

Workshop Report on the European Bone Sarcoma Networking Meeting: Integration of Clinical Trials with Tumor Biology

David M. Thomas, PhD,¹ Miriam Wilhelm, MD,² Anne-Marie Cleton-Jansen, PhD,³ Uta Dirksen, MD, PhD,⁴ Natacha Entz-Werlé, MD, PhD,⁵ Hans Gelderblom, MD, PhD,³ Bass Hassan, DPhil,⁶ Heribert Jürgens, MD,⁴ Jan Koster, PhD,⁷ Heinrich Kovar, PhD,⁸ Arjan C. Lankester, MD, PhD,³ Ian J. Lewis, MB ChB,⁹ Ola Myklebost, DPhil,¹⁰ Michaela H.M. Nathrath, MD,¹¹ Piero Picci, MD,¹² Jeremy S. Whelan, MBBS,¹³ Pancras C. W. Hogendoorn, MD, PhD,³ and Stefan S. Bielack, MD²

A key workshop was held in The Netherlands in June 2011, hosted by several European bone sarcoma networks and with a broad range of stakeholders from Europe and Australia. The purpose of the meeting was to identify the strengths and weaknesses in current clinical trials for bone sarcomas and to make recommendations as to how to accelerate progress in this field. Two areas of particular interest were discussed. First, all participants agreed upon the importance of tumor biology to understanding clinical responses for all types of bone sarcoma. Various barriers to biobanking tumor and germline specimens were canvassed and are outlined in this paper. Second, there was consideration of the particular challenges of dealing with adolescent and young adult cancers, exemplified by bone sarcomas. Participants recommended greater engagement of both pediatric and adult sarcoma trial organizations to address this issue. Specific opportunities were identified to develop biological sub-studies within osteosarcoma, focused on understanding germ line risk and pharmacogenomics defining toxicity and biological responses. In Ewing sarcoma, it was harder to define opportunities for biological insights. There was agreement that the results for insulin-like growth factor pathway inhibition in Ewing family tumors were disappointing, but represented a clear indication of the need for companion biologic studies to develop predictive biomarkers. The meeting ended with broad commitment to working together to make progress in this rare but important subgroup of cancers.

Introduction

THE EUROPEAN BONE SARCOMA NETWORKING MEETING was held June 27–30, 2011, in Leiden, The Netherlands, to discuss the integration of translational research in clinical bone sarcoma trials. The aim of the meeting was to provide a forum for broad-based discussion by key stakeholders regarding the role of translational studies to be integrated into a

program of clinical trials, particularly in osteosarcoma and Ewing sarcoma.

Meeting Organization and Participants

Coordinating networks

The meeting was a joint venture between ENCCA, EuroBoNet, and the ECT EUROCORES program of the

¹Peter MacCallum Cancer Centre, Melbourne, Australia.

²Pediatrics 5, Klinikum Stuttgart Olgahospital, Stuttgart, Germany.

³Medical Oncology, Pathology, and Pediatrics, Leiden University Medical Center, Leiden, The Netherlands.

⁴Pediatric Hematology and Oncology, University Children's Hospital Münster, Münster, Germany.

⁵Pediatric Oncology and Hematology, CHRU Hautepierre, Strasbourg, France.

⁶Oxford University, Oxford, United Kingdom.

⁷Oncogenomics, Academic Medical Center, Amsterdam, The Netherlands.

⁸Children's Cancer Research Institute, Vienna, Austria.

⁹Alder Hey Children's NHS Foundation Trust, University of Leeds, Leeds, United Kingdom.

¹⁰Institute for Cancer Research, Oslo University Hospital, Norwegian Radium Hospital, Oslo, Norway.

¹¹Helmholtz Zentrum and Department of Pediatrics, Technische Universität, Munich; Pediatric Oncology, Klinikum Kassel, Germany.

¹²Laboratory of Experimental Oncology, Istituto Ortopedico Rizzoli, Bologna, Italy.

¹³Department of Oncology, University College Hospital, London, United Kingdom.

