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INTRODUCTION

Due to high rates of bone cancer relapse and chemoresistance, there is an urgent need for innovative therapies that can improve patient outcomes. Gamma delta (γδ) T cells engineered to express IL-15 and featuring an anti-B7H3 opsonin platform are a potential candidate for allogeneic cancer adoptive immunotherapy in bone cancer. γδ T cells are innate immune cells that recognize tumour-associated antigens (TAA) through T-cell receptors and natural killer cell receptors. γδ T cells recognize tumour antigens without restriction by major histocompatibility complex (MHC), and once activated, can kill cancer cells directly by secreting perforin and granzyme B, as well as by enhancing anti-tumour abilities of other immune cells.[1]

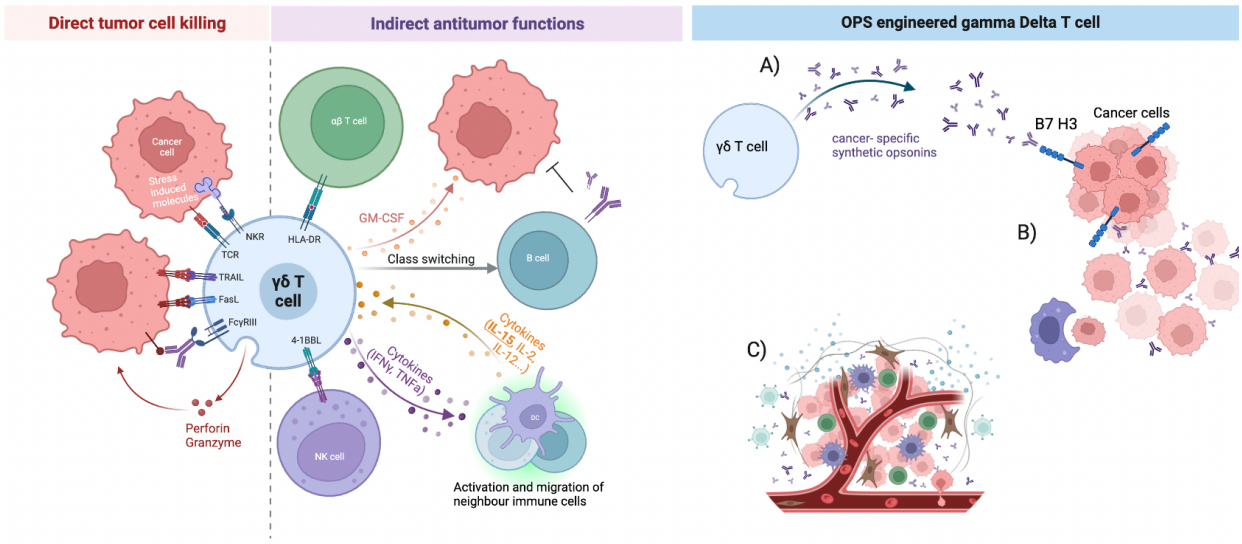


Figure 1: Direct and indirect anti-tumour functions of γδ T cells, and OPS engineered γδ T cell (Figure generated in BioRender)

In addition to secreting cytokines and chemokines, γδ T cells can also directly interact with other immune cells to coordinate the immune response against the tumour. For example, γδ T cells can form conjugates with dendritic cells, which can present tumour antigens to other T cells and activate them to attack the tumour.

A) Secreted opsonins designed to recognise and bind to specific cancer cells or tumour associated antigens.

B) Interaction between the B7-H3 protein and the opsonin molecule, leading to opsonisation of the cancer cell: Opsonins mark cancer cells for destruction by the immune system. Immune cells such as γδ cells can recognize and attach to the marked cancer cells through the opsonin receptors on their surface.

C) Opsonins concentrate in the tumour microenvironment, improving the persistence γδ T cell activity in the tumour, as they can continue to recognize and attack cancer cells marked by opsonins. γδ T cells can secrete interleukin-2 (IL-2), which can activate and recruit other immune cells such as natural killer (NK) cells and T cells to the site of the tumour.

Although γδ T cells have demonstrated the ability to target cancer cells, they typically require genetic engineering to improve their effectiveness.[2] When genetically modified to express receptors that are specific to tumour antigens, they can be even more efficient killers. An innovative technique called OPS-engineering has been developed to adjust lymphocytes to express and excrete cancer-specific synthetic opsonins.

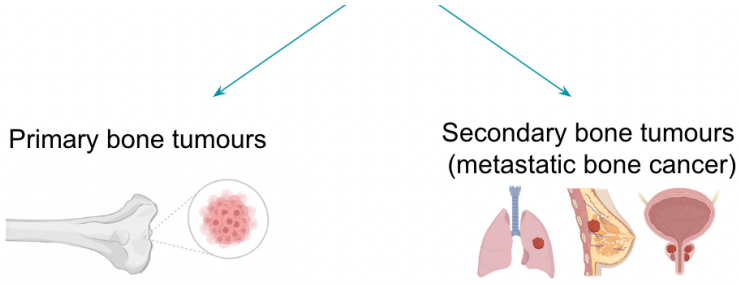
Opsonins work by coating cancer cells, making them more easily recognized and engulfed by phagocytes. Unlike classical CAR-T cells, which directly recognize and kill target cells, opsonins can facilitate antibody-dependent cellular cytotoxicity (ADCC) by immune cells expressing cognate receptors, such as gamma delta T cells, NK cells, and macrophages.[3] This allows for a wider

range of immune cells to participate in the killing of target cells, potentially increasing the efficacy of the therapy.

In the case of gamma delta (γδ) T cells that are engineered to secrete opsonins, these immune cells can recognize and target cancer cells more efficiently. This is because opsonization can increase the ability of γδ T cells to bind and engulf cancer cells, leading to their destruction. Additionally, opsonized cancer cells are more easily recognized by other immune cells, such as natural killer (NK) cells and myeloid cells, which can further enhance the immune response against cancer. OPS-engineered γδ T cells can overcome T cell exhaustion and antigen-negative relapse, which are major obstacles in the treatment of solid tumours.[4] This approach holds great potential for developing engineered γδ T cell therapies for solid tumours, especially for those with low response rates to existing therapies.

In bone cancer specifically, γδ T cells that are engineered to secrete opsonins can home to the tumour from the bloodstream and kill cancer cells without the need for exogenous cytokine support. These γδ T cells can also efficiently inhibit bone cancer invasion and infiltrate and kill patient-derived osteosarcoma.[2] Furthermore, *γδ* T cells could be administered in combination with zoledronic acid, which has a high affinity for mineralised bone to increase honing to the tumour site.[5] The hypothesis is that combining Zoledronic acid with immunotherapy may be a promising strategy for the treatment of bone cancers, which could be either primary tumours or secondary tumours that have metastasised to the bone from other cancer sites.

The premise is to develop a therapy that maximises clinical benefit to as many people as possible, the purpose of this research is to provide data as to where the opportunity would be. This will involve investigating patient populations and the clinical trial landscape to compile real-world data to support the findings when presenting to investors.



Primary Bone Tumours

Primary bone tumours are rare, aggressive, and malignant tumours originating in bone, accounting for 0.2% of all malignancies worldwide.[6] In the United States, it is estimated that 3,970 new cases of primary bone tumours will be diagnosed in 2023, with 2,140 deaths.[7] They are typically treated with surgical excision (amputation) and then very aggressive chemotherapy to treat any metastasis. About 600 people are diagnosed with it in the UK each year.[8]

Most primary bone tumours (56%) are osteosarcomas which usually occur in paediatric patients – making up 2% of cancers in children aged 0 to 14 and 3% of cancers in ages 15-19.[7] Osteosarcoma is characterized by altered osteoid deposition and high rate of metastasis,[9-10] and poses a major challenge to clinicians due to its heterogeneity and resistance to conventional therapies.[11,12] Prior to the 1970s, the 5-year overall survival rate for osteosarcoma patients who underwent surgery alone was approximately 20%.[13] The introduction of chemotherapy increased survival rates to 60-70% for patients with localized osteosarcoma. However, despite the

development of surgical techniques and adjuvant chemotherapies, outcomes have not improved further over the past 30 years. The overall survival rate of patients who have metastatic or recurrent disease remains at a dismal 25%.[14-16] Standard treatment regimens currently include neoadjuvant preoperative chemotherapy, surgical excision of the main tumour, and postoperative adjuvant chemotherapy. The required high dose of anticancer medications frequently results in a variety of acute and chronic side effects, including nephrotoxicity, neutropenia, infective problems, and thrombocytopenia.[17-19] Furthermore, because bone cancer is most common in adolescents, therapy might result in irreversible alterations to a growing body. Even when conformal 3D proton treatment is used, some patients encounter substantial radiation related toxicity.[20] Long-term adverse effects can include organ damage, and some studies have suggested that chemotherapy increases the chance of developing a second solid cancer.[21]

Despite their rarity, primary bone tumours remain an area of significant clinical need, novel therapeutic strategies are desperately needed to improve patient outcomes in this challenging disease area.

