Childhood Cancer Survivor Study
Analysis Concept Proposal

Original Title: Long-term Follow up of Survivors of Childhood Osteosarcoma: A Report from the Childhood Cancer Survivor Study (CCSS)

Working Groups:

Primary working group: Chronic Disease
Secondary working groups: Secondary Malignancies, Cancer Control

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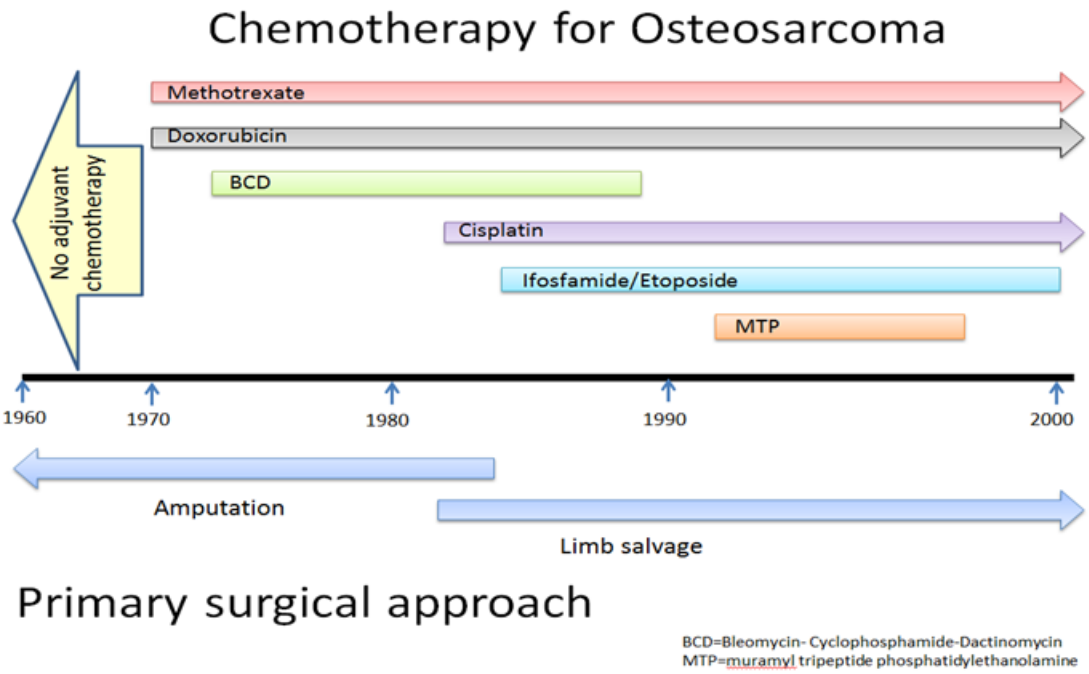
Background and Rationale:
Osteosarcoma (OS) is the most common primary bone tumor occurring in children, with the highest frequency in adolescents. It accounts for approximately 5% of childhood cancer diagnoses [1]. The treatments have evolved since the 1970's, with the addition of chemotherapy and transitioning from amputation to limb salvage procedures. These advances have led to improved outcomes from overall survival from 10-20% to 60-70% [2]. The changes in therapy throughout this time and the consequent late effects are the focus of this proposal.

Evolution of treatment in Osteosarcoma
Prior to the 1970's, the treatment for patients with localized osteosarcoma was surgical, resulting in a 10-20% 5-year survival rate [3]. Radiation therapy as an alternative strategy for local control was also explored prior to the 1970's, but was less effective than amputation [4, 5]. The rapid development of distant metastases occurring in patients following surgery suggested that micro metastases existed at the time of diagnosis for many patients; the addition of systemic chemotherapy addressed this issue [2].

Chemotherapy was introduced into the treatment of osteosarcoma in the early 1970's. The first two chemotherapy agents reported to demonstrate improvement in metastatic tumor regression were doxorubicin and methotrexate [6, 7]. At the same time, larger randomized trials were conducted to evaluate survival outcomes comparing adjuvant chemotherapy plus surgery to surgery alone. Single center studies in the mid 1970's using a combination of chemotherapy agents demonstrated significant improvement in 5-year EFS compared to historical controls. Chemotherapy agents used initially included methotrexate, doxorubicin, and bleomycin-cyclophosphamide-dactinomycin (BCD). Cisplatin was initially added for poor responders, but eventually was incorporated in upfront chemotherapy regimens [8]. The first multi-institutional study evaluating adjuvant chemotherapy (BCD, methotrexate, doxorubicin, and cisplatin) versus surgery alone, demonstrated clear a 2-year EFS of 66% in the chemotherapy group compared to 17% in the surgery only group [9]. A second study completed in the same time period reported similar results, with an improvement in 2-year EFS of 55% with adjuvant chemotherapy (BCD, methotrexate, doxorubicin, and vincristine) compared to 20% with surgery alone [10]. However, a study from the Mayo clinic in the early 1970's reported a surgery only group reaching an overall survival of 50% [11], resulting in a delay in the acceptance of chemotherapy as standard of care in osteosarcoma.

