The role of bone targeted agents in early breast cancer

A thesis submitted for the degree of

Doctor of Medicine

By

Dr Elisavet Theodoulou

Ptychio Iatrikes, MRCP

Supervised by

Prof. Ingunn Holen, Prof. Janet Brown and Prof. Lynda Wyld

The University of Sheffield

Faculty of Medicine, Dentistry and Health

Department of Oncology and Metabolism

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Summary

Breast cancer is the most common cancer among women with almost 56,000 new cases every year in the UK. Most of the new cases are diagnosed as early breast cancer (EBC) which is potentially curable. However, metastatic disease is the main cause of death in advanced breast cancer. Bone has been identified as the most common site of metastasis in breast cancer and is present in 70% of the patients with metastatic disease.

The bone targeted agents bisphosphonates (BPs) have been extensively investigated for their role in early and metastatic disease. Currently, they have been approved for the following settings:

1) Prevent bone metastasis.
2) Prevention and treatment of cancer treatment-induced bone loss (CTIBL).
3) Management of established bone metastasis.

The main aim of this MD thesis is to describe the role of BPs in EBC and give a better understanding of their use within the UK. Multiple studies have shown that if BPs are given after breast cancer surgery offer anticancer benefits. Nevertheless, these are only in postmenopausal women (natural or induced). The ZOLMENO study, a single centre proof of concept study, was set up with the aim to provide further data for the mechanism responsible for these differential effects of adjuvant BPs. Additionally, the use of these agents in EBC has only recently been approved in the UK, providing the opportunity to track their journey within the UK breast cancer practice. Two surveys (breast cancer oncologists and oncology pharmacists) were conducted with plan to describe the use of adjuvant BPs in the UK. An international collaboration was formed between our team and Australian colleagues where the UK physicians' adjuvant BPs survey was compared to the Australian physicians' survey, with the aim to support the adoption of these agents in Australia but also pave the way for other nations to follow the UK example. Also, with the support of Breast Cancer Now (BCN), a UK breast cancer charity, a patients' survey was performed to gather information about the experience of patients receiving adjuvant BPs.

Early breast cancer therapies are known for their effects on bone health. In particular, antioestrogen treatment with aromatase inhibitors (AIs) have been shown to lead to bone loss and increase the risk of fractures. Subsequently, fragility fractures increase mortality and significantly affect quality of life. Therefore, prevention of CTIBL is crucial, to minimise the risk of fractures among breast cancer patients. This is highly important for older (>70 years) EBC patients where bone density is already negatively affected by age and menopause. With the aim to determine the management of bone health of older women receiving AIs for EBC in the

UK, bone health data were collected from the participants of the large multicentre Bridging the Age Gap (BTAG) study .

Dedication

To Mikis, for protecting us from up there.

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Declaration

I hereby declare that the work presented in this thesis is my own original research carried out under the guidance of my supervisors Prof. Ingunn Holen, Prof. Janet Brown and Prof. Lynda Wyld. All of the work described was carried out by myself except for the ZOLMENO study translational studies which were performed with the assistance of Alyson Evans (University of Sheffield). Decalcified bone trephine samples were embedded in paraffin wax and then sectioned and stained independently by Alyson Evans. MicroCT of bone trephine samples and analysis of bone structures (SkyScan) were performed independently by Holly Evans, Senior Research Technician at the University of Sheffield. The Australian physicians' survey was developed by Dr Sally Baron-Hay and Dr Isobel Porter of the Royal North Shore Hospital in Sydney, Australia. Frailty scores for the Bridging the Age Gap sub-study were provided by Olivia Turner of the Healthy Lifespan Institute of the University of Sheffield.

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Publications

Publications arising from MD studies include:

1. George CN, Canuas-Landero V, Theodoulou E, Muthana M, Wilson C, Ottewell P. Oestrogen and zoledronic acid driven changes to the bone and immune environments: Potential mechanisms underlying the differential anti-tumour effects of zoledronic acid in pre- and post-menopausal conditions. J Bone Oncol. 2020 Sep 15;25:100317. doi: 10.1016/j.jbo.2020.100317. PMID: 32995253; PMCID: PMC7516134.

All the publications and presentations related to the work of each chapter have been added at the end of each chapter of this thesis.

List of Abbreviations

ADCs	Antibody- drug conjugates
AEs	Adverse events
AFF	Atypical femoral fracture
AIs	Aromatase inhibitors
AJCC	American Joint Committee on Cancer
APR	Annual Progress Report
aPTT	Activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
ATAC	Arimidex, Tamoxifen, Alone or in Combination
ATLAS	Adjuvant Tamoxifen: Longer Against Shorter
ATP	Adenosine triphosphate
aTTom	Adjuvant Tamoxifen Treatment offers more
BALP	Bone specific alkaline phosphatase
BCN	Breast Cancer Now
BIG	Breast International Group
BMA	Bone modifying agent
BMD	Bone mineral density
BMI	Body mass index
BOPA	British Oncology Pharmacy Association
BPs	Bisphosphonates
BRCA	Breast cancer gene
BTAG	Bridging the Age Gap
BTAs	Bone targeted agents
BV	Bone volume
CCO	Cancer Care Ontario
CCTC	Cancer Clinical Trials Centre
CDK	Cyclin-dependent kinase
CPS	Clinical and pathological stage
CQC	Care Quality Commission
CRF	Case Report Form
CRG	Clinical Reference Group
CT	Computer tomography
CTIBL	Cancer treatment-induced bone loss
CTX	C-terminal telopeptide
DEXA	Dual energy x-ray absorptiometry
DFS	Disease free survival
DMSO	Dimethyl sulfoxide
DSUR	Developmental Safety Update Report

DTCs	Disseminated tumour cells
EBC	Early breast cancer
EBCTCG	Early Breast Cancer Trial Collaborative Group
ECACC	European Collection of Authenticated Cell Cultures
ECOG	Easter Cooperative Oncology Group
EDTA	Ethylenediaminetetraacetic acid
EG	Oestrogen receptor status and histological grade
ER	Oestrogen receptor
ER+ve	Oestrogen receptor positive
ERG	Expert Reference Group
ER-ve	Oestrogen receptor negative
ESMO	European Society of Medical Oncology
ET	Endocrine treatment
FBC	Full blood count
FBS	Foetal bovine serum
FCS	Foetal calf serum
FPPS	Farnesyl pyrophosphate synthase
FSH	Follicle-stimulating hormone
FST	Follistatin
GAIN	German Adjuvant Intergroup Node-Positive
GFR	Glomerular filtration rate
GFs	Growth factors
GnRH	Gonadotrophin releasing hormone
GPs	General practitioners
H&E	Haematoxylin and eosin stain
HER2	Human epidermal growth factor receptor 2
HRA	Health Research Authority
HRT	Hormone replacement therapy
HSC	Haemopoietic stem cell
IDFS	Invasive disease free survival
IES	Intergroup Exemestane Study
IGFs	Insulin-like growth factors
IHC	Immunohistochemistry
IL-1β	Interleukin-1β
INR	International normalised ratio
ISH	In situ hybridization
IV	Intravenous
LH	Luteinising hormone
M	Mean
MDP	Methylene diphosphonate

MDT	Multidisciplinary team
MHRA	Medicines and Healthcare Products Regulatory Agency
MicroCT	Micro computed tomography
MRI	Magnetic resonance imaging
MSCC	Malignant spinal cord compression
NaOH	Sodium hydroxide
NCCN	National Comprehensive Cancer Network
NF-kB	Nuclear Factor Kappa-B
NGS	Next generation sequencing
NHS	National Health Service
NICE	National Institute of Health and Care Excellence
NK cell	Natural killer cell
NPI	Nottingham Prognostic Index
NTX	N-terminal telopeptide
OD	Optical density
ONJ	Osteonecrosis of the jaw
OPG	Osteoprotogerin
OPSAT-Q	Osteoporosis Patient Treatment Satisfaction Questionnaire
OS	Overall survival
OVX	Ovariectomised
P1CP	C-terminal propeptide of type 1 procollagen
P1NP	N-terminal propeptide of type 1 procollagen
PARP	Poly(ADP-ribose)polymerase
PBS	Phosphate-buffered saline
PBS	Pharmaceutical Benefits Scheme
PDGF	Platelet derived growth factor
PET	Primary endocrine treatment/therapy
PFA	Paraformaldehyde
PI	Principal investigator
PIS	Patient information sheet
PPI	Patient and public involvement
PR	Progesterone receptor
PT	Prothrombin time
r	Pearson correlation coefficient
R2	Coefficient of determination
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
REC	Research Ethics Committee
RPMI	Roswell Park Memorial Institute
RR	Recurrence rate

rφ	Phi coefficient
SD	Standard deviation
SDV report	Site Data Verification report
SE	Standard error
SERM	Selective oestrogen receptor modulator
SIOG	International Society of Geriatric Oncology
Spearman's rho	Spearman correlation coefficient
SPECT	Single photon emission computed tomography
SPSS	Statistical Package for the Social Sciences
SREs	Skeletal related events
STH	Sheffield Teaching Hospitals
TAM	Tumour associated macrophage
Tc-99m	Technetium-99
TGA	Therapeutic Goods Administration
TGF-β	Transforming growth factor-beta
TMG	Trial Management Group
TNBC	Triple negative breast cancer
TNM	Tumour, node, metastasis
TRAP	Tartrate-resistant acid phosphatase
Tregs	Regulatory T cells
UKBCG	UK Breast Cancer Group
UoS	University of Sheffield
US	Ultrasound
VEGF	Vascular endothelial growth factor
v/v	Volume per volume
WHO	World Health Organisation
WPH	Weston Park Hospital
w/v	Weight in volume
YCR	Yorkshire Cancer Research
ZOL	Zoledronic Acid

Chapter 1

Introduction

1. Introduction

1.1 General background of breast cancer

Breast cancer is the most common cancer in the UK, with around 56,000 new cases diagnosed every year (1). Over the last decade, breast cancer incidence has increased by around 4%, with 82% of the cases to be in women over the age of 50 (1). Although breast cancer survival is improving and has doubled over the last 40 years, there are around 11,400 breast cancer deaths every year making it the 4th most common cause of cancer death (1). Despite the UK 5-year breast cancer survival being below the European average, data from 2010-11 showed that about two-thirds of women with breast cancer survive their disease for more than twenty years (1).

New breast cancer cases are diagnosed mainly through breast cancer screening or after self-detection by the patient. In the UK, breast cancer screening programme calls all women aged 50 to 70 for a mammogram every 3 years. Tumours detected by screening tend to have more favourable characteristics and better prognosis compared to the ones that are self-detected (2, 3). All primary tumours should be assessed with triple assessment, physical examination, mammography and breast/axillary ultrasound (US). In some cases, such as familiar breast cancer with BRCA (breast cancer gene) mutation or dense breasts, breast MRI (magnetic resonance imaging) scan is advisable. Biopsy of the suspicious tumour is performed to determine its biology [histological type, grade, expression of ER (oestrogen receptor), PR (progesterone receptor) and HER2 (human epidermal growth factor 2) and Ki67 (proliferation marker)] and guide further management.

Almost all breast cancers are adenocarcinomas with invasive ductal carcinoma and lobular carcinoma (15%) the 2 most common types. There are 4 main molecular subtypes of breast cancer (luminal A, luminal B, HER2 positive, basal like), which assist with prognosis and treatment decision making (Table 1.1) (4). Overall, luminal A cancers have the best prognosis and with luminal B cancers require antioestrogen treatment as they are both oestrogen receptor positive (ER+ve). Oestrogen receptor positive cancers are diagnosed in 75% of postmenopausal women and in less than half of premenopausal women. HER2 is expressed in 15-25% of breast cancers and HER2+ve tumours have worse prognosis than luminal and require antiHER2 therapy. HER2 positive tumours are those with HER2 protein overexpression on immunohistochemistry (IHC 3+ score) or ERBB2 gene amplification on in situ hybridization (ISH) (5). Despite being classed as HER2 negative, these tumours still have detectable HER2 protein, with HER2 IHC 1+ or 2+ with ISH not amplified to be defined as HER2 low (6).

Triple negative breast cancers (TNBC) constitute 20% of all breast cancers. They are most common in younger women (<40 years) and among all subtypes they are the most aggressive with the worst prognosis.

Subtype	Luminal A	Luminal B	HER2	'Basal-like'
Definition	**'Luminal A-like'** • E-positive • HER2-negative • Ki67 low [a] • PgR high [b] • Low-risk molecular signature (if available)	**'Luminal B-like (HER negative)'** • ER-positive • HER2-negative • and either Ki67 high, or • PgR low • High-risk molecular signature (if available) **'Luminal B-like (HER positive)'** • ER-positive • HER2-positive • Any Ki67 • Any PgR	**'HER2-positive (non-luminal)'** • HER2-positive • ER and PgR absent	**'Triple-negative'** [c] • ER and PgR absent [c] • HER2-negative

a Ki-67 scores should be interpreted in light of local laboratory values.
b Suggested cut-off value is 20%.
c There is 80% overlap between 'triple-negative' and intrinsic 'basal' subtype.

Table 1.1: *Definition and characteristics of the main breast cancer subtypes (4).*

Familial breast cancer accounts for about 10% of breast cancers (7, 8). The most common inherited mutations are in BRCA1 and BRCA2 genes, responsible for 4-6% of all new cases and for the 20-50% of the familial cases (9, 10). BRCA1 and BRCA2 mutations appear in 1 in 400 to 1 in 800 individuals in the general population (11, 12). Genetic counselling and testing are recommended for women with breast cancer diagnosis if they have a strong family history of breast or ovarian cancer, or they have been diagnosed with TNBC before the age of 50 (13).

Staging of disease is currently performed according to the American Joint Committee on Cancer (AJCC) tumour, node, metastasis (TNM) staging system (8^{th} edition) (14). Stages start from 0 which is the carcinoma *in situ,* followed by stages I to III the invasive breast cancer and Stage IV the metastatic disease when the cancer has spread outside of the breast and the axillary lymph nodes. The 4 stages of the invasive disease (I - IV) are based on the tumour size (T), the axillary lymph nodes involvement (N) and distal metastasis (M). The AJCC TNM staging system is comprehensive with some of the stages further divided into A, B or C stages. This is not discussed in detail here as it is outside of the scope of this thesis. A simple version of the current disease staging system is described in figure 1.1.

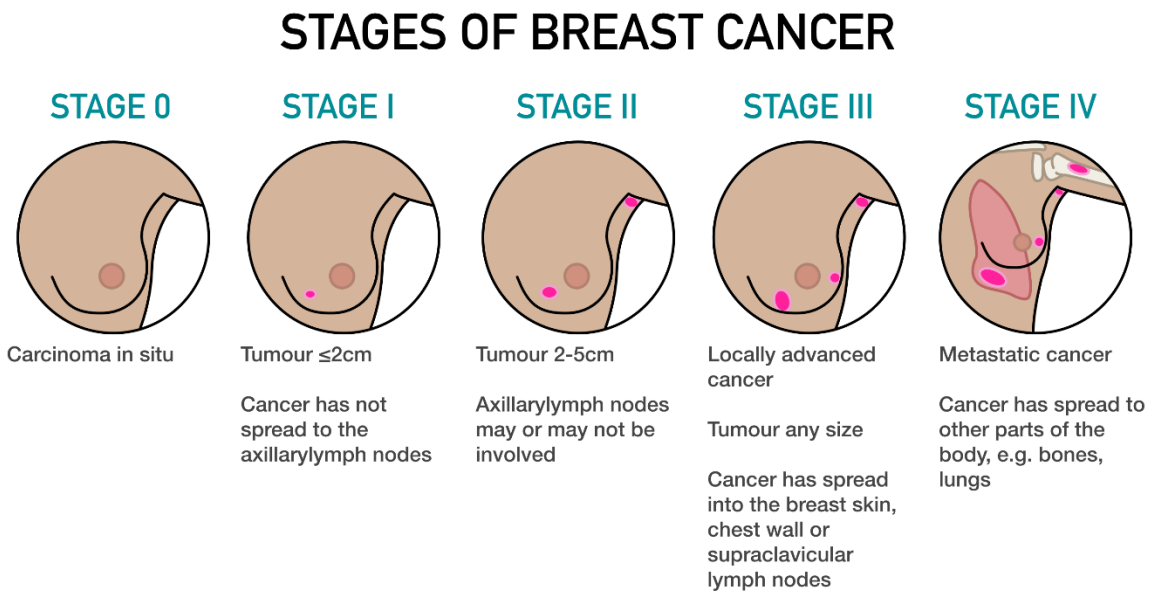

Figure 1.1: *Breast cancer staging.*

In combination with the staging system, the Nottingham Prognostic Index (NPI) is widely used in breast cancer MDT (multidisciplinary team) meetings to determine prognosis following surgery (15, 16). The NPI calculation is based on the tumour size, grade and the number of axillary lymph nodes involved. There are 4 different categories according to the NPI score, category I – excellent ≤2.4, category II – good >2.4, category III – moderate >3.4 and category IV – poor >5.4. Five year survival based on the NPI score ranges between 97% for category I patients and 88% for category IV (17).

The main part of early disease management is breast cancer surgery, combined with neoadjuvant (before surgery) and/or adjuvant (after surgery) treatments to improve mortality and reduce the risk of recurrence. Treatment options for primary disease include chemotherapy, radiotherapy, endocrine therapy (aromatase inhibitors or Tamoxifen), targeted molecular therapies [HER2, poly(ADP-ribose) polymerase (PARP), cyclin-dependent kinases (CDK) 4/6 and immune inhibitors] and bisphosphonates (BPs), and they are recommended based on tumour biology and the individual's risk of recurrence. Breast cancer oncologists are advised to use the prognostic tool PREDICT, which provides information about the benefit that patients could receive with each type of adjuvant treatment (18, 19).

Recently, new therapies have been introduced in the neo-adjuvant/adjuvant setting and have received approval for their use in the UK (20-22). These include CDK4/6, PARP and immune inhibitors. More specifically, 5637 patients with high risk ER+ve, HER-ve early disease were randomised to standard endocrine therapy +/- abemaciclib (CDK4/6 inhibitor, oral 150mg twice daily) for 2 years. The patients that were considered high risk were those who had ≥4 positive nodes, or 1-3 positive nodes and either ≥5cm tumour, or grade 3 disease, or Ki-

67≥20%. Participants had already completed neoadjuvant/adjuvant treatment (surgery, radiotherapy, chemotherapy). The results demonstrated that the addition of abemaciclib to standard endocrine therapy improved invasive disease free survival (IDFS) compared to placebo (92.2% versus 88.7% respectively) (23). Abemaciclib with endocrine therapy is currently recommended by National Institute of Health and Care Excellence (NICE) in the adjuvant setting for patients with high risk ER+ve, HER2-ve, node positive EBC (high risk: ≥4 positive axillary lymph nodes or 1-3 positive axillary lymph nodes and grade 3 disease or 1-3 positive axillary lymph nodes and tumour size ≥5cm) (20).

For the aggressive TNBC disease, treatment options have been so far limited to chemotherapy, due to the lack of expression of targetable receptors. However, a recent large multicentre phase 3 study (n=1174) has presented promising results for these patients (24). Participants were randomised into 2 groups, the chemotherapy and immunotherapy or chemotherapy only group, with the first group to receive neo-adjuvant 3 weekly pembrolizumab (200mg, 8 cycles) with carboplatin/paclitaxel (4 cycles) followed by doxorubicin or epirubicin and cyclophosphamide (4 cycles), and the second group to receive 3 weekly carboplatin/paclitaxel (4 cycles) followed by doxorubicin or epirubicin and cyclophosphamide (4 cycles). Both groups underwent breast cancer surgery followed by adjuvant treatment which was 3 weekly pembrolizumab (9 cycles) for the first and placebo for the second group. Analysis showed that more patients in the immunotherapy/chemotherapy arm (64.8%) had pathological complete response compared to chemotherapy only arm (51.2%), and the difference in event-free survival rate was 6%, favouring the immunotherapy plus chemotherapy arm (immunotherapy/chemotherapy 91.3% vs placebo/chemotherapy 85.3%). Additionally, final overall survival (OS) results showed that OS at 60 months was 86.6% in the pembrolizumab/chemotherapy group compared to 81.7% in the placebo/chemotherapy group (25). Therefore, pembrolizumab has been established as the new neo-adjuvant and adjuvant treatment option for TNBC (22).

In terms of the PARP inhibitors, these were assessed for their role in adjuvant treatment in BRCA positive patients with positive results (26). Participants with BRCA (BRCA 1 and BRCA 2) mutated and HER2-ve early disease were randomised to olaparib (oral 300mg twice daily) or placebo for 1 year, following standard neo-adjuvant/adjuvant therapy. After 3 years, DFS (invasive and distant) was found to be improved in the olaparib group compared to control (IDFS 85.9% and 77.1%, distant DFS 87.5% and 80.4%, respectively). Additionally, with 3,5 years of median follow up, OS was significantly improved in the Olaparib group compared to placebo, with OS at 4 years of 89.8% and 86.4% respectively (27). NICE recommends Olaparib in BRCA positive patients after neo-adjuvant or adjuvant chemotherapy, as a monotherapy in TNBC or in combination with endocrine therapy in ER+ve HER2-ve high risk of recurrence disease (21).

In HER2+ve early disease, trastuzumab, a monoclonal antibody, has traditionally been the adjuvant treatment choice (28-30). More recently, the addition of pertuzumab to trastuzumab and the use of conjugate trastuzumab emtansine in patients with residual disease after neoadjuvant therapy, demonstrated improvement in disease free survival (31-33). The ExteNET trial randomised 2840 patients with HER2+ve EBC to oral neratinib (irreversible pan-HER tyrosine kinase inhibitor) 240mg daily or placebo, after neoadjuvant/adjuvant trastuzumab-based treatment (34). Results showed the absolute 5 year invasive DFS benefit was 5.1% and the 8 year OS benefit was 2.1% in the neratinib group compared to placebo (35, 36). Benefit from addition of neratinib was even higher in the group of patients who did not have pathological complete response after neoadjuvant therapy. In this population 5 year invasive DFS benefit, and 8 year OS were 7.4% and 9.1% respectively.

Multiple studies failed to demonstrate any clinical benefit of antiHER2 therapy in patients with low expression of HER2 (37, 38). However, the recent development of potent anti-HER2 antibody- drug conjugates (ADCs) has allowed the effective targeting of breast cancer with low HER2 expression, with the clinical relevance of HER2 low expression to be established with the publication of the DESTINY-Breast04 trial (39, 40). The phase 3 study randomised 557 patients with HER2 low metastatic breast cancer who had received one or two previous lines of chemotherapy (HER2 low: IHC score of 1+ or 2+ and negative ISH results) to receive trastuzumab deruxtecan or the physician's choice of chemotherapy. Analysis showed OS of 23.4 months in trastuzumab deruxtecan group, compared to 16.8 months in physician's choice group. Currently, trastuzumab deruxtecan is only NICE approved for use as second line in metastatic HER2+ve disease (41, 42). Nevertheless, the relevance of the low expression of HER2 in early breast cancer disease is still unclear and more evidence is required to clarify the role of anti-HER2 therapy in this population.

In metastatic breast cancer the disease has spread outside of the breast and the axilla, and the aim of the treatment changes as the cancer is no longer considered curable. The management of metastatic disease mainly includes chemotherapy, radiotherapy, targeted therapies [e.g. anti-HER2, antioestrogen therapy, immunotherapy, PARP inhibitors, CDK4/6 inhibitors and bone targeted treatments (BPs or denosumab)] and focuses on palliation of symptoms, disease control and prolonging survival.

1.2 General background of bone targeted agents

Bisphosphonates are chemically stable pyrophosphate analogues that are characterised by a PCP (phosphorus–carbon–phosphorus) bond that gives them their high affinity to bone. They were initially synthesised in 1800s, but their first clinical use was only reported in 1969 (43). Bisphosphonates inhibit osteoclast-mediated bone resorption after they enter mature osteoclasts at the bone surface, especially in areas with high activity of bone

resorption. They are classified into two groups, depending on their site chains, known as R1 and R2 chains (Figure 1.2). The non-nitrogen containing BPs with no nitrogen site chain, such as clodronate and etidronate, are the most similar to pyrophosphate and are converted intracellularly into non-hydrolysable ATP (adenosine triphosphate) analogues that inhibit ATP-dependant enzymes, leading to osteoclasts apoptosis. In contrast, the more potent nitrogen containing BPs with nitrogen site chains, such as alendronic acid, zoledronic acid (ZOL) and Ibandronic acid, inhibit enzymes of the mevalonate pathway which steers the cholesterol and other sterols production (Figure 1.2). The main target of nitrogen BPs is farnesyl pyrophosphate synthase (FPPS) which is important for the prenylation of small GTPases (e.g. Ras, Rab, Rho) required for the function and survival of osteoclasts.

Bisphosphonates have very long-lasting effects, supported by the fact that they rapidly bind to bone with high affinity and can be traced in serum and urine of patients many months after their administration. There are also differences in action between BP agents, with alendronate and ZOL shown to have better and more prolonged effects compared to etidronate and risedronate (43). This is explained by the differences in potency and their ability to bind to hydroxyapatite. Additionally, it has been shown that the effects of BPs are strongly dose-dependent, with some BPs to be given in small and frequent doses (e.g. oral daily ibandronate) and others to be given once or twice a year in much higher doses (e.g. ZOL) (44, 45).

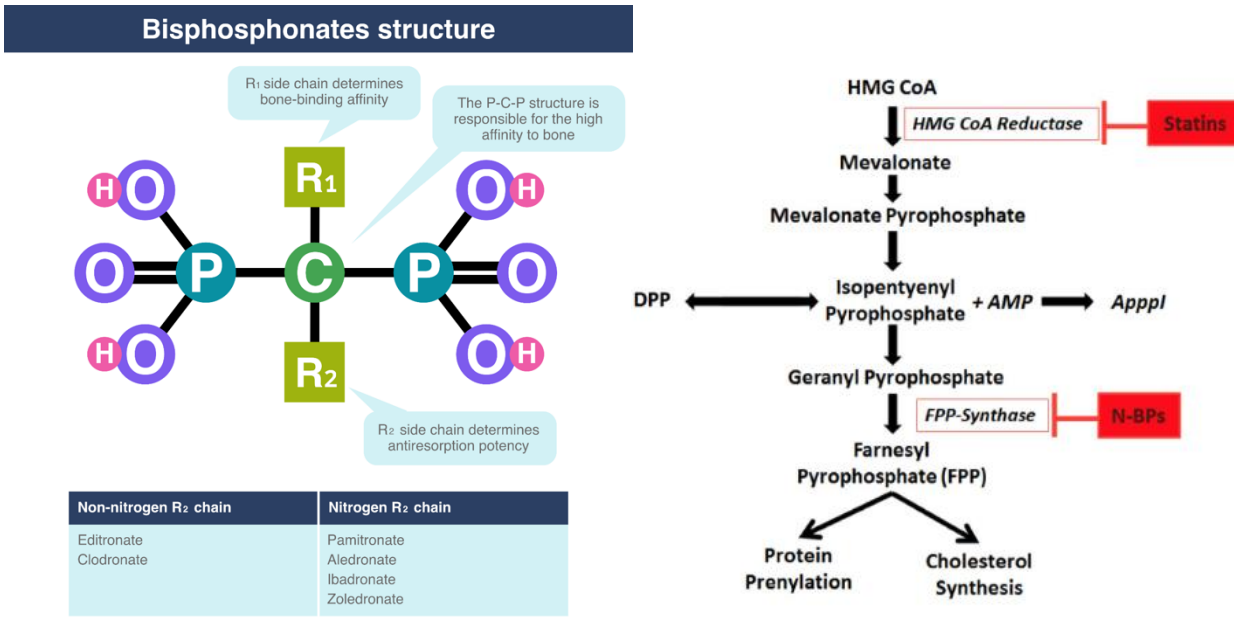

Figure 1.2: *Structure of Bisphosphonates and molecular pathway of nitrogen bisphosphonates.*

In clinical practice, BPs were first used to inhibit calcification and as agents in bone spinthrorography. Their use in bone scanning is still applicable today where they are linked to an isotope (Technetium-99m) to help identify bone lesions and metastasis. Additionally, BPs are the main treatment agents for Paget's disease and since the 1990s they have an established role in the prevention and management of osteoporosis (43, 46). Although, their role in osteoporosis is one of their most widely recognised and apparent clinical applications, their use in this field was introduced well after their other clinical practice uses.

In oncology, BPs have extended and well-established roles in the management of bone metastatic disease and malignant hypercalcaemia, irrespective of the primary cancer site (47). Most specifically, in breast cancer, where this thesis focuses, the use of BPs has been extensively researched and approved for 3 indications (47, 48) (Figure 1.3):

1) To prevent skeletal related events (SREs) such as fractures and spinal cord compression and also to relief bone pain and prevent and/or treat hypercalcemia, in established bone metastasis.
2) To prevent and/or treat cancer treatment induced bone loss (CTIBL), mainly due to systemic anticancer treatment and antioestrogen therapy.
3) To prevent metastasis in early breast cancer.

All the above 3 breast cancer indications of BPs will be described in more detail in the following sections of this introduction.

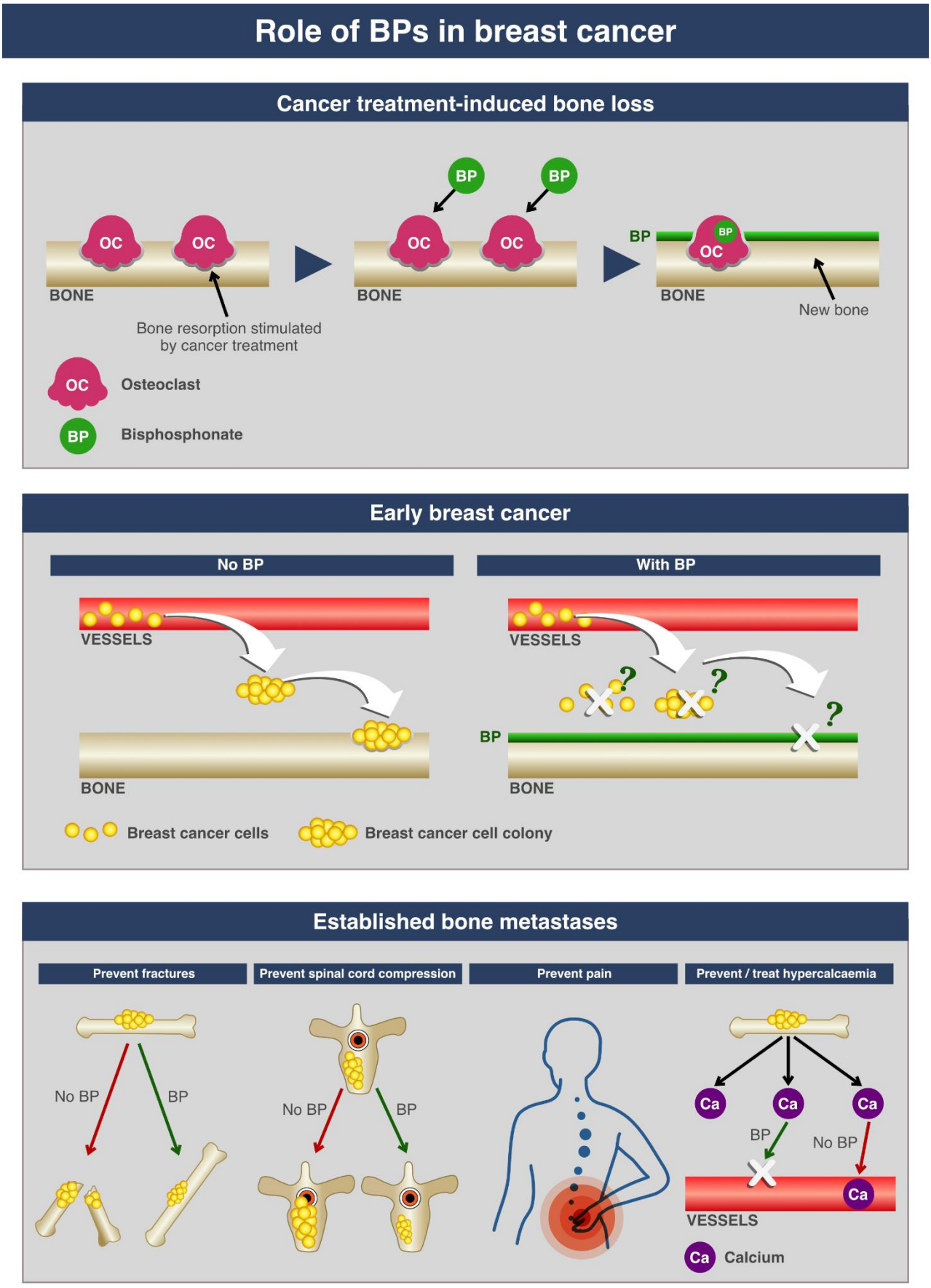

Figure 1.3: *Role of bisphosphonates (BPs) in breast cancer.*

Denosumab, a monoclonal human antibody, is another widely used bone targeted agent in similar clinical situations to BPs. It is a RANKL [receptor activator of Nuclear Factor Kappa-B (NF-κB) ligand] inhibitor, and therefore it blocks the development, activation, and survival of osteoclasts (49). In the case of BPs, they affect osteoclast recruitment, differentiation, and resorptive activity, and cause apoptosis. In comparison to BPs, denosumab has stronger anti-resorptive effects which ceased upon discontinuation of its use. Bisphosphonates continue to act long after they have been stopped as they remain in the bones. Patients receiving denosumab are advised to switch to a short course or even a single dose of BPs after the discontinuation of denosumab to avoid worsening of symptoms (e.g. bone loss), the so called rebound phenomenon (50).

The use of denosumab in breast cancer is similar to BPs and it is an approved agent for the treatment of established bone metastasis and the prevention of fractures in CTIBL (51). Denosumab has been found to be superior in preventing SREs in known metastatic breast cancer and also in preventing fractures in postmenopausal EBC treated with aromatase inhibitors (AIs) (52-54). However, denosumab has failed to show any meaningful anti-metastatic activity, suggesting that its strong osteoclastic inhibition and the resulting bone microenvironment changes, could potentially compromise its effectiveness in reducing breast cancer relapse. Therefore, denosumab is not recommended in the EBC adjuvant setting. In terms of side effects, BPs and denosumab are fairly well tolerated treatments, regardless of their indication or form of administration (54-57). Nitrogen containing BPs such as ZOL, are known to cause acute phase reaction with patients reporting flu-like symptoms and generalised aches and pains which resolved within a few days following administration (56, 58). Additionally, rare cases of renal impairment secondary to ZOL have been reported, especially in patients with pre-existing renal issues. A retrospective cohort study (n=221) in bone metastatic breast cancer reported that in almost 60% of the patients who had renal failure due to BPs, baseline renal function recovered on cessation of therapy (56). Oral BPs, such as ibandronic acid and alendronic acid, are known to cause gastrointestinal toxicity and this is perhaps one of the main reasons why patients stop the oral agents and switch to intravenous alternatives (55). Hypocalcemia (defined as adjusted serum calcium <2.1mmol/L) is another adverse event (AE) reported for both BP and denosumab (54). However, hypocalcemia is preventable by close monitoring of patients' calcium levels. In current clinical practice, patients receiving bone agents are prescribed prophylactic calcium supplements to prevent future hypocalcemia.

Serious but rare AEs that have been described in patients receiving BPs and denosumab are osteonecrosis of the jaw (ONJ) and atypical femoral fractures (AFFs) (54, 59). The AZURE trial which randomised 3360 women with EBC to receive standard adjuvant chemotherapy with or without ZOL 4mg for 19 doses over 5 years, reported incidence of ONJ

in the ZOL group only 2.1% (60). Similarly, a study compared denosumab (n=1020, subcutaneous 120mg/4weekly) and ZOL (n=1013, intravenous 4mg/4 weekly) in metastatic breast cancer (confirmed bone metastasis), showed an incidence of ONJ 2% and 1.3% respectively, after 3 years of treatment (54). Atypical femoral fractures were found in the general population, women and men receiving BPs, to be approximately 3 to 5 per 1000 hip fractures (61, 62). Incidence of AFFs was noted to be higher in long term BP therapies, especially after 5 years (59). Although a concern for patients, the risk of ONJ can be minimised by careful dental examination prior to treatment and the avoidance of invasive dental procedures.

In general, side effects of bone targeted agents tend to resolve on cessation of therapy with benefits for breast cancer patients to outweigh the risk of AEs, especially now that oncologists are well aware of how to minimise the risks.

1.3 Improve breast cancer outcomes and prevent bone metastasis

1.3.1 Anticancer effects of bisphosphonates in breast cancer

Mechanism of action of adjuvant bisphosphonates

The role of BPs in EBC has been extensively investigated in pre-clinical and clinical studies, demonstrating anticancer benefits. However, the mechanisms by which BPs lead to anticancer effects in early disease is still unknown.

Daubine and colleagues in 2007 showed in an *in vivo* model that ZOL and clodronate have the ability to reduce tumour growth in bone (63). Mice were treated with equivalent to clinical doses of ZOL (98-100 μg/kg, daily, weekly or single dose) or clodronate (530 μg/kg, daily) or vehicle, either before or after injection of human breast cancer cells (B02/GFP.2). When BPs were given in treatment protocols (after the injection of tumour cells), ZOL reduced bone tumour burden by 87% (daily) and 90% (weekly) and clodronate (daily) by 53%. When they were given prior to tumour cells (preventive protocols), reduction of bone tumour burden was 83% and 63% with daily and weekly ZOL respectively and 49% with daily clodronate. Single dose of ZOL did not show any benefit in both of the protocols. Similarly, in another animal study, ibandronate (10 μg/kg, daily) was found to reduce skeletal lesions when was given either prior or after the injection of cancer cells (MDA-MB-231) (64).

Ibandronate was also examined in a different murine model where it was given alone or in combination with osteoprotegerin (OPG) (65). Breast cancer cells (MDA-MB-231) were injected in the animals and 10 days later mice received OPG (1mg/kg/day), ibandronate (160μg/kg/day), combination of both or vehicle. Ibandronate and OPG, alone or in combination, prevented progression of the skeletal lesions, reduced cancer cells proliferation and induced their death.

Ottewell et al. treated sham operated (mimics the premenopausal setting) and ovariectomised (OVX) (mimics the postmenopausal setting) mice who had disseminated breast cancer cells (MDA-MB-231) in bone with weekly ZOL (100ug/kg) or saline (66). Zoledronic acid treated OVX animals had no tumour growth suggesting that the disseminated cancer cells remained inactive in postmenopausal conditions. However, this was not observed in the sham operated ZOL treated animals. Further *in vivo* studies from the same group investigated the anti-tumour effects of BPs when combined with conventional chemotherapy (67). Mice with established bone metastases received control, ZOL (100mcg/kg), doxorubicin (2mg/kg), combination of ZOL and doxorubicin or doxorubicin followed by ZOL 24hrs later. All the combinations of BP/chemotherapy led to reduction of skeletal metastases, through reduction of proliferation and increase of apoptosis of tumour cells, but did not affect any extra skeletal tumour sites, suggesting that even the sequential combination offers bone anticancer benefits.

The anti-tumour effects of BPs and chemotherapy combination were also investigated in animal models in the absence of bone metastases (only subcutaneous breast tumours were present) (68). Mice injected with human breast cancer cells (MDA-MB-436) received weekly control, doxorubicin (2mg/kg), ZOL (100mcg/kg), doxorubicin and ZOL or doxorubicin followed by ZOL 24 hrs later or vice versa for 6 weeks. Treatment with single agents or the ZOL followed by doxorubicin combination did not show any significant reduction in the tumour size compared to controls. However, tumour size was smaller by 50% in the doxorubicin and ZOL group when compared to the doxorubicin only treated animals and complete resolution of tumour was observed in the doxorubicin followed by ZOL animals, suggesting the extraosseous anticancer benefits of BPs/chemotherapy combination.

The outcomes of the pre-clinical studies were supported by early phase clinical studies confirming the anti-metastatic effects of BPs. More specifically, a phase 2 study recruited 120 breast cancer patients to receive chemotherapy (neoadjuvant epirubicin and docetaxel x4 and adjuvant epirubicin and docetaxel x2) and to have ZOL (4mg/3weekly, intravenous) or no ZOL for 1 year (69). Bone marrow biopsies were performed at baseline and at 3 months with detectable tumour cells to be present at 3 months in less ZOL treated patients compared to those who did not have ZOL. Additionally, in another clinical study, 172 patients with EBC and known isolated tumour cells in bone marrow, 31 of them received ZOL (4mg/4 weekly, intravenous) for 6 months and 141 received no extra treatment after the completion of breast cancer surgery and adjuvant chemotherapy (70). Results showed that tumour cells were found in only 13% of the ZOL group compared to 27% of the control group.

The synergistic effects of BPs and chemotherapy were clinically demonstrated by a retrospective analysis of 205 patients who were treated with chemotherapy +/-ZOL prior to their breast cancer surgery (71). At completion of treatment the remaining tumour size was

12mm bigger in the chemotherapy only group in comparison to the ZOL group and, more patients reached pathological complete response with ZOL compared to no ZOL patients (6.9% vs 11.7% respectively).

Overall, a review published by Holen and Coleman in 2010 considering all the available pre-clinical data, suggested that anticancer effects of BPs were separated in 2 groups, the skeletal and extra skeletal effects (72). Although, nitrogen BPs can potentially affect all types of cells as they inhibit the cholesterol synthesis pathway, their rapid binding to bone limits their uptake from other cells to those with phagocytic effects. The review described the following mechanisms as potential for the anti-tumour effects of BPs:

1) Anti-angiogenic effects in tumour and bone
2) Activate immune cells
3) Affect cells in bone and tumour microenvironment
4) Direct effects on tumour cells in bone and outside
5) Modify levels of circulating and bone growth factors
6) Reduce tumour macrophage infiltration

Additionally, a European panel summarised the potential effects of adjuvant BPs in 2016 as (Figure 2.1 in chapter 2):

1) Preclude tumour cells from homing to bone (63, 64, 73).
2) *In vivo* combination with chemotherapy can directly cause tumour cell death in bone (74).
3) Preserve tumour cells quiescence in bone (66, 69).
4) Disrupt the bone metastasis vicious cycle and stop the release of bone-derived tumour growth factors (65, 72, 75-77).

Despite the available pre-clinical and clinical evidence, further research is essential to clarify the mechanism of action through which BPs lead to prevention of breast cancer metastasis.

Clinical trials of adjuvant bisphosphonates

The first breast cancer randomised clinical trials that provided data for the use of BPs in the adjuvant setting were investigated clodronate and ZOL (78-81). In 2006, an international multicentre study randomised 1069 women with EBC to have oral clodronate (1.6g/daily) or not for 2 years in addition to standard therapy (78). Results demonstrated the anticancer effects of clodronate, with bone metastasis risk to be less in BP patients compared to non-BP

patients over 5 years (51 vs 73 patients with bone metastasis respectively). In addition, the benefit was observed to be more marked in patients with higher grade disease. Similarly, another clodronate study which randomised 302 patients (adjuvant treatment +/- clodronate 1.6g/daily for 2 years) showed reduction in skeletal and extra-skeletal lesions and improvement in DFS and OS in clodronate arm at 36 and 55 months analysis (79). However, long term analysis (103 +/- 12 months) demonstrated that only clodronate benefits in relation to OS were maintained.

Three studies with the same design, Z-FAST (n=602), ZO-FAST (n=1065) and E-ZO-FAST (n=527), researched the potent ZOL in ER+ve EBC patients who were randomised to adjuvant endocrine therapy with immediate or delayed ZOL (4mg/6monthly) for 5 years (81-85). Although they were primarily designed to assess the effects of ZOL on AI-related bone loss, the trials assessed disease recurrence as a secondary endpoint. The largest of the three trials, ZO-FAST, demonstrated reduction of the risk of DFS events in the upfront ZOL group by 41% and 34% at 3 and 5 years, respectively. In contrast, in Z-FAST the difference in local and distant recurrence between the 2 arms were minimal (2.3% in favour of upfront ZOL) at 3 years and were not significantly different at the final 5 year analysis (similar rates of DFS and death). The E-ZO-FAST failed to show any differences in disease recurrence due to the low incidence of events (only 29).

Oral ibandronate was evaluated by a German group in a phase 3 randomised study (GAIN - German Adjuvant Intergroup Node-Positive study) of 3023 participants with node positive EBC (86). Patients were randomised to 2 different doses of chemotherapy [2 weekly epirubicin 150mg/m^2 x3 followed by paclitaxel 225mg/m^2 x3 followed by cyclophosphamide 2,500 mg/m2 x3 (reduced to 2,000 mg/m^2 after recruitment of approximately 1,200 patients) or epirubicin 112.5 mg/m2 and cyclophosphamide 600 mg/m^2 x4 followed by weekly paclitaxel 67.5 mg/m^2 with capecitabine 2,000 mg/m^2 given daily for 14 days x10 every 3 weeks] with or without ibandronate (oral, 50mg daily) for 2 years. Analysis of DFS and OS in BP and no BP groups found no difference, however DFS was longer in younger (<40 years) and older patients (>60 years).

Zoledronic acid was also assessed for its anti-metastatic effects in a premenopausal population in the ABCSG-12 study which recruited 1803 premenopausal women to receive adjuvant goserelin with endocrine treatment (oral tamoxifen 20mg/day or anastrozole 1mg/day), with or without ZOL (intravenous, 4mg/6 months) for 3 years (87). Long term analysis (94.4 months of median follow up) showed that the relative risks of disease progression were reduced in the ZOL arm compared to control (DFS absolute risk reduction of 3.4%, OS absolute risk reduction of 2.2%), suggesting that adjuvant ZOL is beneficial for this group of patients when combined with standard of care. However, patients were receiving

ovarian suppression with goserelin and were therefore technically in induced menopause, something that should be carefully considered when interpreting the data from this study.

Another large multicentre international trial, the AZURE trial, randomised 3360 women (pre- and post-menopausal) to receive standard adjuvant systemic treatment with or without ZOL (intravenous 4mg 3-4 weekly x6, then 3 monthly x8, followed by 6 monthly x5) for 5 years (88). The overall results showed that DFS and OS were similar in both the ZOL and control groups. Nevertheless, women who were 5 years into menopause appeared to benefit, with IDFS of 78.2% in the ZOL arm compared to 71% in the control arm, and OS at 5-year of 84.6% and 78.7% in ZOL and the control group, respectively (88). These results were the first to identify a differential benefit from adjuvant BPs in pre- and post-menopausal women, but as it was a subgroup analysis this required further confirmation.

In 2015, the Early Breast Cancer Trial Collaborative Group (EBCTGC) published a meta-analysis of 26 randomised trials of adjuvant BPs in EBC which included data from 18766 women (89). In the combined meta-analysis, the most apparent effect of BPs was a reduction in bone recurrence, irrespective of menopausal status. However, subgroup analysis confirmed the AZURE findings, showing a clear benefit in postmenopausal women with 3% reduction in breast cancer recurrence, 2.2% reduction in bone recurrence and 3.3% reduction in breast cancer mortality in overall 10-year risk. In contrast, in premenopausal women no benefit was found in bone recurrence or survival, and extra skeletal recurrence was somehow worse in the BP group (17%) compared to control (15.9%). The benefit in postmenopausal women was independent of the oestrogen receptor status, nodal status, tumour grade, or concomitant chemotherapy and with the exception of oral pamidronate, all the other BPs (clodronate, ZOL ibandronate) produced similar benefit in disease outcomes. A 10 year update meta-analysis form the EBCTGC group is currently under way (personal communication with Prof. Coleman).

No differences in anti-metastatic effects between 3 years of ZOL (intravenous 4mg monthly for 6 months, then 3 monthly), clodronate (oral, 1.6g daily) and ibandronate (oral, 50mg daily) were also reported by an American/Canadian group (n=6097) (90).

Overall, data that are presented here suggest that adjuvant BPs have differential effects based on the menopausal status of the patients. The anticancer benefit of these agents in postmenopausal women is clear and comparable to that seen with the addition of taxanes to anthracycline schedules, or with the use of AIs versus tamoxifen (91, 92). Despite that, in premenopausal patients benefit from adjuvant BPs demonstrated only in patients on ovarian suppression at the start of their adjuvant therapy. The differential effects of adjuvant BPs will be discussed in more detail in the next section.

Another bone modifying agent, the RANKL (receptor activator of nuclear factor kappa-B) inhibitor denosumab was investigated in the same setting. In the phase 3 multicentre ABCSG-18 study, 3425 postmenopausal women with ER+ve EBC treated with AIs were

randomised to denosumab (subcutaneous, 60mg/6 monthly) or placebo for the duration of antioestrogen therapy (52, 93). Final analysis revealed a 9 year DFS benefit with denosumab of 3.5% and a very minimal OS benefit of only 1%. Correspondingly, the D-CARE study recruited 4509 EBC patients from 389 centres in 39 countries to have denosumab (subcutaneous 120mg 3-4 weekly with standard neo-adjuvant or adjuvant therapy for 6 months followed by 12 weekly) for 5 years or placebo (94). The trial failed to show any benefit from adjuvant denosumab in EBC and therefore, this agent is not currently recommended in this setting.

1.3.2 Menopausal status modifies response to adjuvant bisphosphonates, pre-clinical and clinical evidence

The results of the ABCSG-12 and AZURE trials (discussed above) (87, 88) gave the first indications that BPs have different anticancer benefits in pre- and post-menopausal women. This was also supported by the GAIN study (discussed above) which showed longer DFS with BPs in older postmenopausal women (>60 years) (86). Similarly, the NSABP B-34 study (n=3323) which randomised patients post breast cancer surgery to clodronate (oral, 1.6mg daily) or placebo for 3 years reported improved recurrence free interval and skeletal and non-skeletal metastasis free interval in BP participants older than 50 (95).

The large EBCTCG meta-analysis (discussed above) confirmed that menopausal status modifies response to adjuvant BPs, with anticancer effects to only benefit postmenopausal women by reducing risk of recurrence (bone, local, distant and overall) and improve survival (89). However, premenopausal EBC women could also benefit from adjuvant BPs if they were deemed postmenopausal [induced postmenopausal by ovarian suppression with gonadotrophin releasing hormone (GnRH) analogues] at the start of their adjuvant therapy.

These results were supported by early clinical results reporting that premenopausal bone marrow might be less attractive to cancer cells compared to postmenopausal. Braun et al. recruited 4703 breast cancer women from 9 studies and found that risk of bone micrometastasis was higher in younger participants (96). In particular, this was 33.3% in women aged 36-50 and 27.8% in those older than 65 and 32.7% and 29.5% in pre- and post-menopausal respectively. Another early clinical trial of 3141 patients who underwent bone marrow biopsy at the time of breast cancer surgery with the aim to isolate disseminated tumour cells (DTCs), found that although all women (pre- and post-menopausal) with bone marrow DTCs had greater DFS with adjuvant BPs, only DTC positive postmenopausal patients showed improved OS after adjuvant BP therapy (97).

The differential effects of adjuvant BPs have also been described in a number of pre-clinical studies. In a mouse model with breast cancer cells (MDA-MB-231) present in bone, animals underwent sham operation (to mimic the premenopausal environment) or OVX (to mimic the postmenopausal environment) and treated with ZOL (100μg/kg weekly) or saline. Analysis showed that ZOL impeded tumour growth only in OVX mice and had no effect on sham operated mice (66). A further animal study treated OVX mice with pre, peri and postmenopausal doses of oestradiol and ZOL (100μg/kg weekly) or saline (98). Animals were also injected with cancer cells (of MDA-MB-231, 4T1, or E0771). The incidence of extra skeletal metastasis was higher in the premenopausal models, even if they received ZOL, compared to the significant reduction of it in ZOL-treated postmenopausal models.

Despite the robust evidence from clinical and pre-clinical studies supporting the differential effects (depending on menopausal status) of BPs in the adjuvant setting, the mechanism responsible for these effects is still unknown. It is hypothesised that potentially high oestrogen environments inhibit the antitumour effects of BPs and also BPs interact with endocrine and practice factors in the bone microenvironment, leading to impaired survival of breast cancer cells (99, 100). However, further studies are crucial to clarify this hypothesis.

The bone microenvironment differs based on the menopausal status, with endocrine and paracrine factors to be in opposite levels in pre- and post-menopausal women. More specifically, due to the increased levels of oestrogen, progesterone and inhibin in premenopausal patients, the action of paracrine factors activin and TGF-β (transforming growth factor-beta) is reduced, in contrast to postmenopausal women where activin and TGF-β are highly active (99). Activin and TGF-β are paracrine factors which share the same intracellular pathway and have been extensively studied for their role in malignant process. Activin stimulates the production of FSH (follicle-stimulating hormone) and LH (luteinising hormone) hormones from pituitary and the expression of the GnRH gene (101). Transforming growth factor-beta belongs to a family of proteins that regulate many aspects of the cell cycle (102). Both activin and TGF-β are known to compete with inhibit, hence their different activity in pre- and post-menopausal environments.

The intracellular pathway of activin and TGF-β (most commonly Smad 2 and Smad 3) has been shown to have tumour suppressor abilities, as loss of its function has been connected with poor prognosis and reduction in DFS in breast cancer patients (103, 104). Additionally, activin has been found to be an inhibitor of angiogenesis, function which is crucial for tumour growth, and to stimulate the production of macrophages with antitumour structure (M1 macrophages) (99, 105). Transforming growth factor-beta plays an important role in the activation of fibroblasts, with its signalling in this cell type to prevent formation of tumours (99). Taken together, the actions of both activin and TGF-β suggest that they have anticancer benefits. Nevertheless, TGF-β has been also shown to increase factors responsible for the

homing of breast cancer cells in bone and both paracrine factors promote bone turnover, by different mechanism, which subsequently increases the production of bone growth factors supporting the tumour process (106-109).

Overall, the effects of activin and TGF-β described here, in addition to treatment with adjuvant BPs, might partially explain the differential effects of this therapy in EBC although this remains to be determined.

The immune system and its role in breast cancer has been extensively studied, demonstrating that it is crucial for the development and maintenance of neoplastic tumours. Their mechanism of action is complicated and not completely understood in relation to the malignant process. For instance, NK (natural killer) cells, macrophages and T cells have been found to have anticancer properties. The action of NK cells was investigated in a small breast cancer study (n=18) which found that their activity was much higher in healthy women compared to breast cancer participants (62.5% and 24.4% respectively) (110). Regulatory T cells (Tregs) are type of T cells which have a role in regulating or supressing other immune cells. In cancer, Tregs demonstrated neoplastic role by affecting antitumour T cells such as CD8+ and CD4+, leading to more aggressive and with poor prognosis malignancies (111). T cell CD8+ and CD4+ appear to have protective roles against breast cancer cells, with CD8+ to be related with better survival in established breast cancer (112). Macrophages, another important immune cells based on their stimulation they can differentiate in 2 different phenotypes, the macrophages with antitumor activities and the tumour associated macrophages (TAMs) (113, 114).

As described earlier in this introduction, BPs have been found to have effects on the immune system with studies suggesting that potentially their anticancer effects derive through their immune system interactions. Some of their effects described in pre-clinical and clinical studies are as follows:

1) Enhance antitumour immunity (115).
2) Activate γδ T cells leading to cancer cells death (116, 117).
3) Increase polarisation of macrophages towards the antitumour phenotype (118).
4) Reduce Tregs and their polarisation (119, 120).

Oestrogens are known for their immunogenic effects with multiple actions on different immune cells as these express ER receptors (such as T cells and macrophages). Currently available evidence indicate the oestrogen effects on immune system as follows:

1) Increase immune suppression in tumour microenvironment (100).
2) Promote Tregs activation (100, 121).

3) Preclude activation of CD8+ (122).
4) Support polarisation of macrophages towards TAMs (123).

The immune effects of BPs and oestrogens appear to be contradictory, suggesting that it is very likely the differential effects of BPs in pre- and post-menopausal women are driven (at least in part) by these opposing immune abilities. Low oestrogen levels in postmenopausal women lead to enhancement of BPs anti-metastatic action on immune cells, as opposed to the high oestrogen levels of premenopausal women which promote overlapping immune cancer effects with those of BPs'. This is a working hypothesis and further evidence is required.

Moreover, BPs for prevention of metastasis in the adjuvant setting are only currently recommended for EBC patients. Studies failed to show any benefit in other cancer types such as lung or prostate (124, 125), indicating that breast cancer cells interact differently with BPs. It is possible that breast cancer specific factors, in combination with menopausal status, might contribute to the differential effects of BPs.

1.3.3 Current clinical consensus of adjuvant bisphosphonates in early breast cancer

Adjuvant BPs have been adopted by national and international bodies who recommend their use in postmenopausal women (natural or induced) with EBC (13, 51, 126, 127). National and international guidelines, recent and updated versions, are described in detail in chapter 3 of this thesis. This includes patients' characteristics which deem them suitable for this therapy as well as recommended agents and regimes. Table 1.2 shows the national and updated international recommendations for the use of adjuvant BPs for prevention of metastases in EBC. Of note and in view of the Australian international collaboration (chapter 3), no guidelines were available in Australia for the use of adjuvant BPs during the period of interest of this thesis.

Adjuvant Bisphosphonates (BPs) for prevention of metastases in Early Breast Cancer

UKBCG (2016)	ESMO (2020)
Offer adjuvant BPs to postmenopausal or premenopausal women receiving ovarian suppression or had oophorectomy who will be offered chemotherapy or have >12% 10-year risk of breast cancer death. **Recommends:** 3 doses of zoledronic acid (4mg) while patient is receiving chemotherapy and then either 6 monthly zoledronic acid or daily oral ibandronate (50mg) for 3 years.	Offer adjuvant BPs to postmenopausal women with >12% 10-year risk of cancer death and premenopausal women on ovarian suppression. **Recommends:** intravenous zoledronic acid for 3 doses while patient is receiving systemic anticancer treatment followed by either 6 monthly zoledronic acid or a daily oral agent (clodronate 1,600mg or ibandronate 50mg) for a total period of 3 years.
NICE (2018)	**ASCO/CCO (2022)**
Offer adjuvant BPs to postmenopausal patients with node-positive and node-negative breast cancer with high risk of recurrence. **Recommends:** intravenous zoledronic acid (4mg) or oral sodium clodronate (1600mg) for 3-5 years.	Offer adjuvant BPs to postmenopausal and premenopausal on ovarian suppression women who are candidates for adjuvant systemic therapy. **Recommends:** intravenous zoledronic acid (4mg) 6 monthly for 3 years or 3 monthly for 2 years or oral clodronate (1,600 mg daily) for 2-3 years or oral ibandronate (50mg daily) for 3 years.

