Potentiation of Type I Interferon Signaling Leads to In Vivo Efficacy Achieved with Combination of Chemotherapy and the PARP7 Inhibitor RBN-2397

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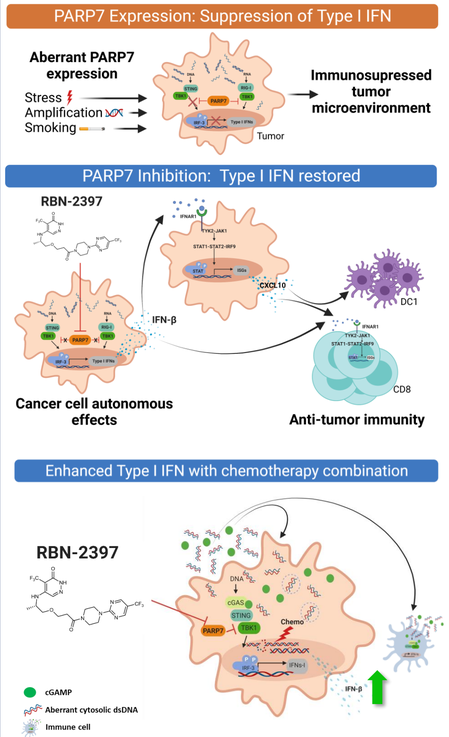
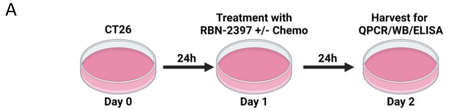
Abstract 3260
AACR 2023

Background



PARP7 is a mono-ART that is upregulated in response to cellular stress (e.g., viral infection, cigarette smoke), and suppresses the Type I interferon (IFN) response following cytosolic nucleic acid sensing. RBN-2397 is a first-in-class PARP7 inhibitor[1], inducing cancer cell autonomous and immune stimulatory effects in preclinical models through enhanced type I IFN signaling in cancer cells. Moreover, RBN-2397 induces $CD8^+$ T cell-dependent tumor-specific immune memory in an immunocompetent mouse cancer model[1]. RBN-2397 is currently being tested in an ongoing Phase I clinical study (NCT04053673)[2] and in combination with pembrolizumab (NCT05127590) and nivolumab (jRCT2031210373). Treatment of tumors with DNA damaging agents like chemotherapy can result in the accumulation of double-stranded (ds) DNA in the cytoplasm. Aberrant levels of cytosolic dsDNA can activate innate immune signaling through the cGAS-STING pathway, leading to increased expression of type I interferons. Since PARP7 acts as a negative break on nucleic acid sensing, the chemotherapy-induced activation of the type I IFN response would be minimized. Combining RBN-2397 with chemotherapeutic agents in PARP7-active tumors would lead to enhanced type I IFN signaling in tumor and immune cells. We found that combining RBN-2397 with cisplatin led to increased cGAMP and pSTAT1 protein as well as CXCL10 gene expression. In vivo, cisplatin alone modestly increased cytosolic DNA and ISGs. Finally, the combination led to increased efficacy and survival of mice harboring CT26 tumors. Increased survival correlated with enhancement of ISGs in the combination groups.

Methodology – In vitro assays



B

Treatments	RBN-2397 (nM)	Chemo dose #1 (μM)	Chemo dose #2 (μM)
RBN-2397	65	-	-
Cisplatin	-	3	1
Cisplatin + RBN-2397	65	3	1
Oxaliplatin	-	3	1
Oxaliplatin + RBN-2397	65	31	
Carboplatin	-	1, 3, 5, 10 dose range	
Carboplatin + RBN-2397	65	1, 3, 5, 10 dose range	
Docetaxel	-	0.3	0.1
Docetaxel + RBN-2397	65	0.3	0.1
Paclitaxel	-	0.3	0.1
Paclitaxel + RBN-2397	65	0.3	0.1

Figure 1. (**A**) The mouse colon cancer tumor cell line CT26 was seeded and allowed to attach for 24 h. Cells were then treated with the indicated single agent or combination treatments listed in table (**B**) for 24 h. Cells were harvested for downstream assays including RT-PCR to assess gene expression changes, protein for western blotting, and cellular lysates to evaluate cGAMP intracellular level. The doses selected for chemotherapy drugs were chosen based on 24 or 72 h viability assays to ensure cell viability was <75% by cell titer glow assay (not shown). All chemotherapies were tested after 24h of treatment and carboplatin was tested at 24h and 72h of treatment at lower doses to ensure high percent cell viability.