European Science Foundation. Meeting organizers were Pauline de Graaf and Pancras Hogendoorn (Leiden, The Netherlands) representing EuroBoNeT and Miriam Wilhelm and Stefan Bielack (Stuttgart, Germany) representing ENCCA Work Package 7 (bone sarcoma) and ECT-EURAMOS.

European Network for Cancer Research in Children and Adolescents (ENCCA; www.encca.eu)

ENCCA's aim is to establish a durable European Virtual Institute for clinical and translational research on childhood and adolescent cancers that will define and implement an integrated research strategy. It will also facilitate the necessary investigator-driven clinical trials to introduce the new generation of biologically targeted drugs into standards of care for children and adolescents with cancer. Work Package 7 (bone sarcoma) has the primary goal of establishing a platform for multinational intergroup bone sarcoma trials with integrated tumor biology research questions.

European Network to Promote Research into Uncommon Cancers in Adults and Children: Pathology, Biology and Genetics of Bone Tumours (EuroBoNeT; www.eurobonet.eu)

EuroBoNeT aims to integrate the different European laboratories performing bone tumor research. The research activities of the consortium focus on cartilaginous tumors (both benign and malignant), osteogenic tumors, giant cell tumors, and Ewing sarcoma, as well as processes involved in chondrogenesis and osteoclastogenesis. Goals of EuroBoNet include the integration of consortium partners' expertise, dissemination of results to the research community, and collaboration with the Network of Excellence Connective Tissue Cancer Network (NoE CONTICANET), among others. The continuing EuroBoNeT network reports that it has established a collection of preclinical resources and technologies for bone cancer research, including well-characterized collections of cell (*in vitro*) and xenograft (*in vivo*) models. The models have been characterized in detail with regard to a number of biological properties, such as tumor initiation, cancer markers, differentiation and invasion potential, genome-wide expression profiling (mRNA and microRNA), DNA methylation, and copy number aberration. These models are compared with a homogeneously treated panel of clinical samples. A wider virtual biobank of available clinical samples across the Network has been established. An important effort has been to introduce quality assurance measures concerning the detailed diagnostic procedures for these tumors and facilitate comparisons across Europe, thus resulting in more consistent clinical results and collaboration.

Pan-European Clinical Trials (ECT; www.esf.org/activities/eurocores/running-programmes/ect.html)

ECT is a European Science Foundation (ESF) program that coordinates funding for pan-European, non-commercial, investigator-driven clinical trials addressing questions that have a strong impact on the quality of life, morbidity, and mortality of the European population. The ECT program provides a framework for the implementation of pan-European clinical trials in compliance with Good Clinical Practice and current national legislation and European regulations. By promoting and sup-

porting networking, ECT fosters synergy with other European and international initiatives. Two collaborative research projects aimed at rare diseases and the pediatric population are funded under this program, including the European and American Osteosarcoma Study (EURAMOS-1; www.esf.org/activities/eurocores/running-programmes/ect/ect-projects.html).

Participants

In addition to members of the coordinating networks, representatives were present from (in alphabetical order): the Australasian Sarcoma Study Group (ASSG), the Cooperative Osteosarcoma Study Group (COSS), the European Osteosarcoma Intergroup (EOI), the European Organisation for Research and Treatment of Cancer Bone and Soft Tissue Groups (EORTC-STBSG), European Clinical Trials in Rare Sarcomas Within an Integrated Translational Trial Network (EuroSarc), the Italian Sarcoma Group (ISG), the Scandinavian Sarcoma Group (SSG), and the Société Française de Lutte contre les Cancers et Leucémies de l'Enfant et de l'Adolescent (SFCE)/Groupe Sarcomes Français et Groupe d'Etude de Tumeurs Osseuses (SFCE/GSF-GETO). In short, the meeting comprised a rich and diverse assembly of current European clinical and basic researchers in bone cancer.

Advance Assessment of Urgent Areas

All complex enterprises present challenges to be overcome, as well as opportunities to be seized. Prior to the meeting, a postal survey sent to all participants to determine where these challenges lie in translational bone sarcoma research received 29 responses. Nine respondents identified themselves as belonging to a clinical trial group, 10 as belonging to a research lab, and another 10 claimed allegiance to both. This survey was prepared and circulated by Miriam Wilhelm and Stefan Bielack (Stuttgart, Germany).

