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SHORT REPORT

Inhibition of the Sec61 translocon overcomes cytokine-induced glucocorticoid resistance in T-cell acute lymphoblastic leukaemia

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Abstract

Glucocorticoid (GC) resistance is a poor prognostic factor in T-cell acute lymphoblastic leukaemia (T-ALL). Interleukin-7 (IL-7) mediates GC resistance via GC-induced upregulation of IL-7 receptor (IL-7R) expression, leading to increased pro-survival signalling. IL-7R reaches the cell surface via the secretory pathway, so we hypothesized that inhibiting the translocation of IL-7R into the secretory pathway would overcome GC resistance. Sec61 is an endoplasmic reticulum (ER) channel that is required for insertion of polypeptides into the ER. Here, we demonstrate that KZR-445, a novel inhibitor of Sec61, potently attenuates the dexamethasone (DEX)-induced increase in cell surface IL-7R and overcomes IL-7-induced DEX resistance.

KEYWORDS
cytokine, glucocorticoids, Sec61 inhibitor, T-cell acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy, with T-cell ALL (T-ALL) accounting for approximately 15% of cases.[1] While outcomes for children diagnosed with T-ALL have improved dramatically over the past several decades, survival rates for patients with relapsed or refractory disease remain dismal.[2] This indicates a need for strategies to enhance the efficacy of frontline treatment protocols in order to induce deeper remissions and decrease the likelihood of disease relapse.

Glucocorticoids (GCs) are a crucial component of T-ALL therapy due to their potent pro-apoptotic effects in lymphoid cells.[3] They also represent a critical target for strategies aimed at improving clinical outcomes in paediatric T-ALL, as GC sensitivity at diagnosis is an important prognostic factor. Specifically, in patients who receive up-front GC monotherapy, those who clear their peripheral blasts over the first eight days have significantly improved event-free survival (EFS) and a decreased risk of relapse relative

Jack Taunton, Christopher J. Kirk, and Michelle L. Hermiston contributed equally.

to those patients with a poor GC response.[4] Similarly, GCs are central to multi-agent induction therapy regimens, and minimal residual disease (MRD) at the end of induction is associated with inferior EFS and overall survival.[5]

Through *ex vivo* analyses, we have demonstrated that approximately one-third of diagnostic T-ALL samples are resistant to dexamethasone (DEX), a synthetic GC used during induction therapy, specifically when cultured in the presence of interleukin-7 (IL-7),[6] a pro-survival cytokine that has previously been implicated in T-ALL pathogenesis and disease maintenance.[7] Mechanistically, we have demonstrated in primary T-ALL samples and in a cell line model of IL-7-induced DEX resistance that DEX paradoxically induces its own resistance by upregulating IL-7 receptor (IL-7R) expression. In the presence of IL-7 ligand, this leads to an increase in pro-survival signalling through the IL-7R/JAK/STAT5 signalling axis and subsequent upregulation of the pro-survival protein BCL-2, which directly antagonizes DEX-induced apoptosis. In these cells, DEX resistance can be effectively overcome with ruxolitinib (RUX), a JAK1/2 inhibitor, and with venetoclax, a BCL-2 inhibitor.[8] Given that increased cell surface IL-7R expression is a critical component of this resistance mechanism, we hypothesized that therapeutic strategies to inhibit the DEX-induced upregulation of cell surface IL-7R would similarly overcome IL-7-induced DEX resistance in T-ALL.

