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Discovery and characterization of novel anti-cancer small molecule inhibitors of Sec61

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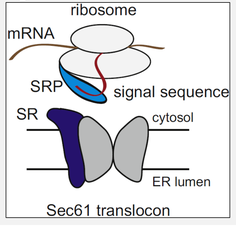
BACKGROUND

- **The Sec61 translocon is the primary mediator for cotranslational translocation of secreted and transmembrane proteins into the endoplasmic reticulum, providing a novel therapeutic target for blocking expression of therapeutically relevant targets such as cytokines, oncogenic receptors, and immune checkpoint molecules (Figure 1)**
- **Previously described inhibitors of Sec61[2-5] have shown anti-tumor activity but lacked adequate pharmaceutical properties or tolerability for clinical development**
- **Through a drug discovery program to identify novel inhibitors of Sec61 we identified three distinct chemical scaffolds with differential selectivity and potential for treatment of malignant diseases**

Figure 1. Sec61 Translocon[1]



METHODS

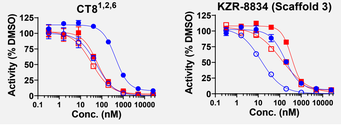
- Inducible luciferase reporter expression plasmids were created by fusing cDNA encoding Gaussia luciferase to the 3' end of signal sequence + 10 amino acids (SS+10AA), transmembrane domain (TMD) or full length (FL) cDNA of proteins of interest. Expression was induced with doxycycline alongside compound treatment for 24 hours (Figures 2-7)
- Mutant R66I[6] or wild type (WT) Sec61 was stably overexpressed in HEK293 cells followed by transient transfection with plasmids expressing luciferase reporters (Figure 3)
- Cell viability was measured by CellTiter-Glo® after the indicated treatment period (CTG; Figures 3, 5, 6, 7)
- HCT116 cells were selected for resistance to CT9 generating R66I mutant Sec61 cells[6]. WT and mutant Sec61 cells were exposed to CT8 (positive control), proteasome inhibitor carfilzomib (negative control), or test compounds for 48 hours followed by viability measurement as described above (Figure 3)
- Luciferase reporter plasmids were transiently transfected into Flp-In T-REx™ 293 cells followed by induction and compound treatment as described above (Figure 4, 8)
- HER3 expression in BT474 breast cancer cells was evaluated after 24 hours of compound treatment by capillary immunoassay (WES-Protein Simple; Figure 6)
- CD4 and PD-1 expression was measured by flow cytometry on T-cells activated with antibodies to CD3 and CD28 alongside compound treatment for 24 hours (Figure 7)
- PD-1, LAG3, TIM3, and CD96 expression was measured by flow cytometry on PBMCs stimulated with antibodies to CD3 and CD28 for 72 hours followed by 24 hour compound treatment (Figure 8)
- Previously frozen human PBMCs and fresh whole blood (n=3 donors) were treated with compound for 20 hours followed by flow cytometry analysis (Figure 9)
- Cytokine levels were measured in human PBMCs (n=3 donors) stimulated with antibodies to CD3 and CD28 (GM-CSF, IFN-γ, TNF-α) or LPS (IL-6, IL-23) alongside compound treatment for 24 hours via Meso Scale Discovery electrochemiluminescent detection (MSD; Figure 9)
- BALB/c mice (n=5) were dosed with KZR-9098 2 hours prior to administration of LPS. Samples were collected 2 hours later and serum TNF-α levels were measured by MSD (Figure 10)
- BALB/c mice were dosed as indicated followed by whole blood collection and flow cytometry analysis 20 hours later (Figure 10)

Screening Workflow

Primary Screen — Inhibitors of Targets 1 & 2 reporters → **Selectivity Screen** — > 40 reporters (e.g. PD-1, IL-2, TNF-α, HER3) → **Counterscreens** — Cytosolic Firefly-luc, Mutant R66I Sec61, Normal cell cytotoxicity → **Potency Evaluation** — Anti-tumor activity, Surface marker expression, Pharmacologic properties

RESULTS

Figure 2. Luciferase Tagged Sec61 Client Proteins Enable Target Selectivity Profiling



IC_{50} (nM)	CT8	KZR-8834
PD-1 (SS+10AA)	56	14
PD-1 (FL)	431	177
TNF-α (TMD)	33	125
TNF-α (FL)	61	361

Figure 3. Cytosolic Firefly Luciferase and Sec61 Mutant Verify On-Target Inhibition

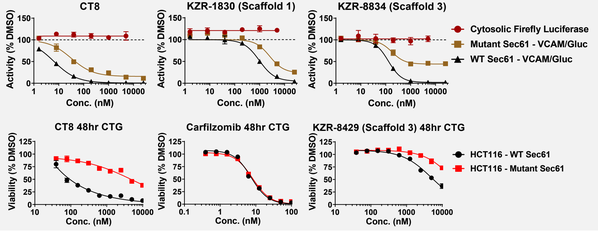


Figure 4. Distinct Chemical Scaffolds Achieve Differential Sec61 Client Selectivity