Table 1.2: *Updated guidelines for the use of adjuvant bisphosphonates in early breast cancer.*

Nevertheless, optimal duration of adjuvant BPs and better selection of patients continues to be debated between breast cancer specialists. In an attempt to support de-scalation of adjuvant BPs courses, the SUCCESS trial, which was published in 2021, compared 5 years to 2 years of adjuvant ZOL (128). This phase 3 study randomised 2987 EBC patients who received adjuvant chemotherapy to ZOL for 5 years (4mg intravenous 3monthly for 2 years and then 6 monthly for the remaining 3 years) or ZOL for 2 years (4mg intravenous 3 monthly for 2 years). Results demonstrated that shorter 2 year therapy in not inferior to longer 5 year therapy, with DFS, OS and distant DFS to be similar in the 2 groups. Therefore, SUCCESS study could encourage the change in current standard of care in adjuvant management of high risk EBC which suggest adjuvant BPs for 3-5 years.

In addition, identification of the best group of EBC patients that benefit from adjuvant BPs will avoid overtreatment or even undertreatment. Adjuvant BPs are currently only recommended for women with high risk of recurrence, a population of patients who are usually heavily treated with chemotherapy and targeted therapies with the aim of improving their survival. Efforts are ongoing to identify biomarkers that will predict the group of patients who will benefit the most from adjuvant bone therapies. Currently, MAF amplification (at 16q23) which is associated with increased risk of breast cancer bone metastases, is under investigation (129). Tumour samples from 1739 AZURE trial patients (standard chemo +/- adjuvant ZOL) were tested for the MAF amplification with 21% found to be MAF positive according to the scoring criteria used (130). Invasive DFS was found to have improved only in patents with non-amplified MAF gene who had ZOL, with ZOL MAF positive patients (non-postmenopausal) to have worse invasive DFS. Further study from Paterson et al., confirmed these findings (131). MAF gene status was assessed in 1833 patients who had adjuvant

chemotherapy with oral clodronate (1.6gr/daily) or placebo for 3 years. Assessment at 5 years demonstrated that MAF negative population who were treated with BPs had better OS and DFS (improved by 30%) and MAF positive patients showed no benefit from BPs.

Despite these intriguing findings, MAF testing is not currently in clinical use to determine which EBC patients would benefit from adjuvant ZOL, with further studies urgently required.

1.4 Cancer Treatment Induced Bone Loss

1.4.1 General background

Bone is a dynamic and active tissue which undergoes lifelong remodelling. Physiological bone remodelling is the balance between bone formation by osteoblasts and bone resorption by osteoclasts which is responsible for the maintenance of mineral integrity and homeostasis. This process is affected by internal factors such as hormones (e.g. oestrogen, parathyroid, calcitonin), growth factors and cytokines and by external factors such exercise and daily activities (132, 133). When the normal bone remodelling is impaired, bone health is interrupted leading to multiple skeletal pathologies, most commonly osteoporosis.

In women, oestrogen is critical for the maintenance of normal bone integrity and mass, with the main role being reduction of bone resorption. Oestrogen receptors (ER-α and ER-β) are found in both osteoblasts and osteoclasts but also on T-cells (134). The functions of the 2 oestrogen receptors are different based on how they affect the RANKL/RANK/OPG pathway (receptor activator of NF-kB ligand/receptor activator of NF-kB/osteoprotegerin). This system is known for its regulatory function on bone resorption, with interactions between RANKL and RANK promoting osteoclast differentiation and activation, leading to bone turnover and OPG blocking osteoclast formation by inhibiting RANKL binding to RANK (135). Therefore, activation of ER-α receptors induce osteoclastic apoptosis (increases RANKL and reduces OPG), as opposed to activation of ER-β receptors which stimulate osteoblastic activity by decreasing RANKL and increasing OPG (136). Reduction in normal oestrogen levels by physiological, pharmacological or pathological factors (e.g. menopause, hormonal treatments, cancer) will alter bone balance and lead to excessive bone turnover .

Women achieve 90% of their adult bone mass by the age of 20 and reach maximum bone mass in their late 20’s/early 30’s. After that, bone formation gradually slows down with age leading to bone loss. However, the bone loss process is accelerated after menopause when the dramatic reduction of oestrogen levels interrupts the bone remodelling balance. Bone resorption outweighs bone formation, precipitating menopausal bone loss which is fast the first 3 years of menopause, 2-5% per year, and it slows down to 0.5-1% per year thereafter (137). Figure 1.4 illustrates the alteration of female bone density over time.

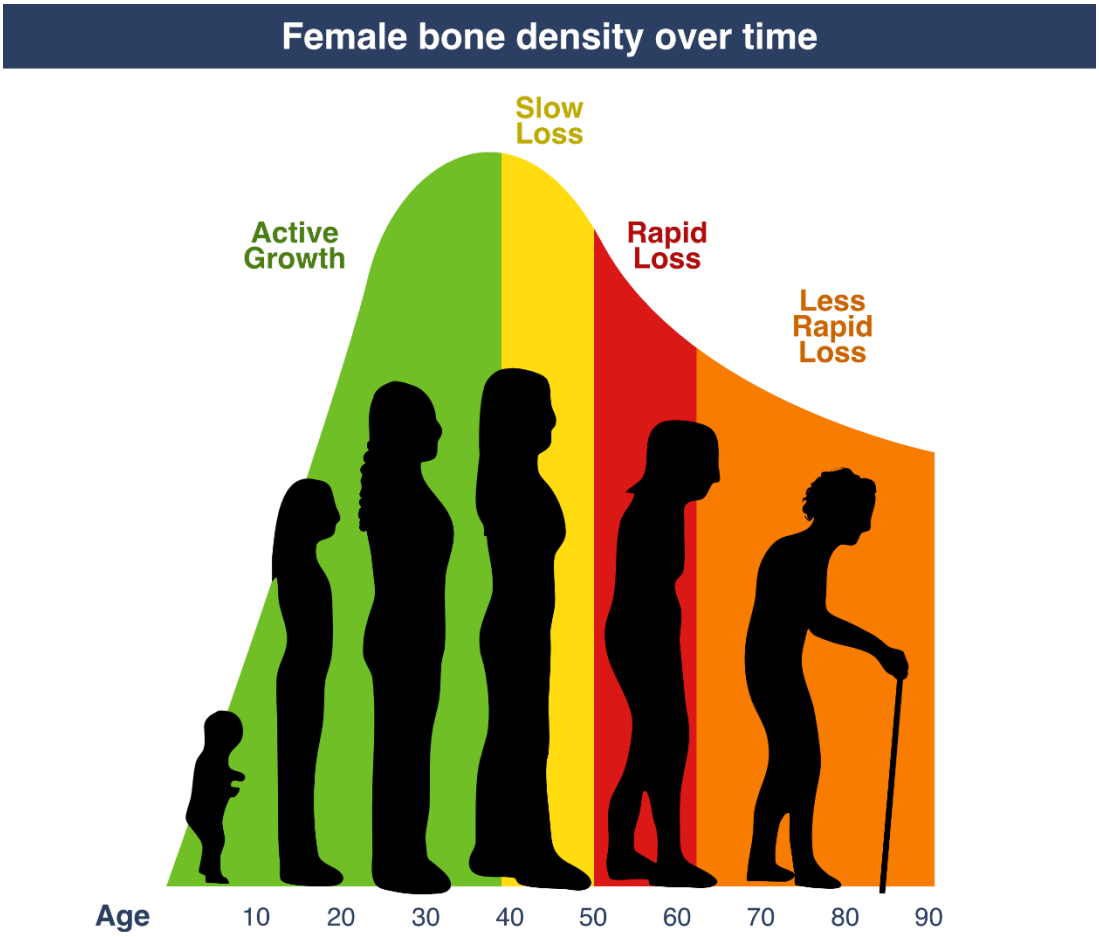

Figure 1.4: *Female bone density alterations over time.*

In the general female population, bone mass is not only affected by age and menopause. Modifiable (nutrition, lifestyle, daily exercise, smoking) and non-modifiable (race, personal and family history of non-traumatic fractures) factors play a crucial role in the maintenance of bone mineral density (BMD). Additionally, many medications and diseases have been shown to affect bone health, with the most apparent example being breast cancer and its treatments. As discussed elsewhere in this chapter, breast cancer is the most common cancer among women, with the majority requiring treatment aimed either at reduction of recurrence risk or to improve survival and control cancer symptoms. The impact of breast cancer treatment on bone health will be discussed in the next section.

1.4.2 Bone effects of anticancer treatment

Recent advantages in breast cancer management and therapies have led to improvements in cancer outcomes and survival. As a consequence, late effects of anticancer treatments have increasingly become a concern within the breast cancer communities.

Treatment-related adverse effects affect quality of life of patients who might otherwise be cancer free and are a considerable economic burden for the health and social systems.

One of the main long-term effects of anticancer treatments is their impact on bone health. Chemotherapy, targeted and hormone treatments have been shown to cause osteopenia and osteoporosis with patients' risk of fractures significantly increased. Many women require close monitoring of their BMD and for some treatment with bone targeted agents to preserve normal bone health is required.

Chemotherapy and Targeted Therapies

The type of chemotherapy agent, duration and frequency of the treatment course are selected based on the histopathology of the breast cancer. Chemotherapy reduces bone mass through indirect and direct effects. Mainly, agents have been described to affect skeletal integrity by causing premature menopause (resulting in reduction of oestrogen levels), but also due to direct effects on bone turnover (138).

Platinum based chemotherapies (e.g. cisplatin) are therapeutic options for TNBC. Among their other documented side effects, nephrotoxicity and hypomagnesaemia are the most well described, with patients requiring good hydration and strict monitoring of their kidney function and magnesium levels prior and after the administration of treatment. Nevertheless, some will still experience issues with low magnesium levels and require replacement, in some cases long term. In relation to bone health, magnesium has a pivotal role. Low magnesium has shown to alter skeletal structure and affect bone cells (reduce osteoblast, increase osteoclasts), but also it has indirect bone effects as interferes with the secretion of PTH and subsequently the synthesis and activation of vitamin D (139).

Other breast cancer chemotherapy agents that have been found to affect BMD are cyclophosphamide and doxorubicin. Cyclophosphamide causes premature ovarian failure in premenopausal women by acting on ovarian follicles, an effect which is dose-dependent, meaning that ovarian function should return soon after the completion of the chemotherapy course (140). It also interferes with the physiological bone remodelling by stopping the differentiation and activation of both osteoclasts and osteoblasts (141). Similarly, doxorubicin have shown to affect bone cells, leading to increase bone resorption (increase osteoclast differentiation and reduce formation of osteoblasts), and has been demonstrated to cause ovarian failure in animal studies (142).

The negative impact on bone caused by chemotherapy has been confirmed in clinical trials. In premenopausal EBC, BMD of 49 women was assessed at 3 time points (4 weeks, 6 months and 1 year) after commencing adjuvant chemotherapy (143). The study results showed a reduction in spine BMD at 6 months which worsened a year after chemotherapy, but only in patients who experienced ovarian failure. Another small study of 41 premenopausal

patients who had chemotherapy for EBC demonstrated reduction in BMD (spine and hip) at 6 months, but this was independent of patient's age, type of chemotherapy, amenorrhoea or oestradiol levels (144). However, 6 months post chemotherapy, bone mass was further reduced only in those women who reported amenorrhoea (during first 6 months of treatment) and had low oestradiol.

Long-term bone effects of breast cancer chemotherapy were investigated by Vehmanen et al. who assessed BMD of 73 premenopausal patients after 5 years of cyclophosphamide, methotrexate, 5-fluoroucil (145). Lumbar spine BMD was +0.6 and -1.3% at 3 and 5 years respectively in the group that maintained menses, compared to -7.5 and -10.4% in the amenorrhoeic group. At 3 and 5 yeas the femoral BMD was +1.7 and -0.3% in the menstruating group, compared to -3.5 and -5.8% in the amenorrhoeic group, respectively. The authors concluded that even after 5 years of chemotherapy, patients' bone health is better in those who preserved menstruation compared to those women who experienced ovarian failure.

In the metastatic setting, Everolimus, a biological targeted therapy, has been shown to be beneficial in ER+ve, HER2-ve disease that has progressed after prior endocrine therapy (146). Resistance to endocrine therapy in breast cancer is associated with activation of the mammalian target of rapamycin (mTOR) intracellular signalling pathway, and therefore Everolimus, an mTOR inhibitor has shown to improve survival. It is given alongside exemestane, and unlike other anticancer agents, it has been shown to have positive bone effects. The phase 3 randomised BOLERO-2 trial (n=724, everolimus and exemestane versus exemestane and placebo) demonstrated that the mTOR inhibitor reduces bone resorption, with the everolimus/placebo group having increased bone markers (formation and resorption markers) at 6 and 12 weeks, in contrast to the combination group which had reduction in bone markers (formation and resorption markers) (146, 147).

Additionally, from the new breast cancer therapies, immunotherapy has been found to affect bone health with patients treated with this therapy to have increased incidence of fractures after 1 year of treatment (148). This is a new reported adverse effect of immunotherapy, with the mechanism responsible to be unclear. Some of this may be related to the high dosage and long steroid courses that immunotherapy patients receive to treat immune related toxicities and/or due to endocrine deficiencies such as development of hypothyroidism secondary to immune inhibitors. However, *in vivo* studies demonstrated that blockage of PD1 affects bone mass by inhibiting osteoclast formation (149, 150). Therefore, further data are needed to clarify the effects of immune inhibitors on skeletal health.

Ovarian Suppression

For younger (premenopausal) women with functioning ovaries who have been diagnosed with primary ER+ve breast cancer, the addition of GnRH analogues to standard chemotherapy is shown to improve survival but also (and most importantly) provide ovarian protection (151). Ovarian protection is crucial to preserve fertility for future pregnancies (childbearing population) and also minimises the risk of permanent early menopause caused by chemotherapy.

Gonadotrophin releasing hormone analogues provide a persistent activation of GnRH which eventually lead to reduction of pituitary gonadotropins (LH and FSH), which in turn lead to decrease in oestrogen production from the ovaries. These effects are temporary and reversible, with menses to resume within a few weeks post discontinuation of the analogues. However, during the course of therapy, hypogonadism affect bone health by reducing skeletal mass.

A sub-study of the original Zoladex Early Breast Cancer Research Association study (n=1640) where EBC patients (<50 years) were randomised to Goserelin (GnRH analogue, 3.6mg/28 days for 2 years) or cyclophosphamide, methotrexate, 5- fluorouracil chemotherapy (6 cycles every 28 days), assessed the participants spine and femoral BMD at different time points (baseline, 12, 24 and 36 months). The sub-study involved 96 patients, with BMD at 24 months showing a reduction in spine and femur (-10.5% and -6.4%, respectively) in the goserelin group compared to -6.5% and -4.5% in the chemotherapy group. Further assessment at 36 months demonstrated improvement in BMD only in patients treated with goserelin (152).

Endocrine Treatment

Endocrine therapies are a therapeutic options for the management of ER+ve EBC; current available options are tamoxifen and AIs. Endocrine treatments are explained in detail in chapter 5 of this thesis.

Tamoxifen, a selective oestrogen receptor modulator (SERM), has a protective role in postmenopausal bone health, but reduces bone mineral density in premenopausal women, although not to a clinically significant level and therefore does not warrant any monitoring or treatment (153). Unlike Tamoxifen, AIs have significant negative effects on bone density, leading to bone loss and increased fracture risk, which in some large breast cancer trials, was over 50% higher than with Tamoxifen (154, 155).

Table 1.3 summarises the clinical trials that investigated the effects of AIs on bone health and shows the difference in fracture risk between experimental and control arms.

Clinical trial	Number of patients	Experimental drug	Control	Fracture incidence	Reference
ATAC	9366	Anastrozole	Tamoxifen	Annual 2.9% vs 1.9%	[137-139]
MA-17	5187	Letrozole	Placebo	Overall 3.6% vs 2.9%	[140]
BIG 1.98	4895	Letrozole	Tamoxifen	Overall 9.3% vs 6.5%	[141]
IES	4724	Exemestane	Tamoxifen	Overall 7% vs 5%	[142]

Table 1.3: *Clinical trials investigating the effects of aromatase inhibitors on bone health and the incidence of fractures.*

A sub-study of the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) trial (original study n=9366) assessed the bone effects of endocrine therapy. Postmenopausal patients who had received either adjuvant anastrozole (1mg/day) or tamoxifen (20mg/day) alone for 5 years were included in the sub-study (n=197, n=108 in final analysis) (156). Bone mineral density (lumbar spine and hip) was assessed at baseline, at 1, 2 and 5 years. At the completion of endocrine treatment (5 years), BMD of patients in the anastrozole group was reduced in both spine and hip, in contrast to the tamoxifen group where BMD was found to be increased. Further analysis, 1 and 2 years post anastrozole, demonstrated some recovery of lumbar spine BMD and no further hip bone loss (157). In addition, a 100-month analysis of the original trial reported higher annual fracture risk in patients receiving anastrozole (2.93%), compared to the tamoxifen group (1.9%) (158).

The MA-17 study recruited 5187 postmenopausal women with breast cancer to either 5 years of letrozole or placebo, following completion of 5 years of tamoxifen (159). Bone health analysis showed higher incidence of osteoporosis among letrozole patients [5.8%, in contrast to placebo patients (4.5%)], but found no difference in the reported fractures between the two groups. Direct comparison of 5 years of tamoxifen versus letrozole (BIG 1-98 study, n=4895) demonstrated 9.3% letrozole related and 6.5% tamoxifen related overall fracture rates (160). Also, more cases of multiple fractures were documented in the letrozole group (0.9%) compared to the tamoxifen group (0.4%).

In 2007, Coleman et al. published the results of the Intergroup Exemestane Study (IES) (n=4724) which randomised women who had received 2-3 years of adjuvant tamoxifen to continue tamoxifen (20mg/day) or switch to exemestane (25mg/day) to complete 5 years (161). Skeletal effects analysis (n=206) reported 2.7% and 1.4% reduction in BMD in lumbar spine and hip respectively, after 6 months of exemestane. However, bone loss in both spine and hip slowed down after 2 years of exemestane (1% reduction in lumbar spine, 0.8% reduction in hip). Fracture risk was assessed in the whole IES population and after 58 months was found to be greater in the exemestane (7%) compared to the tamoxifen group (5%).

Collectively, these studies demonstrate that in the breast cancer setting, AIs cause loss of bone density which subsequently lead to higher rates of osteoporosis and fragility fractures.

1.4.3 Prevention and Management of Cancer Treatment Induced Bone Loss

Assessment of CTIBL

All breast cancer patients, pre- and post-menopausal, who are eligible for any anticancer therapy which can potentially affect bone health should undergo a bone health assessment. Obtaining a thorough patient and family history is important to identify any additional risk of osteoporosis such as alcohol use, smoking, previous fracture after the age of 50, parental history of fracture, low body mass index (BMI), oral steroid use and history of other diseases known to cause osteoporosis (rheumatoid arthritis, ankylosing spondylitis, immobility, diabetes mellitus type 1 or 2) (51, 153, 162). Biochemical blood tests to investigate secondary causes of osteoporosis are also performed [e.g. full blood count (FBC), renal and liver function tests, calcium, phosphate, alkaline phosphate, thyroid function tests, parathyroid hormone]. General osteoporosis prevention advice and lifestyle changes apply to cancer patients with cessation of smoking, limiting alcohol intake and regular weight-bearing and resistance exercise to be encouraged (163).

In addition, maintaining good levels of vitamin D and calcium, either through an enriched diet or supplements, prevent osteoporosis and fractures. More specifically, a National Osteoporosis Foundation meta-analysis (n=970) demonstrated a 15% risk reduction of fractures in a population of non-cancer patients receiving these supplements (164). Calcium ≥1gr and vitamin D ≥800 IU a day are recommended (153).

Baseline (within 6 months of commencing endocrine therapy and/or ovarian suppression) dual energy x-ray absorptiometry (DEXA) scanning is crucial to identify a preexisting bone loss (13, 51, 153, 162). As discussed in chapter 5 of this thesis, DEXA scanning is the "gold standard" for measuring and monitoring BMD. It is mainly used to diagnose and monitor osteoporosis. It is cost-effective, non-invasive, requires minimal preparation from the patient and is performed in an outpatient setting and therefore it has become the examination of choice for prevention and management of CTIBL.

Dual energy x-ray absorptiometry scanners use two different X-ray energies with the radiation source to aim at the radiation detector placed directly opposite to the site to be measured. At the time of the DEXA scanning, patient is lying on a table where the radiation beam will pass through. The source and detector are then scanned the body area in the presence of bone and soft tissue. The different in total attenuation between the two X-ray energies is related to bone density, after subtracting out the absorption by soft tissues and only focusing on the absorption by bone.

Dual energy x-ray absorptiometry technology can potentially measure any skeletal site, but clinical use has been concentrated on the lumbar spine (L1-L4) and the femoral head where osteoporotic fractures are most likely to happen. Other sites such as the forearm might

be used in cases where BMD cannot be assessed in the spine or femur (e.g. due to spine or femoral operation). Dual energy x-ray absorptiometry systems are available as full table systems for multiple skeletal measurements or peripheral systems which are limited in measuring the peripheral skeleton. In current clinical practice, full table DEXA scanner is the preferred choice.

Results of the DEXA investigation are quoted as T-scores, which represent the standard deviation (SD) of the mean BMD of a healthy young adult. The T-score is calculated using the formula: (patient's BMD − young normal mean)/SD of young normal. The clinical interpretation of T-scores, according to the World Health Organisation (WHO) are as follows:

- T-score within ≥-1 SD of the mean indicates a normal BMD.
- T-score between -1 to -2.5 SD from the mean indicates osteopenia.
- T-score below -2.5 SD from the mean indicates osteoporosis.

Along with DEXA scan, vertebrae fracture assessment is part of the CTIBL screening and follow up in breast cancer patients. Lateral radiograms of thoracic and lumbar spine are performed to rule out any asymptomatic vertebrae fractures (165).

The University of Sheffield FRAX® tool is a validated and widely used fracture-risk assessment tool (166). It is mainly used in osteoporotic patients over the age of 40 and provides the 10-year probability of a hip fracture and major osteoporotic fracture (clinical spine, forearm, hip or shoulder fracture) (166, 167). Although the FRAX® tool was not built to assess the fracture risk in cancer patients receiving hormone therapy, its validity has been assessed in cancer clinical trials (168-170). Results are still limited and unclear and therefore FRAX® is not currently recommended for this group of patients.

Bone Targeted Agents

Bisphosphonates and denosumab are the currently approved bone targeted agents for the prevention and treatment of CTIBL in all patients with EBC. Table 1.4 describes the clinical trials that investigated the effects of antiresorptive treatment on breast cancer treatment induced bone loss.

Study	No. of patients	Experimental arm	Control arm	BMD variation at lumbar spine	Fracture risk	Reference
IBIS-II	1410	Anastrozole + Risedronate	Anastrozole + placebo	+1.1% vs -2.6% (at 3 years)	No difference in fracture risk at 5 years	[155-156]
ABCSG-12	404	Goserelin + Anastrozole or Tamoxifen + Zoledronic Acid	Goserelin + Anastrozole or Tamoxifen	-0.4% vs -4.2% (at 5 years) +4% vs -6.3% (at 60 months)	——	[158]
AZURE	3359	Standard adjuvant therapy + Zoledronic Acid	Standard adjuvant therapy	——	3.8% vs 5.9% (at 5 years)	[70,159]
Z-FAST	602	Letrozole + Zoledronic Acid (up front)	Letrozole + Zoledronic Acid (delayed)	+6.18-6.22% vs -2.42% (at 61 months)	9.3% vs 11% (at 61 months)	[65]
ZO-FAST	1065	Letrozole + Zoledronic Acid (up front)	Letrozole + Zoledronic Acid (delayed)	+4.3% vs -5.4% (at 60 months)	No difference in fracture risk	[66]
ABCSG-18	3425	Aromatase inhibitor + Denosunab	Aromatase inhibitor + placebo	+8.1% vs -2.4% Last assessment while on treatment +3.5% vs -1.5% Last assessment after treatment discontinuation	15.9% vs 19.2% (at 11 years)	[75]

Table 1.4: *Clinical trials investigating the effects of bisphosphonates and denosumab on CTIBL in women with early breast cancer.*

Oral BPs were the first to be researched for this indication, with small studies showing that clodronate, risedronate and ibandronate improved BMD when given to patients who were receiving ET for ER+ve EBC (171-173). More specifically, clodronate was tested in patients receiving tamoxifen (20mg/day) or toremifene (60mg/day) whilst both risedronate and ibandronate were given to patients receiving AIs.

Risedronate was further investigated in a large international double-blinded randomised study (IBIS-II) which recruited 3864 postmenopausal breast cancer patients to receive either anastrozole (1mg/day) or placebo (174). Thirty six percent of the participants (n=1410) included in a bone sub-study were separated into 3 groups based on their baseline DEXA scan T-score (174). Group 1 was women with normal DEXA scan (T-score ≥-1) who were not given any risedronate. Group 2 was women with osteopenia (T-score between -1 to -2.5) who were randomised to either risedronate (35mg/week) or placebo. Group 3 was the osteoporotic group (T-score between -2.5 and -4) in which all patients were prescribed risedronate. The IBIS-II sub-study had a further bone assessment at 3 years, showing that group 2 risedronate patients had improvement in lumbar spine BMD (+1.1%) and stable hip BMD (-0.7%), compared to placebo patients who experienced bone loss in both lumbar spine (-2.6%) and hip (-3.5%). Results also showed that patients with normal BMD had significant bone loss in spine and hip (-4% in both), demonstrating the negative effects of anastrozole on bone health. Subsequent assessment after 5 years found no major changes in the lumbar spine BMD (-0.4%) of those who treated with risedronate (group 2) but showed further reduction in the lumbar spine BMD (-4.2%) of the placebo arm. In terms of fracture rates, these were not different between the 2 arms of the osteopenic group after 5 years (175).

Nevertheless, beneficial effects of oral BP agents in preventing AI related fractures were reported by Pineda-Moncusi et al. in 2020 (176). The observational cohort study of 36472 patients with EBC treated with AIs or tamoxifen showed that after 10 years of follow up, patients on AIs who were treated with BPs had a 30% reduction in fracture risk compared to those who did not receive BPs.

Zoledronic Acid is an intravenous and very potent agent which currently is the most widely used and extensively researched BP in pre- and post-menopausal EBC. The bone sub-protocol of the ABCSG-12 study randomised 404 premenopausal women to receive goserelin (3.6mg/28 days) combined with antioestrogen therapy (anastrozole or tamoxifen) with or without ZOL (4mg/6months) for 3 years (177). Participants had DEXA assessments at baseline, 6, 12, 36 and 60 months with BMD of the ZOL group remaining stable whilst on treatment and to continue improving after the discontinuation of ZOL (BMD: 3 years +0.4% lumbar spine and +0.8% hip, 60 months +4.1% lumbar spine and +3.9% hip). In contrast, patients who did not receive ZOL had reduction of their bone mass throughout (BMD: 3 years -11.3% lumbar spine and -7.3% hip, 60 months -6.3% lumbar spine and -4.1% hip).

The large international multicentre phase 3 AZURE trial (n=3360) was mainly investigating the anticancer effects of adjuvant ZOL (standard adjuvant therapy with or without ZOL 4mg for 5 years 3-4weekly x6, 3monthly x8 and 6monhtly x5) (88). However, in 2018 Wilson et al. published that ZOL also reduced the time to first fracture in breast cancer patients with a 5-year fracture incidence 3.8% for the AZURE ZOL group compared to 5.9% for those who did not receive ZOL (178).

The differences between commencing ZOL the same time as AIs, or delay until after documented bone loss or facture, were explored by Z-FAST (n=602) and ZO-FAST (n=1065) trials (83, 84). Both studies demonstrated that upfront ZOL was superior to delayed ZOL in reducing fractures and also improving and maintaining skeletal density (Table 1.4). Additionally, upfront ZOL was found to be beneficial, even for patients with normal baseline bone assessment compared to delayed ZOL, suggesting that AIs could lead to skeletal loss even in this population (BMD lumbar spine +3.9% vs -7.1% upfront and delayed ZOL respectively).

Denosumab was investigated by a few small studies, but only in the postmenopausal setting (85-87). ABCSG-18 was the largest study to demonstrate the benefits of denosumab on CTIBL on breast patients (52, 179-181). Postmenopausal women (n=3425) were randomised to subcutaneous denosumab (60mg/6 months) or placebo during their adjuvant AI therapy. Initial analysis revealed that denosumab significantly delayed the presentation of the first fracture in this cohort with an overall fracture reduction of 50%. Moreover, long term analysis published at the end of 2022, demonstrated a fracture rate of 15.9% in the denosumab group compared to 19.2% in the placebo group, 11 years post

randomisation (93). Although the long-term overall fracture reduction dropped to 30%, this was still high, which could be partially explained by the rebound effects of denosumab. However, the study did not report any rebound in fracture risk after discontinuation of denosumab.

Current guidelines – postmenopausal setting

Currently, for postmenopausal EBC patients both denosumab (60mg/6monthly subcutaneous) and BPs (alendronate 70mg/weekly oral, zoledronic acid 4mg/6monthly or 5mg/12monthly intravenous) are the antiresorptive treatment of choice for the prevention or treatment of CTIBL. However, in view of their oncological benefit (discussed in this chapter), BPs (adjuvant BPs: zoledronic acid 4mg/6monthly, oral clodronate 1,600 mg daily or oral ibandronate 50 mg daily) are generally the preferred option for women with high recurrence risk, hormone receptor positive breast cancer. Denosumab is more suitable for patients whose risk of AI-induced fractures outweighs the (low) risk of breast cancer recurrence. In addition, patients who commenced denosumab but discontinued it prior to the completion of AI therapy are advised to be switched to a BP until the end of their antioestrogen therapy. This is in view of the rebound phenomenon of accelerated bone loss that occurs with the discontinuation of denosumab (50, 162).

Prior to starting any bone therapy, all postmenopausal women commencing endocrine treatment with AIs are advised to have a baseline bone DEXA assessment within the first 6 months of treatment initiation (13, 51, 153, 162). These assessments should be repeated every 1-2 years during the course of endocrine therapy (13, 51, 153, 162). However, UK guidelines suggest that if both spine and hip baseline T-scores are above -1, then further assessment is not needed unless clinically indicated (153). In general, the frequency of follow-up DEXA scans should always be decided based on the individuals' bone loss risk and the use or not of bone protective therapy.

Treatment with a bone targeted agent, Vitamin D and calcium supplementation should be started in all AI patients with a T-score <-2 (13, 51, 153, 162). UK patients older than 75 with at least 1 risk factor for bone loss, such as previous low-trauma fracture after age 50, parental history of hip fracture, alcohol intake of >4 units/day, diseases associated with secondary osteoporosis, prior corticosteroids for >6 months, low BMI (<22), are also advised to commence bone protection therapy with the start of their antioestrogen therapy (153). All other UK postmenopausal ages are advised to receive a bone targeted agent if the baseline T-score is <-2, or they have a vertebrae fracture. Patients with osteopenic T-score (<-1 but >-2) are given lifestyle advice, vitamin D and calcium supplements with plan to have a follow up bone assessment in 2 years. If their BMD drops below -2 or they have annual bone loss >4%, they are commenced on bone antiresorptive therapy (153).

In contrast, the international consensus was initially suggesting bone protection therapy for women older than 65 with 1 additional risk factor for bone loss (T-score <1.5, smoking, BMI<24, family history of hip fracture, history of fragility fracture above age 50, oral glucocorticoid use for >6 months) (51). However, these recommendations have since been updated, advising antiresorptive therapy for all postmenopausal women receiving AIs with ≥2 risk factors or ≥1 risk factor and T-score<-1 (risk factors: previous fragility fractures, parental hip fracture, recurrence falls ≥2 in last year, diabetes (type 1 or 2), rheumatoid arthritis, BMI<20, glucocorticoid use >3 months and >7.5mg/day, current smoking, alcohol >2units/day) (162).

Current guidelines - premenopausal setting

According to UK guidelines, all premenopausal breast cancer patients due to start ovarian suppression therapy or any other therapy that can potentially induced menopause are recommended to have a baseline DEXA scan within 3 months of commencing treatment (153). Treatment with BPs (alendronate 70 mg per week, risedronate 35 mg per week, ibandronate 150 mg oral monthly or 3 mg iv 3-monthly or zoledronic acid 4 mg iv 6-monthly) is advised for those who have been prescribed an AI and their baseline T-score is <-1 or have known vertebrae fracture. For those not receiving AIs, BPs are initiated if the baseline T-score is <-2 or have known vertebrae fracture. In the cases where T-score is >-1 with an AI or between -1 and -2 without an AI, DEXA assessment is repeated in 2 years, and they follow the same medium risk pathway as the postmenopausal women (described above).

For the international consensus, all premenopausal women initiated on AIs +/- ovarian suppression therapy or tamoxifen with ovarian suppression therapy follow the same pathway and risk assessment as the postmenopausal patients (described above) (162). However, ZOL is the only treatment option for this population receiving endocrine therapy, as currently no evidence is available for the use of oral BPs or denosumab.

1.5 Established bone metastasis in breast cancer

1.5.1 General background

Bone metastasis is very common event in breast cancer, up to 70% of the patients with metastatic disease reported to have skeletal involvement (182). Additionally, bone is the most common place of breast cancer relapse with 50% of the recurrences to present in bone (183). Oestrogen receptor positive, HER2-ve disease is the subtype associated with the highest incidence of bone metastasis (184).

Bone metastatic disease, regardless of the primary tumour site, leads to complications called skeletal related events, which dramatically increases mortality and morbidity, patients

have poor quality of life and cancer outcomes, and health care costs rise (185). Breast cancer has the highest rates of SREs among all the cancer types that can potentially metastasise to bones (186). Skeletal related events involve fractures, pain, need for radiotherapy and/or surgery, malignant spinal cord compression and hypercalcemia. However, pain is the most common SRE and pathological fractures (fractures due to bone metastases) in metastatic breast cancer have annual rate of 20-40% (186, 187).

When metastasis occurs in bone then bone metabolism and normal remodelling are disrupted. The actual bone metastasis process is very complex and not completely understood; multiple pre-clinical and clinical studies have been performed in an attempt to clarify the pathophysiology of skeletal metastasis. For the development of any metastatic site (not only bone), cancer cells need to escape the primary cancer side and enter the blood and/or lymphatic circulation. Only a very small percentage (0.02%) of disseminated cancer cells is thought to be able to form metastasis (188).

So far, the bone metastatic process and the events supporting the multiplex action of cancer cells movement to skeleton have been described as below (189):

- Primary tumours secrete growth factors (GFs) and exosomes that induce the formation of appropriate environment for distal metastasis to occur, the so called premetastatic niche (190-192).
- Disseminated tumour cells interact with the haemopoietic stem cell (HSC) mechanism [e.g. E-selectin, IL-1β (interleukin-1β)] which help their homing in bone marrow.
- When cancer cells arrive in bone they bind to extracellular matrix proteins that aid the attachment of cancer cells to bone in specific bone niches.
- Inside the bone microenvironment, disseminated cancer cells can stay inactive (in dormancy) for many years, especially breast and prostate cancer cells. Cancer cells remain in dormancy with the help of the vascular and endosteal niches. Some cancer cells will never become active to establish bone metastasis whereas cancer cells that exit dormancy grow to form bone metastasis when the microenvironment is appropriate.
- Circulating cancer cells reactivated from dormancy due to osteoclastic bone resorption that naturally occur in bones. These cells are able to survive and grow in bone due to osteomimicry.
- Active cancer cells disrupt bone balance. They stimulate osteoclasts and subsequently increase bone resorption, and the same time reduce the action of osteoblasts. This process is also supported by bone marrow immune cells.

- Progression of bone metastasis is encouraged by GFs released by the bone matrix such as TGF-β, IGFs (insulin-like growth factors) and PDGF (platelet derived growth factor).

More studies are needed to clarify the mechanism of bone metastasis and subsequently encourage the development of more targeted therapies for the prevention and treatment of malignant bone disease. In particular, the interactions between cancer cells and the bone microenvironment that maintain or trigger escape from dormancy remain to be fully understood.

Bone metastasis is mainly diagnosed through medical imaging, whereas in a small number of patients diagnosis of bone metastasis is only achievable by bone biopsy, an invasive and not always easy procedure. It is only performed from accessible bone sides and mainly in patients with isolated bone disease where the primary cancer diagnosis in unknown.

Plain x-ray is the preferred imaging in patients with pain as it is easy, cost effective, quick and available in all the hospitals. X-rays give information about the size and location of bone metastatic foci and most importantly can rule out bone fractures within a few minutes. Nevertheless, they have poor sensitivity and bone lesions need to be at least 1 cm and/or cause 50% loss of bone mineral content in order to be visible in plain films (193). Computer tomography (CT) has better sensitivity (74%) than x-rays and helps to assess level of bone destruction, structure of the lesion and the volume of the metastatic bone disease (194). Computer tomography is a staging investigation where the whole cancer disease is assessed. In the cases where bone surgery is planned, CT is the imaging modality of choice in preparation for the intervention. Magnetic resonance imaging (MRI) has 95% sensitivity and 90% specificity, and it is particularly useful in detecting spine lesions and bone marrow infiltration (194).

Nuclear medicine also plays an important role in the diagnosis of bone metastasis. Bone scintigraphy, more widely known as bone scan, is the most commonly used radionuclide imaging. It has high sensitivity (78%) in detection of bone metastasis, but its specificity is very low (194). It uses Tc-99m (Technetium -99) in combination with another agent, usually methylene diphosphonate (MDP) and it provides planar images of the whole skeleton. Areas with active bone turnover release more radioactive material and are shown as areas of increase uptake, also known as hot spots. Similarly, SPECT (single photon emission computed tomography) uses Tc-99m-MDP and provide cross-sectional images of the skeleton with better sensitivity than bone scan. However, the most accurate and with better resolution nuclear medicine imaging is positron emission tomography (PET) which uses radiotracers

(18F FDG or 18FNaF) and identifies bone metastasis based on the high glucose metabolism of cancer cells. Position emission tomography can also be combined with CT scan (PET-CT).

During bone formation and resorption, bone turnover molecules are naturally released into the circulation and can be detected in serum and urine (195). Examples of bone formation markers are P1NP (N-terminal propeptide of type 1 procollagen), P1CP (C-terminal propeptide of type 1 procollagen) and BALP (bone specific alkaline phosphatase), whilst CTX and NTX (C- and N- terminal telopeptide) are bone resorption markers (195, 196).The role of bone biomarkers in detection of bone metastasis, prognosis and monitor of bone disease post initiation of treatment, has been extensively studied (196, 197). However, their clinical role is still unclear. To support the use of bone biomarkers in diagnosis of solid tumours bone metastasis, a large meta-analysis (n=3268) demonstrated that BALP was much higher in patients with bone metastasis compared to those without (198). Also, a different meta-analysis (n=1279) showed that NTX was increased in patients with bone lesions (199). Same biomarkers have been found to be associated with poor prognosis in cancer patients. Studies in patients who were treated with BPs for their bone disease showed that incidence of SREs was higher in participants with high BALP and NTX levels (200, 201).

Although research results are promising, the use of bone biomarkers in clinical practice has been challenging and they are currently not recommended in routine use in any aspects of bone metastatic disease (51). This is mainly due to limitations and accuracy of their levels which are affected by factors such as patient's age, sex, food and medication intake and liver and kidney problems (195). In breast cancer patients, treatments with bone effects, such as antioestrogen therapies, can lead to inaccurate levels of bone biomarkers (195). Additionally, bone turnover markers have seasonal fluctuations making their clinical use even more complicated (202). Clearly, further research is needed to clarify the role of bone turnover markers and potentially open new doors in the diagnosis and management of malignant bone disease.

1.5.2 Treatment of established bone metastasis in breast cancer

1.5.2.1 Local Treatments

Treatment of SREs in patients with known bone metastasis is focused on symptom control and prevention of further SREs. Pain, one of the main SREs, can be difficult to control and therefore lead to poor quality of life which subsequently can affect patients' performance status. Adequate analgesia is crucial in the cases of symptomatic bone disease. This can be initiated by the oncology team and escalated to the palliative or local pain teams, in the cases where pain is complicated or regular monitoring is required. Bone pain can also be treated with radiotherapy, either with one single fraction or multiple. Radiotherapy can relieve pain in

one third of the cases, suggesting that good pain control with analgesia is very important for the majority of the patients (51). Even in the cases where radiotherapy will eventually benefit bone pain, analgesia needs to be continued throughout the course of radiotherapy and for short period afterwards, as the effects of radiotherapy will take from days to weeks to be fully achieved. The decision to offer one or multiple fractions of palliative radiotherapy is taken based on the general performance status of the patient and their tolerability. Although longer regimes tend to be more toxic and require more hospital visits or longer hospitalisation, a meta-analysis of 29 studies (n=6099) showed no difference in pain benefit between the two radiotherapy options (203). In severe pain cases where other pain management techniques failed, bone surgery might be offered.

Malignant spinal cord compression (MSCC) is an oncological emergency. It is diagnosed with a spinal MRI, and it is crucial for patients to be started on high dose of oral steroids (e.g. dexamethasone 16mg/daily) as soon as the diagnosis is confirmed. Treatment options are radiotherapy and/or surgery. Surgery is offered to those with limited metastatic bone disease, good performance status and better prognosis. Multiple fractions of spine radiotherapy are given to patients not suitable for surgery (e.g. extensive spine disease) but fit enough to undergo multiple sessions of treatment, whereas single palliative radiotherapy is for those frail and with very poor prognosis patients.

Pathological fractures are managed surgically, whenever possible, to preserve mobility and minimise complications (e.g. pain). In the cases where surgery is not feasible (e.g. extensive bone metastatic disease), life expectancy is very short or any intervention carries multiple peri- and post-operative risks, the fractures are managed conservative. Impending fractures will be treated surgically if the risk of fracture is imminent, otherwise they remain under close monitoring.

1.5.2.2 Bone Targeted Agents

The current standard of care of the management of established bone metastasis in solid tumours, including breast cancer, are BPs and denosumab (51, 204, 205). The occurrence of one SRE significantly increases the risk for further SREs, making their prevention crucial. Both BPs and denosumab have been shown to prevent SREs (185, 206).

The role of BPs in cancer bone disease was demonstrated well before the development of denosumab. Their first indication in bone metastasis was in the treatment of malignant hypercalcaemia, and up until today, they are the only approved bone targeted agents for the management of this pathology. Clodronate, was the first BP to show reduction in SREs in breast cancer patients (oral 1600mg/day, n=173) (207), followed by pamidronate (intravenous, 90mg/day). Two randomised controlled trials demonstrated that addition of pamidronate in the standard of care (chemotherapy, antioestrogen therapy) of metastatic

breast cancer with at least one bone lesion, can reduce SREs and also increase the time to first SRE by 50% (208, 209). Other BPs such as ibandronate, oral and intravenous, have been found to be effective in malignant bone disease (210, 211).

However, the most effective BP in the treatment of bone metastasis is the potent intravenous agent ZOL. When ZOL was compared to placebo in patients with metastatic breast cancer, it reduced SREs by almost 40% (n=228, ZOL 4mg intravenous for 1 year) (212). Direct comparison with pamidronate and ibandronate showed that ZOL is superior. Patients with bone metastatic breast cancer or multiple myeloma (n=1648) were randomly selected to have either ZOL (4mg) or pamidronate (90mg) 3-4 weekly for 2 years (213). Results demonstrated extra 20% reduction in SREs in breast cancer patients receiving ZOL, compared to those who had pamidronate. In a phase 3 multicentre UK study, 1404 women with metastatic breast cancer were assigned to receive ZOL (4mg, every 3-4 weeks, intravenous) or ibandronate (50mg, daily, oral) for 96 weeks, with ZOL to be greater in preventing skeletal morbidity (214). These results were also confirmed by a meta-analysis (n=2806) of 9 studies (BPs versus placebo versus no BPs), showing that BPs in breast cancer decreased bone metastasis complications by 15%, with ZOL (4mg, intravenous) being the most active agent (215).

The optimal duration of ZOL was assessed in several studies in an attempt to de-escalate treatment and minimise hospital visits for patients with metastatic disease who perhaps have already been on multiple lines of palliative systemic treatments and have spent considerable amount of their time in cancer units. Two studies, the ZOOM and OPTIMIZE-2 examined whether 12 weekly ZOL was inferior to 4 weekly ZOL in the treatment of breast cancer-induced bone disease. Breast cancer patients with established bone metastases were treated with ZOL for at least 12 months prior to their enrolment to the studies. Both trials confirmed the non-inferiority of 3 monthly intravenous ZOL for this cohort of patients (216, 217). Additionally, a large study (n=1822) which recruited patients with metastatic breast and prostate cancer and multiple myeloma demonstrated similar results, showing that 3 monthly ZOL has equal effects to monthly ZOL in preventing SREs (218).

When the monoclonal antibody RANKL inhibitor denosumab became available, it was tested against ZOL. In large, randomised trial of breast cancer with bone metastases, patients were randomised to receive either ZOL (4mg, intravenous) (n=1020) or denosumab (120mg, subcutaneous) (n=1026) monthly (219). Data demonstrated the superiority of denosumab in preventing SREs and delaying the time to the first SRE, making this agent another option for the treatment of malignant bone disease. Additionally, an analysis of three phase 3 randomised studies of metastatic solid tumours and multiple myeloma, in which patients had denosumab (120mg, subcutaneous) or ZOL (4mg, intravenous) for their bone disease (breast cancer patients n=2049), showed again that ZOL was inferior to denosumab in managing

skeletal metastasis (220). Time to first SRE was delayed by a median 8.21 months and risk of SREs was reduced by 17% with denosumab. In terms of side effects, denosumab and ZOL have been demonstrated to have similar profiles and in particular, no differences were found in the rates of ONJ between the two (185, 219). However, acute phase reaction and renal impairment are associated with ZOL treatment (220).

Overall, denosumab has been found superior to ZOL in treatment of bone metastasis and prevention of skeletal morbidity (185, 187, 206). It is also more convenient for patients and oncologists, as it can be given in an outpatient setting (subcutaneous injection), in contrast to ZOL which require chair time in chemotherapy wards (intravenous infusion). In some cancer centres, trials of self-administration of denosumab at home are currently ongoing. Nevertheless, denosumab has been found to be less cost-effective compared to ZOL, but further health economic evaluation for its use in the metastatic breast cancer setting in the UK is needed (221-223).

National and international guidelines for the management of breast cancer bone metastatic disease are currently recommending both BPs (ZOL, clodronate or ibandronate) and denosumab. Factors that could affect the choice of bone targeted agent is local cancer unit capacity, preferred route of administration, patient's wishes and preferences and contraindications such as renal impairment. For patients with known renal issues, denosumab is the preferred choice. Bone targeted agents should be started as soon as bone metastases are diagnosed, even if the patient is asymptomatic. The duration of treatment is indefinite unless issues with tolerability and serious side effects occur. In the UK, NICE advises the use of either denosumab (120mg, 4 weekly subcutaneous) or BPs, with the choice of BP agent to be left with the breast oncologist (204, 224). The European guidelines suggest that oligometastatic bone disease should be treated with 3monthly ZOL (4mg, intravenous), unless life expectancy is less than 3 months. In these cases, use of bone targeted agents should be assessed carefully. Only in oligometastatic bone disease and if good response to treatment is achieved, treatment with bone modifying agents could be interrupted after 2 years. However, bone agents should be restarted soon after disease progression is confirmed. For the breast cancer patients with multiple bone lesions, ESMO (European Society of Medical Oncology) recommends BPs (mainly intravenous ZOL 4mg/3monthly but oral daily ibandronate 50mg or clodronate 1600mg could be used) or denosumab (subcutaneous, 120mg/monthly) (51). Furthermore, the American Society of Clinical Oncology and Cancer Care Ontario (CCO) recommendations for the use of bone targeted agents in breast cancer with established bone metastases were updated in 2017 to suggest the use of subcutaneous denosumab (120mg/ monthly), intravenous pamidronate (90mg/ 3-4 weekly) or intravenous ZOL (4mg/3-4 weekly or 12 weekly) (205) .

Discontinuation of denosumab or intermittent courses of treatments are not advisable. Unlike BPs which stay in bone for a long period of time after their administration, denosumab cessation lead to increase of bone loss and fractures (51, 225). This phenomenon is called rebound. Therefore, in patients where interruption of denosumab is unavoidable, BPs should be commenced to maintain good bone health (51).

Taken together, the clinical data demonstrate that the use of bone targeted agents, in particular ZOL, is beneficial in a range of breast cancer settings, from adjuvant use in postmenopausal EBC through prevention of treatment-induced bone loss to the metastatic setting. This thesis aimed to increase our understanding of how best to use these agents and capture the experience of their use from different stakeholders.

1.6 Thesis aims

The primary aims of the work presented in this thesis were as follows:

- Provide a better understanding of the mechanism responsible of the adjuvant effects of BPs and investigate why adjuvant BPs have anticancer benefits only in postmenopausal EBC.

- Explore the UK and Australian breast cancer oncologists' opinions and routine practice around the use of adjuvant BPs in EBC.

- Evaluate the use of adjuvant BPs in UK oncology centres.

- Describe the UK patients' experience with adjuvant BPs.

- Determine the management of bone health in older women with ER+ve EBC in the UK.

Chapter 2

The ZOLMENO study

The role of ZOLedronic Acid and MENOpausal status in patients with early breast cancer

2.1 Summary

Bisphosphonates (BPs) given in early breast cancer post cancer surgery have been found to reduce the risk of disease recurrence and prevent bone metastasis (78-81, 88, 89, 226). However, their anticancer effects only benefit postmenopausal women (natural or induced), with the mechanism leading to these differential outcomes still to be established.

The ZOLMENO study, a single centre, open label, randomised, proof of concept study was designed in 2014 with the aim to address this research gap and establish the differential effects of adjuvant BPs in women with different menopausal status. Originally, 80 women with EBC were planned to be recruited to the study, 40 premenopausal and 40 postmenopausal, all receiving a single dose of the intravenous BP, Zoledronic Acid (ZOL) either 7 days prior or 21 days post their breast cancer surgery. Serum (blood) and bone marrow biopsy (aspirate and trephine) samples would have been collected from all the participants at four different time points, to allow translational studies to explore the effects of ZOL.

Study sample analysis was intended to describe how hormone levels and bone microenvironment differ between pre- and post-menopausal patients and the implications for tumour growth and response to therapy. Funded by Yorkshire Cancer Research (YCR), the study was approved in 2016 and opened for recruitment in 2018.

However, the ZOLMENO study was significantly affected by the COVID-19 pandemic resulting in lower than planned recruitment, resulting in the translational studies being scaled down and several of the original aims no longer being feasible. This chapter describes the original study plan, the approval process, patients recruitment and samples analysis.

2.2 Introduction

Bisphosphonates are a class of drugs that have been clinically available since the 1990s. They are synthetic analogues of pyrophosphate, all have in common the P-C-P backbone structure and differ from each other only at the two R side chains (Figure 1.2 chapter 1). There are two classes of BPs, the nitrogen-containing such as alendronic acid, ibandronic acid, and ZOL and the non-nitrogen-containing BPs such as etidronate and clodronate. All BPs inhibit bone resorption by attaching to hydroxyapatite binding sites on the bone, especially in areas with active resorption. As osteoclasts resorb bone, the BP embedded in the bone is released and impairs the osteoclasts' ability to continue bone resorption (227-229). Despite of their fast clearing from the circulation, they remain in bone where they continue to act (230, 231).

Bisphosphonates are mainly known for their established role in treatment and prevention of osteoporosis. In breast cancer, they are indicated for both early and metastatic disease. They have been found to have anticancer effects, prevent and treat cancer treatment

induced bone loss (CTIBL), but also treat breast cancer bone metastasis and prevent skeletal related events (SREs) (51, 126) (Table 2.1). This chapter focuses on the role of adjuvant BPs in EBC. A comprehensive review of the use of BPs in breast cancer treatment is described in the introduction of this thesis (chapter 1).

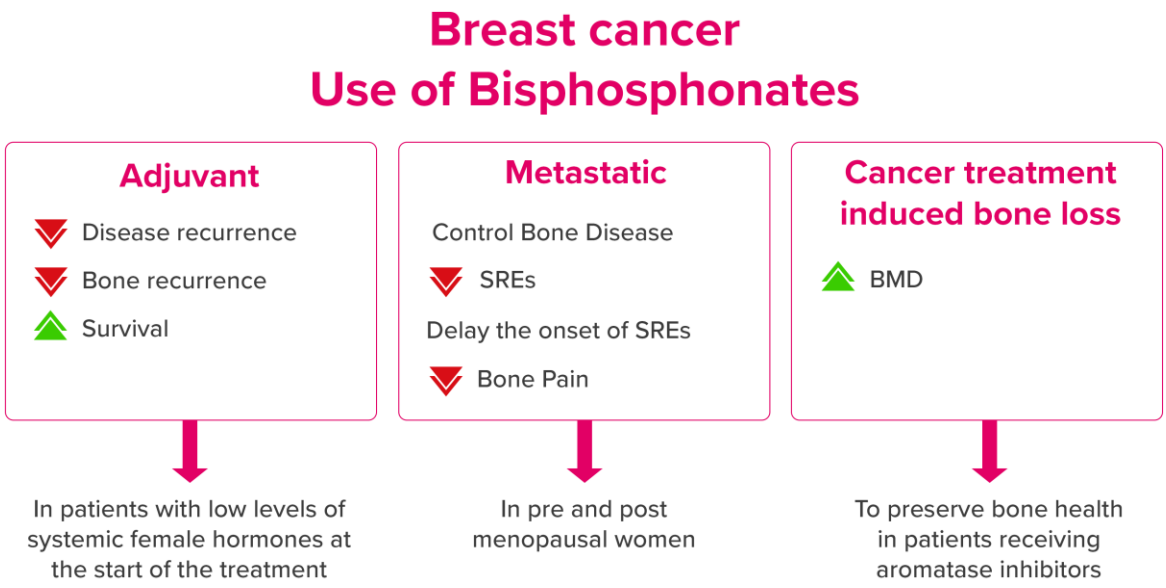

***Table 2.1**: Role of bisphosphonates in breast cancer. SREs – Skeletal- related events, BMD – bone mineral density.*

The role of BPs in EBC has been extensively investigated in preclinical and clinical studies, demonstrating anticancer benefits. Although the mechanism of which BPs lead to anticancer effects in early disease is still unknown, *in vivo* studies have shown the following effects (Figure 2.1):

1) Preclude tumour cells from homing to bone (63, 64, 73).
2) *In vivo* combination with chemotherapy can directly cause tumour cell death in bone (74).
3) Preserve tumour cells quiescence in bone (66, 69).
4) Disrupt the bone metastasis vicious cycle and stop the release of bone-derived tumour growth factors (65, 72, 75-77).

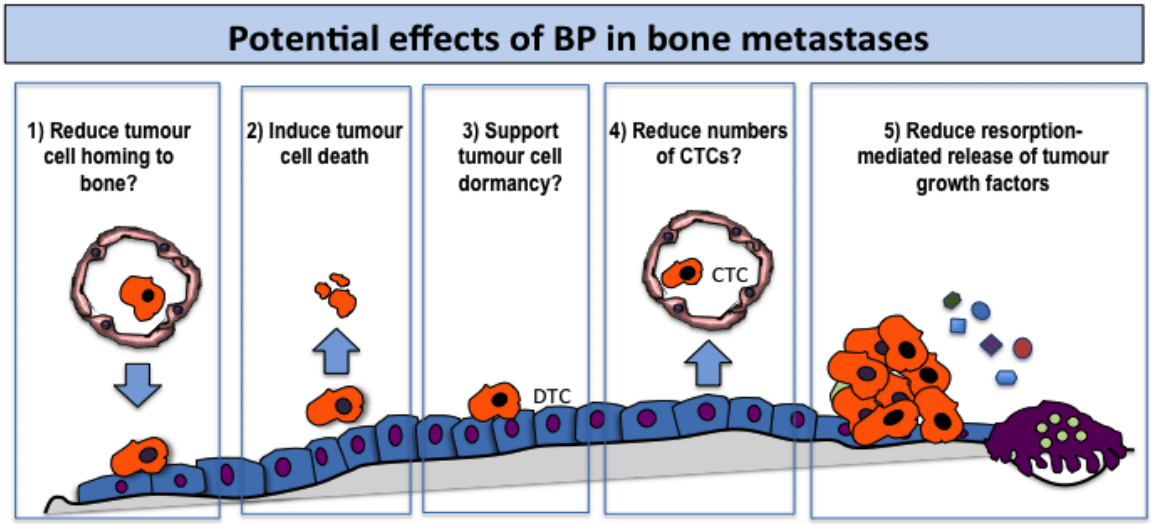

***Figure 2.1**: Potential effects of bisphosphonates (BPs) in bone metastases. Adapted from Hadji et al., 2016 (232).*

Additionally, it has been demonstrated that BPs have negative impact on endothelial cells and angiogenesis which are crucial for the survival of cancer cells. In patients with established metastatic disease, ZOL reduced the levels of serum vascular endothelial growth factor (VEGF) for 21 days post its administration (233). Also, Winter et al. showed that ZOL given during the first cycle of chemotherapy, reduced serum VEGF from day 5 to day 21 of the treatment cycle (234). Animal model studies indicated that BPs increase polarisation of macrophages to M1 antitumour phenotype in mammary tumours and also that they supress paracrine factors such as transforming growth factor-beta (TGF-β) and activin which have potential oncogenic activities (118, 235, 236). Many of these *in vitro* and some *in vivo* studies used high and repeated doses of BPs and therefore, whether these effects are induced by routine clinical use of BPs in patients remain to be established.