Secondary Bone tumours

Secondary bone cancer, also known as metastatic bone cancer, is a type of cancer that has spread to the bones from another primary cancer site in the body. It is a common complication of many types of cancer, particularly breast, prostate, and lung cancer.[22-27]

Bone is the most frequent site for metastasis for many cancers, tumour cells may escape the primary tumour site and colonize the bone microenvironment. The bone microenvironment provides a fertile soil for tumour cells to seed metastases. The chances are high that, in patients who are originally diagnosed with breast or prostate cancers, the bulk of the tumour burden at the time of death will be in bone.[28] 609,820 cancer deaths are projected to occur in the United States in 2023, 34% of which are either breast, lung, or prostate cancer.[7] This means potentially 205,470 deaths in 2023 will occur with bone metastases.

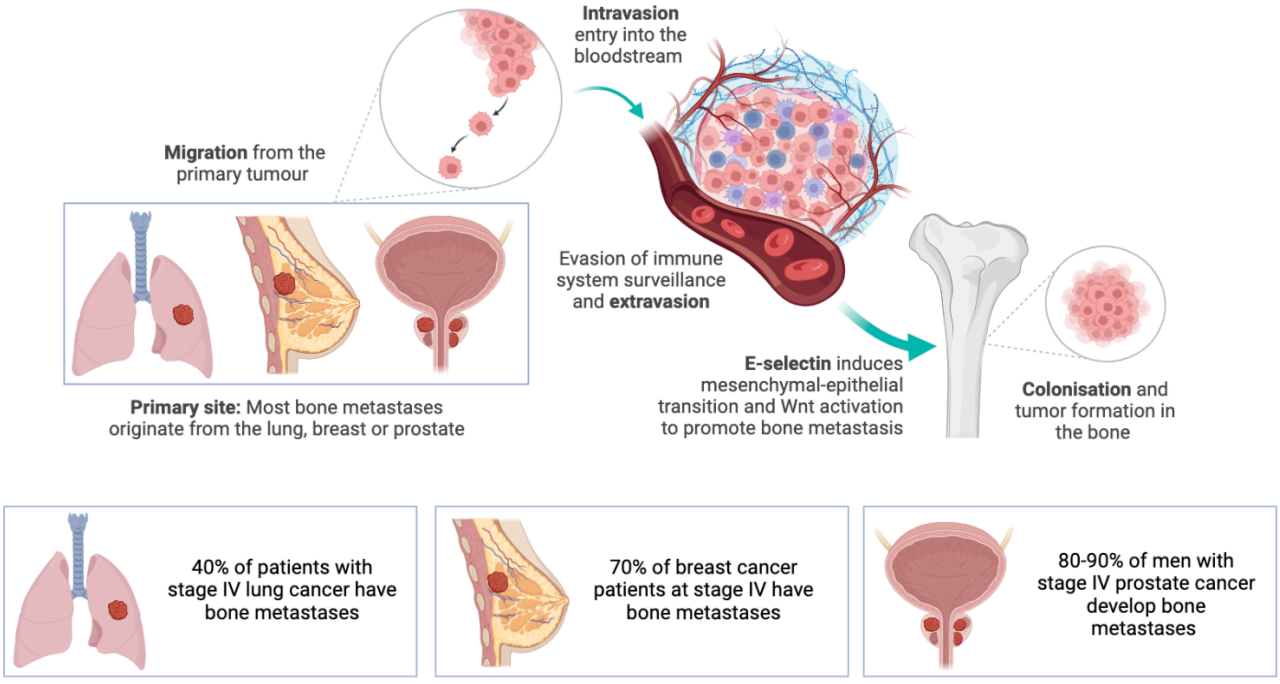


Figure 2: generated in BioRender. Adapted from: Guerrieri et al. (15)

The process of cancer metastasizing to the bones is a complex and multifaceted process. It involves

several different mechanisms, including mesenchymal-epithelial transition, intravasation, adhesion, and extravasation. Once cancer cells reach the bones, they must adhere to the bone surface and then extravasate or leave the bloodstream. This involves the interaction between several molecules, including E-selectin expressed on the surface of endothelial cells in the bone microvasculature, which acts as a "sticky" molecule, allowing cancer cells to bind and infiltrate the bone tissue.[29]

Tumour dormancy refers to the phenomenon where disseminated tumour cells stay in a condition of quiescence or slow progression for a long period of time, before restarting proliferation and generating overt metastasis.[30] This has been observed in various types of cancer, including breast and prostate cancer, where progression to overt metastases often does not occur until many years after diagnosis and treatment of the primary cancer.[31]

As the cancer cells grow and multiply, they can cause damage to the bone tissue. The presence of disseminated tumour cells can activate osteoclast-mediated osteolysis, leading to the destruction of bone tissue.[32] This structural damage can lead to considerable morbidity, including bone pain, weakness, fractures, and hypercalcemia. Skeletal-related events (SREs) are defined as pathological fractures, spinal cord compression, bone pain, or hypercalcaemia in patients with established bone metastases. SREs are associated with increased mortality, decreased quality of life, and considerable medical care expenses.[33] Surgical intervention is necessary to manage structural complications associated with bone destruction or nerve compression. Additionally, radiation therapy and systemic therapies, such as chemotherapy, immunotherapy, and hormonal therapy, can be used. While radiation, chemotherapy, and surgery are helpful in the early stages, they are less effective in the later stages when metastatic tumours are present.

The goal of treatment is to relieve pain, prevent fractures, and improve quality of life. Bisphosphonates and monoclonal antibodies, such as Zoledronic acid and Denosumab, are used to inhibit the activity of osteoclasts, the cells responsible for breaking down bone tissue, and slowing down the progression of bone metastasis. Despite these treatments, current therapies focus on relieving symptoms rather than targeting the cancer itself. Patients with secondary bone cancer have a poor prognosis, and there is an urgent need for therapies to improve patient outcomes.

Breast

Breast cancer has a high frequency of skeletal involvement, and 70% of breast cancer patients who later develop stage 4 cancer have bone metastases.[25] Patients diagnosed with localized breast cancer, even when treated with adjuvant therapy, remain at risk for late metastatic relapse, most commonly in bone. Clinically silent bone marrow micro metastases can be detected in 30% of breast cancer patients with stage I to III disease and predict the likelihood of disease relapse.[34]

Current adjuvant chemotherapies and hormonal therapies reduce the risk of relapse by targeting micro metastatic disease, yet these treatments may fail in part because of the nature of the bone microenvironment. Not only is it avid soil for metastasis localization, but it may also be a protective haven against chemotherapy.[35] In studies of patients receiving neoadjuvant chemotherapy, many patients with complete responses at the primary site demonstrated persistent micro metastatic disease.36 Although it is possible that these micro metastases persist after adjuvant chemotherapy solely because of intrinsic chemoresistance, bone marrow stromal cells can protect breast cancer from chemotherapy-induced apoptosis.[37] Additionally, Cancer cells can become quiescent in the bone microenvironment, rendering them less susceptible to chemotherapy and radiation therapy.

