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LIPOSOMAL MTP

(mifamurtide)

Advisory Committee Briefing Document

NDA 22-092

EXECUTIVE OVERVIEW

Liposomal mifamurtide (MTP) is a fully synthetic lipophilic derivative of muramyl dipeptide (MDP), the smallest naturally-occurring immune stimulatory component of *mycobacterium sp* cell walls. MTP stimulates the innate immune system to kill tumor cells, and when used in combination with surgery and chemotherapy, MTP prevents the recurrence of osteosarcoma and improves long term survival.

Unmet Medical Need

Osteosarcoma primarily affects children and young adults with most cases occurring at the metaphyseal plate in the distal femur, proximal tibia, and proximal humerus. Osteosarcoma is an orphan disease with less than 1,000 new cases diagnosed in the United States (US) annually. Since the introduction of neo-adjuvant and adjuvant chemotherapy to surgery in the early 1980s, the long term survival of patients with osteosarcoma has remained stable at about 60-65%. The primary cause of death is metastatic osteosarcoma to the lung occurring in about 85% of the relapses.

The clinical trial demonstrating the efficacy of MTP (INT-0133) remains the largest and longest randomized controlled trial ever conducted in children and young adult patients with osteosarcoma. The results of INT-0133 address an important unmet need for treatment options for this rare and fatal cancer that affects children who are otherwise healthy and would expect to live a long life.

Product Development

Liposomal MTP is an intravenous formulation developed to minimize the systemic concentration of free MTP and thereby reduce non-target cell and organ toxicity. The liposomes are selectively taken up in tissues rich in macrophages such as lung, spleen, and liver. Once inside the macrophage, the liposomes are slowly metabolized, releasing free MTP intracellularly. The MTP stimulates the NOD2/NF-κB system, activating the macrophage. The activated macrophages become directly and indirectly tumoricidal, engulfing and killing tumor cells, and releasing cytokines and immunostimulatory molecules such as TNFα and IL-6 to recruit and activate other immune cells.

Studies in the dog spontaneous osteosarcoma model have shown that macrophages stimulated with MTP are effective in reducing and/or eliminating microscopic tumors. Following removal of the dog's primary tumor, MTP can provide long term survival and cure in an often fatal disease. Canine osteosarcoma is very similar in etiology and course to human osteosarcoma with early dissemination of metastases to the lung. Though pulmonary micrometastases are usually not apparent after the patient is rendered clinically disease free by surgery, relapse almost always occurs in the lungs.

MTP clinical development began in 1986 with multiple Phase 1/2 dose ranging studies in adults with advanced and measurable malignancies that had failed all prior therapy. Nine US IND studies were conducted in 248 patients at doses from 0.01 to 12 mg/m^2. An additional eight Phase 1/2 studies outside the US included 141 subjects. The maximum tolerated dose of 4-6 mg/m^2 was the dose below which Grade 3 toxicities occurred. The best biological activity was at 0.5-2 mg/m^2 based on *in vitro* measures of *in vivo* stimulation of monocyte tumoricidal activity (MTA) and cytokine release.

In a Phase 2 study (Protocol 8) MTP showed biological activity and an increase in relapse free survival in patients with recurrent osteosarcoma. The encouraging results from this Phase 2 study lead the National Cancer Institute (NCI) and Children's Oncology Group (COG) to initiate and conduct a Phase 3 clinical study (INT-0133).

Efficacy

The Phase 3 Study (INT-0133) is a multicenter, open label, randomized, factorial, four parallel treatment group study. The study included 178 sites mostly in North America and enrolled 793 patients: 678 with non-metastatic resectable disease and 115 with metastatic or unresectable disease. The 678 patients with non-metastatic resectable osteosarcoma are the primary focus of the original investigative plan for INT-0133 and support the efficacy of MTP for this indication. All 793 patients are included in the evaluation of the safety of MTP.

Patients were stratified and randomized prior to any therapy to one of four treatment groups. The three stratification parameters were blood lactate dehydrogenase (LDH), location of primary tumor, and presence or absence of prior amputation. The study tested the addition of neoadjuvant

ifosfamide or adjuvant MTP to cisplatin, methotrexate, and doxorubicin chemotherapy in a factorial design illustrated in the table below.

Summary of Phase 3 Treatment Arms

	Regimen A or A plus MTP			**Regimen B or B plus MTP**		
Drug (dose)	Neoadjuvant Induction Week 0-9	Definitive Surgery Week 10-11	Adjuvant Maintenance Week 12-48	Neoadjuvant Induction Week 0-9	Definitive Surgery Week 10-11	Adjuvant Maintenance Week 12-48
MTP ($2mg/m^2$)	-		48 doses	-		48 doses
Methotrexate ($12\ g/m^2$)	4 doses		8 doses	4 doses		8 doses
Doxorubicin ($25\ mg/m^2$/day x3)	2 courses		4 courses	2 courses		4 courses
Cisplatin ($120\ mg/m^2$)	2 doses		2 doses	-		4 doses
Ifosfamide ($1.8\ g/m^2$/day x5)	-		-	2 courses		3 courses
Duration of Treatment						
Chemotherapy	10 weeks		21 weeks	10 weeks		28 weeks
Surgery		2 weeks			2 weeks	
MTP			36 weeks			36 weeks

MTP was the only investigational drug in the study. Doxorubicin, methotrexate, cisplatin and ifosfamide were all marketed in the United States at the time the study began and were considered active agents for treatment of osteosarcoma.

All patients were assessed for safety and signs of recurrence on a predetermined schedule throughout drug treatment and follow up. The principal investigator at each site was responsible for maintaining the records of protocol-required visits and tests and transferring selected data to COG through case report forms (CRFs).

Disease-free survival (DFS) was the primary efficacy endpoint. The factorial design of the study assessed two separate comparisons: (1) ifosfamide versus non-ifosfamide regimens [Regimens A and A+MTP versus Regimens B and B+MTP] and (2) the MTP versus non-MTP regimens [Regimens A and B versus Regimens A+MTP and B+MTP]. The factorial design of the study

enabled independent evaluation of each comparison. This NDA only addresses the comparison of MTP vs. non-MTP regimens.

The Phase 3 Study, INT-0133, began in 1993 using the accepted cooperative group clinical research and data collection methods of that time. The Gompertz survival model and the disease-free survival endpoint were the basis for determining the sample size and the minimum follow up period. The statistical analysis section of the original study protocol provides the statistical analysis plan but is less detailed than would be expected for current protocols. IDM used independent statistical experts to validate the intended statistical approach for analyzing the MTP versus non-MTP regimens. The INT-0133 protocol was evaluated independently by three expert statisticians who, without knowledge of prior analyses or the interpretation of the other statisticians, agreed that disease-free survival (DFS) is the prospectively planned primary efficacy outcome in the original COG protocol. They also concluded that survival is an implied endpoint since the primary intent of INT-0133 was "To improve the survival of patients with osteogenic sarcoma." DFS is a surrogate endpoint for overall survival (OS), the gold standard for any oncology study.

Enrollment opened in 1993 and closed in 1997. The patients enrolled in INT-0133 represent approximately one third of all children and adolescents newly diagnosed with osteosarcoma in the United States during the study enrollment period. Their demographic characteristics reflect those of the general osteosarcoma population. The mean age was approximately 14 years (range 1.4 to 30.6 years) with slightly more males than females and the majority were white. Most subjects had a primary tumor site in either the femur or tibia. Four hundred and sixty-four (464) patients with non-metastatic resectable disease at diagnosis completed protocol specified treatment. The median time for follow up for the primary final analysis in June 2003 is 4.8 years for patients alive at last contact.

MTP significantly increases disease-free survival. The 6-year probability of surviving without a relapse of osteosarcoma was 66% (95% CI: 61%-72%) among patients who received MTP compared with 57% (95% CI: 52%-64%) among patients who did not. This disease-free survival advantage resulted in a hazard ratio of 0.76 in favor of MTP, or a 24% reduction in the risk of relapse, progressive disease or death. The addition of MTP to multi-agent chemotherapy also

resulted in a clinically meaningful and statistically significant increase in overall survival in patients with non-metastatic resectable osteosarcoma. The 6-year survival probability was 77% (95% CI: 72%-83%) in patients who received MTP compared with 66% (95% CI: 59%-73%) in patients who did not. The study is internally consistent with most subset analyses trending in the same direction as the primary endpoints. The table and figures below further describe the results of the Phase 3 study.

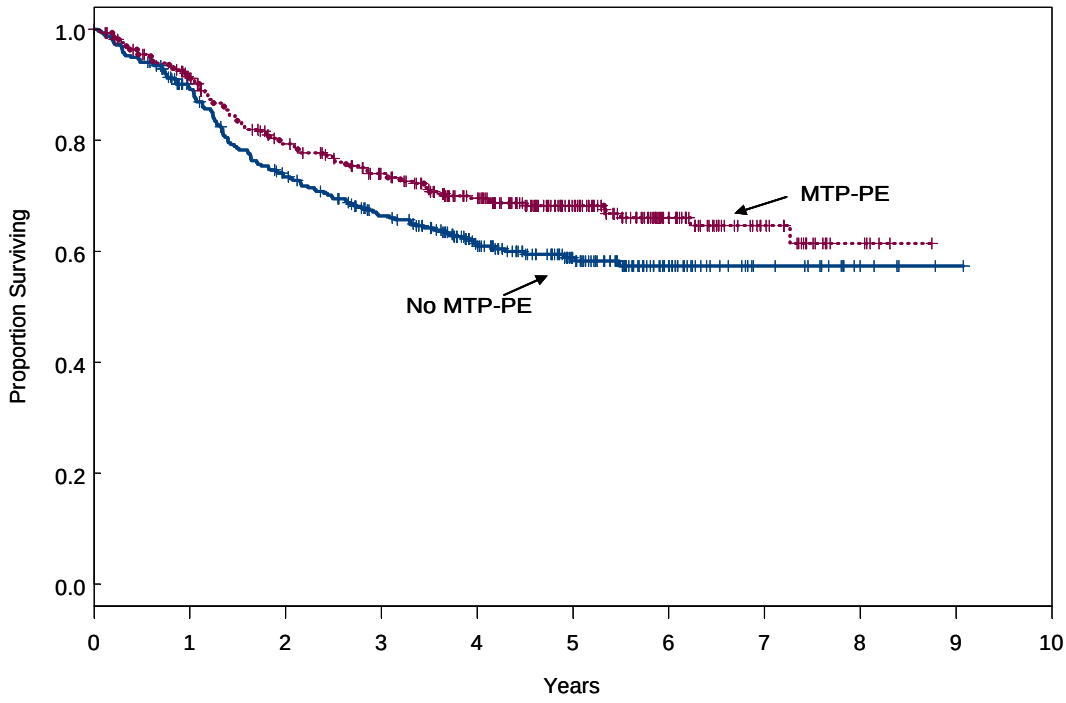
Primary Efficacy Analysis (ITT Data Set)

Variable	# of Pts (events)	Median (years)	P-value	Hazard Ratio	95% CI for HR
Disease-Free Survival					
No MTP (A/B)	340 (126)	NR*	---	1.00	---
MTP (A+/B+)	338 (102)	NR	0.0245[1]	0.76	(0.58, 0.98)
Overall Survival					
No MTP (A/B)	340 (85)	NR	---	1.00	---
MTP (A+/B+)	338 (63)	NR	0.0183[1]	0.68	(0.49, 0.95)

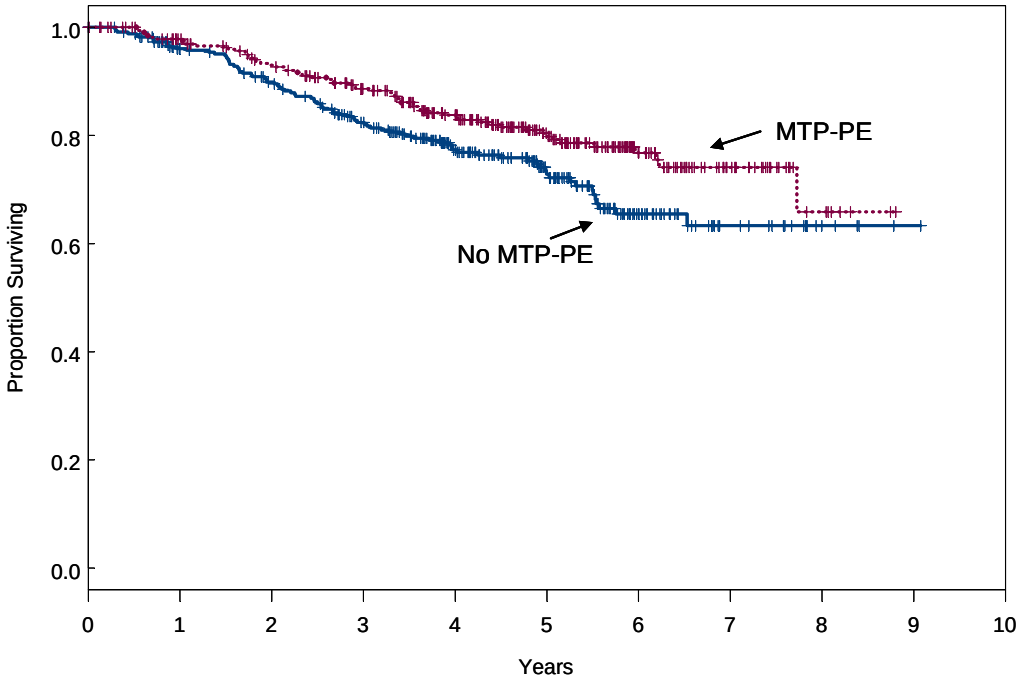
[1] p-value from log-rank test stratified by ifosfamide use and randomization strata.

* NR = Not reached.

Disease-Free Survival ± MTP (ITT Data Set)



Overall Survival ± MTP (ITT Data Set)



The sponsor identified several design challenges and data issues in bringing this large 14 year old multi-center NCI Cooperative Group study into compliance with current clinical research design and documentation standards and making it possible to submit an NDA to the FDA. The three major design challenges were the open label treatment, the factorial design, and the potential for ascertainment bias for the disease-free survival (DFS) endpoint. The data issues included verification of DFS status, evidence of interaction by regimen in DFS, and completeness of follow up. These issues have been addressed and support the use of the Phase 3 Study (INT-0133) as the basis for approving MTP based on the statistically and clinically significant increase in disease-free and overall survival.

Safety

The safety database includes 580 patients who received at least one dose of MTP: 248 oncology patients in the Phase 1/2 US IND studies and 332 patients in the Phase 3 study (INT-0133).

The Phase 1/2 studies provide the primary evidence of safety for MTP as the single treatment agent. The most commonly reported adverse events occurring in more than 50% of patients were

chills, fever, fatigue, nausea, tachycardia, and headache. Most were mild to moderate in severity (Grade 1 and 2) and directly linked to the expected biological activity of MTP.

Only Grade 3 and 4 toxicities were collected in the Phase 3 Study, a practice consistent with other cooperative group chemotherapy-based pediatric oncology trials. The safety database also includes adverse events that occurred during the neoadjuvant and surgical portions of the study prior to any use of MTP. Hearing loss was identified as the only potentially serious adverse event reported more frequently in MTP recipients; however, further examination of other COG data, including objective tests of hearing conducted during the clinical trial, revealed no significant differences across all treatment regimens.

Reports of deaths and treatment discontinuations during the Phase 1/2 and the Phase 3 program were primarily attributed to the underlying disease. Serious adverse events that occurred either with single agent MTP use or in combination with chemotherapy were primarily more severe forms of the most common adverse events. Twenty (8%) of the 248 patients in the Phase 1/2 safety database reported a serious adverse event that was considered by the investigator to be potentially related to MTP. While the Phase 3 data collection and reporting practices for serious adverse events followed NCI guidelines and are different from FDA guidelines, there were 15 out of 332 (5%) MTP exposed patients where a serious adverse event was reported to NCI as potentially related to MTP. In all study phases fever, chills, nausea, dyspnea, hypotension, and pain (myalgia, arthralgia) were the more common serious, potentially related adverse events reported with MTP. Infrequent reports of serum sickness and anaphylactic-like reactions may represent an exaggerated inflammatory reaction to MTP.

The clinical safety of MTP is well characterized as both a single agent in the nine Phase 1/2 studies and in combination with chemotherapy in a large Phase 3 study. Additional support comes from an extensive preclinical program. It can be concluded that the most common adverse events associated with the use of MTP are mild to moderate, transient, and manageable events related to the expected immunostimulatory activity of MTP. There are rare, possibly-related events of greater clinical significance that may represent allergic reactions or an exaggeration of the immunostimulation associated with MTP use.

Overall Clinical Benefit

Osteosarcoma is fatal in approximately a third of the children and young adults in whom it is diagnosed. This mortality rate has not changed in the two decades since the initial introduction of surgery and chemotherapy. The primary cause of death in treated patients is recurrent metastatic disease to the lung. The addition of MTP to surgery and chemotherapy extends survival in non-metastatic resectable osteosarcoma. The results of the largest clinical trial ever conducted in children and young adults with osteosarcoma support this conclusion and represent the first significant increase in survival in more than 20 years.

The increase in survival is accompanied by a modest increase in mild to moderate adverse events. While these events do not represent a safety concern, they are relevant to the tolerability of MTP. Tolerability is important, particularly in a pediatric population already subjected to the toxicities associated with chemotherapy and the trauma of surgery. The mild to moderate events were transient and commonly managed with acetaminophen during the Phase 3 study.

Liposomal MTP was intentionally designed to deliver the immunostimulatory activity of MTP to the tissue macrophages in the lung without unwanted systemic effects. The demonstration of a survival benefit by the Phase 3 study along with the favorable tolerability profile of MTP reflect the substantial clinical benefit of adding MTP to the treatment regimen for osteosarcoma.

TABLE OF CONTENTS

Section **Page**

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

1. BACKGROUND

1.1 Product Description

Liposomal MTP (with the International Nonproprietary Name of mifamurtide), referred to as MTP in the following sections, is a wholly synthetic liposomal formulation of muramyl tripeptide phosphatidyl-ethanolamine developed to stimulate the innate immune system to attack micrometastatic osteosarcoma. Muramyl dipeptide (MDP) is the smallest repeating immunostimulatory unit of the *mycobacterium sp.* cell wall used in the complete Freund's adjuvant. A third amino acid is added to MDP to allow the linkage of phosphatidyl-ethanolamine to make the active drug lipophilic. When combined with other lipids, MTP intercalates into the spontaneously formed multi-lamellar liposomes. The liposomes are actively phagocytosed by tissue macrophages and monocytes and slowly degraded, releasing MTP.

1.2 Pharmacology

1.2.1 Mechanism of Action

Non-liposomal (free) MTP has been shown in several *in vitro* and *in vivo* models to activate monocytes and macrophages to tumoricidal activity. Intercalation of free MTP into liposomes results in a stronger and more sustained cell activation *in vitro*, particularly with human monocytes. The composition and size of the liposomes were optimized to enhance distribution to the lungs and uptake by phagocytic cells of the reticuloendothelial system. As a result, liposome-encapsulation enhances delivery of intravenous MTP to lung, liver, and spleen macrophages after fast disappearance from the vascular system.

