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Blockade of IL-10 Signaling Ensures Mifamurtide Efficacy in Metastatic Osteosarcoma

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Simple Summary: Osteosarcoma is a highly aggressive and metastasizing primary bone neoplasm with poor patient survival rates. Mifamurtide is an immunostimulant drug whose clinical efficacy is still debated. Here we identified IL-10 as a new possible target useful to improve mifamurtide effectiveness on metastatic OS. Indeed, we demonstrated that in patients, high levels of IL-10 correlate with mortality. Moreover, the use of anti-IL-10 antibodies causes a significantly increased mortality rate in highest-grade OS cells and lower formation of lung metastases in an in vivo mouse model. These data suggest a possible clinical application of anti-IL-10 antibody and mifamurtide combined treatment as an effective approach for the treatment of metastatic osteosarcomas.

Abstract: Osteosarcoma (OS) is the most common primary malignancy of the bone, highly aggressive and metastasizing, and it mainly affects children and adolescents. The current standard of care for OS is a combination of surgery and chemotherapy. However, these treatment options are not always successful, especially in cases of metastatic or recurrent osteosarcomas. For this reason, research into new therapeutic strategies is currently underway, and immunotherapies have received considerable attention. Mifamurtide stands out among the most studied immunostimulant drugs; nevertheless, there are very conflicting opinions on its therapeutic efficacy. Here, we aimed to investigate mifamurtide efficacy through in vitro and in vivo experiments. Our results led us to identify a new possible target useful to improve mifamurtide effectiveness on metastatic OS: the cytokine interleukin-10 (IL-10). We provide experimental evidence that the synergic use of an anti-IL-10 antibody in combination with mifamurtide causes a significantly increased mortality rate in highest-grade OS cells and lower metastasis in an in vivo model compared with mifamurtide alone. Overall, our data suggest that mifamurtide in combination with an anti-IL-10 antibody could be proposed as a new treatment protocol to be studied to improve the outcomes of OS patients.

Keywords: osteosarcoma; macrophages; immunotherapy; cytokines; mifamurtide

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1. Introduction

OS, also known as "osteogenic sarcoma", is a highly aggressive and metastasizing primary bone neoplasm derived from malignant mesenchymal cells producing an immature

osteoid matrix [1]. It is the most common cancer diagnosed in children and adolescents [2], and it represents 20–40% of all bone tumors [3]. The incidence of OS at diagnosis is 40% higher for males than females [4].

OS differs greatly in its biology and clinical behavior; indeed, patients' survival rates at 5 and 10 years from diagnosis differ significantly depending on the specific tumor subtype [5]. The incidence of OS is common in the metaphysis of long tubular bones but rare in the spine, pelvis, and sacrum areas [6]. OS has a high tendency to metastasize; the most common sites are the lungs, followed by the bones and, occasionally, the lymph nodes. Some patients develop micrometastases, which cannot be accurately detected by the currently available diagnostic methods. It is well known that the presence of metastases in OS patients is associated with poor prognosis, and it is the primary complexity of tumor therapy [7].

The etiology of OS has not yet been fully clarified; however, some risk factors have been identified and some hypotheses have been formulated on its molecular causes [8–12]. The heterogeneity of OS and the limited understanding of its pathogenesis at present make the diagnosis and therapy really challenging. Currently, the available therapies include intensive multiagent chemotherapy, surgery, and radiation, but often they do not show the expected therapeutic effects, so this points out a real and critical necessity to improve innovative therapeutic approaches, such as immune modulation strategies.

Among immune stimulatory agents, mifamurtide was indicated as one of the most promising for OS treatment. Mifamurtide (muramyl tripeptide ethanolamine (MTP-PE)), a synthetic derivative of muramyl dipeptide (MDP), is the smallest naturally occurring immune stimulatory component of bacterial cell walls [13,14]. Mifamurtide and MDP both stimulate immune responses by binding the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), an intracellular pattern-recognition receptor expressed principally in monocytes, macrophages, and dendritic cells. Mifamurtide activates the nuclear factor (NF)-kB pathway by binding NOD2, which drives an increase in proinflammatory cytokine production (IL-1, IL-6, IL-12, TNF-α) and other serum indicators of immune stimulation (neopterin and C-reactive protein) [15]. Through this pathway, mifamurtide activates macrophages that can target and destroy tumor cells. However, the clinical efficacy of mifamurtide treatment is still debated.

Here, we investigated mifamurtide efficacy in three OS cell lines with increasing features of malignancy through in vitro and in vivo experiments to experimentally test its efficacy and identify new strategies to improve its action.

2. Materials and Methods

2.1. Study Design

In vivo experiments followed the ARRIVE guidelines and included at least 4 mice per group. All experiments and sample collections were carried out according to the European Union (EU) guidelines for animal care procedures and the Italian legislation (DLgs 26/2014) application of the EU Directive 2010/63/EU (Europe). Experimental animal procedures were approved by the Italian ethical committee of the Animal Welfare Office of the Italian Work Ministry (authorization 796/2021-PR). Mice were housed in a temperature- and humidity-controlled vivarium (12 h dark/light cycle, free access to food and water), and 1 h before the in vivo experiments, mice were acclimatized to the experimental RT.

2.2. Cell Culture

MG-63 (CRL-1427), HOS (CRL-1543), and 143B (CRL-8303) human OS cell lines (from (ATCC) were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% Fetal Bovine Serum (FBS), 2 mM L-glutamine, 100 $U \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin. To obtain the 143B complete growth medium, 0.015 mg/mL of 5-bromo-2′-deoxyuridine was added. K7M2 murine OS cells (ATCC, CRL-2836) were cultured in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 $U \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin and were maintained in culture plates at 37 °C in a water-