy only) (21, 22).

Table 1: Overall survival by pathological stage according to TNM 8th edition (14).

Stage	OS at 24 months (%)	OS at 60 months (%)
IA1	97	92
IA2	94	83
IA3	90	77
IB	87	68
IIA	79	60
IIB	72	53
IIIA	55	36
IIIB	44	26
IIIC	24	13
IVA	23	10
IVB	10	0

Abbreviations: OS, overall survival.

1.3 SPECIAL POPULATIONS: NON-SMALL CELL LUNG CANCER AND BONE METASTASES

Bone metastases are common in NSCLC: 30-60% of the patients with advanced disease develop bone metastases during their disease course (23, 24). However, in clinical trials or guidelines less attention is paid to diagnosis and treatment of bone metastases or bone related outcomes of NSCLC (25-30). Before one can decide what the best treatment strategy is for treatment of bone metastases or skeletal related events (SREs) in NSCLC, it is advisable to know more about the incidence, pathophysiology of bone metastases, clinical presentation, and treatment options in patients with (*EGFR+)* NSCLC.

Bone metastases are not always symptomatic and can be an incidental finding on imaging during the work-up of a malignancy. The European Society for Medical Oncology (ESMO) clinical practice guidelines for metastatic NSCLC, the ESMO guideline on bone health, National Institute for Health and Care Excellence (NICE) lung cancer guideline and the Dutch national guideline on NSCLC advise to perform bone imaging if bone metastases are clinically suspected (11, 25-27). Other guidelines such as NSCLC guidelines of the National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) do not specify recommendations regarding bone metastases (28-30). This is in contrast to breast cancer, in which screening for bone metastases is advised in the advanced state and for prostate cancer, screening for bone metastases is recommended in patients with localized prostate cancer and intermediate and high risk profile (31, 32).

1.3.1 Pathophysiology of bone metastases

The bone microenvironment consists of a mineralized extracellular matrix, fenestrated capillaries and different bone cells (e.g., osteoblasts, osteoclasts) and is controlled by local and systemic factors (33). Osteoblasts are "bone-forming cells", they secrete type I collagen and other proteins that are essential for the mineralization of the bone matrix (34). The work of the osteoblasts is balanced by osteoclasts, which are cells that adhere to the bone surface and demineralize the bone matrix and degrade proteins by means of secretion of collagenases and proteases (34). Normal bone remodeling is under strict regulation of numerous growth factors, control mechanisms and signaling pathways. One of the most important signaling pathways is the Receptor activator of Nuclear Factor κB (RANK)/ RANK ligand (RANKL)/ osteoprotegerin (OPG, the decoy receptor and antagonist of RANKL) pathway (Figure 1) (35). RANK is located on osteoclast precursors and binding of RANKL to its receptor RANK, stimulates the osteoclastogenesis. Osteoclast formation and stimulation are also promoted by EGFR signaling. This stimulation is the result of inhibition of OPG expression, along with an increase of monocyte chemoattractant protein 1 (MCP1; which induces osteoclast fusion and activity), as well as an increase in macrophage colony-stimulating factor (MSCF) and RANKL expression (34).

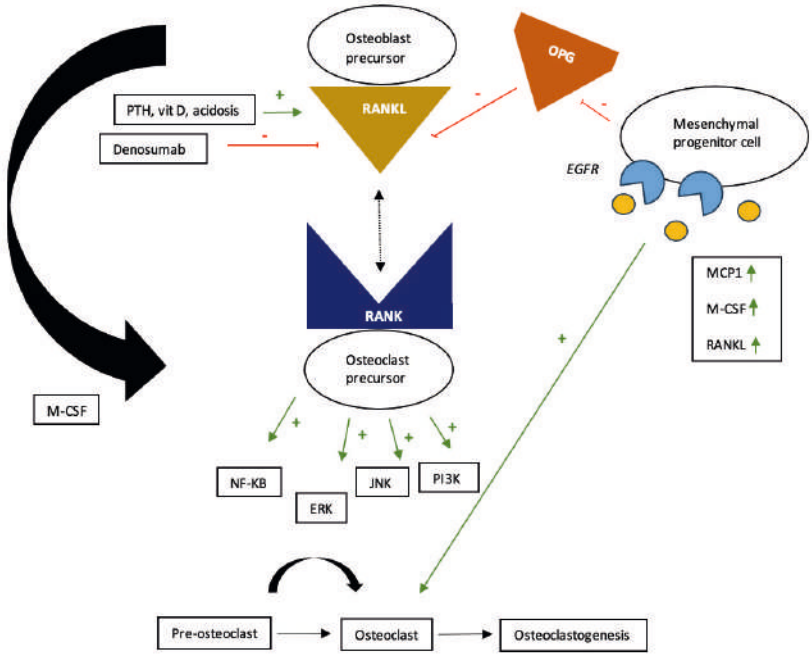


Figure 2: Simplified representation of the RANK/RANKL/OPG axis and its influencing factors.
Abbreviations: OPG: osteoprotegerin; PTH: parathyroid hormone; vit D: vitamin D; RANKL: Receptor activator of Nuclear Factor κB ligand; EGFR: epidermal growth factor receptor; M-CSF: macrophage colony-stimulating factor; RANK: Receptor activator of Nuclear Factor κB; MCRP1: monocyte chemoattractant protein 1.

The preferential colonization of metastatic tumor cells to the bone, relies on the attractiveness of the bone microenvironment by the development of a premetastatic niche. Steven Paget proposed in 1889 the “seed and soil” theory to explain the selective colonization of metastatic cells (“seeds”) to bone (33). Through premetastatic niches (“soil”), it is more feasible for metastatic tumor cells to adhere to bone and then to invade the bone, survive and proliferate. Bone metastasis leads to a disturbed bone turnover. Tumor cells secrete osteolytic factors (e.g., parathyroid hormone-related peptide (PTH-RP), interleukine-11, -6, -8, vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF), Jagged 1 and epidermal growth factor (EGF)-like ligands) that stimulate osteoclastogenic bone resorption. These effects are directly or indirectly by increasing the ratio of RANKL to OPG. Through this bone resorption various growth factors and ions are released from the mineralized bone matrix, which enhance the process of osteoclastogenesis. Besides that, the tumor cells also secrete metalloproteinases or EGF-like growth factors which favor local angiogenesis, tumor homing and osteoclastogenesis (36). In other words, a vicious cycle is created in which tumor growth and bone degradation are continually stimulated. The last years, more attention is paid to the role of extracellular vesicles

(EVs) in normal bone homeostasis and bone metastases development (37-39). EVs are involved in cell-cell communication and play a great role in physiological and pathophysiological functions. EVs are lipid membranous vesicles that are released from every type of cell in the body and are found in body fluids. They can be categorized by routes of biogenesis into exosomes (~100 nm), microvesicles (~1 µm), and apoptotic bodies (>1 µm) (38). The content of EVs consist of lipids, nucleic acids (e.g., micro ribonucleic acid (miRNA), deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA)) and proteins (39). Despite the fact that much remains to be clarified about the role of EVs and bone metastases (development), we know that EVs take part in preparation of the metastatic niche, bidirectional stimulation of bone -and tumor cells, and after priming by cancer cells, exosomes from bone marrow derived mesenchymal stem cells could promote breast cancer cell survival and dormancy by altering their mRNA content (37). In NSCLC, tumor cells secrete EVs containing EGFR ligand and amphiregulin (AREG), both stimulate the osteoclastogenesis and thereby contribute to the vicious cycle of tumor growth and bone degradation (40).

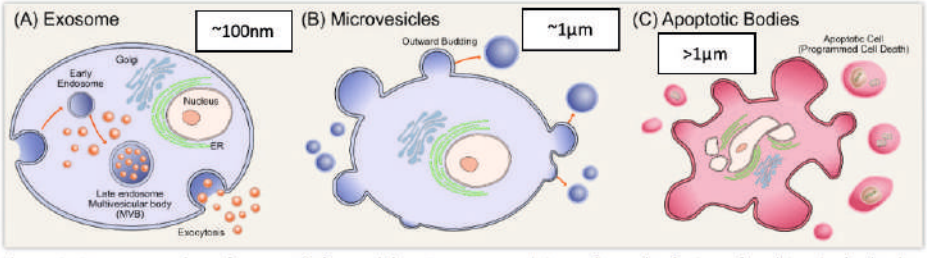


Figure 3: Representation of extracellular vesicles. Exosomes originate from the fusion of multivesicular bodies (MVBs) with the plasma membrane. Microvesicles are vesicles that bud directly from the plasma membrane. Figure adapted from (41).

Multivariate analyses involving 1,025 patients with NSCLC showed that the probability of bone metastases development is affected by age at diagnosis of NSCLC, histological subtype and NSCLC treatment (42-44). Adenocarcinoma of the lung is the histologic subtype in which bone metastases are most common, patients with squamous NSCLC and SCLC have less frequently bone involvement (Hazard Risk (HR) of developing bone metastases in adenocarcinoma compared to other histologies of 1.51; 95% CI 1.06-2.15; p=0.021) (42-44). Another study (n=197) reported stage IV disease, performance status ≥1 and increased bone alkaline phosphatase as predictors of bone metastases (45).

The biological predisposition for bone metastases seems to vary between different molecular subgroups of NSCLC (46, 47). A nationwide Dutch database (n=2052), including all patients with advanced non-squamous NSCLC (ns-NSCLC) at initial diagnosis with data from molecular analysis and metastasis pattern at diagnosis of advanced disease showed that in patients with advanced NSCLC and an EGFR mutation a significantly higher incidence of bone metastases was reported in comparison with other molecular subgroups (54% vs. 33% *KRAS+* vs. 30.5% *ALK+* vs. 31.5% triple negative patients, $p < 0.001$) (47). Other smaller studies (n=189-550) evaluating the incidence of bone metastases in various molecular subgroups, showed conflicting results (23, 46, 48-50). Though, sparce data is known why some patients with NSCLC develop bone metastases and others do not. The presence of an *EGFR* mutation could play a role in the enhanced sensitivity in some patients with NSCLC to develop bone metastases.

1.3.2 Complications of bone metastases

Bone metastases are a clinically relevant problem as these patients are at risk for developing skeletal related events (SREs), which affects quality of life (QoL) and OS (43, 51, 52). SREs are defined as a pathological fracture, spinal cord compression, necessity for radiation to bone (for pain or impending fracture) or surgery to bone, because of bone metastases (53, 54). Sometimes hypercalcemia of malignancy is also part of the SRE definition (54). The natural history of bone metastases and SREs is poorly studied since most of the data are derived from retrospective studies. Retrospective data of patients with NSCLC and bone metastases (n=211-273) showed that ever smoking, non- adenocarcinoma histology, WHO-PS of ≥2, never treated by EGFR-TKI therapy (regardless of presence of *EGFR* mutation) were independent risk factors for development of an SRE, whereas higher tumor stage at initial diagnosis and presence of SREs are independently associated with worse OS (55, 56). The rate of SREs in NSCLC is high: of the patients with bone metastases 30-73% (n=383-1283) experience one or more SREs (43, 51, 53, 57). About thirty percent of the patients with *EGFR+* NSCLC treated with first and second EGFR-TKI develop SREs (52, 58). However, data about SREs in these patients treated with osimertinib, nowadays first-line treatment, is unknown.

Furthermore, up to 80% of the patients with lung cancer and bone metastases experience Cancer Induced Bone Pain (CIBP) (53, 59), but data regarding severity of CIBP is scarce. The nervous system of humans is designed to perceive and regulate pain. Nociceptors (“pain sensors”)

located at the nerve endings in the skin, bones, connective tissues and organs detect pain and transmit the pain stimulus to the spinal cord through A-delta fibers (myelinated, quick fibers) and C-fibers (unmyelinated, slow fibers) (60). The pathophysiology of CIBP is complex and consists of inflammatory, ischemic and neuropathic mechanisms. In case of CIBP, several factors contribute to this both inflammatory and neuropathic pain: 1) Activation of the RANKL/RANK pathway leads to osteoclastic bone resorption. The acidic environment between osteoclasts and bone stimulates excitement of ion channels in the cell membrane of nerve fibers, 2) Nociceptors of the bone sense noxious stimuli produced by cancer and tumor-associated stromal cells, tumor-associated immune cells or other factors from the tumor microenvironment, 3) Mechanosensitive ion channels detect mechanical stimuli which arise with distal aspects of sensory nerve fibers are distended by mechanical pressure due to the growing tumor or by facture of the bone and 4) Forming of newly primary afferent neurons that sprout in response to peripheral nerve injury or forming of neuroma. The spontaneous or by movement evoked discharges of these neurons result in severe bone pain (61, 62). Studies with animals with cancer induced pain show that due to pain, changes in spinal excitatory synaptic transmission emerge, which results in hyperexcitability to stimuli (central sensitization) (61). Ultimately, peripheral and central sensitization causes a vicious circle of CIBP and chronic pain. Patients with CIBP experience (chronic) pain resulting in major impact on physical and social functioning, and CIBP often leads to hospital admission (63). Therefore, early pain reduction is important.

1.3.3 Treatment of cancer induced bone pain and skeletal related events

Treatment of CIBP is multifactorial and treatment decisions are preferably taken by a multidisciplinary team of medical and radiation oncologists, radiologists and palliative care specialists (26). Treatment involves non-drug interventions such as behavior modifications (avoiding strenuous movement, use of appropriate movement aids), prescription of pain killers, radiotherapy, radioisotopes or bone targeted agents (BTAs) (64).

According to the World Health Organization (WHO) pain ladder, bone pain should first be treated with paracetamol and/or non-steroidal anti-inflammatory drugs (NSAIDs), followed if necessary by adding mild, and later strong opioids (65). If patients experience pain despite optimal medical treatment or in case of e.g., impending myelum compression due to bone metastases, local radiotherapy is an effective treatment strategy to control pain and prevent complications. Radiotherapy to painful bone metastases has a pain relief success rate of 70-80%, and 40% of the patients experience an early relief within 10 days after radiotherapy (26). In patients with widespread painful bone metastases, radiotherapy is not feasible. For more diffuse bone pain radioisotopes can be administered. The efficacy of radioisotopes for painful bone metastases has been primarily studied in prostate and breast cancer (66, 67). In NSCLC, data is limited and consists of subgroup analysis from other trials (64, 68). Radioisotopes for painful bone metastases in NSCLC are likely to cause a rapid effect on pain, which lasts one to three months (64). However, in daily practice, radioisotopes are barely used in NSCLC, as they can cause myelosuppression which

can interfere with systemic therapy, and the effect is often short-lived. In case of a pathological fracture surgical fixation is recommended to maintain patient mobility and functionality (26, 69). Prophylactic surgical stabilization is being considered for lesions ≥30mm, lytic destruction of ≥50mm of the cortex of a long bone and persistent pain with weight-bearing after radiotherapy (26). Postoperative radiotherapy should follow surgical stabilization to inhibit local tumor growth (26, 69).

Denosumab and bisphosphonates are BTAs and are recommended in guidelines to prevent or delay the time to SREs (26, 70, 71). The evidence behind this recommendation is mainly based on data from multiple myeloma and solid malignancies other than NSCLC (70, 71). The ESMO Clinical Practice guideline for diagnosis, treatment and follow-up of non-oncogene addicted metastatic NSCLC refers for treatment of bone metastases to the ESMO guideline on bone health (4). This guideline advices BTAs in patients with a life expectancy of >3 months and clinically significant bone metastases (level of evidence I, grade of recommendation B) (26). No specific recommendations exist for patients with *EGFR+* NSCLC. BTA use in breast cancer is associated with less pain due to bone metastases, whereas in lung cancer no clear evidence exist (64, 72). Probably because less attention is paid to BTA use in NSCLC guidelines and because of lack of strong recommendations, BTA are not frequently used in the treatment strategy of NSCLC. Prescription of BTAs varies from 16% of patients with *EGFR+* NSCLC and bone metastases to 38% in unselected NSCLC patients and bone metastases (71, 73, 74).

1.4 TREATMENT OF ADVANCED NON-SMALL CELL LUNG CANCER

In establishing an individualized treatment plan for a patient with advanced NSCLC, different factors should be taken into account, for example: world health organization performance score (WHO-PS), comorbidities, extent of the disease, results from molecular subtyping, PDL-1 status of the tumor and patient's preferences (75). Given that this thesis focuses on patients with *EGFR* mutated lung cancer, we further only describe treatment options for this patient population (4).

1.4.1 EGFR mutations and EGFR-tyrosine kinase inhibitors

EGFR is a member of the ErbB tyrosine kinase family: HER2/neu (or erbB2), HER3 (or erbB3), HER4 (or erbB4) and EGFR (or erbB1) are four closely related receptors in the cell membrane. EGFR signaling plays a role in (tumor) cell proliferation, angiogenesis and survival (76). Binding of EGF to EGFR leads to dimerization of its receptor and autophosphorylation of the cytoplasmic receptor domain and activation of downstream pathways (e.g. RAS/RAF/MEK/ERK and PIK3/AKT/mTOR pathways) (77). Uncontrolled activation of these downstream pathways counteracts with apoptosis of tumor cells and results in increased proliferation and survival. *EGFR* mutations can be subdivided in "common sensitizing mutations" (deletions in *EGFR* exon 19 or substitutions of

leucine for arginine [L858R] in exon 21) and "uncommon sensitizing mutations" (e.g. *EGFR* exon 18 and some of the exon 20 mutations) and the non-sensitizing mutations (mainly *EGFR* exon20 insertions) (4).

The introduction of EGFR tyrosine kinase inhibitors (TKIs) truly changed the landscape for *EGFR* mutated NSCLC. In comparison with platinum-based chemotherapy, treatment with EGFR-TKIs resulted in a significantly longer PFS and a higher objective response rate (ORR) (78-81). EGFR-TKIs, such as gefitinib and erlotinib, bind reversibly to the kinase domain of EGFR and potently inhibit the receptor when it has been activated by common EGFR mutations. EGFR-TKIs interrupt EGFR signaling by competing with the adenosine triphosphate (ATP) at the intracellular catalytic kinase domain of the receptor and thereby preventing of autophosphorylation of the EGFR receptor and activation of downstream pathways (82). Unfortunately, also nonselective inhibition of wildtype EGFR-TKI occur, resulting in treatment related adverse events (TRAEs) such as rash and diarrhea. Response to EGFR-TKIs depends on the mutational subtype of EGFR: patients with EGFR exon 19 deletions have the highest ORR and survival, which decreases in patients with EGFR L858R substitution and further declines in patients with uncommon EGFR sensitizing mutations (83, 84).

The first developed EGFR-TKIs were the so-called "first-generation EGFR-TKIs:" erlotinib, gefitinib and icotinib. All were approved for first-line treatment of *EGFR+* NSCLC as they resulted in a significantly prolonged PFS compared with chemotherapy (80, 85-88). First generation EGFR-TKIs were followed by second generation EGFR-TKIs, such as dacomitinib and afatinib (89). These are EGFR-TKIs which irreversibly inhibit various ErbB receptors to overcome activation of other signal pathways that by-pass EGFR signaling. During treatment, eventually all patients develop resistance to EGFR-TKIs (87). Three main categories of acquired resistance to EGFR-TKI exist: 1) Target alteration due to appearance of other EGFR mutations (e.g. T790M mutation in exon 20), 2) Activation of other signal pathways that by-pass EGFR signaling (e.g. HER2 amplification or MET amplification), 3) Histological changes (e.g. epithelial to mesenchymal transition, transformation to small cell lung cancer) (82, 90). Emergence of T790M is the most frequent escape mechanism to first and second generation EGFR-TKI treatment (82). Osimertinib targets both the common sensitizing EGFR mutations as well as T790M mutant EGFR, while it harbors less activity towards wildtype EGFR, leading to a lower rate of TRAEs (89, 90). Osimertinib was first approved for the treatment of T790M positive disease, and afterwards also for first line treatment as osimertinib was found to be superior to the first generation EGFR-TKI erlotinib and gefitinib regarding PFS and OS (19, 82, 91). Osimertinib has a similar safety profile and less serious TRAEs compared to first/second generation EGFR-TKIs: TRAEs ≥ grade 3 with osimertinib were 34% versus 45% with erlotinib or gefitinib (82). However, QT prolongation is more frequent with osimertinib than with erlotinib or gefitinib (≥ grade 3.2% versus 1%) (82). Importantly, osimertinib has a better central nervous system (CNS) efficacy compared to erlotinib or gefitinib (92). Consequently, osimertinib is the preferred first-line treatment for patients with metastatic *EGFR+* NSCLC (93).

Nowadays, one hopes with improved systemic treatment options for patients with metastatic *EGFR+* NSCLC that that there is also an improved efficacy in bone. To the best of our knowledge EGFR-TKI efficacy in bone is only studied in retrospective series (94, 95). One series of 388 patients treated with first, second and third EGFR TKIs (n=183 erlotinib/gefitinib, n=55 afatinib, n=150 osimertinib) showed a better PFS of 17.0 months in *EGFR* mutated patients with bone metastases treated with osimertinib compared to a PFS of 8.6 months ($p<0.001$) for patients treated with first or second generation EGFR-TKIs, unfortunately no difference in OS exist (94). A larger series of 604 patients with *EGFR* mutated NSCLC (300 with and 304 without bone metastases) confirmed that both PFS and OS are worse for bone metastasized patients (95). These results hold true even when treated with osimertinib. However, possibly there is hope on the horizon as adjuvant osimertinib in resected EGFR mutated NSCLC patients lowers the risk for bone relapse (96). Given these results come from retrospective series and meta-analysis, prospective studies are needed to evaluate the efficacy of EGFR-TKIs on bone and bone related outcomes.

1.5 AIMS AND OUTLINE OF THIS THESIS

As summarized above, bone metastases and SREs are frequent in patients with NSCLC. As bone metastases often result in difficult to treat pain, new treatments to reduce pain are needed. Furthermore, although patients with an *EGFR*+ NSCLC seem to have a higher incidence of bone metastases, the biological mechanisms are not clear. As despite the presence of bone metastases, these patients have a long survival, optimal prevention and treatment of bone metastases is necessary to ensure a good QoL. The overall aim of this thesis was to optimize the treatment of patients with NSCLC and bone metastases, with a specific focus on *EGFR+* NSCLC, including also potential biological mechanisms resulting in the observed increased bone metastases incidence in this subgroup of patients.

Up to 80% of the patients with bone metastases experience CIBP and radiation of a painful bone metastasis is usually a good treatment option. Unfortunately, not all patients benefit from radiotherapy, or radiotherapy is not feasible due to the extent of painful bone lesions. Because of the negative impact of CIBP on daily life, rapid pain reduction is desirable. We performed a systematic review to evaluate non-radiation based treatment options in **chapter 2.**

In patients with breast cancer, prostate cancer and multiple myeloma evidence exist for bisphosphonates on pain reduction in CIBP. In current evidence based guidelines from the ESMO, NCCN, and NICE bone targeted agents such as bisphosphonates are mentioned as a treatment option to prevent SREs. However, data is scarce for NSCLC. We completed a phase II, multicenter study, to investigate the effect of loading doses of ibandronate on bone pain response in **chapter 3**.

The other chapters of this thesis focus on *EGFR+* NSCLC and bone metastases. As the biological mechanism behind the observed higher incidence of bone metastases in *EGFR+* NSCLC is unknown,

we investigated in **chapter 4**, whether there is an association between EGFR gene expression and RANKL, RANK and OPG gene expression in the tumor and presence of bone metastases.

EVs play a role in communication between cells and can play a role in organ specific development of metastases, such as bone metastases. We explored the possibilities to identify and quantify EVs in archival frozen plasma samples of patients with metastatic *EGFR+* NSCLC in **chapter 5.**

As efficacy of EGFR-TKI in patients with *EGFR+* NSCLC and bone metastases is unknown, we first performed a systematic review to evaluate this question (**chapter 6**), and subsequently in **chapter 7** we retrospectively studied the incidence of SREs in patients with *EGFR+* NSCLC treated with Osimertinib.

In **chapter 8** the results of the studies presented in this thesis are discussed and placed in a broader context.

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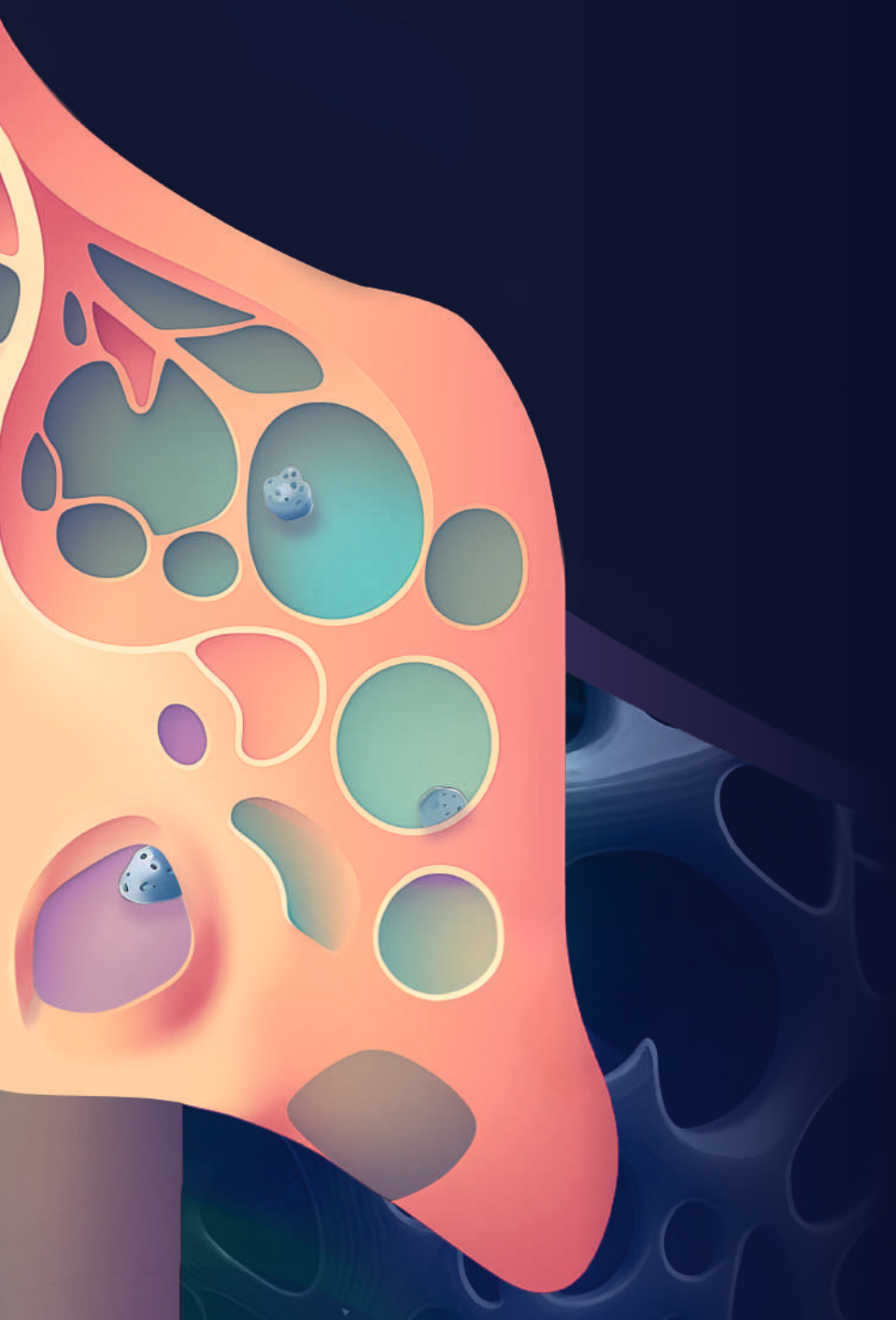
CHAPTER 2



Non-radiation based pain relief treatment options for patients with non-small cell lung cancer and cancer induced bone pain: a systematic review

Anita J.W.M. Brouns, Ben H. De Bie, Marieke H.J. van den Beuken-van Everdingen, Anne-Marie C. Dingemans, Lizza E.L. Hendriks

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ABSTRACT

Introduction

Cancer induced bone pain (CIBP) is frequent in non-small cell lung cancer (NSCLC) patients. Radiation therapy continues to be the gold standard for treatment of painful bone metastases, however only a limited number of metastases can be irradiated. We evaluated non-radiation based early CIBP relief options in NSCLC through a systematic review.

Methods

Systematic review including all prospective articles published between 01-1994 and 06-2020 on Pubmed, Cochrane Library and ClinicalTrials.gov database. Inclusion: non-radiation based trials evaluating CIBP early pain relief options (initially defined as pain score evaluated within two weeks, because of no randomized trials, later inclusion broadened to pain score evaluated within six weeks) in ≥10 NSCLC patients. Radioisotope trials were excluded as these treatments have interactions with systemic anticancer therapy.

Results

188 articles were found; 10 articles (6 randomized controlled (4 double blinded), 1 phase II single-arm, and 3 prospective trials) fulfilled the inclusion criteria. Six of these trials consisted of ≥2 treatment arms, whereas the others were single-arm studies. In total, 554 NSCLC patients were evaluated in these trials. The included trials were very heterogeneous regarding evaluated treatment options, methods of pain measuring, and endpoints. No high-level evidence for specific early pain relief treatment options was found.

Discussion

Non-radiation based studies evaluating treatment options to rapidly reduce CIBP in NSCLC are scarce. This systematic review shows that there is no high-level evidence to recommend a specific treatment for early pain relief. Future research should focus on early pain relief treatment options for CIBP in NSCLC.

INTRODUCTION

Bone metastases are diagnosed in 24-60% of non-small cell lung cancer (NSCLC) patients during the course of the disease (1-3). Up to 80% of these patients experience cancer induced bone pain (CIBP) (3). Unfortunately, scarce data is available describing the severity of bone pain in lung cancer patients; only the incidence of bone pain or usage of analgesics is reported (3, 4). In about one fifth of the patients Quality of Life (QoL) worsens after a diagnosis of bone metastases (6). Furthermore, bone metastases are associated with lower overall survival (OS) (6).

Tumor invasion into bone causes osteoclast and osteoblast recruitment and modulation of genes and proteins involved in the bone microenvironment. Numerous factors are involved in the process of bone pain such as nociceptor activation and sensitization, ectopic sprouting of nerve fibers and central sensitization in the spinal cord and brain. Without treatment of the underlying disease and/or local treatment, no bone healing occurs in bone metastases, leading to a vicious circle of CIBP, central sensitization resulting in more pain, and the development of chronic bone pain (7). Therefore, early pain reduction is important.

Due to the high incidence, chronic character, and negative impact on QoL and OS, CIBP is an important issue that needs to be addressed in metastatic NSCLC. According to the World Health Organization (WHO) pain ladder, (bone) pain should first be treated with paracetamol and/or non-steroidal anti-inflammatory drugs (NSAIDs), followed if necessary by adding mild, and later strong opioids (8). The extended use of NSAIDs and opioids is associated with unwanted side effects (e.g., renal, hepatic or gastro-intestinal) (7). Especially in the older population (i.e., most lung cancer patients), opioids can lead to neurological complaints such as dizziness or cognitive clouding, which in turn increases the likelihood of falling with the risk of for example bone fractures (7). Furthermore, several patients are reluctant to take opioids because of fear to become addicted, or because of the side effects (9). Radiotherapy is another effective treatment strategy for bone pain with a complete pain resolution in approximately 50% of the patients (10). Drawbacks of radiotherapy as treatment option are the time delay, as it takes up to 6 weeks before a maximum treatment effect is obtained (although ≥50% of responders have benefit within 1-2 weeks) and a frequently occurring pain flare-up in the first week after radiotherapy (10,11). In addition, radiotherapy is only feasible in patients with a limited number of painful bone metastases.

The European Society for Medical Oncology (ESMO) guideline on metastatic NSCLC (2018) recommends denosumab or zoledronic acid in patients with NSCLC with bone metastases considered at high risk for skeletal related events (SREs) and with a life expectancy of >3 months (level of evidence I, grade of recommendation B) (12). This recommendation is based on the observation that bone targeted agents (BTAs) reduce SREs. Of note, pain scores are not included in the definition of SRE, although necessity for radiation because of painful bone metastases is included. For denosumab it was found that in patients with bone metastases and no/mild baseline CIBP, time to pain interference with daily life was longer compared with zoledronic acid. The ESMO advice is based on randomized phase III trials that included solid tumors (approximately

50% NSCLC) and early pain relief was not a primary objective of these trials (12). Trials including patients with bone metastases from prostate- or breast- or lung cancer (N=607 of which 1 NSCLC), which evaluated the effect of ibandronate (intravenous or oral) on bone pain showed pain relief within seven days after start of ibandronate (13-15). However, most of the patients received concomitant antineoplastic treatment, therefore a pain relief effect of the systemic anti-cancer therapy cannot be excluded and is it difficult to evaluate the therapeutic effect on CIBP of bisphosphonate therapy alone.

The ESMO guideline on bone health in cancer patients states that multidisciplinary management (e.g., systemic treatments, radiation therapy, surgery and supportive care) is needed for effective treatment of metastatic bone disease. They suggest radiotherapy as treatment of choice in localized CIBP, but no specific treatment recommendations are made for diffuse CIBP (9). The National Comprehensive Cancer Network (NCCN) guideline on NSCLC and National Institute for Health and Care Excellence (NICE) flowcharts on lung cancer mention radiotherapy as pain relief option in CIBP (16, 17).

Survival is improving in patients with NSCLC, partly because of the survival benefit seen with immune checkpoint inhibitors for a large proportion of patients and partly due to the availability of tyrosine kinase inhibitors for the group of patients with an oncogenic driver. As it is possible that these patients live longer with CIBP, effective pain reducing treatment might be more relevant. We performed a systematic review specifically focusing on non-radiation based pain relief options for NSCLC patients with CIBP.

MATERIALS AND METHODS

Search strategy and selection criteria

A systematic search of the literature published between January 1994 and June 2020 was performed using the PubMed, the Cochrane Library and the ClinicalTrials.gov database. Published studies were identified using a search strategy based on the Patient-Intervention-Control-Outcome (PICO) method (shown in Table 1 in the Supplementary Material) (18). PRISMA 2009 checklist for systematic reviews is shown in Table 3 in the Supplemental Material. Our clinical question was to assess the efficacy of CIBP relief treatment options in patients with NSCLC. Initially, we defined early pain relief as pain reduction within two weeks. As we identified only one trial, and to be as inclusive as possible, we expanded the time to six weeks because in this period the maximum effect of radiotherapy occurs. We excluded radiotherapy because aforementioned drawbacks, and radioisotopes since the possible interaction with systemic treatment which is the mainstay of treatment for the majority of patients with NSCLC (19). The main inclusion criteria were 1) prospective trials focusing on treatment options for early pain relief, 2) inclusion of a minimum of 10 patients with NSCLC and with at least one bone metastasis. All inclusion criteria for this systematic review are summarized in Table 2 in the Supplementary Material.

Study Selection

Two authors (A.B. and B.D.B.) independently screened the titles of the selected studies and subsequently the abstracts of the eligible studies. The same authors independently examined the full texts of the selected articles regarding the inclusion criteria. Studies were included if they met the eligibility criteria. To complete the search, the references of all eligible articles were manually searched for additional relevant articles. Also, the excluded review articles were screened for relevant studies which were not represented in the original search. The entire search and selection were independently checked by a third reviewer (L.H.). In case of disagreement during study inclusion, consensus was sought.

Data selection

When available and applicable, the following data were extracted from eligible studies by one author (A.B.) and independently by another author (B.D.B.): year of publication, number of study arms, randomization method, duration of study and follow-up, histological diagnosis, intervention (i.e., type, dose, duration, route and frequency), method of pain score (e.g. bone pain inventory [BPI]), timing of pain score, efficacy of intervention on pain relief, whether results were specifically for NSCLC or for all included patients, and primary and secondary objectives of the trials. Final approval of the extracted data was performed by L.H.

We did not perform a formal risk of bias assessment and a formal test of heterogeneity because of the heterogeneous type of trials included in the systemic review, with one third of the included trials being single arm (i.e., per definition high risk of bias).

RESULTS

Study selection

The literature search identified 186 articles in total without duplicates. As mentioned in the inclusion criteria, reviews were excluded in the search strategy, but to broaden the search results these reviews were manually searched for relevant studies. After checking the reference list of the reviews identified with the systematic search, 2 additional relevant articles were included. Of these 188 articles, 151 were excluded because of non-relevant titles. 18 of the 37 remaining articles were excluded because they did not fulfill the inclusion criteria based on the abstract. After screening of the full text of the remaining 19 articles, 14 articles were excluded because of: no answer on clinical question (N = 4), radiotherapy as treatment modality (N = 1), radioisotopes as treatment modality (N = 1), retrospective study or case report (N = 2), and a language barrier (e.g., Chinese, Japanese or Serbian language, N = 6). After manual search of reference list of included articles 5 other relevant articles were included. The flowchart for article selection is shown in Figure 1.

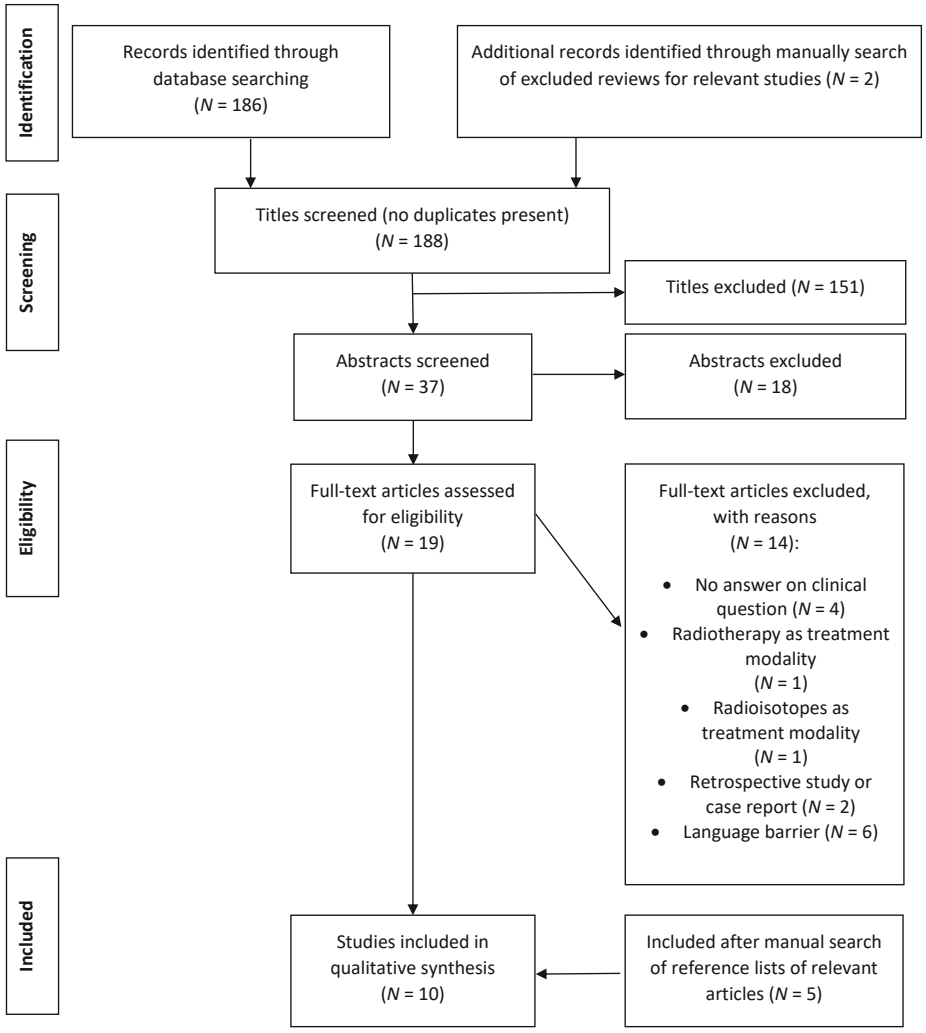

Figure 1: Flowchart for article selection.

Description of studies

One phase II trial (21), six randomized controlled trials (22-27) and three other prospective series (28-30) were included in this review. The randomized controlled trials were double-blinded in four trials (24-27) (of which one was placebo-controlled) and open label in two (22, 23). Three studies were single-arm (21, 28, 29) six were 2-arm (22, 24-27, 30) and one 3-arm (23). In the study of Zarogoulidis et al. the selection was made on the presence of bone pain (30). The main characteristics of the included studies are shown in Table 1. Four studies included only patients with NSCLC, with a minimum of one bone metastasis (21, 22, 28, 30). The other studies also

included patients with CIBP caused by bone metastases of solid malignancies (256 out of 978 patients in total had NSCLC) (23-27, 29). The number of patients with NSCLC enrolled in the studies ranged from 14 (24, 28) to 144 (30), leading to in total 554 patients with NSCLC included in this review. Only in five trials patients received pain modifying therapy alone (23-25, 27, 28), in all other trials other anticancer treatment (mainly chemotherapy) was given (21, 22, 26, 29, 30). The inclusion criteria were quite similar across the included trials. The only difference was the treatment history of the patients e.g., pre-treated with radiotherapy (28), chemonaïve (29) and presumably chemonaïve (22, 30) and pre-treated with chemotherapy and/or radiotherapy (21, 26). In the other trials no information about previous therapy is provided (23-27). The exclusion criteria concerning comorbidities were comparable among eight studies (21-27, 29). In two other studies no exclusion criteria were mentioned (28, 30).

The primary objectives of the included trials varied from efficacy and safety of combined treatment of chemotherapy and zoledronic acid (21, 29) effects of zoledronic acid on bone resorption or formation markers (22), efficacy of treatment on time to progression (TTP), OS (30), reduction in pain intensity (24-27), frequency of breakthrough pain (24, 27), remission rate (24), dose of morphine sulfate (24) and duration-adjusted average change (DAAC) from baseline in the daily NRS worst pain score (26). Six studies had (reduction in) pain score as primary or secondary objective (23-28). Three studies assessed bone pain or change in BPI from baseline as secondary objectives (22, 29, 30). The study of Davidov et al. measured pain scores as an exploratory objective (21). Table 1 provides a detailed summary of all outcome variables of the included studies.