In vivo intravenous administration of liposomal MTP resulted in inhibition of tumor growth in mouse and rat models of lung metastasis, skin, and liver cancer, and fibrosarcoma. Significant enhancement of disease-free survival in dog osteosarcoma and hemangiosarcoma after adjuvant treatment with MTP was demonstrated. However, MTP was not effective in cases of high tumor burden in rodents or in metastatic mammary tumors in cats and dogs. This suggests that macrophage activation may be more or less effective in controlling tumor growth depending on tumor burden and localization. Because the dog osteosarcoma model played an important role in supporting further investigation in human osteosarcoma, the dog studies are described in more detail in the next section (Section 1.2.2).

The anti-tumor effects of MTP are linked to both direct and indirect effects of macrophage activation. Despite numerous demonstrations that macrophages or monocytes activated by MTP kill cancer cells, the only mechanism of direct anti-tumor cytotoxicity fully characterized was TNF-α. An efficient anti-tumor NK cells effector response was observed *in vivo* and was attributed to a secondary activation of a broader immune response by the pro-inflammatory cytokines secreted by macrophages and monocytes.

The precise mechanism of macrophage activation by MTP is being investigated. Free MTP is a specific ligand of NOD2, which is an intracellular receptor found primarily in monocytes, dendritic cells, and macrophages. Activation of mouse, dog, and human monocytes and macrophages is associated with enhanced secretion of pro-inflammatory cytokines such as TNF-α, IL-6, IL-8, IL-1, expression of adhesion molecules (CD54/ICAM-1 and CD11a-CD18/LFA-1) and pro-inflammatory chemokines such as MCP-1. Kinetics of cytokine secretion are generally similar between *ex vivo* models using animal macrophages or human monocytes and serum samples of animals or patients who received MTP. Serum levels of TNF-α and IL-8 peak within 4 hours after administration, while IL-6 peaks within 4 to 16 hours. Cytokine levels generally return to baseline within 24 hours. MTP-activated human monocytes from healthy donors or cancer patients specifically killed tumor cells but were not cytotoxic to normal cells, either autologous or allogeneic.

No serious undesirable pharmacodynamic effects due to exposure in the therapeutic range were seen on the major physiological systems studied in animals. MTP had no important neurological or behavioral effects in mice or rats. In cats, there were marginal and transient reductions of blood pressure and heart rate. MTP caused moderate increase in respiration rate and decrease in tidal volume in cats, as well as minor increase in urine volume and electrolyte content in rats. MTP had no effect on QT interval in multiple models. Although intravenous administration of high doses of liposomes increased rat serum triglycerides and very low density lipoprotein, no effects of MTP were observed on serum lipids and lipoprotein levels, and there were only slight changes in carbohydrate metabolism.

Assessments of MTP in animals showed no antagonism of acetylcholine, barium chloride, histamine, noradrenaline, serotonin, apomorphine, or physostigmine.

Interactions of MTP with various chemotherapeutic drugs were extensively studied *in vitro*. Doxorubicin did not affect MTP-mediated murine macrophage activation, but slightly increased activation of human monocytes. Cisplatin, methotrexate, and cyclophosphamide also did not interfere with monocyte activation. MTP administration reduced the general myelosuppression and depletion of peritoneal and alveolar macrophage number normally seen following doxorubicin administration. These studies indicated that MTP did not interfere with the anti-tumor activity of ifosfamide, cisplatin, or doxorubicin against subcutaneous, kidney, lung, or spleen syngeneic tumors.

Some combinations of MTP and non-steroidal anti-inflammatory drugs (i.e. ibuprofen) demonstrated beneficial effects on the management of MTP side effects in animal studies. However, high doses of ibuprofen were also shown to interfere with the enhancement of macrophage cytotoxic activity mediated by MTP. *In vivo* anti-tumor effects of both MTP and non-steroidal anti-inflammatory drug diclofenac were lost when these two drugs were used in combination. These animal studies suggest that certain combinations with anti-inflammatory drugs may interfere with the mechanism of action of MTP, and that the mechanism of action may be through inflammatory mediators.

1.2.2 Canine Sarcoma Model

Canine osteosarcoma is a spontaneous malignancy which has micrometastasis at the time of diagnosis, most commonly in the lungs, and is considered the best model for human osteosarcoma. Significant efficacy of MTP was demonstrated in this setting in randomized double-blind trials.

Twenty-seven dogs with spontaneously occurring osteosarcoma underwent amputation to remove the primary tumor. Immediately after surgery they were randomized to receive 2 mg/m^2 MTP or empty liposomes twice weekly for eight weeks. The 14 animals treated with MTP had a significantly longer metastasis-free interval ($p < 0.001$) and survival time (median 7.4 vs. 2.6 months, $p < 0.002$) compared to those treated with placebo liposomes. In addition, canine monocytes showed tumoricidal activity against canine osteosarcoma cells after activation with MTP *in vitro* and *in vivo*.

In a complementary trial, after amputation of the affected limb and 16 weeks of cisplatin chemotherapy, 25 dogs with osteosarcoma were randomized to consecutive MTP at 2 mg/m^2

twice weekly for eight weeks or placebo liposomes. The 11 dogs receiving MTP had a significantly longer metastasis-free interval ($p < 0.035$) and survival time (median 14.4 vs. 9.8 months, $p < 0.01$) compared to dogs given placebo liposomes. In another study, concurrent therapy with MTP and cisplatin did not show positive results.

In another trial, 32 dogs with splenic hemangiosarcoma were treated with splenectomy, and randomized to treatment with chemotherapy (doxorubicin and cyclophosphamide) associated with MTP (1-2 mg/m^2 twice weekly for 8 weeks), or placebo liposomes. The 16 dogs receiving MTP had significantly longer disease-free survival ($p = 0.037$) and overall survival (median 9.2 vs. 4.8 months, $p = 0.029$) compared to the placebo group.

1.2.3 Human Pharmacology

1.2.3.1 Pharmacokinetics

Two studies provide pharmacokinetic data on liposomal MTP in humans. In a biodistribution study, a subset of patients received ^{99}Tc labeled liposomes containing MTP. At 6 hours after injection of ^{99m}Tc-labeled liposomes containing 1 mg MTP, radioactivity was found in liver, spleen, nasopharynx, thyroid, and, to a lesser extent, in lung. This radioactivity partially cleared by 24 hours. In 2 of the 4 patients, localization of labeled MTP within lung metastases was also observed.

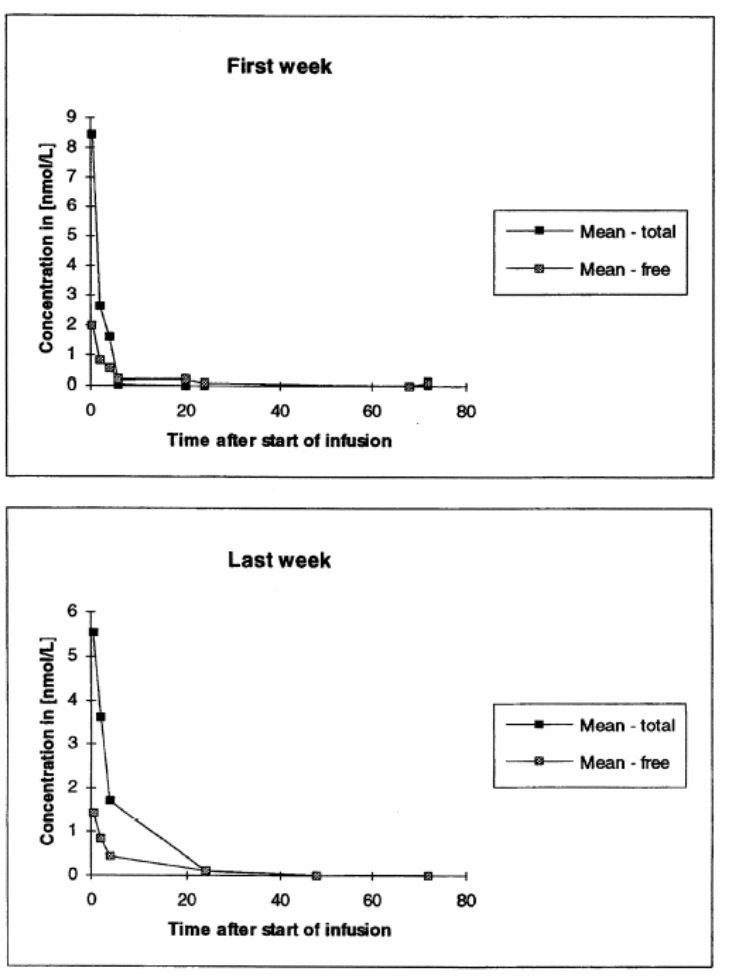
As anticipated by animal studies, the liposomes were rapidly cleared from the blood and mainly phagocytosed by cells of the reticuloendothelial system in the liver, spleen, lung and nasopharynx. The rapid clearance and uptake by the RES make it less likely that MTP is available to affect the cytochrome P450 system.

In a second study, serum-concentration-time profiles of total and free MTP were evaluated in 14 patients given repeated intravenous. infusions of 4 mg of MTP. Serum concentrations of free and total MTP were measured by a chemiluminescence immunoassay that had a limit of quantitation of 0.1 nmol/L for free drug and 1.0 nmol/L for total drug.

Within 24 hours after infusion, serum concentrations of total and free MTP declined to values below the limit of quantitation. Serum concentration-time curves of free drug were lower than those of total drug, indicating the presence of liposomes in circulation. Pretest and trough serum concentrations of total and free MTP were below the limit of quantitation.

Mean serum concentration-time curves of total and free MTP after the first infusion on day 1 ("First week" in figure below) and after the last infusion during week 11 or week 12 ("Last week") were almost superimposable. The mean AUC values of free drug after the first and last infusion were similar (Figure 1). These data indicate that neither total nor free MTP accumulated during the treatment period.

Figure 1: Serum-Concentration-Time Profiles of Total and Free MTP Evaluated in 14 Patients



1.2.3.2 Pharmacodynamics

In most trials of MTP, variable levels of cytokines (e.g., IL-1, IL-6 and TNF-α) and/or other serum indicators of immune stimulation (e.g., neopterin and C-Reactive Protein [CRP]) were

detected. These same markers are used to measure macrophage activation *in vitro* and the clinical measurements are interpreted as an indirect measure of *in vivo* macrophage activation.

There is a fairly consistent pattern of cytokine release in the early studies, though with considerable intra- and inter-subject variability. The detection of one or more cytokines typically early during treatment was interpreted by early investigators as evidence of the biologic activity of MTP and consistent with activation of macrophages.

In vitro, MTP can induce peripheral blood monocytes to display tumoricidal activity. *In vivo* peripheral blood monocyte tumoricidal activity (MTA) can be measured in subjects after intravenous MTP administration with most subjects having elevated MTA for up to 96 hours when compared with MTA activity before treatment. A clear dose response could not be established, but the optimal average dose of MTP necessary for *in vivo* stimulation of MTA, as well as other parameters of biological activation ranged from 0.5 mg/m^2 to 2 mg/m^2. Doses above 2 mg/m^2 tended to provide little increased effects and there was a tendency towards decreasing MTA at high doses (6 mg/m^2).

1.2.3.3 Drug Interactions

Compatibility of MTP with ifosfamide in humans was studied in Protocol 10, a single arm safety study in patients with relapsed osteosarcoma. There was no impact on ifosfamide activity by MTP, no impact on MTP activity by ifosfamide and no change in expected adverse events.

1.2.3.4 Cardiac Electrophysiology

In six Phase 1/2 clinical studies, cardiac effects and ECGs were monitored in 177 of the 248 patients who received MTP. ECGs were obtained either at the start and finish of treatment or at specified intervals during treatment. In all of the studies, no significant ECG abnormalities were attributed to MTP while on treatment as compared to baseline. Of 248 patients who received MTP in Phase 1 or 2 studies, the cardiac events reported in >1% of patients were tachycardia (125 patients, 50%), cyanosis (19 patients, 8%), and palpitations (four patients, 2%). An estimate of cardiac effects potentially associated with MTP is made difficult because of concomitant or prior use of cardiotoxic chemotherapy in the subjects enrolled in studies conducted to date.

1.2.4 Conclusion

Overall, the nonclinical and human pharmacology studies provide support for the safety and efficacy of MTP in combination with chemotherapy agents in the patient population indicated. The nonclinical pharmacology studies demonstrated that systemic administration of MTP in a number of different chemotherapy models affords significant anti-tumor activity in a variety of syngeneic tumor and spontaneous canine osteosarcoma models. The primary pharmacological effect of MTP in these models appears to be activation of macrophages and subsequent induction of cytokines stimulating an effective inflammatory response. The data suggest that macrophage activation may be more effective in controlling tumor growth in limited tumor burden and specific localizations. Distribution studies showed that after intravenous administration of MTP, the drug is rapidly delivered to specific organs such as lung, liver and spleen, as was seen in human pharmacokinetics studies.

2. UNMET MEDICAL NEED

2.1 Pathophysiology and Epidemiology of Osteosarcoma

Sarcoma of the bone is derived from primitive bone-forming mesenchymal stem cells. Osteosarcoma may arise from any bone site, but it occurs most often near the metaphyseal portion of long bones or at sites of increased osteoblastic activity. There is a bimodal age distribution incidence with peaks in early adolescence and then again in a subset of older adults mostly more than 65 years of age. Metaphyseal osteosarcoma most commonly develops in teenagers who are experiencing their adolescent growth spurt. Osteosarcoma observed in elderly patients is generally associated with Paget's disease of bone or arising in previously irradiated tissue. The focus of the development program for MTP is on young osteosarcoma patients.

The incidence of osteosarcoma is very low. It is estimated that less than 1000 cases of osteosarcoma are diagnosed in the US each year with similar numbers for Europe. Osteosarcoma staging is based on tumor grade (low or high), tumor extent (intraosseous involvement only or extraosseous extension) and presence of distant metastases, regardless of the extent of local disease. Low volume tumor size, presence in an extremity, absence of metastases at diagnosis, alkaline phosphatase and blood lactate dehydrogenase (LDH) levels within the normal limits, complete surgical resection and good histologic response to chemotherapy are prognostic factors associated with better outcome.

Osteosarcoma disseminates almost exclusively by hematogenous spread to the lungs resulting in pulmonary metastases (about 15-20% of patients at diagnosis and 85% of patients at relapse) and secondarily by direct bone involvement, generally in more advanced stages (10-15% of patients at relapse). Early lymphatic spread to regional nodes has only rarely been reported and is a poor prognostic sign.

2.2 Current Treatment

The earliest treatment of osteosarcoma was surgical removal of the tumor. This resulted in a long term survival of <20%. The introduction of single agent and then multi-agent dose-intense chemotherapy in the 1970's and 1980's resulted in an increase in overall survival to about 60% (Table 1). There has been no significant advance in the survival rate since that time.

Table 1: Osteosarcoma Survival Rates*

1975-1984	1985-1994	1995-2000
50%	63%	61%

*CureSearch/COG Bone Tumor Committee

The standard treatment for nonmetastatic osteosarcoma starts with 6-16 weeks of multi-agent neoadjuvant chemotherapy. This is followed by complete surgical excision and a careful examination of the tumor histology. If adequate necrosis of the tumor is seen (>90%), then the same chemotherapy is generally given for another 9-36 weeks of adjuvant maintenance. If large areas of the tumor remain viable, the maintenance chemotherapy may be changed.

The combination of surgery and intensive chemotherapy in this young population results in substantial morbidity. While there remains a significant need to improve survival, the ideal interventions would not put these patients at greater risk for the traumatic, serious, and severe events associated with the current best practices.

2.3 Clinical Research Challenges

As a disease with incidence rate of less than 1,000 new cases diagnosed per year, osteosarcoma is considered an orphan disease and a disease where improvement in survival and the morbidity associated with treatment represent significant unmet medical needs. From a research point of view, osteosarcoma, being rare, presents significant logistic difficulties in enrolling large trials. Because of these limitations, few new trials of osteosarcoma can be run at any one time and most of the changes in interventions have yielded only small changes in outcome.

Pediatric oncology trials contrast sharply with research in adult oncology. In adult breast and colorectal cancers multiple large simultaneous trials result in small, incremental advances in care. New therapies may only extend survival by months and cures, if they occur, are difficult to detect because most adults with cancer also have significant cardiovascular or pulmonary co-morbidities of aging which may limit their ability to tolerate chemotherapy and decrease long-term survival, even if the chemotherapy is successful.

Unlike most adult oncology trials, pediatric oncology trials begin with a premise that cures can be detected in this population. Children and adolescents have few other near-term mortality risks. If a subject remains disease-free beyond the period when risk of relapse is highest, then a

cure is presumed and children are unlikely to die from other causes. For this reason, most large pediatric studies are designed using survival models, such as the Gompertz model, that assume cures will be detected as seen by a flattening of the Kaplan-Meier survival curves. Intermediate endpoints such as DFS are often selected as surrogates for overall survival.

Study INT-0133 was initiated in 1993 in the US the clinical research design and logistical challenges. The outcome of that study is described in the following section.

3. EFFICACY

3.1 Study 08

Study 08 was a non-randomized open-label single-center Phase 2 study of 33 patients with recurrent osteosarcoma conducted between 1988 and 1992 at the MD Anderson Cancer Center. The objective of this trial was to determine the activity of MTP in controlling or preventing pulmonary metastases in patients with osteosarcoma who had relapsed while on chemotherapy. There are 4 published reports of this study (Kleinerman ES et al, *Am J Clin Oncol* 18:93-99, 1995; Kleinerman ES et al., *Cancer Immunol Immunother* 34:211-220, 1992; Kleinerman ES et al., *J Clin Oncol* 10:1310-1316, 1992; Asano T et al., *J Immunother* 14:286-292, 1992).

The study enrolled patients with histologically proven osteosarcoma who had pulmonary metastases that had developed or persisted during adjuvant chemotherapy or that had recurred following surgical excision. Prior to study entry, the primary tumor had been resected and the patients were rendered clinically disease-free by surgical resection of any discernible pulmonary tumors.

In the first cohort, 2 mg/m^2 of MTP was infused for one hour twice weekly for 12 weeks for a total of 24 infusions. In a second consecutive cohort, half of the patients received 2 mg/m^2 twice weekly for 12 weeks and then once weekly for 12 weeks, for a total of 36 infusions over 24 weeks. In the other half of patients in the second cohort, the dose was titrated above 2 mg/m^2 until clinical evidence of monocyte activation was seen, such as fever, chills or an increase in C-reactive protein (CRP), to a maximum of 2 mg/m^2 + 2 mg.

Thirty-three (33) patients were enrolled in the trial, 17 males and 16 females with ages ranging from 11 to 61 years. Eighteen patients completed the trial and 15 discontinued prematurely. Fifteen patients were treated twice weekly for 12 weeks, 9 patients were treated twice weekly for 12 weeks and then once weekly for an additional 12 weeks, and 9 patients were treated for 24 weeks and received dose escalation based on evidence of monocyte activation. The patients treated for six months were analyzed as a single cohort. All patients were evaluated for safety and 28 patients were evaluable for efficacy (12 in 3 month group and 16 in 6 month group).

