PRECLINICAL ACTIVITY OF NOVEL TGF BETA RECEPTOR I KINASE INHIBITORS IOA-359 AND IOA-360 FOR TREATMENT OF ANEMIA IN MDS/AML

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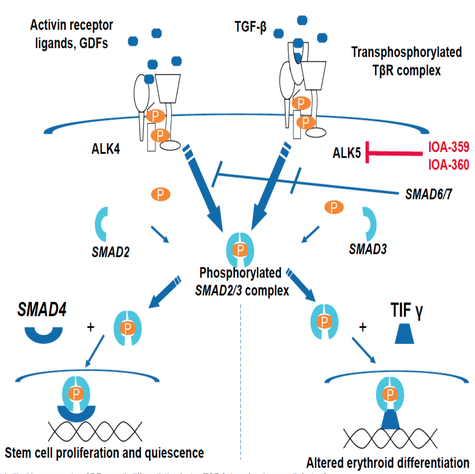
INTRODUCTION

Overactivation of the Transforming growth factor beta (TGF-β1) superfamily has been associated with bone marrow failure in MDS. TGF-β1 binds to set of receptors that include the TGF-receptor I kinase (also known as ALK5), that in turn phosphorylates and activates the downstream SMAD2/3 proteins. Activation of SMAD2/3 transcription factors has been shown to occur in MDS and is associated with anemia. Thus, we wanted to evaluate the preclinical efficacy of novel, clinic-ready, TGF-B1receptor I kinase small molecule ALK5 inhibitors IOA-359 and IOA-360 in MDS models.

AIM

Our goal of this study is to evaluate the efficacy of IOA-359 and IOA-360 in inhibiting downstream phosphorylation and activation of the SMAD 2/3. We also wanted to determine cellular activity of these novel ALK5 inhibitors in MDS models.

TGF-β role in Hematopoiesis



ALK, activin-like kinase receptor; GDF, growth differentiation factor; TGF-β, transforming growth factor β.

RESULTS

Fig 1

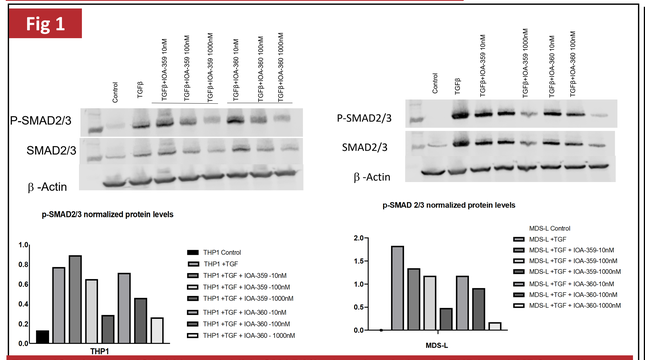


Fig 1: IOA-359 and IOA-360 can inhibit SMAD2/3 phosphorylation in THP1 and MDS-L cells upon TGF stimulation: Immunoblotting of phospho-SMAD2/3 and SMAD2/3 in (A) THP1 and (B) MDS-L cells that were treated with IOA-359 and IOA-360, respectively, at multiple concentrations (10nM, 100nM and 1000nM), followed by 30 minutes stimulation of TGF-β (20 ng/mL). Quantification of anti-psmad2/3, normalized to β-actin, by densitometry analysis, is shown as bar graphs.