Questions addressed the quality of and access to data, the quality of and access to biospecimens, ethics and consent, collaboration, data sharing, and funding. Participants were asked to rate how important each of these areas was to their research and how well they believed these areas were actually performing. There were an equal number of basic, clinical, and translational researchers in the group surveyed.

The survey found that there was broad agreement between all researchers across survey items, regardless of background. As expected, lack of sufficient tumor material for translational research was experienced as the most important obstacle to translational research (Table 1). The survey participants were then asked to grade 27 items according to their importance for the respondent's individual research, and their satisfaction with the current situation accordingly (scale=1–5 where 1="least important or least satisfied," 5="most important or most satisfied"). The answers documented that the items chosen were indeed considered relevant (mean for importance: 4.33 ± 0.33 , 9/27 items >4.50 , 23/27 >4.00). Satisfaction with the way things worked today was rated lower (3.50 ± 0.39 , 0/27 >4.50 , only 2/27 >4.00 , 12/27 <3.50). Concordance between the assessments of clinical and laboratory scientists was very high.

Four areas appeared in need of particular attention, as they were ranked as very important (mean importance score >4.50), yet not working to satisfaction (mean satisfaction score <3.50). These were: (1) the link between clinical and

TABLE 1. MAJOR OBSTACLES TO TRANSLATIONAL BONE SARCOMA RESEARCH IN EUROPE

	<i>Clinical trial group (n=9)</i>	<i>Research lab (n=10)</i>	<i>Both (n=10)</i>	<i>Total (n=29)</i>
Availability of tumor material for translational research	4	7	6	17
Communication between basic research and clinical trials	2	3	3	8
Education of clinicians about the importance of provided information and follow-up	2	0	1	3
Obtaining clinical data about patients	1	0	0	1
Obtaining ethics approval	0	0	0	0

Responses to pre-meeting survey question asking, "What is the most important obstacle for you and your group?" listed according to respondents' professional affiliation. Of the answers, each participant could choose just one.

biological information; (2) the integration of biological research into clinical trials; (3) the transfer of basic research into clinical trials design; and (4) the level of funding available for this work. It became clear that there is a broad perception among clinicians and scientists that clinical trials represent an essential forum for understanding the biology of disease and treatment effects. Several issues related to funding became apparent. First, the current economic climate is likely to pose challenges to research activities in general. Second, a particular challenge is the funding of research into rare cancers (such as bone sarcomas) when that research involves engagement across national boundaries—and even between continents. There are few mechanisms that alone will provide funding for both basic and clinical components of a trial and also fund collaborators in multiple countries. Funding is especially difficult for the mundane but essential components of biospecimen collection, storage retrieval and processing, and shipping. Consortia like EuroBoNeT and now ENCCA have been remarkably effective in providing such support; hopefully other groups will emerge to fill the gap created by the completion of some of these programs.

Proceedings of the Meeting

The timing of this meeting arose in part because there are a gathering series of initiatives in both osteosarcoma (e.g., EURAMOS, EuroSarc) and Ewing sarcoma (e.g., the European Ewing Tumour Working Initiative of National Groups—Euro-EWING, EuroSarc), making the issue of defining and developing translational research within clinical trials a pressing issue. It was agreed that recent technological developments were particularly exciting and presented new challenges and opportunities for shedding light on cancer biology. There was a palpable sense of enthusiasm for massively parallel sequencing and its capacity to transform our understanding of the germline and somatic basis for developing bone cancers. The imminent planning of several studies in this context gave the meeting a sense of urgency and optimism. The possibility of employing the unique samples size of intergroup studies to investigate genes predisposing for bone sarcoma development and affecting pharmacodynamics and adverse effects was generally endorsed. The advantage is that although the experience from previous EURAMOS and Euro-EWING studies was that collecting sufficient tumor samples across so many institutions was challenging, blood samples should be feasible and sufficient for these purposes. It was pointed out that it was imperative that protocols and consents

were designed to allow exchange of these samples for international projects.