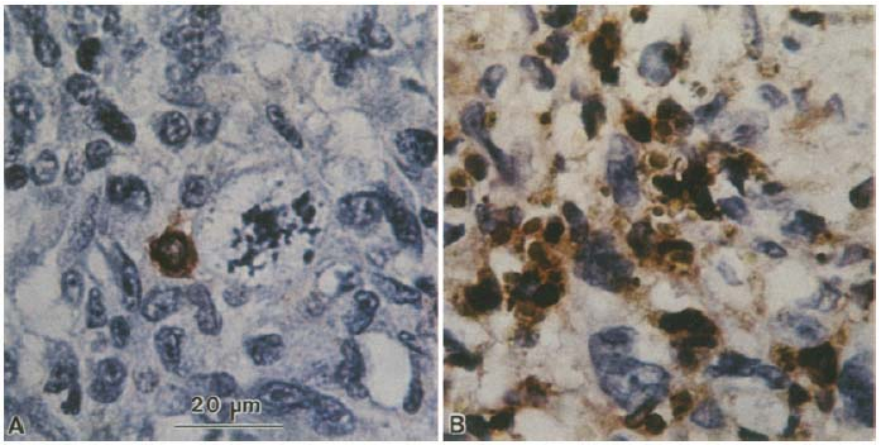
Progression-free intervals were assessed in all patients. The 3- and 6-month treatment groups were compared with a carefully matched historical control group from the same institution with

similar characteristics who would have met the trial eligibility criteria. The charts from 47 patients were reviewed for inclusion and 21 were chosen to be the comparator for the active treatment cohorts.

Patients who received single agent MTP for 12 weeks following surgery had a slightly but not significantly improved time to recurrence. In 5 patients, a single tumor nodule recurred within 6 weeks after completion of therapy. These lesions were resected and compared to tissue specimens obtained before therapy. All patients showed a histological change in the characteristics of the pulmonary tumors, including peripheral fibrosis with inflammatory cell infiltration and neovascularization in three patients. This contrasts to the central necrosis and no inflammatory infiltrate observed in lesions resected after chemotherapy. In a fourth case, there was evidence of early fibrotic changes and in this and a fifth case, there were changes in the malignant characteristics from high grade to low grade after MTP therapy. The figure below (Figure 2) demonstrates the increase in inflammatory macrophages in pulmonary lesions that recurred following treatment with MTP. MRP-14 is a calcium binding protein specific for cells of the myeloid series. Expression of these proteins outside the circulation is characteristic of inflammatory tissue macrophages.

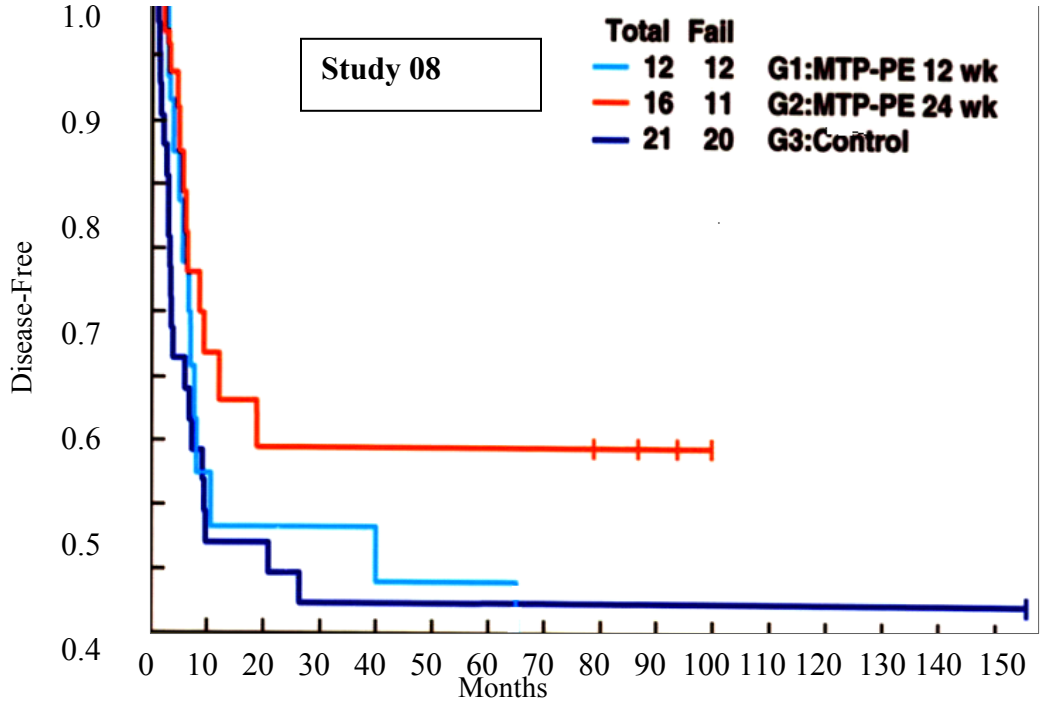
Figure 2: Immunohistochemical Staining of Lung Lesions Before (A) and After (B) MTP Therapy Showing an Increase in Inflammatory Macrophages. Staining With Anti-MRP-14 Reveals Brown to Black Inflammatory Macrophages

Kleinerman et al., *Cancer Immunol Immunother* 34:211-220, 1992



Based on this histologic finding suggestive of activity in pulmonary lesions, the treatment duration was increased to 24 weeks for the next cohort of patients. The patients treated for 24 weeks had a significant improvement in progression-free survival compared to historical controls, suggesting that longer duration of treatment is important. This was the basis for the decision to extend treatment to 36 weeks in the Phase 3 study (INT-0133).

Figure 3: Disease-Free Survival In Patients With Recurrent Pulmonary Osteosarcoma Treated With MTP For 12 or 24 Weeks After Being Rendered Clinically Disease Free By Surgery As Compared To Historical Controls.
Kleinerman *et al.*, *Am J Clin Oncol* 18:93-99, 1995



The patients in the second cohort, whose dose escalated to a maximum of 2 mg /m2 + 2 mg until signs of MTP activity were observed, tolerated the increase in dose. Based upon the tolerability in this study, dose escalation was adopted for the Phase 3 study. Because the *in vitro* monocyte tumoricidal activity (MTA) assay used in Study 08 to titrate the MTP dose was difficult to validate and gave variable results, dose escalation was to be based on the presence or absence of fever, chills and elevated C-reactive protein as biological parameters associated with MTP activity.

3.2 Study INT-0133

3.2.1 Study Rationale

High-dose methotrexate, doxorubicin and cisplatin are highly active against osteosarcoma and were the standard of care at the time of this study. A pilot study by Miser *et al*. reported high

rates of good histological response after 5 cycles of induction with high-dose methotrexate and ifosfamide given in combination with doxorubicin (Miser J et al. *Proc. ASCO* 10:310, 1991). These results provided the basis for selecting the chemotherapy agents used in the treatment groups.

The primary options for incorporating MTP into the study design were to combine it with chemotherapy using chemotherapy alone as the control, or to randomize patients to receive it following completion of chemotherapy with observation as the control. The choice to combine MTP with chemotherapy was based on an understanding of the biology of osteosarcoma, and the probable lack of interaction between MTP and conventional chemotherapy agents.

Osteosarcoma frequently relapses early after chemotherapy and surgery. Because MTP was considered likely to be most effective against clinically undetectable 'microscopic' disease, the ideal time to use MTP is after neo-adjuvant chemotherapy and definitive surgery when patients are clinically disease-free. This time point corresponds to the initiation of maintenance chemotherapy. Prior pediatric cooperative group experience showed that late randomization resulted in significant withdrawal of patients, with 25% or more attrition between primary chemotherapy and enrollment into a subsequent randomized trial. It was therefore considered critical to randomize the patients at the time of entry into the trial and to introduce MTP early in the course of maintenance treatment with concomitant chemotherapy.

Because the effects of MTP are mediated by activated monocytes and macrophages, there was concern that cytotoxic chemotherapy could decrease levels of circulating monocytes and macrophages and blunt the potential benefits of MTP. However, monocyte recovery after cytotoxic chemotherapy generally precedes neutrophil recovery and rebound monocytosis is not uncommon. Based on the available data at the time, it was concluded that sufficient monocytes should be available over the course of treatment to allow a fair trial of MTP.

In addition to decreased levels of circulating monocytes, there was a concern that cytotoxic chemotherapy may impair the response of monocytes and macrophages to MTP. Extensive *in vitro* studies were performed to assess the effects of doxorubicin, cisplatin, methotrexate, and cyclophosphamide on the ability of MTP to activate monocytes (Hudson MH et al. Cancer Res 48:5256-5263, 1998; Kleinerman ES et al. *J Clin Oncol* 9:259-267, 1991). Monocytes incubated with doxorubicin retained the ability to be activated by MTP, including activation of tumoricidal

activity and upregulation of interleukin-1 (IL-1). Neither cisplatin nor methotrexate interfered with the action of MTP on monocytes from children with osteosarcoma, though monocytes obtained after administration of cyclophosphamide had a transiently impaired response (72 hours).

Studies in animals had demonstrated that the simultaneous administration of MTP with chemotherapy (doxorubicin, cisplatin, ifosfamide) did not impact toxicity as assessed by nadirs or recovery time for white blood cells (WBC), neutrophils, platelets, and hematocrit, or impair the antitumor effect of chemotherapy to inhibit tumor growth in murine models. The simultaneous use of chemotherapy and MTP also was explored in a study of dogs with naturally occurring osteosarcoma (MacEwen EG et al. *J Natl Cancer Inst* 81:935-938, 1989) and in a Phase 2 clinical study to assess potential interactions between ifosfamide and MTP in patients (Kleinerman ES et al. *J Immunother* 17:181-193, 1995). There was no evidence that MTP would impair the anti-tumor effects of chemotherapy or exacerbate the toxicities. There was no evidence that chemotherapy would significantly impact the ability of monocytes to be activated by MTP.

The appeal of a factorial design study, especially in a rare disease, is compelling since 2 different treatment questions may be asked of the same pool of patients. A common approach to sample size and analysis for factorial design trials assumes no statistical interactions between the 2 treatments being studies and does not adjust for multiple testing (Green S, Liu P-Y, O'Sullivan J. *J Clin Oncol* 20:3424-3430, 2002). Based on the preclinical and early clinical data, the study design for INT-0133 proceeded on the assumption that there would be no interaction between MTP and chemotherapy, and with the conviction that early introduction of MTP to treat clinically unapparent disease was the optimal way to test the impact of MTP in osteosarcoma.

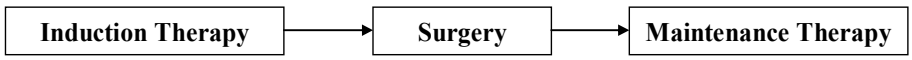
3.2.2 Dose Rationale

The rationale for a randomized study using 2 mg/m^2 MTP (INT-033) is based on the post-treatment immune based biologic effect demonstrated histologically in surgically resected pulmonary metastases in Study 08 and the observation that at least 24 weeks of MTP therapy was necessary to increase progression free survival. The choice of the 2 mg/m^2 dose of MTP is consistent with data from the large series of Phase 1 and Phase 2 studies in other indications that demonstrated that a dose of 0.5-2 mg/m^2 yielded the best biologically active dose and it was well

below the maximal tolerated dose of 4-6 mg/m^2. Thirty six weeks of therapy was chosen based on the suggestion from Study 08 that longer treatment duration may be better and this extension provided some doses of MTP alone after the completion of chemotherapy.

3.2.3 Study Design

This multi-center, randomized, factorial, open-label study was conducted in three steps: induction or neoadjuvant chemotherapy, definitive surgical resection of the tumor and maintenance or adjuvant therapy.



Weeks 0-9 **Weeks 10-11** **Weeks 12-48**

Within 30 days of a new diagnosis of high-grade non-metastatic resectable osteosarcoma, eligible patients were stratified by LDH level, site of disease and prior amputation and then randomly assigned to 1 of 4 treatment groups. All patients received 10 weeks of neoadjuvant induction therapy with 1 of 2 chemotherapy regimens. Regimen A induction therapy consisted of doxorubicin, cisplatin and methotrexate, and Regimen B induction therapy consisted of doxorubicin, ifosfamide, and methotrexate. Definitive surgery was performed during Weeks 10 to 11 while off all chemotherapy and study medication.

Beginning at Week 12, patients in Regimen A received maintenance therapy that consisted of the same agents they had received for induction chemotherapy with or without MTP. Maintenance therapy for patients in Regimen B consisted of the same agents they received for induction therapy with the addition of cisplatin, with or without MTP.

Patients assigned to receive MTP in the maintenance phase received twice weekly IV injections for 12 weeks followed by once weekly IV injections for an additional 24 weeks for a total of 48 injections over 36 weeks. The starting dose of MTP was 2 mg/m^2 which could twice be escalated by 1 mg (i.e., 2 mg/m^2 + 1 mg and then 2 mg/m^2 + 2 mg) until clinical signs of monocyte/macrophage activation were seen. Biological activity was defined by: elevation of oral body temperature to at least 38.1°C within 24 hours of beginning drug administration, the presence of Grade 2 visible rigors lasting 30 minutes, or a significant elevation in CRP (>2x baseline) 24 hours post-dosing. The drugs, doses, and duration of administration for each

regimen in each phase are summarized below. A more detailed description of the schedules for study drug administration is provided in Appendix 1.

Table 2: Summary of Phase 3 Study Treatment Arms

	Regimen A or A plus MTP			Regimen B or B plus MTP		
Drug (dose)	Neoadjuvant Induction Week 0-9	Definitive Surgery Week 10-11	Adjuvant Maintenance Week 12-48	Neoadjuvant Induction Week 0-9	Definitive Surgery Week 10-11	Adjuvant Maintenance Week 12-48
MTP ($2mg/m^2$)	-		48 doses	-		48 doses
Methotrexate ($12\ g/m^2$)	4 doses		8 doses	4 doses		8 doses
Doxorubicin ($25\ mg/m^2$/day x3)	2 courses		4 courses	2 courses		4 courses
Cisplatin (120 mg/m2)	2 doses		2 doses	-		4 doses
Ifosfamide ($1.8\ g/m^2$/day x5)	-		-	2 courses		3 courses
Duration of Treatment						
Chemotherapy	10 weeks		21 weeks	10 weeks		28 weeks
Surgery		2 weeks			2 weeks	
MTP			36 weeks			36 weeks

Efficacy was assessed by monitoring disease status and survival at defined time points. Disease status was assessed at baseline using a combination of clinical and diagnostic tests, including complete history and physical examination (with assessment of pain and swelling prior to diagnosis), imaging studies of the affected bone (x-ray, CT, MRI), imaging studies of the chest for metastatic disease (x-ray, CT), bone scans with radiographic examination of areas that were isotope positive, and review of diagnostic biopsy. Clinical assessment and imaging study efficacy endpoints were completed at designated points during each course of treatment. Post-treatment follow-up continued after treatment every 3 months for 1 year, then every 6 months for 2 years, then once a year indefinitely.

3.2.4 Sample Size, Power and Other Statistical Considerations

INT-0133 was designed using the Gompertz survival model and the disease-free survival endpoint to determine the sample size and the minimum follow up period. The primary aim of the study was to improve overall survival. The statistical section of the protocol indicated that the

study would be statistically powered based upon disease-free-survival (DFS), that the impact of treatment on DFS would be assessed using a log rank test and that patients with metastatic or unresectable disease would not be included in the primary analysis. The statistical analysis of the study by IDM is based on the interpretation of the statistical section of the original study protocol. Because the original protocol was developed in 1993, and did not describe the intended statistical analysis with the rigor of today's customary registration requirements, IDM consulted with 3 independent statisticians with expertise in clinical trial design and analysis.

Without knowledge of prior analyses, the conclusions of the 3 independent statistical reviews were as follows:

1. Disease-free survival (DFS) was the intended prospectively planned primary outcome measure in Study INT-0133. The definition of DFS as used in the protocol is really progression-free survival. DFS assumes all patients are rendered disease-free before time to endpoint; some patients progressed before surgery – these are counted as relapses as are patients who relapsed after incomplete surgical resection. This definition was acknowledged in the study report and was used as such for the purposes of primary analysis.
2. Patients with non-metastatic and resectable disease were the intended primary analysis group.
3. The stratified log rank test was the defined method of analysis.
4. Overall survival is the first stated aim of the study ("to improve the survival of patients with osteogenic sarcoma") and the reference endpoint for DFS. Therefore analysis of survival will also be important.

3.2.5 Data Transfer

The COG statistician provided the INT-0133 data to IDM in June 2003 as SAS data sets that were described as being a mirror image of the entire COG database for this study. The 2003 COG data sets include 228 DFS events with a median follow up of patients alive at last contact of approximately 4.8 years. IDM also obtained copies of the study CRFs.

3.2.6 Results

The ITT data set contains all patients randomized to receive either MTP or no MTP who were declared to have resectable, non-metastatic osteosarcoma at the time of randomization. Unless otherwise indicated, all analyses in this section are based on the ITT data set.

3.2.6.1 Disposition of Patients in ITT Data Set

Table 3: Disposition of Patients (ITT Data Set)

	MTP (n=338)	No MTP (n=340)
Entered Induction Phase	333	334[1]
Withdrawn		
Progressive Disease	9	10
Removed for Toxicity	1	2
Withdrawal by Parent or Patient	8	8
Withdrawal by Physician	1	3
Major Protocol Deviation	7	8
Death	0	2
Lost to Follow-Up	0	0
Other	4	1
Entered Maintenance Phase	303	301
Withdrawn		
Progressive Disease	17[2]	16
Removed for Toxicity	3	5
Withdrawal by Parent or Patient	46	14
Withdrawal by Physician	7	4
Major Protocol Deviation	9	7
Death	2	1
Lost to Follow-Up	2	0
Other	2	1
Deemed Ineligible	1	3
Completed Protocol Therapy	214	250

[1] One patient with prior surgery went directly to maintenance chemotherapy and did not have induction chemotherapy.

[2] One patient had progressive disease prior to surgery. This patient is included among those with progressive disease.

3.2.6.2 Baseline Demographics and Disease Characteristics

The baseline demographics and disease characteristics of the intent-to-treat patients enrolled in the Phase 3 trial are shown below (Table 4).

Table 4: Demographics: Intent-to-Treat Data Set

	No MTP	MTP	Total
Gender			
Male	172	200	372
Female	168	138	306
Total	340	338	678
Age (years)			
Mean	13.7	13.9	13.8
Median	13.3	14.0	13.7
Range	4.4 – 30.6	4.5 – 30.4	4.4 – 30.6
Race			
White	236	214	450
Hispanic	36	49	85
Black	51	46	97
Oriental	4	10	14
Filipino	2	5	7
Other	11	14	25
Primary Tumor			
Arm – Humerus	34	42	76
Arm – Radius	6	5	11
Arm – Ulna	1	1	2
Arm	1	0	1
Leg – Femur	187	182	369
Leg – Tibia	78	90	168
Leg – Fibula	9	6	15
Leg	2	0	2
Other	15	9	24
Unknown	7	3	10

Stratification based on tumor location (involvement or not above the knee or elbow), serum LDH and prior amputation resulted in evenly balanced risk factors across the MTP and No-MTP groups (Table 5).

