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

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Introduction

• Sec61-mediated protein secretion and global modulation of protein homeostasis across multiple tumor cell lines (Table 1 and PBMCs).

• Sec61 inhibitors, particularly KZR-261 (clinical), and KZR-834 will be studied in an upcoming Phase 1 study in solid tumors and multiple myeloma.

• KZR-261 is a broad anti-cancer agent that induces tumor cell specific effects via inhibition of Sec61.

Methods

• Sec61 inhibitors, particularly KZR-261 (clinical), and KZR-834 were identified through a medicinal chemistry campaign and found to have broad anti-tumor activity in vitro and in vivo (Figure 1).

• Preferential inhibition of Secreted and Type I TM proteins relative to MultiTM proteins was seen in multiple tumor cell lines (Table 3).

• A549 cells were treated with 1 µM KZR-261 for 24 hours, results in reduced expression in only 3% of clients detected ≥ 2-fold.

Background

• Subcellular Fractionation: Data Analysis

• 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).

• Secreted and transmembrane (TM) proteins play key roles in carcinogenesis and tumor progression.

Results – F1 (Cytosolic)

• Flow Cytometry: PBMCs ban 5 different markers were treated with 1 µM KZR-261 or different concentrations of KZR-834 for 24 hours. Data analysis was performed using FlowJo software and analysis was performed using FlowJo software.

• Type I clients (Sec61 clients) were preferentially inhibited by KZR-261 and KZR-834, only 9% of clients decreased ≥ 2-fold.

• In vitro: KZR-261 and KZR-834 demonstrated anti-tumor activity in vitro and in vivo with minimal impact on non-tumor cell lines.

Results – F2 (Membrane/Organelle)

• For cellular fractionation, subcellular fractions were collected using a commercial proteoextract kit and analyzed using high sensitivity liquid chromatography and tandem mass spectrometry (Table 1).

• Sec61 clients avoiding the ER were found to have broad anti-tumor activity in vitro and in vivo (Figure 2).

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Results – PBMCs

• In vitro: KZR-261 and KZR-834 demonstrated anti-tumor activity in vitro and in vivo with minimal impact on non-tumor cell lines.

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Conclusions

• A549 cells were treated with 1 µM KZR-261 for 24 hours, results in reduced expression in only 3% of clients detected ≥ 2-fold.

• In vitro: KZR-261 and KZR-834 demonstrated anti-tumor activity in vitro and in vivo with minimal impact on non-tumor cell lines.

• 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 biomarkers.

• Less than 10% of measured Sec61 clients were reduced in expression following exposure to KZR-834 in tumor cells and KZR-261 inhibited ≥5% in PBMCs.

• Preferential inhibition of Sec61 was found in multiple tumor cell lines.

• KZR-261 was well tolerated in an upcoming Phase I study in solid tumors and sensitive cell surface markers identified here represent potential markers of drug activity in patients.

References