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

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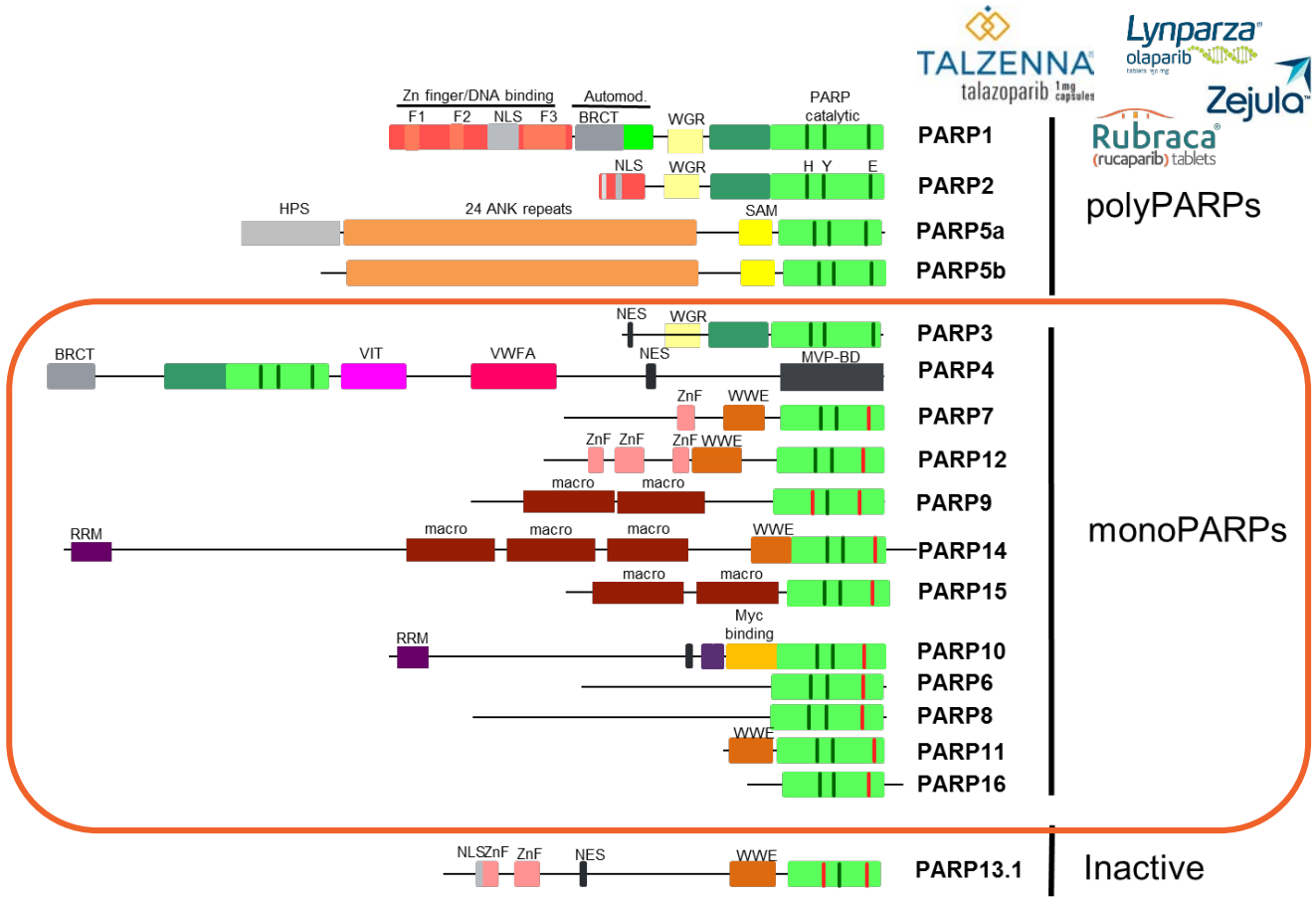
- Ribon Therapeutics

