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A Summary of the Osteosarcoma Banking Efforts: A Report from the Children's Oncology Group and the QuadW Foundation

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Abstract

Background—Survival rates of patients with osteosarcoma have remained stagnant over the last thirty years. Better understanding of biology, new therapeutics, and improved biomarkers are needed. The Children's Oncology Group (COG) addressed this need by developing one of the largest osteosarcoma biorepositories ever, containing over 15,000 tumor and tissue samples from over 1,500 patients.

Procedure—The biology study P9851 and the banking study AOST06B1 has enrolled 1,787 patients (as of September, 2013). Clinical information was lacking on 510 patients on P9851, who were not enrolled on a concurrent therapeutic trial. The value of these specimens was diminished. The lack of statistical support available for biology projects slowed the analysis of several critical studies. The QuadW Foundation, CureSearch, and the COG formed the Childhood Sarcoma Biostatistics and Annotation Office (CSBAO) to provide the infrastructure and address these needs by linking clinically annotated patient data to archived tissue samples and to develop biostatistical support for childhood sarcoma research.

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Results—Originally 5.3% of samples from the 510 patients on P9851 not enrolled on a therapeutic study had full clinical annotation. The efforts of the CSBAO have linked clinical annotation to 90.8% of those specimens and provided statistical analyses to several studies that had used COG samples. As a result, 24 biology studies in osteosarcoma have been completed and published in peer-reviewed journals.

Conclusions—These samples and *in-silico* data are available to the research community for basic and translational science projects to improve the biological understanding and treatment of patients affected by osteosarcoma.

Keywords

Osteosarcoma; Banking; Biology; Bone; Sarcoma

Introduction

Osteosarcoma is the most common malignant bone tumor diagnosed in children. There are approximately 400 new pediatric and adolescent patients with this diagnosis per year in the US [1]. Over 90% of osteosarcomas are considered high-grade neoplasms. Multidisciplinary treatment with both surgery and chemotherapy provides patients with the greatest opportunity for survival. The standard chemotherapeutic regimens used are long and intensive utilizing cisplatin, doxorubicin and high dose methotrexate with or without ifosfamide and etoposide. These agents have a high incidence of late effects including cardiotoxicity, hearing loss, decreased fertility, and development of a secondary malignancy.

Using existing therapy, children with localized disease have approximately a 70-80% chance of being free of disease after 5 years post-treatment. At the time of diagnosis, 15-20% of patients will present with metastatic disease [2]. The presence of metastatic disease at diagnosis is the most important prognostic factor for patients. Children with metastatic disease at diagnosis only have a 10-30% chance of being disease-free after 5 years [3,4].

Another important treatment principle in osteosarcoma therapy is the need to achieve good local control. Many children undergo surgical resection that leaves them with poor cosmetic and/or functional results. A number of children are left with nonfunctional limbs and must undergo a later amputation, which will affect body image throughout life [5]. Currently, there is no reliable method to identify which patients will respond to therapy. Patients would benefit greatly, if biologic markers were developed to risk stratify surgical procedures and chemotherapeutic treatment regimens. An improved understanding of the biology of osteosarcoma is needed to improve patient outcomes.

We are in the nascent stages of understanding what genetic drivers play a part in osteosarcoma oncogenesis in relation to other sarcomas. Unlike Ewing Sarcoma, which has a balanced karyotype with the uniform reciprocal translocation (EWS/FLI1), osteosarcoma appears to be a much more heterogeneous disease with complex karyotypes [6]. The known genetic mutations associated with osteosarcoma include the *P53* gene associated with Li Fraumeni Syndrome, the *RBI* tumor suppressor gene associated with hereditary retinoblastoma, *BLM* gene seen in Bloom syndrome, and *RECQL4* gene associated with

Rothmund-Thomson syndrome. Currently no genetic alterations are effective in predicting clinical outcomes for patients with osteosarcoma. In this age of personalized medicine we need to understand the genetic mechanisms of osteosarcoma development to identify druggable targets, overcome resistance to therapy, and further risk stratify individual patient treatments.

Osteosarcoma researchers have recognized the need to better understand the biology of osteosarcoma to improve outcome since the late 1990's. To facilitate biologic discoveries, the Children's Oncology Group established a biospecimen repository with the goal of having each sample linked to clinically annotated information and patient outcome. Over 15,000 samples (see Table I) of the Osteosarcoma Biospecimen Repository have come from the two prospective studies described below.

