Title: Pilot Study to Prevent the Nephrotoxicity of High-Dose Methotrexate by Prolonging the Infusion Duration and Prevent the Nephrotoxicity and Ototoxicity of Cisplatin with Pantoprazole in Children, Adolescents and Young Adults with Osteosarcoma

Study Key Name: CHP12ST051 OS Nephrotoxicity and Ototoxicity

Protocol Number: CHP12ST051

Drug Name: Pantoprazole

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Sponsor: The Gateway for Cancer Research

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ABBREVIATIONS AND DEFINITIONS OF TERMS

Term	Definition
AKI	Acute kidney injury
AUC	Area under the plasma concentration-time curve
Block	Two consecutive treatment cycles (Cycles 1 & 2 or Cycles 3 & 4)
BMP	Basic mineral panel
BSAP	Bone-specific alkaline phosphatase
CBC	Complete blood counts
CL	Clearance
C_{max}	Peak concentration
CMP	Comprehensive mineral panel
COG	Children's Oncology Group
Course	A course of cisplatin is 60 mg/m^2 daily x 2 days (total dose 120 mg/m^2); a course of doxorubicin is 37 mg/m^2 daily x 2 days (total dose 75 mg/m^2); a course of HDMTX is two 12 g/m^2 infusions administered 1 week apart
Cr	Creatinine
CrCL	Creatinine clearance
CR	Complete Response
Cycle	A course of cisplatin and doxorubicin followed 3 weeks later by a course of HDMTX (cycles 1-4; duration 5 weeks); OR A course of doxorubicin followed 2 weeks later by a course of HDMTX (cycles 5&6; duration 4 weeks)
EOI	End of infusion
EOT	End of therapy
FEMg	Fraction excretion of magnesium
GFR	Glomerular filtration rate
H&P	History and physical exam
HDMTX	High-dose methotrexate (12 g/m^2)
IV	Intravenous
KIM-1	Kidney Injury Molecule-1
LV	Leucovorin
MAP	Methotrexate, Adriamycin (doxorubicin), cisPlatin
Mg	Magnesium
MTX	Methotrexate
NAG	N-Acetyl-β-D-Glucosamidase
NGAL	Neutrophil Gelatinase-Associated Lipocalin
OCT2	Organic cation transporter 2
OS	Osteosarcoma
PK	Pharmacokinetics
Pre-op	Preoperatively
PPI	Proton pump inhibitor
PR	Partial Response

Pre-Rx	Pretreatment
PRO	Patient reported outcome
PS	Performance score (Lansky or Karnofsky)
$T_{1/2}$	Half-life
TDM	Treatment delivery map (roadmap)
UA	Urinalysis

PROTOCOL SYNOPSIS

STUDY TITLE	Pilot Study to Prevent the Nephrotoxicity of High-Dose Methotrexate by Prolonging the Infusion Duration and Prevent the Nephrotoxicity and Ototoxicity of Cisplatin with Pantoprazole in Children, Adolescents and Young Adults with Osteosarcoma
SPONSOR	The Gateway for Cancer Research
CLINICAL PHASE	Pilot clinical trial
PARTICIPATING SITES	The Children's Hospital of Philadelphia
STUDY RATIONALE	Current osteosarcoma treatment regimens include drugs that are nephrotoxic and ototoxic, and the damage to kidneys and cochlear hair cells may be irreversible. Preventing these toxicities will improve the outcome in long-term survivors and may also prevent short-term treatment delays and dose reductions that can compromise the efficacy of the treatment regimen and allow for administration of higher cumulative doses of cisplatin. This pilot study evaluates pharmacologically-based approaches to: • prevent the nephrotoxic effect of high-dose methotrexate (HDMTX) by prolonging the infusion duration and thereby lowering the risk of drug precipitation in renal tubules, and • selectively block the uptake of cisplatin into renal tubular cells and cochlear hair cells by inhibiting the organic cation transporter 2 (OCT2) with the proton pump inhibitor, pantoprazole. These pharmacologically-based approaches to prevent nephrotoxicity and ototoxicity may also be applicable in other cancers treated with cisplatin or HDTX.
STUDY OBJECTIVES	**Primary objective** The primary objective is to identify more rational, pharmacologically-based drug delivery approaches to prevent nephrotoxicity from HDMTX and cisplatin and ototoxicity from cisplatin in patients with OS by: • comparing biomarkers of acute kidney injury (AKI), glomerular function and plasma and urine MTX concentrations after 12 g/m^2 of MTX infused over 4 and 12 hours, • comparing biomarkers of AKI, glomerular function and fractional excretion of magnesium (FEMg) after cisplatin administered with and without the OCT2 inhibitor, pantoprazole, and • comparing audiograms after cisplatin administered with and without pantoprazole. **Secondary objectives** • compare the incidence and severity (grade) of common toxicities from cisplatin/ doxorubicin administered with and without pantoprazole and from HDMTX administered as a 4 and 12 h infusion, • compare radiographic response (log ratio of tumor volumes) and histological response (percent tumor necrosis) to two cycles (10 weeks) of neoadjuvant chemotherapy for the two infusion durations of HDMTX and for cisplatin with and without pantoprazole, • correlate urinary markers of AKI and GFR estimated from serum cystatin C to standard measures of renal function (serum creatinine, urinalysis, estimated creatinine clearance, FEMg), • build a tissue microarray from biopsy, primary resection and resected metastatic tumors to evaluate the expression of proteins that are responsible for resistance

	to the drugs in the MAP regimen and to assess expression of proteins that are targeted by new anticancer drugs under development for childhood cancers, • evaluate serum BSAP as a potential biomarker for OS, • monitor nutritional status (weight, arm circumference, skin fold thickness, pre-albumin) in patients receiving the MAP chemotherapy regimen, and • pilot an osteosarcoma-specific patient reported outcomes survey to assess the incidence and severity of tumor-related and treatment-related symptoms.
TEST ARTICLE	Pantoprazole
STUDY DESIGN	A randomized cross-over, 2 x 2 factorial design, in which patients serve as their own control, will be used to maximize the potential to detect differences in biomarkers of AKI (NAG, NGAL, KIM-1, cystatin C) and audiograms resulting from altering the infusion duration of HDMTX and from adding pantoprazole to cisplatin. Two infusion durations of HDMTX will be studied – the standard 4 h duration and more prolonged 12 h infusion duration. Leucovorin rescue will commence 24 h from the start of the infusion. The HDMTX infusions will be divided into 2 blocks (cycles 1 & 2, cycles 3 & 4), each of which include 4 HDMTX infusions. The order of infusion duration studied in each block will be randomly determined. Pantoprazole will either be administered with the first two courses of cisplatin during cycles 1 & 2 or with the third and fourth courses during cycles 3 & 4, and the order will be assigned by randomization. Cisplatin is administered as a 60 mg/m^2 dose infused over 4 h daily x 2 d. At steady state, 7.2 mg/m^2 of active cisplatin is in the body, and by the end of the 4 h infusion, 88% of the total dose has been cleared. Therefore, adequate concentrations to inhibit OCT2 should be present for at least 4 h to prevent cisplatin nephrotoxicity and ototoxicity. Pantoprazole has a short half-life (1.2 h), and when administered at a dose of 1.6 mg/kg as a standard 15 min infusion, plasma pantoprazole concentration by the end of the 4 h cisplatin infusion is 1.3 mcM. By administering the same dose as a 0.3 mg/kg loading dose infused over 15 min followed by a 4 h infusion of the remaining 1.3 mg/kg, plasma pantoprazole concentration is 5.9 mcM at 4 h. The primary endpoint to assess nephrotoxicity will be the AKI markers (cystatin C, KIM-1, NGAL, NAG) and fractional excretion of magnesium (FEMg). This will allow us to assess each course of HDMTX and cisplatin independently. Standard renal function tests (urinalysis, serum creatinine, estimated creatinine clearance, and FEMg) will also be monitored. Audiograms will be performed after each course of cisplatin to assess ototoxicity. Radiographic and histological response in the resected tumor specimen after neoadjuvant chemotherapy will be assessed to ensure that changes in HDMTX infusion duration and the addition of pantoprazole to cisplatin do not alter the efficacy of chemotherapy. A brief survey to assess the patient's view of disease-related and drug toxicity-related symptoms will be piloted prior to each treatment cycle, post-op and at the end of therapy, and nutritional status will be monitored over the course of therapy. The use of: • sensitive, short-term biomarkers as endpoints to assess the effect of interventions, • continuous, rather than categorical, variables that are analyzed by course or cycle rather than by patient, and • a crossover design with patients serving as their own control, will minimize the number of subjects and time required to complete this pilot study in a limited patient population with a rare form of cancer, increase statistical power, and

	maximize the amount of data collected in this pilot study.
SUBJECT POPULATION	Children, adolescents and young adults with previously untreated osteosarcoma **Inclusion Criteria** • Age: <30 years of age • Diagnosis: histological diagnosis of high-grade osteosarcoma • Site: Extremity or central axis (including craniofacial) primary tumor; localized or metastatic • Prior therapy: No prior chemotherapy or radiation therapy for osteosarcoma. Subjects who develop osteosarcoma as a second cancer are eligible if they have not previously received cisplatin, doxorubicin or MTX • Kidney function: Normal serum creatinine for age and gender • Cardiac function: Shortening fraction on echocardiogram >28% • Hearing: Hearing level threshold ≤25 dB at all frequencies in both ears to be evaluable for assessment of pantoprazole's effect on cisplatin ototoxicity. Patients with hearing loss can be enrolled but will not be evaluable for ototoxicity objective. • Hematological function: Absolute neutrophil count >1,000/mcL and platelet count >100,000/mcL **Exclusion Criteria** • Receiving H_2 antagonists (cimetidine, ranitidine, famotidine, nizatidine) or proton pump inhibitors (lansoprazole, omeprazole, pantoprazole, esomeprazole, raberprazole, dexlansoprazole) AND unable to hold the drug for 24 h prior to and 24 h after each cisplatin course on cycles 1-4. • Pregnant or breastfeeding • Unable to cooperate with research procedures
NUMBER OF SUBJECTS	24
DURATION OF SUBJECT PARTICIPATION	40 weeks
STUDY DURATION	24-36 months
STUDY PHASES	Pilot clinical trial
EFFICACY EVALUATION	Radiographic and histological response to neoadjuvant therapy, survival
PHARMACOKINETIC EVALUATION	Plasma and urine methotrexate concentrations at the end of each HDMTX infusion on cycles 1-4
SAFETY EVALUATION	Nadir neutrophil count and duration of severe (ANC <500/mcL) neutropenia, nadir platelet count and number of platelet transfusions, hospital admissions/days for neutropenia with fever or infection, highest value for serum ALT and total bilirubin, grade 3 or 4 mucositis, treatment delays or missed chemotherapy doses to allow for recovery from toxicity, dose modifications for toxicity, and deaths attributed to chemotherapy toxicity will be monitored and collected by cycle. After the first 12 patients have completed therapy, the incidence and severity of the events listed above as well as the nephrotoxicity and ototoxicity endpoints will be assessed by Cycle according to HDMTX infusion duration (4 h vs. 12 h) and according to whether or not IV pantoprazole was administered with cisplatin to ensure that the experimental dosing methods are not associated with excessive toxicity. After the first 12 patients have undergone resection of their primary tumor, the radiographic response (log ratio) and histologic response (% necrosis) will be assessed according to HDMTX infusion duration (4 h vs. 12 h) during Cycles 1 & 2 and according to whether or not IV pantoprazole was administered with cisplatin during Cycles 1 & 2 to ensure that the anti-tumor effect of the MAP chemotherapy regimen is not compromised by the experimental dosing methods.

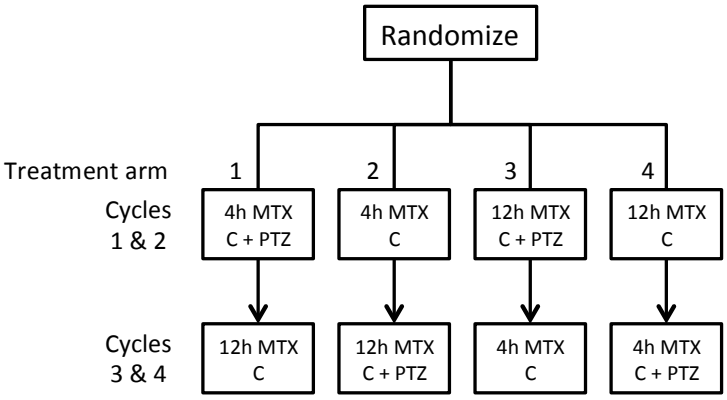
STATISTICAL AND ANALYTIC PLAN	This pilot study uses a 2 x 2 factorial, randomized crossover design, which will allow patients to serve as their own control. Nephrotoxicity data will be analyzed by course for cisplatin and by dose for HDMTX, and ototoxicity data will be analyzed by cycle. The primary endpoints are sensitive urinary biomarkers of AKI, standard renal function tests, and audiograms, and all endpoints will be analyzed as continuous variables. Twenty-four patients will be enrolled and patients serve as their own control. Over the past 5 years, 57 newly diagnosed patients with osteosarcoma were seen and treated at CHOP. This is an average of 11.5 patients per year, indicating that we can complete accrual to this study in about two years. For each primary endpoint, separate linear mixed effects models will be constructed for comparison of cisplatin with vs. without pantoprazole and for comparison of the 4 vs. 12 h infusion durations of HDMTX.
DATA AND SAFETY MONITORING PLAN	The research team led by the protocol PI will monitor adverse events related to the protocol therapy in real time. The progress of every patient actively receiving treatment on this study will be discussed weekly in the Solid Tumor Team Meeting. An interim analysis to assess the safety of the interventions will be performed after the first 12 patients have completed therapy.