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**

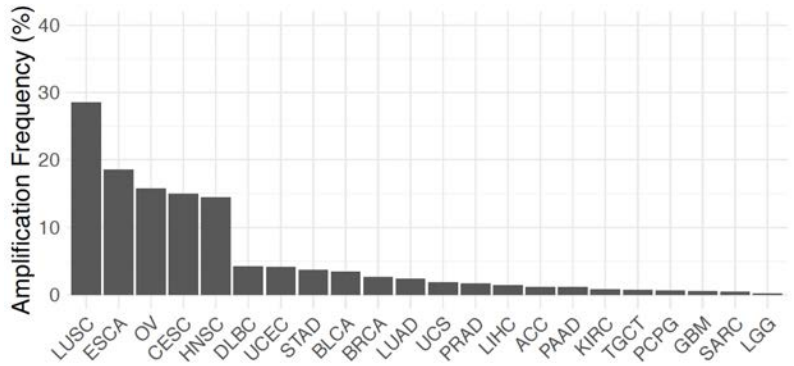


Adapted from Vyas, Chang et. al. Nature Comm. 2013

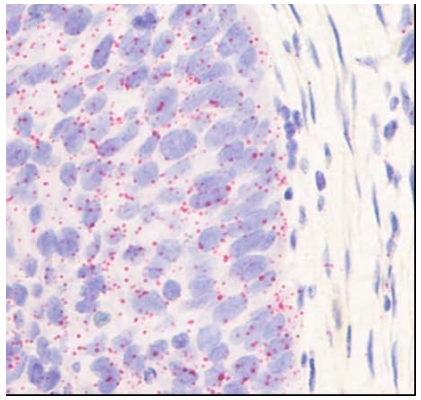
PARP7: A Novel Brake on the Type I Interferon Response and Genetic Alterations in Cancer

- **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

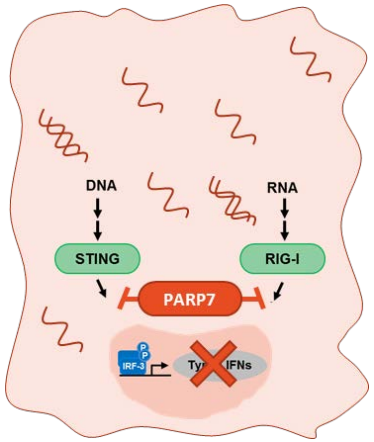


Highly expressed in primary SCCL tumors



PARP7 in situ hybridization

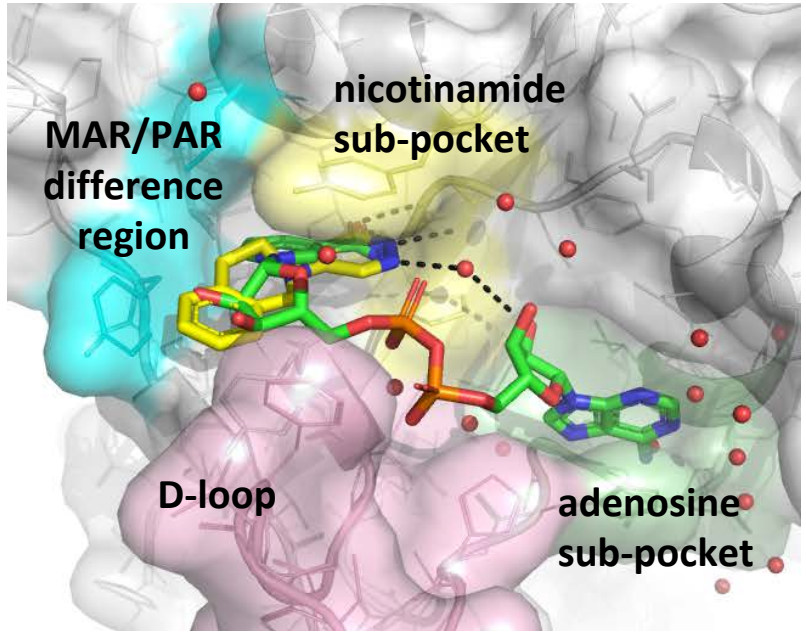
PARP7 "brake" on nucleic acid sensing and Type I IFN response