Both single and multi-institution studies evaluated a variety of combinations and administration timing strategies for chemotherapy along with surgery for patients with osteosarcoma throughout the 1980's resulting in 5-year EFS ranging from 40-66% and 5-year OS ranging from 50-72% [12-17]. Methotrexate and doxorubicin was used throughout these studies, with a slower but eventual standard incorporation of cisplatin. In the mid-1980's ifosfamide and etoposide were introduced into chemotherapy regimens after showing some activity in relapsed osteosarcoma [18, 19]. The BCD combination stopped being a part of the study regimens around that same time and was not used in any large trials in the 1990's. A COG study evaluated the effect of the addition of Muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE) to standard chemotherapy (MTX, doxorubicin, cisplatin, +/- ifosfamide). The results showed a significant improvement in OS from 70% to 78%. No improvement in survival outcomes was noted with the addition of ifosfamide [20], raising questions regarding the utility of ifosfamide as part of upfront therapy. Most recently, the multi-institutional international randomized trial (EURAMOS 1) showed that the addition of pegylated interferon alfa-2b to good responders or ifosfamide and etoposide to poor responders to standard chemotherapy (doxorubicin, cisplatin, and high-dose methotrexate) did not improve patient outcomes [21]. Trends in chemotherapy use over the diagnosis years covered by CCSS are depicted in the figure below.



Local control

Surgery is the treatment of choice for local control in osteosarcoma. In the US, the use of amputation gave way to the limb salvage therapies by the mid to late 1980's, as improvement in pre-surgical chemotherapy treatments, improved imaging modalities, and improved prosthetic surgical materials and techniques became available [22]. Limb salvage was shown to have similar survival rates compared with amputation procedures for osteosarcoma in multiple retrospective analysis using historical controls [23, 24], and in a small randomized study (n=43) [25]. The National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) program has collected basic surgical information on pediatric sarcoma patients since 1988. A SEER report has found increased use of limb salvage therapies over this period, with 70 percent of osteosarcoma patients having this procedure in the second decade of the reporting period compared with 50% in the first decade [22]. Data regarding the long term survival outcomes for limb salvage vs. amputation are limited. It also remains unclear how the surgical approach impacts long-term health status. Long-term complications that may differ for amputation vs. limb salvage include need for further surgeries, pain, and functional limitations. Other approaches to local control such as radiation for those with unresectable disease[26, 27] and rotationplasty as used often in Europe may result in different outcomes. OS is highly radioresistant and radiation doses of less than 6,000 cGy have been associated with only transient tumor control.[28] Higher doses used as adjuvant therapy are likely to have long term local consequences.

Pulmonary Metastases –Surgical approach

Resection of pulmonary metastasis from primary osteosarcoma is now a well-established practice that dates back to a report by Martini and colleagues over four-and-a-half decades ago, when metastasectomy conferred a survival advantage over chemotherapy alone [29]. Approximately 20% of patients present with metastatic lesions to the lung, whereas an additional 20% develop pulmonary metastasis over the course of disease [30]. Numerous other studies have documented the utility of repeated, open thoracic explorations for the removal of all evidence of disease as this confers a survival advantage [31-33]. However, it is not clear that aggressive extirpative procedures are required or indicated in all cases, and the role of pulmonary metastasectomy remains a question for debate. Regardless of the role of surgery for pulmonary tumor clearance, pulmonary metastases from osteosarcoma can be small (<2 mm) lesions and, as such, radiographically underestimated [34]. If surgery is undertaken, it is important to directly inspect and palpate the entirety of the lung parenchyma in order to localize and resect all evidence of disease, as feasible [31]. Parenchymal preserving approaches should be utilized in all cases, as patients may develop metachronous metastatic foci that mandate subsequent repeated resections. Relative contraindications include miliary disease, hilar involvement, and pleural involvement unless complete resection is anticipated [30]. Finally, metastasectomy should not be performed until staging is complete and there is definitive disease control at the primary site in the absence of other metastatic sites.

Outcomes

Chronic Health Conditions

Prior CCSS studies that examined survivors diagnosed 1970-1986 established that these adult survivors of childhood cancers were more likely to report adverse health outcomes, have a high prevalence of chronic medical conditions and often have high utilization of the health care system [35-37]. Survivors of bone tumors experienced many of the same long-term effects, but also have potential outcome challenges that are unique to them. The CCSS has found that survivors of soft tissue sarcomas and bone tumors were more likely to report adverse health status as compared to survivors of leukemia [35].

It is well established that anthracycline exposure increases the risk of chronic cardiac conditions in childhood cancer survivors [36]. Survivors of osteosarcoma have a high rate of exposure to anthracycline dosing throughout many of the treatment eras described above. The addition of dexrazoxane for cardiac protection in the setting of anthracycline administration has been evaluated and found to not interfere with treatment efficacy in patients with sarcomas [38]. Dexrazoxane has also shown promising early effects on acute cardiotoxicity when administered with doxorubicin, however the long-term effects require continued evaluation [39].

