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

Darrell Green[1], Roelof van Ewijk[2], Elisa Tirtei[3,4], Dimosthenis Andreou[5], Fredrik Baecklund[6], Daniel Baumhoer[7], Stefan S. Bielack[8], Rajesh Botchu[9], Kjetil Boye[10], Bernadette Brennan[11], Michael Capra[12], Lucia Cottone[13], Uta Dirksen[14], Franca Fagioli[3,4], Natalia Fernandez[15], Adrienne M. Flanagan[13,16], Marco Gambarotti[17], Nathalie Gaspar[18,19], Hans Gelderblom[20], Craig Gerrand[21], Anne Gomez-Mascard[22,23], Jendrik Hardes[24], Stefanie Hecker-Nolting[8], Edita Kabickova[25], Leo Kager[26,27], Jukka Kanerva[28], Lennart A. Kester[2], Marieke L. Kuijjer[29,30,31], Valérie Laurence[32], Cyril Lervat[33], Antonin Marchais[18], Perrine Marec-Berard[34], Cristina Mendes[35], Johannes H.M. Merks[2,36], Benjamin Ory[37], Emanuela Palmerini[38], Pan Pantziarka[15,39,40], Evgenia Papakonstantinou[41], Sophie Piperno-Neumann[33], Anna Raciborska[42], Elizabeth A. Roundhill[43], Vilma Rutkauskaite[44], Akmal Safwat[45], Katia Scotlandi[46], Eric L. Staals[47], Sandra J. Strauss[48], Didier Surdez[49], Gwen M.L. Sys[50], Marie-Dominique Tabone[51], Maud Toulmonde[52], Claudia Valverde[53], Michiel A.J. van de Sande[54], Klaus Wörtler[55], Quentin Campbell-Hewson[56,‡], Martin G. McCabe[57,58], and Michaela Nathrath[59,60]

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

[1]Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, United Kingdom. [2]Princess Maxima Center for Pediatric Oncology, Utrecht, the Netherlands. [3]Pediatric Oncology, Regina Margherita Children's Hospital, Turin, Italy. [4]Department of Public Health and Pediatrics, University of Turin, Turin, Italy. [5]Department of Orthopaedics and Trauma, Medical University of Graz, Graz, Austria. [6]Pediatric Oncology Unit, Karolinska University Hospital, Stockholm, Sweden. [7]Institute of Medical Genetics and Pathology, University Hospital Basel, Basel, Switzerland. [8]Center for Pediatric, Adolescent and Women's Medicine, Klinikum Stuttgart—Olgahospital, Stuttgart Cancer Centre, Stuttgart, Germany. [9]Department of Musculoskeletal Radiology, Royal Orthopaedic Hospital NHS Foundation Trust, Birmingham, United Kingdom. [10]Department of Oncology, Oslo University Hospital, Oslo, Norway. [11]Paediatric Oncology, Royal Manchester Children's Hospital, Central Manchester University Hospital NHS Foundation Trust, Manchester, United Kingdom. [12]Haematology/Oncology, Children's Health Ireland at Crumlin, Dublin, Ireland. [13]Department of Pathology, UCL Cancer Institute, University College London, London, United Kingdom. [14]Pediatrics III, West German Cancer Center, University Hospital Essen, German Cancer Consortium (DKTK) Site Essen, Cancer Research Center (NCT) Cologne-Essen, University of Duisburg-Essen, Essen, Germany. [15]Patient and Parent Advocacy Group, FOSTER, Washington, District of Columbia. [16]Histopathology, The Royal National Orthopaedic Hospital NHS Trust, Stanmore, United Kingdom. [17]Department of Pathology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy. [18]Department of Oncology for Child and Adolescent, Gustave Roussy Cancer Campus, Université Paris-Saclay, Villejuif, France. [19]U1015, Université Paris-Saclay, Villejuif, France. [20]Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands. [21]Orthopaedic Oncology, The Royal National Orthopaedic Hospital NHS Trust, Stanmore, United Kingdom.

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.

