Quantitative Proteomic Profiling of Novel Anti-cancer Small Molecule Inhibitors of Sec61: Mechanistic Investigation and Biomarker Discovery

Yu Qian, Janet Anderl, Andrea Fan, Henry W. B. Johnson, Christopher J. Kirk, Eric Lowe, Dustin McMinn, Beatriz Millare, Tony Muchamuel, Jennifer Whang and Jinhai Wang
Kezar Life Sciences, South San Francisco, USA

323

KEZAR LIFE SCIENCES

Introduction

- Secreted and transmembrane (TM) proteins play key roles in malignant transformation and tumor growth. The majority of those proteins require translocation through Sec61 translocon into the ER for further processing (Figure 1A).
- >6000 proteins were annotated as Sec61 clients (Uniprot) and categorized into five types: MultiTM (2657), Secreted (1892), Type I (1196), Type II (414), Type III (36) (Figure 1B).
- KZR-261 (clinical) and KZR-834, small molecules which inhibit the Sec61 translocon, were identified through a medicinal chemistry campaign and found to have broad anti-tumor activity *in vitro* and *in vivo* (Figure 2).

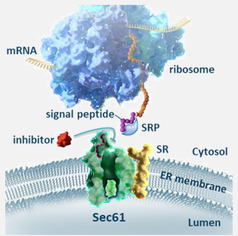


Figure 1A. Sec61 translocon

- Quantitative proteomic methods were utilized to study inhibition of Sec61-mediated protein secretion and global modulation of protein homeostasis across multiple tumor cell lines (Table 1) and PBMCs.

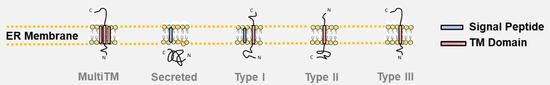


Figure 1B. Types of Sec61 client proteins

Methods

- *Subcellular Fractionation:* Cytosolic Fraction (F1) and Membrane/Organelle Fraction (F2) were obtained using Calbiochem® ProteoExtract® Subcellular Proteome Extraction Kit (Figure 3).
- *Liquid Chromatography:* Liquid chromatography is performed using Thermo Scientific™ EASY-nLC™ 1200 System, utilizing a 2 cm trap column and 50 cm C18 Thermo Scientific™ EASY-Spray™ Column heated at 55°C with 120min LC gradients.
- *Mass Spectrometry:* All samples were analyzed on a Thermo Scientific™ Q Exactive Plus mass spectrometer. For Thermo TMT-6 plex labeled samples, MS-level scans were performed with resolution set to 70,000; AGC target 3e6; maximum injection time 50 ms; intensity threshold 2e4; dynamic exclusion 30 sec. Data-dependent MS2 selection is performed in Top Speed mode with HCD collision energy set to 32%, AGC target 1e5 and maximum injection time 100 ms.
- *Data Analysis:* Raw data files were analyzed using Thermo Scientific™ Proteome Discoverer™ 2.2 and results were filtered using a 1% protein FDR threshold. Further pathway analysis is performed using Qiagen Ingenuity® Pathway Analysis (IPA®).
- *Flow Cytometry:* PBMCs from 3 different donors were treated with 1 μM KZR-261 or different concentrations of KZR-834 for 24 hours. Protein surface expression was examined on live cell populations by flow cytometry. Median fluorescence intensity (MFI) was measured, and % of DMSO was calculated.

Background

Figure 2. KZR-261 and KZR-834 demonstrate anti-tumor activity *in vitro* and *in vivo* with minimal impact on non-transformed cells[1,2]

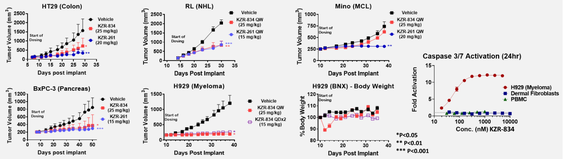


Figure 3. Sub-cellular fractionation workflow to obtain F1 (Cytosolic) and F2 (Membrane/Organelle)

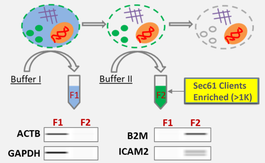
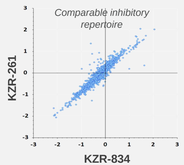
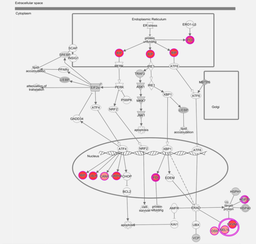


Figure 4. KZR-261 and KZR-834 display a similar proteomic profile in F2 analysis



Results – F1 (Cytosolic)

Figure 5. KZR-834 treatment induces an unfolded protein response in H929 cells



ProteinID	Gene	logFC_H929_F1	adjP.Val_H929_F1
P27797	CALR	2.55	1.78E-07
P11021	BiP	2.41	1.78E-07
P07237	PDI	2.39	1.78E-07
P07339	CTSD	1.63	2.34E-06
P27824	CANX	1.40	3.70E-04

Figure 6. IPA® pathway analysis highlights activating transcription factor 4 (ATF4) in H929 cells following KZR-834 treatment

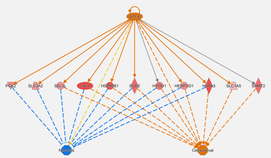
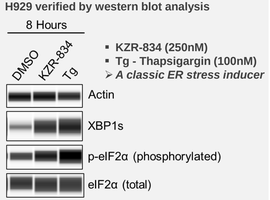