Secreted and membrane-associated proteins, including IL-7R, require processing through the cellular secretory pathway to reach the cell surface. These proteins are targeted to the secretory pathway by the translation of a signal sequence on the nascent peptide, which is subsequently recognized by the Sec61 complex.[9] The Sec61 complex is a component of the mammalian translocon, a protein-conducting channel on the membrane of the endoplasmic reticulum (ER). Upon recognition of the signal sequence, these peptides undergo productive translocation into the lumen of the ER, where post-translational processing is initiated to produce functional secreted or membrane-associated proteins.[9] Small-molecule inhibitors of the Sec61 translocon act in a signal sequence-specific manner to block the interaction of these proteins with Sec61, thereby preventing their translocation into the ER and ultimately their secretion or expression at the cell surface.[10] Sec61 inhibitors have previously shown preclinical efficacy both as antimicrobial and anti-cancer agents,[11,12] and one Sec61 inhibitor, KZR-261, is currently in a phase 1 clinical trial for adults with advanced solid malignancies (NCT05047536). In this study, we asked whether inhibition of the Sec61 translocon can overcome IL-7-induced DEX resistance in T-ALL cells by preventing cell surface localization of newly-synthesized IL-7R.

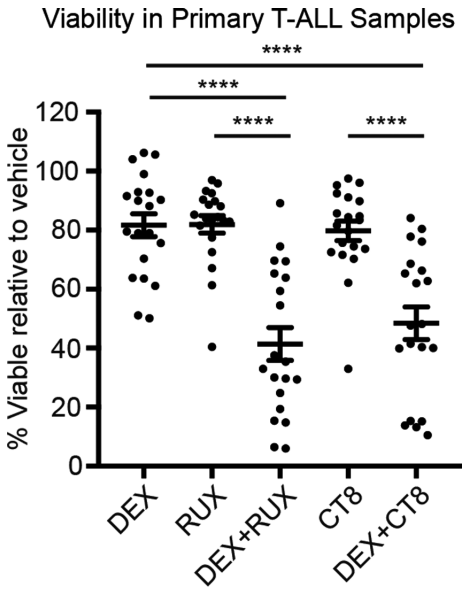
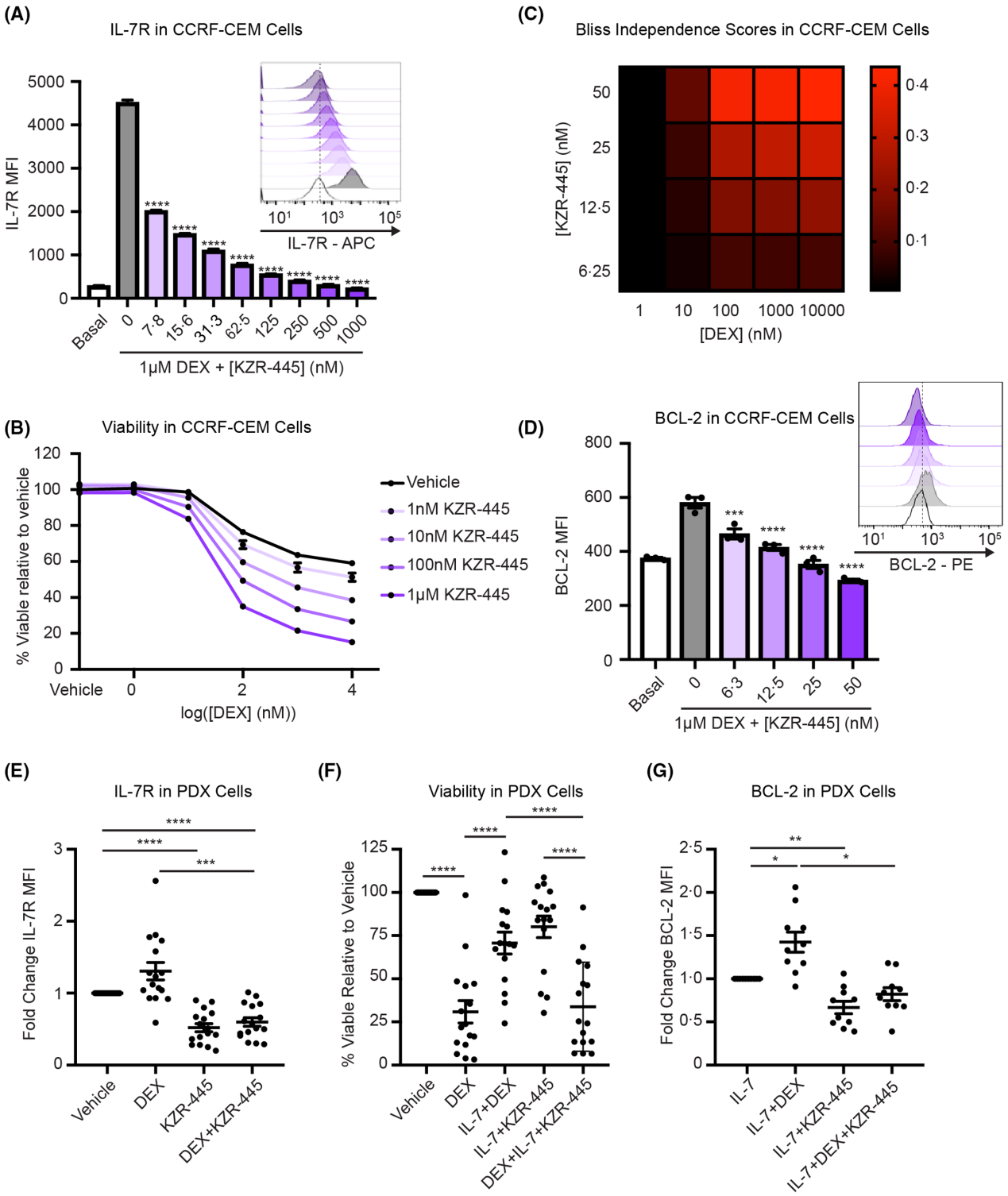


