Prioritizing tumor types for clinical study of novel Sec61 inhibitors by searching for expression profiles of sensitive cell lines in tumor sample databases

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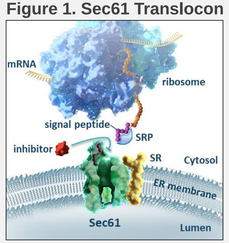
Introduction

- The Sec61 translocon is the primary mediator for cotranslational translocation of secreted and transmembrane proteins into the endoplasmic reticulum and provides a novel therapeutic target for blocking expression of Sec61 client proteins such as cytokines, oncogenic receptors, and immune checkpoint molecules (Figure 1)
- KZR-261 is a novel small-molecule inhibitor of the Sec61 translocon which has been shown to potently inhibit many therapeutically relevant targets with minimal effects on global protein secretion[1]
- KZR-261 displays broad anti-tumor activity in vitro and in vivo at well tolerated doses and has been nominated for clinical development[2]
- Goal: Utilize in vitro sensitivity and gene expression data from cell lines and patient samples to predict tumor-intrinsic vulnerabilities to Sec61 inhibition and inform selection of expansion cohorts for a first-in-human study of KZR-261 in solid tumor malignancies

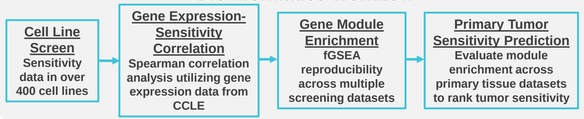
Figure 1. Sec61 Translocon



Methods

- In vitro anti-tumor activity of KZR-834, a KZR-261 analog, was characterized in 2 independent cell line panel screens (N=346, Screen 1; N=174, Screen 2) following treatment for 72 hours and cell viability measured by CellTiter-Blue® or Cell-TiterGlo ® (Figure 3A)
- In vitro anti-tumor activity of KZR-261 and KZR-834 were compared across 47 cancer cell lines (Screen 3) treated for 72 hours followed by cell viability measurement with CellTiter-Glo ® (Figure 3B)
- Sensitivity (AUC) of a subset of cell lines (N=232) was compared to the log expression of each gene (Fragments Per Kilobase of transcript per Million reads; FPKM) by Spearman correlation using available gene expression data in the Cancer Cell Line Encyclopedia (CCLE[3]; Figure 4)
- Sensitivity-expression associations were examined for enrichment of gene modules by performing fGSEA[4] with a large database of modules primarily sourced from mSigDB[5]. A subset of non-redundant modules (Figure 5A) that is reproducibly enriched across the 3 screens (Figure 5B) was carried forward
- A database of gene expression profiles of normal and primary tumor tissues, combining data from TCGA[6], TARGET[7] and GTEx[8] was evaluated for enrichment of modules that associated with sensitivity to KZR-834 in vitro (Figure 6)

Bioinformatics Workflow



Results

Figure 2. Uniform Expression of Sec61 Across Tumor and Adjacent Tissues

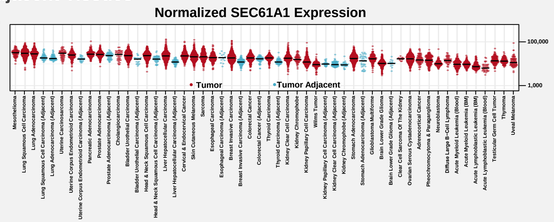


Figure 3. Broad In Vitro Anti-Tumor Activity of KZR-834 and KZR-261

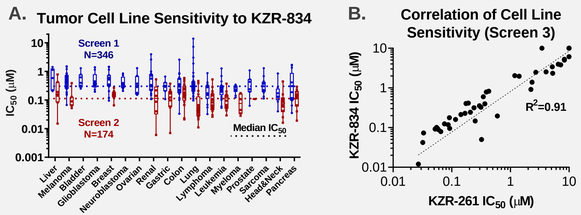
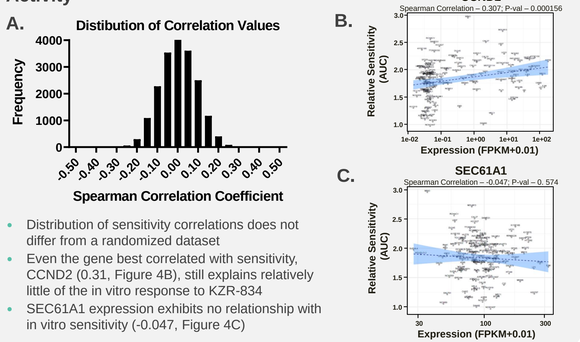