Several early clinical trials showed a potential benefit of BPs in EBC disease. They mainly used clodronate (78, 79) and ZOL (80, 81) and showed that BPs given after breast cancer surgery improved survival and reduced bone metastases. Subsequently, the large AZURE (88) and ABCSG-12 (87) trials demonstrated the benefits of adjuvant ZOL in reducing bone metastasis and improving survival, but only in postmenopausal women (either natural or artificial) and not in premenopausal women.

In ABCSG-12, 1803 premenopausal women received adjuvant goserelin with endocrine treatment (oral tamoxifen 20mg/day or anastrozole 1mg/day), with or without ZOL (intravenous 4mg/6 months) for 3 years and after 94.4 months of median follow up, relative risk of disease progression was reduced in the ZOL arm compared to control [Disease free survival (DFS) absolute risk reduction of 3.4%, Overall Survival (OS) absolute risk reduction

of 2.2%] (87). In the AZURE trial, 3360 women were randomised to receive standard adjuvant systemic treatment with or without ZOL (intravenous 4mg every 3-4 weeks for 6 doses, then every 3 months for 8 doses, followed by every 6 months for five doses) for 5 years (88). The overall results showed that DFS and OS were similar in both the ZOL and control groups. However, women who were 5 years into menopause appeared to benefit, with invasive disease free survival (IDFS) of 78.2% in the ZOL arm compared to 71% in the control arm, and OS at 5-year of 84.6% and 78.7% in ZOL and the control group, respectively (88). These results were the first to identify a differential benefit from adjuvant BPs in pre- and post-menopausal women, but as it was a subgroup analysis this required further confirmation.

In 2015, the Early Breast Cancer Trial Collaborative Group published a meta-analysis of 26 randomised trials of adjuvant BPs in EBC which included data from 18766 women (89). In the combined meta-analysis, the most apparent effect of BPs was a reduction in bone recurrence, irrespective of menopausal status. However, confirming the findings from AZURE, subgroup analysis demonstrated a clear benefit in postmenopausal women with decreased overall recurrence, distant recurrence and mortality.

In premenopausal patients, data from both AZURE trial and the subsequent meta-analysis suggested benefit from adjuvant BPs in those patients who were on ovarian suppression therapy at the start of their BP treatment. In contrast, premenopausal women rendered postmenopausal due to chemotherapy did not have the same benefit from adjuvant BPs. This suggests that menopausal status at the initiation of adjuvant BPs is important. These results were supported by preclinical studies which reported that the first interaction between BPs and endocrine/paracrine factors in the bone microenvironment impacts the survival of disseminated tumour cells (DTCs) in both bone and bone marrow microenvironment at diagnosis (99).

Another bone modifying agent, the RANKL (receptor activator of nuclear factor kappa-B ligand) inhibitor denosumab which inhibits the recruitment, maturation and action of osteoclasts and therefore reduces bone resorption, has been also evaluated in a large international randomised trial for its effects in early disease (D-CARE, n=4509) (94). As opposed to BPs, denosumab failed to show any anticancer benefit, suggesting that osteoclast inhibition is not the reason why adjuvant BPs have a positive effect in the postmenopausal population. Identifying the mechanism behind this differential effect of adjuvant BPs will help to determine the group of patients that benefit most from this therapy and also to inform development of new treatment approaches for those who do not benefit from it.

The clinical study, ZOLMENO, was the first part of my MD project which I started in September 2018. The study was designed aiming to investigate how effects of ZOL are governed by endocrine influences, exploring interactions between hormones, tumour and bone microenvironment which might explain why adjuvant ZOL only has positive survival

effects in postmenopausal breast cancer patients. The first 3 months of my degree were spent in getting the study protocol finalised and study approvals in place.

This single centre study was run at Weston Park Hospital (WPH), Sheffield, UK and was funded mainly by YCR. It aimed to recruit 80 patients (40 pre- and 40 post-menopausal) with EBC. Patients were randomised to receive a single 4mg IV (intravenous) dose of ZOL, either 7 days prior or 21 days post breast cancer surgery. Serum, tumour and bone marrow samples were collected for translational studies, allowing us to establish the differential effects of ZOL in women with different menopausal status.

2.3 My role in the ZOLMENO study

My role as a Clinical Research Fellow in the ZOLMENO study included non-patient and patient facing activities.

Non-patient facing activities were the following:

1) Finalising the ZOLMENO study protocol.
2) Preparing all the substantial and non-substantial ethical amendments for the study.
3) Keeping track of all the study paperwork and making sure the study folder was always up to date.
4) Organising and chairing meetings with the stakeholders.
5) Organising and preparing the 4 monthly Trial Management Group (TMG) meetings. Following the TMG meetings, I was responsible for the preparation of the TMG meeting minutes. Also, sponsor was kept up to date about the TMG meetings by me.
6) Preparing the study Developmental Safety Update Report (DSUR) and the Annual Progress Report (APR) for the study.
7) Acting on all the recommendations and suggestions of the sponsor after their 2 monitoring visits.
8) Completing all the study Case Report Forms (CRFs) and acting on all the findings of the Site Data Verification Reports (SDVs) that were created by the study Data Manager.

The patient facing activities are explained in Figure 2.2.

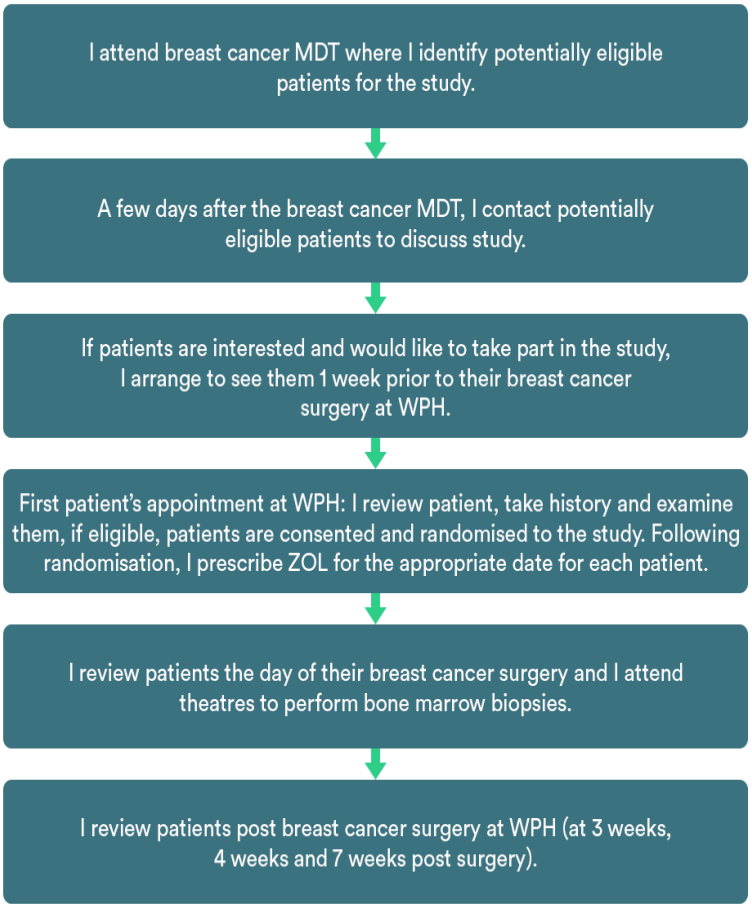

***Figure 2.2**: My patient facing activities in the ZOLMENO study. MDT – Multidisciplinary team.*

2.4 Hypothesis, Aims and Objectives

Hypothesis

- The mechanism responsible for the differential effects of adjuvant ZOL seen in pre- and post-menopausal women with EBC is caused by hormone-driven alterations of the tumour and bone microenvironment.

Aims

- Identify the mechanisms responsible for the above differential effect of ZOL.
- Describe the group of patients who benefit the most from adjuvant BPs.

Primary Objective

- Determine the changes in follistatin levels in premenopausal and postmenopausal women following ZOL administration before and after surgical excision of the primary tumour.

Secondary and Tertiary Objectives

- Compare changes in activin levels following ZOL infusion and determine how these differ depending on menopausal status and timing of ZOL administration (pre- vs. post-surgical).
- Identify histomorphological and immunohistochemical changes in the tumour microenvironment and determine how these relate to menopausal status and ZOL administration.
- Identify genetic and histomorphological changes in the bone marrow microenvironment and determine how these relate to menopausal status and ZOL administration.
- Determine the ability of serum from participants in different treatment groups to modify the aggressiveness of breast cancer cells *in vitro.*
- Identify changes in the bone and tumour biomarkers and proteomic profiles of serum, plasma, tumour and bone marrow samples from the different patient groups.

2.5 Methods

2.5.1 Study design and setting

The ZOLMENO study was a single centre, open label, randomised, proof of concept study, with Professor Janet Brown being the principal investigator (PI). Premenopausal (n=40) and postmenopausal (n=40) women due to undergo primary surgery for EBC were randomised on a 1:1 basis to receive a 4mg infusion of ZOL either pre-surgically (Group A) or post-surgically (Group B). This was aimed to create 4 patient groups for comparison: 20 premenopausal women receiving pre-surgical ZOL; 20 postmenopausal women receiving pre-surgical ZOL; 20 premenopausal women receiving post-surgical ZOL and 20 post-menopausal women receiving post-surgical ZOL (Table 2.2). The protocol of the study can be found in Appendix 2.1.

	Group A **Pre-**surgical ZOL	**Group B** **Post-**surgical ZOL
Premenopausal women	n=20	n=20
Postmenopausal women	n=20	n=20

Table 2.2*: The ZOLMENO study proposed patient recruitment plan showing 4 different groups.*

This was an open label study as it was not ethically justifiable to offer or perform unnecessary additional bone marrow tests on patients, as would be required if patients or

clinicians were to be blinded to the treatment schedule. However, analysis of biological samples and endpoints will be performed without knowledge of treatment allocation, reducing the risk of analytical bias.

During the period September 2018 and November 2018, multiple meetings took place with all the involved parties in order to establish a well-ordered patients' pathway. Diagram 2.1 is illustrating all the parties involved in the ZOLMENO study, their role and the purpose of the meetings.

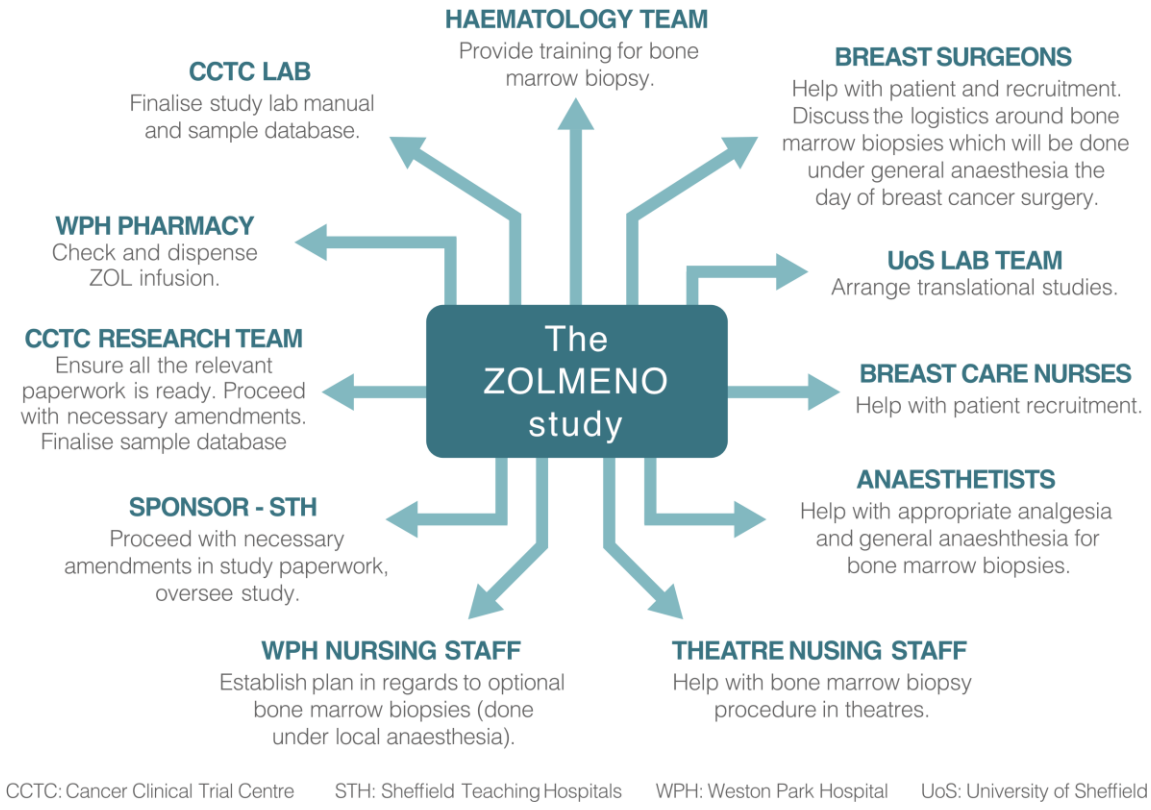

***Diagram 2.1**: Overview of the teams involved in the ZOLMENO study.*

2.5.2 Regulatory approvals

The ZOLMENO study received NHS Research Ethics Committee (REC) approval on 13 May 2016 (REC reference: 16/YH/0151) (Appendix 2.2 & 2.3). Medicines and Healthcare Products Regulatory Agency (MHRA) approval received on 11 July 2016 (Appendix 2.4) and Health Research Authority (HRA) approval was received on 9 May 2017 (Appendix 2.5). The study was then approved by the Research and Development department of Sheffield Teaching Hospitals, NHS Foundtaion Trust. The full ZOLMENO study protocol is included in Appendix 2.1.

2.5.3 Patient eligibility criteria

Inclusion Criteria

- Female patients aged ≥ 40.
- Histologically confirmed early breast cancer.
- Tumour size more than 1cm (≥ T1).
- Any nodal status including unknown (≥N0).
- Scheduled for surgery as primary treatment.
- Any tumour hormone receptor (ER/PR) or HER2 status.
- ECOG (Eastern Cooperative Oncology Group) performance status of 0,1 or 2.
- Menopausal status defined clinically by menstrual and clinical history, or where this is indeterminate patient is willing to have biochemical profile testing following consent.
- Clinical biochemistry:
  - Measured or calculated Glomerular Filtration Rate (GFR) ≥30 ml/min (Cockcroft and Gault formula).
  - Serum corrected calcium ≥2.2mmol/L.
- Clotting screen:
  - aPTT (Activated partial thromboplastin time) ≤30.5 seconds.
  - PT (Prothrombin time) ≤13.2 seconds or INR (International normalised ratio) <1.5.
  - Platelets ≥100 x 10^9/L.
  - Or clotting abnormalities which are due to be reversed as part of standard care by the time of bone marrow sampling (e.g. stopping anticoagulants prior to surgery).
- Potentially fertile women must:
  - have a negative pregnancy test within 72 hours prior to randomisation and not be breastfeeding.
  - agree to use effective, medically approved, barrier contraception from the time of consent to 30 days after their ZOL infusion.
- Potential participants must:
  - be willing to have the required mandatory samples taken, including bone marrow aspiration and trephine at the time of surgery.
  - have the mental capacity to understand the study information, make an informed choice regarding participation and to provide written informed consent.

Exclusion Criteria

- Any previous diagnosis or treatment of cancer that could confound results and endpoints (allowed situations include non-melanomatous skin cancer or superficial bladder cancer).

- Patients with an estimated life expectancy of <6 months.
- Any diagnosis of a bone marrow disorder.
- Any previous BP treatment.
- Use of hormone replacement therapy (HRT) or continued use of oral contraceptives / implant / depo injection in the past 30 days or a diagnosis of hormonal imbalance such as polycystic ovarian syndrome.
- Current active dental problems including dental abscess or infection of the jawbone (maxilla or mandible), any open oral wounds or a current or previous diagnosis of osteonecrosis of the jaw.
- Recent (within 4 weeks) or planned dental or jaw surgery (recent dental fillings, scale and polish or minor gingival surgery do not exclude the patient).
- Any other serious medical or psychiatric condition, which, in the opinion of the investigator, could affect participation in the ZOLMENO study. This may include dehydration, notable electrolyte disturbances, significant use of nephrotoxic, anti-angiogenic or hypocalcaemia-inducing drugs or history of significant renal failure, which would render the patient unsuitable for ZOL or sample collection.

Definition of Menopausal Status

Premenopausal:

- Women 40-54 years of age and
- regular or frequent menses without the use of oral contraceptives or HRT

Postmenopausal:

- Women aged ≥55, or
- Women with an intact uterus and absence of menses for ≥12 months, or
- Women who have undergone bilateral oophorectomy

Women who did not meet any of these criteria, including those who had undergone hysterectomy, thyroidectomy and those who had been receiving HRT, cannot accurately had remaining ovarian function determined by clinical assessment and they therefore had biochemical testing performed. This included serum FSH (follicle-stimulating hormone) and, where the FSH level was indeterminate, LH (luteinising hormone) and oestradiol levels were performed. Women who did not fit all the biochemical criteria of being postmenopausal (perimenopausal patients) were classed as premenopausal.

2.5.4 Recruitment and Patient pathway

Potentially eligible patients for the ZOLMENO study were identified at the weekly breast cancer Multidisciplinary team (MDT) meeting. Patients underwent screening for the ZOLMENO study in order to make sure that they met all the inclusion criteria and none of the exclusion criteria (= screening patients). Screening patients were invited to participate in the study and once they signed the study consent form and have undergone randomisation, they were recruited to the study. Recruited patients followed the patients' pathway based on which group (A or B), they had been randomised to (Figure 2.3).

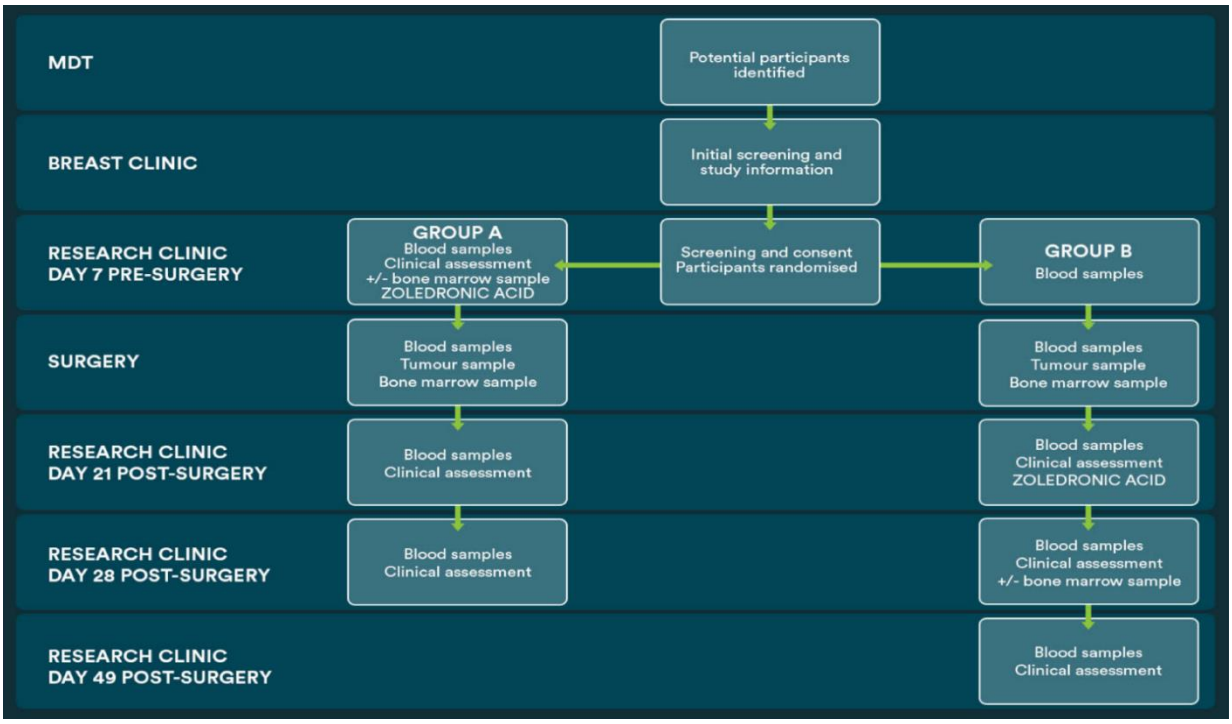

***Figure 2.3**: The ZOLMENO study patient pathway.*

The ZOL was prescribed and authorised by me on the NHS electronic system called Chemocare. Chemocare is an electronic system used by the Sheffield Teaching Hospitals for all the anticancer systemic treatments prescriptions. Following the authorisation of the ZOL infusion, oncology pharmacy was checked the prescription and dispensed the infusion. Then, the ZOL was administrated to the study participants by the chemotherapy nurses at the Clinical Research Unit at WPH.

2.5.5 Promoting the study to aid recruitment

The ZOLMENO study was introduced to the eligible patients by the breast surgeons and breast care nurses at their first outpatient appointment when they received their breast cancer diagnosis. Patients were given the study patient information sheet (PIS) which

explained in detail the study rationale, logistics and also included information about ZOL (Appendix 2.6). Patients were followed up by a telephone call by me to discuss the study.

Following feedback from both patients and breast care nurses, it was decided to design a study post card which would be given to the patients with the PIS, aiming to give quick and easy information about the study. With the support of the Patient and Public Involvement team, I designed the following post card (Figure 2.4).

The post card was approved by both REC and study sponsor (Sheffield Teaching Hospitals, NHS Foundation Trust) on 11th April 2019.

***Figure 2.4**: Study information post card.*

2.5.6 Sample collection

2.5.6.1 Serum samples

All the study participants, pre- and post-menopausal women, provided blood samples which were collected, before and after their breast cancer surgery and also before and after administration of ZOL (Figure 2.5).

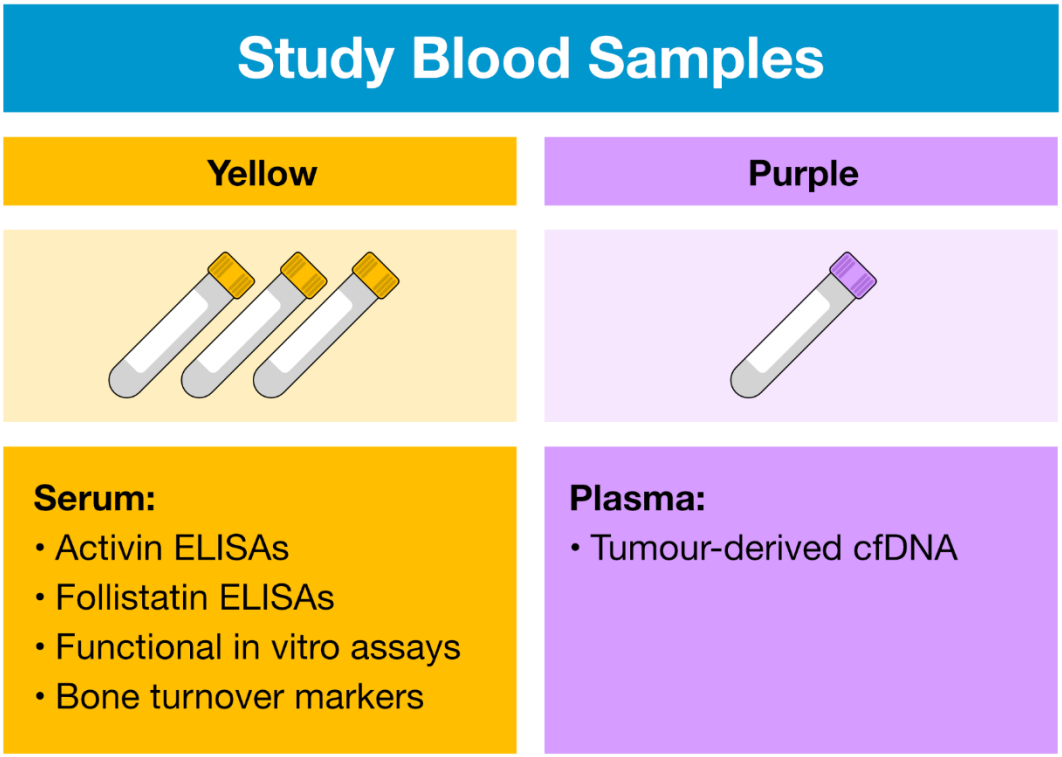

***Figure 2.5**: Study blood sample collection.*

Table 2.3 shows the study times that patients had blood tests based on the study group that they were randomised to.

	GROUP A	GROUP B
Day -7	ZOL administration, Serum samples	Serum samples
Day 0 - Surgery	Serum samples	Serum samples
Day 21 Post-surgery	Serum samples	ZOL administration, Serum samples
Day 28 Post-surgery	Serum samples, End of the study	Serum samples
Day 49 Post-surgery		Serum samples, End of the study

***Table 2.3**: Study serum samples based on study group.*

Blood samples were collected in called serum separator tubes (yellow top vials) which contained a gel to separate blood from serum on centrifugation.

ELISA measurements of reproductive hormones were planned to be used in order to determine the changes in follistatin and activin levels in both pre- and post-menopausal women, before and after ZOL and before and after surgical removal of breast cancer. Commercially available ELISA kits are available, FST (follistatin) ELISA kit for human follistatin and human Activin A ELISA kit for activin.

Additionally, serum was intended for functional *in vitro* assays in order to assess the effect of patient serum to modify breast cancer cells, and whether this was affected by ZOL. Functional *in vitro* assays are explained in more details in section 2.5.7.6. The plan was to match patients' ER and PR (oestrogen/progesterone) status to a human breast cancer cell line and carry out functional studies using the correspond breast cancer cell line. If the patient was ER positive, ER positive human breast cancer cell line (MCF-7) would have been used and if the patient was ER negative, ER negative human breast cancer cell line (MDA-MB-231) would have been used. Breast cancer cells were planned to be cultured in patients' collected serum and proliferation (MTT assay, section 2.5.7.6) of breast cancer cells would have been performed comparing pre and post ZOL serum. Effects found in the functional assays would have been investigated further using reversal experiments where recombinant human endocrine (inhibin) and paracrine (activin, follistatin) factors (commercially available) would have been added to the cultures to identify the factors responsible for the effects. These results would have helped us to understand whether the soluble factors in pre- and post-menopausal serum have different abilities in modifying the aggressiveness of breast cancer cells and whether this is affected by ZOL.

In addition to the serum, plasma samples were collected to allow future detection and analysis of tumour-derived cfDNA.

2.5.6.2 Bone marrow biopsies

Patients underwent a bone marrow biopsy under general anaesthesia on the day of their breast cancer surgery. Bone marrow aspirates and bone marrow trephines were collected. Bone marrow biopsies were performed by me in surgical theatres. This is an interventional procedure for which I received relevant training prior to the opening of the ZOLMENO study by the haematology team of the Sheffield Teaching Hospitals (Dr Andy Chantry, Dr Becky Andrews and Dr Jack Goddard). Bone marrow biopsies are routinely performed by this haematology team under local anaesthesia. Only in exceptional circumstances, for example when multiple samples are needed from an individual, is bone marrow biopsy performed under general anaesthesia.

The equipment which was used for the bone marrow biopsies is shown in Figure 2.6a and Figure 2.6b. Traditionally, bone marrow biopsies are performed using the manual biopsy needles (Figure 2.6a). However, recently the Arrow® OnControl® Powered Bone Marrow

Biopsy System (Figure 2.6b) has been introduced and received approval by the NICE (237). This is a battery-powered device and in contrast to the manual biopsy needles (2 different needles), a single needle technique is used to perform bone aspiration and trephine. The Arrow® OnControl® system is thought to be generally easier to be used which makes the whole experience better for both patients and clinicians and improves the quality and efficacy of the procedure.

The manual biopsy needles were used for the bone marrow biopsies that were performed in 2019. However, due to the advantages of the Arrow® OnControl®, this was preferred for the biopsies that were performed in 2022.

***Figure 2.6a**: Bone marrow biopsy disposable needles. Needle for bone marrow trephines on the left and needle for bone marrow aspirate on the right.*

***Figure 2.6b**: Arrow® OnControl® Powered Bone Marrow Biopsy System (source:teleflex.us.com)*

2.5.6.2.1 Bone marrow aspirate samples

Bone marrow aspirates were collected following the standard procedure for bone marrow biopsies (site: upper pelvic bone – posterior iliac crest). Patients were also asked if they wished to undergo a second optional bone marrow aspirate either 7 days prior to breast surgery or 28 days post breast surgery, which was performed under local anaesthesia. Patients were not asked about the optional bone marrow biopsy during 2021-2022 due the COVID-19 pandemic hospital restrictions.

PAX tubes were used to collect bone marrow aspirates. A subset of storage samples (due to cost limitations probably ~5/group) was planned for gene expression analyses using the latest next generation sequencing (NGS) technologies. Next generation sequencing would have been performed on an Illumina HiSeq2500 platform using standard operating procedures. The gene expression profiles of the bone marrow of each patient would have been compared before and after ZOL treatment and also between pre- and post-menopausal women. RNA sequencing reads are aligned to the reference human genome, by using standard bioinformatics tools (Tophat2), and gene expression is calculated by counting read coverage for each gene (HTSeq-Count). Then, statistical analysis would have been performed to generate a list of genes differentially expressed between the conditions under study (DESeq). The Holen lab will pursue the additional funding needed to complete these studies.

2.5.6.2.2 Bone trephine samples

Bone trephine samples were collected the same time as bone marrow aspirates from the participants. Patients underwent a mandatory for the purpose of the study bone trephine the day of their breast surgery (under general anaesthesia) and they were also offered an additional optional bone trephine under local anaesthesia either 7 days prior to breast surgery or 28 days post breast surgery. The optional bone marrow biopsy was not taking place during 2021-2022 due to the COVID-19 pandemic hospital restrictions. Bone trephines were collected following the standard procedure for bone marrow biopsies (site: upper pelvic bone – posterior iliac crest).

Samples were collected in paraformaldehyde (PFA; 4%) and after 48 hours, the solution was changed to phosphate-buffered saline (PBS). PFA was used for fixation of the samples (preserve samples for future use), and it was prepared as follow: A volume of 1X PBS (pH 7.4) equal to the desired final volume of PFA was measured and heated to 45^0C. A quantity of paraformaldehyde powder that would make up to 4% (w/v) solution to the heated PBS solution (for example 100ml = 4g PFA) followed by heating to 55^0C with stirring until the solution became clear and all PFA was dissolved. Upon removal from heat, the solution was cooled on ice and then was stored at 4^0C or aliquoted into tubes and frozen for later use.

Following microCT (micro computed tomography) analysis (section 2.5.6.3), trephines were decalcified by using Ethylenediaminetetraacetic acid (EDTA) for a period of 2 weeks and then processed for histology. EDTA was prepared as follows: 1L of PBS (one tablet/100ml distilled water) was prepared and approximately 700 ml transferred of it to a clean 1L empty bottle. A few pellets of sodium hydroxide (36) or concentrated NaOH solution were added under stirring. A hundred and eighty six point six grams (186.6g) of EDTA powder [quantity(g)=half of molecular weight of EDTA(g)] were added in several stages until dissolved. The optimal pH for EDTA is 8 as EDTA will not otherwise dissolve, hence additional NaOH pellets/solution is added if required. Once the EDTA was completely dissolved, the solution was made up to 1L by adding PBS.

The decalcification process was necessary in order for the samples to be sectioned for histological analysis. Decalcified samples were embedded in paraffin wax and then sectioned by using a microtome and the section thickness is 3u. Sectioned samples were stained with haematoxylin and eosin stain (H&E) and tartrate-resistant acid phosphatase (TRAP). Haematoxylin and eosin stain stains cell nuclei blue and extracellular matrix and cytoplasm pink, and TRAP is a histochemical marker of osteoclasts. Osteomeasure software was intended to be used to detect and quantify osteoclasts and osteoblasts per mm bone surface.

2.5.6.3 Micro computed tomography (MicroCT) for bone trephine

Bone trephines underwent microCT which gave us a three-dimensional volume of the sample and allowed us to evaluate the morphometric characteristics of the bone trephines. The basic principles of MicroCT are similar to those of medical computer tomography with the sample to be placed in the path of an x-ray beam which is producing a projection image on the scintillator or other x-ray sensitive detector array (238). The trephine sample is rotated and imaged at a large number of angles, and the sequence of projection images is "back-projected" to reconstruct the x-ray absorption at each point within the scanned volume (238).

Bone volume (BV in mm^3), percent bone volume (BV/TV in %), trabecular thickness (in mm) and trabecular number (in mm^{-1}) were analysed using a SkyScan 1272 (SkyScan). Samples were scanned using a currency of 200mA, 51kV, a 0.5 mm aluminium filter and medium camera resolution of 2016x1344. Pixel size for all scans is set at 4.3µm. For each sample, images were reconstructed using NRecon software and bone parameters were calculated using CTAn software.

2.5.7 Cell culture

Part of the project was to determine if administration of ZOL modified soluble factors reducing cancer cell growth. To explore this, breast cancer cells would be grown in medium containing serum collected from patients before and after ZOL administration. As these studies would be carried out with batched serum, they could not be carried out until recruitment was completed and all samples collected. To prepare the design of these studies I carried out some preliminary work to establish the growth conditions and seeding densities of human breast cancer cell lines, using standard medium containing foetal calf serum (FCS), that would form the basis for similar studies using serum samples collected from patients. For the tissue culture studies, human breast cancer cell lines were used. Handling of human breast cancer cells was performed in a category 2 laboratory inside a microbiological safety class 2 cabinet. All the techniques were fully aseptic, and all the biosafety requirements of the European Collection of Authenticated Cell Cultures (ECACC) were followed.

2.5.7.1 MDA-MB-231 human breast cancer cell line

The MDA-MB-231 cell line is one of the most commonly used breast cancer cell lines in laboratory medicine. It is an epithelial human breast cancer cell line which was first descripted in the 1970s, isolated from a pleural effusion of a 51 year old woman with metastatic breast adenocarcinoma (239). MDA-MB-231 cells are triple negative breast cancer (TNBC) cells as they don’t express oestrogen receptors (ER), progesterone receptors (PR) or human

epidermal growth factor receptor 2 (HER2), fact which is making them aggressive and poorly differentiated (240, 241).

MDA-MD-231 cells were planned to be added to the serum of patients with ER-ve (oestrogen receptor negative) breast cancer for the purposes of the tissue culture studies.

2.5.7.2 MCF-7 human breast cancer cell line

MCF-7 human breast cancer cell line is one of the most studied ER+ve (oestrogen receptor positive) breast cancer cell lines. It was initially established in a pleural effusion of a female patient with breast adenocarcinoma (242, 243). This cell line has characteristics similar to epithelium and expresses oestrogen and progesterone receptors (244-246). Hence, MCF-7 cells were planned to be exposed to the serum collected from the patients with ER+ve breast cancer for the purpose of the tissue culture studies.

2.5.7.3 Routine maintenance of monolayer cell cultures

Both MDA-MD-231 and MCF-7 cells were maintained *in vitro* as monolayer cell cultures grown in growth medium, containing RPMI (Roswell Park Memorial Institute)-1640 basal medium (11mM glucose, 0.14mM L-glutamate) supplemented with 10% foetal bovine serum (FBS), in sterile 75cm^2 tissue culture flasks (T75). The total volume of both cells and growth medium was kept at 10ml regardless of the cell density and flasks were incubated in a water-jacketed incubator at 37^{0}C with 95% air, 5% CO_2 and 100% relative humidity. The growing cells were monitored daily and were divided when cell coverage occupied around 80% of the available flask.

2.5.7.4 Retrieval of adherent monolayer cultures

When cells were ready to be sub-cultured, medium was removed from the flask and adherent cell monolayer cultures were washed twice with PBS (pH 7.4, 37^{O}C). One millilitre (1ml) of 0.25% trypsin-EDTA solution (2.5 g porcine trypsin and 0.2 g EDTA) was used in order for both MDA-MD-231 and MCF-7 cells to be detached from the flask which was incubated for 2-5 minutes (water-jacketed incubator at 37^{0}C with 95% air, 5% CO_2 and 100% relative humidity). Then, 5ml of fresh growth medium was added in order to stop the action of trypsin, with the total volume to be transferred to a centrifuge tube and centrifuged for 5 minutes in 800rpm. Supernatant was removed and cells were resuspended in 10ml of fresh growth medium. Finally, 0.5-1ml of the cell suspension transferred to a new T75 flask containing 9-9.5ml of growth medium (total volume 10ml).

2.5.7.5 Haemocytometric counting of cell suspensions

Haemocytometric counting was used to determine cellular concentration. Process started by washing the cells which were in a T75 flask with sterile PBS (pH 7.4, 37^{O}C) twice followed by addition of 1ml of 0.25% trypsin - EDTA solution and incubated for 2-5 minutes in order for the cells to be detached from the flask (water-jacketed incubator at 37^{0}C with 95% air, 5% CO_2 and 100% relative humidity). Five millilitres (5ml) of fresh growth medium was added to the cells which then were transferred to a sterile tube using a sterile pipette ensuring single cell suspension. Nine microlitres (9µl) of suspension were loaded into the haemocytometer chamber and by using an inverted microscope cells were counted in the four primary squares of grid of the haemocytometer (cells counted within the grid, along the top and left side of it only, cells were not counted on the right and bottom side of the grid) (Figure 2.7).

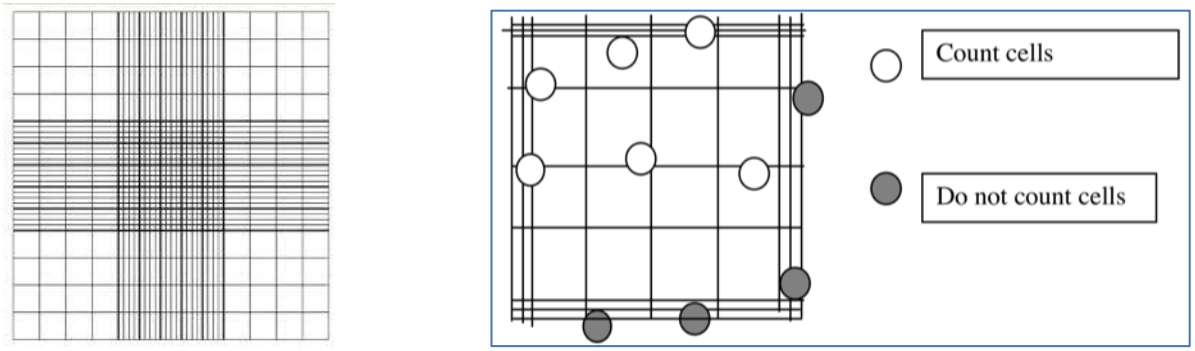

Figure 2.7: *Haemocytometric counting of cell suspensions.*

The total number of the cells was calculated after dividing the total number of cells counted in the four primary squared by four, then multiplied that number by the original volume from which the cell sample was initially removed and then multiplied again by 10000.

2.5.7.6 MTT assay (proliferation)

The MTT assay is an *in vitro* assay which is widely used to measure cell proliferation and viability. The yellow water-soluble tetrazolium MTT salt [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] is converted to an insoluble purple formazan by the mitochondrial enzymes of the actively respiring, the formazan is then solubilised with dimethyl sulfoxide (DMSO) and its concentration can be read on a spectrophotometer by optical density.

A basic MTT assay was performed by seeding the cells into a sterile 96 well plate at the appropriate cell densities. The following 4 cell concentrations: 1x10^{3}/100µl, 2x10^{3}/100µl, 3x10^{3}/100µ, 4x10^{3}/100µl were used (Figure 2.8). The different cell densities were prepared by

using the haemocytometric counting technique (section 2.5.7.5) to count the cell concertation and then resuspended the pallet of cells in fresh growth medium to a final cell density of $1x10^6$ million cells per ml. From the final cell density of $1x10^6$ million cells per ml, 10µl, 20µl, 33µl, and 40µl were removed and added to 1ml of fresh growth medium (in 4 different sterile tubes) in order to achieve the above cell concentrations. The plate then was incubated for 48 hours (water-jacketed incubator at 37^0C with 95% air, 5% CO_2 and 100% relative humidity).

The MTT is stock in 5mg/ml of sterile PBS and is stored in -20^0C. Prior to use, the required number of aliquots of the MTT were removed, protected from the light and maintained at 37^0C. Aliquots of the MTT were then diluted in 1:5 [v/v in complete growth medium to a final working concentration of 1mg/ml (w/v)].

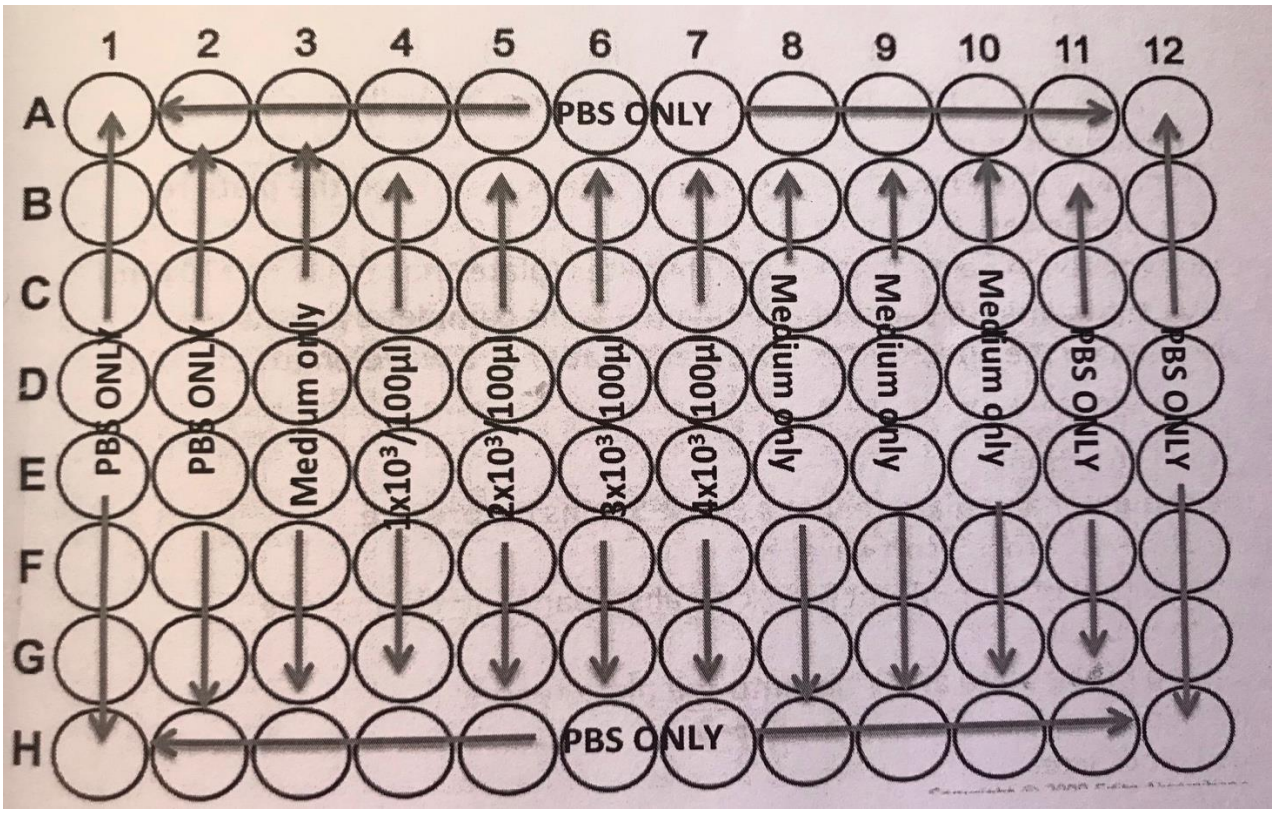

Figure 2.8. *Basic MTT assay – 96 wells plate.*

After the 48hours of incubation, the growth medium was removed from all the wells and 100µl of MTT (1mg/ml) was added to all wells and the plate was covered to protect form the light and incubated (water-jacketed incubator at 37^0C with 95% air, 5% CO_2 and 100% relative humidity) for 3 hours. Following incubation, the MTT was removed and 100µl of DMSO was added to all wells. The plate was re-covered and was agitated in dark for 15 minutes (plate shaker set to 170rpm) in order to ensure the formazan contained within the wells was completely dissolved. The plate was read on the SpectraMax 5Me plate reader at a wavelength of 570nm.

This basic MTT assay has been performed with both cell lines in order to identify the cell density in which cells proliferate the most. Further MTT assays were performed by adding to the appropriate cell density FCS concentrations of 2.5% (100μl of FCS in 4ml of growth medium), 5% (200μl of FCS in 4ml of growth medium), 7.5% (300μl of FCS in 4ml of growth medium) and 10% (400μl of FCS in 4ml of growth medium) in order to identify which FCS concentration caused the greatest cell proliferation (Figure 2.9).

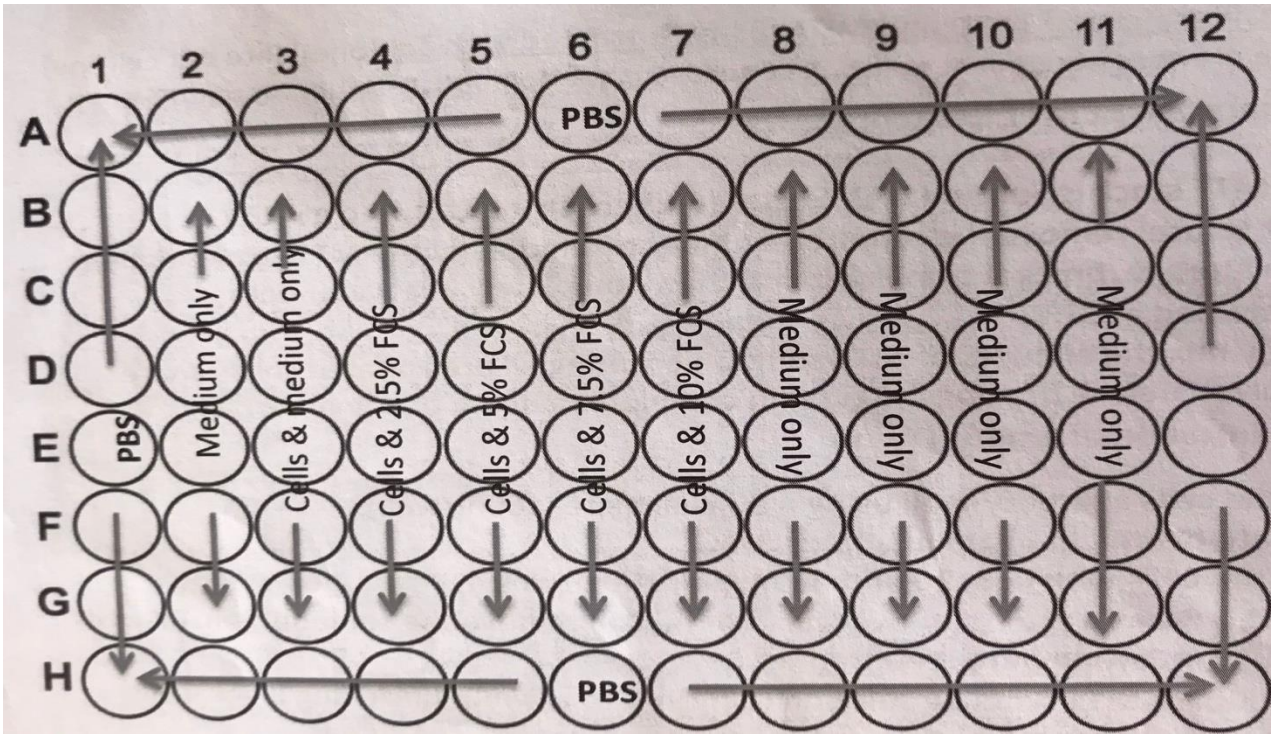

Figure 2.9*: Plate layout - MTT assay with different concentrations of FCS.*

MTT assay was also performed with the presence of ZOL to indicate the concentration of ZOL that led to the lowest cell proliferation. Cells were seeded at 2,000 cells/well in a 96 well plate (100μl/well). Cell concentration was calculated using haemocytometric counting, technique which was descripted in section 2.5.7.5. Well plate was incubated overnight (water-jacketed incubator at 37^0C with 95% air, 5% CO_2 and 100% relative humidity). Cells were treated with 0,5,10 and 25nM ZOL (100μl/well) and incubated initially for 48hrs and 72hrs (water-jacketed incubator at 37^0C with 95% air, 5% CO_2 and 100% relative humidity) (Figure 2.10). After the desired period of exposure to ZOL, solution was removed from all wells and the same MTT assay process that has been described above was followed.

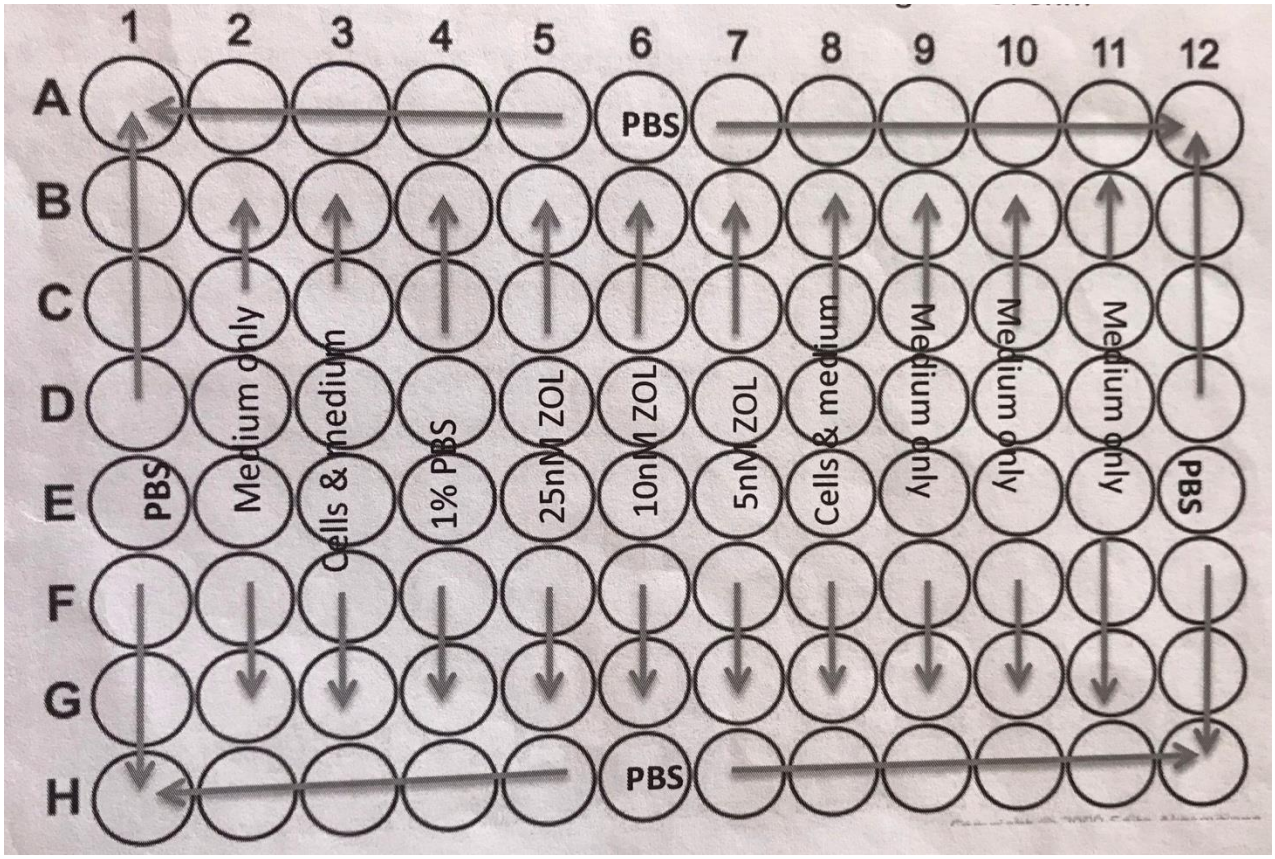

Figure 2.10: *Plate layout - MTT assay testing different concentrations of ZOL.*

2.6 COVID-19 pandemic impact on the study

The study remained open to recruitment until 30th November 2019. No patients were recruited to the study during the period December 2019 to December 2020 due to my absence (maternity leave). My return and therefore the restart of the recruitment period was initially planned for January 2021. Due to the COVID-19 pandemic, this was not feasible as NHS hospital restrictions made the reopening of the study very difficult. With the support of my supervisors, I redeployed to NHS for a period of 9 months (January 2021 – September 2021), with the study recruitment to also remain paused.

In October 2021, I returned to my studies, but the ZOLMENO study did not reopen to recruitment until 17th December 2021. This time was given to allow for the NHS sponsor (Sheffield Teaching Hospitals, NHS Foundation Trust) to perform an interim monitoring visit. Despite the reopening of the study, the patient recruitment was slower than expected mainly due to 3 reasons:

1) The NHS hospital COVID-19 restrictions were still in place affecting the smooth running of the study.
2) Competing breast cancer clinical trials had been approved and started at Sheffield Teaching Hospitals.

3) Due to the backlog of breast cancer patients, many now received endocrine therapy prior to their surgery which made them ineligible for inclusion to the ZOLMENO study.

In addition, for the completion of the proposed translational studies extra funding was required. This was not able to be secured due to the COVID-19 pandemic. Therefore, this had significant implications for the study translational work and also in the amount of data this project could generate. With the support of YCR the study was therefore scaled down, as it was clear that it would not have been able to recruit the number of patients originally planned. In particular, too few premenopausal patients were recruited to allow the comparison of ZOL effects between pre- and post-menopausal groups. However, a large amount of work was carried out to open the trial and recruit as many patients as possible, which is described in the first part of this chapter.

The sample analysis and translational studies which were practical to be performed are described in the next section of this thesis.

2.7 Results

2.7.1 Recruitment to the ZOLMENO study

The ZOLMENO study opened to patient recruitment in December 2018 and 109 patients were screened and 11 were recruited to the study by the end of 2019 (Figure 2.11a). Study remained close to patient recruitment for 2 years and it was reopened in December 2021. The actual patients screening by the breast cancer MDT did not start until January 2022. In 2022, 53 patients were screened and 8 were recruited to the study (Figure 2.11b). In total, 162 patients were screened and 19 recruited to the study. From the study participants, 15 were postmenopausal and 4 premenopausal. Eighteen (18) patients completed the study and 1 withdrew early (soon after breast cancer surgery) due to acute medical issues unrelated to the study.

The reasons that screening patients failed to be recruited to the study are the following (Table 2.4):

1) Patient not interested in participating (47 patients)
2) Not eligible (28 patients, reasons: receiving contraception, other comorbidities, receiving alendronic acid, previous cancer history, for neo-adjuvant chemotherapy, receiving primary endocrine therapy, lack of mental capacity).
3) No time to recruit to the study due to the breast cancer surgery date being too close to the date patient was first contacted (18 patients).
4) Unable to contact patient (9 patients).
5) Busy lifestyle (2 patients).

6) Private patient (1 patient).
7) Unknown reasons (9 patients).

The numbers in the brackets indicate the number of screening patients that failed to be recruited to the study due to each listed reason.

ZOLMENO study screening failure reasons	
Reasons	Number of patients
Patient not interested	47
Not eligible	28
No time to recruit due to breast cancer surgery being too close to the patient first contact	18
Unable to contact patient	9
Busy lifestyle	2
Private patient	1
Unknown reasons	38

Table 2.4: ZOLMENO study screening failure reasons. Table shows the number of screening patients that failed to be recruited to the study due to each listed reason.