Methods

P9851 Osteosarcoma Biology Protocol: Companion to Group-Wide Therapeutic Studies

P9851 is a legacy biology study that intended to increase the knowledge of osteosarcoma biology and accelerate the discovery of novel therapeutic targets, uncover prognostic indicators and biomarkers of outcome. Other goals of P9851 were to develop a reliable centralized biospecimen repository with appropriate quality controls that could distribute tissues to scientific investigators. P9851 also served as a companion biology study to the contemporaneous treatment studies P9754 (Protocol for Patients with Newly Diagnosed, Non-metastatic Osteosarcoma: A Pilot Study) and AOST0121 (A Group-wide Phase II Study of Trastuzumab Herceptin in Metastatic Osteosarcoma Patients with Tumors that Overexpress HER2). Subsequently, P9851 served as a companion to AOST0331 (A Randomized Trial of the European and American Osteosarcoma Study Group to Optimize Treatment Strategies for Resectable Osteosarcoma Based on Histological Response to Pre-Operative Chemotherapy) until the opening of the current and active banking study AOST06B1.

From 1999 to 2008, P9851 enrolled 1,110 (1,105 eligible) patients from 211 COG institutions (Figure 1). During the time span that P9851 was accruing tumor samples derived from patients for biologic banking purposes, there were periods of time when there was no actively open accompanying treatment study for patients with localized or metastatic disease. Additionally, the requirement for clinical follow up and data tracking was not explicitly mandated for patients not co-enrolled on a treatment study. As a result thorough clinical data collection was suboptimal and incomplete. Indeed, although 5,369 tissue and tumor samples were collected on 1,105 patients, 46.1% of the patients enrolled on P9851 were not enrolled on any accompanying treatment study. As such, the majority of these patients had no clinically linked follow up data, which diminished the value of the tumor bank for conducting biology driven investigations. Ultimately, P9851 was closed on July 7, 2008, with the opening of its successor study AOST06B1.

AOST06B1 A Children's Oncology Group Protocol for Collecting and Banking Osteosarcoma Specimens

AOST06B1 was developed as a tumor banking study to collect biological specimens from osteosarcoma patients from COG institutions, and to provide a repository for long term storage of the tumor and related tissue samples. The main goal of AOST06B1 was to prospectively collect tissue specimens and make these biologic samples available to qualified scientific researchers to understand the biology of osteosarcoma and to be able to correlate the results with the patients' clinical data.

AOST06B1 did not have the specific biological aims entailed with its predecessor P9851, and therefore had the advantage of open-ended sample acquisition protocol. As of September 13, 2013, AOST06B1 had enrolled 689 patients with 682 being eligible (Figure 1). AOST06B1 is an active study and continues to enroll patients. Institutional Review Board approvals were current for 143 institutions, not current for 11 institutions and terminated at 2 institutions. Approximately 75% of these patients at present are concurrently enrolled on AOST0331, which is still partially under the purview of its Data and Safety Monitoring Committee, so treatment and survival data cannot currently be released for some patients. The recent results of the "good-responder" arm of AOST0331 have been presented, and the clinically correlative results of the "poor responder" arm are anticipated in the near future.

Both AOST06B1 and AOST0331 studies required IRB approved informed consent by all participating institutions prior to enrolling patients. Institutions were required to submit either formalin-fixed paraffin-embedded (FFPE) tissue block or 30 slides derived from initial diagnostic biopsy, definitive surgery, recurrence, and autopsy. The collection of pretreatment serum, plasma and whole blood were required. In addition, the collection of snap frozen tissue and fresh tissue in culture media submitted at diagnostic biopsy, definitive surgery, recurrence, and autopsy was strongly encouraged if applicable. Clinical data that was obtained for each patient included age at enrollment; primary tumor site; metastasis site (if any); sex, ethnicity, and race of the patient; time to last patient contact; time to relapse; time to second malignant neoplasm; and time to death. For patients co-enrolled on a therapeutic study, additional data (e.g., treatment details, percent necrosis, and surgery details) are available depending on the therapeutic study.

Results/Discussion

As outlined above, Surveillance Epidemiology and End Result (SEER) data approximates that in the U.S. 400 new cases per year of osteosarcoma are diagnosed in the pediatric and adolescent population [7]. From the opening of P9851 on September 15, 1999 to September 30, 2013, 3,935 children were treated for osteosarcoma at U.S. COG institutions (302 children per year). During these 13 years 1,337 children (33.9 % of children treated at U.S. COG institutions) were enrolled on a frontline treatment study, and 1,586 children (40.3 % of children treated at U.S. COG institutions) were enrolled on biology/banking studies described in this paper.