Prostate

If prostate cancer spreads to other parts of the body, it nearly always goes to the bones first, with nearly 90% of cases affected.[27] Metastatic prostate cancer cells may remain dormant in the bone

marrow for several years before proliferating and driving metastatic progression.[38] Most men with prostate cancer who develop bone metastases will do so several years after primary tumour removal, suggesting a delay between interventional therapy and the indication of biochemical recurrence prostate specific antigen relapse.[39-40] The presence of bone metastases is associated with poorer survival rates, and patients who had bone metastases had a 1.5-fold higher probability of death compared to men with lymph node involvement only.[41] Histological examination of bone metastases from prostate cancer has shown an osteoblastic response, with a wide variation in osteoclast response present from patient to patient and, consequently, in the amount of structural damage.[42] Patients with prostate cancer often have high bone resorption rates, and SREs are common.

Lung

In lung cancer patients, bone metastases are common, with approximately 40% of patients developing bone metastases[23,24] and 22-60% showing bone marrow micro metastases.[43] SREs are also prevalent in lung cancer patients with bone metastases, with about 25% of patients presenting with an SRE as the first sign of skeletal involvement and 40% experiencing an SRE during the clinical course of their metastatic disease.[44-45] With new treatments improving overall survival, the relevance of skeletal morbidity in lung cancer is becoming increasingly important. However, the prognosis for lung cancer patients with bone metastases is poor, with cumulative survival rates of 59.9% at 6 months, 31.6% at 1 year, and 11.3% at 2 years.[46]

Hypotheses

Our hypothesis is that that cancer incidence of secondary bone cancers will be greater than primary bone tumours and that it will occur in an adult population. However, the lack of clear data limits the validity of these claims. The epidemiological analysis will provide data to support the rationale for selecting a patient population in an initial clinical trial. Moreover, analysing the trial landscape for both cancer groups will demonstrate the areas of unmet need. We predict that the broader disease area of secondary bone cancer will exhibit a more extensive drug development landscape, featuring a greater number of active trials. The discussion will consider the opportunities and benefits observed in the population and clinical trial data. Additionally, reviewing the literature for the development of adoptive immunotherapies and their targets in the context of these patient groups will offer insights into a potential development plan for an engineered gamma delta T-cell therapy in bone cancer.

MATERIALS AND METHODS

The study requires an assessment of patient populations and the clinical trial space. Global data, the NHS cancer statistics database, and the SEER (Surveillance, Epidemiology, and End Results) registry were searched to identify the potential patient population for a therapy.

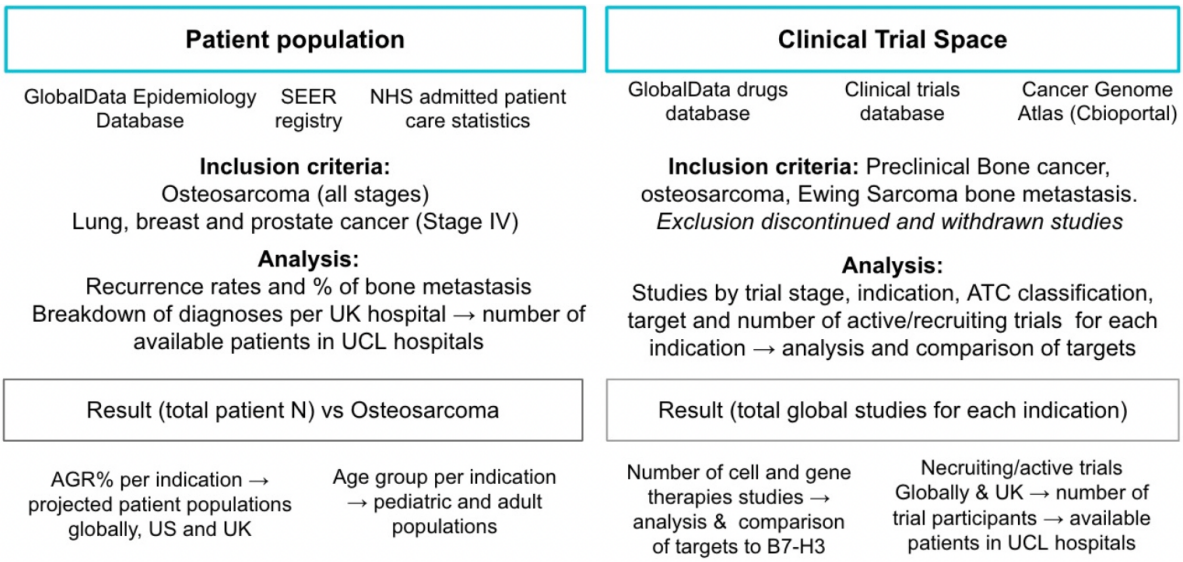


Figure 3: Search strategy and criteria

The stage IV incidence was taken for cancers with the highest incidence of bone metastasis[22] to determine the potential patient population for a therapy. Multiple sources were used to calculate the percentage of stage IV patients with bone metastasis (Figure 4).

Table 1: Search Strategy Global Data 50-63

Patient stratification & search criteria from the Global Data Epidemiology database (2022)

Indication:	Data type:	Inclusion criteria:	N (2022)	% BM	N (Stage IV BM)
Osteosarcoma	Incidence (N)	All stages	32,049	-	-
Breast	Incidence (N) by stage	Stage IV	454,915	40%	36,200
Lung	Incidence (N) by stage	Stage IV	42,667	70%	5,936
Prostate	Incidence (N) by stage	Stage IV	149,821	80%	18,869
Total N Stage IV estimated to have bone metastases (BM)			*647,403*	-	*61,005*

The bone cancer preclinical landscape was analysed by refining the drug search database in global data for pipeline Drugs (Oncology) in development, and indications for Bone Cancer, Bone Metastasis, or Osteolytic Bone Metastasis. The study's preclinical analyses of bone cancer were conducted by development stage, indication, molecule type, and Anatomical Therapeutic Chemical (ATC) classification.

In the analysis of preclinical targets in drug development, a search strategy was developed to identify relevant studies from the Medline database. The following MESH terms and keywords were used to identify studies:
The inclusion of multiple search terms and MeSH terms were used to reduce the risk of missing trials in the global data trials database and the possibility of missing relevant studies that could introduce bias.

RESULTS

Globally, over 19 million cancers are registered each year, with over 50% of cases developing metastatic disease.[47] The incidence of bone metastasis was researched to estimate secondary bone cancer incidence, the three most common cancers presenting with bone metastasis are described in figure 4. Bone is the most frequent site for metastasis for many cancers, but most often for tumours originating in the lung, breast, and prostate. In 2020, 9.9 million cancer deaths were recorded globally; cancers of the lung (2.2 million cases; 1.8 million deaths), breast cancer (2.3 million cases; 684,000 deaths) and prostate cancer (1.4 million cases; 375,000 deaths) were the most common types.[47] Given the high prevalence of breast, lung, and prostate cancers, they account for over 80% of all patients with metastatic bone disease.[48,49]

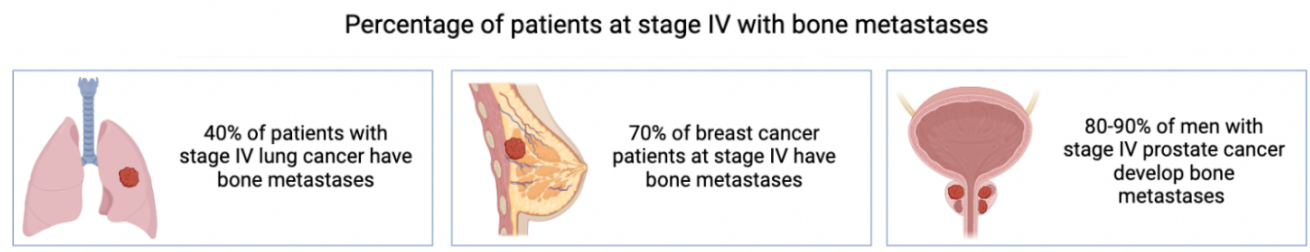


Figure 4: generated in BioRender. Metastases rate data: (22-27)

According to the Global Data analysis, it is estimated that approximately 331,690 patients with stage IV lung, breast, and prostate cancer have bone metastasis across 16MM countries. On the other hand, there is a much lower incidence of osteosarcoma, a primary bone cancer, with an estimated 32,000 cases across the same 16MM countries. In the UK, it is estimated that in 2022, there will be 52,000 patients with bone metastasis from lung, breast, and prostate cancers, while there will only be 188 cases of osteosarcoma.

