Oral TGF-beta receptor1 inhibitor, Vactosertib promotes osteosarcoma regression by targeting tumor proliferation and enhancing anti-tumor immunity

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Abstract

Osteosarcoma (OS) is an aggressive malignant bone cancer, with the lung as the most frequent site of metastasis. Unresectable pulmonary metastasis remains a significant challenge with a survival rate of less than 20%. Identification of novel therapeutic strategies are desperately needed. Transforming growth factor-β1 (TGF-β) is a potent immune suppressive cytokine in OS tumor microenvironment (TME). TGF-β1 expression is increased in the sera and tumor tissues of OS patients and this increase is associated with high-grade OS and lung metastases. Therefore, blocking TGF-β1 signaling may be a novel therapy for OS treatment. In this study, we show that blocking TGF-β1 signaling using the orally bioavailable small molecule TGF-βR1 inhibitor, Vactosertib, significantly inhibited OS proliferation in vitro and in vivo. Notably, Vactosertib inhibits c-Myc expression in the OS cells and oral administration of Vactosertib significantly reduces OS growth in vivo. Vactosertib increased immune effectors (e.g., IFNγ+CD8+cells and NK cells) and inhibited immune suppressors (e.g., M2-like TAM, MDSC) in the OS TME. Our results suggest that inhibition of TGF-β1 signaling is an effective therapeutic strategy against OS through a multi-pronged approach that targets tumor intrinsic and extrinsic factors to achieve optimal immune-effector functions and maximal clinical response.

Introduction

- Osteosarcoma (OS) is an aggressive malignant bone cancer, with the lung as the most frequent site of metastasis (1). Unresectable pulmonary metastasis remains a significant challenge with a survival rate of less than 20%. Identification of novel therapeutic strategies are desperately needed (2).
- Transforming growth factor-β1 (TGF-β) is a potent immune suppressive cytokine in OS tumor microenvironment (TME) (3). TGF-β1 expression is increased in the sera and tumor tissues of OS patients and this increase is associated with high-grade OS and lung metastases (4). Therefore, blocking TGF-β1 signaling may be a novel therapy for OS treatment.
- Vactosertib is a highly selective and a potent small molecule inhibitor against Type 1 TGF-β Receptor (TGF-βR1). Vactosertib is orally available and more potent than another ALK5 inhibitor (5). Vactosertib shows minimal side effects in adults (6). Treatment with Vactosertib reduces cancer cell migration, invasion and metastasis as demonstrated in various animal cancer models, including lung metastasis and melanoma mouse models (7-8).
- Therefore, a higher potency and less toxic TGF-β inhibitor, Vactosertib can be promising new therapeutic reagent for OS treatment.

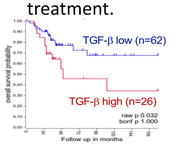


Figure 1. Kaplan-Meier analysis of OS patients with TGF-β1 high (n=26) vs TGF-β1 low (n=62). Data were obtained from "R2: Genomics analysis and visualization platform" [http://r2.amc.nl]. Datasets provided by Kuijer (n=88).

Results

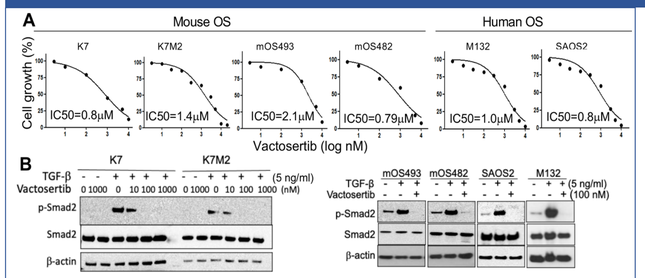


Figure 2. Vactosertib inhibits OS cell growth and TGF-β1 pathways. A) Vactosertib inhibits mouse and human OS proliferation. Various doses of Vactosertib (10 nM–10 mM) were incubated with mOS or hOS over a 4 day period using the IncuCyte Imaging System. B) Vactosertib inhibits TGF-β1 signaling pathway in OS cells. Vactosertib (10-1000 nM) were used to treat in K7, K7M2, mOS493, mOS482, SAOS2 and M132 cells 15 minutes before TGF-β1 (5ng/ml) treatment. 1 hour after TGF-β1 treatment, cells were harvested and p-Smad2, Smad2 and β-actin expressions were measured by Western blot analysis.

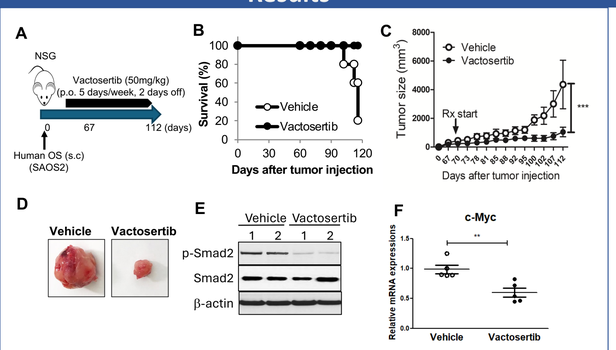


Figure 4. Vactosertib improves survival and inhibits human OS tumor growth *in vivo*. A) NSG mice were inoculated with 1x10^6 human SAOS2 hOS cells (s.c.) and were treated with Vactosertib (50 mg/kg, p.o. 5 days/week), starting on day 67 after tumor injection. B) Survival rate (n=5). C) tumor sizes were measured by caliper. D) Representative tumor pictures, E) p-Smad2, Smad2 and β-actin expressions in tumors, F) mRNA expressions of c-Myc in vehicle or Vactosertib treated groups.