Results of individual studies

Method and timing of pain score

The studies of Yoh et al. and Zarogoulidis et al. used the BPI as method of measuring pain score (29,30). In Zarogoulidis's study the BPI was scored each clinical visit (the interval between study visits was not further specified) (30), whereas in Yoh's et al. study this assessment was performed at baseline and after six weeks of treatment (29). Three studies used the visual analogue scale (VAS) expressed in figures or millimeters as method of measuring pain score (23, 24, 27). The pain assessments took place within the first week of treatment (23, 24, 26, 27) till four weeks (23, 26, 27). Two other studies were not focused on a direct pain score, they evaluated pain response indirectly with the use of analgesics (21, 28). This was evaluated after one and three cycles of chemotherapy (e.g. first measurement at three-four weeks after start of anticancer treatment) (21) and within 48 h after the intervention with a re-evaluation after two weeks (28). One study used the McGill-Melzack pain score, which was performed at baseline and after one and three months of treatment (22). The numeric rating scale (NRS) as method of measuring pain score was used by two trials (25, 26), one double blind randomized trial only recorded till day three of treatment (25), whereas the other recorded daily till day 35. Details on methods and timing of pain score are in Table 2.

Table 1: Main characteristics of included studies.

Study (year)	Trial type	Total pts/ NSCLC pts	Treatment arm	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Yousef (2019) (27)	Phase NR, prospective Randomized, 2 arms, single center	19/100	Sublingual fentanyl[%]	Piroxicam fast-dissolving tablets[%]	1	Pain intensity reduction, frequency of BTP attacks, onset of pain relief	Functional interference items of BPI
Liu (2017) (23)	Phase NR, prospective Randomized 3 arms, single center	95/342	Diclofenac + celecoxib + morphine sulfate*	Diclofenac + morphine sulfate* Celecoxib + morphine sulfate*	NR	VAS score, remission rate, breakthrough pain, dose of morphine sulfate	Tox
Davidov (2013) (21)	Phase NR, prospective Single-arm, single center	53/53	Cycles of Gem (1250mg/m2) on d1+8, CDDP (80mg/m2) on d1 and ZOL 4mg IV once Q3-4w	None	NR 14 (mean)	Efficacy, safety of ZOL IV	NA
Sjöland (2013) (26)	Phase NR, prospective Randomized, double, 2 arms, multicenter	39/152	Flexible dose pregabalin[$] as add-on to stable opioid analgesic therapy	Placebo as add-on to stable opioid analgesic therapy	NR	DAAC from baseline in daily worst pain on first day stable dose to day 28	DAAC for average pain, NRS sleep interference scores, change in mBPI-sf, HADS change, change in PGIC score
Sima (2012) (25)	Phase NR, prospective Randomized 2 arms, multicenter	75/246	1-3 Oxycodone/ paracetamol (5/325mg) Q6H d1-3[#] + morphine/ fentanyl patches	Placebo + morphine/ fentanyl patches	NR	PID	Breakthrough pain, rescue morphine consumption
Yoh (2012) (29)	Phase II Single-arm, single center	35/35	1-4 cycles of CDDP (80mg/m2), D (60mg/m2) and ZOL 4mg IV on d1 Q3-4w	None	16	Feasibility of CDDP, D and ZOL	Tox, SRE, pain scores, best ORR, OS
Francini (2011) (22)	Phase NR Randomized 2 arms, single center	55/55	ZOL 4mg IV Q4w and CTX (CDDP/GEM, CDDP/VBN, CDDP/GEM + BEV)	IBA 50mg q.d. and CTX (CDDP/GEM, CDDP/VBN, CDDP/GEM + BEV)	NR	Effect ZOL and IBA on s-CTX and B-ALP	Bone pain, SRE, time to first SRE, TTP of BM, OS

Table 1: Main characteristics of included studies.

Study (year)	Trial type	Total pts/ NSCLC pts	Treatment arm	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Zarogoulidis (2009) (30)	Phase NR, prospective Non-randomized, 2 arms, single center	144/144	Pts with bone pain: ZOL 4mg IV Q4w and 1-8 cycles of D (100mg/m2), carbo (AUC = 6) In case of response to CTX: 50Gy RTX to primary site between 2-3rd cycle	Pts without bone pain: 1-8 cycles of D (100mg/m2), carbo (AUC = 6) In case of response to CTX: 50Gy RT to primary site between 2-3rd cycle	NR	TTP, OS	Bone lesion response, change in BPI, change in biochemical markers of bone resorption
Rodríguez (2003) (24)	Phase NR, prospective Randomized, 2 arms, multicenter	14/113	Dexketoprofen trometamol 25mg q.i.d.	Ketorolac 10mg q.i.d.	NR	Pain intensity at d 7 +1	Pain intensity at d 3 ± 1, %pts reaching PID ≥20mm from baseline/ pain level <30 mm on VAS at d7, QoL, analgesic efficacy, %pts withdrawn from study, %pts needed rescue medication
Gangi (1994) (28)	Phase NR Observational, prospective study, single center	14/25	Percutaneous injection of 3-25ml ethanol 95%	None	NR	Pain score	NA

Abbreviations: NR: not reported; BTP: break through pain; VAS: Visual Analogue scale; Tox: toxicity; Gem: gemcitabine; CDDP: cisplatin; D: docetaxel; ZOL: zoledronic acid; IV: intravenous; d: day; Q: every; SRE: Skeletal Related Event; ORR: objective response rate; OS: overall survival; NA: not applicable; DAAC: duration-adjusted average change; NRS: numeric rating scale; mBPI-sf: modified Brief Pain Inventory Short Form; HADS: Hospital Anxiety and Depression Scale; PGIC: Patient Global Impression of Change; Q6H: every six hours; PID: pain intensity difference; w: week; CTX: chemotherapy; VBN: vinorelbine; BEV: bevacizumab; IBA: ibandronate; q.d.: once a day; s-CTX: serum C-telopeptide of collagen type I; B-ALP: bone-alkaline phosphatase; TTP: time to progression; Pts: patients; BM: bone metastasis; carbo: carboplatin; AUC: area under the curve; RT: radiotherapy; BPI: bone pain inventory.

* Dosage of painkillers was as follows: diclofenac 100mg/12h; celecoxib 400mg/day; morphine sulphate 10mg/12h, with a reduction of 50% or addition of 25% each time until the VAS score was <5.

$ Dosage of pregabalin was as follows: 100, 150, 300 or 600mg/day.

The amount of placebo or oxycodone/paracetamol tablets was titrated step by step based on the pain assessment, up to 12 tablets per day maximum.

% Doses were adjusted individually. The effective dose was defined as the dose needed to control BTP (pain reduction by 50% in each pain episode without the occurrence of relevant adverse events.

Table 2: Overview of reported items on early bone pain relief.

Study (y)	Method of pain score	Timing of pain score	Pain score on baseline	Efficacy treatment on pain	Results specified for NSCLC only	Duration of response
Yousef (2019) (27)	VAS	3d, 1, 2, 3, 4 w	Fentanyl: 8.09 ± 0.75 Piroxicam: 8.3 ± 0.75	At 1 m Fentanyl: 3.37 ± 0.74 Piroxicam: 3.47 ± 0.76	No	NR
Liu (2017) (23)	VAS	1st, 2nd, 4th w after treatment	Dicl+ Cele: 8.48 ± 1.06 Dicl: 8.53 ± 1.06 Cele: 8.50 ± 1.06	At d 28[2] Dicl+ Cele: 2.40 ± 1.20 Dicl: 3.50 ± 0.70 Cele: 3.40 ± 0.70	No	NR
Davidov (2013) (21)	NR	After 1 and 3 cycles	NR	*After 1 cycle:* 5/53 pts reduced their analgesic need, 14/53 pts needed more pain medication, 34/53 pts showed no change in pain medication	Yes	NR
Sjöland (2013) (26)	NRS	Each day	NR	Mean change DAAC pregabalin: -1.53 (1.81) Mean change DAAC (SD) placebo: -1.23 (1.74)	No	NR
Sima (2012) (25)	NRS	d 1-3	Placebo: 5.3 Oxycodone/ paracetamol: 5.2	At d 3 PID placebo: 0.3 Oxycodone/ paracetamol: 1.5	No	NR
Yoh (2012) (29)	BPI	Baseline, after 6 w	mean BPI 2.6 ± 0.2	mean BPI 1.0 ± 0.3 at 6 w (p<0.0001)	Yes	NR
Francini (2011) (22)	McGill-Melzack pain questionnaire	Baseline, after 1 and 3 m	ZOL 1.98 ± 1.12, IBA 1.88 ± 0.89	"Trend for more rapid decrease in bone pain score in favor of ZOL"	Yes	NR

Table 2: Overview of reported items on early bone pain relief.

Study (y)	Method of pain score	Timing of pain score	Pain score on baseline	Efficacy treatment on pain	Results specified for NSCLC only	Duration of response
Zarogoulidis (2009) (30)	BPI	Each clinical visit	78 pts ≤ 4, 8 pts 4 - 6, 1 pt > 8	"no significant difference between treatment arms in pain effect of ZOL compared to baseline"	Yes	NR
Rodríguez (2003) (24)	VAS in millimeters	d 3 ± 1, d 7 + 1	Dexketoprofen: 69 ± 15 Ketorolac: 75 ± 16	At d 7 Dexketoprofen: 32 ± 24 Ketorolac: 40 ± 30	No	NR
Gangi (1994) (28)	Indirect measured by scale according to reduction of opiate analgesics[1]	<48h after intervention, re-evaluation after 2w	"Pain relief insufficient after treatment with opiate analgesics and RTX and/or CTX"	55% of pts score ≥3, 74% of pts score ≥2, 26% of pts score 1[1]	No	10-27 w

Abbreviations: VAS: Visual Analogue scale; w: weeks; Dicl: diclofenac; Cele: celecoxib; d: days; NR: not reported; pts: patients; NRS: numeric rating scale; BPI: brief pain inventory; m: months; RTX: radiotherapy; CTX: chemotherapy.

[1] Scale consists of the following items: Score 4: complete relief (opiate analgesic drugs no longer necessary), score 3: very good but incomplete relief (75% reduction of analgesic requirement), score 2: good relief (25-50% reduction of analgesic requirement), score 1: little of no relief (<25% reduction or no change of analgesic requirement).

[2] Gr 1: diclofenac and celecoxib, Gr 2: diclofenac, Gr 3: celecoxib.

[3] VAS scores at day 7 and 14 are shown in the original article, they showed superiority in pain reduction the diclofenac and celecoxib group.

Efficacy of treatment on pain and duration of response

Five studies (including 217 patients with NSCLC, out of 554 patients included in total) showed a significant treatment effect on pain score (23-25, 27, 29). One double blind randomized controlled trial, including 14 patients with NSCLC out of 113 included patients (10%) evaluating dexketoprofen trometamol versus ketorolac showed superior of the former on pain rating index (secondary outcome, p = 0.04) (24). One open label, randomized controlled trial, showed that diclofenac combined with celecoxib and morphine sulfate was superiority to NSAID monotherapy combined with morphine sulfate in CIBP reduction, measured with VAS (average VAS score at 28 days: 2.40 ± 1.20 vs 3.50 ± 0.70 (diclofenac monotherapy plus morphine) or 3.40 ± 0.70 (celecoxib monotherapy plus morphine), p = 0.006) (23). Another double blind randomized controlled trial, including 75 patients with NSCLC out of 246 included patients (30%) showed an additional effect of the combination of short acting oxycodone/paracetamol versus placebo, added to standard long-acting opioids on reducing bone pain (pain intensity difference (PID) after three days in the placebo group 0.3, compared with 1.5 in the oxycodone/paracetamol group, p<0.001) (25). One, double blind randomized controlled, trial, including 19 patients with NSCLC out of 100 included patients (19%), evaluating fentanyl versus piroxicam for CIBP reduction, reported for both drugs a significant decrease in VAS score at 1 month. No significant difference in efficacy was found between the treatment arms (27). The only study with bisphosphonates, which found a significant effect of treatment on pain score was the single-arm study of Yoh et al. (29). They showed that treatment of both chemotherapy and zoledronic acid reduced pain score at six weeks compared to baseline. In another study no significant difference in pain effect of zoledronic acid between the treatment arms (docetaxel and carboplatin +/- zoledronic acid) was observed (30). Another double blind randomized controlled trial, including 39 patients with NSCLC out of 152 included patients (26%), reported a nonsignificant effect of pregabalin treatment on pain compared placebo (DAAC from baseline in the daily NRS worst pain score -1.53 vs. -1.23). The study of Francini et al. showed a trend for more rapid decrease in bone pain score at one month in favor of zoledronic acid compared to oral ibandronate (22). Davidov et al. found only a reduced analgesic need in five out of 53 patients (10%), whereas most of the patients (34 out of 53 patients [64%]) had no change in pain medication after one treatment cycle (21). One study showed a reduction of minimal 25%-50% of analgesic requirement in 74% of the patients and 55% of the patients had a reduction of 75% of analgesic requirement after treatment with ethanol injections (28). The duration of treatment response was only reported in one study and was ten to 27 weeks (28).

Of note, none of the studies including patients with different primary tumor histologies reported results for the subgroup of patients with NSCLC. Table 2 provides an overview for reported items on bone pain relief.

DISCUSSION

CIBP is a clinically relevant problem in metastatic NSCLC due to the high prevalence of bone metastases, the chronic character and the negative impact on QoL and OS (6). Survival is improving for NSCLC: five-year survival improved from 5% to 31% for patients without targetable mutations, treated with immune checkpoint inhibitors and five-year survival rates are over 40% in patients with an *EGFR* mutation or ALK fusion (31-37). It is possible that some of these patients survive a prolonged time with CIBP that impairs QoL, making effective pain reducing strategies necessary.

To obtain more insight in possible treatment options for early pain relief in patients with NSCLC with bone metastases and CIBP, we performed a systematic review on this topic excluding radioisotopes and radiotherapy for the reasons mentioned above. The initial scope of this review was early pain relief (pain relief evaluated within two weeks of start of treatment), but this resulted in limited number of eligible trials. To be more inclusive, we broadened the time of "early pain relief" to a maximum of six weeks. Even then, only ten studies were eligible. Of note, the included trials were very heterogeneous regarding treatments evaluated, primary endpoints, methods of pain measurement and timing of assessment. Importantly, the randomized trials included patients with different histologies, and patients with NSCLC only comprised a subgroup in these randomized trials (554 [44%] of included patients). Importantly, not all treatments evaluated are comparable with recommended pain treatment in clinical guidelines. For example, according to international and national guidelines for breakthrough cancer pain, shorting-acting morphine should be added to standard dose long-acting morphine to treat breakthrough pain (38). Three of the included studies indeed underscore the importance of adding breakthrough medication to continuous release medication (23, 25, 27). As the comparator arms of these trials included a non-optimal treatment according to current guidelines, the results found in these trials have limited value in daily clinical practice. Another study excluded patients previously or currently treated with a scheduled regimen of painkillers, except acetaminophen and acetylsalicylic acid, which is also not according to the WHO pain ladder (8, 24).

In most other studies (21, 22, 29, 30), systemic therapy and pain relief therapy were administered concurrently, therefore conclusions on the specific efficacy of pain relief therapy were difficult as it cannot be excluded that the systemic therapy also causes a reduction in pain. For zoledronic acid, only one study showed an early pain reduction, but this pain reduction disappeared at three months despite continuous bone targeted agent use (22). While out of the scope of this review, information on long-term pain reduction is also particularly important. Only two studies provided follow-up of more than one year (21, 29). As CIBP is a chronic problem, it is also of interest to know information about the pain efficacy in the long term. However, only the studies of Davidov and Yoh had a follow-up of more than a year (21, 29). Duration of pain response and the recurrence rate of CIBP was lacking.

What are other possible treatment options for CIBP in NSCLC? The first step to achieve early pain relief in CIBP is analgesics according the WHO pain ladder (8). This advice is based on general

pain management recommendations for patients with cancer. As was found in this review, opioids are indeed effective in the treatment of CIBP. Palliative radiotherapy is frequently used in the treatment of CIBP, because of the high response rate (around 85%). Drawbacks are the possibility of a pain flare-up and the limited use in multiple painful bone metastases (10). Besides that, there are disparities in the access to radiotherapy facilities in high and low-income countries. For example, in Central Africa 0.05 machines are available per million people versus 11.4 machines in North America (39). Furthermore, even if there is access, older, multi-fractionated radiotherapy schedules for treatment of painful bone metastases are often used, instead of the recommended single-fraction radiotherapy, as was shown in a survey on radiation facilities in African countries (40). This further limits the access to (up-to date) radiotherapy facilities and strengthens the need for other "early pain relief options for patients with NSCLC." Bisphosphonates and denosumab are also used to tread CIBP. Trials including patients with breast and prostate cancer with uncontrolled CIBP indeed showed a reduction in pain scores with (loading doses) of bisphosphonates. However, data in NSCLC is scarce and results found in our systematic review do not show a clear reduction in CIBP in NSCLC. After our search, the NVALT-9 trial was published, and in contrast to the studies including patients with breast- or prostate cancer, loading doses of ibandronate did not lead to rapid bone pain relief in patients with NSCLC and uncontrolled bone pain (41). Denosumab was compared with zoledronic acid in a randomized phase III trial (1596 patients with solid tumors and at least one bone metastasis, 702 patients had NSCLC, patients with breast or prostate cancer were excluded). Primary endpoint was time to first on-study SRE, pain worsening was one of the other endpoints. Denosumab significantly delayed the time to pain worsening (HR, 0.83; 95% confidence interval, 0.71-0.97) in patients with no/mild baseline pain, compared to zoledronic acid (42). Results regarding early pain reduction in patients with baseline CIBP are not available. Unfortunately, the recently published randomized phase III Splendour trial, including only patients with advanced NSCLC (inclusion irrespective of presence of bone metastases), did not report data on the effect of denosumab on pain relief in the subgroup of patients with painful bone metastases (43). The trial design was to evaluate whether the addition of denosumab to standard first-line treatment improved OS; the primary endpoint was not met (12).

To the best of our knowledge, there is no explanation why for example bisphosphonates showed a reduction of CIBP in some malignancies but not in lung cancer (13–15, 41, 44, 45). Possible explanations are differences in tumor histology/biology or bone metastasis metabolism [although bone turnover markers are comparable between for example breast and lung cancer (46)], which leads to different response on bone pain relief options, are probably the most obvious. Also, the usage of different concomitant (systemic) therapies, which differs among malignancies, could strengthen pain control (47). Therefore, specific recommendations for (bone) pain relief are needed for different malignancies and findings cannot be extrapolated.

Radioisotopes (e.g., samarium, strontium, and rhenium) are an alternative treatment for CIBP. Radioisotopes have a rapid onset of action, but data on NSCLC are limited and consist only of subgroup analyses (19). Zoledronic acid combined with radioisotopes is another treatment option.

The efficacy of adding a radioisotope (choice at discretion of investigator) to zoledronic acid was evaluated in the randomized phase III RTOG 0517 trial [26/262 included patients had lung cancer] (48). Primary endpoint was time to SRE development, pain control was a secondary endpoint. Only patients with stable or no bone pain were included. As a subgroup of patients did not have CIBP, and one of the treatment arms consisted of radioisotopes, we excluded this study in our article selection. The addition of radioisotopes resulted in superior pain control at one month, compared with zoledronic acid alone (median pain score of 0 versus 1, p=0.02). Subgroup analysis regarding the primary tumor histology or the presence of baseline CIBP were not performed (48). Because the relatively short duration of action of radioisotopes, it is expected that this treatment must be repeated several times if the patient has a prolonged survival.

Immune checkpoint inhibitors have become standard of care treatment for most patients with advanced NSCLC and result in durable responses in a subgroup of patients. For the subgroup of patients with oncogenic drivers, tyrosine kinase inhibitors often result in early and prolonged responses. For both classes of drugs, effects on CIBP have not been specifically reported. It is possible that in some patients, immune checkpoint inhibitors will not be very active in pain relief for CIBP, as Schmid et al. reported that efficacy of immunotherapy depends on the metastatic location: the treatment efficacy is less in bone lesions compared to lymph nodes (49). However, CIBP related outcomes have not been reported. Denosumab in combination with nivolumab, a programmed death-1 (PD-1) inhibitor, is currently under evaluation in patients with NSCLC and bone metastases (NCT03669523) with the overall response rate as primary outcome measurement. Time to first SRE is one of the secondary outcome measurements but there is no specific focus on pain relief. A phase II study with AL2846, a multi-target tyrosine kinase receptor inhibitor versus zoledronic acid in bone metastasized NSCLC (NCT04325776) is not yet recruiting. The primary endpoint is time to first SRE, and effectiveness of improving average daily pain (not specifically CIBP) is one of the secondary outcomes. Another, not yet recruiting, phase IV, study is zoledronic acid combined with radiotherapy for bone metastasis of NSCLC (NCT02480634). The primary outcome of this study is the percentage of patients who reach objective bone pain response.

Experimental studies in animal models of CIBP have shown alterations in e.g., astrocytes or in the sphingolipid metabolism in the spinal cord or showed the importance of connexins in the cell-cell communication with probable effects on CIBP. Recently, different studies focused on therapeutic options to block or alter these pathophysiological changes. Blockade of interleukine-6 signaling is promising as it could lead to prevention or delay of bone remodeling as well as decreased pain intensity (4).

Some possible drawbacks for this systematic review exist. A point for discussion could be the chosen definition of early pain reduction (pain reduction within six weeks). We chose this upper limit to be as inclusive as possible to include treatment options that resulted in pain reduction within a relatively short term. Of note, for pain reduction treatment options within a shorter time frame (e.g., one or two weeks), even less data is available. As for example bisphosphonates have

different activity on early CIBP reduction in breast- and prostate cancer compared with NSCLC, we did not broaden our inclusion criteria to include other tumor types (13-15, 41).

Furthermore, as expected with over half of the included trials being single arm and/or not blinded as shown by the Jadad score the methodological quality of most included trials is poor. Last, we did not include a formal test of heterogeneity, as only very heterogeneous trials met our inclusion criteria.

CONCLUSION

In conclusion, despite the frequent occurrence of CIBP combined with the negative effects on QoL and OS, literature on the optimal treatment of CIBP in NSCLC is lacking. Most of the recommendations given in current guidelines are mainly based on data obtained in other tumors such as breast and prostate. Therefore, randomized trials evaluating treatment options with early pain relief for CIBP are necessary in lung cancer patients.

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SUPPLEMENTARY MATERIAL

Table 1: Search Strategy based on PICO method.

PICO		Free search terms		MESH terms
Patient		Carcinoma Non-Small-Cell Lung Carcinoma non small cell lung Non-small-cell lung carcinoma Non-small-cell lung cancer NSCLC Lung neoplasm Lung neoplasms	OR	Carcinoma, Non-Small-Cell Lung Lung neoplasms
	AND	Distant metastasis Distant metastases Bone metastasis Bone metastases Stage IV Metastatic disease	OR	Neoplasm metastasis
	AND	Pain Neuropathic pain Palliative care Cancer palliative therapy Cancer pain Bone pain	OR	Musculoskeletal pain Nociceptive pain Cancer pain Neuralgia Pain Acute pain Palliative care
Intervention	AND	Non opioids Paracetamol Acetaminophen APAP Non steroidal anti inflammatory drugs NSAID Morphine Opioid Antiepileptic Anticonvulsant Antidepressant Anti-depressant Tricyclic antidepressant Selective serotonin and noradrenalin reuptake inhibitor Selective serotonin reuptake inhibitor Serotonin inhibitor N methyl d aspartate receptor antagonist NMDA receptor antagonist Corticosteroid Glucocorticoid Bisphophonate Disphophonate	OR	Analgesics, non-narcotic Buprenorphine Fentanyl Hydromorphone Oxycodone Tapentadol (supplementary concept) Tramadol Methadone Anticonvulsants Gabapentin (supplementary concept) Pregabalin Valproic acid Phentoin Carbamazepine Lamotrigine (supplementary concept) Topiramate (supplementary concept) Antidepressive agents, Antidepressive agents, tricyclic Antidepressive agents, second-generation

Table 1: Search Strategy based on PICO method.

PICO		Free search terms		MESH terms
Intervention	AND	Denosumab	OR	Adrenergic uptake inhibitors
		Cannabinoid		Serotonin and noradrenaline reuptake inhibitors
		Cannabis sativa		
		Cannabis		Serotonin uptake inhibitors
		Local anesthetics		Imipramine
		Lidocaine		Venlafaxine hydrochloride
		Capsaicin		Bupropion
		Botulinum toxin A		Citalopram
		Botulinum toxin		Clomipramine
		BTX-A		Desipramine
		Aceclofenac		Fluoxetine
		Benzydamine		Mirtazpine (supplementary concept)
		Diclofenac		Paroxetine
		Ibuprofen		Sertraline
		Indometcin		
		Meloxicam		
		Naproxen		
		Piroxicam		
		Tiaprofenic acid		
		Buprenorphine		
		Fentanyl		
		Hydromorphone		
		Oxycodone		
		Tapentadol		
		Tramadol		
		Gabapentin		
		Pregabalin		
		Valproate		
		Valproic acid		
		Phenytoin		
		Carbamazepine		
		Topiramate		
		Levetiracetam		
		Oxcarbazepine		
		TCA		
		SNRI		
		SSRI		
		Nortriptyline		
		Duloxetine		
		Imipramine		
		Venlafaxine		
		Bupropion		
		Citalopram		
		Clomipramine		
		Desipramine		
		Fluoxetine		
		Mirtazapine		

Table 1: Search Strategy based on PICO method.

PICO		Free search terms		MESH terms
Intervention	AND	Paroxetine	OR	
		Sertraline		
		Ketamine		
		Memantine		
		Amantadine		
		Methadone		
		Cortisone		
		Dexamethasone		
		Fludrocortisone		
		Hydrocortisone		
		Prednisolone		
		Prednisone		
		Triamcinolone		
		Alendronic acid		
		Alendronate		
		Ibandronic acid		
		Ibandronate		
		Pamidronic acid		
		Pamidronate		
		Zoledronic acid		
		Clodronic acid		
		Zoledronate		
		Ropivacaine		
		Bupivacaine		
		Levobupivacaine		
		Risedronic acid		
		Lamotrigine		
		Amitriptyline		
		Doxepin		
		Dextromethorphan		
Comparator		Not specified in search strategy in order to include single arm studies		
Outcome	AND	Acute pain relief	OR	Pain measurement
		Pain intensity		Pain Management
		Pain control		
		Pain measurement		
		Pain response		

Abbreviations: NSCLC, non-small-cell lung cancer; APAP, acetaminophen; NSAID, non-steroidal anti-inflammatory drugs, NMDA, N-methyl-D-aspartate; BTX-A, botulinum toxin-A.

Table 2: Inclusion criteria.

Criterion	Definition
Subjections included	Human only.
Language	No restrictions.
Article type	Original articles; reviews excluded.
Study phase	No restrictions, but retrospective series were excluded.
Year of publication	1994 - September 2018.
Site of primary tumor	NSCLC with at least one bone metastasis, at least 10 NSCLC patients included in the study.
Age	≥ 18 years.
Treatment	No restrictions on treatment for NSCLC. Treatment for pain should be focused on acute pain relief. All pain relief options, except radiotherapy and radioisotopes, are allowed.
Follow-up period	No lower or upper limit.
Outcome	Efficacy of analgesics on early pain relief (< 6 weeks) in patients with bone metastasized NSCLC.

Abbreviations: NSCLC, non-small-cell lung cancer.

Table 3 - PRISMA 2009 checklist for systematic reviews

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	28-30.
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	28, 30-31.
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	29-30.
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	30, Table 1 Supplementary Material.
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	30-31, Table 1.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	30, 31.

Table 3 - PRISMA 2009 checklist for systematic reviews

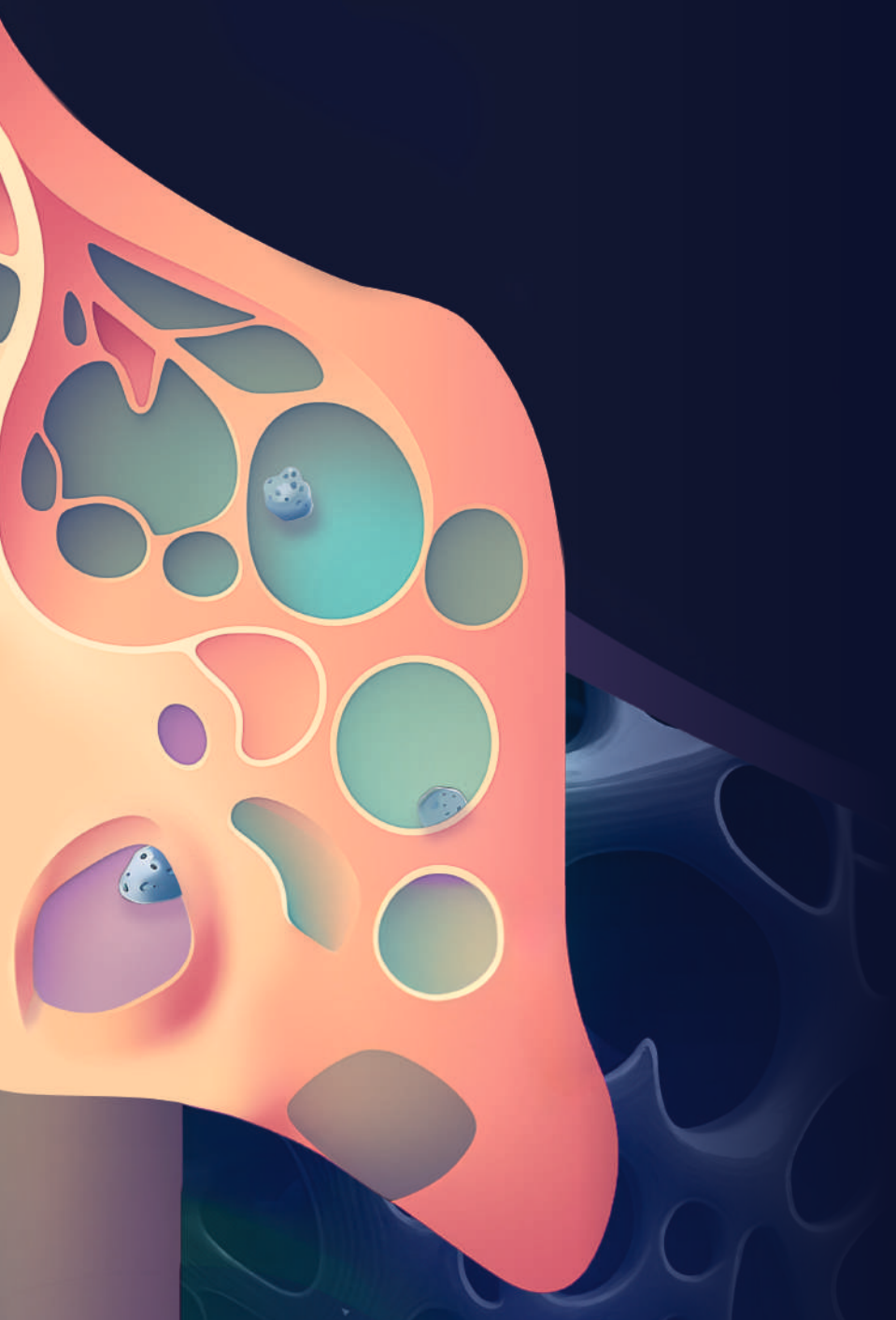
Section/topic	#	Checklist item	Reported on page #
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Table 1 Supplementary Material.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	30-31.
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	31.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	30.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	30.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA.
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA.
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	30.
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	31, Figure 1.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	32, 33, Table 1 + 2.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA.
RESULTS			
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	31.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA.

Table 3 - PRISMA 2009 checklist for systematic reviews

Section/topic	#	Checklist item	Reported on page #
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	39-42.
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	39-42.
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	39-42.
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA.

Abbreviations: NA: not applicable.
From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

CHAPTER 3



Efficacy of ibandronate loading dose on rapid pain relief in patients with non-small cell lung cancer and cancer induced bone pain: the NVALT-9 trial

Anita J.W.M. Brouns, Lizza E.L. Hendriks, Vincent van der Noort, Ben E.E.M van de Borne, Franz M.N.H. Schramel, Harry J.M. Groen, Bonne Biesma, Hans J.M. Smit, Anne-Marie C. Dingemans

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ABSTRACT

Introduction

Approximately 80% of non-small cell lung cancer (NSCLC) patients with bone metastases have cancer induced bone pain (CIBP).

Methods

The NVALT-9 was an open-label, single arm, phase II, multicenter study. Main inclusion criterion: bone metastasized NSCLC patients with uncontrolled CIBP (brief pain inventory (BPI) ≥ 5 over last 7 days). Patients were treated with six milligram ibandronate intravenously (day 1-3) once a day. Main exclusion criteria: active secondary malignancy, systemic anti-tumor treatment and radiotherapy ≤4 weeks before study start, previous bisphosphonate treatment. Statistics: Simon's Optimal two-stage design with a 90% power to declare the treatment active if the pain response rate is ≥ 80% and 95% confidence to declare the treatment inactive if the pain response rate is ≤ 60%. If pain response is observed in ≤12 of the first 19 patients further enrollment will be stopped. Primary endpoint: bone pain response, defined as 25% decrease in worst pain score (PSc) over a 3-day period (day 5-7) compared to baseline PSc with maximum of 25% increase in mean analgesic consumption during the same period. Secondary endpoints: BPI score, quality of life, toxicity and World Health Organization Performance Score.

Results

Of the 19 enrolled patients in the first stage, 18 were evaluable for response. All completed ibandronate treatment according to protocol. In 4 (22.2%), a bone pain response was observed. According to the stopping rule, further enrollment was halted.

Discussion

Ibandronate loading doses lead to insufficient pain relief in NSCLC patients with CIBP.

INTRODUCTION

Cancer induced bone pain (CIBP) is an important issue in metastasized non-small cell lung cancer (NSCLC). During the course of the disease, 24-60% of NSCLC patients are diagnosed with bone metastases and up to 80% will experience CIBP (1-3). Furthermore, bone metastases have a negative influence on quality of life (QoL) and are associated with a poorer overall survival (OS) (4). Radiotherapy is an effective treatment for CIBP with a 50% chance of complete pain resolution, but it unfortunately has several drawbacks. Examples are a time delay before the maximum treatment effect is obtained, the possibility of a pain flare-up, and it is only feasible in patients with a limited number of bone metastases (5). In general, pain management, according to the World Health Organization (WHO) pain ladder (6), frequently results in treatment with opioids. Especially in this vulnerable population, opioid use can result in neurologic, renal, hepatic and/or gastro-intestinal toxicity (7).

In current guidelines e.g., European Society for Medical Oncology [ESMO], National Comprehensive Cancer Network [NCCN] and National Institute for Health and Care Excellence [NICE]) bone targeted agents such as bisphosphonates are mentioned as an option to prevent skeletal related events (SREs) in NSCLC patients (5, 8-10). However, actual data on (rapid) pain relief of bisphosphonates are scarce in NSCLC (11). Trials including patients with bone metastases from prostate- or breast- or lung cancer (N=607 of which only one NSCLC patient), which evaluated the effect of ibandronate (intravenous or oral) on bone pain showed pain relief within seven days to twelve weeks after start of ibandronate (12-14). Therefore, we performed a multicenter phase II study to evaluate the effect of intravenous loading doses of ibandronate on acute pain response in NSCLC patients with uncontrolled CIBP.

MATERIALS AND METHODS

The primary aim of this open label single arm phase II study (NVALT-9, EudraCT number 2007-000885-20, NTR1602) was to establish the efficacy of intravenous loading doses of ibandronate to achieve acute bone pain relief in NSCLC patients with CIBP. The trial was approved by the appropriate ethics committee (METC 07-2-035.6/ivb).

The trial was performed in eight Dutch hospitals (see Supplementary Data, paragraph 1). The main inclusion criteria were: I) pathologically proven NSCLC with pathologically and/or radiologically confirmed bone metastases with a patient life expectancy of at least one month; II) the pain scored for bone metastases had to correspond to known locations of bone metastases (based on imaging); III) mean bone pain score ≥ five over the last seven days before inclusion on the worst pain scale on the brief pain inventory (BPI), IV) use of nonsteroidal anti-inflammatory drugs (NSAIDs) or a weak opioid base on the WHO analgesic ladder step 2, V) adequate renal function (creatinine clearance as calculated by Cockcroft-Gault method

> 50 ml/min). The main exclusion criteria were I) active secondary malignancies, II) start of anti-tumor treatment within four weeks before study entry, III) bone radiotherapy in the preceding four weeks, IV) bisphosphonate treatment in the previous two months, V) hypocalcemia (serum albumin corrected calcium concentration <2 mmol/L) or hypercalcemia (serum albumin corrected calcium ≥ 2.7 mmol/L).

With the aim of assessing the efficacy of ibandronate on acute bone pain relief, the primary endpoint was acute bone pain response over a seven-day period. This was defined as a 25% decrease in worst bone pain score over day five, six and seven compared to bone pain score at baseline (determined by the "worst pain scale" of the BPI), with no more than a 25% increase in mean analgesic consumption over the same three-day period compared to baseline analgesic consumption. Secondary endpoints were mean worst bone pain scale of the BPI in the first seven days, interference scales of the BPI, analgesic consumption, WHO-Performance Score (WHO-PS), QoL and safety. In the context of safety spontaneous adverse events (scored by Common Terminology Criteria for Adverse Events (CTCAE) version 4.0) were collected.

Patients were treated with six mg ibandronate once a day intravenously on day one, two and three. Concomitant analgesic use was assessed using the WHO pain ladder. On day one to seven patients recorded their worst bone pain score of the BPI and their analgesic consumption in a diary. Patients were evaluated for BPI, WHO-PS and QoL on day one and seven. On day seven, a serum chemistry panel (including serum creatinine) was performed to assess the renal safety of ibandronate. Adverse events were recorded on day one to three, on day seven, and at the end of follow-up (day 28).

The study was designed following a Simon's Optimal two-stage design with a 90% power to declare the treatment active if the pain response rate was ≥ 80% and 95% confidence to declare the treatment inactive if the pain response rate was ≤ 60%. The 60% pain response rate is comparable to expected response rates of radiotherapy and opiates on cancer related pain5, 15. In the first stage, 19 patients were treated and evaluated. If ≤ 12 pain responses were observed the study was stopped. Otherwise, 34 patients would subsequently be enrolled and the treatment would be declared active if ≥ 38 of 53 included patients had a positive pain response.

RESULTS

Between December 2007 and November 2010, 19 NSCLC patients were enrolled in the first stage. 18 out of 19 patients were evaluable for response. The patient characteristics are shown in Table 1. One patient received only one day of study medication, as it was discovered that the patient was ineligible because of previous bisphosphonate use. All other patients received all doses of ibandronate without dose reductions or dose delays.

Table 1: patient characteristics.

Patient characteristics		N=18
Gender, male (%)		12 (67)
Age, mean (range)		58.7 (42-74)
WHO-PS at baseline (%)	0	1 (6)
	1	11 (61)
	2	2 (11)
	3	2 (11)
	Unknown	2 (11)
Prior treatment for malignancy	Surgery for BM, yes (%)	2 (11)
	Chemotherapy, yes (%)	12 (67)
	Palliative radiotherapy to BM, yes (%)	5 (28)
Current analgesics (n/N)	Analgesics according to WHO pain ladder[1]	15/15
	Analgesics according to WHO pain ladder[1] + anti-epileptics	4/15
	Analgesics according to WHO pain ladder[1] + anti-epileptics + methadone	1/15
Patient status at day 28 (%)	Alive, yes	9 (64)
Patient characteristics		N=18
Patient status at day 28 for bone pain responders (%)	Alive, yes	4 (100)

Abbreviations: WHO-PS: World Health Organization Performance Score; BM: bone metastases.
[1] Analgesics according to WHO pain ladder means a one to three step, which starts with non-opioids with or without any adjuvant therapy and increases to opioids for moderate to severe pain with or without any non-opioids or adjuvant therapy.

Patients were treated with analgesics according the WHO pain ladder6. Before study entry, four patients were also treated with anti-epileptics and one patient with methadone because of uncontrolled pain. Except for two patients, none of the patients were able to reduce their analgesic use during the study period and in two other patients, the opioid doses increased. For the primary endpoint of bone pain response, four out of 18 patients (22%) had a ≥25% decrease in worst pain score over day 5-7, therefore the endpoint was not met and the study was discontinued. Figure 1 shows the observed and estimated worst bone pain scores for patients grouped by outcome.

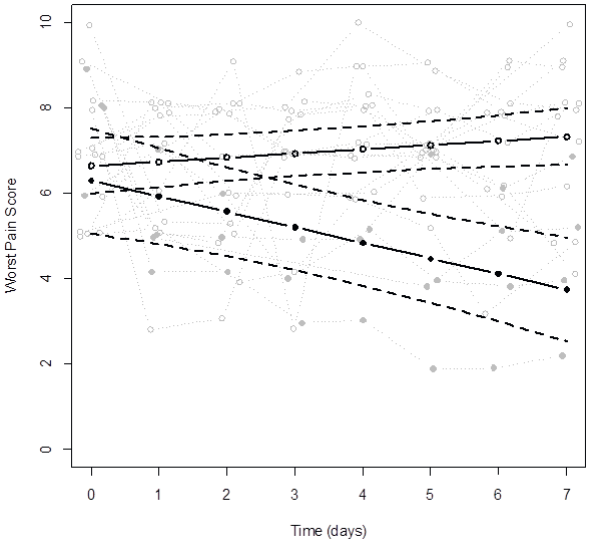

Figure 1: The observed and estimated worst pain scores for patients grouped by outcome (no bone pain responder vs. bone pain responder).
No bone pain response is depicted by open circles (N=14), bone pain response is depicted by solid circles (N=4).

In Table 2 primary and secondary endpoints were shown. For secondary endpoints, two patients (11.1%) reported an improvement of WHO-PS during treatment while 33.3% and 16.7% reported no change or a worsening of WHO-PS, respectively. In seven patients (38.9%) change in WHO-PS was not recorded. No relation between change in WHO-PS and bone pain response was recorded. After 28 days follow-up 5 patients (27.8%) had died. Mean worst bone pain scale of the BPI, interference scales of the BPI, analgesic consumption and QoL revealed no significant or clinically relevant differences between study participants (see Supplementary Data, paragraph 2). No serious adverse events were reported. Two patients experienced ≥ grade 2 hypophosphatemia (one grade 2, one grade 3), which was possibly treatment related.

Table 2: Primary and secondary endpoints.