Challenges to successful translational bone sarcoma research

We next reviewed the experience of the EURAMOS-1 and Euro-EWING 99 trials to see what could be learned for the future. Jeremy Whelan (London, United Kingdom) summarized the experience of EURAMOS in translational research. He proposed that the failure to define *a priori* specific questions contributed to less than optimal translational productivity. The translational components within EURAMOS were, by contrast, intended to provide a resource for unspecified future research. The subsequent difficulties with biospecimen exchange, particularly across the Atlantic, was agreed to be unfortunate and undesirable. There was agreement that hard work to prevent this from recurring, if at all possible, was essential for future trials. Heinrich Kovar (Vienna, Austria) presented the detailed experience of translational research in Euro-EWING 99. He strongly recommended that a research question integrated into any trial be kept as simple and precise as possible, as trying to answer too many questions may result in none being answered well. He also recommended centralization of specific assays, as it appeared that there was remarkable variability between designated laboratories, even in apparently simple molecular procedures. A contributor to this effect is the longevity of large-scale clinical trials in rare cancers, with changes to technique, protocols, and personnel affecting the outputs of various laboratories over the study period. Comment was made that the variability across different labs may have the advantage of defining the reproducibility of a particular assay (important in clinical development), as well as providing replicates for assays with controversial implications. Other factors may dictate a less centralized laboratory structure. In this case, Dr. Kovar suggested that it was critical to define sensitivity and specificity controls to be used across all sites and with regular audit of performance.

Biospecimen collection and quality

A most important observation was that, for tumor specimens, there was a real challenge in obtaining consistently high-quality material from a majority of trial participants. For example, investigators from the Euro-EWING 99 trial reported during the meeting that only about one-quarter of patients had adequate material for molecular tumor studies.

There was considerable variability in sample quality even for paraffin-embedded diagnostic material, which is available in greater abundance. It was agreed that this represented a problem, but it was less clear how this could be solved. One issue is that different centers use varying diagnostic algorithms. For some centers, core biopsies are the basis of diagnosis, whereas for others open biopsies are the rule. Core biopsies are small, often insufficient for additional molecular studies, and may be limiting for diagnostic purposes. Resection specimens for both Ewing sarcoma and osteosarcoma are almost always affected by neoadjuvant therapy and of limited use biologically. It appeared that mandating collection of pre-treatment tumor material for biological studies would preclude participation of some centers and many patients. Given the importance of large-scale trials to clinical outcomes, both within the study and as a standard of care, it was agreed (but not universally) that mandating biospecimen collection was not practical. However, it was agreed that sub-studies could still be developed using tumor material involving key sites wherever this was feasible.

One issue raised was how to engage the enthusiasm of more sites in biological studies. While it is obviously critical to have funding for such activities, it was not generally felt that financial compensation would solve this problem. One suggestion was the partitioning of biological material, with some of the material held at the site (or by the group representing those sites), while the remainder is shipped to centralized sites for common molecular studies of agreed importance. Use of the locally held material would then be solely at the group's discretion, thereby providing an incentive for the group to support sample banking. This strategy would also encourage smaller, innovative research studies on these samples, which would enrich the trial's overall productivity. A limitation of this approach is that it can only readily be applied to samples that are collected easily and in abundance (i.e., can be split and feasibly used for research), and where they can be shipped centrally for molecular studies.

Database integration

A discussion was held regarding the integration of databases. At the "front" end, it was noted to be desirable for the clinical, biospecimen, and molecular databases to be linked. The ultimate form of this linkage was not defined, but the key elements were identified. Such a database may be customized to accommodate all data types, representing a shift from the conventional case record form used in phase 3 trials. More likely, data could be linked by common identifiers so that clinical data could be readily extracted when relevant to molecular analyses. This would necessitate a common unique identifier to be assigned to both the clinical dataset and the biospecimens. At the "back" end, a single readily usable interface was envisioned that enables approved external users to access all of the data on demand. An excellent example was presented to the group by Jan Koster (Amsterdam, The Netherlands) of an internet-based visualization tool for array-based molecular data linked to various datasets (R2, developed by the Department of Oncogenomics at the Academic Medical Center in Amsterdam, <http://r2.amc.nl>). The applicability of such systems to molecular datasets from trials was discussed. One key issue is the de-identification of individual patients for ethical reasons. EuroBoNeT was discussed as a

resource of relevant tools for future studies. In particular, the virtual biobank structure, standard operating procedures, and technology platform coordination were of interest in this respect.

