RBN-2397: A First-in-Class PARP7 Inhibitor Targeting a Newly Discovered Cancer Vulnerability in Stress-Signaling Pathways

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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)
- Use common cofactor (NAD⁺) to post-translationally ribosylate substrates
- Outside of the conserved catalytic domain PARPs have limited homology and reflect diverse function
- MonoPARPs offer a mechanistically distinct and untapped opportunity beyond PARP1

Adapted from Vyas, Chang et. al. Nature Comm. 2013
PARP7: A Novel Brake on the Type I Interferon Response and Genetic Alterations in Cancer

- PARP7 is induced by cancer relevant stress (e.g., aryl hydrocarbon receptor ligands such as chemicals found in cigarette smoke and kynurenine)
- PARP7 gene locus is amplified in cancers with strong smoking association (e.g., squamous cell carcinoma of the lung (SCCL), head and neck and esophageal squamous cancers)
- PARP7 acts as a tumor cell brake in cytosolic nucleic acid sensing and the Type I interferon (IFN) response

PARP7 is frequently amplified in cancers of the upper aerodigestive tract

Highly expressed in primary SCCL tumors

PARP7 “brake” on nucleic acid sensing and Type I IFN response
PARP7 Hit Identified in Cross Screening of Ribon Library

Co-crystals of NAD⁺ and PARP7 hit bound to PARP16

Small molecule PARP inhibitors HTS and fragment screening hits
Crystal structures across PARP family
Structure based drug design

<table>
<thead>
<tr>
<th>PARP</th>
<th>Biochemical IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP7</td>
<td>9</td>
</tr>
<tr>
<td>PARP1</td>
<td>300</td>
</tr>
<tr>
<td>PARP16</td>
<td>3</td>
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Developed Biochemical and Cellular Assays which Enabled Optimization of PARP7 Inhibitors

High Quality PARP7 Protein

Biochemical Assay (TR-FRET)

Cell Biochemical Assay (MARylation)

Phenotypic Assay (Proliferation)

Wigle, et. al. SLAS Discovery, 2019

PARP7 inhibition in cells by measuring MARylation

NCI-H1373 lung cancer cells

Correlation Between Biochemical, Cell Biochemical, and Cell Phenotypic Assays
Optimization of Hit Led to Potent and Selective PARP7 Inhibitors

**Biochemical IC\(_{50}\) (µM)**

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<tr>
<th>PARP</th>
<th>PARP7</th>
<th>PARP1</th>
<th>PARP16</th>
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<td></td>
<td>9</td>
<td>300</td>
<td>3</td>
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**PARP**

- **PARP7**
  - Pan monoPARP fragment hit
  - IC\(_{50}\) = 9 µM

- **PARP1**
  - 300 µM

- **PARP16**
  - 3 µM

**Biochemical IC\(_{50}\) (µM)**

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<tr>
<th>PARP</th>
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<tr>
<td>PARP7</td>
<td>0.007</td>
</tr>
<tr>
<td>PARP1</td>
<td>0.3</td>
</tr>
<tr>
<td>All other monoPARPs similar potency to PARP7</td>
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**PARP12 RBN011082**

- Pan monoPARP selective inhibitor
- MAR/PAR difference region
- D-loop
- Adenosine sub-pocket

**PARP12/7 RBN011364**

- PARP7 potent and selective inhibitor
- Adenosine sub-pocket

**Biochemical IC\(_{50}\) (µM)**

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<tr>
<td>PARP7</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>PARP1</td>
<td>1</td>
</tr>
<tr>
<td>All other monoPARPs &gt;20 fold selective</td>
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</table>
Discovery of Development Candidate RBN-2397

Co-crystal structure of RBN-2397 bound to PARP12/7

- Lead optimization efforts targeted interactions in key areas of the NAD$^+$ binding pocket
  - Adenosine sub-pocket: exploit positive interaction with PARP7 Gly541 and clash with bulky residues in other PARPs
  - Removed 2 aromatic rings which improved solubility and microsomal stability
- Optimization of physicochemical properties to identify development candidate
  - Cell MARylation EC$_{50}$ = 1 nM
  - >50-fold selective vs. PARPs
  - Low predicted human clearance

PARP12 was used as a surrogate for PARP7. Four labeled residues were mutated from PARP12 to match the PARP7 sequence.
RBN-2397 – PARP7 Development Candidate Summary