[22]Department of Pathology, Institut Universitaire du Cancer de Toulouse-Oncopole, Toulouse, France. [23]EQ ONCOSARC, CRCT Inserm/UT3, ERL CNRS, Toulouse, France. [24]Tumour Orthopaedics, University Hospital Essen, German Cancer Consortium (DKTK) Site Essen, Cancer Research Center (NCT) Cologne-Essen, University of Duisburg-Essen, Essen, Germany. [25]Paediatric Haematology and Oncology, University Hospital Motol, Prague, Czech Republic. [26]Pediatrics, St Anna Children's Hospital, Medical University Vienna, Vienna, Austria. [27]St Anna Children's Cancer Research Institute, Vienna, Austria. [28]Hematology-Oncology and Stem Cell Transplantation, HUS Helsinki University Hospital, New Children's Hospital, Helsinki, Finland. [29]Computational Biology and Systems Medicine Group, Centre for Molecular Medicine Norway, University of Oslo, Oslo, Norway. [30]Pathology, Leiden University Medical Center, Leiden, the Netherlands. [31]Leiden Center for Computational Oncology, Leiden University Medical Center, Leiden, the Netherlands. [32]Department of Medical Oncology, Institut Curie, Paris, France. [33]Department of Pediatrics and AYA Oncology, Centre Oscar Lambret, Lille, France. [34]Institute of Hematology and Pediatric Oncology, Léon Bérard Center, Lyon, France. [35]Portuguese Institute of Oncology of Lisbon, Lisbon, Portugal. [36]Division of Imaging and Oncology, University Medical Center Utrecht, Utrecht, the Netherlands. [37]School of Medicine, Nantes Université, Nantes, France. [38]Bone and Soft Tissue Sarcomas and Innovative Therapies Unit, IRCCS Istituto Orthopedico Rizzoli, Bologna, Italy. [39]Anticancer Fund, Meise, Belgium. [40]The George Pantziarka TP53 Trust, London, United Kingdom. [41]Pediatric Hematology-Oncology, Ippokratio General Hospital of Thessaloniki, Thessaloniki, Greece. [42]Oncology and Surgical Oncology for Children and Youth, Institute of Mother and Child, Warsaw, Poland. [43]Children's Cancer Research Group, Leeds Institute of Medical Research, University of Leeds, Leeds, United Kingdom. [44]Center for Pediatric Oncology and Hematology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania. [45]The Danish Centre for Particle Therapy, Aarhus University Hospital, Aarhus, Denmark. [46]Laboratory of Experimental Oncology, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy. [47]Orthopaedics and Trauma, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy. [48]Department of Oncology, University College London Hospitals NHS Foundation Trust, UCL Cancer Institute, London, United Kingdom. [49]Balgrist University Hospital, Faculty of Medicine, University of Zurich (UZH), Zurich, Switzerland. [50]Department of Orthopaedic Surgery and Traumatology, Ghent University Hospital, Belgium. [51]Department of Hematology and Oncology, A. Trousseau Hospital, Sorbonne University, APHP, Paris, France. [52]Department of Medical Oncology, Institut Bergonié, Bordeaux, France. [53]Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Spain. [54]Orthopaedic Oncology, Leiden University Medical Centre, Leiden, the Netherlands. [55]Musculoskeletal Radiology, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany. [56]Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom. [57]Division of Cancer Sciences, School of Medical Sciences, The University of Manchester, Manchester, United Kingdom. [58]The Christie NHS Foundation Trust, Manchester, United Kingdom. [59]Children's Cancer Research Center, Klinikum Rechts der Isar, Technical University of Munich, Munich, Germany. [60]Pediatric Oncology, Klinikum Kassel, Kassel, Germany.

D. Green, R. van Ewijk, and E. Tirtei are joint first authors to this article.

Q. Campbell-Hewson, M.G. McCabe, and M. Nathrath are joint senior authors to this article.

Corresponding Author: Martin G. McCabe, The Christie NHS Foundation Trust, Wilmslow Road, Withington, Manchester M20 4BX, United Kingdom. E-mail: martin.mccabe@manchester.ac.uk

Clin Cancer Res 2024;30:3395–406

doi: 10.1158/1078-0432.CCR-24-0101

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 "*BRCA*ness" 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

[a]Assumes successful completion of ongoing research.
[b]Aided 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 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 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:

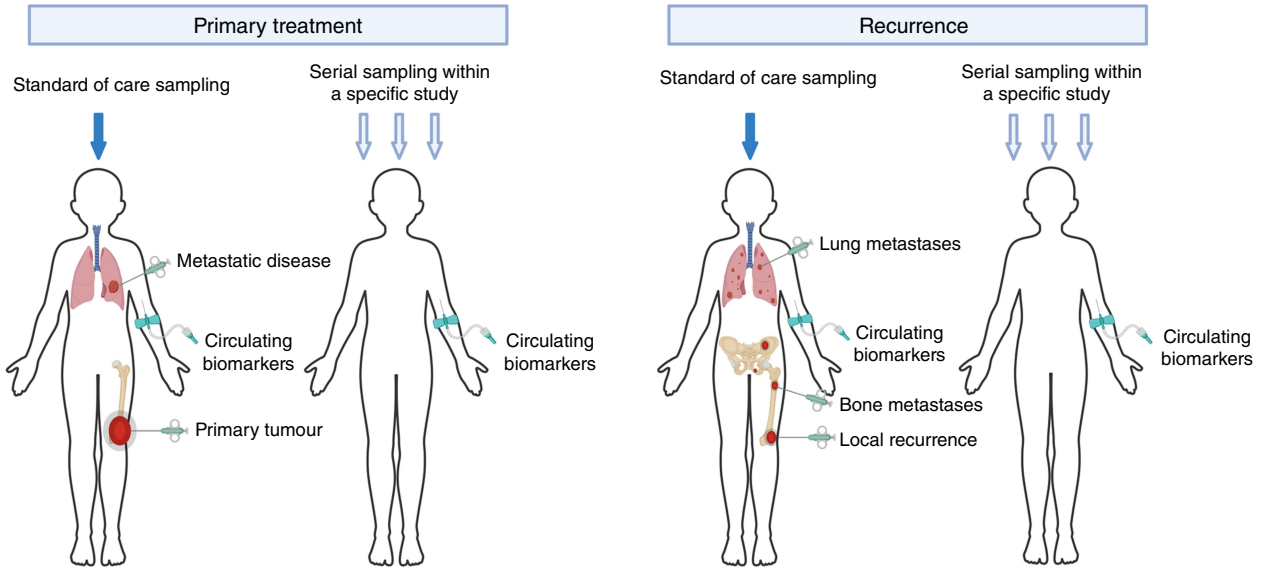

Figure 1.
Overview of the sample types to be collected. To maximize the advantages of sample collection, in order to obtain a comprehensive biological understanding of bone sarcoma and host-related factors, different sample types at sequential stages of the clinical pathway should be collected. (Created 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		
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 one core or tumor piece snap-frozen in liquid N_2 or immediately stored in −80°C	Diagnostic and 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 × 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
Optimal 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

[a]Procedures and volumes for children and adults in accordance with the WHO guidelines on drawing blood: best practices in phlebotomy.
[b]Germline 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.
[c]Blood 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 micrometastases 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.

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

	Osteosarcoma			Ewing sarcoma		
Question	**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.

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.

Authors' Disclosures

D. Andreou reports other support from PharmaMar outside the submitted work. S.S. Bielack reports personal fees from Y-mAbs, MAP BioPharma, SERB SAS, and Zschimmer and Schwarz Mohsdorf GmbH & Co. KG outside the submitted work. K. Boye reports personal fees from Bayer, GSK, Deciphera, Incyte, and NEC Oncoimmunity, as well as grants and personal fees from Eli Lilly and Company, personal fees from Novartis, and nonfinancial support from Merck outside the submitted work. N. Gaspar reports other support from ISPEN, Eisai, and Innovative Therapies for Children with Cancer outside the submitted work. J. Hardes reports grants from implantcast, Buxtehude, outside the submitted work. S. Hecker-Nolting reports grants from Deutsche Kinderkrebsstiftung, Bonn, Germany, and Foerderverein Krebskranke Kinder, Stuttgart, Germany, outside the submitted work. E. Palmerini reports personal fees from Deciphera Pharmaceutical, SynOX Therapeutics, Daiichi Sankyo, and Eusa Pharma outside the submitted work. S.J. Strauss reports personal fees from Ceridwen Oncology, Boehringer Ingelheim, Tessellate BIO, Inhibrx, and Adaptimmune outside the submitted work. C. Valverde reports grants and personal fees from PharmaMar, personal fees from Roche, GSK, Mundipharma, Eli Lilly and Company, Boehringer Ingelheim, and Ipsen, other support from Adaptimmune, Karyopharm, Foghorn Therapeutics, Ayala therapeutics, and Inhibrx, grants from Bayer, and nonfinancial support and other support from GEIS (Spanish Sarcoma Group) outside the submitted work. M.A.J. van de Sande reports grants from CarboFix and other support from SynOX and Deciphera outside the submitted work. M.G. McCabe reports other support from Eisai outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

This position paper was mainly written by **D. Green**, **R. van Ewijk**, **E. Tirtei**, **Q. Campbell-Hewson**, **M.G. McCabe**, and **M. Nathrath**. Data from the sarcoma experience survey were generated by **P. Pantziarka**. Authors are young investigators, work package and nation leads who all contributed to the content of the manuscript from the perspective of the group that they represent as well as commenting on general aspects of this policy review.