SCHEDULE OF STUDY PROCEDURES

PROCEDURE	Baseline	Cycle 1	Cycle 2	Surgery	Cycle 3	Cycle 4	Cycle 5	Cycle 6	End therapy
Standard of care procedures									
Tumor biopsy	X								
H&P, PS, weight, height	X	D1, 22, 29	D1, 22, 29	Pre-op	D1, 22, 29	D1, 22, 29	D1, 15, 22	D1, 15, 22	X
CBC + Differential	X	D1 & weekly	D1 & weekly	Pre-op	D1 & weekly	D1 & weekly	D1 & weekly	D1 & weekly	X
CMP + PO_4, Mg	X	D1, 22	D1, 22	Pre-op	D1, 22	D1, 22	D1, 15	D1, 15	X
BMP		D29	D29		D29	D29	D22	D22	
UA w/o micro	X	D1, 22[1], 29[1]	D1, 22[1], 29[1]		D1, 22[1], 29[1]	D1, 22[1], 29[1]	D1, 15[1,] 22[1]	D1, 15[1], 22[1]	X
Urine pregnancy test[2]	X								
Serum creatinine[3]		D22, 29	D22, 29		D22, 29	D22, 29	D15, 22	D15, 22	
Serum MTX[3]		D22, 29	D22, 29		D22, 29	D22, 29	D15, 22	D15, 22	
Creatinine clearance[4]	X				Pre-Rx		Pre-Rx		X
Echocardiogram[5]	X				Pre-Rx		Pre-Rx		X
Audiogram	X		Pre-Rx		Pre-Rx	Pre-Rx			X
Plain X-ray of primary	X			Pre-op	Pre-Rx				X
MRI of primary	X			Pre-op	Pre-Rx[6]				X[6]
Chest CT scan	X			Pre-op			Pre-Rx[7]		X
Bone or PET scan	X			Pre-op			Pre-Rx[7]		X[7]
Dexrazoxane		D1, 2	D1, 2		D1, 2	D1, 2	D1, 2	D1, 2	
Doxorubicin		D1, 2	D1, 2		D1, 2	D1, 2	D1, 2	D1, 2	
Cisplatin		D1, 2	D1, 2		D1, 2	D1, 2			
Pegfilgrastim		D3	D3		D3	D3	D3	D3	
HDMTX + LV rescue		D22, 29	D22, 29		D22, 29	D22, 29	D15, 22	D15, 22	
Tumor resection				X					
% Tumor necrosis				X					
Research procedures									
IV Pantoprazole		D1, 2[8]	D1, 2[8]		D1, 2[8]	D1, 2[8]			
Urinary AKI markers, Cr[9]	X	D1, 2, 7, 22, 23, 29, 30	D1, 2, 7, 22, 23, 29, 30	Pre-op	D1, 2, 7, 22, 23, 29, 30	D1, 2, 7, 22, 23, 29, 30	D1		
Serum Cystatin C[10]	X	D1, 7, 22, 29	D1, 7, 22, 29	Pre-op	D1, 7, 22, 29	D1, 7, 22, 29	D1		X
Plasma/Urinary Mg, Cr[11]	X	D1, 2, 7	D1, 2, 7		D1, 2, 7	D1, 2, 7	D1		X
BSAP	X			Pre-op	D1				X
Pre-albumin	X		D1	Pre-op	D1	D1	D1	D1	X
Urine MTX[12]		EOI D22, 29	EOI D22, 29		EOI D22, 29	EOI D22, 29			
PRO survey[13]	X		D1	Pre-op	D1	D1	D1	D1	X
Arm circum/Skin fold	X		D1	Pre-op	D1	D1	D1	D1	X

[1] Check urine pH prior to the start of HDMTX and every shift (q8h) until serum MTX <1 mcM.
[2] For females ≥11 years of age
[3] At the end of the HDMTX infusion, hour 24, and daily until MTX <0.1 mcM
[4] 12 or 24 hour urine collection to measure creatinine excretion plus serum creatinine
[5] Repeat prior to every course of doxorubicin if SF <30% on prior cycle
[6] Substitute CT scan if metal in endoprothesis does not allow for MRI to be performed
[7] Perform chest CT or bone scan only if positive for metastatic disease at baseline
[8] Pantoprazole will be administered either on cycles 1 & 2 or cycles 3 & 4 based on the treatment arm to which the subject is randomized
[9] Baseline; prior to cisplatin on day 1, at end of cisplatin infusion on day 2, and day 7; prior to HDMTX and 24 h after the start of the infusion on days 22/23 & 29/30 (cycles 1-4); pre-op and day 1 of cycle 5. A separate aliquot of same urine specimen saved for urine creatinine. See Section 4.0 for sample collection, processing and storage instructions.
[10] Baseline; prior to each course of cisplatin and each dose of HDMTX (cycles 1-4) and post-treatment IF serum creatinine increases by ≥50% compared to pretreatment value; pre-op and day 1 of cycle 5. Draw simultaneous BMP with serum cystatin C. See Section 4.1 for sample collection, processing and storage instructions
[11] Serum Mg and creatinine and spot urine for Mg and creatinine at baseline, prior to the start of the cisplatin infusions on day 1 and day 2 and on day 7 of cycles 1-4, day 1 of cycle 5, and at the end of therapy
[12] End of each HDMTX infusion (cycles 1-4). See Section 4.1 for sample collection, processing and storage instructions
[13] Appendix 3

STUDY DIAGRAM



MTX: High-dose methotrexate by 4h or 12h infusion

C: Cisplatin

PTZ: Pantoprazole

1. BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Conventional chemotherapy administered neoadjuvantly (prior to surgical resection of the primary tumor) and adjuvantly after tumor resection improves survival in children, adolescents and young adults with non-metastatic osteosarcoma from 15% with surgery alone to 65% by combining chemotherapy with surgery.[2,7] However, survival has not improved since the efficacy of adjuvant chemotherapy was demonstrated in the mid-1980s, as illustrated in Figure 1.[2,8-10] Survival in the 10 to 20% of patients who present with overt metastatic disease at diagnosis is poor despite intensive treatment,[11-13] and the subsequent development of overt metastatic disease is the primary factor limiting survival in patients who present with localized disease. Conventional cytotoxic chemotherapy eradicates micrometastatic disease in about half the patients with localized osteosarcoma, but the most effective known treatment regimens cause substantial acute and long-term toxicity, including nephrotoxicity, cardiotoxicity, ototoxicity and neurotoxicity.[7,14-17] Improving the outcome for patients with osteosarcoma in the short term can be achieved by lessening the toxicity of standard cytotoxic treatment regimens, but we must also identify new treatment targets in tumors cells from patients with osteosarcoma in order to develop more effective and less toxic drugs for this disease.

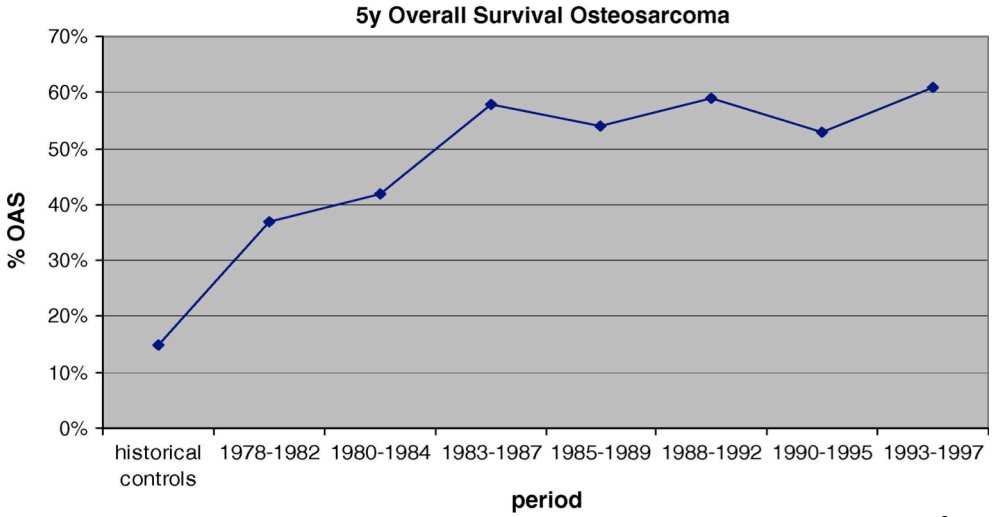


Figure 1 Overall 5-yr survival for osteosarcoma diagnosed during different time periods.[2]

1.2 Compliance Statement

This study is being conducted in full accordance with all applicable The Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and State laws and regulations including 45 CFR 46, and the HIPAA Privacy Rule. Any episode of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent when appropriate, and will report unexpected problems in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all Federal requirements.

Collecting, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

1.3 Relevant Literature and Data

1.3.1 Osteosarcoma

Osteosarcoma is the most common type of bone cancer in children and adolescents under the age of 20 yrs. Four hundred cases occur annually in the U.S., and the peak incidence occurs at the time of the adolescent growth spurt.[18] The cell of origin for osteosarcomas is believed to be primitive bone-forming mesenchymal stem cells. Osteosarcomas arise primarily in the metaphyses of long bones – 78% in the lower extremities (64% around the knee) and 10% in the humerus.[19] More than 85% of patients present with localized disease. The most common sites of metastases at presentation are the lung (85%) and other bones (20%).[19] Pain and swelling are the most common presenting symptoms, and the median duration of symptoms prior to diagnosis is 69 days.

The standard management of osteosarcoma starts with a biopsy of the primary tumor for a histological diagnosis. Suspected metastatic lesions in the lung may also be resected at presentation. Patients then receive pre-operative (neoadjuvant) multi-agent chemotherapy followed by surgical resection of the primary tumor, and then additional adjuvant chemotherapy for a total of 6 to 8 months of treatment.[20] Limb salvage procedures, in which the tumor and involved bone are replaced by an endoprosthesis, are the preferred surgical approach when feasible, rather than amputation. The chemosensitivity of the tumor to the neoadjuvant chemotherapy is assessed in the resected specimen by microscopically quantifying the percent of the tumor that is necrotic, which is predictive for outcome.[21] The efficacy of adjuvant chemotherapy for localized osteosarcoma has been demonstrated in randomized clinical trials.[7,22]

Four conventional cytotoxic anticancer drugs have activity in osteosarcoma (Table 1). Adjuvant regimens include 2, 3, or all 4 of these agents. A meta-analysis comparing event-free and overall survival for regimens containing 2 drugs (n=4), 3 drugs (n=8) or 4 drugs (n=6) demonstrated better survival for 3 drug regimens than regimens containing 2 drugs (HR 0.70 [95% CI 0.68-0.93] for EFS) but no additional advantage for 4 drug regimens.[2] The 3 drug combination used in 7 of the 8 regimens in this analysis included high-dose methotrexate (HDMTX), doxorubicin and cisplatin (MAP), which is also the standard regimen in the recently completed COG trial (AOST0331). The prior COG trial, which evaluated the addition of ifosfamide and MTP-PE to the standard MAP regimen using a 2 x 2 factorial design failed to show a survival advantage for the addition of ifosfamide, but the results were confounded by a possible interaction between ifosfamide and MTP-PE.[23,24] A

Table 1 Response rates for drugs used to treat osteosarcoma.[2]

Drug	Dose Range [mg/m^2/course]	No. of Patients	Response Rates [%]	
			CR	PR
Doxorubicin	35-90	108	13	30
Ifosfamide	5,000-15,000	246	12	20
Methotrexate	80-15,000	164	16	16
Cisplatin	120-625	174	10	16

recent Italian study did not find an advantage to adding ifosfamide to MAP.[25] The current international study, which recently completed enrollment, is evaluating the utility of adding ifosfamide post-operatively in patients with a poor histological response (≤90% tumor necrosis) to neoadjuvant MAP chemotherapy and adding interferon-α to MAP in patients who experience a good histological response. Until the results of this most recent study are available, MAP (Figure 2) remains the standard of care for osteosarcoma.

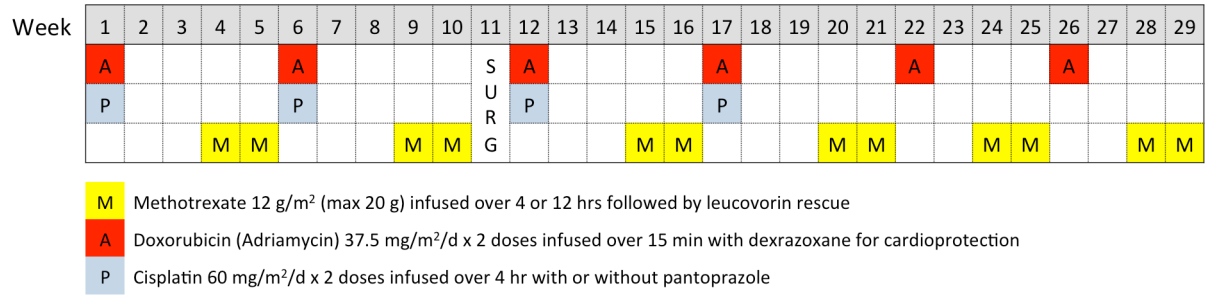


Figure 2 Schema of the MAP regimen used in the treatment of osteosarcoma. The first two 5-week cycles are administered neoadjuvantly. Surgery is performed at week 11, followed by 4 additional treatment cycles (MAP x 2 and MA x 2). Numbers at the top are the week of treatment regimen.

1.3.2 Nephrotoxicity

Osteosarcoma chemotherapy regimens are associated with acute and long-term nephrotoxicity from cisplatin, HDMTX, and ifosfamide.[26] The mechanism of kidney injury differs for the three drugs. MTX can transiently alter glomerular filtration but is not tubulotoxic, cisplatin directly damages renal tubular epithelium resulting in decreased glomerular filtration and electrolyte wasting, and ifosfamide is toxic to renal tubules and primarily affects the proximal renal tubules but can also alter glomerular function. Hydration, alkalinization (HDMTX), and slowing the dose rate (cisplatin) can lessen (but not prevent) nephrotoxicity from cisplatin and HDMTX. A prospective evaluation of long term (post-treatment) renal function in patients with osteosarcoma treated with HDMTX, cisplatin and ifosfamide demonstrated persistent glomerular and tubular impairment.[27] Half had a reduction in creatinine clearance and proteinuria and 25% had glycosuria and impaired phosphate reabsorption.

1.3.2.1 High-Dose Methotrexate

Methotrexate inhibits dihydrofolate reductase (DHFR) and depletes intracellular pools of tetrahydrofolates, which are required for the synthesis of thymidine and purines. MTX is administered in high doses by prolonged intravenous infusion because prolonged exposure to a cytotoxic drug concentration is the primary determinant of MTX's antitumor effect.[28] High doses of MTX are also thought to overcome mechanisms of resistance to MTX, such as increased expression of DHFR and decreased expression of the reduced folate carrier, which is responsible for uptake of the drug into tumor cells. HDMTX can also enhance the intracellular formation of MTX-polyglutamates, which are retained in cells and prolong the drug's effect.[28,29] HDMTX requires the subsequent administration of leucovorin (5-formyl-tetrahydrofolate) to rescue patients from the potentially lethal toxic effects of HDMTX. Leucovorin is administered

until plasma concentrations of MTX fall below 0.1 mcM. MTX is eliminated by renal excretion – more than 90% of the administered dose appears in the urine as parent (unchanged) drug.[30] A small fraction of methotrexate is metabolized to 7-OH-MTX.[6]

Table 2 pH-dependent solubility of MTX and its metabolite (7-OH-MTX) at 37°C.[6]

	Solubility [mM]		
Compound	**pH 5.0**	**pH 6.0**	**pH 7.0**
MTX	0.97	3.5	19.6
7-OH-MTX	0.28	0.79	3.3

HDMTX nephrotoxicity is dose related and is thought to result from precipitation of MTX in urine in renal tubules as a result of MTX's limited solubility in acidic solutions.[31-33] MTX-induced nephrotoxicity results in delayed excretion of MTX, sustained and elevated plasma MTX concentrations, which may not be effectively rescued by leucovorin, and marked enhancement of MTX's other systemic toxicities, such as myelosuppression, mucositis, dermatitis, and hepatotoxicity. The incidence of severe nephrotoxicity from HDMTX in osteosarcoma is 1.8% and in 4% of cases it is fatal.[32] Severe MTX nephrotoxicity is a medical emergency that requires high doses of leucovorin to protect the patient and glucarpidase (carboxypeptidase-G_2 – metabolizes MTX to an inactive metabolite) or hemodialysis to remove MTX. Less severe nephrotoxicity is more common[34] and requires more prolonged leucovorin rescue, which can compromise the antitumor effect of MTX.[35] This may result in delays in subsequent therapy, thus reducing dose intensity. At CHOP in 2011, 240 courses of high dose methotrexate (HDMTX ≥1 g/m^2) were administered to children with osteosarcoma (n=15) or leukemia/lymphoma (n=45); 5% of courses were associated with delayed excretion of methotrexate, likely due to acute changes in renal function. Infusion of HDMTX over 4 hours in osteosarcoma regimens puts patients at higher risk for nephrotoxicity because of the high peak plasma concentrations and high urinary concentrations of the drug. Standard methods to prevent kidney damage from HDMTX include vigorous hydration and alkalinization, which improves MTX solubility in the urine (Table 2).[6] At a pH of 7.0, MTX (pKa, 4.8 and 5.7) is more than 98% ionized, so further increases in the pH are not likely to improve solubility.