PARP7 Hit Identified in Cross Screening of Ribon Library

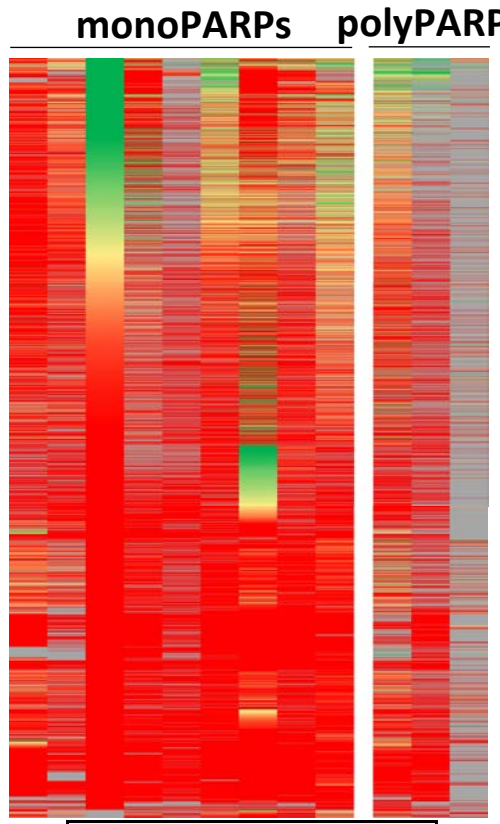
Ribon proprietary library

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

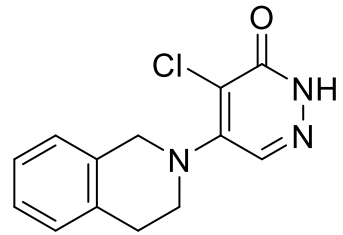


Small molecule PARP inhibitors
HTS and fragment screening hits
Crystal structures across PARP family
Structure based drug design

Cross screening



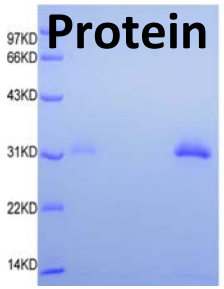
PARP7 hit



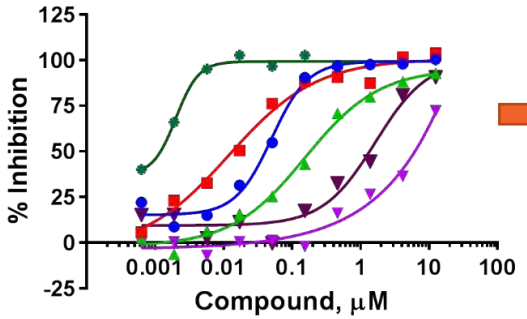
PARP	Biochemical IC_{50} (μM)
PARP7	9
PARP1	300
PARP16	3

Developed Biochemical and Cellular Assays which Enabled Optimization of PARP7 Inhibitors

High Quality PARP7 Protein

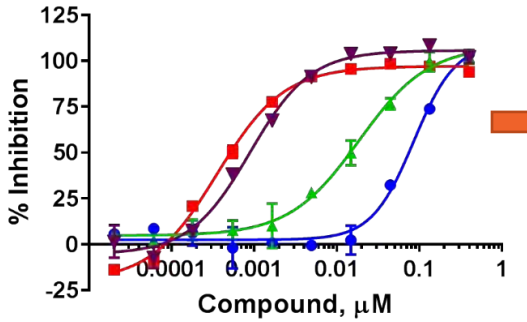


Biochemical Assay (TR-FRET)



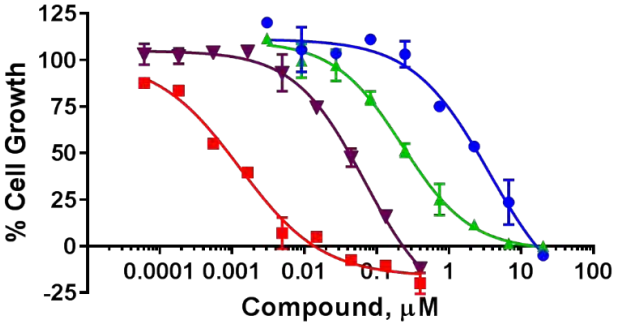
Wigle, et. al. SLAS Discovery, 2019

Cell Biochemical Assay (MARylation)