Table 5: Stratification Distribution: Intent-to-Treat

	No MTP	MTP	Total
LDH < ULN; no involvement; no amputation	79	80	159
LDH < ULN; involvement; no amputation	134	134	268
LDH > ULN; no involvement; no amputation	29	30	59
LDH > ULN; involvement; no amputation	88	89	177
LDH < ULN; no involvement; amputation	2	1	3
LDH < ULN; involvement; amputation	3	2	5
LDH > ULN; no involvement; amputation	2	0	2
LDH > ULN; involvement; amputation	3	2	5
Total	340	338	678

3.2.6.3 Follow Up

Regardless of treatment status all patients were followed for as long as possible for disease and survival status. Limited follow-up was available on 14 of the 678 patients in the ITT data set because they were considered ineligible for the study. However, they are included in all analyses of the ITT data. Table 6 summarizes the follow-up data for the ITT data set.

Table 6: Follow-Up (years) for Patients Alive at Last Contact (ITT)

	No MTP n=340	MTP n=338	Total
Mean	4.4	4.6	4.5
Median	4.6	4.9	4.8
Range	0 – 9.1	0 – 8.8	0 – 9.1

3.2.6.4 Disease-Free Survival

MTP significantly increased disease-free survival. The 6-year probability of surviving without a relapse of osteosarcoma was 66% (95% CI: 61%-72%) among patients who received MTP compared with 57% (95% CI: 52%-64%) among patients who did not. This disease-free survival advantage resulted in a hazard ratio of 0.76 in favor of MTP, or a 24% reduction in the risk of relapse, progressive disease or death.

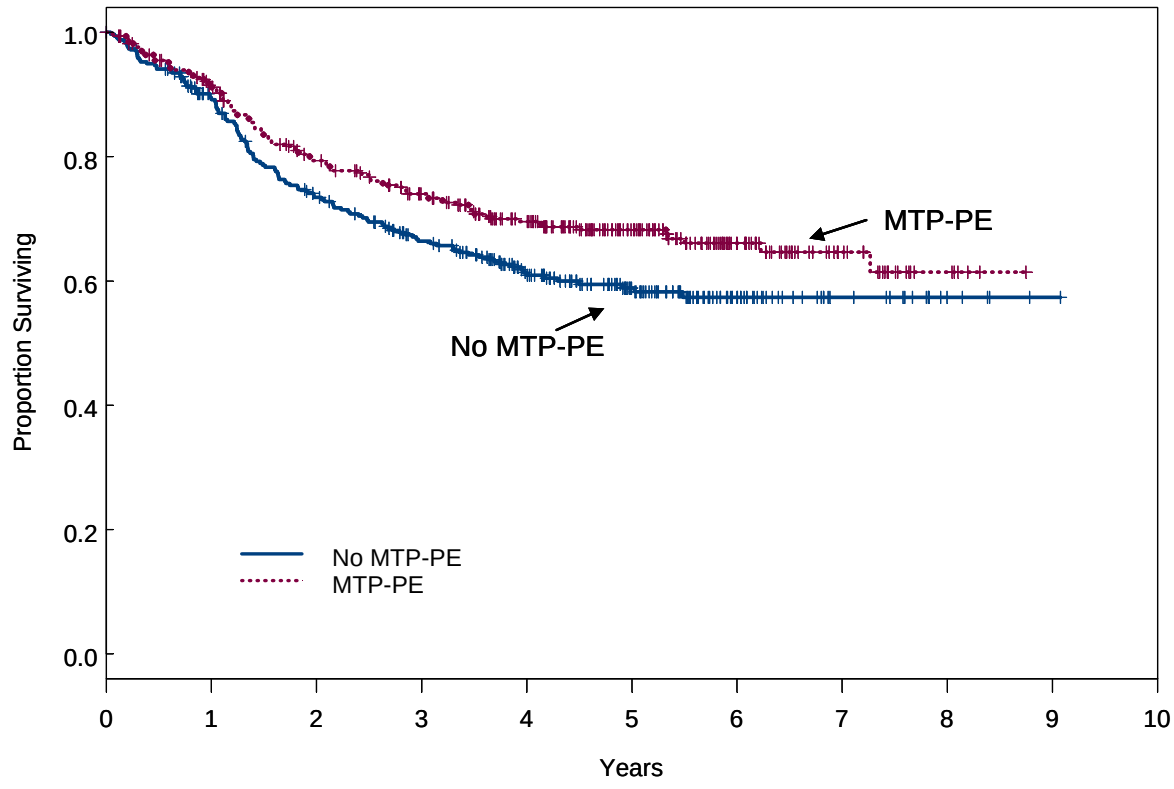
Table 7A: Disease-Free Survival Probability (95% CI)

Treatment	# of Pts (events)	4-Year	6-Year	P-value	Hazard Ratio	95% CI for HR
No MTP (A/B)	340 (126)	0.609 (0.556, 0.667)	0.574 (0.517, 0.636)	---	1.00	---
MTP (A+/B+)	338 (102)	0.696 (0.646, 0.750)	0.661 (0.607, 0.720)	0.0245*	0.76	(0.58, 0.98)

*p-value from log-rank test stratified by ifosfamide use and randomization strata.

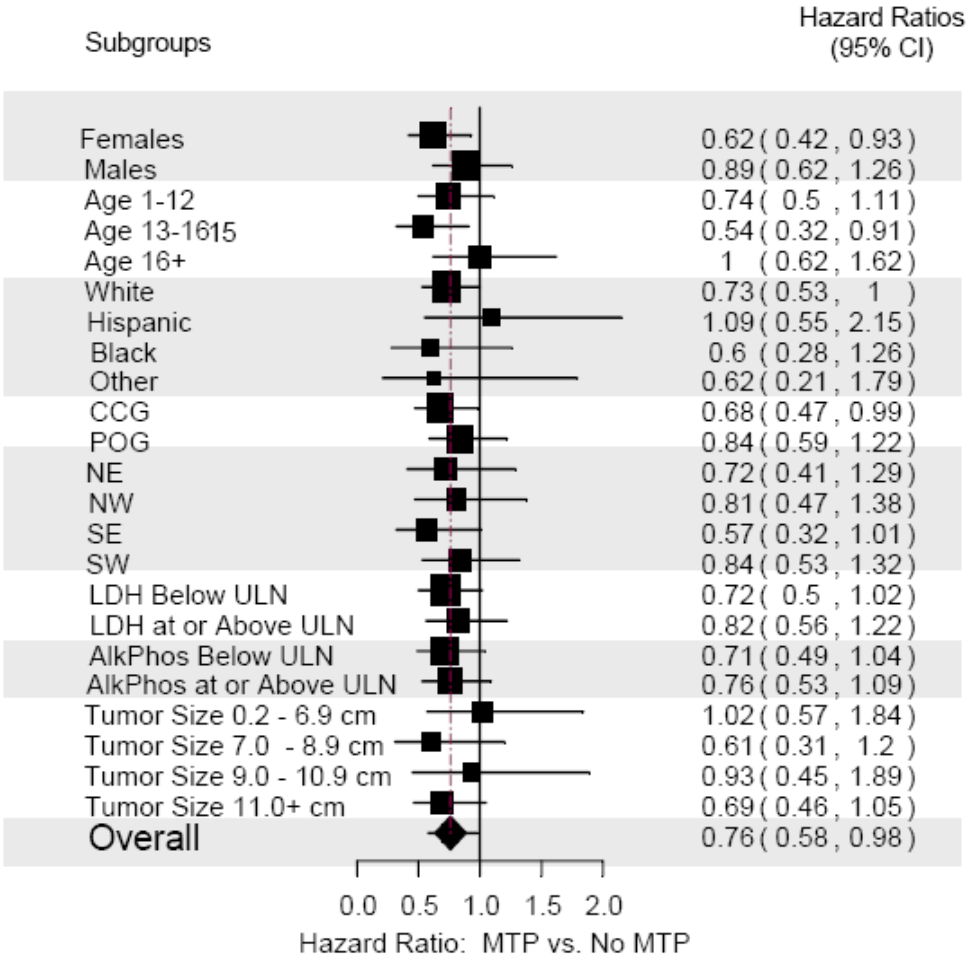
The Kaplan-Meier survival curves for DFS (Figure 4) show a clear separation and flattening and are consistent with the expectation of observing cure as in the Gompertz model used to design the study. Note that at 7 years there are only 17 patients at risk of recurrence or death in the no-MTP treatment arm and only 24 patients at risk of recurrence or death in the MTP treatment arm.

Figure 4: Disease-Free Survival ± MTP (ITT Data Set)



When evaluated by randomization to MTP or no MTP, the supportive analyses by subgroups of race, age, gender, tumor location, LDH, alkaline phosphatase and tumor size also reinforced the conclusion of MTP benefit on DFS and further demonstrated internal study consistency. These supportive analyses are best summarized by the following Forest plots in which it can be seen that regardless of the subgroup, the preponderance of results favor MTP (Figure 5).

Figure 5: Disease-Free Survival: Hazard Ratios for Various Risk Groups



3.2.6.5 Overall Survival

The addition of MTP to multi-agent chemotherapy in study INT-0133 resulted in a clinically meaningful and statistically significant increase in overall survival in patients with non-metastatic resectable osteosarcoma. The 6-year survival probability is 77% (95% CI: 72%-83%) in patients who received MTP compared with 66% (95% CI: 59%-73%) in patients who did not.

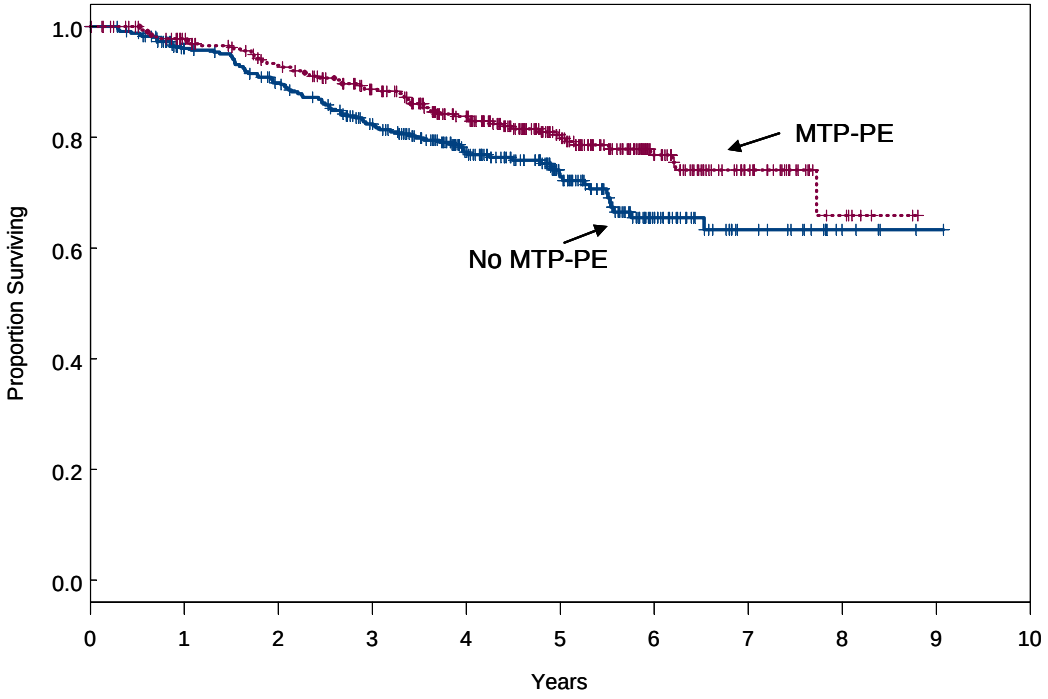
Note that at 7 years there are only 19 patients at risk of death in the no-MTP treatment arm and only 28 patients at risk of death in the MTP treatment arm.

Table 7B: Overall Survival Probability (95% CI)

Treatment	# of Pts (events)	4-Year	6-Year	p-value	Hazard Ratio	95% CI for HR
No MTP (A/B)	340 (85)	0.773 (0.726, 0.823)	0.655 (0.591, 0.726)	---	1.00	---
MTP (A+/B+)	338 (63)	0.838 (0.796, 0.881)	0.768 (0.715, 0.826)	0.0183*	0.68	(0.49, 0.95)

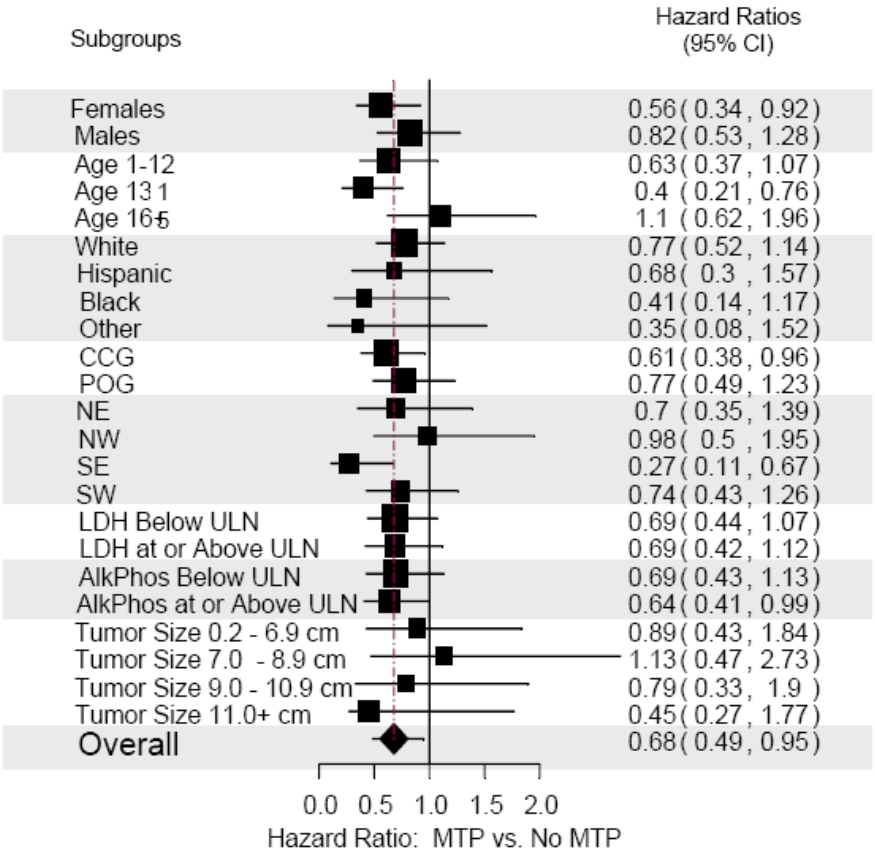
*p-value from log-rank test stratified by ifosfamide use and randomization strata.

Figure 6: Overall Survival ± MTP (ITT Data Set)



When evaluated by randomization to MTP or no MTP, the supportive analyses by subgroups of age, race, gender, location, LDH, alkaline phosphatase and tumor size reinforce the conclusion of MTP efficacy and internal consistency. These supportive analyses are best summarized by the following Forest plots in which it can be seen that regardless of the subgroup, the direction of benefit almost always favors MTP (Figure 7).

Figure 7: Overall Survival: Hazard Ratios for Various Risk Groups



3.2.6.6 Critique of Study INT-0133

Study IND-0133 is the largest controlled trial ever conducted in osteosarcoma patients, enrolling approximately one third of newly diagnosed cases over the time period of study in major childrens' oncology centers in the US. The study conformed to NCI and COG clinical research standards. Current regulatory requirements for registration of a new product for marketing differ from those used when the study was conducted. This difference posed several challenges which the sponsor has attempted to effectively address.

3.2.6.6.1 Design of INT-0133

Three major design challenges identified by the sponsor include open label treatment, factorial design and the use of DFS as a primary endpoint.

1. INT-0133 was not blinded. There is concern that the open label nature of the study may introduce bias. Neither patients nor investigators were blinded to the treatment assignment for two major reasons. First, the MTP dose escalation was dependent upon the observation of a clinical biological effect. Since there is a clear biologic effect in most patients, it is likely that the blind would have been quickly compromised. Second, clinicians and IRBs are reluctant to consider placebo use in pediatric trials. They have considerable difficulty justifying 48 IV placebo injections concurrent with and for 10 weeks after chemotherapy. While understanding the desirability to minimize bias through the use of blinding, it was felt that the additional IV infusions in these children and young adults were not acceptable. While an unbiased assessment of disease relapse is difficult to implement in an unblinded study, the possibility that the survival endpoint is biased in an open study is generally considered to be nonexistent.

2. Factorial design complicated the analysis. INT-0133 was designed to answer two independent questions using the same data set: "did the addition of MTP offer a clinical advantage?" and "did the addition of ifosfamide offer a clinical advantage?" This design is based on the assumption that there is no interaction between the two questions. Only the question of MTP efficacy is addressed in this application.

   Another consequence of the factorial design was the requirement to randomize all patients before any therapy was given. This resulted in patients being randomized to receive MTP almost 3 months before MTP was added to the treatment regimen.

3. DFS may be subject to ascertainment bias. The lung is the first site of recurrence in 85% of patients. These metastases are typically asymptomatic and detected on chest X-ray or CT scan. Although most relapses were documented by subsequent surgery, a blinded review of periodic radiologic findings leading to the diagnosis of relapse was not mandated by protocol. Coupled with the lack of treatment blinding, this makes elimination of potential ascertainment issues difficult. However, there was a specified schedule of follow up assessments at 3, 6, 9, 12, 18, 24, 30 and 36 months and then yearly. The follow up visits were documented at the clinical sites and compliance was audited consistent with COG practices.

Although there is no evidence of bias or inequality in the frequency of evaluation of patients in different study arms, reliance on the survival endpoint as the basis for demonstration of efficacy mitigates potential ascertainment issues associated with DFS.

3.2.6.6.2 Data Collection and Analysis for INT-0133

It is important to note that the handling of data for this study was in accordance with the standards of the cooperative groups and differs from industry. There are a few data management practices and data analysis issues of note:

1. Database closure: According to COG no study ever 'officially' ends, i.e. the database is not closed/locked, but rather COG continues to collect survival and disease status indefinitely. Therefore any data sets provided by COG to IDM simply represent a snapshot of the data received and entered into their database as of that time point.
2. Selective data entry: IDM created new data sets only for drug doses in the phase 3 study since these were not computerized by COG. These were entered into a separate database created for IDM by a contract organization and to facilitate safety analysis.
3. Selective data collection: Much data that could be collected in CRFs in a study of this type, e.g. all visit dates during follow up and all assessment results, are not collected on CRFs and entered into a centrally managed database. Rather it is the responsibility of the sites to document this data in records retained at the site. The lack of this information in a central database necessitates the auditing at clinical sites for confirmation of protocol compliance and data quality.

   IDM audited key data from the phase 3 study by comparing the COG data set to the source documents at the sites. Data were reviewed by an independent contract audit team that included medical oncologists who visited 5 sites and audited key data from 69 patients to source documents (10% of the population in the study).