Upcoming studies in osteosarcoma and Ewing sarcoma

After the broader discussions about the general principles and priorities, the group then reviewed imminent studies in both osteosarcoma and Ewing sarcoma. These studies provided concrete examples for considering many of the issues discussed above.

Osteosarcoma. The next incarnation of EURAMOS (EURAMOS-2) clearly presents the largest intergroup study of first-line osteosarcoma treatment and a remarkable opportunity for biological research. As with EURAMOS-1, both European (COSS, EOI, SSG) and North American (Children's Oncology Group, COG) groups intend to undertake this study. A number of additional groups, including those from Italy (ISG) and Australasia (ASSG), as well as the Sarcoma Alliance for Research through Collaboration (SARC) and the Spanish Sarcoma Group (GEIS), have also stated interest in consortium participation. At the time of preparation of this manuscript, the clinical study design, asking whether bisphosphonates provide benefit when added to standard chemotherapy, was rejected by COG's scientific council, creating uncertainty as to the final study proposal. However, there remains a strong commitment to continuing the alliance, which has proved so successful with EURAMOS. Regardless of the final form of the next intergroup study, David Thomas (Melbourne, Australia) presented plans for biological sub-studies in this trial.

In accordance with the principles defined above, the translational biology questions associated with EURAMOS-2 are "simple." Blood samples will be obtained from as many patients as possible and used for germline whole exome (and perhaps ultimately whole genome) sequencing. The second study presents an opportunity to define, in a large population of patients, both germline risk factors for osteosarcoma and genetic determinants of chemotherapy toxicity and response. The high-quality clinical dataset available from a clinical trial and the standardization of treatment for a single disease provide the opportunity for these studies, which could not be performed on retrospectively collected samples. Blood is readily collected on all (or nearly all) patients, bypassing some of the challenges encountered by studies using tumor material. However, it was agreed that somatic sub-studies on the subset of patients from whom suitable tumor material could be collected would nicely complement the germline data.

There were noted to be several implications of this proposal that affect the protocol's development. Germline genetic studies mandate careful consideration of informed consent and management of high-risk scenarios. The most likely of these represent the identification of risk alleles in potent tumor suppressor genes like *TP53*. Optional consents that allow the participant (and/or their families) to be notified of clinically significant findings may represent an important aspect of such studies.

Other osteosarcoma-specific proposals were discussed. Michaela Nathrath (Kassel and Munich, Germany),

representing COSS, suggested an initiative to develop tissue microarrays for validation of biomarkers and newly identified molecular targets. The future potential of serum samples, collected serially on patients treated within adjuvant studies, was noted. Either proteomic or genomic techniques aimed at quantitating circulating molecules could provide convenient measures of tumor burden, and collecting samples of this kind would assist in the development of such tools. Bass Hassan (Oxford, United Kingdom) presented a EuroSarc study examining the role of mifamurtide in advanced osteosarcoma. The rationale behind this study is to understand the molecular basis of the action of mifamurtide in osteosarcoma, which remains enigmatic despite much basic and clinical research.

Notably, immunologic aspects of cancer were prominent in discussions of both Ewing sarcoma and osteosarcoma. For example, Arjan Lankester (Leiden, The Netherlands), Anne Marie Cleton-Jansen (Leiden, The Netherlands), and David Thomas (Melbourne, Australia) presented intriguing pre-clinical data supporting the role of the immune system in osteosarcoma and Ewing sarcoma, suggesting that this field will prove fertile as we search for compelling strategies to improve survival for these patients. Other interesting aspects of study design included the use of Bayesian designs to accelerate progress in trials of rare cancers.

