Sec61 inhibitor KZR-834, an anti-cancer agent, exhibits immunomodulatory activity and combines with PD-1 blockade to further enhance immune responses

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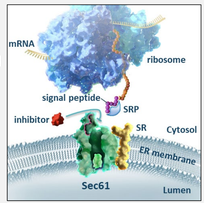
5592

Introduction

- The Sec61 complex facilitates the cotranslational translocation of secreted and transmembrane proteins by directing nascent polypeptides through the channel into the endoplasmic reticulum (ER) for expression and function (Figure 1).[1,2]
- Blockade of Sec61 is a novel therapeutic approach to targeting diverse pro-tumorigenic proteins.
- KZR-261 and KZR-834, a structural analog, are small molecule Sec61 inhibitors that potently block various cancer and immune checkpoint-associated proteins as well as activate the ER stress pathway in tumor cell lines sensitive to Sec61 inhibition.[3,4]
- KZR-261 and KZR-834 have broad anti-cancer activity *in vitro* and *in vivo* across multiple solid and hematologic tumor types.[4,5]
- KZR-261 is currently in a Phase I clinical trial in patients with advanced solid tumors (NCT05047536).
- The MC38 tumor model, a model responsive to PD-1 blockade, was selected to characterize the immunomodulatory potential of Sec61 inhibitors.

Figure 1. Sec61 translocon



Methods

MC38 tumor model

- C57BL/6 mice were implanted with MC38 cells, and mice were randomized when tumors reached a mean volume between 140-175 mm^3 (Study Day 1). Animals were dosed with vehicle or 25 mg/kg KZR-834 IV QWx3 and/or 5 mg/kg anti-PD-1 (RMP1-14) IP BIWx2 (n=10 per group). Animals were euthanized when tumors reached >2000 mm^3 or Study Day 45 (end of study). The percent tumor growth delay (%TGD) was calculated as the median time to endpoint in a treatment group compared to the control group. Statistical significance was determined with the logrank test.

Flow cytometric analysis

- MC38 bearing mice were treated with 25 mg/kg KZR-834 IV on Day 1 and 8 and/or 5 mg/kg anti-PD-1 IP on Day 1, 4, and 8. Tissues were collected on Study Day 9. Single cell suspensions were generated from dissociated tissues. Cell populations were identified by flow cytometry. Statistical significance was determined with the Kruskal-Wallis test. Alternatively, tumor tissues were evaluated for immune landscape signatures by RNA-seq analysis (Q^2 Solutions EA Genomics). Scores were standardized to the mean score of the study.

Immunogenic cell death

- MC38 cells were treated *in vitro* with the indicated concentrations of KZR-834 for 48 hours. Apoptosis was measured by flow cytometry with Annexin V and 7AAD labeling.
- Calreticulin (CRT) expression was assessed on 7AAD negative cells by flow cytometry.
- Culture supernatants were collected to evaluate lactate dehydrogenase (LDH) and high mobility group box 1 (HMGB1) release. LDH was measured with LDH-Glo™ cytotoxicity assay. HMGB1 was measured with Lumit™ HMGB1 immunoassay kit.

Results

Figure 2. KZR-834 and anti-PD-1 demonstrate enhanced anti-tumor response in the syngeneic MC38 colon carcinoma model

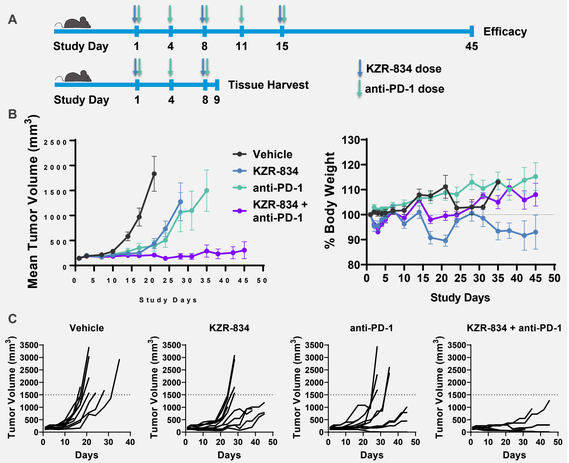
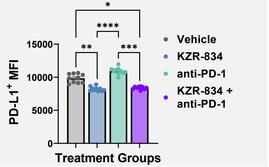


Table 1. Significant tumor growth delay observed with KZR-834 and anti-PD-1 combination treatment

		Statistical Significance Versus			Regression	
Groups	%TGD	Vehicle	KZR-834	anti-PD-1	PR	CR
Vehicle	-	-	*	**	0	0
KZR-834	40%	*	-	ns	0	0
anti-PD-1	66%	**	ns	-	0	1
KZR-834 + anti-PD-1	127%	***	*	*	0	3