   The conclusion from these audits was that the rate of observed discrepancies (between 2.5-5% including data differences, missing data in CRF and data not found at site) were consistent with published data from other unmonitored cooperative group trials (Favalli, G et al. *Eur J Cancer* 36 :1125-1133, 2000 ; Steward WP et al. *Eur J Cancer* 29A : 943-947, 1993 ; JK Mauer et al. *Cancer Treat Rep* 69:1177-1187, 1995). The auditors also concluded

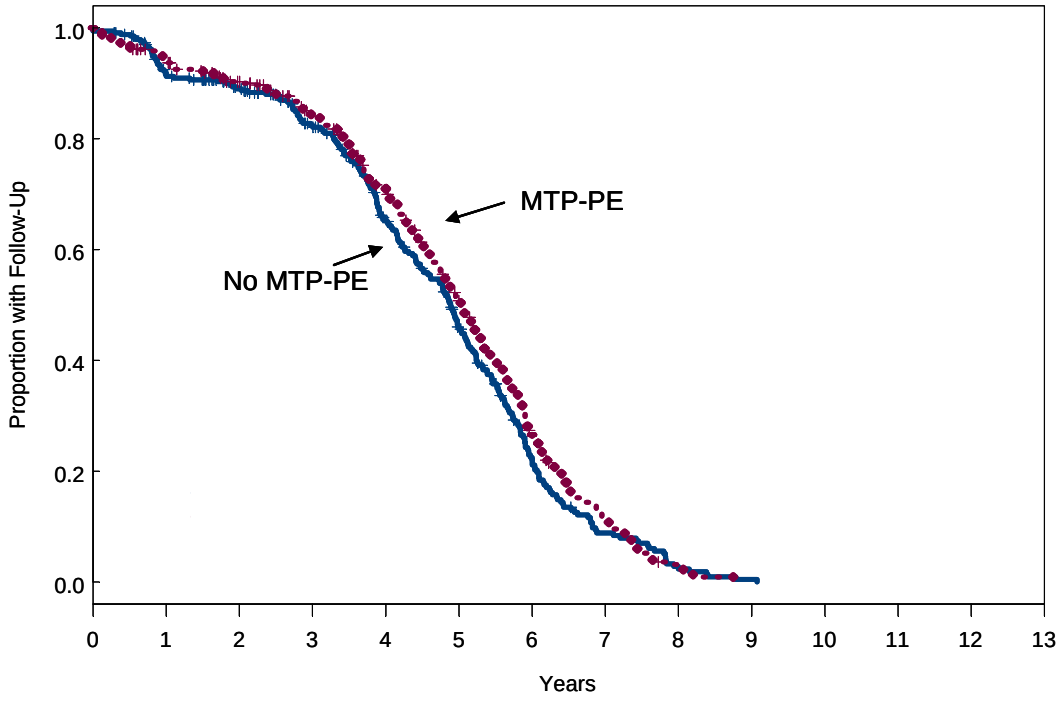
that the data are reliable and that the discrepancies found should not impact the efficacy or safety conclusions.

4. Completeness of follow-up: IDM made the decision not to retrospectively modify the 2003 data sets provided by COG as they were the least biased way to use the data for the primary analyses. However, IDM was aware of limited follow-up beyond 5 years in the 2003 data set. This limited follow-up was attributed, at least in part, to a loss of interest by investigators caused by the closure of the company that held the rights to MTP. Pediatric follow-up is further complicated in general because of the transition from pediatrician to internists or family practitioners in adulthood, the frequency that US families move and the number of patients referred from primary care practices and physicians outside the US. The following table and curves illustrate the comparable follow up for survival in the MTP and no-MTP groups in the 2003 data set.

Table 8: Overall Survival: Number of Patients Alive at the Beginning of the Interval and Number of Deaths in the Interval: 2003 Data

	No MTP			**MTP**		
Year	# at Risk	# of Deaths	# Lost to FU	# at Risk	# of Deaths	# Lost to FU
0.0-0.9	340	13	29	338	7	20
1.0-1.9	298	19	7	311	15	12
2.0-2.9	272	22	19	284	13	19
3.0-3.9	231	13	46	252	13	37
4.0-4.9	172	8	51	201	8	57
5.0-5.9	113	9	55	137	4	64
6.0-6.9	49	1	29	69	2	39
7.0-7.9	19	0	14	28	1	20
8.0-8.9	5	0	4	7	0	7
9.0-9.9	1	0	1	0	0	0
Total		85	255		63	275

Figure 8: Length of Follow-Up (ITT Data Set)



In the ITT data set (2003), sixty one patients (15%) in the MTP group and fifty-five patients (16%) in the no-MTP group have less than three years of follow up. Forty-three percent of patients in the MTP group (145/338) and 45% of patients (152/340) in the no-MTP group have less than 5 years of follow. While the follow up was considered suboptimal, it was not selective.

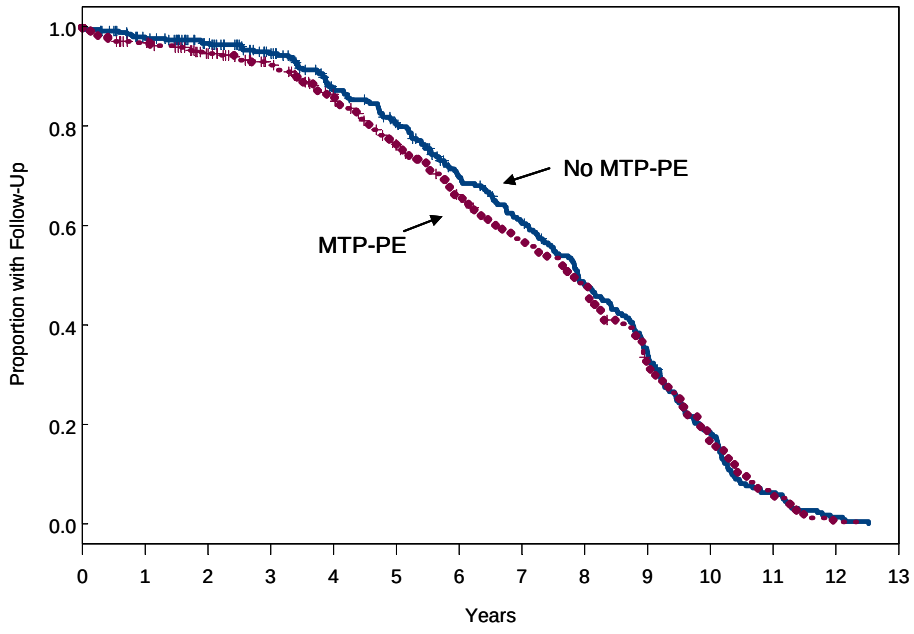
IDM requested and COG agreed to update the follow up for disease and survival status in 2006, providing a second more complete snapshot of the data to provide confidence in the conclusions based on the 2003 data.

COG provided an updated data set in August 2006 in which the median survival of patients alive at last follow-up was 7.7 years compared to 4.8 years in the 2003 data set. In the 2006 data set, only 7% (25) of patients in the MTP group and 5% (17) in the no-MTP group have less than three years of follow up and patients with less than five years of follow up have been reduced to 21% (71) in the MTP group and 16% (55) in the no-MTP group.

Table 9: Overall survival: Number of Patients Alive at the Beginning of the Interval and Number of Deaths in the Interval: 2006 Data

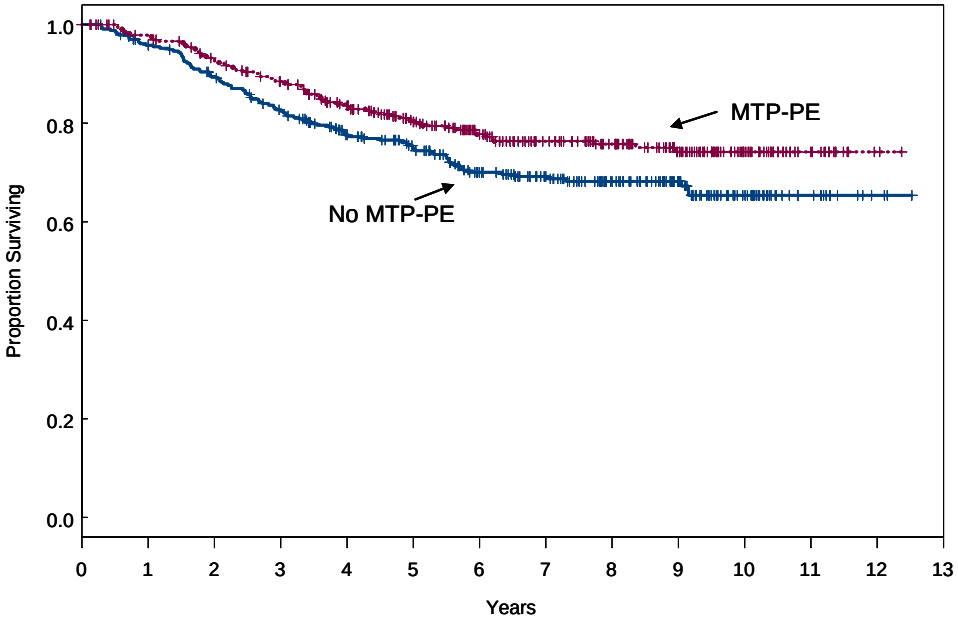
	No MTP			MTP		
Year	# at Risk	# of Deaths	# Lost to FU	# at Risk	# of Deaths	# Lost to FU
0.0-0.9	340	14	8	338	7	11
1.0-1.9	318	21	3	320	16	7
2.0-2.9	294	22	6	297	14	7
3.0-3.9	266	16	19	276	15	17
4.0-4.9	231	8	19	244	9	29
5.0-5.9	204	12	26	206	6	27
6.0-6.9	166	2	22	173	3	23
7.0-7.9	142	2	29	147	1	23
8.0-8.9	111	0	31	123	2	39
9.0-9.9	80	3	36	82	0	40
10-10.9	41	0	27	42	0	26
11-11.9	14	0	11	16	0	14
12-12.9	3	0	3	2	0	2
Total		100	240		73	265

Figure 9: Length of Follow-Up (2006 Data Set)



The overall survival (HR = 0.72, 95% CI: 0.53-0.97) from the updated 2006 data demonstrate that the curves remain apart with extended follow up. The analyses of these updated data provide high confidence in the conclusions based on the 2003 data.

Figure 10: Overall Survival ± MTP (2006 Data Set)



The more complete follow up data confirm the clear survival benefit associated with MTP.

5. Type I error: The type I error probability for the DFS endpoint may be very modestly compromised by the introduction of mandated interim analyses. An NCI policy change required the addition of data monitoring committees and interim safety analyses for all cooperative group studies and the ongoing protocol was amended to add 3 interim analyses. This concern was compounded when the original statistical plan outlined in the protocol was not executed at the planned time (i.e. after 2 years of follow up).

   To address these issues, a detailed simulation study to estimate the potential impact on the Type I error probability of the interim analyses and the delayed final analysis was performed. This investigation found that any increase in Type I error probability was modest for DFS.

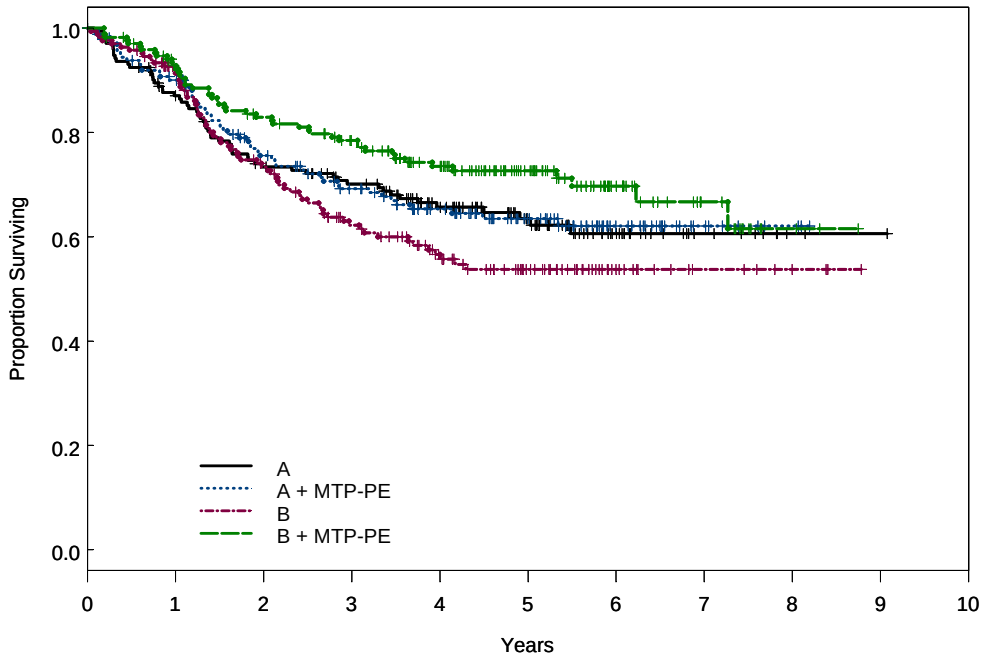
6. Potential for interaction: COG published an analysis of the INT-0133 study results in March 2005 in the *Journal of Clinical Oncology* (Meyers PA et al. *J Clin Oncol*. 23:2004-2011, 2005). COG did not use the prospectively planned factorial design analysis, but analyzed the

data by individual study arm because of their interpretation of a potential interaction between chemotherapy and MTP. Based on this unplanned analysis, the published data and those presented today differ.

Though neither analysis by study arm nor analysis of interaction was prospectively planned for the INT-0133 data sets, IDM performed exploratory analyses based on the COG publication.

When assessed by treatment arm, it appears that Regimen A+ is very similar to Regimen A, suggesting little or no advantage of the addition of MTP. In contrast study Regimen B appears worse than Regimen A and Regimen B+ appears much better, suggesting considerable advantage when adding MTP to study arm B. This is the basis for suggesting that there may be an interaction between MTP and treatment regimen. The 2003 Kaplan-Meier curves for disease-free survival by individual study arm illustrate this point.

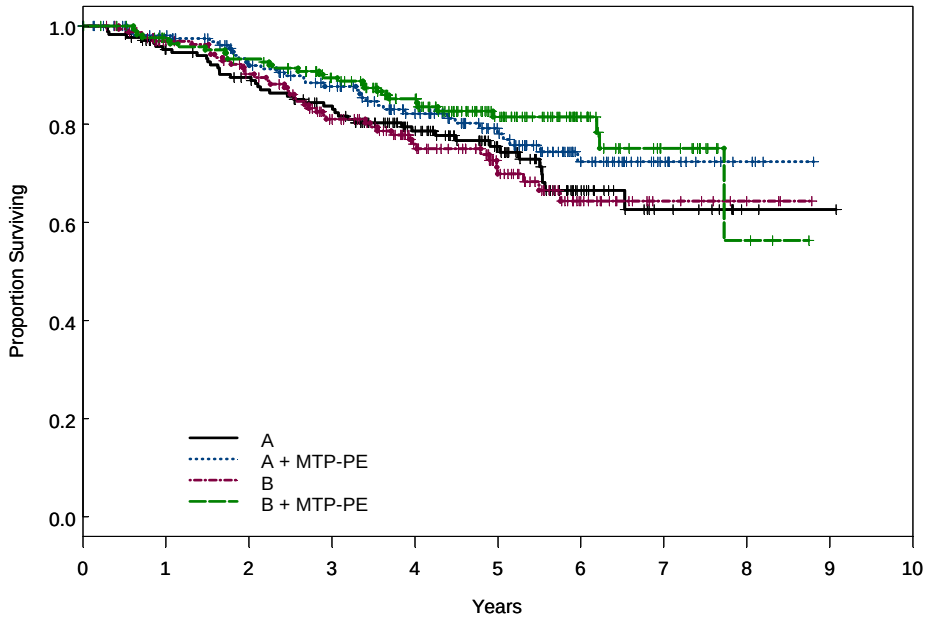
Figure 11: Disease-Free Survival (ITT Data Set)



In contrast, analysis of overall survival by individual study arm shows no interaction. The hazard ratios and survival probabilities for the two MTP arms are favorable compared to the two no-MTP arms. This is also illustrated by the 2003 survival Kaplan-Meier curves, where

the two most favorable curves are the two MTP arms. Since the study was neither powered nor planned for these exploratory analyses, differences are not significant.

Figure 12: Overall Survival (ITT Data Set)



The statistical tests for drug interaction support these differences between DFS and OS. While there is suggestion of an interaction for DFS using a Cox proportional hazards regression model, (p=0.06), interpretation is complicated by the observation that there was a potential imbalance in histologic response (Arm A+ had a higher number of poor responders) among the individual arms entering adjuvant maintenance chemotherapy and higher toxicity in the 4 drug chemotherapy arm compared to the 3 drug arm even before MTP was started. However, for OS, there is no statistical support for any interaction (p=0.51) between MTP and chemotherapy in individual study arms.

The evidence for interaction between MTP and treatment regimen with respect to DFS is illustrated in the following table (Table 10). When the DFS endpoint is assessed comparing A to A+ and B to B+, the MTP hazard ratios are very different. This is reflected in the p-value (0.06) that is suggestive of an interaction. In contrast the MTP hazard ratios for survival comparing A to A+ and B to B+, are quite similar. This is reflected in the p value (0.51) that indicates there is no interaction.

Table 10: Interaction Analyses for DFS and Survival

	DFS		Survival	
	HR	**p-value**	**HR**	**p-value**
Regimen A v A+	0.97	p = 0.06	0.76	p = 0.51
Regimen B v B+	0.59		0.61	
	Implies MTP HR may not be equal across regimens		No evidence of MTP by chemotherapy interaction	

Thus, the use of the survival endpoint mitigates issues of potential MTP by treatment regimen interaction.

3.3 Conclusions

Disease-free survival was significantly improved by the addition of MTP to adjuvant maintenance chemotherapy (p=0.0245; HR = 0.76, 95% CI: 0.58 to 0.98). DFS is a surrogate for overall survival, the gold standard in oncology studies. The addition of MTP to adjuvant combination chemotherapy in the treatment of resectable osteosarcoma without metastases results in a clinically meaningful and statistically significant increase in overall survival with a p-value of 0.0183 and a hazard ratio of 0.68 (95% CI: 0.49-0.95). At six years, the probability of survival when MTP is added to the standard of care is 77% compared to 66% without MTP, a clinically meaningful finding in a pediatric population where the longer the survival, the greater chance that the patient is cured of cancer.

4. SAFETY

The clinical safety of MTP is demonstrated in 9 Phase 1/2 studies and a large Phase 3 study with support from an extensive preclinical program. The results show that the most common adverse events associated with the use of MTP are mild to moderate, transient, and manageable events related to the expected immunostimulatory activity of MTP. There are rare, possibly-related events of greater clinical significance that may represent allergic reactions or an exaggeration of the immunostimulation associated with MTP use.

4.1 Methods of Assessment and Analysis

Seven hundred twenty one (721) subjects received MTP in clinical studies from 1986 to 1997. The safety database includes 580 patients who received at least one dose of MTP. US Phase 1/2 studies enrolled 248 subjects. For the Phase 3 study, 395 subjects were randomized to MTP treatment arms and 332 received MTP. Case report forms are available for the 248 subjects enrolled in the US Phase 1/2 and for all subjects enrolled in the Phase 3 study. An additional 141 subjects were treated with MTP in Phase 1/2 studies conducted in Europe. Only summary study reports are available for the EU studies so these subjects are not included in the safety database.

