KZR-540 is a novel oral small molecule inhibitor of Sec61 cotranslational translocation that potently and selectively blocks PD-1 expression

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Introduction

• Most secreted and transmembrane proteins utilize unique amino-terminal signal sequences (ss) to enable translocation through the Sec61 complex into the endoplasmic reticulum (ER).

• Targeting Sec61 with small molecule inhibitors such as KZR-261, currently in a clinical trial for solid tumors (NCT01204735), has shown anti-tumor effects through inhibiting the expression of multiple therapeutic targets, including PD-1, in preclinical models.

• Inhibition of PD-1/PD-L1 is effective in the treatment of a variety of tumors, but current PD-1 inhibitors are IV administered biologics, which have limitations.

• Herein we describe KZR-540, an orally bioavailable small molecule Sec61 inhibitor that potently and selectively blocks PD-1 ss and inhibits protein surface expression, which may expand the potential of anti-PD-1 therapy.

Methods

• Flp-in T-REx® HDK-253 cells were transfected with constructs containing a fused to a luciferase reporter. Pretreated expression was induced with doxycycline and cells were treated with the compounds for 24 hours.

• Human T cells were isolated from healthy donors using negative magnetic separation, activated with ImmunoCult® Human CD3/28 activator and expanded. Cells were treated with compounds and stimulated with 24-hour, and protein surface expression analysis was analyzed on live cells by flow cytometry (FC). Alternatively, membrane protein levels were assessed using mass spectrometry (MS)-based proteomic profiling after sub-cellular fractionation.

• Mixed lymphocyte reactions (MLR) were performed using monocyte-derived dendritic cells as stimulator cells and human T cells as responder cells. Nextolyn bioadhesive (N90) was used as a control. L-2 and IFN-γ supernatants were quantified with a Millipore Discovery electrochemiluminescence detection kit.

• PD-1 mediated cancer cell killing was analyzed in co-culture assays of A375 melanoma cells and human T cells. Change in A375 cell number, determined by percentage of confluency, was assessed in an Incucyt® live-cell imager.

• Pharmacokinetics (PK) was assessed using liquid chromatography-tandem MS performed on plasma collected from Balb/c mice dosed orally.

- In vivo effect of KZR-540 treatment on PD-1 expression was assessed in human-PD-1 knock-in (huPD-1) mice and in human PBMC NSG mice dosed orally with KZR-540 and injected with mouse or human anti-CD3 antibody. Splenocytes were collected and processed 24 hours later, and membrane PD-1 expression was analyzed by flow cytometry (FC).

- Tumor efficacy assessment was performed in huPD-1 mice bearing human PD-1+L1 Meso colon carcinoma cells. Mice were treated daily with KZR-540 PO or twice weekly with 10 mg/kg KZR-430 (anti-PD-1 antibody) for 21 days, and tumor volume and body weight were monitored 3 times per week. Tumors and tumor draining lymph nodes (axLNs) were collected at the end of the study, and PD-1 surface expression was measured on live T cells by FC.

- One-way ANOVA was used for statistical analyses (p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) compared to vehicle groups.

Results

Figure 2. Potency and selectivity profiles of Sec61 inhibitors (A), KZR-540 selectively inhibits PD-1 (B)

Figure 3. KZR-540 blocks expression of PD-1 in primary human T cells while other T cell activation markers remain unchanged, as measured by flow cytometry (A) or proteomics profiling (B)

Figure 4. Co-culturing A375 melanoma cells with human T cells in the presence of KZR-540 increases T cell-dependent cell death

Figure 5. KZR-540 increases T cell activity, observed as augmented production of IL-2 and IFNγ in MLR

Figure 6. Oral administration of KZR-540 in mice results in sustained and dose-proportional exposure

Figure 7. T cells in humanized PBMC NSG® mice (A) and huPD-1 mice (B) dosed orally with KZR-540 show decreased levels of PD-1 expression after in vivo anti-CD3 stimulation

Figure 8. KZR-540 demonstrates a dose-dependent anti-tumor response (A and B) and decrease in PD-1 expression (D) in a syngeneic MC38 colon carcinoma model established in huPD-1 mice

Conclusions

• KZR-540 is an orally bioavailable small molecule Sec61 inhibitor that potently and selectively blocks PD-1 expression.

- In vitro treatment of human T cells with KZR-540 decreases post-activation upregulation of PD-1 without affecting other activation markers and increases T cell responses.

- Oral administration of KZR-540 is well tolerated and results in dose dependent anti-PD-1 activity and selective inhibition of PD-1 expression in vivo.

- Oral treatment with KZR-540 of huPD-1 mice bearing huPD-L1 MC38 tumors inhibits tumor growth in a dose-dependent manner and decreases PD-1 expression in tumor infiltrating lymphocytes and PD-1.

- In this model, KZR-540 performance is comparable to that of a commercially available biologic anti-PD-1 agent.

References

2. et al. JCI Insight 2015; 1(19):

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