Fig 2

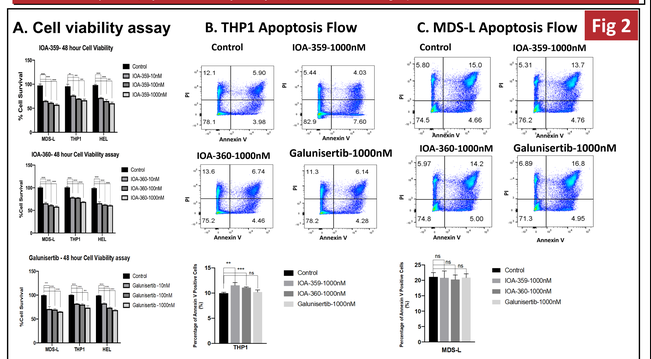


Fig 2: IOA-359 and IOA-360 ALK5 inhibitors do not increase leukemic cell proliferation – (A) MDS-L, THP1 and HEL cell lines were treated with IOA-359, IOA-360 and Galunisertib at multiple concentrations (10nM, 100nM and 1000nM), respectively, for 48 hours. Cell viability was assessed at 48 hours with Cell-Titer Blue assay. **(B)** THP1 and **(C)** MDS-L cells treated with IOA-359, IOA-360 and Galunisertib, (1000 nM) respectively, for 72 hours. Cells were harvested and stained with Annexin V and PI and analyzed by FACS. Representative bar plots are also shown.

Fig 3

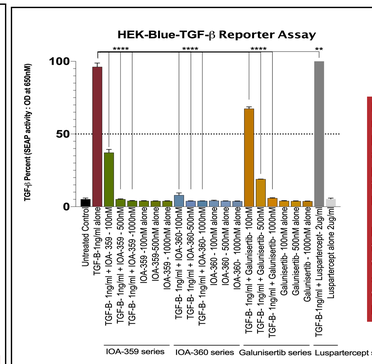


Fig 3 - IOA-359 and IOA-360 inhibit TGF-β mediated gene expression. (A) HEK-293-Blue-TGF-β reporter cell line was used to detect TGF-β ligand activity and monitor the activation of the TGF-β /SMAD pathway. HEK-293-Blue-TGF-β reporter cells were treated with IOA-360, IOA-359 and Galunisertib for 24 hours at multiple concentrations (100nM, 500nM and 1000nM), respectively, and co-stimulated with TGF-β ligand (1 ng/mL). HEK-293-Blue-TGF-β reporter cells treated with IOA-360, IOA-359 and Galunisertib, without TGF-β ligand stimulation, were used as control. HEK-293-Blue-TGF-β reporter cells treated with Luspartecept (2 ug/mL) with or without TGF-β ligand (1 ng/mL) stimulation were used as control.

Fig 4

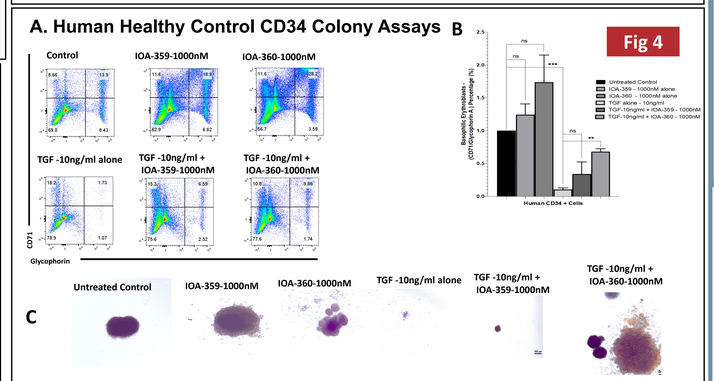


Fig 4: IOA-359 and IOA-360 can reverse growth inhibitory effects of TGF-β in healthy human CD34 cells – (A) Healthy Human CD34+ cells were grown on methylcellulose to determine the effects of drugs IOA-359 and IOA-360 respectively and /or TGF-β stimulation. Cells were harvested after 14 days for erythroid differentiation by FACS analysis. TGF-β inhibited erythroid differentiation. An increase in mature erythroid and basophilic progenitors was observed in TGF-β stimulated cells treated with IOA-359 and IOA-360.**(B)** A representative flow bar plot shows percentage of Basophilic erythroblasts (CD71/Glycophorin A) after 14 days. (C) A representative picture of colonies treated with drugs TGF-β and IOA-359 and IOA-360 were larger in size when compared to TGF-β alone.