The Children's Oncology Group, the QuadW Foundation, American Association of Cancer Research, and CureSearch recognized that a substantial proportion of samples derived from P9851 and AOST06B1 in the biorepository lacked clinically linked data, and the overall value of the repository was diminished for conducting biology-driven studies. Despite the accrual success, samples collected within P9851 were of limited biological value since clinical annotation was available for only 5.3% of samples from the 510 patients not enrolled on a therapeutic study. To address this issue, the CSBAO interfaced with all of the treating institutions that had patients lacking clinically annotated data in order to recover critical information. The CSBAO had P9851 added to the COG Scheduled and Delinquent Data (SADD) report, and contacted relevant sites requesting enrollment data not covered by the SADD report. Sites with large amounts of missing data or particularly valuable data were directly contacted by members of the CSBAO advisory committee and encouraged to submit their missing data. Through these efforts the CSBAO has now successfully linked clinical annotation to 90.8% of those initial 510 specimens and provided statistical analyses. (Figure 2) We believe that the maximum amount of data from this subset of P9851 has been recovered.

The lack of clinical annotation to samples had also stalled the biostatistical analysis of several studies that had requested and used samples from the bank. This backlog of studies required biostatistical support to advance the translational goals and publication of many of these studies. Through the CSBAO, twenty four biology projects that had been back-logged have been completed using samples from P9851 or AOST06B1. All of those have been successfully published in peer-reviewed journals (Table II) [6,8-29]. The CSBAO has also provided support to two large genomic and genetic studies in osteosarcoma. Briefly, these include the TARGET and SPECS studies in osteosarcoma genomics and the first Genome-Wide Association Studies (GWAS) in osteosarcoma.

Emerging genetic studies- TARGET and SPECS Discovery

Banked tumor tissue samples from 72 patients have been given to the NCI's Therapeutically Applicable Research to Generate Effective Treatments (TARGET) program to improve the understanding of osteosarcoma biology through comprehensive genomic approaches. Data derived from the TARGET initiative will include genome-wide analysis of matched tumor and normal samples, which have been performed using gene expression (Affymetrix Human Exon 1.0 STR arrays), copy number and loss of heterozygosity (affymetrix SNP 6.0 arrays), DNA methylation (Illumina Infinuim 450K methylation assay), miRNA analysis (MegaPlex Taq Man platform), whole genome, whole exome, and transcriptome sequencing (mRNA-seq). The TARGET program data from 72 COG patients with osteosarcoma is being sequentially released and is available at: http://target.nci.nih.gov/dataMatrix/TARGET_DataMatrix.html

Genome-wide Association Study Identifies Novel Loci Associated with Osteosarcoma

Recently GWAS were conducted through an international multi-institutional collaborative effort using samples from our biorepository and assistance from the CSBAO [27]. As a result, two loci achieved genome wide significance, rs1906953 at p621.3, as well as and the glutamate receptor metabotropic 4 (*GRM4*), rs7591996, and rs10208273 in a gene desert on

p2p25.2. *GRM4* is expressed in osteoblasts, osteoclasts, and human osteosarcoma cell lines. Additionally *GRM4* expression is associated with poor prognosis in colorectal cancer, multiple myeloma, rhabdomyosarcoma and other pediatric CNS tumors. Hopefully these recently identified loci will be new targets for which novel therapeutics may be developed.

Tissue Microarray Development and Distribution

In addition to patient samples we have developed a tissue microarray from the P9851 study population, which is available to investigators. There are 52 patient samples on the array as well as 10 healthy tissues as controls. Thirty-eight of the 52 samples have clinically annotated data available. Although the tissue microarray has a smaller percentage of patients with metastatic disease, we believe the 38 osteosarcoma patients with follow-up on the TMA are representative of the general patient population of P9851 patients with follow-up.

High Dimensional Database Development

An imperative ongoing direction for the COG Osteosarcoma Biospecimen Repository will be to link biological data generated by investigators to the physical and clinical data for banked samples. We have begun to collect this data into a High Dimensional Database (HDD) that will be available for qualified researchers to conduct *in silico* studies. The HDD is a scalable data management platform customized to support the complexities of a wide diversity of data types collected within the work of a cooperative group. The hope is that a number of future requests will be for data on completed studies rather than requests for tissues. This will decrease shipping costs and benchside expenses. The HDD currently contains data from projects of ezrin immunostaining, serum proteomics, ErbB-2 status, telomerase expression, IGF-1/IGF-1R axis, chromosomal instability, and Fas expression allelotype/genotype. Eventually it will also contain data including Strategic Partnering to Evaluate Cancer Signatures (SPECS) gene expression data, genome wide association study data, and array data from the TARGET project.

