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**STUDY TITLE:**

The Effect of Muramyl Tripeptide Phosphatidyl-Ethanolamine on the Durability and Function of Orthopaedic Limb Reconstructions in Patients with Osteogenic Sarcoma Undergoing Limb Salvage Surgery

**BACKGROUND:**

A recent meeting of the National Institute of Health, Orthopaedic Research and Education Foundation, and the American Academy of Orthopaedic Surgeons concluded that one of the most important research priorities for the field of musculoskeletal oncology was the determination of the effect of muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE) on the durability of orthopedic limb reconstructions.<sup>1</sup>

High grade osteosarcoma is the most common primary malignancy of bone, and in most instances treatment involves chemotherapy, surgical resection, and a limb-preserving reconstruction. Two recently published studies by the Children's Oncology Group (COG) demonstrated that the five-year patient survival rate for osteogenic sarcoma can be increased by the addition of MTP-PE to a standard regimen of cisplatin, high-dose methotrexate, and doxorubicin. There was a statistically significant 30% reduction in mortality for patients presenting with localized disease and a reduction in mortality approaching statistical significance for patients presenting with disseminated disease.<sup>2,3</sup> MTP-PE, a synthetic lipophilic analog of muramyl dipeptide (a component of the cell wall of *Bacille Calmette-Guerin*), functions by activating macrophages to a tumoricidal state, a phenomenon caused by and/or associated with increased levels of IL-1, IL-6, and TNF- $\alpha$  cytokine production.<sup>4</sup>

Interestingly, macrophage activation has also clearly been implicated as a causative factor in loosening of endoprosthetic reconstructions. Polyethylene particle debris generated at the joint is ingested by macrophages and results in their activation, which is associated with production of characteristic cytokines, including IL-1, IL-6, and TNF- $\alpha$  (a similar profile of cytokines produced by MTP-PE). Macrophage activation results in bone resorption (i.e., osteolysis), which can cause periprosthetic bone loss and prosthetic loosening.<sup>5</sup> This pathway and the resultant osteolysis-associated loosening is widely believed to be the most common method of failure of most joint reconstructions.<sup>6</sup>

The activation of macrophages induced by MTP-PE naturally raises the question of whether MTP-PE, although improving oncologic outcome, adversely effects the durability of orthopaedic reconstructions. If this is indeed the case, then secondary measures to prevent implant loosening can potentially be utilized, such as antiinflammatory medications and antiresorptive agents like bisphosphonates or denosumab. Alternative methods of prosthetic fixation could also be considered for the patient. Finally, the orthopedic oncologist could better counsel patients about the projected durability of the reconstruction.

**NULL-HYPOTHESIS:**

There is no difference in survival rates of pure endoprosthetic, allograft-prosthetic, or pure structural allograft reconstructions between patients that received MTP-PE at the time of resumption of chemotherapy after limb-sparing surgery for extremity osteosarcoma and those that did not receive MTP-PE.

## STUDY DESIGN:

This study will analyze prosthetic and allograft results from the Intergroup-0133 prospective, randomized controlled trial on MTP-PE performed by the COG.<sup>2,3</sup>

## RATIONALE:

The study group is the largest ever treated for osteogenic sarcoma. The randomized, controlled trial design minimizes the differences between the treatment arms. The number of osteosarcoma patients in the United States is insufficient to perform an independent, single-institution orthopaedic trial. Therefore, it is most appropriate to use the same chemotherapy cohort to evaluate the orthopaedic outcomes and determine how multidisciplinary treatment influences prosthetic and allograft survivorship.

