# Biological sample collection to advance research and treatment: a Fight Osteosarcoma Through European Research (FOSTER) and Euro Ewing Consortium (EEC) statement.

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## STATEMENT OF TRANSLATIONAL RELEVANCE

Most patients with osteosarcoma and Ewing sarcoma have minimal sampling performed at clinical presentation, sufficient for diagnosis but not for comprehensive molecular analysis. Mechanistic understanding of tumorigenesis, metastasis and treatment resistance has progressed little. Standard management involves upfront biopsy, frequently by an interventional radiologist, followed by chemotherapy +/- definitive resection, by which time post-treatment necrotic tumour may be less informative for cellular analysis and model generation. Few patients have fresh or frozen tissue stored for patient-specific or unspecified molecular research. Treatment has changed little in decades and outcomes are poor. Here, the European osteosarcoma and Ewing sarcoma patient and professional communities set out minimum standards for tissue sampling, sufficient for histological and molecular evaluation and for all patients to have the opportunity to donate samples for research. The proposed core samples will facilitate a revolution in biologically rational treatment of paediatric-type bone sarcomas.

**ABSTRACT**

Osteosarcoma and Ewing sarcoma are bone tumours mostly diagnosed in children, adolescents and young adults. Despite multi-modal therapy, morbidity is high and survival rates remain low, especially in the metastatic disease setting. Trials investigating targeted therapies and immunotherapies have not been ground-breaking. Better understanding of biological subgroups, the role of the tumour immune microenvironment, factors that promote metastasis and clinical biomarkers of prognosis and drug response are required to make progress. A prerequisite to achieve desired success is a thorough, systematic and clinically linked biological analysis of patient samples but disease rarity and tissue processing challenges such as logistics and infrastructure have contributed to a lack of relevant samples for clinical care and research. There is a need for a Europe-wide framework to be implemented for the adequate and minimal sampling, processing, storage and analysis of patient samples. Two international panels of scientists, clinicians and patient and parent advocates have formed the Fight Osteosarcoma Through European Research (FOSTER) consortium and the Euro Ewing Consortium (EEC). The consortia shared their expertise and institutional practices to formulate new guidelines. We report new reference standards for adequate and minimally required sampling (time points, diagnostic samples, liquid biopsy tubes), handling and biobanking to enable advanced biological studies in bone sarcoma. We describe standards for analysis and annotation to drive collaboration and data harmonisation with practical, legal and ethical considerations. This position paper provides comprehensive guidelines that should become the new standards of care that will accelerate scientific progress, promote collaboration and improve outcomes.

## INTRODUCTION

Osteosarcoma and Ewing sarcoma are malignant bone tumours affecting ~1,800 people annually in Europe [1]. Despite continuous efforts and the investigation and intensification of treatment modalities, the prognosis for patients is poor when compared to other cancers [2,3]. Repeated attempts by large international cooperative groups to improve outcomes through randomised clinical trials have not led to survival improvement in osteosarcoma [4-11] and brought only modest benefits in Ewing sarcoma [12-18]. A lack of available high-quality biological samples for omics (e.g. genome-wide profiling) assessments has meant that we still have poor understanding of the molecular basis of observed heterogeneous clinical phenotypes and mechanisms of chemoresistance and metastasis. Acquisition of snap frozen and fresh tissue is recommended in international clinical guidelines [19-22], but is frequently not achieved and the absence of standardised procedures for sampling has hampered compliance.

Two international panels of scientists, clinicians and patient and parent advocates formed the Fight Osteosarcoma Through European Research (FOSTER) consortium (www.fosterconsortium.org) and the Euro Ewing Consortium (EEC) (https://www.ucl.ac.uk/cancer/research/centres-and-networks/euro-ewing-consortium/euro-ewing-consortium) to promote European collaboration and to accelerate clinical and scientific progress. The consortia have already delivered benefits by bringing together multiple – previously disparate – national clinical trial groups and scientists to develop and deliver collaborative trial protocols [14,17,23,24], share samples [25,26] and expertise [27] to perform collaborative research. A major goal of both consortia is the refinement and intensification of translational research. Systematic acquisition of high-quality biological samples from children and adults across multiple sites with associated clinical metadata should enable the identification and characterisation of disease subgroups and tumour and germline genetic, biological, immunological and cellular environmental factors that can be used for the stratification of disease subgroup-specific therapies.

This position paper complements international clinical guidelines and provides comprehensive procedures for the adequate minimal sampling, handling and storage of bone sarcoma samples that

should be adopted across European centres. Although this statement has been drafted by the osteosarcoma and Ewing sarcoma communities, the principles discussed apply equally to other bone sarcoma histotypes and perhaps other cancers where a lack of samples hinders translational research and clinical progress.

## UNMET CHALLENGES IN OSTEOSARCOMA AND EWING SARCOMA BIOLOGY

Key features of osteosarcoma biology include *in utero* loss-of-imprinting at chr.14q32 [28,29], postnatal *TP53* loss-of-function [30] (or possibly mutant gain-of-function [31]) and complex genome rearrangements via chromoplexy and chromothripsis ([32,33] and Valle-Inclan JE, Noon SD, Trevers K, Elrick H, Tanguy M, Butters T, et al: Mechanisms underpinning osteosarcoma genome complexity and evolution. bioRxiv:2023.12.29.573403, 2023). Specific molecular alterations in some cases include *MYC* amplification [34], *RB1* deletion and mutation and a '*BRCA*ness' phenotype [35]. Ewing sarcoma cells are characterised by gain-of-function gene rearrangements between FET (FUS, EWSR1, TAF15) RNA binding proteins and ETS (FLI1, ERG, FEV) transcription factors, most commonly *EWSR1::FLI1* [36]. The *FET::ETS* fusions encode oncogenic chimeric transcription factors with neomorphic features that reprogramme the transcriptome [37], binding to GGAA microsatellites that become neoenhancers [38,39], which leads to ectopic gene expression and tumour development. Additional *STAG2* and *TP53* cooperative mutations are associated with poorer survival [40-43].

Although the key driver mutations and recurrent alterations present in a subset of cases have been identified in both tumours, fragmented data from multiple small series and a lack of sufficient and appropriate solid and liquid tissue biopsies have hindered the development of molecular classifications and risk stratifications. Current and recent European clinical trials in Ewing sarcoma (ISRCTN92192408, ISRCTN36453794, NCT00987636) have collected prospective liquid biopsies and accessed clinical diagnostic tissue samples to validate previously reported prognostic biomarkers, but none include specific molecular analysis of pre- and post-treatment tumour samples and clinical trials are not representative of all patient groups. For osteosarcoma, there have been no large

prospective clinical trials since the closure of the EURAMOS-1 study and clinical trial samples do not inform individual patient treatment decisions. A culture of more universal prospective tissue collection is needed.

## 'REPRESENTATIVENESS' OF CURRENT RESEARCH MODELS

Preclinical models are a central component of translational research. Model systems such as patient-derived cell lines, ex vivo engineered models [44-46]and spheroids / tumoroids [47,48], in addition to in vivo rodent (e.g. mice, rats), non-rodent (e.g. canine, zebrafish, xenopus) [49] and chicken chorioallantoic membrane [50,51] models, allow researchers to mimic bone sarcoma including its genetics and molecular biology, local microenvironment, systemic dissemination and drug response. Most bone sarcoma deaths occur because of the emergence of drug-resistant lung, bone and/or bone marrow metastases. Orthotopic and patient-derived xenograft [52] and engineered mouse models [53] recapitulating disseminated disease are essential. Sampling paired treatment-naive and relapsed material is critical for the development of relevant models to avoid unfavourable scenarios where preclinical drug efficacy data generated using less relevant models appear promising [54-56] but the subsequent clinical trials show no patient benefit [23,57,58].

Historical cell lines, recent patient-derived cells and orthotopic xenograft mouse models have been developed for osteosarcoma [52,53,59-63] and Ewing sarcoma [53,56,64,65], but they typically over-represent the higher-risk end of the disease spectrum. The Innovative Therapies for Children with Cancer (ITCC) consortium has generated patient-derived xenografts for *in vivo* compound testing from children with relapsed disease and includes some bone sarcoma models [66-68], but more representative and accessible patient-derived cell lines, xenograft and genetically engineered autograft models that allow simultaneous examination of the tumour, immune, extracellular and structural microenvironment are needed [69-72].

