Small molecule inhibitors of Sec61 cotranslational translocation regulate the phagocytosis checkpoint molecule CD47

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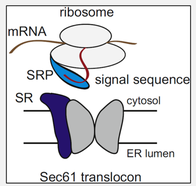
Introduction

- 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 (Figure 1).[2]
- CD47, a multi-pass transmembrane protein, is overexpressed on tumor cells and binds SIRPα expressed on macrophages to dampen phagocytic activity.
- Blockade of CD47/SIRPα interactions enhances macrophage phagocytosis.
- We identified a novel method to target CD47 and enhance macrophage phagocytosis with small molecule inhibitors of Sec61.

Figure 1. Cotranslational targeting of precursor polypeptides



Methods

Signal sequence screening

- Flp-In T-REx™ 293 cells 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.

Flow cytometric analysis of cell surface expression

- Cell lines were treated with compounds for 72 hours. Protein surface expression was analyzed on live cells by flow cytometry.

Cell viability and apoptosis

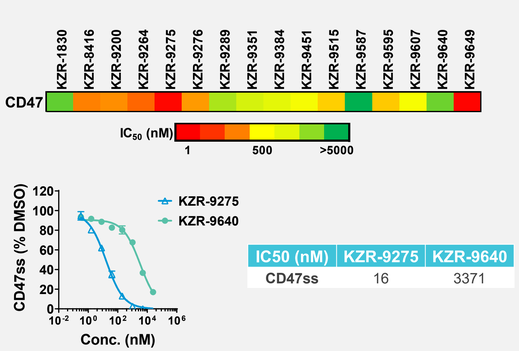
- Cell viability and apoptosis was measured by flow cytometry with a viability dye or Annexin V (apoptosis) and 7AAD (dead) labeling.

Macrophage phagocytosis assay

- Macrophages were differentiated *in vitro* from human CD14⁺ monocytes or mouse bone marrow cells. Cells were cultured in M-CSF to differentiate into macrophages and further differentiated into M1- and M2-type macrophages with IFN-γ + LPS or IL-4 + IL-13, respectively. Tumor cells were treated with DMSO or 333 nM KZR-9275 for 72 hours. Cells were washed prior to labeling with pHrodo™ Green. For antibody opsonization, pHrodo-labeled cells were incubated with anti-CD47 (B6.H12) and/or anti-CD3 (OKT3) for 30 minutes and washed before co-culturing with macrophages. Macrophages and tumor cells were co-cultured for 4 hours, and phagocytosis was measured by flow cytometry. % phagocytosis was measured by the % CD11b⁺pHrodo⁺ population.

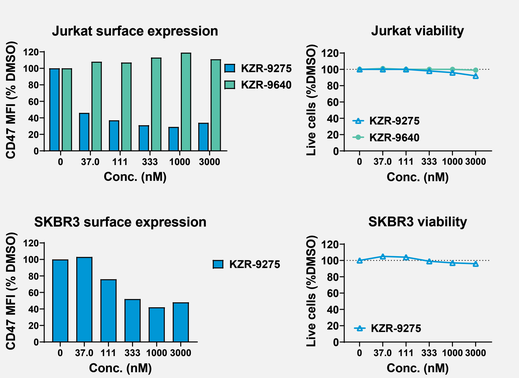
Results

Figure 2. Sec61 inhibitor analogs demonstrate different potency on the CD47 signal sequence



IC50 (nM)	KZR-9275	KZR-9640
CD47ss	16	3371

Figure 3. KZR-9275 incubation for 72 hours reduces CD47 surface expression on Jurkat and SKBR3 tumor cell lines



Results

Table 1. Apoptosis is not increased in Jurkat (A) and SKBR3 (B) cells following incubation with KZR-9275 for 72 hours

A

Jurkat cells		
Mean %	DMSO	KZR-9275
Live	98%	96%
Annexin V+	0.9%	1.3%
7AAD+	1.4%	2.7%

B

SKBR3 cells		
Mean %	DMSO	KZR-9275
Live	95%	86%
Annexin V+	0.7%	1.2%
7AAD+	3.4%	12%

Figure 4. Treatment of SKBR3 tumor cells with KZR-9275 enhances macrophage phagocytosis

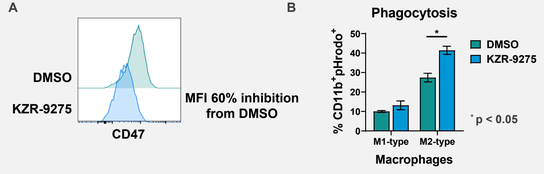
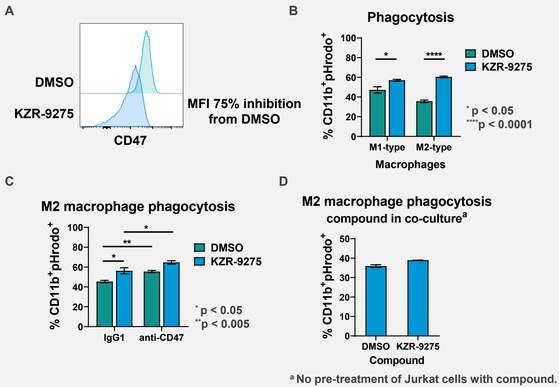


Figure 5. KZR-9275 treatment of Jurkat cells increases macrophage phagocytosis



[a] No pre-treatment of Jurkat cells with compound.

Results

Figure 6. Mouse macrophage phagocytosis of KZR-9275-treated Jurkat cells is further enhanced with antibody opsonization

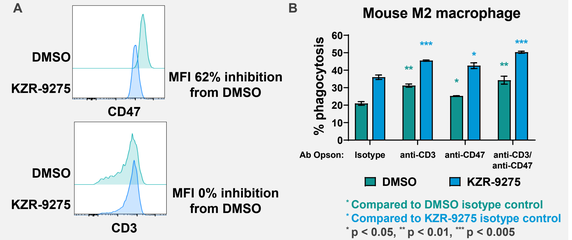
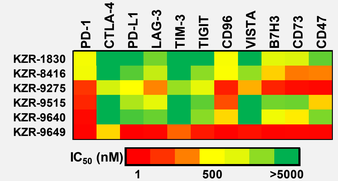


Figure 7. Sec61 inhibitors exhibit distinct selectivity profiles on immune checkpoint signal sequences



Conclusions

- Inhibitors of Sec61 translocation demonstrate activity on the CD47 signal sequence.
- KZR-9275 blocks CD47 surface expression on tumor cell lines without inducing apoptosis.
- Exposure of tumor cells to KZR-9275 leads to increased macrophage phagocytosis.
- Small molecule inhibitors of Sec61 provide an opportunity to target multiple checkpoint proteins on various cell populations thus possibly offering combination-like therapy in a single compound.

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.

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