Background Secreted and transmembrane proteins are integral in promoting cancer cell proliferation as well as tumor growth, immune evasion, and metastasis. Most of these proteins require translocation through the Sec61 translocon complex (Sec61) for entry into the endoplasmic reticulum (ER) and progression to the cell membrane. Inhibiting programmed cell death 1 (PD-1), a Sec61 client protein, has been effective in treating many cancer types. Previously we reported the anti-tumor activity of KZR-540, an orally bioavailable small molecule that binds to Sec61 and selectively inhibits the expression of PD-1, inducing a T-cell dependent anti-tumor effect. Here we utilized LC/MS-based quantitative proteomic profiling to study the unique selectivity of KZR-540 in contrast to the promiscuous Sec61 inhibitor KZR-261 and its analog KZR-834.

Methods Secretome of stimulated human T-cells were obtained through albumin depletion of the culture media using the BioMag® ProMax Albumin Removal Kit. The membrane/organelle proteome of stimulated human T-cells were extracted using the ProteoExtract® Subcellular Proteome Extraction Kit (Policysciences). All samples were subsequently labeled with Tandem Mass Tag (Thermo Scientific) and analyzed through tandem mass spectrometry to determine the relative protein abundance.

Results We quantified 510 Sec61 client proteins in the secretome samples treated with therapeutically relevant doses of KZR-540 and KZR-834. KZR-834 treatment inhibited the secretion of 27 out of 510 total quantified Sec61 clients by a minimum of 1.5-fold. In contrast, KZR-540 exhibited no statistically significant impact on the secretome of stimulated T-cells. Furthermore, we quantified 1305 Sec61 clients in the membrane/organelle samples. KZR-834 treatment inhibited translocation of 45 Sec61 client proteins, including PD-1, in membrane/organelle fraction by at least 2-fold. In contrast, KZR-540 treatment inhibited expression of only 3 Sec61 client proteins in the membrane/organelle fraction of stimulated T-cells by at least 2-fold. Among those 3 proteins, PD-1 was the only one inhibited by more than 4-fold. Overall, KZR-540 exhibited a far more selective impact compared to KZR-834, inducing the inhibition of less than 1% of the total quantified Sec61 clients.

Conclusions Inhibition of Sec61 via both the selective inhibitor KZR-540, and the promiscuous inhibitors KZR-261/834 have demonstrated anti-tumor potential in preclinical studies. KZR-261 is currently being evaluated in a phase-1 clinical trial in solid tumors (NCT05047536). Follow-up in vivo studies will examine the differential impact of KZR-540 and KZR-261/834 on the tumor-draining lymph nodes (TDLNs) and plasma proteomes of human-PD-1 knock-in mice bearing human-PD-L1 MC38 tumors.

REFERENCES