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<th>RBN-2397</th>
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| **Target potency**       | NAD⁺ competitive inhibitor  
                          PARP7 IC₅₀ <3 nM  
                          Kᵦ <0.001 µM, t₁/₂ 325 min  
                          Cell MARylation EC₅₀ = 1 nM  
                          Cell Proliferation (NCI-H1373) GI₅₀ = 20 nM |
| **Selectivity**          | >50-fold selective vs. PARP family  
                          No inhibition in kinase panel (1 µM) |
| **Compound properties**  | MW: 523 / Solubility pH 7.4 PBS: 0.07 mg/mL  
                          cLogP: 1.8 / tPSA: 112  
                          Protein Binding 63% in human |
| **ADME**                 | Good in vitro / in vivo correlation across species  
                          Eliminated predominantly by metabolism  
                          Orally bioavailable |
| **Toxicology**           | CYP P450 inhibition (>100 µM)  
                          hERG (> 10 µM)  
                          No inhibition in CEREP panel (1 µM) |
| **Pharmacology**         | Complete tumor regressions as single agent in human tumor model  
                          Complete responses with tumor-specific adaptive immune memory in murine syngeneic model |
PARP7 Inhibitors Block Proliferation in a Subset of Cancer Cell Lines

Subset of cancer cell lines exhibit dependency on PARP7 for proliferation

- Cell line panel screen consisting of 125 cancer cell lines derived from multiple cancer types
- Clear differentiation compared to a PARP1 inhibitor
- Sensitive cell lines were enriched with genes involved in Type I interferon response and antigen presentation
RBN-2397 Restores Cytosolic Nucleic Acid Sensing and Blocks Cell Proliferation in a Human Lung Cancer Cell Line

- PARP7 inhibition “releases the brake” on cytosolic nucleic acid sensing and induces Type I IFNs in tumors
- Restoration of Type I IFN response is measured by an increase in STAT1 phosphorylation
- PARP7 inhibition blocks cell proliferation

**PARP7 inhibitor RBN-2397 reverses block in Type I IFN response**

**PARP7 inhibitor RBN-2397 potently inhibits cell proliferation**
RBN-2397 Causes Complete Regressions in Human NSCLC NCI-H1373 Xenografts and Dose-Dependent Pharmacodynamic Effects

**Antitumor activity of RBN-2397**

- 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

**Exposure-PD relationship**

- Single oral dose of RBN-2397 in CB17 SCID mice with NCI-H1373 xenografts
- Exposure-dependent effects on ADP-ribosylation (MAR/PAR) and CXCL10 mRNA levels
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

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**CT26 re-challenge**

**4T1 re-challenge**

*All groups co-dosed with ABT*
CRISPR-Cas9 Used to Ablate either TBK1 or IFNAR1 in CT26 Cells to Investigate the Mechanism of Action of RBN-2397

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

- Ablation of tumor TBK1 prevents the antitumor activity of RBN-2397 in the CT26 tumor model
- IFN-β release by tumor cells is crucial for RBN-2397 mediated antitumor response

No PARP7i-mediated IFN-β release
No effects on cancer or immune cells
• IFNAR1 knockout initially attenuates antitumor activity of RBN-2397, but a subset of tumors start responding after Day 12
• Suggests onset of antitumor immunity around Day 12, induced by effects of tumor-derived IFN-β on immune cells
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 neutralizing antibodies on the background of tumoral IFNAR1 KO prevents antitumor activity of RBN-2397
- Suggests contribution of immune system through activation of IFN-β signaling in immune cells

All groups co-dosed with ABT
Engaging Cytosolic Nucleic Acid Sensing in the Tumor Cell as an Emerging Therapeutic Strategy

**Adaptive immunity**

- T-cell

**Cytosolic nucleic acid sensing - innate immune pathways**

- Tumor microenvironment (TME)
  - (DC, MΦ)
  - STING
  - TLR7/9
  - RIG-I

- Tumor cell
  - PARP7

New cancer treatment strategy
RBN-2397 – A Novel Cancer Therapeutic Being Tested in Clinical Trials

- Discovered first potent and selective PARP7 inhibitor
  - Novel first-in-class therapy
- RBN-2397 inhibits PARP7 reactivating effective nucleic acid sensing, leading to:
  - Arrest of cancer cell proliferation and tumor regression
  - Increased signaling to the immune system
  - Development of immune memory
- 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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ribon therapeutics