Ewing sarcoma. For Ewing sarcoma, the situation was different. It was more difficult to agree upon a single dominant intervention that could form the basis of an adjuvant study. Bass Hassan (Oxford, United Kingdom) presented a proposal to study the IGF-1R pathway based on the tantalizing (but ultimately somewhat disappointing) results of recent phase 2 studies using IGF-1 pathway inhibitors. It was strongly argued that the pharmaceutical industry's reluctance to pursue the basis for sometimes striking responses was shortsighted. The study proposed by Hassan and colleagues in the context of EuroSarc is intended to test the proposition that co-targeting of the insulin and IGF-1 receptors may increase the response rate for patients with Ewing sarcoma, and includes an intense schedule of serial biopsies for study participants. In general, the research on IGF-1 inhibitors in Ewing sarcoma illustrates the importance of biological correlative science in identifying mechanisms of action and predictive biomarkers for response.

There was a consensus that the aims of current preclinical and clinical studies in Ewing sarcoma are to optimize chemotherapy dose-intensity and to identify novel biomarkers and therapeutic targets to integrate into future clinical trials. For example, the role of minimal residual disease (MRD) testing is being evaluated. National and international networks (e.g., the German Translational Sarcoma Research Network, [TranSaRNet], including the Sarcoma Relapse Registry, [SAREZ]) are working to collect data and optimize biobanking to evaluate prognostic factors as innovative biomarkers, and in their preclinical research evaluate candidate therapeutic targets and cellular therapy strategies.

In addition to IGF-1R, several candidate strategies have been identified. The EWS-FLI1 fusion protein, in principle, presents a unique tumor cell-specific therapeutic target. However, while long-debated as predictive biomarker, a recent prospective Euro-EWING 99-associated study failed to

confirm the EWS-FLI1 fusion sequence as a prognostic marker, underscoring the importance of trial-associated translational studies. While previously considered undruggable, first approaches start to exploit the fusion protein as tumor-specific target. Nanoparticle delivery approaches, such as for RNAi moieties, may facilitate this in the future. With receptor and intracellular kinases such as IGF-1R emerging as EWS-FLI1-cooperating pathways, available small molecule inhibitors and antibodies warrant translational evaluation. Immune-targeting strategies are also being explored through the targeting of surface molecules such as CD99 or GD2, as well as vaccine- or cell-based approaches. The recent identification of putative Ewing sarcoma cancer stem cells and of molecular mechanisms of sarcoma metastasis may open targeting avenues for these crucial tumor cell populations, such as the targeting of involved kinases and epigenetic mechanisms (e.g., inhibition of EZH2 activity through histone deacetylase inhibitors).

Taken together, basic research has identified potential therapeutic targets within almost all the fundamental mechanisms of cancer: angiogenesis, proliferation, loss of tumor suppressor function, and others. While a clear prioritization of candidates for future concerted translational studies was not determined during this meeting, it provided an important discussion platform. Also, with the emerging notion that single-agent clinical activities of targeted inhibitors may be limited, further challenging clinical study designs such as platform data will be of particular importance in the future.

Conclusion

Overall, there was a striking mood of optimism at the meeting with many fruitful ideas discussed; future translational and clinical research proposals are likely to emerge from the process. In addition to the importance of biology to clinical outcomes for patients with these cancers, another key theme was the importance of collaboration. It was heartening to see representatives at the meeting from 10 European countries, as well as partners from overseas, in total representing more than a dozen translational and clinical research organizations. Only by such a collaborative effort can progress in the treatment of rare cancers be achieved. The delegates all agreed that repeating the meeting would be beneficial to see the ideas of this meeting turned into future research studies.

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Disclosure Statement

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Address correspondence to:
Stefan S. Bielack, MD
Cooperative Osteosarcoma Study Group (COSS)
Klinikum Stuttgart Olgahospital
Pediatrics 5 (Oncology, Hematology, Immunology; General
Pediatrics Gastroenterology, Rheumatology)
Bismarckstrasse 8
D-70176 Stuttgart
Germany

E-mail: coss@olgahospital-stuttgart.de