Cisplatin has been associated with both short and long-term ototoxic effects, most notably hearing loss and vertigo [40]. There are also documented long term renal effects, most notably hypomagnesemia, which are estimated to be near 10% in sarcoma survivors [41].

Osteosarcoma patients who received alkylating agents (ifosfamide or cyclophosphamide) are at risk for dose related gonadal dysfunction, in both males and females [37, 42, 43]. One study estimated that 6% of osteosarcoma patients experience early menopause, but no large cohorts

of osteosarcoma survivors have been evaluated for long term fertility and pregnancy outcomes [44].

While bleomycin is no longer used in conventional osteosarcoma chemotherapy, a substantial population of osteosarcoma survivors received this agent. Bleomycin is associated with long-term adverse pulmonary outcomes, often reported as subclinical changes on pulmonary function tests (PFTs) [45]. Assessment of clinical symptoms related to bleomycin have not been evaluated in a population of long-term osteosarcoma survivors; a short-term follow study reported transient changes in PFTs during therapy [46] which warrants further follow up. Given that osteosarcoma patients may often have lung surgery (for pulmonary metastases), those who were also exposed to bleomycin may be at particularly high risk of additive late pulmonary complications.

Secondary Malignancies

Within chronic disease outcomes, secondary malignant neoplasms (SMNs) represent a clinically significant late effect. A report from the CCSS on the initial cohort of participants found a statistically significant excess of SMNs in all childhood cancer diagnoses [47]. Identified risk factors for greatest risk of development of SMNs included radiation exposure, being diagnosed at a younger age, being female, and having an initial diagnosis of Hodgkin Lymphoma [47-49]. Fortunately, only a small proportion of osteosarcoma survivors receive radiation therapy, however they still experience exposures of concern. One publication demonstrated an increased risk of SMNs in bone tumor survivors, RR 1.31 (0.76-2.24) [47]. Increased risk of SMNs with anthracycline and alkylating agent exposure have been reported, which is important as anthracyclines are a consistent exposure in the OS population [47, 50].

The other consideration in sarcoma patients is the possibility of an underlying genetic predisposition, particularly a *p53* gene mutation. Childhood osteosarcoma diagnoses can be associated with *p53* mutations in up to 9% of patients with a new diagnosis [51], however testing is often not routinely done without other clinical indications (prior cancer diagnoses, strong family history of certain cancers). Osteosarcoma can be the first cancer suggestive of a p53 mutation in families that are ultimately identified as having Li-Fraumeni syndrome (LFS) [52]. Retrospective testing of patients with second malignancies demonstrated that 7% had a *p53* mutation without any family history suggestive of that diagnosis [53]. This raises the concern that there may be an increase in late secondary malignancies in osteosarcoma patients, as they are at risk of having a previously unknown genetic predisposition. A second CCSS analysis reported an increased incidence of breast cancer in sarcoma survivors [54] without a known history of LFS. Detailed analysis of SMNs in OS survivors with a focus on LFS-related tumors has not been reported to date, and may provide insights into whether this population might benefit from more routine germline testing in the future.

Self-Reported Health Status

The self-reported health and functional status of survivors of osteosarcoma are important to evaluate. This population faces a unique set of challenges in regard to both physical and emotional outcomes, as their treatments involve significant surgical interventions that can have

a profound effect on body image, mobility, and function. Prior CCSS reports describe outcomes of self-reported health status across six major domains: general health, mental health, functional status, activity limitations, cancer-related pain and cancer-related anxiety/fear. A report from the initial cohort by Hudson, et al, in 2003 reported that compared to leukemia survivors, bone tumor survivors had increased risk for adverse health status across many domains including general health, functional status, activity limitations, pain and anxiety [35]. A more recent report that described temporal changes over three treatment era decades by Ness, et al, found that overall there has been no significant improvement in self-reported health status over time from 1970-1999 despite treatment changes and improved outcomes [55]. Both studies demonstrated continued increased risk of adverse health outcomes (particularly pain) in osteosarcoma survivors compared to other survivors across this time period. Another prior CCSS study examined the effect of limb salvage vs amputation in sarcoma survivors treated from 1970-1986 and found no differences in self-reported health status [57]. Many other studies have evaluated self-reported health status in bone sarcoma survivors, but a meta-analysis of these studies concluded that while there seems to be a trend in lower status among bone sarcoma patients compared to other diagnoses, the measures used were not uniform and were difficult to interpret [56]. Overall, given the changes in osteosarcoma treatment, including the growing use of limb salvage in place of amputation, there is need to understand the impact of more contemporary treatment on self-reported health status.

Summary

This study of the entire CCSS cohort will evaluate the late morbidity and mortality in osteosarcoma survivors, including chronic medical conditions and secondary malignancies, and the associated QOL and health status. The study will evaluate how the outcomes differ based upon the type of therapy patients received, as the therapies have changed over time. The notable changes in medical and surgical therapy for the sarcoma patients have potential to have many effects on long term effects. This study will be valuable in helping oncologists and surgeons educate current patients on expected long-term outcomes, as the current osteosarcoma treatment does not largely differ from the latter half of the patients included in this analysis.