Infrastructure Development and Pursuing Other Childhood Sarcomas

In addition to the primary goals of the CSBAO a new tracking platform, JIRA was instituted to manage biospecimen requests for osteosarcoma samples. Samples (including tissue microarray samples) may be requested at: <https://ccrod.cancer.gov/confluence/display/OSTEOSR/Osteosarcoma+Sample+Request>

Also a standard operating procedure was initiated for collecting high quality specimens for AOST06B1. The next direction the CSBAO will take is to expand its efforts to aid in biostatistical support and improve the clinical annotation to other childhood sarcomas, most recently Ewing Sarcoma and Soft Tissue Sarcomas. There are proactive plans to enrich the number of metastatic childhood sarcoma (Ewings, Soft Tissue Sarcoma, and Osteosarcoma) samples and paired primary and metastatic samples over the next three years [30]. In that time we aim to collect paired primary and metastatic samples from at least 15 patients, frozen metastatic tumor samples from 30 patients and paraffin embedded tumors from at least 50 patients. Finally we hope to develop tissue microarrays that contain paired primary and metastatic tissues from the same patient. We also hope to increase the number of

samples obtained from autopsy, as they are eligible for collection per AOST06B1, however to date these have not been large sources of patient samples.

Conclusions

As a result of the two osteosarcoma banking studies P9851 and AOST06B1 prospectively initiated and supported by the efforts of COG, CureSearch, QuadW Foundation and the CSBAO, one of the world's largest tumor banks of osteosarcoma samples has been established. 40.3% of patients with osteosarcoma treated at COG institutions have enrolled on biology/banking studies. Additionally the value the COG Osteosarcoma Biospecimen Repository has been increased significantly by the efforts of the CSBAO by obtaining missing clinical annotation. A focus of the bone tumor committee at COG is to develop pre-clinical and human datasets that are focused on metastatic progression as it is the most important cause of mortality in osteosarcoma. Our goal is to continue to provide high quality specimens of tumor and related tissue samples to qualified researchers with projects that advance the understanding and treatment of patients with osteosarcoma. These samples are collected with the objective of being distributed to the scientific and research community for the discovery of new genetic targets that may be used to better define the likelihood of survival, track response to therapy, and explain the development of metastasis.

We will continue to provide biostatistical support to projects that utilize the repository. Additionally we have created a High Dimensional Database that will allow researchers to answer biological questions from existing biorepository data. We encourage the scientific community to utilize all of these resources. It is our hope that the continued efforts of the CSBAO and its efforts to foster childhood sarcoma research will improve the understanding of metastatic disease, mechanisms of resistance, development of biomarkers, and discovery of novel therapies.

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P9851

1,110 patients enrolled

1,105 patients eligible

3 ineligible due to misdiagnosis

2 ineligible due to administrative reasons

5,639 tissue and tumor samples

AOST06B1

689 patients enrolled

682 patients eligible

2 ineligible due to misdiagnosis

5 ineligible due to administrative reasons

10,839 tissue and tumor samples

Figure 1.

Distribution of the patients enrolled and samples obtained on P9851 and AOST06B1 through September 13, 2013.

Figure 2.

Recovery rate of clinical data for patients enrolled on P9851 not contemporaneously enrolled on a therapeutic trial is shown above. The recovery of data was much quicker than expected. As of April 2011 any additional data beyond the 90.8% was determined to be irretrievable.

Table I

Distribution of available tissues to researchers.

	P9851	AOST06B1
Blood Ficoll WBC	187	298
Blood Fresh	34	14
Blood Frozen	1	94
Bone Marrow Ficoll WBC	4	2
DNA	6	3
Fresh Unstained Touch Prep Primary	130	7
Frozen OCT Stained H&E Normal	1	
Frozen OCT Stained H&E Primary	61	99
Frozen Tissue RNAlater Primary		2
OCT Embedded Tissue Metastatic	3	2
OCT Embedded Tissue Normal	4	
OCT Embedded Tissue Primary	304	101
Other	11	94
Paraffin Block Core Biopsy Primary		1
Paraffin Block Metastatic	7	7
Paraffin Block Normal	11	
Paraffin Block Primary	304	188
Paraffin Scroll Metastatic	1	1
Paraffin Scroll Primary	67	6
Paraffin Stained H&E Core Biopsy Primary		1
Paraffin Stained H&E Metastatic	2	9
Paraffin Stained H&E Primary	1,719	971
Paraffin Unstained Metastatic	58	124
Paraffin Unstained Primary	1,288	5,251
Paraffin Unstained Core Biopsy Metastatic		29
Paraffin Unstained Core Biopsy Primary		30
Plasma Frozen	14	1,487
Serum Frozen	826	1,077
Snap Frozen Tissue Metastatic	16	7
Snap Frozen Tissue Normal	7	
Snap Frozen Tissue Primary	295	150
Total Samples	5,396	10,839