## SUPPORTING PRELIMINARY DATA:

MTP-PE has been shown to induce macrophage activation and specifically result in the elaboration of various cytokines from macrophages, including IL-1, IL-6, and TNF- $\alpha$ <sup>4</sup>. Osteolysis and subsequent loosening around prosthetic implants has been clearly shown to involve activation of macrophages and cytokine elaboration including IL-1, IL-6, and TNF- $\alpha$ <sup>5</sup>. The high degree of similarity in these pathways of macrophage activation lends strong credence to the hypothesis that MTP-PE administration can result in osteolysis and subsequent loosening around implants. Similar theoretical concerns exist for failure of allograft reconstructions secondary to resorption, non-union, or fracture; for example, overexpression of TNF- $\alpha$  secondary to inflammation has been shown to increase bone resorption and result in bone loss<sup>7-11</sup> and is also known to inhibit bone-forming activities such as production of bone matrix proteins and alkaline phosphatase, osteoblast differentiation, and mineralization of osteoblast nodules in vitro<sup>12,13</sup>.

## STUDY OBJECTIVE:

We will test the hypotheses that 1) MTP-PE as used in Intergroup-0133 adversely effects the durability and function of endoprosthetic and/or allograft-prosthetic reconstructions in patients undergoing limb salvage surgery and 2) MTP-PE adversely effects the durability and function of pure allograft reconstructions.

## ELIGIBILITY/EXCLUSION CRITERIA:

Patients eligible for this study will be all participants in the Intergroup-0133 study treated from 1993 to 1997, which was a randomized, controlled trial involving 752 patients looking at the oncologic effect of MTP-PE as an additional chemotherapeutic agent. Any patient undergoing amputation will be excluded from this series.

## STUDY OUTLINE:

We will obtain all appropriate clinical and radiographic documents of participants from the Children's Oncology Group and the respective institutions and review and record the necessary data.

The primary outcome measure for this study will be clinical or radiographic failure of the reconstruction. Clinical failure will be defined as revision for any reason. Radiographic failure will be defined as definite loosening (indicated by implant migration or clear progression of radiolucent lines), periprosthetic fracture, implant fracture, massive osteolysis, massive resorption of allograft, allograft non-union, and allograft fracture. Secondary outcome measures will include Musculoskeletal Tumor Society Score at time of last follow-up or at time of implant failure, submassive osteolysis,

submassive allograft resorption, delayed allograft union, and development of deep or superficial infection at the reconstruction site.

Patients will be divided into groups based upon anatomic site of reconstruction. Knee reconstructions (i.e., distal femur and proximal tibia) will be regarded as one group, and upper extremity reconstructions will be regarded as a second group. All other reconstructions will be regarded as a third group. We will assess for differences in the primary and secondary outcomes measures between the MTP-treated and untreated patients among the entire collection of patients and also within the various anatomic groups.

#### PRELIMINARY STATISTICS:

Based on extrapolation of existing data,<sup>14-18</sup> we surmise that the 10-year failure rate of all endoprosthetic, allograft-prosthetic, and allograft reconstructions in patients treated and patients not treated with MTP-PE is 45% and 15%, respectively. A (two-sided) chi-squared analysis using  $\alpha = .05$  and  $\beta = 0.20$  reveals that the study will require at least 43 patients treated with MTP-PE and 43 patients not treated with MTP-PE. Assuming that approximately 30% of patients will not survive 10 years (e.g. due to disease), and about 10% will have an amputation (and thus be excluded from the study), approximately 136 total patients will be required for the most basic analysis (i.e., grouping all anatomic sites of reconstructions together). If the data is to be analyzed based on anatomic site, a larger number of subjects will be required. If, for example, enough patients must be accrued to compare MTP-treated and untreated patients with knee reconstructions specifically, then, assuming that 50% of osteosarcomas will occur at the knee, a total of 272 patients must be included in the study. This will be the preferred method of analysis for this study. Since the Intergroup 0133 study included 752 patients, there should be ample power to answer the study questions.

#### FEASIBILITY:

All patients were consented for treatment and follow-up and their study involvement approved by the local institutional review board (IRB) as part of their participation in Intergroup-0133.

In order to maximize power, patients with similar operations (e.g. knee reconstructions) will be regarded as one group (with MTP-treated and untreated patients within the group being compared to one another). Since these groups were not the basis of the initial randomization of patients in the COG study, the groups defined in this study may not be entirely comparable. However, recent studies have shown analogous prosthesis survival among distal femoral and proximal tibial reconstructions,<sup>19,20</sup> making the grouping clinically reasonable.

## References

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