1. Gozgit et al. Cancer Cell. 2021; 39(9):1214-1226
2. Falchook et al. ASCO 2021 oral presentation

RBN-2397 Plus Chemotherapy Potentiates Type I IFN In Vitro

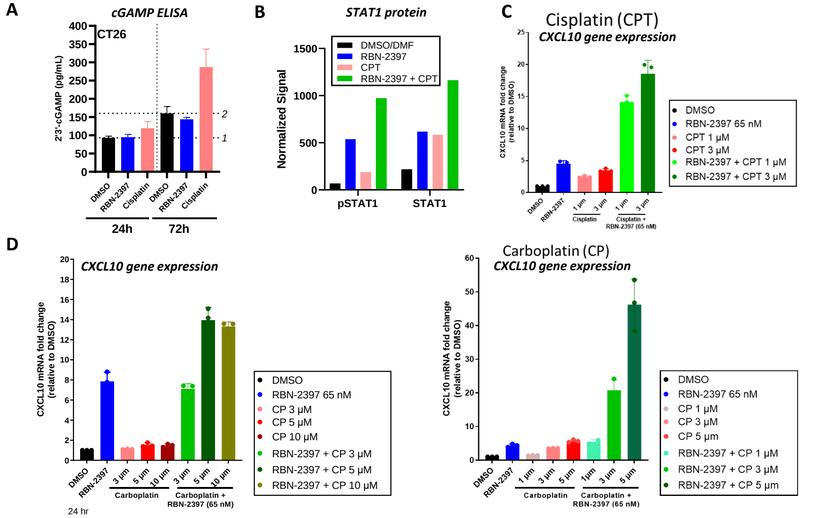


Figure 2. (A) CT26 cells were treated as in figure 1 and harvested to determine intracellular cGAMP by ELISA. Single agent cisplatin increased cGAMP from baseline (***1***: 24h baseline; ***2***: 72h baseline) demonstrating a link between DNA damage and the nucleic acid sensing pathway. (B) pSTAT1 and STAT1 protein level from western blots of CT26 cells treated as indicated for 24 h. Protein was normalized to actin. (C-D) CT26 cells were treated as in Figure 1 and harvested for RT-PCT of several ISGs including CXCL10, MX1, IFN-β, and CCL5 (not shown). Single agent RBN-2397 increased CXCL10 as expected. Single agent cisplatin (CPT) (C) and carboplatin (CP) (D) modestly increased CXCL10 to a lower extent relative to RBN-2397. The combination of RBN-2397 with either cisplatin or carboplatin robustly enhanced expression of CXCL10 and other ISGs (not shown).

Cisplatin Increased Cyto-DNA and IFN Induced Genes

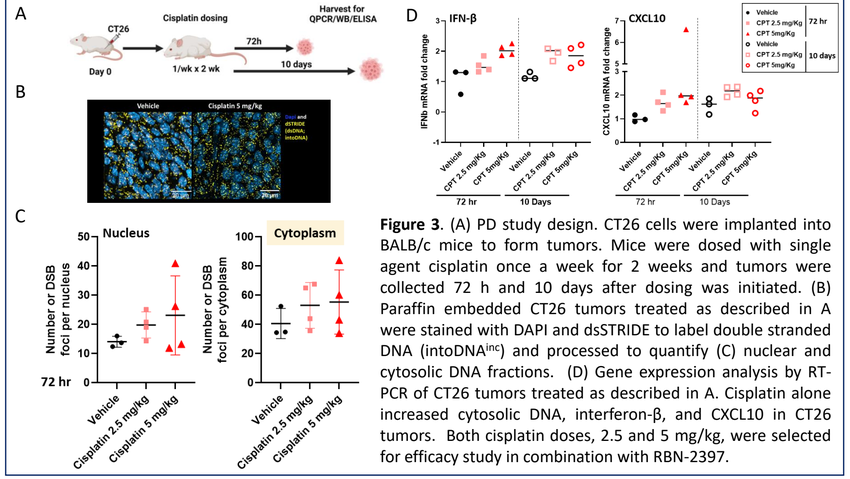
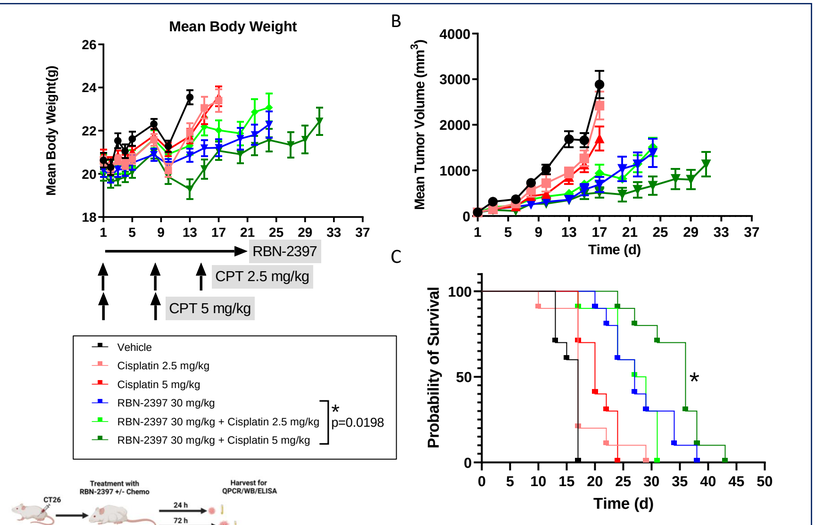