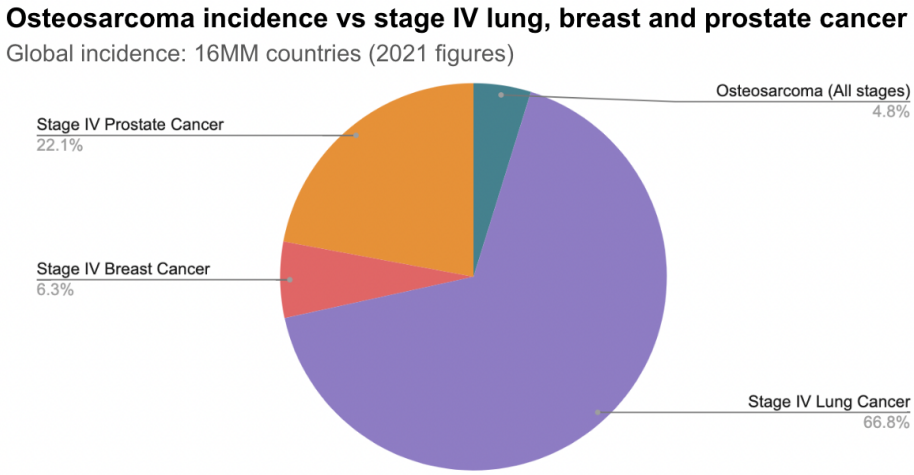


Figure 5: Data source: Global Data epidemiology database (50-63)

*16MM = the US, France, Germany, Italy, Spain, the UK, Japan, Australia, Brazil, Canada, China, India, Mexico, Russia, South Africa, and South Korea.

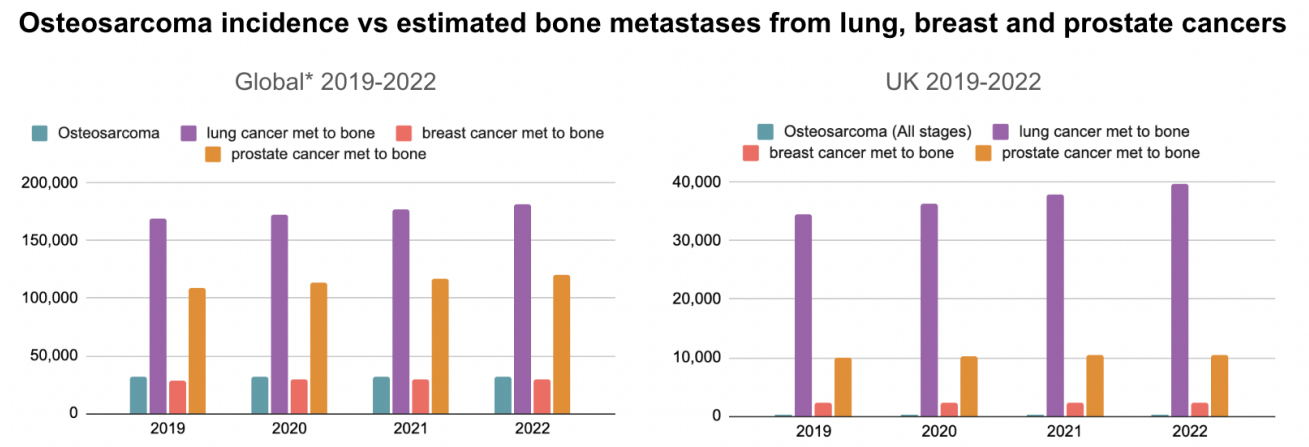


Figure 6: Data source: Global Data epidemiology database

The results confirm that the annual incidence growth for stage IV lung, breast and prostate cancers is significantly higher than that of osteosarcoma. Therefore, by taking the Annual Growth Rate (AGR) from 2019-2022, we can forecast the patient populations and apply the proportion of bone metastasis to project patient populations (Table 2). Projections should be interpreted with caution as they were limited by the availability of data. Specifically, the study relied on historical data up until 2019 and incidence rates of various cancers, including lung, breast, and prostate cancers, have been changing over time. A more extensive historical dataset would have improved the accuracy of the projections presented in this study.

Table 2: Incidence rates and forecast created in excel. Data source: Global Data epidemiology database

UK	2019	2020	2021	2022	AGR%	2023	2024	2025	2026	2027	2028	2029	
Osteosarcoma	185	186	187	188	*0.54%*	189	190	191	192	193	194	195	
		0.54%	0.54%	0.53%									
Stage IV Lung Cancer	86,397	90,763	94,833	99,191	*4.71%*	103,864	108,756	113,880	119,244	124,861	130,743	136,902	
		5.05%	4.48%	4.60%									
lung cancer met to bone	**34,559**	**36,305**	**37,933**	**39,676**		**41,545**	**43,503**	**45,552**	**47,698**	**49,945**	**52,297**	**54,761**	**40%**
Total Breast Cancer Stage IV	3,196	3,222	3,252	3,279	*0.86%*	3,307	3,336	3,364	3,393	3,422	3,452	3,481	
		0.81%	0.93%	0.83%									
breast cancer met to bone	**2,237**	**2,255**	**2,276**	**2,295**		**2,315**	**2,335**	**2,355**	**2,375**	**2,396**	**2,416**	**2,437**	**70%**
Stage IV Prostate Cancer	11,156	11,345	11,533	11,731	*1.69%*	11,929	12,131	12,336	12,544	12,756	12,971	13,191	
		1.69%	1.66%	1.72%									
prostate cancer met to bone	**10,040**	**10,211**	**10,380**	**10,558**		**10,736**	**10,918**	**11,102**	**11,290**	**11,480**	**11,674**	**11,872**	**90%**

Figure 7 presents an analysis of NHS data focusing on the UK, which shows all primary malignant neoplasms of bone and articular cartilage (C40-41) and secondary malignant neoplasms of bone and bone marrow (C79.5). The trend of greater incidence in the secondary bone cancer group is consistent with the estimated incidence from global population data.

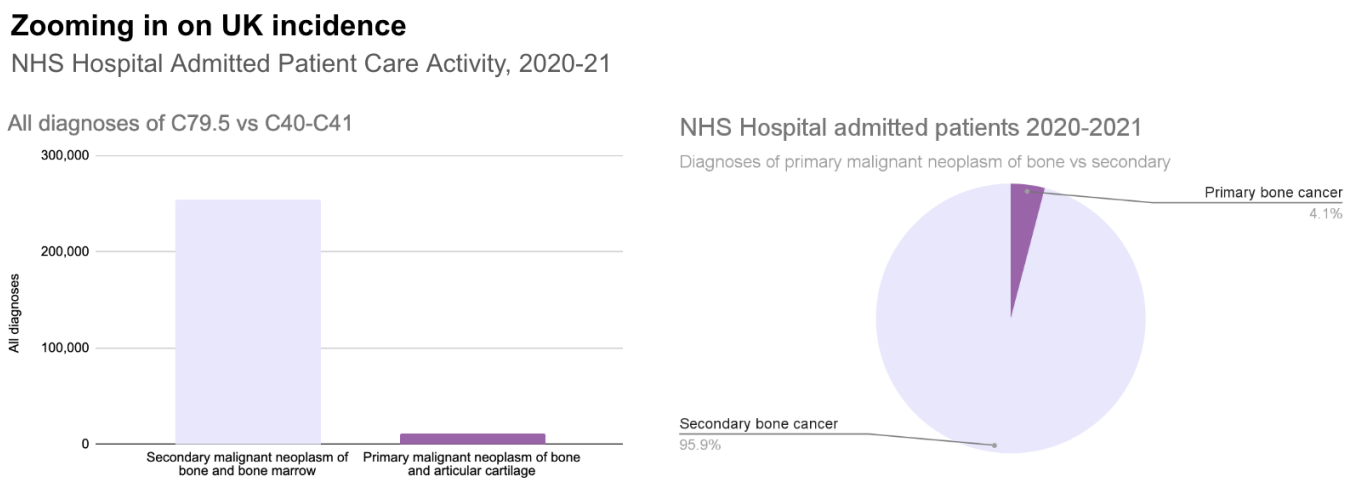


Figure 7: Data presented in google sheets, data source: NHS patient care statistics (64)

The data can be analysed by age groups, which shows that the patient groups for these two types of cancer clearly fall into paediatric and adult populations. Figure 8 provides a breakdown of the admitted patients for each cancer indication by age, indicating that primary bone cancer primarily affects the paediatric population while secondary bone cancer is more commonly observed in adults. This age-specific data can help in understanding the clinical features and management of each cancer type, as well as in informing prevention and treatment strategies tailored to different age groups.