4.2 Phase 1/2 Studies

The Phase 1/2 studies characterize the single agent tolerability and safety of MTP. Single and multiple dose studies were conducted through a range of doses to identify the maximal tolerated dose (MTD) and the optimal biological dose.

4.2.1 Demographics

A total of 248 patients were treated in 9 studies under an Investigational New Drug application (IND) in the United States. These uncontrolled Phase 1/2 studies were conducted in patients with advanced malignancies. The median age of these 248 patients was 54, the mean daily dose of MTP was 1.9 mg/m^2, and the mean cumulative dose was 52.3 mg.

Two of the 9 Phase 1/2 studies were conducted in osteosarcoma patients (n=45). Six patients in the osteosarcoma studies were enrolled twice, i.e. they were allowed to be re-enrolled if they relapsed within a specified period and could be rendered clinically disease free prior to retreatment. These 6 patients were assigned a new registration number upon re-enrollment and are counted twice in the descriptions of adverse reactions below. In the osteosarcoma studies,

the median patient age was 17 and the average daily and cumulative doses for a complete treatment regimen were 2.1mg/m^2 and 91.8 mg, respectively.

Table 11: Baseline Demographic Characteristics; Phase 1/2 Studies

	Osteosarcoma Patients: Protocols 08 and 10 (N=51)	All Patients (N=248)
Gender		
Male	27 (53%)	153 (62%)
Female	24 (47%)	91 (37%)
Not Reported	0	4 (2%)
Total	51	248
Age (years)		
Mean (SD)	21.4 (11.4)	48.4 (18.4)
Median	17.0	54.0
Min, Max	9, 61	9, 81
Race		
White	44 (86%)	232 (94%)
Black	3 (6%)	6 (2%)
Other	4 (8%)	7 (3%)
Not Reported	0	3 (1%)

4.2.2 Exposure

MTP doses from 0.01 mg to 12 mg/m^2 were investigated as single and multiple injections in the Phase 1/2 studies. Exposure and dose intensity in the Phase 1/2 studies are shown in the following table (Table 12).

Table 12: MTP Exposure; All Phase 1/2 Studies and Phase 2 Osteosarcoma Studies

Cumulative Dose				
	0-50 mg n (%)	**51-100 mg n (%)**	**101-150 mg n (%)**	**>150 mg n (%)**
Phase 1/2 (n=248)	151 (61%)	52 (21%)	24 (10%)	21 (8%)
Osteosarcoma Protocols 8 & 10 (n=51)	10 (20%)	20 (39%)	14 (27%)	7 (14%)
Maximum Individual Dose*				
	< 0.5 mg/m^2	**0.5-< 2.0 mg/m^2**	**2.0-< 4.0 mg/m^2**	**≥ 4.0 mg/m^2**
Phase 1/2	50 (20%)	39 (16%)	85 (34%)	44 (18%)
Osteosarcoma Protocols 8 & 10	0	0	51 (100%)	0

Number of Doses Received (weekly or twice weekly)					
	1-3	**4-10**	**11-20**	**21-40**	**>40**
Phase 1/2 (n=248)	27 (11%)	98 (40%)	59 (24%)	59 (24%)	5 (2%)
Osteosarcoma Protocols 8 & 10 (n=51)	0 (0%)	7 (14%)	8 (16%)	33 (64%)	3 (6%)

*Thirty (30) patients received fixed doses of MTP and are not included in this analysis

4.2.3 Common Adverse Events

Adverse events were graded using the Common Toxicity Criteria (or its precursors; see Appendix 2) in the Phase 1/2 studies. All reported adverse events recorded on the Phase 1/2 case report forms (CRF) were entered by IDM into an integrated safety database and coded to Medical Dictionary for Regulatory Activities (MedDRA).

All patients in a Phase 1/2 study experienced at least one adverse event. One hundred one (41%) of the 248 patients reported at least one Grade 3 adverse event and 16 (6%) reported at least one Grade 4 adverse event. The most frequently reported adverse events are thought to be related to the biological activity of MTP. The majority of these events were reported as either mild or moderate in severity. This profile is consistent whether including all uncontrolled studies (n=248) or only those studies in osteosarcoma (n=51). The following table lists adverse events by System Organ Class, regardless of causality, including severe (Grade 3) or life-threatening events (Grade 4) reported in ≥10% of all patients in uncontrolled studies (n=248) and in parentheses in ≥ 10% of the patients in the two osteosarcoma uncontrolled studies (n=51). Events reported as Grade 3 or Grade 4 in severity are listed separately in the table. Adverse events

reported in more than 50% of the patients enrolled in a Phase 1/2 study or the Protocol 8 and 10 osteosarcoma studies are highlighted in the table below (Table 13).

Table 13: Adverse Events Reported by >10% of Patients: Total and Grade 3 or Grade 4; All Patients in Phase 1/2 Studies (Osteosarcoma Patients in Protocol 8 and 10)

System Organ Class Adverse Event	Total		Grade 3		Grade 4	
	N 248 (51)	%	N	%	N	%
Blood and Lymphatic System Disorders						
Anemia	24 (6)	10 (12)	3 (2)	1 (4)	1 (0)	0 (0)
Leucopenia	10 (10)	4 (20)	4 (4)	2 (8)	4 (4)	2 (8)
Thrombocytopenia	6 (5)	2 (10)	4 (3)	2 (6)	0 (0)	0 (0)
Granulocytopenia	5 (5)	2 (10)	1 (1)	0 (2)	3 (3)	1 (6)
Cardiac Disorders						
Tachycardia	125 (31)	50 (61)	2 (0)	1 (0)	1 (1)	0 (2)
Gastrointestinal Disorders						
Nausea	142 (37)	57 (73)	2 (1)	1 (2)	0 (0)	0 (0)
Vomiting	109 (32)	44 (63)	6 (2)	2 (4)	0 (0)	0 (0)
Constipation	43 (4)	17 (8)	5 (0)	2 (0)	0 (0)	0 (0)
Diarrhea	32 (8)	13 (16)	1 (0)	0 (0)	0 (0)	0 (0)
Abdominal pain	27 (7)	11 (14)	6 (2)	2 (4)	0 (0)	0 (0)
General Disorders and Administration Site Conditions						
Chills	220 (49)	89 (96)	26 (9)	10 (18)	2 (1)	1 (2)
Pyrexia	210 (50)	85 (98)	15 (3)	6 (6)	3 (1)	1 (2)
Fatigue	132 (44)	53 (86)	10 (3)	4 (6)	1 (0)	0 (0)
Hypothermia	56 (8)	23 (16)	0 (0)	0 (0)	0 (0)	0 (0)
Pain	36 (2)	15 (4)	4 (0)	2 (0)	0 (0)	0 (0)
Malaise	32 (5)	13 (10)	7 (0)	3 (0)	0 (0)	0 (0)
Asthenia	31 (2)	13 (4)	5 (0)	2 (0)	1 (0)	0 (0)
Chest pain	27 (6)	11 (12)	2 (1)	1 (2)	0 (0)	0 (0)
Chest discomfort	15 (5)	6 (10)	1 (1)	0 (2)	0 (0)	0 (0)
Catheter site pain	6 (5)	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)
Infections and Infestations						
Pharyngitis	6 (5)	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)
Injury, Poisoning and Procedural Complications						
Post procedural pain	20 (7)	8 (14)	0 (0)	0 (0)	0 (0)	0 (0)
Metabolism and Nutrition Disorders						
Anorexia	51 (10)	21 (20)	2 (0)	1 (0)	0 (0)	0 (0)
Musculoskeletal and Connective Tissue Disorders						
Myalgia	78 (39)	31 (76)	2 (1)	1 (2)	1 (0)	0 (0)
Back pain	37 (3)	15 (6)	5 (1)	2 (2)	0 (0)	0 (0)
Pain in extremity	29 (5)	12 (10)	8 (2)	3 (4)	1 (0)	0 (0)
Arthralgia	24 (7)	10 (14)	4 (0)	2 (0)	0 (0)	0 (0)
Shoulder pain	17 (5)	7 (10)	0 (0)	0 (0)	0 (0)	0 (0)
Nervous System Disorders						
Headache	124 (47)	50 (92)	12 (9)	5 (18)	0 (0)	0 (0)

Table 13: Adverse Events Reported by >10% of Patients: Total and Grade 3 or Grade 4; All Patients in Phase 1/2 Studies (Osteosarcoma Patients in Protocol 8 and 10)

System Organ Class Adverse Event	Total		Grade 3		Grade 4	
	N 248 (51)	%	N	%	N	%
Dizziness	43 (12)	17 (24)	1 (0)	0 (0)	0 (0)	0 (0)
Respiratory, Thoracic and Mediastinal Disorders						
Dyspnea	53 (8)	21 (16)	7 (0)	3 (0)	2 (1)	1 (2)
Cough	44 (14)	18 (27)	3 (0)	1 (0)	0 (0)	0 (0)
Tachypnea	32 (1)	13 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Nasal congestion	13 (10)	5 (20)	0 (0)	0 (0)	0 (0)	0 (0)
Pharyngolaryngeal pain	12 (6)	5 (12)	0 (0)	0 (0)	0 (0)	0 (0)
Skin and Subcutaneous Tissue Disorders						
Hyperhidrosis	27 (2)	11 (4)	1 (1)	0 (2)	0 (0)	0 (0)
Vascular Disorders						
Hypotension	73 (14)	29 (27)	6 (1)	2 (2)	1 (0)	0 (0)
Hypertension	65 (0)	26 (0)	2 (0)	1 (0)	0 (0)	0 (0)

4.2.4 Serious Adverse Events

Because the CRFs used in the Phase 1/2 studies did not capture which adverse events met the regulatory criteria for serious adverse events (SAE), the data were carefully reviewed by the sponsor to identify events that might meet the regulatory definition of an SAE (i.e. fatal, life-threatening, requiring medical or surgical intervention). The following criteria were used to identify events that could potentially represent SAE:

- Any Grade 4 adverse events
- Adverse events that lead to hospitalization or prolonged hospitalization,
- Adverse events with an outcome of death.

Using these criteria, 67 of the 248 patients in the Phase 1/2 studies had at least one potential SAE. The table below (Table 14) summarizes the events by System Organ Class that occurred in more than one patient.

Table 14: Potential Serious Adverse Events Occurring in >1 Patient; Phase 1/2 Studies and Osteosarcoma Studies

System Organ Class / Preferred Term	Osteosarcoma Protocols 08 and 10 N=51			All Patients N=248		
	N	%	Number of Events	N	%	Number of Events
Patients Reporting at Least One Serious Adverse Event	**14**	**27**	**75**	**67**	**27**	**307**
General Disorders And Administration Site Conditions	**6**	**12**	**17**	**32**	**13**	**78**
Pyrexia	5	10	9	15	6	27
Chills	3	6	3	11	4	16
Asthenia	0	0	0	10	4	11
Chest Pain	2	4	2	6	2	6
Fatigue	0	0	0	5	2	5
Edema	0	0	0	2	1	5
Pain	0	0	0	2	1	3
Respiratory, Thoracic And Mediastinal Disorders	**2**	**4**	**2**	**18**	**7**	**29**
Dyspnea	1	2	1	7	3	10
Dyspnea Exacerbated	0	0	0	3	1	5
Cough	0	0	0	2	1	2
Hemoptysis	0	0	0	2	1	2
Pleural Effusion	1	2	1	2	1	2
Gastrointestinal Disorders	**0**	**0**	**0**	**13**	**5**	**37**
Abdominal Pain	0	0	0	5	2	7
Abdominal Distension	0	0	0	4	2	7
Nausea	0	0	0	4	2	8
Vomiting	0	0	0	4	2	6
Constipation	0	0	0	2	1	2
Small Intestinal Obstruction	0	0	0	2	1	3
Infections And Infestations	**4**	**8**	**6**	**12**	**5**	**18**
Sepsis	2	4	3	4	2	6
Cellulitis	0	0	0	3	1	3
Catheter Site Infection	2	4	2	2	1	2
Vascular Disorders	**0**	**0**	**0**	**12**	**5**	**19**
Hypotension	0	0	0	7	3	12
Hemorrhage	0	0	0	2	1	2
Hypertension	0	0	0	2	1	3
Blood And Lymphatic System Disorders	**5**	**10**	**41**	**9**	**4**	**48**
Anemia	0	0	0	4	2	7
Leukopenia	4	8	19	4	2	19
Granulocytopenia	3	6	17	3	1	17
Neutropenia	2	4	5	2	1	5

Table 14: Potential Serious Adverse Events Occurring in >1 Patient; Phase 1/2 Studies and Osteosarcoma Studies

System Organ Class / Preferred Term	Osteosarcoma Protocols 08 and 10 N=51			All Patients N=248		
	N	%	Number of Events	N	%	Number of Events
Metabolism And Nutrition Disorders	**1**	**2**	**1**	**9**	**4**	**14**
Dehydration	0	0	0	5	2	7
Anorexia	0	0	0	2	1	2
Nervous System Disorders	**0**	**0**	**0**	**9**	**4**	**17**
Headache	0	0	0	2	1	2
Lethargy	0	0	0	2	1	3
Musculoskeletal And Connective Tissue Disorders	**2**	**4**	**3**	**8**	**3**	**12**
Arthralgia	0	0	0	2	1	2
Pain In Extremity	0	0	0	2	1	3
Cardiac Disorders	**2**	**4**	**2**	**6**	**2**	**7**
Tachycardia	1	2	1	5	2	5
Psychiatric Disorders	**0**	**0**	**0**	**6**	**2**	**9**
Confusional State	0	0	0	4	2	5
Anxiety	0	0	0	2	1	2
Renal And Urinary Disorders	**0**	**0**	**0**	**3**	**1**	**7**
Hematuria	0	0	0	2	1	4
Skin And Subcutaneous Tissue Disorders	**0**	**0**	**0**	**3**	**1**	**3**
Erythema	0	0	0	2	1	2

The relationship to MTP was considered unlikely by the investigator for the majority of events. However 20 of the 67 patients with potentially serious adverse events had at least 1 event considered to be possibly, probably, or definitely related to study drug. The most frequent serious events reported were dyspnea and hypotension. Because the Phase 1/2 studies were single agent dose finding studies, some of the serious adverse events occurred at doses that were higher than those studied in Phase 3.

4.2.5 Deaths

The Phase 1/2 studies primarily enrolled subjects with advanced malignancies who had failed all prior therapy. The majority of the participants in these studies were withdrawn because of disease progression and died shortly thereafter. No deaths were specifically attributed to MTP. Three deaths occurred during study participation. Two of the 3 deaths were reported to be

disease-related. The specific cause of the third death was not reported, however this was a 70 year old male patient with lung cancer (squamous cell) who after the 13th cycle of MTP was hospitalized with severe obstructive airway disease reported as anorexia, cough, exacerbated dyspnea, respiratory disorder, anxiety, and edema.

Seventeen (17) deaths were reported after the patient had completed Phase 1/2 study participation or had withdrawn from study. The cause of death was reported as progressive disease for 13 patients and as unknown for 4 other patients. One of the latter patients had anorexia, dyspnea, insomnia, bronchospasm, constipation, tachycardia, and anxiety all temporally associated with death. All of these adverse events were reported by the investigator as having a remote relationship to study drug. Another patient was noted to have a rapidly deteriorating performance status and general condition at the time of study withdrawal. The cause of death for a third was reported as uncertain, but was suspected to be doxorubicin toxicity. The cause of death for the fourth was unknown, but the patient was noted as being elderly and frail with multiple medical problems.

Of the 20 deaths described above for the 9 Phase 1/2 studies, 7 occurred in patients enrolled in the two Phase 2 osteosarcoma studies. Four of 5 deaths that occurred in protocol 08 were due to progressive disease and the fifth death was associated with the potential doxorubicin toxicity described above.

4.2.6 Other Significant Adverse Events

For the Phase 1/2 studies, other significant adverse events were defined as events that were considered the primary reason for the patient's termination from the study or adverse events that resulted in a change in MTP treatment such as dose discontinued, interrupted or changed.

Six patients were terminated from a Phase 1/2 study with a primary reason of adverse reaction. The adverse event(s) leading to termination are listed below along with the dose at the time of discontinuation

- rash (0.05mg/m²)
- myalgia, pyrexia, tachypnea (**4 mg/m²**)
- fatigue, edema, vomiting, chills, fever, dehydration (**6 mg/m²**)

- asthenia, hypotension, cough, dyspnea (**6 mg/m²**)
- hypotension, edema, cough, ↓ urine output, pain, asthenia, dyspnea, malaise (**4 mg/m²**)
- pyrexia, chest pain, pericardial and pleural effusions (2mg/m²)

Four of these six patients were receiving doses of MTP of 4-6 mg/m^2, at or above the maximal tolerated dose and below the dose eventually used in the Phase 3 study.

Adverse events that were reported to result in discontinuation from MTP dosing were reported in 32 patients. Eighteen adverse events in 14 patients were considered possibly, probably or definitely related to MTP by the investigator. The table below summarizes the events that were considered potentially related to MTP.

Table 15: Discontinuations Due to an Adverse Event; All Phase 1/2 Studies

Protocol and Patient ID	Adverse Event	Grade	Relationship	Treatment Required	Outcome
1-2	Maculopapular Rash Both Hands	1	Possible	Rx	Still Present
1-5	Rash	2	Definite	Rx	Recovered
2-7	Nausea	1	Possible	Rx	Still Present
	Fatigue	1	Possible	None	Still Present
3-1	Fatigue	2	Possible	None	Still Present
3-2	Nausea & Vomiting	1	Possible	Rx	Still Present
	Vomiting	1	Possible	Rx	Still Present
	Bilateral Hip Pain	2	Possible	Hosp.	Still Present
3-4	Fever Intermittent	2	Probable	OTC	Still Present
3-5	Fever	2	Possible	Hosp.	Still Present
3-7	Tachycardia	1	Possible	None	Still Present
3-13	Fatigue	2	Possible	None	Still Present
3-15	General Weakness	3	Possible	None	Recovered
3-26	Hemoptysis	2	Possible	Rx	Still Present
8-20	Increased Pain After Tx	3	Probable	Rx	Recovered
8-21	Back Pain	3	Possible	Rx	Still Present
10-303	Pericardial-Effusion	3	Probable	Hosp	Unchanged
	Pleural Effusion	2	Probable	Hosp	Unchanged

Most MTP attributed adverse events that were associated with discontinuation of therapy were low grade and have emerged from the safety data as part of the constellation of expected responses to the immune stimulation of MTP, including fever, chills, fatigue, nausea and vomiting, myalgia, arthralgia and tachycardia. In a few instances these were more severe than was typically reported. The report of pericardial/pleural effusion in a patient in protocol 10 (osteosarcoma; chemotherapy + MTP) is an uncommon event but may also be a consequence of immune/inflammatory stimulation. Although rash has not previously been recognized as part of the constellation of typical symptoms that result from immune stimulation with MTP, the occasional reports of this in association with MTP administration may be another manifestation of general immune and inflammatory responses.