Acknowledgments

The Bone Cancer Research Trust (https://www.bcrt.org.uk/) funds FOSTER and EEC structure and activities. E.A. Roundhill and L. Cottone received funding from the Bone Cancer Research Trust (grant numbers BCRT/8422 and /7721). S. Hecker-Nolting received funding from Foerderkreis Krebskranke Kinder e.V. Stuttgart and the German Child Cancer Foundation (grant number DKS 2021.13). The consortia also thank Esperanza Perez, the Institut Gustave Roussy, Société Française des Cancers et leucémies de l'Enfant et de l'adolescent, ATTRACT, Enfants Cancers Santé, INTERSARC, une Main Vers l'Espoir, Imagine for Margo, The George Pantziarka TP53 Trust, German Cancer Aid (DKH), German Child Cancer Foundation (DKS), Society for Pediatric Oncology and Hematology (GPOH), Scandinavian Sarcoma Group, Trust Paola Gonzato Rete Sarcoma Onlus, Myrovlytis Trust, Ludovica's Parente, InfoCilento, and The Christie Charity for more specific support.

Received January 9, 2024; revised March 27, 2024; accepted June 11, 2024; published first June 13, 2024.

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 2013;49:684–95.
2. Gill J, Gorlick R. Advancing therapy for osteosarcoma. Nat Rev Clin Oncol 2021;18:609–24.
3. Gaspar N, Hawkins DS, Dirksen U, Lewis IJ, Ferrari S, Le Deley M-C, et al. Ewing sarcoma: current management and future approaches through collaboration. J Clin Oncol 2015;33:3036–46.
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 2016;17:1396–408.
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 2015;33:2279–87.
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 2016;34:3031–8.
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 1995;24:6–12.
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 1997;350:911–7.
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 1992;10:1579–91.
10. Gaspar N, Occean B-V, 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 2018;88:57–66.
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 2022;128:1958–66.
12. Ladenstein R, Pötschger 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 2010;28:3284–91.
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 2021;39:4029–38.

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 2022;400:1513–21.
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 2023;41: 2098–107.
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 2022;40: 2307–20.
17. Dirksen U, Brennan B, Le Deley M-C, 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 2019;37:3192–202.
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 2012;30:4148–54.
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 2021;32: 1520–36.
20. Bovée JVMG, 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 2023;82:531–40.
21. Bovee JF, Nielsen A, Bloem G, Akihiko Y, Bloem J. The WHO classification of tumours editorial board: WHO classification of tumours: Soft tissue and bone tumours. 5th ed. Lyon, France: 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 Paediatr Oncol 2023;2:100029.
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. J Clin Oncol 2020;38:11523.
24. McCabe M, Kirton L, Khan M, Fenwick N, Strauss SJ, Valverde C, 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). J Clin Oncol 2022;40(suppl 17):abstr LBA2.
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 2021;12: 3230.
26. Sheffield NC, Pierron G, Klughammer J, Datlinger P, Schönegger A, Schuster M, et al. DNA methylation heterogeneity defines a disease spectrum in Ewing sarcoma. Nat Med 2017;23:386–95.
27. van Ewijk R, Cleirec M, Herold N, le Deley M-C, 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 2023;120:102625.
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 2016;7: 21298–314.
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 2019; 51:113–6.
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 2014;7:104–12.
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 2024;15:180.
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 2023;83:3796–812.
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 2021;254:556–66.
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 2023;7:e2200334.
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 2015;6:8940.
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 1992;359:162–5.
37. Grünewald TGP, Cidre-Aranaz F, Surdez D, Tomazou EM, de Álava E, Kovar H, et al. Ewing sarcoma. Nat Rev Dis Primers 2018;4:5.
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 2008;105:10149–54.
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 2009;4:e4932.
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 2022;127:2220–6.
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 2021;39:827–44.e10.
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 2021; 39:810–26.e9.
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 Precis Oncol 2022;6:65.
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 2023;120: e2302101120.
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 2018;150:150–61.
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 2020;6: 539–52.
47. Lawlor ER, Scheel C, Irving J, Sorensen PHB. Anchorage-independent multicellular spheroids as an *in vitro* model of growth signaling in Ewing tumors. Oncogene 2002;21:307–18.
48. Nguyen HTL, Soragni A. Patient-derived tumor organoid rings for histologic characterization and high-throughput screening. STAR Protoc 2020;1: 100056.
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 2021;27:100307.
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 2015;17:10.
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 2017;7:4991.
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 2023;13:1166063.
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 2023;39:100474.