Fig 5A

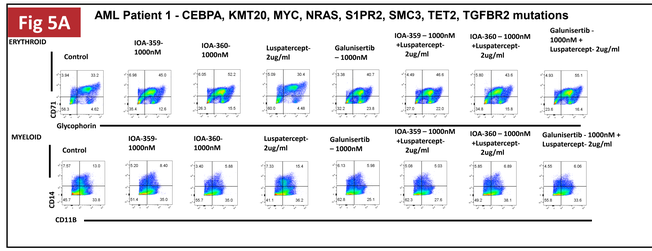


Fig 5B

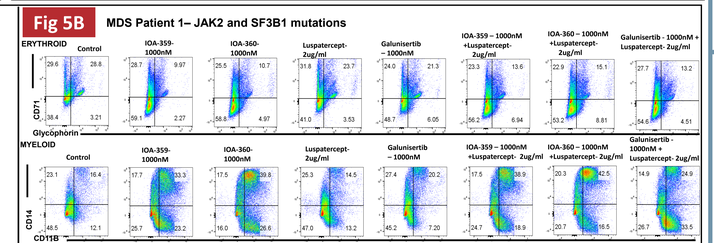


Fig 5C

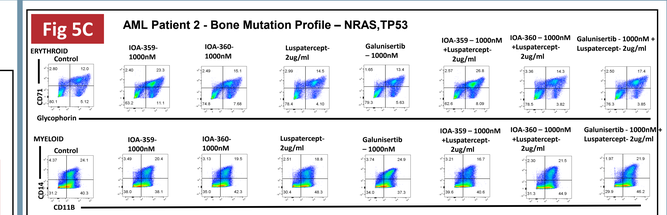


Fig 5D

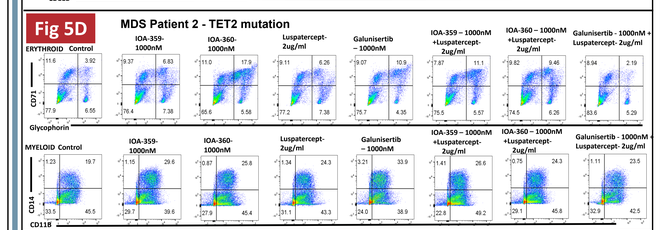


Fig 5 – IOA-359 and IOA-360 augments erythroid differentiation in AML and MDS patient samples– The efficacy of the novel ALK5 inhibitors IOA-359 and IOA-360 was next tested in primary patient samples from MDS and AML and compared to Galunisertib in colonogenic assays. Increased erythroid colonies was observed in majority of MDS samples treated with both the ALK5 inhibitors IOA-359 and IOA-360. Colonies were picked and analyzed for erythroid and myeloid differentiation by FACS analysis. A representative flow plot of diverse patient samples (Fig 5A-D) shows enhanced erythroid and myeloid differentiation. Moreover, in specific cases, the inclusion of Luspatercept resulted in enhanced erythrocyte maturation. Representative flow cytometry plots shows enhanced erythroid differentiation with Luspatercept combinatorial therapy of AML1 with CEBPA, KMT20, MYC, NRAS, S1PR2, SMC3, TET2 and TGFBR2 mutations (5A). Representative flow cytometry plot shows erythroid and myeloid differentiation with Luspatercept combinatorial therapy of MDS Patient 1 with JAK2 and SF3B1 mutations (5B). Representative flow cytometry plot shows erythroid and myeloid differentiation with Luspatercept combinatorial therapy of AML Patient 2 with NRAS, TP53 mutations (5C). Representative flow cytometry plot shows erythroid and myeloid differentiation with Luspatercept combinatorial therapy of MDS Patient 2 with TET2 mutation (5D).

CONCLUSIONS

- ALK5 inhibitors IOA-359 and IOA-360 effectively inhibit TGF-β / SMAD2/3 downstream activation in a dose dependent manner in MDS and AML cell lines. Functionally, the ALK5 inhibitors did not lead to any increase in viability or proliferation in leukemic cell lines.
- ALK5 inhibitors reverse the growth inhibitory effects exerted by TGF-β on normal hematopoietic stem cells (HSCs), as demonstrated through CFU assay. Furthermore, treatment enhanced erythroid differentiation in MDS and AML patient samples with diverse mutational profiles.
- Our current results demonstrate the preclinical in vitro efficacy of ALK5 inhibitors IOA-359 and IOA-360, highlighting their potential for further development and clinical testing in MDS/AML.

CONTACT INFORMATION

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