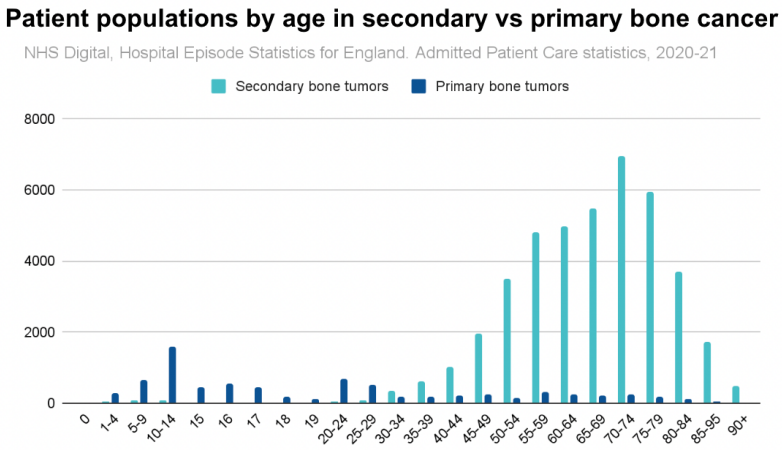


Figure 8: Data presented in google sheets, data source: NHS patient care statistics (25)

Conducting drug development for paediatric populations poses several unique challenges, including ethical considerations, and the fact that children often require different dosages and formulations of drugs compared to adults which can impact the design and execution of a clinical trial. Fewer patients also mean that there are fewer tumour samples available for study, hindering the molecular characterization of the disease and resulting in fewer clinical trials that can be conducted within a given time. This leads us to consider what the current drug development landscape looks like for both patient groups.

Based on the analysis of the drug development landscape, 37.7% of drug candidates for bone cancer are in the preclinical stage. Out of the total 285 therapies in development, 149 have indications for Osteosarcoma and/or Ewing Sarcoma, while only 35 have indications for bone metastasis.

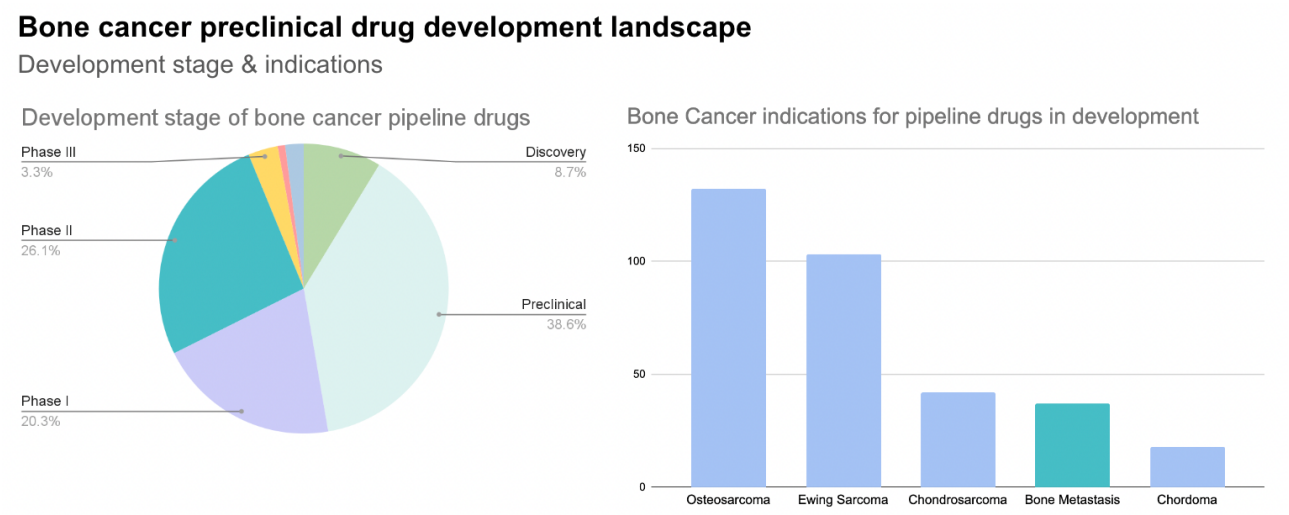


Figure 9: Data source: Global Data drug search database

Most drugs in development for bone cancer fall under the ATC classification of antineoplastic agents (chemotherapy), monoclonal antibodies, and protein kinase inhibitors. Currently, approved therapies for secondary bone cancer, include antiresorptive drugs (Bisphosphonates) such as Zoledronic acid, which are used to inhibit the activity of osteoclasts and slow down the progression of bone metastasis. And the monoclonal antibody Denosumab targeting RANKL, which is involved in the formation and activity of osteoclasts, used for the prevention of SREs.

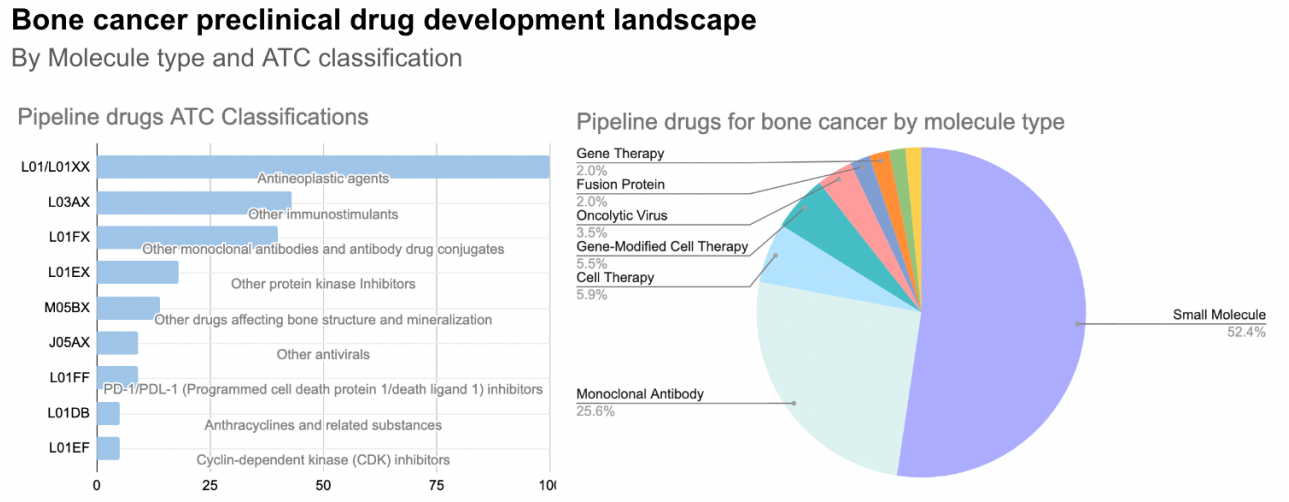


Figure 10: Data source: Global Data drug search database

Both approved drugs and drugs in clinical trials for secondary bone cancer are focused on improving the quality of life and reducing the risk of bone fractures in patients with bone metastasis. There is significant unmet need in working towards curative therapies. However, there are only 15 gene modified cell therapies contributing to 5.5% of preclinical trials in bone cancer.