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 2016;7:86594–607.
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 2012;483:570–5.
56. Odri G, Kim P-P, 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 2014; 14:169.
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 2019;66: e27524.
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 2014;14:813.
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 2020; 39:5553–69.
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. J Bone Oncol 2021;31:100398.
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 2016;35:2852–61.
62. Roundhill EA, Jabri S, Burchill SA. ABCG1 and Pgp identify drug resistant, self-renewing osteosarcoma cells. Cancer Lett 2019;453:142–57.
63. Landuzzi L, Manara MC, Lollini P-L, Scotlandi K. Patient derived xenografts for genome-driven therapy of osteosarcoma. Cells 2021;10:416.
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 2022; 69:e29959.
65. Surdez D, Landuzzi L, Scotlandi K, Manara MC. Ewing sarcoma PDX models. Methods Mol Biol 2021;2226:223–42.
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 Res 2023;83(Suppl 7):234.
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 throughput *in vivo* testing. Neuro-Oncol 2022;24(Suppl 1):i189.
68. Marques Da Costa ME, Zaidi S, Scoazec J-Y, 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 2023;6:949.
69. Sayles LC, Breese MR, Koehne AL, Leung SG, Lee AG, Liu H-Y, et al. Genome-informed targeted therapy for osteosarcoma. Cancer Discov 2019;9: 46–63.
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 2023;30:849–64.
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 2019;9:12174.
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) 2021;44:1065–85.
73. Vassal G, Blanc P, Copland C, Pearson A. Will the revised class waiver list make it? Lancet Oncol 2015;16:e425–6.
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 2021;10:1589–604.
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 2016;63:1761–70.
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 2011;29:4534–40.
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 2011;29:4541–7.
78. Pappo AS, Vassal G, Crowley JJ, Bolejack V, Hogendoorn PCW, 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 2014;120:2448–56.
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 2012;30:1849–56.
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 2014;61:452–6.
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 2012;23:508–16.
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 2019;20:120–33.
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 2021;6:100250.
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 2023;12:1532–9.
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 2020;21:446–55.
86. Duffaud F, Blay J-Y, 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. Ann Oncol 2020;31(Suppl 4):S1199.
87. 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 2019;30:582–8.
88. 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 2020;38:2543–51.
89. 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 2011;22:1228–35.
90. Blay J-Y, Soibinet P, Penel N, Bompas E, Duffaud F, Stoeckle E, et al. Improved survival using specialized multidisciplinary board in sarcoma patients. Ann Oncol 2017;28:2852–9.
91. 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 2007;27:189–205.
92. Oliveira MP, de Andrade Lima PM, de Mello RJV. Tumor contamination in the BIOPSY path of primary malignant bone tumors. Rev Bras Ortop 2012; 47:631–7.

93. 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 2017;475:511–18.
94. Toomayan GA, Robertson F, Major NM. Lower extremity compartmental anatomy: clinical relevance to radiologists. Skeletal Radiol 2005; 34:307–13.
95. Toomayan GA, Robertson F, Major NM, Brigman BE. Upper extremity compartmental anatomy: clinical relevance to radiologists. Skeletal Radiol 2006;35:195–201.
96. Tomasian A, Hillen TJ, Jennings JW. Bone biopsies: what radiologists need to know. AJR Am J Roentgenol 2020;215:523–33.
97. Saifuddin A, Palloni V, du Preez H, Junaid SE. Review article: the current status of CT-guided needle biopsy of the spine. Skeletal Radiol 2021;50: 281–99.
98. Taupin T, Decouvelaere A-V, Vaz G, Thiesse P. Accuracy of core needle biopsy for the diagnosis of osteosarcoma: a retrospective analysis of 73 patients. Diagn Interv Imaging 2016;97:327–31.
99. 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 2020;126:1917–28.
100. 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 2022;82:974–85.
101. 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 2008;248:962–70.
102. Dietz MS, Whiteway S, Gupta S, Davis JL, 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. In: Presented at the Connective Tissue Oncology Society 2023 Annual Meeting; Dublin, Ireland; 2023.
103. Tassé AM, Budin-Ljøsne I, Knoppers BM, Harris JR. Retrospective access to data: the ENGAGE consent experience. Eur J Hum Genet 2010;18:741–5.