3582

Preclinical evaluation of KZR-261, a novel small molecule inhibitor of Sec61

Eric Lowe, R. Andrea Fan, Jing Jiang, Henry W.B. Johnson, Christopher J. Kirk, Dustin McMinn, Beatriz Millare, Tony Muchamuel, Yu Qian, Brian Tuch, Jennifer A. Whang, Patricia Zuno

Kezar Life Sciences, Inc. South San Francisco, CA

Contact: elowe@kezarbio.com

BACKGROUND

- **The Sec61 translocon regulates the translocation of nearly all secreted and transmembrane proteins into the endoplasmic reticulum (ER)**
- **Recognition of unique signal peptides by the signal recognition particle (SRP) targets nascent peptides to the SRP receptor (SR) for translocation through Sec61 into the ER (Figure 1A)**
- **Blockade of Sec61 represents a novel therapeutic target for inhibition of angiogenic factors, oncogenic receptors, and immune checkpoint molecules (Figure 1B)**
- **Known inhibitors of Sec61[2-5] have demonstrated anti-tumor activity but lack adequate pharmaceutical properties or tolerability for clinical development**
- **KZR-261 and KZR-834 are closely related analogs which represent our lead series of novel Sec61 inhibitors and show increased selectivity and tolerability for treatment of solid tumors and hematologic malignancies**

Figure 1A. Sec61 translocon[1]

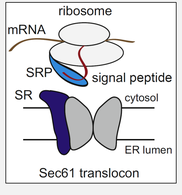


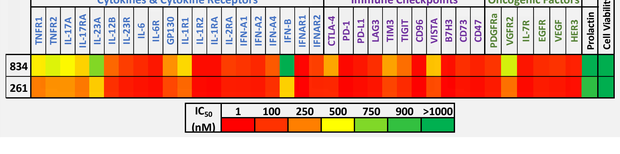
Figure 1B. Types of Sec61 client proteins

METHODS

- Target protein secretion was monitored using a reporter expression system in Flp-In T-REx™ 293 cells induced with doxycycline alongside compound treatment for 24 hours (Figure 2)
- Global proteomic analysis was performed on cell lines (H929, U266 – Myeloma; CAL27, SNU899 – HNSCC) treated with 250nM KZR-261 or KZR-834 for 24 hours. Lysates were trypsin digested followed by TMT labeling and LC-MS/MS analysis (Figures 3, 4)
- Viability was assessed in 346 human cancer cell lines treated with KZR-834 for 72 hours (Figure 5)
- Cell viability (CellTiter-Glo®) and Caspase 3/7 activity (Caspase-Glo®) were measured after 24 hours (Figures 5, 6)
- Gene expression profiling of cell lines treated with 250nM KZR-834 for 4 hours was conducted by RNA-Seq followed by fGSEA analysis (Figure 6)
- Expression of the GO_RESPONSE_TO_ENDOPLASMIC_RETICULUM_STRESS gene module was analyzed across cell lines and primary tumors profiled in the Cancer Cell Line Encyclopedia (CCLE) and The Cancer Genome Atlas (TCGA; Figure 7)
- Female BNX or athymic nude mice were implanted with $5x10^6$ H82, HT29, BxPC-3, 22Rv1, $5x10^6$ Mino, $1x10^7$ RL (athymic nude) or $3x10^7$ H929 (BNX) cells with Matrigel® (1:1) subcutaneously in the right flank. When tumors reached the appropriate tumor volume, mice were size matched into treatment and control groups (n=10 per treatment group). KZR-261 and KZR-834 were administered IV once weekly. Etoposide was administered IP every other day (Figures 8, 9)
- Female C57BL/6 mice were implanted with $5x10^5$ MC38 cells subcutaneously in the flank. KZR-834 was administered IV once weekly. Anti-PD-1 RMP1-14 antibody was administered IP biweekly (Figure 10)
- Tumor growth delay calculated as the increase in time to endpoint compared to vehicle control group (Table 1)

RESULTS

Figure 2. Sec61 inhibitors KZR-261 and KZR-834 block expression of many therapeutically relevant targets in reporter screen



RESULTS

Figure 3. KZR-834 has a limited effect on overall Sec61 client expression

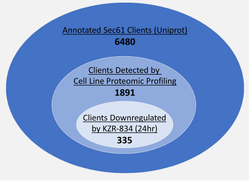


Figure 4. KZR-261 and 834 have equivalent effects on global protein expression

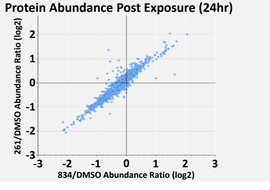


Figure 5. KZR-834 exhibits broad anti-tumor activity with minimal effect on non-transformed cells

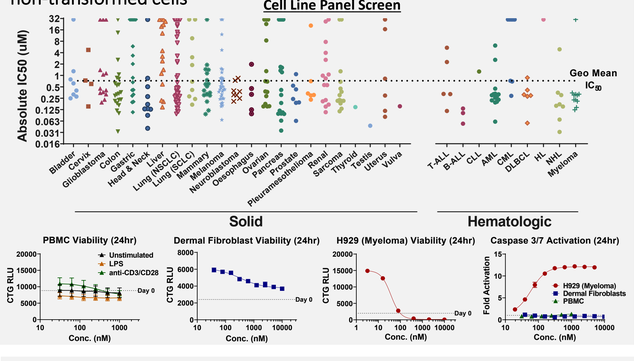


Figure 6. Gene expression profiling reveals differential ER stress response in KZR-834 treated sensitive (H929) and resistant (U266) myeloma cell Lines