Primary endpoint		
Bone pain response[1] N, %	Bone pain response, yes	4 (22)
Secondary endpoint		
Mean worst pain score over last seven days at baseline[2] (%)	5	4 (22)
	6	2 (11)
	7	5 (28)
	8	4 (22)
	9	2 (11)
	10	1 96)
Interference scales of the BPI	No significant or clinically relevant difference between scores at day 1 compared to day 7 on al interference scales of the BPI.	
Analgesic consumption	No significant or clinically relevant difference between analgesic consumption at day 1 compared to day 7.	
Change in WHO-Performance Score day 1 compared to day 6 N, %	No change	6 (33)
	Improvement	2 (11)
	Worsening	3 (17)
	Not reported	7 (39)
Quality of Life[3]	No significant or clinically relevant difference between scores at day 1 compared to day 7 on all dimensions of QLQ-C30 questionnaire.	
Spontaneous adverse events N, %	CTCAE grade 2 hypophosphatemia	1 (6)
	CTCAE grade 3 hypophosphatemia	1 (6)

Abbreviations: WHO-PS: World Health Organization Performance Score; BM: bone metastases.
[1] Analgesics according to WHO pain ladder means a one to three step, which starts with non-opioids with or without any adjuvant therapy and increases to opioids for moderate to severe pain with or without any non-opioids or adjuvant therapy.

DISCUSSION

This study of intravenous loading doses of ibandronate in NSCLC patients with CIBP did not show adequate pain reduction in most patients. However, in four out of 18 patients a sufficient pain response was observed. In contrast to most other trials in which patients used concomitant anti-cancer therapy (making evaluation of the effect of the bone targeted agent more difficult), patients in this study were only eligible if they did not start an anti-cancer therapy (systemic therapy and/or radiotherapy) in the four weeks before study entry. To not unnecessarily expose patients to a treatment that was not beneficial enough, a Simon 2-stage design was used, and the trial was stopped due to futility.

CIBP remains important in NSCLC. Despite these older data of ibandronate loading dose for acute pain relief in lung cancer patients with CIBP, no new treatment options with rapid pain relief are currently available. Furthermore, phase II/III trials in metastasized NSCLC evaluating

systemic anti-cancer treatment modalities (i.e., chemotherapy with or without immunotherapy and tyrosine kinase inhibitors) are not focused on rapid bone pain reduction and only report pain as an adverse event of therapy. We show that loading doses of bisphosphonates do not induce a rapid reduction of CIBP and that other strategies should be pursued.

There is evidence of the effects of bisphosphonates on pain reduction for breast cancer, multiple myeloma and prostate cancer (12-14, 16). Analogous to a previous pilot study (13), in which opioid refractory bone pain was relieved by ibandronate loading doses within seven days, ibandronate was the bisphosphonate of choice in our study.

It is unclear why breast or prostate cancer patients responded to loading doses of bisphosphonates in a previous studies (12-14, 17, 18), while the NSCLC patients in our study did not. There could be different explanations why NSCLC patients in this study do not respond to bisphosphonates: I) Influence of tumor histology on the chance of bone pain reduction by bisphosphonates, II) Possible differences in the metabolism of bone metastases between breast cancer and lung cancer (although not shown when evaluating bone turnover markers) [19] or III) Lack of concomitant systemic therapy which resulted in reduced pain control, as in a systematic review and meta-analysis the combination of bisphosphonates and systemic therapy was superior compared with one of the two treatment modalities alone (20).

We observed that bone pain response was not associated with an improvement in WHO-PS; only one of the bone pain responding patients improved while two patients with a reduction in pain had a deteriorating WHO-PS, probably due to progression of cancer. Bone pain response was associated with survival as all four patients in the bone pain responding group were alive at study completion, whereas five of the 14 patients in the no bone pain responding group already had died because of progressive disease.

27.8% of all enrolled patients died within one month, although only patients with a life expectancy of more than one month could be enrolled. This stresses that it is difficult for physicians to accurately estimate the prognosis of a patient. It is already known that physicians tend to overestimate survival of patients in 27-42% of patients (21, 22). However, 2/3 of the included patients in our study had a good performance status (WHO PS 0-1), and in general this is associated with a survival of more than one month (23). Compared with the literature, QoL was lower for our patients, especially on the domains of role, cognitive and social functioning (24-26). It could be that the high pain scores influenced these parts of QoL.

Limitations and strengths

A limitation of this study is the BPI as assessment tool of bone pain because the BPI does not exclude pain from other causes. To the best of our knowledge, there are no tools or questionnaires to fully discriminate between bone pain and pain from other causes. We attempted to minimize bias due to pain from other causes by only including patients with bone metastases diagnosed by imaging studies, bone pain corresponding with a location of bone metastases on imaging, or investigator judgement that the reported pain was indeed caused by bone metastases. Lacking

a placebo arm is another limitation of the study. However, in light of insufficient pain relief by ibandronate, this probably did not have any influence on the interpretation of the results (i.e., low chance of placebo effect). Furthermore, we assessed changes in WHO-PS at seven days after ibandronate infusion. Additional collection of WHO-PS through day 28 would have likely added value in identifying long-term changes in performance status.

A strength of this study is the separation of systemic treatment and treatment for CIBP as there is no potential interaction in efficacy on rapid pain relief.

In conclusion, loading doses of ibandronate do not lead to rapid bone pain relief in a sufficient number of NSCLC patients with uncontrolled CIBP to constitute its use. Studies evaluating other treatment options for rapid bone pain relief in this patient population are necessary.

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SUPPLEMENTARY MATERIAL

Participating centers of the NVALT-9 trial.

Participating centers of the NVALT-9 trial are:

1. Maastricht University Medical Center+ (MUMC+), Maastricht, The Netherlands.
2. Catharina Ziekenhuis, Eindhoven, The Netherlands.
3. Sint Antonius Ziekenhuis, Nieuwgein, The Netherlands.
4. Viecuri, Venlo, The Netherlands.
5. Rode Kruis Ziekenhuis, Beverwijk, The Netherlands.
6. Jeroen Bosch Ziekenhuis, 's Hertogenbosch, The Netherlands.
7. Leids Universitair Medisch Centrum, Leiden, The Netherlands.
8. Rijnstate Ziekenhuis, Arnhem, The Netherlands.

Analysis of secondary endpoints.

1.1 Mean worst bone pain scale of the BPI.

Table 1: Mean worst bone pain scale of the BPI.

	BPI day 1 n/N=18/18	**BPI day 7 n/N=16/18**
Worst pain Median (range)	7.5 (4.0-9.0)	7.0 (2.0-9.0)

Abbreviation: BPI: brief pain inventory.
To conclude: no significant or clinically relevant difference.

1.2 Interference scales of the BPI.

Table 2: Interference scales of the BPI.

	BPI day 1 n/N=18/18	**BPI day 7 n/N=16/18**
Average pain Median (range)	5.0 (2.0-9.0)	5 (2.0-7.0)
General activity Median (range)	6.0 (3.0-10.0)	8.0 (2.0-9.0)
Mood Median (range)	5.0 (1.0-10.0)	6.0 (0.0-8.0)
Ability to walk Median (range)	7.0 (1.0-10.0)	8.0 (1.0-10.0)
	BPI day 1 n/N=18/18	**BPI day 7 n/N=16/18**
Normal operation Median (range)	8.0 (2.0-10.0)	8.5 (3.0-10.0)
Relationships with others Median (range)	5.0 (0.0-10.0)	4.0 (0.0-9.0)
Sleep Median (range)	7.0 (0.0-10.0)	4.0 (0.0-9.0)
Pleasure in life Median (range)	5.0 (1.0-10.0)	5.0 (1.0-8.0)

Abbreviation: BPI: brief pain inventory.
To conclude: no significant or clinically relevant difference.

1.3 Analgesic consumption.

Patients were treated with analgesics according the WHO pain ladder. Before study entry, four patients were also treated with anti-epileptics (4 patients) and methadone (1 patient).

Two patients were able` to reduce their analgesic use during the study period (one stopped opioid treatment, the other the dose decreased with 25%). In two other patients the opioid doses increased substantially (times 2 in one patient, times 5 in the other patient).

To conclude: no significant or clinically relevant difference.

1.4 WHO-PS.

Table 3: WHO-PS change in general.

		n/N=18/18 (%)
WHO-PS change	No change	6 (33)
	Improvement	2 (11)
	Worsening	3 (17)
	Not reported	7 (39)

Abbreviation: WHO-PS: World Health Organization Performance Score.

Table 4: WHO-PS change subdivided in no bone pain responder vs. bone pain responder*.

		No bone pain responder n/ N=14/18 (%)	Bone pain responder n/ N=4/18 (%)
WHO-PS change	No change	6 (33)	0 (0)
	Improvement	2 (11)	1 (25)
	Worsening	3 (17)	2 (50)
	Not reported	7 (39)	1 (25)

Abbreviations: WHO-PS: World Health Organization Performance Score.
*Definition of a bone pain responder: a 25% decrease in worst bone pain score over day five, six and seven compared to bone pain score at baseline (as determined by the "worst pain scale" of the BPI), with no more than a 25% increase in mean analgesic consumption over the same three-day period compared to baseline analgesic consumption.
To conclude: no significant or clinically relevant difference.

1.5 Quality of life

Table 5: QLQ-C30 scores in general.

	QLQ-C30 day 1 n/N=18/18	QLQ-C30 day 7 n/N=16/18
Global QoL Median (range)	33.3 (0.0-66.7)	33.3 (16.7-66.7)
Physical functioning dimension Median (range)	40.0 (13.3-86.7)	33.3 (6.7-80.0)
Role functioning dimension Median (range)	16.7 (0.0-50.0)	0.0 (0.0-66.7)
Emotional function dimension Median (range)	50.0 (0.0-91.7)	58.3 (8.3-83.3)
Cognitive functioning dimension Median (range)	66.7 (0.0-100.0)	66.7 (0.0-100.0)
Social functioning dimension Median (range)	41.7 (0.0-100.0)	33.3 (0.0-100.0)

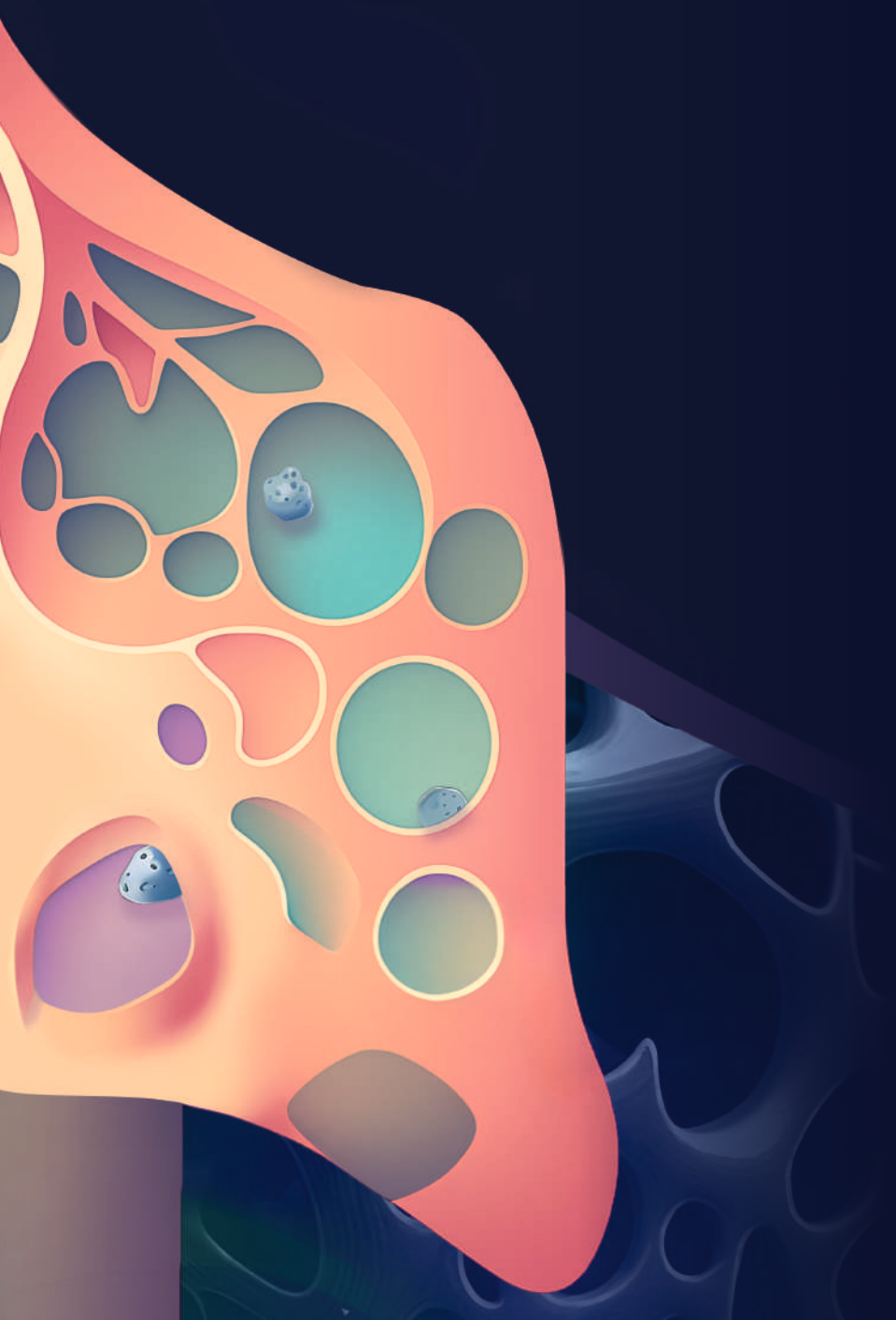
Abbreviations: QLQ-C30: Quality of Life Questionnaire-Core 30; QoL: Quality Of Life.
To conclude: no significant or clinically relevant difference.

Table 6: QLQ-C30 scores subdivided in QLQ-C30 symptom scores of greater than 50% maximum attainable value.

	QLQ-C30 day 1 n/N=18/18 (%)	QLQ-C30 day 7 n/N=16/18 (%)
Nausea > 50%	1 (5)	1 (5)
Pain > 50%	14 (74)	11 (58)
Dyspnea > 50%	6 (32)	6 (32)
Insomnia > 50%	10 (53)	6 (32)
Appetite loss >50%	7 (37)	7 (37)
Constipation >50%	6 (32)	6 (32)
Diarrhea >50%	1 (5)	3 (16)
Financial difficulties > 50%	4 (21)	3 (16)

Abbreviations: QLQ-C30: Quality of Life Questionnaire-Core 30.
To conclude: no significant or clinically relevant difference.

CHAPTER 4



Association of RANKL and EGFR gene expression with bone metastases in patients with metastatic non-small cell lung cancer

Anita J.W.M. Brouns, Lizza E.L. Hendriks, Iris J. Robbesom-van den Berge, Annemariek J.H.M. Driessen, Guido M.J.M. Roemen, Britt L.J. van Herpen, Zoë Dekkers, Bas Heitzer, Daphne J.G. Leunissen, Laura Moonen, Ragnar Lunde, Marcel Westenend, Marjolein van Driel, Ernst-Jan M. Speel, Anne-Marie C. Dingemans

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ABSTRACT

Introduction

Bone metastases are frequent in patients with non-small cell lung cancer (NSCLC). The receptor activator of Nuclear Factor κB (RANK)/ RANK ligand (RANKL)/osteoprotegerin (OPG) pathway is important in bone metastases development. Furthermore, epidermal growth factor receptor (EGFR) signaling promotes osteoclast formation and stimulation. The understanding of the biological mechanism of bone metastases development might have implications for treatment strategies. Therefore, we studied whether there is an association between EGFR, RANKL, RANK and OPG gene expression in the tumor and presence of bone metastases in patients with NSCLC.

Methods

From an updated multicenter study, including patients with *EGFR* mutated (*EGFR*+), Kirsten rat sarcoma *(KRAS+)* and *EGFR/KRAS* wildtype metastatic NSCLC, all patients with available formalin-fixed paraffin-embedded (FFPE) tumor samples were selected. Ribonucleic Acid (RNA) was isolated from these samples and gene expressions of EGFR, RANKL, OPG and RANKL were determined via quantitative Polymerase Chain Reaction (qPCR). Data on demographics, histology and molecular subtyping, sample origin, presence of bone metastasis, SREs and bone progression were collected. Primary endpoint was relation between EGFR, RANK, RANKL, OPG gene expression, RANKL:OPG ratio and bone metastases.

Results

In 73/335 (32% *EGFR+*, 49% *KRAS+*, 19% *EGFR/KRAS* wildtype) samples from unique patients, gene expression analysis could be performed. Of these 73 patients, 46 (63%) had bone metastases at diagnosis or developed bone metastases during the disease course. No association was found between EGFR expression and presence of bone metastases. Patients with bone metastases had a significantly higher RANKL expression and RANKL:OPG ratio compared to those without. An increased RANKL:OPG ratio resulted in a 1.65x increased risk to develop bone metastases, especially in the first 450 days after diagnosis of metastatic NSCLC.

Conclusion

Increased RANKL gene expression and RANKL:OPG ratio, but not EGFR expression, was associated with presence of bone metastases. Additionally, an increased RANKL:OPG gene ratio was associated with a higher incidence of bone metastases development.

INTRODUCTION

The skeleton is a common site for tumor metastases of several malignancies. For example, 30-60% of patients with metastatic lung cancer develop bone metastases (1, 2). In patients with bone metastases, bone turnover is disturbed. Normal bone remodeling requires a perfect balance between osteoblasts, osteoclasts and numerous signaling pathways, growth factors and control mechanisms. An important role is reserved for the Receptor activator of Nuclear Factor κB (RANK)/ RANK ligand (RANKL)/ osteoprotegerin (OPG, the decoy receptor and antagonist of RANKL) pathway in bone development (3). By binding of RANKL to RANK, an ongoing cascade is set in motion, in which cancer cells stimulate osteoclasts, which in turn degrade the bone. During osteoclastogenic bone resorption different growth factors and cytokines are released from the bone, which stimulate the cancer cells to expansive growth (4).

Epidermal growth factor receptor (EGFR) signaling is involved in the proliferation of osteoclast precursors. Signaling via EGFR promotes osteoclast formation and stimulation by inhibition of OPG expression and by increasing monocyte chemoattractant protein 1 (MCP1; which induces osteoclast fusion and activity), macrophage colony-stimulating factor (MSCF) and RANKL expression (5, 6). An *in vitro* study showed that the addition of EGFR-tyrosine kinase inhibitors (EGFR-TKIs) completely blocked RANKL-dependent osteoclast formation and led to apoptosis in matured osteoclasts. These observations suggest an essential role for EGFR signaling in RANKL-mediated osteoclast differentiation and survival (7).

EGFR protein expression, determined by immunohistochemistry, in non-small cell lung cancer (NSCLC) is up-regulated in 40-80% of the tumors (8, 9). Conflicting results exist regarding the association of EGFR protein expression and *EGFR* mutations in NSCLC: some studies showed a higher EGFR protein expression in tumor samples (n=133-970) of patients with *EGFR* mutated (*EGFR+*) NSCLC (10, 11), while others (n=102-159) showed no association (12, 13). The upregulated EGFR protein expression in the tumor (which possibly results in increased EGFR signaling) that was observed in some studies evaluating *EGFR+* NSCLC, could be an explanation for our previously reported higher incidence of bone metastases in *EGFR+* NSCLC compared with Kirsten rat sarcoma (*KRAS+*) and *EGFR/KRAS* wildtype NSCLC (14).

To the best of our knowledge, it has never been studied in a clinical setting whether there is an association between EGFR, RANKL, RANK and OPG gene expression in the tumor and presence of bone metastases in patients with NSCLC. In this study, we tried answering this question since understanding the biological mechanism of bone metastases development might have implications for adequate bone metastasis screening and (prophylactic) treatment decisions.

MATERIALS AND METHODS

Data from a study of patients with metastatic NSCLC were used (1). In this case-control study, for every patient with *EGFR*+ NSCLC (i.e., exon 19 deletion or exon 21 point mutation), the consecutive patients with a *KRAS*+ and *EGFR/KRAS* wildtype NSCLC were included as a case-control group. Wildtype was defined as *EGFR* and *KRAS* mutation negative NSCLC, as extensive molecular testing was not standard of care at that time. The established database covered the period from 01-10-2008 to 01-08-2012 and was updated (additional patients as well as updated data) till 01-09-2017 (1). For the current study, all patients with available formalin-fixed paraffin-embedded (FFPE) tissue samples were selected. This study was approved by the ethics committee of Maastricht UMC+ (METC 2017-0318) and the need for informed consent was waived.

Data collection

The in-and outpatient medical records of all patients were retrieved. Eligible patients were patients with metastatic NSCLC, with data regarding molecular analysis and follow-up and sufficient FFPE tumor tissue available. The following data were collected: demographics, date of diagnosis of metastatic NSCLC, smoking status, histology, mutation status, site of biopsy (e.g., pathology obtained from bone, lung, lymph node, adrenal lesion), baseline bone metastasis, development of bone metastases during treatment, treatment, skeletal related events (SREs) and time of death. SREs were defined as pathological fracture, spinal cord compression, necessity for radiation to bone (for pain or impending fracture) or surgery to bone (15).

Measurement of EGFR, RANKL, RANK and OPG gene expression

EGFR, RANKL, RANK and OPG expression was measured by reverse transcriptase quantitative real time Polymerase Chain Reaction (RT-qPCR) on ribonucleic acid (RNA) extracted from FFPE tissue. Data were presented as relative mRNA levels calculated by the equation $2^{-\text{delta cycling time (Ct)}}$. Delta CT is CT of target gene minus CT of housekeeping gene. Data were expressed on a logarithmic scale. See Supplementary Material for a more detailed explanation of the measurement of gene expression.

Statistics

Statistical analysis was conducted with SPSS (v20; SPSS Inc., Chicago, IL) and SAS 9.4. Descriptive statistics of demographic and clinical variables were obtained. Categorical variables were compared using chi-square tests and continuous variables were compared using the Mann-Whitney U Test or the Kruskal-Wallis test. Reverse Kaplan-Meier was used for calculating median follow-up time. Due to small sample sizes, bone metastases at baseline or development of bone metastases during disease were grouped together and classified as “bone metastases present.” EGFR gene expression was represented in quartiles, as there is no standard cut-off for high or low EGFR gene expression.

Competing risk analysis was used for the association between RANKL:OPG ratio and time to development of bone metastases for patients without bone metastases. The proportional hazards assumption was tested using time-dependent Cox regression analyses with interaction between RANKL:OPG ratio and time. Due to violation of this assumption the analysis was separated in two time intervals and the -2LogLikelihood was compared between models with different time-cut-off points to identify the best cut-off (i.e., the model with the lowest -2LogLikelihood).

The relation of EGFR, RANK, RANKL, OPG gene expression, RANKL:OPG ratio and bone metastases was the primary endpoint of this study. Secondary endpoints were 1) Association between sample origin (primary site, non-bone metastasis, metastasis in general except bone, bone) and expression of EGFR, RANK, RANKL and OPG and RANKL:OPG ratio, 2) Expression of EGFR, RANK, RANKL and OPG and RANKL:OPG ratio in different molecular subgroups (*EGFR+*, *KRAS+*, *EGFR*/*KRAS* wildtype) in relation to bone metastases.

RESULTS

Patient characteristics

From 169 patients (50%) of the total group of 335 patients, FFPE tumor samples were available. Ultimately, sufficient RNA could be extracted from 73 samples (Flowchart in Figure 1). In 52 out of 73 patients (81%), the pathology samples were obtained at diagnosis of metastatic disease. The other 21 patients were primarily diagnosed with early-stage NSCLC and had a median time to detection of metastatic disease of 550 days (range 87-2196 days). Patient characteristics are shown in Table 1. Median follow-up from diagnosis of metastatic NSCLC was 58.5 months (95% confidence interval (CI): 34.8-82.2 months).

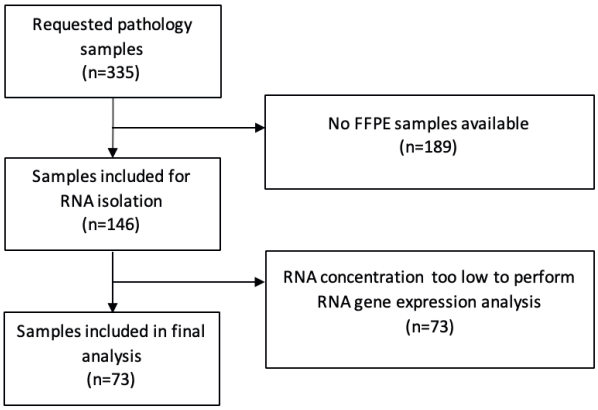


Figure 1: Flowchart of pathology sample selection.
The flowchart showed the process of sample selection and reasons for exclusion of samples. Abbreviations: n: number; FFPE: formalin-fixed paraffin-embedded; RNA: Ribonucleic acid.

Table 1: Patient characteristics.

Characteristics	**Total n=73**
Female n (%)	46 (63)
Never smoker n (%)	8 (11)
Mean age at diagnosis metastatic NSCLC, years (range)	62.8
	(32-84)
Molecular subgroup n (%)	23 (32)
EGFR+	36 (49)
KRAS+	14 (19)
EGFR/*KRAS* wildtype	
Origin of pathology sample n (%)	29 (40)
Lung (primary tumor)	9 (12)
Bone	35 (48)
Other metastasis	
Metastatic disease at diagnosis n (%)	47 (64)
Bone metastases at diagnosis stage IV n (%)	27 (37)
Bone metastases at diagnosis or during course of disease n (%)	46 (63)
SRE n (%)[*]	26 (57)
Type of SRE n (%)[#]	25 (96)
Radiotherapy	4 (15)
Pathologic fracture	6 (23)
Surgery	2 (8)
Spinal cord compression	
BTA use in all patients n (%)[$]	9 (12)
Denosumab	1 (1)
Bisphosphonate	8 (11)

Abbreviations: n, number; EGFR+, Epidermal Growth Factor Receptor mutation; KRAS+, Kirsten rat sarcoma mutation; SRE, skeletal related event; BTA, bone targeted agent.
[*] Percentages were calculated by group of patients with bone metastases.
[#] Percentages were calculated by subgroup of all pts with SREs (n=26). Some patients experienced more than one SRE.
[$] Denosumab was used in one patient without bone metastases, all patients who used bisphosphonates had bone metastases.

EGFR, RANKL, RANK, OPG gene expression

EGFR, RANKL, RANK and OPG gene expressions were non-normally distributed (data not shown). The median EGFR expression was 0.84 (interquartile range (IQR) 1.67), the median RANKL expression was 0.02 (IQR 0.05), the median OPG expression was 0.09 (IQR 0.10) and the median RANK expression was 0.02 (IQR 0.03) [Figure 2].

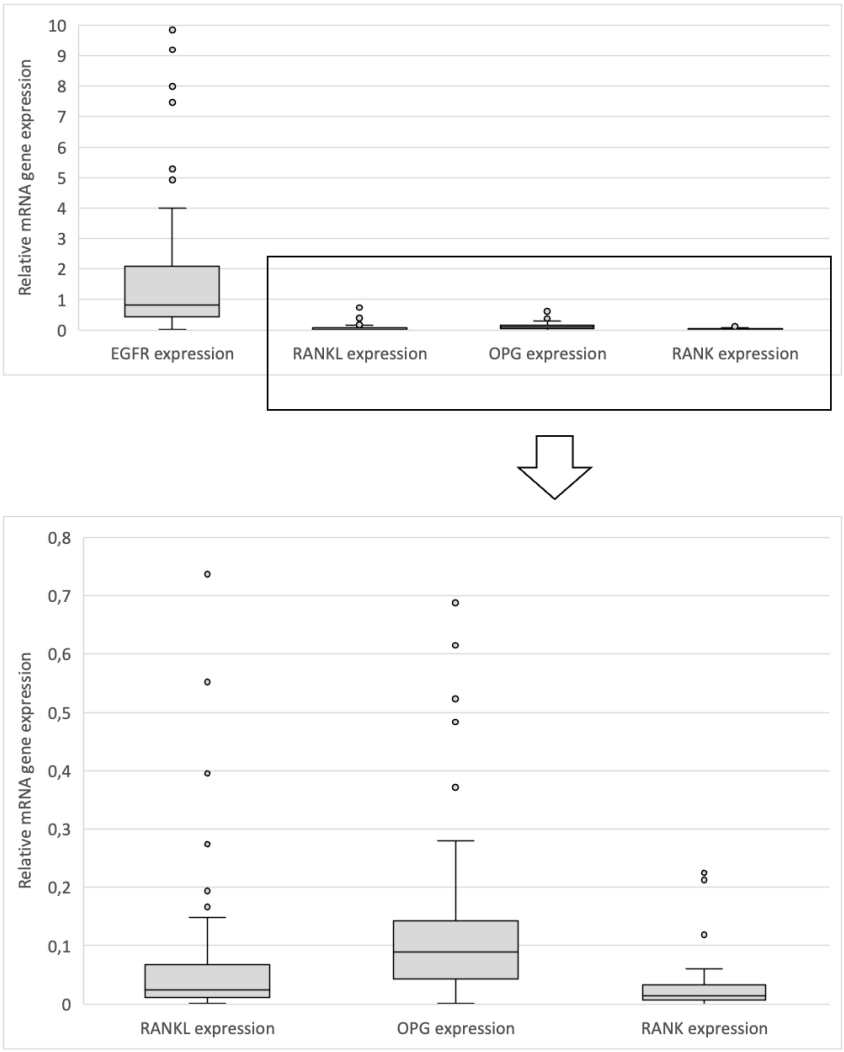


Figure 2: EGFR, RANKL, OPG, RANK gene expression in all patients.
This figure shows the relative EGFR, RANKL, OPG and RANK gene expression measured on pathology samples of all patients.
Abbreviations: EGFR: Epidermal Growth Factor Receptor; RANKL: Receptor Activator of Nuclear Factor κB ligand; OPG: osteoprotegerin; RANK: Receptor Activator of Nuclear Factor κB.

Association between EGFR gene expression and RANKL, RANK and OPG gene expression or RANKL:OPG ratio and presence of bone metastases

EGFR expression was similar for patients with and without bone metastases (p=0.479). The percentage of patients with and without bone metastases was comparable between all EGFR quartiles (p=0.174, Figure 3A). Patients with bone metastases had an increased tumor RANKL

expression and increased RANKL:OPG ratio, compared to those without bone metastases (p=0.002 and p=0.026 respectively).

Subdividing patients based on EGFR quartiles showed that RANKL gene expression was numerically higher in all EGFR quartiles for patients with bone metastases and statistically higher in the second and third EGFR quartile (Figure 3C). In the different EGFR quartiles, no significant differences for OPG, RANK gene expressions and RANKL:OPG ratio and presence of bone metastases were observed (Figures 3B, 3D-E).

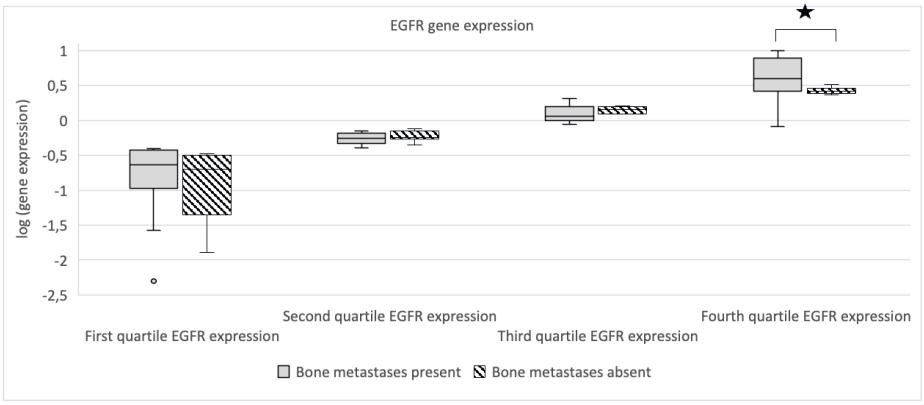
EGFR, RANKL, RANK and OPG gene expression or RANKL:OPG ratio in primary tumors and metastases

The obtained tumor samples were subdivided based on site of origin: primary tumor (n=29), non-bone metastases (e.g., lymph node, liver, adrenal grand, parietal pleura, brain, other; n=35) and bone metastases (n=9). No difference was found for the percentage of tumor cells in the pathology sample and RANKL gene expression (data not shown). For the whole population of patients with and without bone metastases, significantly higher RANKL gene expression was observed in bone samples than in samples derived from the primary tumor (p=0.025). Pathology samples of both non-bone as well as bone metastases had a significant higher RANKL:OPG ratio in comparison to samples of the primary tumor (p=0.004 and p=0.028). The OPG gene expression was significantly lower in samples of bone metastases compared to non-bone metastases (p=0.043). RANK gene expression was significantly higher in samples of non-bone metastases in comparison to the primary tumor (p=0.047). Figure 4 shows the different gene expression of the pathology samples. In the group of patients with bone metastases, no significant differences were observed between the various sample origins, only a trend to significance for OPG gene expression and RANKL:OPG ratio (p=0.072, p=0.079).

Gene expression of EGFR, RANKL, RANK and OPG or RANKL: OPG ratio in different NSCLC molecular subgroups in relation to bone metastases

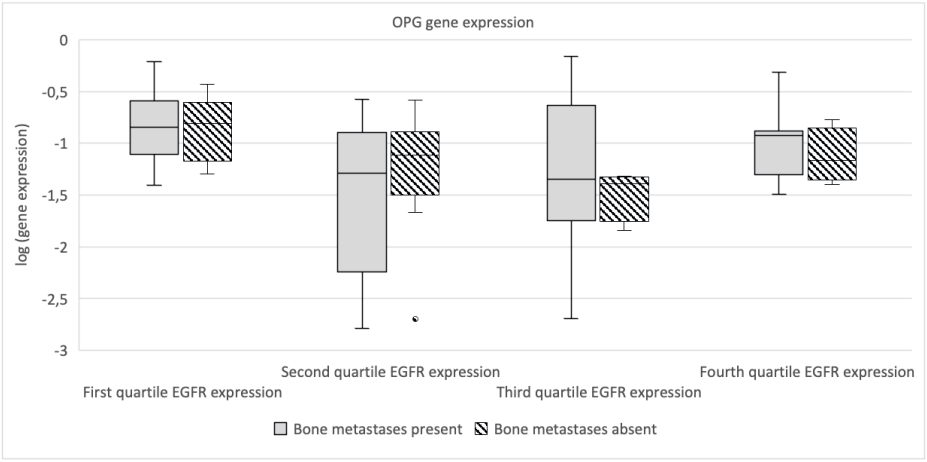
Independent of the presence of bone metastases, patients with an *EGFR* mutation had a significantly higher EGFR expression, compared to patients with a *KRAS+* or *EGFR/KRAS* wildtype NSCLC (p<0.001) (Supplementary Material, Figure 1A).

Patients with *KRAS*+ NSCLC and bone metastases had a significantly higher RANKL expression and higher RANKL:OPG ratio (p=0.002) compared to patients with *KRAS+* NSCLC without bone metastases (p=0.017). This was not found for the other molecular subgroups. The OPG expression was significantly higher for patients with bone metastases in the subgroup of patients with *EGFR+* and *EGFR/KRAS* wildtype NSCLC (p=0.021 and p=0.028) (Supplementary Material, Figures 1 B-E). No significant difference was observed between the different expression levels and presence of SREs in patients with bone metastases (data not shown).



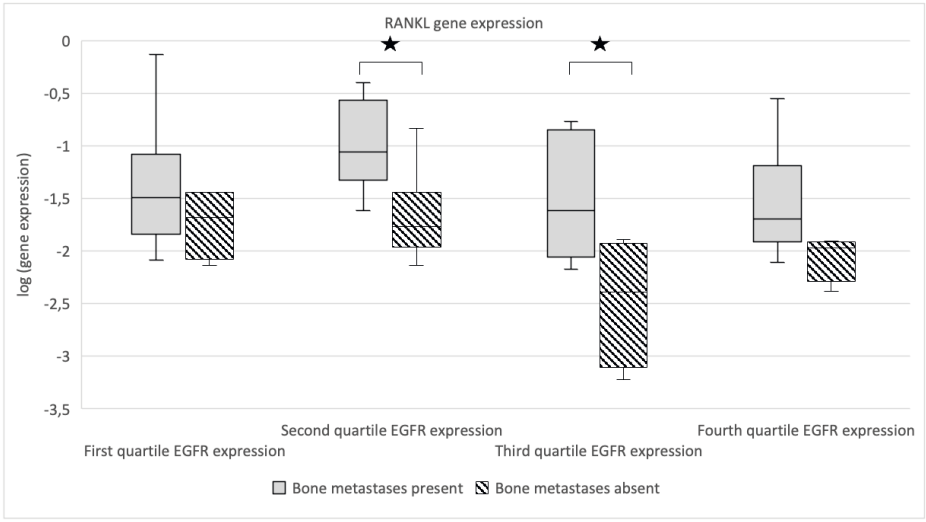
A)

n=18	n=19	n=18	n=18
13BM+ 5BM-	8BM+ 11BM-	13BM+ 5BM-	12BM+ 6BM-

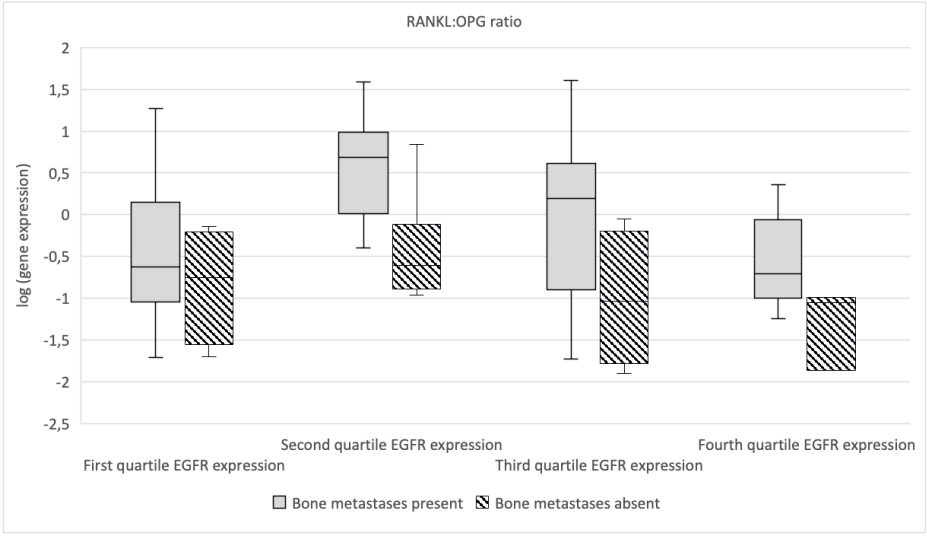


B)

n=18	n=19	n=18	n=18
13BM+ 5BM-	8BM+ 11BM-	13BM+ 5BM-	12BM+ 6BM-



C) n=18 13BM+ 5BM- n=19 8BM+ 11BM- n=18 13BM+ 5BM- n=18 12BM+ 6BM-



D) n=18 13BM+ 5BM- n=19 8BM+ 11BM- n=18 13BM+ 5BM- n=18 12BM+ 6BM-

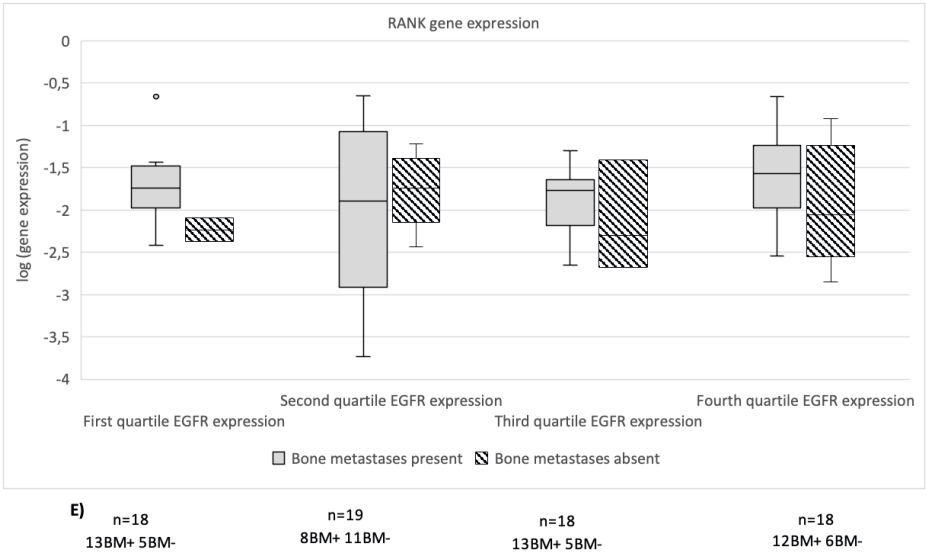
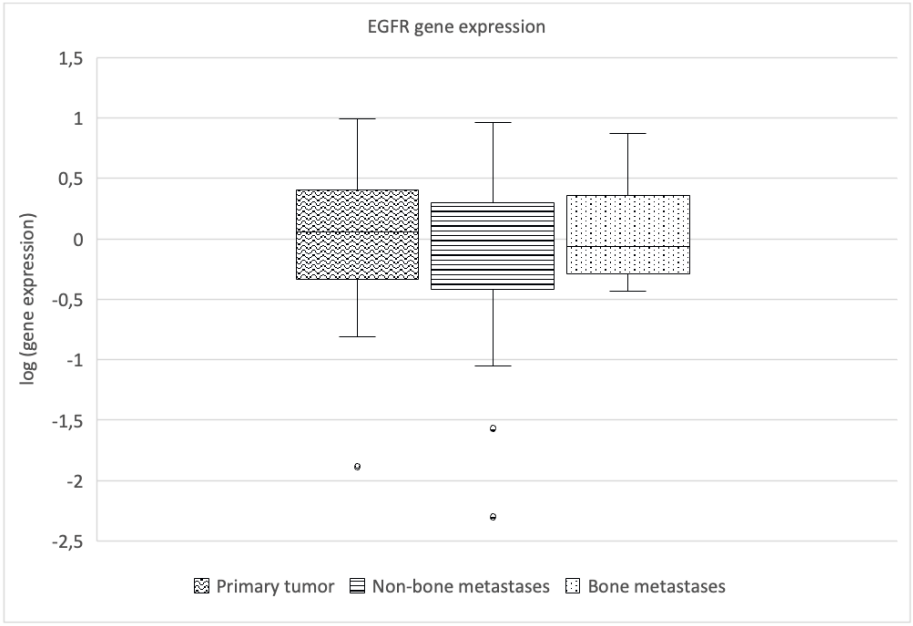


Figure 3 A-E: EGFR, OPG, RANKL gene expression, RANKL:OPG ratio and RANK gene expression in relation to presence of bone metastases.
Patients were subdivided in groups by EGFR expression. The first quartile is the lowest and the fourth quartile is the highest EGFR gene expression. A) EGFR gene expression, B) OPG gene expression, C) RANKL gene expression, D) RANKL:OPG ratio, E) RANK gene expression. An asterisk denotes a significant difference between groups.
Abbreviations: EGFR: Epidermal Growth Factor Receptor; OPG: osteoprotegerin; RANKL: Receptor Activator of Nuclear Factor κB ligand; RANK: Receptor Activator of Nuclear Factor κB.