Specific Aims:

Aim 1: Estimate all-cause late mortality for all 5-year survivors of OS diagnosed from 1970-1999. We will compare late mortality for cohorts based on treatments of interest including

a. Chemotherapy (methotrexate+doxorubicin+cisplatin (MA) vs. methotrexate+doxorubin (MA) vs. methotrexate+doxorubicin+BCD (MA-BCD))
b. Primary surgical approaches (limb salvage vs. amputation; metastatectomy vs. no metastatectomy).

We will estimate mortality from competing causes of death: cancer related causes (related to recurrence or progression of original cancer), health related causes (conditions that exclude recurrence or progression of original cancer and external causes but include late effects of cancer therapy), and external causes (unrelated to the cancer diagnosis),

Hypothesis: Improvement in mortality from recurrence will be seen with-use of MAP chemotherapy combination vs MA or MA-BCD chemotherapy combinations; an impact on health related outcomes may be identified. No change in mortality from recurrence will be seen between survivors based on the initial surgical approach to extremity tumor.

Aim 2: Determine the prevalence and incidence of chronic health conditions (adapted CTCAE grades 1-5 and grades 3-5 categories) for survivors of osteosarcoma and identify risk factors based on specific treatment exposure. Subgroup analysis will be conducted to determine specific chronic health conditions related to cumulative doses of treatment, and based upon exposure groups of interest (MA, MA-BCD, MAP). (For example, pulmonary outcomes with exposure to bleomycin, cardiomyopathy-related outcomes with exposure to anthracyclines, ototoxicity with exposure to cisplatin, venothromboembolic disease with limb-sparing surgery)

Hypothesis: Dose dependent relationships will be observed for chronic health outcomes related to specific chemotherapy exposures.

Aim 3: Determine the incidence of secondary malignant neoplasms (SMN) and compare according to treatment exposures in OS survivors. While it is captured within chronic health conditions, we are interested in looking at details of SMNs in this population. Exposures to be evaluated: alkylating agents, anthracyclines, cisplatin, etoposide, primary tumor radiation, and whole lung radiation. Additionally, will evaluate SMNs based on major treatment groups (MA, MA-BCD, MAP).

SMNs other than basal cell carcinomas will be compared by cumulative dosing categories to the exposures of interest. Additionally, we will determine if there are any relationships between patient characteristics (diagnosis of localized vs metastatic disease, age at diagnosis) and incidence of SMNs. We will specifically evaluate the incidence of secondary cancer types that are known to be related to germline mutation in the p53 gene (see appendix 1) given the known association of OS with this genetic condition. Additionally, we will evaluate for any effects therapeutic exposures of interest may have had on this group of interest (specifically radiation therapy, anthracycline exposure, and alkylating agent exposure).

Hypotheses:

1) *Increased SMNs will occur in patients with increased cumulative dosing categories of anthracyclines, and in those patients exposed to alkylating agents in addition to anthracyclines.*
2) *The standard incidence rate (SIR) for secondary malignancies associated with p53 mutations will be more significantly increased than those for other secondary malignancies (with a possible exception of leukemia that may be induced by standard chemotherapies used in OS).*

Aim 4: Evaluate the effect of the changes in OS treatment on adverse health status for OS survivors, across six domains: general health, mental health, functional status, activity limitations, cancer-related pain, and cancer-related anxiety/fears.

Hypothesis: Risk factors for worse adverse health status outcomes in OS survivors will be associated with escalating anthracycline doses, alkylating agents and bleomycin exposure, and an increase in total number of surgeries. We hypothesize that there will be no differences in

functional status or activity limitations when compared by initial surgical approach to extremity tumors, consistent with outcomes in prior studies.

Aim 5: Evaluate the treatment profiles of the CCSS survivors, and identify the population who received treatment that is consistent with the current standard treatment approach (Methotrexate, Cisplatin and Doxorubicin with surgical resection). Describe the chronic health outcomes and adverse health status outcomes for this population, which can be used by clinicians to help inform current patients of long-term risks. Specifically, we will examine all-cause late mortality, SMN incidence, incidence of grades 3-5 chronic conditions, and health status in this subset of survivors.

Analysis Framework:

Population of interest:
The study population will include individuals in the CCSS diagnosed with osteosarcoma and treated from 1970-1999 (both the original and expansion cohorts, N=1205; N=~1050 with treatment data). For Aim 1, analyses will be among all eligible Osteosarcoma survivors (N=1771).