Table II

Publications of osteosarcoma biology projects produced with either samples from P9851 and AOST06B1 or with support from the CSBAO

Publications produced from samples collected on P9851	Journal
Biology of childhood osteogenic sarcoma and potential targets for therapeutic development: meeting summary	Clin Cancer Res. 2003 Nov 15; 9(15): 5442-53.
The membrane-cytoskeleton linker ezrin is necessary for osteosarcoma metastasis.	Nat Med. 2004 Jan 4 Epub 2004 Jan 04
Determination of a minimal region of loss of heterozygosity on chromosome 18q21.33 in osteosarcoma	Int J Cancer. 2003 Jun 10; 105(2): 285-8.
Sequence alterations in the reduced folate carrier are observed frequently in osteosarcoma tumor samples	Clin Cancer Res 2003; 9:837-844.
PHC3, a component of the hPRC-H complex, associates with E2F6 during G(0) and is lost in osteosarcoma tumors	Oncogene 2007; 26:1714-22. Epub Sep 2006.
Wnt/LRP5 signaling by a soluble receptor modulates the epithelial to mesenchymal transition and suppresses met and metalloproteinases in osteosarcoma Saos-2 cells	J Orthop Res. 2007; 25:964-971. Epub Feb 2007.
Multiple Drug Resistance in Osteogenic Sarcoma (INT0133)	J Clin Oncol 2007; 25:2057-2062.
Over-expression of parathyroid hormone type 1 receptor confers an aggressive phenotype in osteosarcoma	Int J Cancer. 2007; 121:943-954. Epub April 2007.
The folate receptor alpha is frequently over-expressed in osteosarcoma samples and plays a role in the uptake of the physiological substrate, 5-methyl-tetrahydrofolate	Clin Cancer Res 2007;13:2557-2567
Platelet-derived growth factor receptor as a prognostic marker and a therapeutic target for imatinib mesylate therapy in osteosarcoma	Cancer. 2008; 112:2119-2129. Epub Mar 2008.
Quantitative correlation between promoter methylation and messenger RNA levels of the reduced folate carrier	BMC Cancer. 2008;8:124.
Hypoxia markers in human osteosarcoma: An exploratory study	Clin Orthop Related Res. Epub Jun 2008.
Polymorphisms and methylation of the reduced folate carrier in osteosarcoma	Clin Orthop Related Res. Epub Jun 2008.
Cell cycle regulator gene CDC5L, a potential target for 6p12-p21 amplicon in osteosarcoma	Cancer Res. 2008;6:937-946
Blocking Wnt/LRP5 signaling by a soluble receptor modulates the epithelial to mesenchymal transition and suppresses met and metalloproteinases in osteosarcoma Saos-2 cells	J Orthop Res. 2007 Jul;25(7):964-71.
The actin-cytoskeleton linker protein ezrin is regulated during osteosarcoma metastasis by PKC	Oncogene. 2009; 28: 792-802. Epub Dec 2008
Publications produced with assistance from the CSBAO	Journal
Investigation of the insulin-like growth factor-1 signaling pathway in localized Ewing sarcoma.	Cancer 117 (21): 4966–4976.
Detectable clonal mosaicism and its relationship to aging and cancer.	Nature Genetics 44 (6): 651–658.
Poor Survival for Osteosarcoma of the Pelvis: A Report from the Children's Oncology Group.	Clinical Orthopaedics and Related Research 470 (7): 2007–2013
Phase II Trial of Trastuzumab in Combination With Cytotoxic Chemotherapy for Treatment of Metastatic Osteosarcoma With Human Epidermal Growth Factor Receptor 2 Overexpression: A Report From the Children's Oncology Group.	J Clin Oncol 30 (20): 2545–2551.
Outcome for adolescent and young adult patients with osteosarcoma.	Cancer 118 (18): 4597–4605
Analysis of serum Insulin Growth Factor-1 concentrations in localized osteosarcoma: A Children's Oncology Group Study	Pediatric Blood & Cancer 61 :4: 749–752
High-BMI at diagnosis is associated with inferior survival in patients with osteosarcoma: A report from the Children's Oncology Group.	Pediatric Blood & Cancer 60 (12): 2042–2046
Genome-wide association study identifies two susceptibility loci for osteosarcoma.	Nature Genetics 45 (7): 799–803