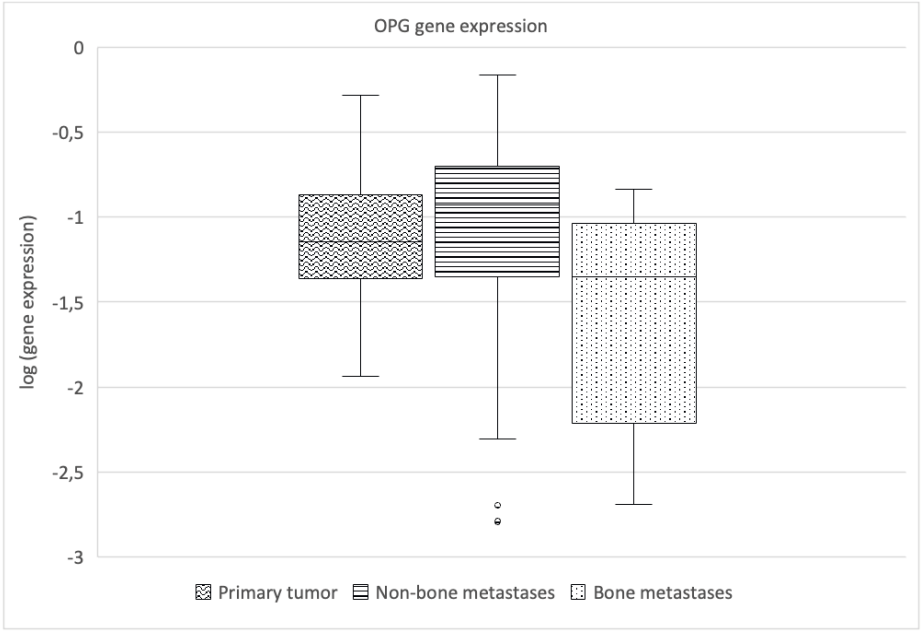
Association between RANKL:OPG ratio and time to development of bone metastases

The RANKL:OPG ratio in relation to bone metastases development violated the proportional hazards assumption, therefore an early and late effect was determined. The hazard ratio (HR) of the RANKL:OPG ratio in the first 450 days after diagnosis of metastatic NSCLC was 1.65 (95% CI: 0.66-4.12) and decreased to 0.17 (95% CI: 0.03-0.95) thereafter.



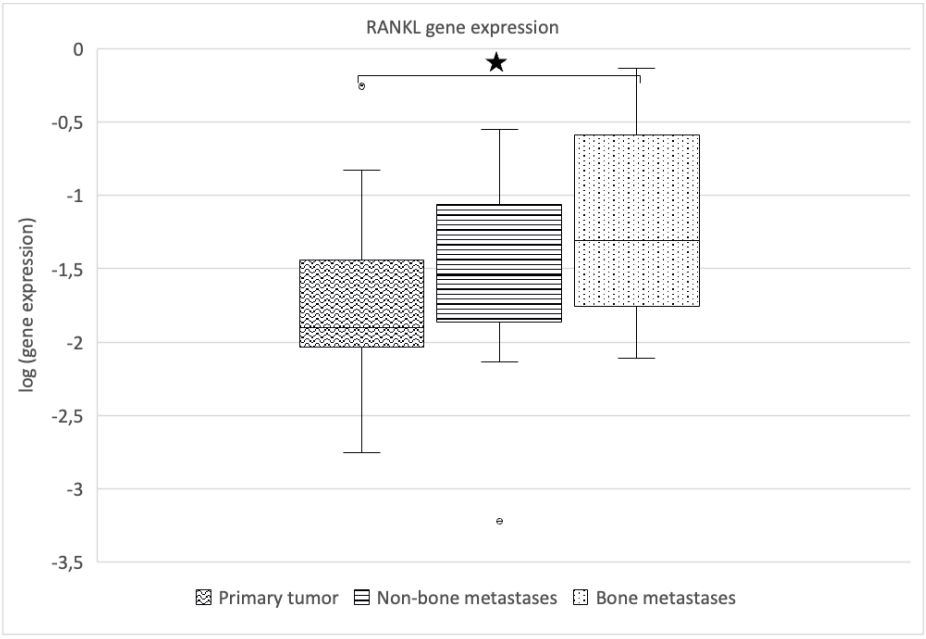
A)

n=29 n=35 n=9



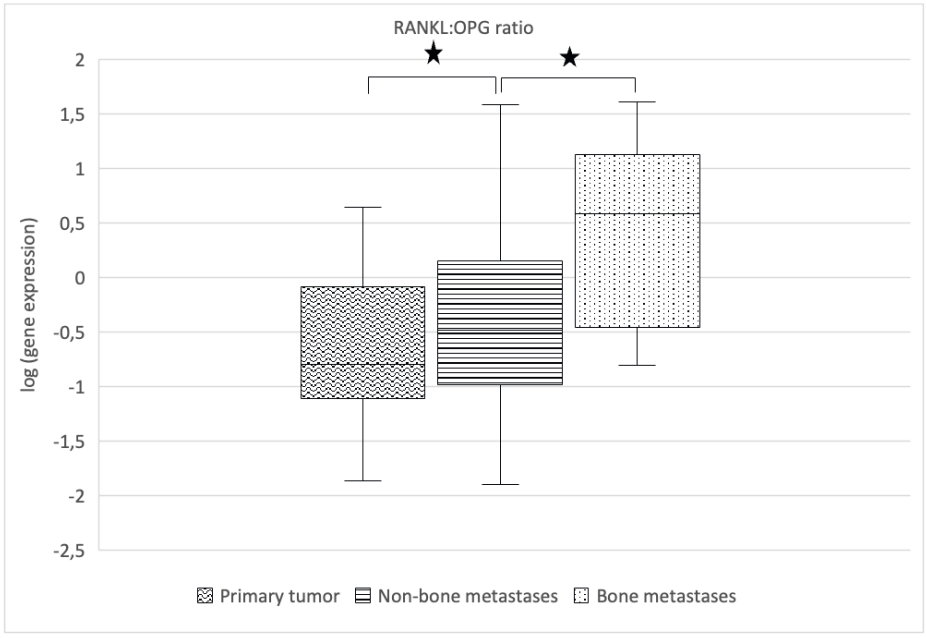
B)

n=29 n=35 n=9



C)

n=29 n=35 n=9



D)

n=29 n=35 n=9

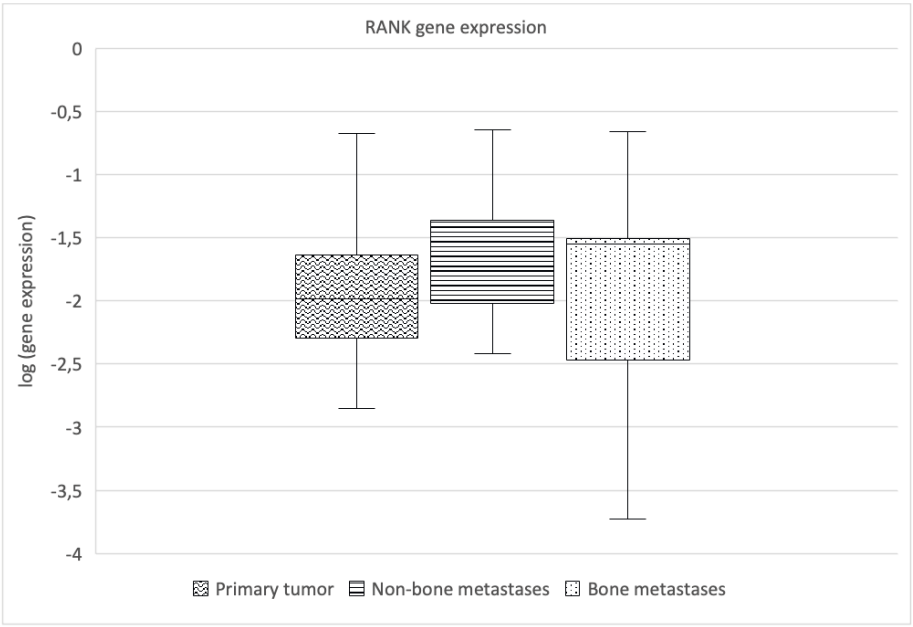


Figure 4 A-E: EGFR, RANKL, OPG gene expression, RANKL:OPG ratio and RANK gene expression in relation to origin of pathology sample.
A) EGFR expression, B) OPG expression, C) RANKL expression, D) RANKL:OPG ratio, E) RANK expression, all expressions are shown in primary tumor, non-bone metastases and bone metastases. An asterisk denotes a significant difference between groups.
Abbreviations: EGFR: Epidermal Growth Factor Receptor; OPG: osteoprotegerin; RANKL: Receptor Activator of Nuclear Factor κB ligand; RANK: Receptor Activator of Nuclear Factor κB.

DISCUSSION

Previously, we showed that bone metastases were more frequent in patients with *EGFR+* metastatic NSCLC than in patients with *KRAS+* or *EGFR/KRAS* wildtype NSCLC, and that post bone metastases survival was significantly longer in patients with *EGFR+* NSCLC (1, 14). Based on preclinical data, showing that EGFR expression inhibits OPG expression and increases RANKL expression(5, 6), we hypothesized that the earlier observed increased EGFR gene expression in *EGFR+* NSCLC (8, 9) may lead to an altered shift of RANKL expression or RANKL:OPG ratio and thereby promote bone metastases in *EGFR+* NSCLC. In the current study, we indeed found that *EGFR+* NSCLC had a significantly higher EGFR gene expression as compared to *KRAS+* or *EGFR/KRAS* wildtype NSCLC. We could not demonstrate any association between EGFR gene expression level and the presence of bone metastases. However, patients with bone metastases had a significantly

higher RANKL expression and RANKL:OPG ratio compared to those without bone metastases; possibly because the bone microenvironment in those with bone metastases released cytokines or growth factors which induced RANKL expression also in the tumor. This increased RANKL and RANKL:OPG ratio is in line with observations in an *in vitro* study in three human NSCLC cell lines and in 127 NSCLC tumor samples (52 primary tumors and 75 bone metastasis samples) in which the expression of RANKL, RANK and OPG was estimated by RT-PCR in cell lines and by immunohistochemistry (IHC) on tumor tissue (16). In addition, both *in vitro* and *in vivo* an increased RANKL expression and elevated RANKL:OPG ratio was associated with an enhanced potential of NSCLC to metastasize to the bone (16). Our data confirmed that patients with NSCLC with a higher RANKL:OPG ratio more often developed bone metastases, primarily in the first 450 days after diagnosis of metastatic NSCLC. Various studies have been performed to investigate biomarkers related to bone metastases or skeletal related event development. Examples are bone specific alkaline phosphatase in serum, urine N-terminal telopeptide in urine and C-X-C- Motif Chemokine Receptor 4 on the tumor (17). However, most of these are not used in daily practice as there is no recognized standard because of inconsistent study results. We showed an increased RANKL expression especially in patients with *KRAS+* NSCLC and bone metastases. As far as we know, no data about RANKL expression in this subgroup exists. Human lung adenocarcinoma data sets only showed that RANKL expression was significantly higher in *KRAS+* lung adenocarcinoma compared to *KRAS* wildtype lung adenocarcinoma (18).

As previously reported in breast or renal cell carcinoma, RANKL triggers the migration and metastasis of RANK expressing cancer cells (19, 20). A retrospective analysis in patients with non-metastatic breast cancer (n=509) showed a positive association between higher RANKL serum levels (measured by enzyme linked immune sorbent assay [ELISA]) and presence of disseminated tumor cells in the bone marrow and also with the development of bone metastases (21). Moreover, patients within the highest quartile of RANKL had a 4.6 increased risk for developing bone metastases compared to those within the lowest quartile (21). This is in line with our observation that patients with bone metastases had higher RANKL expression, especially in *KRAS+*NSCLC. It is not known whether the effect of RANKL inhibition (e.g., denosumab) on bone metastases related outcomes in patients with high versus low RANKL expression is different. In the Splendour trial no survival benefit was found when denosumab was added to first-line chemotherapy in patients with metastatic NSCLC (2). However, these patients were unselected for the presence of bone metastases and bone related outcomes were not reported. It would be of interest to explore the outcomes in patients with bone metastases and evaluate whether there is a relation between bone metastases related outcomes and RANKL expression (tumor or serum) as well as RANKL/OPG ratio (2).

In the current study, we could not find an explanation for our previously observed higher incidence of bone metastases in patients with *EGFR+* NSCLC (14). Although EGFR gene expression was higher, no association with a higher RANKL gene expression or RANKL:OPG ratio in tumor samples was observed. It could be that the tumor tissue is not the correct place to measure these

values. Nowadays, more and more studies point on the role of extracellular vesicles (EVs) in bone metastases development in multiple types of cancer (22-24). An *in vitro* study showed that CRL-2868 NSCLC cells containing an *EGFR* 19 deletion, secrete exosomes containing EGFR ligand and Amphiregulin. These EVs were able to induce *in vitro* osteoclast differentiation of murine RAW264.7 cells by activation of EGFR phosphorylation and induction of matrix metalloproteinase-9 and tartrate-resistant acid phosphatase expression. These results were confirmed *ex vivo* by the finding that patient derived EVs were able to modulate osteoclastogenesis in human osteoclast precursors (23). Therefore, future studies should also focus on EVs in patients with (*EGFR+*) NSCLC to unravel the biological mechanism of bone metastases formation.

This study has its limitations. First, due to unavailability of tumor samples or impossibility to perform the gene expression analysis, the sample size was not large enough to have sufficient power for subgroup analysis. A second limitation is the different origin of the pathological samples, which could create bias in expression analysis as, by nature, RANKL expression in bone is higher than in lung tissue (25). However, as we had only nine bone samples in our analysis, we think this did not significantly affected our results. Third, not all patients underwent a 2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computer tomography scan (FDG-PET-CT scan) or bone scintigraphy, therefore it could be that presence of bone metastases is underestimated as not all asymptomatic bone metastases will have been diagnosed by regular computed tomography of the chest and upper abdomen. Finally, patients with bone metastases and development of bone metastases during disease were grouped together and in doing so, one can ask whether the biological behavior of the tumor is the same in both groups. However, when analyzing both groups separately, the results remained similar (data not shown).

In conclusion, our study showed no association between EGFR gene expression and presence of bone metastases in patients with NSCLC; however, patients with bone metastases had a higher RANKL gene expression and RANKL:OPG ratio. An elevated RANKL:OPG ratio was associated with a higher incidence of bone metastases development, especially in the first year after diagnosis of metastatic NSCLC.

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SUPPLEMENTARY MATERIAL

Supplementary Materials and methods

EGFR gene expression

RNA was extracted from FFPE tissue using the automated Maxwell® RSC RNA FFPE Kit (AS1440; Promega USA) and subsequently quantified using the Quantifluor RNA System (E3310; Promega USA) on a Quantus Fluorometer (E6150; Promega USA).

Multiplex one step RT-qPCR were designed to determine the EGFR RNA expression levels. Importin 8 (IPO8) and Polymerase II polypeptide A (POLR2a) were used as reference genes to control the variability of clinical samples [25]. The expression of EGFR was determined one reaction: IPO8, POLR2a and EGFR. One step RT-qPCR analysis was performed (2 µl (1-40 ng) RNA input/reaction) on a Bio Rad Cfx96 instrument using the TaqPath™ 1-Step Multiplex Master Mix (No ROX) (A28522; Thermo Fisher Scientific USA) according to the manufacturer instructions, 400 nM of each primer and 100-150nM of each probe (Table 1, supplementary data 1). Normal lung tissue served as a positive control for EGFR expression.

OPG, RANKL and RANK gene expression

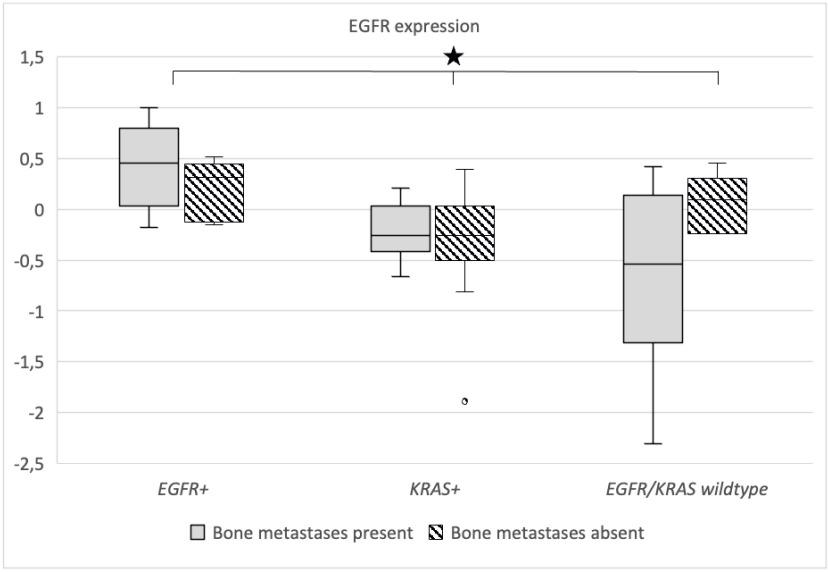
RNA was reverse transcribed into cDNA, using the SuperScript™ VILO™ cDNA Synthesis Kit according to the protocol of the manufacturer (Invitrogen, Carlsbad, California, United States). RT-qPCR was conducted using the Quantstudio Flex 7 system (Thermo Fisher Scientific, Waltham, Massachussetts, United States). Reactions were performed in 10 µl volumes using a SYBR green mastermix (GoTaq® qPCR Master Mix by Promega, Madison, Wisconsin, United States) in a 384 wellsplate (MicroAmp® Optical 384-Well Reaction Plate with Barcode, Applied biosystems®, Waltham, Massachussetts, United States). Reaction mixes contained 1 µl cDNA, 5 µl SYBR green mastermix, primers and RNAse free water. Primer sets were designed using the Primer-BLAST tool by the National Center for Biotechnology Information of the U.S. National Library of Medicine and manufactured at Integrated DNA Technologies (IDT), Coralville, Iowa, United States (Table 1). Cycling conditions were 50°C for two minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Data are presented as relative mRNA levels calculated by the equation $2^{-\text{delta cycling time (CT)}}$. Delta CT is CT of target gene minus CT of housekeeping gene (36B4).

SUPPLEMENTARY FIGURES AND TABLES

Table 1: Primer and probe-sequences.

RANKL	• Forward-primer: 5' GATGGTGGATGGCTCATGGT 3' • Reverse-primer: 5' GGAACCAGATGGGATGTCGG 3' • Probe: 5' [FAM] TCTGGCCAAGAGGAGCAAGC [BHQ1] 3'
EGFR	• Forward-primer: 5'GCAGCGATACAGCTCAGACC 3' • Reverse-primer: 5'CTTTTGGGAACGGACTGGTTT 3' • Probe: 5'[FAM] CGCCTTGACTGAGGACAGCA [BHQ1] 3'
RANK	• Forward-primer: 5' GTACCACTGGAGCCAGGACT 3' • Reverse-primer: 5' CTTGTTGAGCTGCAACGGGT 3'
OPG	• Forward-primer: 5' CCATGTTCGTGGCCCTCC 3' • Reverse-primer: 5' TAGGATCCATCTGCGCTCTG 3'
Housekeeping genes	
IPO8	• Forward-primer: 5' CGTTCCTCCTGAGACTCTGC 3' • Reverse-primer: 5' TGCAGTGCCCACTTCTTACA 3' • Probe: 5' [HEX] TGATAGACCAGAACTGGTATGGTGGA [BHQ1] 3'
POLR2a	• Forward-primer: 5'GCATTGACTTGCGTTTCCA 3' • Reverse-primer: 5' TGCCGTTCCACCTTATAGCC 3' • Probe: 5'[Cyanine 5] CCCAGTGACCTTCACCTGCA [BHQ3] 3'
36B4	• Forward-primer: 5' GTCCTCGTGGAAGGCCC 3' • Reverse-primer: 5' AGGAGAGACAGGGAGCTCAG 3'

Abbreviations: RANKL: Receptor activator of NF- κB ligand; EGFR: Epidermal Growth Factor Receptor; RANK: Receptor activator of NF- κB, OPG: osteoprotegerin, IPO8: Importin 8; POLR2a: Polymerase II polypeptide A.



A)

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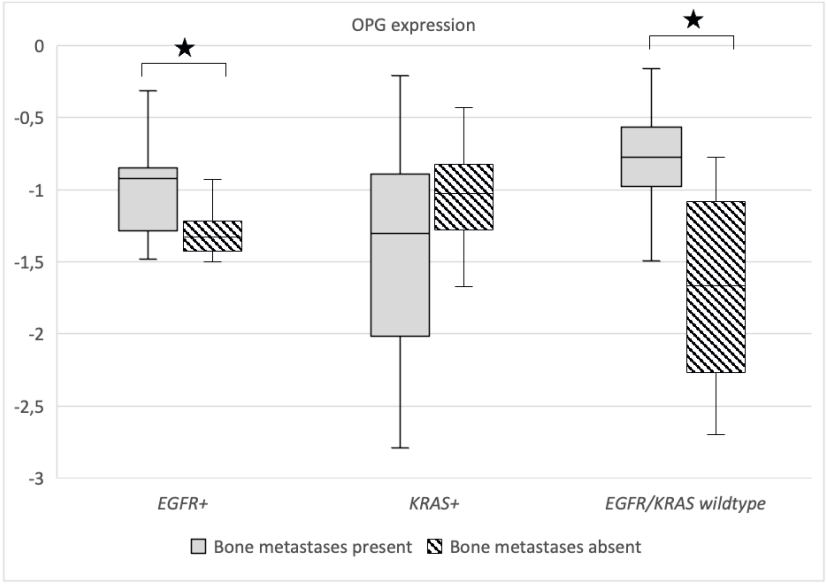
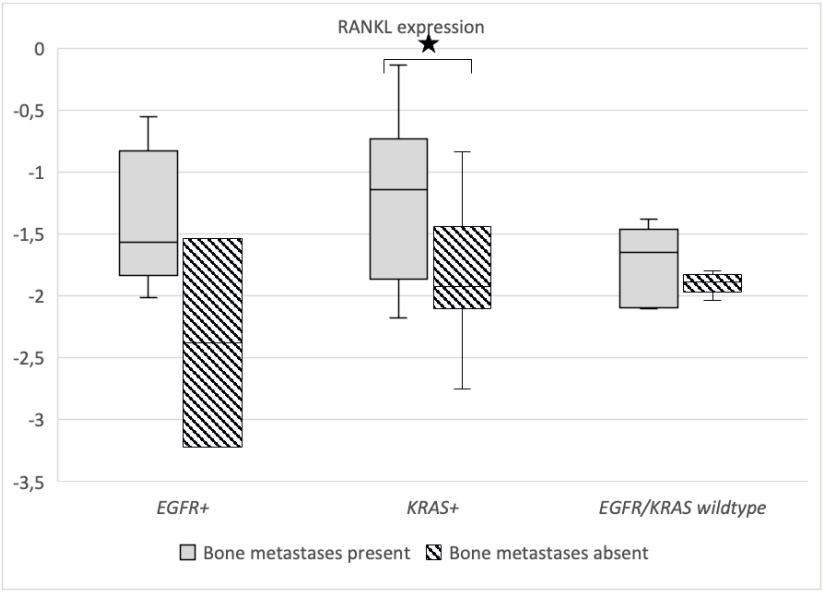


B)

n=23	n=36	n=14
17BM+ 6BM-	20BM+ 16BM-	9BM+ 5BM-



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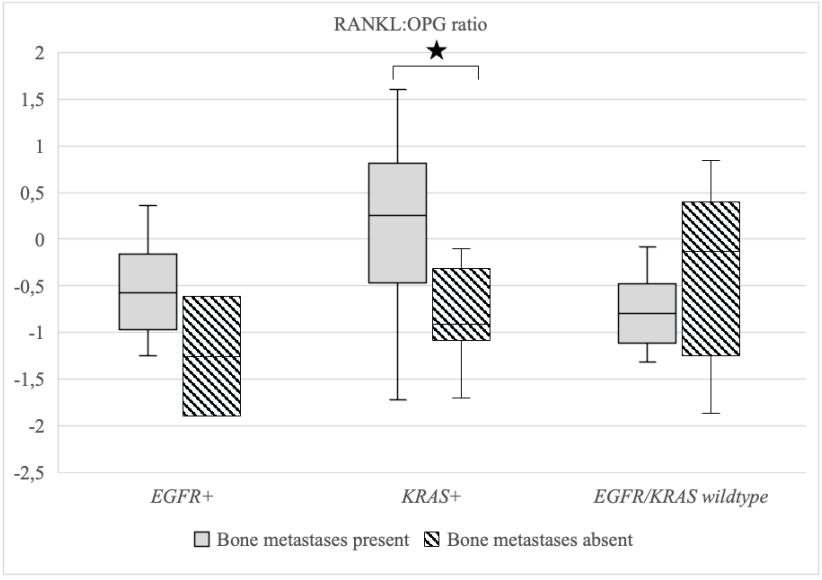
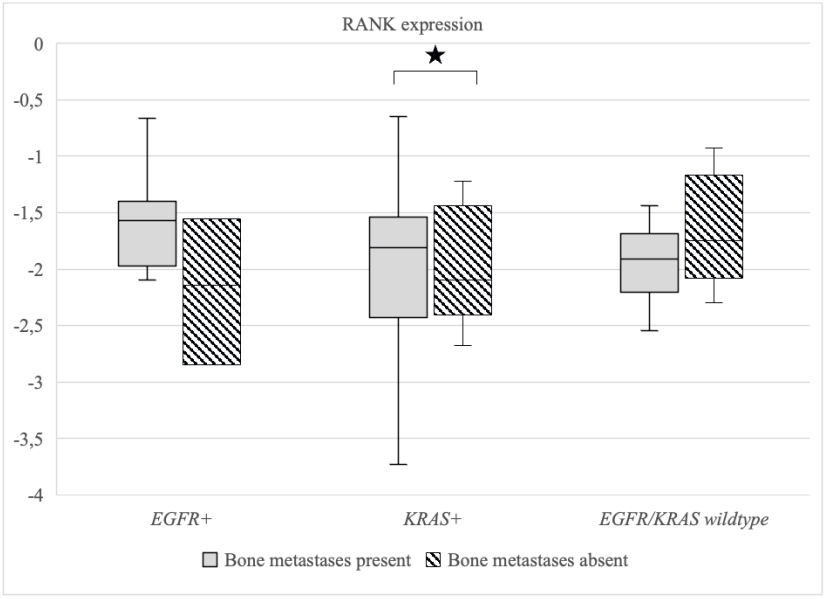


D)

n=23	n=36	n=14
17BM+ 6BM-	20BM+ 16BM-	9BM+ 5BM-



E)

	n=23 17BM+ 6BM-	n=36 20BM+ 16BM-	n=14 9BM+ 5BM-

Figure 1 A-E: Relation between EGFR, RANKL, RANK and OPG expression or RANKL:OPG ratio and molecular subgroup.

A) EGFR expression, B) OPG expression, C) RANKL expression, D) RANKL:OPG ratio, E) RANK expression. An asterisk denotes a significant difference between groups.

Abbreviations: EGFR: Epidermal Growth Factor Receptor; OPG: osteoprotegerin; RANKL: Receptor Activator of Nuclear Factor κB ligand; RANK: Receptor Activator of Nuclear Factor κB.

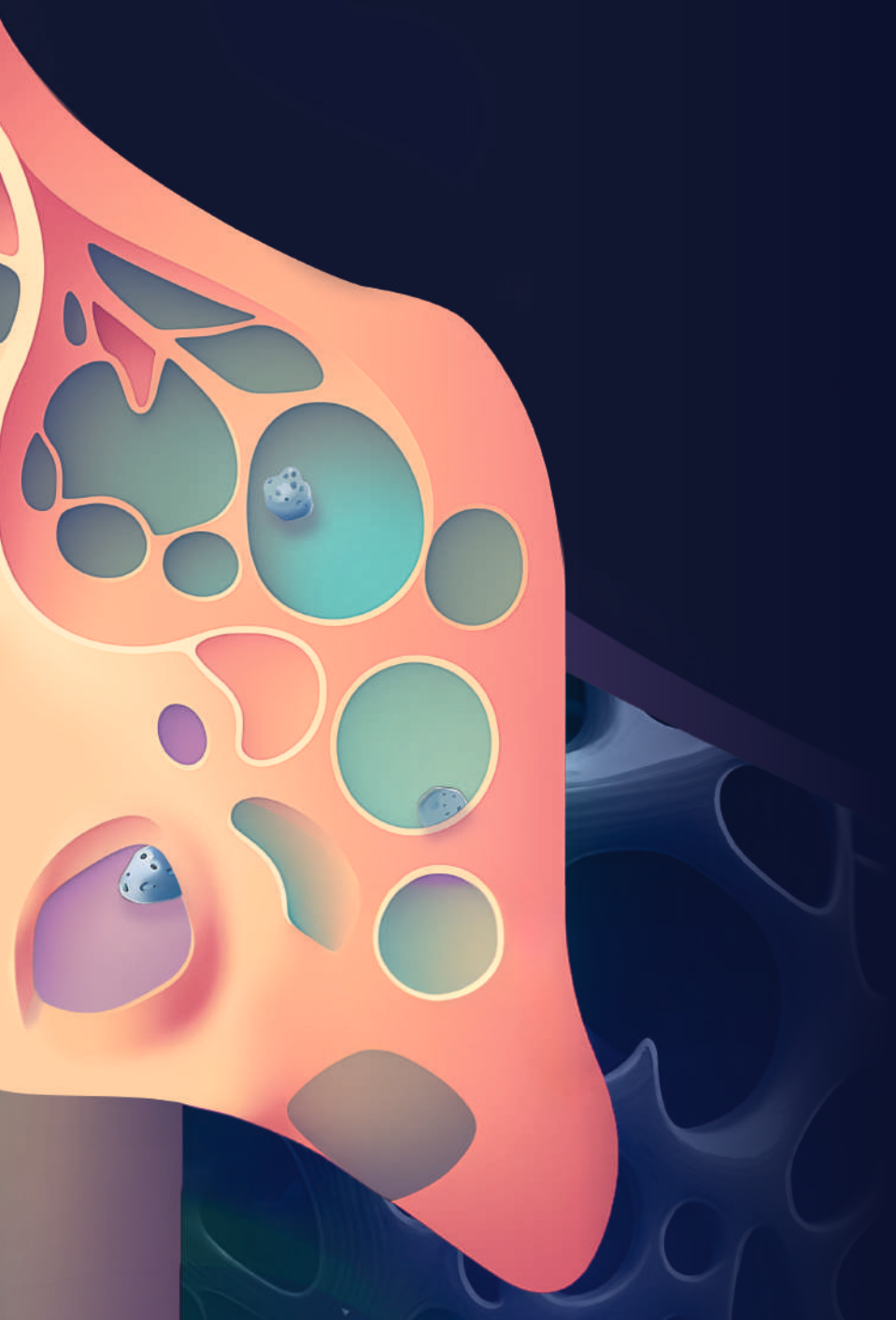
CHAPTER 5

Connecting the dots: (RANKL^{+}) extracellular vesicle count in blood plasma in relation to bone metastases, skeletal related events and osimertinib treatment in patients with EGFR mutated NSCLC

Anita J.W.M. Brouns, Iris J. Robbesom-van den Berge, Sophie M. Ernst, Christi M.J. Steendam, Wouter W. Woud, Liang Wu, Anne-Marie C. Dingemans, Lizza E.L. Hendriks, Marjolein van Driel

Submitted

CHAPTER 6



Reporting of Incidence and Outcome of Bone Metastases in Clinical Trials Enrolling Patients with Epidermal Growth Factor Receptor Mutated Lung Adenocarcinoma - A Systematic Review

Anita J.W.M. Brouns, Safiye Dursun, Gerben P. Bootsma, Anne-Marie C. Dingemans, Lizza E.L. Hendriks

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ABSTRACT

Bone metastases occur in 30-60% of patients with non-small cell lung cancer (NSCLC), are associated with decreased survival, cancer induced bone pain and skeletal related events (SREs). Those with an activating epidermal growth factor mutation (*EGFR*+) seem to be more prone to develop bone metastases. To gain more insight into bone metastases related outcomes in *EGFR*+ NSCLC, we performed a systematic review on Pubmed (2006-2021). Main inclusion criteria: prospective, phase II/III trials evaluating EGFR-tyrosine kinase inhibitors, ≥10 *EGFR*+ patients included, data on bone metastases and/or bone related outcomes available. Out of 663 articles, 21 (3176 *EGFR+* patients) met the eligibility criteria; 4 phase III (one double blind), 17 phase II trials (three randomized) were included. In seven trials dedicated bone imaging was performed at baseline. Mean incidence of bone metastases at diagnosis was 42%, 3-33% had progression in the bone upon progression. Except for one trial, it was not specified whether the use of bone target agents was permitted, and in none of the trials, occurrence of SREs was reported. Despite the high incidence of bone metastases in *EGFR+* adenocarcinoma, there is a lack of screening for, and reporting on bone metastases in clinical trials, as well as permitted bone targeted agents and SREs.

SIMPLE SUMMARY

Around 30-60% of the patients with lung cancer develop bone metastases, which are associated with decreased survival, bone pain and skeletal related events such as need for radiation. Patients with an epidermal growth factor mutation (*EGFR*), a subgroup of the patients with lung cancer, seem to develop more bone metastases than other patients with lung cancer. Due to prolonged survival of these patients, they live longer with bone metastases and/or skeletal related events, therefore optimal management is warranted. The aim of our systematic review is to gain more insight in reporting of bone metastases, skeletal related events and bone-specific outcome of treatment in clinical trials enrolling patients with *EGFR* mutated lung cancer. We found that data on bone metastases and bone related outcomes is largely lacking in clinical trials. There should more focus on reporting and preventing of skeletal related events in these patients.

INTRODUCTION

Activating epidermal growth factor mutations (*EGFR+*) are found in approximately 10% of the Caucasian and 50% of the Asian patients with lung adenocarcinoma (1-3). *EGFR* mutations are prognostic and are also predictive for efficacy of EGFR-tyrosine kinase inhibitors (TKI). For patients with advanced lung adenocarcinoma and an *EGFR+* mutation, treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs), the five-year survival rate is 40-50% (4,5). This is more favorable than the historical (i.e., before the introduction of immune checkpoint inhibitors) 5.8% five-year survival rate of patients without oncogenic-driven non-small cell lung carcinoma (NSCLC) (6). Immune checkpoint inhibitors (ICI) as monotherapy result in disappointing outcomes in patients with EGFR mutated lung adenocarcinoma, with low responses rates and low survival and should only be considered after exhaustion of other systemic therapies (7). ICI combined with EGFR-TKI has been evaluated in clinical studies but the combinations were either too toxic or did not provide an advantage over EGFR-TKI alone (7).

Importantly, the biological predisposition for distant metastases seems to vary between the different molecular subgroups of non-squamous NSCLC (8,9). The largest series is a nationwide Dutch database analysis (n=2052), including all patients with metastasized non-squamous NSCLC (ns-NSCLC) at initial diagnosis with data from molecular analysis and metastasis pattern at diagnosis of stage IV disease. A significantly higher bone metastases incidence was reported in patients with an *EGFR* mutation compared with other molecular subgroups (54% vs. 33% Kirsten rat sarcoma [*KRAS+*] vs. 30.5% anaplastic lymphoma kinase fusion [*ALK+*] vs. 31.5% triple negative patients, $p< 0.001$) (9). However, other studies (n=189-1063) evaluating the incidence of bone metastases in different molecular subgroups, i.e., *EGFR* mutated, *KRAS* mutated, *ALK* rearranged or wildtype patients with non-squamous NSCLC, showed conflicting results (8,10-13).

The currently available clinical trials evaluating bone targeted agents (BTAs) and clinical guidelines (European Society for Medical Oncology (ESMO), National Comprehensive Cancer Network (NCCN)) providing recommendations for the management of bone metastases and skeletal related events (SREs), do not focus on specific molecular subgroups of NSCLC lung adenocarcinoma (14-18). The guidelines state that it is advised to treat patients with bone metastases and a favorable survival (specified as at least three months) with BTAs. However, a more personalized advise is important, as in clinical trials especially the patients with a prolonged overall survival (OS) (i.e., historically mainly patients with metastatic breast and prostate cancer) benefit the most from BTAs (i.e., significant reduction of SREs) such as bisphosphonates or denosumab (19-21). As the clinical behavior of EGFR mutated lung adenocarcinoma resembles metastatic breast and prostate cancer, with a real possibility for prolonged survival, data for this subgroup is also needed. However, to the best of our knowledge, clinical trials evaluating BTAs specifically in *EGFR* mutated lung adenocarcinoma do not exist.

The risk of a negative influence on quality of life (QoL) and OS, caused by SREs, could be significant in patients with EGFR mutated lung adenocarcinoma and bone metastases (22,23).

In one retrospective case-control study (n=189, no use of BTAs) survival post bone metastases diagnosis was superior for patients with an *EGFR* mutation compared to patients with a *KRAS* mutation or those without an *EGFR/KRAS* mutation, while time to first SRE was not significantly different (12). As a result, these patients live longer with SREs. Therefore, optimal management, treatment, and outcome of bone metastases in this specific patient population is necessary and should be further evaluated.

As large prospective series on bone metastases related outcomes are lacking for patients with *EGFR* mutated lung adenocarcinoma, we performed a systematic review to gain more insight in the reporting of bone metastases and/or SREs, and bone-specific outcomes in patients with *EGFR*-mutated lung adenocarcinoma included in phase II/III EGFR-TKI trials. Improved knowledge about bone-related events in *EGFR*-mutated tumors can lead to better advice about the use of BTA in this subgroup of patients.

MATERIALS AND METHODS

Search strategy and selection criteria

A systematic search was performed using the Pubmed database. The search period was limited to January 2006 until January 2021 (search data January 8th 2021). The start date of January 2006 was chosen, as in 2006 EGFR-TKIs were approved by the United Status of America's Food and Drug Administration (FDA) and became standard treatment for patients with lung adenocarcinoma and an *EGFR* mutation. Published studies were identified using a search strategy based on the patient intervention comparator outcome (PICO) method (shown in Table S1) (24). Because of the outcome variable of interest (i.e., bone metastases and SRE incidence) was an undefined endpoint in EGFR-TKI trials, we decided to exclude this outcome variable in the search strategy to prevent missing data. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 checklist for systematic reviews is shown in Table S2. Furthermore, the control intervention was not included in the search strategy to include single arm EGFR-TKI trials. Trials had to include a minimum of 10 patients with non-squamous NSCLC and an *EGFR* mutation, as trials in the beginning of the TKI era also included patients with wildtype *EGFR*. Only prospective, phase II and III trials were included. All inclusion criteria are summarized in Table 1.

To minimize missing articles, in the same time period, we searched for relevant articles in the meeting libraries of the American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO) and International Association for the Study of Lung Cancer (IASLC).

Table 1: Inclusion criteria.

Criterion	Definition
Subjects included	Human only
Language	English
Article type	Original article; reviews excluded
Study phase	II or III
Year of publication	January 2006 - Jan 2021
Site of primary tumor	NSCLC, ≥ 10 patients with *EGFR* mutation
Tumor stage	IIIB or IV
Age	≥ 18 years
Treatment	At least one of the trial arms was treatment with EGFR-TKI
Follow-up period	No lower or upper limit
Dosing, route and frequency or duration of treatment	No restrictions
Outcome	Bone metastases and SREs at baseline or during the course of the disease, and/or their outcome. Regardless of whether they were primary, secondary, or no pre-specified endpoint of the trial

Abbreviations: NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor; SREs, skeletal related events.

Study Selection

The titles of the retrieved studies, and the abstracts of the eligible studies based on title screening, were evaluated independently by two reviewers (A.B. and S.D.). The same reviewers independently examined the full text of the remaining articles regarding the inclusion criteria. Studies were included if they met the pre-specified inclusion criteria as shown in Table 1. To complete the search, the references of all eligible articles were manually searched for additional relevant articles. In case of disagreement during study inclusion, consensus was sought.

Data selection

Two reviewers (A.B. and S.D.) independently extracted relevant characteristics of each eligible study. When available and if applicable, the following data were extracted: year of publication; phase II or III trial, number of study arms, randomization method, blinding method, duration of study and follow-up, histological diagnosis, method of staging, number of patients and number of patients with an EGFR+ mutation, intervention (i.e., type, dose, duration, route and frequency of administration of TKIs), bone metastases (i.e., incidence, outcome, treatment), SREs (i.e. incidence, outcome, treatment), secondary and primary objectives of the trial and OS.

The Jadad scale was used to assess the methodological quality of the included trials (25). We did not perform a formal test of heterogeneity because of the heterogeneous type of trials included in the systemic review, with three quarter of the included trials being single arm (i.e., per definition high risk of bias).

RESULTS

Study selection

The literature search identified 663 unique articles in total. 317 articles were excluded because of non-relevant titles. About 160 of the 346 remaining articles were excluded because they did not fulfill the inclusion criteria based on the abstract. The full text of the remaining 186 articles was screened; 166 articles were excluded due to: no information about bone metastases or SREs (n=139), unknown EGFR mutational status (n=10), unknown if the patients with NSCLC and bone metastases were patients with an EGFR mutation or if they were wildtype patients (n=9), insufficient number of patients with an EGFR mutation (n=4), other reasons (n=4). A manual search of the reference list of the included articles revealed one additional relevant article. No additional studies were identified by searching the meeting libraries of the ASCO, ESMO and IASLC conferences in the period 2006-2021. Ultimately, 21 articles were included in this review. The flowchart for article selection is shown in Figure 1.

Description of studies

Four phase III trials (26-29), of which one double blind randomized (29), and 17 phase II trials (30-46), of which three randomized (37,39,40), were included. The main characteristics of the included studies are shown in Table 2.

