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**The ENCCA-WP7 / EuroSarc / EEC / PROVABES / EURAMOS 3rd
European Bone Sarcoma Networking Meeting / Joint Workshop of
EU Bone Sarcoma Translational Research Networks; Vienna,
Austria, September 24-25, 2015. Workshop Report.**

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ABSTRACT

This report summarizes the results of the 3rd Joint ENCCA-WP7, EuroSarc, EEC, PROVABES, and EURAMOS European Bone Sarcoma Network Meeting, which was held at the Children's Cancer Research Institute in Vienna, Austria on September 24-25 2015. The joint bone sarcoma network meetings bring together European bone sarcoma researchers to present and discuss current knowledge on bone sarcoma biology, genetics, immunology as well as results from preclinical investigations and clinical trials to generate novel hypothesis for collaborative biological and clinical investigations. The ultimate goal is to further improve therapy and outcome in patients with bone sarcomas.

Keywords: Osteosarcoma, Ewing sarcoma, Bone sarcoma, Immunotherapy, Next generation sequencing, Pharmacogenomics, Translational research

ORGANIZATION

The 3rd European bone sarcoma networking meeting was held at the Children's Cancer Research Institute in Vienna, Austria on September 24-25 2015. It was organized by Stefan Bielack, Stuttgart, and Leo Kager, Vienna, and supported by the European Network for Cancer research in Children and Adolescents Work Package 7 (ENCCA-WP7, represented by Stefan Bielack), the EUROpean Clinical Trials in Rare SARComas initiative (EuroSARC, represented by Bass Hassan, Oxford), the European Ewing Consortium (EEC, represented by Jeremy Whelan, London), the PROspective VALidation of Biomarkers in Ewing Sarcoma network (PROVABES, represented by Uta Dirksen, Münster), and the European branch of the EUROpean and AMerican Osteosarcoma Study Group (EURAMOS, represented by Stefan Bielack and Jeremy Whelan).

HIGH-GRADE OSTEOSARCOMA

Genomics

High-grade osteosarcomas (HGOS) have complex karyotypes showing abundant structural and numerical aberrations. Michaela Nathrath, Kassel, presented data from the Cooperative Osteosarcoma Biology Study Group. Exomes of 31 HGOS were sequenced and their evolutionary landscape was deciphered by inferring clonality of the individual mutation events. Exome findings were interpreted in the context of mutation and single-nucleotide polymorphism (SNP) array data from a replication set of 92 tumors. 14 genes were identified as the main drivers, of which some were formerly unknown in the context of HGOS. Tumor protein 53 (TP53) and molecular pathways functionally similar to TP53 seem to drive genomic instability in OS. More than 80% of HGOSs exhibited a specific combination of single base substitutions, loss of heterozygosity (LOH), or large-scale genome instability signatures characteristic for breast cancer 1/2, early onset (BRCA1/2)-deficient tumors. The findings imply that multiple oncogenic

pathways drive chromosomal instability during osteosarcoma evolution and result in acquiring BRCA-like traits, which could be therapeutically exploited.

The Norwegian Sarcoma Consortium (<http://kreftgenomikk.no/en/sarkom/>), a national collaboration collecting samples from all sarcoma patients in Norway for next generation sequencing (NGS)-based analysis and search for new therapies, was introduced by Ola Myklebost, Oslo. He presented preclinical studies of a panel of osteosarcoma cell lines using NGS of DNA and RNA. They found hundreds of fusion transcripts, of which only a small fraction corresponded to fusion genes, suggesting a phenotype of trans-splicing, by which transcripts from different genes are joined during splicing. Whereas only few of the genomic fusions gave fusion transcripts, the trans-spliced mRNAs should give large numbers of neo-antigens that could support immune checkpoint therapies. Furthermore, all cell lines had completely abrogated p53 response, largely caused by copy number-neutral fusions and aberrations of *TP53*.

Modulation of drug effects in osteosarcoma cells, drug resistance and pharmacogenomics

Given the plateau in survival since the last three decades, Anne-Marie Cleton-Jansen, Leiden, discussed new opportunities for HGOS therapy. Improving the efficacy of chemotherapy may provide one opportunity and might be achieved, for example, by modulating the pharmacodynamic effects of drugs in HGOS cells. Using a small interfering RNA (siRNA) screen, the Aven-ATM serine/threonine kinase-checkpoint kinase 1 (ATM-CHEK1) pathway was identified as a target to sensitize osteosarcoma cells to conventional chemotherapeutic agents; and modulation of Aven-ATM-CHEK1 may provide a novel strategy towards improving HGOS therapy.