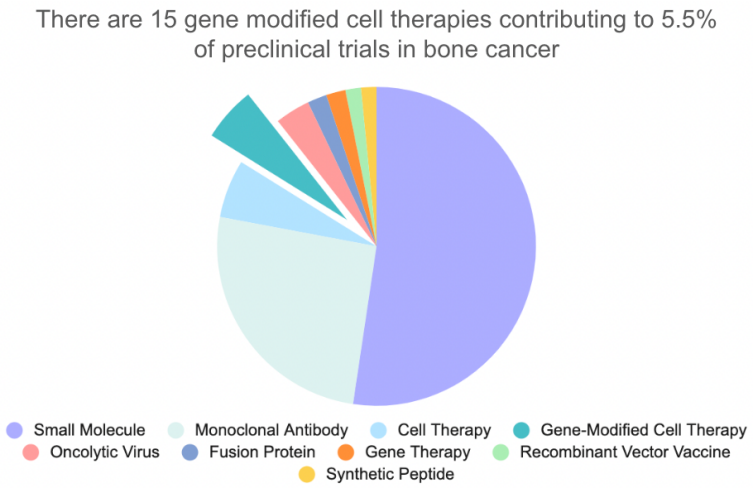


Figure 11: Data source: Global Data drug search database

DISCUSSION

Considering the opportunities and benefits observed in the patient population and clinical trial data is crucial when assessing the potential of a new therapy. By analysing the trial landscape for both primary and secondary bone cancers, we can identify areas of unmet need and determine where a new therapy could have the greatest impact.

Cell and gene therapy drugs being developed for bone cancer show promise for targeted therapy. By analysing clinical trials by stage and target, we can gain insights into the most researched targets and their potential therapeutic benefits. The cell and gene therapies in clinical trial are presented by their stage of development and their target in figure 12. AXL and HER2 are the most researched targets, each with three therapies in development.

- AXL knockdown has shown to reduce the generation and progression of bone metastases in prostate and breast cancer cells. As a result, therapeutic Axl targeting may reduce tumor spread to the bones via neoplastic and host cell signaling axes.
- HER2: The prognostic value of HER-2 in bone sarcomas has varied results and is under some debate; in many studies it was expressed in osteosarcoma but was not shown to have prognostic value. One small study with HER2-targeted CAR T cells demonstrated activity.

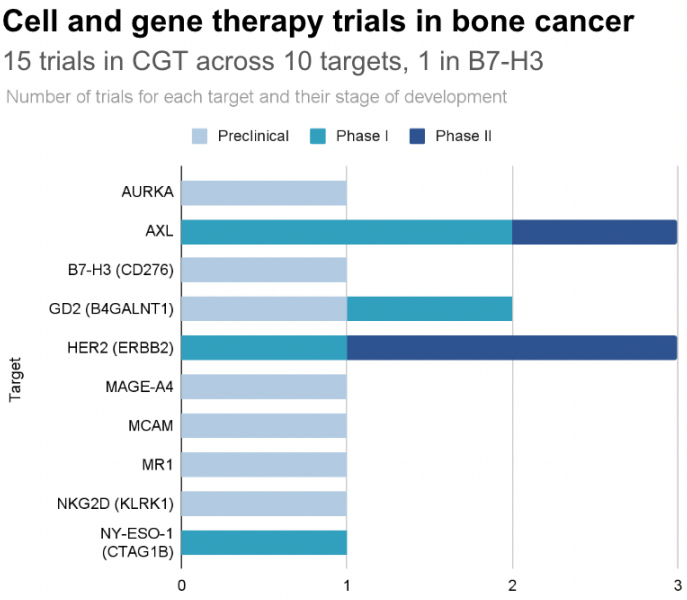


Figure 12: Data source: Global Data drug search database

B7-H3 is overexpressed in osteosarcoma, one study finding B7-H3 to be expressed in 91.8% of osteosarcoma lesions, which was found to be negatively associated to the quantity of tumour infiltrating CD8(+) T cells.[65] In comparison, HER2 expression in osteosarcoma is generally lower, with just a small percentage of patients demonstrating overexpression.[66] AXL expression is also variable, with some studies finding high levels of expression in osteosarcoma and others reporting low or missing expression.[67]

There is one cell and gene therapy trial found targeting B7-H3 (Gene Therapy to Target B7-H3 for Bone Sarcomas and Medulloblastoma - Stanford University School of Medicine) [68] in Preclinical stage. One other trial outside of cell and gene therapies found targeting B7-H3 indicated for bone cancer is the Monoclonal antibody (enoblituzumab - MacroGenics Inc) [69] in Phase I.

The B7-H3 immunological checkpoint molecule is found to have a co-inhibitory role on T-cells, contributing to tumour cell immune evasion and thus a role in modulating cancer progression.[70] Investigations of several cancers are beginning to show evidence that B7-H3 plays a tumour-promoting role, in processes such as proliferation, migration, and invasion.[71-73] It is hypothesised to increase metastasis by activating the EMT process [74] and promote cancer stemness by lowering E-cadherin expression. Studies have shown B7H3 knockdown inhibited cell migration and invasion.[75] According to data from the Cancer Genome Atlas, B7-H3 expression is higher in various types of cancer and highest in sarcomas (Figure 13).

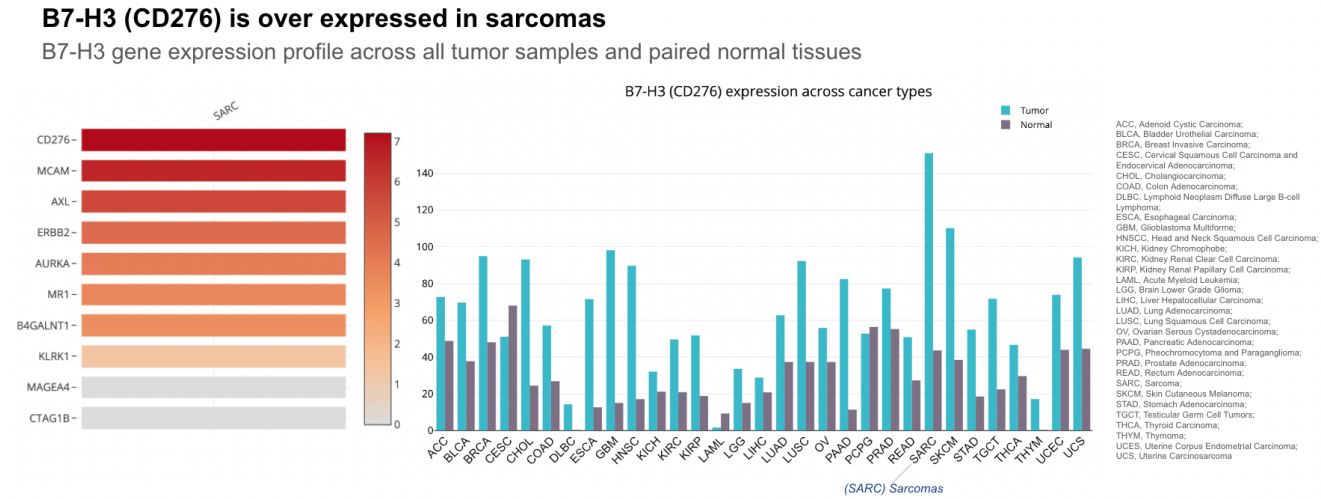


Figure 13: Figure generated in plotly chart studio in python, data source: 76

Table 3: B7-H3 Immunohistochemistry, data source: (77-79)

Expression of B7-H3 on metastatic (stage IV) tumours by immunohistochemistry	
Tumour type	**BH-73 Expression %**
Breast	80.55%
Lung	83.70%
Prostate	80%
Osteosarcoma	75.8%

Benefit of targeting B7-H3 in bone cancers and in combination with zoledronic acid may set it apart from competitors, even in the busier trial landscape for primary bone cancers

B7-H3 expression in osteosarcoma is a significant factor that impairs host T cell-mediated immunity by negatively regulating T lymphocyte infiltration. This expression is negatively associated with the intensity of infiltrating CD8+ T lymphocytes in tumour sites.[65] Studies have shown that decreased numbers of tumour-infiltrating immune cells, including T, B, and natural killer (NK) cells, correlate with decreased survival times in human osteosarcoma.[80] B7-H3 is expressed more selectively than HER2 and AXL.[81] AXL protein is expressed in normal tissues, particularly in bone marrow stroma and myeloid cells, while HER2 is expressed in various tissues including the nervous system, epithelial cells, or the mammary gland.[82,83] B7-H3 has a broader expression pattern in bone cancer, making it a more versatile target for treatment.

