Abstract #1556

Targeting Ewing Sarcoma with A Novel RBM39 Degrader: DNA Damage Repair Pathway Effects

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ABSTRACT

Patients with metastatic Ewing Sarcoma have a five-year survival rate of 15-30%. For 30 years, there are limited approved therapies, including chemotherapy, radiotherapy and surgery, which are associated with significant morbidities and secondary cancers, supporting the need for new impactful therapies. Analysis of the RNA splicing factor RNA Binding Motif Protein 39 (RBM39) in the Cancer Target Discovery and Development (CTD²) Network showed that Ewing sarcoma cell lines are especially sensitive to RBM39 loss.

A next generation RBM39 degrader ST-00937 was designed through structure-based drug design and prioritized based on in vitro anti-cancer potency, metabolic stability and oral pharmacokinetics in mice. Here we demonstrated that ST-00937 degraded RBM39 by 6 hours of treatment and reduced in vitro A673 Ewing sarcoma cell viability with an IC50 of 170nM. These in vitro effects translated to complete A673 tumor regressions in mice by the 11th day of twice daily ST-00937 oral dosing at 30mg/kg, without an effect on body weight. By the third day of treatment, RBM39 was undetectable in A673 tumors.

Mechanically both the EWS-FLI1 driver gene fusion in A673 cells and RBM39 degradation are reported to reduce DNA damage repair pathways. Here we report RBM39 degradation in A673 cells significantly increases phosphorylated H2Ax, a marker of DNA double strand breaks. This DNA damage is thought to contribute to the induction of cell apoptosis detected as increased cleaved caspase-3. Increased DNA double strand breaks is furthermore associated with negative effects on 3 proteins involved in homologous recombination repair of DNA double strand breaks; mis-splicing of BRCA1 and ATM RNA, and reduced protein levels of RAD51D.

The RBM39 molecular glue degrader ST-00937 therefore demonstrates total tumor regression in a model of Ewing sarcoma. Mechanistic studies indicate that the robust activity of ST-00937 involves deficient homologous recombination repair. Continued research in our lab will add further insights into the mechanism of action of RBM39 degraders in this cancer of high unmet need.

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INTRODUCTION

1. Five-year patient survival rate is 15-30%[1] for metastatic Ewing Sarcoma. For 30 years, no effective treatments are approved by FDA supporting the need for new impactful therapies.
2. RBM39 is an RNA-binding motif protein that plays a vital role in tumorigenesis. Overexpression of RBM39 promotes cancer cell proliferation and migration. Robust anti-tumor efficacy was evidenced for RBM39 degrader in multiple cancers[2,3], but not for the Ewing sarcoma.
3. SEED's lead RBM39 degrader ST-00937 was identified through structure-based drug design and prioritized based on in vitro anti-cancer potency, metabolic stability and oral pharmacokinetics in mice.
4. Anti-tumor effects of ST-00937 was evaluated in Ewing Sarcoma A673 in vitro and in vivo. Mechanism of Action (MOA) studies revealed that the robust anti-tumor efficacy of ST-00937 was associated with homologous recombination (HR) DNA repairing.

METHODS AND MATERIALS

Human Ewing sarcoma A673 cells (ATCC) were utilized to explore the effects of ST-00937 in this cancer type. Tumor cell viability was evaluated by CTG assay (The CellTiter-Glo® Luminescent Cell Viability Assay Kit)(Promega), and anti-tumor efficacy was evaluated in NOD SCID mice bearing tumors established with A673 cells (Pharmaron, the treatment was performed in compliance with the guidance AAALAC). Protein levels in cell lysates and tumor homogenate were measured by Western Blot (anti-RBM39 from Atlas; antibody for phosphor and total H2X, RAD51D, Caspase 3 and cleavage Caspase 3 from Cell Signaling). Target gene RNA splicing was evaluated by standard RT-PCR.

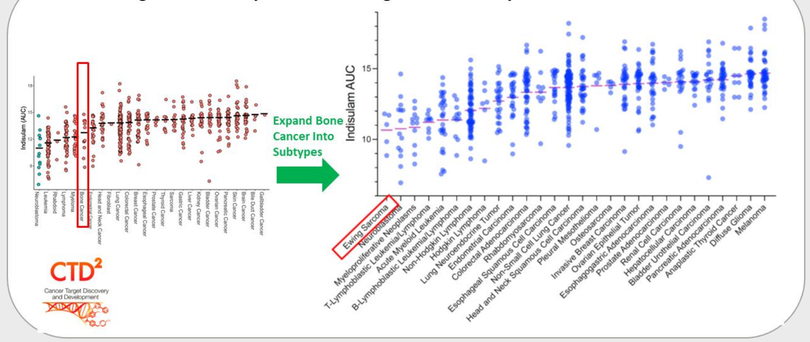
REFERENCES

1. Tsibulnikov, S., Fayzullina, D., Karlina, I. et al. Ewing sarcoma treatment: a gene therapy approach. Cancer Gene Ther 30, 1066–1071 (2023).
2. Kohsaka, S., Yagishita, S., Shirai, Y. et al. A molecular glue RBM39-degrader induces synthetic lethality in cancer cells with homologous recombination repair deficiency. npj Precis. Onc. 8, 117 (2024).
3. Nijhuis, A., Sikka, A., Yogev, O. et al. Indisulam targets RNA splicing and metabolism to serve as a therapeutic strategy for high-risk neuroblastoma. Nat Commun 13, 1380 (2022).
4. https://portals.broadinstitute.org/ctrp.v2.1/?page=#ctd2BodyHome