		1970-1979		1980-1989		1990-1999	
		N	%	N	%	N	%
Osteosarcoma							
Alkylating agent, cyclophosphamide equivalent dose (mg/m^2)	None	163	56.2	126	32.2	104	30.3
	1-<4000	22	7.6	71	18.2	42	12.2
	4000-<8000	82	28.3	160	40.9	39	11.4
	8000-<12000	15	5.2	20	5.1	82	23.9
	12000-<16000	5	1.7	2	0.5	49	14.3
	≥16000	3	1.0	12	3.1	27	7.9
Anthracycline, doxorubicin equivalent dose (mg/m^2)	None	56	20.1	51	13.1	20	5.8
	1-<300	53	19.0	100	25.7	66	19.1
	300-<450	90	32.3	165	42.4	197	56.9
	≥450	80	28.7	73	18.8	63	18.2
Cisplatin (mg/m^2)	None	273	88.6	138	34.9	52	15.0
	1-<400	13	4.2	67	17.0	100	28.8
	≥400	22	7.1	190	48.1	195	56.2
Methotrexate, IV (mg/m^2)	None	94	32.9	91	22.9	57	16.5
	1-<12000	45	15.7	59	14.8	61	17.6
	≥12000	147	51.4	248	62.3	228	65.9

Descriptive characteristics of the cohort will be obtained, as outlined in Table 1

Analysis framework by specific aim:

Aim 1: Estimate all-cause mortality for survivors of osteosarcoma diagnosed from 1970-1999, and compare according to major treatment changes.

1. To assess Mortality: Vital status (alive/dead) will be obtained from the National Death Index, and used to identify:
    a. Cumulative incidence of mortality
    b. Standardized mortality ratios (SMRs) and excess absolute risk (EAR)
        i. Will be calculated using age-sex and calendar year specific mortality rates for the US population from the National Center for Health Statistics to evaluate expected counts.
        ii. Overall and cause specific SMRs will be calculated by dividing the observed number of deaths among survivors by the expected number of deaths in the general population
        iii. Multivariable Poisson regression will be used to assess the simultaneous impact of multiple factors on the cause-specific SMRs (including specific chemotherapy agent cumulative doses: anthracyclines, platinum, alkylating agents, and combination chemotherapy treatment regimens: Methotrexate+doxorubicin, Methotrexate+ doxorubicin+BCD, Methotrexate+doxorubicin+cisplatin), potentially adjusting for sex, age at diagnosis, year of diagnosis, current age and/or years since diagnosis (Table 1a)
2. Among survivors, hazard ratios will be evaluated for associations of chemotherapy, radiation and surgery exposure with overall mortality and cause-specific mortality using Cox proportional hazards models with age as the time scale.
3. Non-parametric estimate of the cumulative incidence function will be used to estimate cause specific mortality by major treatment groupings of interest, accounting for the competing risk of death from other causes.
4. Overall survival will be estimated by Kaplan-Meier method.
5. Since treatment data is not available for all non-participant subjects in the CCSS multiple imputation procedures will be utilized to generate missing treatment data similar to methodology utilized in Armstrong et al (NEJM 2016).

Aim 2: Determine the prevalence and incidence of chronic health conditions for survivors of osteosarcoma and identify risk factors based on specific treatment exposure.

1. Chronic conditions – CTCAE Grades completed by Oeffinger and Armstrong using questionnaire data that is organized into a fixed matrix managed by the CCSS team. Severity of conditions is scored using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03: grade 1 (mild), 2 (moderate), 3 (severe), 4 (life-threatening), or 5 (fatal). Severity (grade 3-5) of conditions (overall) and grade 3-5

conditions in certain organ systems based on therapeutic exposures (below) will be assessed.

a. Cardiac conditions (congestive heart failure, myocardial infarction, arrhythmia, coronary artery disease, hypertension, complications requiring heart transplant)
b. Respiratory conditions (asthma, chronic cough, oxygen requirement, lung fibrosis, complications requiring lung transplant)
c. Hearing conditions (hearing loss not requiring hearing aid, hearing loss requiring hearing aid, tinnitus, dizziness/vertigo)
d. Neurologic outcomes (problems with learning or memory, migraines, problems with balance, special education program needs, graduated high school)
e. Venothromboembolism outcomes (blood clot in head, lung, arm, leg or pelvis)

2. The chronic conditions of interest will be described by evaluating the hazard ratio (HR) of each condition for survivors compared with siblings using age/sex/race adjusted Cox regression models. Cumulative incidence curves for chronic condition outcomes will be evaluated using death as a competing risk and as a function of age, for both survivors and siblings.

3. Among survivors, Cox proportional hazards models will be used to evaluate associations between treatment factors (detailed below) and chronic conditions of interest (listed above). Cumulative incidence curves will be also displayed based on treatment exposures of interest
    a. Exposures of Interest:
        i. Anthracyclines (yes/no/cumulative dose categories), with dexrazoxane (yes/no)
        ii. Alkylating agents (yes/no/cumulative dose categories reported as cyclophosphamide equivalent dose)
        iii. Platinums (yes/no/cumulative dose categories)
        iv. Bleomycin (yes/no/cumulative dose categories)
        v. Methotrexate+doxorubicin combination (yes/no)
        vi. Methotrexate+doxorubicin+BCD combination (yes/no)
        vii. Methotrexate+doxorubicin+cisplatin combination (yes/no)

Aim 3: Determine the incidence of secondary malignant neoplasms (SMN) and compare according to treatment exposures in OS survivors.