## ACCESS TO NOVEL THERAPIES

There is limited commercial incentive for the development of novel therapies for bone sarcoma. The European Medicines Agency (EMA) implemented the European Union (EU) Paediatric Regulation in 2006, requiring the investigation of new therapies in children before marketing authorisation was granted. A waiver system in the initial legislation was modified in 2015 [73], strengthening the legal requirement to investigate all therapies with a relevant mechanism of action for childhood cancer. United States (US) Congress approval of the Research to Accelerate Cures and Equity for Children Act ('RACE Act') enacted in 2020 gave the Food and Drug Administration (FDA) powers to mandate paediatric clinical trials for new oncology drugs with a molecular target relevant to childhood cancers. There is considerable alignment between the EMA and FDA and this concerted regulatory approach has and will lead to greater opportunities for access to novel targeted therapies in children.

Although peaking in incidence in the 2nd and 3rd decades and occurring in older adults as well as children, osteosarcoma and Ewing sarcoma are frequently considered 'paediatric' cancers and are represented in early phase paediatric drug trials. The regulatory coordination between the EMA and FDA presents an opportunity to utilise the inclusion of patients with osteosarcoma and Ewing sarcoma in early phase trials to study drug response and to develop predictive biomarkers. However, the number of patients with bone sarcomas recruited to each early phase trial is typically small [27,74], sampling is not standardised, correlative biomarker studies are typically published long after conclusion of the trial, if at all, and together these factors have led to an extreme paucity of high-quality predictive biomarker evidence relevant to bone sarcoma. IGF1R inhibitors in Ewing sarcoma are an example of a failed opportunity to identify why only some patients responded to treatment. Across multiple early phase trials, multiple agents and over 400 patients, IGFR1 inhibitors resulted in response rates of 5-15%, including some sustained responses [75-80], but no predictive biomarkers were identified. As a result, no patient enrichment was possible in the Children's Oncology Group AEWS1221 study comparing standard interval compressed VDC/IE with or without ganitumab. There was no significant difference in survival between the arms.

## THE IMPORTANCE OF OPTIMISING SAMPLE COLLECTION

Several factors have converged to limit translational progress in osteosarcoma and Ewing sarcoma including recurrent molecular alterations not being validated, a consensus on molecular classification being made, a burgeoning of preclinical models but with an over-emphasis on high-risk disease, a relative paucity of models for some disease settings, limited access to samples, almost non-existent validated information about predictive biomarkers of response to cytotoxic chemotherapy and molecularly directed treatment plus poor recruitment to early phase trials. In particular, while the key molecular drivers of osteosarcoma and Ewing sarcoma are relatively well understood, there is a fundamental lack of understanding of how genetic and epigenetic modifiers and tumour-host interactions affect disease progression and treatment response. This lack of understanding is largely driven by the absence of comprehensive, serial, annotated tumour tissue, normal tissue stroma and liquid biopsy. At the level of clinical trials and collaborative large-scale research, there is a need for more, high-quality, tumour and normal tissue (solid and liquid) biopsies, ideally, serial biopsies to facilitate research into the molecular drivers and inhibitors of treatment response. At the level of individual patients, tissue acquisition needs to meet the needs of modern, multi-omic analysis to monitor disease response and facilitate options for molecularly targeted, personalised medicine and critically for osteosarcoma, to identify patients with underlying cancer predisposition syndromes. Taking tyrosine kinase inhibitors (TKIs) as an example, several TKIs have shown promise as single agents in osteosarcoma and Ewing sarcoma [81-86] but despite responses in up to 40% of patients, there are as yet no validated predicted biomarkers and the TKI mechanism of action remains obscure. Ongoing trials are evaluating combinations of TKIs with chemotherapy in front-line and relapse settings (e.g. the INTER-EWING-1 and rEECur trials developed by the EEC and NCT05691478 in the USA) and the FOSTER consortium was recently awarded ATTRACT funding to investigate the TKI cabozantinib as 12-month maintenance therapy following first-line standard therapy in osteosarcoma. All include sampling timepoints designed to investigate biomarkers predictive of TKI response.

A decades-long limitation to resolving some of the challenges discussed above is that there are no consistent or systematic Europe-wide practices for sample collection. Standard operating procedures

(SOPs) for biopsies and other sample types, storage and sharing are either absent or only developed at local or national level. Exacerbating the problem is that there is little infrastructure and few dedicated staff to obtain bone sarcoma biopsies for both clinical care and translational research, although recent initiatives are working towards changing this landscape.

Across Europe, the stakeholders engaged in obtaining biopsy material have different practices. The amount, quality and availability of viable tumour material is variable and frequenetly inadequate for molecular analyses. Because of the lack of a framework for sampling, much tumour tissue research is performed on postoperative, necrotic material obtained after induction therapy meaning there is 'tainted' data and knowledge on tumorigenesis, clonal evolution, metastasis and experimental drug response. There is evidence that the chromoplexy attribute of osteosarcoma results in dramatically different genetic alterations in different regions of the same tumour [87], making a strategic approach to tissue biopsy critical to understanding patient-specific tumour biology and target actionability (Table 1).

We present consensus guidelines on the appropriate type and timing of tissue and liquid samples to facilitate research for future patients and to inform the treatment and future surveillance of current patients. Where this dedicated approach has taken place in other cancers, for example melanoma, the 10-year survival rate has improved from ~10% [88] to ~56% [89] because high-quality samples are made available for routine testing of the *BRAF* gene, which dictates first-line immunotherapy decision. Cytotoxic chemotherapy is now disregarded as first-line therapy in melanoma.

## GUIDELINES AND RECOMMENDATIONS

### Introduction

Cooperative effort from all involved disciplines is required. Routinely obtained written informed consent, collection and storage of patient material for advanced biological studies is recommended in international clinical guidelines [19,20,22] but non-compliance exists because of a lack of standard procedures for biological sampling. Our position, complementing the clinical guidelines, is that all

patients with bone sarcoma should have snap frozen and fresh tissue samples (in addition to the conventional diagnostic samples) taken at diagnosis, surgery and relapse regardless of their inclusion in research initiatives or clinical trials.

Biopsies should be performed at specialist bone sarcoma units [90,91]. Within research groups, clear definitions of the sample types and relevant SOPs should be used. Solutions for ethical, legal and practical issues should be widely shared. To maximise the advantages of sample collection, to obtain a comprehensive biological understanding of bone sarcoma and host-related factors, different sample types at sequential stages of the clinical pathway should be collected (Figure 1; Table 2). To enhance fundamental understanding of bone sarcoma clonal evolution and chemoresistance, tumour tissue collection at relapse and autopsy (e.g. PEACE study, NCT03004755) is essential. Metastases often comprise different genetics to the original primary tumour so sampling metastatic lesions is recommended to ensure that the maximal amount of biological information is collected.

**Diagnostic biopsy**

Treatment-naive core or open biopsies should be obtained from suspected bone sarcoma cases at sarcoma specialist centres with the infrastructure to take, process and store (or send to a centralised national centre) snap frozen and fresh tissue in addition to the biological material placed in formalin. Fine needle aspiration is not adequate. Biopsies and their position should be determined at a multidisciplinary team meeting with discussion on what the suspected lesion is expected to be, which tumour zones the biopsies should be taken from and by which approach to avoid unnecessary contamination. The procedure should be performed by a musculoskeletal or interventional radiologist experienced in the diagnosis of bone tumours or by a specialist surgeon and reported in line with the International Collaboration on Cancer Reporting [20] (https://www.iccr-cancer.org/). The biopsy tract should be considered contaminated and resected en bloc during local therapy or be included in the radiotherapy field to minimise the risk of local recurrence [19,21,92-94]. The surgeon who will perform the tumour resection should be involved in defining the optimal approach for the biopsy. The biopsy tract is preferably marked and described according to compartmental anatomy [92,95,96]. In many cases,

image-guided percutaneous biopsy using 8-, 11- or 14-gauge needles represents a well-established alternative to open biopsy in terms of safety and diagnostic results [21,92-94]. Advantages and (contra)indications have been described for both procedures [92,97-100].

