KZR-540 IS A NOVEL ORAL SMALL MOLECULE INHIBITOR OF SEC61 COTRANSLATIONAL TRANSLLOCATION THAT POTENTLY AND SELECTIVELY BLOCKS PD-1 EXPRESSION

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Background Blocking the PD-1/PD-L1 pathway with antibodies has been effective in treating multiple types of cancers. However, orally administered small molecules can allow for better tumor penetration and more convenient dosing than antibody-based therapies. Most secreted and transmembrane proteins utilize unique signal sequences (ss) to enable translocation through the Sec61 complex into the endoplasmic reticulum. Targeting Sec61 with small molecule inhibitors such as KZR-261, currently in a clinical trial for solid tumors (NCT05047536), has anti-tumor effects through inhibiting the expression of multiple therapeutic targets, including PD-1, in preclinical models. Here we describe KZR-540, an oral small molecule Sec61 inhibitor that potently and selectively blocks PD-1 expression.

Methods Inhibition of Sec61 clients was assayed in HEK-293 cells overexpressing the ss of interest fused to a luciferase reporter. Human T cells pre-treated in vitro with test compounds and stimulated were monitored for expression of PD-1 and other markers by flow cytometry and mass spectrometry (MS)-based proteomic profiling. Cytokine production was measured from allogeneic mixed lymphocyte reaction (MLR). T cell-mediated killing was assessed in co-culture assays with A375 cells. Pharmacokinetics (PK) was assessed using liquid chromatography-tandem MS. In vivo evaluation of PD-1 expression was conducted in humanized PBMC NSG™ and B-hPD-1 mice dosed orally with KZR-540 and treated with anti-CD3 antibody. Human PD-1 expression on T cells was assessed by flow cytometry of splenocytes.

Results We have discovered a series of Sec61 inhibitors that potently and selectively block ssPD-1 expression. In human T cells treated with these compounds in vitro prior to activation, surface expression of PD-1 was inhibited while other T cell activation markers were unaffected. KZR-540 demonstrated good liver microsome stability and a robust oral PK profile and was selected for further studies. The selectivity of KZR-540 was also demonstrated by global Sec61 client proteomic profiling of treated T cells. Treatment with KZR-540 increased IL-2 and IFNgamma production in MLR and enhanced killing of A375 melanoma cells by human T cells. Furthermore, oral dosing of KZR-540 potently suppressed anti-CD3-induced PD-1 expression in both humanized NSG mice and human-PD-1 knock-in mice.

Conclusions KZR-540 is a small molecule inhibitor of Sec61 that can potently and selectively block PD-1 expression and increase T cell activity in vitro, as well as inhibit surface PD-1 expression when dosed orally in vivo. Future studies will examine whether there is an enhanced anti-tumor response in a tumor model.

Ethics Approval Animal studies were approved by the Institutional Animal Use and Care Committee.