Aurélie Dutour, Lyon, demonstrated that chemotherapeutic treatments trigger an increase of netrin-1 (NTN1) expression which is accompanied by netrin-1 dependence receptors DCC and UNC5H increase; and that combining chemotherapeutic agents and netrin-1 interference potentiates cancer cell death. In an orthotopic and metastatic rat osteosarcoma model, the effect of anti-Ntn1 antibody combined with doxorubicin was investigated. The combination slows down osteosarcoma progression, significantly prolongs animal survival and prevents metastatic dissemination. Therefore, combining conventional drugs with Ntn-1 interference could lead to superior efficacy as well as a reduction of chemotherapy doses for HGOS treatment.

The blockade of mammalian target of rapamycin (mTOR) represents a promising approach to treat patients with HGOS, although mTOR monotherapy has met with mixed results. Winette van der Graaf, London, suggested that combination therapy might be the key to success. Using two *in vivo* osteosarcoma models, van der Graaf et al. demonstrated that the activity of the mTOR-inhibitor temsirolimus is significantly enhanced by the addition of either cisplatin or bevacizumab. Moreover, extensive immunohistochemical and 3'-deoxy-3'-¹⁸F-fluorothymidine positron emission tomograph/computed tomography (¹⁸F-FLT-PET/CT) analyses of tumor response indicated that tumor volumes underestimated treatment efficacy, and that ¹⁸F-FLT-PET/CT can potentially be used to measure response in the early phases of treatment. These findings suggest the further exploration of temsirolimus combined with either cisplatin or bevacizumab for HGOS, with the incorporation of ¹⁸F-FLT-PET scans.

Stefano Ferrari, Bologna, pointed out that novel approaches to the treatment of HGOS should include patients risk stratification. For example, the over-expression of the ATP-binding cassette transporter B1 (ABCB1) at diagnosis is involved in the processes of resistance to the classic antineoplastic drugs and identified patients with a poor prognosis in Italian Sarcoma Group (ISG) studies. The ISG and the Spanish Sarcoma Group are presently carrying out a trial (ISG/OS-2,

ClinicalTrials.gov number: NCT01459484) where patients are stratified in different regimens based on ABCB1 expression. Besides ABCB1, also overexpression of the DNA excision repair protein ‘excision repair cross-complementation group 1’ (ERCC1) was identified to be associated with a high relapse rate and poor EFS and OS. The co-evaluation of ERCC1 and ABCB1 protein expression showed that patients positive for both markers had a significantly worse prognosis.

Pharmacogenomics, which aims to tailor drug therapy based on the genomic ‘make-up’ of normal host cells and cancer cells, can help to improve drug therapy; and there is an increase in pharmacogenomic investigations in patients with HGOS. Dr. Ferrari pointed out that a special effort should be made to prospectively undertake pharmacogenetic profiling of patients entering clinical trials.

From bench to bedside, and back to bench

The beneficial effect of combining chemotherapy with the bisphosphonate zoledronate in syngenic models of rat osteosarcoma constituted the rationale for the French OS2006 trial (NCT00470223). Laurence Brugières, Villejuif, reported on the final results of the OS2006 trial which was conducted in France between 2007 and 2014. 315 patients with HGOS (83% localised and resectable) were randomised to receive or not 10 injections of zoledronate in addition to chemotherapy. The final analysis of this trial showed that the addition of zoledronate to pre- and post-operative chemotherapy did not improve event-free survival and overall survival of patients with previously untreated osteosarcoma. Furthermore, this analysis showed a slight excess of events, and deaths in patients treated with zoledronate as compared to the patients without zoledronate leading to a non- significant difference in EFS and OS.

Françoise Rédini, Nantes, provided information on how the French group will explore the biological background for the unexpected results of the OS2006 trial. Several hypotheses have been raised which include involvement of infiltrating macrophages, receptor activator of nuclear factor-kappa B (RANK)-expressing osteosarcoma cells, tartrate-resistant acid phosphatase (TRAP) expressing cells in the tumor bone niche, and impact of the hormone microenvironment. Ancillary studies performed on OS2006 biological samples have the objectives to elucidate whether the results are linked to the disease itself (HGOS), to the bone microenvironment, or to the age of the patients.

Immunology and Immunotherapy in HGOS

David Thomas, Darlinghurst, provided an overview on immunotherapeutic approaches, which hold great promise for cancer treatment. HGOS is noted to be an immunologically interesting disease, for multiple reasons. For example, HGOS is assumed to derive from osteoblasts, a unique cell type that, together with the osteoclast, plays an important role in bone development and physiology. Dr. Thomas reviewed the complex signalling molecules and interdependencies that link the osteoblast and osteoclast, and their overlap with the immune system. There is an association between mutational load and tumor neo-antigens, apparently important to the effectiveness of a new generation of immunotherapies (e.g., immune checkpoint inhibitors); and HGOSs are noted to be genotypical complex and unstable, perhaps making them good targets for immunotherapies. There is also a long history of immunologic approaches in HGOS (e.g., muramyl-tripeptide and interferons), which reinforces optimism about their susceptibility to new developments in immune therapies.

