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Targeting multiple immune checkpoint proteins with novel small molecule inhibitors of Sec61-dependent cotranslational translocation

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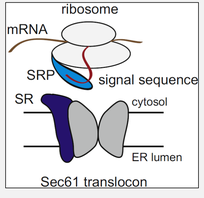
BACKGROUND

- **The expression of secreted and transmembrane proteins, including immune checkpoint molecules, involves cotranslational translocation, which is facilitated by the ribosome-signal recognition particle-Sec61 complex.[1]**
- **Amino-terminal signal sequences, which are unique to each protein, direct nascent polypeptides through the Sec61 channel into the endoplasmic reticulum for expression and function.**
- **While many Sec61-targeting compounds have demonstrated anti-tumor effects by broadly inhibiting protein translocation, some compounds have been identified to exert signal sequence-specific blockade.[3]**
- **We identified small molecule inhibitors of Sec61 capable of selectively targeting multiple co-inhibitory immune checkpoint proteins.**

Figure 1. Cotranslational targeting of precursor polypeptides[2]



METHODS

- **Flp-In T-REx™ 293 cells or HEK293 stable cell lines overexpressing wild-type (WT) or mutant R66I[4] Sec61α were transiently transfected with signal sequence (ss) luciferase reporter constructs. Plasmid expression was induced with doxycycline, and cells were treated with compounds for 24 hours.**
- **Cell lines were treated with compounds for 24 hours or Jurkat T cells or PBMCs were stimulated with anti-CD3 and anti-CD28 antibodies and treated with compounds for 48 or 72 hours. Protein surface expression was analyzed on live cells by flow cytometry.**
- **Cell viability was measured by CellTiter-Glo® or by flow cytometry with a viability dye.**
- **Mixed lymphocyte reactions (MLR) were completed using enriched T cells as responder cells and monocyte-derived dendritic cells (human MLR) or allogeneic splenocytes (mouse MLR) as stimulator cells. IFNγ or IL-2 levels in the supernatant were measured with a Meso Scale Discovery electrochemiluminescence detection kit.**
- **SKOV3 cells were treated with compound for 24 hours, and 200 µM AMP was added for 1 hour. The hydrolysis of AMP was indirectly measured using the AMP-Glo™ assay kit.**
- **PBMCs were stimulated with anti-CD3/CD28 beads in the presence of exogenous 200 µM AMP and compound. IFNγ levels were measured.**

RESULTS

Figure 2. Sec61 compound analogs exhibit distinct and species-specific selectivity profiles for immune checkpoint signal sequences

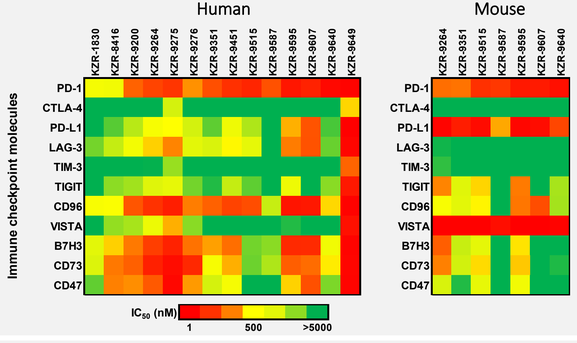
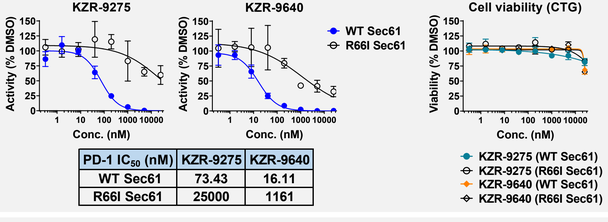
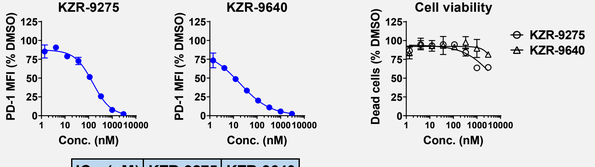


Figure 3. Sec61-dependent blockade of PD-1 signal sequence luciferase reporter



PD-1 IC_{50} (nM)	KZR-9275	KZR-9640
WT Sec61	73.43	16.11
R66I Sec61	25000	1161

Figure 4. Dose-dependent reduction of PD-1 surface expression on stimulated Jurkat T cells