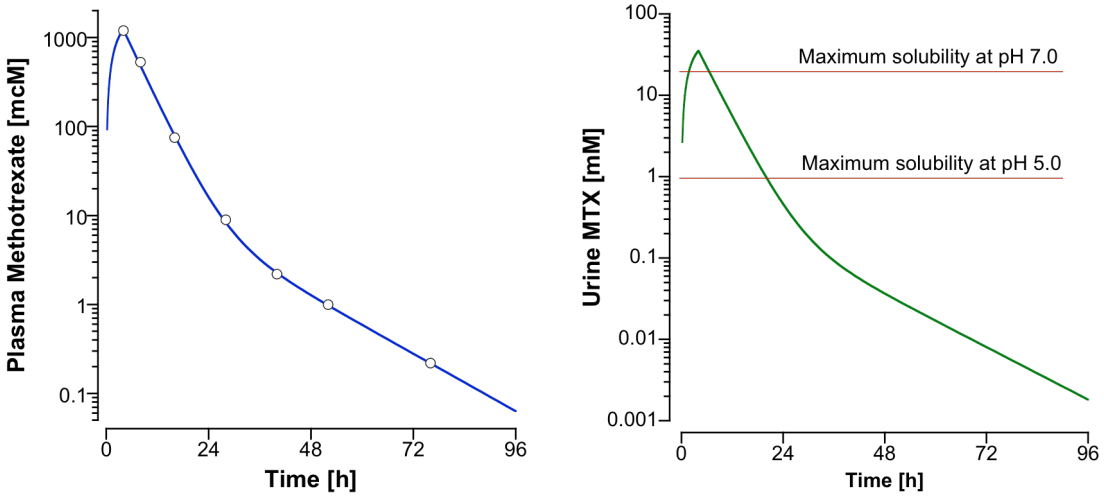


Figure 3 Plasma (in mcM) and urine (in mM) MTX concentrations with 12 g/m^2 infused over 4 h. Horizontal lines represent the solubility limit of MTX at a pH of 5.0 and 7.0.

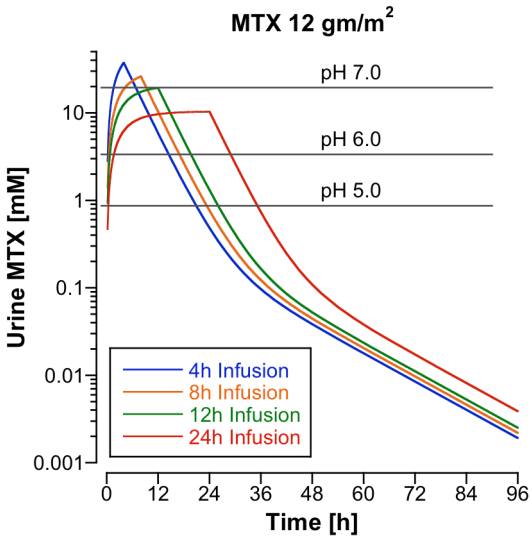


Figure 4 Simulations of urine MTX concentrations with 12 g/m^2 infused over 4, 8, 12 and 24 h.

We fit a 2-compartment pharmacokinetic model to the mean plasma MTX concentrations from 1,045 infusions of 12 g/m^2 over 4 h (Figure 3).[5] The urinary concentration-time profile could then be simulated assuming that the rate of urine production was equal to the IV fluid hydration rate of 100 ml/m^2/h used at CHOP with the equation:

$$C_{Urine}^{MTX} = \frac{C_{central}^{MTX} \bullet V_{central} \bullet k_{el}}{IV\ rate} \bullet 0.9$$

where C_{urine} is the MTX in urine, $C_{central}$ is the MTX concentration in the central compartment, $V_{central}$ is the volume of the central compartment, k_{el} is the elimination rate constant and 0.9 accounts for the fraction of the MTX dose excreted in the urine unchanged. The urine MTX concentration-time profile is shown in Figure 3. The urine MTX concentration exceeds the solubility limit in urine at a pH of 7.0 for 4.8 h, putting the kidneys at risk for injury from MTX precipitation. Figure 4 shows the urine MTX concentration-time profiles for 12 g/m^2 infused over 4, 8, 12 and 24 h. With the longer infusion durations, urine MTX concentrations are below the solubility limit, and the risk of MTX precipitation should be lower.

In addition to lowering peak plasma and urine concentrations and potentially lowering the risk of nephrotoxicity, prolonging HDMTX infusions has a strong pharmacological rationale because the duration of exposure to a cytotoxic MTX concentration is the primary determinant of the drug's anti-tumor effect.[28] Retrospective analyses from European studies have demonstrated an association between high peak (end of infusion) plasma MTX concentration and improved survival.[5,36,37] However, the role of peak plasma MTX concentration in determining outcome is not supported by the observations that:

- peak plasma concentrations were highly correlated with area under the curve (AUC) and other pharmacokinetic parameters in these studies,[5,36] making difficult to ascertain whether the peak concentration was the critical determinant;
- the critical peak MTX concentration varied depending on the dose and infusion duration. For 12 g/m^2 of MTX infused over 4 h, peak concentrations >1,000 mcM were associated with better survival, whereas for doses of 8-10 gm/m^2, the cut point for peak concentration was 700 mcM, indicating that an absolute peak concentration is not the critical determinant of outcome;[37]
- in other osteosarcoma studies, no relationship was found between peak MTX concentration and survival,[38-40] and in one study, very high MTX exposures were associated with a poorer outcome;[41] and

- disease-free survival was similar for 7,500 mg/m^2 of MTX infused over 6 h and 690 mg/m^2 infused over 42 h in a randomized clinical trial in OS.[42]

These observations along with data that demonstrate that prolonged infusions of MTX are more efficacious for other cancers[29] do not support the importance of peak concentration as the critical determinant of HDMTX efficacy in OS.

1.3.2.2 Cisplatin

Cisplatin is an inorganic compound that has a broad spectrum of activity in cancers occurring in adults and children, including osteosarcoma. Its mechanism of action, platination of DNA, is analogous to alkylation by the nitrogen mustards and other bifunctional alkylating agents. A reactive intermediate, which is formed spontaneously in solution through aquation by the loss of chloride (leaving group), non-discriminately forms a covalent bond with any nearby nucleophilic group. The platination of a nucleotide in DNA damages the DNA template and can lead to inter- and intra-strand crosslinks.[43]

Cisplatin is neurotoxic, ototoxic, highly emetogenic and nephrotoxic, and these toxicities can limit our ability to administer the drug to some patients. Cisplatin nephrotoxicity is acutely manifested as lower glomerular filtration rate and electrolyte (magnesium, potassium, phosphate) wasting in urine,[44] and these effects appear to be cumulative and only partially reversible.[45,46] The mean decrease in glomerular filtration rate for a dose of 100 mg/m^2 was 8% per dose but was highly variable across patients in one study.[46] In some cases, the severity of nephrotoxicity compromises our ability to administer subsequent doses of cisplatin and can also effect HDMTX elimination by the kidneys. Current methods of attenuating cisplatin nephrotoxicity can reduce its severity but do not prevent it. Intravenous fluid hydration with normal saline prior to and after cisplatin infusion and fractionating the dose over several days or prolonging the duration of drug infusion have been the most successful methods.[47] The role of diuretics (mannitol or furosemide) remains controversial.

Cisplatin-related renal tubular and vascular injury is a complex, multi-factorial process.[44] Cisplatin uptake into and efflux from cancer cells appears to be mediated by copper transporters (CTR1, ATP7B).[44] However, in the renal tubule, the polyspecific organic cation transporters (OCTs), and specifically the renal isoform OCT2, mediate cisplatin uptake.[48] OCTs in the kidney mediate basolateral to apical transport of cations, and on the apical surface, cations are secreted into urine by MATEs (multidrug and toxin extrusion transporters).[49] Cisplatin is not transported by MATEs, and therefore it accumulates in renal tubular cells. Several lines of evidence indicate that this interaction of cisplatin with OCT2 accounts for its selective nephrotoxic effects. The less nephrotoxic platinum analog, carboplatin is not an OCT2 substrate; OCT2 inhibitors (e.g., cimetidine) can block cisplatin renal tubular cell uptake and damage *in vitro* and *in vivo*; and OCT2-deficient animals and humans with OCT2 polymorphisms are not as susceptible to cisplatin nephrotoxicity.[48-52] Tissue expression of OCTs is limited, and they are not expressed on most human cancers, including childhood cancers.[51,52] Therefore, inhibiting OCT2 could selectively rescue the kidney from the toxic effects of cisplatin without interfering with the drug's antitumor effect.[50] Patients treated with cisplatin may also be at

higher risk to develop renal cancer later in life,[53] but it is not clear whether this is related to cisplatin accumulation in the renal tubule cells.

Cisplatin pharmacokinetics is difficult to quantify because it is chemically unstable. The parent drug spontaneously forms a reactive aquated intermediate that rapidly reacts with a nearby nucleophilic group. In plasma, the reactive intermediates react with plasma proteins forming a covalent bond, which inactivates the drug. Protein binding increases over time, reflecting the non-reversible nature of the protein binding. The platinated proteins are slowly removed from circulation and degraded, which releases platinum in a form that is no longer active. Studies of cisplatin pharmacokinetics (PK) have measured free (non-protein bound) and total platinum (bound + free) using atomic absorption spectroscopy. The free form of platinum, which includes both active drug species and inactive forms, has a biexponential plasma concentration-time curve with half-lives of 20 min and >24 h in children.[54,55] The former likely represents the disappearance of the active species of the drug, and the latter, more prolonged half-life likely reflects the disappearance of inactive, non-protein bound forms. Although renal excretion is the primary route of platinum elimination, it accounts for only 27% of total clearance,[55] and a substantial fraction of platinum in urine is inactivated before it is excreted. Therefore, renal excretion is not the major route of elimination for the active species of the drug as evidenced by the fact that cisplatin clearance in an anephric patient was not substantially different than that of patients with normal renal function.[56] Co-administration of an OCT2 inhibitor with cisplatin should not interfere with cisplatin clearance, because cisplatin is not secreted after it is taken up into renal tubular cells (see above) and because renal excretion of the active forms of the drug is not a major route of elimination.

1.3.3 Biomarkers of Acute Kidney Injury

Current clinical laboratory tests of renal function in children with cancer do not detect early, subtle changes in glomerular filtration rate (GFR) or renal tubular function. Limitations of serum creatinine (sCr) as a marker of GFR[57] include:

- Non-renal factors (diet, muscle mass, tubular secretion) can alter the sCr but do not affect GFR,
- sCr can be within the normal range with a GFR as low as 40 mL/min/1.73 m^2,
- sCr is imprecise in small children and infants because it is rounded to the first decimal place, resulting in a significant potential error in estimating creatinine clearance (eCrCL),
- a recent change in the method used to quantify sCr has invalidated formulas used for eCrCL.[58]

More sensitive and specific biomarkers to accurately quantify small changes GFR and to rapidly detect renal tubular damage are being developed. These biomarkers may be useful for detecting and monitoring renal toxicity of nephrotoxic anticancer drugs, such as MTX and cisplatin. We plan to use a panel of new biomarkers (described below) in combination with traditional laboratory tests of renal function to assess the impact of altering the schedule of HDMTX and adding pantopazole as a cisplatin rescue agent on nephrotoxicity of these drugs on this trial. These new biomarkers will include:

- **Cystatin C** is a 13kDa cysteine protease inhibitor produced by all nucleated cells. It is freely filtered, reabsorbed and metabolized in proximal tubules but not normally secreted in urine by the kidney. Serum cystatin C is used to estimate GFR and urine cystatin C is a marker of tubular damage. Cystatin C is not influenced by muscle mass, and it has improved temporal discrimination in detection of kidney injury compared to sCr. Cystatin C is measured by a FDA approved nephelometry method in clinical laboratories.[59-68] CrCl can be estimated from sCr using the Schwartz formula and GFR is calculated from a sCr and Cystatin C based formula developed in the Children with Chronic Kidney Disease Cohort:[58]

  CrCL=0.413•[height (cm)]/sCr

$$GFR = 39.1 \bullet \left[\frac{Hgt}{Cr_{serum}}\right]^{0.516} \left[\frac{1.8}{CyC}\right]^{0.294} \left[\frac{30}{BUN_{serum}}\right]^{0.169} \left[1.099\right]^{male} \left[\frac{Hgt}{1.4}\right]^{0.188}$$

Chemotherapy induced renal tubular dysfunction manifests as renal wasting of minerals and electrolytes. In current clinical practice, renal tubular dysfunction is diagnosed when serum electrolyte concentrations fall below normal and require oral supplementation. Sensitive biomarkers of renal tubular damage or dysfunction could detect toxicity earlier and contribute to the evaluation of strategies to attenuate nephrotoxicity. New biomarkers of renal tubular damage or dysfunction include:

- **Kidney Injury Molecule-1 (KIM-1)** is a 50 kDa trans-membrane protein expressed on proximal tubule cells. Urine KIM-1 is a marker of proximal tubular damage that may discriminate pre-renal azotemia from ischemia. Urine concentrations are not effected by chronic kidney disease. ELISA kits are commercially available for research, and clinical laboratory micro-bead assays are under development.[69-73]
- **Neutrophil Gelatinase-Associated Lipocalin (NGAL)** is a 25 kDa protein expressed in intestine lung, liver, and renal tubular epithelial cells. Elevations in plasma NGAL occur promptly after renal ischemic or tissue injury. Plasma and urine NGAL are early predictors of acute kidney injury (AKI) and may discriminate pre-renal from intrinsic AKI. ELISA kits are commercially available for research applications and clinical laboratory assays are available.[74-85]
- **N-acetyl-β-D-glucosamidase (NAG)** is a 130 kD enzyme within lysomomes of proximal tubular cells of the kidney. Urinary NAG concentrations are increased during oxidative stress and renal tubular damage including from cisplatin and gentamicin.[52,86] Urinary NAG increases in the presence of oxidative stress and proximal tubular damage regardless of development of AKI.

Prospective systematic evaluation of glomerular and tubular function using these new, more sensitive biomarkers should allow us to detect more subtle changes in GFR and tubular function and assess the impact of the interventions to attenuate HDMTX and cisplatin nephrotoxicity.

The **fractional excretion of magnesium** will also be monitored during cisplatin administration to assess renal tubular function. FEMg = (100•(Urine Mg • Serum Cr))/(0.7•Serum Mg • Urine Cr). Multiplying serum Mg by 0.7 accounts for 30% protein binding to albumin.