Piotr Rutkowski, Warsaw, showed that overexpression of the programmed cell-death ligand 1 (PD-L1) on tumor cells, which interact with PD-1 on cytotoxic T-lymphocytes, impedes anti-tumor immunity and results in immune evasion. Therefore, interruption of the PDL1/PD-1 pathway represents an attractive therapeutic strategy to reinvigorate tumor-specific T-cell immunity. Dr. Rutkowski reported that PD-L1 had been observed to be significantly expressed in different subtypes of sarcomas including osteosarcoma (36%), leiomyosarcoma (97%) and Ewing's sarcoma (39%). Kim et al. found PD-L1 expression in 70% of leiomyosarcomas, 67% of Ewing sarcomas and 75% synovial sarcomas. These levels of expression, together with the poorer outcome of sarcoma patients with PD-L1 positivity, justify the further exploration of the role of PD-L1 antibody for the treatment of sarcomas. Currently, the European Organisation for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group and several pharmaceutical companies are planning phase II studies of anti-PD-L1 antibodies in advanced sarcomas including HGOS.

The focus of the immunoSARC project, which was presented by Javier Martin-Broto, Sevilla, is to explore immunomodulation as a therapeutic approach in sarcomas. The perspective is not only to investigate the impact of immune target drugs (e.g., PD-1 or PD-L1 inhibitors), but also to focus on the role of immunomodulation via combinations of these drugs with anti-angiogenic agents. Whereas the link between angiogenesis of tumors and immune evasion (e.g., via the vascular endothelial growth factor A (VEGFA)) is well known, this connection has been poorly studied in sarcomas even though some tumors are highly vascularised. A “pick the winner” randomized phase II trial, comparing the anti-PD-1 antibody nivolumab alone versus the combination of nivolumab plus pazopanib (a multi-targeted tyrosine kinase inhibitor that blocks angiogenesis) in cohorts of patients with bone sarcomas and soft tissue sarcomas, has been designed. This investigation aims to explore whether combination arms prove to be synergistic in

efficacy in selected sarcoma subtypes and if some immune targets (e.g., PD-1/PDL-1) could be predictive at least in some sarcoma subtypes.

Franca Fagioli, Turin, provided preclinical data on two different adoptive immunotherapy strategies for the treatment of sarcomas including HGOS, namely human leukocyte antigen (HLA)-unrestricted and HLA-restricted strategies, with cytokine-induced killer (CIK) cells and sarcoma-specific cytotoxic T-lymphocytes (CTLs), respectively. The results of her investigations provided preclinical proof-of-concept for an effective strategy to attack sarcomas with CIKs; and a Phase I study with CIKs for patients with high-risk sarcomas including HGOS is being prepared.

Bass Hassan, Oxford, reviewed current knowledge on the macrophage activating drug mifamurtide in the context of osteosarcoma and provided an overview on the MEMOS ('A mechanistic study of mifamurtide in patients with metastatic and/or recurrent osteosarcoma') trial (EudraCT number: 2012-000615-84; <http://www.oncology.ox.ac.uk/trial/memos>) of the EuroSARC group (<http://eurosarc.eu/>). The primary objective of this trial is to analyze biological effects of mifamurtide, and some centers have begun recruiting patients.

CHONDROSARCOMA and SPINDLE/PLEOMORPHIC BONE

SARCOMAS

Judith Bovee, Leiden, updated on the biology of chondrosarcoma (CS). For peripheral CS a novel mouse model has emphasized that in addition to the biallelic inactivation of exostosin glycosyltransferase (*EXT1* or *-2*) in chondrocytes, additional alterations affecting either the TP53 or cyclin-dependent kinase inhibitor 2A (*CDKN2A*) cause malignant transformation towards

secondary peripheral chondrosarcoma. For central tumours, it was shown that mutations in isocitrate dehydrogenase (*IDH1* or *-2*) are an early event: the oncometabolite D-2-hydroxyglutarate that is induced by the mutation inhibits osteogenic differentiation and promotes chondrogenic differentiation, causing enchondromas of bone. In central chondrosarcomas, the *IDH* mutation is, however, no longer a driver mutation, as inhibition of the mutant protein has no effect on the tumorigenic properties of chondrosarcoma cell lines. Instead, other additional genetic alterations, affecting amongst others the TP53 and retinoblastoma (Rb) pathway, as well as collagen, type II, alpha 1 (*COL2A1*) and neuroblastoma RAS viral oncogene homolog (*NRAS*), and other signaling pathways (e.g. hedgehog, mTOR, Bcl-2, survivin) are involved.