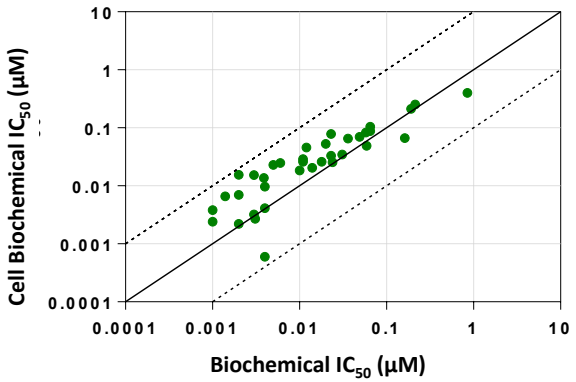
PARP7 inhibition in cells by measuring MARylation

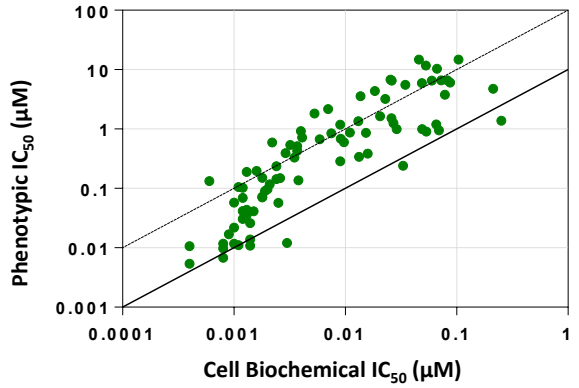
Phenotypic Assay (Proliferation)



NCI-H1373 lung cancer cells

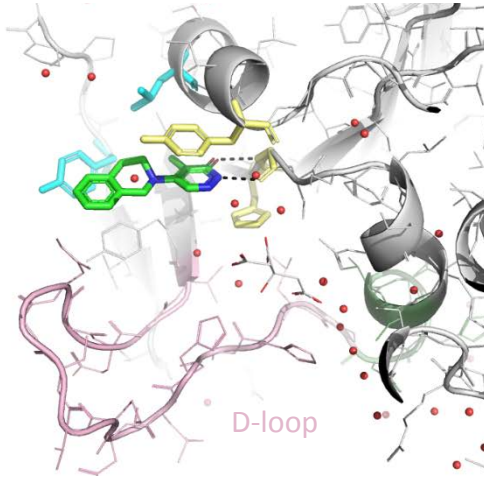
Correlation Between Biochemical, Cell Biochemical, and Cell Phenotypic Assays





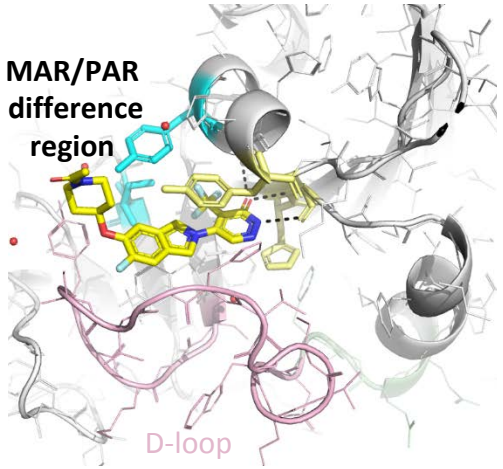
Optimization of Hit Led to Potent and Selective PARP7 Inhibitors