Sampling focused purely on histological diagnosis, usually from decalcified FFPE tissue, does not consider the developing prognostic technologies that require snap frozen and/or fresh tissue that are becoming standards of care, for example, the macrophage expression phenotype in osteosarcoma [101]. The equivalent of three 11- or 14-gauge needle biopsy samples have typically been sufficient to provide diagnostic yield [102] when paired with conventional histology. Our position is that where a core biopsy is performed, five samples should be collected where possible, of which at least one must be snap frozen (Table 2). The 4$^{th}$ and 5$^{th}$ sample should be designated for research but can be used for diagnostic purposes where a diagnosis could not be made using the FFPE samples. In many cases, the 4$^{th}$ and 5$^{th}$ sample will also be snap frozen and stored but depending on active research studies, one or both could be formalin-fixed for use in spatial transcriptomics or used fresh for the isolation of live tumour cells for cell line generation, organoid development and/or engraftment into immunocompromised animals (Table 2). For open biopsy, a minimum of 1 cm$^3$ of tissue cut into multiple 0.2 cm$^3$ sections is recommended. Where there are detectable oligometastases at presentation, consideration should be given to obtaining metastatic tissue at the time of the biopsy. For reference, recent Children's Oncology Group guidance advocates up to 20 core biopsies for bone sarcomas with a soft tissue component (or up to 7 core biopsies where there is no soft tissue) plus up to 3 cores of underlying osteoid [103].

**Primary tumour resection and metastasectomy**

There are three surgical specimens where resection serves as both performing standard of care and obtaining research samples: (i) primary tumour, (ii) matched adjacent normal tissue and (iii) metastatic lesions. Samples should be prioritised by the pathologist to collect, depending on the availability of biobanking and specific research initiatives: (1) FFPE as the standard of care and

neoadjuvant chemotherapy assessment, (2) snap frozen and stored, (3) fresh and placed into an RNA-preserving medium, (4) fresh and placed into a culture-compatible medium (Table 2).

**Relapsed disease**

Samples from relapsed disease are particularly valuable if they can be paired with tissue from the primary diagnosis. As most bone sarcoma recurrences develop early and there is usually little doubt about the diagnosis, pre-treatment biopsy material is scarcer than at initial diagnosis. Given the poor outcomes of relapsed disease and the limited treatment options, consideration should be given to obtaining snap frozen and fresh and/or fixed tumour tissue at recurrence. These samples should be appropriately processed for omics assessment, other research or biobanking. Irrespective of whether there are currently recruiting and/or routinely commissioned omics initiatives available at the time of recurrence, relapsed tissue is highly valuable if stored for future assessment.

**Blood samples**

Blood samples should be obtained at (i) diagnosis, (ii) before and after surgery and (iii) at follow-up. Blood can be used as a liquid biopsy for the identification of circulating tumour DNA (ctDNA) and RNA (ctRNA), circulating cell-free DNA (cfDNA) and circulating tumour cells (CTCs). For specific diagnostic, monitoring and biomarker studies, urine and other body tissues (e.g. tears, hair) may be collected. Blood samples should be processed according to the relevant study, for example, CTC studies to be collected in cell-free Streck, PAXgene or EDTA blood collection tubes (BCTs) and processed immediately. Streck and PAXgene both have BCTs specifically designed for ctDNA and ctRNA capture. EDTA tubes can be used for either analytes, proteins or live cells. There are pros and cons to each BCT related to the need for immediate versus delayed processing, plasma volume yield and transport and storage costs. There is no consensus between European centres on which, if any, is best overall. We recommend that EDTA is used as a minimum for storage as these BCTs enable most analyses. But other more specific BCTs can be used according to research studies taking place at the time of collection. Blood samples may be key to detect micrometastases as well as allowing for the analysis of metastatic tumour-derived DNA, RNA (including microRNA) or proteins in circulation.

## Technical considerations

Technical aspects of collection and storage need to be considered to obtain minimum amounts of high-quality samples (Table 3), which may require a fundamental change in clinical practice in individual centres. Radiologists, surgeons and pathologists have critical roles in the collection of adequate samples for histological and molecular diagnostics and for translational research. The biopsy, operative and histology procedures need to allow sufficient time to be devoted to sample collection and processing. These procedures should be appropriately funded. If diagnostic centres are unable to adequately process and store relevant material, consideration should be given by national bodies to restrict diagnostic biopsies to centres with adequate infrastructure or establish regulated delivery channels to central repositories.

## Standard operating procedures (SOPs)

SOPs for tissue processing should be implemented by designated staff other than the radiologist or surgeon because the tissue needs to be processed at the same time as the procedure being performed, which requires the full attention of the radiologist or surgeon. After collection, material allocated by the pathologist for diagnostic procedures will be processed as standard. Samples to be frozen should be transferred to sterile vials and immediately snap frozen and stored in -80 °C freezers or in liquid nitrogen. Fresh samples for cell and organoid cultures or animal engraftment need to be placed under sterile conditions into appropriate vials with a culture-compatible medium. The logistics and reagents may require pre-planning with the research group for material transfer to the laboratory within 24 h.

## Infrastructure and personnel

Sampling requires a team effort. Some centres will require changes to current care pathways, for example, automatic reminders to collect samples and duplicating processes so the biobank sample pathway is parallel with the pathology sample pathway. The radiologist's and surgeon's focus will be on the clinical procedure so it is important to establish a tissue processing pipeline as an interdisciplinary effort and adapt it to local conditions, which may include oncology, pathology,

biobanking and theatre staff. For SOPs to work, theatre staff must be well informed, prepared and adequately resourced to undertake the extra work. All personnel involved should recognise that tissue processing for research is pertinent to future patients being cured. Understanding the importance of their new role in tissue sampling could increase personnel efficiency and reliability.

## PATIENT AND PUBLIC SUPPORT

Patients and their families overwhelmingly support research sample donation surplus to diagnostic requirement. FOSTER together with the Sarcoma Patient Advocacy Global Network (SPAGN) have undertaken an international survey. The survey includes questions on diagnosis, treatment and survivorship experiences, plus assessment of patient and family priorities for future research. Four questions are specific to sample donation. As of 2 February 2024, there were 372 combined osteosarcoma and Ewing sarcoma respondents (n=234 osteosarcoma, median age 16 y; n=138 Ewing sarcoma, median age 14 y). Just over half of respondents with Ewing sarcoma (52.2%) and less than half with osteosarcoma (46.6%) were asked to donate research samples (Table 4). Of those asked, 97% consented to donate (Table 4). For the half of respondents who were not asked, almost two-thirds reported that they would like to have been asked (Table 4).

## ETHICAL, LEGAL, PRIVACY AND PRACTICAL CONSIDERATIONS

Responding to patient-led direction involves important ethical, legal, privacy and practical consideration (Table 5). Patients or their families must provide written informed consent for the collection, storage and use of research samples. Lawful protocols should be in place to ensure that patient confidentiality and personally identifiable data are protected. Consent and protocols need to navigate the range of legal frameworks of different European nations. Age-appropriate information sheets for patients and their guardians must explain the purpose of the planned tissue storage and/or research, the recipients of the material (either now or in future) and the use of pseudonymised clinical data prior to providing forms for informed consent. Pairing sample data with pseudonymised clinical data including treatment and imaging findings and where the law allows, explicit linkage to regional

and national cancer registries, should be possible. Ethical approval from international, national or local authorities to study samples previously collected should be obtained.

Advantages of centralised versus decentralised (virtual) tumour banking and procedures to check for appropriate tissue representation for interpretable biological results should be considered. Whether sample availability should be defined as a mandatory inclusion criterion for patients going into clinical trials should be evaluated by regulatory bodies and ethics panels. For clinical trials, responsibilities of trial coordinators and local centres should be defined and adapted to applicable laws and regulations. Adequate coverage of the local costs and shipment of samples by research grants or national initiatives can help to facilitate the compliance of local institutions, particularly where there are financial challenges faced by sample collection units. However, in some cases, financial constraints will prevent the collection of samples for unspecified research. Reusable tumour box devices can facilitate the shipment of frozen and unfrozen material. Practical aspects of exchange (including transborder) and the use of material should be defined by material transfer agreements (MTAs) between research centres.