Jeremy Whelan, London, discussed the therapy of mesenchymal CS, which is a very rare subtype accounting for approximately 5% of chondrosarcoma. The diagnosis is supported by identification of a translocation involving the transcription factor *HEY1* and the nuclear receptor coactivator 2 (*NCOA2*); and it mostly affects young adults and can arise at multiple anatomical sites, either in soft tissue or bone. A recent publication by Frezza et al. of a case series derived from the experience of European centres of excellence has supported the prognostic significance of metastatic disease and proposed a survival advantage for the use of adjuvant chemotherapy. A second recent publication by Xu et al., using data extracted from subsets of patients reported in multiple retrospective series, drew opposite conclusions. Examples of where observational data had influenced clinical practice, but were refuted by data from randomised trials, were used to emphasize the importance of randomised studies even in very rare sarcoma subtypes. The challenges of undertaking a randomised study of chemotherapy in mesenchymal chondrosarcoma were described using a recently published typology for access to trials and were concluded to be too formidable.

Hans Gelderblom, Leiden, reported on the COSYMO Study (EudraCT No: 2013-005155-32), in which three cohorts of patients (i.e., conventional chondrosarcoma, dedifferentiated/mesenchymal CS, and myxoid/roundcell liposarcoma (LS)) are treated with a combination of mTOR inhibition and cyclophosphamide. The rationale to use this drug combination in patients with CS and LS derives from preclinical and clinical investigations.

Piero Picci, Bologna, stressed the importance of a more accurate classification for spindle/pleomorphic bone sarcomas, as it was recently done for soft tissue sarcomas. This would permit more specific chemotherapy regimens for the different entities within collaborative studies, necessary given the rarity of these sarcomas.

EWING SARCOMA

Biology of Ewing sarcoma and Ewing-like sarcomas

Heinrich Kovar, Vienna, reported on novel players in the pathogenesis of Ewing sarcoma (ES). *EWS-FLII* affects a number of nicotinamide adenine dinucleotide (NAD) metabolizing enzymes, resulting in reduction of NAD levels in ES cells. Drugs, which interact with the NAD metabolome (e.g., Nicotinamide phosphoribosyltransferase (NAMPT)) may be considered for the preclinical development in ES.

David Herrero-Martin, Barcelona, provided data on non-coding RNAs in ES. Analysis of the ES methylome showed micro RNA miR-10a (5p) hypermethylation; and miR-10a was found to be very low expressed in both ES cell lines and patient samples. Reintroduction of miR-10a in two ES cell lines reduced migratory capacity and decreased clonogenic growth; and isobaric tags for

relative and absolute quantification (iTRAQ) analysis of miR-10a transfected cells allowed identifying several proteins that could be related to the described phenotype.

Oscar Martinez Tirardo, Barcelona, and co-workers uncovered a role for ephrin A receptor 2 (EphA2) in the progression of ES. This receptor participates, most of all, in the migratory capacity of ES cells by ligand-independent means; and work is in progress to decipher the molecular mechanisms associated to such effects.

Franck Tirode, Paris, reported that four Ewing-like sarcoma entities have been described as small round cell tumors presenting morphological characteristics of ES but carrying a different chromosomal translocation (either one of *BCOR-CCNB3*, *CIC-DUX4*, *EWSR1-NFATc2* or *EWSR1-PATZ1*). The resemblance of these Ewing-like tumors both with ES and between each other's was examined; and first demonstrated that ES presenting rare fusion variants such as *FUS-ERG* or *FUS-FEV* are transcriptionally undistinguishable from classical *EWSR1-FLI1* or *EWS-ERG* positive tumors. Second, expression profiling and careful examination of clinical and pathological data indicated that the four Ewing-like sarcomas are far to resemble ES and are distinct entities. Tirode et al. propose to consider ES as tumors carrying only FET family proteins (*EWSR1* or *FUS*)-ETS transcription factor (*ERG* and *PEA3* types) fusions. While *CIC-DUX4* and *BCOR-CCNB3* are now classified as undifferentiated/unclassified sarcomas in the latest WHO sarcoma classification, they propose adding to this category all the other type of Ewing-like translocations such as the *EWSR1-NFATc2* and *EWSR1-PATZ1*. Finally, one might even recommend a different nomenclature for Ewing-like sarcomas such as small round cell sarcoma with *X-X* translocation.