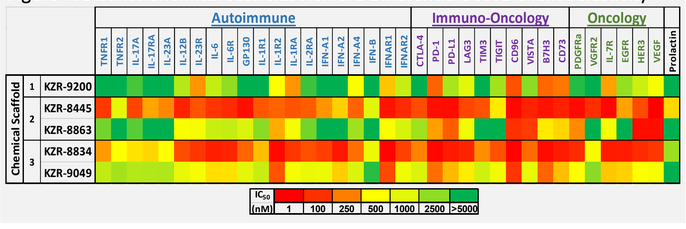
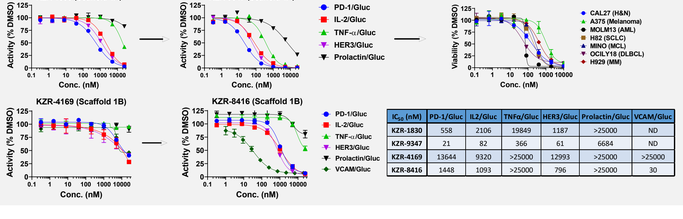


Figure 5. Scaffold 1: Broad Sec61 Inhibition and Anti-Cancer Activity or Target (VCAM1) Selective Inhibition



IC_{50} (nM)	PD-1/Gluc	IL2/Gluc	TNFα/Gluc	HER3/Gluc	Prolactin/Gluc	VCAM/Gluc
KZR-1830	558	2106	19849	1187	>25000	ND
KZR-9347	21	82	366	61	6684	ND
KZR-4169	13644	9320	>25000	12993	>25000	>25000
KZR-8416	1448	1093	>25000	796	>25000	30

Figure 6. Scaffold 2: Broad Anti-Cancer Activity or Selective HER3 Blockade

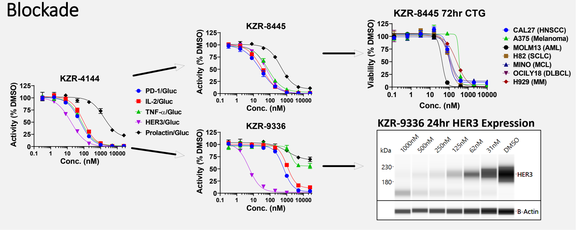


Figure 7. Scaffold 3: Broad Anti-Cancer Activity or Selective PD-1 Inhibition

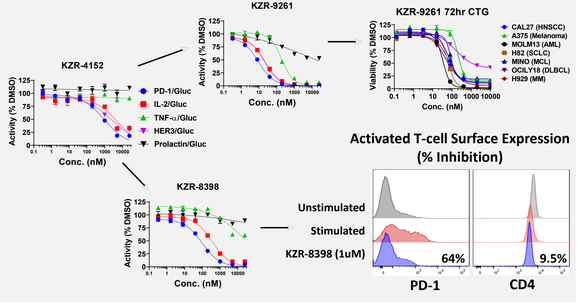


Figure 8. Scaffolds 1 and 3 Exhibit Potent Blockade of Multiple Immune Checkpoint Proteins

Immune Checkpoint Luciferase Reporter IC_{50} (nM)

			CTLA-4	PD-1	PD-L1	LAG3	TIM3	TIGIT	CD96	VISTA	B7H3	CD73	CD47
Chemical Scaffold	1	KZR-9275	1261	106	500	260	2331	933	62	345	68	25	16
	3	KZR-9039	8	72	20	41	90	90	7	138	10	29	32

PBMC Surface Expression (% Inhibition)

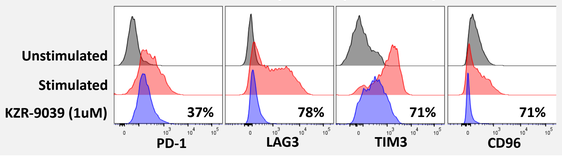
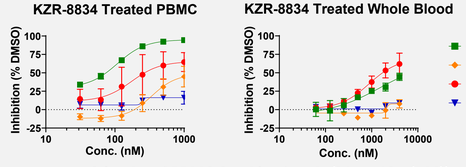


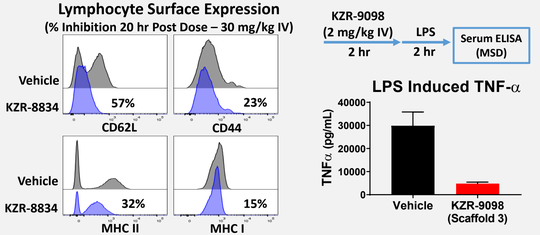
Figure 9. Inhibition of Sec61 Clients in Primary Cell Assays



Stimulated PBMC Cytokine Levels (Mean IC_{50} (nM))

GM-CSF	IFNγ	TNFα	IL-6	IL-23
165	152	82	61	200

Figure 10. Modulation of Basal and Inducible Sec61 Client Proteins in Mice



CONCLUSIONS

- **Reporter-based screening assays were successfully developed to discover broad and selective inhibitors of Sec61 client proteins**
- **Small molecule targeting of therapeutically relevant cell surface and secreted proteins can be achieved in-vitro and in-vivo**
- **Multiple chemical scaffolds are able to act as broad and selective inhibitors of Sec61 function resulting in reduced expression of key targets in autoimmune/inflammatory, immuno-oncology, and oncology indications**

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