Figure 4. No Individual Gene Level Correlation with Anti-Tumor Activity



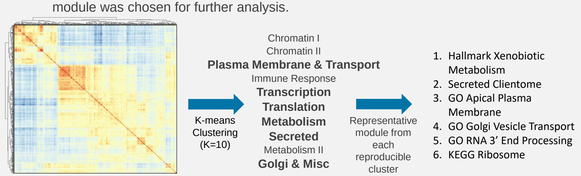
- Distribution of sensitivity correlations does not differ from a randomized dataset
- Even the gene best correlated with sensitivity, CCND2 (0.31, Figure 4B), still explains relatively little of the in vitro response to KZR-834
- SEC61A1 expression exhibits no relationship with in vitro sensitivity (-0.047, Figure 4C)

Results

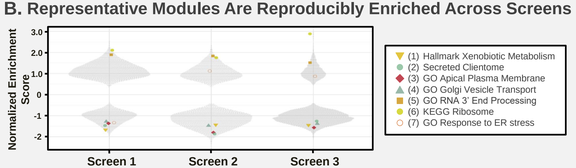
Figure 5. Gene Module Expression Reproducibly Associated with Cell Line Sensitivity

A. Selection of Representative Modules Associated with Sensitivity

- 663 modules enriched in both Screen 1 and Kezar screens were clustered based on gene overlap to identify common themes
- From each cluster that reproduced in Screen 2 (**bolded below**), a representative module was chosen for further analysis.

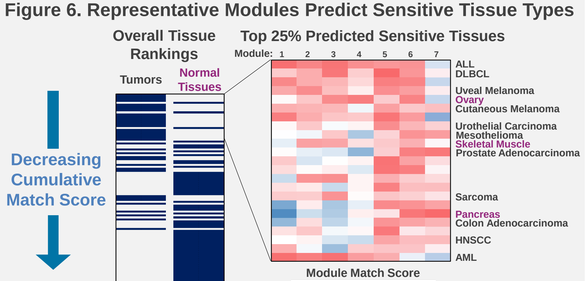


B. Representative Modules Are Reproducibly Enriched Across Screens



- Although modules related to ER stress were not enriched, previous studies have found strong activation of the pathway in cells sensitive to Sec61 inhibition[9] leading to its inclusion in subsequent analysis

Figure 6. Representative Modules Predict Sensitive Tissue Types

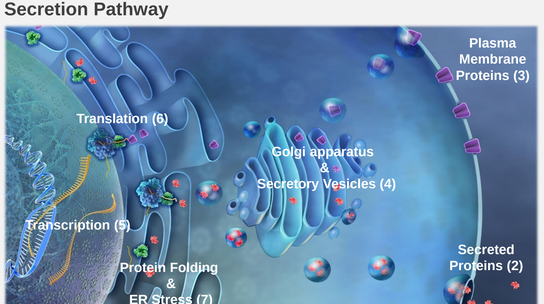


- Tissue types were ranked by their expression of each of the 7 representative modules and ranks were summed to generate a Cumulative Match Score (CMS) for each tissue
- Tumors tend to have higher CMSs than normal tissues
- The tissues with the highest CMSs include many solid tumors for future study

Conclusions

- KZR-261 and KZR-834 exhibit broad anti-cancer activity across many different tumor types in vitro
- No single gene (including SEC61A1) sufficiently predicts tumor sensitivity
- The expression of 6 representative gene modules is reproducibly associated with sensitivity across 3 screens
- Representative modules identified through mechanism agnostic analysis show high overlap with key processes involved in protein secretion (Figure 7)
- Examination of these modules in primary tumor and tissue expression datasets predicts that tumors will be more sensitive than normal tissues
- These gene modules were used to rank tumor types, prioritizing them for clinical trials
- A Phase 1 study is planned in multiple solid tumors to understand the safety and anti-tumor activity of KZR-261 in patients

Figure 7. Representative Modules Overlap with the Protein Secretion Pathway



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