1.3.4 Ototoxicity

Cisplatin causes dose- and schedule-dependent permanent, sensorineural hearing loss by damaging cochlear hair cells.[87] Initially, osteosarcoma patients experience high frequency hearing loss at 4 to 8 kHz at cumulative doses exceeding 300 mg/m^2, but hearing at lower frequencies becomes impaired as the cumulative dose increases, and a substantial fraction of patients are affected.[14,88-90] Younger patients are at greater risk for hearing loss from cisplatin.[91,92] With long-term follow-up, there may be worsening or progression of hearing loss at lower frequencies.[93]

Fractionating the cisplatin dose appears to lessen the risk of ototoxicity,[88] but more effective strategies to prevent damage to the hair cells are needed. Sodium thiosulfate is being tested as a rescue agent in an ongoing COG trial, but thiosulfate is a non-selective rescue agent and can interfere with the anti-tumor effect of cisplatin. Cochlear hair cells also express OCT2, and inhibition of OCT2 during cisplatin therapy may selectively block the uptake of cisplatin into hair cells without impacting the drug's anti-tumor effect.[48,50]

1.3.5 Pantoprazole

Pantoprazole (Figure 5) is a proton pump inhibitor (PPI) that blocks gastric acid secretion by irreversibly binding to the proton pump.[94] It is indicated for treatment of GERD with erosive esophagitis and hypersecretion associated with Zollinger-Ellison syndrome. Pantoprazole is commercially available in an intravenous formulation that can be administered as an intermittent bolus or continuous infusion.

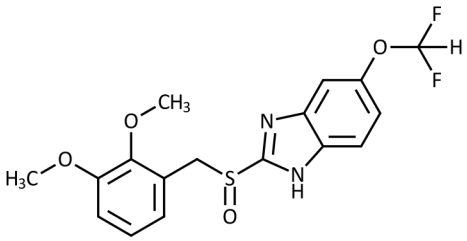


Figure 5 Structure of pantoprazole.

Pantoprazole and other PPIs are potent OCT2 inhibitors (Figure 6).[1] The IC_{50} for pantoprazole inhibition of OCT2 is 2.8 mcM and the C_{max} of the drug in children after 0.8 mg/kg (equivalent to 40 mg in adults) was 15 mcM and after a dose of 1.6 mg/kg (equivalent to 80 mg in adults) was 27 mcM,[3] which would inhibit OCT2 by >80%.[1]

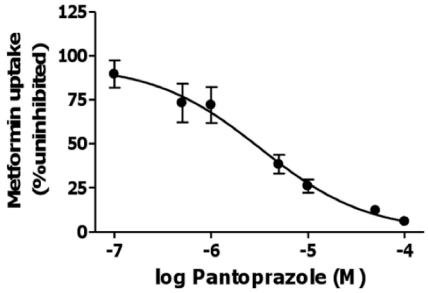


Figure 6 Inhibition of OCT2 by pantoprazole.[1]

Cimetidine (histamine H_2-receptor antagonist) also inhibits OCT2. In a small study of 9 patients with testicular cancer treated with cisplatin (20 mg/m^2/d x 5 days), cimetidine plus verapamil appeared to prevent cisplatin nephrotoxicity (assessed as change in effective renal plasma flow and GFR) compared to a similar historical control population (n=9) treated with cisplatin alone (Figure 7).[4] Cimetidine does not interfere with the anticancer effect of cisplatin *in vitro* and *in vivo* in an osteosarcoma cell line, indicating that uptake of cisplatin

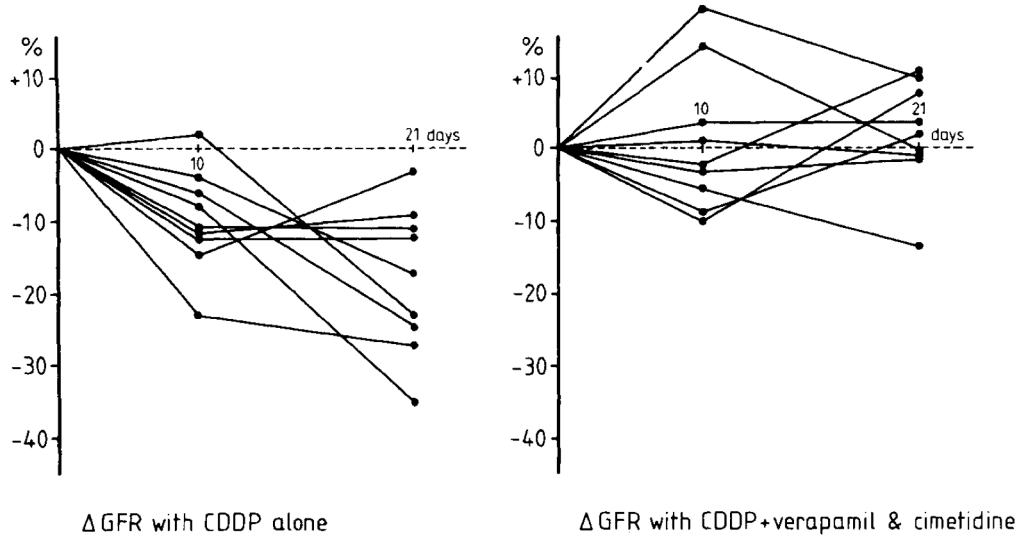


Figure 7 Change in GFR 10 and 21 days after 100 mg/m^2 cisplatin administered over 5 days without (left) and with cimetidine and verapamil.

into tumor cells is not mediated by OCT2 in osteosarcoma.[95] Pretreatment of human tumor cells with a proton pump inhibitor enhanced the cytotoxic effect of multiple anti-cancer drugs including cisplatin, and omeprazole pretreatment in mice harboring human tumor xenografts induced sensitivity of the tumors to cisplatin.[96,97]

The PK and tolerability of single intravenous doses of 0.8 and 1.6 mg/kg of pantoprazole have been studied in children.[3] The C_{max} and AUC of IV pantoprazole at 0.8 and 1.6 mg/kg in children aged 2 to 16 years were similar to these PK parameters in adults at 40 and 80 mg (Figure 8). The clearance of pantoprazole in children was 0.2 L/h/kg and the half-life was 1.2 h. Although the $t_{1/2}$ is short, it pharmacodynamic effect is more long lasting.[94] PPIs interfere with MTX excretion,[98] but by 20 days after the last cisplatin dose when HDMTX is administered, pantoprazole will be completely cleared.

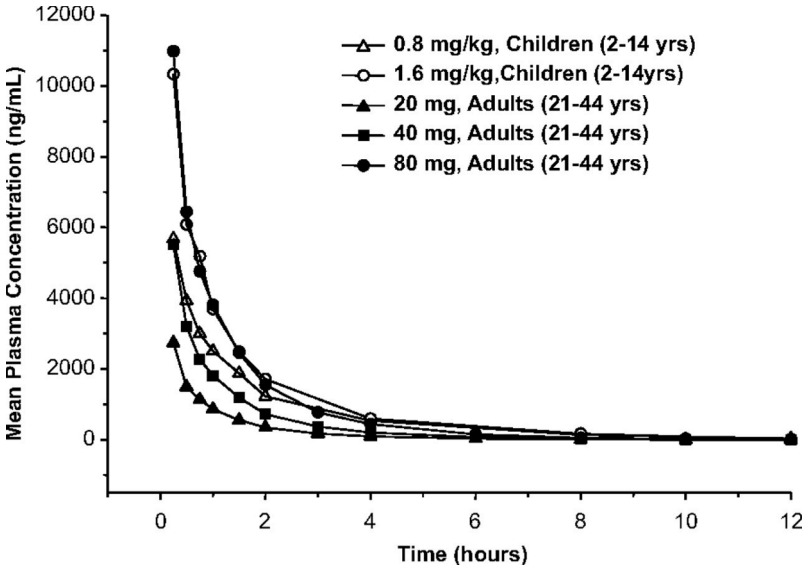


Figure 8 PK of intravenous pantoprazole in children (2-16 yrs) at doses of 0.8 and 1.6 mg/kg compared to adults at 20, 40 and 80 mg.[3,4]

Pantoprazole is primarily metabolized by CYP2C19, which is genetically polymorphic. CYP2C19 activity is absent in 3% of whites and 20% of Asians, and children with CYP2C19 polymorphisms have slower clearance and longer $t_{1/2}$s.[3]

The single IV doses of pantoprazole (0.8 and 1.6 mg/kg) were well tolerated. Three adverse events occurred in 18 patients, all of which were mild and judged to be unrelated to pantoprazole.[3] The most common toxicities associated with PPIs are diarrhea, nausea, headaches, abdominal pain, fatigue and dizziness.[94]

1.3.6 Serum biomarkers for osteosarcoma

Serum biomarkers for diagnosing osteosarcoma, for monitoring response to treatment, and for detecting disease recurrence would be valuable for the clinical management of the disease and as endpoints for clinical trials. Osteosarcoma is a matrix-producing tumor and radiographic response (tumor shrinkage) to neoadjuvant chemotherapy may not reflect the extent of tumor necrosis, histologically, after neoadjuvant chemotherapy.

Elevated serum alkaline phosphatase (AP) is an independent prognostic factor predicting for poor outcome in osteosarcoma,[25,99] but serum AP may be derived from other organs and is therefore not specific. Bone-specific AP (BSAP), which is the isoform of AP derived from bone, is a more specific measure of osteoblastic activity.[100-102] BSAP is elevated in patients with OS and is correlated with tumor burden.[102,103] However, BSAP is also higher in adolescents reflecting periods of bone growth during the adolescent growth spurt. We will investigate the potential role of BSAP as a potential biomarker for OS by measuring serum levels at diagnosis, after neoadjuvant chemotherapy, after surgical resection, and at the end of treatment.

1.3.7 Study Rationale

Current osteosarcoma treatment regimens include drugs that are nephrotoxic and ototoxic, and the damage to kidneys and cochlear hair cells may be irreversible. Preventing these toxicities will improve the outcome in long-term survivors and may also prevent short-term treatment delays and dose reductions that can compromise the efficacy of the treatment regimen and allow for administration of higher cumulative doses of cisplatin. This pilot study evaluates pharmacologically-based approaches to:

- prevent the nephrotoxic effect of HDMTX by prolonging the infusion duration and thereby lowering the risk of drug precipitation in renal tubules, and
- selectively block the uptake of cisplatin into renal tubular cells and cochlear hair cells by inhibiting the organic cation transporter 2 with the proton pump inhibitor, pantoprazole.

A randomized cross-over design, in which patients serve as their own control, will be used to maximize the potential to detect differences in markers of acute kidney injury (NAG, NGAL, KIM-1, cystatin C) and audiograms resulting from altering the infusion duration of HDMTX and from administering pantoprazole with cisplatin.

Two infusion durations of HDMTX will be studied – the standard 4 h duration and more prolonged 12 h infusion duration. Leucovorin rescue will commence 24 h from the start of the infusion. The HDMTX infusions will be divided into 2 blocks (cycles 1 & 2, cycles 3 & 4, see Figure 2), each of which include 4 HDMTX infusions. The order of infusion duration studied in each block will be randomly determined.

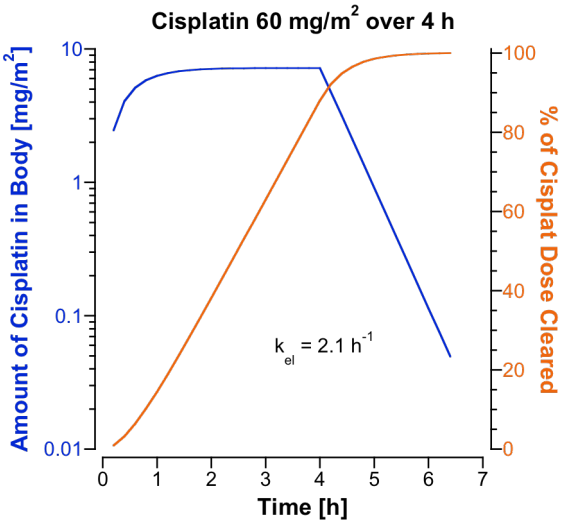


Figure 9 Amount of cisplatin in the body (blue) and cumulative percent of the dose cleared (orange) for a 60 mg/m^2 dose infused over 4 h.

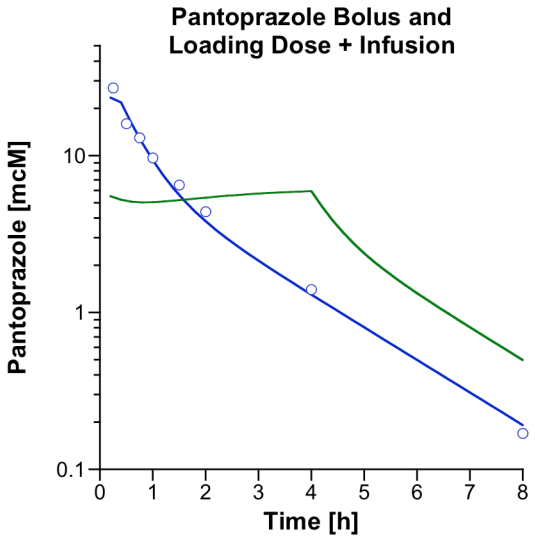


Figure 10 Plasma concentration-time profile of 1.6 mg/kg pantoprazole infused over 15 min (blue)[3] or administered as a 0.3 mg/kg loading dose over 15 min followed by 1.3 mg/kg infused over 4 h (green).

Pantoprazole will either be administered with the first two courses of cisplatin during cycles 1 & 2 or with the third and fourth courses during cycles 3 & 4, and the order will be randomly determined. Cisplatin is administered as a 60 mg/m^2 dose infused over 4 h daily x 2 d, and Figure 9 is a PK simulation showing the amount of active drug in the body (assuming a half-life of 20 min) and the percent of the total dose cleared. At steady state, 7.2 mg/m^2 of active cisplatin is in the body, and by the end of the 4 h infusion, 88% of the total dose has been cleared. Therefore, adequate concentrations to inhibit OCT2 should be present for at least 4 h to prevent cisplatin nephrotoxicity and ototoxicity. Pantoprazole has a short half-life (1.2 h),[3] and when administered at a dose of 1.6 mg/kg as a standard 15 min infusion (Figure 10), plasma pantoprazole concentration by the end of the 4 h cisplatin infusion is 1.3 mcM. By administering the same dose as a 0.3 mg/kg loading dose infused over 15 min followed by a 4 h infusion of the remaining 1.3 mg/kg, plasma pantoprazole concentration is 5.9 mcM at 4 h (Figure 10).

The primary endpoint to assess nephrotoxicity will be the AKI biomarkers (cystatin C, KIM-1, NGAL, NAG) described in Section 1.3.3. This will allow us to assess each dose of HDMTX and each course of cisplatin independently. Standard renal function tests (urinalysis, serum creatinine, creatinine clearance, and fractional excretion of magnesium) will also be monitored. Audiograms will be performed after each course of cisplatin to assess ototoxicity. Radiographic response and histological response in the resected tumor specimen after neoadjuvant chemotherapy will be assessed to ensure changes in HDMTX infusion duration and the addition of pantoprazole to cisplatin do not alter the efficacy of chemotherapy. The toxicity of the regimen will be closely monitored, and we will pilot a disease-specific patient reported outcome survey to assess the impact of tumor-related and drug toxicity-related symptoms.