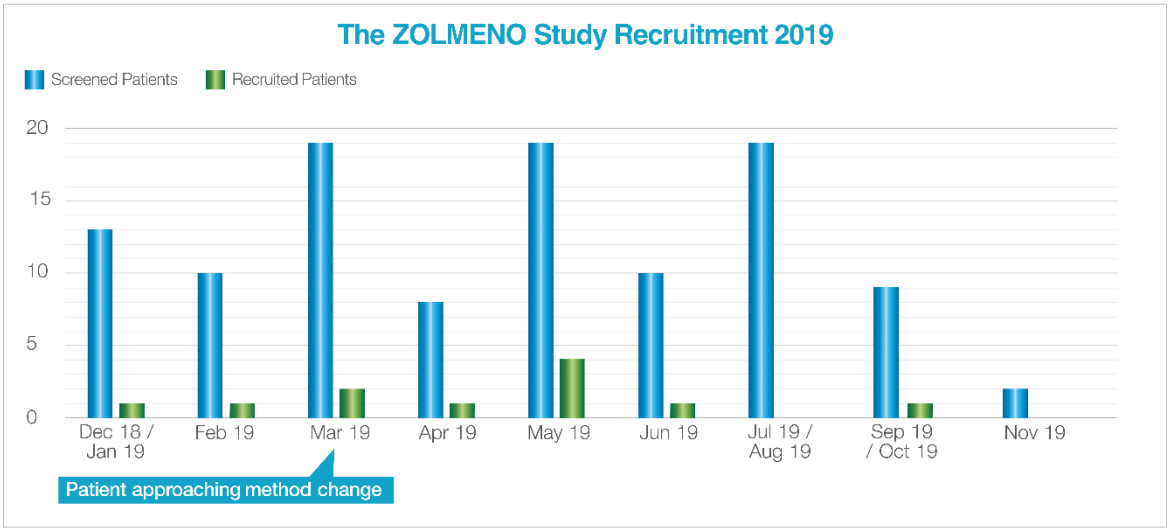

Figure 2.11a. *The ZOLMENO study patient recruitment in 2019.*

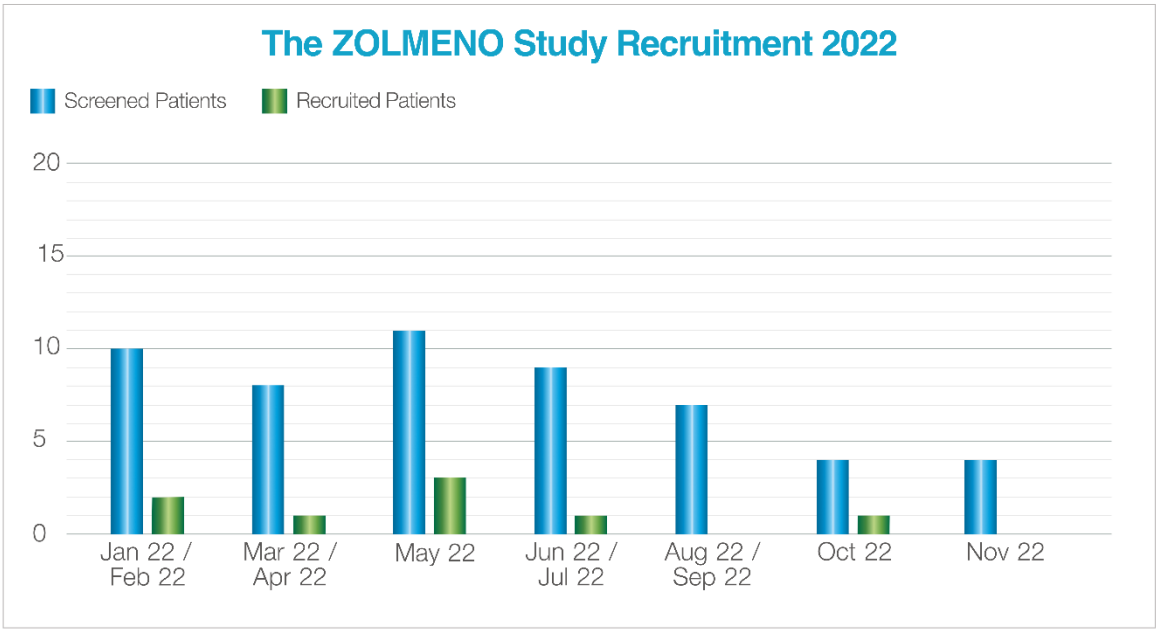

Figure 2.11b. *The ZOLMENO study patient recruitment in 2022.*

Potentially eligible patients were identified at the weekly breast cancer MDT meeting. Before March 2019, patients would have been approached by the surgical team and the breast care nurses at one of their first appointments. Patients were given the study PIS (Appendix 2.6) and if they were interested in participating to the study, I would have contacted them to discuss the study further. Recruitment was slower than expected and the recruitment pathway was assessed to establish if this could be improved. Following discussions with members of the breast cancer MDT, the method for approaching patients was changed in March 2019, introducing changes to the system to improve recruitment. From then onwards, patients identified from the MDT were given the study PIS and received a call from me within a few

days from their breast cancer diagnosis to discuss the study. This new approach improved recruitment significantly and was also positively received by patients as they now had the chance to discuss study in detail with the responsible researcher and helped inform the decision about their potential participation to the study.

All of the patients (18/18) donated the required blood samples. The patient who withdrew from the study only donated blood samples on her first visit. On the day of the breast cancer surgery, bone marrow aspirates were performed for 11 patients (11/18) and bone marrow trephines for 9 patients (9/18). Only one patient (1/18) consented and underwent the optional bone marrow and therefore the total number of bone marrow aspirates that were collected was 12 and the total number of bone marrow trephines was 10.

The study did not report any serious side effects related to ZOL and no serious complications due to bone marrow biopsies were recorded.

2.7.2 Translational studies

Patients' serum was planned to be used for functional *in vitro* assays and for ELISA measurements of hormones. Patients' plasma was collected for future detection and analysis of tumour-derived cfDNA. Bone marrow aspirates were collected for gene expression analysis and bone trephines for microCT and histological analysis. Overall, these translational studies aimed to identify whether ZOL modifies the bone microenvironment differently in pre- and post-menopausal women.

Table 2.5 shows an overview of the samples that I collected from the study participants.

			Patients who had blood samples					Bone Aspirates	Bone Trephines
		Patients recruited	Day -7	Day 0 Surgery	Day 21	Day 28	Day 49		
Premenopausal	Group A	2	2	2	2	2	X	2	1
	Group B	2	2	2	2	2	2	1	1
Postmenopausal	Group A	6	6	6	6	6	X	5	4
	Group B	8	8	8	8	8	8	4	4

***Table 2.5**: Overview of the ZOLMENO study collected samples. Day -7, Day 0 Surgery, Day 21, Day 28 and Day 49 represent the different time points of the study. Only 1 patient (postmenopausal group) provided bone aspirate and bone trephine on Day 28. All the other bone aspirate and trephine samples were collected on Day 0 surgery. The samples obtained from the 1 patient who withdrew from the study are not included on this table.*

2.7.3 Cell proliferation of ER+ve and ER-ve cell lines and effects of increasing FCS and different ZOL concentrations on these cell lines

As described in section 2.5.7.6, both ER+ve and ER-ve cell lines were used for MTT assays to determine the optimal experimental design for use of the serum samples obtained from patients. The results that are described here are results from 3 assays for both cell lines, basic MTT, MTT with different FCS concentrations and MTT with different concentrations of ZOL. Incubation period was 48hrs for basic MTT and MTT with different FCS concentrations, and 48hrs and 72hrs for the MTT with the different concentrations of ZOL.

Figure 2.12 is showing the proliferation results of MDA-MB-231 and more specifically is showing that the concentration of cells with the highest proliferation was $4x10^3$/100µl. The assay was performed twice as the first time (blue bars – Figure 2.12) the 2 medium alone bars were not equal which was unexpected and unexplained. Therefore, the assay was repeated, and results confirmed that the concentration of cells with the highest proliferation was $4x10^3$/100µl (green bars - Figure 2.12).

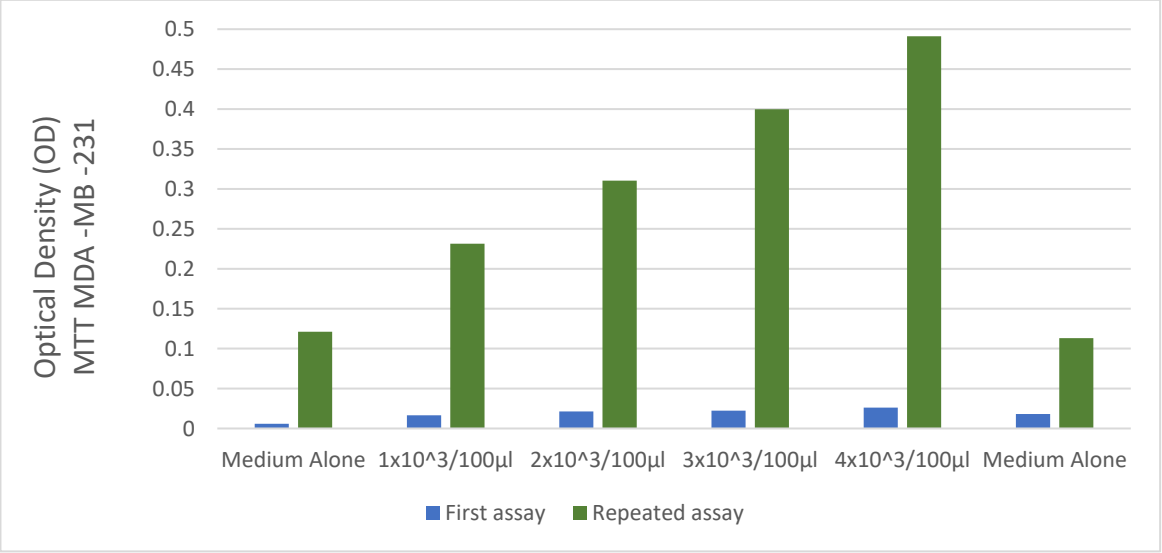

***Figure 2.12**: MTT assays using different densities of MDA-MB-231 cells. Graph shows the OD (optical density) of the different cell concentrations after 48 hours in medium containing 10% FCS. Blue colour represents the first MTT assay and green colour the repeated assay.*

The MTT assay of MDA-MB-231 cells was also performed with the presence of different concentrations of FCS and results are shown in Figure 2.13. The FCS concentration which led to the most cell proliferation was 10%; this concentration should be used for future MTT assays of this cell line.

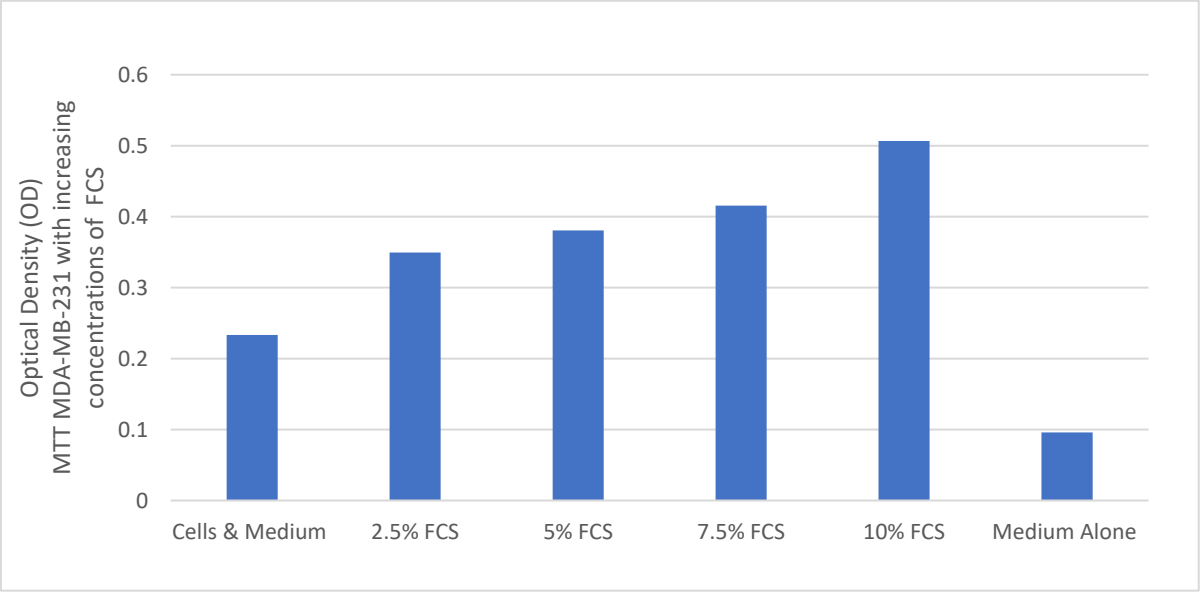

Figure 2.13: *MTT assay of MDA-MB-231 cells in different concentration of FCS. Graph shows the OD of the cells after 48 hours in different concentration of FCS.*

The results from the basic MTT assay with MCF-7 cell line are shown in Figure 2.14. Similar to the results obtained for the MDA-MB-231 cell line, MTT for MCF-7 revealed that the cell concentration with the most proliferation was $4x10^3$/100µl. This cell concentration should be used for future MTT assays for this cell line.

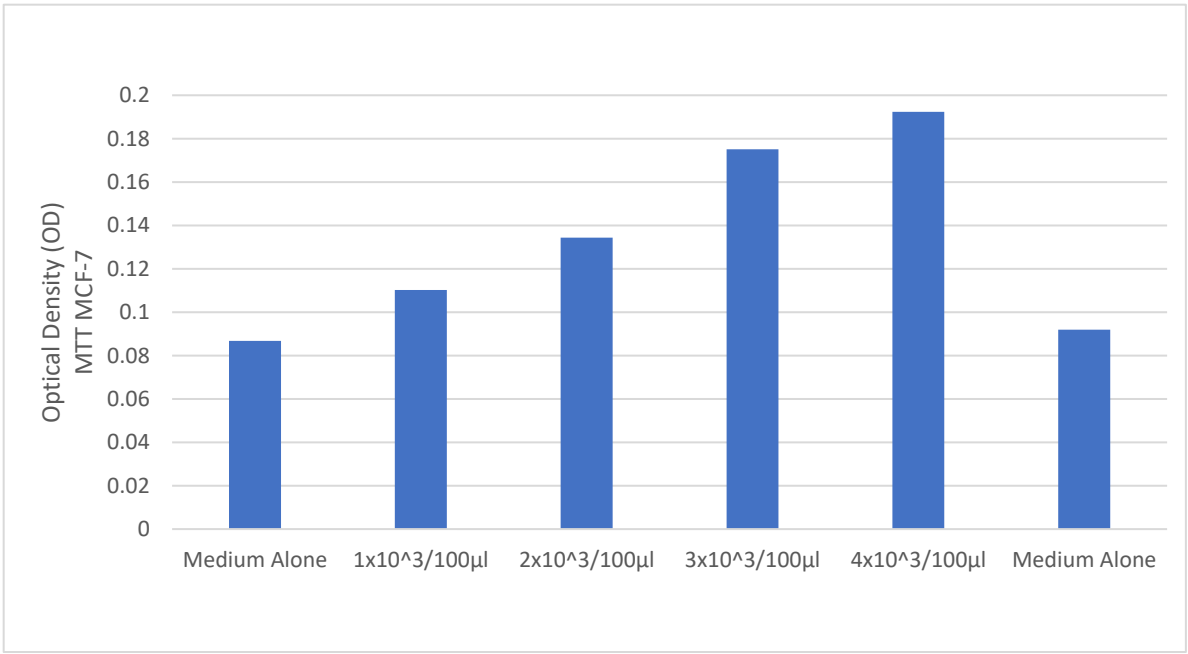

Figure 2.14: *MTT assays using different densities of MCF-7 cells. Graph shows the OD of the different cell concentrations after 48 hours in medium containing 10% FCS.*

The MTT assay of MCF-7 cells was also performed with the presence of FCS and results are showing below (Figure 2.15) The assay was performed 3 times and the FCS concentration which led to the most cell proliferation was 7.5% of increasing concentrations of FCS and this concentration should be used for future MTT assays of this cell line.

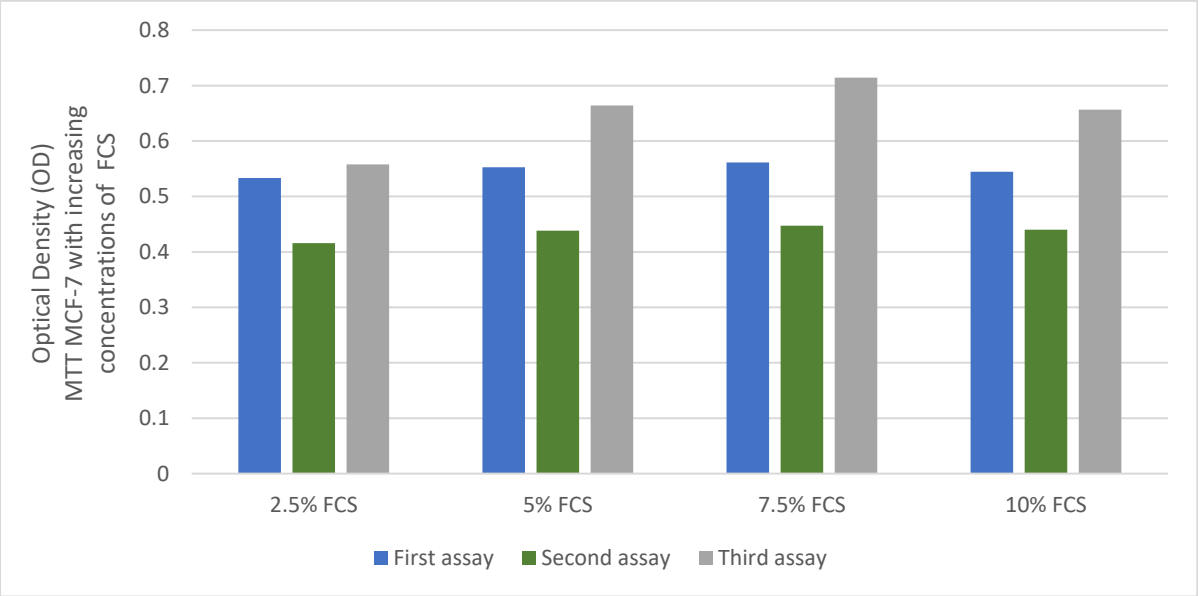

Figure 2.15: *MTT assay of MCF-7 cells in different concentration of FCS. Graph shows the OD of the cells after 48 hours in different concentration of FCS. The experiment was repeated 3 times (blue – first assay, green – second assay, grey – third assay).*

MTT assays for both cell lines were also performed with difference concentrations of ZOL over 48hrs and 72hrs with the aim to identify the concentration of ZOL and incubation period that led to the lowest cell proliferation. For the MDA-MB-231 cell line the MTT ZOL assays over 48hrs and 72hrs are demonstrated in Figure 2.16. For the MCF-7 cell line the MTT ZOL assays over 48hrs and 72hrs are demonstrated in Figure 2.17. For both cell lines, the concentration of ZOL that led to the lowest cell proliferation was 25μM in 48hrs incubation. After 72hrs of incubation, 25μM of ZOL was the concentration that had the lowest cell proliferation of MCF-7. However, in the 48hrs assays for both the cell lines, cells only bars showed lower cell proliferation compared to those that were treated with ZOL, which were unexpected findings. Additionally, no ZOL concentration was identified as the one leading to the lowest cell proliferation in 72hrs incubation for the MDA-MD-231 cell line. Therefore, these assays needed to be repeated but this was unable due to the difficulties that study experienced during the COVID-19 pandemic.

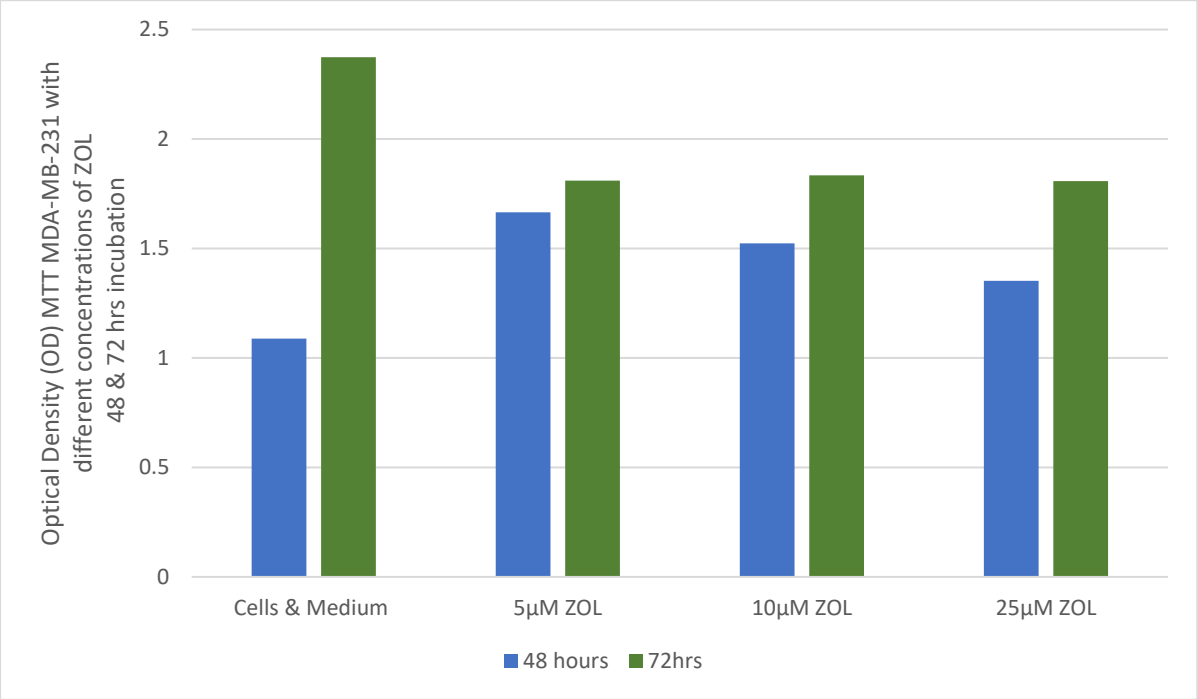

Figure 2.16: *MTT assay of MDA-MB-231 cells in different concentration of ZOL. Graph shows the OD of the cells after 48 (blue) and 72 (green) hours in different concentration of FCS.*

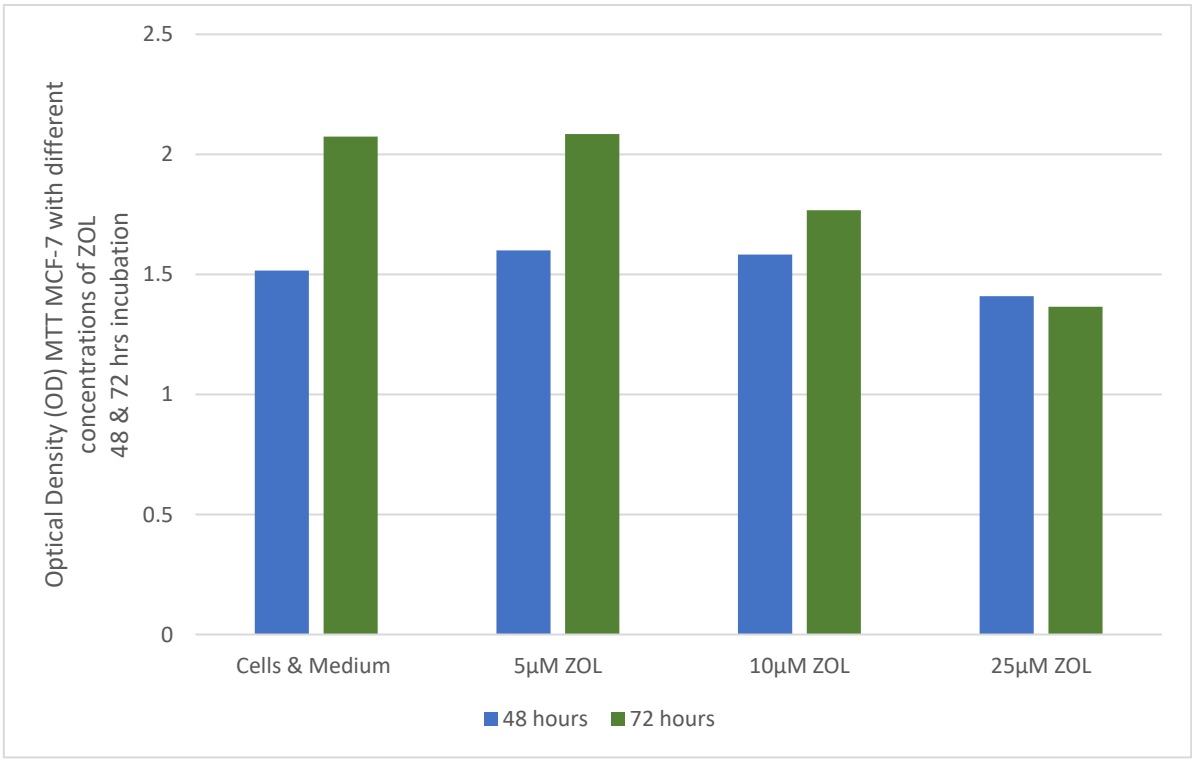

Figure 2.17*: MTT assay of MCF-7 cells in different concentration of ZOL. Graph shows the OD of the cells after 48 (blue) and 72 (green) hours in different concentration of FCS.*

2.7.4 MicroCT analysis of bone trephines

Samples of bone trephines were collected from 9/18 patients. In total, 10 samples were collected with 2 of them to be from the same patient. Only 6 of the samples (2 from the same patient) underwent microCT, as two of the collected samples were unable to undergo microCT due to their limited size, and two were processed without prior microCT. The samples that underwent microCT were all from postmenopausal women. Samples were one post ZOL infusion, 3 prior to ZOL infusion and 2 were from the same patient pre and post administration of ZOL.

The following figure shows the 3D reconstructions microCT images of the bone trephines that they were performed, carried out by Holly Evans, Senior Research Technician at the University of Sheffield (Figure 2.18). A and B images are from the same patient (patient 1), with A obtained the day of breast cancer surgery (D0 on the ZOLMENO study) and prior to the administration of ZOL, and B was donated 28 post-surgery and 1 week post administration of ZOL (D28 on the ZOLMENO study). Image C is from patient 2 and bone trephine was taken the day of the breast cancer surgery (D0 on the ZOLMENO study) and prior to the administration of ZOL. Image D is from patient 3 and bone trephine was taken the day of the breast cancer surgery (D0) and after the administration of ZOL. Image E is from patient 4 and image F from patient 5 and both trephines were taken the day of the breast cancer surgery (D0) and before the administration of ZOL. Images D and F represent smaller samples but otherwise differences are not apparent between the samples.

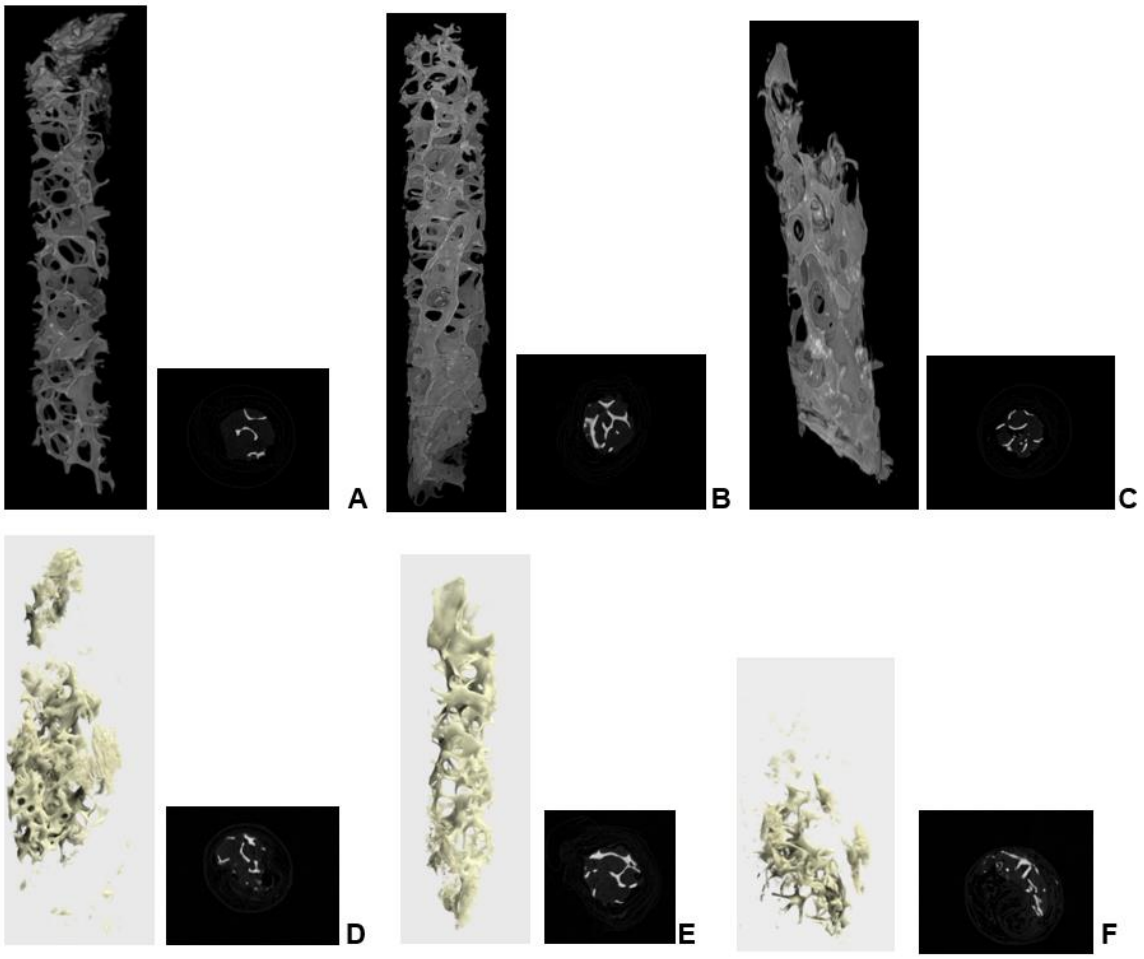

Figure 2.18: *3D reconstructions MicroCT images of bone trephines. Patient 1 D0 (A) and day 28 (B), Patient 2 D0 (C), Patient 3 D0 (D), Patient 4 D0 (E) and Patient 5 D0 (F). No apparent differences between the samples.*

MicroCT results were further analysed to quantify the bone structures. Bone volume, percent bone volume, trabecular thickness and trabecular number were analysed for 5 of the bone trephine samples. Sample F from patient 5 was not able to be further analysed due to its small size. A section in the middle of the samples was used to generate these results. The graphs below (Figure 2.19) represent the results. A/B from patient 1, C from patient 2, D from patient 3 and E from patient 4 used as per the figure above. The numbers in brackets inside the graphs represent the patients' age.

In general, changes between the samples are very minimal. Differences between A and B which are from patient 1, before and after administration of ZOL and before and after breast cancer surgery, are very minimal and all the values are slightly greater in the post ZOL sample (B). Bone volume and percent bone volume appear to be lower in younger patients (sample A patient 1 age 65 and sample E patient 4 age 60). However, trabecular thickness was lower in

the older patient (sample D patient 3 age 70). These results suggest that there is variability between the bone content of the samples obtained from different patients, as expected. Although, it is difficult to establish a true range due to the number of collected samples, results demonstrate that the bone trephines do contain sufficient bone for microCT analysis and quantification of bone content as well as structure.

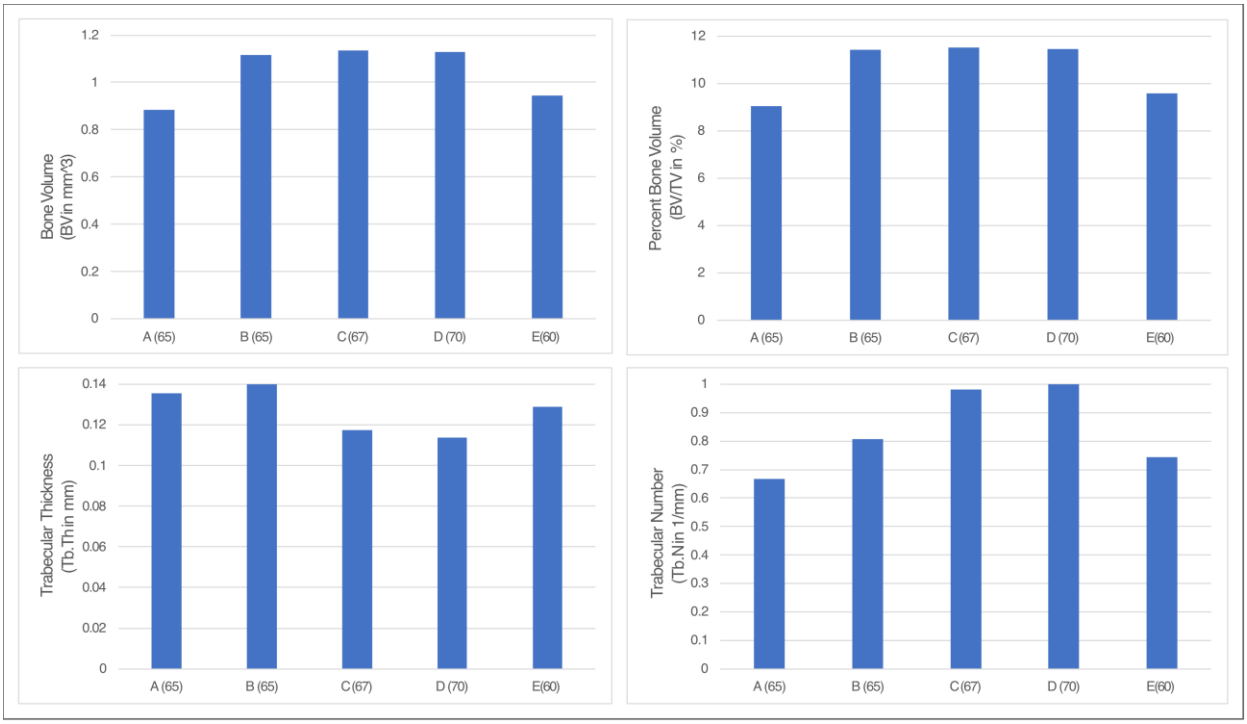

***Figure 2.19**: MicroCT data of bone trephines. Patient 1 D0 (A) and day 28 (B). Patient 2 D0 (C). Patient 3 D0 (D) and patient 4 D0 (E). The numbers in the brackets in the graphs represent the patients' age.*

2.7.5 Histological analysis

Post microCT analysis the bone trephine samples processed for histological analysis (section 2.5.6.2.2). Bone trephines were sectioned in longitudinal and transverse axis and sections thickness was 3u. From each bone trephine 50 sections could be obtained. Histological staining was performed in each section (section 2.5.6.2.2) and the following images were obtained for histological analysis (Figure 2.20). Histological analysis was not completed. This pilot sample (Figure 2.20) was used to establish that the best way to section the bone trephine was longitudinally and also sufficient material can be taken from a trephine sample. Note the high fat content of the bone marrow and the lack of obvious osteoblasts/osteoclasts (osteocytes can be seen).

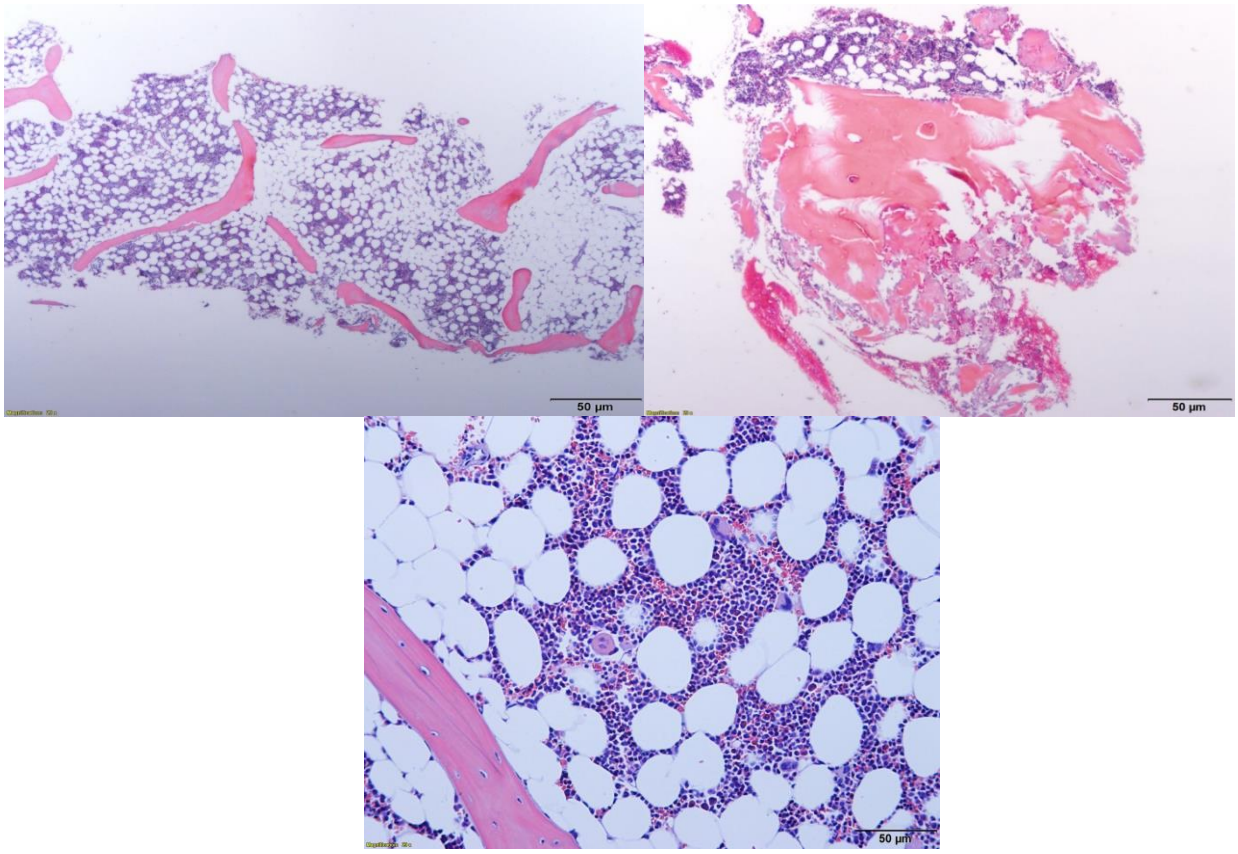

Figure 2.20. *H&E histological staining of bone trephines. Longitudinal and transverse axis sections. Bone and adipose tissue can be seen in the histological pictures.*

2.8 Discussion

The ZOLMENO study was a single centre, open label, randomised, proof of concept study which aimed to clarify the mechanism of action of adjuvant ZOL in EBC. Adjuvant ZOL and BPs in general, have been found to have anticancer effects in postmenopausal EBC (78-81, 88, 89, 226), and they are currently standard of care in early disease (13, 51, 126). The ZOLMENO study had recruitment target of 80 patients with EBC, 40 premenopausal and 40 postmenopausal, who were planned to have one single dose of intravenous ZOL (4mg) either before or after their breast cancer surgery. Participants would require having blood samples and bone marrow biopsies with the aim samples to proceed to translational studies. Although, this project started in September 2018, the first patient was only recruited in February 2019, as this time was required to finalise the study protocol and approvals and establish a viable study pathway for patients and all the teams involved. For multiple reasons which will be discussed in this section, the study only recruited 19 patients, 4 premenopausal and 15 postmenopausal, with 1 patient to withdraw early due to medical issues unrelated to the study. Eighteen (18) participants completed the study.

The study drug, ZOL, is a drug in routine use in oncology and other medical specialties. However, for the ZOL to be given as a study drug a multidisciplinary team needed to be assembled in order to cover all the elements around the prescription, dispensing process and administration of the drug. Additionally, the study was run across 2 different clinical teams, oncology and breast cancer surgery, and this needed a careful coordination as the teams run in very different ways. Following multiple meetings with all the parties involved the study was opened to recruitment. Despite this, only after running the study I could actually identify the gaps in the recruitment process and establish a smooth process for the patients and the teams involved. Additionally, in terms of the translational studies, these were planned to be carried out at the end of the recruitment period, to avoid both pooling of samples and batch differences by analysing samples as they became available. Therefore, collection of the study samples was a priority.

Initially, the breast cancer surgery team was responsible to discuss the study with the potential participants who were prior to this identified by the breast MDT as eligible. This was used to be done at the first patients' appointment with the team where they were receiving their diagnosis. However, this was proven challenging and perhaps inappropriate for the team as the focus of that first appointment was to give patients their breast cancer diagnosis and discuss the disease management rather than discussing a clinical trial. After raising the issue with the surgical team, we agreed that the responsibility of discussing the study should be passed to me and the breast cancer surgeons and breast care nurses were only ensuring that PIS was given to the eligible patients. After this change, the study recruitment improved significantly, and this was welcomed by both departments.

At the first stages of the study multiple issues were isolated as potential cause of delays in patients' recruitment process, with the most important to be listed below:

1) Clinical research team needed to be available the day of patient's review at WPH to perform the randomisation.
2) A chair needed to be available at the WPH chemotherapy suite for ZOL infusion to be given.
3) Post randomisation of the patient, surgical team needed to ensure that patient's surgery date remained the same as any changes would have affected their participation to the study.
4) Extra theatre time was important to be allocated for the study participants to allow for the bone marrow biopsy.

Some patients who expressed interest in participating to the study, failed to be recruited due to the above challenges, especially during the first 2 months of the study. Following

identification of these issues, study pathway was improved leading to uncomplicated run of the study until the end of 2019 with 11 patients recruited.

COVID-19 pandemic had a tremendous impact on cancer care and research. In particular, Cancer Research UK paused recruitment in almost all of its studies and an independent report described a 60% reduction in new cancer clinical trials (247, 248). For the ZOLMENO study, pandemic and the NHS hospital restrictions stopped its reopening after my return from maternity leave in January 2021, leading to my redeployment to the NHS for a period of 9 months. Study received sponsor's green light for restarting recruitment in December 2021, however ongoing COVID-19 NHS hospital restrictions were considerably affecting the process. Nevertheless, eligible patients were keener to participate in the study in 2022 compared to 2019, most likely due to the wide media coverage of the importance of research behind the development of COVID-19 vaccines. This is supported by the fact that 53 patients were screened in 2022 and 8 were recruited (15%), in contrast to 2019 where 109 were screened and 11 were recruited (10%) (Figure 2.11a&b*)*.

In 2022, in addition to COVID-19 pandemic, the ZOLMENO trial was affected by competing trials ran also by the STH breast cancer directorate and changes in clinical practice. Competing trials aimed to recruit from the same cohort of patients which was not only challenging for the teams running the studies but also for the patients themselves. Studies like the ZOLMENO study that do not have any direct benefit for the participants are likely to lose out when they are other trials on offer. Ethically patients needed to be informed for all the available clinical studies, but the decision was not easy for them as the participation in one study meant their ineligibility to the other. Future better communication within the directorate is needed to avoid similar issues and improve patients' participation and experience with clinical trials. Furthermore, current crisis in the NHS has been driving changes in clinical practice which subsequently have an impact on clinical research. In view of the long backlog of breast cancer patients, those suitable were given neo-adjuvant endocrine therapy, deeming them ineligible for the study. These were only a proportion of EBC women with ER+ve disease but considering that the majority of breast cancers are ER+ve (75%) and that COVID-19 pandemic NHS hospital restrictions were still in place, the pool of patients from where I could identify potential participants was significantly restricted. This is going to be a continuous issue given the current NHS environment within the cancer departments, making future proof and design of studies very challenging. Clinical projects will need to be more flexible and perhaps where possible have longer recruitment periods in order to achieve their targets.

In general, patients' participation to clinical trials is very low with international report to suggest that less than 5% of the adult patients approached ends up being enrolled in studies (249). However, for cancer patients the percentage appears to be much higher with 25% of them to be recruited in clinical trials (249). This is similar to the UK data which showed that

more than 1 in 5 cancer patients take part in clinical studies (250). This could partially explain the low patient recruitment of the ZOLMENO study considering that patients were asked to consent to a study that involved extra interventions with no direct benefit to them. Additionally, the study was used to be discussed with potential patients at a very sensitive time, around the time of their breast cancer diagnosis. The majority had to decide whether they wished to participate within a few days which was very challenging for them, given the life changing diagnosis they had just received.

The number of postmenopausal (15 patients) women that recruited to the study was almost 4 times higher compared to premenopausal (4 patients). Throughout the ZOLMENO study recruitment period, screening and recruitment of premenopausal women was difficult. Premenopausal pool of patients is anyway smaller compared to the postmenopausal one, with only 18% of new breast cancers to be diagnosed in women under 50 (82% in those ≥50 years) (9). Premenopausal women are in childbearing age with most of them to be receiving some form of hormonal contraception, making them ineligible for the study. In addition, premenopausal participants are younger with busier lifestyles (for example: working, caring for children) which is often a barrier for their participation in any clinical trial, especially in one that requires extra hospital visits like this study. Understandably, particularly low recruitment of premenopausal women has implications in the analysis of the study samples. The comparisons between pre- and post-menopausal serum and bone marrow samples will be done in a much lower scale and in some cases won't be feasible at all.

Although too few samples were obtained to allow comparisons to be made and conclusions drawn, the study did establish that:

It is possible to obtain bone trephines and bone marrow aspirates during breast cancer surgery and these contain sufficient material for *ex vivo* microCT analysis and 3D reconstruction of trabecular structures. Pre and post treatment bone trephines could not be obtained. However, it was unlikely that a single dose of ZOL would cause a change in bone volume within the timeframe of the study, whereas a change in serum bone turnover markers (in progress) and potentially in bone cell numbers would have been detectable.

Additionally, histological analysis of the trephines showed that there were few bone cells (osteoblasts and osteoclasts) present, bone histomorphometry and quantification of bone cells could therefore not be carried out.

Overall, the study sample analysis has been difficult, mainly due to the long recruitment period suggesting that time was focused on sample collection rather than sample analysis. Having said that, part of the translational analyses are still planned to be completed in the near future. Despite the difficulties and challenges I faced running the ZOLMENO trial, this was a great and valuable experience which put me in a position to carry out future NHS cancer

clinical trials. Lessons learned from study and lab protocols writing to approval, sample collection, ethics applications and process and professional publications will support my future career goals and help me run similar projects with better inside and understanding of the research process.

2.9 Conclusion

In view of multiple reasons but mainly due to the COVID-19 pandemic, the ZOLMENO study was unable to reach its recruitment target. Recruitment of patients to proof of concept cancer clinical studies, like the ZOLMENO study, has been proven very difficult, however further work in a local and national level need to be undertaken in order to improve patients' and clinicians' awareness for the clinical need of such trials.

2.10 Future work

- The samples that were collected from the ZOLMENO study participants will be analysed. This will not include all the translational studies. As a first step, the aim is to analyse the bone marrow aspirates and some of the serum samples. The serum samples will be sent to a different lab within the University of Sheffield for ELISA bone markers. Additionally, functional studies will be performed for the serum samples by the laboratory team of Professor Ingunn Holen. Funding and appropriately trained staff are currently limiting the sample analysis.

- The results of the translational studies are aiming to be published in peer review journals and presented in local, national and international meetings with special interests in breast cancer and the anticancer effects of BPs. Disseminations of the results will help both scientists and clinicians to improve their understanding of the effects of ZOL in EBC and support further research which will aim to clarify the mechanism responsible for the effects of adjuvant BPs with ultimate aim to improve breast cancer care.

2.11 Presentations and publications arising from this project

Poster presentations

1. **Theodoulou E***., Wilson C., Brown J.E., Holen I., The ZOLMENO study, exploring the effects of ZOLedronic Acid and MENOpausal status in patients with early breast cancer. Bisphosphonates 50th Anniversary meeting, 2019, July 2019, Sheffield, UK.

2. **Theodoulou E***., Wilson C., Brown J.E., Holen I., Does menopausal status modify response to bone-targeted therapy in early breast cancer? British Association of Cancer Research (BACR) conference 2019, October 2019, Newcastle, UK.

Published abstracts

1. **Theodoulou E**., Wilson C., Brown J.E., Holen I., The ZOLMENO study, exploring the effects of ZOLedronic Acid and MENOpausal status in patients with early breast cancer. Bisphosphonates 50th Anniversary meeting, 2019, July 2019, Sheffield, UK.

***Indicates presenting author

Chapter 3

UK experience with Adjuvant Bisphosphonates in Early Breast Cancer

3.1 Summary

Multiple clinical studies and a large meta-analysis have shown that adjuvant bisphosphonates (BPs) in early breast cancer can reduce the risk of disease recurrence (local, distance and in bones) and improve survival, but only in postmenopausal (natural or induced by ovarian suppression) women (78-81, 88, 89, 226). Adjuvant BPs were first introduced in the UK when they were included as a recommendation in the breast cancer CRG (Clinical Reference Group) service specification and were endorsed as a priority for implementation by the UK Breast Cancer Group (UKBCG) in November 2015 (C. Harper-Wynne oral presentation at UKBCG annual meeting, Nov 2015). From 2018, they are part of the UKs NICE (National Institute for Health and Care Excellence) recommendations for EBC treatment, advising cancer physicians to offer adjuvant BPs (zoledronic acid or sodium clodronate) to postmenopausal women with node-positive and node-negative breast cancer with high risk of recurrence (251). NICE recommendations exist to guide and help UK physicians and also to inform whether drugs should be available on the National Health Service (NHS). However, they don't automatically result in change in practice, especially with BPs traditionally being considered as anti-metastatic drugs. Therefore, change of UK breast cancer practice to include routine use of adjuvant BPs may not be immediate. We have conducted a national cancer physicians' survey (2019) and an oncology pharmacists' survey (2021) to evaluate the experience of NHS oncologists and hospitals and increase the understating of the degree of national uptake.

Adjuvant BPs are now included in guidelines for the management of early disease in Europe and America but despite this, their adoption still varies in many countries (47, 51, 126, 232, 251-254). In Australia, BPs are still not recommended in the adjuvant setting and they are not included in the Pharmaceutical Benefits Scheme (PBS), with eligible patients missing to receive this life saving treatment. Through an international collaboration, the UK physicians' survey was compared with a similar Australian physicians' survey, with intention to assess the pathway taken for adjuvant BP implementation in the UK and how it might inform changes in Australian practice and potentially guide other countries with similar issues with the ultimate aim of improving the care of women with EBC globally.

3.2 Introduction

In addition to their established role in bone metastasis and prevention of cancer treatment induced bone loss (CTIBL), BPs have been found to have anticancer effects in EBC disease. As it has been described in the introduction section of this thesis, several trials have shown that both clodronate and zoledronic acid (ZOL) in the adjuvant setting improve breast cancer survival and reduce the risk of bone metastasis (78-81). Although, addition of oral

ibandronate to chemotherapy showed no difference in DFS and OS between the BP and no BP groups, DFS was longer in younger (<40 years) and older patients (>60 years) (86).

Subsequently, the 2 large randomised ABCSG-12 and AZURE trials demonstrated that adjuvant ZOL only benefit postmenopausal women (natural or induced by ovarian suppression) (88, 226). The ABCSG-12 trial (n=1803) randomised premenopausal women with ER (oestrogen receptor) positive EBC to receive endocrine treatment (tamoxifen or anastrozole) and ovarian suppression with or without ZOL 6 monthly for 3 years, showed reduction in disease progression and recurrence of 36% and 35%, respectively (226). In the large multicentre international trial AZURE, 3360 women with high risk EBC were randomised to standard adjuvant systemic treatment with or without ZOL for 5 years. A pre-planned subgroup analysis showed that women who were 5 years into menopause benefitted from ZOL, with IDFS of 78.2% in the ZOL arm compared to 71% in the control arm, and OS at 5 years of 84.6% and 78.7% in ZOL and the control group, respectively (88).

In 2015, the EBCTCG published a meta-analysis of 26 randomised trials of adjuvant BPs in EBC which included data from 18,766 women (89). In the combined, meta-analysis, the most apparent effect of BPs was in bone recurrence, irrespective of menopausal status. However, subgroup analysis showed a clear benefit in postmenopausal women with 3% reduction in breast cancer recurrence, 2.2% reduction in bone recurrence and 3.3% reduction in breast cancer mortality in overall 10-year risk. The 10-year risk of death was 14.7% in those treated with adjuvant BPs versus 18.0% in the standard therapy group. This benefit was seen regardless of treatment schedule, oestrogen receptor status, nodal status, tumour grade, or concomitant chemotherapy. There was no difference in outcome between patients receiving nitrogen or non-nitrogen BPs and, with the exception of oral pamidronate, all the other BPs (clodronate, ZOL, ibandronate) produced similar benefit in disease outcomes. Based on these results, the survival benefit of adjuvant BPs in postmenopausal women is comparable to that seen with the addition of taxanes to anthracycline schedules, or with the use of aromatase inhibitors (AIs) versus tamoxifen (91, 92). Therefore, translation of the benefit percentages into real patient numbers will mean that, if adjuvant BPs treatment is given to all eligible patients (>35,000), it has the potential to save over 1000 lives every year in the UK (255).

Denosumab, a RANK ligand inhibitor, has also been studied in the same setting, with somewhat inconsistent results. The ABCSG-18 trial (n=3425) demonstrated reduced fractures and improved DFS in postmenopausal women with hormone-receptor positive EBC receiving adjuvant AI therapy (52, 53). In contrast, the D-CARE study (n=4509) failed to show improved disease-related outcome from adjuvant use of denosumab, including in postmenopausal women (94). As a result, denosumab is not recommended for this indication in EBC.

Following the results of the meta-analysis, ESMO (European Society of Medical Oncology) published in 2014 the first available international guidelines for the use of adjuvant

BPs, suggesting their use only for postmenopausal women with EBC (47). Early breast cancer is potentially curable, as the disease has not spread beyond the breast or the axillary lymph nodes. Since then, many international guidelines have advised the use of adjuvant BPs (usually intravenous ZOL every six months or oral clodronate) for postmenopausal (natural or induced) EBC for 3 to 5 years (126, 232, 251-254), with the aim to reduce recurrence and mortality, particularly in patients considered at high risk of breast cancer recurrence. Women with high risk disease are defined as those who warrant standard adjuvant systemic chemotherapy/HER-2 targeted therapy, and/or have greater that 12% 10-year risk of disease relapse.

In the UK, the journey for the adoption of adjuvant BPs in the national breast cancer practise started at the end of 2015, when they were included as a recommendation in the breast cancer CRG service specification and were endorsed as a priority for implementation by the UKBCG, promoting national uptake, guidance, and funding arrangements through local commissioning agreements (C. Harper-Wynne oral presentation at UKBCG annual meeting, Nov 2015). The same year, Sheffield Teaching Hospitals, NHS Foundation Trust shared through UKBCG the first national guidelines (based on the Annals of Oncology publication) (47), for the use of these agents in EBC. Additionally, Breast Cancer Now (BCN), the leading UK breast cancer charity, was heavily engaged in raising awareness of the benefits of adjuvant BPs amongst patients lobbying the Department of Health and Social Care and NHS England to clarify commissioning responsibility and advice, helping hospital trusts to make the case for their use, and worked to generate significant media coverage on this issue (255).

In March 2016, UKBCG and BCN conducted the first physicians' survey with the aim to give an insight into the initial uptake of adjuvant BPs for EBC in the UK. The results from the initial survey showed that in the period covered by the survey (November 2015 to March 2016), only 24% of oncologists were prescribing adjuvant BPs for this indication, with the number increased to 44% in a follow up survey in October of the same year (C. Harper-Wynne personal communication). In November 2017, a subsequent survey was performed at the annual UKBCG meeting, with 77% of the attendees answering positively to the question about offering adjuvant BPs in EBC (C. Harper-Wynne personal communication).

Adjuvant BPs were fully adopted by the national UK regulatory body NICE in 2018, when they were included in its recommendation for the management of EBC (251). NICE suggests the use of oral clodronate (1,600mg daily) or intravenous ZOL (4mg/ 6 monthly) in postmenopausal women with node positive and node negative high risk of recurrence breast cancer for 3 to 5 years. This is similar to the 2017 ASCO/CCO (American Society of Clinical Oncology / Cancer Care Ontario) recommendation which suggests 6 monthly ZOL (4mg, intravenously) for 3-5 years or daily clodronate (1,600 mg, orally) for 2-3 years for postmenopausal and premenopausal receiving ovarian suppression women who are

candidates for adjuvant systemic therapy (252). However, in 2022, ASCO/CCO guidelines were updated to suggest ZOL (4mg, intravenously) 6 monthly for 3 years or 3 monthly for 2 years or oral clodronate (1,600 mg daily) for 2-3 years or oral ibandronate (50mg daily) for 3 years (126). The 2016 European guidelines also recommended intravenous ZOL (4 mg every 6 months) or oral clodronate (1,600 mg daily) for 3 to 5 years, but these have now been updated to recommend ZOL for 3 doses while patient is receiving systemic anticancer treatment followed by either 6 monthly ZOL or a daily oral agent (clodronate 1,600mg or ibandronate 50mg) for a total period of 3 years (51, 232). ESMO is suggesting adjuvant BPs for postmenopausal women with >12% 10-year risk of cancer death and for premenopausal women on ovarian suppression.

The variation in international guidelines for the use of these agents highlights the need to identify the optimal adjuvant BP regime (agent, dose, frequency, and duration). Despite this, the EBCTCG meta-analysis showed that all the agents, apart from oral pamidronate, offer the same anticancer benefit (89). Most clinical trials have included clodronate, ZOL or ibandronate. Patients with busy lifestyles and those who want to avoid hospital visits, typically opt for oral BP. However, gastrointestinal side effects such as oesophagitis are more common with the oral agents, leading to BP intolerance and change to intravenous ZOL which usually causes mild flu like symptoms for a short period of time. Additionally, ZOL is preferred in the cases where patients' adherence is an issue as it is less frequent (mostly every 6 months) compared to the daily oral alternatives and administered clinically.

NICE approval for the use of adjuvant BPs in the routine management of EBC disease has been an important step in the endorsement of these agents in UK breast cancer practice. However, NICE approval does not automatically mean that every clinician or NHS Trust will change their practice immediately. The implementation process usually takes several years as changes at a national, local, and personal (individual clinicians) level are required. A National evaluation study published in 2004 showed that general implementation of NICE guidelines is variable (256). Barriers such as lack of education, local guidance, and leadership, as well as lack of financial support and resources or staff, have been identified in the implementation of NICE guidelines (257, 258). Results from clinical trials might be positive with clear benefit for patients, but barriers need to be addressed for the intervention to be used in real life settings. Therefore, NICE has introduced an implementation programme which focuses on providing education and support to local NHS Trusts. Financial support is provided through the local Clinical Commissioning Groups where hospitals need to apply for every new intervention they wish to implement. In the case of adjuvant BPs, advice as to how to approach the local Clinical Commissioning Groups with positive outcomes was shared by Sheffield within the first national guidelines, encouraging other NHS Trusts to follow this path and adopt adjuvant BPs. Additionally, the Care Quality Commission (CQC) is the national body

responsible to monitor and support the implementation process through its inspection checks against national standards. NICE also advice patients and carers to be actively involved in the whole process, as pressure from these groups will further encourage change.

In the UK, the journey of adjuvant BPs to become part of the standard of breast cancer care has been marked by two milestones. Firstly, their endorsement by national bodies paved the way for many to change their adjuvant management habits as was captured by the first 3 national surveys. Secondly, it was the inclusion of BPs into the adjuvant NICE guidelines and here we are presenting the UK experience with these bone agents since then. However, we have conducted two separate national surveys, a UK oncologists' survey in 2019 and an NHS oncology pharmacists' survey in 2021 with aim to describe the current consensus and give an insight into the national status of the adjuvant use of BPs.