FIGURE 1 CT8 overcomes IL-7-induced DEX resistance in patient-derived T-ALL cells. Viability relative to vehicle control of cells from 20 fresh diagnostic T-ALL samples cultured in the presence of 25 ng/ml IL-7 with or without 2.5 μM DEX and/or 500 nM RUX or 2 μM CT8 for 48 h. Error bars represent SEM. Statistical significance was assessed using one-way ANOVA with Tukey's method for adjustment for multiple comparisons. ****, $p < 0.0001$

First, we assessed the efficacy of this strategy using CT8, a tool compound belonging to a class of cyclic heptadepsipeptides known as cotransins, which function to inhibit cotranslational translocation via the Sec61 translocon. We studied 20 diagnostic T-ALL samples (Table S1) that demonstrate DEX resistance in the presence of IL-7, which we defined as retaining greater than 50% viability relative to the vehicle control condition. We exposed these cells to DEX plus IL-7, either alone or in combination with RUX or CT8. In this analysis, RUX and CT8 both sensitized cells to DEX with minimal single-agent toxicity ($p < 0.0001$ for the addition of RUX or

FIGURE 2 KZR-445 modulates cell surface IL-7R expression and overcomes IL-7-induced DEX resistance in CCRF-CEM cells and primary patient samples. (A) Median fluorescent intensity (MFI) and representative histograms of cell surface IL-7R in CCRF-CEM cells treated with or without 1 μM DEX and/or increasing concentrations of KZR-445 for 24 h in technical triplicate. (B) Viability relative to vehicle control of CCRF-CEM cells treated with increasing concentrations of DEX in the presence of 25 ng/ml IL-7 and the indicated concentrations of KZR-445 for 72 h in technical triplicate. (C) Heatmap of Bliss independence scores calculated as the average of technical triplicates for the combination of DEX and KZR-445 in CCRF-CEM cells cultured in the presence of 25 ng/ml IL-7 for 72 h, in which positive values, indicated in red, are indicative of a synergistic interaction. (D) MFI and representative histograms of BCL-2 protein expression in CCRF-CEM cells treated with 100 ng/ml IL-7 with or without 1 μM DEX and/or the indicated concentration of KZR-445 for 24 h in technical triplicate. Statistical significance is relative to the DEX-treated condition in the absence of KZR-445. (E) Fold change in the MFI of IL-7R in cells from 16 patient-derived T-ALL samples treated with 1 μM DEX with or without 50 nM KZR-445 for 24 h. (F) Viability relative to vehicle control of cells from 16 patient-derived T-ALL samples treated in the presence or absence of 25 ng/ml IL-7 with or without 1 μM DEX and/or 50 nM KZR-445 for 48 h. (G) Fold change in the MFI of BCL-2 in cells from 10 patient-derived T-ALL samples cultured in the presence of 100 ng/ml IL-7 and treated with or without 1 μM DEX and/or 50 nM KZR-445 for 24 h. Error bars represent SEM. Statistical significance was assessed using one-way ANOVA with Tukey's method for multiple comparisons adjustment. All cell line data are representative of three independent experiments. ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$ [Colour figure can be viewed at wileyonlinelibrary.com]



CT8 to IL-7 + DEX; Figure 1), suggesting that Sec61 inhibition, like JAK/STAT pathway inhibition, may be effective at augmenting DEX sensitivity in the presence of IL-7.

Based on these promising initial findings with CT8, we pursued further evaluation of this therapeutic strategy utilizing KZR-445, a novel analogue of CT8 that includes side chain modifications of the cyclic peptide intended to decrease lipophilicity and improve pharmaceutical properties. Specifically, KZR-445 is a fluorinated analogue of the CT8 derivative PS3061.[13] For these studies, we first used the CCRF-CEM T-ALL cell line, which we have shown to closely recapitulate the IL-7-induced DEX resistance phenotype observed in primary patient samples.[8] In these cells, the DEX-induced upregulation of cell surface IL-7R protein was effectively reduced by nanomolar concentrations of KZR-445 ($p < 0.0001$ for all concentrations of KZR-445;

Figure 2A). When we analysed expression of the *IL7R* transcript, we found that it was similarly induced upon exposure to DEX, and that this was unchanged in the presence of KZR-445 (Figure S1A), confirming that KZR-445 downregulates cell surface IL-7R expression via a post-translational mechanism.

All common γ-chain cytokine receptors, including IL-7R, and many other cell surface proteins important in T-cell biology, are Sec61 clients. To determine whether KZR-445 similarly modulates expression of these other cell surface proteins, we evaluated their expression in activated primary human CD8 T-cells or CCRF-CEM cells exposed to increasing concentrations of KZR-445. In this analysis, we found that the relative potency of KZR-445 for reducing cell surface expression of these proteins was significantly greater for IL-7R relative to other common γ-chain cytokine receptors (Figure S1B) and relative to the majority of the other cell surface proteins we evaluated (Figure S1C). This suggests that there may be a therapeutic window for targeting IL-7R in T-ALL without significant modulation of other proteins in healthy T-cells.

We next evaluated the effect of KZR-445 on DEX-induced cell death in CCRF-CEM cells. Here, we observed a dose-dependent effect of KZR-445 to overcome IL-7-induced DEX resistance (Figure 2B). Using Bliss independence analysis,[14] we further demonstrated potent synergy between DEX and KZR-445 in the presence of IL-7 (Figure 2C). To determine whether this effect is specific to DEX, we also exposed CCRF-CEM cells to other chemotherapies used in T-ALL treatment in the presence or absence of IL-7 and KZR-445. As previously reported,[6] we observed no effect of IL-7 to confer resistance to these other agents, and saw no significant change in drug-induced cell death with the addition of KZR-445 (Figure S2), suggesting specificity for activity in the setting of IL-7-induced DEX resistance.