Epigenetics, inhibition of signalling pathways and circulating tumor DNA in ES

Günther Richter, Munich, reported that next-generation sequencing (NGS) data confirm *EWS/ETS* translocations as the crucial driver event of ES tumorigenesis. *EWS/FLII* induces altered epigenetic marks, and targeting ‘epigenetic readers’ such as bromodomain-containing proteins (e.g., BRD3/4) in ES reduces *EWS/FLII* expression. Treatment with bromodomain and extra-terminal motif (BET) inhibitors like JQ1 blocks an ES typical expression profile. Moreover, JQ1 inhibits proliferation, induces apoptosis and reduces ES tumor growth. Together with phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitor BEZ235 inhibition of BRD3/4 may offer new opportunities for combination therapy in ES.

Emmy Fleuren, Nijmegen, focused on the topic of multi-receptor tyrosine kinase targeting in ES. Unfortunately, inhibitors targeting a single receptor tyrosine kinase (RTK) such as Insulin-like growth factor 1 receptor (IGF-1R) appeared insufficient for ES treatment in the clinic. Because research indicates that tumors often do not rely on a single RTK and different RTKs can compensate for one another to maintain tumor growth, Fleuren et al. hypothesized that the targeting of multiple RTKs at once may be a more promising approach. Therefore, they investigated the co-expression patterns and co-targeting effects of various oncogenic RTKs implicated in ES in 30 primary ES patient samples. Significant, strong, positive correlations and co-expression patterns were observed between IGF-1R and MET, IGF-1R and AXL, and MET and AXL receptors. Co-targeting of these RTKs was synergistic or at least additive in 6/6 ES cell lines in vitro, with most pronounced effects in IGF-1R-targeted combinations, indicating that IGF-1R deserves further investigation, particularly in combinations.

Jenny Potratz, Münster, provided data that showed that the receptor tyrosine kinase (RTK) ‘*recepteur d'origine nantais*’ (RON) is expressed and active in Ewing sarcomas, with over-expression in tumors derived from metastatic disease. Evidence of function in pro-metastatic cellular features supports RON as a potential therapeutic target. However, targeting strategies are

challenged by a short-form RON isoform lacking the extracellular antibody-binding domain and thereby bypassing antibody inhibition. This exemplifies that isoforms, principally described in diverse RTKs, require attention and further understanding to adapt targeting strategies.

Bass Hassan discussed the rationale for dual inhibition of IGF-1R and insulin receptor (IR) in ES. Moreover, he presented the LINES trial (<http://www.oncology.ox.ac.uk/trial/lines>); a Bayesian single-arm phase II trial in which the dual anti-IGF-1R/IR drug linsitinib is investigated in patients with relapsed and/or refractory ES.

Katia Scotlandi, Bologna, reported on the interaction between the cytotoxic drug trabectedin (Yondelis®) and IGF-1 signalling. In ES, trabectedin may not only inhibit but also enhance the binding of *EWS-FLII* to certain target genes, leading to upregulation of IGF1R. Combination of trabectedin and anti-IGF-1R inhibitors represents a potential therapeutic option for patients with ES.

Markus Metzler, Erlangen, provided information on the EFACT (EWS-FLI sequence analysis from ctDNA) project, which assesses circulating tumor DNA (ctDNA) as response marker in ES. EFACT is implemented as accompanying research project to the ongoing EWING2008 trial. Extended application to relapsed ES appears particularly informative given the individual treatment schedules often necessary in this cohort. Analysis of ctDNA copy numbers is suitable to be integrated in preclinical studies and in Phase I/II trials as additional response marker.

DELIVERING TRIALS EFFECTIVELY FOR BONE SARCOMA

What can be learned from recent sarcoma trials?

Hans Gelderblom provided an overview on recently published clinical trials in HGOS, ES and CS; and discussed the difficulties in conducting investigator initiated clinical trials in bone sarcomas. Collaboration, creation of centres of expertise for bone sarcomas, innovative statistical

designs, lowering administrative burden, inventive funding, molecular tumor boards, etc. might help to boost investigator initiated research in bone sarcomas.

Marie-Cecile LeDeley, Villejuif, summarized results from the EURO-EWING 99 trial (NCT00020566) and discussed how to improve international cooperation to be more efficient, based on the experience made in the EURO-EWING 99 trial.

Uta Dirksen, Münster, provided an update on the EWING 2008 trial (NCT00987636; <https://www.clinicaltrialsregister.eu/ctr-search/trial/2008-003658-13/DE>); and focused on the administrative barriers, the implementation of an online registration and data entry system (MARVIN; <https://www.xclinical.com/en/products/marvin>), and the prospective validation of biomarkers in ES (PROVABES, <http://provabes.uni-muenster.de>) project.