2. STUDY OBJECTIVES

The **primary** objective is to identify more rational, pharmacologically-based drug delivery approaches to prevent nephrotoxicity from HDMTX and cisplatin and ototoxicity from cisplatin in patients with OS by:

- comparing biomarkers of acute kidney injury (KIM-1, NAG, NGAL), glomerular function (cystatin C, creatinine) and plasma and urine MTX concentrations after 12 g/m^2 of MTX infused over 4 and 12 hours,
- comparing biomarkers of acute kidney injury (KIM-1, NAG, NGAL) and glomerular and tubular function (cystatin C, creatinine, FEMg) after cisplatin administered with and without the OCT2 inhibitor, pantoprazole, and
- comparing hearing loss (audiograms) after cisplatin administered with and without the OCT2 inhibitor, pantoprazole.

Secondary objectives include:

- Comparing the incidence and severity of common toxicities from cisplatin/doxorubicin administered with and without pantoprazole and from HDMTX administered as a 4 and 12 h infusion,
- comparing radiographic response (log ratio of tumor volumes) and histological response (percent tumor necrosis) to two cycles (10 weeks) of neoadjuvant chemotherapy for the two infusion durations of HDMTX and for cisplatin with and without pantoprazole,
- correlating urinary biomarkers of AKI and GFR estimated from serum cystatin C to standard measures of renal function (serum creatinine, urinalysis, estimated creatinine clearance, FEMg),
- building a tissue microarray from biopsy, primary resection and resected metastatic tumors to evaluate the expression of proteins that are responsible for resistance to the drugs in the MAP regimen and to assess expression of proteins that are targeted by new anticancer drugs under development for childhood cancers,
- evaluating serum BSAP as a potential biomarker for OS,
- monitoring nutritional status (weight, arm circumference, skin fold thickness, pre-albumin) in patients receiving the MAP chemotherapy regimen, and
- piloting an osteosarcoma-specific patient reported outcomes survey to assess the incidence and severity of tumor-related and treatment-related symptoms.

3. INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

Patients with previously untreated osteosarcoma will receive 6 cycles of the standard MAP (Methotrexate, Adriamycin [doxorubicin], cisPlatin) chemotherapy regimen. The first two cycles will be administered neoadjuvantly followed by surgical resection of the primary tumor if

feasible, and then 2 additional cycles of MAP followed by 2 cycles of MA (Figure 2). A randomized, 2 x 2 factorial, crossover design (see Study Diagram) will be used to:

- study the effect of prolonging the methotrexate infusion duration from 4 h to 12 h on nephrotoxicity as measured by biomarkers of acute renal injury and
- study the effect of the OCT2 inhibitor, pantoprazole, on cisplatin nephrotoxicity as measured by biomarkers of acute renal injury and on ototoxicity as assessed on serial audiograms.

Each patient will serve as his/her own control, and 24 evaluable patients will be studied. Randomization to one of 4 treatment arms (see Study Diagram) will be performed after enrollment onto the trial and prior to the start of neoadjuvant chemotherapy. The use of sensitive, short-term biomarkers as endpoints to assess the effect of these interventions, continuous (rather than categorical) endpoints analyzed by dose, course or treatment cycle rather than by patient, and a cross-over design that allows patients to serve as their own controls will minimize the number of subjects and time required to complete this pilot study, increase the statistical power, and maximize the amount of data collected.

3.1.1 Screening, Diagnosis, and Staging

Patients with osteosarcoma typically present to the orthopedic or oncology clinical service with a history of pain and swelling at the primary tumor site and radiographic evidence of a destructive bone lesion. The standard of care at CHOP is to perform an open biopsy to ensure that an adequate amount of viable tumor tissue is obtained to make a diagnosis. **Histological diagnosis** is based on morphology and the presence of osteoid (unmineralized bone matrix). There are no specific immunohistochemical stains that can be used to confirm the diagnosis of osteosarcoma.

The standard radiographic **staging** for osteosarcoma to assess tumor extent in the involved bone and to document the presence of overt metastatic disease in the lungs or skeleton includes:

- AP and lateral radiographic of the entire involved bone
- MRI with contrast of the entire involved bone, including joints proximal and distal to the tumor (e.g., for a distal femoral tumor, the scan should include the entire femur with the hip and knee joints)
- Chest CT scan
- ^{99m}Tc-MDP Bone scan OR ^{18}FDG PET scan

Other standard clinical and laboratory test performed prior to initiation of treatment in patients with osteosarcoma include:

- History and physical examination, including measurement of the primary tumor (circumference for limb tumors) and performance status,
- CBC with differential, comprehensive mineral panel, calcium, phosphate, magnesium,

urinalysis, 12-24 h urine collection for creatinine clearance, urine pregnancy test,

- Echocardiogram with quantitation of left ventricular shortening fraction prior to administration of doxorubicin, and
- Audiogram to document baseline hearing prior to administration of cisplatin.

These procedures are standard of care for patients with osteosarcoma and can be performed prior to informed consent and enrollment on this clinical trial. Results of these studies will be used as baseline values and entered into the clinical trial database after patients have consented and enrolled onto the trial.

3.1.2 Baseline (Pretreatment) Research Studies

See Section 4.1 for research sample collection, processing, and storage instructions.

- Urinary AKI Markers (NAG, NGAL, KIM-1)
- Plasma and urine magnesium and creatinine for FEMg.
- Serum cystatin C
- Serum bone-specific alkaline phosphatase (BSAP)
- Plasma pre-albumin
- Patient-reported outcomes survey
- Upper arm (mid-way between the shoulder [acromion] and elbow [humeral lateral condyle]) circumference with elbow extended and triceps skin fold thickness 1 cm above site circumference was measured in arm NOT involved with tumor. These measurements are performed to estimate nutritional status, muscle mass, and creatinine production.

3.1.3 Treatment with MAP Regimen

All patients will receive 6 cycles of the 3-drug MAP chemotherapy regimen (Methotrexate, Adriamycin [doxorubicin] and cisPlatin) that is shown in Figure 2. Cycles 1 & 2 will be administered neoadjuvantly (before resection of the primary tumor). After recovery from surgery, patients will resume treatment with 2 cycles of MAP followed by 2 cycles of MA (no cisplatin).

Treatment arm specific treatment delivery maps (TDM) will be generated for each patient after randomization and placed in the clinic chart to serve as a guide. Appendix 1 shows an example of a TDM.

Standard treatments: Cycles 1-4 of MAP are 5 weeks (35 days) in duration. Cisplatin and doxorubicin are administered on days 1 & 2 and HDMTX + leucovorin rescue is infused on days 22 & 29. cisplatin is not administered in Cycles 5 & 6 and the duration of Cycles 5 & 6 is 4 weeks (28 days). During cycles 5 & 6, doxorubicin is administered on days 1 & 2 and HDMTX + leucovorin rescue is infused on days 15 & 22. Supportive care/rescue agents that are administered with MAP to alleviate toxicity include antiemetics (see CHOP Cancer Center

antiemetic guidelines), dexrazoxane as a cardioprotectant, pegfilgrastim to facilitate neutrophil recovery after doxorubicin, and leucovorin as a HDMTX rescue agent. Intravenous fluid hydration is administered with cisplatin and HDMTX to prevent nephrotoxicity, and urine alkalinization is used to increase the solubility of MTX in urine.

Research Interventions: Pantoprazole will be administered intravenously with cisplatin on 2 consecutive treatment cycles (either Cycles 1 & 2 or Cycles 3 & 4), and the order of pantoprazole administration will be determined by randomization (see Study Diagram). Patients will receive HDMTX as 4 and 12 h infusions on 2 consecutive treatment cycles (Cycles 1 & 2 or Cycles 3 & 4), and the order of the infusion durations will be determined by randomization.

Drug doses, schedules, and administration routes: for the chemotherapy and supportive care/rescue medications are listed below:

- **Dexrazoxane** 375 mg/m^2/dose IV over 15 min immediately prior to the doxorubicin doses on days 1 & 2 (total dose 750 mg/m^2/cycle) of treatment cycles 1-6;
- **DOXOrubicin** 37.5 mg/m^2/dose IV over 15 min on days 1 & 2 (total dose 75 mg/m^2/cycle) of treatment cycles 1-6 (total cumulative dose 450 mg/m^2);
- **Pantoprazole** 0.3 mg/kg IV over 15 min immediately prior to cisplatin as a loading dose on days 1 & 2 followed by 1.3 mg/kg IV infused over 4 h concurrent with the 4 h cisplatin infusion on days 1 & 2 of treatment Cycles 1 & 2 (Treatment Arms 1, 3) OR Cycles 3 & 4 (Treatment Arms 2, 4);
- **CISplatin** 60 mg/m^2/dose infused IV over 4 h on days 1 & 2 (total dose 120 mg/m^2/cycle) of treatment cycles 1-4;
- **Pegfilgrastim** 0.1 mg/kg (6 mg for patients weighing ≥45 kg) SC on day 3 or 4 of treatment cycles 1-6;
- **Methotrexate** 12 g/m^2 (maximum dose 20 g) infused IV over 4 OR 12 h on days 22 & 29 of Cycles 1-4 and on days 15 and 22 of Cycles 5 & 6 (HDMTX infusion duration on Cycles 5 & 6 based on patient tolerance on cycles 1-4);
- **Leucovorin** 15 mg/m^2 IV or PO every 6 h until serum MTX <0.1 mcM on days 23 and 30 of cycles 1-4 and days 16 & 23 of cycles 5 & 6, **starting 24 h after the start of the HDMTX infusion**.

Intravenous fluid hydration guidelines: Intravenous fluid hydration should be administered prior to, during and after cisplatin and HDMTX. In addition, the urine should be alkalinized to a pH of ≥7 for HDMTX until serum MTX <1 mcM by including sodium bicarbonate or equivalent base in the IV fluids.

Ci**splatin**:

- Prior to the first dose of cisplatin on day 1 of the cycle, infuse a normal saline (0.9% NaCl) fluid bolus of 750 mL/m^2 over 1 h.
- Cisplatin is diluted in 0.9% NaCl to a final cisplatin concentration of 100 mg/L. Therefore,

patients will receive 150 mL/m^2/h of this solution over the 4 hour drug infusion.

- After the cisplatin infusion is completed, infuse 100 mL/m^2/h D_5 0.45% NaCl + 20 mEq/L KCl until at least 12 h after the second cisplatin infusion is completed and the patient is tolerating PO fluids.
- Monitor Input and Output, at 3hr or 6hr after start of cisplatin
    - If urine Output > total Input by 100 mL/m^2/hr over the previous 3 hr, consider 0.9% NaCl bolus (10-15 cc/kg)
    - If total Input > urine Output by 100 mL/m^2/hr over the previous 3 hr, consider furosemide (0.5 mg/kg, MAX dose 20 mg) to promote urine output.[104,105]

HDMTX:

- Prior to the start of a HDMTX infusion, infuse D_5 0.22% NaCl with 40 mEq/L $NaHCO_3$ (sodium biocarbonate) or equivalent base as a fluid bolus of 750 mL/m^2 over 1 h.
- Continue hydration with D_5 0.22% NaCl with 40 mEq/L $NaHCO_3$ (sodium bicarbonate) or equivalent base at 100 mL/m^2/h until serum MTX <1 mcM and PO fluids are tolerated. Acetate can be substituted if bicarbonate is not available at a 1:1 dose conversion.
- If urine pH <7 administer 0.5 mEq/kg $NaHCO_3$ IV. Recheck urine pH ≥30 min after $NaHCO_3$ bolus is complete. Repeat $NaHCO_3$ bolus until urine pH ≥7 and consider increasing dose of bicarbonate in maintenance fluids if urine pH consistently <7.
- Once urine pH ≥7 start HDMTX infusion. Continue to check urine pH every 8 hours and administer 0.5 mEq/kg $NHCO_3$ IV if urine pH <7, until serum MTX <1 mcM.
- HDMTX solution (25 mg/mL) is piggy-backed into the hydration fluids and infused over 4 OR 12 h depending on the treatment arm and cycle (see Study Diagram).

Leucovorin dosing guidelines including guidelines for delayed MTX excretion:

- The dose and duration of leucovorin rescue are determined by the plasma MTX concentration. Leucovorin rescue should start no later than 24 h after the **start** of the HDMTX infusion and continue until the plasma MTX concentration is <0.1 mcM.
- **Leucovorin** 15 mg/m^2 IV or PO every 6 h **starting 24 h after the start of the HDMTX**

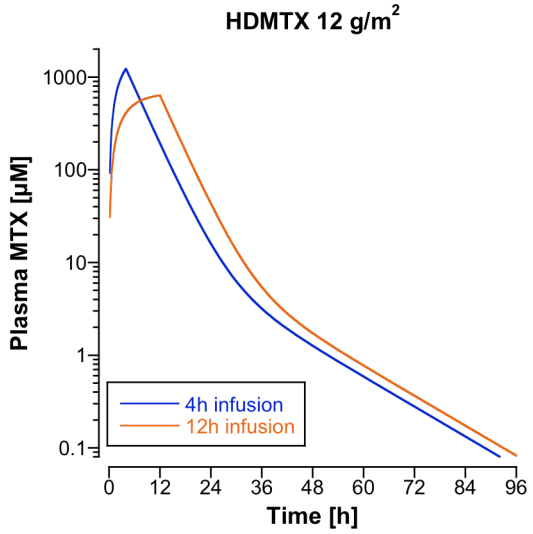


Figure 11: Concentration time curves for MTX (12 g/m^2) infusions over 4 and 12 h generated by fitting a 2-compartment pharmacokinetic model to the mean plasma MTX concentrations from 1,045 infusions of 12 g/m^2 over 4 h.[5]

infusion on days 23 and 30 of cycles 1-4 and days 16 & 23 of cycles 5 & 6 and administered until serum MTX <0.1 mcM.

- Serum MTX levels will vary based on the duration of the HDMTX infusion (Figure 11). Table 3 lists the expected plasma MTX concentrations at various times after a 4 and 12 h infusion. **Please contact the PI or study Co-investigators <u>at any time</u> for questions or concerns about MTX levels.**

Table 3. Expected MTX levels by infusion duration. (EOI, end of infusion)

	Plasma MTX [mcM]	
Sample time (h)	**4 h Infusion**	**12 h Infusion**
EOI	1200	600
24	15	40
36	3	5
48	1	2
60	0.5	1
72	0.3	0.4
96	<0.1	<0.1

- If a patient experiences MTX nephrotoxicity and delayed MTX clearance, the leucovorin dose or schedule may need adjustment based on plasma MTX concentration, or the patient may require glucarpidase to lower plasma MTX concentrations. **The study PI or a co-Investigator should be contacted immediately if the plasma MTX concentration is >100 mcM at hour 24, >50 mcM at hour 28-40, or >10 mcM at hour 48 or later. Leucovorin should be escalated to 15 mg/m^2 IV or PO every 3 h if the plasma MTX concentration is >2-fold higher than expected (Table 3) AND >1 mcM. The every 6 h schedule can be resumed when the plasma MTX concentration is <1 mcM.**

3.1.4 Criteria for Starting Treatment

CISplatin/DOXOrubicin (Cycle 1-4) or DOXOrubicin alone (Cycle 5&6):

Delay treatment until the following conditions are met:

- ANC >750/mcL and platelet count >75,000/mcL
- Oral mucositis grade <2
- Hyperbilirubinemia (total) grade <3 and ALT/AST elevation grade <4
- Normal serum creatinine for age and gender (see Table in eligibility criteria) OR creatinine clearance >70 mL/min/1.73 m^2 (Cycles 1-4 only). If renal function does not recover after a one week delay, administer doxorubicin alone.
- Adequate wound healing (determined by the surgeon) after resection of the primary tumor (prior to Cycle 3) or after resection of pulmonary metastases.