1. We will report cumulative incidence and cumulative burden curves of SMNs, treating death as a competing risk event.
    a. All subsequent neoplasms combined
    b. Secondary neoplasms that may be more closely related to p53 mutations (leukemia, breast cancer, sarcoma, adrenocortical carcinoma, CNS tumors)
2. Standardized incidence ratios (SIRs) will be reported by type of SMN, including a grouping of p53 associated cancers (see Appendix 1), using U.S. Surveillance,

Epidemiology, and End Results (SEER) cancer incident rates to evaluate the expected numbers of cancers for comparisons (Table 3b).

3. Compare SIRs for individual SMNs (both in total, and in subgroup evaluating p53 mutations) based on certain therapeutic exposure groups of interest reported by attained age:
    a. Alkylating agents (yes/no/cumulative dosing categories)
    b. Etoposide (yes/no/cumulative dosing categories)
    c. Methotrexate+doxorubicin combination (yes/no)
    d. Methotrexate+doxorubicin+BCD combination (yes/no)
    e. Methotrexate+doxorubicin+cisplatin combination (yes/no)
    f. Primary site radiation (yes/no)
    g. Chest radiation (yes/no)
4. Among survivors, multivariable Cox proportional hazards regression will be performed to look at the impact of the above therapeutic exposures (or combinations of exposures) on rates of SMNs (overall and by p53 related tumor grouping). Candidate factors for adjustment in models will be [sex, age at diagnosis, year of diagnosis, current age and/or years since diagnosis]. (Table 3a)

Aim 4: Evaluate the effect of changes of OS treatment on adverse health status, across six domains: general health, mental health, functional status, activity limitations, cancer-related pain, and cancer-related anxiety/fears.

1. The prevalence of each health status outcome in the study population (detailed below) will be calculated and reported using the same methodology that has been used in prior CCSS papers (Hudson et al, 2003, Ness et al, 2017). Health status from the most recently available survey will be used for each subject. Comparisons of prevalence of health status outcomes will be compared based on specific treatment exposures and surgical procedures of interest in multivariable log binomial regression models adjusting for age/sex/race. (Table 4). There will not be a sibling comparison for this aim, as our goal is to evaluate how treatment changes have impacted OS survivors.
    a. Health Status Outcome Measures
        i. Poor general health (fair or poor/ good vs very good or excellent)
        ii. Poor mental health (Score of 63 or higher on the GSI index of the Brief Symptom Inventory [BSI], or a score of 53 or higher on any two of the subscales [depression, anxiety, somatization])
        iii. Activity limitations (if limited for more than three months over past 2 years to any of the three questions)
        iv. Functional impairment (if yes to any of the three questions vs no to all three questions)
        v. Cancer Related Pain (if a lot, very bad excruciating pain, medium amount of pain vs no or small amount of pain)
        vi. Cancer related anxiety (answers a lot, very many/extreme, medium amount of anxiety/fears vs no or small amount of anxiety/fears
    b. Treatment exposures of interest

i. Alkylating agents (yes/no/cumulative dosing)
ii. Anthracyclines (yes/no/cumulative dosing)
iii. Cisplatin (yes/no/cumulative dosing)
iv. Etoposide (yes/no/cumulative dosing)
v. Methotrexate+doxorubicin combination (yes/no)
vi. Methotrexate+doxorubicin+BCD combination (yes/no)
vii. Methotrexate+doxorubicin+cisplatin combination (yes/no)
viii. Surgical Procedure
    1. Initial surgical approach to tumor: amputation vs limb-sparing procedure vs resection of axial primary tumor vs no surgery
    2. Total number of surgeries (0,1,2,3-5,>5)
    3. Lung metastasectomy
    4. Other metastasectomy

Aim 5: Evaluate the treatment profiles of the CCSS survivors, and identify the population who received treatment that is consistent with the current standard treatment approach (Methotrexate, Cisplatin and Doxorubicin with surgical resection). Describe the chronic health outcomes and adverse health status outcomes for this population, which can be used by clinicians to help inform current patients of long-term risks.

1. Identify patients in the cohort who received treatment that was limited to the chemotherapy agents that are currently the standard therapy for osteosarcoma: Methotrexate, doxorubicin and cisplatin (without defining dose limitations).
2. Describe the outcomes of this population of patients
    a. All cause late mortality
    b. Incidence of secondary malignant neoplasms
    c. Incidence of grade 3-5 chronic health conditions (described in Aim 3) for this patient population
    d. Health Status outcome measures by domain (6 domains described in Aim 4) for this patient population

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Table 1. Characteristics of OS survivors

Characteristic	**Survivor (n=)**	**Siblings (n=)**
Demographic		
Gender		
Male		
Female		
Race or ethnic group		
Non-Hispanic white		
Non-Hispanic black		
Hispanic		
Other		
Educational attainment		
Completed <12 grades of high school		
High school graduate		
College graduate		
Post-Graduate Training		
Insurance Status		
Yes		
No		
Body Mass Index		
Underweight		
Normal		
Overweight		
Obese		
Smoking		
Current		
Past		
Never		
Heavy Drinking		
Yes		
No		
Age at diagnosis (years)		NA
0-10		
11-15		
16-20		
Age at last follow-up (years)		
<20		
20-29		
30-39		
40-49		
>50		