3.3 Introduction to the UK/Australian international collaboration

As has been described above, adjuvant BPs are included in many international guidelines, with ASCO/CCO and ESMO to recommend their use in postmenopausal EBC (47, 51, 126, 232, 251-254). Despite this, wide variation still exists in their adoption. Bisphosphonates are off patent with generic formulations being manufactured. Therefore, pharmaceutical lobbying for BPs to gain regulatory approval for this indication is lacking in many countries. This may have negative impact on the prescribing of adjuvant BPs, resulting in EBC patients not receiving the intervention and thereby missing out on the significant clinical benefit.

In the UK, NICE is the national regulatory body which approves new medicines for use within the NHS and subsequently their funding comes from the NHS. In some cases, drugs which have been shown clinical benefit but lack NICE approval, could still be used within the NHS but only as off-label drugs. This was the case for BPs before 2018, when they were not licenced for use in prevention of disease recurrence in breast cancer. At that point, the NHS Trusts that would like to offer adjuvant BPs had to gain funding through presenting a successful business case to the local commissioning group.

In Australia, new medicines need to be approved by the Therapeutic Goods Administration (TGA) before they can be listed on the PBS, which is a nationally funded programme. For drugs not PBS listed, patients have to pay the full cost. The PBS approval process is long; it can take up to 35 weeks for a medicine to be approved. Bisphosphonates are available on the PBS for treating osteoporosis, reducing the risk of skeletal related events in patients with breast cancer metastatic to the bone and managing hypercalcemia of malignancy (259). However, despite the evidence and recommendation in international guidelines (47, 51, 126, 153, 232, 251-254, 260), adjuvant BPs are not PBS listed for preventing CTIBL, reducing breast cancer recurrence or improving survival for patients with

early disease. On a private script in Australia, the most commonly used BP (ZOL 4 mg) costs between AUD $50 - $200 per dose (261). In addition, Australian states have different funding mechanisms in place. For example, in South Australia, postmenopausal women with a >12% 10-year recurrence risk can access funded ZOL via their Statewide High Costs Medicines Formulary, whereas no such funding mechanism exists in New South Wales or other Australian states (262).

Unlike UK, until my project, no surveys had been conducted to document adjuvant BP prescribing practices of oncologists in Australia. Therefore, through an international collaboration between our team and two Australian oncologists, an Australian physicians' survey (similar to the UK) was created, and the results compared with the UK responses, with aim to evaluate the current Australian practise and indicate barriers to uptake, in order to aid translation of the UK experience of adjuvant BP implementation to Australian practice.

3.4 Hypothesis, Aims and objectives

Hypothesis

- Use of adjuvant BPs in women with EBC is variable in the UK and Australia. The underlying causes of the variation are multifactorial.

Aims

- To provide real world data for the current use of adjuvant BPs in the UK and Australia.
- Increase understanding of the causes of this variation.
- Raise awareness of this issue and thereby reduce underutilisation of this therapy.

Objectives

National UK oncologists' survey:

1) Evaluate the use of adjuvant BPs for EBC in the UK.
2) Assess any barriers in prescribing these agents in the NHS.
3) Identify the current adjuvant prescribing habits of UK cancer physicians.

National UK pharmacists' survey:

1) Confirm continued adjuvant BP use for postmenopausal EBC in the UK.
2) Evaluate the use of adjuvant BPs in the UK during the COVID-19 pandemic.
3) Explore the prescribing pathway of the oral adjuvant BPs within the NHS.

Australian oncologists' survey and the international collaboration:

1) Evaluate the use of adjuvant BPs for EBC in Australia.

2) Identify any barriers in adjuvant BP implementation.
3) Compare the Australian with the UK experience with adjuvant BPs.

3.5 Methods and Materials

3.5.1 Survey research

Survey research has been around since 1930, and it is described as the collection of data through individuals' answers to specific survey questions. It can produce quantitative, qualitative, or mixed type data. In the past, surveys were mainly conducted via post, but this has now been replaced by electronic surveys with researchers able to distribute their surveys via email, websites, and/or social media. Electronic surveys can be cost effective, easily distributed and answers can be tracked in real time. Additionally, web-based software such as SurveyMonkey and Microsoft Forms are available either for free or on a subscription basis, to support researchers create effective surveys. Despite these, debates still exist in the usefulness of this kind of research (263, 264), and many might think that surveys are easy and effortless to develop but appropriate steps should be considered in order for survey research to produce unbiased, replicable, and robust data.

Traditionally, healthcare professional surveys have had very low response rates, usually less than 50% (265) and attempts to improve this issue have been descripted in the literature (264, 266). Use of closed-ended rather than opened-ended questions, careful consideration of sample size, reminders and the offer of small monetary incentives have been showed to increase surveys response rates. Informed by this, the surveys that are presented here, were designed to include mainly closed-ended questions, completion time was less than 5 minutes, and one reminder was sent to all potential participants. No monetary incentives were offered in our surveys.

3.5.2 UK Physicians' Survey

3.5.2.1 Survey Population

The target study population was consultant medical and clinical oncologists who were treating patients with breast cancer in the UK. The 2019 UK workforce census report, published by Royal College of Radiologists, showed that there were 1,506 consultant oncologists in the UK, 568 medical oncologists and 938 clinical oncologists (267). In terms of tumour site specific specialists, the report showed that UK had 274 clinical oncologists treating breast cancer patients in 2019. However, the actual number of breast cancer consultants is much higher as the report did not include the tumour site specialties for medical oncologists.

3.5.2.2 Power calculation/sample size

This was an exploratory survey and consequently a power calculation was not appropriate, but we aimed to ensure that we adequately sampled the target population to ensure the generalisability of the findings. To this end the survey was sent out to all breast specialist oncologists identified via the UKBCG members list with the expectation of a 20-40% response rate based on previous medical professional surveys.

3.5.2.3 Survey Development

Survey content validity was assured by using 3 sources of information to guide questionnaire content: review of the existing literature, an expert reference group (ERG) comprising members of the core research team plus several other medical oncologists and lastly the draft version was reviewed by several further medical oncologists. Face validity, useability and acceptability were confirmed by piloting the questionnaire with several medical oncologists to ensure the questions were appropriate and making any modifications before the final survey was distributed. A key aim was to keep the questionnaire short and to the point to encourage survey completion.

The survey was then imported into SurveyMonkey, an online survey software platform. This software helps the users to develop an online survey and to analyse data, select samples and present their data. It offers the option of including any type of question (open-ended and closed-ended) but in our survey, we avoided open-ended questions, as they are much more complex to analyse and time consuming to complete and therefore, not particularly popular with responders. Upon completion of the survey development, an online link is created which can be sent to the potential responders either through the SurveyMonkey website or by email.

The survey was a 15-item self-administered survey, and it was developed to cover three broad themes: 1) current practice, 2) patient selection and monitoring, and 3) choice of bone modifying agent (BMA) regimen (Figure 3.1).

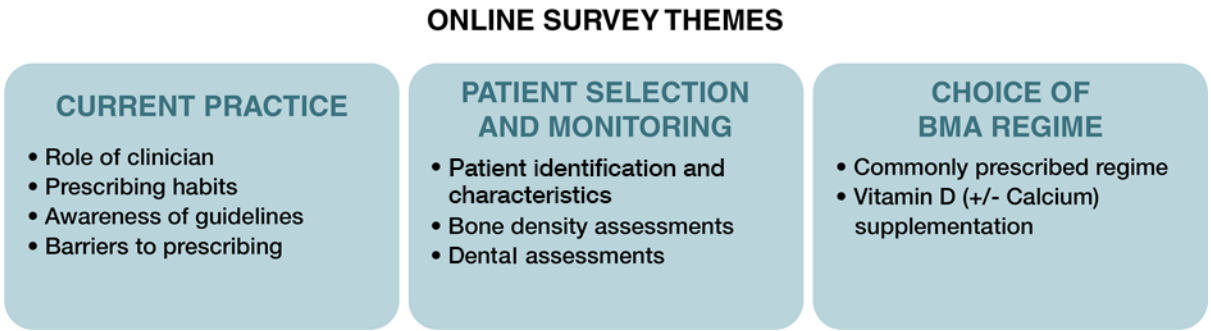

***Figure 3.1**: Themes of the online UK and Australian physicians' survey.*

3.5.2.4 Survey Implementation

The survey link was distributed via email through the UKBCG to all the medical and clinical oncologists treating breast cancer patients and were members of the UKBCG. UKBCG has members in all 4 UK countries. The initial invitation was sent in March 2019, followed by a reminder email in May 2019. The survey remained open between March 2019 and June 2019, no incentive to participate was provided.

3.5.2.5 Data Analysis

Descriptive statistics were used to summarise the responses to the survey. The frequency of each choice was calculated as a proportion of the total number of responders. The data were compiled using Microsoft Office Excel (© 2018 Microsoft Corporation). Percentages were rounded to nil decimal point.

3.5.2.6 The Survey

1. In which centre do you practice Oncology?

2. What is your role?

☐ Consultant Medical Oncologist

☐ Consultant Clinical Oncologist

☐ Other – Specify

3. Which guidelines regarding the prescription of ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE in women with early breast cancer do you follow?

☐ UKBCG

☐ ASCO / CCO

☐ NICE

☐ ESMO

4. Do you currently prescribe ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE in women with early breast cancer?

☐ Yes go to question 6

☐ No go to question 5

5. What are the reasons for NOT prescribing ADJUVANT bone modifying agents for PREVENTIONF OF DISEASE RECURRENCE in early breast cancer?

- ☐ Lack of convincing evidence
- ☐ Cost / Reimbursement
- ☐ Side effects
- ☐ Access to an infusion chair
- ☐ Not enough time to discuss data with patients
- ☐ Local protocol guidance
- ☐ Other

6. How do you select women with early breast cancer for ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

- ☐ All premenopausal women
- ☐ Premenopausal women on GnRH analogue
- ☐ All postmenopausal women
- ☐ Postmenopausal women at high risk for disease recurrence
- ☐ None of the above

7. If you offer ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE in postmenopausal women at high risk for disease recurrence, what do you deem high risk?

- ☐ All those who would be offered chemotherapy irrespective of if they go on to receive it
- ☐ Other – Free text box under other

8. What ADJUVANT bone modifying agent(s) do you prescribe for PREVENTION OF DISEASE RECURRENCE?

- ☐ Zoledronic Acid
- ☐ Clodronate
- ☐ Ibandronic Acid
- ☐ Other

9. For how long do you recommend ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRECE?

- ☐ 2 years
- ☐ 3 years
- ☐ ≥ 3 years

10. Do you recommend calcium and Vitamin D supplements in patients on ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

- ☐ Yes
- ☐ No

11. Is the use of ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE discussed at your breast multi-disciplinary meeting (MDT)?

- ☐ Yes
- ☐ No

12. Do you routinely perform BONE DENSITY ASSESSMENT prior to starting ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

- ☐ Yes
- ☐ No

13. How frequently do you perform BONE DENSITY ASSESMENTS in women with early breast cancer on ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

- ☐ Only at baseline
- ☐ Yearly
- ☐ 2 yearly
- ☐ At completion of duration of adjuvant bone modifying agents
- ☐ Other

14. After completion of ADJUVANT bone modifying agents for PREVENTION OF DIESEASE RECURRENCE, how often do you perform BONE DENSITY ASSESSMENTS, if patients are on extended endocrine therapy?

- ☐ Not measured
- ☐ At completion of duration of adjuvant bone modifying agents
- ☐ Yearly
- ☐ 2 yearly
- ☐ At completion of duration of endocrine therapy
- ☐ Other

15. Do you mandate a baseline DENTAL ASSESSMENT prior to starting ADJUVANT bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

☐ Yes

☐ No

3.5.3 UK Pharmacists' Survey

3.5.3.1 Survey Population

The target survey population was oncology pharmacists who were practising within the NHS.

3.5.3.2 Power calculation/sample size

This was an exploratory survey and consequently a power calculation was not appropriate, but we aimed to ensure that we adequately sampled the target population to ensure the generalisability of the findings. To this end the survey was sent out to all oncology pharmacists identified via the BOPA (British Oncology Pharmacy Association) members list with the expectation of a 20-40% response rate based on previous similar surveys.

3.5.3.3 Survey Development

Survey content validity was assured by using 2 sources of information to guide questionnaire content: review of the existing literature and an ERG comprising members of the core research team and an oncology pharmacist. Face validity, useability and acceptability were confirmed by piloting the questionnaire with members of the core team and an oncology pharmacist to ensure the questions were appropriate and making any modifications before the final survey was distributed. The key aim again was to keep the questionnaire short to encourage survey completion.

The on-line survey was created by using the same on-line software platform SurveyMonkey which has been described above. It was an anonymous 5-item self-administered survey, and it was consisted by one free text question and four multiple choice questions. The survey focused on collecting information for the use of adjuvant BPs in EBC patients within the NHS cancer centre that pharmacists were practising oncology (Figure 3.2).

For Pharmacists

Place of work

For Pharmacists' local NHS Oncology Centre

Use of adjuvant BPs

Use of intravenous and/or oral adjuvant BPs

Prescribing pathway for oral agents

***Figure 3.2**: Topics of the National Pharmacists' Survey.*

3.5.3.4 Survey Implementation

The survey was distributed to the BOPA members. The on-line survey link was sent to the BOPA members by email, was shared at the association social media page and was included at the weekly email newsletter that all members receive. The initial invitation was sent in November 2021 followed by a reminder email 3 weeks later. The survey remained open for one month and no incentive to participate was provided.

3.5.3.5 Data Analysis

Descriptive statistics were used to summarise the responses to the survey. The frequency of each choice was calculated as a proportion of the total number of responders. The data were compiled using Microsoft Office Excel (© 2018 Microsoft Corporation). Percentages were rounded to nil decimal point.

3.5.3.6 The Survey

1. Which hospital do you work at?

2. Does your hospital currently use ADJUVANT Bisphosphonates in Early Breast Cancer in any of the following groups of patients?

☐ Postmenopausal women

☐ Premenopausal women on ovarian suppression

☐ Both of the above

☐ Don't use them at all

☐ Other

3. Which ADJUVANT Bisphosphonate(s) does your hospital use?

☐ Zoledronic acid

☐ Zoledronic acid whilst on SACT then oral Bisphosphonates

☐ Both of the above

☐ Not applicable

4. Which oral Bisphosphonate(s) does your hospital use?

☐ Ibandronic acid

☐ Sodium clodronate

☐ Both of the above

☐ Not applicable

5. For oral Bisphosphonates, who prescribes these?

☐ Initiated by Oncologist then GP

☐ Oncologist

☐ GP

☐ Pharmacist

☐ Not applicable

3.5.4 Australian Physicians' Survey

3.5.4.1 Survey Population

The target survey population was Australian consultant medical oncologists and medical oncology advanced trainees. In 2016, 568 medical oncologists were employed in Australia and 158 trainees (268).

3.5.4.2 Power calculation/sample size

The Australian physicians' survey was developed by Dr Sally Baron-Hay and Dr Isobel Porter (Royal North Shore Hospital, Sydney, Australia) and they followed similar steps and had similar response rate expectations to the UK survey. The survey was sent out to all medical oncologists and medical oncology advanced trainees identified via workplace e-mails, the Breast Cancer Trials Group and advanced trainee social media page.

3.5.4.3 Survey Development

Survey content validity, face validity, useability and acceptability were ensured by following similar steps to the UK Survey.

It was an anonymous, electronic, 17-item self-administered survey. Most of the questions were closed-ended questions and apart from 3 questions about use of bone modifying agents (BMAs) in cancer treatment induced bone loss, all the other questions were focused on the same themes as the UK physicians' survey (current practice, patient selection and monitoring, and choice of BMA regimen) (Figure 3.1).

3.5.4.4 Survey Implementation

The survey was distributed via email and social media to medical oncologists and medical oncology advanced trainees via the Breast Cancer Trials Group (256 recipients), workplace e-mails (38 recipients) and advanced trainee social media page (150 recipients), between December 2018 and April 2019. The total number of participants reached via these avenues was 444, although it was anticipated that there would have been considerable overlap in recipients. The survey was not limited to one distribution list so as to allow more responses and try and achieve input from a broad mix of medical oncologists and medical oncology trainees. Medical oncology advanced trainees were included as insight into the prescribing habits of their consultants and of those soon to be entering the work force as new consultants. No incentive was provided.

3.5.4.5 Data Analysis

Although the Australian survey was created by the two Australian clinicians, I participated in the data analysis and to the comparison of the Australian with the UK physicians' survey. Descriptive statistics were used to summarise the responses to the survey. The frequency of each choice was calculated as a proportion of the total number of responders. The data were compiled using Microsoft Office Excel (© 2018 Microsoft Corporation). Percentages were rounded to nil decimal point.

3.5.4.6 The Survey

1. What is your role?

☐ Consultant medical oncologist in the private sector

☐ Consultant medical oncologist in the public sector

☐ Consultant medical oncologist in both private and public sectors

☐ Medical oncology advanced trainee

2. How many patients with a new diagnosis of early breast cancer do you treat each year?

☐ Less than 20

☐ 21- 50

☐ 51-80

☐ More than 80

3. Which guidelines regarding the prescription of ADJUVANT bone modifying agents to PREVENT DISEASE RECURRENCE in women with a history of early breast cancer are you familiar with?

☐ ASCO/CCO

☐ NCCN

☐ ESMO

☐ UKBCG

☐ NICE

☐ Other

4. If a bone-modifying agent was listed on the PBS for the ADJUVANT management of breast cancer to PREVENT DISEASE RECURRENCE, would you prescribe it based on the current literature?

☐ Yes

☐ No

☐ Not sure

5. Do you currently prescribe bone modifying agents for women with a history of early breast cancer?

☐ Yes go to question 6

☐ No go to question 10

6. For PREVENTION OF CANCER TREATMENT INDUCED BONE LOSS, which women with a history of early breast cancer do you select for adjuvant bone modifying agents?

- ☐ All premenopausal women
- ☐ All postmenopausal women
- ☐ Premenopausal women at high risk for fracture
- ☐ Postmenopausal women at high risk for fracture
- ☐ All women with osteoporosis
- ☐ None of the above
- ☐ Other

7. For PREVENTION OF DISEASE RECURRENCE, which women with a history of early breastcancer do you select for adjuvant bone modifying agents?

- ☐ All premenopausal women
- ☐ Premenopausal women on GnRH analogue
- ☐ All postmenopausal women
- ☐ Postmenopausal women at high risk for disease recurrence
- ☐ None of the above

8. What bon-modifying agent(s) do you prescribe for PREVENTION OF CANCER TREATMENT INDUCED BONE LOSS?

- ☐ Intravenous Zometa
- ☐ Oral Clodronate
- ☐ Other oral bisphosphonate
- ☐ Denosumab

Please document what dosing regimen you prescribe:

9. What bone modifying agent(s) do you prescribe for PREVENTION OF DISEASE RECURRENCE?

- ☐ Intravenous Zometa
- ☐ Oral Clodronate
- ☐ Other oral bisphosphonate
- ☐ Denosumab

Please document what dosing regimen you prescribe:

Go to question 10

10. What are your reasons for NOT prescribing adjuvant bone modifying agents in naturally/biochemically POSTMENOPAUSAL women for PREVENTION OF DISEASE RECURRENCE?

- [ ] I am not convinced by the data
- [ ] Costing
- [ ] Access to an infusion suite chair
- [ ] Not enough time to discuss the data with patients
- [ ] Other

11. What do you see as the major barriers to prescribing bone modifying agents for PREVENTION OF DISEASE RECURRENCE?

- [ ] Lack of awareness of the current data
- [ ] Lack of convincing data
- [ ] Costing
- [ ] Side effects
- [ ] Patient uptake
- [ ] Local protocol guidance
- [ ] Other

12. Is the use of adjuvant bone sparing agents for PREVENTION OF DISEASE RECURRENCEdiscussed at your breast multidisciplinary meeting?

- [ ] Yes
- [ ] No

13. Do you routinely perform a BONE MINERAL DENSITY prior to starting an aromatase inhibitor?

- [ ] Yes
- [ ] No

14. Do you routinely perform a BONE MINERAL DENSITY prior to starting adjuvant bone modifying agents for PREVENTION OF DISEASE RECURRENCE in women with history of early breast cancer?

- [ ] Yes
- [ ] No
- [ ] N/A

15. How frequently do you perform a Bone Mineral Density?

☐ Only at baseline

☐ Yearly
a. 2 yearly
b. Other

16. Do you prescribe any of the following alongside bone modifying agents?
a. Calcium
b. Vitamin D
c. Other

17. Do you have any further comments?

3.6 Results of the UK Physicians' Survey

The potential sources of bias for this survey include:

1) Sampling bias
2) Non-response bias
3) Social desirability bias
4) Acquiescence bias

Sampling bias from distribution via specialist breast cancer membership lists and also social desirability and acquiescence bias (in view of the NICE guidelines) could potentially over-estimate the current prescription of adjuvant BPs in the UK. However, the low response rate and therefore the non-response bias could affect the generalisability of the survey.

3.6.1 Current Practice

Role of Clinician

The survey was sent to 277 UKBCG members, and we received 68 replies (25% response rate). Ninety-six percent of the participants were consultant oncologists (50% medical oncologists, 46% clinical oncologists) and 4% were at a non-consultant level. Replies were received from 35 centres from all four UK countries, with the vast majority of responders (99%) working as NHS oncologists. Twenty nine of the responders were practising in England, 2 in Scotland, 2 in Wales and 1 in Northern Ireland. One responder was an Oncologist in private sector without specifying location.

Prescribing Habits

At the time of this 4th UKBCG survey (2019), almost all of the UK cancer physicians (99%) were offering adjuvant BPs to patients with EBC demonstrating that pressure from

national bodies, national guidelines and funding decisions have been critical to implementation, as the number of UK oncologists who prescribe adjuvant BPs has significantly increased (by 75%) since the first survey in March 2016 (Figure 3.3).

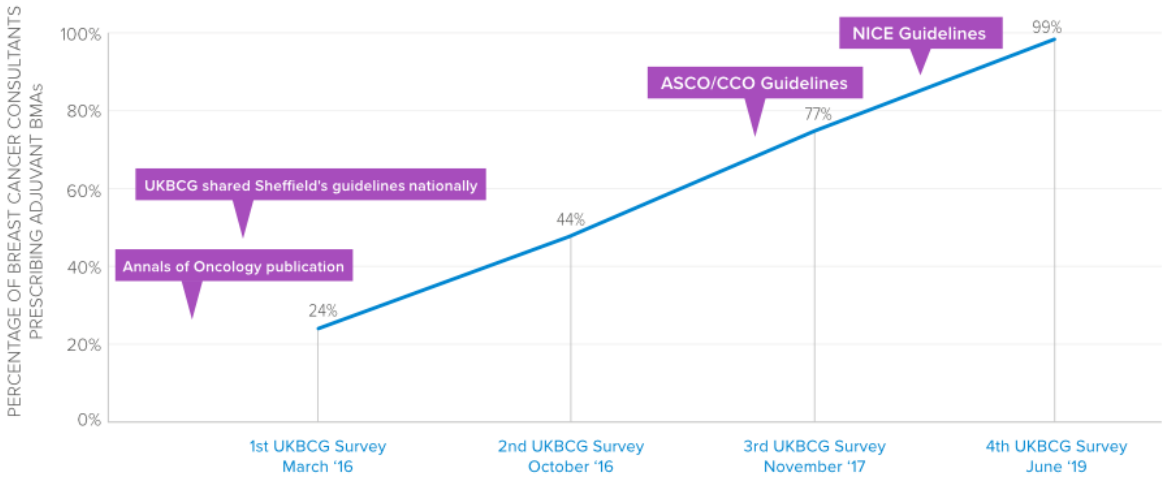

***Figure 3.3**: The use of adjuvant bisphosphonates for EBC in the UK from 2016 to 2019.*

Awareness of Guidelines

Participants were asked about their awareness for the following guidelines: UKBCG 2015, ESMO 2016, ASCO/CCO 2017, and NICE 2018 (Figure 3.4). All these international guidelines recommend adjuvant BPs for postmenopausal and premenopausal women on ovarian suppression treatment with high risk EBC for up to 5 years. Most (85%) of the UK oncologists were following the UKBCG guidelines regarding the prescription of adjuvant BPs to prevent disease recurrence in women with EBC, with 26% following NICE guidelines showing that UKBCG guidelines capture more patients. UK oncologists were also familiar with the ASCO/CCO recommendations and ESMO guidelines, but only 9% and 5% respectively stated that they were following them.

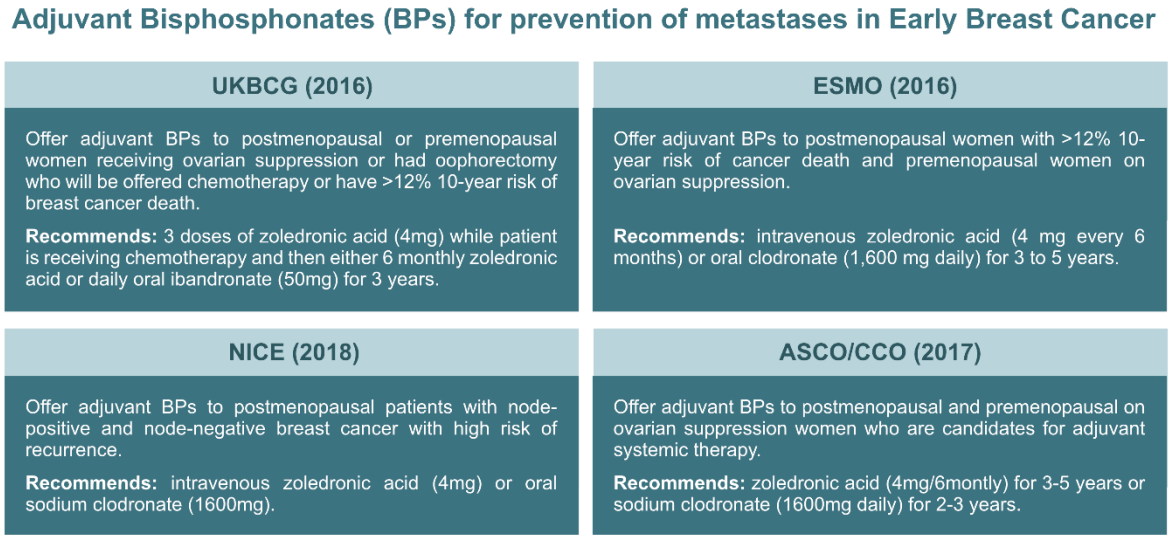

***Figure 3.4**: National and international guidelines for the use of adjuvant BPs in EBC.*

Barriers to Prescribing

The barriers to prescribing adjuvant BPs that were identified by this survey were the lack of local protocol guidance and the difficulties in accessing an infusion chair (required for administration of iv ZOL) and these were the reasons given only by one centre which does not offer these agents in women with EBC. This shows that despite the fact that most of the UK cancer centres have overcome any educational or financial barriers in adopting adjuvant BPs, some centres still facing difficulties and therefore the efforts from national bodies to support and guide these centres should continue.

3.6.2 Patient selection and monitoring

Patient Identification and Characteristics

Multidisciplinary Team (MDT) meetings are crucial part of the breast cancer care in the UK and worldwide, as they incorporate the treatment schedule and decisions for every new patient. Despite this, only 67% of the physicians were discussing the use of adjuvant BPs in their local MDTs, indicating that adaptation of this treatment is the responsibility of the individual breast cancer oncologist.

The majority of the responders suggested that they were offering adjuvant BTAs (bone targeted agents) for prevention of recurrence to postmenopausal women with high-risk disease (97%), and for most of the UK cancer physicians (85%) ‘high risk patients’ were those who would be offered chemotherapy, irrespective of whether they receive it or not. Other reasons for considering a postmenopausal breast cancer patient at high risk of recurrence were node positive (3%) or large node positive disease (2%), and >2% (2%) and >10% (2%) benefit on PREDICT scoring. PREDICT score is an online prognostication tool that assist

breast cancer physicians, as well as patients, in the adjuvant treatment decision process (269). It was created using data from the UK cancer registry and based on patients' (for example age) and cancer characteristics (for example tumour size, grade) provide the user with the predicted cancer survival and also the percentage benefit that anticancer treatments (chemotherapy, endocrine and anti- HER2 treatment, BPs) could add if received. A high risk of recurrence is a major determinant for prescribing, as only 3% of the UK oncologists were prescribing adjuvant BPs to all post-menopausal women regardless of their risk of disease recurrence (Figure 3.5).

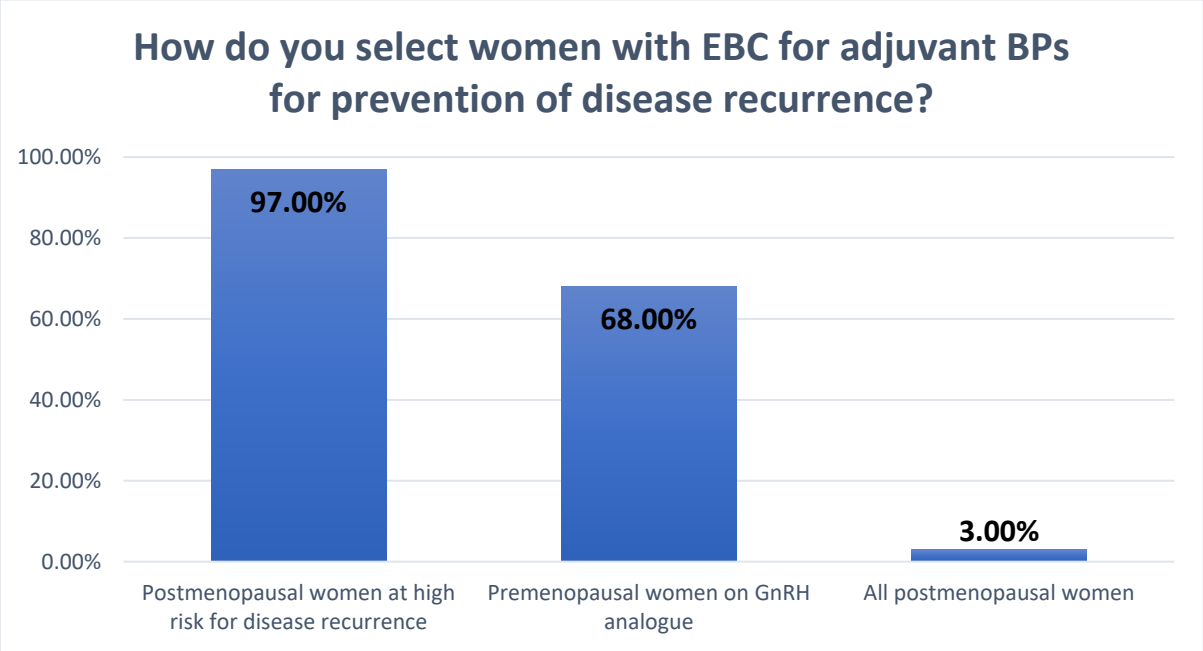

***Figure 3.5**: Selection of patients for adjuvant BPs in the UK (national oncology physicians' survey 2019).*

In terms of the premenopausal group (20% of breast cancers is diagnosed before the age of 50 and 7% before the age of 40) evidence is clear that adjuvant BPs are beneficial to this group only when patients receive ovarian suppression (induced menopause) (88, 89, 226). The responses mirrored this with 68% of the participants offering these agents to premenopausal women receiving GnRH (gonadotrophin realising hormone) analogues.

Bone Density Assessments

Anticancer treatments such as chemotherapy and endocrine treatment are known to increase the risk of CTIBL (51, 138, 153, 260, 270). Cancer treatment-induced bone loss is defined as osteoporosis (loss of bone mass) due to cancer treatment and dual energy X-ray absorptiometry (DEXA) scan is the gold standard test for its diagnosis. DEXA scan measures bone mineral density (BMD) and the results are reported as T score, with T score <-2.5 to indicate osteoporosis (T score is the standard deviation from the mean in comparison to the

BMD of a 30 year old healthy individual). National and international guidelines are available for the prevention and management of CTIBL, with treatment options to be either BPs or the anti-RANKL antibody denosumab (51, 153, 260, 270). Therefore, cancer patients who are already on the optimal treatment for CTIBL as part of their anticancer regime (adjuvant BPs), do not require bone density assessments before being considered for adjuvant BP therapy. As a result, 82% of the UK cancer clinicians did not perform bone density assessments prior to commencing adjuvant BPs and 35% of them performed these assessments at the completion of the adjuvant bone treatment (up to 3 years), mainly to use them as a baseline for future reference. Additionally, after the end of the adjuvant BP treatment (up to 3 years) and if the patient was receiving extended endocrine treatment (for 7 to 10 years) where the risk of CTIBL is higher, 39% of the cancer physicians assessed bone density every 2 years, with similar percentage (36%) stating that they never measured bone density in this group of patients. These findings demonstrate the necessity for development of a global UK consensus regarding the optimal frequency of bone density assessments post adjuvant BPs for women on extended endocrine therapy.

Dental Assessments

Bisphosphonate treatment is associated with osteonecrosis of the jaw (ONJ) and the incidence varies based on the type of treatment, dose, and frequency (271-273). A BPs' review (n=10,694 from 15 trials) indicated that ONJ in early disease is a rare event and it is more common in patients receiving ZOL (274). In the AZURE trial where the ZOL regime was more intense, compared to other EBC trials, the ONJ incidence was 2% (60). Therefore, oncologists are asked to perform dental assessments prior to commencing BP treatment in order to reduce the risk of such complications. In this survey, 92% of the responders said that they mandate a baseline (prior to starting treatment) dental assessment.

3.6.3 Choice of Bone Modifying Agent Regime

Commonly Prescribed Regime

The optimal adjuvant BP regime (agent, dose, frequency, and duration) is still an active international debate between the breast cancer experts. In the UK, the most commonly prescribed agent was ZOL (91%), and it was the only used adjuvant BP by 62% of the responders. The second most common regime was ZOL for the duration of intravenous chemotherapy and then a switch to oral Ibandronic acid (27%). In terms of duration, the majority answered that they offer adjuvant bone targeted treatment for 3 years (86%).

Vitamin D (+/- Calcium) Supplementation

The NICE guidelines state that vitamin D 400units daily and calcium 500mg daily should be taken by patients who are on adjuvant BPs, in order to prevent hypocalcaemia and undiagnosed vitamin D deficiency. Most of the responders (89%) followed this advice and offered both vitamin D and calcium alongside BMAs.

3.7 Results of the UK Pharmacists' Survey

The potential sources of bias for this survey include:

1) Sampling bias
2) Non-response bias
3) Social desirability bias
4) Acquiescence bias

Sampling bias from distribution via specialised list and also social desirability and acquiescence bias (in view of the NICE guidelines) could potentially over-value the current UK practise. Although, the response rate for this survey was not able to be calculated, low number of replies, non-response bias, could affect the generalisability of the survey.

Oncology Pharmacists Demographics

For the national oncology pharmacists' survey, we received 41 replies from 34 different UK centres. Five replies from international centres were not included in the final analysis as they did not practise oncology in the NHS. The response rate of this survey was unable to be calculated as there was no information on the actual recipients' number or the country of their oncology practice. A 2019 report suggested that there were 62 UK cancer centres indicating that responses were received from more than 50% of the national cancer centres (267).

Use of Adjuvant Bisphosphonates at the NHS Cancer Centres

Almost all of the UK cancer centres (93%) offered adjuvant BPs, confirming their continued use in preventing breast cancer recurrence. Sixty nine percent were prescribing BTAs for prevention of breast cancer recurrence to both postmenopausal and premenopausal on ovarian suppression women, followed by 45% who were prescribing these agents only to postmenopausal women, demonstrating that adjuvant BP is now standard of care for EBC patients in the UK (Figure 3.6).

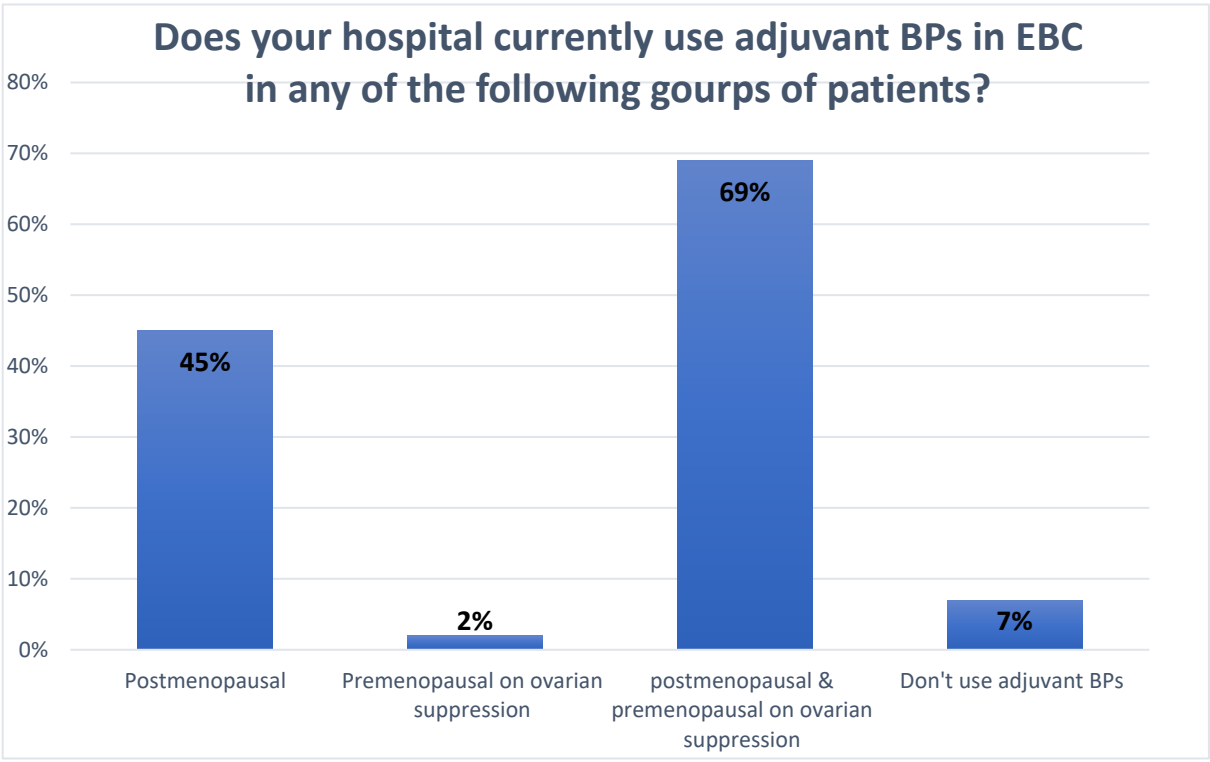

***Figure 3.6**: Selection of patients for adjuvant BPs in the UK (national pharmacists' survey 2021).*

Choice of Adjuvant Bone Targeted Agent

The most commonly used adjuvant agent was ZOL (83%). It was the only used agent by 64% of the cancer centres, and it was offered either alone or in combination with oral agents, or only in combination with oral agents by 12% and 7% of the hospitals, respectively. In the centres where ZOL was used in combination with oral agents, ZOL was given to patients during systemic anticancer treatment followed by a change to an oral alternative (Figure 3.7).

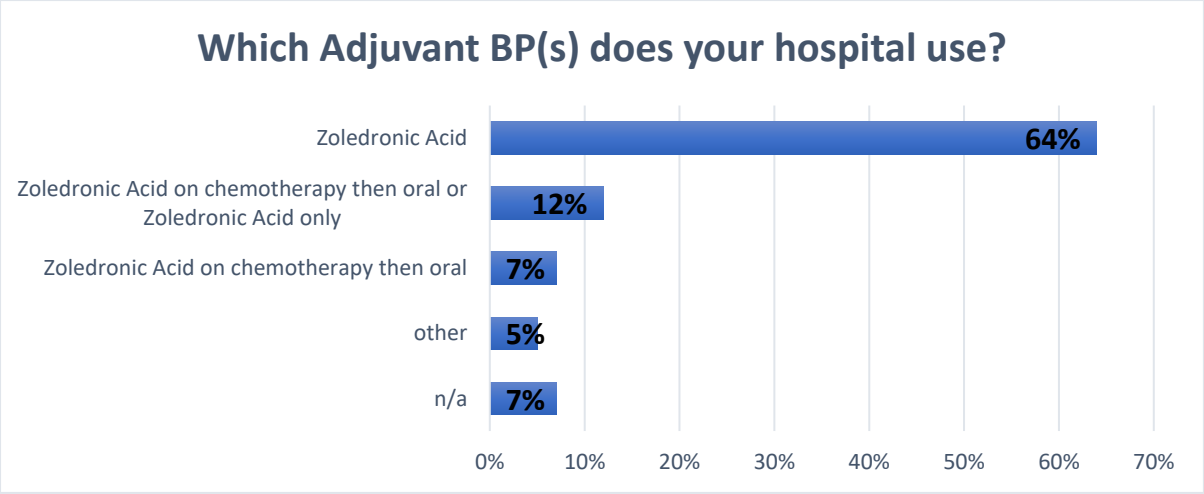

***Figure 3.7**: Choice of adjuvant bone agent by NHS cancer centers 2021.*

Prescribing Pathway for Oral Adjuvant Bisphosphonates

After the completion of their post-surgery systemic anticancer treatment and radiotherapy, EBC patients enter a 5 year follow up period in which a large percentage will continue receiving oral treatments such as endocrine treatment and adjuvant BPs, without the need for any oncological review. For these patients, the NHS has introduced the 'shared care agreement', where clinical responsibility for prescribing and monitoring long-term treatments is transferred from a hospital/specialist service to general practice, to improve patients experience and make best use of clinical time and NHS resources in all care.

For the oncology centers that replied to the survey, only 43% [33% started by oncologist and transferred to general practitioner (GP), 10% started only by GP)] were sharing the prescribing and monitoring of adjuvant BP treatments with GP practices. These results indicate that more national support and education are needed for GPs to feel able to include oral adjuvant BPs into their shared care agreements. For 12%, the care of the oral agents started and stayed with the oncologists. Pharmacists and advanced nurse practitioners were also responsible for the oral BPs' prescription in 7% and 5% of the NHS oncology centers respectively.

3.8 Results of the Australian Physicians' Survey and Comparison with the UK Physicians' Survey

The potential sources of bias for this survey include:

1) Sampling bias
2) Non-response bias

Sampling bias from distribution via specialist breast cancer membership lists could potentially over-estimate the use of BPs in Australian breast cancer practice. However, the low response rate and therefore the non-response bias could affect the generalisability of the survey.

3.8.1 Current Practise

Role of Clinician

The Australian survey was distributed via email and social media. Email distribution was facilitated via the Breast Cancer Trials Group (256 recipients), workplace e-mails (38 recipients) and advanced trainee social media page (150 recipients). The total number of participants reached via these avenues was 444, although it is anticipated that there would have been considerable overlap in recipients.

Survey received 60 responses (14% response rate, not accounting for overlap). Of the 60 participants, 22 (37%) were consultant medical oncologists working in both the public and private sectors, 20 (33%) were consultant medical oncologists working in the public sector only, 11 (18%) were medical oncology advanced trainees and 7 (12%) were consultant medical oncologists working in the private sector only. Overall, the participants were experienced in treating EBC, with 33% treating >80 patients with a new diagnosis of EBC each year, 25% treating between 51 and 80, 33% treating between 21 and 50 and only 8% treating less than 20 new patients with EBC each year. Although geographic data were not specifically captured, there was evidence of participation from multiple Australian states noted in the comments of respondents.

Prescribing Habits

In contrast to the UK survey that illustrated almost universal uptake of adjuvant BPs within the last few years, only 48% of the Australian cancer physicians were prescribing these agents, with the majority of them (83%) reporting that they would prescribe them if they were nationally funded (listed on the Australian PBS) for preventing of breast cancer recurrence.

Awareness of Guidelines

Australian participants were asked about the awareness of the following guidelines for the use of adjuvant BPs: UKBCG 2015, ESMO 2016, ASCO/CCO 2017, National Comprehensive Cancer Network (NCCN) and NICE 2018. National Comprehensive Cancer Network guidelines were only included in the Australian survey.

The majority of Australian responders (83%) were familiar with the joint ASCO/CCO guidelines. Other guidelines with familiarity included NCCN (39%) and ESMO (32%). Only one participant reported not being familiar with any guidelines on the topic. As opposed to these results, in the UK most of the cancer physicians (85% UKBCG and 26% NICE guidelines) follow national guidelines, highlighting the importance of established national and international consensus for the use of adjuvant BPs (Figure 3.8).

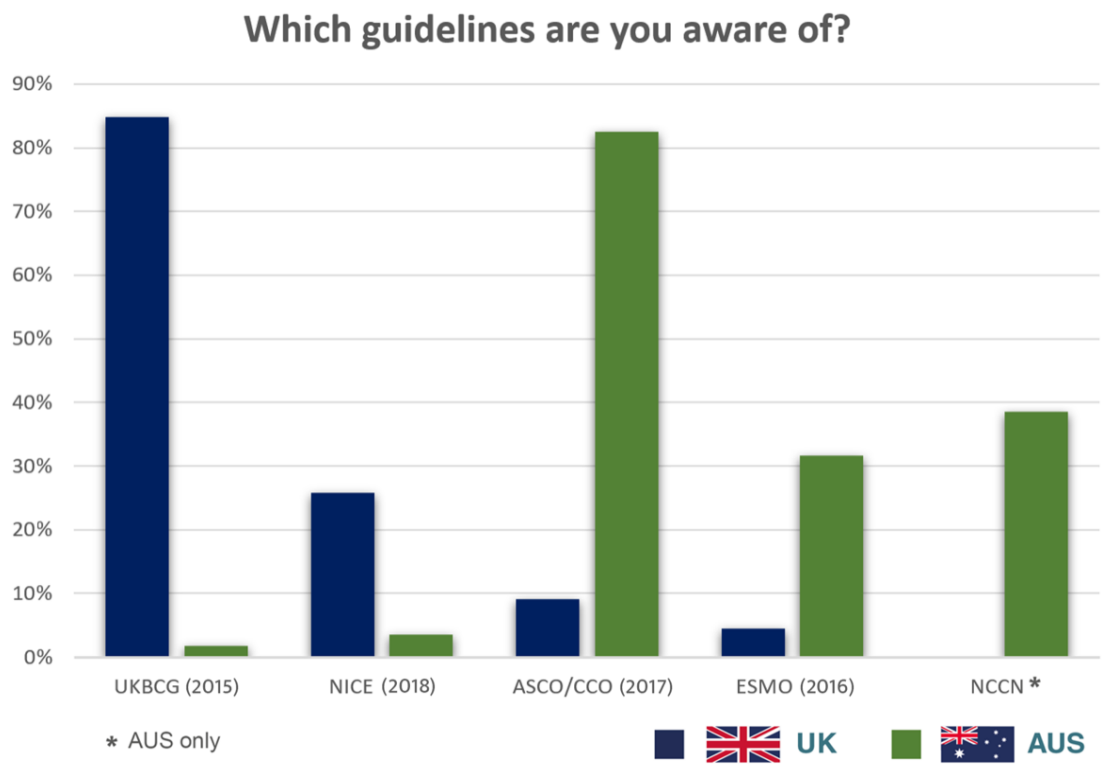

***Figure 3.8**: Cancer physicians' awareness of guidelines for the use of adjuvant BPs in EBC in the UK and Australia.*

Barriers to Prescribing

For the Australian clinicians, of the 21 participants who did not currently prescribe adjuvant BPs, 16 (76%) reported that it was due to cost concerns, whereas 8 (38%) were not convinced by the available data regarding significant patient benefit (Figure 3.9). For the entire population surveyed, major barriers to prescribing BPs in Australia were identified as being cost (80%), local protocol guidance (36%) and lack of awareness of the current data (31%). Side effects (29%) and patient reluctance to additional treatment (22%) were also considered barriers. Only 3 responders (5%) stated that they would not prescribe BMAs for the adjuvant management of EBC, even if they were part of the Australian PBS, with an additional 7 (12%) stating that they were unsure (Figure 3.9).

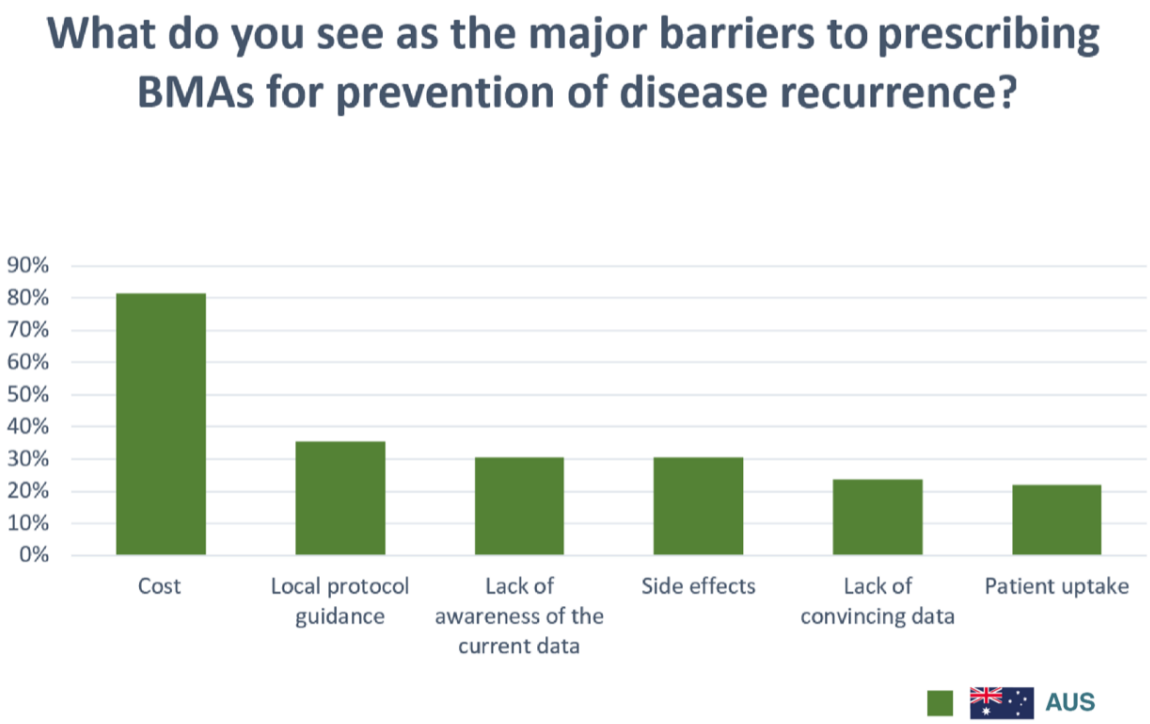

***Figure 3.9**: Barriers to prescribing adjuvant BPs in EBC identified by the Australian participants in 2019.*

3.8.2 Patient Selection and Monitoring

Patient Identification and Characteristics

Multidisciplinary Team meetings are crucial part of the breast cancer care as they incorporate the treatment schedule and decisions for every new patient. They run for the same purpose in both the UK and Australia, and they have similar structure. Despite this, only 18% of the Australian participants discuss the use of adjuvant BPs in their local MDTs (68% in the UK), demonstrating that Australians, as well as the UK, cancer physicians, leave the decision for adjuvant BP treatment for the individual breast oncologists.

Similar to the UK, the majority of the Australian physicians (79%) were offering adjuvant BPs to postmenopausal women with high risk early breast cancer. Only 17% were offering them to all of the postmenopausal women (3% in the UK), showing again how crucial high risk disease is for decision making by cancer physicians (Figure 3.10).

Australian participants were not asked to clarify what they deemed high risk EBC. The surveys were similar but the wording of the two questionnaires was not identical. However, the questions address the same issues of how a selection is made for prescription of adjuvant BTAs and therefore the comparison of the two surveys is valid.

Premenopausal women receiving GnRH analogues were prescribed BMAs for prevention of disease recurrence by 31% of the Australian clinicians (68% in the UK) (Figure 3.10).

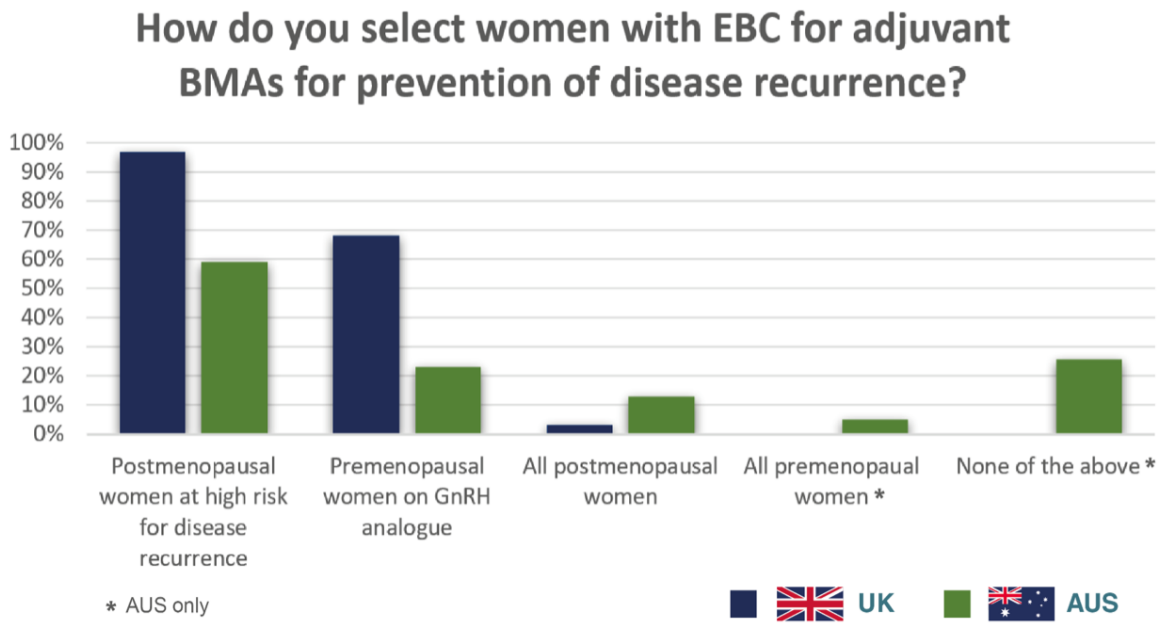

Figure 3.10: *Patient selection for adjuvant BMAs in the UK and Australia.*

Bone Density Assessments and Dental Assessments

As described above, BMA also prevent BMD loss from systemic anticancer therapies and BMD monitoring is recommended in patients at risk of BMD decline. Ninety three percent of the Australian clinicians ordered a BMD assessment prior to starting an AI for women with EBC. When BMAs were prescribed to prevent breast cancer recurrence, 35% of the Australian oncologists perform BMD assessment prior to commencing adjuvant BPs, while 82% of the UK oncologists did not. There was a wide spectrum of practice (in Australia) when it came to the frequency of ordering BMDs, with the majority between 1 and 3 years and many taking into consideration the baseline result.

Australian cancer physicians were not asked about their habits in regard to the dental assessments.

3.8.3 Choice of BMA Regime

Commonly Prescribed Regime

Intravenous ZOL (4 mg/6monthly) was the most commonly prescribed adjuvant BMAs in both countries (89% UK, 81% Australia) and most responders offered BMAs for prevention of disease recurrence for a duration of 3 years. In Australia, the second most commonly prescribed agent was denosumab (36%), while in the UK, denosumab is only used in metastatic setting and for CTIBL, as it has not been shown to prevent disease recurrence. The Australian survey was performed prior to publication of the results of the D-CARE study which

showed that denosumab does not have any anticancer benefit in EBC disease (94). Therefore, changes to Australian practise in regard to the adjuvant use of denosumab are expected.

Vitamin D and Calcium Supplementation

In the Australian survey, 98% of participants prescribed vitamin D and 83% prescribed calcium alongside BMAs (in the UK 89% prescribed both vitamin D and calcium). Only 3% reported that they recommended exercise alongside BMAs, there was no question about prescribed exercise in the UK survey.

3.8.4 Outcome of UK and Australian collaboration

This international collaboration gave an insight into UK's successful pathway of adjuvant BP implementation and aimed to suggest steps (Table 3.1) that Australia, as well as other countries with similar barriers, could follow to achieve the same outcome and improve breast cancer care. The results from these 2 surveys were published in Journal of Bone Oncology in 2021 (275).

Proposed steps to increase update of adjuvant bisphosphonates in Early Breast Cancer
Raise awareness of the adjuvant BPs benefits by engaging with the oncology community (oncology conferences etc)
Encourage the discussion of adjuvant BPs in local breast cancer MDT meetings
Work with national bodies to produce clear guidelines for the use of adjuvant BPs in EBC
Develop a convincing business case to demonstrate value for money
Work with breast cancer charities (local, regional, and national) to raise awareness among patients and lobby decision makers

***Table 3.1**: Proposed steps to increase update of adjuvant BPs in EBC.*

3.9 Discussion

Multiple clinical studies and a subsequent large meta-analysis demonstrated that adjuvant BPs (given after breast cancer surgery) can improve cancer outcomes only in postmenopausal women (natural or induced by ovarian suppression) with EBC, as they offer better survival and reduce cancer recurrence (78-81, 88, 89, 226). As a result, international guidelines for the management of EBC disease have included these agents, encouraging their use by cancer physicians (47, 51, 126, 232, 251-254). However, despite the clear evidence and the international recommendations, changes in breast cancer practice are not always

straight forward. The implementation process is usually lengthy and time consuming as barriers in national, local, and individual level need to be overcome in order for practise to change. The introduction of BPs from their traditional use to treat bone metastases to the adjuvant setting was only started in 2015, providing an ideal opportunity to track their uptake in national and international level, follow their implementation journey and describe barriers and the ways these were addressed.

