RBN-2397: A potent and selective small molecule inhibitor of PARP7 that induces tumor-derived antitumor immunity dependent on CD8 T cells

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• Ribon Therapeutics
Disclosure Statement

- I am an employee and shareholder of Ribon Therapeutics
Targeting PARP7 to Restore Tumor-Derived Type I Signaling is a Novel Therapeutic Strategy in Cancer

- Engaging cytosolic nucleic acid sensing and the Type I interferon (IFN) response is an emerging therapeutic strategy
  - Stimulate production of cytokines to promote an adaptive immune response
  - Currently, most approaches involve agonistic modulation of the tumor microenvironment
- PARP7 is a monoPARP regulated by cancer relevant stresses
  - Amplified in cancers with strong smoking association
  - Acts as a “brake” on cytosolic nucleic acid sensing and suppresses Type I IFN signaling

Targeting a negative regulator of tumor-produced Type I IFN is a novel therapeutic strategy

<table>
<thead>
<tr>
<th>Adaptive Immunity</th>
<th>Innate Immunity: Type I IFN Response</th>
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<tr>
<td>T-cell</td>
<td>Tumor microenvironment (TME)</td>
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<td>(DC, MΦ)</td>
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**PARP7 acts as a fundamental regulator of intrinsic stress support pathways and is a novel tumor vulnerability in cancer cells**
PARP7 Acts as a Brake on Cytosolic Nucleic Acid Sensing and the Type I IFN Response

Aberrant nucleic acids can be detected by various sensing mechanisms including cGAS/STING and RIG-I

Overexpression of PARP7 suppresses IFN-β response to dsDNA

PARP7 has been reported to negatively regulate the Type I response by interacting with TBK1 during viral infection (Yamada et al., 2016)

Suppression of IFN-β by PARP7

HEK293T cells transfected with PARP7 treated with synthetic double stranded (ds)-DNA for 24 hours
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

- **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 Potently and Selectively Inhibits PARP7-Dependent Activity Compared to PARP1

- PARPs regulate their cellular function by modifying target proteins with ADP-ribose
  - PolyPARPs (e.g., PARP1) attach polymers of ADP-ribose units (PARylation)
  - MonoPARPs (e.g., PARP7) modify proteins with a single unit of ADP-ribose (MARylation)

PARP family consists of 17 members:
2 subclasses based on catalytic activity

Vyas et al., 2013

PARP1 inhibitor: Niraparib

IC_{50} = 0.003 µM

PARP7 inhibitor: RBN-2397

IC_{50} = 0.002 µM

PARP1-H_{2}O_{2} activated Hela cells (PAR)

PARP7-overexpressing SK-MES-1 cells (MAR)

Lu et al., 2019

24-hour treatment
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

Selective induction of CXCL10

DO NOT POST
RBN-2397 Restores Cytosolic Nucleic Acid Sensing Dependent on Pattern Recognition Receptor Signaling

Pharmacological inhibitors used to investigate the role of PARP7 in suppressing Type I IFNs

RBN-2397 restores Type I IFN signaling through pattern recognition receptor pathway

Blockade of: TBK1 | JAK | STING

24-hour treatment

RX795: TBK1 inhibitor
Ruxolitinib: JAK inhibitor
C-178: STING inhibitor
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

TF: Tumor free mice
All groups co-dosed with ABT

DO NOT POST
Adaptive Immune Response is Indispensable for RBN-2397 Antitumor Activity

Characterization of immune cell populations present in BALB/c and NOG mice

<table>
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<tr>
<th>CT26 mouse model</th>
<th>Innate immune cells</th>
<th>Adaptive immune cells</th>
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<tr>
<td>Mouse Strain</td>
<td>RBN-2397 Efficacy</td>
<td>NK</td>
</tr>
<tr>
<td>BALB/c</td>
<td>Tumor regression</td>
<td>+</td>
</tr>
<tr>
<td>NOG</td>
<td>~50% TGI</td>
<td>-</td>
</tr>
</tbody>
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+: Present; -: Absent
a: Reduced macrophage and dendritic cell function

RBN-2397 shows substantially reduced activity in CT26 tumor-bearing immunodeficient mice

**BALB/c: Immunocompetent**

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

**NOG: Immunodeficient**

- Vehicle
- 30 mg/kg
- 100 mg/kg
Robust Depletion of Immune Cell Populations in CT26 Tumor-Bearing Mice

Scheme for immune cell depletion in CT26-tumor-bearing BALB/c mice

- Administration of blocking antibodies to deplete CD4, CD8 and NK cells
- Tumor implantation
- RBN-2397 dosing
- Efficacy readout

Specific depletion of immune cell populations

**Blood absolute counts**

- Isotype control
- Anti-CD4
- Anti-CD8
- Anti-asialoGM1
- Anti-CD4 + anti-CD8 + anti-asialoGM1

**Tumor absolute counts**

- Isotype control
- Anti-CD4 + anti-CD8 + anti-asialoGM1
CD8 T Cells Are Essential for the Antitumor Immunity Induced by RBN-2397 in the CT26 Syngeneic Model

CD4 T cell depletion had no effect on antitumor activity

NK cell depletion had no effect on antitumor activity

CD8 T cell depletion attenuated antitumor activity

Triple depletion was not different than CD8 T cell single depletion

All groups co-dosed with ABT
RBN-2397 Induces Type I IFN Signaling and Enhances Immune Markers in CT26 Tumors

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

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

CT26 tumor-bearing mice administered as single oral dose. All groups co-dosed with ABT

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

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Type I IFN Produced by Tumors in Response to RBN-2397 Plays a Major Role in the Development of Durable Antitumor Immunity in CT26 Model

- Ablation of tumor TBK1 nearly eliminates the antitumor activity of RBN-2397
  - Tumor-produced IFN-β is the source of the innate immune activation and crucial for antitumor activity
- Blockade of tumor and host IFNAR1 signaling prevents the antitumor activity of RBN-2397
  - Suggests contribution of immune system through activation of IFN signaling in immune cells by tumor-produced IFN-β
RBN-2397 is the First Potent and Selective PARP7 Inhibitor to Enter Clinical Development

• Targeting PARP7 to restore tumor-derived Type I signaling is a novel therapeutic strategy in cancer
• Inhibition of PARP7 induces antitumor immunity dependent on tumor-produced Type I IFN and CD8 T cells
• RBN-2397 is the first agent targeting this cancer vulnerability to enter clinical development

PARP7 acts as a “brake” on cytosolic nucleic acid sensing and suppresses the Type I IFN response

Complete regressions and antitumor immunity as a single agent

Ribon PARP7 abstracts at AACR 2021
#381: PARP7 expression in cancer
#1021: PARP7 inhibitor mechanism of action studies
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