The number of patients included in the studies ranged from 10 (38) to 556 (Flaura (29)), leading to in total 3176 patients with advanced NSCLC and an *EGFR* mutation included in this review. One trial also enrolled patients with advanced non-squamous NSCLC and *EGFR* wildtype, the results were specified per molecular subgroup (46). The inclusion criteria were generally similar across the included trials (i.e., pathological proven locally advanced NSCLC not suitable for treatment with radical radio-chemotherapy or metastatic NSCLC). All studies, except one in which also patients with non-squamous NSCLC and *EGFR* wildtype were included [46), enrolled exclusively patients with an activating *EGFR* mutation. The other exception in inclusion criteria was systemic treatment history of the patients. The exclusion criteria concerning comorbidities were comparable among all studies. The Aura 3 was the only trial in which explicitly a statement about bone targeted agents (BTAs) was added, it was permitted for patients to use medication (e.g., denosumab) for painful bone metastases (26). The other trials provided no information about BTAs (26-34,36-46).

In seven trials the primary endpoint was objective response rate (ORR) on EGFR-TKI treatment (30-34,36) or chemotherapy combined with EGFR-TKI treatment (43). ORR was evaluated in different patient categories: patients with NSCLC and *EGFR* mutation pretreated with chemotherapy or TKI (Aura 2 (32), (30), KCSG-Lu15-09 (31)), irrespective of previous chemotherapy (36), patients that were chemotherapy or TKI-naïve (33,34) and treatment naïve (43). Progression free survival (PFS) was the primary outcome in 11 trials (26-29,35,37-39,41,42,45). PFS was evaluated in different patient categories: patients with NSCLC and an *EGFR*

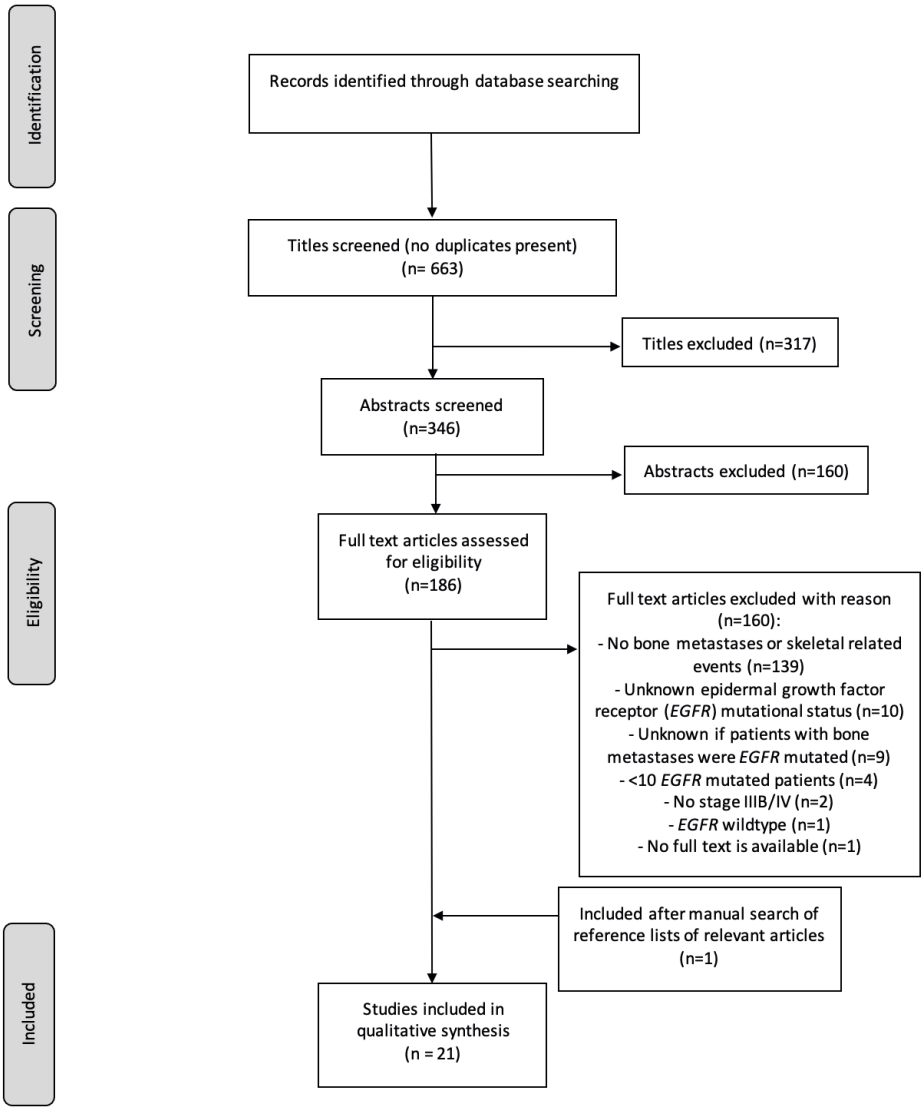

Figure 1: Flowchart.

mutation who were treatment naïve in one trial (45) or treatment naïve for advanced disease in five trials (Lux-Lung 7 (40), Jo22903 and Jo25567 trial (39), Flaura (29), (27,38)). In the other trials patients with NSCLC and *EGFR* mutation were pretreated with EGFR-TKIs (Aura 3 (47), (35,42)), were chemotherapy naïve (Aspiration study (41), (46)) and chemotherapy naïve for advanced disease (Eurtac (28), Insight study (37)). The study of Yoshimura (2013) in which patients with NSCLC and an EGFR mutation who previously were treated with EGFR-TKIs and in the trial were

treated with pemetrexed in combination with erlotinib or gefitinib had disease control rate as primary outcome. Two trials had co-primary endpoints: PFS and response to treatment (46) and PFS, time to treatment failure, OS in Lung-Lux 7 (40). None of the trials had bone metastases related outcomes as primary or secondary endpoint

Assessment of the risk of bias within studies

A formal test of heterogeneity was not performed, due to the heterogeneous type of trials included in this systematic review: only four trials were randomized controlled trials, whereas two-third of the trials being single arm had per definition a high risk of bias. Instead, we used the Jadad scale to assess the methodological quality of the included studies [25]. The methodological quality of four of 21 studies were assessed as high (i.e., Jadad score ≥3) [27-29,40]. The other 17 studies were assessed as poor methodological quality (i.e., Jadad score ≤2) [26,30-39,41-46].

Results of individual studies

Imaging and incidence of bone metastases at baseline

In 12 out of 21 studies the mandated imaging at study entry was described [28,30-33,36,39,42-46]. Dedicated bone imaging was performed in seven out of 21 trials, by means of a bone scintigraphy [33,36,39,43,44] or a 2-deoxy-2-[fluorine-18]fluoro-D-glucose Positron Emission Tomography-Computer Tomography scan (FDG-PET-CT scan) [45,46].

The incidence of bone metastases at baseline was reported in 14 studies (total 1196 patients) [27,28,31,34-38,40,42-46]. Out of these 1196 patients, 502 (42%) had bone metastases at baseline (range 14-90%). In none of these studies the bone metastasis was a stratification factor.

Imaging and incidence of bone metastases during follow-up

In two out of 21 studies dedicated bone imaging during follow-up was performed: with FDG-PET-CT scan [45,46] or with a bone scintigraphy [33]. Bone scintigraphy was performed in the study of Reguart when clinically indicated [42].

In ten studies (total 2378 patients) the incidence of bone metastases as site of progressive disease (PD) was reported ([27,30,33,38,39,46], Flaura [29], Aura 2 [32], Aura 3 [26], Aspiration study [41]). Three to 26% of the patients had development or progression of bone metastases as site of PD (215 of 2378 patients). In none of the included studies data were provided whether bone progression was the only site of progression or not.

Skeletal related events

In none of the included studies information about SREs was provided. In Table 3 a summary of the reported imaging, incidence of bone metastases and SREs is shown.

Table 2. Main characteristics of the included studies.

Study (y)	Trial type	Jadad score	Total pts/ EGFR+ pts	Histological diagnosis (%)	Stage (%)	Treatment arm (dose)	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Sunaga (2007)	Phase II, single-arm, multicenter study	1	21/21	AdC (100)	IIIB (24) IV (76)	Gefitinib (250 mg q.d.)	-	12.6	ORR	PFS, tolerability
Inoue (2009)	Phase II, single-arm study	1	29/29	AdC (93) Adenosquamous (3) Undifferentiated (3)	IV (93) Other (7)	Gefitinib (250 mg q.d.)	-	17.8	ORR	PS improvement rate, toxicity, PFS, OS
Rosell (2012) [Eurtac]	Phase III, open-label, multicenter RCT	3*	173/173	AdC (92) BAC (1) LCC (2) SCC (0.5) NOS (3)	IIIA (1) IIIB (6) IV (92)	Erlotinib (150 mg q.d.)	3-week cycles of chemotherapy[1]	Erlotinib arm: 18.9 Chemotherapy arm: 14.4	PFS	OS, ORR, serum analysis *EGFR* mutation
Yoshimura (2013)	Phase II, single-arm, study	1	27/27	AdC (96) SCC (1)	IV (100)	3-weekly cycles of pemetrexed d1 (500mg/m^2) and erlotinib/gefitinib d2-16 (dose NR)	-	11.4	DCR	ORR, PFS, OS, toxicity, safety
Reguart (2014)	Phase I-II, single-arm, multicenter study	1	25/25	AdC (84) SCC (1) LCC (8)	IIIB (4) IV (96)	Erlotinib (150mg q.d.) + vorinostat (400mg q.d.)	-	NR	PFS at 12 weeks	Median PFS, OS
Zwitter (2014)	Phase II, single-arm, study	1	53/38	AdC (100)	IIIB (4) IV (96)	3-weekly cycles of gemcitabin 120mg/m^2 d1, cisplatin 75mg/m^2 d2, gemcitabin 1250mg/m^2 d4, erlotinib 150mg q.d. d5-15	-	NR	PFS, response to treatment	OS, toxicity, metabolic response only from 2010

Table 2. Main characteristics of the included studies. (continued)

Study (y)	Trial type	Jadad score	Total pts/ EGFR+ pts	Histological diagnosis (%)	Stage (%)	Treatment arm (dose)	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Yoshimura (2015)	Phase II, open-label, single-arm study	1	26/26	AdC (100)	III (4) IV (96)	3-weekly cycles of pemetrexed d1 (500mg/m^2) and gefitinib 250mg q.d. d2-16	-	19.7	ORR	-
Park (2016a) [Aspiration study]	Phase II, single-arm, multicenter study	1	207/207	AdC (97) SCC (1) NOS (2)	IV (85) Recurrent (16)	Erlotinib 150mg q.d.	-	11.3	PFS-1[2]	PFS-2[3], ORR, DCR, PFS-1[2] in exon 19 del and L858R subsets, OS, safety
Park (2016b) [Lux-lung 7]	Phase IIB, open-label, multicenter RCT	3*	319/319	AdC (99) NOS (1)	IIIB (3) IV (97)	Afatinib (40mg q.d.); dose escalation to 50mg q.d. allowed after 4 weeks without AE	Gefitinib (250mg q.d.)	27.3	PFS, time-to-treatment failure, OS	ObR, time to and duration of ObR, % pts that achieved DCR, duration of DCR, tumor shrinkage, QoL
Zwitter (2016)	Phase II, open-label, single-arm, study	1	38/38	Non-SCC (100)	IIIB (3) IV (97)	3-weekly cycles of gemcitabin (1250mg/m^2) d1+4, cisplatin 75mg/m2 d2, erlotinib 150mg q.d. d 5-15	-	35	PFS	-
Atagi (2016)	Combined results of 2 phase II studies: JO22903 (single-arm) and JO25567 study (randomized)	JO22903: 1 JO25567: 2	177/177	NSCLC (100)	IIIB/IV (78) Recurrent (22)	JO22903: erlotinib 150mg q.d. JO25567: erlotinib 150mg q.d.	JO22903: - JO25567: bevacizumab 15mg/kg 3-weekly cycles + erlotinib 150mg q.d.	JO22903: 20.4 JO25567: at minimum 20	PFS both studies	JO22903 and JO25567: ORR, DCR, OS. JO25567: also QoL, symptom improvement[4], safety

Table 2. Main characteristics of the included studies. (continued)

Study (y)	Trial type	Jadad score	Total pts/ EGFR+ pts	Histological diagnosis (%)	Stage (%)	Treatment arm (dose)	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Hirano (2016)	Phase II, single-arm, multicenter study	1	11/11	AdC (100)	IV (100%)	Erlotinib (25mg q.d.); dose escalation to 150mg q.d. in case of PD	-	NR	ORR	PFS, OS, safety
Goss (2016) [Aura 2]	Phase II, open-label, multicenter single-arm study	1	199/199	AdC (95) SCC (1) Adenosquamous (1) NOS (3)	IIIB (6%) IV (94%)	Osimertinib (80 mg q.d.)	-	13.0	ORR	PFS, duration of response, DCR, tumor shrinkage, OS, safety, QoL, pharmacokinetics
Mok (2017) [Aura 3]	Phase III, open-label, multicenter RCT	2	419/419	AdC NOS (86)	IIIB (NR) IV (NR)	Osimertinib 80mg q.d.	3-weekly cycles of pemetrexed (500mg/m^2) + carboplatin (AUC 5) or cisplatin (75mg/m^2)	8.3	PFS	ORR, DoR, DCR, OS, tumor shrinkage, PROMS, safety, side-effect profiles
Soria (2018) [FLAURA]	Phase III, multicenter, double-blind, RCT	4*	556/556	AdC (97) Other (3)	IIIB (5) IV (95) Missing (<1)	Osimertinib 80mg q.d.	Erlotinib (150mg q.d.) or Gefitinib (250mg q.d.)	15	PFS	OS, ORR, DoR, DCR, depth of response[5], safety
Lim (2018)	Phase II, single-arm, study	1	49/49	NSCLC NOS (100)	IV (98.7) Recurrent (10.2)	Gefitinib 250mg q.d.	-	At minimum 6	PFS-2[3]	PFS-1[2], difference between PFS-2-PFS-1[6], OS, safety

Table 2. Main characteristics of the included studies. (continued)

Study (y)	Trial type	Jadad score	Total pts/ EGFR+ pts	Histological diagnosis (%)	Stage (%)	Treatment arm (dose)	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Ahn (2019)	Combined results of 2 phase II studies (AURA extension and AURA 2 trial), both single-arm	Aura extension trial: 1 Aura 2 trial: 1	411/411	AdC (96) SCC (<1) Adenosquamous (<1) Other (3)	IIIB (4) IV (96)	Osimertinib 80mg q.d.	-	NR	ORR	DoR, DCR, PFS, OS, safety
Zheng (2019)	Phase II, single-arm study	1	10/10	AdC (100)	IV (100%)	Erlotinib 150mg q.d. or Gefitinib 250mg q.d. plus thoracic radiotherapy[7]	-	12	PFS at 12 months	PFS, OS, safety, ORR, time to progression of irradiated lesion
Cho (2019) [KCSG-Lu15-09]	Phase II, open-label, single arm, study	1	36/36	AdC (97) SCC (3)	IV (64) Recurrent (36)	Osimertinib 80mg q.d.	-	20.6	ORR	PFS, OS, DoR, safety
Noronha (2020)	Phase III, open-label, study	3*	350/350	Gefitinib+chemo arm: AdC (98) Adenosquamous (2) SCC (1) Gefitinib arm: AdC (97) Adenosquamous (2) SCC (1) Sarcomatoid carcinoma (1)	Gefitinib+chemo arm: IIIB (2) IV (98) Gefitinib arm: IIIB (3) IV (97)	3-weekly cycles of Gefitinib 250mg q.d. and pemetrexed 500mg/m^2 + carboplatin (AUC 5) on d1, (up to four cycles), followed by 3-weekly cycles maintenance pemetrexed	Gefitinib 250mg q.d.	17	PFS	PS, RR, toxicity, QoL

Table 2. Main characteristics of the included studies. (continued)

Study (y)	Trial type	Jadad score	Total pts/ EGFR+ pts	Histological diagnosis (%)	Stage (%)	Treatment arm (dose)	Comparator arm	Median follow-up (months)	Primary objective(s)	Secondary objective(s)
Wu (2020) [Insight study]	Phase Ib/II, open-label, study	2	55/55	Teponitinib plus gefitinib arm: AdC (97) SCC (3) Chemotherapy arm: AdC (100)	NR	Teponitinib 500mg q.d. + gefitinib 250mg q.d.	Pemetrexed 500mg/m^2+ cisplatin 75mg/ m^2 or carboplatin (AUC 5-6) on d1 ≤6 cycles or 4 cycles + pemetrexed maintenance	21.8	Investigator-assessed PFS	OS, safety

Abbreviations: *EGFR+*; activating mutation in the epidermal growth factor receptor (EGFR), pts; patients, AdC; adenocarcinoma, q.d.; once a day, - ; not applicable, ORR; overall response rate, PFS; progression free survival, OS; overall survival, RCT; randomized controlled trial, BAC; bronchoalveolar adenocarcinoma, LCC; large cell carcinoma, SCC; squamous cell carcinoma, NOS; not otherwise specified, NSCLC; non-small-cell lung carcinoma, DCR; disease control rate, RR; response rate, PS; performance score, DoR; duration of response, ObR; objective response, QoL; quality of life, d; day, AE; adverse events, RECIST; response evaluation criteria in solid tumors, PD; disease progression, NR; not reported, AUC; area under the curve, PROMS; patient reported outcome measures. * High Jadad score, i.e., ≥3. [1]Cisplatin 75 mg/m2 on day 1 plus docetaxel (75 mg/m2 on day 1) or gemcitabin (1250 mg/m2 on days 1 and 8). In patients with contra-indications for cisplatin, carboplatin (AUC 6 with docetaxel 75 mg/m2 or AUC 5 with gemcitabin 1000 mg/m2) was allowed. [2]PFS-1; time from first study dose to first RECIST, PD or death. [3]PFS-2; time from first study dose to off-erlotinib PD in subset of pts who continued erlotinib therapy beyond RECIST 1.1 PD. [4]Measured by the Functional Assessment of Cancer Therapy Lung (FACT-L) scale. [5]Defined as change in target-lesion size from baseline. [6]Defined as time from RECIST 1.1 progression until off-gefitinib progression. [7]54–60 Gray/27–30 fractions/5.5–6 weeks.

Table 3: Summary of the reported imaging, incidence of bone metastases and SREs of the included studies.

Study (y)	Required imaging at baseline	Method of imaging during follow-up	BM at baseline (%)	BM at progression (%)	Number of SRE (%)	BTA use
Sunaga (2007)	Chest X-ray, chest+ abdominal CT scan, brain MRI scan, radionuclide bone scan	NR	24	NR	NR	NR
Inoue (2009)	NR	NR	41	NR	NR	NR
Rosell (2012) [Eurtac]	Ct scan, optional PET-CT scan	CT scan (not further specified)	Erlotinib arm: 33 Chemotherapy arm: 33	NR	NR	NR
Yoshimura (2013)	Chest X-ray, chest+ abdominal CT scan, brain MRI or CT scan, radionuclide bone scan	NR	59	NR	NR	NR
Reguart (2014)	Chest+ abdominal CT scan. Brain CT scan and bone scintigraphy on indication	Chest CT scan, abdominal CT scan. Brain CT scan and bone scintigraphy on indication	40	NR	NR	NR
Zwitter (2014)	Chest X-ray, brain+chest+ upper abdominal CT scan From 2010 PET-CT scan	Before 2010 NR, from 2010 PET-CT scan	63	*EGFR+* group: 26	NR	NR
Study (y)	**Required imaging at baseline**	**Method of imaging during follow-up**	**BM at baseline (%)**	**BM at progression (%)**	**Number of SRE (%)**	**BTA use**
Yoshimura (2015)	Chest X-ray, chest+abdominal CT scan, brain MRI or CT scan, radionuclide bone imaging or PET-CT scan	CT scan not further specified every 6 wks for first 24 wks, thereafter every 8 wks till PD or new therapy	31	NR	NR	NR

Table 3: Summary of the reported imaging, incidence of bone metastases and SREs of the included studies. (continued)

Study (y)	Required imaging at baseline	Method of imaging during follow-up	BM at baseline (%)	BM at progression (%)	Number of SRE (%)	BTA use
Park (2016a) [Aspiration study]	NR	NR	NR	8.2	NR	NR
Park (2016b) [Lux-lung 7]	NR	CT scan (not further specified) or MRI scan	Afatinib arm: 50 Gefitinib arm: 46	NR	NR	NR
Zwitter (2016)	PET-CT scan	PET-CT scan	63	"bone (10) most frequent site of PD." Number of pts with PD NR.	NR	NR
Atagi (2016)	Chest+abdominal scans (CT/MRI), brain scan (CT/MRI), bone scans (bone scintigraphy, PET-CT, MRI)	NR	NR	16	NR	NR
Hirano (2016)	Chest X-ray, chest +abdominal/pelvis CT scan, brain MRI, bone scintigraphy	CT, MRI, bone scan every 2 months	NR	12.5	NR	NR
Goss (2016) [Aura 2]	CT scan or MRI scan (not further specified)	CT scan or MRI scan (not further specified)	NR	13.8	NR	Permitted, no further information
Mok (2017) [Aura 3]	Chest+abdominal scans (CT/MRI), any other areas of disease involvement based on patients' signs or symptoms	Chest+abdominal scans (CT/MRI), any other areas of disease involvement based on patients' signs or symptoms	NR	Osimertinib arm: 3, Platinum/ pemetrexed arm: 4	NR	NR

Table 3: Summary of the reported imaging, incidence of bone metastases and SREs of the included studies. (continued)

Study (y)	Required imaging at baseline	Method of imaging during follow-up	BM at baseline (%)	BM at progression (%)	Number of SRE (%)	BTA use
Soria (2018) [Flaura]	Chest+abdominal scans (CT/MRI), any other areas of disease involvement based on patients' signs or symptoms	Chest+abdominal scans (CT/MRI), any other areas of disease involvement based on patients' signs or symptoms	NR	Osimertinib arm: 4, Gefitinib or erlotinib arm: 4	NR	NR
Lim (2018)	NR	Tumor assessments every 8 weeks by CT-scan (not further specified)	18	NR	NR	NR
Ahn (2019)	AURA extension: CT scan or MRI scan (not further specified), AURA2 study: NR	AURA extension: CT scan or MRI scan (not further specified), AURA2 study: NR	NR	7	NR	NR
Zheng (2019)	NR	NR	90	20	NR	NR
Cho (2019) [KCSG-Lu15-09]	CT scan or MRI scan, not further specified	Chest X-ray every 3 weeks, CT scan every 6 weeks	28	NR	NR	NR
Noronha (2020)	NR	Every 9 wks by CT scans (not further specified)	Gefitinib+chemo arm: 14 Gefitinib arm: 14	Gefitinib+chemo arm: 3 Gefitinib arm: 5	NR	NR
Wu (2020) [Insight study]	NR	NR	Teponitinib plus gefitinib arm: 23 Chemotherapy arm: 37.5	NR	NR	NR

Abbreviations: BM; bone metastasis, CT scan; computer tomography scan, MRI scan; magnetic resonance scan, PET-CT scan; 2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computer tomography scan, wks; weeks, SRE; skeletal-related event, NR; not reported.

DISCUSSION

Bone metastases with their risk of SRE development and resulting impact on QoL, can become a clinically relevant problem in patients with lung adenocarcinoma and an *EGFR* mutation because of their prolonged post-bone metastases diagnosis survival [4,12,23,48,49]. To gain more insight into bone metastases and their outcomes, we performed a systematic review focusing on screening, treatment, and reporting of bone metastases and/or SREs and bone-specific outcomes in EGFR-TKI trials.

In none of the trials, primary or secondary outcomes related to bone metastases and/or its complications were mentioned. A 42% median baseline incidence of bone metastases in patients with NSCLC and an *EGFR* mutation was reported, which is slightly lower compared to the 54% baseline incidence reported in the Dutch nationwide database study and other retrospective studies [9,12,50]. Of note, in only seven of the included trials specific bone imaging was performed at baseline, possibly resulting in an underestimation of the real incidence of bone metastases. Up to 26% of patients had progression in the bone upon PD. This probably is also underestimated as in only two of the trials standardized follow-up bone imaging was performed. Patients in the Aura 2 trial were permitted to use BTAs in case of painful bone metastases, but further information on actual BTA use and outcome was not provided. In all other trials, all data regarding BTA was lacking.

EGFR-TKIs have a high efficacy in patients with lung adenocarcinoma and an *EGFR* mutation and bone metastases [51,52]. Their efficacy in bone is mediated by blockade of receptor activator of NF-κB ligand (RANKL)-mediated osteoclast activation and by inhibiting epidermal growth factor (EGF) signaling in bone stromal cells [53]. Therefore, it could be that in this specific patient population, bone metastases do not frequently lead to SREs. Indeed, a retrospective study in patients with lung adenocarcinoma and bone metastases (n = 410) reported a preventive effect of EGFR-TKIs on the development of SREs: 23.5% of the patients with lung adenocarcinoma who were treated with

EGFR-TKIs experienced SREs compared with 61.7% of patients without EGFR-TKI treatment (information about specific treatment in this group is not provided) ($p < 0.001$) [54]. However, even with EGFR-TKI use almost a quarter of the patients experienced SREs in this study, and in other studies, the reported frequency of SREs is even higher (37.3% to 58%) in patients with *EGFR*-mutated lung adenocarcinoma mainly treated with EGFR-TKIs [12,49,54–57]. A recently published retrospective study, which evaluated the type and frequency of SREs in patients with *EGFR*-mutated lung adenocarcinoma and bone metastases (n = 274, of which 148 treated with EGFR-TKI), showed that one-third of these patients developed their first SRE before start of EGFR-TKI treatment, the other two-third of the patients developed SREs in the first year of EGFR-TKI treatment [49]. The above summarized SRE percentages were observed in patients with *EGFR*-mutated lung adenocarcinoma, treated with first or second generation EGFR-TKIs. To the best of our knowledge, no data are available for the different generation EGFR-TKIs (i.e., first/second

versus third) regarding efficacy specifically on bone metastases. Mouse models were set up to investigate the efficacy of osimertinib with or without bevacizumab on bone metastases of NSCLC. Treatment with osimertinib (with and without bevacizumab), showed tumor regression and bone remodeling [58]. Based on these results, it is not clear whether osimertinib is superior to earlier generation TKI in humans in the treatment of bone metastases.

In the abovementioned retrospective studies, use of BTAs varied from 0 to 65% [12,49,54–57]. Interestingly, in vitro and in vivo studies showed that bisphosphonates can act synergistically with EGFR-TKIs [54,55,59]. The in vitro study of Chang on the HCC827 NSCLC cell line expressing mutated *EGFR*, suggested that the combination of gefitinib and zoledronic acid caused more tumor suppression [59]. A small retrospective study of Cui et al. (n = 38) studied the efficacy of bisphosphonates in patients with *EGFR*-mutated lung adenocarcinoma and bone metastases, treated with EGFR-TKIs. They showed a significant additive effect of bisphosphonates on OS post-bone metastases diagnosis: post-bone metastases OS in EGFR-TKI + bisphosphonate group: 28.3 months versus 22.0 months in the EGFR-TKI only group, p = 0.0587 [55]. Another small retrospective study studied the effects of bisphosphonates in patients with EGFR-mutated lung adenocarcinoma and bone metastases (n = 62) and found comparable results (PFS and OS prolonged in the bisphosphonate + EGFR-TKI group compared with the EGFR-TKI group) [54]. As these are retrospective, small series, these data are only hypothesis generating. To the best of our knowledge, no data on denosumab combined with EGFR-TKI are available and it would be interesting to prospectively evaluate this.

Due to the increasing number of treatment options (e.g., EGFR-TKI in combination with chemotherapy, or combination of EGFR-TKI with angiogenesis inhibition), survival is further improving for patients with an *EGFR* mutation [47,51,52,60,61]. In the Flaura trial, the median OS with first line osimertinib was 38.6 months, in the NEJ009 trial (combination gefitinib with carboplatinum/pemetrexed versus gefitinib alone), median OS was 50.9 months [51,60]. Five-year survival rates for these trials have not been reported yet, but with a median OS of 50 months, 5-year survival rates resemble that of advanced breast or prostate cancer in which 28.1–30.2% of the patients are alive five years after the diagnosis [48,62,63]. This is important, as it is suggested in retrospective series that patients with *EGFR*-mutated NSCLC have a long post-bone metastatic survival of 15.5 to 28.0 months [12,49], implying that these patients live long with SREs.

Despite the similarities in the incidence and nature of *EGFR*-mutated lung adenocarcinoma and breast cancer bone metastases, current guidelines (e.g., ESMO, National Comprehensive Cancer Network [NCCN], and National Institute for Health and Care Excellence [NICE], ASCO) provide different recommendations for screening of bone metastases for different primary tumors [18,64–67]. The most important difference between the guidelines is the recommendation to screen all breast cancer patients, whereas for NSCLC only the Lung Cancer South East French Guidelines recommend to screen for bone metastases in NSCLC [18,64–68]. The French guideline also recommends to evaluate each bone metastasis for pain, neurological risk, and fracture risk to aid in defining the optimal bone metastasis management in harmony with the oncological

treatment [68]. BTAs demonstrated benefit in reducing SREs and providing better pain control, in advanced breast patients diagnosed with bone metastases [19]. In the ESMO guideline on advanced breast cancer it is recommended to use BTAs in these patients (level of evidence I, grade of recommendation A [65]. Guidelines for lung cancer are less clear in their recommendations: the NCCN NSCLC guideline advises to consider BTAs in patients with NSCLC and bone metastases [16]. The ESMO guideline on bone health further specifies and recommends using BTAs in patients with a life expectancy of >3 months (i.e., almost all patients with an EGFR mutation) [18,67]. No specific recommendations for patients with *EGFR* mutated lung adenocarcinoma were found in these guidelines. Probably because of the historically poor OS of NSCLC compared to advanced breast cancer, only 15–33% of patients with NSCLC and bone metastases are treated with BTAs in daily practice [69,70].

To the best of our knowledge no trials are ongoing that evaluate BTAs in patients with an *EGFR* mutation, although trials in patients without an oncogenic driver are ongoing [(NCT03669523 trial: denosumab in combination with nivolumab, NCT01951586 trial: denosumab in combination with chemotherapy (recently finished, results are not published)].

Drawbacks for this systematic review are: (1) The heterogeneity of the included trials with differences in populations (e.g., ethnicity) and/or follow-up which could have led to the observed differences in reported incidences of SREs; (2) the lack of primary or secondary outcomes related to bone metastases and/or related complications in studies could have led to underreporting of these outcomes.

CONCLUSIONS

Despite long (post-bone metastatic) OS of patients with *EGFR*-mutated NSCLC, and the high incidence of bone metastases in this patient population, occurrence of SRE and outcome of bone metastases is barely reported in clinical trials. Based on in vitro data found and retrospective series there might be synergistic activity of EGFR-TKI and BTA. However, prospective research is needed to validate these observations. Furthermore, the results of this systematic review stress on the importance of screening for bone metastases and reporting of clinical outcomes of treatment on bone metastases future trials for patients with *EGFR*-mutated NSCLC.

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APPENDIX A

Table S1: Search strategy based on PICO method.

PICO		Search terms	
Patient	AND	Carcinoma Non-Small-Cell Lung	OR
		Carcinoma non small cell lung	
		Non-small-cell lung carcinoma	
		Non-small-cell lung cancer	
		NSCLC	
		Lung neoplasm	
		Lung neoplasms	
		Carcinoma, Non-Small-Cell Lung [MeSH]	
		Lung neoplasms [MeSH]	
		Epidermal growth factor receptor	
		Epidermal growth factor receptor mutation	
		EGFR	
		EGFR mutation	
		EGFR mutant	
		ERBB receptor [MeSH]	
		Receptor, epidermal growth factor [MeSH]	
Intervention	AND	Tyrosine kinase inhibitor	OR
		Tyrosine kinase inhibitors	
		TKI	
		EGFR TKI	
		EGFR-TKI	
		Protein Kinase Inhibitors [MeSH]	
		Protein Kinase Inhibitors [Pharmacological Action]	
		Erlotinib	
		Tarceva	
		Gefitinib	
		Iressa	
		Icotinib	
		BPI-2009H	
		Erlotinib Hydrochloride [MeSH]	
		Gefitinib [Supplementary Concept]	
		Afatinib	
		Giotrif	
		Dacomitinib	
		PF-00299804	
		Neratinib	
		HKI-272	
		Lapatinib	
		Tykerb	
		PF 00299804 [Supplementary Concept]	
		Lapatinib [Supplementary Concept]	
		Osimertinib	
		Tagrisso	
		AZD9291	
		Rociletinib	
		CO-1686	

PICO		Search terms	
Intervention	AND	Olmutinib HM61713 Nazartinib EGF816 ASP 8273 Avitinib AC 0010 osimertinib [supplementary Concept] AZD9291 [Supplementary Concept] Rociletinib [Supplementary Concept] Olmutinib [Supplementary Concept] Antineoplastic agent Antineoplastic agents Protein kinase inhibitors Protein kinase inhibitor Antineoplastic Agents [Pharmacological Action] Antineoplastic Agents [Mesh]	OR
Comparator		Not specified in search strategy in order to include single arm studies	
Outcome		Not specified in search strategy in order to include studies in which SRE was not a primary outcome	

Abbreviations: PICO, patient intervention comparator outcome; NSCLC, non-small-cell lung cancer; EGFR, epidermal growth factor receptor; TKI, tyro-sine kinase inhibitor; EGFR-TKI, epidermal growth factor receptor tyrosine kinase inhibitor.

Table 3: PRISMA 2009 checklist for systematic reviews

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	118, 120.
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	118.
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	119-120
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	120, Table S1, appendix.

Table 3: PRISMA 2009 checklist for systematic reviews (continued)

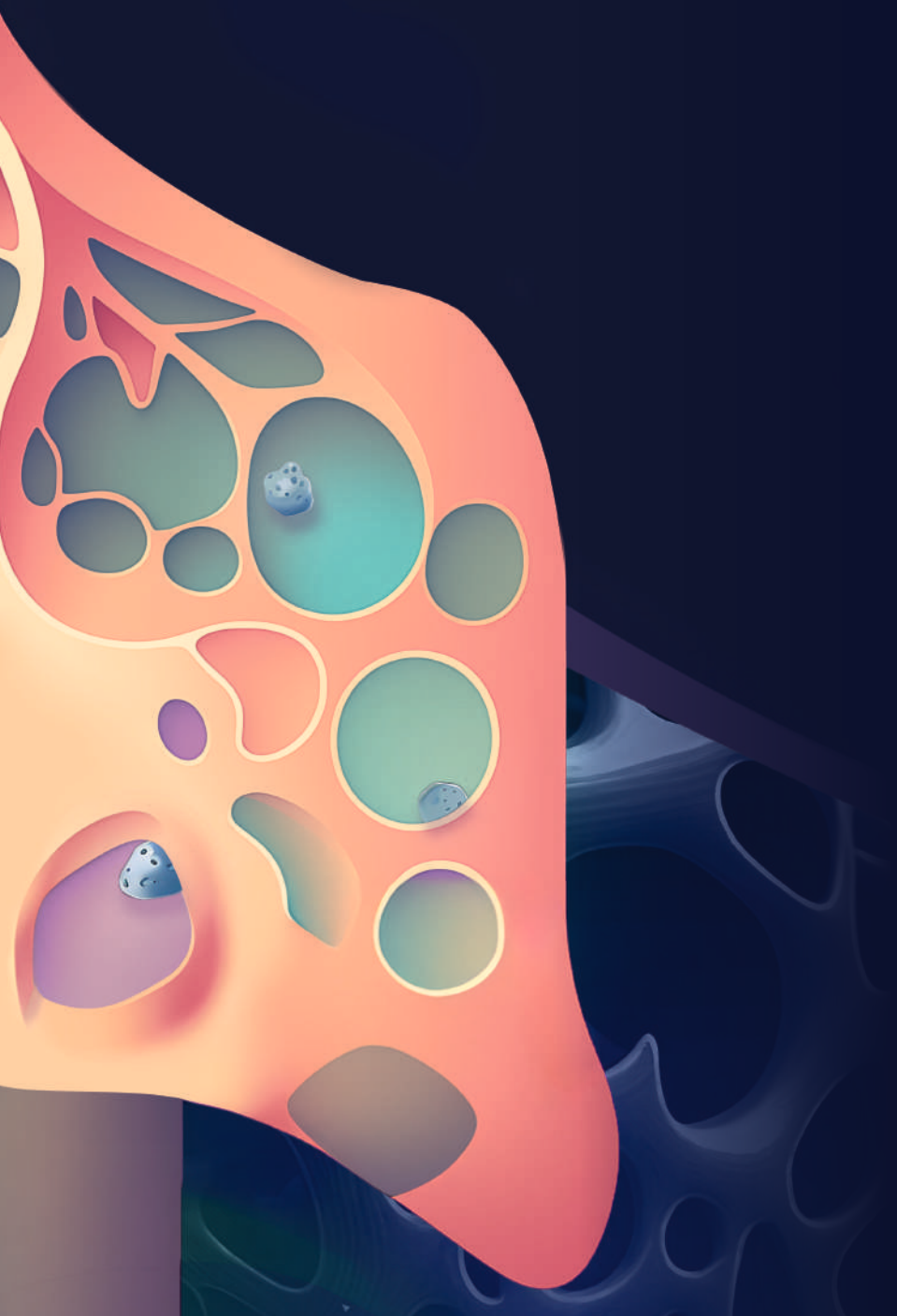
Section/topic	#	Checklist item	Reported on page #
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	120-121, Table 1.
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	120-121.
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Table S1, appendix.
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	120, Table 1 and Figure 1.
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	121.
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	120, 121.
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	121, Table 2.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA.
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA.
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA.
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1.
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 2, 121.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA.

Table 3: PRISMA 2009 checklist for systematic reviews (continued)

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	121.
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA.
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	133-135.
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	121, 135.
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	133-135.
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA.

Abbreviations: NA: not applicable.
From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097 .

CHAPTER 7



Incidence of bone metastases and skeletal related events in patients with epidermal growth factor receptor mutated non-small cell lung cancer treated with osimertinib

Anita Brouns, Ard van Veelen[#], G.D. Marijn Veerman[#], Christi M.J. Steendam, Safiye Dursun, Cor van der Leest, Sander Croes, Anne-Marie C. Dingemans, Lizza E.L. Hendriks

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[#]Ard van Veelen and Marijn Veerman equally contributed to the manuscript

ABSTRACT

Background

Bone metastases are frequent in patients with epidermal growth factor receptor mutated (EGFR+) non-small cell lung cancer (NSCLC). Skeletal related events (SREs) are common in these patients, however no data on SRE in osimertinib treated patients are reported. We investigated the development of bone metastases and SREs in patients with EGFR+ NSCLC treated with osimertinib.

Materials and methods

Retrospective multicenter cohort study, including patients with metastatic EGFR+ NSCLC who were treated with osimertinib between 02-2016 and 09-2021. Demographics, bone metastases related outcomes, SREs, treatment efficacy and overall survival (OS) were collected.

Results

In total, 250 patients treated with osimertinib (43% first line) were included. Fifty-one percent of patients had bone metastases at initiation of osimertinib. Sixteen percent of patients with bone metastases used bone targeted agents (BTAs). Median follow-up from initiation of osimertinib was 23.4 months (95% confidence interval [CI] 19.9-26.9 months). During osimertinib treatment, 10% developed new bone metastases or bone progression. Thirty-nine percent of patients with bone metastases had ≥1 SREs: 28% developed first SRE before osimertinib treatment, one percent after and 11% during. Median OS post bone metastasis was 30.8 months (95% CI 21.9-39.7). Median OS after first SRE was 31.1 months (95% CI 15.8-46.5).

Conclusion

Bone metastases and SREs are frequent before and during treatment with osimertinib in EGFR+ NSCLC. Because of these findings and the long OS post bone metastases, we advocate prescription of BTAs in these patients and recommend adding bone-specific endpoints in clinical trials.

INTRODUCTION

Bone metastases occur in 30%-60% of patients with advanced non-small cell lung cancer (NSCLC) (1,2). Patients with bone metastases are at risk for skeletal related events (SREs), with subsequently a possible negative impact on quality of life (QoL) and overall survival (OS) (3-5). The term SRE is a composite end point consisting of pathologic fracture, spinal cord compression, necessity for radiation to bone (for pain or impending fracture) or surgery to bone, because of bone metastases. Sometimes hypercalcemia of malignancy is also part of the SRE definition (6). On the basis of data of a nationwide registry (N=2052) we have revealed that at diagnosis of metastatic disease, 54% of patients with NSCLC and an epidermal growth factor receptor mutation (*EGFR+*) have bone metastases, which is the highest incidence compared with 33% in those with Kirsten rat sarcoma (*KRAS*+), 31% in those with anaplastic lymphoma kinase fusion (*ALK*+) and 32% in those with *EGFR/KRAS/ALK* wildtype (7). However, in other mainly small retrospective series (N=137-209) no differences were observed (8,9).

In patients with *EGFR*+ advanced NSCLC, treatment with first and second generation EGFR-tyrosine kinase inhibitors (TKIs) results in superior progression free survival (PFS) compared with chemotherapy (10). The incidence of SREs in this patient population is high (24%-58%) (1,11). In a retrospective series (N=189), incidence and time to first SRE were similar between patients with *EGFR*+, *KRAS*+ and *EGFR/KRAS* wildtype NSCLC when treated with first/second generation EGFR-TKI or chemotherapy, respectively (1). Nevertheless, patients with *EGFR*+ NSCLC had a significantly longer post metastatic bone disease survival compared with the other patients (median 15 mo [*EGFR*+], 9.0 mo [*KRAS*+] and 3.2 mo [*EGFR/KRAS* wildtype] (*EGFR+ - KRAS+*, p = 0.049, *EGFR+ - EGFR+/KRAS+ wildtype*, p = 0.004)) (1). Consequently, patients with an *EGFR* mutation are longer at risk for new SREs and live longer with SREs which might affect QoL. Nowadays, osimertinib is the preferred first-line treatment for patients with *EGFR+* NSCLC, with a median PFS of 18.9 months. The prevalence and incidence of SREs during osimertinib treatment are unknown (12).

Denosumab and bisphosphonates are bone targeted agents (BTAs), that inhibit normal osteoclast induced bone resorption. Bisphosphonates are ingested by osteoclasts during bone resorption, which causes cell death of the osteoclast. Denosumab binds to the receptor activator of nuclear factor κB ligand (RANKL) and prevents the interaction with its receptor, receptor activator of nuclear factor κB (RANK), with reduction of bone resorption as result. Both denosumab and bisphosphonates are supposed to have (in)direct antitumor effects, but their precise role has to be elucidated (13). BTAs prevent SREs or delay the time to SREs in solid tumors and multiple myeloma (14-16). Although BTA use in breast cancer is associated with reduction of pain owing to bone metastases, in lung cancer this evidence is less clear, and BTA use is low in patients with lung cancer (17-22).