## BIOBANKING

Biological material can be stored centrally by an academic tissue bank with software systems allowing for maximal up-to-date information about the stored materials. The materials can also be stored in local tumour banking facilities and later shipped in batches, as required, for use in further analyses. Both centralised and decentralised material storage allow for their use in big data analyses with bioinformatics support. Regardless of storage location (e.g. accredited laboratories with alarm monitoring versus research lab freezers), proper evaluation by experienced bone sarcoma pathologists should ensure appropriate tissue representation before being used in specific projects. Biological material storage in aliquots allows for the tissue to be used for multiple research projects. Within existing legal frameworks of some European countries it has been possible in some clinical units to store fresh and snap frozen material from the biopsy before a diagnosis is obtained for a limited time prior to explicit patient consent for biobanking [104]. This practice requires the appropriate infrastructure

to be in place at the time of the biopsy and some bureaucracy to ensure adequate record keeping. Across most European centres, it is the tissue bank where the samples were collected that owns the biological material. Tissue banks are typically non-specific repositories for all patient materials collected at a local institution or within a region, or sometimes can be a study-specific biobank.

Transparent criteria for the regulation of access to larger material series by researchers from local contributing institutions could positively influence the cooperation of local centres. MTAs and SOPs for material shipment and adequate cost coverage (e.g. research grants) could further facilitate cooperative tumour banking. It is also important to establish procedures for the coupling of tumour material data to patient data. Genomic, transcriptomic, methylomic and metabolomic data from tumour biopsies plus data from experiments on patient-derived cell cultures and xenografts should ideally be stored in an international bone sarcoma registry together with comprehensive anonymous clinical, radiological and pathological data. It is worth investing in the collection of large amounts of retrospective clinical data regarding baseline characteristics, treatment and survival from multiple international groups and to correlate these data with the analysis of genomic and epigenomic data from corresponding banked tumour samples. FOSTER, the EEC and clinical trial groups should consider aspects of data collection and sample storage and discuss early in the planning phase of collaborative projects so that specific national requirements and future projects linking datasets can be implemented in a timely manner. Data sustainability beyond individual projects and connection of data at overarching levels should be considered.

## CONCLUSION

Tangible progress in bone sarcoma has been bottlenecked by insufficient biological assessment and investigation, which in significant part has been caused by limitations in sample collection. Routine collection of decalcified and formalin-fixed tissue for histological examination will not support diagnostic and prognostic technologies that evolve from translational research, for example NGS, in large part because fresh and snap frozen tissue is not routinely stored. The benefits of obtaining fresh and snap frozen samples at biopsy exceed the risks of complications of taking more tissue. Changing

the process in which we collect biological samples and link patient data will lead to new molecular-based standards of care as well as new targeted therapies with fewer side effects.

Metastatic sites are not routinely sampled. Liquid biopsies are not routine. Screening for germline predisposition syndromes is not routine. The availability of properly sampled and stored biological materials will confer multiple scientific and clinical advantages including allowing identification and validation of new and reported prognostic factors and druggable targets. We need to ensure that children, teenagers and young adults with bone sarcoma are not left behind while precision oncology offers new treatment solutions for more common, typically older adult, cancers. Because paediatric sarcomas are clinically and biologically highly distinct from adult cancers, precision medicine approaches should be adapted to make the best use of samples that are as informative as possible. Appropriate sample collection, storage and sharing can only be achieved successfully if all the relevant steps are optimised at each local centre. Collection and storage procedures could be adapted by local institutions to suit their individual structures, defined and assigned to dedicated individuals who are specifically educated and trained. FOSTER, EEC and institutional researchers should actively collaborate, share data, methods and samples and disseminate good practice. These approaches will advance progress in bone sarcoma.

## AUTHORS' CONTRIBUTIONS

This paper was mainly written by DG, RVE, ET, QCH, MGM and MN. Data from the sarcoma experience survey were generated by PP. Authors are Young Investigators, Work Package and Nation Leads who all contributed to the content of the paper from the perspective of the group that they represent as well as commenting on general aspects of this Policy Review.

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## REFERENCES

1. Stiller CA, Trama A, Serraino D, Rossi S, Navarro C, Chirlaque MD, et al: Descriptive epidemiology of sarcomas in Europe: report from the RARECARE project. Eur J Cancer 49:684-95, 2013
2. Gill J, Gorlick R: Advancing therapy for osteosarcoma. Nat Rev Clin Oncol 18:609-624, 2021
3. Gaspar N, Hawkins DS, Dirksen U, Lewis IJ, Ferrari S, Le Deley MC, et al: Ewing Sarcoma: Current Management and Future Approaches Through Collaboration. J Clin Oncol 33:3036-46, 2015
4. Marina NM, Smeland S, Bielack SS, Bernstein M, Jovic G, Krailo MD, et al: Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial. Lancet Oncol 17:1396-1408, 2016
5. Bielack SS, Smeland S, Whelan JS, Marina N, Jovic G, Hook JM, et al: Methotrexate, Doxorubicin, and Cisplatin (MAP) Plus Maintenance Pegylated Interferon Alfa-2b Versus MAP Alone in Patients With Resectable High-Grade Osteosarcoma and Good Histologic Response to Preoperative MAP: First Results of the EURAMOS-1 Good Response Randomized Controlled Trial. J Clin Oncol 33:2279-87, 2015
6. Lagmay JP, Krailo MD, Dang H, Kim A, Hawkins DS, Beaty O, 3rd, et al: Outcome of Patients With Recurrent Osteosarcoma Enrolled in Seven Phase II Trials Through Children's Cancer Group, Pediatric Oncology Group, and Children's Oncology Group: Learning From the Past to Move Forward. J Clin Oncol 34:3031-8, 2016
7. Bielack SS, Wulff B, Delling G, Göbel U, Kotz R, Ritter J, et al: Osteosarcoma of the trunk treated by multimodal therapy: experience of the Cooperative Osteosarcoma study group (COSS). Med Pediatr Oncol 24:6-12, 1995
8. Souhami RL, Craft AW, Van der Eijken JW, Nooij M, Spooner D, Bramwell VH, et al: Randomised trial of two regimens of chemotherapy in operable osteosarcoma: a study of the European Osteosarcoma Intergroup. Lancet 350:911-7, 1997
9. Bramwell VH, Burgers M, Sneath R, Souhami R, van Oosterom AT, Voûte PA, et al: A comparison of two short intensive adjuvant chemotherapy regimens in operable osteosarcoma of limbs in children and young adults: the first study of the European Osteosarcoma Intergroup. J Clin Oncol 10:1579-91, 1992
10. Gaspar N, Occean BV, Pacquement H, Bompas E, Bouvier C, Brisse HJ, et al: Results of methotrexate-etoposide-ifosfamide based regimen (M-EI) in osteosarcoma patients included in the French OS2006/sarcome-09 study. Eur J Cancer 88:57-66, 2018