Next, we evaluated the effect of KZR-445 on expression of the STAT5 target protein BCL-2, which is significantly upregulated upon the addition of DEX to cells exposed to IL-7 ($p < 0.0001$). The addition of KZR-445 inhibited the increase in BCL-2 protein expression in a dose-dependent manner (Figure 2D). Based on these findings, we asked whether KZR-445 and venetoclax, a BCL-2 inhibitor, would demonstrate combinatorial effects on cell viability in the presence of DEX and IL-7. While low nanomolar concentrations of venetoclax showed moderate single-agent efficacy, we saw no synergistic effect of these two compounds with the addition of increasing concentrations of KZR-445, consistent with the reduction in BCL-2 protein expression following exposure to KZR-445 (Figure S3).

Finally, following the observed efficacy of KZR-445 in CCRF-CEM cells, we assessed the utility of this compound in cells from 16 patient-derived xenografts (PDXs) established from diagnostic T-ALL samples (Table S1). In these cells, we found that *ex vivo* exposure to KZR-445 effectively inhibited the DEX-induced increase in cell surface IL-7R expression ($p = 0.0001$ for DEX + KZR-445 relative to DEX alone; Figure 2E). As observed with CT8, KZR-445 also overcame IL-7-induced DEX resistance ($p < 0.0001$ for the addition of KZR-445 to IL-7 + DEX), restoring DEX sensitivity to that observed in the absence of IL-7 (Figure 2F). This improvement in DEX sensitivity was further associated with a reduction in BCL-2 protein expression in the presence of DEX and IL-7 ($p = 0.01$ for the addition of KZR-445 to IL-7 + DEX; Figure 2G).

Taken together, these data suggest that small-molecule inhibitors of the Sec61 translocon represent an effective alternative to signal transduction inhibitors and BH3 mimetics for targeting cytokine-mediated pro-survival signalling in T-ALL. The IL-7R pathway plays a well-established role in leukemogenesis, with its importance underscored by the prevalence of gain-of-function mutations in IL-7R or components of the downstream signal transduction machinery.[15] We have further demonstrated that IL-7 induces GC resistance in up to one-third of paediatric T-ALL patients at diagnosis,[6] indicating the potential clinical impact of such a strategy. Despite the vast number of proteins that require trafficking through the cellular secretory pathway, the selectivity of Sec61 inhibitors is modifiable through medicinal chemistry approaches.[16,17] Consistent with this, we demonstrate relative selectivity of KZR-445 for IL-7R over other common γ-chain cytokine receptors. This supports the potential for further evaluation of Sec61 inhibitors for clinical use in T-ALL and other diseases with unique pathophysiologic or therapeutic dependencies on secreted or membrane-associated proteins.

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CONFLICT OF INTERESTS

Phillip P. Sharp holds patents and royalties at Kezar Life Sciences. David T. Teachey is a consultant for Janssen, Sobi, and BEAM Therapeutics and receives research funding from NeoImmune Tech and BEAM Therapeutics. Jack Taunton holds patents and royalties at Kezar Life Sciences, equity ownership at Global Blood Therapeutics, Principia Biopharma, and Cedilla Therapeutics, and receives research funding from Pfizer. Michelle L. Hermiston is a consultant for Novartis and Sobi.

AUTHOR CONTRIBUTIONS

Lauren K. Meyer, Cristina Delgado-Martin, Benjamin J. Huang, Christopher J. Kirk, Jack Taunton, and Michelle L.

Hermiston designed the experiments and analysed the data. Lauren K. Meyer and Cristina Delgado-Martin performed the experiments. Phillip P. Sharp designed and synthesized KZR-508445. Dustin McMinn provided KZR-508445 for experimental use. Tiffaney L. Vincent, Theresa Ryan, Terzah M. Horton, Brent L. Wood, and David T. Teachey provided patient cells for *ex vivo* analysis. Lauren K. Meyer and Michelle L. Hermiston wrote the manuscript. All authors reviewed the manuscript.

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SUPPORTING INFORMATION

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