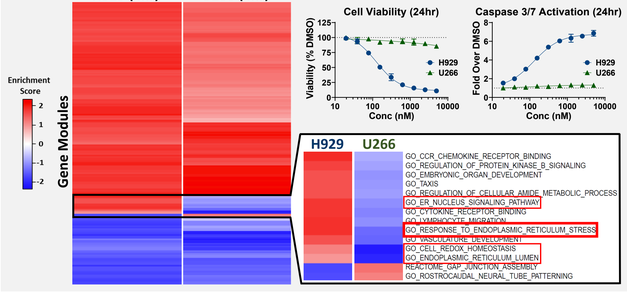


Figure 7. ER stress response gene module expression in cell line and primary tumor samples suggests multiple potential sensitive tumor types

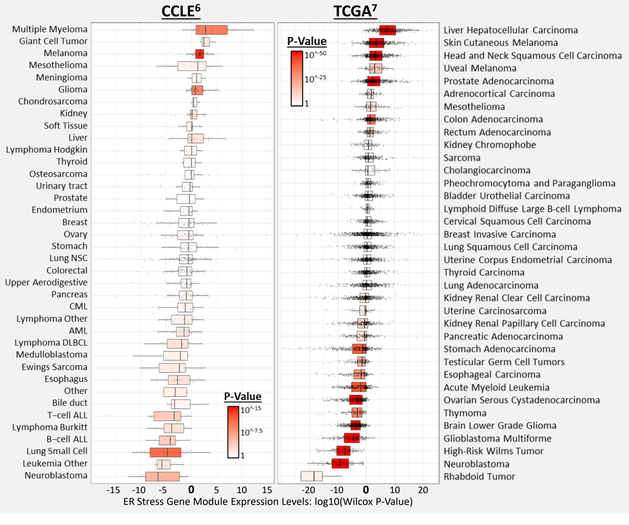


Figure 8. Weekly administration of KZR-261 and KZR-834 results in dose dependent tumor growth inhibition in solid tumor xenograft models

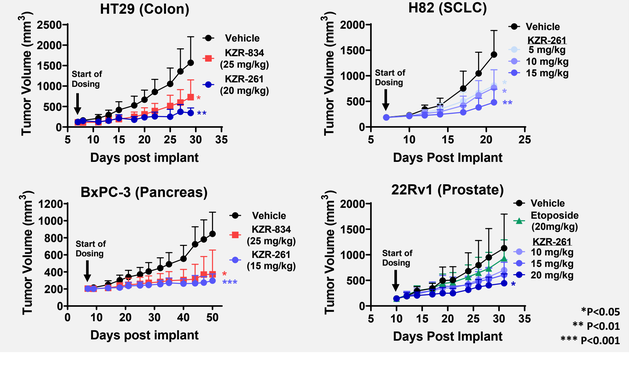


Figure 9. KZR-261 and KZR-834 induce anti-tumor response in multiple hematologic xenograft models at well tolerated doses

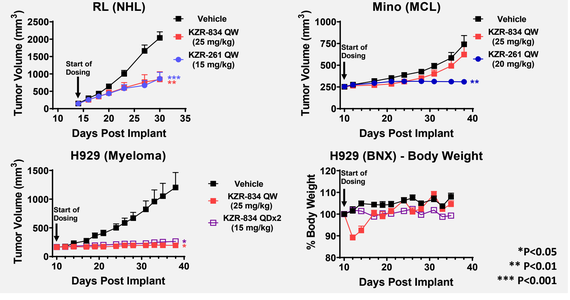


Figure 10. KZR-834 synergizes with anti-PD1 therapy in a syngeneic mouse model of colorectal adenocarcinoma

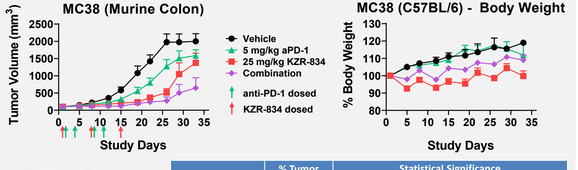


Table 1. Response summary of MC38 model

Treatment	% Tumor Growth Delay	Statistical Significance		
		vs Vehicle	vs 25mg/kg KZR-834	vs anti-PD-1
25 mg/kg KZR-834	52	**	-	ns
anti-PD-1	22	*	ns	-
Combo	114	***	**	***

ns – not significant; * P<0.05; ** P<0.01; *** P<0.001

CONCLUSIONS

- **Sec61 is a promising therapeutic target for multiple solid tumors and hematologic malignancies**
- **Sec61 inhibition results in blockade of several therapeutically relevant proteins involved in tumor growth, metastatic spread and immune evasion**
- **Sec61 inhibition results in an ER stress response in sensitive, but not resistant tumor cells, and warrants further study as a potential biomarker**
- **Novel Sec61 inhibitors exhibit broad anti-cancer activity with minimal adverse effects in vitro and in vivo**
- **Clinical trials with KZR-261 are being planned to understand safety and efficacy in patients with solid tumors**

REFERENCES

1. Maifeld, et al. *Cell Chem. & Bio.* 2011, 18: 1082
2. Garrison, et al. *Nature.* 2005, 436: 285
3. Paatero, et al. *Cell Chem. & Bio. 2016, 23: 561*
4. Juune, et al. *J. Cell Sci.* 2015, 128: 1217
5. Baron, et al. *J. Exp. Med.* 2016, 213: 2885
6. Cancer Cell Line Encyclopedia. *https://portals.broadinstitute.org/ccle*
7. The Cancer Genome Atlas. *https://www.cancer.gov/tcga*