PARP16 RBN010206



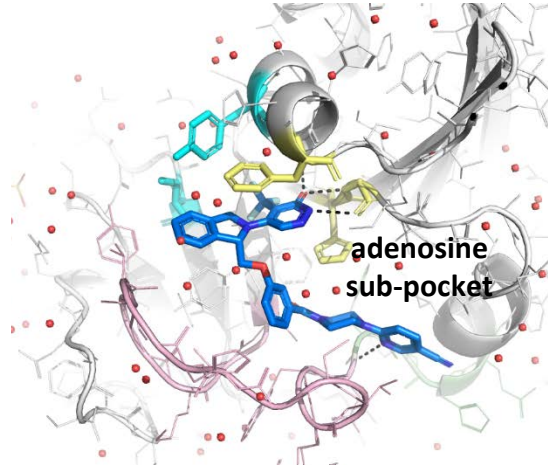
Pan monoPARP fragment hit

PARP12 RBN011082



Pan monoPARP selective inhibitor

PARP12/7 RBN011364



PARP7 potent and selective inhibitor

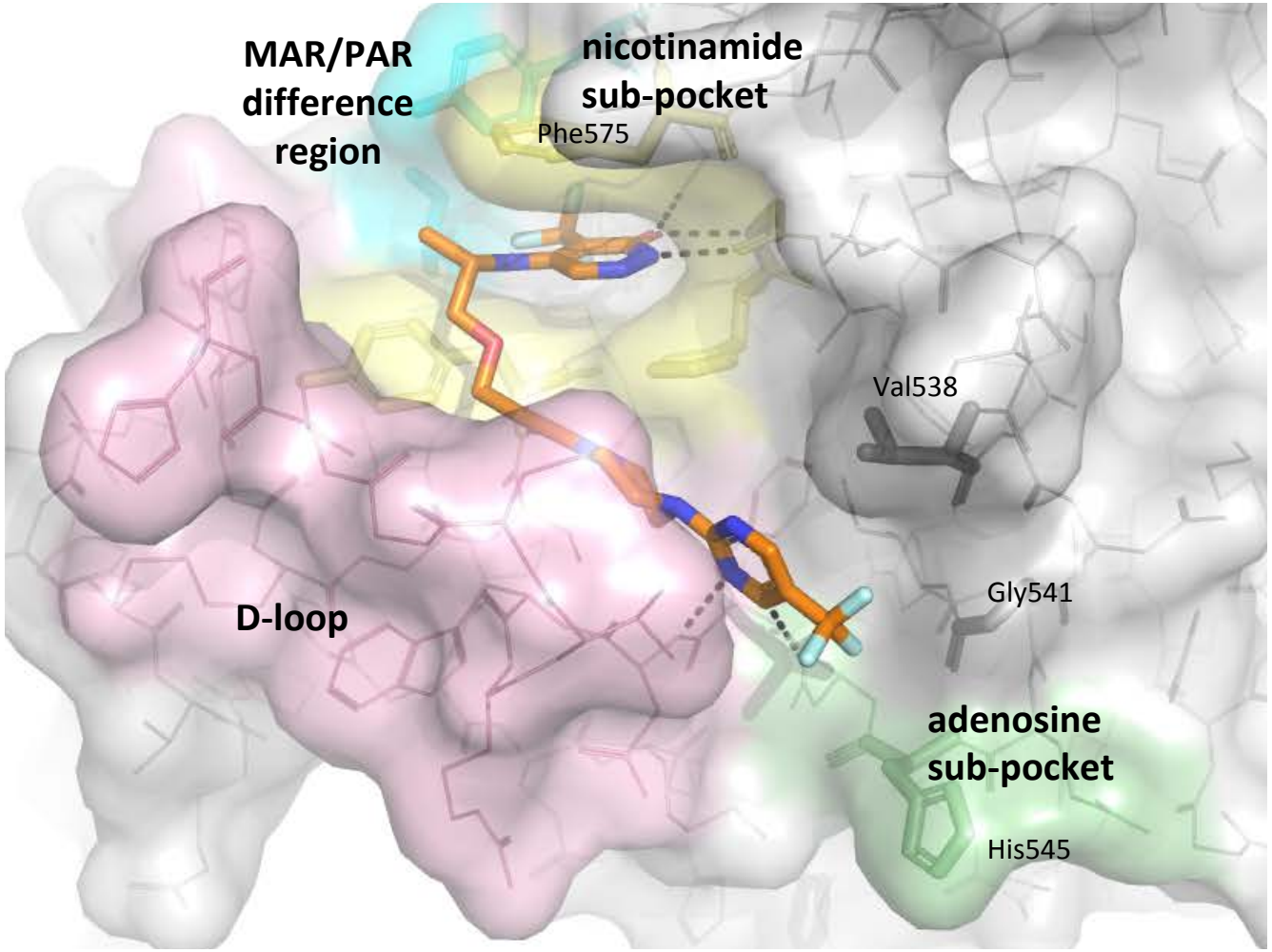
PARP	Biochemical IC_{50} (µM)
PARP7	9
PARP1	300
PARP16	3

