



Biological Sample Collection to Advance Research and Treatment: A Fight Osteosarcoma Through European Research and Euro Ewing Consortium Statement

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ABSTRACT

Osteosarcoma and Ewing sarcoma are bone tumors mostly diagnosed in children, adolescents, and young adults. Despite multimodal therapy, morbidity is high and survival rates remain low, especially in the metastatic disease setting. Trials investigating targeted therapies and immunotherapies have not been groundbreaking. Better understanding of biological subgroups, the role of the tumor 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 consortium and the Euro Ewing Consortium. 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, and 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 harmonization 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 tumors 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 with other cancers (2, 3). Repeated attempts by large international cooperative groups to improve outcomes through randomized clinical

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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 tumor 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 patient and professional communities with osteosarcoma and Ewing sarcoma set out minimum standards for tissue sampling, sufficient for histologic 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 pediatric-type bone sarcomas.

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 standardized 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 characterization of disease subgroups and tumor and germline genetic, biological, immunologic, 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 centers. 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 in which a lack of samples hinders translational research and clinical progress.

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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 (or possibly mutant gain-of-function; refs. 30, 31) and complex genome rearrangements via chromoplexy and chromothripsis [(32, 33) and bioRxiv 2023.12.29.573403]. Specific molecular alterations in some cases include *MYC* amplification (34), *RB1* deletion and mutation, and a “*BRCAness*” phenotype (35). Ewing sarcoma cells are characterized by gain-of-function gene rearrangements between *FET* (*FUS*, *EWSR1*, and *TAF15*) RNA binding proteins and *ETS* (*FLI1*, *ERG*, and *FEV*) transcription factors, most commonly *EWSR1::FLI1* (36). The *FET::ETS* fusions encode oncogenic chimeric transcription factors with neomorphic features that reprogram the transcriptome (37), binding to GGAA microsatellites that become neoenhancers (38, 39), which leads to ectopic gene expression and tumor 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 tumors, 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, and 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 pretreatment and posttreatment tumor 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 and rats), nonrodent (e.g., canine, zebrafish, and *Xenopus*; ref. 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-naïve and relapsed material is critical for the development of relevant models to avoid unfavorable scenarios in which preclinical drug efficacy data generated using less-relevant models seem 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 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 tumor, 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 Paediatric Regulation in 2006, requiring the investigation of new therapies in children before marketing authorization 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. US Congress approval of the Research to Accelerate Cures and Equity for Children Act (“RACE Act”) enacted in 2020 gave the FDA powers to mandate pediatric 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 second and third decades and occurring in older adults as well as children, osteosarcoma and Ewing sarcoma are frequently considered “pediatric” cancers and are represented in early phase pediatric drug trials. The regulatory coordination between the EMA and FDA presents an opportunity to utilize 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 standardized, 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 more than 400 patients, IGF1R inhibitors resulted in response rates of 5% to 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 Optimizing 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 overemphasis on high-risk disease, a relative paucity of models for some disease settings, limited access to samples, almost nonexistent 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 tumor-host interactions affect disease progression and treatment response. This lack of understanding is largely driven by the absence of comprehensive, serial, annotated tumor 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, tumor 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, multiomic analysis to monitor disease response and facilitate options for molecularly targeted, personalized medicine and critically for osteosarcoma, to identify patients with underlying cancer predisposition syndromes. Taking tyrosine kinase inhibitors (TKI) 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 first-line and relapse settings (e.g., the INTER-EWING-1 and rEECur trials developed by the EEC and NCT05691478 in the United States) 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 (SOP) 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 toward changing this landscape.

Across Europe, the stakeholders engaged in obtaining biopsy material have different practices. The amount, quality, and availability of viable tumor material is variable and frequently inadequate for molecular analyses. Because of the lack of a framework for sampling, much tumor 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 tumor (bioRxiv 2023.12.29.573403), making a strategic approach to tissue biopsy critical to understanding patient-specific tumor 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% (87) to ~56% (88) 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 noncompliance exists because of the 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 (89, 90). 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 maximize 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 (Fig. 1; Table 2). To enhance fundamental understanding of bone sarcoma clonal evolution and chemoresistance, tumor tissue collection at relapse and autopsy (e.g., PEACE study, NCT03004755) is essential. Metastases often comprise different genetics to the original primary tumor, so sampling metastatic lesions is recommended to ensure that the maximal amount of biological information is collected.

Diagnostic biopsy

Treatment-naïve core or open biopsies should be obtained from suspected bone sarcoma cases at sarcoma specialist centers with the infrastructure to take, process and store (or send to a centralized national center) 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 tumor 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 tumors or by a specialist surgeon and reported in line with the International Collaboration on Cancer Reporting (<https://www.iccr-cancer.org/>; ref. 20). The biopsy tract should be considered contaminated and resected *en bloc* during local therapy or be included in the radiotherapy field to minimize the risk of local recurrence (19, 21, 91–93). The surgeon who will perform the tumor resection should be involved in defining the optimal approach for the biopsy. The biopsy tract is preferably marked and described according to compartmental anatomy (91, 94, 95). 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, 91–93). Advantages and (contra) indications have been described for both procedures (91, 96–99).