The results of the two physicians' surveys are representative of a group of UK and Australian cancer physicians with significant experience in treating women with EBC (58% of Australian responders seeing >50 new EBC patients per year) and knowledge of the current international guidelines on the topic. The Australian questionnaire also included advanced medical oncology trainees as insight into the prescribing habits of their consultants and of those soon to be entering the work force as new consultants. In the UK, almost all of the oncologists offer adjuvant BPs in postmenopausal women with EBC (99%), especially in those considered high risk of recurrence, demonstrating that BPs have now fully endorsed and incorporated into the national management of EBC. These results were confirmed by the follow-up pharmacists survey which showed similar outcomes (used by 93% of cancer centres), supporting the statement that the national UK uptake of adjuvant BPs was massively accelerated since their introduction in 2015 and that adjuvant BPs are now standard of care in the UK (Figure 3.3). In contrast, although their adjuvant use in EBC is supported by the majority of the Australian oncologists (83%), their uptake remains heterogenous and sub-optimal, with less than 50% of respondents currently prescribing adjuvant BPs.

Education, national and local guidance, and funding arrangements have been crucial in the implementation of adjuvant BPs in the UK. This was highlighted in the answers of Australian clinicians who suggested that main barriers in the use of BPs are lack of national funding, local protocol guidance and physician awareness. Additionally, UK responders indicated that most of them follow UKBCG and NICE guidelines, showing that the first essential step in the successful transition of a new treatment form clinical trials to standard of care, is its inclusion in the guidelines that most physicians follow. Surprisingly, 14% of the UK oncologists said that they are following ASCO/CCO and ESMO guidelines (9% and 5% respectively).

Considering the absolute OS benefit of adjuvant BPs, their addition to the entire eligible Australian population (15,000) has the potential to reduce annual breast cancer deaths by about 400 (276-278), suggesting that their implementation should be a priority.

An Australian business case and financial impact statement could be modelled on those done in the UK, to support the implementation process and increase the likelihood of obtaining national funding. Until then, the international collaboration presented here, recommend that development of national guidelines endorsing BP use in the adjuvant setting

should be prioritised, as well as improving awareness of the data amongst clinicians and patients (Table 3.1).

Optimal recommended adjuvant regime (strength, frequency, type of agent and duration) is an active debate between breast cancer specialists. Although, studies have not showed any difference in disease outcomes between agents (89), physicians showed clear preference towards intravenous ZOL in both the UK and Australia. This was even the case for the UK during the COVID-19 pandemic, where the need for switching from intravenous treatments to oral alternatives was inevitable, in order to reduce hospital visits. This might well be due to the fact that intravenous treatment is less frequent (6 monthly) compared to daily oral agents and also hospital administered treatment guarantees adherence. Despite this, a patient survey published by Sheffield researchers showed good patient adherence to oral adjuvant BPs (84% the first year of treatment), with only 16% discontinuing the treatment and less than 10% reporting to be extremely bothered by the side effects (55). The same survey showed that discontinuation of treatment was more likely to happened during the first 6 months of use. Furthermore, a more recent survey of Canadian EBC patients treated or undergoing adjuvant BP therapy (92% ZOL) demonstrated that the therapy is tolerable with a 94% completion rate, despite 60% of patients experiencing one or more side effects (279). Data on patients experience with these agents are limited, highlighting the need of exploring this in more details. Patients experience with adjuvant BP can guide the decision making for the optimal regime and subsequently maximise the benefit that patients receive.

UK oncologists preferred use of ZOL could also be explained by the lack of established share-care agreements between the oncology centres and GPs. As the pharmacists' survey showed, less than half of the cancer centres have shared-care agreement in place for oral adjuvant BPs making the daily oral choice less favourable for the already busy cancer physicians. If the prescription and monitoring of daily oral agents is shared with local GP practices, then UK oncologists might switch to oral adjuvant BPs, at least for after the completion of systemic anticancer treatment.

Despite the lack of clear guidelines to support the use of adjuvant denosumab to improve overall survival, 18% of Australian clinicians surveyed were prescribing it, compared with none of the surveyed UK clinicians. The rationale for such significant use of denosumab in the adjuvant setting in Australia was not explored by the survey but may at least in part be due to outcome benefits in terms of greatly reduced fracture rate and improved DFS from 6 monthly use of denosumab in addition to adjuvant AIs therapy reported in the ABCSG-18 trial (52, 53). In addition, there may have been a lack of awareness of the D-CARE trial data as the final results were published after the completion of this survey (94). Finally, there may have been extrapolation from the use of denosumab in CTIBL, where it has performed as well

as/better than BPs and been more convenient for patients and does not require chair time in busy outpatient chemotherapy treatment facilities (52).

Most international guidelines suggest adjuvant BP treatment should be given for 3 to 5 years, with a recent study showing that cancer outcomes do not improve after 2 years of treatment (compared 2 years to 5 years of adjuvant BPs) and less treatment comes with less problems for the patients (128). Canadian physicians and patients expressed interest in de-escalation clinical trials in terms of the adjuvant BPs duration (279, 280). Current UK consensus sees patients receiving adjuvant BTAs for 3 years, but this might soon change as more clinical evidence become available to inform the optimal required treatment period to obtain drug benefit. In the osteoporosis setting, real-world patients' adherence to oral BPs has been found to decline significantly with time. A review from 55 studies (n=4033731) has reported adherence at 6 months between 56% to 90% and at 3 years 23% to 48% (281), indicating that this might be also happening at the adjuvant cancer cohort where treatment duration is 3 years. Sheffield report only captured patients' experience for just over a year (55) and therefore more real-world data describing patients' adherence for the whole duration of adjuvant treatment is needed. Of the concerns, ONJ is the main, arising from the long term BP use. Data suggest that the risk in the adjuvant setting is generally small (up to about 2%) and with the introduction of prober dental assessment (performed by the majority of the UK responders, 92%), the risk could be prevented (60, 274).

In addition to their survival benefit, BPs also prevent CTIBL (51, 80, 81, 153, 260, 270, 282). DEXA scan is the test of choice for assessing BMD, especially in patients receiving chemotherapy and/or endocrine treatment (mainly AIs) who are at increased risk of CTIBL (51, 138, 153, 260, 270, 282). Pharmacological choices for prevention and/or treatment of CTIBL differ from those used to prevent bone metastases. For this setting, denosumab had recently become the first choice and BPs doses are less frequent and more comparable to the osteoporosis doses (51, 153, 260, 282). Therefore, offering adjuvant BPs to patients receiving endocrine treatment (mainly AIs) for endocrine positive EBC, will benefit them for both bone metastases prevention and CTIBL. In the cases where BPs were given for prevention of disease recurrence, a considerable percentage of Australian oncologists were experiencing the same barriers in routinely performed BMD assessment prior to commencing BMAs, demonstrating a potential avenue for cost saving. In the UK, the frequency of bone density assessments in patients receiving adjuvant BPs and extended endocrine treatment, was an area that was lacking national consensus. Oncologists were not very clear as to when and how often bone density assessments were needed in this population, therefore this topic requires further review to avoid unnecessary investigations or mistakes from misdiagnosis.

There are several limitations to these studies. All surveys had a relatively low response rate which may limit their generalisability to practice as a whole requiring caution in

interpretation although, in the Australian survey, as explained, it's anticipated that there would have been considerable overlap between the various platforms used to distribute the survey to recipients. Sampling bias from distribution via specialist breast cancer membership lists could potentially over-estimate the current prescription of adjuvant BPs given the high rates of breast cancer specialisation, penchant for research and international collaboration within the group. However, the data in Figure 3.11 clearly show the increase in monthly doses of adjuvant ZOL administered in England between January 2017 and January 2020 (data source: NHS Systemic Anticancer Therapy data set). These data support the conclusion that prescribing has changed dramatically over the years, presumably due to the national education campaign and NICE and UKBCG endorsement. A similar Canadian clinicians' survey published in 2019 also had a low response rate at 11% (68/618), suggesting that this is not uncommon for this type of survey-based study (283). However, a further Canadian physician survey exploring the same topic had 41% response rate (52/127) which may well be due to the more targeted group of participants (280).

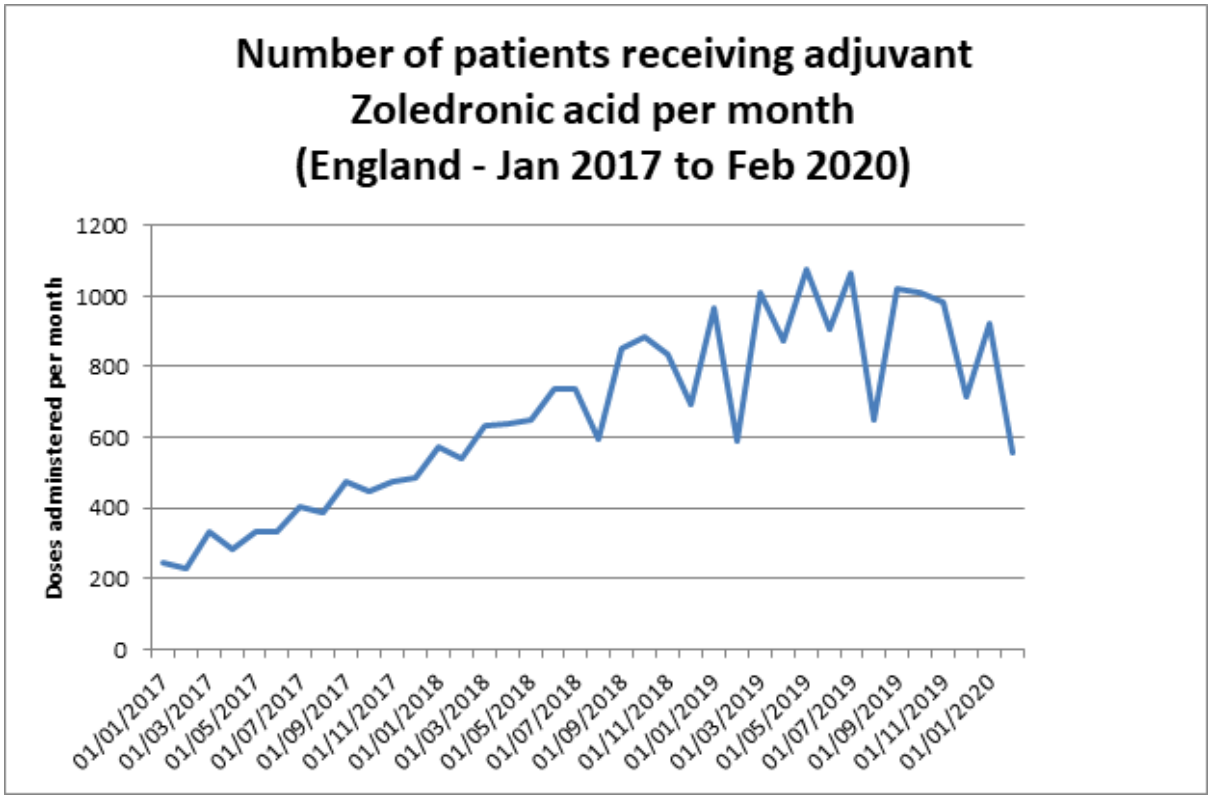

Figure 3.11: *Monthly use of adjuvant zoledronic acid in NHS England from 2017 to 2020 (data source: NHS Systemic Anticancer Therapy data set).*

Currently, data on adoption rates from other countries do not appear to be available, with the exception of the 2021 clinician survey from Canada in which most responders (77.4%) recommending adjuvant BMAs (mainly ZOL 4 mg/6monthly) for postmenopausal patients with

high-risk breast cancer (280). However, this high rate amongst the survey responders was not reflected by prescription data, e.g. in Ontario only 20% of eligible patients received adjuvant BPs. This may be due to selection bias in terms of survey responders, which may also be an element in our data, resulting in higher uptake rate than in the general oncology community. Interestingly, in this study the main barriers to wider uptake were identified as increased risk of toxicities from BMAs and the need for additional follow up and treatment.

3.10 Conclusion

Adjuvant BPs are now in routine use in the UK for postmenopausal EBC. Pressure from national bodies and breast cancer charities, education and health economics have been crucial for supporting and encouraging UK physicians to change their breast cancer care. More evidence is needed to identify the optimal adjuvant BP regime with aim to improve patients and physicians experience with these agents. Furthermore, we believe by translating the methodology for adjuvant BPs implementation in the UK to Australia, that this may pave the way for other nations struggling with similar barriers to adopt this life saving treatment and ultimately improve outcomes for women with EBC globally.

3.11 Future work

- Further similar surveys and real-world data from other countries (European or not) are needed to explore how different health care systems approach the use of adjuvant BPs in EBC. These will provide a better insight and understanding of the global use of these agents and support future research needs towards the identification of the optimal adjuvant BP regime.

- In view of the Australian survey results and the low uptake of adjuvant BPs, the survey will need to be repeated in the near future. This will aim to reassess Australian EBC practice, explore the effects that the proposed steps may have had on the EBC care and describe any potential increases in the use of adjuvant BPs. It is of particular interest to reassess the use of denosumab in this setting.

3.12 Presentations and publications arising from this project

Poster Presentations

2. I Porter*, **E Theodoulou**, C Wilson, JE Brown, S Baron-Hay, Adjuvant bone modifying agents for women with early breast cancer: documenting Australian prescribing habits. Medical Oncology Group of Australia, Annual Scientific Meeting 2019, Canberra, Australia.

3. S Baron-Hay*, **E Theodoulou**, I Porter, I Holen, C Harper-Wynne, JE Brown & C Wilson, Inclusion of adjuvant bone-modifying agents for early breast cancer into standard clinical practice: challenges and lessons learnt from an international collaboration. San Antonio Breast Cancer Symposium (SABCS), December 2019, San Antonio, Texas, USA.

4. **E Theodoulou***, N Masters, I Holen, JE Brown, Pharmacists' Experience with Adjuvant Bisphosphonate use in Early Breast Cancer. A National UK survey. Mellanby Centre Research Meeting 2022, March 2022, Sheffield, UK.

5. **E Theodoulou***, N Masters, I Holen, JE Brown, Use of Adjuvant Bisphosphonates in Early Breast Cancer. A National UK Survey Describing the Experience of Oncology Pharmacists, British Oncology Pharmacy Association (BOPA) Conference 2022, October 2022, Liverpool, UK.

Oral Presentations

1. **E Theodoulou***, N Masters, I Holen, JE Brown, Pharmacists' Experience with Adjuvant Bisphosphonate use in Early Breast Cancer. A National UK survey. The Cancer and Bone Society (CABS) Young Investigator Symposium 2022 - Virtual meeting, February 2022.

Publications

1. Porter I, **Theodoulou E**, Holen I, Harper-Wynne C, Baron-Hay S, Wilson C, Brown J. Adoption of adjuvant bisphosphonates for early breast cancer into standard clinical practice: Challenges and lessons learnt from comparison of the UK and Australian experience. J Bone Oncol. 2021 Nov 1;31:100402. doi: 10.1016/j.jbo.2021.100402. PMID: 34804788; PMCID: PMC8581365.

Published Abstracts

1. S Baron-Hay, **E Theodoulou**, I Porter, I Holen, C Harper-Wynne, JE Brown & C Wilson, Inclusion of adjuvant bone-modifying agents for early breast cancer into standard clinical practice: challenges and lessons learnt from an international collaboration. The San Antonio Breast Cancer Symposium (SABCS), December 2019, San Antonio, Texas, USA.

***Indicates the presenting author

Chapter 4

Patients Experience with Adjuvant Bisphosphonates.

A Breast Cancer Now Survey

4.1 Summary

Having explored the use of adjuvant Bisphosphonates (BPs) from the cancer physicians and oncology pharmacists point of view in chapter 3, I also wanted to understand more about how patients receive the use of these agents in early breast cancer (EBC) and what is their general understanding of the therapy. In order to do this I developed a patient survey.

This patient survey aimed to gather real world data for the use of adjuvant BPs in EBC. With the support of Breast Cancer Now (BCN), an anonymous online survey was created and disseminated to the members of the Breast Cancer Voices, a BCN patient group. The survey was seeking information for the patients' experience with these agents, their understanding of the purpose of this therapy, any side effects they had and their overall opinion about BPs in breast cancer.

In view of their demonstrated anticancer benefits (78-81, 88, 89, 226) BPs are recommended by national and international bodies, but only for postmenopausal EBC (13, 51, 126). Since 2018, they have been fully endorsed in the management of early disease in the UK after NICE added them in its EBC recommendations (13). UK physicians and pharmacists surveys (chapter 3) showed that almost all of the eligible patients are offered adjuvant BPs. However, UK patients perspective for this therapy has only been explored in a study that was solely focused on oral agents (55), whereas zoledronic acid (ZOL, iv infusion) is the most commonly used adjuvant BP.

Therefore, identifying the gap in evidence and knowledge, a patients survey was set up in collaboration with BCN. Survey outcomes will give us a better understanding of patients experience and views about adjuvant BPs with, ultimately aiming to obtain information that can improve patients overall experience with this therapy.

4.2 Introduction

Bisphosphonates are pyrophosphate analogues that inhibit osteoclast mediated bone resorption. In clinical practice, BPs are traditionally used for the prevention and treatment of osteoporosis (284). In breast cancer, they have an established role in prevention of complications from bone metastasis such as fractures and pain and they are recommended for the management of cancer treatment-induced bone loss (CTIBL) (51). The use of BPs in breast cancer treatment is described in more detail in the introduction of this thesis.

In recent years, BPs have gained extra interests for their anticancer effects in postmenopausal (natural or induced by ovarian suppression) EBC. Multiple clinical studies have demonstrated that BPs can reduce the risk of disease recurrence and improve survival, but only in postmenopausal women (78-81, 88, 89, 226). The full reasons for their positive

effects in EBC are still unknown, but the evidence of their beneficial effect in this setting were robust enough for BPs to become standard of care for postmenopausal EBC in the UK, Europe and America (13, 51, 126).

In the UK standard clinical practice, adjuvant BPs were first introduced at the end of 2015 when they were included as a recommendation in the breast cancer CRG (Clinical Reference Group) service specification and were endorsed as a priority for implementation by the UK Breast Cancer Group (UKBCG) (127). They were fully adopted in the UK breast cancer care in 2018 when NICE included them in its recommendation for the management of early disease (13).

The UK national physicians survey, described in chapter 3 of this thesis, demonstrated that the uptake of adjuvant BPs has increased significantly within the last few years, with almost all breast cancer physicians offering these agents to patients with postmenopausal EBC. The most commonly prescribed BP in the UK is the intravenous agent ZOL (4mg/6montly for 3 years). However, adjuvant BPs still remain a fairly new treatment for EBC, meaning that many patients are unfamiliar with these agents and have limited understanding of why they are prescribed in this setting. I therefore wanted to explore patients' journeys, understand and monitor any potential issues they are having, with the ultimate aim to gather information that can be used to improve their experience with adjuvant BPs.

Traditionally, patient perspectives for a treatment have not been prioritised as clinical studies tend to focus on reporting side effects, toxicities and tolerability. Therefore, data descripting the patients' experience with adjuvant BPs are limited, in particular outside clinical trials. So far, the only available real-world data for the use of these agents are from a cross sectional study published by a Sheffield group in 2019 (n=295/389, questionnaire response rate 76%) (55) followed by a Canadian patient survey published 2 years later (n=165/255, survey response rate 64.3%) (279). The UK study explored adherence and patient reported toxicity for oral BPs, whilst the Canadian survey reported toxicity and tolerability for both oral and intravenous agents. The Sheffield participants were asked to complete the Osteoporosis Patient Treatment Satisfaction Questionnaire (OPSAT-Q) and demonstrated that oral Ibandronic Acid (50mg/daily) was very well tolerated, and the incidence of severe side effects was very low. Similarly, the Canadian EBC patients who were mostly receiving ZOL (92%) 6 monthly for 3 years (73%), showed that adjuvant BPs were well tolerated despite some reported side effects.

Acknowledging the gap and the need for further data, with the support of BCN (the largest UK breast cancer charity with a leading role in the promotion of adjuvant BPs in the UK), a patient survey was created (Figure 4.1). The survey aimed to gather real-world data about patients' experience with adjuvant BPs and focused on patients' general understanding

of why they were offered adjuvant BPs, tolerability of treatment, side effects and patients' opinions and suggestions about the use of bone targeted agents in EBC.

4.3 Hypothesis, Aims and objectives

Hypothesis

- UK breast cancer patients have limited knowledge around the use of adjuvant BPs.

Aims

- To provide real-world data about patients' experience with adjuvant BPs.
- To describe patients' general understanding of adjuvant BPs.

Objectives

- Evaluate the use of adjuvant BPs in the UK from the patients' perspective.
- Determine any difficulties or side effects that patients experienced with adjuvant BPs.
- Describe patients' opinions and suggestions about adjuvant BPs.

4.4 Methods

4.4.1 Survey Population

The target study population was EBC patients who were members of the BCN group, Breast Cancer Voices and were offered adjuvant BPs, irrespective of if they go on to receive the therapy. Members of this group are people whose lives have been affected by early or metastatic breast cancer, whether they have been diagnosed themselves, a friend or a family member. The Breast Cancer Voices group had 925 members in February 2023 (285), but at the time of the survey the exact number of EBC patients participating in Breast Cancer Voices was unknown.

Participants had to be able to provide an online written consent and be willing and able, to complete the survey, which was available only in English. With the help of BCN, the survey was shared in the monthly charity newsletter that emailed to the Breast Cancer Voices group. The main survey exclusion criteria was metastatic breast cancer.

4.4.2 Power calculation/sample size

This was an exploratory survey and consequently a power calculation was not appropriate, but we aimed to ensure that we adequately sampled the target population to ensure the generalisability of the findings. To this end the survey was sent out to all the members of the Breast Cancer Voices by BCN with the expectation of a response rate 30-50% based on other cancer patients surveys.

4.4.3 Survey Development, Collaboration with Breast Cancer Now and Patient and Public Involvement.

The survey content validity was assured by using 3 sources of information to guide questionnaire content: review of the existing literature, an expert reference group (ERG) comprising members of the core research team and lastly the draft version was reviewed by a Patient and Public Involvement (PPI) group and the BCN group responsible for the dissemination of the Breast Cancer Voices newsletter. Face validity, useability and acceptability of the survey were confirmed by piloting the questionnaire with several patients from the PPI group, to ensure the questions were appropriate and making any modifications before the final survey was distributed. A key aim was to keep the questionnaire short and to the point to encourage survey completion, whilst capturing meaningful information about the broader experience with adjuvant BPs.

I first approached BCN who were very enthusiastic and keen to support a project with potential to positively impact patients' experience with breast cancer. They agreed to help with the development and dissemination of the survey and connected me with their patient groups (Breast Cancer Voices and Breast Cancer Louder Voices) which are a great resource for patient-centred projects and surveys. Subsequently, BCN supported me in reaching patients who then formed the PPI group and extensively helped in the development of this survey. More specifically, the survey was revised following input form the PPI group through several rounds of iteration; the final version was piloted by members of the same PPI group prior to becoming live and available to the Breast Cancer Voices members (Figure 4.1).

The survey was imported into SurveyMonkey, an online survey software platform. This software helps the users to develop an online survey and to analyse data, select samples and present their data. It offers the option of including any type of question, open-ended and closed-ended, both types of questions were used. Questions were 1 choice, multiple choice or free text box questions. The majority of questions had also the option of "other" as a free box for participants to use if needed. The Likert rating scale was used for 2 of the questions where the aim to gather peoples' attitudes and opinions. Upon completion of the survey development, an online link is created which can be sent to the potential responders either through the SurveyMonkey website or by email. This survey link was included in the monthly Breast Cancer Voices newsletter and sent by email to potential participants.

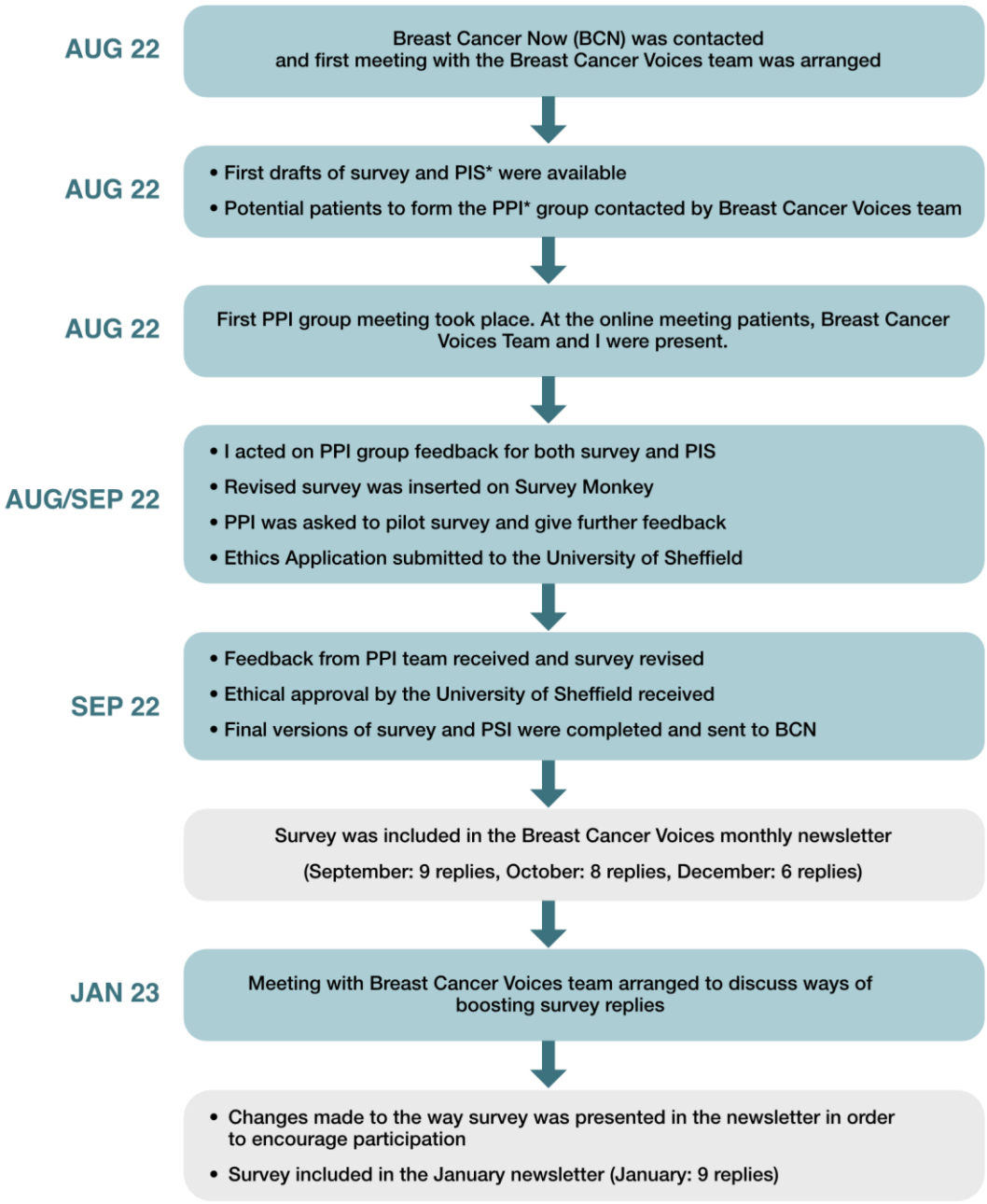

Figure 4.1: *Timeline of patients' survey preparation including number of replies received the 4 times survey was sent out. The number of replies demonstrated here include all the participants prior to any analysis.*
**PIS – Patient Information Sheet, PPI – Patient and Public Involvement*

A Patient Information Sheet (36) (Appendix 4.1) was generated and also shared with the survey link, explaining the purpose of the survey and what was expected from participants. On the PIS, participants could also find information about confidentiality, personal data handling and the team contact details. The PIS was also reviewed by the PPI group and revised accordingly.

The survey was a 30-item self-administered survey covering three broad themes: 1) Demographics, 2) Breast Cancer treatment, 3) Information about BP treatment (Figure 4.2).

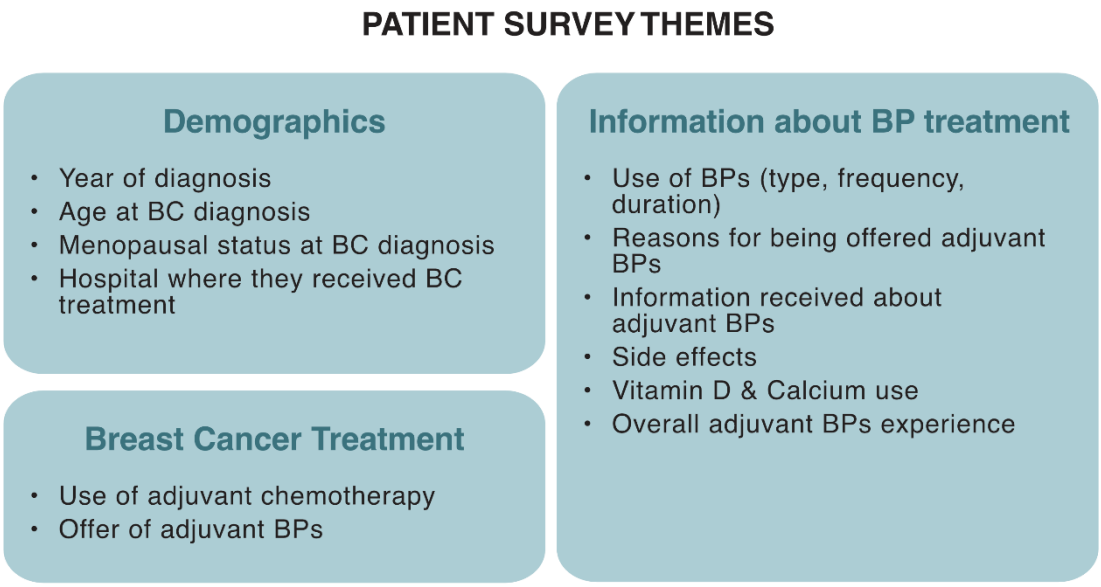

Figure 4.2: *Themes of the online patients' survey. The first survey question was the written consent.*

4.4.4 Ethical Approval and Survey Implementation

The survey received ethical approval by the University of Sheffield ethics committee on 9th September 2022 (Application 049602). NHS ethical approval was not required as no NHS organisation was involved with this project.

The survey link was distributed via email to the members of the Breast Cancer Voices in the monthly newsletter of the group. The survey was shared 4 times, in September, October, December 2022 and in January 2023. The survey link was accompanied by a small summary explaining the project and the PIS (Appendix 4.1). No incentive to participate was provided. Figure 4.3 shows how the survey link was shared in the Breast Cancer Voices newsletter in November 2022.

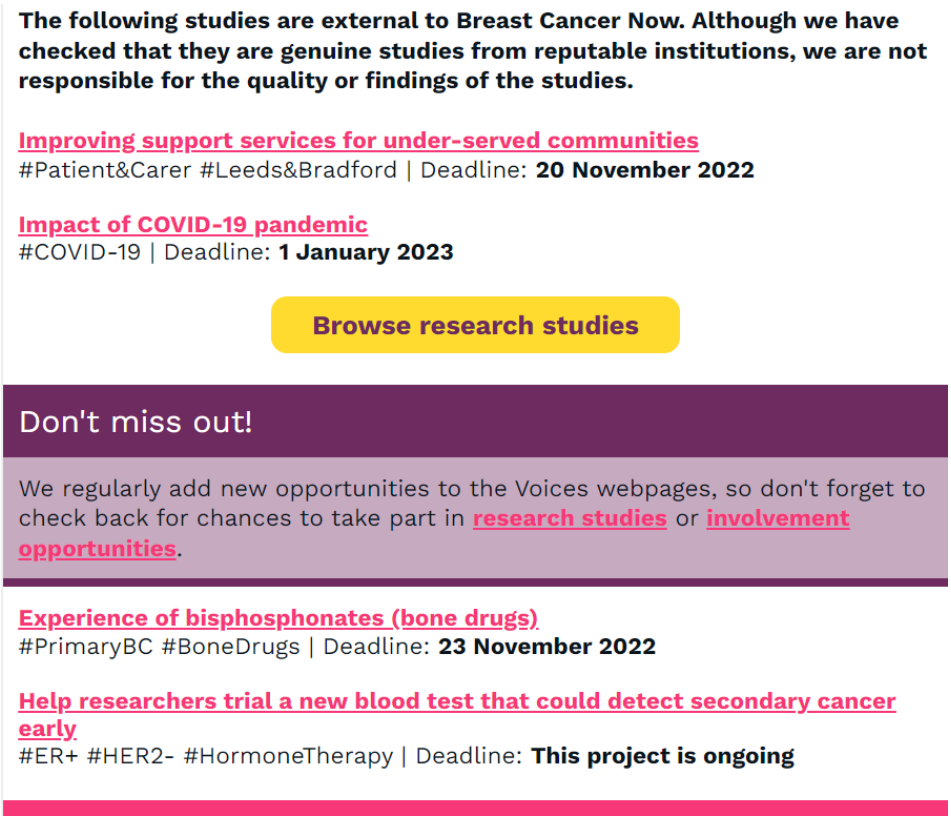

Figure 4.3: *How survey link was shared in the Breast Cancer Voices newsletter in November 2022.*

4.4.5 The Survey

The final survey consisted of 27 questions with free text permitted. Where possible and in order to avoid invalid answers, replies to some of the free text questions were validated for a specific format. Participants had to provide an online anonymous consent prior to being able to take part to the survey. If participants were not offered adjuvant BPs (answered "NO" to question 6), they automatically completed the survey. For participants who were offered adjuvant BPs, but they did not receive them (answered "Not at all" to question 7), the survey was completed at question 10. Additionally, patients who stated that had no side effects from the treatment, they were not asked any further questions about side effects (question 23 and 24). The full survey questions are listed below:

1. What year were you diagnosed with breast cancer?
Free text box

2. How old you were when you were diagnosed with breast cancer?
Free text box

3. When you were diagnosed with breast cancer were you?
    a. Premenopausal (before the menopause)
    b. Postmenopausal (gone through the change / after menopause)
    c. Perimenopausal (around menopause / menopause transition)
    d. I don't know / I don't remember
    e. Other -Free text box

4. In which hospital(s) did you receive treatment for your breast cancer?
Free text box

5. Did you receive chemotherapy after your breast cancer surgery?
    a. Yes
    b. No
    c. I don't know / I don't remember
    d. Other – Free text box

6. Have you been offered bisphosphonates (e.g. Zoledronic acid, Ibandronic acid, Alendronic acid, Clodronate) after your breast cancer surgery?
    a. Yes
    b. No
    c. I don't know /I don't remember
    d. Other-Free text box

7. If you were offered bisphosphonates after your breast cancer surgery, have you taken them?
    a. Yes
    b. Yes, but not all
    c. Not at all
    d. I don't know/I don't remember
    e. Other – free text box

8. If you were offered bisphosphonates, but you did not wish to receive them, what was the reason(s) for your decision? Tick all that apply
    a. I am not convinced they would have been beneficial
    b. Possible side effects
    c. Family/Friends/Somebody I know had bad experience with these medications
    d. I don't want to take more medications
    e. Busy lifestyle
    f. I don't know/I don't remember
    g. I don't want to say
    h. Other – Free text box

9. If you were offered bisphosphonates, do you know why and what they are for? Tick all that apply
    a. Prevent breast cancer returning (recurrence)
    b. Prevent breast cancer spreading to the bones (metastases)
    c. Prevent bone loss unrelated to breast cancer treatment
    d. Prevent bone loss from some breast cancer treatments (such as hormone treatment)
    e. I don't know /I don't remember
    f. Other – Free text box

10. If you were offered bisphosphonates, do you feel you received enough information about the treatment?
    a. Yes, I received enough information
    b. Yes, I received some information
    c. Yes, I received minimal information
    d. No, I didn't receive enough information
    e. I received no information
    f. I don't know/ I don't remember
    g. I don't want to say
    h. Other – Free text box

11. Which bisphosphonate(s) do/did you take? Tick all that apply
    a. Zoledronic Acid
    b. Ibandronic Acid
    c. Clodronate
    d. Zoledronic Acid when I was receiving chemotherapy and then it was changed to Ibandronic Acid
    e. Zoledronic Acid when I was receiving chemotherapy and then it was changed to Clodronate
    f. I don't know/ I don't remember
    g. Other – Free text box

12. If you do/did take bisphosphonates, who prescribes/prescribed them? Please do not give any names of individuals. Tick all that apply
    a. Cancer Doctor
    b. GP
    c. Pharmacists
    d. I don't know /I don't remember
    e. Other – Free text box

13. Do/did you take vitamin D and calcium (e.g. Adcal, Calcichew) with your bisphosphonates?
    a. Yes
    b. No
    c. I was not advised to take or prescribed vitamin D and calcium with my bisphosphonates
    d. I don't know/I don't remember
    e. Other – Free text box

14. If you are receiving or have received bisphosphonate through the vein (given in hospital), how often do/did you have them?
    a. Not been offered bisphosphonates through the vein. Had only tablets.
    b. 6 monthly
    c. 3 monthly
    d. once a month
    e. I don't know/I don't remember
    f. Other – Free text box

15. If you are taking or have taken bisphosphonate tablets, how often do or did you take them?
    a. Not been offered tablets bisphosphonates. Had only through the vein
    b. Daily- never missed one
    c. Missed the odd tablet
    d. Every now and then
    e. Never taken
    f. I don't know/I don't remember
    g. Other – Free text box

16. How long did you take or expect to take bisphosphonates for?
    a. 2 years
    b. 3 years
    c. More than 3 years
    d. I don't know/I don't remember
    e. Other - Free text box

17. Have you completed your bisphosphonate treatment?
    a. Yes, I have completed as planned
    b. I plan to complete
    c. No, I have stopped early
    d. I consider stopping early
    e. Other – Free text box

18. If you have stopped your bisphosphonate treatment, or you are planning to stop early, what are the reason(s)? Tick all that apply
    a. Side effects
    b. Negative effect on my quality of life
    c. I don't have time to take treatment
    d. I regularly don't remember to take the treatment so may just stop
    e. I don't think I get any benefit
    f. I don't think I need to take them for this long
    g. I don't want to say
    h. I don't know/I don't remember
    i. Other – Free text box

19. If you have stopped your bisphosphonate treatment, or you are planning to stop early, have you discussed this with your doctor? Please do not give names of individuals. Tick all that apply
    a. Yes with my cancer doctor
    b. Yes with my breast care nurse
    c. Yes with Advanced Nurse Practitioner
    d. Yes with my GP
    e. No, I haven't discussed it with anybody
    f. I don't want to say
    g. I don't know/I don't remember
    h. Other – Free text box

20. If you have stopped your bisphosphonate treatment, or you are planning to stop early and you discussed this with your health care provider (for example cancer doctor, nurse, GP), how helpful was the conversation?
    a. I didn't discuss it
    b. Very helpful
    c. Quite helpful
    d. Neither helpful nor unhelpful
    e. Quite unhelpful
    f. Very unhelpful
    g. I don't know
    h. Other – Free text box

21. If you have stopped your bisphosphonate treatment, or you are planning to stop early and you discussed this with your health care provider (for example cancer doctor, nurse, GP), what was the decision?
    a. I didn't discuss it
    b. We agreed to change the bisphosphonate tablets to a drip (hospital treatment)
    c. Benefits were explained but I decided to stop or planning to stop
    d. We agreed it is best for me to stop
    e. I don't know/ I don't remember
    f. I don't want to say
    g. Other – Free text box

22. Have you experience any of the following side effects with your bisphosphonate treatment? Tick all that apply.
    a. No side effects
    b. Joint pain
    c. Muscle pain
    d. Extreme tiredness (Fatigue)
    e. Constipation
    f. Diarrhoea
    g. Dizziness
    h. Low calcium (needing to take supplements)
    i. Fever
    j. Stomach pain
    k. Headache
    l. Flu-like symptoms
    m. Nausea/Vomiting
    n. Food pipe (oesophagus) problems like ulcers
    o. Kidney problems
    p. Skin problems
    q. Reduced appetite
    r. Low blood count (low haemoglobin / anaemia)
    s. Dental issues (including jaw issues)
    t. I don't know/I don't remember
    u. Other – Free text box

23. If you have/had any side effects from your bisphosphonate treatment, have you discussed these with your doctor or someone else? Please do not give names of individuals.
    a. Yes with my cancer doctor
    b. Yes with my breast care nurse
    c. Yes with my GP
    d. No, I didn't discuss it
    e. I don't want to say
    f. I don't know/I don't remember
    g. Other – Free text box

24. Thinking about your side effects from your bisphosphonate treatment, which statement describes your experience best?
    a. I have been affected significantly and I have stopped/ consider stopping taking the drugs
    b. I have been affected significantly but I am managing
    c. I have had many problems and I have stopped/ consider stopping taking the drugs
    d. I have had many problems, but I am managing
    e. I have had some problems and I have stopped/consider stopping taking the drugs
    f. I have had some problems, but I am managing
    g. I don't know/I don't remember
    h. Other – Free text box

25. How would you rate your overall experience with bisphosphonates?
    a. Very good
    b. Good
    c. Average
    d. Poor
    e. Very poor
    f. I don't know/I don't remember
    g. Other – Free text box

26. How likely would you be to recommend treatment with bisphosphonate to a relative or friend with breast cancer if this was offered to them?
    a. Very likely
    b. Quite likely
    c. Neither likely nor unlikely
    d. Quite unlikely
    e. Very unlikely
    f. I don't know
    g. Other – Free text box

27. Anything else you would like to share with us to help us improve other patients' experience with bisphosphonates after breast cancer surgery, please use the Free text box here
Free text box

4.4.6 Data analysis

Descriptive statistics were used to summarise the responses to the survey. The frequency of each choice was calculated as a proportion of the total number of responders. The data were compiled using Microsoft Office Excel (© 2018 Microsoft Corporation). Percentages were rounded to nil decimal point.

4.5 Results

The survey was received by the members of the Breast Cancer Voices via email, the group had 925 members in February 2023. Thirty two participants agreed to take part in the survey by giving their consent**.** As the Breast Cancer Voices group consists of patients, friends and family members, the response rate could not be calculated. Additionally, information about patients' primary breast cancer diagnosis is not held by Breast Cancer Now. Only participants who were offered adjuvant BPs were included in the analysis of this survey. Seven patients that were offered BPs for bone health (not for their anticancer benefit) were excluded from this analysis, indicating that patients may be unclear about why they are prescribed BPs.

Potential sources of bias for this survey include:

1) Sampling bias: Members of the Breast Cancer Voices were purposely selected to take part in this survey as they are a group of patients who had already agreed to take part in research and surveys aiming to improve breast cancer care and patients' experience.
2) Non-response bias: The number of responders was small.
3) Social desirability bias: Due to the sensitive topic of the survey and the potential need to avoid disappointing or expressing negative opinions for their breast cancer team.
4) Acquiescence bias: Due to the sensitive topic of the survey and the potential need to avoid disappointing or expressing negative opinions for their breast cancer team.
5) Extreme response bias.
6) Demand characteristic bias.

Sampling bias could not be avoided in this survey as it was developed with the support of BCN, with plan to be distributed within the Breast Cancer Voices. However, non-response bias may have an impact on the generalisability and interpretation of the survey results. Social desirability bias, acquiescence bias and demand characteristics bias could have affected the positivity of the results, whilst extreme response bias could have potentially affected the way side effects have been reported by the participants.

4.5.1 Demographics

Year of breast cancer diagnosis

The majority of the participants were diagnosed with EBC after 2018 (71%) followed by 21% who were diagnosed in 2016-2017 period and 8% who were diagnosed before 2015.

Age at breast cancer diagnosis

Most of the responders (66%) were older than 50 years when they were diagnosed with EBC (44% 50-60 years, 22% 60-70 years). Seven patients (30%) were found to have EBC when they were 40-50 years old and only 1 patient (4%) was diagnosed when she was younger than 40 years. The survey did not have any participants older than 70, mainly explained by the fact that women older than 75 is the least represented group within the Breast Cancer Voices community (285).

Menopausal status at breast cancer diagnosis

Women were mainly postmenopausal when they received the breast cancer diagnosis (61%), with 30% to be premenopausal and only 9% to be perimenopausal.

Hospital where they received breast cancer treatment

Almost all of the participants received treatment for their EBC in an NHS hospital (96%) and only 1 patient (4%) had breast cancer treatment in a private centre. Replies were received from patients who had breast cancer treatment in England (83%), Scotland (13%) and Wales (4%). More specifically, answers were received from 12 different geographical areas in England, 1 area in Wales and 3 in Scotland, suggesting that the survey captured a wide geographical group of breast cancer patients.

4.5.2 Breast Cancer Treatment

Use of adjuvant chemotherapy

Anticancer systemic therapy is offered after the completion of breast cancer surgery, with the aim to improve survival and reduce the risk of recurrence. This is mainly offered to patients with higher risk of recurrence EBC (13). Responses suggested that the 70% of the participants received adjuvant chemotherapy, with only 17% replying that they were not offered any chemotherapy post breast cancer surgery. A small number of participants (13%) were offered chemotherapy prior to surgery which is designed to downsize tumours and offers better surgical outcomes.

Offered adjuvant BPs

Since 2018, adjuvant BPs are included in NICE recommendations for the management of postmenopausal EBC (13). The majority of the survey participants (96%) were offered this therapy. In line with the national guidelines, only 1 (4%) responder was not offered adjuvant BPs as she was premenopausal when she was diagnosed with EBC.

4.5.3 Bisphosphonate Treatment

Use of BPs

Of the patients who were offered adjuvant BPs, 90% indicated that they received the course of therapy, 5% received it but did not complete the full course, and 5% were still receiving adjuvant BPs when they participated in the survey. No patients stated that they were offered adjuvant BPs but did not wish to receive them. The most commonly offered adjuvant agent was intravenous ZOL (90%), whereas oral Ibandronic Acid was only given to 10% of the patients (Figure 4.4). Both intravenous and oral adjuvant BPs were mainly offered for 3 years (80%) although a small number of patients received it for either 2 years (10%) or more than 3 years (10%). In terms of frequency, ZOL was mainly given 6 monthly (80%) and only 1 patient (5%) indicated that they received it 3 monthly (had only oral BPs 10%, don't know/don't

remember 5%). Women who were receiving the daily oral agent Ibandronic Acid stated that they completed the recommended course of treatment and only missed the odd tablet. Almost all of the patients had their adjuvant BPs prescribed by their breast cancer oncologist (95%), while none of the patients received these agents by their General Practitioner (GP) (don't know/don't remember 5%).

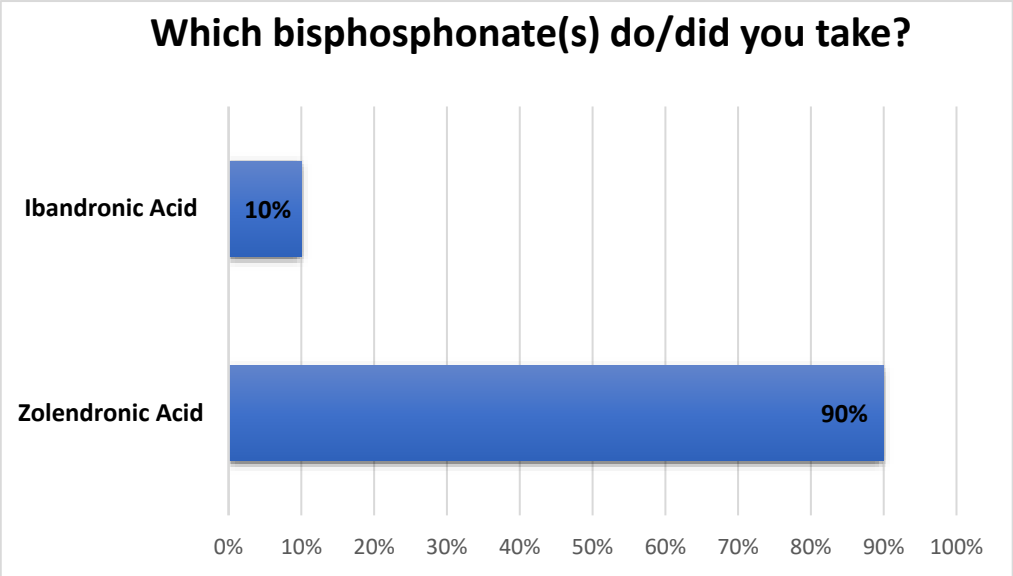

Figure 4.4: *Choice of adjuvant bone agent according to patients' survey results in 2023.*

Reasons for being offered adjuvant BPs

Participants were asked to explain the reasons why they were offered BPs after their breast cancer surgery, as communicated to them by their breast cancer specialists (Figure 4.5). All participants replied that adjuvant BPs prevent bone metastasis (100%), in contrast with only half of the responders (55%) who indicated that adjuvant BPs were offered to them for prevention of breast cancer recurrence. Additionally, patients answered that adjuvant bone agents prevent bone loss related and unrelated to breast cancer treatments (55% and 23% respectively), highlighting the need for better patient education.

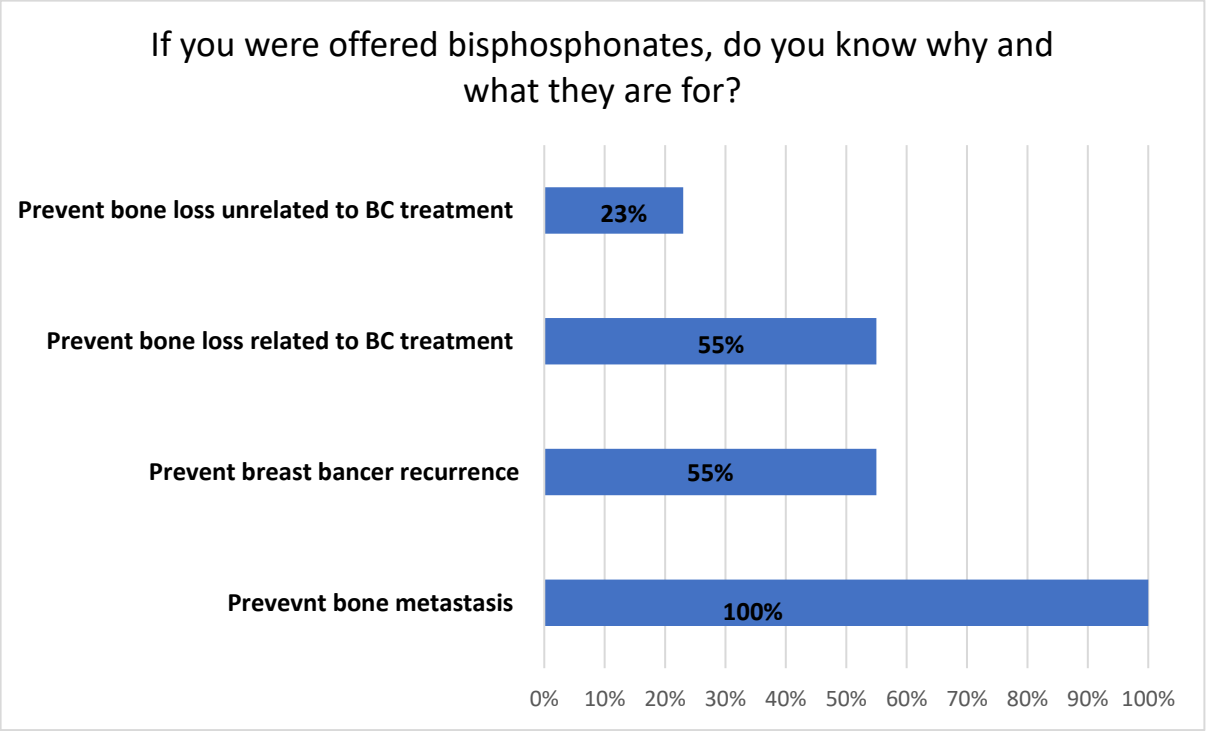

Figure 4.5: *Reasons for being offered adjuvant BPs. according to patients' survey results in 2023.*

Information received about adjuvant BPs

Less than half of the participants (41%) stated that they received enough information for the use of BPs after their surgery, while 32% replied that they only had some information and 14% had minimal information for the use of these agents (Figure 4.6). In contrast, 9% of the responders felt that they did not receive enough information and 5% had no information at all about adjuvant BPs (Figure 4.6).

Irrespective of the information that patients received about these agents, the majority (62%) suggested that more information about benefits, side effects and rationale for the frequency and duration of therapy was needed. In particular, participants suggested the introduction of a national booklet for the use of adjuvant BPs in EBC to improve patients understanding and information about bone agents in early disease. Although, adjuvant BPs are widely used and recommended by national and international guidelines, survey results and suggestions highlight that patients' knowledge, understanding and awareness are still lacking.

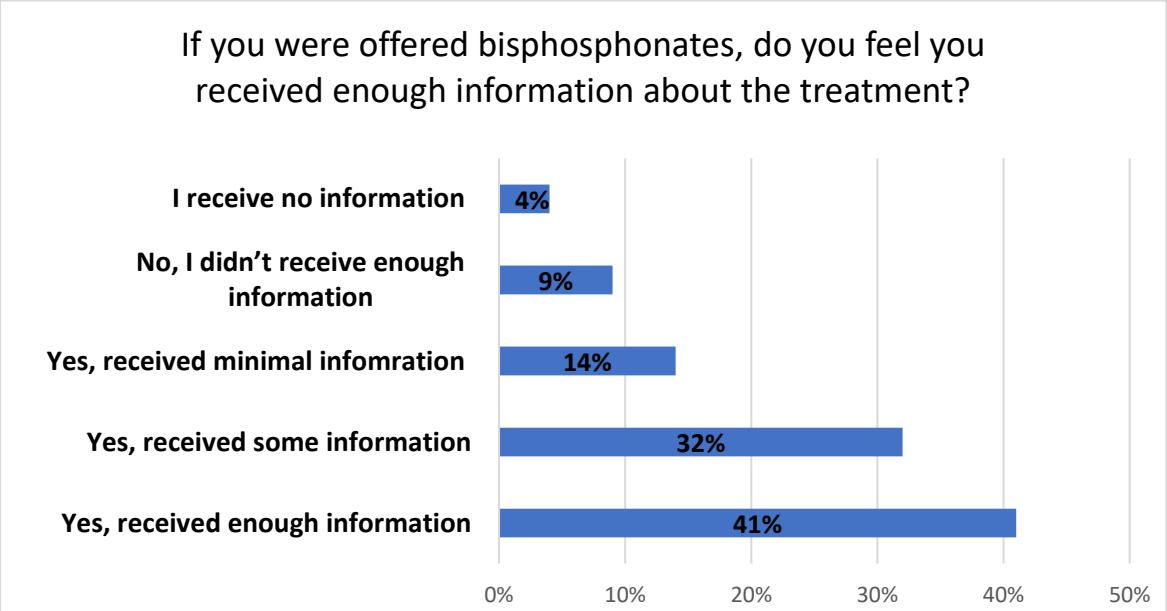

Figure 4.6: *Information that patients received for the use of adjuvant BPs in EBC, according to patients' survey results in 2023.*

Completion of adjuvant BP therapy

Most of the patients who had been started on adjuvant bone agents had either completed the course of their therapy (15%) or were planning to complete it (75%), suggesting that treatment is well tolerated. Only 10% indicated that they were considering stopping their BPs early.

From those who stopped or planned to stop adjuvant BPs early (4 replies), the main reason was that they were experiencing side effects (50%) followed by the negative effects on quality of life (25%) and concerns about possible future side effects, especially dental issues (25%). Patients stated that they discussed their concerns with their oncologist (67%) and breast care nurse (33%) but found the discussion neither helpful nor unhelpful (50%) or helpful (50%, helpful breast cancer nurse – unhelpful oncologists). Despite the discussion they had with their breast cancer specialist, 50% understood the anticancer benefits of adjuvant BPs but still decided to stop or planned to stop early, with the other 50% stating that they had to stop due to side effects.

Side effects

The most common side effects that patient experience with adjuvant BPs were joint pain and fatigue (both 50%), followed by flu-like symptoms (45%) and muscle pain (35%). However, 30% of the survey participants reported no side effects at all. Headaches were experienced by 20% of the women, constipation by 15% and diarrhoea but 10%. Dental

issues, including osteonecrosis of the jaw (ONJ) were reported by 10% of the responders. Dizziness, stomach pain, skin problems, nausea/vomiting, joint stiffness, urine infections and reduced appetite were only reported by 5% of the patients.

In an attempt to get a better understanding of where patients sought help when they had issues with their bone therapy, participants were asked to state the health care professional that they discussed their concerns with. Despite the NHS crisis and the busy schedules of cancer teams, EBC patients preferred to discuss the adjuvant BPs issues with their breast oncologists (69%). Some patients discussed their concerns with their breast care nurses (25%), GP (25%) or chemotherapy nurses (6%), compared to 25% of the patients who in spite of the fact that they experienced side effects from adjuvant BPs, they choose not to discuss these. This was a multiple choice question.

Vitamin D and Calcium use

The NICE guidelines state that vitamin D (400units daily) and calcium (500mg daily) should be taken by patients who are on adjuvant bisphosphonates, in order to prevent hypocalcaemia and undiagnosed vitamin D deficiency. Most of the EBC patients (85%) were advised to take vitamin D and calcium with their BPs. Only 1 patient (5%) stated that they were not advised to take these supplements, whilst one patient (5%) was advised against vitamin D and calcium due to hypercalcaemia and one (5%) had to stop them early due to side effects.

Overall adjuvant BPs experience

Overall, although patients had some problems with their course of adjuvant BPs, most reported that they were managing (56%). However, a 13% of the responders appeared to have significant issues and still managed to receive their treatment. Patients who had problems and had to stop or considered stopping BPs represented the 13% of the replies (13% had issues only with first ZOL infusion and no issues with subsequent infusion, 6% no issues but significant worries about possible future ONJ).

Most of the participants rated their adjuvant BPs experience as average (30%) followed by very good (25%) and good (20%). Interestingly, 10% of the patients found the experience very poor at the beginning but good thereafter. Five percent (5%) of the patients felt that treatment was part of their breast cancer therapy and therefore needed to take it. However, a similar proportion of patients (5%) rated their overall experience as “treatment with no anticancer benefit” (5% were still having adjuvant BPs and did not want to comment).