11. Palmerini E, Meazza C, Tamburini A, Bisogno G, Ferraresi V, Asaftei SD, et al: Phase 2 study for nonmetastatic extremity high-grade osteosarcoma in pediatric and adolescent and young adult patients with a risk-adapted strategy based on ABCB1/P-glycoprotein expression: An Italian Sarcoma Group trial (ISG/OS-2). Cancer 128:1958-1966, 2022
12. Ladenstein R, Potschger U, Le Deley MC, Whelan J, Paulussen M, Oberlin O, et al: Primary disseminated multifocal Ewing sarcoma: results of the Euro-EWING 99 trial. J Clin Oncol 28:3284-91, 2010
13. Leavey PJ, Laack NN, Krailo MD, Buxton A, Randall RL, DuBois SG, et al: Phase III Trial Adding Vincristine-Topotecan-Cyclophosphamide to the Initial Treatment of Patients With Nonmetastatic Ewing Sarcoma: A Children's Oncology Group Report. J Clin Oncol 39:4029-4038, 2021
14. Brennan B, Kirton L, Marec-Bérard P, Gaspar N, Laurence V, Martín-Broto J, et al: Comparison of two chemotherapy regimens in patients with newly diagnosed Ewing sarcoma (EE2012): an open-label, randomised, phase 3 trial. Lancet 400:1513-1521, 2022
15. DuBois SG, Krailo MD, Glade-Bender J, Buxton A, Laack N, Randall RL, et al: Randomized Phase III Trial of Ganitumab With Interval-Compressed Chemotherapy for Patients With Newly Diagnosed Metastatic Ewing Sarcoma: A Report From the Children's Oncology Group. J Clin Oncol 41:2098-2107, 2023
16. Koch R, Gelderblom H, Haveman L, Brichard B, Jürgens H, Cyprova S, et al: High-Dose Treosulfan and Melphalan as Consolidation Therapy Versus Standard Therapy for High-Risk (Metastatic) Ewing Sarcoma. J Clin Oncol 40:2307-2320, 2022
17. Dirksen U, Brennan B, Le Deley MC, Cozic N, van den Berg H, Bhadri V, et al: High-Dose Chemotherapy Compared With Standard Chemotherapy and Lung Radiation in Ewing Sarcoma With Pulmonary Metastases: Results of the European Ewing Tumour Working Initiative of National Groups, 99 Trial and EWING 2008. J Clin Oncol 37:3192-3202, 2019
18. Womer RB, West DC, Krailo MD, Dickman PS, Pawel BR, Grier HE, et al: Randomized controlled trial of interval-compressed chemotherapy for the treatment of localized Ewing sarcoma: a report from the Children's Oncology Group. J Clin Oncol 30:4148-54, 2012
19. Strauss SJ, Frezza AM, Abecassis N, Bajpai J, Bauer S, Biagini R, et al: Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN PaedCan Clinical Practice Guideline for diagnosis, treatment and follow-up. Ann Oncol 32:1520-1536, 2021
20. Bovée J, Webster F, Amary F, Baumhoer D, Bloem JLH, Bridge JA, et al: Datasets for the reporting of primary tumour in bone: recommendations from the International Collaboration on Cancer Reporting (ICCR). Histopathology 82:531-540, 2023
21. Bovee JF, A. Nielsen, G. Akihiko Y. Bloem, J. The WHO Classification of Tumours Editorial Board: WHO Classification of Tumours: Soft Tissue and Bone Tumours. IARC Press, 2020
22. van Ewijk R, Herold N, Baecklund F, Baumhoer D, Boye K, Gaspar N, et al: European standard clinical practice recommendations for children and adolescents with primary and recurrent osteosarcoma. EJC Paediatric Oncology 2:100029, 2023
23. Dirksen U, Koch R, Bhadri V, Brichard B, Butterfass-Bahloul T, Cyprova S, et al: Efficacy of maintenance therapy with zoledronic acid in patients with localized Ewing sarcoma: Report from the international Ewing 2008 trial. Journal of Clinical Oncology 38:11523-11523, 2020
24. McCabe MG KL, Khan M, et al: Phase III assessment of topotecan & cyclophosphamide and high-dose ifosfamide in rEECur, an international randomised controlled trial of chemotherapy for the treatment of recurrent and primary refractory Ewing sarcoma (RR-ES). . Presented at the ASCO 2022 Annual Meeting, Chicago, US, 2022
25. Peneder P, Stutz AM, Surdez D, Krumbholz M, Semper S, Chicard M, et al: Multimodal analysis of cell-free DNA whole-genome sequencing for pediatric cancers with low mutational burden. Nat Commun 12:3230, 2021

26. Sheffield NC, Pierron G, Klughammer J, Datlinger P, Schonegger A, Schuster M, et al: DNA methylation heterogeneity defines a disease spectrum in Ewing sarcoma. Nat Med 23:386-395, 2017
27. van Ewijk R, Cleirec M, Herold N, le Deley MC, van Eijkelenburg N, Boudou-Rouquette P, et al: A systematic review of recent phase-II trials in refractory or recurrent osteosarcoma: Can we inform future trial design? Cancer Treat Rev 120:102625, 2023
28. Shu J, Li L, Sarver AE, Pope EA, Varshney J, Thayanithy V, et al: Imprinting defects at human 14q32 locus alters gene expression and is associated with the pathobiology of osteosarcoma. Oncotarget 7:21298-314, 2016
29. Green D, Singh A, Sanghera J, Jeys L, Sumathi V, Dalmay T, et al: Maternally expressed, paternally imprinted, embryonic non-coding RNA are expressed in osteosarcoma, Ewing sarcoma and spindle cell sarcoma. Pathology 51:113-116, 2018
30. Chen X, Bahrami A, Pappo A, Easton J, Dalton J, Hedlund E, et al: Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep 7:104-12, 2014
31. Zhao M, Wang T, Gleber-Netto FO, Chen Z, McGrail DJ, Gomez JA, et al: Mutant p53 gains oncogenic functions through a chromosomal instability-induced cytosolic DNA response. Nat Commun 15:180, 2024
32. Kinnaman MD, Zaccaria S, Makohon-Moore A, Arnold B, Levine MF, Gundem G, et al: Subclonal Somatic Copy-Number Alterations Emerge and Dominate in Recurrent Osteosarcoma. Cancer Res 83:3796-3812, 2023
33. Kovac M, Ameline B, Ribi S, Kovacova M, Cross W, Barenboim M, et al: The early evolutionary landscape of osteosarcoma provides clues for targeted treatment strategies. J Pathol 254:556-566, 2021
34. Marinoff AE, Spurr LF, Fong C, Li YY, Forrest SJ, Ward A, et al: Clinical Targeted Next-Generation Panel Sequencing Reveals MYC Amplification Is a Poor Prognostic Factor in Osteosarcoma. JCO Precis Oncol 7:e2200334, 2023
35. Kovac M, Blattmann C, Ribi S, Smida J, Mueller NS, Engert F, et al: Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. Nat Commun 6:8940, 2015
36. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, et al: Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature 359:162-5, 1992
37. Grünewald TGP, Cidre-Aranaz F, Surdez D, Tomazou EM, de Álava E, Kovar H, et al: Ewing sarcoma. Nat Rev Dis Primers 4:5, 2018
38. Gangwal K, Sankar S, Hollenhorst PC, Kinsey M, Haroldsen SC, Shah AA, et al: Microsatellites as EWS/FLI response elements in Ewing's sarcoma. Proc Natl Acad Sci U S A 105:10149-54, 2008
39. Guillon N, Tirode F, Boeva V, Zynovyev A, Barillot E, Delattre O: The oncogenic EWS-FLI1 protein binds in vivo GGAA microsatellite sequences with potential transcriptional activation function. PLoS One 4:e4932, 2009
40. Shulman DS, Chen S, Hall D, Nag A, Thorner AR, Lessnick SL, et al: Adverse prognostic impact of the loss of STAG2 protein expression in patients with newly diagnosed localised Ewing sarcoma: A report from the Children's Oncology Group. Br J Cancer 127:2220-2226, 2022
41. Adane B, Alexe G, Seong BKA, Lu D, Hwang EE, Hnisz D, et al: STAG2 loss rewires oncogenic and developmental programs to promote metastasis in Ewing sarcoma. Cancer Cell 39:827-844.e10, 2021
42. Surdez D, Zaidi S, Grossetête S, Laud-Duval K, Ferre AS, Mous L, et al: STAG2 mutations alter CTCF-anchored loop extrusion, reduce cis-regulatory interactions and EWSR1-FLI1 activity in Ewing sarcoma. Cancer Cell 39:810-826.e9, 2021