Patient survival and recurrence

A diagnosis of bone metastases is associated with varying median survival rates depending on the primary cancer type (Table 4). Patients with breast cancer and prostate cancer have a median survival of 3-5 years, with a 1-year survival rate of 51% and 35%, respectively. However, patients with lung cancer and bone metastases have a significantly lower median survival of approximately 1 year, with a 1-year survival rate of only 10%.

The primary indication of osteosarcoma has a significantly higher 3-year survival rate compared to secondary indications such as bone metastases. This indicates that clinical trials in the primary indication may have a higher likelihood of success as it allows for better follow-up with patients.

Table 4: Survival and recurrence, data source: (84-87)

One-year, 3-year and 5-year survival estimates with 95% CI after bone metastasis diagnosis (all) by primary cancer type				
Cancer type	1-year survival % (95% CI)	3-year survival % (95% CI)	5-year survival % (95% CI)	Recurrence rate (Stage IV)
Breast	51 (50 to 53)	25 (23 to 26)	13 (11 to 14)	20 to 30%
Lung	10 (9 to 11)	2 (1 to 2)	1 (0.5 to 1)	30% to 55%
Prostate	35 (34 to 37)	12 (11 to 13)	6 (5 to 7)	20 to 30%
Osteosarcoma	-	59 (57 to 61)	54 (52 to 56)	30 – 50%

Patient availability, a key consideration for successful development of the therapy

Another consideration is that the smaller patient numbers in osteosarcoma also mean that fewer trials can be conducted within a given time. There are currently 6 active or recruiting clinical trials in the UK for primary bone cancer shown here, exactly half of these trials include UCL hospitals as locations. All are running trials in paediatric populations.

Despite the larger patient populations, only one active but not recruiting trial was found in the UK for treating metastases to the bone, with Radium-223, a bone-targeted radioisotope for males with prostate cancer and painful bone metastases; to lower the incidence of SREs. Globally there are 202 active or recruiting trials found with the osteosarcoma indication, and 93 in bone metastases (Appendix 1).

Table 5: Active/recruiting bone cancer trials in the UK (84)

Active Primary Bone Cancer Trials UK	Status	Locations
ICONIC: Improving Outcomes Through Collaboration in OsteosarComa	Recruiting	University College Hospitals London NHS Foundation Trust & Multi-centre UK
A Study to Compare the Efficacy and Safety of Ifosfamide and Etoposide With or Without Lenvatinib in Children, Adolescents and Young Adults With Relapsed and Refractory Osteosarcoma	Active, not recruiting	UCL Cancer Institute, Multi-centre UK & global
Massive Implants the Next Generation	Recruiting	Royal National Orthopaedic Hospital NHS Trust, London
Indocyanine Green (ICG) in Paediatric Oncology MIS	Recruiting	Birmingham children's hospital, Birmingham, United Kingdom
Trial of Sunitinib and/or Nivolumab Plus Chemotherapy in Advanced Soft Tissue and Bone Sarcomas	Recruiting	University College Hospitals London NHS Foundation Trust, multi centres in Italy and Spain
Dose Escalation and Cohort Expansion Study of Niraparib and Dostarlimab in Pediatric Participants With Solid Tumors (SCOOP)	Recruiting	GSK Investigational Site, London, United Kingdom and Europe
Cancer of the Prostate When Medical or Surgical Castration Does Not Work and When the Cancer Has Spread to the Bone, Has Not Been Treated With Chemotherapy; Mild-no Symptoms		
Radium-223 Dichloride and Abiraterone Acetate Compared to Placebo and Abiraterone Acetate	Active, not recruiting	Multi-centre UK & global

What incentivises such a busy trial landscape in primary tumours considering the smaller patient population?

There is a busy trial landscape in primary tumours despite the smaller patient population. This may be driven by several factors such as regulatory incentives, including the Fast Track, Breakthrough Therapy, and Priority Review programs established by the FDA in the United States.[89] In Europe, the Priority Medicines (PRIME) scheme provides early and enhanced support for promising medicines that have the potential to address unmet medical needs.[90] The UK Medicines and Healthcare products Regulatory Agency (MHRA) offers the Early Access to Medicines Scheme (EAMS) for drugs that show early promise in treating patients with limited treatment options.[91] Orphan drug status is another important factor that encourages the development of treatments for rare diseases or conditions, providing companies with financial incentives to invest in clinical trials for treatments for rare primary tumours.

However, there are still challenges in conducting clinical trials in paediatric patients due to ethical and legal considerations, limited access to specialised care centres, and the smaller patient population. These factors may result in slower recruitment for clinical trials.[92] Although the patient population may be smaller and paediatric trials may result in higher costs, targeting a rare disease area can be balanced by various incentives. The potential for orphan drug designations in the primary bone cancer indication may present opportunities to offset higher costs with extended patent life and faster clinical trial enrolment.

In contrast, treating secondary bone cancer may offer several benefits, including a larger patient pool, which may help to increase the potential market for the therapy, and provide valuable information about the therapy's efficacy in different populations. However, this indication also presents challenges, such as a higher heterogeneity of patient populations, more diverse disease states, and potential comorbidities that could affect treatment outcomes. Metastases often involve multiple organs and tissues, which may make it difficult to deliver the targeted therapy to all affected areas. Metastatic cancer cells may harbour different molecular characteristics than the primary cancer cells contributing to treatment resistance. Acquired traits that enable colonisation of a different cancer site may mean that therapies that were effective for the primary cancer may not work as well against the metastatic cancer.[93]

Comorbidities associated with later age in adult populations might pose significant challenges in the efficacy. A compromised immune system may affect the γδ T cell recruitment of resident

immune cells to the tumour site. And the presence of comorbidities such as heart disease, diabetes, and hypertension would limit the delivery of the therapy. This may be overcome by the novel strategy proposed to enhance immune cell recruitment and function, such as the use of combination therapy with Zoledronic acid and the engineering of γδ T cells to better target the tumour microenvironment.

There is a reluctance of oncologists to replace or modify standard care, which has a proven track record of clinical efficacy. Traditional chemotherapy regimens have been used for decades and have established protocols and guidelines for dosing and administration. In contrast, a novel cell therapy is a new and complex treatment modality, requiring specialized delivery mechanisms and posing unique challenges for dosing, monitoring, and patient selection. The reluctance to adopt cell and gene therapy in oncology may be more easily overcome with a focus on the potential benefits of these therapies in paediatric patients, who are particularly vulnerable to the long-term effects of traditional chemotherapy regimens.[17-19]

While there are opportunities and challenges in both primary bone cancer and secondary bone cancer indications, regulatory incentives and orphan drug status can encourage investment in rare primary tumours, whereas the secondary bone cancer indication provides a broader market and potentially easier regulatory route.

Long-term view: the need and impact of the therapy

Bone cancer incidence has remained stable over the past few decades, but environmental exposures, genetic predisposition, and demographic changes may influence future incidence. The field of bone cancer treatment is complex and constantly evolving. Surgery and chemotherapy are likely to remain the standard of care for the foreseeable future. While immunotherapy has shown potential in treating other types of cancer, its application to bone cancer is still in early stages, as evidenced by the limited number of trials in this area. Replacing or modifying the standard of care poses challenges due to its proven clinical efficacy and ethical considerations surrounding clinical trials.

The challenge remains in determining how immunotherapy can fit in with the existing arsenal of drugs used for bone cancer. Despite its potential, immunotherapy presents legal and ethical challenges that must be carefully navigated to bring this treatment option to patients. Since cancer is such a deadly disease it is difficult to replace or modify standard care due to proven clinical efficacy. There's almost no scenario in which a doctor would not carry out surgery as first line treatment for bone cancer.[94] It is difficult to replace chemotherapy as a relapse refractory treatment. Therefore, cell and gene therapy are only used once surgery and chemotherapy have failed, to provide an alternative option for patients who have exhausted all standard treatments. The irony is that these patients are often heavily pre-treated with chemotherapy, which may make the immune system less receptive to immunotherapy.