Irrespective of the side effects that patients reported with adjuvant BPs, they indicated that it was very likely (38%) or likely (19%) to recommend this treatment to friends and family if this was offered to them. Neither likely nor unlikely to recommend this therapy was given by

24%, whilst 5% answered that they would have advised friends and family to accept BPs due to their benefits. Patients who would not recommend adjuvant BPs (14%) explained that this was an individual's choice, and they also felt it was more appropriate for patients to discuss this with family and friends or oncologists, rather than themselves.

4.5.4 Patients' Quotes

The last survey question was a free text box question for participants to share anything they thought was important for the research team to know. Here are some of the patients' quotes (Figure 4.7 a&b&c).

Some of the participants highlighted the importance of clear and adequate information about the use of adjuvant BPs in EBC (Figure 4.7a):

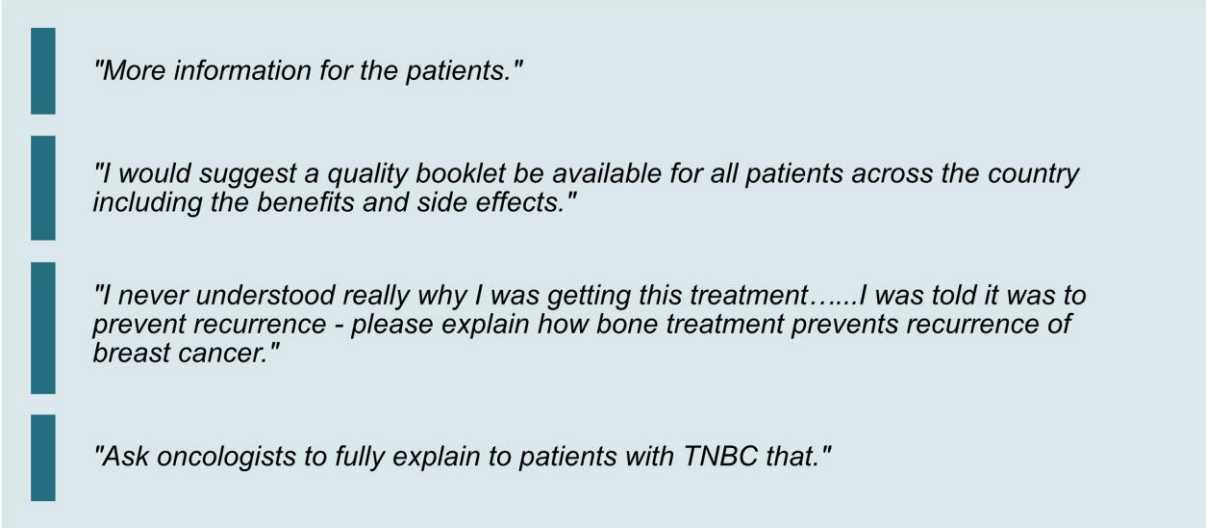

Figure 4.7a: *Patients' quotes for the information given about adjuvant BPs.*

For some survey participants, the anticancer and bone health benefits of adjuvant BPs outweigh any potential issues arising from this therapy (Figure 4.7b):

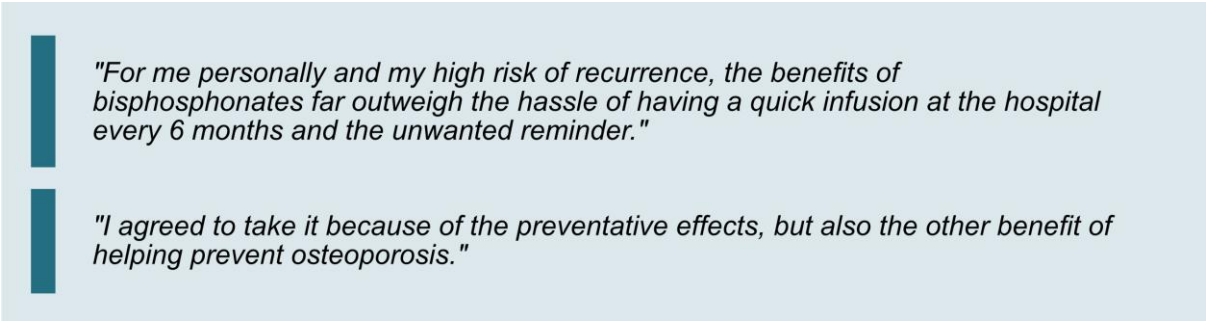

Figure 4.7b: *Patients' quotes showing the importance of adjuvant BPs.*

A small number of participants described adjuvant BPs as “treatment with no anticancer benefit” (Figure 4.7c):

> *"It could have been a lot worse, but I think it’s meant to improve my prognosis by about 1% along with letrozole so why bother?"*

> *"I feel annoyed that I am being given treatments that have marginal benefit according to the Predict tool and a bad impact on quality of life for five years so why bother?"*

Figure 4.7c: *Patients’ quotes describing adjuvant BPs as “treatment with no anticancer benefit”.*

4.6 Discussion

The survey captured real-world data for the use of adjuvant BPs in women with EBC across 3 UK countries (England, Scotland and Wales). In line with the national and international recommendations, adjuvant BPs were only offered to postmenopausal women (13, 51, 126), with the majority considered to be at high risk of recurrence as they were also offered adjuvant chemotherapy. The data validate the physicians’ survey results (chapter 3), which showed that adjuvant BPs were mainly offered to those women who were also offered chemotherapy, irrespective of whether they received it or not. The most commonly used agent was intravenous ZOL 6 monthly for 3 years, which demonstrates again that breast cancer oncologists prefer ZOL over oral alternatives. Since the publication of the SUCCESS trial in 2022 (128), which showed that there was no difference in benefit between patients receiving 2 or 5 years of adjuvant BPs, general consensus has moved away from longer adjuvant BPs regimes, with the survey results confirming that UK breast cancer care is following.

Many patients understood the reasons they were offered adjuvant BPs, but there was a proportion of patients who at the time of the survey were still uncertain. The reasons for this are potentially the way the information about adjuvant BPs was given to them at the time, patients did not associate bone metastasis with recurrence, or they thought that breast recurrence only refers to local recurrence. Additionally, some of the patients might need re-enforcement of information if this was done some time ago. Despite this, they were willing to receive and complete the course of treatment. Participants were well aware that adjuvant BPs prevent bone metastasis, but only a number of them appeared to be informed that treatment could also reduce the risk of recurrence. Some patients believed that adjuvant BPs aim to prevent bone loss from other therapies, related or not to breast cancer. Although BPs regimes (ZOL 4mg/6 monthly or ZOL 5mg/yearly or Alendronic Acid 70mg/weekly) for prevention or treatment of CTIBL are different from those of adjuvant BPs (ZOL 4mg/6monthly or Ibandronic

Acid 50mg/daily), women receiving BPs for their anticancer effects could also benefit from their bone protection effects for the period they receive them (usually 3 years). This is something that is very often discussed with patients receiving antioestrogen therapies, mainly AIs, for ER+ve EBC. However, adjuvant BPs would not be offered to breast cancer patients for prevention of bone loss unrelated to breast cancer therapies, as this is outside of the oncologists' expertise.

Information for the use of adjuvant BPs intended for patients, family and friends is currently available by many national and international bodies. The majority of the NHS hospitals are able to provide written information about this treatment to all the eligible patients. In addition, for the survey cohort who were patients closely related to BCN, the charity website has extensive information for the use of BPs in primary breast cancer (286). Nevertheless, the information does not seem to reach the right people at the right time, with the survey participants to suggest clarity and guidance for the use of these agents. Further work, perhaps in the form of updated national leaflets, webinars and social media clips and talks, are clearly needed to ensure that information for BPs in EBC is disseminated and get to the right group of patients, with the aim to potentially improve acceptance and adherence to this beneficial treatment.

Adjuvant BPs course was completed by most of the patients, with side effects reported only by half or less than half of the participants, suggesting that this therapy was well tolerated. Good tolerability to bone targeted therapy by EBC was also reported by the other 2 patient-focussed studies (55, 279). The side effect profile was as expected with the majority reporting fatigue and flu-like symptoms. Dental issues, including ONJ, were reported by a relatively high percentage of patients (10%), compared to much lower ONJ incidence reported to the literature (60, 274, 287). This should be interpreted with caution, as side effects were patient-reported in this limited survey.

In general, patients had a reasonable experience with adjuvant BPs, with the majority of them willing to recommend this therapy to other patients, if this was offered to them. Interestingly, some patients felt that their experience was less important than advice from family or friends with no experience of receiving the treatment. On one hand, this was somehow perhaps not expected from a cancer charity associated cohort who aim to support fellow patients. On the other hand, patients understand that breast cancer diagnosis is a life changing event with its treatment to be an individual's choice after discussion with their oncologist.

The survey has a number of limitations. It was only available in English and therefore non English speaking patients were unable to participate. The response rate could not be calculated as the actual number of patients with EBC being members of the Breast Cancer Voices was unknown. In spite of that, the survey only had a limited number of replies. This

could well be due to the fact that survey link was not directly sent to potential participants, but it was part of a monthly newsletter email. Although a number of active steps were taken to make the survey stand out, patients could still miss the survey link or even the entire newsletter. Additionally, in contrast to the other 2 published patient surveys (55, 279), the group of patients that was asked to participate to this survey was not specifically selected, targeted or known to the research team.

In conclusion, adjuvant BPs are widely used in the UK and are offered to almost all of the eligible postmenopausal EBC patients. The responses to the survey suggest that despite some expected side effects, treatment is well tolerated, and patients were motivated to complete the course of therapy. However, some patients did not have a good understanding of the purpose of the treatment and would welcome better information. Further work in a local and national level is needed to ensure narrowing of patients' knowledge gap related to the benefits of adjuvant BPs and why they were offered the treatment.

4.7 Conclusion

Adjuvant BPs for postmenopausal EBC is standard of care in the UK. The treatment is well tolerated and accepted by patients. Nevertheless, patients' understanding and knowledge for the use of these agents in primary breast cancer are lacking, with further efforts and work focusing on appropriate information and communication to be crucial.

4.8 Future Work

- In collaboration with BCN, the future aim is to increase patients' awareness for the use of adjuvant BPs and what these agents offer to patients with EBC and why they are used. This will be achieved through in person and online talks, social media clips and webinars designed specifically for patients with EBC. Additionally, the survey results will be published within the BCN community, and a report will be available in one of the future Breast Cancer Voices newsletters. Publication of the results was a request which was strongly expressed within the PPI group during the preparation of the survey. Although, the majority of the patients seem to prefer to receive information about adjuvant BPs from their oncologists, patients should also be made aware that BCN website provide useful information and help for the use of these agents. This perhaps might not be the way that information reach patients who are not very computer literate and who do not think of the BCN website as a source of info. For this group of patients, future efforts to ensure appropriate and adequate education for the use of adjuvant BPs in EBC should be made in local and national level, outside of the collaboration with BCN.

- The survey will be published in peer review journals in order to ensure dissemination of the results within the breast cancer community in the UK. This will encourage oncologists to improve the information that is shared with patients in an individual's level in oncology clinics. In addition, the results are aimed to be discussed with the UKBCG which was actively involved in the efforts of adopting adjuvant BPs in the UK breast cancer practise. This will enable us to find ways to improve oncology consultations and ensure that adequate information reach eligible patients in a local level. At the first oncology appointment, where adjuvant anticancer treatment is discussed, breast cancer oncologists should be made aware that patients would like to know more about the rational of the use of BPs in EBC, have a comprehensive discussion about the possible treatment side effects and also would like to know where to reach for support if this was needed. Future collaboration with the UKBCG will ensure that written information (local and national available leaflets) shared with patients include these points of discussion.

Chapter 5

Bone Health in Older Women with Breast Cancer.

A study nested within the Age Gap Cohort study.

5.1 Summary

This observational sub-study aimed to understand the management of bone health in older patients receiving endocrine therapy treatment for oestrogen receptor positive (ER+ve) EBC. Bone health data were collected from the participants of a pre-existing, large observational cohort study (Bridging the Age Gap - BTAG) which recruited patients older than 70 years of age (n=3456) with EBC between 2013 and 2018 from 56 UK hospitals. The BTAG study aimed to optimise breast cancer care for older women, so treatment could be better tailored to the patients' health and fitness levels and also to help to reduce some of the wide practice variation that exists across the UK. For the purposes of this sub-study 5 UK hospitals were included. During the original BTAG study, no data were collected on bone health.

About 75% of newly diagnosed EBC are ER+ve and these patients should receive endocrine therapy either as primary or adjuvant treatment. Primary endocrine treatment (PET) is offered as a therapeutic alternative to patients who are not fit for breast cancer surgery. The majority of such women are significantly older, with a poor performance status, and multiple comorbidities compared to women who are offered surgery. Adjuvant (post breast cancer surgery) endocrine treatment is also offered to the majority of ER+ve patients after surgery, with the aim to improve survival and reduce the risk of disease recurrence.

There are two antioestrogen therapies currently available: aromatase inhibitors (AIs) and Tamoxifen. Unlike Tamoxifen, AIs are known for their negative effects on bone health. Women receiving AIs are at risk of bone loss which may subsequently lead to fractures. The bone mineral density (BMD) of older women is already compromised due to menopause, age and other medical health issues. Therefore, older women receiving AIs for EBC are at greater risk of bone loss and fractures. Appropriate management of bone health in older populations is crucial to prevent fractures which significantly increase the mortality and morbidity of this group of patients.

National and international recommendations suggest that older women receiving AIs, who already have evidence of bone loss or who are at increased risk of bone fractures should be advised to exercise more, take calcium and vitamin D supplements and also to be commenced on antiresorptive therapy (denosumab or BPs).

This bone health sub-study aimed to increase our understanding of how bone health is monitored, preserved and treated in older women with EBC and to identify areas of improvement with the ultimate aim to optimise bone health care in older women with EBC.

5.2 Introduction

5.2.1 Breast cancer in older women

Breast cancer is the most common cancer in the UK with over 55,000 new cases every year (1). The risk of developing breast cancer increases with age, with 25% of new cases being diagnosed in women aged 75 and over (1). Overall, breast cancer accounts for 7% of all cancer deaths in the UK, with mortality rising with age such that almost half of the deaths are observed in patients older than 75 (1).

The stage of breast cancer in older women is higher, with larger primary tumour sizes, higher rates of nodal metastases, although biology is less aggressive (288). Tumour expression of oestrogen (ER) and progesteron (PR) receptors is higher in this population, in contrast with the lower expression of human epidermal growth factor receptor 2 (HER2) (289). In terms of histological subtype, invasive ductal carcinoma is the most common, followed by lobular carcinomas (290). Results from the BTAG, which recruited more than 3000 women aged >70 years, showed that 84% of the patients had ER+ve disease and only 12% had HER2+ve disease (291). Despite the favourable biology in older women, studies have shown that they usually present with more advanced disease mainly due to lack of screening (292, 293). In the UK, breast cancer screenning programme stops when women turn 71 (294). Although, women above 71 can request 3 yearly screening through self-referral.

The older population has increased healthcare needs with comorbidities, polypharmacy and often lack of social support being much more common. Despite the recent improvements in the diagnosis and treatment of breast cancer, management of early disease in patients >70 years has lagged behind, with women in this group more likely to receive non-standard care. Evidence-based oncology is very limited, as older patients are often excluded from clinical trials, making the optimal management of breast cancer in this population very challenging. The International Society of Geriatric Oncology (SIOG) defines this population as heterogenous and recommends that treatment should be tailored to the individual's needs and preferences and that decision-making should not be driven only by patients' age (295). Clinicians are advised to perform geriatric assessments, consider patients' general health, life expectancy and wishes (295).

More recently, research has focused on de-escalation of treatment for older patients with breast cancer. In view of the increased incidence of multiple other medical issues and the increased risk of adverse events from surgery and anticancer treatments, less therapy is desirable for this population, provided there is no oncological disadvantage. The BTAG study demonstrated that for women over 70 (n=3456) with early disease and a predicted life expectancy of less than 5 years, it is oncologically safe to omit breast cancer surgery and opt for primary endocrine therapy (296). Additionally, no survival benefit from axillary clearance in

older patients with no axillary involvement was found (n=671), making sentinel lymph node biopsy the standard of care in this group of patients (295, 297). More recently the ASCO/Ontario guidelines have suggested that for women over age 70, with low risk, node negative, ER+ve cancer and comorbidities, even sentinel node biopsy confers no advantage and may be omitted (298). In 2017, the St Gallen Panel suggested omission of adjuvant radiotherapy in older women (>65 years) with low-risk ER+ve, HER2-ve EBC, in view of the lack of additional clinical benefit (253). However, more data are needed to support this de-escalation pathway and reassure oncologists that this approach is not leading to undertreatment of older patients with breast cancer.

5.2.2 Antioestrogen use in older women

The current management of EBC includes surgery for all patients plus varying combinations of chemotherapy, radiotherapy, endocrine therapy, targeted molecular therapies (HER2, CDK4/6, immune and PARP inhibitors) and BPs, depending on disease stage, biology and patient tolerance (chapter 1) (13). About 75% of newly diagnosed early breast cancers are ER+ve and these patients should receive endocrine therapy either as primary or adjuvant treatment. Primary endocrine treatment is offered as a therapeutic alternative to patients who are not fit for breast cancer surgery and undoubtedly the majority of them are significantly older, with a poor performance status, and multiple comorbidities. In contrast, adjuvant (post breast cancer surgery) endocrine treatment is offered to the majority of patients with ER+ve disease, with the aim to improve survival and reduce the risk of disease recurrence.

Antioestrogens have also an important role in the management of the metastatic ER+ve disease. Considering their benefit and the relative lack of significant toxicities, they are the preferred palliative treatment modality for older women. They are mainly offered in combination with other anticancer agents such as Palbociclib, a CDK4/6 inhibitor, to extend progression free survival (299-301).

In the adjuvant setting, the addition of CDK4/6 inhibitors to standard endocrine therapy was evaluated by four major clinical trials (23, 302-304). The MONARCH-E trial recruited 5637 patients with high risk ER+ve, HER-ve early disease to standard endocrine therapy +/- abemaciclib (CDK4/6 inhibitor, oral 150mg twice daily) for 2 years. High risk were those with ≥4 positive nodes, or 1-3 positive nodes and either ≥5cm tumour, or grade 3 disease, or Ki-67≥20%. Participants had already completed neoadjuvant/adjuvant treatment (surgery, radiotherapy, chemotherapy). The results demonstrated that the addition of abemaciclib to standard endocrine therapy improved invasive disease free survival (IDFS) compared to placebo (92.2% versus 88.7% respectively) (23). Abemaciclib with endocrine therapy is currently recommended by NICE in the adjuvant setting for patients with high risk ER+ve,

HER2-ve, node positive EBC (high risk: ≥4 positive axillary lymph nodes or 1-3 positive axillary lymph nodes and grade 3 disease or 1-3 positive axillary lymph nodes and tumour size ≥5cm) (20).

Both the PALLAS and PENELOPE-E trials explored the role of adjuvant palbociclib (302, 303). The PALLAS trial (n= 5796) randomised ER+ve, HER2-ve EBC patients to endocrine therapy with or without 2 years of palbociclib (125 mg orally daily, 3 weeks on, 1 week off) (302). Analysis failed to show any improved outcomes over endocrine therapy alone (IDFS at 4 years: 84.2% palbociclib and endocrine therapy , 84.5% endocrine therapy alone). The PENOLOPE-B trial (n=1250) randomised ER+ve, HER2-ve EBC patients with residual invasive disease after standard neoadjuvant chemotherapy and high risk of relapse disease [clinical, pathological stage, oestrogen receptor, grading (CPS-EG) score of ≥3 or 2 with positive lymph nodes after neoadjuvant treatment (ypN+)] to receive palbociclib (125mg orally daily, 3 weeks on, 1 week off) or placebo for 1 year (13 cycles) with standard endocrine therapy (303). No significant difference in IDFS was found between the palbociclib and placebo groups (3-year IDFS: 80.6% palbociclib, 78.3% placebo). However, subgroup analysis revealed that premenopausal women receiving tamoxifen and ovarian suppression benefitted from the addition of palbociclib (3-year IDFS: 83% palbociclib versus 74.1% placebo group).

The NATALEE trial (n=5101) assessed the role of ribociclib in patients with ER+ve, HER-ve early disease (304). Participants were randomised to nonsteroidal aromatase inhibitor (letrozole 2.5mg daily or anastrozole 1mg daily for ≥5 years) with or without the addition of ribociclib (400mg orally daily, 3 weeks on, 1 week off) for 3 years. The published interim analysis showed significant benefit in IDFS in patients receiving ribociclib compared to those receiving endocrine therapy alone (3-year IDFS: 90.4% ribociclib arm, 87.1% endocrine therapy alone arm). Endocrine therapy in early ER+ve breast cancer, which is the focus of this thesis chapter, is offered based on the menopausal status of the patient. Premenopausal women with early ER+ve disease receive Tamoxifen, a selective oestrogen receptor modulator and postmenopausal women are offered AIs (13). Women over 70 are always postmenopausal and therefore are predominantly offered AIs. Aromatase inhibitors act by blocking the peripheral synthesis of oestrogen (305). The major source of oestrogen in postmenopausal women are the adrenal glands and adipose tissue making AIs the preferred antioestrogen therapy in this group. In premenopausal women, where AIs are thought to be beneficial, they must only be used alongside ovarian suppression therapy, as a compensatory physiological response to the action of AIs will induce ovarian oestrogen production. All currently used AIs are third generation drugs, represented by letrozole, anastrozole, and exemestane. Non-steroidal AIs, letrozole and anastrozole, bind reversibly to the aromatase, whereas the steroidal AI, exemestane, binds irreversibly to the aromatase enzyme. Aromatase

is the enzyme that converts androgens to oestrogens which is the main source of oestrogen in post-menopausal females.

Tamoxifen has historically been the standard of care for all women with hormone receptor positive EBC. In 1992, the EBCTCG reviewed data from 30000 women receiving adjuvant Tamoxifen, showing that treatment for at least 2 years offers a survival benefit over no adjuvant treatment or chemotherapy alone (306). Further studies successfully reported that extension of Tamoxifen to 5 years offered an extra benefit (307). This was confirmed in 2011 in the EBCTCG meta-analysis of 20 adjuvant Tamoxifen trials from 21457 women (308). In addition, the meta-analysis demonstrated that the anticancer effects of Tamoxifen in early disease continue well after its discontinuation at 5 years, reporting a reduction of the 15-year risk of breast cancer recurrence (308). As a result, studies were conducted to explore the benefit of extended antioestrogen therapy, with the UK aTTom (adjuvant Tamoxifen Treatment offers more) trial and its international counterpart ATLAS (Adjuvant Tamoxifen Longer Against Shorter) trial comparing 5 versus 10 years of adjuvant Tamoxifen (309, 310). The aTTom trial recruited 6953 women (2755 confirmed ER+ve and 4198 ER untested) who had completed 5 years of adjuvant tamoxifen and who were randomised to either stop tamoxifen or continue for an additional 5 years (310). Continuing tamoxifen showed a 4% absolute benefit in 15-year recurrence-free survival (72% vs 68%) and a 2% absolute benefit in 15-year breast cancer-specific survival (79% vs 74%), compared to no further treatment. The ATLAS trial enrolled 12894 women with EBC, treated with 5 years of tamoxifen and randomly assigned to either continue tamoxifen for 10 years (n=3428) or stop at 5 years (n=3418) (309). Among those who continued Tamoxifen the risk of recurrence at 15 years was 21.4% compared to 25.1% in those who stopped, and 15 year breast cancer mortality was 12.2% in those who had 10 years Tamoxifen compared to 15% in the control group. It should be noted that, similarly to the EBCTGC meta-analysis, the aTTom and ATLAS trials demonstrated that the actual anticancer benefit of adjuvant Tamoxifen increases significantly after 10 years of treatment.

Since the clinical introduction of AIs in the management of postmenopausal EBC, multiple studies have tried to compare these agents with the standard of care Tamoxifen. The Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial was the first large study to show the superiority of AIs against Tamoxifen in the postmenopausal population (154). Patients with early-stage breast cancer were randomised to receive anastrozole (1mg) (n=3125, ER+ve n=2618) or tamoxifen (20mg) (n=3116, ER+ve n=2698) for 5 years. Ten year analysis demonstrated a reduction of disease recurrence, distant recurrence and the incidence of contralateral breast cancer in the anastrozole group. Patients with ER+ve disease who were treated with anastrozole had an absolute reduction of recurrence of 2.7% at 5 years and 4.3% at 10 years, and a 2.6% absolute difference in distant recurrence at 10 years, compared to the Tamoxifen treated patients. Overall analysis showed that the observed anticancer benefits

of anastrozole increased overtime, even after the completion of hormone therapy. In addition, reported treatment-related serious adverse events were less in the anastrozole than the tamoxifen group (223 anastrozole vs 369 tamoxifen).

Similar favourable results to AIs were reported by the Breast International Group (BIG) 1-98 study which randomly assigned postmenopausal women with hormone receptor positive early breast cancer to receive 5 years of letrozole, letrozole followed by tamoxifen, tamoxifen, or tamoxifen followed by letrozole (155). Statistical analysis compared the letrozole (n=4003) with the tamoxifen (n=4007) groups showing an advantage for letrozole in 5 year DFS of 3.6% (84% letrozole and 81.4% tamoxifen groups).

In a large meta-analysis by the EBCTCG demonstrated for the first time that AIs reduce not only disease recurrence but also breast cancer mortality (311). Data from 31920 postmenopausal women with ER+ve EBC who had either AIs or Tamoxifen alone or in various combinations (e.g. Tamoxifen for 2-3 years followed by AI to complete 5 years) were included. Outcomes were reported for the first 10 years as no patients were followed up beyond that point. Overall, the recurrence rate (RR) was better with AIs during the first 4 years of treatment (year 0-1: RR 0·64 and year 2–4: RR 0·80) but similar to Tamoxifen for the next 5 to 6 years. Differences in RRs were more apparent when endocrine treatments were different but similar when groups were receiving identical therapies, independent of their previous therapy in the cases where AIs and Tamoxifen were combined. Interestingly, recurrence was further reduced in the patients who proceeded to have AIs after Tamoxifen, in contrast to those who never received Tamoxifen. In terms of breast cancer mortality, this favoured AIs throughout the 10-year analysis (RR 0·86).

In view of the increased incidence of late breast cancer recurrences and similar to Tamoxifen, multiple studies have attempted to investigate the benefit of extended AI therapy. Although, DFS was found to be increased in the group receiving AIs for 10 years, results were not statistically significant (312-314). The MA.17R trial randomly assigned patients who completed 5 years of AI alone or in combination with Tamoxifen to receive letrozole (n=959) or placebo (n=959) for an additional 5 years (315). However, results failed to demonstrate any OS benefit, although extended AI therapy improved both DFS (letrozole: 95%, placebo: 91%) and contralateral breast cancer incidence (letrozole: 0.21%, placebo: 0.49%) compared to placebo (315). A large number of study participants were older and in an attempt to identify the benefit of letrozole in this older age group, patients were divided in 3 age groups (<60 years, 60-69 years and >70 years) (316). Subgroup analysis showed a significant difference in DFS favouring letrozole only in patients younger than 60 years. Nevertheless, there was no interaction between age and treatment suggesting similar effects of letrozole across all age groups. No differences in toxicity or quality of life were reported in those older than 70 between

those taking letrozole and the control group, indicating that healthy older patients should be considered for extended endocrine therapy with AIs.

Currently, the optimal antioestrogen therapy and its duration are still unclear. Both Tamoxifen and AIs are recognised by national and international bodies as standard of care in postmenopausal patients with hormone receptor positive EBC and they are offered based on the individual's recurrence risks (13, 317, 318). Tamoxifen is mainly offered to those with small, low-grade tumours and axillary node negative disease who often complete endocrine treatment after 5 years. Women with a higher recurrence risk, ER+ve disease (bigger and higher-grade tumours, axillary node positive) are offered AIs and if no issues with tolerability, this is advised to be extended to 10 years. However, evidence suggests that all patients, regardless of their risk of recurrence should be offered an AI during the course of their endocrine therapy.

5.2.3 Effects of antioestrogens on bone health

Antioestrogens are generally well tolerated but prolonged and extended use increases side effects, which negatively affects patient adherence/compliance. The adverse effects are well described. Tamoxifen increases the risks of endometrial cancer and thromboembolic events, whilst AI complications are mainly related to the musculoskeletal system (319). Although musculoskeletal issues are also reported by patients receiving Tamoxifen, these are far more common in those treated with an AI (154, 155, 311, 316). Tamoxifen has a protective role in postmenopausal bone health, but reduces bone mineral density in premenopausal women, although not to a clinically significant level and therefore does not warrant any monitoring or treatment (153). Unlike Tamoxifen, AIs have significant negative effects on bone density, leading to bone loss and increased fracture risk, which in some large breast cancer trials, was over 50% higher than with Tamoxifen (154, 155).

A bone sub-study of the ATAC trial assessed the changes in BMD and bone turnover markers in 308 postmenopausal women (≤70 years) who received adjuvant anastrozole (1mg daily) or tamoxifen (20 mg daily), alone or in combination, for 5 years (320). Bone mineral density of the lumbar spine and hip was assessed at baseline and at 1 and 2 years. Bone turnover markers were measured at baseline, and at 3, 6 and 12 months. The different bone effects of anastrozole and tamoxifen were clearly seen. Anastrozole led to BMD reduction and an increase in bone turnover, whilst tamoxifen improved BMD and decreased bone turnover. Further analysis of BMD at the completion of antioestrogen therapy (anastrozole or tamoxifen or combination) revealed significant anastrozole-related bone loss over the 5-year treatment period. In spite of this, none of the women with normal baseline bone density were diagnosed with osteoporosis after 5 years of AI therapy (156).

Women achieve 90% of their adult bone mass by the age of 20 and reach maximum bone mass in their late 20's/early 30's. Bone mass is affected by modifiable (nutrition, lifestyle, daily exercise) and non-modifiable (sex, race, hormone status) factors. In the female population, bone loss is an unavoidable process which starts with menopause. Oestrogens have a protective role towards bones and are the major regulators of bone metabolism. They maintain bone formation, inhibit bone remodelling and reduce bone resorption by effecting osteoblasts, osteoclasts, osteocytes and T-cells (321). With the start of menopause, oestrogen levels begin to fall, and women experience a reduction of their BMD leading to postmenopausal osteoporosis and an increased risk of fractures. The bone loss process is accelerated in women with hormone receptor positive breast cancer receiving endocrine treatment with an AI. Aromatase inhibitor therapy increases the rate of bone loss 2-4 fold (161, 322-327), with the overall risk of fractures estimated to be 18-20% in the first 5 years of AI therapy and for those who receive extended endocrine treatment (10 years), fracture risk increases by 2-3% every year (82, 328-331).

Older women have a significantly higher risk of fractures, not only due to osteoporosis but also due to polypharmacy, poor vision and balance. Osteoporotic-related fractures are a major heath issue in the older population as they dramatically increase mortality and morbidity. Estimates indicate that 1 in 3 women aged over 50 years will experience an osteoporotic-related fracture at some point in their life (332). Therefore, older women with ER+ve EBC, who require antioestrogen therapy with an AI, are at greater risk of fractures, making the prevention and treatment of pre-existing or AI-related osteoporosis in this group crucial.

5.2.4 Prevention and management of aromatase inhibitor-induced bone loss

Bone targeted therapies, denosumab and BPs, have an established role in the prevention and treatment of CTIBL (51, 89, 153, 162). Denosumab, a RANK ligand inhibitor, significalty reduced fractures in postmenopausal women with early hormone receptor positive breast cancer treated with AIs in a large phase 3 trial (n=3420, denosumab 60mg 6monthly vs placebo) (52). Oral and intravenous BPs have been extensivley studied in hormone-receptor positive EBC showing a reduction in AI-induced bone loss (82, 174, 333-335). The benefits of BPs for reducing fracture incidence were first reported in the AZURE trial (n=3359), which evaluated the effects of adjuvant ZOL (intravenous, potent BP) on fractures in women with EBC (178). Women were randomised to receive standard of care with or without ZOL 4mg (3-4 weekly/6 doses, then 3 monthly/8 doses and 6 monthly/5 doses) for 5 years. This study demonstrated a reduction in fractures in the ZOL (3.8%) group compared to the control group (5.9%)

In addition to antiresorptive therapies, maintaining good levels of Vitamin D and calcium, either through an enriched diet or supplements, prevent osteoporosis and

subsequent fractures. More specifically a National Osteoporosis Foundation meta-analysis (n=970) demonstrated a 15% risk reduction of fractures in a population of non-cancer patients receiving these supplements (164).

Dual energy x-ray absorptiometry scanning is the "gold standard" for measuring and monitoring bone mineral density. It is mainly used to diagnose and monitor osteoporosis. It is cost-effective, non-invasive, requires minimal preparation from the patient and is performed in an outpatient setting. Bone density is mainly measured and reported at the lumbar spine (L1-L4) and the femoral head, where osteoporotic fractures are most likely to happen. Other sites such as the forearm might be used in cases where BMD cannot be assessed in the spine or femur (e.g. due to spine or femoral operation). Results are quoted as T-scores, which represent the standard deviation (SD) of the mean BMD of a healthy young adult. The clinical interpretation of T-scores, according to the World Health Organisation (WHO) are as follows:

- T-score within ≥-1 SD of the mean indicates a normal BMD.
- T-score between -1 to -2.5 SD from the mean indicates osteopenia.
- T-score below -2.5 SD from the mean indicates osteoporosis.

FRAX® is a validated and widely used fracture-risk assessment tool, created by the University of Sheffield (166). It is mainly used in osteoporotic patients over the age of 40 and provides the 10-year probability of a hip fracture and major osteoporotic fracture (clinical spine, forearm, hip or shoulder fracture) (166, 167). However, although the FRAX® tool was not built to assess the fracture risk in cancer patients receiving hormone therapy, it's validity has been assessed in cancer clinical trials (168-170). Results are still limited and unclear and therefore FRAX® is not currently recommended for this group of patients.

Current national and international guidelines for the prevention and management of AI-induced bone loss recommend that a DEXA scan is performed in all women commencing endocrine treatment with AIs within the first 6 months of treatment initiation (baseline assessment) (13, 51, 153, 162). This will allow any pre-existing bone loss to be appropriately treated before any further deterioration occurs due to the AIs. This is particularly important for older postmenopausal women whose bone density is already affected by age and menopause. DEXA scans should be repeated every 1-2 years during the course of endocrine therapy (13, 51, 153, 162). However, UK guidelines suggest that if both spine and hip baseline T-scores are above -1 then further assessment is not needed, unless clinically indicated (153). In general, frequency of follow-up DEXA scans should always be decided considering the individuals' bone loss risk and the use or not of bone protective therapy.

Treatment with a BTA, Vitamin D and calcium supplementation should be started in all AI patients with a T-score <-2 (13, 51, 153, 162). UK patients older than 75 with at least 1 risk factor for bone loss, such as previous low-trauma fracture after age 50, parental history of hip

fracture, alcohol intake of >4 units/day, diseases associated with secondary osteoporosis, prior corticosteroids for >6 months, low BMI (body mass index) (<22), are also advised to commence bone protection therapy with the start of their antioestrogen therapy (153). In contrast, the international consensus was suggesting bone protection therapy for women older than 65 with 1 additional risk factor for bone loss (T-score <1.5, smoking, BMI<24, family history of hip fracture, history of fragility fracture above age 50, oral glucocorticoid use for >6 months) (51). However, these recommendations have since been updated advising antiresorptive therapy for women receiving AIs with ≥2 risk factors or ≥1 risk factor and T-score<-1 (risk factors: previous fragility fractures, parental hip fracture, recurrence falls ≥2 in last year, diabetes (type 1 or 2), rheumatoid arthritis, BMI<20, glucocorticoid use >3 months and >7.5mg/day, current smoking, alcohol >2units/day) (162).

Both denosumab (60mg/6monthly subcutaneous) and BPs (alendronate 70mg/weekly oral, ZOL 4mg/6monthly or 5mg/12monthly intravenous) are the antiresorptive treatment of choice for the prevention or treatment of AI related bone loss. However, in view of their oncological benefit (discussed in the introduction section of this thesis), BPs (adjuvant BPs: ZOL 4mg/6monthly, oral clodronate 1,600 mg daily or oral ibandronate 50 mg daily) are generally the preferred option for women with high recurrence risk, hormone receptor positive breast cancer. Denosumab is more suitable for low-risk patients whose risk of AI-induced fractures outweighs the risk of breast cancer recurrence. In addition, patients who commenced denosumab but discontinued it prior to the completion of AI therapy are advised to be switched to a BP until the end of their antioestrogen therapy. This is in view of the rebound phenomenon of accelerated bone loss that occurs with the discontinuation of denosumab (50, 162).

Bone protection and use of antiresorptive therapy are crucial for older women receiving AIs for EBC. Despite the importance of maintaining good bone health and the national and international recommendations, bone management in this population still varies. Additionally, real-world data on the management of bone health in older women with ER+ve EBC are lacking. The BTAG study cohort (women >70 years with EBC) should provide a better understanding on this topic. Therefore, the data collected as part of the BTAG bone health sub-study offered an opportunity to investigate UK practice.

5.3 Hypothesis, Aims and Objectives

Hypothesis

- Variation exists between UK centres in the management of bone health of older women with EBC.
- Patient age and frailty may impact the bone health of older women with EBC.

Aims

- To understand how bone health is monitored and managed in older women with EBC.
- To map bone health screening and use of DEXA scans in this population.
- To map use of BTAs in this population.
- To determine how age and frailty affect bone health in older women with EBC.

Objectives

Bone Health Screening

- To describe how bone health screening is performed in older women with EBC and to assess the use of bone density scans.
- To determine bone mineral density loss in older women with EBC.
- To describe the patient's characteristics which predict BMD loss in older patients with EBC.
- To describe the correlation between patient's age and frailty and bone mineral density loss.

Bone Targeted Agents

- To assess the use of BTAs in older women with EBC.
- To describe the patient's characteristics that predict treatment with these agents.
- To describe the correlation between use of BTAs with patients' age and frailty scores.
- To describe the changes in BMD in relation to BTAs use.

Vitamin D and Calcium

- To describe the use of vitamin D and calcium supplements in older women with EBC.

Bone Fractures

- To describe the rate of bone fractures in older women with EBC.
- To determine patient characteristic which predict bone fractures in older women with EBC.
- Bone fracture incidence will be correlated with patient's age and use of BTAs, vitamin D and calcium.

5.4 Methods

5.4.1 Study design

The Age Gap study was a prospective, multicentre observational study of older women with EBC (336) . This bone health sub-study is an unplanned secondary analysis supplemented with new data obtained from a limited number of study sites. No new patients were recruited to the sub-study. The sub-study involved collecting additional data about bone health relating to the existing Bridging the Age Gap cohort, using electronic patient records held by the NHS Trusts.

Data were collected from participants of the Age Gap study from the following 5 participating sites:

- Sheffield Teaching Hospitals NHS Foundation Trust
- Liverpool University Hospitals NHS Foundation Trust
- Leeds Teaching Hospitals NHS Trust
- Doncaster and Bassetlaw Teaching Hospitals NHS Foundation Trust
- Chesterfield Royal Hospital NHS Foundation Trust

These were selected based on geographic convenience to permit the researcher easy access to each Trust to collect new data. This was an exploratory analysis with limited previously published data relating to this issue and therefore no sample size was determined.

5.4.2 Regulatory approvals and study specific amendment

The sub-study received ethical approval by the University of Sheffield (Reference Number: 045920) and from the National NHS Research Ethics Committee (REC reference: 12/LO/1808, Amendment number: 1145/2022/NCTS). The Ethics application to the University of Sheffield was made on 24 March 2022 and it was approvied on 4 April 2022. The sub-study was submitted to the National NHS Research Ethics Committee as a submstantial amendment to the original Bridging the Age Gap study on 11 February 2022 and received a favourable opinion on 22 April 2022 (Appendix 5.1). The main Bridging the Age Gap study protocol (336) was then amended to include the bone health sub-study. The main study protocol amendment was submitted on 24 April 2022, as a non-substantianl amendment to the Health Research Authority (HRA) only, as further approval by REC was not needed. HRA approval was received on 9 May 2022 (Appendix 5.2). The sub-study was then approved by the Research and Development departments of the 5 participating NHS Trusts. The bone health sub-study protocol is included in appendix 5.3.

5.4.3 Patient eligibility criteria

Eligibility

- All the Bridging the Age Gap study participants from the above-mentioned 5 centres who met all the inclusion and none of the exclusion criteria were included in this sub-study.

Inclusion Criteria

- Female patients recruited to the main Bridging the Age Gap study with ER+ve EBC who had been offered endocrine therapy with an AI or tamoxifen, either as primary or adjuvant treatment, with or without subsequent chemotherapy.

Exclusion Criteria

- Patients who had requested withdrawal from the study since initial recruitment.
- Patients who had declined or never received endocrine therapy.
- Patients with known metastatic breast cancer at diagnosis.
- Patients who consented to the main study via proxy as a result of cognitive impairment.

Defining ER+ve participants

- From the existing database of the Bridging the Age Gap study patients with hormone positive (ER+ve) EBC were identified. Hormone receptor positive (ER+ve) cancers were defined as those with an immunohistochemistry ER Allred score of ≥ 3 (positive when 3-8), or an H score of >50/300 or a notation that their disease was categorised by the host breast unit as ER+ve.

5.4.4 Recruitment

Patients already recruited to the Bridging the Age Gap study from Sheffield, Liverpool, Leeds, Doncaster and Chesterfield, were identified from the study database and screened. Patients who met the inclusion criteria and none of the exclusion criteria, were included to the sub-study. Eligible patients were not contacted. Upon recruitment to the sub-study, additional data were collected from existing hospital electronic records. Patients who took part in the original Bridging the Age Gap study had already signed a consent form which permitted further data collection about patients' cancer diagnosis, treatment and follow up.

The main Bridging the Age Gap study recruited a proportion of patients with severe cognitive impairment with consent obtained via a proxy. This consent form did not permit application of the sub-study and so these patients were excluded from the study.

5.4.5 Data collection

Data used in this sub-study were collected from data entered into the following sources:

- Type of breast cancer primary treatment (breast cancer surgery or primary endocrine therapy) and patients' age had already been collected by the main Age Gap study and therefore these data were obtained from the main study database.
- NHS Trusts electronic systems.
- Frailty scores were kindly provided by Olivia Turner, Healthy Lifespan Institute, University of Sheffield, UK. These had been derived using a modified Rockwood frailty index.

Sub-study data were recorded in an electronic case report form (CRF) (Appendix 5.4).

5.4.6 Addition study specific data collection

The existing Age Gap dataset already holds some of the data required by this bone health sub-study including primary breast cancer treatment, patient's age and frailty score.

Additional data that were collected:

- Type of endocrine therapy treatment.
- The performance of a DEXA scan within 6 months of starting endocrine therapy treatment (baseline).
- The result of the baseline DEXA scan if performed in terms of T-scores at the hip and spine.
- The performance of any further follow-up DEXA scans and their results (hip and spine T-scores).
- The offer of BPs, calcium and vitamin D supplements at any point during the course of antioestrogen therapy.
- The incidence of fractures during or after (until Q3/2022) the course of antioestrogen therapy and when this occurred.

5.4.7 Statistical analysis

Data analysis was only performed for the patients who received AIs as an endocrine therapy treatment for EBC. Descriptive statistics were used to describe the sub-study findings. Means (M) and standard errors (SE) are reported for continuous normally distributed data and medians and ranges for continuous non-normally distributed data. Categorical data were compared using chi-squared tests. Statistical analysis to calculate p-values and correlation

coefficients was performed using SPSS (Statistical Package for the Social Sciences) statistical software. Correlation analysis was performed to describe the correlation between age, frailty, use of BPs and bone health in older women with EBC. Correlation analysis was done by using SPSS statistical software or Microsoft Excel using Pearson correlation coefficient (r), Spearman correlation coefficient (Spearman's rho) and phi coefficient (r_φ). Pearson correlation coefficient was used to measure the degree of association between two continuous normally distributed variables. Spearman correlation coefficient was used to measure the degree of association between two continuous non-normally distributed variables and Phi coefficient was used to measure the degree of association between two categorical variables. The level of correlation (r value) that was accepted as confirming a correlation was 0.7 (or -0.7).

Box plots were created in Microsoft Excel to describe the differences in BMD according to group analysis.

Scatter plots were created in Microsoft Excel and simple linear regression was used to describe the correlation of patient's age and baseline hip and spine T-scores and also the correlation of frailty scores and baseline hip and spine T-scores. In this two linear regression models, the patient's age and frailty scores were the independent variables, where baseline hip and spine T-scores were the dependant variables. Standard errors and coefficients of determination (R^2) are reported for simple linear regressions.

5.4.8 Calulation of frailty scores

Baseline frailty was assessed during the patients' participation in the original BTAG study. I was not involved in the collection and/or calculation of the frailty scores as these were not part of my degree. Frailty scores were kindly provided, for the purpose of this analysis, by Olivia Turner, Healthy Lifespan Institute, University of Sheffield, UK.

The frailty score was developed based on Rockwood's accumulation of deficits model and was calculated by selecting 75 frailty-related variables (e.g. bathing, pulmonary comorbidities) from the comprehensive geriatric assessment taken at baseline. The patient responses to the variables were dichotomised onto a binary scale whereby if the patient was positive for the deficit to any extent they were given a 1 for that variable, if they had no association with that variable they were allocated a 0. An average was calculated across all 75 variables to derive a frailty score between 0 and 1 for each patient. A score of <0.08 is considered robust/not frail, 0.08-0.25 is pre-frail and a score over 0.25 is frail.

5.5 Results

5.5.1 Participants

This is a sub-study of a larger observational cohort study (BTAG) which recruited patients older than 70 years old (n=3456) with primary breast cancer between 2013 and 2018 from 56 UK hospitals. For the purposes of this sub-study, 5 UK hospitals were included. Of these total recruits, not all were suitable for inclusion in this sub-study. A strobe diagram showing patient dispositions within the parent and sub-study and reasons for exclusion is shown in Figure 5.1.

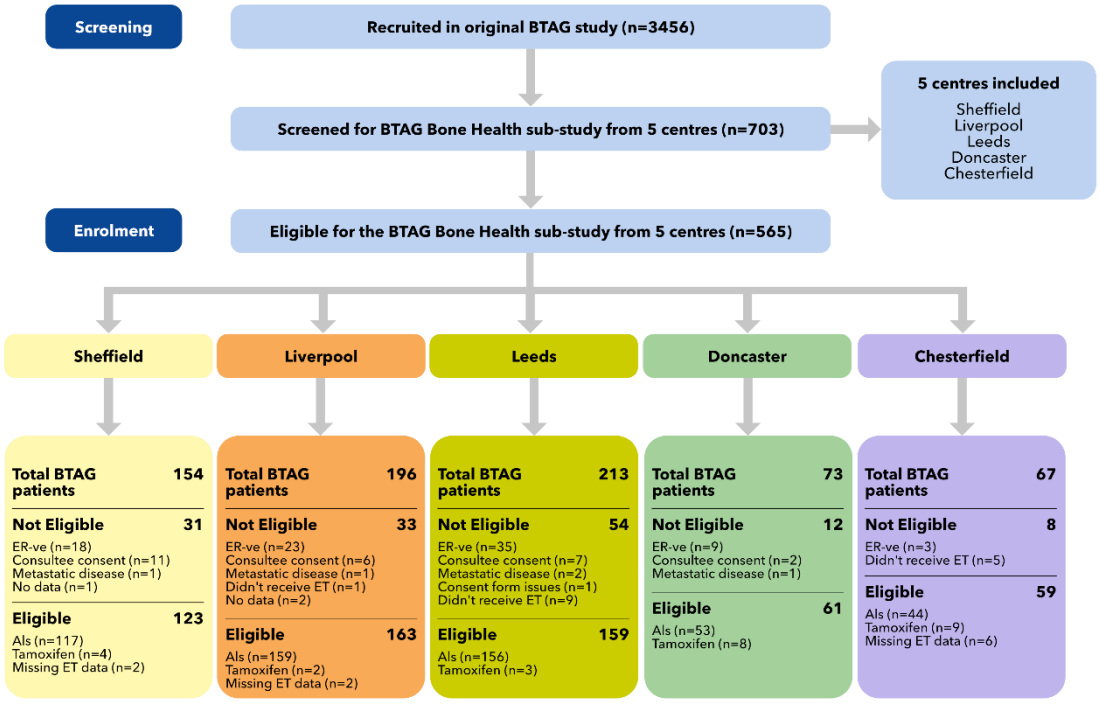

Figure 5.1: *Strobe diagram showing recruitment to the original BTAG study and sub-study and reasons for patients inclusion and exclusion per centre.*

Only patients who were diagnosed with ER+ve EBC and who received endrocrine therapy as part of their anticancer treatment were eligible for this sub-study. From the total of 703 patients who were recuited to the BTAG study at the 5 hospitals, 565 were diagnosed with ER+ve breast cancer and therefore were eligible for this sub-study (Sheffield n=123, Leeds n=159, Doncaster n=61, Chesterfield n=59, Liverpool n=163) (Figure 5.1). One hundred and thirty eight patients (n=138) were excluded from the sub-study due to having ER-ve disease (n=88), consultee consent (n=26), metastatic disease at the time of recruitment to the BTAG study (n=5), not receiving endocrine treatment (ET) (n=15), issues with their consent form (n=1) and missing data (n=3) (see Strobe diagram for full details, Figure 5.1).

The parent Age Gap study recruited a proportion of patients with severe cognitive impairment with consent obtained via a proxy. This consent form did not permit application of the sub-study and so these patients (n=26) were excluded from the study.

Of 565 eligible patients, 529 were prescibed aromatase inhibitors, 26 Tamoxifen and for 10, information about their ET was missing (Figure 5.1). Some women were prescribed endocrine therapy after surgery and for others the endocrine therapy was used as primary treatment.

Aromatse inhibitors reduce bone density and increase the risk of fractures, especially in older women whose bone denisty is already affected by the menopause. The effects of AIs on bone health are discussed in detail in the introduction of this chapter. The focus of this sub-study was the group of patients who received aromatase inhibitors (n=529) and in particular whether bone health was monitored during AI therapy, the impact of AI therapy on bone density and fracture risk and any practice variation between centres.

The sub-study population (AI patients) was separated into 2 age groups; 70 to 79 years old (342/529, 65%) and over 80 years old (187/529, 35%), with the median age of 77 years (70-98 years).

Full baseline demographics of the Age Gap population are available in the main trial publications (296, 337). The ER+ve EBC patients (n=2854) who were recruited to the main study from all the 56 UK centres, were 69 to 102 years of age (median 77 years). Although, the study recruited patients from all the ability groups, the majority were fully active (66.7%) and only 0.3% were completely disable (restricted in physically strenuous activity 24.1%, ambulatory and capable of all self-care 5%, capable of only limited self-care 3.9%).

5.5.2 Bone density monitoring with DEXA scans

DEXA Scan utilisation

In view of the effects of AIs on bone health, patients receiving this therapy are advised to have a baseline bone density assessment within 6 months of the initiation of the treatment and every 2-3 years thereafter (13, 51, 153, 162).

For the 529 women who received adjuvant or primary endocrine AI therapy, baseline DEXA scans were requested for 354/529 (67%) and only half of these had a further follow-up scan (179/354, 51%). Sheffield was the centre with the highest percentage of AI patients having a baseline DEXA assessment (89/117, 76%), followed by Leeds (117/156, 75%), 64% (101/159) for Liverpool and 59% (31/53) for Doncaster participants, compared to only 36% (16/44) of patients treated in Chesterfield.

Although, the difference between centres and the request for a baseline DEXA scan was statistically significant, analysis showed no correlation between the 5 centres and the number of AI patients who had a baseline DEXA scan ($p<0.001$, phi coefficient $r_{\varphi}<0.001$) (Table 5.1). The statistical test that was used to calculate the p-value was chi-squared test, and the variables were the request for a baseline DEXA scan (Yes or No) and the number of patients per centre that had or did not have a baseline DEXA scan.

The percentage of patients who had a second bone density assessment, compared to the percentage of patients who had a baseline DEXA remained almost the same in Chesterfield (6/16, 38%) and Doncaster (19/31, 61%) but dropped significantly in the 3 bigger centres (Sheffield 48/89, 54%, Leeds 55/117, 47%, Liverpool 51/101, 51%).

	AI patients	Baseline DEXA scan	Surgery	PET	F/U DEXA scan
Total	529	354 (67%)	319 (90%)	33 (9%)	179 (51%)
Centres					
Sheffield	117	89 (76%)	70 (79%)	18 (20%)	48 (54%)
Liverpool	159	101 (64%)	91 (90%)	10 (10%)	51 (51%)
Leeds	156	117 (75%)	113 (97%)	4 (3%)	55 (47%)
Doncaster	53	31 (59%)	29 (94%)	1 (3%)	19 (61%)
Chesterfield	44	16 (36%)	16 (100%)	0 (0%)	6 (38%)

Table 5.1: *Number of BTAG sub-study AI patients (per centre) who had a baseline and a follow up DEXA scan per primary treatment (Surgery or PET). For two patients who had baseline DEXA scan (1 patient in Sheffield and 1 in Doncaster), primary treatment was unknown.*

Baseline bone density assessment was more likely to be performed if the patient had breast cancer surgery (319/354, 90%) as opposed to PET (33/354, 9%). This very low rate for PET patients was consistent across units and may link to the increased age of this patient cohort. For 2 patients who had baseline DEXA scans, 1 from Sheffield and 1 from Doncaster, the primary treatment type was unknown (2/354, 1%). There was considerable variation in practice between units (Table 5.1).

Age was a significant determinant of DEXA scanning with younger women more likely to have a scan, (70-79, 251/354, 71%, compared to 80+, 103/354, 29%). The oldest patient to have a DEXA scan was 95 years old. Some units have upper age thresholds for DEXA scans.

Bone Density Results

Bone density is mainly measured and reported at the lumbar spine (L1-L4) and the femoral head, where osteoporotic fractures are most likely to happen. Other sites such as the forearm might be used in the cases where BMD cannot be assessed in the spine or femur (e.g. due to spine or femoral operation). Results are quoted as T-scores which represents the SD of the mean BMD of a healthy young adult (332). The clinical interpretation of T scores, according to the WHO are as follows (332):

- T-score ≥-1 SD of the mean indicates a normal BMD.
- T-score between -1 to -2.5 SD from the mean indicates osteopenia.
- T-score below -2.5 SD from the mean indicates osteoporosis.

In our sub-study, of those that were scanned, 42% (148/354) had osteopenia, 37% (132/354) had normal results and 18% (64/354) had osteoporosis. For 3% (10/354) of the patients, DEXA T-score results were unknown.

Half of the patients who had a baseline DEXA scan (n=354) had their bone density re-assessed by a follow-up DEXA scan (179/354, 51%). The majority of these patients were known to be osteopenic (87/179, 49%) and 36% (65/179) had normal BMD at the first scan. Only 14% (25/179) of those who had a follow-up DEXA assessment were known to have baseline osteoporosis. For 1% (2/179) for those who had a follow-up DEXA the results of their baseline assessment were missing.

Hip T-scores in the AI patients were generally lower in both baseline and follow-up assessments than the spine T-scores (Figure 5.2). Patients' experienced a minor degree of bone loss in both hip and spine when bone density was re-assessed at a follow-up DEXA scan (Figure 5.2), however age group analysis demonstrated an increase in spine bone density in women >80 years (Figure 5.4). Hip T-scores (baseline and follow-up) were higher in patients aged 70 to 79 years compared to those over age 80 (Figure 5.3). In contrast, spine T-scores (baseline and follow-up) were higher in the older (>80) population (Figure 5.4).