Ten patients in the Phase 1/2 studies reported adverse events associated with MTP dosing modification (i.e., temporary suspension, dose decrease). These events were typically mild to moderate and similar to the constellation of expected responses to the immune stimulation by MTP including fever, chills, fatigue, headache, pain and weakness.

4.3 Phase 3 Study

4.3.1 Demographics

The Phase 3 study enrolled 793 children and young adults with osteosarcoma. Half of the subjects were randomized to treatment arms that did not include MTP. The original intent of INT-0133 was to enroll patients within 30 days of diagnosis who had resectable disease that was not metastatic. One of the Cooperative Groups also enrolled subjects with unresectable or metastatic disease; these were not planned to be included in the ITT data set. For the purposes of the integrated safety analysis, all Phase 3 subjects, including both the ITT and the metastatic patients were grouped together and separated by assignment to MTP or No MTP. Seven hundred eighty one patients received treatment with at least one drug (safety population) and 332 received treatment with MTP. The demographics of all Phase 3 patients are summarized in Table 16.

Table 16: Demographics; All Phase 3 Patients

	No MTP	MTP	Total
Age (years)			
N	398	395	793
Mean (SD)	13.8 (4.6)	13.9 (4.4)	13.9 (4.5)
Median	13.7	14.0	13.8
Range	4 – 30.6	1.4 – 30.4	1.4 – 30.6
Gender [N(%)]			
Male	213 (54%)	230 (58%)	443
Female	185 (46%)	165 (42%)	350
Race [N(%)]			
White	275 (69%)	251 (64%)	526
Hispanic	46 (12%)	58 (15%)	104
Black	55 (14%)	55 (14%)	110
Asian	5 (1%)	10 (3%)	15
Other	17 (4%)	21 (4%)	38
Weeks Since Diagnosis			
N	398	395	793
Mean (SD)	1.1 (0.9)	(0.8)	(0.8)
Median	0.9	0.9	0.9
Range	0 – 5.1	0 – 5.7	0 – 5.7

4.3.2 Exposure

In the Phase 3 study, the 2 mg/m^2 starting dose could be escalated twice by 1 mg increments until chills, fever or a rise in CRP was seen to assure that a biological effect was being induced. Only 34 subjects (10%) in the Phase 3 trial required dose escalation and many of these subjects had their dose reduced back to 2 mg/m^2 over time.

Exposure and dose intensity in the Phase 3 study are shown in the following table. 390 patients were randomized to receive and 332 received at least one dose. Exposure and dose intensity of the two Phase 2 osteosarcoma studies are shown for comparison

Table 17: MTP Exposure; Phase 3 Patients and Phase 1/2 Osteosarcoma Studies

Cumulative Dose				
	0-50 mg N (%)	**51-100 mg N (%)**	**101-150 mg N (%)**	**>150 mg N (%)**
Osteosarcoma Protocols 8 & 10 (n=51)	10 (20%)	20 (39%)	14 (27%)	7 (14%)
Phase 3 Patients (n=332)	39 (12%)	75 (22%)	115 (35%)	103 (31%)
Maximum Individual Dose				
	< 0.5mg/m^2	**0.5-< 2.0 mg/m^2**	**2.0-< 4.0 mg/m^2**	**≥ 4.0 mg/m^2**
Osteosarcoma Protocols 8 & 10	0	0	51 (100%)	0
Phase 3 (n=332)	0	0	332 (85%)	0

Number of Doses Received (weekly or twice weekly)					
	1-3	**4-10**	**11-20**	**21-40**	**>40**
Osteosarcoma Protocols 8 & 10 (n=51)	0 (0%)	7 (14%)	8 (16%)	33 (64%)	3 (6%)
Phase 3 (n=332)	9 (3%)	16 (6%)	18 (6%)	77 (26%)	212 (62%)

4.3.3 Adverse Events

4.3.3.1 Grade 3 and Grade 4 Adverse Events

Grade 1 and Grade 2 adverse events were not reported during the Phase 3 study. Only Grade 3 and Grade 4 adverse events were recorded and entered into the COG database. Grade 4 myelotoxicity was only to be reported as an adverse event if it caused a delay, decrease, or change in therapy.

To integrate the safety from the Phase 3 study into a common coding format, the Phase 3 Grade 3 and Grade 4 adverse events were recoded to preferred term and system organ classes based on MedDRA. The table below compares the Grade 3 and Grade 4 adverse events for MTP plus chemotherapy to the chemotherapy alone arms. Only adverse events occurring in more than 3% of the patients are included in the table (Table 18).

Table 18: Grade 3 or 4 Adverse Events Reported by ≥3% of Patients: Phase 3 Patients

System Organ Class / Preferred Term	No MTP (N=391)			MTP (N=390)			P-value
	N	%	Number Of Events	N	%	Number Of Events	
Patients Reporting at Least One Adverse Event	**346**	**88**	**2567**	**348**	**89**	**2541**	
Investigations	**308**	**79**	**1809**	**310**	**79**	**1702**	
Alanine Aminotransferase Increased	221	57	496	207	53	479	
Neutrophil Count Decreased	176	45	409	182	47	382	
Aspartate Aminotransferase Increased	139	36	246	136	35	252	
Platelet Count Decreased	109	28	193	112	29	197	
White Blood Cell Count Decreased	100	26	178	95	24	173	
Blood Bilirubin Increased	41	10	58	33	8	46	
Hemoglobin Decreased	32	8	48	39	10	47	
Blood Glucose Abnormal	33	8	52	20	5	26	0.087
Blood Potassium Abnormal	27	7	37	23	6	26	
Creatinine Renal Clearance Decreased	16	4	22	6	2	8	0.0492
Blood Magnesium Decreased	5	1	7	14	4	14	0.0389
Blood Pressure Diastolic Abnormal	10	3	13	4	1	5	
Gastrointestinal Disorders	**202**	**52**	**447**	**206**	**53**	**493**	
Stomatitis	174	45	305	172	44	328	
Vomiting	66	17	100	70	18	116	
Ileus	13	3	15	15	4	15	
Diarrhea	7	2	8	16	4	16	0.0599
Abdominal Pain	9	2	11	12	3	14	
Infections And Infestations	**94**	**24**	**152**	**85**	**22**	**152**	
Infection	92	24	148	84	22	149	
Ear And Labyrinth Disorders	**23**	**6**	**33**	**47**	**12**	**68**	
Deafness	23	6	33	47	12	68	0.0026
Skin And Subcutaneous Tissue Disorders	**24**	**6**	**27**	**19**	**5**	**25**	
Rash Generalised	18	5	20	15	4	18	
Nervous System Disorders	**19**	**5**	**25**	**21**	**5**	**25**	
Cerebellar Ataxia	12	3	12	10	3	13	
Psychiatric Disorders	**10**	**3**	**13**	**12**	**3**	**18**	
Depression	9	2	11	12	3	17	
General Disorders And Administration Site Conditions	**8**	**2**	**9**	**10**	**3**	**13**	
Pyrexia	8	2	9	10	3	13	

Grade 3 and Grade 4 adverse events in the Phase 3 study have also been assessed in subgroups. Analysis by dose is not particularly informative, since the cumulative dose is related to duration of treatment with MTP, which generally correlates with duration of treatment of chemotherapy, and most adverse events reported in this study were known effects of chemotherapy. The analyses by age, gender and race indicate that the Grade 3 and Grade 4 adverse events were consistent with those expected during treatment with high dose multiple agent chemotherapy, and were reported at similar rates regardless of subgroup and treatment assignment.

4.3.3.2 NCI Reportable Adverse Events

During the Phase 3 study investigators were instructed to report adverse events that were "Life threatening (Grade 4), or fatal unknown reactions" and "Grade 4 (except myelosuppression) and Grade 2 and Grade 3 unknown reactions" using an NCI Adverse Reaction Form.

Twenty seven patients (27) with 51 adverse events were reported to the NCI. Of the 27 patients, 26 were randomized to an MTP containing treatment group. Multiple events occurred in each of the 26 patients randomized to an MTP group, only some of which were attributed by the investigator to MTP. The following table reports on the 15 patients randomized to an MTP treatment group where at least one of the adverse events was considered to be possibly, probably, or definitely related to MTP.

Table 19: Events Reported to NCI as Definitely, Probably or Possibly Related to MTP

Pt ID Age/ Gender	Adverse Event	Grade	Relationship
19012	chest pain	3	Probable for MTP
	pain	3	Probable for MTP
60243	bronchospasm	2	Possible for MTP
	dyspnea	2	Possible for MTP
60621	constitutional symptoms (fever in the absence of neutropenia)	3	Definite for MTP
	musculoskeletal (arthritis)	2	Possible for MTP
60734	headache	0	Possible for MTP
	pain	0	Possible for MTP
	hypertension	1	Possible for MTP
	fever w/o neutropenia	2	Possible for MTP
61540	Cardiac arrhythmia	2	Probably related to anthracycline; possibly related to MTP
61388	convulsion	4	Probable for MTP
63109	abdominal pain	3	Probable for MTP
	pain	3	Probable for MTP
	vomiting	4	Probable for MTP
62321	Vomiting	3	Definitely related to methotrexate; possibly related to MTP
62523	Vomiting	3	Definitely related to methotrexate; possibly related to MTP
63283	headache	3	Probable for MTP
	Decreased neutrophils	4	Probably related to doxorubicin and methotrexate; possibly related to MTP
65464	myalgia	2	Definite for MTP
	pain	2	Definite for MTP
	visual disturbance	2	Probable for MTP
66676	urticaria	3	Probable for MTP
68081	chills	3	Definite for MTP
68129	pericardial effusion	2	Possibly related to MTP; possibly related to viral infection
			possible
97815	fever w/o neutropenia	1	probable

The patient randomized to a no-MTP arm had neurologic symptoms probably related to a concomitant medication, imipenem. Only one event was considered reportable to FDA in an IND safety report. After induction chemotherapy and surgery, a 16 year old male completed

maintenance course one with no Grade 3 or 4 toxicities noted. During maintenance course two and during infusion of the 20th doe of MTP, he experienced a Grade 4 seizure. EEG and MRI were normal and the patient continued treatment. During course 4 and several days following methotrexate and MTP, the patient experienced several grand mal seizures requiring intubation. EEG showed epileptic activity and dilantin was started. Protocol therapy was continued including MTP and the patient completed protocol therapy and entered the follow up phase. Although both seizure events were considered probably attributable to MTP, brain damage, including seizures, is a rare but recognized side effect of high dose methotrexate and were described as a potential side effect in the clinical protocol.

Most events were expected side effects of chemotherapy and/or MTP, though perhaps occasionally exaggerated in those patients receiving both agents.

4.3.3.3 Ototoxicity

Deafness was the only one of the coded Grade 3 or 4 adverse events reported in Table 18 that was considered potentially serious and clinically important. Table 20 compares that the uncoded Grade 3 or Grade 4 reports of hearing loss between the randomized treatment arms.

Table 20: Uncoded Grade 3 or 4 Adverse Events of Hearing Loss; Treatment Groups with MTP (Regimen A+ and Regimen B+) versus Treatment Groups without MTP (Regimen A and Regimen B) by Individual Treatment Arm; All Phase 3 Patients

	A N=203		A+ N=195		P-value	B N=195		B+ N=200		P-value
	N	%	N	%		N	%	N	%	
Hearing – Objective	8	3.9	27	13.8	0.0006	18	9.2	14	7.0	0.4638
Hearing – Subjective	1	0.5	10	5.1	0.0049	1	0.5	2	1.0	0.9999
Hearing – Objective/Subjective	9	4.4	37	19.0	<0.0001	19	9.7	16	8.0	0.5975

Ototoxicity was found to be significantly increased in Regimen A + MTP compared to Regimen A alone. There was no difference in ototoxicity in Regimen B with or without MTP. To explore this further, audiogram data and other reports of auditory deficit in the COG data were examined. No significant differences could be found using these expanded data sets in either the number of patients for whom hearing loss was reported or the medians of the maximum hearing loss in each group, compared either by study arm or MTP assignment.

Table 21: Ototoxicity Summary – Study INT-0133 (ITT Data Set)

Treatment Assignment	A (174)	A+ (167)	B (166)	B+ (171)	No MTP	MTP	p-value
Maximum hearing loss at 2,000 Hz (decibels)							
N	47	54	56	55	102	110	0.51*
Median (range)	15 (5,75)	25 (2,90)	15 (3,90)	20 (5,90)	15 (3,90)	20 (2,90)	0.11**
Significant Events – Auditory Deficit							
Auditory deficit	39 (22.4%)	48 (28.7%)	48 (28.9%)	47 (27.5%)	87 (25.6%)	95 (28.1%)	0.49*
Cumulative Cisplatin Dose by Individual Study Arm							
Mean (SD)	611.5 (207.7)	614.5 (242.0)	546.1 (286.1)	515.8 (264.2)			
Median	649.0	658.0	604.0	510.0			
Range	0.0 – 1116.0	0.0 – 1167.0	0.0 – 1065.0	0.0 – 1163.0			

* Fisher's Exact Test

** Wilcoxon Sum Rank Test

Since even a single dose of cisplatin is known to be associated with ototoxicity, exposure to cisplatin was evaluated. There was no statistical difference in the mean or median dose of cisplatin between groups.

COG defined significant events of auditory deficit were collected in the Phase 3 study separately from the Grade 3 and 4 toxicities. There were between 22% and 29% of patients reporting an auditory deficit among treatment groups and no difference between those receiving MTP and those not receiving MTP.

Objective assessment of Grade 3 or 4 toxicity as defined by >40 decibel (DB) hearing loss was observed in 16% of patients in Group A in combination with MTP versus 5-10% in the other treatment groups. The median maximum DB hearing loss was mild, at 15DB in the non-MTP groups and 20-25 DB in the MTP group. The reason for the higher rate of ototoxicity in the A+ group and not in the B+ group is not clear.

The dose of cisplatin administered was 120 mg/m^2 for 4 doses. This is more than twice the single dose of 50 mg/m^2 associated with ototoxicity in 30% of patients receiving a single dose of cisplatin. It is likely that the ototoxicity observed in this study is related to cisplatin and the observed higher incidence in the A+ group may be variability due to the small numbers of reports. There are no reports of ototoxicity in the single agent Phase 1/2 studies of MTP nor is

there any likely mechanism to explain an effect on the ear or on cisplatin's potential for ototoxicity.

4.3.4 Deaths

In the Phase 3 study, investigators were asked to report the main cause of death for patients based on the following categories: progressive disease, infection, hemorrhage, toxicity, graft-versus-host disease, operative complications, unrelated, or other. A total of 196 deaths were reported. One hundred sixteen deaths were reported for patients receiving chemotherapy without MTP and 84 deaths were in patients treated with MTP and chemotherapy. As shown in the following Table, 172 of the 196 deaths (88%) were due to progressive disease. In the remaining 24 cases, the main causes of death were other (8), infection (6), toxicity (3), operative complication (3), hemorrhage (2), unrelated (1), and missing (1). In patients randomized to MTP, there were four deaths due to infection, one unrelated accidental death and four deaths listed as other including AML. Two subjects died within 90 days of the last dose of MTP, in both cases due to septic shock.

Table 22: Deaths; All Phase 3 Patients

	Total (N=793)	No MTP (N=398)	MTP (N=395)
Number of Patients who Died	200	116	84
Progressive Disease	176	103	73
Infection	6	2	4
Hemorrhage	2	2	0
Toxicity	3	3	0
Operative Complication	3	2	1
Unrelated	1	0	1
Missing	1	0	1
Other	8	4	4

*p-value = 0.011

4.3.5 Discontinuations

Three hundred and seventy four (374) patients were withdrawn during the treatment phase, primarily due to progressive disease. It is COG's policy to continue to follow patients withdrawn from treatment for disease and survival status.

Table 23: Discontinuations; All Phase 3 Patients

	No MTP N (%)	MTP N (%)	Total N (%)
Total Enrolled	398	395	793
Total Treated	391	390	781
Number Withdrawn	181 (46)	193 (49)	374 (48)
Reason for Withdrawal:			
Progressive Disease	122 (31)	93 (24)	215 (27)
Removed for Toxicity	8 (2)	5 (1)	13 (2)
Withdrawal by Parent/Patient	27 (7)	66 (17)	93 (12)
Withdrawal by Physician	9 (2)	10 (3)	19 (2)
Major Protocol Break	15 (4)	19 (5)	34 (4)

More subjects were withdrawn by patient/parent in the MTP group compared to the no MTP group. This appeared to be due to unpleasant but not serious or life-threatening side effects of MTP.

Most of the withdrawals from the MTP arms by parent/patient occurred in the absence of significant toxicities. It was assumed that many parents withdrew subjects from MTP since it was experimental and contributed fevers, chills, nausea, fatigue and headache to the already severe side effects of chemotherapy.

4.3.6 Other significant adverse events

Thirteen patients were withdrawn from treatment by investigators due to toxicity of the regimen. Five patients who were withdrawn from the study due to toxicity were in MTP treatment arms. One of these patients was withdrawn during the induction phase of therapy and prior to starting MTP. Therefore, 4 patients were withdrawn from the study due to toxicity after receiving treatment with MTP. Although the specific toxicity leading to termination was not specified in the CRF, the following toxicities were temporally associated with study termination.

- During the last maintenance course, one patient (Regimen A+) had therapy stopped permanently due to unacceptable abdominal pain and nausea and vomiting.
- During the last maintenance course, one patient (Regimen B+) had therapy omitted due to unacceptable magnesium toxicity.

- One patient (Regimen B+) had therapy stopped permanently due to severe potassium toxicity during the third maintenance course.
- One patient (Regimen B+) had therapy stopped permanently due to unacceptable creatinine clearance during the fourth course of maintenance therapy.

These events are anticipated toxicities of the chemotherapy agents.

4.4 Rare Adverse Events in Phase 1/2 and Phase 3 Studies

A complete review of the adverse event database for all phases of investigation revealed rare events in several areas that may be related to the pharmacologic properties of MTP.

Serum sickness and anaphylaxis adverse events were reported in the Phase 3 study as Grade 3 or 4 adverse reactions. Six reports of serum sickness and two reports of anaphylactic reactions in a total of 7 patients were reported for MTP regimens compared with one serum sickness and four anaphylaxis reports for chemotherapy alone regimens. Two of the reports involving an MTP regimen occurred prior to initiation of MTP treatment. For the reactions that occurred during MTP treatment, one was attributed to methotrexate, one to Bactrim, two to MTP, and one was unattributed.

In the Phase 1/2 studies, 64 reports of mild to moderate rash or pruritus were noted when MTP was used alone. In the Phase 3 study, there was no difference in reports of Grade 3 or Grade 4 rash or exfoliative dermatitis between MTP or non-MTP arms and most of the reported rashes were attributed to methotrexate use.

It may be difficult to distinguish a true allergic reaction from the inflammatory stimulation by MTP. Rashes, alone or in combination with other symptoms, may be occasionally associated with MTP use.

Approximately 50% of Phase 1/2 subjects reported at least one respiratory symptom or sign including dyspnea, tachypnea, cough, nasal congestion, pharyngitis/ laryngitis, wheezing/ asthma, and occasional pleural effusion. Grade 3 or Grade 4 dyspnea was occasionally observed, especially in patients with extensive malignant disease in the chest. Two patients were removed from Phase 1/2 studies after experiencing severe dyspnea within 1.5-24 hours after MTP treatment. No Grade 3 or Grade 4 dyspnea or chest pain was reported in the Phase 3 study.