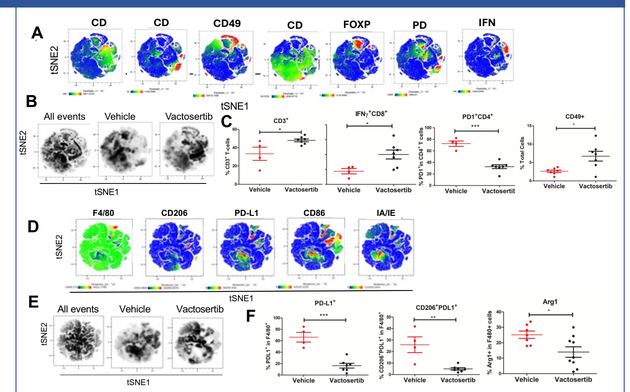


Figure 6. The effects of Vactosertib on tumor extrinsic T-cell and myeloid cell profile. A,D) tSNE heatmap plots show the MFI expression level and the distribution of each indicated marker. Low to high levels of protein expression are depicted in the gradient from blue (low) to red (high). B,E) tSNE density plots of live cells in vehicle or Vactosertib treated samples. C) The frequency of T cell markers and F) The frequency of myeloid cell markers by conventional FACS analysis.

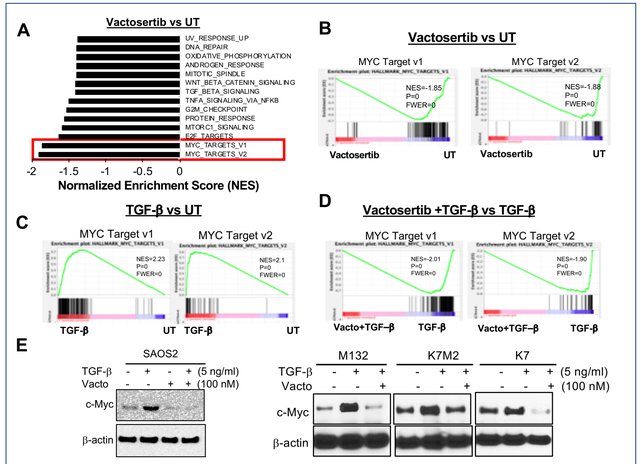


Figure 3. TGF-β1-induced c-Myc signaling pathways are suppressed by Vactosertib in OS cells. A) Hallmark Pathways from Gene Set Enrichment Analysis (GSEA) of RNA-sequencing analysis of human OS (SAOS2) cells after Vactosertib (100 nM) or untreated (UT) for 24 hours. B-D)GSEA enrichment plot of MYC target v1 and v2 pathway in A)Vactosertib treatment versus untreated treatment (UT), C)TGF-β1 treatment versus untreated and D)TGF-β1+ Vactosertib (Vacto) vs TGF-β1. E) c-Myc and β-actin protein expressions after treatment of TGF-β1 or TGF-β1/ Vactosertib (Vacto) on OS cells for 24 hours.

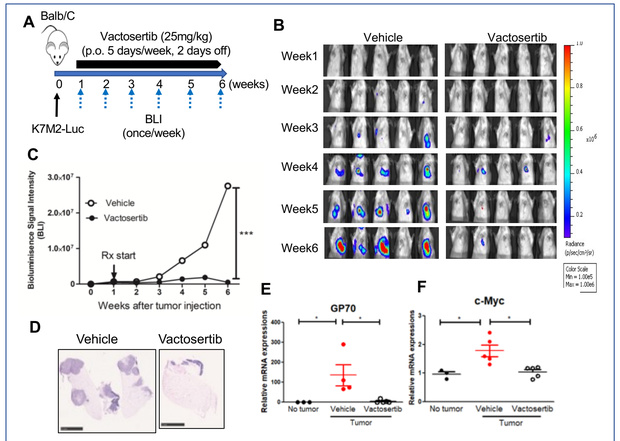
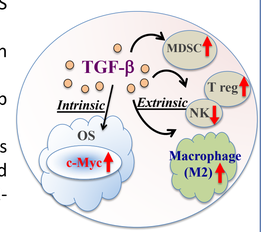


Figure 5. Vactosertib inhibits pulmonary metastatic mouse OS development *in vivo*. A) BALB/c mice were inoculated with 1x10^6 K7M2-Luc (i.v.) on day 0, and then treated with vehicle (p.o) or Vactosertib (25 mg/kg p.o. 5 days/week) starting on day 7. B) Bioluminescence image intensity (BLI) was measured once a week. C) Graph of BLI over time. D) Representative H&E staining of vehicle-treated or Vactosertib-treated lung samples day 42 day after tumor injection. Scale bar = 5 mm. Relative mRNA expression of E) GP70, and F) c-Myc in lung samples of vehicle or Vactosertib-treated mice on day 42 days after tumor injection compared with that of control lungs of no tumor bearing mice.

Conclusions

- Vactosertib, significantly inhibited OS proliferation *in vitro and in vivo*.
- Vactosertib inhibits c-Myc expression in the OS cells.
- Oral administration of Vactosertib significantly reduces OS growth *in vivo*.
- Vactosertib increased immune effectors (e.g., IFNγ$^+$CD8$^+$ cells and NK cells) and inhibited immune suppressors (e.g., M2-like TAM) in the OS TME.
- Our results suggest that inhibition of TGF-β1 signaling is an effective therapeutic strategy against OS through a multi-pronged approach that targets tumor intrinsic and extrinsic factors to achieve optimal immune-effector functions and maximal clinical response.



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