PARP	Biochemical IC_{50} (µM)
PARP7	0.007
PARP1	0.3
All other monoPARPs similar potency to PARP7	

PARP	Biochemical IC_{50} (µM)
PARP7	<0.003
PARP1	1
All other monoPARPs >20 fold selective	

Discovery of Development Candidate RBN-2397

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

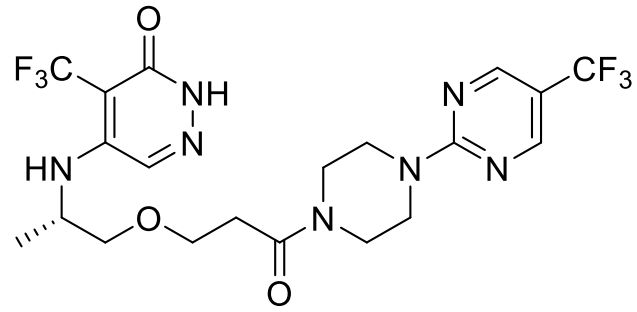


- **Lead optimization efforts targeted interactions in key areas of the NAD$^+$ binding pocket**
  - Adenosine sub-pocket: exploit positive interaction with PARP7 Gly541 and clash with bulky residues in other PARPs
  - Removed 2 aromatic rings which improved solubility and microsomal stability
- **Optimization 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

	RBN-2397
Target potency	NAD⁺ competitive inhibitor PARP7 IC_{50} <3 nM K_D <0.001 µM, $t_{1/2}$ 325 min Cell MARylation EC_{50} = 1 nM Cell Proliferation (NCI-H1373) GI_{50} = 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