It could be hypothesized that because of the superior efficacy of osimertinib, less bone metastases and consequently less SREs develop during osimertinib therapy, with as a result less need for the use of BTAs. Reporting of prevalence of bone metastases, SREs, and bone-

specific outcomes in patients with *EGFR+* NSCLC in clinical trials evaluating EGFR-TKIs, including osimertinib, is lacking (11). Therefore, we performed this multicenter cohort study to evaluate bone metastases related outcomes in patients treated with osimertinib.

MATERIAL AND METHODS

In this multicenter cohort study, data from patients with *EGFR+* NSCLC in two tertiary referral university hospitals and one teaching hospital in the Netherlands (Maastricht University Center+ [MUMC+], Erasmus Medical Center Cancer Institute [Erasmus MC]) and Amphia Hospital were analyzed.

Patient selection and data collection

In MUMC+ all patients with metastatic *EGFR+* NSCLC treated with osimertinib as part of regular care between February 2, 2016 and September 22, 2021 were identified using dispensing data from the pharmacy. In Erasmus MC, all patients with metastatic *EGFR+* NSCLC treated with osimertinib between January 18, 2017 and September 22, 2021, were retrieved from a prospective cohort study (START-TKI, NCT05221372). Patients were excluded if no follow-up data were available (at least one follow-up visit after initiation of osimertinib was required).

The inpatient and outpatient medical records of all patients were retrieved. The following data were collected: demographics, date of diagnosis of metastatic NSCLC, smoking status, pathological subtyping of NSCLC, mutational status, presence of bone metastasis at diagnosis of metastatic NSCLC and development of bone metastases during the course of the disease, date of initiation of osimertinib treatment including treatment line, duration of osimertinib treatment and date of progression on osimertinib, presence of SREs in patients with confirmed bone metastases on imaging and if applicable date and type of first SRE, use of bone targeted agents, and date of death or last follow-up. SREs were defined as: either the occurrence of a pathologic fracture, spinal cord compression, necessity for radiation to bone (for pain or impending fracture) surgery to bone because of bone metastases and hypercalcemia (in patients with bone metastases). SRE at diagnosis of bone metastases was defined as an SRE within 2 months before and 2 months after diagnosis of bone metastases, SRE at initiation of osimertinib was defined as an SRE within 2 months before and 2 months after initiation of osimertinib. Dispensing data from the pharmacy were used to evaluate BTA prescription. Standard radiological evaluation was performed every two till three months by chest and upper abdomen computer tomography (CT) scans with iodine contrast. The last date of follow-up was October 1, 2021.

Medical ethical committee approval was obtained in accordance with local regulations (METC: 2021-2989 and START-TKI, MEC 2016-643, NCT05221372). The ethics committee waived the need for informed consent for 2021-2989, for the START-TKI study all patients provided informed consent.

Statistical analysis

Patient demographics and baseline characteristics are summarized using descriptive statistics. Categorical variables were compared using chi-square tests or Fisher exact probability tests, and continuous variables were compared using the Mann-Whitney U test, Kruskall Wallis test or analysis of variance. Cox regression analysis was used for univariate and multivariate analyses. The cumulative incidence function, taking the competing risk of mortality into account was used to calculate the cumulative incidence of bone progression. Survival analysis was performed by Kaplan Meier analysis. Statistical analyses were performed using SPSS (IBM statistics, version 20).

RESULTS

Patient characteristics

All patients treated with osimertinib (n=64) in MUMC+ were included. In addition, 186 patients treated with osimertinib from Amphia Hospital and Erasmus MC were enrolled in the START-TKI study. As a result, 250 patients were included in this analysis. Patient characteristics were found in Table 1.

Median follow-up from diagnosis of metastatic NSCLC was 43.0 months (95% confidence interval [CI]: 38.8-47.3 mo). Median follow-up from initiation of osimertinib was 23.4 months (95% CI: 19.9-26.9 mo). In 107 of 250 patients (43%) osimertinib was administered as a first-line treatment.

Bone metastases

In total, 112 of 250 patients (45%) had synchronous bone metastases at diagnosis of metastatic NSCLC. Of 250 patients, 15 (6%) developed bone metastases before initiation of osimertinib treatment. As a result, 127 of 250 patients (51%) were already diagnosed with having bone metastases at initiation of osimertinib (Fig. 1). Thereafter, 15 of 250 patients (6%) developed bone metastases (14 during and one after osimertinib treatment), resulting in a total of 142 patients (57%) of the whole study population being diagnosed with having bone metastases at the last follow-up.

Of the 250 patients, 25 (10%) developed bone progression or new bone metastases during osimertinib treatment with a median time to event of 6.4 months (95% CI: 2.3-10.6 mo). In three patients this was the first diagnosis of bone metastases. The cumulative incidence of bone progression at 1 year after diagnosis of stage IV NSCLC was 8.8% (95% CI: 5-12.9) and increased to 14.2% (95% CI: 9.4-19.9) at 5 years after diagnosis of stage IV NSCLC. The cumulative incidence of bone progression at one year after initiation of osimertinib was 8.8% (95% CI: 5.5-12.9) and increased to 14.2% (95 CI: 9.4-19.9) at five years after initiation of osimertinib.

Table 1: Patient characteristics

Characteristics	Total (n=250)	1st line osimertinib (n=107)[a]	≥2nd line osimertinib (n=143)[a,b]	p-value
Female *N (%)*	165 (67)	71 (66)	94 (66)	NS
Never smoker *N (%)*	100 (40)	44 (41)	56 (39)	NS
Mean age at diagnosis metastatic NSCLC, years (range)	65.1 (33-87)	67.2 (37-87)	63.6 (33-84)	<0.05
WHO-PS	180 (72)	80 (75)	100 (70)	NS
0-1	54 (22)	26 (24)	28 (20)	
>2	16 (6)	1 (1)	15 (11)	
Unknown				
EGFR mutation	60 (24)	57 (53)	3 (2)	<0.001
Exon 19 deletion	28 (11)	25 (23)	3 (2)	
Exon 21 L858R	8 (3)	7 (7)	1 (1)	
Two mutations simultaneously				
Uncommon	17 (7)	16 (15)	1 (1)	
Original exon 19 del or L858R and exon 20 T790M mutation	129 (52)	1 (1)	127 (88)	
Original uncommon and exon 20 T790M mutation				
	8 (3)	1 (1)	8 (6)	

Abbreviations: N, number; NS, not statically significant; WHO-PS, world health organization - performance score; exon 21 L858R, single point mutation that substitutes leucine for arginine at position 858 in exon 21, T790M, point mutation that substitutes methionine for threonine at position 790 in exon 20; NSCLC, non-small cell lung cancer.
[a] Percentages were calculated by subgroup.
[b] All patients received first or second generation EGFR-TKIs. One hundred twenty-three patients received osimertinib as second line treatment.

Skeletal related events

Of the 142 patients with bone metastases, 21 (15%) presented with a first SRE at diagnosis of metastatic NSCLC, and in total 56 patients (40%) developed one or more SREs during the course of their disease. Furthermore, 28% of the patients developed their first SRE before, 11% during osimertinib treatment and 1% after discontinuation of treatment (Table 2 and Fig 2). The median time to first SRE for patients who did not have an SRE at metastatic NSCLC diagnosis was 10.1 months (95% CI: 6.9-13.3 mo). In the group of patients with the first SRE during osimertinib treatment (15 of 56 patients), the median time to SRE was 4.8 months (95% CI: 2.1-7.6 mo).

Survival outcomes

The median PFS from initiation of osimertinib was 16.5 months (95% CI: 14.2-18.9 mo) for the total study population. Although numerically higher, there was no significant difference in median PFS between patients treated with osimertinib in first-line or in second line and beyond (median PFS of 18.9 mo (95% CI: 14.3-23.5 mo) vs. 16.3 mo (95% CI: 14.5-18.1 mo); p=0.575, respectively).

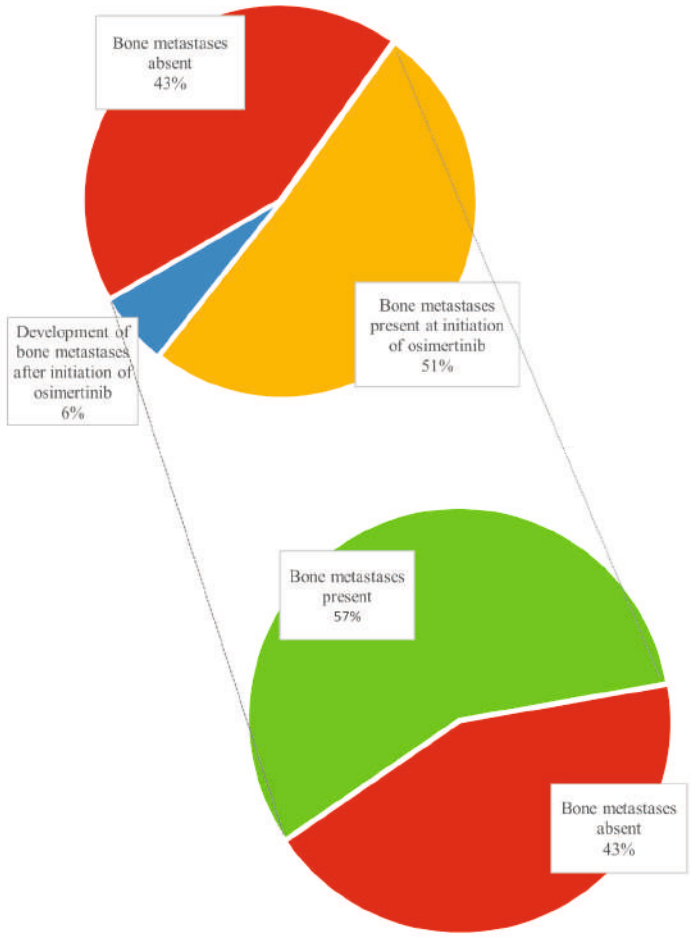


Figure 1: Presence of bone metastases. Time frame of development of bone metastases during NSCLC disease course.

At data cutoff, 106 of 250 patients (42%) had deceased. The median OS from diagnosis of metastatic NSCLC was 48.5 months (95% CI: 39.8-57.2) and was significantly shorter for patients with bone metastases during the course of their disease than for those without: 37.2 months (95% CI: 33.3-41.1 mo) versus 66.6 months (95% CI: 55.9-77.2 mo) ($p<0.0001$, hazard ratio (HR) 2.4 [95% CI: 1.6-3.6]). The median OS for patients with bone metastases and a minimum of one SRE was not significantly different compared with those without SREs: 41.1 months (95% CI: 27.3-54.9 mo) versus 36.5 months (95% CI: 29.4-43.5 mo) ($p=0.585$, HR 1.1 (95% CI: 0.7-1.8)). Multivariate analysis revealed uncommon mutations, presence of bone metastases and bone progression, or development of new bone metastases during osimertinib treatment as independent negative prognostic factors for OS ($p=0.009$, $p=0.001$ and $p=0.02$ respectively) (Table 3).

The median OS from initiation of osimertinib treatment was 28.0 months (95% CI: 23.8-32.2 mo) and was significantly shorter for patients with bone metastases than for patients without bone metastases during the course of their disease: 23.6 months (95% CI: 17.1-30.0 mo) versus 38.3 months (95% CI: 23.9-52.7 mo) for patients without bone metastases (p<0.0001, HR 2.1 (95% CI: 1.4-3.2). The median OS for patients with bone metastases and a minimum of one SRE was not significantly different compared with those without SREs: 26.1 months (95% CI: 18.2-34.1 mo) versus 22.5 months (95% CI: 14.7-30.3 mo) (p=0.939, HR 1.0 (95% CI: 0.6-1.6)). In Figure 3A and B the median OS for the study population with/without bone metastases, subdivided by the different treatment lines is illustrated. The median OS after development of bone metastasis was 30.8 months (95% CI: 21.9-39.7 mo). The median OS after development of the first SRE was 31.1 months (95% CI: 15.8-46.5 mo).

Table 2: Bone metastases and bone related outcomes

Characteristics	**Total (n=250)**	**First-line osimertnib (n=107)[a]**	**≥Second- line osimertinib (n=143)[a,b]**	**p value**
Bone metastases at diagnosis stage IV *N* (%)	112 (45)	55 (51)	57 (40)	NS
Bone metastases at initiation of osimertinib *N* (%)	127 (51)	56 (52)	71 (50)	NS
New bone metastases or bone progression during osimertinib *N (%)*	25 (10)	10 (10)	15 (11)	NS
Presence of minimum one SRE in NSCLC patients with bone metastases *N (%)*	56 (40)	22 (36)	34 (42)	<0.05
First SRE at diagnosis NSCLC in NSCLC patients with bone metastases[c]	19 (13)	11 (8)	8 (6)	<0.05
First SRE before initiation of osimertinib in NSCLC patients with bone metastases[c,d]	20 (28)	1 (9)	19 (19)	NS
First SRE during osimertinib in NSCLC patients with bone metastases[c]	15 (11)	8 (6)	7 (5)	NS
Type of first SRE *N (%)*[a]	45 (80)	17 (30)	28 (50)	<0.05
Radiotherapy *N (%)*	4 (7)	2 (4)	2 (4)	
Pathologic fracture due to bone metastasis *N (%)*	6 (11)	3 (5)	3 (5)	
Surgery *N (%)*	1 (2)	0 (0)	1 (2)	
Spinal cord compression *N (%)*				
BTA use in pts with bone metastases *N (%)*[c]	23 (16)	5 (4)	18 (13)	NS

Abbreviations: N, number; NS, not statically significant; NSCLC, SRE, skeletal related event; non-small cell lung cancer; BTA, bone targeted agent.

[a] Percentages were calculated by subgroup.

[b] All patients received first or second generation EGFR-TKIs. One hundred twenty-three patients received osimertinib as second line treatment.

[c] Percentages were calculated by all patients with bone metastases (n=142).

[d] Numbers were calculated minus patients with SRE at diagnosis of advanced NSCLC. For example, one patient developed an SRE between diagnosis of advanced NSCLC and initiation of osimertinib.

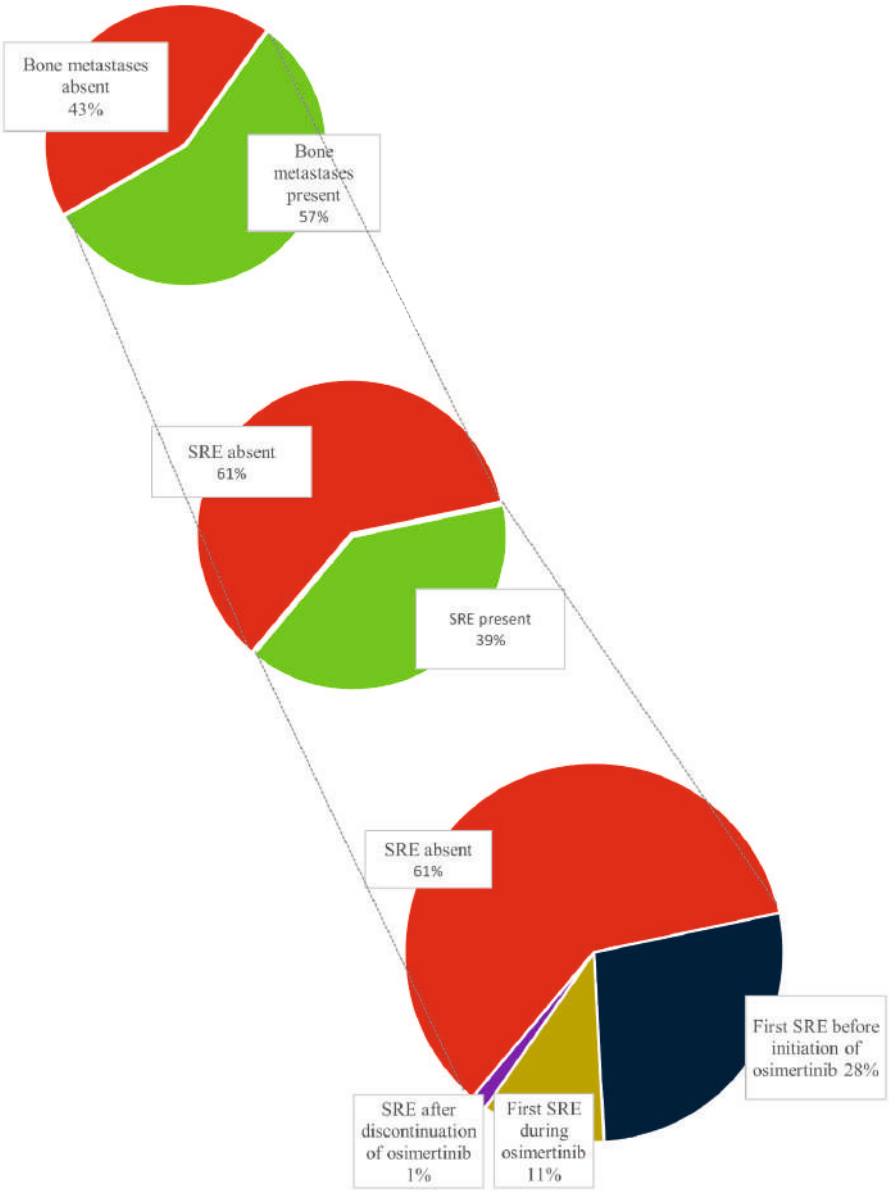


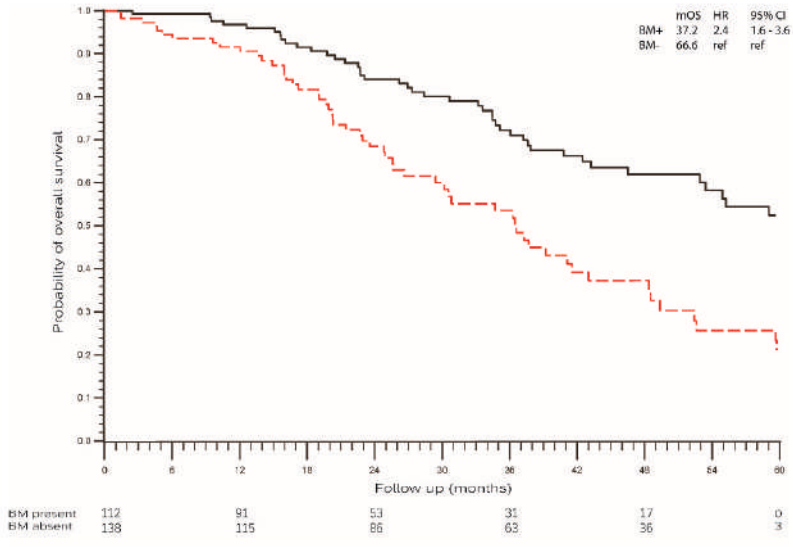
Figure 2: Presence of skeletal-related events. (A) Bone metastases during NSCLC disease course. (B) Presence of SRE in patients with bone metastases. (C) Time frame of SRE development in patients with bone metastases during NSCLC disease course. SREs are presented as percentage of the study population with bone metastases, for example, 39 patients have an SRE before initiation of osimertinib. Abbreviations: SRE, skeletal-related event.

Table 3: Univariate and multivariate analysis for overall survival from diagnosis of stage IV NSCLC

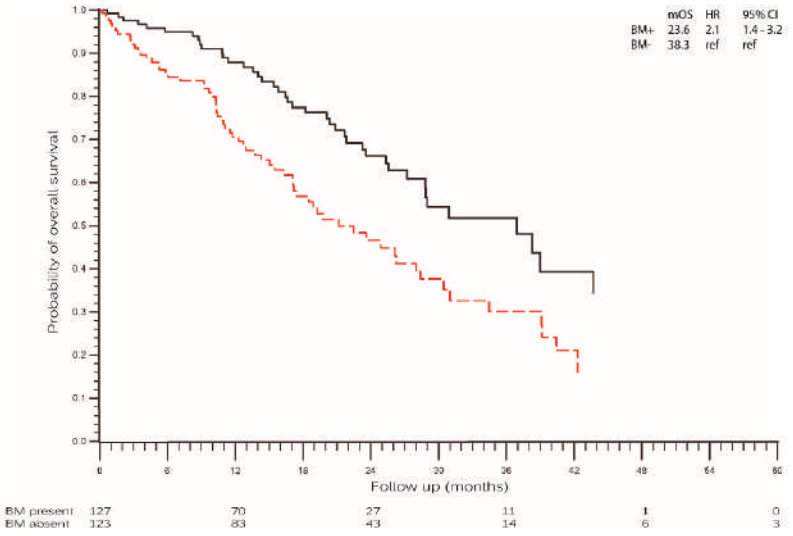
Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	p value
Sexe	1 (reference)	0.160	-	-
- Female	1.33 (0.89-1.98)			
- Male				
Smoke	1 (reference)	0.681	-	-
- Never	1.24 (0.44-3.51)	0.015[a]		
- Current	1.67 (1.11-2.53)			
- Former				
EGFR Mutation	1 (reference)	0.028[a]	1 (reference)	0.057
- Exon 19 deletion and exon 20 T790M mutation		0.146		0.462
- Exon 21 L858R and exon 20 T790M mutation	1.62 (1.05-2.48)	0.001[a]	1.54 (0.99-2.40)	0.009[a]
- Two mutations simultaneously	2.39 (0.74-7.75)		1.59 (0.46-5.51)	
- Uncommon and exon 20 T790M mutation	2.57 (1.45-4.55)		2.26 (1.23-4.17)	
TKI line	1 (reference)	0.048[a]	1 (reference)	0.196
- First line	0.63 (0.40-1.00)		0.71 (0.43-1.19)	
- Second line				
Mean age at diagnosis metastatic NSCLC	1.00 (0.98-1.02)	0.825	-	
Bone metastasis	1 (reference)	<0.001[a]	1 (reference)	0.001[a]
- Absent	2.39 (1.57-3.65)		2.32 (1.43-3.74)	
- Present				
Bone progression or new bone metastases during osimertinib	1 (reference)	<0.001[a]	1 (reference)	0.020[a]
- Absent	2.42 (1.48-4.0)		1.93 (1.11-3.35)	
- Present				
Skeletal related event	1 (reference)	0.103	0.69 (0.42-1.15)	0.155
- Absent	1.42 (0.93-2.16)			
- Present				
Bone targeted agent use in patients with bone metastases	1 (reference)	0.485	-	-
- Absent	1.22 (0.70-2.12)			
- Present				

[a] Data indicate a $p < 0.05$.

Abbreviations: HR, Hazard ratio; CI, confidence interval; exon 21 L858R, single point mutation that substitutes leucine for arginine at position 858 in exon 21; T790M, point mutation that substitutes methionine for threonine at position 790 in exon 20; TKI, tyrosine kinase inhibitor.



A)



B)

Figure 3: A) Overall survival from diagnosis of metastatic NSCLC. Black line: patients with metastatic NSCLC without bone metastases; red dashed line: patients with metastatic NSCLC with bone metastases. **B)** Overall survival from initiation of osimertinib. Black line: patients with metastatic NSCLC without bone metastases; red dashed line: patients with metastatic NSCLC with bone metastases. Abbreviations: mOS, median overall survival; HR, hazard ratio; CI, confidence interval; BM +, bone metastases present; BM -, bone metastases absent; ref, reference.

DISCUSSION

Baseline and cumulative incidence of bone metastases and SREs is high in patients with *EGFR+* metastatic NSCLC treated with first and second generation EGFR-TKIs and therefore better treatment options are necessary (11). We found that the majority of patients (45%) already had bone metastases at first diagnosis of metastatic NSCLC and this percentage increased to 51% at initiation of osimertinib if patients were treated with osimertinib in second line and beyond. At diagnosis of metastatic *EGFR+* NSCLC 15% of patients with bone metastases were diagnosed with an SRE, the cumulative incidence increased to 39%. Consequently, both prevention of progression of existing bone metastases and SREs as well as prevention of new events is important. We found that during osimertinib treatment 10% of the patients developed new bone metastases or progression of existing bone metastases. In other series (including a systematic review evaluating EGFR-TKI trials (N=1,196) and several retrospective series evaluating patients (N=126-1081) treated with EGFR-TKI the percentage of patients with bone metastases at diagnosis of metastatic NSCLC was similar to our study (Supplementary Table 1) (11, 23). However, data about bone progression and development of SREs during EGFR-TKI treatment is scarce (11). The percentage of patients who develop bone progression during osimertinib in our series is comparable to a smaller series (N=126) evaluating outcomes on first-line osimertinib (10% vs. 12%) and with trials evaluating first and second generation EGFR-TKIs (11% vs. 3%-26%) (Supplementary Table 1) (23-33). The highest percentages of bone progression were found in two studies (n=38-53) in which regularly a 2-deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computed tomography scan (FDG-PET-CT scan) was made during follow-up. This is not surprising as FDG-PET has a high sensitivity to detect bone metastases (24, 26, 34). Another small series (n=101) in patients treated with osimertinib in second line (78% of patients) and beyond also reported a 22% bone progression rate. Radiological tumor assessment during follow-up was comparable to our series (35).

We are the first to report the incidence of SREs during osimertinib treatment (11% of the patients with bone metastases developed their first SRE during osimertinib treatment), which is more than half compared with the 25.9-28% observed in series (N=274-552) evaluating first or second generation EGFR-TKI (4, 36).

In our series, we show a relatively long median OS of 48.5 months, and although shorter, the majority of patients with bone metastases survived more than three years (median OS 37.2 mo). Development of SREs did not significantly impair OS (median OS after first SRE was 41.1 mo vs. 36.5 mo in patients without SREs, p=0.585)). As our population consists of a mixture of treatment naïve patients and pre-treated patients, other patient and/or tumor characteristics (e.g., more resistant tumor cells, older age, increased WHO-PS) could also influence OS. Most SREs occurred already at diagnosis or developed during the first year after a diagnosis of bone metastases. Previous studies showed that SREs have an impact on patient reported outcomes with a decline in patients' physical and emotional well-being, ability to perform basic functions

of daily living and quality of life (37, 38). Furthermore, we know that previous SREs are a risk factor for development of new SREs and patients with *EGFR* mutated NSCLC have a long post metastatic bone disease survival (1, 39). That is why any reduction in SREs, even if it does not lead to improvement in OS, is important too. BTAs are not specifically recommended in Dutch NSCLC or bone metastases guidelines (40, 41). In clinical practice, BTAs are not frequently used in the treatment strategy of NSCLC, as is also reflected in the low percentage of use (only 16% in patients with bone metastases) in our series. Data is also lacking on BTA use in other series evaluating *EGFR*+ NSCLC. In series (N=114-10,982) evaluating patients with NSCLC unselected for oncogenic drivers, uptake of BTA use was also limited (15-38%) (19-21). This low BTA usage is in contrast with the European Society for Medical Oncology (ESMO) clinical practice guideline on bone health in which it is recommended to start a BTA in the vast majority of patients as soon as bone metastases are diagnosed, whether they are symptomatic or not (13). In metastatic breast and prostate cancer, two solid malignancies with a similar favorable prognosis as *EGFR*+ NSCLC, the majority of the patients with bone metastases received a BTA, which translated into a significant SRE reduction by bisphosphonates in patients with breast cancer and bone metastases (relative risk 0.86, 95% CI 0.78-0.95, p = 0.003) (14, 21).

Based on our data as well as the international guideline recommendations, we strongly recommend to prospectively evaluate and consider the use of BTA (as in daily practice they are barely used) in this specific oncogenic driven subgroup with a favorable survival, also post bone metastases diagnosis, to reduce the burden of SREs (42, 43). Other arguments for the use of BTA are small, hypothesis generating, *in vivo* (N=62-129) and *in vitro* series that reveal synergy between bisphosphonates and EGFR-TKIs with effects on tumor suppression, PFS and OS post bone metastases (44-46). This synergistic effect should be evaluated prospectively. Currently, one trial (NCT03958565) is enrolling patients with bone metastasized NSCLC to evaluate the percentage reduction of bone markers in urine or serum while treated with zoledronic acid or denosumab. This study population is subdivided in patients with any oncogenic driver treated with a TKI and in patients without actionable mutations treated with chemotherapy and/or immunotherapy. The incidence of SREs in both groups is a secondary outcome measurement.

This study has its limitations. First, part of the data was retrospectively collected. Nevertheless, bone metastases and SREs are relevant clinical events that are captured in the medical records. Second, not all patients underwent an FDG-PET-CT scan or bone scintigraphy to detect asymptomatic bone metastases and we did not have detailed information on location and burden of bone metastases. Nevertheless, there was no underreporting of SREs as these per definition cause complaints. Third, we included all lines of osimertinib treatment as although osimertinib is the preferred first line treatment, not all patients worldwide have access to first line osimertinib, and data on osimertinib in second line and beyond remain therefore important (47. 48). Finally, as it was a retrospective study, we could not evaluate the impact of SREs on patient reported outcomes. Nevertheless, other studies already revealed the impact of SREs on patients' QoL (37, 38).

To conclude, bone metastases and SREs are frequent events both before and during treatment with osimertinib in patients with *EGFR*+ NSCLC. These findings together with the long OS after the occurrence of bone metastases and SREs advocate the prescription of BTAs in *EGFR*+ NSCLC with bone metastases and the use of bone-specific end points in clinical trials.

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Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies.

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Sunaga (2007) (49)	Phase II, single-arm, multicenter study	21/21	Gefitinib 250 mg q.d. (100)	-	12.6	5/21 (24)	NR	NR	NR	NR
Inoue (2009) (50)	Phase II, single-arm study	29/29	Gefitinib 250 mg q.d. (100)	-	17.8	12/29 (41)	NR	NR	NR	NR
Rosell (2012) [Eurtac] (10)	Phase III, open-label, multicenter RCT	173/173	Erlotinib 150 mg q.d. (50)	3-week cycles of chemotherapy[a] (50)	Erlotinib arm: 18.9 Chemotherapy arm: 14.4	Erlotinib arm: 28/86 (33) Chemotherapy arm: 29/87 (33)	NR	NR	NR	NR
Yoshimura (2013) (51)	Phase II, single-arm, study	27/27	3-weekly cycles of pemetrexed d1 500mg/m^2 and erlotinib/ gefitinib d2-16 dose NR (100)	-	11.4	16/27 (59)	NR	NR	NR	NR
Reguart (2014) (52)	Phase I-II, single-arm, multicenter study	25/25	Erlotinib 150mg q.d. + vorinostat 400mg q.d. (100)	-	NR	10/25 (40)	NR	NR	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zwitter (2014) (24)	Phase II, single-arm, study	53/38	3-weekly cycles of gemcitabin 120mg/m^2 d1, cisplatin 75mg/m^2 d2, gemcitabin 1250mg/m^2 d4, erlotinib 150mg q.d. d5-15 (100)	-	NR	24/38 (63)	*EGFR+* group: "bone (10) most frequent site of PD." Number of pts with PD NR	*EGFR+* group: 10/38 (26)	NR	NR
Yoshimura (2015) (53)	Phase II, open-label, single-arm study	26/26	3-weekly cycles of pemetrexed d1 500mg/m^2 and gefitinib 250mg q.d. d2-16 (100)	-	19.7	8/26 (31)	NR	NR	NR	NR
Park (2016a) [Aspiration study] (25)	Phase II, single-arm, multicenter study	207/207	Erlotinib 150mg q.d. (100)	-	11.3	NR	14/171 (8)	14/207 (21)	NR	NR
Park (2016b) [Lux-lung 7] (54)	Phase IIB, open-label, multicenter RCT	319/319	Afatinib 40mg q.d.; dose escalation to 50mg q.d. allowed after 4 weeks without AE (50)	Gefitinib 250mg q.d. (50)	27.3	Afatinib arm: 80/160 (50) Gefitinib arm: 73/159 (46)	NR	NR	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zwitter (2016) (26)	Phase II, open-label, single-arm, study	38/38	3-weekly cycles of gemcitabin 1250mg/m^2 d1+4, cisplatin 75mg/m2 d2, erlotinib 150mg q.d. d 5-15 (100)	-	35	24/38 (63)	"Bone (10) most frequent site of PD." Number of pts with PD NR.	10/38 (26)	NR	NR
Atagi (2016) (27)	Combined results of 2 phase II studies: JO22903 (single arm) and JO25567 study (randomized)	177/177	JO22903: erlotinib 150mg q.d. (56) JO25567: erlotinib 150mg q.d. (22)	JO22903: - JO25567: bevacizumab 15mg/kg 3-weekly cycles + erlotinib 150mg q.d. (22)	JO22903: 20.4 JO25567: at minimum 20	NR	20/125 (16)	20/177 (11)	NR	NR
Hirano (2016) (28)	Phase II, single-arm, multicenter study	11/11	Erlotinib 25mg q.d.; dose escalation to 150mg q.d. in case of PD (100)	-	NR	NR	1/8 (13)	1/11 (9)	NR	NR
Goss (2016) [Aura 2] (29)	Phase II, open-label, multicenter single-arm study	199/199	Osimertinib 80 mg q.d. (100)	-	13.0	NR	9/65 (14)	9/199 (5)	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Mok (2017) [Aura 3] (30)	Phase III, open-label, multicenter RCT	419/419	Osimertinib 80mg q.d. (6S)	3-weekly cycles of pemetrexed 500mg/m^2 + carboplatin AUC 5 or cisplatin 75mg/m^2 (33)	8.3	NR	Osimertinib arm: 9/97 (9) Platinum/ pemetrexed arm: 6/101 (6)	Osimertinib arm: 9/277 (3) Platinum/ pemetrexed arm: 6/149 (4)	NR	NR
Soria (2018) [Flaura] (12)	Phase III, multicenter, double-blind, RCT	556/556	Osimertinib 80mg q.d. (50)	Erlotinib 150mg q.d. or Gefitinib 250mg q.d. (50)	15	NR	Osimertinib arm: 11/NR Gefitinib or erlotinib arm: 11/NR	Osimertinib arm: 11/278 (4) Gefitinib or erlotinib arm: 11/278 (4)	NR	NR
Lim (2018) (56)	Phase II, single-arm, study	49/49	Gefitinib 250mg q.d. (100)	-	At minimum 6	9/49 (18)	NR	NR	NR	NR
Ahn (2019) (57)	Combined results of 2 phase II studies (AURA extension and AURA 2 trial), both single arm	411/411	Osimertinib 80mg q.d. (100)	-	NR	NR	28/NR	28/411 (7)	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zheng (2019) (31)	Phase II, single-arm study	10/10	Erlotinib 150mg q.d. or Gefitinib 250mg q.d. plus thoracic radiotherapy[b] (100)	-	12	9/10 (90)	2/7 (29)	2/10 (20)	NR	NR
Cho (2019) [KCSG-Lu15-09] (58)	Phase II, open-label, single arm, study	36/36	Osimertinib 80mg q.d. (100)	-	20.6	10/36 (28)	NR	NR	NR	NR
Noronha (2020) (32)	Phase III, open-label, study	350/350	3-weekly cycles of Gefitinib 250mg q.d. and pemetrexed 500mg/m^2 + carboplatin AUC 5 on d1, (up to four cycles), followed by 3-weekly cycles maintenance pemetrexed (50)	Gefitinib 250mg q.d. (50)	17	Gefitinib+ chemo arm: 24/174 (14) Gefitinib arm: 25/176 (14)	Gefitinib + chemo arm: 3/97 (3) Gefitinib arm: 7/136 (5)	Gefitinib + chemo arm: 3/175 (2) Gefitinib arm: 7/175 (4)	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Wu (2020) [Insight study] (59)	Phase Ib/II, open-label, study	55/55	Teponitinib 500mg q.d. + gefitinib 250mg q.d. (66)	Pemetrexed 500mg/ m^2+ cisplatin 75mg/m^2 or carboplatin AUC 5-6 on d1 ≤6 cycles or 4 cycles + pemetrexed maintenance (34)	21.8	Teponitinib plus gefitinib arm: 15/49 (23) Chemotherapy arm: 9/24 (38)	NR	NR	NR	NR
Lagana (2020) (4)	Retrospective multicenter study	274/274	First-line gefitinib 250mg q.d. (67), erlotinib 150mg q.d. (16), afatinib 40mg q.d. (17), osimertinib 80mg q.d. (0.4)	-	23	274/274 (100)	NR	NR	77/274 (28)	NR
Luo (2021) (60)	Prospectively observed cohort study	417/417[c]	≥2nd line osimertinib 80mg q.d. (100)	-	49.2	76/154 (49)[d]	NR	NR	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Dal Maso (2021) (35)	Retrospective multicenter Study	139/139	≥2nd line osimertinib 80mg q.d. (73), any systemic treatment (27)	-	14.1	Osimertinib arm: 46/101 (46) Any systemic treatment arm: 8/38 (21)	Osimertinib arm: 22/71 (31) Any systemic treatment arm: 6/33 (18)	Osimertinib arm: 22/101 (22) Any systemic treatment arm: 6/21 (29)	NR	NR
Lorenzi (2021) (23)	Real-world. Prospective study	126/126	First-line osimertinib 80mg q.d. (100)	-	12.3	59/126 (47)	15/44 (34)	15/126 (12)	NR	NR
Gen (2022) (61)	Retrospective cohort study	388/388	First-line gefitinib 250mg q.d./ erlotinib 150mg q.d. (47), afatinib 40mg q.d. (14), osimertinib 80mg q.d. (39)	-	NR	160/388 (41)	NR	NR	NR	NR

Supplementary Table 1: Summary of reported bone metastases and SREs of EGFR-TKI studies. (continued)

Study (y)	Trial type	Total pts/ *EGFR+* pts	Treatment arm dose (% of treatment arm)	Comparator arm dose (% of treatment arm)	Median follow-up (months)	BM at baseline n (%)	Number of pts with BM progression of total pts with PD n (%)	Number of pts with BM progression of total study population n (%)	SRE at baseline in pts with BM n (%)	SRE during treatment in pts with BM n (%)
Zeng (2022) (33)	Retrospective cohort study	1081/1081	First-line gefitinib 250mg q.d./ erlotinib 150mg q.d. (86), afatinib 40mg q.d. (5), osimertinib 80mg q.d. (9)	-	35	485/1081 (45)	58/619 (9)	58/1081 (5)	NR	NR

Abbreviations: SREs; skeletal related events, EGFR-TKI; epidermal growth factor receptor tyrosine kinase inhibitors, y; year, pts; patients, EGFR+; activating mutation in the epidermal growth factor receptor (EGFR), BM; bone metastasis, q.d.; once a day,SNR; not reported, RCT; randomized controlled trial, AE; adverse events, AUC; area under the curve.

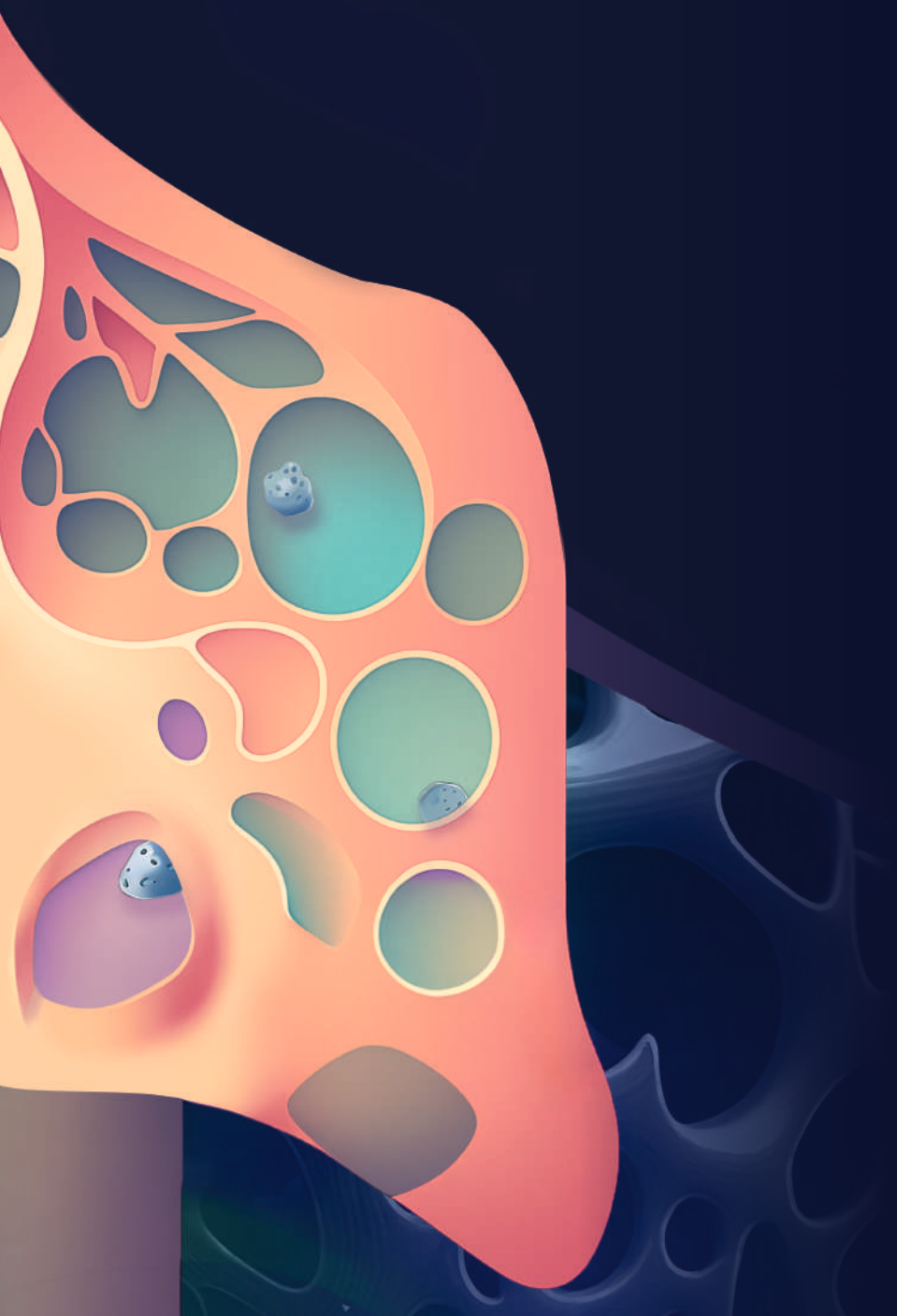
[a] Cisplatin 75 mg/m² on day 1 plus docetaxel (75 mg/m² on day 1) or gemcitabin (1250 mg/m² on days 1 and 8). In patients with contra-indications for cisplatin, carboplatin (AUC 6 with docetaxel 75 mg/m² or AUC 5 with gemcitabin 1000 mg/m²) was allowed.