Bernadette Brennan, Manchester, reported on the Euro Ewing 2012 trial (EudraCT number 2012-002107-17, <http://www.euroewing.eu/clinical-trials/ee2012-trial>); which has been opened and recruiting patients since December 2013 in UK, Spain and France. Thus far, 54 European centres are open with plans to open centres in Australia and Israel in 2016. The opening of all international centres, sponsored by the Birmingham ‘Cancer Research UK Clinical Trial Unit’ (CRUK CTU), involving every country’s interpretation of the EU trials regulations, has not been quick or easy. The CTU perhaps should have considered that protracted contracts with other countries would occur and hence dealt with this much earlier to prevent this. The presentation of data in another bone tumour, using the same investigational medical product (IMP) has caused issues of concern for some investigators which are not taken lightly but may effect recruitment. Lastly the “R3” cohort of ES were not included in this trial and every effort must now be made by the EEC to rectify this as their prognosis remains dismal.

Martin McCabe, Manchester, reported on the 'rEECur' trial (<http://www.euroewing.eu/clinical-trials/reecur/index>), which aims to compare four commonly used chemotherapy regimens for efficacy and toxicity in refractory and relapsed ES. It utilises a multi-arm, multi-stage trial design to exclude less active and/or more toxic arms at an early stage, and therefore will avoid the recruitment of large numbers of patients to these less active and more toxic chemotherapy regimens. rEECur represents the most extensive collaboration yet between European Ewing sarcoma clinical trial groups. This collaboration has been made easier through the acquisition of European Commission funding. However, despite a keen willingness to collaborate and a desire to successfully answer the study questions', opening the study has been hampered by the requirement of each EU member state to individually interpret the EU Clinical Trials Directive through its Competent Authority, and to assess the ethical issues of the study. Two years in to a five-year grant, the study is open in six out of thirteen planned countries. None of the queries raised by Competent Authorities or ethics committees have yet resulted in any changes to the design or implementation of the study protocol or patient consent process, yet the bureaucracy required to repeat the process in thirteen countries has contributed significantly to the resource and time needed to open the study. Major European funders should consider this time delay as an inevitable component of cross-border, European clinical trials when allocating resources for running international trials.

Stefano Ferrari commented on two ISG and Scandinavian Sarcoma Group (SSG) ES trials. ISG/SSG3 (1999-2006) enrolled 300 patients with non metastatic ES, and ISG/SSG4 (1999-2008) enrolled 102 with metastatic Ewing sarcoma. Both protocols foresaw the use of high-dose chemotherapy (HDCHT) and peripheral blood stem cell (PBSC) support for high-risk patients (poor responders to induction chemotherapy or metastatic patients). In patients with non-metastatic ES, patients with a poor response to the induction treatment with the VACA-IE

regimen can achieve the same probability of EFS of good responders thanks to the addition of HDCHT. Surgery was the main option for local control. The use of postoperative radiotherapy should be reserved in case of inadequate surgical margins and the histological response does not affect the local control. The female gender and very young pediatric patients experience higher bone marrow toxicity. In patients with synchronous metastases (lung or only one bone metastasis) a 5 year OS of 50% can be achieved with an aggressive treatment with HDCHT and total lung irradiation. A complete radiological response of lung metastases is predictive of survival. In recurrent patients the post relapse survival is very poor in spite of an intensive chemotherapy treatment with high-dose ifosfamide and high-dose busulfan and melphalan. Second complete remission is the main factor predictive post relapse survival. The role of HDCHT in this subset of patients is uncertain.

Sandra Strauss, London, reported on the SARcoma Research through Collaboration SARC025/SP1 trial (<http://sarctrials.org/SARC025>), which is a collaborative US/European phase I study of a combination of the poly ADP ribose polymerase (PARP) inhibitor, Niraparib, with Temozolomide in ES. Dr. Strauss commented on problems that arise if the sponsor of a trial (the US SARC group) is not based in the European Economic Area (EEA); and models were discussed that may help to overcome such obstacles.

Stefan Bielack commented on similarities between the experiences made by clinical osteosarcoma trialists with those presented from the Ewing sarcoma trials. He used the EURAMOS-1 study of the European and American Osteosarcoma Study Group as an example to describe how the multiple challenges arising during the past one and a half decades of Pan-European and transatlantic cooperation were met. He emphasized the need for constant communication within such a large consortium, the benefits of joint training activities for site staff, the necessity to follow predefined common rules as well as to find compromises where

necessary. The EURAMOS consortium succeeded in addressing the primary objectives of their intergroup trial, and also used its visibility to lobby for a better European environment for investigator-initiated clinical trials.

Statistical issues

Joachim Gerß, Münster, commented on how statistics can help towards more effective bone sarcoma research. With adaptive clinical trial designs in the face of new discoveries or information collected in the course of a trial, flexible design modifications can be performed, such as sample size adjustment or the selection of the target population. Flexible-adaptive clinical trial designs usually adhere to established quality criteria such as the type I error, in contrast to the completely different Bayesian statistical approach. A Bayesian statistical approach allows including prior knowledge, hence an increased amount of information is utilized in statistical analyses, and the knowledge resulting from statistical evaluation is displayed very clearly.