43. Shulman DS, Whittle SB, Surdez D, Bailey KM, de Álava E, Yustein JT, et al: An international working group consensus report for the prioritization of molecular biomarkers for Ewing sarcoma, NPJ precision oncology, 2022, pp 65
44. Griffin KH, Thorpe SW, Sebastian A, Hum NR, Coonan TP, Sagheb IS, et al: Engineered bone marrow as a clinically relevant ex vivo model for primary bone cancer research and drug screening. Proc Natl Acad Sci U S A 120:e2302101120, 2023
45. Marturano-Kruik A, Villasante A, Yaeger K, Ambati SR, Chramiec A, Raimondi MT, et al: Biomechanical regulation of drug sensitivity in an engineered model of human tumor. Biomaterials 150:150-161, 2018
46. Molina ER, Chim LK, Salazar MC, Koons GL, Menegaz BA, Ruiz-Velasco A, et al: 3D Tissue-Engineered Tumor Model for Ewing's Sarcoma That Incorporates Bone-like ECM and Mineralization. ACS Biomater Sci Eng 6:539-552, 2020
47. Lawlor ER, Scheel C, Irving J, Sorensen PH: Anchorage-independent multi-cellular spheroids as an in vitro model of growth signaling in Ewing tumors. Oncogene 21:307-18, 2002
48. Nguyen HTL, Soragni A: Patient-Derived Tumor Organoid Rings for Histologic Characterization and High-Throughput Screening. STAR Protoc 1, 2020
49. Jarvis S, Koumadoraki E, Madouros N, Sharif S, Saleem A, Khan S: Non-rodent animal models of osteosarcoma: A review. Cancer Treat Res Commun 27:100307, 2021
50. Manjunathan R, Ragunathan M: Chicken chorioallantoic membrane as a reliable model to evaluate osteosarcoma-an experimental approach using SaOS2 cell line. Biol Proced Online 17:10, 2015
51. Jefferies B, Lenze F, Sathe A, Truong N, Anton M, von Eisenhart-Rothe R, et al: Non-invasive imaging of engineered human tumors in the living chicken embryo. Sci Rep 7:4991, 2017
52. da Costa MEM, Droit R, Khneisser P, Gomez-Brouchet A, Adam-de-Beaumais T, Nolla M, et al: Longitudinal characterization of primary osteosarcoma and derived subcutaneous and orthotopic relapsed patient-derived xenograft models. Front Oncol 13:1166063, 2023
53. Green D, Singh A, Tippett VL, Tattersall L, Shah KM, Siachisumo C, et al: YBX1-interacting small RNAs and RUNX2 can be blocked in primary bone cancer using CADD522. J Bone Oncol 39:100474, 2023
54. Sampson VB, Vetter NS, Zhang W, Patil PU, Mason RW, George E, et al: Integrating mechanisms of response and resistance against the tubulin binding agent Eribulin in preclinical models of osteosarcoma. Oncotarget 7:86594-86607, 2016
55. Garnett MJ, Edelman EJ, Heidorn SJ, Greenman CD, Dastur A, Lau KW, et al: Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 483:570-5, 2012
56. Odri G, Kim PP, Lamoureux F, Charrier C, Battaglia S, Amiaud J, et al: Zoledronic acid inhibits pulmonary metastasis dissemination in a preclinical model of Ewing's sarcoma via inhibition of cell migration. BMC Cancer 14:169, 2014
57. Isakoff MS, Goldsby R, Villaluna D, Krailo MD, Hingorani P, Collier A, et al: A phase II study of eribulin in recurrent or refractory osteosarcoma: A report from the Children's Oncology Group. Pediatr Blood Cancer 66:e27524, 2019
58. Choy E, Butrynski JE, Harmon DC, Morgan JA, George S, Wagner AJ, et al: Phase II study of olaparib in patients with refractory Ewing sarcoma following failure of standard chemotherapy. BMC Cancer 14:813, 2014
59. Green D, Eyre H, Singh A, Taylor JT, Chu J, Jeys L, et al: Targeting the MAPK7/MMP9 axis for metastasis in primary bone cancer. Oncogene 39:5553-5569, 2020
60. Tattersall L, Shah KM, Lath DL, Singh A, Down JM, De Marchi E, et al: The P2RX7B splice variant modulates osteosarcoma cell behaviour and metastatic properties. Journal of Bone Oncology 31:100398, 2021

61. Weekes D, Kashima TG, Zandueta C, Perurena N, Thomas DP, Sunters A, et al: Regulation of osteosarcoma cell lung metastasis by the c-Fos/AP-1 target FGFR1. Oncogene 35:2852-61, 2016
62. Roundhill EA, Jabri S, Burchill SA: ABCG1 and Pgp identify drug resistant, self-renewing osteosarcoma cells. Cancer Lett 453:142-157, 2019
63. Landuzzi L, Manara MC, Lollini PL, Scotlandi K: Patient Derived Xenografts for Genome-Driven Therapy of Osteosarcoma. Cells 10, 2021
64. Brookes MJ, Roundhill EA, Jeys L, Parry M, Burchill SA, Rankin KS: Membrane-type 1 matrix metalloproteinase as predictor of survival and candidate therapeutic target in Ewing sarcoma. Pediatr Blood Cancer 69:e29959, 2022
65. Surdez D, Landuzzi L, Scotlandi K, Manara MC: Ewing Sarcoma PDX Models. Methods Mol Biol 2226:223-242, 2021
66. Gopisetty A, Federico A, Surdez D, Iddir Y, Zaidi S, Saint-Charles A, et al: Abstract 234: ITCC-P4: Genomic profiling and analyses of pediatric patient tumor and patient-derived xenograft (PDX) models for high throughput in vivo testing. Cancer Research 83:234-234, 2023
67. Kool M, Federico A, Surdez D, Gopisetty A, Saberi-Ansari E, Saint-Charles A, et al: INSP-15. ITCC-P4: A sustainable platform of molecularly well-characterized PDX models of pediatric cancers for high throughputin vivo testing. Neuro-Oncology 24:i189-i189, 2022
68. Marques Da Costa ME, Zaidi S, Scoazec JY, Droit R, Lim WC, Marchais A, et al: A biobank of pediatric patient-derived-xenograft models in cancer precision medicine trial MAPPYACTS for relapsed and refractory tumors. Commun Biol 6:949, 2023
69. Sayles LC, Breese MR, Koehne AL, Leung SG, Lee AG, Liu HY, et al: Genome-Informed Targeted Therapy for Osteosarcoma. Cancer Discov 9:46-63, 2019
70. Schott CR, Koehne AL, Sayles LC, Young EP, Luck C, Yu K, et al: Osteosarcoma PDX-Derived Cell Line Models for Preclinical Drug Evaluation Demonstrate Metastasis Inhibition by Dinaciclib through a Genome-Targeted Approach. Clin Cancer Res:Of1-of16, 2023
71. Nanni P, Landuzzi L, Manara MC, Righi A, Nicoletti G, Cristalli C, et al: Bone sarcoma patient-derived xenografts are faithful and stable preclinical models for molecular and therapeutic investigations. Sci Rep 9:12174, 2019
72. Roundhill EA, Chicon-Bosch M, Jeys L, Parry M, Rankin KS, Droop A, et al: RNA sequencing and functional studies of patient-derived cells reveal that neurexin-1 and regulators of this pathway are associated with poor outcomes in Ewing sarcoma. Cell Oncol (Dordr) 44:1065-1085, 2021
73. Vassal G, Blanc P, Copland C, Pearson A: Will the revised class waiver list make it? Lancet Oncol 16:e425-e426, 2015
74. Felix A, Berlanga P, Toulmonde M, Landman-Parker J, Dumont S, Vassal G, et al: Systematic review of phase-I/II trials enrolling refractory and recurrent Ewing sarcoma: Actual knowledge and future directions to optimize the research. Cancer Med 10:1589-1604, 2021
75. Anderson PM, Bielack SS, Gorlick RG, Skubitz K, Daw NC, Herzog CE, et al: A phase II study of clinical activity of SCH 717454 (robatumumab) in patients with relapsed osteosarcoma and Ewing sarcoma. Pediatr Blood Cancer 63:1761-70, 2016
76. Juergens H, Daw NC, Geoerger B, Ferrari S, Villarroel M, Aerts I, et al: Preliminary efficacy of the anti-insulin-like growth factor type 1 receptor antibody figitumumab in patients with refractory Ewing sarcoma. J Clin Oncol 29:4534-40, 2011
77. Pappo AS, Patel SR, Crowley J, Reinke DK, Kuenkele KP, Chawla SP, et al: R1507, a monoclonal antibody to the insulin-like growth factor 1 receptor, in patients with recurrent or refractory Ewing sarcoma family of tumors: results of a phase II Sarcoma Alliance for Research through Collaboration study. J Clin Oncol 29:4541-7, 2011
78. Pappo AS, Vassal G, Crowley JJ, Bolejack V, Hogendoorn PC, Chugh R, et al: A phase 2 trial of R1507, a monoclonal antibody to the insulin-like growth factor-1 receptor (IGF-1R), in patients with recurrent or refractory rhabdomyosarcoma, osteosarcoma, synovial sarcoma, and

other soft tissue sarcomas: results of a Sarcoma Alliance for Research Through Collaboration study. Cancer 120:2448-56, 2014

79. Tap WD, Demetri G, Barnette P, Desai J, Kavan P, Tozer R, et al: Phase II study of ganitumab, a fully human anti-type-1 insulin-like growth factor receptor antibody, in patients with metastatic Ewing family tumors or desmoplastic small round cell tumors. J Clin Oncol 30:1849-56, 2012

80. Weigel B, Malempati S, Reid JM, Voss SD, Cho SY, Chen HX, et al: Phase 2 trial of cixutumumab in children, adolescents, and young adults with refractory solid tumors: a report from the Children's Oncology Group. Pediatr Blood Cancer 61:452-6, 2014

