PARP7 Negatively Regulates the Type I Interferon Response in Cancer Cells and its Inhibition Leads to Tumor Regression

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- Ribon Therapeutics
Disclosure Statement

- I am an employee and shareholder of Ribon Therapeutics
Not All PARPs Are Alike – Outside of PolyPARPs the PARP Family Is Unexplored for Therapeutic Development

• PARP family consists of 17 members
  • Three subfamilies based on catalytic activity (polyPARPs, monoPARPs and inactive)
  • PARPs regulate their cellular function by modifying target proteins with ADP-ribose
  • monoPARPs transfer a single unit of ADP-ribose onto their substrates where polyPARPs attach polymers of ADP-ribose units

• PARP7 is a monoPARP
  • Target gene of the aryl hydrocarbon receptor (AHR) that can be induced by cancer relevant stresses (e.g. chemicals in cigarette smoke)
  • Gene locus is amplified in cancers with strong smoking association (e.g., squamous cell carcinoma of the lung (SCCL), esophageal and head and neck squamous cancers)
Aberrant nucleic acids can be detected by various sensing mechanisms including cGAS/STING and RIG-I.

Type I IFN signaling plays a key role in antitumor immunity by inducing both innate and adaptive immune mechanisms.

Hypothesis: High levels of PARP7 in tumors blocks interferon production resulting in an immunosuppressive environment.

Targeting a negative regulator “brake” of Type I IFN signaling is a novel therapeutic strategy in cancer.
RBN-2397 is a Potent and Selective Inhibitor of PARP7

- **RBN-2397 is a potent inhibitor of PARP7**
  - Binds to PARP7 in the NAD+ binding pocket with key interactions in adenosine sub-pocket driving potency and selectivity
  - Sub-nanomolar biochemical activity
  - Potent inhibition of cellular MARylation
- **RBN-2397 displays selectivity to PARP7**
  - >50-fold selective vs. PARP family
  - No inhibition in kinase panel (1 µM)
- **Drug-like properties support oral dosing in humans**
- **First in human Phase I multi-center clinical trial is underway (NCT04053673)**
RBN-2397 Restores Cytosolic Nucleic Acid Sensing in the Mouse CT26 Cancer Cell Line

PARP7 inhibition “releases the brake” on cytosolic nucleic acid sensing and induces Type I IFNs

- Restoration of Type I IFN response is measured by an increase in STAT1 phosphorylation and interferon stimulated genes (ISGs)

RBN-2397 restores Type I IFN response in CT26 cells

Induction of pSTAT1

Induction of CXCL10 mRNA
RBN-2397 Induces Tumor-Specific Adaptive Immune Memory in CT26 Syngeneic Model with Durable Complete Responses

Primary Efficacy: RBN-2397 induces durable regressions

- Once daily oral dosing of RBN-2397 in CT26 tumor-bearing BALB/c mice
- Tumor-free mice were monitored for 60 days

Re-challenge of tumor-free mice: Rejection of CT26 cells

- Tumor-free mice re-challenged with CT26 and subsequently 4T1 cells
- All tumor-free mice rejected CT26 cells but not 4T1, demonstrating induction of tumor-specific adaptive immune memory
RBN-2397 Induces Type I IFN Signaling and Enhances Immune Markers in CT26 Tumors

RBN-2397 shows exposure-dependent effects on PD markers in CT26 tumors

RBN-2397 enhances antigen presentation and T cell activation in tumor-infiltrating immune cells

Increase in MHCII on DC1 cells

Increase in CD86 on DC1 cells

Increase in GrzB on CD8 T cells

CT26 tumor-bearing mice administered as single oral dose.

CT26 tumor-bearing mice dosed with RBN-2397 100 mg/kg + ABT for 15 Days.

CT26 tumor-bearing mice dosed with RBN-2397 500 mg/kg. Tumors collected on days 3, 6 & 12.

RBN-2397 increases tumor antigen-specific T cells:
Increase in the number of splenic T cells producing IFN-γ in response to the CT26 antigen

Vehicle + AH1

RBN-2397

RBN-2397 + AH1

Vehicle

RBN-2397

0
1000
10000
100000
1000000

IFN-γ spots/5x10^4 cells

P = 0.0326

0
1000
5000
10000
15000
20000

CD86 MFI Day 3 (on DC1 cells)

P = 0.0064

0
20000
40000
60000
80000
100000

GZMB MFI Day 12 (on CD8+ cells)

P < 0.0001

0
10000
20000
30000
40000
50000
60000
70000
80000

MHCII MFI Day 3 (on DC1 cells)

P = 0.0243

0
5000
10000
15000
20000
25000
30000
35000
40000

DO NOT POST
Combination of Anti-PD1 with RBN-2397 Increases the Number of Tumor-Free Mice in the CT26 Syngeneic Model

Increase in markers of immune feedback regulation following treatment with RBN-2397 in CT26

**Increase in PD-L1 on macrophages**

![Graph showing increase in PD-L1 on macrophages](image)

**Increase in LAG3 on CD8 T cells**

![Graph showing increase in LAG3 on CD8 T cells](image)

Enhanced antitumor immunity with combination with anti-PD1 in CT26

**Enhanced antitumor immunity with combination with anti-PD1 in CT26**

![Graph showing tumor volume over time](image)

*DO NOT POST*
CRISPR-Cas9 Used to Ablate either TBK1 or IFNAR1 in CT26 Cells to Investigate the Mechanism of Action of RBN-2397

- TBK1 knockout (KO) prevents both IRF3 & STAT1 phosphorylation by RBN-2397
- IFNAR1 KO prevents STAT1 phosphorylation by RBN-2397
Tumor-derived Interferon Is Key for Antitumor Activity of RBN-2397