Carlo Lancia, Jakob Anninga, and Marta Fiocco, Leiden, discussed multi-drug regimens and dose-intensity in HGOS. The focus is on the decision-making process of dynamically adjusting therapy based on toxicity. Toxicity is a time-dependent confounder since it is both a mortality risk-factor and a predictor of following exposure to cytotoxic agents. In the presence of a time-dependent confounder, the classical Cox model leads to biased estimates of the hazard ratios for the variables under investigation; and marginal structural Cox models are a possible solution to this problem.

How to construct and perform informative bone sarcoma trials?

Nathalie Gaspar, Villejuif, discussed strategies to prioritize agents for further development in bone sarcoma. In a world where an increasing number of new drugs with increasing various mechanisms of action are in development each year, international networks should think in strategies to prioritize agents for further development in bone sarcoma, with 4 axes. 1- To define appropriate drugable targets relevant with bone sarcoma biology at diagnosis and relapse. 2- To insure strong preclinical evidence on drug efficacy alone and in combination in multiple bone sarcoma models, taking into account microenvironment (bone, neoangiogenesis, immune system). 3- To favour drugs with appropriate toxicity profile that might be compatible with relapse but mainly with first line chemotherapy regimens. 4- To anticipate when introduce the drug according to this mechanism of action, on bulky tumour or in minimal residual disease. Collaboration of clinicians with biologists but also statisticians is crucial, as the main objective remains to introduce new drugs in first line treatment of bone sarcomas whatever the age to improve patient outcome.

Lindsey Bennister, London, a charity representative, and Ornella Gonzato, Udine, a patient representative, discussed that patients expect an appropriate trial to be suggested by their clinician as part of their treatment plan. The National Cancer Patient Experience Survey (England, 2014) identified that only 35% of sarcoma patients were asked by their clinician to participate in research. However, of these, 64% went on to participate, showing there is an interest and willingness to take part if asked by their clinician. Lindsey Bennister provided recommendations on how to improve information on trials to patients. This included the raise of awareness of the importance of recommending trials to patients via a European campaign targeting clinicians, supported by charities, patient groups and research networks, as well as the setup of a one-stop sarcoma clinical trials portal for trials (Europe), rather than having multiple places to search. Moreover, clinical trials should get smarter about ‘marketing’ themselves in

order to attract and retain participants, e.g., by sharing the excitement of what difference the trial could make in the future and clearly set out the benefit to individual patients.

Christopher Copland, York, as a consumer representative, introduced a network of groups and individuals from across Europe (<http://unite2cure.org>), which is calling for better treatment and better access to treatment for children and young people with cancer. They decided to take the opportunity of Childhood Cancer Awareness month to build support for reform of the Paediatric Medicines Regulation (2007). They set the target of getting 1000 signatures for an e-petition by the end of September 2015, a goal that they easily exceeded. Their website now lists amongst its supporters many prominent professionals and numerous well-respected organisations in the field. They carried the initiative forward at meetings with the Cancer Drugs Development Forum and the European Medicines Agency.

Gilles Vassal Villejuif, reported that the Cancer Drug Development Forum (CDDF) with Innovative Therapy for Children with Cancer Consortium (ITCC), European Society for Pediatric Oncology (SIOPE) and European Network for Cancer Research in Children and Adolescents (ENCCA) has created a unique Pediatric Oncology Platform, involving multiple stakeholders and the European Union (EU) Commission, with an urgent remit to improve pediatric oncology drug development.

Virtual tumor-boards and trans-border sarcoma therapy

Craig Gerrand, Newcastle upon Tyne, presented the UK ‘National Ewings Multidisciplinary Panel’, which began as a pilot project in 2011 as a forum to discuss the local treatment of patient with ES of bone. The aim was to improve the consistency of treatment decisions through

discussion and peer review. In the following 4 years, over 295 patients have been discussed and the concept has been proved. More work is required to move beyond the pilot phase if the panel is to fulfil its potential, and support data collection and clinical trials in this and possibly other sarcoma types.

Stephanie Klco-Brosius, Münster, presented the virtual ES tumor board, which has been set up in Münster.

Ruth Ladenstein, Vienna, reported on the European Expert Paediatric Oncology Reference Network for Diagnostics and Treatment (ExPO-r-Net) (<http://www.expornet.eu>). The main aim of ExPO-r-Net is to address and improve health inequalities for children with cancer in Europe by building a European Reference Network for Paediatric Oncology.