IC_{50} (nM)	KZR-9275	KZR-9640
PD-1	149.8	18.45

Figure 5. Surface expression of checkpoint molecules is potently blocked on activated T cells

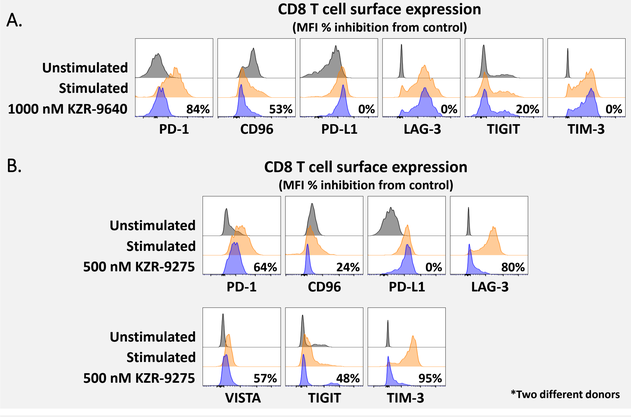


Figure 6. Sec61 inhibitors are capable of potentiating IFNγ and IL-2 production in mixed lymphocyte reactions

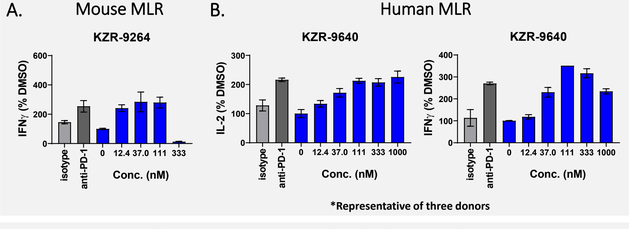


Figure 7. CD47 surface expression is modestly decreased on Ramos B lymphoma cells

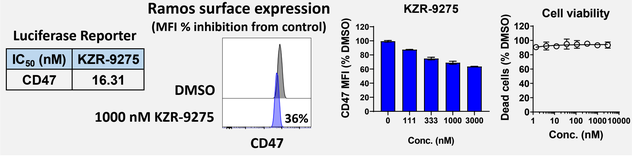


Figure 8. Decreased CD73 expression reduces AMP conversion

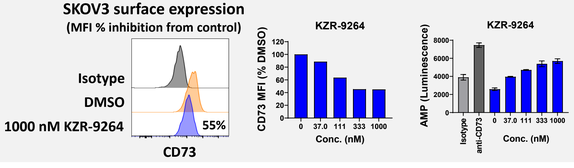
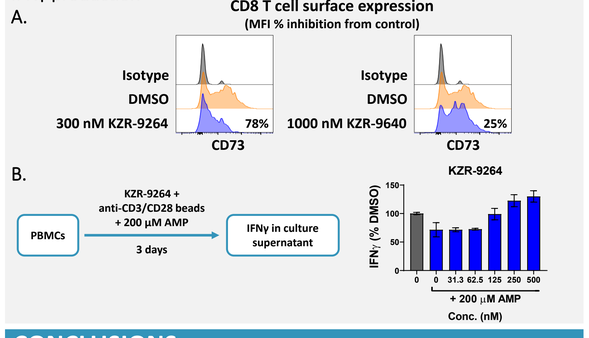


Figure 9. CD73 blockade overcomes AMP-mediated suppression of IFNγ production



CONCLUSIONS

- **Small molecules targeting Sec61-dependent cotranslational translocation provide a new approach for blocking multiple immune checkpoint proteins with a single agent.**
- **We have identified novel Sec61 inhibitors that selectively block PD-1 or widely target multiple immune checkpoint molecules.**
- **These compounds have the capacity to regulate T cell functions and/or the tumor microenvironment.**
- **Future *in vivo* studies will further elucidate the efficacy of Sec61 inhibitors to enhance anti-tumor immune responses.**

REFERENCES

1. Park E, Rapoport TA. *Annu Rev Biophys.* 2012; 41: 1-20.
2. Maifeld SV, MacKinnon AL, Garrison JL, et. al. *Chem Biol.* 2011; 18: 1082-1088.
3. Van Puyenbroeck V, Vermeire K. *Cell Mol Live Sci.* 2018; 75: 1541-1558.
4. MacKinnon AL, Paavilainen VO, Sharma A, et al. *eLIFE.* 2014; 3:e01483.