Amiloride Derivative Compound 10357 in the Treatment of B-Cell Acute Lymphoblastic Leukemia

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Background

Compound 10357 is an amiloride derivative with potential promise as an adjunct to current chemotherapeutics. Previous studies by our collaborator Dr. Fredric Gorin's group showed that compound 10357 demonstrated mitochondrial depolarization with the release of apoptosis-inducing factor (AIF) and caspase-independent cell death in glioma cells.

Our group investigated the therapeutic efficacy of compound 10357 in B-cell acute lymphoblastic leukemia (B-ALL). Our prior studies demonstrated that compound 10357 was cytotoxic in a dose dependent manner in two B-ALL cell lines.

The binary paradigm of necrosis and apoptosis initially defined by its mechanism has shifted to include other models in between. For instance, necroptosis **(Figure 1)**, is a form of programed cellular death independent of caspase activity with the morphology of necrosis.

Objectives

(1) Investigate therapeutic efficacy of compound 10357 in a human leukemia xenograft mouse model.
(2) Investigate the Mechanism of compound 10357

Methods

(1) JM1 B-ALL mouse model
JM1 cells were injected into both tibias of healthy 14-17-week-old male NOD/SCID/IL2rg-/-(NSG) mice using our institutionally-approved animal protocol. 16 leukemia transplanted mice were randomly enrolled into either compound 10357 or vehicle control treatment group.

(2) Apoptosis Activity Assay
Cells were treated with compound 10357 or H_2O_2 (positive control). Caspase activity was measured using 3/7 Glo Assay Kit.

Methods (Cont.)

(3) Necroptosis assay
RIPK1 inhibitor, Necrostatin-1 or MLKL inhibitor, Necrosulphonamide was added to culture media with compound 10357.

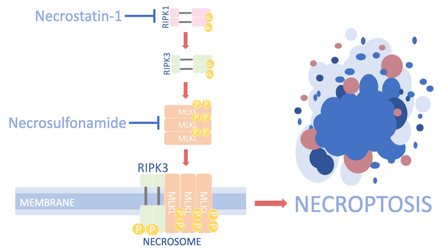

Figure 1. Necroptosis Pathway
RIPK1 phosphorylates RIPK3, which binds to and phosphorylated MLKL in the formation of the Necrosome. The necrosome integrates within the cellular membranes forming a pore, triggering the release of various metabolic compounds and necroptosis.

(4) Apoptosis Induction Factor Analysis
Cells were denatured and separated into nuclear and total protein extractions. Extractions were blotted against AIF and cytoplasmic and nuclear controls.

Results

(1) Compound 10357 Improves survival in a B-ALL xenograft mouse model.
Median survival time of Compound 10357 treated group was 49.0 days vs. 45.5 days in the control (**Figure 2**).

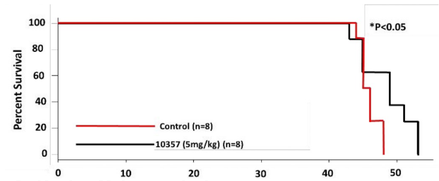

Figure 2. Kaplan-Meier survival curve
Compound 10357 treatment prolongs survival time significantly in a JM1 human B-ALL mouse model ($p < 0.05$). Human Leukemia was confirmed by HLA Class I positivity of harvested bone marrow cells of mice.

Results (Cont.)

(2) Compound 10357's cytotoxicity is caspase independent.
Compound 10357 treatment did not increase caspase activity in comparison to control in B-ALL cells **(Figure 3)**

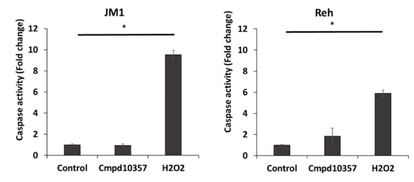

Figure 3. Caspase 3/7 Glo assay results
Caspase 3/7 Glo showed that compound 10357 did not increase caspase activity whereas H2O2 did. (n=3) All data are presented as the mean ± SD. * $p<0.05$

(3) Compound 10357 shows RIPK1 mediated non-apoptotic cytotoxicity.
JM1 and Reh cells treated with compound 10357 was rescued with necrostatin-1 but not necrosulfonamide, making Necroptosis pathway less likely.

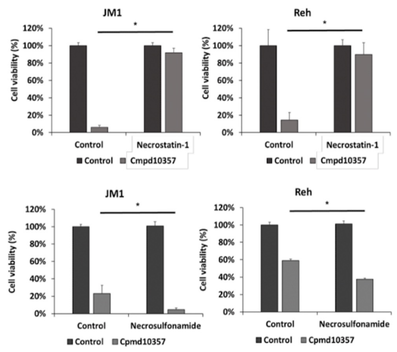

Figure 4. MTS assay results with necroptosis pathway inhibitors
Cytotoxicity of compound 10357 is reversed in the presence of Necrostatin-1 (n=3), but not Necrosulfonamide (n=3). All data are presented as the mean ± SD. * $p<0.05$

Results (Cont.)

(4) Compound 10357 induces AIF nuclear translocation.
The nuclear expression of AIF was significantly increased in both JM1 and Reh cells treated with compound 10357 compared to control cells (**Figure 5**).

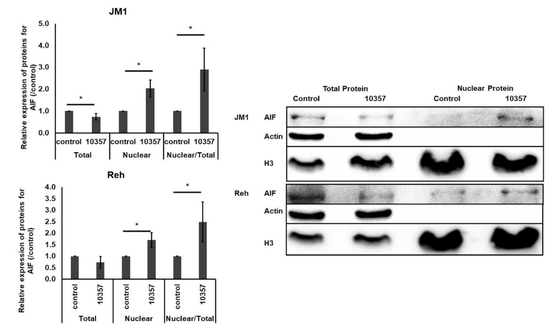

Figure 5. Immunoblot of total protein and nuclear protein fractions
Bands from the blot depicted in were digitally quantified, and the relative expression of AIF was plotted for each condition. AIF migrated into the nucleus 24 hours after exposure to compound 10357 (n=3). Data are presented as the mean ± SD. * $p<0.05$.

Summary/Next Steps

(1) Compound 10357 shows dose dependent cytotoxicity in two B-ALL cell lines and increased the median life expectancy *in vivo*.
(2) The cytotoxicity of compound 10357 seems to be via non-apoptosis, involving RIPK1 and AIF.

Next steps:
(1) Investigate further mechanism of action of compound 10357.
(2) Assess compound 10357 in combination therapy with other chemotherapeutic agents.

Acknowledgements

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