Sampling focused purely on histologic diagnosis, usually from decalcified formalin-fixed, paraffin-embedded (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 (100). The equivalent of three 11- or 14-gauge needle biopsy samples have typically been sufficient to provide diagnostic yield (101) when paired with conventional histology. Our position is that where

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^a
- Assignment to molecular strata^a
- Therapeutic use for immuno-oncology approaches such as tumor vaccines^a

For future research

- Identification and validation of molecular stratification
- Identification of mechanisms of pathogenesis, drivers of tumor growth, and resistance mechanisms
- Analysis of biological drivers of relapse, particularly if paired diagnostic/relapse samples available
- Analysis of tumor microenvironment and immunologic 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^b
- Cross-validation of liquid biopsy, molecular classification, and prognostic and predictive biomarkers between independent cohorts

^aAssumes successful completion of ongoing research.

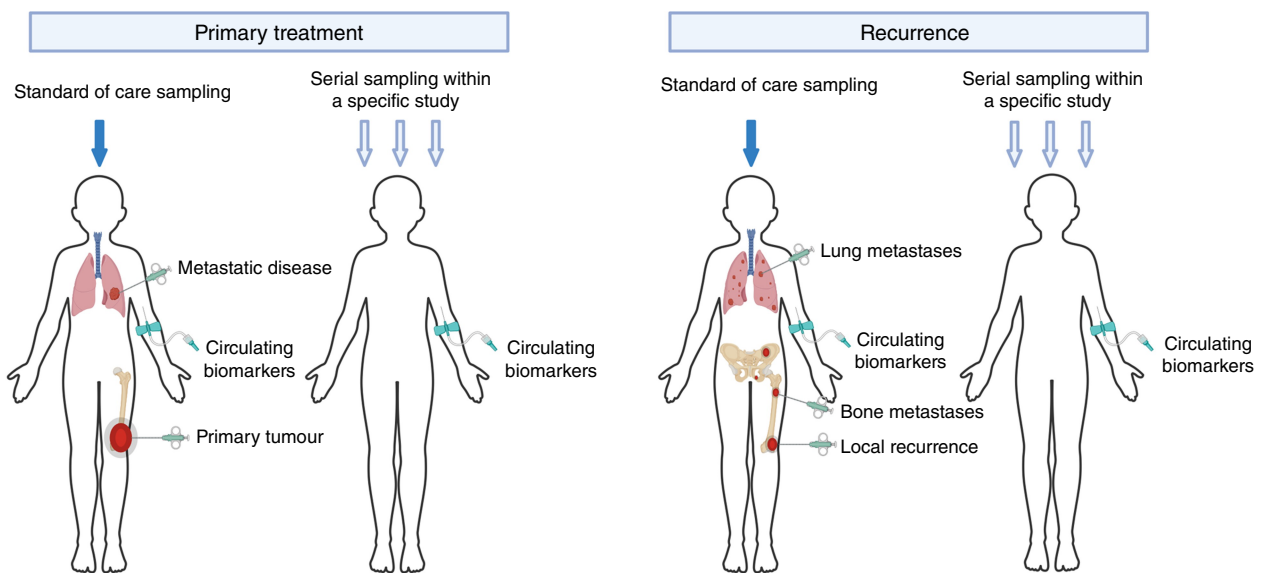
^bAided by ongoing Pediatric Cancer Data Commons initiatives (<https://commons.cri.uchicago.edu/pcdc/>).

a core biopsy is performed, five samples should be collected where possible, of which at least one must be snap-frozen (Table 2). The fourth and fifth sample should be designated for research but can be used for diagnostic purposes in which a diagnosis could not be made using the FFPE samples. In many cases, the fourth and fifth 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 tumor cells for cell line generation, organoid development, and/or engraftment into immunocompromised animals (Table 2). For open biopsy, a minimum of 1 cm³ of tissue cut into multiple

0.2 cm³ 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 seven core biopsies in which there is no soft tissue) plus up to three cores of underlying osteoid (102).

Primary tumor resection and metastasectomy

There are three surgical specimens in which resection serves as both performing standard of care and obtaining research samples:

**Figure 1.**

Overview of the sample types to be collected. To maximize 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.)