Figure 7. Activation of ER stress response in H929 verified by western blot analysis



Results – F2 (Membrane/Organelle)

Table 1. Tumor cell lines used in global proteomic study and sensitivity to KZR-834 and KZR-261

Cell Line	Type	IC50 [KZR-834] (nM)	IC50 [KZR-261] (nM)
H929	Multiple myeloma	177	91
U266	Multiple myeloma	>10000	3451
CAL27	Head & Neck	124	75
SNU899	Head & Neck	786	389
A549	Non-small cell lung cancer	89	59
KYSE180	Esophageal tumor	44	32
BxPC-3	Pancreatic cancer	95	92
MIA PaCa-2	Pancreatic cancer	316	239

Figure 8. Following 24 hours of exposure to 250 nM KZR-834, only 9% of clients decreased ≥ 2-fold

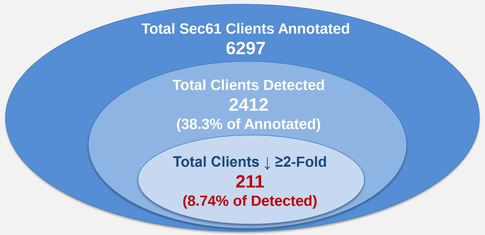


Figure 9. Dose dependent inhibition of Sec61 Clients in CAL27 cells F2 fraction

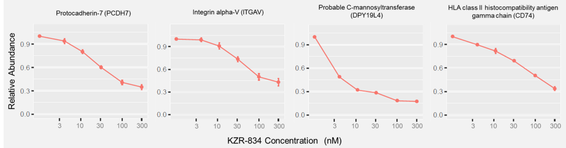


Table 2. KZR-834 preferentially targets Type I and Secreted TM proteins relative to Multi-pass TM proteins in multiple tumor cell lines

	BxPC-3		KYSE180		MIA PaCa-2		A549	
	# Detected	↓ ≥2-fold %	# Detected	↓ ≥2-fold %	# Detected	↓ ≥2-fold %	# Detected	↓ ≥2-fold %
Type I Clients	264	**11.0%**	238	**12.2%**	208	**8.2%**	240	**8.3%**
Other Clients (Total - Type I)	1128	**1.5%**	1060	**4.3%**	1012	**1.4%**	1051	**2.4%**
Secreted Clients	343	**3.5%**	325	**7.7%**	310	**3.2%**	304	**4.3%**
Other Clients (Total - Secreted)	1049	**3.2%**	973	**5.1%**	910	**2.3%**	987	**3.2%**
MultiTM Clients	627	**0.5%**	595	**2.0%**	565	**0.2%**	601	**1.0%**
Other Clients (Total - MultiTM)	765	**5.6%**	703	**9.0%**	655	**4.6%**	690	**5.7%**

Results – PBMCs

Figure 10. Treatment of PBMCs with 1 μM KZR-261 for 24 hours, results in reduced expression in only 3% of detected Sec61 clients

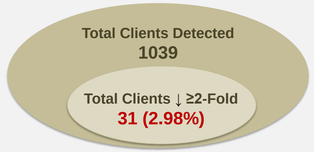


Table 3. Flow cytometric analysis of select Sec61 clients in PBMCs

Molecule	Gene	Sec61	log2FC_F2
CD127	IL7R	type I	-1.8
CD62L	SELL	type I	-1.0
ICAM1	ICAM1	type I	-1.3
B2M	B2M	secreted	-1.0
HLA-DR	HLA-DRB3	type I	-1.1
	HLA-DRB4	type I	-1.0

Figure 11. KZR-261 treatment blocks surface expression of sensitive Sec61 clients on different immune cell populations

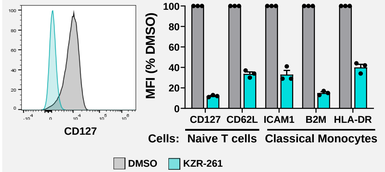
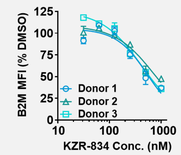


Figure 12. KZR-834 induces a dose-dependent reduction of B2M surface expression



Conclusions

- KZR-261 is a broad anti-cancer agent that induces tumor cell specific effects via inhibition of Sec61
- Quantitative proteomic profiling was used to profile Sec61 client inhibition in tumor cells and non-transformed cells to elucidate anti-cancer mechanism and identify potential pharmacodynamic markers
- Less than 10% of measured Sec61 clients were reduced in expression following exposure to KZR-834 in tumor cells and KZR-261 inhibited <5% clients in PBMCs
- Preferential inhibition of Secreted and Type I TM proteins relative to Multi-pass TM proteins was seen in multiple tumor cell lines
- KZR-261 will be studied in an upcoming Phase 1 study in solid tumors and sensitive cell surface markers identified here represent potential markers of drug activity in patients

References

1. Lowe, et al. ASH Annual Meeting 2019. Abstract 408
2. Lowe, et al. ASCO Annual Meeting 2020. Abstract 3582
3. Puyenbroeck, et al. Cellular and Molecular Life Sciences. (2018) 75: 1541-1558.
4. Morel, et al. Mol Cell Proteomics. (2018) 17(9):1750-1765.
5. Jiang, et al. Thermo Fisher Scientific Application Note 649. (2016).
6. Guo, et al. Tumour Biology. (2015) 36(3): 1711-1720.

Presented at [Virtual AACR Annual Meeting 2021] • [South San Francisco], [CA / USA] • [April 10th – June 21st , 2021]