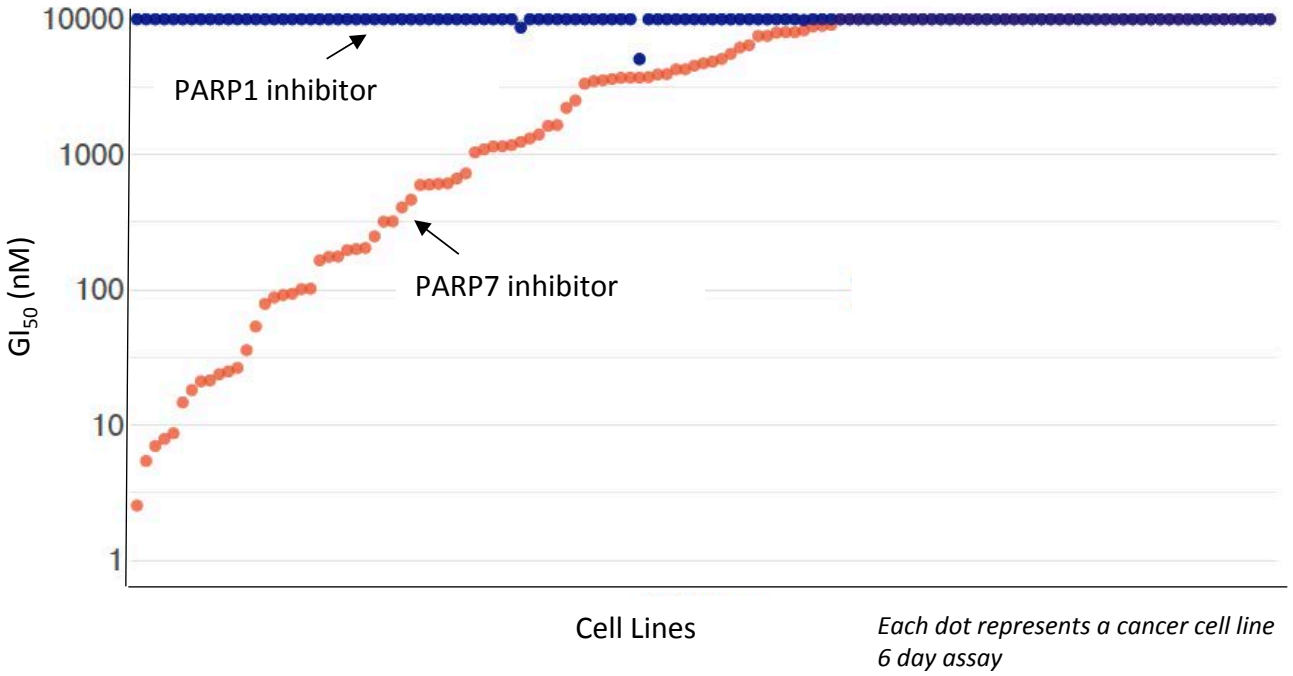
Potent and selective inhibitor

Drug-like properties support oral dosing in humans

On target activity in preclinical models

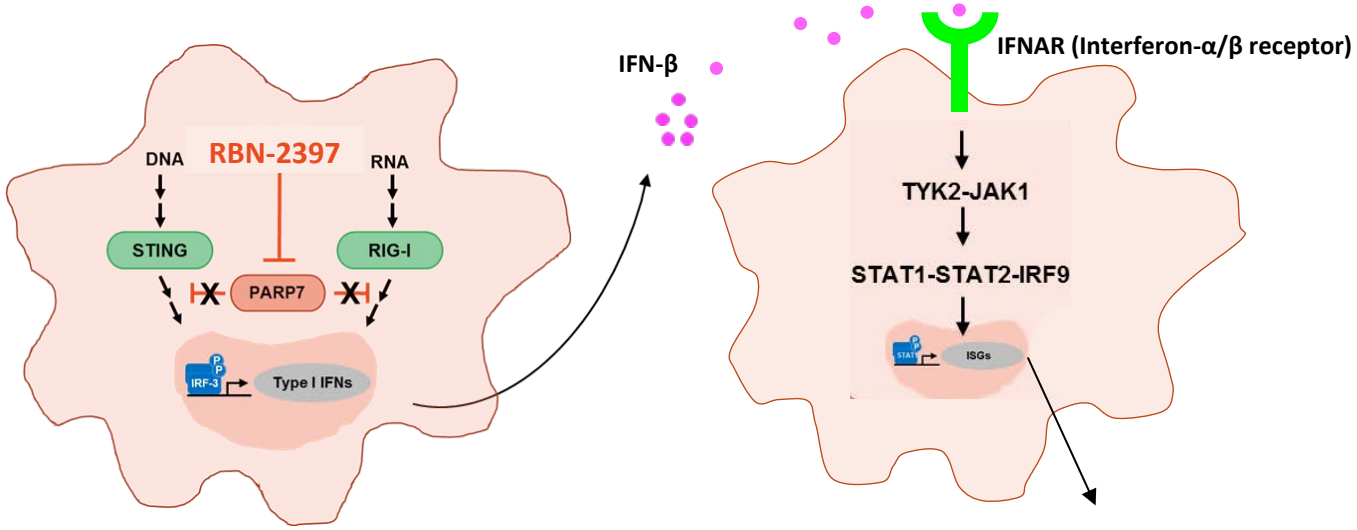
PARP7 Inhibitors Block Proliferation in a Subset of Cancer Cell Lines