Follow-up time (years)		
5-9		
10-14		
15-19		
20-24		
>25		
Primary site		NA For all Cancer/Treatment
Lower extremity		
Upper extremity		
Axial		
Treatment Details		
Chemotherapy		
Yes		
No		
Chemotherapy Combination		
MA		
MA-BCD		
MAP		
Anthracycline (mg/m2)		
None		
0-100		
101-300		
>300-450		
>450		
Alkylating agents (CED dose mg/m2)		
None		
1-4000		
4001-<8000		
8000-<12,000		
≥12,000		
Cisplatin (mg/m2)		
None		
1-400		
≥400		
Etoposide (mg/m2)		
None		
0-1000		
1001-3000		
>3000		
Bleomycin (mg/m2)		
None		

1-50		
51-100		
>100		
Methotrexate (mg/m2)		
None		
+exposure*		
Primary Site Radiation therapy		
Yes		
No		
Pulmonary Radiation		
Yes		
No		
Surgical approach to tumor		
Amputation		
Limb-sparing procedure		
Rotationplasty		
Resection of axial tumor		
No surgery		
Total number of major surgeries (excluding line placements, and biopsies)		
0		
1		
2		
3		
3-5		
>5		
Lung metastastectomy		
Yes		
No		

*For methotrexate we will obtain the distribution of cumulative dose as a continuous variable and then analyze these variables categorically using either quartiles or other percentile cutpoint (decided after examining the range and distribution of these values).

Aim 1:

Table 1a: All-cause and cause-specific standard mortality ratios by treatment era and treatment exposure

	All Causes			Subsequent Malignancy			Recurrent/ Progression of OS			Other nonrecurrent/non-external causes		
	No. of deaths	**SMR**	**95% CI**	**No. of deaths**	**SMR**	**95% CI**	**No. of deaths**	**SMR**	**95% CI**	**No. of deaths**	**SMR**	**95% CI**
All survivors												
Chemotherapy												
Anthracycline (mg/m^2)												
None												
0-100												
101-300												
>300-450												
>450												
Cisplatin (mg/m^2)												
None												
1-<400												
≥400												
Alkylator (CPM equiv. in grams)												
None												
1-<4000												
4000-<8000												
8000-<12,000												
>12,000												
Chemotherapy Combinations												
MA												
MA-BCD												
MAP												

Tables & Figures for mortality outcomes will include: SMRs for mortality, Mortality curves

Aim 2.

Table 2 (a-c). Chronic Medical Conditions

	OS Survivors (n=)	**Siblings (n=)**
	Number (%)	
Health Condition		
None		
Grade 1 (mild)		
Grade 2 (moderate)		
Grade 3 (severe)		
Grade 4 (life threatening)		
Grade 5 (fatal)		

Multiple health conditions		
>/= 2		
>/= 3		
Organ systems		
Heart and Circulatory Grade 2-5 conditions		
Respiratory Grade 2-5 conditions		
Hearing Grade 2-5 conditions		

Table 2a. Heart and Circulatory Conditions and Anthracycline exposure

		Anthracycline exposure (mg/m2)			
	None	**0-<100**	**100-<300**	**300-<450**	**≥450**
Cardiac Condition					
Congestive Heart Failure					
Myocardial Infarction					
Arrhythmia					
Coronary Artery Disease					
Hypertension					
Complications requiring heart transplant					
Thrombotic events					

Table 2b. Respiratory outcomes and Bleomycin Exposure

	Bleomycin exposure (mg/m2)			
	None	**1-<50**	**50-<100**	**≥100**
Respiratory Condition				
Asthma				
Chronic cough				
Oxygen requirement				
Lung fibrosis				
Complications requiring lung transplant				

Table 2c. Hearing complications and Cisplatin exposure

	Cisplatin Exposure (mg/m2)			
	None	**1-<400**	**400-<600**	**≥600**
Hearing Complications				
Hearing loss not requiring hearing aid				
Hearing loss requiring hearing aid				
Tinnitus				
Dizziness/Vertigo				

Table 2d: Hazard (and 95% CI) of having a grade 3-5 chronic condition

	Univariate	Multivariate
Variables	**HR (95% CI)**	**HR (95% CI)**
All survivors		
Chemotherapy		
Anthracycline (mg/m^2)		
None		
0-<100		
100-<300		
300-<450		
≥450		
Cisplatin (mg/m^2)		
None		
1-400		
401-600		
>600		
Alkylator (CPM equiv. in grams)		
None		
1-<4000		
4000-<8000		
8000-<12,000		
>12,000		
Etoposide (mg/m2)		
None		
0-1000		
1001-3000		
>3000		
Chemotherapy Combinations		
MA		
MA-BCD		
MAP		

Additional tables will be used for chronic health condition outcomes: Survivor sibling Hazard Ratios for Chronic conditions, Cumulative incidence of CC

Aim 3:

Table 3a. Multivariable analysis of subsequent neoplasms (SMN) other than basal cell carcinoma, overall and by subtypes