[b] 54-60 Gray / 27-30 fractions / 5.5-6 weeks.

[c] Only 154 out of 417 patients received 1st/2nd generation TKI with subsequent osimertinib, the other 263 patients received 1st/2nd generation TKI without subsequent osimertinib (n=203) or no EGFR-TKI treatment (n=60)

[ds]Percentage bone metastases at initiation of osimertinib, percentage at baseline is not reported.

CHAPTER 8



General discussion and future perspectives

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Bone metastases are diagnosed in approximately 30-60% of patients with advanced non-small cell lung cancer [NSCLC] (1, 2). Patients with bone metastases are at risk of developing SREs, which can cause a decline in their physical or emotional well-being, ability to perform basic daily functions and quality of life (QoL) (3, 4). Previous SREs are a risk factor for the development of new SREs (5). The incidence of bone metastases in patients with epidermal growth factor receptor mutated (*EGFR+*) NSCLC is probably higher than in other molecular subgroups, although conflicting results exist (6-8). In patients with *EGFR+* NSCLC, the majority of SREs occur within the first year of treatment with EGFR tyrosine kinase inhibitors (EGFR-TKIs) (9). Time to first SRE is similar for patients with metastatic *EGFR+,* Kirsten rat sarcoma (*KRAS*+) and EGFR/KRAS wildtype NSCLC, while those with an *EGFR*+ NSCLC have a longer post-bone metastases diagnosis survival (1). Therefore, these patients are at risk for multiple SREs with their potential detrimental effects on QoL (1, 5). Understanding the biological mechanism of bone metastases in patients with (*EGFR*+) NSCLC could have implications for prophylactic treatment strategies.

Aims of this thesis are to optimize the treatment of patients with NSCLC and bone metastases, with a specific focus on *EGFR+* NSCLC, and to evaluate the potential biological mechanisms resulting in the observed increased incidence of bone metastases in patients with *EGFR+* NCSLC. These aims were intended to provide tools for prophylactically treating patients at high risk for bone metastases in order to prevent bone related complications and to optimize pain management for bone metastases.

In this final chapter, the results of this thesis are discussed and placed in a broader scientific and clinical context, taking into account the latest developments in lung cancer treatment. Finally, suggestions for future research will be presented.

Part I: Cancer induced bone pain

In **chapter 2** we performed a systematic review, evaluating the efficacy of non-radiation based early pain relief options for patients with NSCLC and cancer induced bone pain (CIBP). We concluded that despite the high incidence of CIBP, there is scarce literature on early pain reduction and that there is no high level recommendation for a specific treatment option. In **chapter 3**, we showed in the single arm phase II NVALT-9 study that ibandronate loading doses of six milligrams once a day for three consecutive days, did not lead to a relevant reduction in worst pain score (defined as at least 25% reduction) in 18 patients with NSCLC and uncontrolled CIBP; i.e., brief pain inventory (BPI) ≥ 5 over the last seven days. We defined that at least 12 out 19 patients had to have a relevant pain reduction. However, this goal was not reached as only four out of 18 patients (one patient was not evaluable for response) obtained relevant pain reduction. Contrary to what one might expect, the four patients who actually had a bone pain response (in this trial defined as at least a 25% decrease in worst pain score over a three-day period with a maximum of 25% increase in mean analgesic consumption) did not show an improvement in QoL, and only

one patient had an improvement in World Health Organization-Performance Score (WHO-PS). The four patients who exhibited a bone pain response were still alive at the end of the follow-up period (day 28), whereas almost 30% of the enrolled patients had died within one month, indicating a poor prognosis.

Based on these two chapters, it is clear that there should be more efforts and research on how to reduce CIBP. Several strategies can be pursued (see Figure 1). First, although it is known that pain is common in patients with cancer, with a prevalence ranging from 33% in patients after curative intent treatment to up to 64% in patients with advanced disease, health care professionals (HCP) should more proactively be involved in pain management in general and management of CIBP specifically (10). Emphasizing pain is important given its substantial impact on health-related QoL (HRQOL) as well as prognosis (11). This is especially relevant for CIBP as it is one of the most common causes of cancer related pain (10). Second, we have to explore how to improve the use of current treatments for CIBP (e.g., analgesics, bone targeted agents [BTAs]) and as these often do not reduce CIBP sufficiently, research should focus on new therapeutic options. In the paragraphs below, we will go into detail in these potential areas for improvement and research.

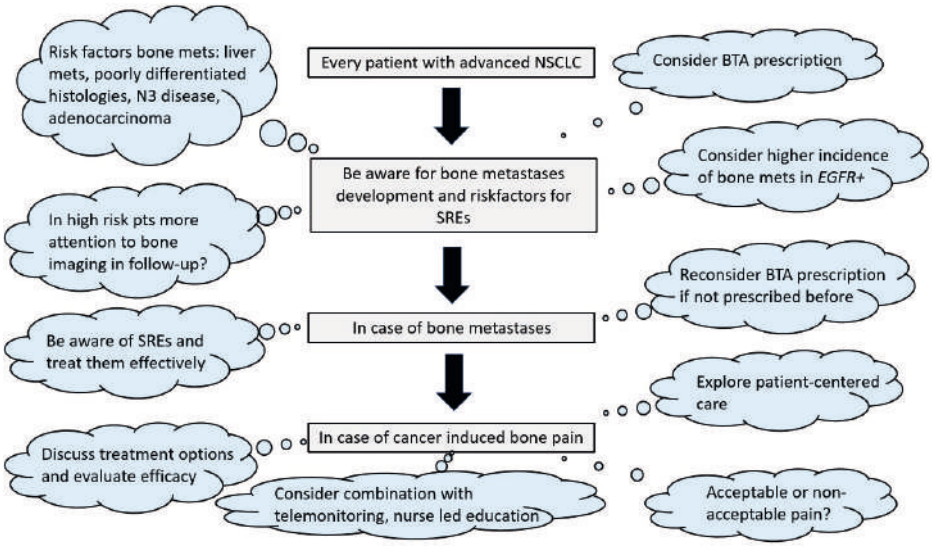


Figure 1: Strategies in approaching a patient with advanced NSCLC with regard to development of bone metastases.

Abbreviations: mets, metastases; N3, involvement of contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene or supraclavicular lymph nodes by metastases; NSCLC, non-small cell lung cancer; BTA, bone targeted agents; pts, patients; SREs, skeletal related events; *EGFR+*, activating mutation in the epidermal growth factor receptor.

To the best of our knowledge, the physiology of CIBP is comparable to that of pain caused by cancer in general. Pain can be broadly categorized into nociceptive and neuropathic pain. Nociceptive pain is triggered by ongoing tissue damage, either somatic (such as bone pain) or visceral (such as gut or hepatic pain). On the other hand, neuropathic pain is caused by damage or dysfunction in the nervous system. Understanding the nature of pain aids in making better choices for therapy (10). In recent years, there has been a growing emphasis on the provision of adequate pain relief in patients with cancer, resulting in a 25% reduction on the Pain Management Index (PMI) between 1994 and 2013 (12). However, despite these efforts, 32% of patients with cancer still experience insufficient pain relief (12).

The first key point is the interpretation and discussion of their pain score with the patients. A study conducted in 2021 on pain management in an oncology outpatient clinic included consecutive patients with an oncologic diagnosis. Treatment could be with curative or palliative intent. After the initial intended evaluation period of approximately five months, the study duration was extended to one year with three additional evaluation periods, as pain was not adequately addressed during the consultations. The study involved 37,580 patients, of whom 23,163 provided numeric rating scale (NRS) scores for pain assessment and indicating whether the pain was (non)acceptable, throughout the entire study period. Of these 23,163 patients, 2,153 (~10%) encountered pain that was deemed non-acceptable; in this study, non-acceptability was defined by a mean NRS score of 6.5. This cutoff was established by considering the NRS scores of all patients who reported their pain as non-acceptable at any given point during the whole study period (13). In the subset of patients with non-acceptable pain, pain was addressed during a consultation in only 40% of the cases at the beginning of the study. However, by the end of the study, this rate increased to 80% of cases. This discussion about pain led to an intervention in approximately three-quarters of these patients (13). When patients with non-acceptable pain were asked who they considered responsible for the lack of intervention, almost 50% of them attributed the responsibility to their physician (14). To improve the discussion of pain with patients, several approaches can be taken. Firstly, HCP should educate patients about pain, therapeutic options and encourage them to take an active role in their own care (10). Second, as pain treatment is often not adequately addressed by HCP, education should focus on awareness and how to optimize pain management within the limited time available in the outpatient clinic, as a significant portion of the consultation time is typically dedicated to discussing treatment options. In a recent randomized clinical trial involving 688 patients with advanced cancer (13% lung cancer) and a worst pain severity NRS score of ≥2 out of 10, patients were randomized between usual care and care from clinicians with an additional pain management training (15). The pain management training introduced guideline implementation strategies with the support of a “super user.” The primary objective was to assess the impact of additional pain management training on reduction of pain scores by 30% among those with a worst NRS ≥5 out of 10 at week 1. However, the trial did not find a significant difference in pain score reduction between the two groups (15). Of note, despite the trial being negative for its primary endpoint, in both arms

patients reported reduced mean and worst pain outcomes, likely due to the Hawthorne effect, which suggests that participation in the trial itself had a positive effect on pain (16).

Indeed, the studies mentioned earlier do not demonstrate a significant improvement in the management of CIBP through enhanced training of HCPs. However, it is noteworthy that personalized education provided to patients about both pharmacological and non-pharmacological pain treatment, correcting erroneous beliefs, and promoting self-management can lead to better pain control. This is highlighted by a recently published trial involving 308 patients with solid tumor bone metastases who reported a worst pain intensity of ≥5 on NRS. In this trial, alongside the administration of radiotherapy for treating CIBP, a nurse-led pain education was implemented (17). The program focused on pain control and QoL and included follow-up sessions for up to 12 weeks after radiotherapy. The results showed that this intervention led to a significant reduction of nearly 50% in the number of patients with uncontrolled pain ($p=0.008$), and that the patients achieved pain relief at a faster rate ($p=0.003$) (17). Based on the promising results of implementing a pain education program alongside radiotherapy, further exploration of the possibilities of integrating this combination into daily care is warranted. While integrating a pain education session alongside the existing intake process (with instructions regarding the radiotherapy planning and expected adverse events) may seem straightforward, it is important to acknowledge and address potential challenges related to scheduling and staff availability. Exploring these factors becomes crucial in order to ensure the successful implementation.

One of the most crucial factors in managing CIBP is that the clinician's communication matches that of the patient, and that HCPs have to explore whether a patient experiences pain and wants to discuss treatment options. As part of regular care it has become common in many hospitals to ask patients, before they are seen by a HCP, to complete a questionnaire focused on pain and to score their mean pain level on the NRS scale. However, many patients struggle to accurately express their pain scores. For instance, they may describe their pain as 'the worst ever' but rate it as a 5 out of 10 on the NRS pain scale (18). Therefore, a suggestion is to use the terms "acceptable pain" or "unacceptable pain" or ask the patient, "Does your pain hinder your daily activities?" instead of relying solely on the NRS scale. The rapid growth and development of mobile applications and telemonitoring systems provide an opportunity to enhance patient education, monitor pain levels, generate alerts if pain scores exceed a specified threshold, and provide electronic consultations when needed. A part of the HCPs in the Netherlands is participating in the "Dichterbij app," which enables patients to conveniently communicate with their clinician through text or video (19). Despite the potential benefits of these mobile applications, questions are raised about the feasibility. One out of five patients in the age group 55-65 years is low-literate in the Netherlands, and this percentage increases with older age (20). Additionally, older and frail patients may not be accustomed to using electronic devices, posing an inevitable barrier. Future research should also focus on whether pain scores improve when using these apps as the results of home monitoring in clinical trials are mixed (21, 22). Moreover, there should be escape options

for patients who cannot or do not want to use electronic devices; such as help from a nurse to complete questionnaires or the option of completing questionnaires by voice instead of online.

Pain relief options focused on CIBP

Although optimized pain management in general in patients with CIBP is important, there are also specific treatments that should be further explored on top of the optimized pain management in general as described above, as based on **chapter 2 and 3**, current treatment options are not always sufficient. Clinical practice has shown that CIBP typically becomes more severe as the disease progresses, making it increasingly challenging to manage effectively. Currently, radiotherapy is frequently employed in the treatment of CIBP due to its high response rate ranging from 50% to 85% in effectively treating peripheral and vertebral bone metastases (23). Despite this high efficacy, a subgroup of patients with CIBP may not experience satisfactory pain relief, leading to observed re-treatment rates up to 20% when using single-fraction palliative radiation schedules compared to 8% with multifractioned schemes (23). It is important to note that the efficacy of re-irradiation is lower, with an overall pain response of 58% which falls towards the lower end of the range of efficacy observed for first-time radiotherapy (24). The most significant drawback of radiotherapy is its infeasibility in cases of diffuse CIBP. Theoretically, BTAs could serve as an alternative for alleviating diffuse CIBP as denosumab and bisphosphonates (BPs) have shown efficacy in treating bone pain in advanced breast and prostate cancer (25-28). Though, the use of BTAs for treating bone pain in patients with NSCLC lacks substantial evidence (29). Furthermore, our previous finding have indicated that loading doses of ibandronate did not provide adequate pain relief in these patients (**chapter 3**). At this time, there is no ongoing clinical trial that is actively recruiting patients with a specific focus on non-radiation based early pain relief options for patients with NSCLC and CIBP. Only one clinical trial (NCT04307914) is currently enrolling patients to investigate the efficacy of magnetic resonance image-guided high-intensity focused ultrasound (MR-HIFU) as an alternative or additional treatment to external beam radiation therapy (EBRT). MR-HIFU is a non-invasive treatment approach that utilizes acoustic energy to heat tissue to temperatures exceeding 60 degrees Celsius, resulting in tissue ablation. This innovative treatment combines focused ultrasound technology with magnetic resonance imaging (MRI) for precise targeting and real-time temperature monitoring using MR thermometry. The primary outcome of this trial is pain response at 14 days after completion of treatment, with pain response at 14 days post-inclusion, pain scores, QoL, and survival as secondary outcomes. An important limitation of this trial is that MR-HIFU (as well as radiotherapy) cannot be utilized for non-localized CIBP.

In patients with NSCLC there is limited evidence available regarding the use of BTAs and the results were frequently obtained from subgroup analyses (29). Studies with different designs (e.g. bisphosphonate vs. placebo, bisphosphonate vs. alternate bisphosphonate, denosumab vs. bisphosphonate) demonstrated efficacy of ibandronate, zoledronic acid and denosumab in delaying the time to the first SRE (30-36) as well as reducing the annual incidence of SREs in

patients with NSCLC (30, 31, 33, 34, 36). For patients with *EGFR+* NSCLC only small retrospective series (n=62-356) exist in which addition of bisphosphonates (BPs) to EGFR-TKIs led to a significantly longer median PFS compared to patients treated with TKI alone (37-39). Results about overall survival (OS) were conflicting with some studies reporting a significantly longer OS (37, 40), while another did not (39). Unfortunately, all prospective trials with BTAs that allowed the enrollment of patients with NSCLC were performed before the wide-spread introduction of targeted therapy, and therefore it is not known whether the results of the previous trials can be extrapolated to the current treatment landscape of patients with *EGFR+* NSCLC that has metastasized to the bone. The survival of *EGFR+* NSCLC has increased significantly due to the introduction of EGFR-TKI, and survival of patients not eligible for targeted treatment has significantly improved due to the introduction of immune checkpoint inhibitors (ICI) (41-43). Data can also not be obtained from the fairly recent phase III randomized Splendour trial, evaluating the addition of denosumab to standard first-line chemotherapy in patients with NSCLC, as barely half of the patients underwent molecular testing. Of these who underwent molecular testing, only 0.6% had a documented *EGFR* mutation or anaplastic lymphoma kinase (*ALK*) rearrangement. Therefore, further subgroup efficacy analysis could not be conducted (2).

From our daily practice, we observe that only a minority of the patients with metastasized (*EGFR+*) NSCLC receive prescriptions for BTAs. This observation is supported by a recently published study conducted in the United Kingdom, which examined the utilization of BTAs in patients diagnosed with bone metastases from breast cancer, prostate cancer, and NSCLC (44). The study revealed that 87% of the patients with NSCLC and bone metastases did not receive any form of BTA treatment. Among the remaining 13% who did receive BTAs, the initiation of treatment occurred at a median of 60 days following the diagnosis of bone metastases (44). The efficacy of BTAs in reducing SREs is meaningful, especially considering that patients with (*EGFR+*) NSCLC often have long-term survival after the development of bone metastases, as we demonstrated in our previous studies (1, 45). Therefore, HCPs should consider the prescription of BTAs as an integral part of managing patients with bone metastases. Implementation of a pop-up feature in the prescribing system for anti-cancer treatment enhances awareness and facilitates the appropriate utilization of BTAs in patients with NSCLC. This pop-up would prompt healthcare providers to consider prescribing BTAs alongside anti-cancer treatment. Taking into account the high prevalence of bone metastases in NSCLC it is worthwhile to consider BTA treatment in every patient, regardless of the presence of bone metastases, as early prescription of BTAs may yield clinical benefits concerning the management of CIBP (46). Osteonecrosis of the jaw (ONJ) is a rare but potentially serious adverse event associated with BTA treatment (47, 48). The cumulative incidence of ONJ increases with higher cumulative doses (up to 2.8% of patients at year three), with the highest incidence observed in multiple myeloma and the lowest in breast cancer (48). Risk factors for ONJ development include poor dentition, dentures, prior oral surgery, and current smoking (47). It is crucial to be vigilant about this rare adverse event, educate patients and encourage patients to undergo regular dental evaluations. Clinical outcomes associated with

the utilization of BTAs in (*EGFR+*) NSCLC could be assessed by conducting a real-life observational study within the different molecular subgroups of NSCLC, ideally within the Trial with Cohorts (TwiCs) design (Figure 2). As discussed in **chapter 4**, patients with *KRAS+* NSCLC exhibited higher RANKL gene expression in their tumors, suggesting that these patients might potentially derive greater benefit from BTAs. The TwiCs design offers the advantage of easier patient recruitment compared to other clinical trials. This is because patients are first given the standard treatment, and later, they have the option to receive another new treatment (the intervention) if they choose to do so. But if more patients than expected refuse the intervention, the sample size will be too low, and the recruitment period will have to be extended (49). The proposed study would involve all patients, both those with and without bone metastases, in order to monitor and assess the impact of BTAs on bone-related outcomes in this specific patient population. By incorporating these findings into guidelines on bone health, this would ultimately contribute to improving the overall care and outcomes for individuals with (*EGFR+)* NSCLC in terms of bone health.

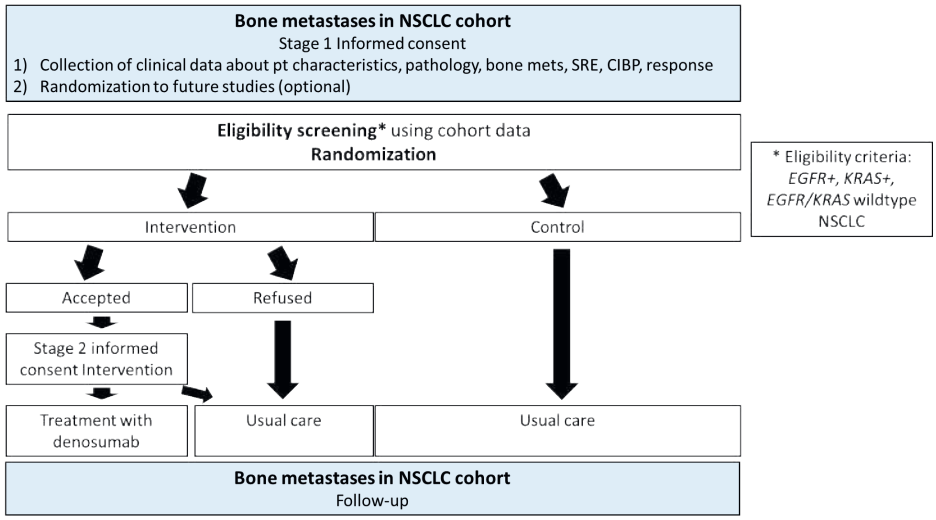


Figure 2: Proposed flow diagram of study evaluating the clinical outcomes of bone-targeted agents in patients with NSCLC.
Abbreviations: NSCLC, non-small cell lung cancer; pt, patients; mets, metastases; SREs, skeletal related events; CIBP, cancer induced bone pain, *EGFR+*, patients with NSCLC and an activating Epidermal Growth Factor mutation, *KRAS+*, patients with NSCLC and an activating Kirsten rat sarcoma mutation.

To further improve management of CIBP, better understanding of the pathophysiology and treatment options for CIBP is needed. Mouse models of CIBP can aid in understanding the pathophysiology and highlight the importance of treating CIBP as early as possible while also focusing on treatments specific for CIBP (50, 51). A sham-controlled mouse model study of breast cancer-induced bone pain was conducted, in which 33 mice received intramedullary injections of human breast cancer cells into the femur, while 33 mice received a sham injection

(50). Injection of cancer cells led to evident bone resorption and first signs of osteolytic lesions in the proximal and distal metaphysis on radiographs, 28 days after injection. The mice also exhibited CIBP behavior from the beginning of osteolytic lesions, which continued to increase in severity. Histologically, pathological nerve sprouting was observed in the periosteum of tumor bearing mice, which was stimulated by the binding of nerve growth factor (NGF) released by tumor or stromal cells to its receptor Tropomyosin receptor kinase A (TrkA) (50). The inappropriate sprouting of sensory nerve fibers, as well as continuous peripheral stimulation of nociceptors and local inflammation can lead to hyperalgesia (50, 52). Continuous stimulation can result in subthreshold depolarization and facilitate the easier transmission of pain stimuli (peripheral sensitization). Central sensitization occurs in the spinal cord through neurochemical changes that induce hypertrophy of astrocytes and increased expression of dynorphin and c-Fos protein which decreases the pain threshold (52). Although substantial progress has been made in understanding the pathological mechanisms associated with CIBP, numerous questions still remain unanswered. For instance, why do some patients experience intense pain due to bone metastases while others remain pain-free? And is there a distinction in the underlying pathology between persistent pain and breakthrough pain? Answering these inquiries is challenging due to the multifactorial nature of pathophysiology of pain and individual pain experiences. To shed light on the first question, conducting a detailed examination of the bone microenvironment on pathological specimens of bone tissue in patients both with and without CIBP might be interested. Ideally, a small clinical trial should be set up, involving patients who undergo a bone biopsy, and the residual pathology specimens should be used to conduct a detailed examination of the bone microenvironment. Data such as patient characteristics, presence of SREs, or CIBP could be retrieved from inpatient and outpatient medical records. Notably, factors such as the extent of local acidity and the presence of specific receptors, such as transient receptor potential channel-vanilloid (TRPVs) and acid-sensing ion channels (ASICs), have been identified as playing important roles in CIBP (53). It is plausible that patients with bone metastases who do not experience CIBP may exhibit lower expression levels of these receptors, potentially attributable to higher local acidity in their bone microenvironment. Given the complexity and breadth of the factors involved, artificial intelligence (AI) could be helpful in interpreting the results and establishing connections. In order to mitigate the need for invasive techniques such as bone biopsies, an alternative approach is to examine serum markers of bone turnover as we did in **chapter 5**. However, it is not clear whether markers of bone turnover also correlate with the occurrence and intensity of CIBP and this should be further explored. Depending on the outcomes obtained, they have the potential to generate hypotheses and open avenues for further investigation.

NGF plays a crucial role in the pathogenesis of CIBP. Apart from its involvement in pathological nerve sprouting, increased expression or impaired degradation of NGF can lead to the development of mechanical pain and thermal hyperalgesia in animal models of pain. Besides that, NGF activates nociceptors and ion channels by upregulations of proteins, thereby contributing to the manifestation of CIBP (54). Anti-NGF therapy should be a new target in treating (bone) pain.

Anti-NGF therapy in mice attenuated the pathological sprouting and reduced pain behavior (50). Unfortunately, it takes many years to translate promising preclinical results to trials in humans; with the first-in-human administration of DS002 (CTR20210155, randomized, double-blind, single-dose escalation, placebo-controlled design), an anti-NGF monoclonal antibody reported in 2022 (55). Three anti-NGF drugs are currently under development: tanezumab [NCT00545129 (phase 2 trial) and NCT00830180 (phase 2 trial, extension study of NCT00545129), studied in prostate- and, breast cancer, renal cell carcinoma and multiple myeloma (56)], fulranumab [NCT00993018, studied in diabetic peripheral neuropathic pain (57)] and fasinumab [NCT02620020, studied in chronic low back pain (58)]. It is important to note that among the mentioned anti-NGF drugs, only tanezumab has been tested in the context of CIBP. The primary objective of this trial (NCT00545129) was to evaluate the efficacy of a single dose of intravenous tanezumab in patients with CIBP treated with opioids. However, the study of tanezumab showed only numeric improvements in daily pain scores, which raises the question of whether these improvements have a clinically relevant effect (56). Therefore, their potential for treating CIBP in NSCLC remains uncertain.

Part 2: Bone metastases in patients with *EGFR* mutated NSCLC

Instead of focusing solely on the treatment of already present CIBP, it may be more advantageous to explore options for early prediction and recognition of bone metastases in order to prevent bone-related complications. In light of previous research demonstrating a higher incidence of bone metastases in patients with *EGFR+* NSCLC (6), we investigated whether we could identify the underlying biological mechanism in **chapter 4**. We hypothesized that patients with *EGFR+* NSCLC and increased EGFR expression might exhibit altered RANKL gene expression or an abnormal RANKL:OPG ratio, potentially resulting in the stimulation of bone metastases. In our study, patients with *EGFR+* NSCLC had a significantly higher EGFR expression ($p<0.001$) compared to patients with *KRAS+* or *EGFR/KRAS* wildtype NSCLC. However, we did not find any association between the presence of bone metastases and EGFR, OPG and RANK gene expression. Nevertheless, we observed that patients with bone metastases exhibited a significantly higher expression of the RANKL gene and an elevated RANKL:OPG ratio in their tumor samples compared to patients without bone metastases. Notably, an increased RANKL:OPG ratio was associated with a 1.65-fold higher risk of developing bone metastases specially within the first 15 months after the diagnosis of metastatic NSCLC. As outlined in **chapter 4**, patients with *KRAS+* NSCLC and bone metastases demonstrate an upregulated expression of RANKL in their tumors. To ascertain the clinical significance of this observation, it is essential to validate these findings in a larger cohort of patients with *KRAS+* NSCLC.

More research is needed to identify why some patients do, and some do not develop bone metastases and how to predict which patients will develop bone metastases. Bone metastasis is associated with a poor prognosis and independent risk factors for bone metastasis development are liver metastases (odds ratio [OR]= 4.53, 95% CI=4.38–4.69), followed by poorly/

undifferentiated lung cancer histology (OR=2.74, 95% CI=2.52–2.99), N3 stage (OR=2.28, 95% CI=2.18–2.39), and adenocarcinoma (OR=2.07, 95% CI=2.00–2.14) (59-61). Currently, there is a growing emphasis on predictive preventive personalized medicine (PPPM), which involves analyzing large cohorts of patients to identify epidemiological trends and risk factors. In a recently published study involving analysis of 204,001 patients with lung cancer and bone metastases, an individualized nomogram was developed for estimating the risk of developing bone metastasis (59). However, the study did not provide specific details regarding molecular subtyping or treatments received by the patients. The proposed nomogram incorporated various clinical factors, including the patient's age, sex, site of lung cancer, laterality, histology, differentiation, tumor stage, nodal stage, as well as the presence of lung, liver, and brain metastases. As indicated by the nomogram, a patient with a bone metastasis probability of ≥70% is considered as high-risk individual for development of bone metastasis (area under the curve (AUC)=0.874, 95% CI=0.781-0.786) (59). In daily clinical practice, the results of such a nomogram, especially when further refined with molecular and treatment data, can be utilized to inform and guide the follow-up care of patients at high risk for developing bone metastasis.

Previous research on development of bone metastasis, focused on identification and clinical validation of bone turnover markers (BTM) too (62). Serum bone alkaline phosphatase (BALP) and N-telopeptide of type 1 collagen (NTX) have been identified as predictive factors in the context of bone metastases derived from solid tumors. Furthermore, elevated levels of serum cross-linked carboxy-terminal telopeptide of type 1 collagen (ICTP) were associated with the presence of bone metastases in lung cancer ($p<0.00001$) (40, 62). A noteworthy point is that type I collagen is not exclusively present in bone. Therefore, elevated levels of ICTP may also be caused by non-skeletal diseases and may not solely predict the presence of bone metastases. Also higher levels of the β isomer of serum C-telopeptide of type 1 collagen (β -CTX), or urinary N-telopeptide of type 1 collagen (uNTX) have been associated with the diagnosis of bone metastases in lung cancer (both $p<0.00001$) (62). The reliable routine application of these BTMs is hindered by various patient-related factors, including age, sex, natural diurnal variation, and comorbidities such as liver or kidney disease. Additionally, concomitant treatments that interfere with bone turnover (e.g., treatment with BTAs) and physiologically seasonal variations of BTM levels can contribute to significant inter-and intra-assay variations, further complicating their practical implementation (62). Currently, there is a lack of reliable and clinically practical prognostic or diagnostic BTMs found in serum, tumor samples, or urine. Thus, there is a need for further research and explore new techniques. To the best of our knowledge, we reported as first on the total concentration of extracellular vesicles (EVs), $RANKL^+$ EVs, and RANKL and OPG values in plasma and their association with the presence of bone metastases in humans with *EGFR+* NSCLC. In **chapter 5,** we observed no association between the total concentration of EVs or $RANKL^+$ EVs or plasma values of RANKL and OPG , and presence of bone metastases. However, patients undergoing treatment and especially those with a tumor response, regardless of the presence of bone metastases, exhibited a lower concentration of total EVs and $RANKL^+$ EVs. This finding is probably related to

a more favorable prognosis (i.e., disease response), as high total EV concentrations previously were linked to reduced OS (63, 64). A possible explanation for the fact that we did not find any association between the concentration of $RANKL^+$ EVs and presence of bone metastases, is that (chemo)therapy also induces modifications in the cargo of EVs (65). Therefore (chemo)therapy could stimulate different types of EVs, that show functionality related to tumor response. It is of interest to explore whether the total concentration of EVs or their cargo, could serve as a marker for treatment response. If there is a decrease in the total EV concentration or their cargo resembles to that "what is known in a patient with a favorable response in plasma during cancer therapy," a potentially strategy to future investigations is whether this also could be used instead of a CT, thereby reducing the frequency of scans. However, this is still only for research objectives, considering that the "favorable response cargo of EVs" is unknown and the determination of EVs cannot yet be carried out routinely in every laboratory.

Since we did not establish any association between EVs and bone metastases through total concentration of EVs or $RANKL^+$ EVs, and RANKL or OPG values in plasma, future experiments should place greater emphasis on investigating the involvement of EGFR and its ligands (e.g., amphiregulin (AREG), epidermal growth factor, eipiregulin) in EVs given the role they play in osteoclastogenesis (66). Beyond osteoclastogenesis, *in vitro* experiments suggest a role for EGFR in modulating the immune response. Tumor-derived EVs carrying EGFR contribute to an immunosuppressive tumor microenvironment by reducing the interferon-β response in monocytes and macrophages (67). This immunosuppressive microenvironment may potentially exert a pro-tumorigenic effect.

So far, our discussion has encompassed various aspects of CIBP. We have explored the general understanding of CIBP, including its pathophysiology and contributing factors. We have also delved into the importance of effective communication regarding bone pain and the available treatment options for patients. The last part of the thesis focused on detection of bone metastases and SREs.

In **chapter 6,** we presented that a significant proportion of 42% of the patients with *EGFR+* NSCLC enrolled in clinical trials had bone metastases at diagnosis of NSCLC. Additionally, up to 33% of these patients developed bone progression during their disease. These figures were further supported by data from a real-life population of patients with *EGFR+* NSCLC who were treated with osimertinib (**chapter 7**). In this population, 51% of the patients had bone metastases at the initiation of osimertinib therapy. Furthermore, during the treatment period, 10% of the patients developed new bone metastases or experienced progression of existing bone metastases. Considering the risk of SREs in patients with bone metastases, it is important to explore the feasibility of screening for bone metastases during treatment as a means of preventing bone-related complications. However, before considering such screening, it is important to acknowledge the challenges associated with diagnosing bone metastases, particularly for radiologists. The introduction of deoxy-2-[fluorine-18] fluoro-D-glucose positron emission tomography-computed tomography (FDG-PET-CT) scans has improved the detection of bone metastases (68). A potential

approach to enhance the detection of bone metastases through PET-CT imaging is the use of a bone-seeking radiotracer. One example of such a radiotracer is ^{68}Ga-P15-041, which combines a gallium-68-chelating bifunctional agent with a bisphosphonate. Comparing ^{68}Ga-P15-041 PET-CT with technetium bone scintigraphy, it has been found to have higher sensitivity (91.1% versus 81.8%) and accuracy (90.7% versus 88.4%) in detecting bone metastases (69). To the best of our knowledge, a direct comparison between ^{68}Ga-P15-041 PET-CT and 18-FDG-PET-CT scans has not been conducted, and ^{68}Ga-P15-041 PET-CT is not currently utilized in routine clinical practice. In addition to the conventional FDG-PET-CT scan, diffusion-weighted whole body magnetic resonance imaging (WB-MRI) scan with apparent diffusion coefficient (ADC) quantifications could be used in detection and quantification of response of bone metastases (70). Unfortunately, it is essential to acknowledge that not all patients have access to MRI or FDG-PET-CT scans, and their use in routine follow-up is limited due to availability and substantial costs associated with the procedure. Therefore, it would be more advantageous to optimize the detection of bone metastases through the use of computer tomography (CT) scans, which are commonly employed for follow-up purposes. As AI technology continues to receive significant attention, researchers have explored its application in the detection of bone metastases and its potential to enhance the performance of radiologists in identifying such metastases. A recently published retrospective single institution center study examined CT scans of 126 patients with lung cancer, both with and without bone metastases (71). In this study, researchers trained and developed a deep convolutional neural network (DCNN) model to detect bone metastases in lung cancer CT scans. The clinical efficacy of the DCNN model was then evaluated in an observer study involving both experienced radiologists (with three to eight years of CT diagnosis experience) and junior radiologists (with one to three years CT diagnosis experience). The results of the study demonstrated that the DCNN model achieved a comparable sensitivity rate of 0.89 (95% CI 0.87-0.90) when compared to experienced radiologists, whose sensitivity rate was 0.87 (95% CI 0.84-0.90). In addition to the promising results, the utilization of the DCNN model helped to improve the detection accuracy of junior radiologists and reduced the time they spent interpreting each case (71). Before implementing DCNN models in routine clinical practice, several challenges need to be addressed. Firstly, it is crucial to validate the results obtained from DCNN models in large patient cohorts representing diverse tumor histologies. This ensures the generalizability and reliability of the model across different clinical scenarios. Secondly, the integration of new computer programs into existing hospital systems is necessary to facilitate the seamless use of DCNN models in radiological workflows. Furthermore, HCPs will require training to effectively interpret and utilize the outputs generated by these models. Additionally, it is essential to ensure the privacy and security of patient data when employing AI algorithms. But in our opinion, most important is to emphasize that the use of AI is intended to complement and assist HCPs rather than replace their expertise, as judgment of HCPs remain invaluable in clinical decision-making.

At the time of NSCLC diagnosis, screening for bone metastases is generally recommended only if there is clinical suspicion of such metastases (72-75). However, it is common for patients, who

do not initially present with signs of metastatic disease, to undergo a PET-CT scan as part of their diagnostic work-up and bone metastases are often discovered incidentally. In routine follow-up, unless a patient presents with symptoms or complaints related to bone metastases, no specific attention is directed towards the detection or monitoring of bone metastases. The effectiveness of routine skeletal surveillance in improving patient outcomes, such as reducing pain or enabling more targeted treatment for bone metastases, is unknown. Moreover, one of the limitations in the follow-up of bone metastases is accurately assessing the response to systemic or local therapy in pre-existing bone lesions. Over the course of treatment cycles, morphological changes can occur within the bone metastases, such as sclerosis, fibrosis, or necrosis (70). These changes can make it challenging to differentiate between the presence of new lesions and alterations in the existing lesions that are being treated. Currently, there is a lack of clinical trial data reporting about the efficacy of routine bone follow-up in NSCLC. In our opinion, a more focused approach would involve conducting a thorough patient history with a specific emphasis on bone pain or any signs of unacceptable pain. Based on this assessment, further radiological investigations can be considered if deemed necessary. Furthermore, it is important to enhance the detection of bone metastases on available CT scans, possibly with the assistance of AI as suggested earlier. This combination of targeted patient evaluation and AI-enhanced radiological analysis can optimize the identification and management of bone metastases in follow-up care. In patients with bone metastases, we strongly recommend the initiation of BTAs through shared decision making, unless there are contra indications for such treatment. This recommendation is based on the promising results indicating the delay of SREs and the potential improvement of OS (37-40). Moreover, in patients with bone metastases from hormone refractory prostate cancer and breast cancer, there is evidence to suggest that early initiation of BTAs before the onset of bone pain can yield greater clinical benefits (46). Furthermore, in patients without bone metastases, it is important to consider BTA treatment too. Studies have shown that up to 60% of patients eventually develop bone metastases, relying solely on imaging for their identification may not always be straightforward (1, 2).

While improving systemic therapy is considered crucial in the management of bone metastases, identifying the most effective treatment approach remains a challenge. In the context of *EGFR+* NSCLC, a retrospective cohort study involving 388 patients evaluated the clinical efficacy of osimertinib compared to first- and second-generation EGFR-TKIs (76). They found that osimertinib significantly improved PFS in patients with bone metastases (p=0.0004) when compared to first- and second generation EGFR-TKIs. However, there was no significant difference in OS between the treatment groups (76). Despite the initial promising efficacy of osimertinib on bone metastases compared to first-and second generation EGFR-TKIs, additional evidence confirmed the worse prognosis despite treatment with osimertinib (60). The survival rate for patients with bone metastasized NSCLC is comparable to those from our real life population, as shown in **chapter 7**. A small retrospective study including 129 patients with bone metastases investigated the combination of EGFR-TKI or ALK-TKI with BPs compared to TKIs alone (37). Among

the study participants, 32 received TKIs and BPs concurrently, while 17 patients were treated with TKIs alone. Other patients received chemotherapy with or without BPs. They reported a longer PFS (11.2 versus 6.9 months, HR=0.13, 95% CI 0.05-0.35, P<0.0001) and OS (31 versus 22 months, HR=0.31, 95% CI 0.10-0.96, p=0.04) in patients treated with TKIs and BPs compared to those receiving TKIs alone (37). However, it is important to interpret these results with caution due to the small sample size, no details about which EGFR or ALK TKI was given and the lack of molecular analysis for all patients (approximately 30% of the included patients had unknown *EGFR/ALK* status). The retrospective nature of the aforementioned studies also raises concerns when extrapolating the findings to clinical practice. In our view, these results may not immediately alter the treatment options for patients with *EGFR+* NSCLC and bone metastases, but they do highlight the potential benefits of BTAs in combination with targeted therapies.

In **chapter 7**, we highlighted that SREs are common in patients with *EGFR+* NSCLC and bone metastases. At the time of metastatic NSCLC diagnosis, 15% of patients with bone metastases were already diagnosed with an SRE. This proportion continued to rise over the course of their disease, reaching a cumulative incidence of 39%. The close association between bone metastases and SREs is well-established. Recognizing and effectively managing bone metastases is crucial in attempting to prevent the occurrence of SREs. Analogous to the utilization of AI in prediction models for bone metastasis, AI is also being employed in predicting SREs in cancer patients with bone metastases. As expected, machine learning techniques such as decision tree and support vector machine have demonstrated high accuracies compared to traditional logistic regression models when prediction outcomes such as SREs (77).

Part 3: Conclusions and summary of future outlook

Clinical research and the translation of its findings by HCPs are essential for generating evidence and play indispensable roles in advancing our understanding and improving patient care. In Figure 2 a flowchart is presented in approaching a patient with advanced NSCLC with regard to development of bone metastases. Most importantly, HCPs should be vigilant and proactive in monitoring for the development of bone metastases and potential complications in patients with advanced NSCLC.

Based on the findings of this thesis, the future outlook for research should be focused on exploring of the potential usefulness of RANKL inhibition in patients with and without bone metastases, with a particular emphasis on patients with *KRAS*-mutated NSCLC, considering the observed increased RANKL gene expression in this subgroup. Furthermore, exploring the feasibility of initiating BTAs before the manifestation of clinical overt bone metastases could offer improved management of CIBP. Additionally given the challenges and uncertainties in interpreting current available bone turnover markers, it is crucial to validate the predictive value of RANKL:OPG ratio in serum with regard to the development of bone metastases across different patient populations, including other solid tumors and non-metastasized stages. EV research is currently mainly conducted through *in vitro* experiments and its application *in vivo* still needs to

find its place. By imaging flow cytometry, we proposed a reliable method to identify and quantify EVs in plasma of patients with NSCLC for expanding experiments. . However, the role of EVs in development of bone metastases in (*EGFR+*) NSCLC has to be further explored.

In conclusion, this thesis has provided evidence of the high incidence of bone metastases, CIBP and SREs in patients with (*EGFR+*) NSCLC. The clinical challenges associated with the detection and management of these conditions are evident. However, there is still a significant knowledge gap regarding non-radiation-based treatment options for CIBP and in clinical trials less attention is paid on bone metastases and bone related outcomes. We demonstrated that patients with bone metastases exhibit significantly higher RANKL gene expression and a higher RANKL:OPG ratio in their tumors compared to patients without bone metastases. Additionally, we did not demonstrate any association between total concentration of EVs or $RANKL^+$ EVs, and RANKL and OPG values in plasma and presence of bone metastases. Despite these results, the biological mechanisms underlying the increased incidence of bone metastases in *EGFR+* NSCLC patients remain unclear. The findings of this thesis underscore the importance of conducting further research in order to enhance the treatment and prognosis of patients with bone metastasized NSCLC.

