TGF-β as a Promising Therapeutic Target for Treating Osteosarcoma
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Abstract

Osteosarcoma (OS) is an aggressive malignant primary bone cancer with a high propensity for lung metastasis. Originating from primitive mesenchymal bone-forming cells in the long bones, OS represents the most prevalent bone cancers affecting children and adolescent and young adults (AYA), with ~400-600 cases a year in the United States. Despite aggressive chemotherapy and surgery, the outcome for metastatic OS remains dismal and the overall survival has not improved significantly over the past 3 decades. A high proportion of OS patients develop metastatic disease either at the time of diagnosis (20%) or after initiation of multimodal therapy (in ~30% of patients). The lung accounts for ~80% of all OS metastatic sites. Unfortunately, almost all of the patients who develop surgically unresectable pulmonary metastatic OS (pOS) invariably succumb to this devastating disease. Therefore, OS represents a disease with urgent unmet needs. As OS contains extremely complex and heterogeneous chromosomal and genetic alterations, recent advances in molecular precision medicine or gene replacement therapy approaches to target OS-specific mutations will likely be challenging. Immunotherapy offers a potential new therapy option for treating pOS. TGF-β is one of the most potent immune suppressive molecules produced by OS cells, fibroblasts and immune cells in the OS tumor microenvironment (TME). TGF-β also conditions OS TME through the recruitment and induction of immune suppressive myeloid cells and regulatory T cells, each known to dynamically suppress the function of tumor-reactive cytotoxic T cells and NK cells. Therefore, TGF-β constitutes a major impediment to both active and passive immunotherapies in solid tumors. In OS, TGF-β expression increases in the sera of patients compared to those of healthy donors. This increase in TGF-β production is correlated with high-grade OS and associated with the presence of lung metastases. Here we describe the therapeutic potential of small molecule inhibitor of TGF-β type I receptor, TEW-7197 (Vactosertib) in OS treatment. Varying doses of TEW-7197 (0.1-50 μM) inhibited the cellular proliferation of mOS (K7M2, mOS482, mOS493) at various time points using IncuCyte. Furthermore, following TEW-7197 treatment of mOS cell lines phosphorylations of Smad2 were inhibited in a dose dependent fashion. Metastasis marker, p-Ezrin and proto-oncogene, c-Myc expressions were also dose dependently inhibited by TEW-7197 treatment. Taken together, these data demonstrate that TEW-7197 has direct anti-tumor effects in OS and has the potential to ameliorate the progression of OS in vivo. However, the latter may involve both tumor-intrinsic effects described above as well as indirect effects mediated through disruption of TGF-β responses in stromal and immune cell populations in the TME.

Results

Figure 1. Chemical structure of TEW-7197 (Vactosertib), a small molecule inhibitor of TGF-β Type I receptor.

Figure 2. TEW-7197 inhibits TGF-β signaling pathway in osteosarcoma cells. TEW-7197 were treated in A) K7, K7M2, B) mOS482, mOS493, mOS482 and mOS493 cells 15 minutes before TGF-β (5 ng/ml) treatment. 1 hour after TGF-β treatment, cells were harvested and p-Smad2, Smad2 and β-actin expressions were measured by Western blot analysis.

Figure 3. The effects of TEW-7197 on osteosarcoma cell proliferation. Various doses of TEW-7197 (10 nM – 1 μM) were incubated with A) K7M2, B) mOS482, C) mOS483 and D) mOS483 and phase-contrast images were obtained every 12 hours using IncuCyte live-Cell Imaging System. After 92 hours, cell proliferation was quantified using phase object confluance.

Figure 4. The effect of TEW-7197 on proto-oncogene expressions in osteosarcoma cells. A) TEW-7197 (10-100 nM) were treated in K7M2 cells 15 minutes before TGF-β (5ng/ml) treatment. Cells were harvested 1 hour after TGF-β treatment, p-Smad2, Smad2 and β-actin expressions were measured by Western blot analysis. B) TEW-7197 (10 nM -10μM) were incubated in K7M2 cells for 24 hours and measured the c-Myc, p-Akt, p-38, p-Ezrin and β-actin by Western blot analysis.

Conclusion

• TEW-7197 inhibits TGF-β signaling in various osteosarcoma cell lines.
• TEW-7197 dose dependently suppresses various osteosarcoma cell proliferation.
• TEW-7197 can inhibit c-Myc and p-Ezrin expressions in K7M2 cells.
• TEW-7197 has direct anti-tumor effects in OS.

Future Plan

• TEW-7197 has the potential to ameliorate the progression of OS in vivo. However, it may involve both tumor intrinsic effects as well as indirect effects mediated through disruption of TGF-β responses in stromal and immune cell populations in the tumor microenvironment.
• Therefore, we will investigate the efficacy of TEW-7197 in enhancing lymphoid and myeloid activation within OS tumor microenvironment as a single drug alone, and its potential synergistic effect with anti-PD-1/L1 or anti-PD-1 inhibitors in preclinical immune-competent murine OS model systems.

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Goal/Hypothesis

• The primary hypothesis for this study is that TGF-β inhibition can be an effective immunotherapy against pOS through a multi-pronged approach that addresses tumor-intrinsic and extrinsic immune-related factors (e.g. modulating the myeloid and cytotoxic milieu in the tissue-specific TME) to achieve optimal conditions for superior immune-effector function and maximal clinical response in pOS
• Our objective is to demonstrate that the dramatic inhibition of CTL and NK cell cytotoxic activity occurring upon prolonged exposure to TGF-β in the tumor microenvironment is reversed by treatment with a TGF-β inhibitor.
• We propose to demonstrate efficacy of orally bioavailable small molecule inhibitor of TGF-β type I receptor, TEW-7197 (Vactosertib) from Medpacto in enhancing CTL and NK cell activity in single drug and its synergistic effect with anti-PDL-1 or anti-PDL-1 inhibitors in preclinical immune-competent murine OS model systems.

Scheme: Immune suppression by OS. TGF-β production by OS, including that associated with releases exosomes, induces immune suppressive Treg and myeloid derived suppressor (MDSC) cells, and provide a tolerogenic dendritic cells (DC) phenotype.