RESULTS

Subtype Analysis of Bone Cancer Identifies Ewing Sarcoma as Particularly Sensitive to an RBM39 Degrader[4]

Figure 1: Re-analysis of RBM39 Degrader Sensitivity in Bone Cancer



ST-00937 Degraded RBM39 and Reduced Ewing Sarcoma Cell Viability

Figure 2a: Reduction of A673 Cell Viability by ST-00937

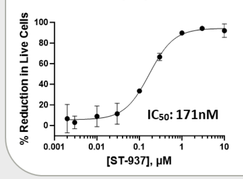


Figure 2b: Total RBM39 Degradation after 6h ST-00937 Treatment

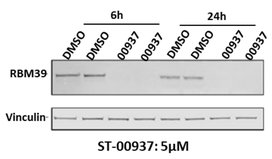
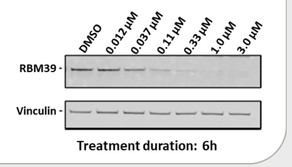


Figure 2c: Dose Response RBM39 Degradation by ST-00937



ST-00937 degraded RBM39 in Ewing Sarcoma Tumors, Leading to Complete Tumor Regressions Without Body Weight Loss

Figure 3a: Dose Response Tumor Growth Inhibition

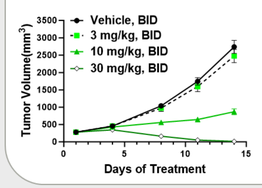


Figure 3b: No Significant Mouse Body Weight Change

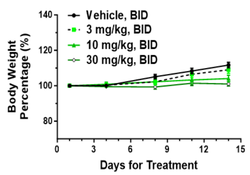
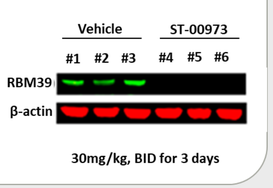


Figure 3c: Tumor Expressed RBM39 Degradation by ST-00973



RESULTS (Continued)

ST-00937 Caused DNA Double Strand Breaks Associated with Apoptosis in Ewing Sarcoma Cells

Figure 4a: Phosphor H2AX Detection by Western Blot

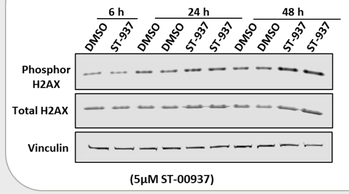
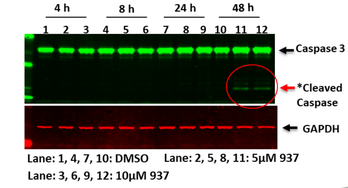


Figure 4b: Cleaved Caspase 3 Detection by Western Blot



ST-00937 Caused Significant Mis-splicing of BRCA1 , and Reduced the Expression of ATM and RAD51D

Figure 5a: RNA Mis-splicing of BRCA1 and ATM

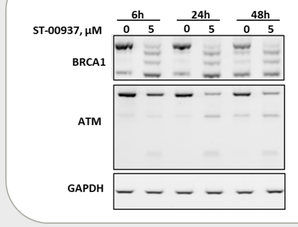
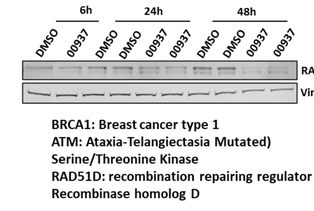


Figure 5b: RAD51D Protein Downregulation



BRCA1: Breast cancer type 1
ATM: Ataxia-Telangiectasia Mutated) Serine/Threonine Kinase
RAD51D: recombination repairing regulator Recombinase homolog D

CONCLUSIONS

ST-00937, SEED's lead oral dosing molecular glue, is a potent RBM39 degrader and has demonstrated robust anti-tumor efficacy in Ewing Sarcoma A673 model in vitro and in vivo:

1. RBM39 degradation to undetectable level in ST-00937 treated A673 cells in vitro and in vivo.
2. Reduction of A673 in vitro cell viability number with an IC_{50} 170nM.
3. Complete A673 tumor regression at 30 mg/kg, BID.

Mechanistic studies indicate that the robust activity of ST-00937 involves deficient homologous recombination repair. Continued research in our lab will add further insights into the MOA of RBM39 degraders in Ewing sarcoma. SEED expects to file an IND for its next generation RBM39 degrader to address the high unmet need in Ewing sarcoma.