HDMTX:

Delay treatment until the following conditions are met:

- ANC >250/mcL and platelet count >50,000/mcL
- Oral mucositis grade <2
- Hyperbilirubinemia grade <3 and ALT/AST elevation grade <4
- Normal serum creatinine for age & gender OR creatinine clearance >70 mL/min/1.73 m^2

3.1.5 Dose Modifications

Modification of the doses of cisplatin, doxorubicin, and HDMTX is required if toxicity for the drugs exceeds the thresholds listed in the Tables below on a prior treatment cycle. The percent of full protocol prescribed dose should be recorded on the TDM in the column labeled "% Dose". If the dose is reduced by 25%, then the % Dose should be recorded as 75% (75% of the full dose).

Cisplatin:

Toxicity	Severity	Modification
Ototoxicity	>30 dB threshold at 2 kHz	Discontinue cisplatin
Nephrotoxicity	Serum creatinine >ULN for age and gender OR creatinine clearance <70 mL/min/1.73 m^2	Hold cisplatin until criterion in Section 3.1.4 is met
Neuropathy*	Grade >2 motor or sensory neuropathy	Hold cisplatin until resolved to grade ≤2

* See Appendix 2 for toxicity grading scale

Doxorubicin:

Toxicity	Severity	Modification
Myelosuppression	Planned day 22 HDMTX is delayed by >10d (cycle day >32) due to ANC <250/mcL or platelet count <50,000/mcL	Reduce doxorubicin dose by 20%
Cardiotoxicity	SF <27% (confirmed with repeat Echocadiogram 1 week later)	Hold doxorubicin until recovery to SF >28%
Mucositis	Grade 4 related to doxorubicin	Reduce doxorubicin dose by 20%

HDMTX:

Toxicity	Severity	Modification
Nephrotoxicity	Serum creatinine >ULN for age and gender OR creatinine clearance <70 mL/min/1.73 m^2	Hold HDMTX. Omit dose if renal function has not recovered after a 7-day delay
Mucositis	Grade 4 related to HDMTX	Reduce HDMTX by 50%

3.1.6 Local Control of Primary Tumor

Complete resection of the primary tumor is the optimal form of local therapy and is performed after two 5-week cycles of neoadjuvant MAP chemotherapy (11 weeks after the start of therapy). A limb salvage procedure is indicated if the neurovascular bundle is not extensively

involved, the tumor can be resected *en bloc* with tumor-free margins, and reconstruction with an endoprosthesis to replace the involved bone and joint is feasible. If a limb salvage procedure is not indicated, an amputation of the involved portion of the limb will be performed.

Radiation therapy is reserved for patients who have unresectable primary tumors (e.g., spine tumors). The timing of radiation therapy will be based on symptoms, such as pain, response to neoadjuvant chemotherapy, and ability to deliver full dose intensity of the chemotherapy regimen. Radiation is less effective than surgery but can achieve local control in some patients.[106,107]

3.1.7 Management of Metastatic Tumors

Complete resection of overt pulmonary metastases that are present at diagnosis can enhance survival chances.[11,108] Median sternotomy or bilateral thoracotomies with manual palpation of both lungs is recommended even in patients with unilateral pulmonary metastases on CT scan. Timing of resection of metastatic disease is left to the clinical judgment of the oncologist and surgeons.

3.2 Randomization

Patients will be randomized to one of 4 treatment arms (see Study Diagram), which will determine the order in which they receive pantoprazole with cisplatin (Cycles 1&2 vs. Cycles 3&4) and the order in which they will receive HDMTX as a 4 or 12 h infusion (Cycles 1&2 vs. Cycles 3&4). A block randomization procedure will be used so that 3 of the first 12 subjects will be randomly assigned to each of the 4 treatment arms (three patient per arm) and 3 of subjects 13 to 24 will be randomly assigned to each of the 4 arms (six patients per arm when enrollment is completed). Subjects who are unable to complete 4 cycles of therapy may be replaced, and the new subject will be placed on the same treatment arm as the subject being replaced.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study

The duration of study participation will be approximately 8 months. The screening process including biopsy of the primary tumor for diagnosis will take up to 2 weeks. The first 4 treatment cycles are 5 weeks each (total, 20 weeks) and cycles 5 and 6 are 4 weeks each (total, 8 weeks). The time for recovery from surgery to resect the primary tumor after Cycle 2 is usually 1-2 weeks. Endpoints for this trial are measured during therapy and at the end of therapy visit after the last HDMTX infusion.

3.3.2 Number of Subjects and Number of Study Sites

This pilot study will be conducted at CHOP. We plan to study 24 subjects. Patients who do not complete 4 cycles of treatment for any reason may be replaced. We may enroll up to 28 subjects in order to have 24 fully evaluable subjects. Based on the number of children with OS seen at CHOP annually, we expect accrual to be complete in 2 to 3 years.

3.4 Study Population

3.4.1 Study Enrollment/Registration

Subjects are enrolled on study after fulfilling the eligibility criteria listed below. Treatment arm will be assigned by the Study PI based on a spreadsheet provided by the Study Biostatistician. Demographic information, results of screening and baseline studies, tumor site(s) and histology, and treatment arm will be entered into OnCore CRM. **Patients must be enrolled and randomized prior to starting neoadjuvant chemotherapy.**

3.4.2 Inclusion Criteria

- **Age**: <30 years of age
- **Diagnosis**: histological diagnosis of high-grade osteosarcoma (Section 3.1.1)
- **Site**: Extremity or central axis (including craniofacial) primary tumor; localized or metastatic
- **Prior therapy**: No prior chemotherapy or radiation therapy for osteosarcoma. Subjects who develop osteosarcoma as a second cancer are eligible if they have not previously received cisplatin, doxorubicin or other anthracyclines, or MTX
- **Kidney function**: Serum creatinine at or below the upper limit of normal (ULN) for age and gender:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 mo to < 6 months	0.4	0.4
6 mo to < 1 year	0.5	0.5
1 year to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4
Derived for Schwartz et a. J Peds 106:522, 1985 utilizing child length and stature data published by the CDC		

- **Cardiac function**: Shortening fraction on echocardiogram >28%
- **Hearing**: Hearing level threshold ≤25 dB at all frequencies in both ears to be evaluable for evaluation of pantoprazole's effect on cisplatin ototoxicity. Patients with hearing loss can be enrolled but will not be evaluable for ototoxicity objective.
- **Hematological function**: Absolute neutrophil count >1,000/mcL and platelet count >100,000/mcL

3.4.3 Exclusion Criteria

- Receiving H_2 antagonists (cimetidine, ranitidine, famotidine, nizatidine) or proton pump inhibitors (lansoprazole, omeprazole, pantoprazole, esomeprazole, raberprazole, dexlansoprazole) AND unable to hold the drug for 24 h prior to and 24 h after each cisplatin course on cycles 1-4.
- Inability to tolerate a PPI
- Pregnant or breastfeeding
- Unable to cooperate with research procedures

4. RESEARCH PROCEDURES

Refer to Treatment arm specific TDMs for schedule of drug administration and Schedule of Study Procedures for timing of standard of care and research procedures.

4.1 Research Studies during Treatment

See Schedule of Study Procedures table for timing of standard of care and research procedures.

Test	Specimen	Tube	Volume	Collection	Processing	Storage	Lab/Assay
KIM-1	Spot urine	Urine container	>5 mL	Prior to cisplatin D1, EOI D2, D7; prior to each HDMTX & 24 after the start of infusion; pre-op; C5D1	Centrifuge	-70°C	CTSU Core
NGAL							
NAG							
Cystatin C	Serum	Gold top SST-clot activator	3 mL	Prior to cisplatin and HDMTX; D7; pre-op; C5D1; post-inf IF sCr ≥50% above baseline	Centrifuge	-70°C	UPenn Core
Mg, Cr	Plasma	Light green top	0.5 mL	Prior to cisplatin D1, D2 & D7; C5D1	Send to Clinical Lab	Room temp	Clinical Lab
Mg, Cr	Spot urine	Urine container	>2 mL	Prior to cisplatin D1, D2 & D7; C5D1	Send to Clinical Lab	Room temp	Clinical Lab
BSAP	Serum	Red top	2 mL	Baseline, pre-op, C3D1 & EOT	Clot, centrifuge, separate serum	-70° C	CTSU Core
Pre-albumin	Plasma	Light green top	1 mL	Baseline, D1 C2-6, Pre-op, EOT	Send to Clinical Lab	Room temp	Clinical Lab
MTX	Urine	Conical tube	1 mL	EOI of each HDMTX C1-4	Pipette 1 mL urine into conical tube w/ 9 mL buffer	-70° C	PK Core HPLC/MS/MS

Abbreviations: EOI, end of infusion; Mg, magnesium; Cr, creatinine; BSAP, bone-specific alkaline phosphatase; inf, infusion; EOT, end of therapy; MTX, methotrexate concentration

4.2 Post-Therapy Follow-up

Periodic follow-up and restaging is standard of care for patients who have completed treatment. Guidelines for following patients after completion of treatment are provided in the table below:

	Months Post-Treatment													
Procedure	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**30**	**36**	**42**	**48**	**54**	**60**
History, physical, PS	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC, CMP, Mg, PO_4	X	X	X	X	X	X	X	X						
Chest CT	X	X	X	X	X	X	X	X						
MRI of primary[1]	X	X		X		X		X						
Chest X-ray									X	X	X	X	X	X
X-ray of primary									X	X	X	X	X	X
Bone or PET scan[2]		X		X		X		X						
Audiogram				X				X						X
Echocardiogram				X				X		X		X		X
BSAP[3]	Draw sample if subject has evidence of tumor recurrence													
Arm circum/Skin fold[3]	X	X		X				X		X		X		X

[1] Substitute CT scan if metal in endoprothesis does not allow for MRI to be performed

[2] Only if positive for metastatic disease at baseline

[3] Research procedure

4.3 Concomitant Medications

H_2-antagonists (cimetidine, ranitidine, famotidine, nizatidine) and **proton pump inhibitors** (lansoprazole, omeprazole, pantoprazole, esomeprazole, raberprazole, dexlansoprazole) should NOT be administered for 24 h prior to and 24 h after cisplatin on Cycles 1-4. Patients will receive pantoprazole with cisplatin on Cycles 1&2 OR Cycles 3&4.

Probenecid, NSAIDS, sulfonamides (including co-trimoxazole), and penicillins should NOT be administered concurrently with HDMTX. These drugs can interfere with the secretion of MTX by the renal tubule.

Patients should receive **co-trimoxazole** 2.5 mg/kg/dose twice daily on 2 consecutive days per week to prevent pneumocystis pneumonia. Co-trimoxazole should NOT be administered concurrently with HDMTX.

Antiemetics should be administered prior to and as needed during and after chemotherapy according to the CHOP Antiemetic guidelines available on the Cancer Center Website.

4.4 Subject Completion/Withdrawal

Subjects will be followed (Section 4.2) until:

- Five years after completing treatment
- Relapse

- Death from any cause

Subjects may voluntarily withdraw from this study at any time but should complete the standard of care chemotherapy regimen and resection of their primary and metastatic tumor as described in this protocol.

5. STUDY ENDPOINTS AND EVALUATIONS

The timing of study endpoints, as well as standard of care procedures, is shown in the Schedule of Study Procedures.

5.1 Primary Endpoints

- Urinary biomarkers of acute kidney injury (KIM-1, NAG, NGAL) after each course of cisplatin (days 2 and 7) and each HDMTX infusion (24 h and day 7) on cycles 1-4 compared to baseline (pre-infusion) values
- Serum cystatin-C and creatinine prior to each course of cisplatin and each HDMTX infusion on cycles 1-4 and post-infusion cystatin-C and BMP if serum creatinine increases by ≥50% compared to pre-infusion baseline value
- FEMg (simultaneous serum and spot urine for Mg and creatinine) prior to the start of the cisplatin infusions on days 1&2 and day 7 of cycles 1-4
- Plasma and urine MTX concentrations at the end of each HDMTX infusion on cycles 1-4
- Audiograms prior to cycles 1-4 and at the end of therapy.

5.2 Secondary Endpoints

- The incidence and severity (CTCAE v.4 grade) of safety events listed in Section 5.4,
- Response of the primary tumor to the first 2 treatment cycles will be assessed by quantifying the change in tumor volume on MRI after treatment relative to the pre-treatment tumor volume using the log ratio method [$\log(V_t/V_0)$, where V_t is tumor volume post-treatment and V_0 is tumor volume at baseline][109] and quantifying the percent tumor necrosis in the resected tumor specimens histologically,
- The new biomarkers of AKI (KIM-1, NAG, NGAL) and GFR (cystatin C) will be correlated with the current standard clinical laboratory methods of assessing renal function with serum creatinine, estimated creatinine clearance, urinalysis and FEMg,
- A tissue microarray will be constructed from the paraffin blocks containing the initial biopsy specimen, the resected primary tumor and resected metastatic lesion (if available) in the Pathology Core Laboratory. A slide cut from the top of the block will be used to identify areas of viable tumor, which will then be marked on the block. IHC staining will be performed for proteins responsible for resistance to MAP agents and for protein targets of new agents under development,
- Serum bone specific alkaline phosphatase will be measured at baseline, pre- and post-op, end of treatment and at the time of relapse and correlated with tumor volume,

- An osteosarcoma-specific patient reported outcome survey (Appendix 3) developed for this study will be administered prior to each treatment cycle, prior to surgery and at the end of therapy,
- Weight, arm circumference (muscle mass), skin fold thickness (body fat), pre-albumin, and use of supplemental feedings via NG tube or G-tube will be used to assess the impact of treatment on nutritional status over the course of therapy.

5.3 Efficacy Evaluations

- The effect of prolonging the duration of the HDMTX infusion to 12 h will be assessed by comparing urinary biomarkers of AKI and serum cystatin C and creatinine measured prior to, 24 h and 7 days after the start of each HDMTX infusion (n=8). All subjects will receive four HDMTX infusion durations of 4 h and four HDMTX infusion durations of 12 h in this crossover design, and subjects will serve as their controls. Measurement of the urine MTX concentration at the end of the infusion will be used to determine whether urinary MTX concentrations exceed the solubility limit for each HDMTX infusion.
- The effect of IV pantoprazole on cisplatin nephrotoxicity will be assessed by comparing urinary biomarkers of AKI, FEMg and serum cystatin C and creatinine measured prior to, at the end of the day 2 infusion, and on day 7 each cisplatin course (n=4). All subjects will receive cisplatin with and without pantoprazole in this crossover design, and subjects will, therefore, serve as their controls.
- The effect of IV pantoprazole on cisplatin ototoxicity will be assessed by comparing audiograms performed prior to each cisplatin infusion and at the end of therapy. All subjects will receive cisplatin with and without pantoprazole in this crossover design, and subjects will, therefore, serve as their own controls.
- Event-free and overall survival will be monitored but are not scientific objectives on this trial. An event is defined as tumor progression, relapse or death from any cause.