Occasional cases of pleural and pericardial effusions have been reported with the use of MTP, with or without chemotherapy. Some were attributed to infectious etiologies and at least one was malignant in origin.

The etiology of these pulmonary symptoms is not clear, but the association with the other pharmacologic effects of MTP, especially in subjects with extensive malignant disease in the chest, suggests that the constellation of chest discomfort, dyspnea, and cough is a rare but serious adverse events associated with the use of MTP.

4.5 Conclusions

MTP has a well characterized safety profile that is directly related to its pharmacologic effects. The optimal biological dose of MTP results in mild to moderate symptoms of cytokine release such as fever, chills, nausea, vomiting, fatigue, tachycardia, myalgia and headache as defined in the Phase 1/2 studies. More serious (Grade 3 or Grade 4), infrequent, but potentially MTP related adverse events occurring in the Phase 1/2 studies were hypotension and dyspnea. Because the Phase 1/2 studies were single agent dose finding studies, some of the serious adverse events occurred at doses that were higher than those studied in the Phase 3 study.

Individual dose selection in the Phase 3 study was based on a demonstration that fever, chills or an increase in CRP occurred within 24 hours of therapy starting. If no signs of biological activity were noted, the 2 mg/m^2 starting dose was increased in 1 mg increments to a maximum of 2 mg/m^2 + 2 mg MTP. Dose escalation was only needed in about 10% of subjects and many had their doses reduced during therapy. Only Grade 3 and Grade 4 adverse events and selected serious adverse events were routinely reported during the Phase 3 study. With the exception of ototoxicity and serum sickness, none of the Grade 3 and Grade 4 reporting imbalances between the MTP or non-MTP regimens were considered clinically important. Serious reportable adverse events that were potentially related to MTP were similar to those seen in the single agent Phase 1/2 studies.

The safety profile of MTP is well delineated and is very benign particularly when compared to that of other cancer chemotherapeutic agents.

5. OVERALL CLINICAL BENEFIT

Osteosarcoma is fatal in approximately a third of the children and young adults in whom it is diagnosed. This mortality rate has not changed in the two decades since the initial introduction of surgery and chemotherapy. The primary cause of death in treated patients is recurrent metastatic disease to the lung. The Phase 3 Study of MTP has demonstrated that the addition of MTP to the standard treatment regimen increases survival, reducing the mortality from approximately one third to one quarter at six years. This reduction in mortality translates into cure in this young population.

Pediatric oncology studies are designed with the expectation of observing cure. Because of the few concomitant non-cancer associated causes for mortality in this population, patients who live past the period of highest relapse risk are considered cured. Pediatric oncology studies frequently use survival models to make sure that the study is of sufficient size and duration to demonstrate a difference between arms before the Kaplan-Meier survival curves reach this plateau. The Gompertz survival distribution model was used to design the Phase 3 Study of MTP. The disease-free survival (DFS) and overall survival (OS) results track very closely to the expected distributions supporting the conclusion that the increase in survival is real.

The increase in survival is accompanied by a modest increase in mild to moderate adverse events. While these events do not represent a safety concern, they are relevant to the tolerability of MTP. Tolerability is important, particularly in a pediatric population already subjected to the toxicities associated with chemotherapy and the trauma of surgery. MTP was specifically developed to minimize adverse events and the commonly reported mild to moderate adverse events are transient and manageable.

There were no evident increases in Grade 3 and Grade 4 adverse events associated with the addition of MTP to cytotoxic chemotherapy. A careful examination of the adverse events reported in the Phase 1/2 and Phase 3 studies revealed a few rare, serious adverse events associated with MTP. These included dyspnea and chest pain, reported more frequently in patients with extensive metastatic pulmonary disease, and potentially allergic or anaphylactic events. These effects may have been true allergic responses or exaggerated immunostimulation. None of these events are as frequent or severe as the toxicities associated with chemotherapy. Physicians must weigh the potential for these events against the survival benefit of MTP.

The benefit risk assessment for treatments of fatal diseases often weighs months of increased survival against the potential for severe and serious adverse events that are sometimes themselves fatal. MTP is different in that it has the potential to provide long term survival and cure without the risk of an increased rate of severe or serious events against a background of standard surgery and high dose chemotherapy.

Liposomal MTP was intentionally designed to deliver the immunostimulatory activity of MTP to the tissue macrophages in the lung without unwanted systemic effects. The demonstration of a survival benefit by the Phase 3 study along with the favorable tolerability profile of MTP reflect the substantial clinical benefit of adding MTP to the treatment regimen for osteosarcoma.

6. REFERENCE

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Appendix 1: Chemotherapy Schema

	Week of Treatment																			
	Induction							Maintenance												
	0	3	4	5	8	9		12	15	16	17	20	21	22	25	26	27	30	31	32	35	38
Regimen A	CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX		CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX	DOXO x 72 hrs	MTX	MTX	DOXO x 72 h	MTX	MTX	-	-	-
Regimen A + MTP	CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX		CDDP DOXO x 72 h MTP	MTX MTP	MTX MTP	CDDP DOXO x 72 h MTP	MTX MTP	MTX MTP	DOXO x 72 hrs MTP	MTX MTP	MTX MTP	DOXO x 72 h MTP	MTX MTP	MTX MTP	MTP	MTP	MTP
Regimen B	IFOS x 5d DOXO x 72h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	Sur-gery	CDDP DOXO x 72 h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	CDDP	IFOS x 5d	CDDP
Regimen B + MTP	IFOS x 5d DOXO x 72h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX		CDDP DOXO x 72 h MTP	MTX MTP	MTX MTP	IFOS x 5d DOXO x 72h MTP	MTX MTP	MTX MTP	CDDP DOXO x 72 h MTP	MTX MTP	MTX MTP	IFOS x 5d DOXO x 72h MTP	MTX MTP	MTX MTP	CDDP MTP	IFOS x 5d MTP	CDDP MTP

Appendix 2: CCG Toxicity and Complications Criteria

The toxicity of MTP was assessed in the Phase 3 study using the CCG toxicity and complications criteria shown below.

Appendix 2: Children's Cancer Group Toxicity and Complications Criteria

Site	Measure	GRADE				
		0/WNL	**1 (Mild)**	**2 (Moderate)**	**3 (Severe)**	**4 (Unacceptable)**
A. Blood	1. WBC/µl	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	<1.0
	2. ANC/µl	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
	3. PLT/µl	WNL	75.0-normal	50.0-74.9	25.0-49.9	<25.0
	4. HGB g/dl	WNL	10.0-normal	8.0-10.0	6.5-7.9	<6.5
	5. LYMPHS/µl	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	<0.5
B. Marrow	1. CELLULARITY	normal	mildly hypo. 25%↓	mod. hypo. 50%↓	marked hypo. 75%↓ 3 wks to recovery	aplastic >3 wks to recovery
C. Liver	1. SGOT	WNL	≤2.5xN	2.6-5.0xN	5.1-20.0xN	>20.0xN
	2. SGPT	WNL	≤2.5xN	2.6-5.0xN	5.1-20.0xN	>20.0xN
	3. ALK PHOS-PHATASE	WNL	≤2.5xN	2.6-5.0xN	5.1-20.0xN	>20.0xN
	4. TOTAL BILI	WNL	--	<1.5xN	1.5-3.0xN	>3.0xN
	5. LIVER-CLIN.	WNL	--	--	precoma	hepatic coma
D. Pancreas	1. Amylase/Cr.Cl.	WNL	<1.5xN	1.5-2.0xN	2.1-5.0xN	>5.0xN
	2. Amylase	WNL	<1.5xN	1.5-2.0xN	2.1-5.0xN	>5.0xN
	3. Glu mg/dl	WNL	55-64/116-160	40-54/161-250	30-39/251-500	<30/>500/ketoacid
	4. Ultrasound size & sonolucency	normal normal	normal increased	increased incr. localized	increased incr. generalized	pseudocyst hermorrhagic
E. Renal and Genitourinary	1. BUN	<20	20 - 39	40 - 59	60 - 79	≥80
	2. Creatinine	WNL	<1.5xN	1.5-3.0xN	3.1-6.0xN	>6.0xN
	3. Creatinine Clearance	WNL	75%	50 - 74%	25-49	<25%
	4. Blood pressure-systolic	baseline	±10%	±20%	±30%	±40%
	5. Blood pressure-diastolic	baseline	±5%	±10%	±15%	±20%
	6. Proteinuria	neg	1+/or <3 g/l	2-3+/or 3-10 g/l	4+/or >10g/l	nephritic synd.
	7. Hematuria	neg	micro only	gross+clots	gross+clots	trans. req'd
	8. Bladder – frequency & dysuria	none	slight	moderate responses to Rx	severe, no response to Rx	Incapacitating with severe hemorrhage
F. Gastro-instestinal	1. Stomatitis	none	erythema, or mild soreness	painful/edema can eat	cannot eat or drink	requires parental or enteral support

Appendix 2: Children's Cancer Group Toxicity and Complications Criteria

Site	Measure	GRADE				
		0/WNL	**1 (Mild)**	**2 (Moderate)**	**3 (Severe)**	**4 (Unacceptable)**
	2. Abdominal pain: severity treatment	none --	mild not required	moderate required-helps	moderate-severe required-no help	severe hospitalization, heavy sedation
	3. Constipation	no chg	mild ileus	mod. ileus	severe ileus	ileus>96 hrs
	4. Diarrhea	none	↑2-3stools/day	↑4-6 stools/day or mod. cramps	↑7-9 stools.day or severe cramps	↑≥10 stools/day bloody, parenteral support required
F. Gastro-instestinal (cont.)	5. Nausea Vomiting	none none	reasonable intake 1x/day	decreased intake 2-5x/day	no sig. intake 6-10x/day	-- >10x/d or IV req'd
G. Pulmonary	1. Vital cap.	WNL	10 - 20%↓	21-35%	36-50%↓	>51%
	2. p AO2	>90	80 - 89	65-79	50-64	<49
	3. Functional	normal	tachypnea	dyspnea	O2 required	assist vent.
	4. DLCO	100-75%	74 - 65%	64-55%	54-40%	<40%
	5. Clinical	no chg	abn PFTs/asympt.	dyspnea on sig. exert.	dyspnea at N. active.	dyspnea at rest
H. Cardiac	1. Card. Rhythm	WNL	asympt./transient no Rx required	recur./persist. No Rx required	requires treatment	hypotens./V tach/ fibrillation
	2. Echo: %FS	>30	24-30	20-24	<20	--
	%STI	<0.35	--	<.40	>0.40	--
	3. -Ischemia	none	non-specific T-wave flattening	asymptomatic/EKG chg sugg ischemia	angina/without evidence of infarct.	acute myocardial infarction tamponade;
	-Pericard. Effusion	none	asympt. effusion no Rx required	pericarditis	drainage required	drainage urgently required
	4. Card. Function	WNL	asymptomatic/ ↓ej. Fr. <20	asymptomatic/ej. fr. <80% baseline	mild CHF/ responds to Rx	severe or refractory CHF
	5. hypertension	no chg	asympt./transient ↑20% no Rx req'd	recur./persist. ↑20%, no Rx req.	requires therapy	hypertensive crisis
	6. Hypotension	no chg	no Rx. req'd.	Rx but no hosp	Rx+ hosp. <48hrs after stop agent	Rx+ hosp. >48 hrs after stop agent
I. Nervous System	1. Peripheral: Sensory	no chg	mild paresthesias, loss tendonreflex	mod. sensory loss, mod. paresthesias	interferes with function	--
	Motor	no chg	subj. weakness/no obj. findings	mild obj.weakness/ no signif. impair	obj. weakness/ function impar	paralysis

Appendix 2: Children's Cancer Group Toxicity and Complications Criteria

Site	Measure	GRADE				
		0/WNL	**1 (Mild)**	**2 (Moderate)**	**3 (Severe)**	**4 (Unacceptable)**
	2. Central: Cerebellar	no chg	slight incoodination/ dysdiadokinesis	intention tremor/ dysmetria/ slurred speech/ nystagmus	locomotor ataxia	cerebellar necrosis
	CNS-general	no chg	drowsy/nervous	confused	seizures/psychosis	comatose
	-headache	no chg	mild	transient/mod/severe	severe, unrelenting	--
I. Nervous System (cont.)	Cortical	no chg	mild somnolence/ agitation	mod. somnolence/ agitation	severe somnolence/ agitation/ confusion/ hallucination	coma/seizures/ toxic psychosis
J. Skin	1. Skin	no chg or WNL	scattered eruption or erythema, asympt.	urticaria/scattered erupt, sympt.	generalized eruption, req. Rx	exfol/ulcer dermatisis
	Alopecia	no loss	mild hair loss	marked/total hair loss	--	--
K. Allergy		none	transient rash	mild bronchospasm	mod. bronchospasm, serum sickness	hypotension, anaphylaxis
L. Coagulation	1. Fibrinogen	WNL	0.99-0.75xN	0.74-0.50xN	0.49-0.25xN	≤0.24xN
	2. PT	WNL	1.01-1.25xN	1.26-1.50xN	1.51-2.00xN	>2.00xN
	3. PTT	WNL	1.01-1.66xN	1.67-2.33xN	2.34-3.00xN	>3.00xN
	4. hemorrhage (clin)	None	mild/no tranf	gross- 1-2 trans/episode	gross- 3-4 trans/ episode	massive->4 trans/ episode
M. Hearing	1. Objective	no chg	20-40db loss >4Khz	>40db loss >4 Khz	>40db loss <2 Khz	>40db loss<2 Khz
	2. Subjective	no chg	loss of audiometry only	tinnitus, soft speech	loss correctable with hearing aide	deafness not correctable
N. Electrolytes	1. Na mEq/l	WNL	↓130-134/ ↑146-149	125-129/ 150-155	116-124/ 156-164	<115/ >165
	2. K mEq/l	WNL	↓3.1-3.4/ ↑5.5-5.9	2.6-3.0/ 6.0-6.4	2.1-2.5/ 6.5-6.9	<2.0/ >7.0
	3. Ca mg/dl	WNL	8.4-7.8/ 10.6-11.5	7.7-7.0/ 11.6-12.5	6.9-6.1/ 12.6-13.5	≤6.1/ ≥13.5
	4. Mg mEq/l	WNL	1.4-1.2	1.1-0.9	0.8-.06	≤0.5
O infection		none	mild	moderate	severe	life threatening
P. Fever		<38°C	38°-40°C	>40°C <24hrs	>40°C>24 hrs	--
Q. Local		none	pain	pain/ swelling with inflammation/	ulceration	plastic surgery indicated

Appendix 2: Children's Cancer Group Toxicity and Complications Criteria

Site	Measure	GRADE				
		0/WNL	1 (Mild)	2 (Moderate)	3 (Severe)	4 (Unacceptable)
				phlebitis		
R. Mood		no chg	mild anxiety or depression	moderate anxiety or depression	severe anxiety of depression	suicidal ideation
S. Vision		no chg	--	--	subtotal vision loss	blindness
T. Weight Change		<5.0%	5.0-9.9%	10-19.9%	≥20%	--
U. Performance (Karnofsky %)		normal (90-100)	mild restriction (70-<90)	ambulatory up 50% (50-<70)	bed or wheelchair (30-<50)	no self care (<30)

There were two toxicity scales used during the Phase 1/2 studies of MTP. These include "Recommendations for Grading of Acute and Subacute Toxicity", which was appended to the earliest clinical studies conducted by Ciba-Geigy and the "Common Toxicity Criteria" appended to later Phase 2 clinical studies. These appear to reflect the evolution of the grading of adverse events in clinical trials in oncology during this time frame.

These two toxicity scales are compared to the Phase 3 criteria below.

CCG Phase III Criteria	Early CBG	Common Toxicity Criteria
Blood		**Leukopenia/Thrombocytopenia/Anemia**
WBC	same*	same
ANC	same	same
PLT	same	same
HGB	same	same
Lymphs	not included**	same
Marrow		
Cellularity	not included	not included
Liver		**Liver**
SGOT	absolute values instead of relative	same
SGPT	absolute values instead of relative	same
Alk Phos	absolute values instead of relative	same
Total Bili	'looser' scale	same
Liver/Clin	same	same
Pancreas		**Metabolic**
Amylase	not included	same
Glu	not included	similar but hypo/hyperglycemia

CCG Phase III Criteria	Early CBG	Common Toxicity Criteria
Ultrasound – size and sonolucency	not included	not included
Renal & Genitourinary		**GU**
BUN	not included	not included
Creatinine	same	same
Cr Cl	not included	not included
Blood press/systolic	not included	not included
Blood press/diastolic	not included	not included
Proteinuria	same	same
Hematuria	same	same
Bladder freq & dysuria	not included	not included
Gastrointestinal		**Gastrointestinal**
Stomatitis	= oral	same
Abdominal pain	treatment related pain	not included
Constipation	gen'l – not ileus or neuroconstipation	same as neuroconstipation/ileus
Diarrhea	same	same
Nausea	incl with vomiting	same
Vomiting	similar	same
Pulmonary		**Pulmonary**
Vital cap.	not included	not included
pAO2	not included	not included
Functional	not included	not included
DLCO	not included	not included
Clinical	= pulmonary	= pulmonary
Cardiac		**Cardiac**
Card. Rhythm	different scale descriptions	same
Echo	not included	not included
Ischemia	not included	same
Pericard. Effusion	not included	same
Card. Function	same	same
Hypertension	not included	same
Hypotension	not included	same
Nervous System		**Neurologic**
Peripher/sensory	combined with motor	same
Peripher/motor	combined with sensory	same
Central: cerebellar	not included	same
Central: CNS gen'l	not included	not included
Central: CNS headache	treatment related pain	same
Central:cortical	= state of consciousness	same
Skin		**Skin**
Skin	= cutaneous	same
Alopecia	not included	same

CCG Phase III Criteria	Early CBG	Common Toxicity Criteria
Allergy		**Allergy**
Allergy	same	same
Coagulation		**Coagulation**
Fibrinogen	not included	same
PT	not included	same
PTT	not included	same
Hemorrhage (Clin)	same	same
Hearing		**Neurologic**
Objective	not included	not included
Subjective	same	same
Electrolytes		**Metabolic**
Na	not included	not included
K	not included	not included
Ca	not included	= hyper/hypocalcemia/same scale
Mg (hypo)	not included	= hypomagnesemia/same scale
Infection		**Infection (ECOG specific)**
Infection	same	same
Fever		**Fever in Absence of Infection**
Fever	tighter scale (by one grade)	tighter scale (by one grade)
Local		**Local**
Local	not included	same
Mood		**Neurologic (mood)**
Mood	not included	similar
Vision		**Neurologic (vision)**
Vision	not included	same
Weight Chg		**Weight Gain/Loss**
Weight Change	not included	same
Performance		
Performance (Karnofsky %)	not included	not included

*same indicates that there is no difference between this scale and the CCG Phase 3 scale for this measure; where the measure is included but named or assessed differently, the difference is noted

** not included indicates that the particular CCG measure was not included in this