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SUMMARY

In approximately 30-60% of all patients with metastatic non-small cell lung carcinoma (NSCLC), bone metastases are present at the time of diagnosis or develop during the course of the disease. Research indicates that patients with NSCLC harboring an activating mutation in the epidermal growth factor receptor gene (*EGFR+*) may develop bone metastases even more frequently than other NSCLC patients. These bone metastases can lead to bone-related complications, known as skeletal related complications (SREs), such as (the risk of) pathological fractures (resulting in the need for surgery), compression of the spinal cord nerves, or the necessity for bone radiation (due to impending fractures or pain). Patients with bone metastases may experience a reduced quality of life compared to those without bone metastases. Furthermore, these patients have a poorer overall survival (OS) compared to those without bone metastases. This thesis aims to enhance the treatment of patients with NSCLC and bone metastases and to provide a biological explanation for why patients with *EGFR+* NSCLC develop bone metastases more frequently.

Part 1: cancer induced bone pain

A substantial portion of patients with NSCLC and bone metastases develop symptoms; up to 80% experience cancer-induced bone pain (CIBP). The management of this pain involves pain control (pain medication with or without local treatment) and systemic therapy to control the underlying malignancy. Radiotherapy can be an effective method for pain reduction in painful bone metastases, particularly when this pain is localized to one or multiple specific areas. If radiotherapy proves to be ineffective or if there are numerous painful bone metastases that cannot all be irradiated, alternative treatment options should be considered. In **chapter 2**, we conducted a systematic review to investigate the possibilities for rapid pain relief in patients with NSCLC experiencing pain due to bone metastases. We did not consider radiotherapy and radioisotopes as treatment options in this context because they are not feasible for diffuse pain complaints (radiotherapy) and due to interactions with systemic therapy (radioisotopes). The definition of the time frame for “rapid pain relief” was originally set as within two weeks after the intervention. Due to a lack of sufficient suitable studies to include in this review, we later adjusted this timeframe to assess pain within six weeks after the intervention. In total, ten articles were included in this review (comprising a total of 554 NSCLC patients). The studies were highly diverse in terms of treatment options, methods of pain assessment, and outcome measures. Furthermore, it was often challenging to differentiate between the effect of systemic therapy and the specific effect of pain management, as these treatments were frequently administered concurrently. Based on this review, we are unable to express a specific preference for a particular pain relief therapy.

In the current treatment guidelines for bone metastases, recommendations regarding the use of bone targeted agents (BTAs), including denosumab and bisphosphonates, primarily focus on their application in breast cancer, prostate cancer, and multiple myeloma. This is because studies

assessing the effectiveness of these agents in terms of skeletal-related events (SREs), survival, or pain reduction mainly target these patient categories. The impact of BTAs on bone metastases resulting from NSCLC is only investigated in small subgroups or post-hoc analyses. Denosumab and bisphosphonates are more frequently prescribed to patients with breast and prostate cancer than to NSCLC patients. Bisphosphonates have demonstrated significant pain reduction in cases of bone pain and a decrease in the number of SREs and/or time to SRE in patients with bone metastases from breast and prostate cancer. However, limited literature is available that examines the effect of bisphosphonates on painful bone metastases in NSCLC.

To investigate the effect of a loading dose of ibandronate (a bisphosphonate) on uncontrolled pain due to bone metastases in NSCLC patients, a single-arm phase 2 multicenter study (NVALT9) was conducted, as described in **chapter 3**. The selection of this loading dose was based on the results of a pilot study examining the effect of a loading dose of ibandronate on opioid-resistant bone pain in patients with various tumors. In this study, patients with uncontrolled pain due to bone metastases (measured using the Brief Pain Inventory [BPI] with a score of ≥5 in the last seven days) were included. These patients were treated with six milligrams of intravenous ibandronate over three days. What made this study unique was the exclusion of the effect of radiotherapy or systemic therapy because patients received ibandronate exclusively during the study. The primary outcome measure was "bone pain response" measured over seven days. A bone pain response was defined as a 25% reduction in the worst pain on days 5, 6, and 7 compared to the pain score at the start of the study. Patients were not allowed to use more than 25% additional pain medication during this period compared to what they were already using at the beginning of the study for it to be considered a bone pain response. The study was designed according to Simon's Optimal Two-Stage Design, evaluating the bone pain response after the inclusion of 19 patients. If 12 patients or fewer out of these 19 achieved a bone pain response, the inclusion was to be halted. Upon evaluation, it was found that a bone pain response existed in four out of 18 (22%) evaluable patients, leading to the cessation of inclusion. We concluded that an ibandronate loading dose does not provide sufficient pain relief for NSCLC patients with uncontrolled pain due to bone metastases. The reason why bisphosphonates lead to pain reduction in other solid tumors but not adequately in NSCLC remains unknown. A potential explanation could be differences in tumor histology, disparities in the local bone microenvironment, or the influence of concomitant systemic therapy.

Part 2: bone metastases in patients with *EGFR+* NSCLC

Steven Paget already discussed the "seed and soil" theory in 1889, in which he attempted to explain the origins of bone metastases. Various processes create a "niche" in the bone to which wandering tumor cells can easily attach and penetrate the bone to establish themselves. In natural bone formation, there exists a strict balance between osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells), regulated by growth factors, control mechanisms, and signaling cascades. One of the most crucial signaling cascades is the Receptor Activator of Nuclear Factor

κB (RANK)/ RANK Ligand (RANKL)/ Osteoprotegerin (OPG) cascade. Binding of RANK to RANKL stimulates osteoclastogenesis, leading to bone resorption. OPG acts as a decoy receptor: when it binds to RANKL, it prevents the binding to RANK, thereby inhibiting bone resorption. The EGFR signaling pathway is essential for osteoclast formation and is under the control of OPG, monocyte chemoattractant protein 1 (MCP1), macrophage colony-stimulating factor (MSCF), and RANKL expression. It is known that 40-80% of NSCLC patients have increased EGFR protein expression in their tumors. The literature is not consistent regarding whether this is associated with an activating EGFR mutation. Preclinical data indicate that EGFR expression leads to inhibition of OPG expression and an increase in RANKL expression. We have previously demonstrated that patients with metastatic *EGFR+* NSCLC have a higher incidence of bone metastases than those without EGFR mutations. Consequently, we suspected that in *EGFR+* NSCLC with high EGFR expression, there is a change in RANKL gene expression or the RANKL:OPG ratio, which can lead to the stimulation of bone metastases. In **chapter 4**, we investigated whether there was a relationship between EGFR, RANKL, RANK, and OPG gene expression in the tumor and the presence of bone metastases in patients with metastatic NSCLC. A database was established, including every patient with metastatic NSCLC and an activating *EGFR+* mutation (exon 19 deletion or exon 21 point mutation), along with the next available Kirsten rat sarcoma (*KRAS+*) and *EGFR/KRAS* wild-type patient as a control. In total, 335 patients were included, and biopsies from NSCLC diagnosis were requested. Ultimately, this material was available for 169 patients (50%), and the gene expression of EGFR, RANKL, RANK, and OPG could be measured in 43% of them (73 out of 169 patients). We found that patients with *EGFR+* NSCLC had higher EGFR gene expression in the tumor compared to *KRAS+* and *KRAS/EGFR* wild-type patients. However, no correlation was found between EGFR gene expression and the presence of bone metastases. Patients with bone metastases, on the other hand, had significantly higher RANKL gene expression and an increased RANKL:OPG ratio compared to patients without bone metastases. Patients with an increased RANKL:OPG ratio developed bone metastases more frequently, especially in the first 450 days after the diagnosis of metastatic NSCLC. Both previous preclinical and clinical research suggest that increased RANKL gene expression and RANKL:OPG ratio are associated with a higher risk of bone metastases due to NSCLC. The elevated RANKL gene expression in tumors of *KRAS+* patients with bone metastases is a noteworthy finding, but it needs confirmation in a larger study population.

Extracellular vesicles (EVs) play a role in cellular communication and contribute to creating the potential for tumor cells to reach a specific site in the body and grow there. Much of what we know about EVs in cancer comes from preclinical research. Translational research on pathological tissue is often hindered by the insufficient availability of tumor tissue to perform all tests, resulting in (relatively) small study populations that reduce the statistical reliability of the results. This was also one of the issues we faced in the study from **chapter 4**. In **chapter 5**, we investigated whether it was possible to identify and quantify EVs in frozen plasma from patients with metastatic NSCLC by means of imaging flow cytometry (IMF). The main objectives of this study were to assess whether there is an association between the total concentration of EVs and EVs with

RANKL expression (RANKL$^+$ EVs) and the presence of bone metastases. As secondary outcomes, we evaluated the plasma levels of RANKL and OPG in relation to bone metastases and the concentration of EVs and OS. We showed the reliability of imaging flow cytometry for identifying and quantifying EVs in plasma. In a cohort of 40 patients with metastatic *EGFR+* NSCLC, wherein 63% had bone metastases at the time of sample collection, no association was observed between the total concentration of EVs, RANKL$^+$ EVs and presence of bone metastases. Additionally, plasma levels of RANKL and OPG showed no association with the presence of bone metastases. The total concentration of EVs and RANKL$^+$ EVs decreased during osimertinib treatment. This likely reflects a response to therapy, considering that most patients had an ongoing response.

In addition to understanding the biological mechanism behind the development of bone metastases in (*EGFR+*) NSCLC, it is interesting to determine the effectiveness of EGFR tyrosine kinase inhibitors (TKIs) on bone metastases in this population, given the frequent occurrence of bone metastases in these patients. There are no prospectively randomized trials investigating bone metastases or bone-related outcomes in patients with *EGFR+* NSCLC. Therefore, we conducted a systematic review, focusing on the reported incidence of bone metastases, skeletal-related events (SREs), and bone-related outcomes in phase 2 and phase 3 EGFR-TKI studies from January 2006 to January 2021 (**chapter 6**). The search strategy was structured according to the Patient Intervention Control Outcome (PICO) strategy. The patient was defined as an individual with metastatic *EGFR+* NSCLC, with treatment with EGFR-TKIs as the intervention. Control and outcome were not included in the search strategy, as it narrowed down the number of articles found too much. The primary inclusion criteria for this review were as follows: 1) a study population of 10 or more patients with NSCLC and an activating *EGFR* mutation, 2) at least one of the treatment arms consisting of an EGFR-TKI, and 3) reporting of data on bone metastases and SREs at diagnosis or during the disease course. Initially, 663 unique articles were found, and after the selection process, 21 articles were ultimately included in this review. In none of the studies were bone metastases, SREs, or bone-related outcomes described as primary or secondary outcomes. The incidence of bone metastases at the time of diagnosis in these studies was 42%. It should be noted that the actual incidence may be higher, as specific bone imaging was performed only in seven of the 21 studies at the time of the diagnosis of metastatic NSCLC. SREs or other bone-related outcomes were not reported in any of the studies. At the time of disease progression, more than a quarter of patients (26%) had progressive disease in the bone. However, there is again a chance that this number is inaccurate, as only two studies performed specific bone imaging during follow-up. In conclusion, bone metastases, SREs, or bone-related outcomes are barely reported in clinical trials.

Patients with *EGFR+* NSCLC are preferably treated with EGFR-TKIs as targeted therapy. The development of EGFR-TKIs from first to third generation has led to a higher level of effectiveness and better tolerance for the patient. It is unknown what the effectiveness of osimertinib, a third-generation EGFR-TKI, is on the development of bone metastases and SREs. **Chapter 7** describes the results of a retrospective, multicenter study in which patients with metastatic *EGFR+* NSCLC

treated with osimertinib were prospectively followed. A total of 250 patients were included in this study between February 2016 and September 2021. Of the patients, 41% were treated with osimertinib as first-line therapy. At the diagnosis of metastatic *EGFR+* NSCLC, 45% of the patients had one or more bone metastases. This percentage increased to 51% when patients were treated with osimertinib in the second or further line. The number of patients with one or more SREs was also high: 15% of the patients had a first SRE at the diagnosis of metastatic disease, while 11% developed their first SRE during osimertinib treatment, and the cumulative incidence increased to 39%. Compared to the development of SREs during treatment with first or second-generation EGFR-TKIs, this 11% is more than half lower: the number of SREs during treatment with earlier-generation EGFR-TKIs was previously reported between 26% and 28%. Ten percent of the patients developed new bone metastases or progression of pre-existing bone metastases during treatment with osimertinib. This number is comparable to a smaller retrospective study (N=126) describing bone progression during first-line osimertinib (10% vs. 12%) and with other trials describing bone progression during treatment with first and second-generation EGFR-TKIs (10% vs. 3-26%, N=2,109). In total, 16% of the patients with bone metastases (23/142) were prescribed denosumab or a bisphosphonate at least once. The median survival of patients with *EGFR+* NSCLC after the diagnosis of bone metastases was more than three years, namely 37 months. This survival was not shortened by the presence of SREs. We also know that experiencing an SRE is a risk factor for developing a subsequent SRE. This means that patients with *EGFR+* NSCLC are exposed to the risk of developing SREs for a long time, with a negative impact on quality of life and prognosis. Therefore, prospective research into the effectiveness of BTAs in this patient group is desirable. Despite the lack of this data, we advocate for the prescription of BTAs in patients with *EGFR+* NSCLC regardless of the presence of bone metastases, given the reduction in the number of SREs, the increase in time to the first SRE, and the potential impact on cancer-induced bone pain.

Chapter 8 presents a general discussion of this thesis.

NEDERLANDSTALIGE SAMENVATTING

Ongeveer 30-60% van alle patiënten met een uitgezaaid niet-kleincellig longcarcinoom (NSCLC) heeft botmetastasen bij diagnose of ontwikkelt deze tijdens de ziekte. Uit onderzoek blijkt dat patiënten met NSCLC en een activerende mutatie in het epidermal growth factor gen (*EGFR+*) mogelijk nog vaker botmetastasen ontwikkelen dan andere patiënten met NSCLC. De botmetastasen kunnen leiden tot bot gerelateerde complicaties (skeletal related complications, SREs) zoals (risico op) een pathologische breuk (en hierdoor noodzaak tot operatie), compressie van de ruggenmergszenuwen of noodzaak tot bestraling van het bot (door een dreigende breuk of door pijnklachten). Patiënten met botmetastasen kunnen hierdoor een verminderde kwaliteit van leven ervaren ten opzichte van patiënten zonder botmetastasen. Ook hebben deze patiënten, vergeleken met degenen zonder botmetastasen, een slechtere overleving. In dit proefschrift wordt getracht de behandeling van patiënten met NSCLC en botmetastasen te verbeteren en daarbij een biologische verklaring te geven waardoor patiënten met *EGFR+* NSCLC vaker botmetastasen lijken te ontwikkelen.

Deel 1: kanker-geïnduceerde botpijn

Een groot deel van de patiënten met NSCLC en botmetastasen ontwikkelt klachten; tot wel 80% ervaart kanker-geïnduceerde botpijn (cancer induced bone pain, CIBP). De behandeling van deze pijn bestaat uit pijnbestrijding (pijnmedicatie met of zonder lokale behandeling), en systemische therapie om de onderliggende maligniteit onder controle te krijgen. Radiotherapie kan een effectieve methode zijn voor pijnreductie bij pijnlijke botmetastasen, wanneer deze pijn gelokaliseerd is op één of meerdere specifieke plekken. Als radiotherapie niet effectief blijkt te zijn of als er sprake is van veel pijnlijke botmetastasen die niet allemaal bestraald kunnen worden, dient men alternatieve behandelopties te overwegen. In **hoofdstuk 2** hebben we een systematische review uitgevoerd om de mogelijkheden voor snelle pijnverlichting bij patiënten met NSCLC en pijn door botmetastasen te onderzoeken. Hierbij hebben we radiotherapie en radio-isotopen als behandeloptie niet meegenomen, omdat dit onmogelijk is bij diffuse pijnklachten (radiotherapie) en door de interactie met systemische therapie (radio-isotopen). De definitie voor de tijdsduur voor “snelle pijnverlichting” werd oorspronkelijk gesteld als binnen twee weken na de interventie. Vanwege een gebrek aan voldoende geschikte studies om te includeren in dit review, hebben we deze termijn later aangepast naar het beoordelen van pijn binnen zes weken na de interventie. In totaal zijn tien artikelen geïncludeerd in dit review (met in totaal 554 patiënten met NSCLC). De studies waren erg divers wat betreft behandelopties, meetmethoden voor pijnklachten en uitkomstmaten. Bovendien was het vaak moeilijk om onderscheid te maken tussen het effect van systemische therapie en het specifieke effect van pijnbestrijding, aangezien deze behandelingen vaak gelijktijdig werden gegeven. Op basis van deze review kunnen we geen specifieke voorkeur uitspreken voor een bepaalde pijn verlichtende therapie.

In de huidige richtlijnen voor behandeling van botmetastasen ligt de nadruk bij aanbevelingen over het gebruik van bone targeted agents (BTAs), waaronder denosumab en bisfosfonaten, vooral op de toepassing ervan bij borstkanker, prostaatkanker en multipel myeloom. Dit komt doordat studies over de effectiviteit van deze middelen met betrekking tot SRE's, overleving of pijnvermindering zich voornamelijk richten op deze categorieën van patiënten. Het effect van BTAs op botmetastasen als gevolg van NSCLC wordt slechts in kleine subgroepen of post-hoc analyses onderzocht. Denosumab en bisfosfonaten worden vaker voorgeschreven aan patiënten met borstkanker en prostaatkanker dan aan patiënten met NSCLC. Bisfosfonaten hebben aangetoond dat ze leiden tot een significante pijnreductie in het geval van botpijn en tot een afname van het aantal SREs en/of tijd tot SRE bij patiënten met botmetastasen als gevolg van borstkanker en prostaatkanker. Er is echter beperkte literatuur beschikbaar die het effect van bisfosfonaten op pijnlijke botmetastasen bij NSCLC heeft onderzocht. Om het effect van een oplaaddosis ibandronaat (een bisfosfonaat) op ongecontroleerde pijn door botmetastasen bij patiënten met NSCLC te onderzoeken, werd er een single arm fase 2, multicenter studie opgezet (NVALT9), die beschreven wordt in **hoofdstuk 3**. Voor deze oplaaddosis werd gekozen op basis van de resultaten in een pilotstudie waarin het effect van een oplaaddosis ibandronaat op opioïd-resistente botpijn bij patiënten met verschillende tumoren werd onderzocht. In deze studie werden patiënten met ongecontroleerde pijn door botmetastasen (gemeten met de brief pain inventory [BPI] en een score van ≥ 5 in de laatste zeven dagen) geïncludeerd. Deze patiënten werden behandeld met zes milligram ibandronaat intraveneus gedurende drie dagen. Wat deze studie uniek maakte, is dat het effect van radiotherapie of systemische therapie werd uitgesloten, omdat de patiënten tijdens de studie uitsluitend ibandronaat kregen toegediend. De primaire uitkomstmaat was "botpijnrespons" gemeten over zeven dagen. Een botpijnrespons werd gedefinieerd als een 25% afname van de ergste pijn op dag 5, 6 en 7 in vergelijking met de pijnscore aan het begin van de studie. Patiënten mochten tijdens deze periode niet meer dan 25% extra pijnmedicatie gebruiken, dan ze al gebruikten bij aanvang van de studie om nog van een botpijnrespons te kunnen spreken. De studie was opgezet volgens Simon's Optimal two-stage design, waardoor de botpijnrespons werd geëvalueerd na de inclusie van 19 patiënten. Als 12 patiënten of minder van deze 19 patiënten een botpijnrespons hadden, werd de inclusie gestaakt. Bij evaluatie bleek een botpijnrespons te bestaan in vier van de 18 (22%) evalueerbare patiënten, waardoor de inclusie werd gestaakt. We concludeerden dat een ibandronaat oplaaddosis niet tot voldoende pijnverlichting zorgt voor patiënten met NSCLC en ongecontroleerde pijn door botmetastasen. Waarom bisfosfonaten wel tot pijnreductie leiden in andere solide tumoren en onvoldoende bij NSCLC is onbekend. Een mogelijke verklaring is verschil in tumor histologie, verschil in lokaal botmilieu of invloed van concomitante systemische therapie.

Deel 2: botmetastasen in patiënten met *EGFR+* NSCLC

Steven Paget sprak in 1889 al over de "seed and soil" theorie, waarin hij probeerde uit te leggen hoe botmetastasen ontstaan. Verschillende processen creëren een "voedingsbodem" ("niche")

in het bot, waaraan rondzwervende tumorcellen zich gemakkelijk kunnen hechten en het bot kunnen binnendringen om zich daar te vestigen. In de natuurlijke botopbouw bestaat er een strikte balans tussen osteoblasten (botvormende cellen) en osteoclasten (botafbrekende cellen), gereguleerd door groeifactoren, controlemechanismen en signaalcascades. Een van de belangrijkste signaalcascades is de Receptor activator of Nuclear Factor κB (RANK)/ RANK ligand (RANKL)/ osteoprotegerin (OPG) cascade. Binding van RANK aan RANKL stimuleert de osteoclastogenese, wat leidt tot botafbraak. OPG fungeert als een soort beschermkapje: wanneer het bindt aan RANKL, kan de binding met RANK niet plaatsvinden en wordt botafbraak niet gestimuleerd. De *EGFR*-signaalroute is van belang voor stimulatie van osteoclastvorming en staat onder controle van OPG, monocyte chemoattractant protein 1 (MCP1), macrophage colony-stimulating factor (MSCF) en RANKL-expressie. We weten dat bij 40-80% van de patiënten met NSCLC de eiwitexpressie van *EGFR* in de tumor verhoogd is. De literatuur is niet eenduidig over de vraag of dit samenhangt met een activerende *EGFR* mutatie. Uit preklinische gegevens blijkt dat EGFR-expressie leidt tot remming van de OPG-expressie en toename van de RANKL-expressie. We hebben al eerder aangetoond dat patiënten met uitgezaaid *EGFR+* NSCLC vaker botmetastasen hebben dan degenen zonder *EGFR* mutatie. Daardoor vermoedden wij dat er bij *EGFR+* NSCLC met een hoge EGFR expressie een verandering optreedt in de RANKL- genexpressie of de RANKL:OPG ratio, wat kan leiden tot de stimulatie van botmetastasen. In **hoofdstuk 4** onderzochten we of er een relatie was tussen *EGFR*, RANKL, RANK en OPG-gen expressie in de tumor en de aanwezigheid van botmetastasen in patiënten met uitgezaaid NSCLC. Er is een database opgesteld waarin elke patiënt met uitgezaaid NSCLC en een activerende *EGFR+* mutatie (exon 19 deletie of exon 21 punt mutatie) werd geïncludeerd, samen met telkens de eerstvolgende Kirsten rat sarcoma (*KRAS+*) en *EGFR/KRAS* wildtype patiënt als controle. In totaal werden 335 patiënten geïncludeerd, waarvan het biopt van NSCLC diagnose werd opgevraagd. Uiteindelijk was dit materiaal beschikbaar voor 169 patiënten (50%), en bij 43% van hen (73 van de 169 patiënten) kon de genexpressie van EGFR, RANKL, RANK en OPG worden gemeten. We vonden dat patiënten met *EGFR+* NSCLC een hogere EGFR-gen expressie in de tumor hadden in vergelijking met *KRAS+* en *KRAS/EGFR* wildtype patiënten. Er werd echter geen verband gevonden tussen de *EGFR*-gen expressie en de aanwezigheid van botmetastasen. Wel bleek dat patiënten met botmetastasen een significant hogere RANKL-genexpressie en een verhoogde RANKL:OPG ratio hadden in vergelijking met patiënten zonder botmetastasen. Patiënten met een verhoogde RANKL:OPG ratio ontwikkelden vaker botmetastasen, vooral in de eerste 450 dagen na de diagnose van uitgezaaid NSCLC. Zowel eerder preklinisch als klinisch onderzoek suggereert dat een verhoogde RANKL-genexpressie en RANKL:OPG ratio verband houden met een groter risico op botmetastasen door NSCLC. De verhoogde RANKL-gen expressie in tumoren van patiënten met *KRAS+* met botmetastasen is een opvallende bevinding, maar dit dient bevestigd te worden in een grotere studiepopulatie.

Extracellulaire vesicles (EVs) spelen een rol in cellulaire communicatie en dragen bij aan het creëren van de mogelijkheid voor tumorcellen om op een specifieke plaats in het lichaam terecht te komen en daar te groeien. Wat we weten over EVs in kanker komt vooral uit preklinisch

onderzoek. Translationeel onderzoek op pathologisch weefsel wordt vaak belemmerd door onvoldoende beschikbaarheid van tumorweefsel om alle onderzoekstesten uit te kunnen voeren, wat resulteert in (relatief) kleine studiepopulaties die de statistische betrouwbaarheid van de resultaten verkleinen. Dit was ook een van de problemen waarmee we werden geconfronteerd in de studie uit **hoofdstuk 4**. In **hoofdstuk 5** hebben we onderzocht of het mogelijk was om EVs te detecteren in resterend bevroren bloedplasma van patiënten met uitgezaaid NSCLC, dit werd gedaan middels imaging flow cytometrie. Het primaire doel van deze studie was om te beoordelen of er een verband bestaat tussen de concentratie circulerende EVs en EVs die RANKL tot expressie brengen (RANKL$^+$ EVs) en de aanwezigheid van botmetastasen. Als secundaire doelen beoordeelden we of er een relatie bestaat tussen RANKL en OPG spiegels in het plasma en de aanwezigheid van botmetastasen en keken we naar de associatie tussen de concentratie van EVs en de overleving. Imaging flow cytometrie is een betrouwbare manier om EVs te identificeren en te tellen in humaan plasma. In een populatie van 40 patiënten met uitgezaaid *EGFR+* NSCLC, waarvan 63% botmetastasen had op het moment van bloedafname voor de studie, zagen we geen relatie tussen het totale concentratie EVs of RANKL$^+$ EVs en de aanwezigheid van botmetastasen. Daarnaast waren de plasma spiegels van RANKL en OPG niet geassocieerd met de aanwezigheid van botmetastasen. Wel zagen we dat de totale concentratie EVs en RANKL$^+$ EVs daalde tijdens behandeling met osimertinib. Dit ontstaat mogelijk als gevolg van een behandeleffect van de therapie, gezien bij het merendeel van de patiënten een therapierespons bestond op het moment van bloedafname.

Naast de vraag wat het biologische mechanisme is achter het ontstaan van botmetastasen in (*EGFR+*) NSCLC is, is het interessant om te weten wat de effectiviteit is van EGFR-tyrosine kinase remmers (TKI's) op botmetastasen in deze populatie gezien het frequente voorkomen van botmetastasen in deze groep van patiënten. Voor patiënten met *EGFR+* NSCLC zijn er geen prospectief gerandomiseerde onderzoeken waarbij botmetastasen of bot gerelateerde uitkomstmaten worden onderzocht. Daarom hebben we een systematisch review verricht, met focus op de gerapporteerde incidentie van botmetastasen, SREs en botgerelateerde uitkomstmaten in fase 2 en fase 3 EGFR-TKI studies in de periode januari 2006 tot januari 2021 (**hoofdstuk 6**). De zoekstrategie werd opgezet volgens de Patiënt Interventie Controle Outcome (PICO) strategie. De patiënt werd gedefinieerd als een persoon met uitgezaaid *EGFR+* NSCLC, waarbij behandeling met EGFR-TKI's als interventie werd vastgesteld. De controle en uitkomst werden niet meegenomen in de zoekstrategie, doordat dit het aantal gevonden artikelen te veel inperkte. De belangrijkste inclusie criteria van dit review waren als volgt: 1) studie populatie van 10 of meer patiënten met NSCLC en een activerende *EGFR* mutatie, 2) minstents één van de behandelarmen bestaat uit een EGFR-TKI en 3) gegevens over botmetastasen en SREs bij diagnose of ziektebeloop worden gerapporteerd. In eerste instantie werden 663 unieke artikelen gevonden, en na het selectieproces werden uiteindelijk 21 artikelen geïncludeerd in dit review. In geen enkele studie werden botmetastasen, SREs of bot gerelateerde uitkomstmaten als een primaire of secundaire uitkomstmaat beschreven. De incidentie van botmetastasen bij diagnose

van de ziekte in deze studies was 42%. Een kanttekening hierbij is dat de werkelijke incidentie hoger kan zijn, omdat slechts in zeven van de 21 studies specifieke beeldvorming van het bot werd uitgevoerd bij de diagnose van uitgezaaid NSCLC. SREs of andere bot gerelateerde uitkomstmaten werden in geen van de studies gerapporteerd. Op het moment van progressie had ruim een kwart van de patiënten (26%) progressieve ziekte in het bot. Er bestaat echter opnieuw de kans dat dit getal onjuist is, gezien slechts twee studies specifieke beeldvorming van het bot uitvoerden tijdens de follow-up. In conclusie: botmetastasen, SREs of bot gerelateerde uitkomstmaten worden slechts minimaal worden gerapporteerd in klinische onderzoeken.

Patiënten met *EGFR+* NSCLC worden bij voorkeur behandeld met EGFR-TKI's als doelgerichte therapie. De ontwikkeling van EGFR-TKI's van eerste tot derde generatie heeft geleid tot een hogere mate van effectiviteit en een betere verdraagzaamheid voor de patiënt. Het is onbekend wat de effectiviteit is van osimertinib, een derde generatie EGFR-TKI, op ontwikkeling van botmetastasen en SREs. In **hoofdstuk 7** worden de resultaten beschreven van een retrospectieve, multicenter studie waarin patiënten met uitgezaaide *EGFR+* NSCLC die behandeld werden met osimertinib prospectief gevolgd werden. Er zijn tussen februari 2016 en september 2021 in totaal 250 patiënten geïncludeerd in deze studie. Van de patiënten werd 41% behandeld met osimertinib als eerstelijns therapie. Bij diagnose van uitgezaaid *EGFR+* NSCLC had 45% van de patiënten één of meerdere botmetastasen. Dit percentage steeg naar 51% wanneer patiënten in de tweede of verdere lijn behandeld werden met osimertinib. Ook het aantal patiënten met één of meerdere SREs was hoog: 15% van de patiënten had een eerste SRE bij de diagnose van uitgezaaide ziekte, terwijl 11% hun eerste SRE ontwikkelde tijdens behandeling met osimertinib en de cumulatieve incidentie opliep naar 39%. In vergelijking met ontwikkeling van SREs tijdens behandeling met eerste of tweede generatie EGFR-TKI's is deze 11% meer dan de helft lager: het aantal SREs tijdens behandeling met eerdere generatie EGFR-TKI's werd eerder tussen 26% en 28% gerapporteerd. Tien procent van de patiënten ontwikkelt nieuwe botmetastasen of progressie van pre-existente botmetastasen tijdens behandeling met osimertinib. Dit getal is vergelijkbaar ten opzichte van een kleinere, retrospectieve studie (N=126) die botprogressie tijdens eerstelijns osimertinib beschrijft (10% vs. 12%) en met andere trials die botprogressie tijdens behandeling met eerste en tweede generatie EGFR-TKI's beschrijven (10% vs. 3-26%, N=2,109). In totaal 16% van de patiënten met botmetastasen (23/142) werd minimaal één keer denosumab of een bisfosfonaat voorgeschreven. De mediane overleving van patiënten met *EGFR+* NSCLC na diagnose van botmetastasen was langer dan drie jaar, namelijk 37 maanden. Deze overleving werd niet verkort door de aanwezigheid van SREs. We weten ook dat doormaken van een SRE een risicofactor is voor het ontwikkelen van een volgende SRE. Dit maakt dat patiënten met *EGFR+* NSCLC langdurig blootstaan aan het risico op het ontwikkelen van SREs met een negatieve invloed op kwaliteit van leven en prognose. Daarom is prospectief onderzoek naar de effectiviteit van BTAs in deze patiëntengroep wenselijk. Ondanks het ontbreken van deze data, willen wij een lans breken voor het voorschrijven van BTAs bij patiënten met *EGFR+* NSCLC ongeacht aanwezigheid van

botmetastasen, gezien de daling van het aantal SREs, het vergrooten van de tijd tot de eerste SRE en de mogelijke invloed op kanker-geïnduceerde botpijn.

In **hoofdstuk 8** wordt een algemene discussie over deze thesis gehouden.

IMPACT

In this impact chapter, we present the most significant findings of our research and discuss their potential scientific impact and relevance for patients, healthcare providers (HCPs), and society. Additionally, we address the dissemination of our work.

Aims and conclusion of this thesis

The overall aim of this thesis was to optimize the treatment of patients with non-small cell lung cancer (NSCLC) and bone metastases, with a specific focus on patients with epidermal growth factor mutated (*EGFR+*) NSCLC. Moreover, we explored potential biological mechanisms that may contribute to the observed increased incidence of bone metastases in this subgroup of patients.

Patients with bone metastases are at risk for developing skeletal related events (SREs) and cancer induced bone pain (CIBP), yet we showed that there is sparce literature available on non-radiation based early pain relief options (1). Unfortunately, loading doses of ibandronate do not lead to significant bone pain reduction in patients with NSCLC and uncontrolled bone pain as we demonstrated in the NVALT9 trial (2). Specifically for patients with *EGFR+* NSCLC and bone metastases, we investigated the biological mechanism behind the previously observed higher incidence of bone metastases in this patient subgroup. One of the most important signaling pathways in bone metabolism is the Receptor activator of Nuclear Factor κB (RANK)/ RANK ligand (RANKL)/ osteoprotegerin (OPG, the decoy receptor and antagonist of RANKL) pathway (3). The binding of RANKL to its receptor RANK on osteoclast precursors leads to the stimulation of osteoclastogenesis. On the other hand, OPG is capable of blocking the binding of RANKL to RANK, resulting in the inhibition of osteoclastogenesis. Osteoclast formation and stimulating are also promoted by *EGFR* signaling. This stimulation is the result of the inhibition of OPG expression, along with an increase in monocyte chemoattractant protein 1 (MCP1), which induces osteoclast fusion and activity, as well as an increase in macrophage colony-stimulating factor (M-CSF) and RANKL expression (4). We found that tumors of patients with bone metastases exhibit significantly higher RANKL gene expression and a higher RANKL:OPG ratio compared to tumor samples of patients without bone metastases. Contrary to what we expected, not the *EGFR+* NSCLC tumors but the NSCLC tumors with an activating mutation in Kirsten rat sarcoma viral oncogene (*KRAS+*) demonstrated the highest RANKL gene expression, and once again, the highest levels were found in tumors of patients with bone metastases. Furthermore, we showed that an increased RANKL:OPG ratio is associated with a higher risk to develop bone metastases. No association was found between tumor *EGFR* gene expression and presence of bone metastases (5). Additionally, we explored whether we could identify and quantify extracellular vesicles (EVs) and measure RANKL and OPG values in deep-frozen plasma of patients with metastatic *EGFR+* NSCLC, as it is known that EVs play a role in metastasis formation, but there is a lack of data specifically for NSCLC bone metastases. We found that the total concentration of EVs, $RANKL^+$ EVs, or plasma values of RANKL and OPG are not associated with the presence of bone metastases. However,

the total concentration of EVs and concentration of $RANKL^+$ EVs decreased significantly during osimertinib treatment.

Although we could not elucidate the biological mechanism, we confirmed that the incidence of bone metastases is high in *EGFR+* metastatic NSCLC. Up to 42% of the patients with *EGFR+* NSCLC enrolled in clinical trials evaluating EGFR tyrosine kinase inhibitors have documented bone metastases at time of NSCLC diagnosis (6). This percentage is even higher (51%) at initiation of osimertinib in a daily clinical practice patient population (7). Despite a substantial number of patients having or developing bone metastases, we demonstrated that there is limited attention paid to bone-related outcomes or SREs in clinical trials (6). Even in patients treated with osimertinib, which is currently the preferred first-line therapy, the incidence of SREs remains high. We found that approximately 40% of patients with bone metastasized *EGFR+* NSCLC treated with osimertinib experienced one of more SREs (7).

Scientific impact

NSCLC, along with prostate cancer and breast cancer, ranks among the most common cancers worldwide (8). Despite different pathologies, these cancers share several similarities; they exhibit comparable dissemination patterns, and the prognosis upon metastatic disease diagnosis, particularly in *EGFR+* NSCLC, is relatively favorable, akin to the prognosis of most patients with prostate cancer and breast cancer. Nevertheless, the results of this thesis showed that the approach to bone metastases in NSCLC in general, as well as in *EGFR+* NSCLC, differs from that in breast cancer or prostate cancer. Patients with bone metastasized NSCLC are less likely to be treated with bone targeted agents (BTAs), and BTAs may not lead to the same pain relief in NSCLC, as they do in bone metastasized breast and prostate cancer. These findings open up opportunities for research to enhance the daily care of patients with bone metastasized NSCLC. Open research questions include: are there subgroups of patients (e.g., patients with *KRAS+* NSCLC) that would benefit the most from BTAs? Or would a pain education program in combination with palliative radiotherapy lead to improved early pain relief in patients with CIBP from bone metastases compared to radiotherapy alone? Ideally, patients should be included in randomized clinical trials (RCTs) to validate the efficacy of new treatments or education programs. Historically, even less than five percent of eligible patients are enrolled in clinical trials and combined with the relatively small patient population of *EGFR+* NSCLC, this poses an increased risk of slow accrual (9). Therefore, the Trial with Cohorts (TwiCs) design, as suggested in chapter 8, could be more suitable to set up studies (10).

To improve the research focused on NSCLC bone metastases, we have established a collaboration with the laboratory for Calcium and Bone metabolism of the Erasmus Medical Center to further investigate the results of the gene expression study on tumor specimens of NSCLC (chapter 4) (5). This collaboration allows us to complement each other's expertise, as the laboratory is experienced in in-depth analysis of (patho)physiology, while we, as HCPs, can

translate the findings into daily practice and patient care. For the future, we are planning new experiments aimed at characterizing the load of EVs in relation to bone metastases.

Relevance for patients with NSCLC, healthcare providers and society

Previous studies have clearly shown that patients with bone metastases are at risk for SREs, which impact patient-reported outcomes by leading to a decline in physical functioning and quality of life (11, 12). Additionally, patients with *EGFR+* NSCLC exhibit a long post-bone metastatic survival (7, 13). The results of this thesis show that there is less attention paid to bone metastases and bone related outcomes in clinical trials (6). Therefore, it is important to enhance the awareness of HCPs in detecting bone metastases and CIBP and to provide optimal treatment. Considering the devastating impact of bone metastases and their complications on QoL and patients' daily care, alleviating patients' complaints becomes even more critical than merely improving survival. Increased focus of HCPs on CIBP and SREs could lead to the utilization of tools such as pain education programs, home monitoring or considering of BTA prescription, resulting in more effective management of patients' complaints. When patients and their caregivers have a better understanding of their disease or situation, they are more likely to adhere to prescribed medications and follow advice from their HCPs. In turn, this could prevent a deterioration in quality of life and daily functioning (including also paid and unpaid work - volunteering).

The results of this thesis are relevant to HCPs as we have attempted to provide more insight into the pathophysiology of bone metastases in patients with *EGFR+* NSCLC, aiming to optimize treatment based on individual patient characteristics, such as prescription of BTAs in patients with an increased level of RANKL gene expression. However, this hypothesis needs to be validated in larger and prospective patient cohorts. Our next research plans are focused on exploring the role of EVs, a yet-to-be-fully understood area, and their relation to bone metastases. We hope that the findings from this research will provide new insights and possibilities to enhance individual patient care.

The incidence of lung cancer in the Netherlands is still rising. It is projected that the number of new lung cancer cases per year will increase to 16,671 by 2032, compared to 14,337 new diagnoses that were registered in 2019. Approximately 7% of all patients with advanced non-squamous NSCLC have an activating *EGFR* mutation in the Netherlands (14). Osimertinib is currently the preferred treatment option for first-line therapy, but it comes at a high cost, with an incremental cost-effectiveness ratio of €324,006 per quality-adjusted life year (QALY) compared to gefitinib, erlotinib, and afatinib (15). Due to the increasing number of patients with lung cancer, the emergence of new targeted treatment options, and improved overall survival, it is crucial to allocate healthcare costs efficiently to ensure the affordability of healthcare services. As preventing SREs would be feasible, it could lead to longer employment participation for patients with NSCLC who were part of the labor force at diagnosis, or to improved social well-being for retired patients. The results of this thesis underscore the importance of personalized medicine

for individual patients and the combination of different modalities, such as the integration of radiotherapy with pain education, to achieve the best possible outcomes.

Dissemination of knowledge

In order to disseminate our results to other researchers, we published the findings of our studies in international peer-reviewed journals. The results were also presented through poster presentations at the Dutch "Week van de longen" in 2019 and at the European Lung Cancer Congress in 2022 and 2023. As mentioned earlier, we have established a collaboration with the Calcium and Bone laboratory of the Erasmus Medical Center to discuss the results of our research and explore new research opportunities. Our goal is to gain more information about bone metabolism and the behavior of osteoclasts and osteoblasts, along with their contributing factors in bone metastases of lung cancer.

In addition to this PhD trajectory, I am working as a pulmonologist with a special focus on patients with *EGFR+* NSCLC and palliative care at Zuyderland Medical Center. The insights I have gained during this research trajectory allow me to apply them daily in the care of patients with lung cancer, aiming to alleviate the complaints caused by their malignancy and enhance their quality of life as much as possible. Additionally, I am involved in teaching our residents about *EGFR+* NSCLC, the risk of bone metastases and how to accurately manage CIBP. I am planning to extend this lectures to our colleagues in the ONCOZON, an oncology network comprising nine hospitals and one radiotherapy institute in the South-East of the Netherlands.

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CURRICULUM VITAE

Anita Brouns was born on December 6th 1986 in Roermond, the Netherlands. In 2004 she finished her secondary education at the Gymnasium of Sint Ursula Scholengemeenschap, Horn. After completing her secondary school, she obtained a propaedeutic degree in molecular life sciences at the Maastricht University. As of 2006, she started her medical studies at the Faculty of Health, Medicine and Life Sciences at Maastricht University and she graduated in 2012. Subsequently, Anita worked as a resident at the pulmonary department of the Atrium Medical Center (now Zuyderland Medical Center) in Heerlen, the Netherlands. On December 31st, 2012 she started her training as a pulmonologist with a special interest inthoracic oncology and finished this training in 2018. During the latter part of her residency, she started a PhD trajectory on bone metastases and bone related outcomes in patients with non-small cell lung cancer (NSCLC) with a focus on patients with epidermal growth factor mutated NSCLC (promotor: A-M. C. Dingemans, co-promotors L.E.L. Hendriks and G.P. Bootsma). In 2018 Anita received a grant for her research from Five4Five Foundation, Cancer Research Fund Limburg. She presented abstracts on national and international (oncology) conferences. From 2018, Anita works as a pulmonologist with a fprimary focus on diagnosing and treating patients suffering from lung cancer, in Zuyderland Medical Center.

LIST OF PUBLICATIONS

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