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

	Processing	Purpose
Standard of care at: diagnosis, primary tumor resection, metastasectomy, recurrence		
<i>Minimum essential</i> 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 At least one core or tumor piece snap-frozen in liquid N ₂ or immediately stored in –80°C	Diagnostic Diagnostic and research
<i>Optimal</i> 5–7 core biopsies using 8-, 11-, or 14-gauge needles; or larger cores divided into pieces; or 2-cm ³ (or 2 × 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, tumor organoids, primary cultures, etc.	Research
<i>Optimal</i> whole blood ^a in EDTA or other normal tissue for germline sequencing ^b	PBMCs, plasma, and serum	Research
Samples for specific research studies and/or biobanking^c		
Live cells in a culture-compatible medium/organ transplant preservation solution	Tumor cells	Research
Whole blood ^a in EDTA or PAXgene tubes	Circulating tumor cells	Research
Whole blood ^a in EDTA or cell-free Streck tubes	ctDNA, plasma, serum, and PBMCs	Research
1–5 mL other biofluids	Saliva and urine	Research
Samples at death/autopsy		
Oligometastases samples	Snap-frozen in liquid N ₂ or immediately stored in –80°C	Research

^aProcedures and volumes for children and adults in accordance with the WHO guidelines on drawing blood: best practices in phlebotomy.

^bGermline sequencing is not currently international standard of care, but many European countries have ongoing standard-of-care next-generation sequencing studies that include germline sequencing.

^cBlood samples may be taken serially during and after treatment in which specific research projects are available.

(i) primary tumor, (ii) matched adjacent normal tissue, and (iii) metastatic lesions. Samples should be prioritized by the pathologist to collect, depending on the availability of biobanking and specific research initiatives: (i) FFPE as the standard of care and neoadjuvant chemotherapy assessment, (ii) snap-frozen and stored, (iii) fresh and placed into an RNA-preserving medium, and (iv) 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, pretreatment 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 tumor 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 ctDNA and circulating tumor RNA (ctRNA), circulating cell-free DNA, and circulating tumor cells (CTC). For specific diagnostic, monitoring and biomarker studies, urine and other body tissues (e.g., tears and 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 (BCT) 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 among European centers 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 micro-metastases as well as allowing for the analysis of metastatic tumor-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 centers. Radiologists, surgeons, and pathologists have critical roles in the collection of adequate samples for histologic 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 centers are unable to adequately process and store relevant material, consideration should be given by national bodies to restrict diagnostic biopsies to centers with adequate infrastructure or establish regulated delivery channels to central repositories.

Standard operating procedures

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

Table 3. SOPs to be considered in local institutions.

- 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
- Orthopedic surgical considerations (frozen section, infiltration zone, and margin material); freezing and fixation of maximal amounts of material
- Orthopedic and pathologic diagnosis and reference assessments
- Sending MRI data via digital route or anonymized and coded external drive
- Providing adequate short-term storage of tumor tissue and other samples
- Transferring materials to long-term storage or shipping samples according to SOPs
- Ensuring trial-specific requirements are met (e.g., tumor sections not sent to pathology for analysis but straight from the operating theater 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 prioritization of pathology in case of sparse material

Staff from all involved disciplines (e.g., interventional radiologists, surgeons, operating room staff, pathologists, pediatric 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.

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 hours.

Infrastructure and personnel

Sampling requires a team effort. Some centers 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 theater staff. For SOPs to work, theater staff must be well informed, prepared, and adequately resourced to undertake the extra work. All personnel involved should recognize 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.

Table 4. The Patient and Parent Advocacy Group and Sarcoma Patient Advocacy Global Network international survey on sarcoma experiences.

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%)

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.

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 has 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 February 2, 2024, there were 372 combined osteosarcoma and Ewing sarcoma respondents ($n = 234$ osteosarcoma, median age 16 years; $n = 138$ Ewing sarcoma, median age 14 years). Just more than 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,

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 anonymized or pseudonymized clinical data
- Coupling of tumor 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 centralized vs. decentralized (virtual) tumor 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 tumor 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

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 pseudonymized clinical data prior to providing forms for informed consent. Pairing sample data with pseudonymized 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 centralized versus decentralized (virtual) tumor 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 centers 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 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 tumor 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 (MTA) between research centers.

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 tumor banking facilities and later shipped in batches, as required, for use in further analyses. Both centralized and decentralized material storage allow for their use in big data analyses with bioinformatics support. Regardless of storage location (e.g., accredited laboratories with alarm monitoring vs. 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 (103). 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 centers, it is the tissue bank where the samples were collected that owns the biological material. Tissue banks are typically nonspecific 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 centers. MTAs and SOPs for material shipment and adequate cost coverage (e.g., research grants) could further facilitate cooperative tumor banking. It is also important to establish procedures for the coupling of tumor material data to patient data. Genomic, transcriptomic, methylomic, and metabolomic data from tumor 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 pathologic data. It is worth investing in the collection of large amounts of retrospective clinical data about 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 tumor 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 histologic examination will not support diagnostic and prognostic technologies that evolve from translational research, for example, next-generation sequencing, 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 pediatric 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 optimized at each local center. 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.

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Authors' Contributions

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