Abbreviations: TGD = tumor growth delay, PR = partial regression, CR = complete regression, ns = not significant, *p<0.05, **p<0.01, ***p<0.001, compared to indicated group

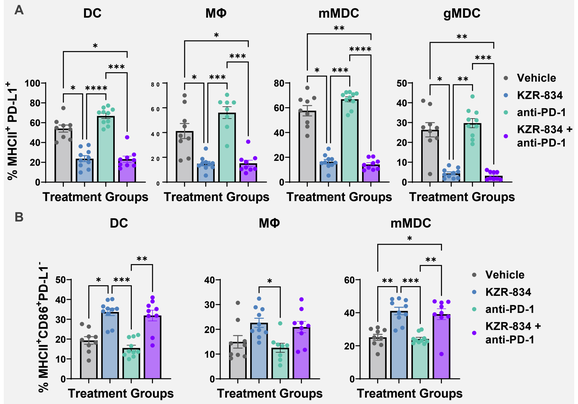
Figure 3. KZR-834 treatment reduces PD-L1 expression on tumor cells



Abbreviations: MFI = median fluorescence intensity, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to indicated group

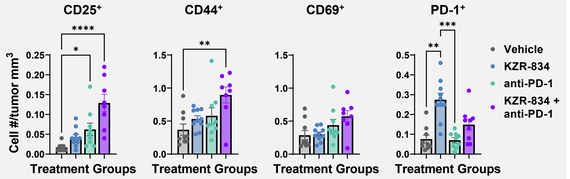
Results (cont'd)

Figure 4. Reduced frequencies of MHCII$^+$PD-L1$^+$ myeloid cells (A) and increased frequencies of MHCII$^+$CD86$^+$ PD-L1$^-$ myeloid cells (B) were detected in the tumor with KZR-834 treatment



Abbreviations: DC = CD11c$^+$ dendritic cells, MΦ = CD11b$^+$F4/80$^+$ macrophages, mMDC = CD11b$^+$Ly6C$^+$ monocytic myeloid-derived cells, gMDC = CD11b$^+$Ly6G$^+$ granulocytic myeloid-derived cells, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to indicated group

Figure 5. Combination treatment with KZR-834 and anti-PD-1 increased numbers of activated CD8 T cells in the tumor

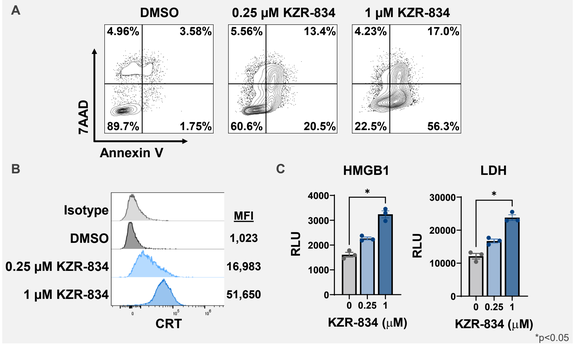


*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared to indicated group

Figure 6. Anti-tumor gene signatures increased with combination treatment

Results (cont'd)

Figure 7. *In vitro* treatment with KZR-834 induces immunogenic cell death with CRT expression and HMGB1 release



*p<0.05

Conclusions

- KZR-834 is a potent anti-cancer agent that demonstrated immunomodulatory effects in the MC38 syngeneic tumor model.
- KZR-834 and anti-PD-1 combination treatment significantly delayed tumor growth, and complete tumor regression was observed in 3 of 10 animals.
- KZR-834 treatment alone decreased PD-L1 expression while increasing the frequency of MHCII$^+$CD86$^+$PD-L1$^-$ antigen presenting cells.
- Such combination treatment elevated the numbers of activated CD8 T cells that were detected within the tumor.
- With combination therapy, an increase in anti-cancer gene signatures was identified via RNA-seq analysis using an exploratory immune landscape signature.
- Immunogenic programmed cell death characterized by CRT expression and HMGB1 release was detected after *in vitro* KZR-834 treatment.
- Taken together, the enhanced immune profile with KZR-834 and anti-PD-1 combination treatment supports further investigation into combining KZR-834 with other immune checkpoint inhibitors to potentiate anti-tumor activity.

References

1. Park E, et. al. Annu Rev Biophys. 2012, 41: 1
2. Maifeld SV, et. al. Cell Chem & Bio. 2011, 18: 1082
3. Lowe, et al. ASH Annual Meeting 2019. Abstract 408
4. Lowe, et al. ASCO Annual Meeting 2020. Abstract 3582
5. Lowe, et al. AACR Annual Meeting 2019. Abstract 3087

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