81. Grignani G, Palmerini E, Dileo P, Asaftei SD, D'Ambrosio L, Pignochino Y, et al: A phase II trial of sorafenib in relapsed and unresectable high-grade osteosarcoma after failure of standard multimodal therapy: an Italian Sarcoma Group study. Ann Oncol 23:508-16, 2012

82. Duffaud F, Mir O, Boudou-Rouquette P, Piperno-Neumann S, Penel N, Bompas E, et al: Efficacy and safety of regorafenib in adult patients with metastatic osteosarcoma: a non-comparative, randomised, double-blind, placebo-controlled, phase 2 study. Lancet Oncol 20:120-133, 2019

83. Gaspar N, Campbell-Hewson Q, Gallego Melcon S, Locatelli F, Venkatramani R, Hecker-Nolting S, et al: Phase I/II study of single-agent lenvatinib in children and adolescents with refractory or relapsed solid malignancies and young adults with osteosarcoma (ITCC-050)(☆). ESMO Open 6:100250, 2021

84. Attia S, Bolejack V, Ganjoo KN, George S, Agulnik M, Rushing D, et al: A phase II trial of regorafenib in patients with advanced Ewing sarcoma and related tumors of soft tissue and bone: SARC024 trial results. Cancer Med 12:1532-1539, 2023

85. Italiano A, Mir O, Mathoulin-Pelissier S, Penel N, Piperno-Neumann S, Bompas E, et al: Cabozantinib in patients with advanced Ewing sarcoma or osteosarcoma (CABONE): a multicentre, single-arm, phase 2 trial. Lancet Oncol 21:446-455, 2020

86. Duffaud F, Blay JY, Mir O, Chevreau CM, Rouquette PB, Kalbacher E, et al: LBA68 Results of the randomized, placebo (PL)-controlled phase II study evaluating the efficacy and safety of regorafenib (REG) in patients (pts) with metastatic relapsed Ewing sarcoma (ES), on behalf of the French Sarcoma Group (FSG) and UNICANCER. Annals of Oncology 31:S1199, 2020

87. Espejo Valle-Inclan J, de Noon S, Trevers K, Elrick H, Flanagan A, Cortes-Ciriano I: Mechanisms and evolutionary trajectories of complex genome rearrangements in osteosarcoma. Presented at the Connective Tissue Oncology Society 2023 Annual Meeting, Dublin, Ireland, 2023

88. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al: Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. Ann Oncol 30:582-588, 2019

89. Garbe C, Keim U, Suciu S, Amaral T, Eigentler TK, Gesierich A, et al: Prognosis of Patients With Stage III Melanoma According to American Joint Committee on Cancer Version 8: A Reassessment on the Basis of 3 Independent Stage III Melanoma Cohorts. J Clin Oncol 38:2543-2551, 2020

90. Andreou D, Bielack SS, Carrle D, Kevric M, Kotz R, Winkelmann W, et al: The influence of tumor- and treatment-related factors on the development of local recurrence in osteosarcoma after adequate surgery. An analysis of 1355 patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. Ann Oncol 22:1228-1235, 2011

91. Blay JY, Soibinet P, Penel N, Bompas E, Duffaud F, Stoeckle E, et al: Improved survival using specialized multidisciplinary board in sarcoma patients. Ann Oncol 28:2852-2859, 2017

92. Liu PT, Valadez SD, Chivers FS, Roberts CC, Beauchamp CP: Anatomically based guidelines for core needle biopsy of bone tumors: implications for limb-sparing surgery. Radiographics 27:189-205; discussion 206, 2007

93. Oliveira MP, Lima PM, de Mello RJ: TUMOR CONTAMINATION IN THE BIOPSY PATH OF PRIMARY MALIGNANT BONE TUMORS. Rev Bras Ortop 47:631-7, 2012

94. Barrientos-Ruiz I, Ortiz-Cruz EJ, Serrano-Montilla J, Bernabeu-Taboada D, Pozo-Kreilinger JJ: Are Biopsy Tracts a Concern for Seeding and Local Recurrence in Sarcomas? Clin Orthop Relat Res 475:511-518, 2017

95. Toomayan GA, Robertson F, Major NM: Lower extremity compartmental anatomy: clinical relevance to radiologists. Skeletal Radiol 34:307-13, 2005

96. Toomayan GA, Robertson F, Major NM, Brigman BE: Upper extremity compartmental anatomy: clinical relevance to radiologists. Skeletal Radiol 35:195-201, 2006

97. Tomasian A, Hillen TJ, Jennings JW: Bone Biopsies: What Radiologists Need to Know. AJR Am J Roentgenol 215:523-533, 2020

98. Saifuddin A, Palloni V, du Preez H, Junaid SE: Review article: the current status of CT-guided needle biopsy of the spine. Skeletal Radiol 50:281-299, 2021

99. Taupin T, Decouvelaere AV, Vaz G, Thiesse P: Accuracy of core needle biopsy for the diagnosis of osteosarcoma: A retrospective analysis of 73 patients. Diagn Interv Imaging 97:327-31, 2016

100. Birgin E, Yang C, Hetjens S, Reissfelder C, Hohenberger P, Rahbari NN: Core needle biopsy versus incisional biopsy for differentiation of soft-tissue sarcomas: A systematic review and meta-analysis. Cancer 126:1917-1928, 2020

101. Marchais A, Marques da Costa ME, Job B, Abbas R, Drubay D, Piperno-Neumann S, et al: Immune Infiltrate and Tumor Microenvironment Transcriptional Programs Stratify Pediatric Osteosarcoma into Prognostic Groups at Diagnosis. Cancer Res 82:974-985, 2022

102. Wu JS, Goldsmith JD, Horwich PJ, Shetty SK, Hochman MG: Bone and soft-tissue lesions: what factors affect diagnostic yield of image-guided core-needle biopsy? Radiology 248:962-70, 2008

103. Dietz M, Whiteway S, Gupta S, Davis J, Montgomery N, Cohen-Gogo S, et al: Optimising Ewing sarcoma and osteosarcoma biopsy acquisition: consensus recommendations from the Children's Oncology Group Bone Tumor Committee. Presented at the Connective Tissue Oncology Society 2023 Annual Meeting, Dublin, Ireland, 2023

104. Tassé AM, Budin-Ljøsne I, Knoppers BM, Harris JR: Retrospective access to data: the ENGAGE consent experience. Eur J Hum Genet 18:741-5, 2010

## FIGURE LEGEND

**Figure 1: Overview of the sample types to be collected.** To maximise the advantages of sample collection, in order to obtain a comprehensive biological understanding of bone sarcoma and host-related factors, different sample types at sequential stages of the clinical pathway should be collected. (Created with BioRender.com)