• Ablation of tumor TBK1 nearly eliminates the antitumor activity of RBN-2397 in the CT26 tumor model
• Tumor-derived IFN-β is the source of the innate immune activation and crucial for RBN-2397 mediated antitumor response

No PARP7i-mediated IFN-β release
No effects on cancer or immune cells
IFNAR1 Knockout in CT26 Tumor Cells Partially Attenuates Antitumor Activity of RBN-2397 in the CT26 Tumor Model

- IFNAR1 KO initially attenuates antitumor activity of RBN-2397, but a subset of tumors start responding after Day 12
- IFNAR1 KO does not prevent IRF3 phosphorylation by RBN-2397
- Suggests onset of antitumor immunity around Day 12, induced by effects of tumor-derived IFN-β on immune cells

No ISGs
No tumor intrinsic effect
Immune response

Mean Tumor Volume (mm³)

CT26 Parental
Vehicle
RBN-2397 100 mg/kg

CT26 IFNAR1 KO
Vehicle
RBN-2397 100 mg/kg

All groups co-dosed with ABT
IFNAR1 Blockade on Tumor and Immune Cells Is Necessary to Prevent Antitumor Activity of RBN-2397 in the CT26 Tumor Model

• Dosing of anti-IFNAR1 AB on the background of CT26 IFNAR1 KO tumors prevents the antitumor activity of RBN-2397

• Suggests contribution of immune system through activation of IFN-β signaling in immune cells

• Tumor-produced Type I IFN by RBN-2397 plays a major role in the development of durable antitumor immunity

Tumor cells

Immune cell

• No ISGs
• No tumor intrinsic effect

• Immune response

CT26 Parental

CT26 IFNAR1 KO

All groups co-dosed with ABT
RBN-2397 Shows Cancer Cell Autonomous Effects and Restores Type I IFN Signaling in Human Cancer Cell Lines

- Responder cell lines are enriched for high expression of genes involved in Type I IFN response

**Subset of cancer cell lines exhibit dependency on PARP7 for proliferation**

![Graph showing PARP1 and PARP7 inhibitors](image)

Each dot represents a cancer cell line in a 6-day assay.

**Dependency on PARP7 for proliferation and suppression of Type I IFN signaling in NSCLC NCI-H1373 cells**

- **Proliferation: RBN-2397 GI50**
- **RBN-2397 induction of pSTAT1**
- **Proliferation: PARP7 KO**
- **Transcriptional changes by RBN-2397: Increase in multiple ISGs**

**Graphs and data showing**

- Relative Cell Viability
- Expression Fold-change (log2)

**CRITICAL INFORMATION**: DO NOT POST.
CRISPRi/a Screen Highlights Innate Immune Response Genes in Driving RBN-2397 Activity in NCI-H1373 Cells

Comparison of CRISPRi/a phenotype scores highlights genes with opposing functionality upon RBN-2397 treatment

Significant enrichment of genes involved in innate immune response that affect RBN-2397 activity

Effects of individual genes on RBN-2397 activity
- Resistance when silenced
- Sensitivity when activated

Genetic screen using whole genome CRISPRi/a libraries
- CRISPRi: interference to silence gene expression
- CRISPRa: activation to increase gene expression

GSEA of genes that affect RBN-2397 activity
- Resistance when silenced
- Sensitivity when activated

GSEA: Gene set enrichment analysis

Innate immune response
- Reg. chemokine production
- Cation channel complex
- Toll-like receptor signaling
- Reg. of microtubule based move.

Ribosome
- DNA replication
- RNA splicing
- DNA repair
- Homologous recombination
- Proteasome
- Chromosome organization

DO NOT POST
RBN-2397 Shows Antitumor Activity in Human Cancer Cell Lines that Show Induction of ISGs

Reactivation of tumor Type I IFN signaling is a major determinant for RBN-2397 antitumor activity

<table>
<thead>
<tr>
<th>No increase in ISGs</th>
<th>Increase in ISGs</th>
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<table>
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<tr>
<th>No antitumor activity</th>
<th>Antitumor activity (50-100% TGI)</th>
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<tbody>
<tr>
<td></td>
<td>• Lung cancer</td>
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<tr>
<td></td>
<td>• Pancreatic cancer</td>
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<td>• Oral squamous cancer</td>
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Transcriptomic changes by RBN-2397 mRNA levels (log2 fold change)

RBN-2397 efficacy in CB17 SCID xenograft mouse model (*)

RBN-2397 causes complete regressions in NCI-H1373 xenografts

- Once daily oral dosing of RBN-2397 in CB17 SCID mice with NCI-H1373 xenografts
- Dose-dependent effects on tumor growth
- Tumor regression at dose levels of ≥30 mg/kg

Vehicle

- 3 mg/kg
- 10 mg/kg
- 30 mg/kg
- 100 mg/kg
- 300 mg/kg

Mean Tumor Volume (mm$^3$) vs Time (d)

- 0 4 8 12 16 20 24 28 32
- 0 500 1000 1500 2000 2500

- Lung cancer
- Pancreatic cancer
- Oral squamous cancer

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RBN-2397 – A Novel Cancer Therapeutic Being Tested in Clinical Trials

- Discovered first potent and selective PARP7 inhibitor
  - Novel first-in-class therapy
- PARP7 is a novel therapeutic target and its inhibition induces both antitumor immunity and cancer cell autonomous effects
  - Increased signaling to the immune system
  - Development of immune memory
  - Arrest of cancer cell proliferation and tumor regression
  - Antitumor activity in multiple cancer types where reactivation of Type I IFN signaling is observed
- Identified PARP7 as a fundamental regulator of intrinsic stress support pathways and a novel tumor vulnerability in cancer cells
- First in human Phase I multi-center clinical trial underway (NCT04053673)
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