	SMN	p53 related SMNs	Other SMN of interest^
Variable	RR (95% CI) P	RR (95% CI) P	RR (95% CI) P
Sex			
Male			
Female			
Age at diagnosis			
0-10			
11-15			
16-20			
CED (mg/m2)			
None			
1-<4000			
4000-<8000			
8000-<12,000			
>12,000			
Etoposide			
None			
0-1000			
1001-3000			
>3000			
Anthracycline (mg/m^2)			
None			
0-<100			
100-<300			
300-<450			
≥450			
Primary Site Radiation therapy			
Yes			
No			
Chest Radiation			
Yes			
No			

CED: cyclophosphamide equivalent dose. p53 related SMNs: breast cancer, sarcoma, adrenocortical carcinoma, CNS tumors.

^ SMNs of interest may include breast cancer, thyroid cancer, sarcomas, lung cancer – analysis will dependent on frequencies of SMNs in initial analysis

Proposed Table 3b: SIR, cumulative incidence at 15 years and AER for SMNs

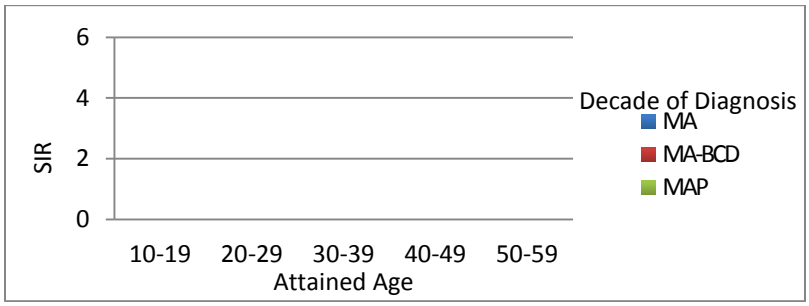
Treatment Exposures	**SIR (95% CI)**	**CI at 15 years**	**AER/1000 person-years**
All OS Patients			
Anthracycline (mg/m^2)			
None			
0-<100			
100-<300			
300-450			
≥450			
Alkylator exposure (CED dose)			
0 mg/m2			
1-<4000			
4000-<8000			
8000-<12,000			
>12,000			
Etoposide exposure			
0 mg/m2			
0-1000 mg/m2			
1001-3000 mg/m2			
>3000 mg/m2			
Combination therapy exposure			
MA			
MA+BCD			
MAP			
Radiation-Primary site			
Yes			
No			
Radiation - Chest			
Yes			
No			

Figures A-B**:** Cumulative incidence of second neoplasms, with years from initial cancer diagnosis as the x-axis time scale

A. Overall cohort, with curves for Any SMN, and for p53 related SMNs

B. Treatment exposures demonstrating differences for Any SMN, and for p53 related SMNs (if applicable)

Figure C. SIRs for SMN, by attained age and treatment combination exposures of interest.



Aim 4:

Table 4. Percentage of patients with adverse health status by therapeutic exposures

		Poor general Health	Poor Mental Health	Activity Limitation	Functional Impairment	Cancer Related Pain	Cancer Related Anxiety
	N	N (row%)	N (row%)	N (row%)	N (row%)	N (row%)	N (row%)
Age at diagnosis							
0-10							
11-15							
16-20							
Treatment Exposures							
Chemotherapy Combination							
MA							
MA-BCD							
MAP							
Anthracycline (mg/m2)							
None							
0-<100							
100-<300							
300-<450							
≥450							
Cisplatin (mg/m2)							
None							
1-400							
≥400							
Alkylating agents (CED dose mg/m2)							
0 mg/m2							
1-<4000							

4000-<8000							
8000-<12,000							
>12,000							
>600							
Etoposide (mg/m2)							
None							
0-1000							
1001-3000							
>3000							
Primary Site Radiation therapy							
Yes							
No							
Chest Radiation							
Yes							
No							
Initial surgical approach to tumor							
Initial amputation							
Initial limb-sparing procedure							
Resection of axial tumor							
No surgery							
Total number of surgeries (excluding line placements, biopsies)							
0							
1							
2							
3							
3-5							
>5							
Lung metastastectomy							
Yes							
No							

Appendix 1:
***p53* related tumors (Li-Fraumeni Syndrome related tumors)[58, 59]**

Most common tumors: 70% of all LFS related tumors

1. Sarcomas
    a. Soft tissue and bone sarcomas
2. Breast cancer
    a. Pre-menopausal breast cancers
3. Brain tumors
    a. Astrocytoma, glioblastoma, medulloblastoma, choroid plexus carcinoma
4. Adrenocortical carcinomas (ACC)
5. Leukemia
    a. Acute Lymphoblastic Leukemia

Less common tumors: 30% of all LFS related tumors

6. Gastrointestinal Cancers
    a. Colorectal, esophageal, pancreatic, stomach
7. Genitourinary Cancers
    a. Renal Cell Carcinoma
8. Thyroid Cancer
    a. Non-medullary thyroid cancer
9. Liver cancer