CONCLUSION

Bone sarcomas are ultra orphan-diseases with stagnation in survival for three decades. To further improve outcomes, international collaboration propelled by initiatives like the European Bone Sarcoma Network is essential. Novel insights from basic and clinical research that may help to improve sarcoma therapy have been discussed during the meeting and included results from investigations on tumor cell genomics, - epigenetics, - metabolism, and – altered signalling pathways, sarcoma immunology as well as the field of pharmacodynamics and pharmacogenomics and measurement of circulating tumor DNA. Moreover, experiences from recent sarcoma trials were reviewed and perspectives from patient and parent's representatives, clinical researchers, statisticians and charity representatives were summarized in order to provide knowledge that can help to make the designing of future clinical sarcoma trials easier. The

discussions generated many novel, exciting ideas, which now need to be taken forward to proposals for innovative trials, which are urgently needed. Such proposals for collaborative research and trials should be a major focus for the next European Bone Sarcoma Networking Meeting, which is scheduled to take place in London in 2017.

ABBREVIATIONS

ABCB1, ATP-binding cassette transporter B1; ATM-CHEK1, ataxia-telangiectasia-mutated serine/threonine kinase-checkpoint kinase 1; AXL, AXL receptor tyrosine kinase; BCOR, BCL6 corepressor; BRCA1/2, breast cancer 1/2, early onset; BRD, bromodomain-containing proteins; CCNB3, cyclin B3; CDDF, cancer drug development forum; CDKN2A, cyclin-dependent kinase inhibitor 2A; CIC, capicua transcriptional repressor; CIK, cytokine-induced killer; COL2A1, collagen, type II, alpha 1; CRUK, Cancer research UK; CS, chondrosarcoma; ctDNA, circulating tumor DNA; CTL, cytotoxic T-lymphocyte; CTU, clinical trials unit; DCC, DCC netrin-1 receptor; DUX4, double homeobox 4; EEA, European economic area; EEC, European Ewing consortium; EFACT, EWS-FLI sequence analysis from ctDNA; ENCCA-WP7, European network for cancer research in children and adolescents work package 7; EphA2, ephrin A receptor 2; ERCC1, excision repair cross-complementation group 1; ERG, v-Ets avian erythroblastosis virus E26 oncogene homolog; ES, Ewing sarcoma; EURAMOS, European and American osteosarcoma study II group; EuroSARC, European clinical trials in rare sarcomas initiative; EWSR1, EWS RNA-binding protein 1; ExPO-r-Net, European expert paediatric oncology reference network for diagnostics and treatment; EXT1, exostosin glycosyltransferase 1; FEV, (FEV) ETS oncogene

family; ¹⁸F-FLT-PET/CT, 3'-deoxy-3'-¹⁸F-fluorothymidine positron emission tomograph/computed tomography; FLI1, Fli-1 proto-oncogene, FUS, FUS RNA binding protein; ETS transcription factor; HDCHT, high-dose chemotherapy; HEY1, Hes-related family BHLH transcription factor with YRPW motif 1; HGOS, High-grade osteosarcoma; HLA, human leukocyte antigen; IDH1, isocitrate dehydrogenase 1; IGF-1R, insulin growth factor 1 receptor; IMP, investigational medical product; IR, insulin receptor; ISG, Italian sarcoma group; ITCC, innovative therapy for children with cancer consortium; iTRAQ, isobaric tags for relative and absolute quantification; LOH, loss of heterozygosity; LS, liposarcoma; MET, proto-oncogene, receptor tyrosine kinase; miRNA, micro RNA; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NCOA2, nuclear receptor coactivator 2; NFATc2, nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2; NGS, next-generation sequencing; NRAS, neuroblastoma RAS viral oncogene homolog; NTN1, netrin-1; PARP, poly ADP ribose polymerase; PATZ1, POZ (BTB) and AT hook containing zinc finger 1; PBSC, peripheral blood stem cells; PD-1, programmed cell-death 1; PD-L1, programmed cell-death ligand 1; PEA3, protein PEA3 (Ets variant 4); PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PROVABES, Prospective validation of biomarkers in Ewing sarcoma network; RANK, receptor activator of nuclear factor-kappa B; RB, retinoblastoma; RON, recepteur d'origine nantais (macrophage stimulating 1 receptor); RTK, receptor tyrosine kinase; SIOPE, European society for pediatric oncology; siRNA, small interfering RNA; SARC, sarcoma research through collaboration; SNP, single-nucleotide polymorphism; SSG, Scandinavian sarcoma group; TP53, tumor protein 53; TRAP, tartrate-resistant acid phosphatase; UNC5H, Unc-5 homolog C; VEGFA, vascular endothelial growth factor A.

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Authors' contributions

LK and SB participated in the conception and design of the manuscript, collected the data from all coauthors, led the drafting of the manuscript, and revised the article critically.

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