5.4 Safety Evaluations

- More than 90% of patients receiving MAP chemotherapy will experience severe (CTCAE grade 3 or 4) acute toxicity. The most common acute toxicities are nausea and vomiting, neutropenia, thrombocytopenia, fever/infection, oral mucositis, and hepatotoxicity (elevated serum transaminases and bilirubin).[14,16,23] Neutropenic fever or infection necessitates hospital admission and empirical antibiotics.
- The following adverse events will be tracked by treatment cycle:
    - Nadir neutrophil count and duration of severe (ANC <500/mcL) neutropenia
    - Nadir platelet count and number of platelet transfusions
    - Hospital admissions/days for neutropenia with fever or infection
    - Highest value for serum ALT and total bilirubin
    - Grade 3 or 4 mucositis

- Treatment delays or missed chemotherapy doses to allow for recovery from toxicity
- Dose modifications for toxicity
- Deaths attributed to chemotherapy toxicity

- After the first 12 patients have completed therapy, the incidence and severity of the events listed above as well as the nephrotoxicity and ototoxicity endpoints will be assessed by Cycle according to HDMTX infusion duration (4 h vs. 12 h) and according to whether or not IV pantoprazole was administered with cisplatin to ensure that the experimental dosing methods are not associated with excessive toxicity.
- After the first 12 patients have undergone resection of their primary tumor, the radiographic response (log ratio) and histologic response (% necrosis) will be assessed according to HDMTX infusion duration (4 h vs. 12 h) during Cycles 1 & 2 and according to whether or not IV pantoprazole was administered with cisplatin during Cycles 1 & 2 to ensure that the anti-tumor effect of the MAP chemotherapy regimen is not compromised by the experimental dosing methods.

6. STATISTICAL CONSIDERATIONS

This is a pilot study to evaluate pharmacologically-based dosing approaches to prevent the nephrotoxic effects of HDMTX and cisplatin and ototoxic effect of cisplatin in children, adolescents and young adults with osteosarcoma treated with the standard MAP chemotherapy regimen. Acute biomarkers of AKI (KIM-1, NAG, NGAL) and renal function tests (creatinine, cystatin C, FEMg) will be used to assess the incidence and severity of nephrotoxicity after each course of cisplatin (with and without pantoprazole) and HDMTX (infused over 4 and 12 h), and audiograms will be used to measure hearing loss prior to cycles 1-4 and at the end of therapy.

A 2 x 2 factorial, randomized crossover design will allow patients to serve as their own control. Nephrotoxicity data will be analyzed by course for cisplatin and by dose for HDMTX, and ototoxicity data will be analyzed by treatment cycle. All endpoints will be analyzed as continuous variables.

6.1 Statistical Analysis Plan

6.1.1 Primary Endpoints

- **Urinary KIM-1, NAG and NGAL** will be normalized to the urinary creatinine concentration measured in the same urine specimen and expressed as ng/mg Cr. The end of infusion and 7-day post values for each biomarker will be analyzed by course for cisplatin with vs. without pantoprazole and by dose for HDMTX administered as a 4 h vs. 12 h infusion.
- **GFR** will be estimated from serum creatinine and from the cystatin C (see Section 1.3.3) prior to each course of cisplatin and each dose of HDMTX and 7-days post treatment. A decrease in the GFR will reflect toxicity from the immediately preceding course of

cisplatin or dose of HDMTX. Change in GFR will be analyzed by course for cisplatin with vs. without pantoprazole and by dose for HDMTX administered as a 4 h vs. 12 h infusion.

- Change in **FEMg** from pre-dose 1 (day 1 of cycles 1-4) of cisplatin to pre-dose 2 (day 2 of cycles 1-4) of cisplatin and day 7 will be measured for each cycle containing cisplatin (n=4). Change in FEMg will be analyzed by course for cisplatin with vs. without pantoprazole to assess renal tubular toxicity.
- The **urinary MTX concentration** at the end of each HDMTX infusion on cycles 1-4 will be tabulated for the 4 and 12 h infusion durations and compared to the MTX solubility limit for the urine pH to determine whether the solubility limit was exceeded. End infusion urine MTX concentration will also be correlated with simultaneous serum concentration and with the severity of renal toxicity as measured by AKI biomarkers and serum creatinine and serum cystatin C.
- An **audiogram** will be performed prior to Cycles 1, 2, 3 and 4 and at the end of therapy (after cycle 6). The average hearing level (HL) threshold in dB over the frequency range of 4,000-8,000 Hz will be derived separately for each ear from each audiogram. The increase in the average HL threshold in dB from the pre-cycle 1 to pre-cycle 2, from pre-cycle 2 to pre-cycle 3, from pre-cycle 3 to pre-cycle 4, and from pre-cycle 4 to end of therapy audiograms will be derived and this increase in HL threshold will be compared for cisplatin with vs. without pantoprazole.

For each primary endpoint, we will construct separate models for comparison of cisplatin with vs. without pantoprazole and for comparison of the 4 vs. 12 h infusion durations of HDMTX. Linear mixed effects model will be used for this crossover design, including all repeated measures as the outcome, order type and period number as the fixed effects, and a subject level random intercept to account for within-subject correlation (or unstructured correlation matrix for the repeated measures if needed). Appropriate contrasts will be constructed to test for treatment effect. For nephrotoxicity outcomes using AKI biomarkers, little carryover effect is expected. For ototoxicity outcomes, carryover effect is possible, but unlikely to be differential between comparison arms because we randomized the order of treatment. Nevertheless, we will conduct a statistical test for differential carryover effect, acknowledging limited power with this test. If differential carryover is present in the analysis we will only use the data from cycle 1.

6.1.2 Secondary Endpoints

- **Common grade ≥2 toxicities** from cisplatin + doxorubicin (other than cisplatin nephrotoxicity and ototoxicity), including myelosuppression, mucositis, nausea and vomiting leading to dehydration requiring IV fluid hydration, peripheral neuropathy, cardiac dysfunction, and treatment delays due to toxicity will be collected and graded according to CTCAE v.4. Similarly, HDMTX-related grade ≥2 toxicities (other than nephrotoxicity), including myelosuppression, mucositis, dermatitis, hepatotoxicity and neurotoxicity will be collected and graded. The incidence and severity (grade) of toxicities will be tabulated and compared by treatment arm (with vs. without

pantoprazole for cisplatin + doxorubicin and by infusion duration for HDMTX).

- **Response** to 2 cycles of neoadjuvant chemotherapy will be assessed radiographically and histologically.
    - The volume of the tumor on MRI will be measured pretreatment and pre-operatively (post-cycle 2). The log ratio (Section 5.2) of the two measurements will be derived and compared using a non-parametric analysis for cisplatin with (patients on treatment arms 1 and 3) vs. without (patients on treatment arms 2 and 4) pantoprazole and for HDMTX administered as a 4 h infusion (treatment arms 1 and 2) vs. a 12 h infusion (treatment arms 3 and 4).
    - The tumor specimen that is removed after 2 cycles of neoadjuvant chemotherapy will be evaluated histologically and the percent of the tumor that is necrotic will be estimated from review of multiple sections. The percent necrosis will be compared using a non-parametric analysis for cisplatin with (patients on treatment arms 1 and 3) vs. without (patients on treatment arms 2 and 4) pantoprazole and for HDMTX administered as a 4 h infusion (treatment arms 1 and 2) vs. a 12 h infusion (treatment arms 3 and 4.).
- **GFR** estimated from serum creatinine using the modified Schwartz formula will be correlated with GFR estimated from serum cystatin C (Section 1.3.3, the equation also includes serum creatinine and BUN). The presence and amount (normalized to creatinine concentration) of biomarkers of AKI in urine will be correlated with proteinuria, serum creatinine, and change in FEMg.
- **BSAP** levels will be correlated with tumor volume and presence or absence of metastatic disease at diagnosis. BSAP after 2 cycles of neoadjuvant chemotherapy will be correlated with tumor volume and percent tumor necrosis post-cycle 2. BSAP drawn post-operatively, at the end of therapy and at the time of relapse will be correlated with disease status.
- An osteosarcoma-specific **Patient Reported Outcome survey** will be used to assess disease-related and treatment-related symptoms and tested in the patients enrolled on this trial. The patient reported outcomes will be compared to toxicity data collected from the medical record and to physician assessments of patient status (performance score).

6.2 Sample Size and Power

The primary endpoints are sensitive urinary biomarkers of AKI and audiograms, and all endpoints are continuous variables. The study is designed to detect a signal sufficient to justify a randomized trial that would be conducted in a cooperative group setting.

Twenty-four patients will be enrolled and data will be analyzed by dose or treatment cycle with patients serving as their own control. Over the past 5 years 57 newly diagnosed patients with osteosarcoma were seen and treated at CHOP. This is an average of 11.5 patients per year, indicating that we can complete accrual to this study in about two years.

- For the analyses of nephrotoxicity using biomarker endpoints of AKI (Urinary KIM-1, NAG and NGAL), data will be the analyzed by course or by dose. Each patient will have 2 courses of cisplatin with pantoprazole and 2 courses without pantoprazole. A power calculation was conducted under the framework of a multivariate general linear hypothesis for general linear models, using the Wilks Lambda test with a significance level of 0.05.[110,111] Because the comparison will be within subject, a larger within-subject correlation will provide greater power for the test. Based on the most conservative assumption that all repeated measures are independent, we will have 80% power to detect a 0.60SD difference for with vs. without pantoprazole. Assuming a moderate correlation of 0.5, we will have 80% power to detect a smaller difference of 0.42SD. Variability (SD) for these biomarkers in children with AKI are not available, but in a healthy adult population,[112] the mean value of KIM-1 was 0.228 with a SD of 0.094, so assuming a similar mean and SD for pediatric patients, we will have 80% power to detect a 0.056 difference (about 25% decrease) in the mean KIM-1 value with pantoprazole, assuming independence among repeated measures. We will have 80% power to detect a difference of 0.039 (about 17% decrease) if the correlation is moderate (e.g. 0.5).
- For comparison of HDMTX infusion durations, each patient will have 4 doses with 4 h infusion duration and 4 doses with 12 h infusion duration. Using the Wilks Lambda test with a significance level of 0.05 under the framework of general linear models, with 24 subjects of 8 repeated measures (4 measures with 4 h duration and 4 measures with 12 h duration), we will have 80% power to detect a 0.42SD difference assuming a within-subject correlation of 0, and will detect a 0.30SD difference assuming a correlation of 0.5.
- For the analyses of ototoxicity data (hearing level thresholds from 4 to 8 kHz in dB), treatment cycle will be the analysis unit. Each patient will have 2 cycles with pantoprazole and 2 cycles without pantoprazole. Using the Wilks Lambda test with a significance level of 0.05 under the framework of general linear models, with 24 subjects of 4 repeated measures (2 measures with and 2 measures without pantoprazole), we will have 80% power to detect a 0.60SD difference assuming a within-subject correlation of 0, and will detect a 0.42SD difference assuming a correlation of 0.5. Moreover, if differential carryover is present, we will only use the data from the first two cycles, so that the comparison will be between subjects, with 12 subjects in each comparison group. We will then have 80% power to detect a 1.2SD difference, based on a two-sided, two-sample t test with a significance level of 0.05.

6.3 Interim Analysis

The sample size in this pilot study is too small to derive a statistically valid stopping rule with sufficient sensitivity. However, after the first 12 patients have completed treatment, the investigators will evaluate the following parameters to determine whether pantoprazole or prolonging the HDMTX infusion affect toxicity or anti-tumor response to MAP:

- The incidence and severity (CTCAE grade) of toxicities will be compared for

cisplatin/doxorubicin with vs. without pantoprazole and for HDMTX infused over 4 h vs. 12 h within and across patients.

- Radiographic response (log ratio) and histological response (% tumor necrosis) following 2 cycles of neoadjuvant chemotherapy will be compared in patients who received pantoprazole vs. patients who did not receive pantoprazole and in patients who received HDMTX over 4 h vs. 12 h.

If the toxicity from MAP is clinically significantly worse when cisplatin is administered with pantoprazole or when HDMTX is infused over 12 h OR if radiographic or histological response to neoadjuvant chemotherapy is clinically significantly less in patients receiving pantoprazole or a prolonged HDMTX infusion, then the intervention found to have the worse outcome will be discontinued.

7. STUDY MEDICATIONS

All medications prescribed in this protocol are commercially available. The MAP chemotherapy regimen, which includes cisplatin, doxorubicin and methotrexate, is the standard of care for osteosarcoma, and the doses, routes of administration and schedules are also standard, except for the alternative infusion duration for HDMTX. Intravenous pantoprazole is being studied to determine whether co-administration with cisplatin can selectively prevent cisplatin nephrotoxicity and ototoxicity.

7.1 Intravenous Pantoprazole (PROTONIX I.V.)

- **IUPAC Name**: (RS)-6-(Difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzo[*d*]imidazole
- **Molecular formula & weight**: $C_{16}H_{15}F_2N_3O_4S$ - 383.4 g/mol (Fig 5, chemical structure)
- **Manufacturer**: Pfizer Injectables (Distributed by Wyeth Pharmaceuticals Inc.)
- **Drug supply**: purchased from commercial sources
- **Indications**: gastroesophageal reflux associated with a history of erosive esophagitis and pathological hypersecretory conditions (e.g., Zollinger-Ellison syndrome)
- **Dosage forms and strength**: supplied in a vial containing 40 mg of pantoprazole as a freeze dried powder
- **Storage and Handling**: store vials at 20-25°C (USP controlled room temperature); excursions permitted to 15-30°C.
- **Dosing (Section 3.1.3)**: 0.3 mg/kg IV over 15 min immediately prior to each dose of cisplatin as a loading dose followed by 1.3 mg/kg IV infused over 4 h concurrent with each 4 h cisplatin infusion on either Cycles 1 & 2 OR Cycles 3 & 4 depending on the treatment arm. See Section 1.3.8 for the rationale for pantoprazole administration schedule.
- **Compatibility**: the compatibility of the cisplatin and pantoprazole drugs solutions was

tested at UPenn by Dr. Suzanne Wehrli, and at the concentrations used for drug administration, there was no evidence of an interaction – indicating that the pantoprazole solution can be infused into the same intravenous tubing through which the cisplatin is being infused.

- **Clinical pharmacology**: see Section 1.3.5 for pediatric pharmacokinetic data and tolerability of single doses in children
- **Adverse reactions**: reactions reported in clinical trials in adults (n=1473) with GERD at a frequency >2% included headache (12%), diarrhea (8.8%), nausea (7%), abdominal pain (6.2%), vomiting (4.3%), flatulence (3.9%), dizziness (3%), arthralgia (2.8%). The use of PPIs is associated with a moderately increased risk of *C. difficile* colitis.[113,114]
- **Drug accountability**: a separate drug supply will be purchased for this study using the research funds, and NCI DARFs will be used to track drug supply.
- **IND status**: this pilot study will evaluate pantoprazole for a non-approved indication. The use of pantoprazole in this study meets the five criteria for IND exempt status:
    - The study is not intended to support FDA approval of a new indication or a significant change in the product labeling
    - The study is not intended to support a significant change in the advertising for the product
    - The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risk (or decreases the acceptability of the risks) associated with the use of the drug product
    - The study is conducted in compliance with institutional review board (IRB) and informed consent regulations set forth in 21 CFR parts 56 and 50
    - The study is conducted in compliance with § 312.7 (promotion and charging for investigational drugs)

8. SAFETY MANAGEMENT

Patients with osteosarcoma that will be treated with surgery (or radiation if surgery is not feasible) and a standard 3-drug MAP chemotherapy will be enrolled on this pilot trial to assess two interventions designed to reduce the nephrotoxicity and ototoxicity from cisplatin and HDMTX. The primary endpoints on this trial will monitor the incidence and severity of these known toxicities. Secondary endpoints include monitoring the overall toxicity of the regimen and response to neoadjuvant chemotherapy to determine whether the interventions increase the incidence or severity of the other expected toxicities of the regimen or alter the response to neoadjuvant treatment. Toxicities of the regimen will be categorized and graded according to the standard NCI CTCAE v.4 toxicity criteria.