Detecting bone cancer at earlier stages would lead to more successful treatment. Currently, primary bone cancers, such as osteosarcoma and Ewing sarcoma, often go undiagnosed for extended periods, resulting in more advanced stages of the disease at diagnosis.[95] According to data estimates from the UK, 76% of primary bone cancer cases are initially misdiagnosed.[96] Unfortunately, metastatic bone disease is often only diagnosed after a skeletal related event (SRE) has occurred, for example, a pathologic fracture, spinal cord compression, or severe bone pain requiring radiation therapy. This is because bone metastases can be hard to detect early on, and symptoms may not be noticeable until the cancer has progressed. However, not all patients with

metastatic bone disease will experience SREs or exhibit noticeable symptoms. This can limit available treatment options and result in worse outcomes for patients.[97]

Recent developments in cancer medicine have shown exciting progress through research into circulating tumour cells (CTCs) to enable liquid biopsies that can better predict tumour recurrence and shift from chemosensitivity to chemoresistance.[98] CTC analyses have also identified correlations that can predict bone metastases, such as PD-1 expression or RANKL-OPG axis alterations, and potentially B7-H3. This capacity to identify high-risk individuals may open opportunities for innovative medicines, such as γδ T-cells, to be introduced into the bone metastasis therapy paradigm. Since bone metastases originate from a broad number of primary tumour sites, any new screening and diagnostic interventions introduced for those cancers, such as liquid biopsy, is likely to increase detection of secondary bone cancer. However, for rare bone cancers like osteosarcoma, which typically occur in young people who are not routinely screened, opportunities for earlier diagnosis may be more limited.

In recent years, several expanded sequencing analyses have enabled the osteosarcoma to be clustered based on the discovery of putative driver genes. The TARGET Osteosarcoma project [99] used molecular characterisation to identify genetic alterations that cause the development and progression of high-risk or difficult-to-treat childhood malignancies. Investigating the formation of physiologically different subgroups of osteosarcoma. These initiatives speed up discovery and have the potential to propel the creation of targeted therapies.

CONCLUSION

Summary

Table 6: Summary of findings

Patient group	Patient Populations Incidence (N)		AGR (2019-22)		Projected patient population (2030)		Preclinical studies per indication	Active/recruiting trials		Median age group (NHS data)
	Global	UK	Global	UK	Global	UK		Global	UK	
Primary bone cancer (Primarily osteosarcoma)	32,049	188	1.98%	1.62%	37,499	214	132	202	6	10-14 years
Secondary bone cancer (stage IV breast, lung and prostate cancer with bone metastasis)	331,690	51,357	7.17%	7.52%	638,375	138,363	32	93	1	40 years and above

Cancer type	1-year survival % (95% CI)	3-year survival % (95% CI)	5-year survival % (95% CI)	Recurrence rate (Stage IV)	BH-73 Expression %
One-year, 3-year and 5-year survival estimates with 95% CI after bone metastasis diagnosis (all) by primary cancer type					*Expression of B7-H3 on metastatic (stage IV) tumors by immunohistochemistry*
Breast	51 (50 to 53)	25 (23 to 26)	13 (11 to 14)	20 to 30%	80.55%
Lung	10 (9 to 11)	2 (1 to 2)	1 (0.5 to 1)	30% to 55%	83.70%
Prostate	35 (34 to 37)	12 (11 to 13)	6 (5 to 7)	20 to 30%	80.00%
Osteosarcoma	-	59 (57 to 61)	54 (52 to 56)	30 - 50%	75.80%

In summary, there are more patients in the secondary bone cancer indication with an adult group rather than the paediatric group. Both cancer groups face poor prognosis with limited treatment options and significant unmet need. It is worth noting that the primary cancer indication has a busier trial space, especially in the UK, which further limits the availability of patients from an already small group. While the primary indication has a smaller patient population, there are incentives including fast-tracked regulatory processes and orphan drug status. It is likely the reason that the indication already has such a high number of drugs in active development, and therefore may face greater competitive pressures. The surprising lack of therapies in development

for the broader indication of metastatic bone cancer or secondary bone cancer clearly would provide easier patient recruitment and market size, however the more diverse disease states may be a significant challenge in the development of the therapy. Younger people generally have a more robust and active immune system compared to adults, which may allow for better responses.

The report has also given an insight into the other targets of cell and gene therapies developed for bone cancer. Targeting B7-H3 in bone cancers, in combination with zoledronic acid, may set opsonin-secreting γδ cells apart from competitors, even in the busier trial landscape for primary bone cancers. The purpose of this analysis was to provide real-world data to aid in the design of a clinical trial while acknowledging the limitations of the available data. The analysis aimed to identify challenges and opportunities for different patient groups; however, several factors were unaccounted for. For example, the lead supervisor of this project, Dr Jonathan Fisher, is in the clinic at Great Ormond Street Hospital daily, interacting with patients. This factor alone may influence the potential launch of a trial first in secondary bone cancer indications, as it would make sense to deliver a therapy to the patients with whom he is in contact for potential recruitment. The report has also given an insight into the other targets of cell and gene therapies developed for bone cancer. Targeting B7-H3 in bone cancers, in combination with zoledronic acid, may set it apart from competitors, even in the busier trial landscape for primary bone cancers.

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LIST OF ABBREVIATIONS

γδ	Gamma delta
TAA	Tumour-associated antigens
ADCC	Antibody-dependent cellular cytotoxicity
NK	Natural killer
MHC	Major histocompatibility complex
SRE	Skeletal related events
CTC	Circulating tumour cells

APPENDIX

Appendix 1

Active/Recruiting trials in the UK

Active Primary Bone Cancer Trials UK	Status	Locations	UCL hospitals?	Interventions	Indications
ICONIC: Improving Outcomes Through Collaboration in OsteosarComa	Recruiting	Multi-centure UK	University College Hospitals London NHS Foundation Trust, London, Greater London, United Kingdom	observational only, no interventions are prescribed in protocol.	Osteosarcoma
A Study to Compare the Efficacy and Safety of Ifosfamide and Etoposide With or Without Lenvatinib in Children, Adolescents and Young Adults With Relapsed and Refractory Osteosarcoma	Active, not recruiting	Multi-centre UK & global	UCL Cancer Institute, London, United Kingdom	Lenvatinib Ifosfamide Etoposide Lenvatinib	Osteosarcoma
Massive Implants the Next Generation	Recruiting	Royal National Orthopaedic Hospital NHS Trust, London, Middx, United Kingdom	No	custom made device	Bone Cancer Bone Diseases
Indo-cyanine Green (ICG) in Paediatric Oncology MIS	Recruiting	Birmingham children's hospital, Birmingham, United Kingdom	No	Indocyanine green	Pediatric Renal Tumor Metastatic Osteosarcoma Metastatic Ewing Sarcoma Pulmonary Metastasis Rhabdomyosarcoma Non-Rhabdo. Soft Tissue Sarcoma
Trial of Sunitinib and/or Nivolumab Plus Chemotherapy in Advanced Soft Tissue and Bone Sarcomas	Recruiting	University College London Hospitals NHS Foundation Trust, London, United Kingdom, Multi centres in Italy and Spain	University College London Hospitals NHS Foundation Trust, London, United Kingdom	Sunitinib 37.5 MG, Sunitinib 25 MG [Sutent] Nivolumab 100 MG/10 ML [Opdivo] Epirubicin Ifosfamide Doxorubicin Dacarbazine Cisplatin Methotrexate	Soft Tissue Sarcoma Bone Sarcoma
Dose Escalation and Cohort Expansion Study of Niraparib and Dostarlimab in Pediatric Participants With Solid Tumors (SCOOP)	Recruiting	GSK Investigational Site, London, United Kingdom and Europe	No	Niraparib Dostarlimab Niraparib	Bone Cancer

Active Secondary bone cancer trials	Status	Locations	UCL hospitals?	Interventions	Indications
Radium-223 Dichloride and Abiraterone Acetate Compared to Placebo and Abiraterone Acetate for Men With Cancer of the Prostate When Medical or Surgical Castration Does Not Work and When the Cancer Has Spread to the Bone, Has Not Been Treated With Chemotherapy and is Causing no or Only Mild Symptoms	Active, not recruiting	Multi-centre UK & global	No	Radium-223 dichloride (Xofigo, BAY88-8223) Matching placebo (normal saline) Abiraterone Prednisone/Prednisolone	Prostatic Neoplasms with bone metastases

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