Figure 1

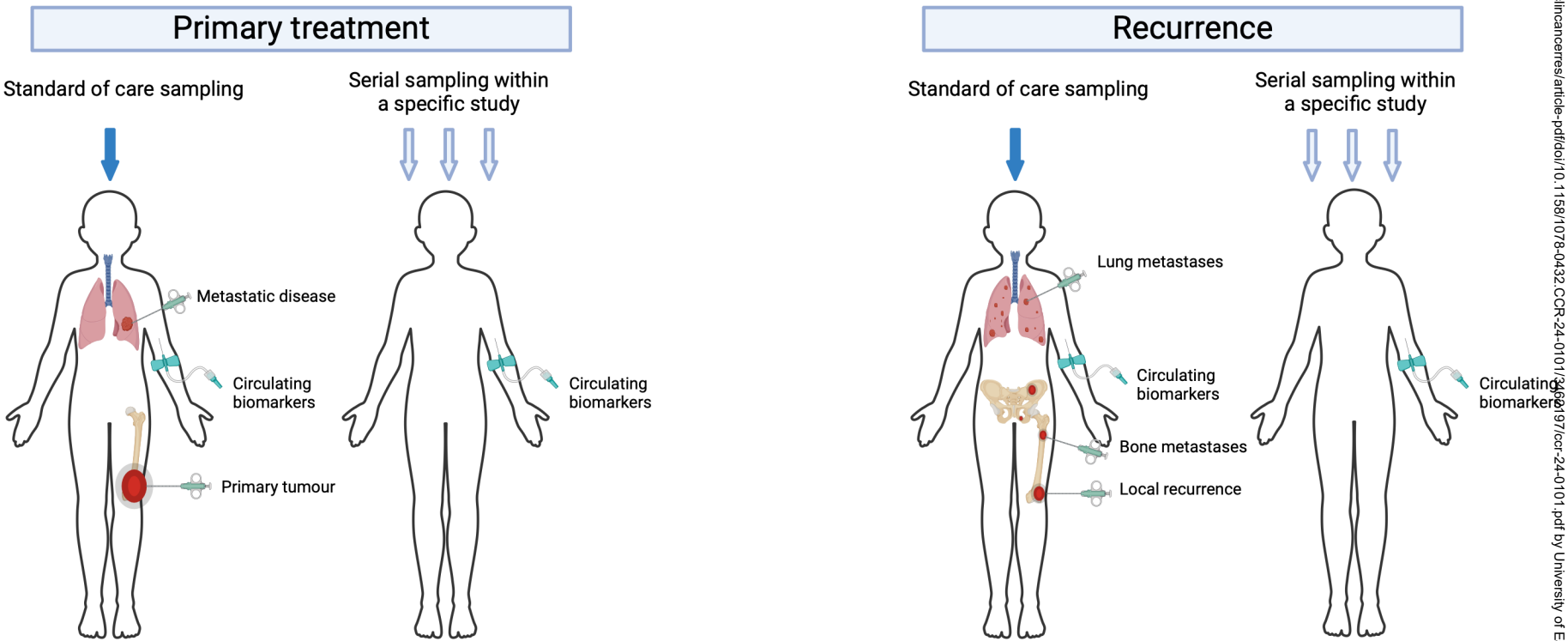

**Table 1. Advantages of appropriately consented, collected and/or biobanked samples.**

| |
|---|
| **For individual patients** |
| Druggable target identification and screening for early phase trials |
| Identification of germline predisposition syndromes |
| Monitoring of minimal residual disease* |
| Assignment to molecular strata* |
| Therapeutic use for immuno-oncology approaches such as tumour vaccines* |
| **For future research** |
| Identification and validation of molecular stratification |
| Identification of mechanisms of pathogenesis, drivers of tumour growth and resistance mechanisms |
| Analysis of biological drivers of relapse, particularly if paired diagnostic/relapse samples available |
| Analysis of tumour microenvironment and immunological aspects |
| Prognostic and predictive biomarker development and validation |
| Validation of liquid biopsy methodologies and development of minimal residual disease biomarkers |
| Identification and validation of SNVs associated with pharmacokinetic properties and treatment-induced early and late toxicities |
| Establishment of representative preclinical models and patient-derived cell lines |
| **For future research – particular benefits of prospective clinical trial samples** |
| Uniform sample processing, homogeneously treated patients |
| Uniform clinical datasets within and between trials** |
| Cross-validation of liquid biopsy, molecular classification, prognostic and predictive biomarkers between independent cohorts |

* Assumes successful completion of ongoing research

** Aided by ongoing Pediatric Cancer Data Commons initiatives (https://commons.cri.uchicago.edu/pcdc/)

**Table 2. Guidelines for sample collection ensuring diagnostic and translational research efficiency**

**Standard of care at: diagnosis, primary tumour resection, metastasectomy, recurrence**

| | Processing | Purpose |
|---|---|---|
| **Minimum essential** 3-5 core biopsies using 8-, 11- or 14-gauge needles. **OR** larger cores divided into two or three pieces **OR** 1 $cm^3$ open biopsy cut into multiple 0.2 $cm^3$ pieces. | FFPE | Diagnostic |
| | At least 1 core or tumour piece snap frozen in liquid $N_2$ or immediately stored in -80 $^oC$ | Diagnostic & Research |
| **Optimal** 5-7 core biopsies using 8-, 11- or 14-gauge needles. **OR** larger cores divided into pieces **OR** 2 $cm^3$ (or 2 x 1 $cm^3$) open biopsy cut into multiple 0.2 $cm^3$ pieces plus normal tissue comparator. | Material to be snap frozen or fresh material used in ongoing research projects to develop PDXs, tumour organoids, primary cultures, etc. | Research |
| **Optimal** whole blood* in EDTA or other normal tissue for germline sequencing** | PBMCs, plasma, serum | Research |
| **Samples for specific research studies and/or biobanking*** | | |
| Live cells in a culture-compatible medium/organ transplant preservation solution | Tumour cells | Research |
| whole blood* in EDTA or PAXgene tubes | Circulating tumour cells | Research |
| whole blood* in EDTA or cell-free Streck tubes | Circulating tumour DNA, plasma, serum, PBMCs | Research |
| 1-5 mL other biofluids | Saliva, urine | Research |
| **Samples at death/autopsy** | | |
| Oligometastases samples | Snap frozen in liquid $N_2$ or immediately stored in -80 $^oC$ | Research |

*=procedures and volumes for children and adults in accordance with the WHO guidelines on drawing blood: best practices in phlebotomy. **=germline sequencing is not currently international standard of care but many European countries have ongoing standard of care NGS studies that include germline

sequencing. ***=blood samples may be taken serially during and after treatment where specific research projects are available

**Table 3. SOPs to be considered in local institutions.** Staff from all involved disciplines (e.g. interventional radiologists, surgeons, operating room staff, pathologists, paediatric and medical oncologists and research nurses) should be aware of the importance of the availability of adequate biological samples and define the practical steps of collection, storage and shipment of samples according to local structures.

- Obtain information and written informed consent from patients or their legal guardians.
- Determining the amount and types of tissue, blood and other material to be collected.
- Orthopaedic surgical considerations (frozen section, infiltration zone, margin material); freezing and fixation of maximal amounts of material.
- Orthopaedic and pathological diagnosis and reference assessments.
- Sending MRI data via digital route or anonymised and coded external drive.
- Providing adequate short-term storage of tumour tissue and other samples.
- Transferring materials to long-term storage or shipping samples according to SOPs.
- Ensuring trial-specific requirements are met (e.g. tumour sections not sent to pathology for analysis but straight from the operating theatre to the research lab).
- Supplying material for cell culture in specific sterile cell culture medium.
- Filing documentation of collected materials per study in institution-specific lists or databases.
- Confirming received materials at research institute.
- Establishing procedure for prioritisation of pathology in case of sparse material.

**Table 4. The Patient and Parent Advocacy Group and Sarcoma Patient Advocacy Global Network international survey on sarcoma experiences.** The survey has so far included 598 respondents with bone sarcoma experience, of which there are 234 with osteosarcoma and 138 with Ewing sarcoma. Questions were asked on diagnosis, treatment and survivorship as well as priorities for future research. Four questions were specific to research sampling.

| Question | Osteosarcoma | | | Ewing sarcoma | | |
|---|---|---|---|---|---|---|
| | Yes (%) | No (%) | Other (%) | Yes (%) | No (%) | Other (%) |
| Were you or your child/family asked to donate tissue samples for research? | 109 (46.6%) | 84 (35.9%) | 41 (17.5%) | 72 (52.2%) | 39 (28.3%) | 27 (19.6%) |
| If you or your child/family member were not asked to donate tissue, would you have liked to be asked? | 82 (65.6%) | 3 (2.4%) | 40 (32%) | 40 (60.6%) | 2 (3%) | 24 (36.4%) |
| If asked, did you or your child/family member agree to donate tissue? | 106 (97.2%) | 0 (0%) | 3 (2.8%) | 68 (94.4%) | 0 (0%) | 4 (5.6%) |
| If you or your child/family member consented, was tissue successfully collected/donated? | 63 (59.4%) | 0 (0%) | 43 (40.6%) | 42 (61.8%) | 1 (1.5%) | 25 (36.8%) |

**Table 5. Ethical, legal, privacy and practical aspects of sample storage, sharing and shipment between research groups.**

- Age-appropriate information sheets must explain the purpose of the planned research, the recipients of the material and the use of anonymised or pseudonymised clinical data.
- Coupling of tumour material data to patient data, including treatment and imaging findings.
- Ethical approval and permissions from international, national or local authorities.
- A monitoring system for available samples and for associated informed consents per local hospital.
- Ownership issues relating to biological tissue and clinical data, which might be different between countries, should be considered.
- Advantages of centralised versus decentralised (virtual) tumour banking and procedures to check for appropriate tissue representation for interpretable biological results should be considered.
- Adequate coverage of the local costs and shipment of samples by research grants can facilitate the compliance of local institutions.
- Integrated, reusable tumour box devices can facilitate the shipment of frozen and unfrozen materials.
- Practical aspects of exchange and use of biological samples should be defined by MTAs between institutions.