8.1 Clinical adverse events

In addition to nephrotoxicity and ototoxicity, the MAP chemotherapy regimen is expected to cause substantial toxicity in all patients undergoing treatment for osteosarcoma, including myelosuppression, febrile neutropenia and infection, nausea and vomiting, mucositis, anorexia, weight loss, fatigue, hepatotoxicity, and neurotoxicity.

Clinical adverse events (AEs) related to the research interventions, which are administration of pantoprazole with cisplatin and prolonging the infusion duration of HDMTX, will be monitored throughout this study.

8.2 Adverse Event Reporting

SAEs will be promptly reported to the IRB in accordance with CHOP IRB policies. AEs that are not serious but that might involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

AEs (including SAEs) that are grade ≥2 in severity and related to the treatment will be collected in an eCRF in OnCore including CTCAE category, grade, date of onset, duration, attribution (causality), and outcome of the event (resolution).

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but would not be an SAE. On

the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention or procedure

The relationship of each SAE to the study intervention will be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.4.2 IRB/IEC Notification of SAEs

The IRB will be promptly notified of all on-site AEs that are (1) serious, (2) unexpected and (3) related to the research procedures. Other unanticipated problems involving risk to subjects or others will also be reported promptly using the CHOP Internal SAE reporting form and in accordance with the following timeline.

Type of Internal Adverse Event	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Summary of AEs Reported at Time of Continuing Review

8.4.3 Follow-up report

If an SAE has not resolved at the time of the initial report, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. All SAE should be followed until either resolved or stable.

9. STUDY ADMINISTRATION

9.1 Data collection and management

9.1.1 Confidentiality

Demographic data including each patient's name, MRN, DOB, and a unique patient study number is entered and stored in Oncore CRM (Forte Research), the CCCR's clinical trial management system. Data regarding markers of Acute Kidney Injury (Cystatin-C, KIM-1, NGAL, NAG) will be collected using secure OnCore electronic case report forms (eCRFs). Results of standard renal function tests, audiograms, bone-specific alkaline phosphatase assays, nutritional assessments, and response assessments (radiographic and histologic) as well as toxicity data will also be entered into Oncore eCRFs. Patient related outcomes forms

completed by a parent will be stored in a locked file cabinet in the CTRB. These forms will have patient identifiers (name, DOB, MRN).

9.1.2 Security

OnCore is maintained on a Research IS server that is password protected and backed up frequently. File cabinets in CTRB with paper forms will be kept locked.

9.1.3 Anonymization, de-identification, or destruction

The PHI in OnCore will be kept indefinitely.

9.2 Confidentiality

All data and records generated during this study will be kept confidential in accordance with CHOP Institutional policies and HIPAA on subject privacy and the Investigator and other site personnel will not use the data and records for any purpose other than conducting the study. Records with identifiers will be retained indefinitely in a secure, password protected commercial clinical trial management system (OnCore).

Safeguards to maintain subject confidentiality are described under Data Collection and Management (Section 9.1).

9.3 Regulatory and ethical considerations

9.3.1 Data safety and monitoring plan

The research team led by the protocol PI will monitor adverse events related to the protocol therapy in real time. The progress of every patient actively receiving treatment on this study will be discussed weekly in the Solid Tumor Team Meeting. An interim analysis to assess the safety of the interventions will be performed after the first 12 patients have completed therapy.

9.3.2 Risk assessment

There is risk associated with the addition of pantoprazole to cisplatin therapy, however PPIs are widely used in pediatric medicine and there is substantial experience with the toxicities associated with these agents (Section 7.1, Adverse Events). Single IV doses of pantoprazole are very well tolerated in children. An association between the use of PPIs and *C. difficile* colitis was recently reported, but the use of pantoprazole in this study is limited to 4 doses. The use of cisplatin and pantoprazole together has not been studied in children. As described in the background, we do not expect pantoprazole to alter the excretion of cisplatin. PPIs are frequently administered chronically to patients receiving chemotherapy for symptoms of reflux. The risk associated with administration of pantoprazole with cisplatin must be considered greater than minimal.

Prolonging the infusion duration may alter the toxicity profile of HDMTX because the pharmacologic effect of MTX is determined by the duration of exposure to the drug. Leucovorin rescue will start 24 h after the start of the HDMTX infusion for both infusion durations. The PK simulations (Figure 4) show that plasma concentrations at later time points are similar for the two infusion durations. There is considerable experience

demonstrating tolerability of HDMTX in children with leukemia treated with higher doses of MTX (e.g., 33 g/m^2) administered as prolonged infusions with leucovorin rescue. Administration of 12 g/m^2 HDMTX infused over 12 h instead of 4 h must be considered greater than minimal risk.

9.3.3 Potential benefits of trial participation

The crossover design ensures all patients will receive pantoprazole and the prolonged infusions of HDMTX. Therefore, all patients have the potential for direct benefit from participation in this study. Pantoprazole may mitigate the nephrotoxicity and hearing loss associated with cisplatin exposure. Prolonged administration of methotrexate may decrease the risk of acute kidney injury in patients receiving this agent. In addition results from this study could benefit future patients with osteosarcoma and other cancers treated with cisplatin and HDMTX if these more rational dosing methods and sensitive biomarkers of toxicity and response prove to be better.

9.3.4 Risk-benefit assessment

This study is associated with greater than minimal risk, however there is the prospect of direct benefit to the subjects. The results of this study may provide pilot data for a larger definitive clinical trial. There is the potential that such a study may result in adoption of new and safer dosing administration schedules and modifications of supportive care recommendations for children receiving methotrexate and cisplatin for osteosarcoma and other cancer types. As a result, this study and a potential successor study may benefit children who receive these agents in the future.

9.4 Recruitment strategy

Potential subjects are identified from the list of children undergoing evaluation of a suspected primary bone tumor. Investigators will ask each potential subject's primary physician at CHOP whether the subject is appropriate for this study, and, if so, the PI or a Co-I will meet with the family after the diagnosis of osteosarcoma is confirmed but prior to the initiation of therapy. The PI or a Co-I will discuss the study and obtain informed consent if a potential subject is willing to participate.

9.5 Informed consent/assent

The protocol PI or a co-investigator will discuss participation in the study with each potential subject or the parents or legal guardian of each potential subject. Written informed consent will be obtained. The discussion will take place in a private room in the Oncology clinic or in the patient's room on the inpatient unit. The family (and patient) are allowed time to ask questions and to read the informed consent form before signing. Participation in this study is entirely voluntary and has no influence on whether the potential subject will receive clinical treatment for osteosarcoma at CHOP. The consent process will be documented in a brief note in the subject's medical record and by the signed informed consent document. Assent will be obtained in the presence of the potential minor subject's parents/guardian when appropriate based on the age and level of maturity and understanding of the subject. Assent will be documented on the consent form.

9.6 Payments to subjects/families

Subjects and families will not receive payments for participation in this trial.

10. PUBLICATION

This is a single Institution pilot study and measurement of the endpoints for the primary objectives will be completed when the treatment is completed. The team of CHOP Investigators plan to publish the results separately for the effect of prolonging the HDMTX infusion duration on MTX nephrotoxicity and the effect of IV pantoprazole on cisplatin nephrotoxicity and ototoxicity.

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APPENDIX 1 SAMPLE TREATMENT DELIVERY MAP

Protocol No.	CHP12STXX
Version Date	June 13, 2012

Name	0
MRN	0
DOB	0

TREATMENT DELIVERY MAP

Cycles	1 and 2
Treatment Arm	1

	C1/D1	C1/D22	C2/D1	C2/D22
Weight (kg)				
Height (cm)				
BSA (m^2)				

Drug	Dose	Route	Schedule	Cycle	Cycle Days	Comment
Dexrazoxane	375 mg/m^2	IV	over 15 min	1 and 2	1 and 2	infuse prior to DOXOrubicin
DOXOrubicin	37.5 mg/m^2	IV	over 15 min	1 and 2	1 and 2	
Pantoprazole	0.3 mg/kg	IV	over 15 min	1 and 2	1 and 2	infuse prior to cisplatin
	1.3 mg/kg	IV	over 4 h	1 and 2	1 and 2	infuse with cisplatin
CISplatin	60 mg/m^2	IV	over 4 h	1 and 2	1 and 2	
Pegfilgrastim	0.1 mg/kg	SC		1 and 2	3	MAX 6 mg
Methotrexate	12 gm/m^2	IV	over 4 h	1 and 2	22 and 29	MAX 20 gm
Leucovorin	15 mg/m^2	IV or PO	q6h until [MTX]<0.1mcM	1 and 2	23 and 30	leucovorin must start 24 h after the start of the MTX infusion

Date	Cycle	Day	Drug	% Dose*	Dose	Units	Comment
	1	1	Dexrazoxane			mg	
			DOXOrubicin			mg	
			Pantoprazole (loading)			mg	
			Pantoprazole (infusion)			mg	
			CISplatin			mg	
	1	2	Dexrazoxane			mg	
			DOXOrubicin			mg	
			Pantoprazole (loading)			mg	
			Pantoprazole (infusion)			mg	
			CISplatin			mg	
	1	3	Pegfilgrastim			mg	
	1	22	Methotrexate over 4 h			mg	
		23	Leucovorin			mg	
	1	29	Methotrexate over 4 h			mg	
		30	Leucovorin			mg	
	2	1	Dexrazoxane			mg	
			DOXOrubicin			mg	
			Pantoprazole (loading)			mg	
			Pantoprazole (infusion)			mg	
			CISplatin			mg	
	2	2	Dexrazoxane			mg	
			DOXOrubicin			mg	
			Pantoprazole (loading)			mg	
			Pantoprazole (infusion)			mg	
			CISplatin			mg	
	1	3	Pegfilgrastim			mg	
	2	22	Methotrexate over 4 h			mg	
		23	Leucovorin			mg	
	2	29	Methotrexate over 4 h			mg	
		30	Leucovorin			mg	

* % of standard dose. 100% is full dose; 75% is a 25% dose reduction.

SAMPLE CORRELATIVE STUDIES MAP

Protocol No.	CHP12ST051
Version Date	January 10, 2013

Name	,
MRN	0
DOB	1/0/1900

CORRELATIVE STUDIES MAP - CYCLES 1 AND 2

Date	Time	Cycle	Day	Specimen	Test	Instructions	Comment
		1	1	Urine Urine Plasma Serum	AKI, Cr Mg, Cr Mg, Cr Cystatin C	Prior to D1 cisplatin infusion	
		1	2	Urine Plasma	Mg, Cr Mg, Cr	Prior to D2 cisplatin infusion	
				Urine	AKI, Cr	End of D2 cisplatin infusion	
		1	7	Urine Urine Plasma Serum	AKI, Cr Mg, Cr Mg, Cr Cystatin C		
		1	22	Urine Serum	AKI, Cr Cystatin C	Prior to D22 HDMTX infusion	
				Urine	MTX	End of D22 HDMTX infusion	
		1	23	Urine	AKI, Cr	24 h after start of D22 HDMTX infusion	
		1	29	Urine Serum	AKI, Cr Cystatin C	Prior to D29 HDMTX infusion	
				Urine	MTX	End of D29 HDMTX infusion	
		1	30	Urine	AKI, Cr	24 h after start of D29 HDMTX infusion	
		2	1	Urine Urine Plasma Serum Plasma - -	AKI, Cr Mg, Cr Mg, Cr Cystatin C Pre-albumin PRO Arm circ/ Skin fold	Prior to D1 cisplatin infusion	
		2	2	Urine Plasma	Mg, Cr Mg, Cr	Prior to D2 cisplatin infusion	
				Urine	AKI, Cr	End of D2 cisplatin infusion	
		2	7	Urine Urine Plasma Serum	AKI, Cr Mg, Cr Mg, Cr Cystatin C		
		2	22	Urine Serum	AKI, Cr Cystatin C	Prior to D22 HDMTX infusion	
				Urine	MTX	End of D22 HDMTX infusion	
		2	23	Urine	AKI, Cr	24 h after start of D22 HDMTX infusion	
		2	29	Urine Serum	AKI, Cr Cystatin C	Prior to D29 HDMTX infusion	
				Urine	MTX	End of D29 HDMTX infusion	
		2	30	Urine	AKI, Cr	24 h after start of D29 HDMTX infusion	

APPENDIX 2 PEDIATRIC NEUROTOXICITY GRADING SCALE

Motor neuropathy:

- Grade 1: Subjective weakness, but no deficits detected on neurological exam
- Grade 2: Weakness that alters fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait without abrogating ability to perform these tasks.
- Grade 3: Unable to perform fine motor tasks (buttoning shirt, writing or drawing, using eating utensils) or unable to ambulate without assistance.
- Grade 4: Paralysis

Sensory neuropathy :

- Grade 1: Paresthesias, pain, or numbness that do not require treatment or interfere with extremity function.
- Grade 2: Paresthesias, pain, or numbness that is controlled by non-narcotic medications, or alter (without causing loss of function) fine motor skills (buttoning shirt, writing or drawing, using eating utensils) or gait*, without abrogating ability* to perform these tasks.
- Grade 3: Paresthesias or pain that is controlled by narcotics, or interfere with extremity function (gait, fine motor skills as outlined above), or quality of life (loss of sleep, ability to perform normal activities severely impaired).
- Grade 4: Complete loss of sensation or pain that is not controlled by narcotics

APPENDIX 3 PATIENT REPORTED OUTCOME SURVEY

OSTEOSARCOMA PATIENT SURVEY

PATIENT ID_____ **DATE_____**

Since the last time I filled this form out . . .	**Never** (0 times)	**Rarely** (less than once a week)	**Sometimes** (1 to 3 times a week)	**Often** (almost daily)	**Almost Always** (daily)
I feel too tired to get out of bed					
I feel like I'm going to throw up					
I throw-up or vomit					
My stomach hurts					
I do not feel as hungry as I use to					
Foods taste different or funny					
I have diarrhea					
I have constipation					
I have a ringing sound in my ears					
I have trouble hearing some things					
My mouth or throat hurts					
I am in pain					
I have trouble keeping up with school work because of being in the hospital or feeling too sick					
I have trouble falling asleep					
I wake up at night because of pain					
I don't see my friends as much anymore because I am too busy with doctors and hospital visits					
I don't do as many fun things anymore because I feel tired or sick					
I don't have fun doing things I used to like to do					
I feel nervous, worried, or anxious					
I have trouble doing daily activities like eating, drinking, putting on clothes					
I have answered these questions before (circle one) YES NO I Don't Know					
Compared to the last time I answered these questions, I feel (circle one) BETTER WORSE THE SAME I DON'T KNOW					
I would like to meet with someone at the hospital talk about how I feel (circle one) YES NO I DON'T KNOW I AM ALREADY MEETING WITH SOMEONE					