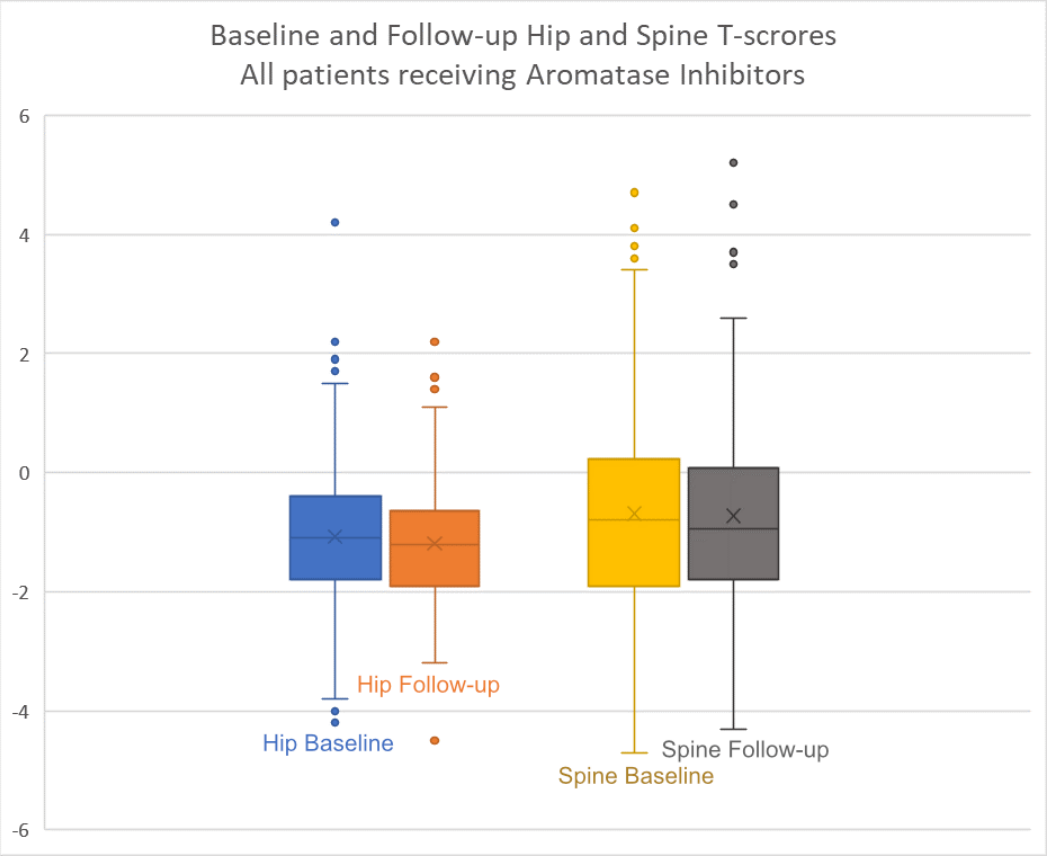

Figure 5.2: *Box plot comparing the baseline and follow-up DEXA hip and spine T-scores in all the patients receiving aromatase inhibitors (For all age groups: Hip Baseline n=354, Hip Follow-up n=179, Spine Baseline n=354, Spine Follow-up n=179). The baseline and follow-up DEXA scans were reported for different patient groups. Repeat DEXA scans were mainly performed in patients with baseline osteopenia but also in patients with normal baseline and baseline osteoporosis.*

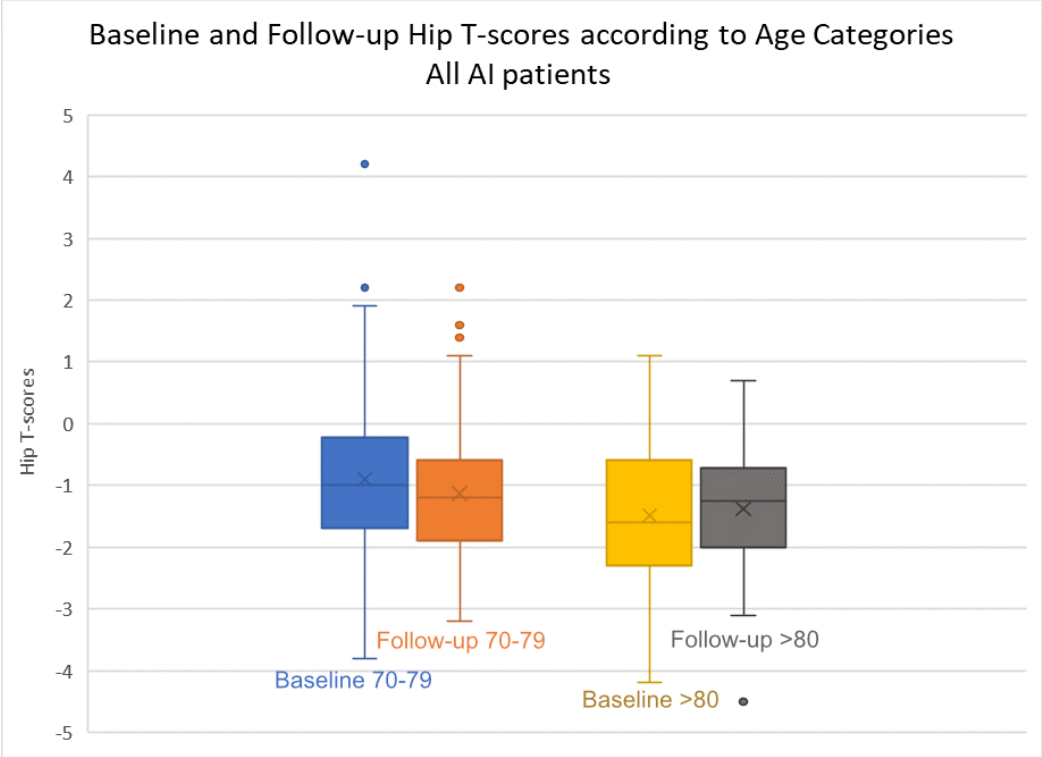

Figure 5.3: *Box plot showing baseline and follow-up DEXA spine T-scores in the 2 age groups (70-79 and >80 years (Baseline 70-79 n=251, Follow-up 70-79=143, Baseline >80 n=103, Follow-up >80 n=36). The baseline and follow-up DEXA scans were reported for different patient groups. Repeat DEXA scans were mainly performed in patients with baseline osteopenia but also in patients with normal baseline and baseline osteoporosis.*

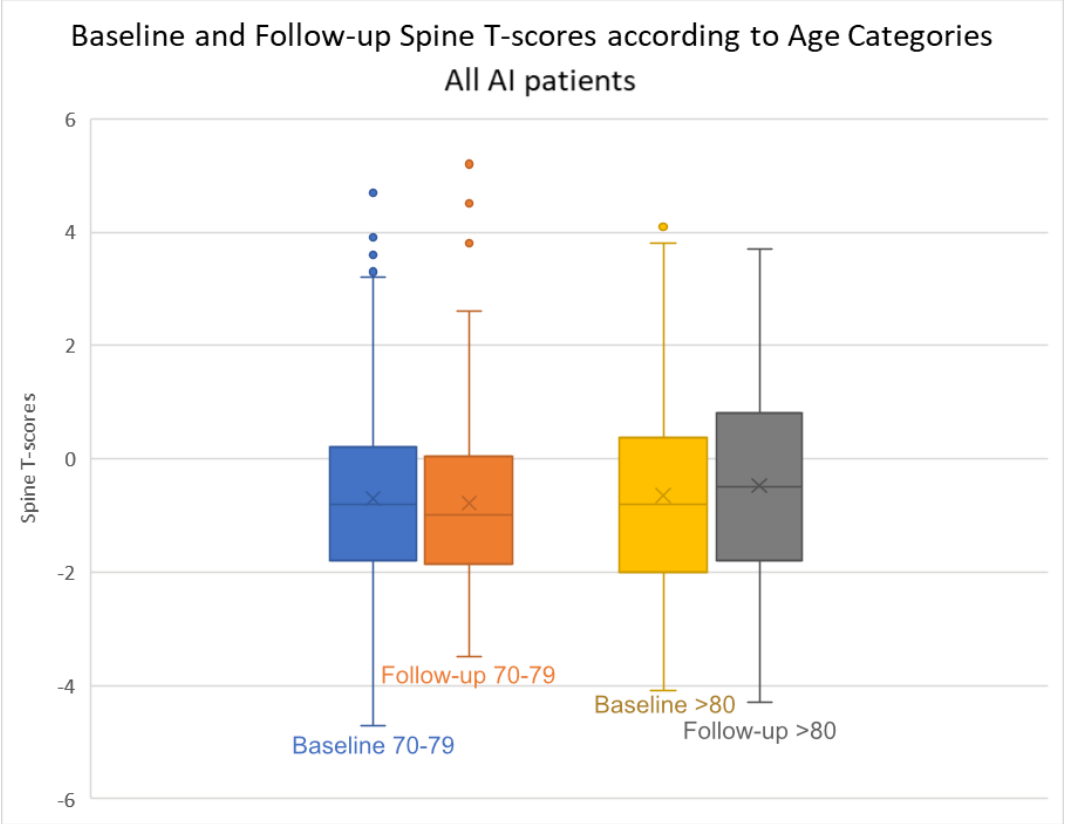

Figure 5.4: *Box plot showing baseline and follow-up DEXA hip T-scores in the 2 age groups (70-79 and >80 years) (Baseline 70-79 n=251, Follow-up 70-79=143, Baseline >80 n=103, Follow-up >80 n=36). The baseline and follow-up DEXA scans were reported for different patient groups. Repeat DEXA scans were mainly performed in patients with baseline osteopenia but also in patients with normal baseline and baseline osteoporosis.*

Analysis demonstrated that patient's age had a minimal effect on baseline hip T-scores (R^2 = 0.0874, SE=0.51) (Figure 5.5) and no effect on baseline spine T-scores (R^2 = 0.0063, SE=0.63) (Figure 5.6). Spearman's rank correlation was used to assess the relationship between baseline hip T-scores and follow-up hip T-scores in patients 70-79 years old. There was a positive correlation between the two variables (Spearman's rho=0.904, p<0.001). Spearman's rank correlation was also used to assess the relationship between baseline hip T-scores and follow-up hip T-scores in patients >80, with the correlation to be positive between the two variables (Spearman's rho=0.923, p<0.001). Similarly, Spearman's rank correlation was used to assess the relationship between baseline spine T-scores and follow-up spine T-scores in 70-79 and between baseline spine T-scores and follow-up spine T-scores in >80. In both age groups, there was a positive correlation between the two variables (Spearman's rho=0.938, p=0.019 for 70-79, Spearman's rho=0.967, p=0.033 for >80).

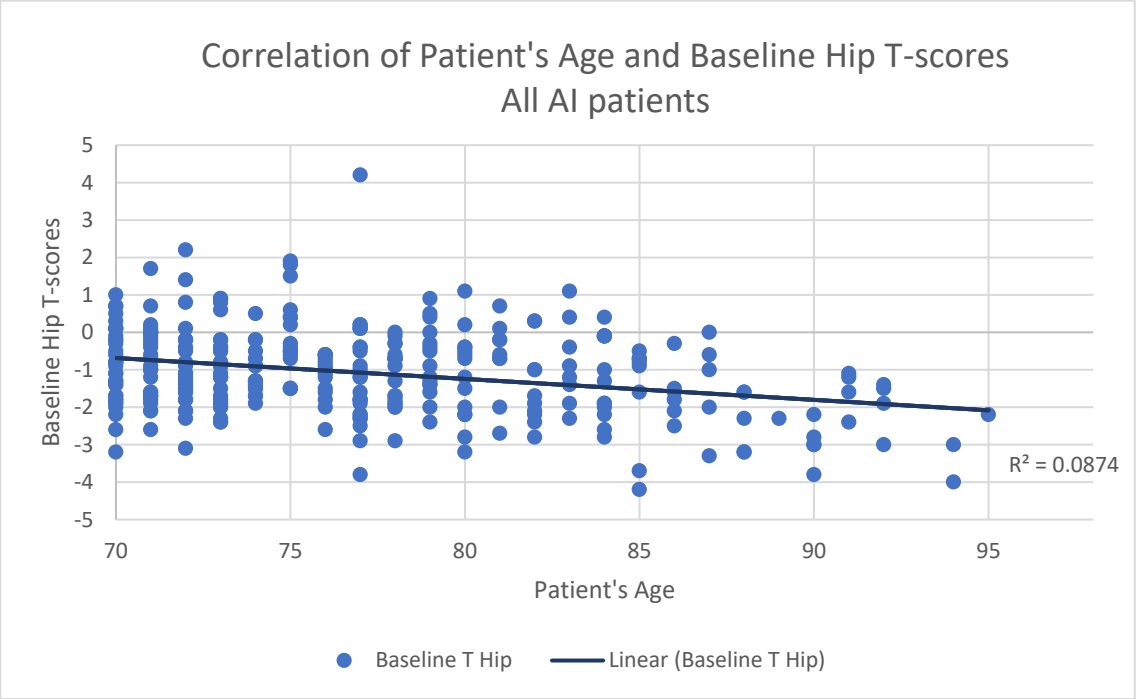

Figure 5.5: *Scatter plot showing the correlation of patient's age and baseline hip T-scores in patients receiving AIs for EBC.*

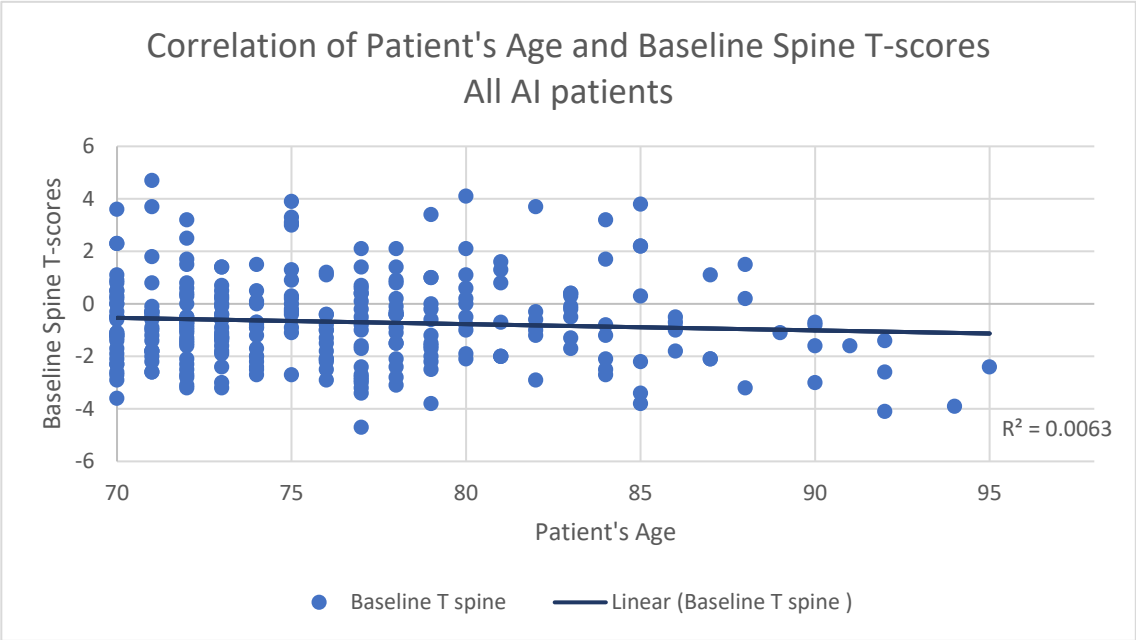

Figure 5.6: *Scatter plot showing the correlation of patient's age and baseline spine T-scores in patients receiving AIs for EBC.*

Overall, bone density measurements demonstrate a variability in BMD within this population, supporting that a more personalised approach to bone health of older women with EBC is warranted.

5.5.3 Use of Bisphosphonates

In this sub-study, only 43% (226/529) of the patients who were prescribed aromatase inhibitors were also offered BPs. There was no use of BPs for 283/529, (53%) and unknown use of BPs for 20/529, (4%). The majority of patients (122/226, 54%) were commenced on BPs with the AIs, while 15% (33/226) were already on the BPs prior to the breast cancer diagnosis and they continued having them with the AIs.

Adjuvant BPs were started to be introduced into routine UK breast cancer care in 2016, after guidelines for their use were shared nationally by the UKBCG and Sheffield Teaching Hospitals, although UK wide NICE guidelines were not published until 2018, and these guidelines always take time to diffuse across clinical sites. The same year (2016), the first BTAG participants were offered BPs for cancer prevention. In total, 71/226 (31%) women, from the 5 hospitals that participated in this sub-study, received adjuvant BPs.

Sheffield was the centre with the highest percentage (75/117, 64%) of patients receiving BPs, either for bone health or as an oncological adjuvant, followed by Chesterfield (22/44, 50%). All the other 3 centres had a similar percentage of patients having BPs (Doncaster 18/53, 34%, Leeds 55/156, 35%, Liverpool 56/159, 35%). The reasons for the wide variation are not clear but may related to the establishment of a specialist metabolic bone unit and the academic interest in BP research in Sheffield, with several research active clinicians regularly taking part in breast cancer MDT meetings.

Bisphosphonates were more likely to be offered in patients who had breast cancer surgery (from 226 who had BPs 195 (86%) had surgery and 30 (13%) had PET, for 1 (1%) patient information for primary treatment is missing) and were younger than 80 years (see below) , despite the higher prevalence of osteoporosis in this older age group. This may relate to the fact that women who had AI therapy in the adjuvant setting (433/529, 82%) were more likely to get BPs than those who had their AI therapy as PET (92/529, 17%) (unknown breast cancer primary treatment 4/529, 1%). This would introduce an age bias, as PET is usually offered to older, frailer and more comorbid women.

Breaking down BP use according to indication, 78 patients out of the 122 (64%) who were prescribed BPs for bone health, were younger than 80 years, whilst 36% (44/122) were over the age of 80. Similarly, 83% (59/71) of those who had BPs for prevention of breast cancer recurrence were 70 to 79 years old and only 17% (12/71) were >80 years old.

Patients who had PET wouldn't have been eligible for adjuvant treatment and therefore primary treatment analysis was not performed for this group of patients. Neither age nor primary treatment analysis was performed for the patients who were commenced on BPs prior to the diagnosis of breast cancer as this was outside of this sub-study's scope.

Almost all of the patients who were diagnosed with osteoporosis at their first DEXA scan were offered BPs (58/64, 91%), compared to 39% (58/148) of the osteopenic patients

(Figure 5.7). Bone protection was also offered to a small number of patients with normal baseline bone density (8/132, 6%). It is not known whether these patients had other risk factors for bone loss as these data were not available (a FRAX score for example). Ten percent (14/148) of the patients with baseline osteopenia and 2% (1/64) with osteoporosis were offered BPs for both bone health and cancer prevention (Figure 5.7). However DEXA scans are generally not recommended for patients who are eligible for adjuvant BPs, as they add no extra clinical benefit.

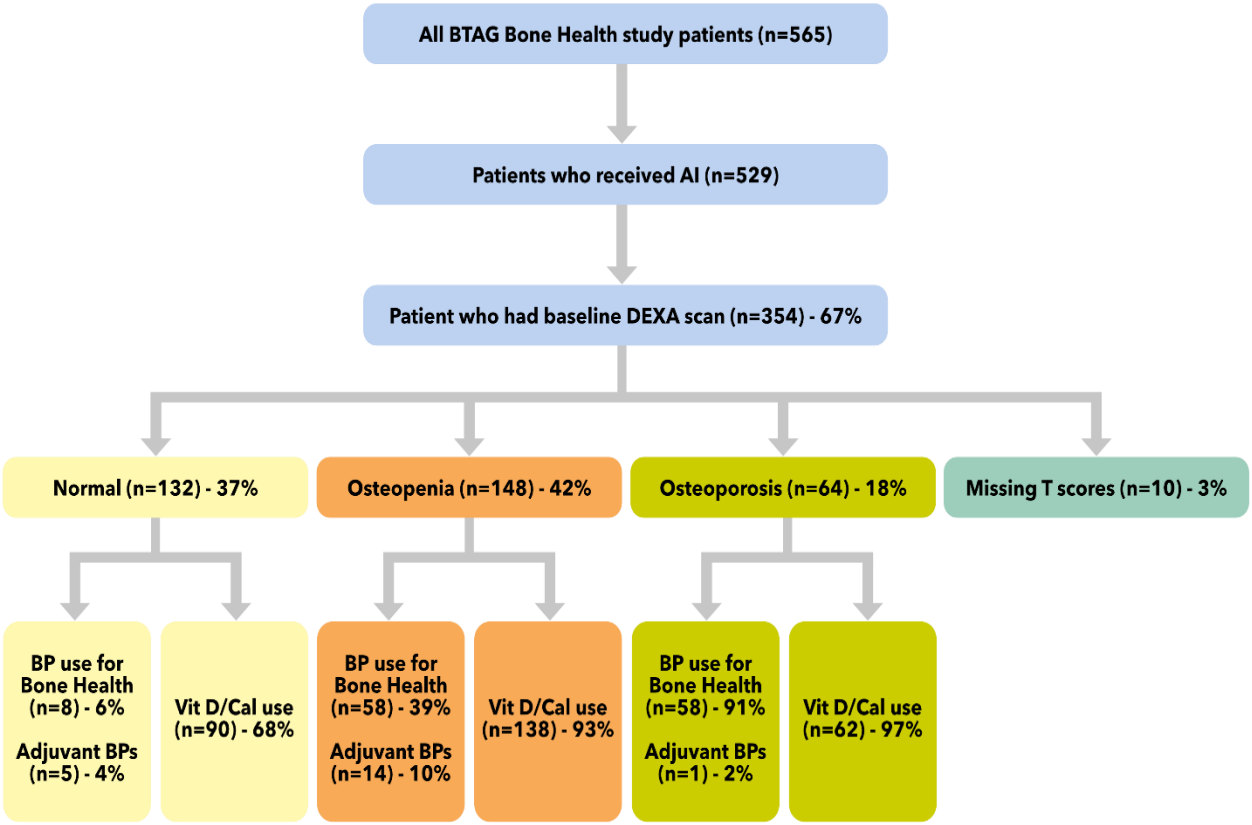

Figure 5.7: *Use of bisphosphonates (BPs) and Vitamin D/Calcium based on patients baseline DEXA scan results.*

As expected, the group of patients who were offered BPs (for bone health and/or adjuvant therapy) had lower hip and spine T-scores in both baseline and follow-up DEXA assessments, compared to the group of patients who did not receive these agents (Figure 5.8 & 5.9). Additionally, the hip and spine bone density remained stable in the BP group, as opposed to those who did not have BPs and experienced bone loss. This suggests that BP use resulted in a slight gain in bone density whereas women who did not have BP, whilst having higher starting scores did show a slight reduction (Figure 5.8 & 5.9). In women receiving BPs, there was no significant difference between baseline hip T-scores (M=-1.6,

SD=0.877) and follow-up hip T-scores (M=-1.7, SD=0.878); (t(65)=1.917, p=0.06 calculated using paired sample t-test) or between baseline and follow-up spine T-scores (p=0.409 calculated using Wilcoxon Signed Rank Test). In contrast, in the non-BP group, there was a significant difference between baseline and follow-up T-scores in both hip (p<0.001 calculated using Spearman's rho) and spine (p<0.001 calculated using Wilcoxon Signed Rank Test).

Taken together, these data demonstrate the need for further and continuous education of breast cancer specialists regarding the use of BPs in this population. This will improve bone health management of older women receiving AIs, especially of those in the primary endocrine treatment group, and offer better breast cancer outcomes for those eligible for adjuvant BPs.

Figure 5.8: *Box plot comparing baseline with follow-up spine DEXA results in AI patients based on bisphosphonate (BP) use (Baseline BP group n=144, Follow-up BP group n=78, Baseline no BP n=204, Follow-up no BP n=99, Baseline but missing information for BP use n=6, Follow-up but missing information for BP use n= 2). Baseline and follow-up DEXA scans were reported for different patient groups. Repeat DEXA scans were mainly performed in patients with baseline osteopenia but also in patients with normal baseline and baseline osteoporosis.*

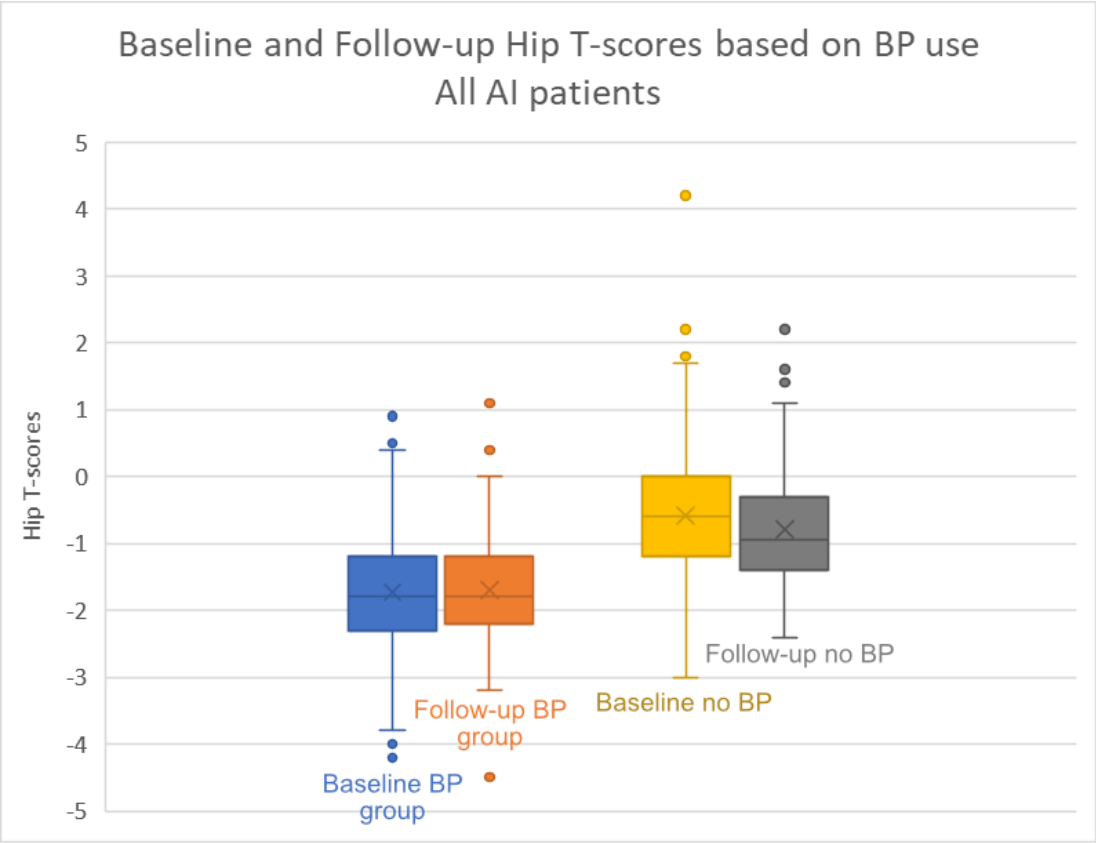

Figure 5.9: *Box plot comparing baseline with follow-up hip DEXA results in AI patients based on bisphosphonate (BP) use (Baseline BP group n=144, Follow-up BP group n=78, Baseline no BP n=204, Follow-up no BP n=99, Baseline but missing information for BP use n=6, Follow-up but missing information for BP use n= 2). Baseline and follow-up DEXA scans were reported for different patient groups. Repeat DEXA scans were mainly performed in patients with baseline osteopenia but also in patients with normal baseline and baseline osteoporosis.*

5.5.4. Use of Vitamin D and Calcium

National guidelines state that patients who are offered BPs, either for bone health or as an adjuvant, should also be advised to take vitamin D and calcium supplements in order to avoid hypocalcaemia and maintain normal vitamin D levels which are often low in these patients. The minimum daily recommended dose is calcium 500 mg and vitamin D 10 µg (400 international units).

In this sub-study cohort, 32% (171/529) of the patients had only vitamin D and calcium (without BPs) and 33% (173/529) had vitamin D and calcium with BPs. Twenty six (26/529, 5%) patients were already on vitamin D and calcium and BPs prior to being diagnosed with breast cancer.

Almost all of the BTAG Sheffield participants (105/117, 90%) who received AIs were advised to take vitamin D and calcium, followed by 76% (121/159) in Liverpool and 67%

(104/156) in Leeds. Less than half of the BTAG AI participants in both Chesterfield (18/44, 41%) and Doncaster (22/53, 42%) were asked to take vitamin D and calcium supplements ($p<0.001$, phi coefficient $r_\varphi<0.001$). The statistical test that was used to calculate the p-value was chi-squared test, and the variables were the use of vitamin D and calcium (Yes or No) and the number of patients per centre that had or did not have vitamin D and calcium. Again the reasons for this variation are not clear but show that despite national guidelines some patients still do not receive recommended care.

Overall, vitamin D and calcium were recommended to the majority of the osteopenic (138/148, 93%) and osteoporotic (62/64, 97%) patients after their first DEXA assessment, whilst 68% (90/132) of women with normal bone density also received these supplements (Figure 5.7).

5.5.5 Fractures

From the 529 patients who were offered AIs, 23% (122/529) were diagnosed with at least one fracture from the date they were recruited to the BTAG study until 2022. The total number of fractures was 161, with some patients having more than one fracture since they were commenced on AIs. Data to differentiate symptomatic from non-symptomatic fractures, were not collected.

The centre with the highest number of fractures in BTAG AI group was Chesterfield (18/44, 41%) followed by Sheffield (30/117, 26%) and Leeds (36/156, 23%). Nine (9/53, 17%) patients in Doncaster and 29 (29/159, 18%) in Liverpool presented with at least one fracture.

Although more women aged 70 to 79 presented with at least one fracture (70/122, 57%), compared to those older than 80 years (52/122, 43%), overall the fracture frequency was slightly higher in the older population (>80 years: 52/187, 28%) (70-79 years: 70/342, 20%). A chi-squared test was performed to assess the relationship between age (70-79 and >80) and fracture frequency. There was no significant association between the two variables ($p=0.055$) and no correlation between fractures and patient age has been identified ($r_\varphi=-0.083$).

Only 38% (46/122) of the patients with fractures were given a prior BP but a much higher percentage (84/122, 69%) had vitamin D and calcium prior to presenting with a fracture. Statistical analysis showed that there was no significant difference or any association or correlation ($r_\varphi=-0.029$, $p=0.515$) between the use of BPs and fractures in this sub-study cohort. A chi-squared test was used to calculate the p-value, and the variables were the use of BPs (Yes or No) and the occurrence or not of a fracture.

5.5.6 Frailty

Baseline frailty was assessed during the patients' participation to the original BTAG study. I was not involved in the collection and/or calculation of the frailty scores as these were not part of my degree. Frailty scores were kindly provided, for the purpose of this analysis, by Olivia Turner, Healthy Lifespan Institute, University of Sheffield, UK.

Some BTAG participants were excluded from the frailty score calculation and therefore not all of the sub-study patients had their frailty score calculated. From the 529 patients in the AIs group, 87% of them (461/529) had a baseline frailty assessment. Results showed that frailty (184/461, 40%) or pre-frailty (247/461,54%) was present in 94% of the women (30/461, 6% had normal assessment).

A baseline DEXA scan was performed for the majority of the prefrail (179/247, 73%) and not frail patients (23/30, 77%) compared to only 57% of the frail patients (105/184) (Figure 5.10). Although, analysis demonstrated no effect of frailty on baseline hip (R^2=0.0098, SE=0.060) or spine (R^2=0.00007, SE=0.058) T-scores (Figure 5.11 & 5.12), prefrail and frail patients were mainly osteopenic (prefrail: 72/179, 40%, frail: 45/105, 43%) or had normal bone density (prefrail: 72/179, 40%, frail: 35/105,33%), in contrast with the patients with normal frailty assessment who were mainly osteopenic (11/23, 48%) or osteoporotic (7/23, 30%). Osteoporosis was present only in 15% (26/179) of the prefrail and 22% (23/105) of the frail women (Figure 5.10).

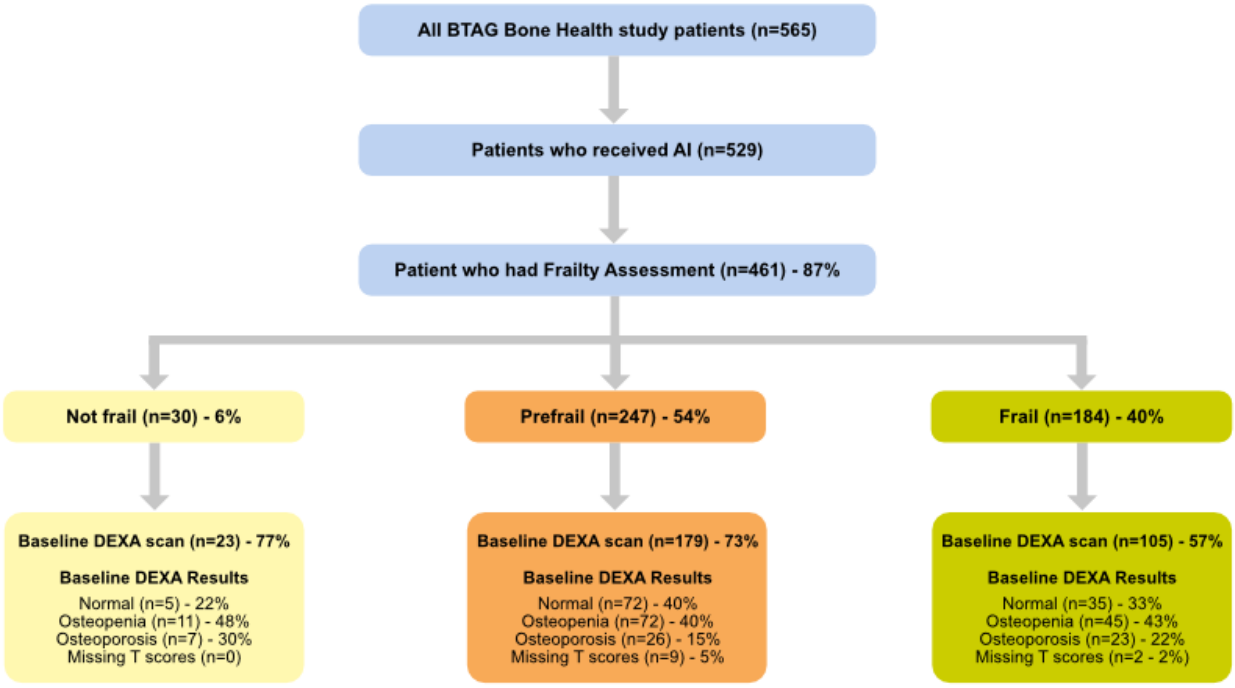

Figure 5.10: *Baseline DEXA results of AI patients based on their frailty assessment outcomes.*

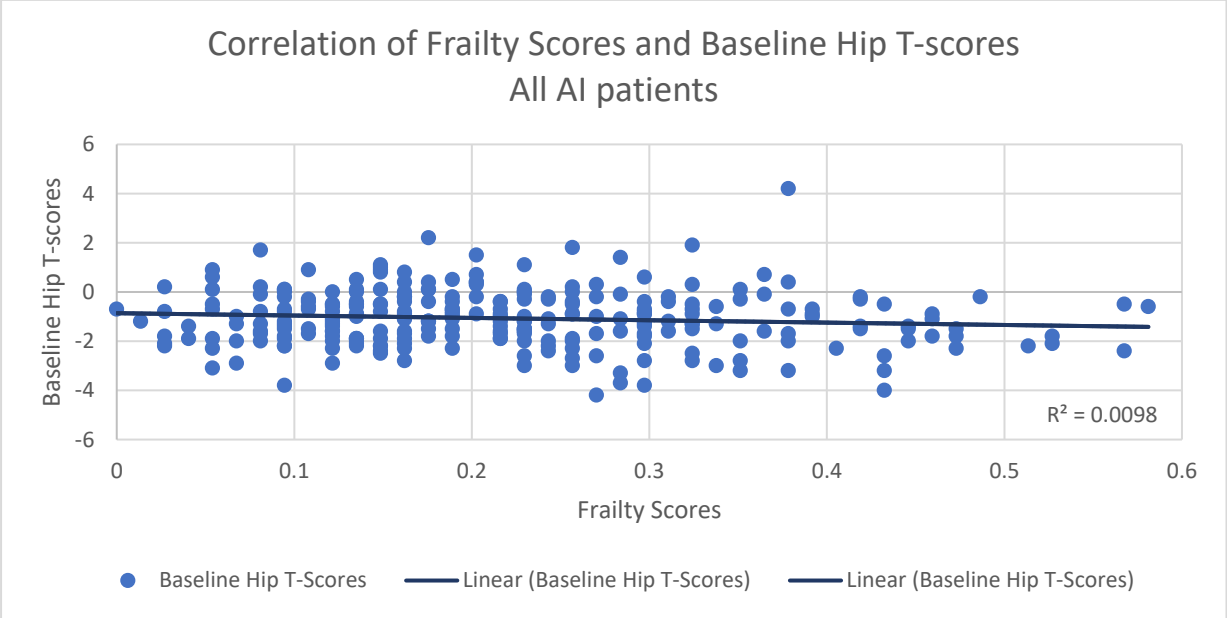

Figure 5.11: *Scatter plot showing the correlation of frailty assessment scores and baseline hip T-scores in patients receiving AIs for EBC.*

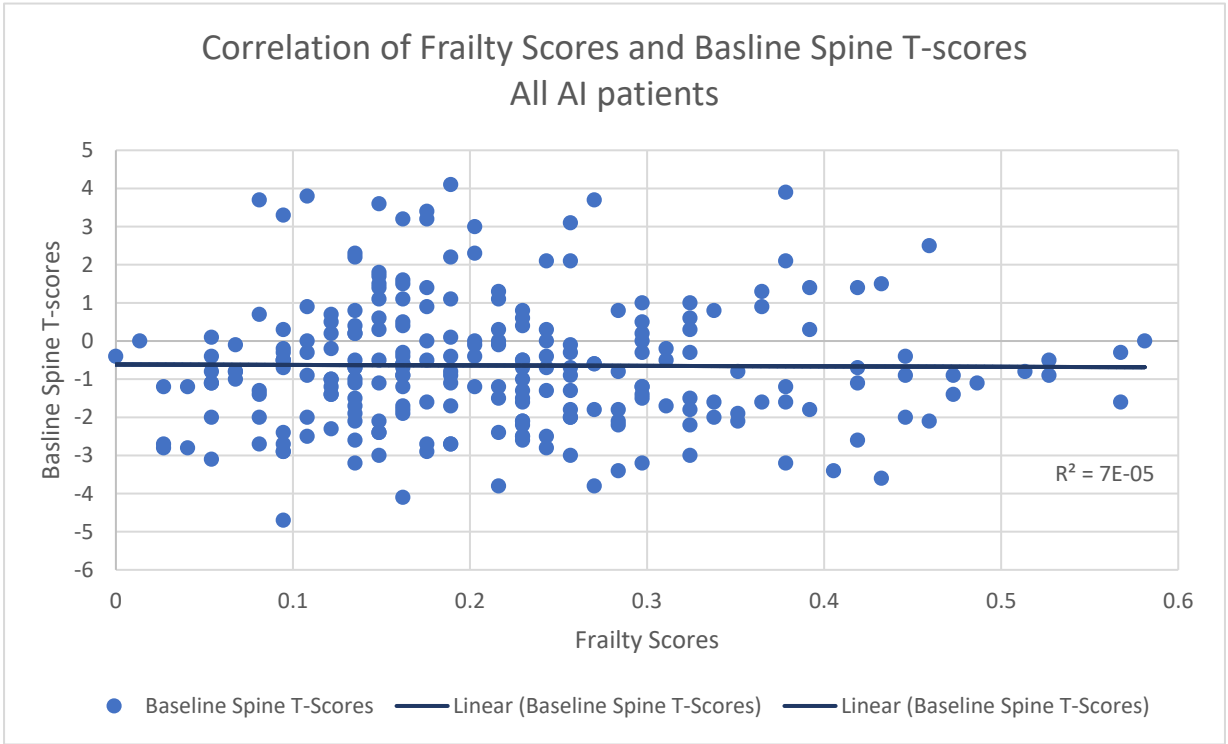

Figure 5.12: *Scatter plot showing the correlation of frailty assessment scores and baseline spine T-scores in patients receiving AIs for EBC.*

In addition, a chi-squared test was performed to assess the relationship between frailty (frail, pre frail, not frail) and the use or not of BPs. There was significant association between

the two variable (p=0.21). Analysis showed that the majority of the non-frail women (21/30,70%) were prescribed BPs with AIs for bone health or to prevent breast cancer recurrence, as opposed to only 43% (107/247) of the prefrail and 47% (87/184) of the frail women (Figure 5.13).

Figure 5.13: *Use of bisphosphonates in patients receiving AI for EBC based on their baseline frailty scores.*

5.6 Discussion

National and international guidelines for the management of bone health in women receiving endocrine therapy for EBC had been available well before the first patient was recruited to the BTAG study in 2013, recommending bone protection in women older than 65(international) or 75(UK) with 1 additional risk factor for bone loss (153, 338). Subsequently, recommendations have been updated but the advice concerning bone heath of older women with ER+ve EBC remains the same, as older age (≥65) is an independent risk factor for fractures (51, 162, 339).

Despite the dissemination of the guidelines, uptake by UK breast cancer specialists still varies significantly. This study has demonstrated a clear variation in the investigation, monitoring and treatment of AI-related bone loss within the 5 participating centres. Rates of baseline DEXA scans were 36-76% whilst rates of BPs use were 34-64%. Even the use of vitamin D and calcium supplements, which are widely available and do not require medical prescription, varied between 41 to 90%. Although the reasons for this wide variation are

unknown, Sheffield was the centre with the highest number of requested DEXA scans and use of antiresorptive therapy. This may well be due to the establishment of a specialist metabolic bone unit and the long-standing academic interest in bone research in Sheffield, with several research active breast cancer clinicians. In addition, two of the participating centres had an age limit in DEXA scans, limiting use to patients over the age of 80 to have the intervention. This was mainly due to the lack of BMD reference values for patients over the age of 84 and also due to the assumption that all patients older than 75 receiving AIs would have been offered BPs, as per NICE guidelines, and therefore DEXA scan would have had no impact on their course of treatment regarding the prevention of osteoporosis. The impact of this on the sub-study results is unclear as one centre stated that requests for DEXA scan could potentially be accepted if clinically indicated regardless of the patient's age and the second centre has now lifted the age limit.

Women receiving AIs were more likely to have their BMD monitored and be offered bone protection with a BP, if their primary breast cancer treatment was surgery and they were younger than 80. Patients who opt for breast cancer surgery as opposed to PET are generally younger with fewer health issues, suggesting that bone health might not be a priority in older and more frail patients. However, poor bone health management in older and frail breast cancer patients receiving AIs, make this population extremely vulnerable and significantly increases their risk of fractures and subsequently their mortality and morbidity. Bone health decision making should be based on holistic geriatric assessments, patients general health, life expectancy and wishes (295).

Patients treated with PET are generally managed by the breast surgeons. In contrast, the majority of the breast cancer surgery group will have some form of adjuvant anticancer treatment (e.g. chemotherapy, anti-HER2 therapy, radiotherapy). Therefore, the latter group will be under the care of medical and clinical oncologists until the end of their breast cancer follow up period, which is currently 5 years (13). In a published clinicians survey in 2006, 307 breast cancer specialists (medical oncologists, clinical oncologists and breast surgeons) indicated that oncologists were considered to be the most appropriate to treat and monitor the bone effects of AI therapies (57%), whilst only 19% thought that responsibility should be shared between oncologists and breast surgeons (340). In the same survey, oncologists were the most confident in interpreting DEXA results and treating AI-related bone loss, with 45% of the responders having no confidence at all (340). This might partially explain the reasons for inadequate bone health care in patients receiving PET, highlighting the need for further education. Additionally, cancer specialists awareness might have improved since the publication of UK guidelines in 2008 (153), however the sub-study findings demonstrate that management of bone health in older patients is still inadequate.

Since the introduction of adjuvant BPs in breast cancer care in 2016 and the adoption by the NICE in 2018 (13, 232), their uptake has massively increased (275). More and more patients are receiving BPs to reduce the risk of disease recurrence and subsequently prevent bone loss, in cases where AIs are offered. For these patients, DEXA scans within 6 months of the start of AIs are not recommended as they have no clinical benefit. Therefore, some reduction in the number of requested baseline DEXA scans is expected during the last 2 years of the BTAG study recruitment period (2016-2018). Nevertheless, adjuvant BPs are not indicated for all the postmenopausal EBC patients. Currently, NICE recommends adjuvant BPs only for postmenopausal women with high risk of recurrence disease (please see chapter 1 for full details) (13), suggesting that even in the present (2023) many of these patient will not fit the adjuvant guidelines. This highlights that bone health still needs to be monitored in this population. Additionally, adjuvant BPs duration does not match the duration of antioestrogen therapy. Adjuvant BPs are only offered for up to 3 years compared to AIs which can be taken for 5 to 10 years, underlining the need for an ongoing bone health care in EBC, especially after the completion of adjuvant BPs. This should be guided by DEXA bone density monitoring once the 3-year adjuvant course of BPs has been completed.

The majority of sub-study participants were found to be osteopenic (37%), in comparison to osteoporosis which was only found in the 18% of the scanned AI women. This might have been expected in reverse, as osteoporosis incidence is 20% in women over 70 and doubles in women over 80 (341). However, the sub-study cohort was mainly 70 to 79 years old (n=342) and additionally some of the older participants (>80) did not have a BMD assessment. From those older patients (>80) who were scanned, the incidence of osteoporosis was almost doubled compared to younger patients (22% and 13% respectively), confirming the importance of baseline DEXA scan and prevention of further bone loss.

Patients who were commenced on bone protection with a BP had reduced baseline and follow-up BMD, compared to those who did not receive these agents. This is to be expected, as those who did not receive BPs are mainly patients with normal BMD. In addition, BMD in both hip and spine of the women who were having antiresorptive therapy appeared to remain stable between DEXA assessments, whereas the group who did not have BPs experienced bone loss, suggesting that BP use resulted in a slight gain in bone density. Women who did not have BP, whilst having higher starting scores did show a slight reduction. In the age group analysis, spine BMD of older women (>80) improved in the follow-up DEXA scans which could be due to the use of bone protection therapy. However, hip BMD of this population reduced, which is contradictive and therefore, results should be interpreted with caution.

The sub-study has several limitations. Although, the original BTAG study recruited 3456 patients, the bone sub-study could only be carried out in a much smaller population.

Bone health data were collected from over 500 patients, but analysis was performed with smaller samples and therefore careful interpretation of findings is needed. In particular, the sub-study did not demonstrate any correlation between patient's age and fractures, fractures and BPs use or baseline DEXA scan T-scores and frailty. Reasons for this might be the small number of patients available for the correlation analysis and the results should therefore be reported carefully.

Despite these limitations, my study supports that further education, increased awareness and encouragement regarding the importance of good bone health in older women is still needed. Reminders about adequate bone health care in older ER+ve EBC should be sent to individuals and local level education should be provided. Efforts should not only focus on oncologists and breast surgeons but should include other health care professionals such as breast care nurses, oncology pharmacists and advanced nurse practitioners , whose involvement in the care of older breast cancer patients is increasingly important. Better bone health will improve patients' experience with breast cancer treatments and ultimately will improve the care we offer to older EBC women.

5.7 Conclusion

Patient's age and general health influence the bone health decision making with older and frail patients to receive non-standard of care. Despite national and international recommendations, there is still wide variation in bone health management, highlighting the need for further education and standardised bone health care in older EBC.

5.8 Future Work

- The results of the BTAG bone health sub-study will be published in peer review journals in order to disseminate findings within the bone health, breast cancer and oncogeriatrics communities. This will encourage specialists to imporve their bone health management of older women with ER+ve EBC. In particular, publication of these data is aiming to support clinicians in being more proactive and less bias in the way they manage bone health of older women receivig primary endocrine therapy.

- The sub-study data will be further presented in local, national and international meetings with special interest in bone health of cancer patients and oncogeriatrics. These meetings will give me the opportunity to discuss these findings in person with health care professionals treating women from this population. My focus will be to remind professionals about the current national and international guidelines for the managemnet of bone health in women with EBC. Additionally, I will discuss the importance of good

management of bone health of older women receiving AIs for EBC and how this could be improved in the future

5.9 Presentations arising from this project

Poster Presentations

1. **Theodoulou E***., Martin C., Morgan J., Turner O., Hartup S., Achuthan R., Azmy I., Henderson J., Reed M., Holen I., Brown J., Wyld L., Management of bone health of older women with oestrogen receptor-positive early breast cancer receiving endocrine treatment. A sub-study of the Bridging the Age Gap study. Mellanby Centre Research Day 2023, March 2023Sheffield, UK.

2. **Theodoulou E.***, Martin C., Morgan J., Turner O., Hartup S., Achuthan R., Azmy I., Henderson J., Reed M., Holen I., Brown J., Wyld L., Bridging the Age Gap: How is bone health of older women with early breast cancer managed?. Bone Research Society (BRS) Annual Meeting 2023, April 2023, Liverpool, UK.

3. **Theodoulou E.***, Martin C., Morgan J., Turner O., Hartup S., Achuthan R., Azmy I., Henderson J., Reed M., Holen I., Brown J., Wyld L., Bridging the Age Gap: How is bone health of older women with early breast cancer managed?. 50th European Calcified Tissue Society (ECTS) Congress 2023, April 2023, Liverpool, UK.

*Indicates presenting author

Chapter 6

General Discussion

6.1 General Discussion

Bisphosphonates are bone targeted agents with multiple roles in the management of breast cancer (51, 342). The work in this thesis focused on their anticancer effects in EBC and their role in prevention and management of CTIBL. Clinical trials have demonstrated that BPs given after EBC surgery (adjuvant setting) improve survival and reduce the risk of disease recurrence, but only in postmenopausal women (88, 89). An anticancer benefit in premenopausal women was only reported in those deemed to actually be menopausal at the start of their adjuvant therapy, either after ovariectomy or introduction of GnRH analogues (87, 89). Although the mechanisms underpinning the differential effects according to menopausal status remain to be established, national and international EBC practices have changed to include adjuvant BPs, with recommendations to advise the use of these agents in postmenopausal (natural or induced) early disease (13, 51, 126).

The ZOLMENO study (chapter 2) was a proof of concept single centre study which aimed to provide further evidence and clarification for the mechanism responsible for the differential effect of adjuvant BPs in EBC. However, the study did not reach its recruitment target, which is a common problem in clinical research with reports suggesting that 86% of clinical trials failing to reach their recruitment targets within their proposed time frame (343). In the case of ZOLMENO, lack of recruitment was mainly due to the COVID-19 pandemic and the subsequent hospital restrictions resulting from this, but also the ongoing NHS crisis which led to practice changes meaning fewer patients were eligible for the study. As much as the recent pandemic is not currently an issue for the UK health service, staff shortages, care backlog and increased demand for services are having a knock-on effect on clinical research. In 2018, a questionnaire was distributed to the members of the Association of Breast Surgery with the aim to identify the challenges and barriers in recruiting women to breast cancer studies (n=48) (344). Results showed that busy breast cancer clinics and service pressures were the main reasons for low patient recruitment to clinical studies. This is particularly important for studies like ZOLMENO that have no direct benefit for the participants, suggesting that under the current environment these are not a clinical priority. Therefore, relevant local support needed to be provided to the breast cancer units to encourage the set up and successful completion of early phase trials.

In the case of the ZOLMENO study, it was particularly difficult to recruit younger (premenopausal) patients. The majority 79% (n=15) of the patients recruited to the study were postmenopausal and only the 21% (n=4) were premenopausal. This is a recognised issue affecting research recruitment in general (345). Younger patients have busy schedules (e.g. full time job, care for young families) and it is very challenging for them to have the extra time to participate in clinical trials that most of the time require additional hospital visits. Improving clinical trials flexibility, e.g. offering late or weekend appointments whenever possible, could

potentially improve participation of young adults in studies. Nevertheless, this option was never possible for the ZOLMENO study which was running to a very strict time schedule in relation to the scheduled surgery and not all the study visits could take place outside of normal working hours as multiple members of the research team needed to be present (e.g. data manager for patient recruitment).

Despite the limitations, the ZOLMENO study has shown that it is feasible to recruit patients to an early phase trial with no direct benefit to the participants which also required patients to undergo an invasive procedure like a bone marrow biopsy. Patients who did enrol were keen to take part to the study and provide all the needed samples. Additionally, the study demonstrated that is possible to obtain both bone marrow aspirate and trephine samples for research purposes during breast cancer surgery, even in unprecedented times like the COVID-19 pandemic.

The UK physicians and oncology pharmacists' surveys showed that adjuvant BPs have been fully adopted in the management of EBC in the country, with 99% of the physicians and 93% of the UK cancer centres offering these agents (chapter 3). These data were confirmed by the BCN patients' survey indicating that 96% of the participants were offered adjuvant BPs (chapter 4). However, the same uptake was not demonstrated by the Australian physicians' survey (48% prescribed adjuvant BPs), suggesting that adoption of BPs in EBC is not universal and there are still countries facing difficulties and challenges. So far, the only published real world data demonstrating the cancer physicians' experience with adjuvant BPs are the data presented in this thesis and two Canadian physicians' surveys. The first Canadian survey was published in 2019 and reported use of adjuvant BPs at 52.2%, with the second survey (published in 2021) to demonstrate an increase in use as the majority of the participants (77.4%) were prescribing bone agents for prevention of disease recurrence (280, 283). Clearly, further evidence is needed to map the use of adjuvant BPs globally, with the aim to increase the number of women receiving this intervention and improve the EBC outcomes.

Currently, adjuvant BPs are offered only to postmenopausal women and premenopausal women on ovarian suppression with high risk of disease recurrence. This is based on the available published data and the subsequent large meta-analysis (87-89). Notwithstanding, since the publication of these large clinical trials and the meta-analysis the standard of care for the management of EBC has changed significantly. Although HER2 status was not part of the sub-group analysis of the EBCTCG meta-analysis, new treatments have now been introduced for the management of HER2+ve early disease, improving the survival of these patients (31, 33, 89, 346, 347). More recently, addition of immunotherapy to the standard neoadjuvant chemotherapy in patients with TNBC, significantly improved the number of patients who had a pathological complete response (24, 348). Additionally, CDK4/6 and PARP inhibitors have now been introduced in the adjuvant management of early disease (20,

21). The CDK4/6 inhibitor abemaciclib when given for 2 years with endocrine therapy, following standard neoadjuvant/adjuvant treatment (e.g. surgery, chemotherapy, radiotherapy), showed to improve IDFS of high risk early disease patients (ER+ve, HER2-ve, node positive) (23). Adjuvant olaparib (PARP inhibitor) showed to offer better DFS (invasive and distant) and to significantly improve OS in BRCA1 and BRCA2 carriers with HER2-ve EBC, when it was given for 1 year after the completion of surgery and chemotherapy (neoadjuvant/adjuvant) (26, 27). All these new adjuvant therapies have now been approved by NICE (20, 21, 346-348). Taken together, addition of new and effective therapies in the adjuvant setting which were not available when adjuvant BPs were first described, suggests that reassessment of BPs in combination with the new targeted molecular therapies (HER2, CDK4/6, immune and PARP inhibitors) is crucial to provide an update and reassurance for their benefit in this setting.

In 2016, Coleman et al. calculated the cost for the adjuvant use of BPs and the annual saving for the national health system that comes from the prevention of advanced disease and the omission of DEXA scanning in this population (255). However, these estimations are perhaps outdated and underestimated as the incidence of breast cancer has since increased and the health costs have changed. In general, cancer care is a considerable financial burden for health systems with data indicating that the first 6 months after cancer diagnosis are the most expensive for the UK system (349). For the adjuvant/curative breast cancer setting, it is perhaps expected that the economic burden will decline with the completion of adjuvant therapies, which in the majority of the cases last 6 to 12 months. In spite of this, Marti et al. showed that the financial implications for the national health system remain substantial, even a year after breast cancer diagnosis (350). Therefore, new cost analysis for the use of bone agents in the adjuvant breast cancer management is important to clarify the actual cost of this therapy. This should carefully take into consideration the results of the 3 UK surveys (physicians, oncology pharmacists and patients) presented in this thesis, demonstrating that intravenous ZOL is the BP of choice, suggesting that the need of a hospital chair twice a year for a period of 3 years will markedly increase the health cost. This is in contrast to the oral alternatives, such as clodronate, which come with no extra cost related to the need for an administration hospital chair. However, ZOL would not have the adherence issues that the oral agents have as a result of patients not coping with the schedule and the common gastrointestinal side effects. Therefore, more patients would receive the full anticancer benefits of these agents if they were given ZOL, and this needs to be factored in.

In addition to their preventative role in early disease, BPs have an established use in the management of CTIBL (51, 342). This is particularly important in patients receiving endocrine therapy with AIs, due to their known effects on bone health (discussed elsewhere in this thesis), especially for older women whose bone health is already compromised due to age and menopause. In comparison to the use of adjuvant BPs, which are fully endorsed in

the UK EBC care pathway, use of BPs to prevent/treat bone loss in the older population receiving AIs varied considerably between UK centres (chapter 5). It was more likely for women to receive BPs for bone health and undergo bone assessments (DEXA scans) if they were younger than 80 and had surgery as their primary breast cancer treatment, implying that older age and health comorbidities led to compromises in bone health care. Although, the data presented in chapter 5 of this thesis were collected from patients diagnosed with EBC between 2013 and 2018 in 5 UK centres, they have provided a good understanding of the bone care that older women with EBC received during that period, and they will support future efforts for the improvement of this populations' care.

According to the national and international guidelines, DEXA scans for assessment of bone health should be carried out within 6 months of the initiation of AIs in all the patients. Current estimates indicate that 1 in 3 women aged over 50 years will experience an osteoporotic-related fracture at some point in their life (332), indicating that a big proportion of this population will have osteopenia or osteoporosis due to natural causes like menopause and older age. Are DEXA assessments then necessary for the older EBC population receiving AIs, or would synchronous commencement of BPs with the start of endocrine therapy be more reasonable and cost-effective? As BTAG sub-study showed, frail, older breast cancer patients with many health issues tend to miss on these interventions and subsequently do not receive the appropriate therapy to prevent future fractures due to bone loss. Further research is needed to answer the questions raised here and to clarify the usefulness of bone assessment to guide the use of bone health interventions in this population.

In conclusion, the projects presented in this thesis demonstrated the importance of BPs in various aspects of breast cancer management. Adjuvant BPs are a well-recognised and accepted therapy by both cancer physicians and patients and continuing of their use should be strongly encouraged. Also, sharing the positive experiences with these agents from the UK is important, as even countries like Australia with an excellent health care system has failed to adopt adjuvant BPs as standard of care. In contrast, the bone health care that older women with ER+ve early disease received, did in many cases not meet the national expectations, with further education and standardised practice to be vital.

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Appendix

Appendix 2.1: The ZOLENO Study Protocol.

Appendix 2.2: National NHS Research Ethics Committee (REC) approval for the ZOLMENO study.

Appendix 2.3: National NHS Research Ethics Committee (REC) acknowledgement of documentation for the ZOLMENO study.

Appendix 2.4: Medicines and Healthcare Products Regulatory Agency (MHRA) approval for the ZOLMENO study.

Appendix 2.5: Health Research Authority (HRA) approval for the ZOLMENO study.

Appendix 2.6: Patient Information Sheet (36) of the ZOLMENO study.

Appendix 4.1: Participants Information Sheet for the Breast Cancer Now patients' survey.

Appendix 5.1: National NHS Research Ethics Committee approval for BTAG sub-study.

Appendix 5.2: Health Research Authority (HRA) approval for BTAG sub-study.

Appendix 5.3: BTAG Bone health sub-study protocol.

Appendix 5.4: BTAG Bone health sub-study CRF.