Figure 3. (A) PD study design. CT26 cells were implanted into BALB/c mice to form tumors. Mice were dosed with single agent cisplatin once a week for 2 weeks and tumors were collected 72 h and 10 days after dosing was initiated. (B) Paraffin embedded CT26 tumors treated as described in A were stained with DAPI and dsSTRIDE to label double stranded DNA (intoDNA^{inc}) and processed to quantify (C) nuclear and cytosolic DNA fractions. (D) Gene expression analysis by RT-PCR of CT26 tumors treated as described in A. Cisplatin alone increased cytosolic DNA, interferon-β, and CXCL10 in CT26 tumors. Both cisplatin doses, 2.5 and 5 mg/kg, were selected for efficacy study in combination with RBN-2397.

Increased Efficacy of RBN-2397 with Cisplatin Combination



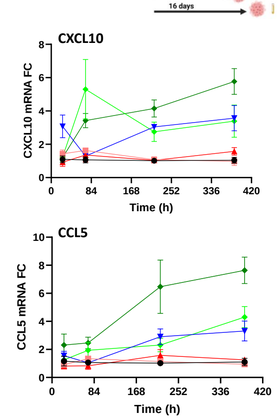


Figure 4. (A) CT26 cells were implanted into the flank of BALB/c mice to form tumors. Mice were dosed as indicated in the presence of 50 mg/kg ABT. Single agent cisplatin and RBN-2397 combination was well tolerated by mean body weight assessment. (B) Mean tumor volume measurements show significantly smaller tumors in mice treated with single agent RBN-2397 and in combination with cisplatin (5 mg/kg). (C) Survival benefit determination in CT26 tumor bearing mice treated as indicated. Single agent RBN-2397 extended survival over vehicle and cisplatin. The combination of the highest cisplatin concentration (5 mg/kg) plus RBN-2397 led to a significant extension of survival over RBN-2397 alone. (D) PK/PD study design of CT26 mice treated with RBN-2397 plus cisplatin as indicated. Tumors were collected at a range of timepoints and processed for gene expression changes. (E and D) Gene expression of the interferon induced genes CXCL10 and CCL5 was determined by RT-PCR. Data is represented as fold change (FC) relative to vehicle. CXCL10 and CCL5 gene expression is highest in the cisplatin 5 mg/kg plus RBN-2397 combination tumors, which also correlates to decreased tumor growth and increased survival. Potentiation of type I IFN genes observed in tumors with RBN-2397 plus cisplatin combination.

Conclusions

- **Single agent cisplatin generates cytosolic DNA that produces cGAMP by engaging the nucleic acid sensing pathway and leads to activation of the type I IFN response in CT26 tumor cells in vitro and in vivo**
- **Combination of RBN-2397 with cisplatin or carboplatin robustly enhance type I IFN signaling**
- **Decreased tumor burden and extended survival observed with cisplatin plus RBN-2397 combination in CT26 tumor bearing mice**
- **Enhanced expression of CXCL10 and CCL5 in combination (CPT 5) group correlated to highest efficacy**