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**

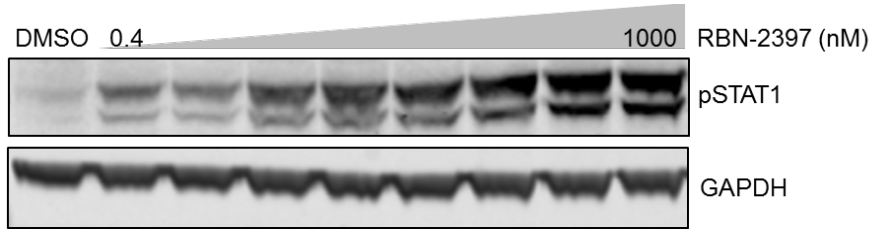
RBN-2397 Restores Cytosolic Nucleic Acid Sensing and Blocks Cell Proliferation in a Human Lung Cancer Cell Line



Cancer cells stop growing
Immune response, e.g. via CXCL10

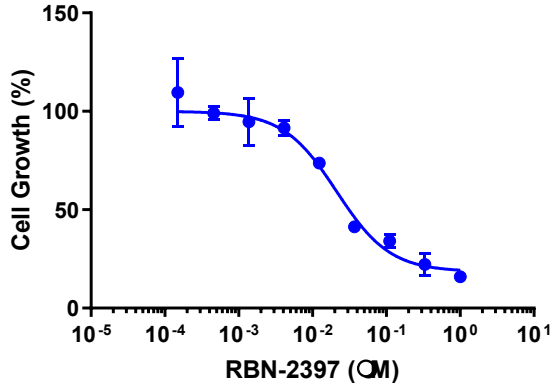
- **PARP7 inhibition "releases the brake" on cytosolic nucleic acid sensing and induces Type I IFNs in tumors**
- **Restoration of Type I IFN response is measured by an increase in STAT1 phosphorylation**
- **PARP7 inhibition blocks cell proliferation**

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



NCI-H1373 human lung cancer cells, 24 h

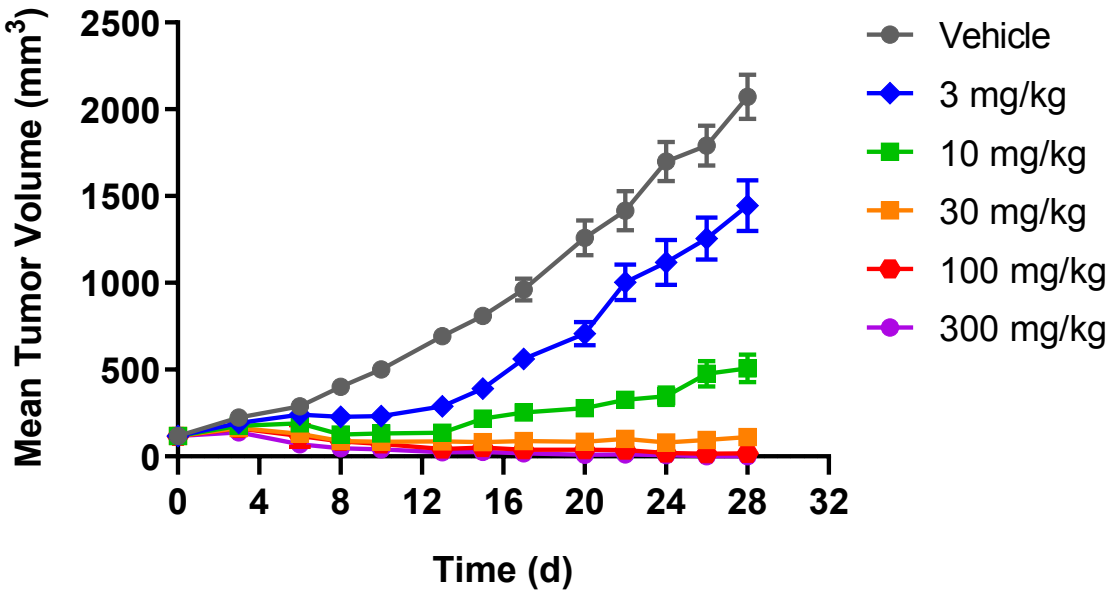
PARP7 inhibitor RBN-2397 potently inhibits cell proliferation



NCI-H1373 lung cancer cells, 6-day assay

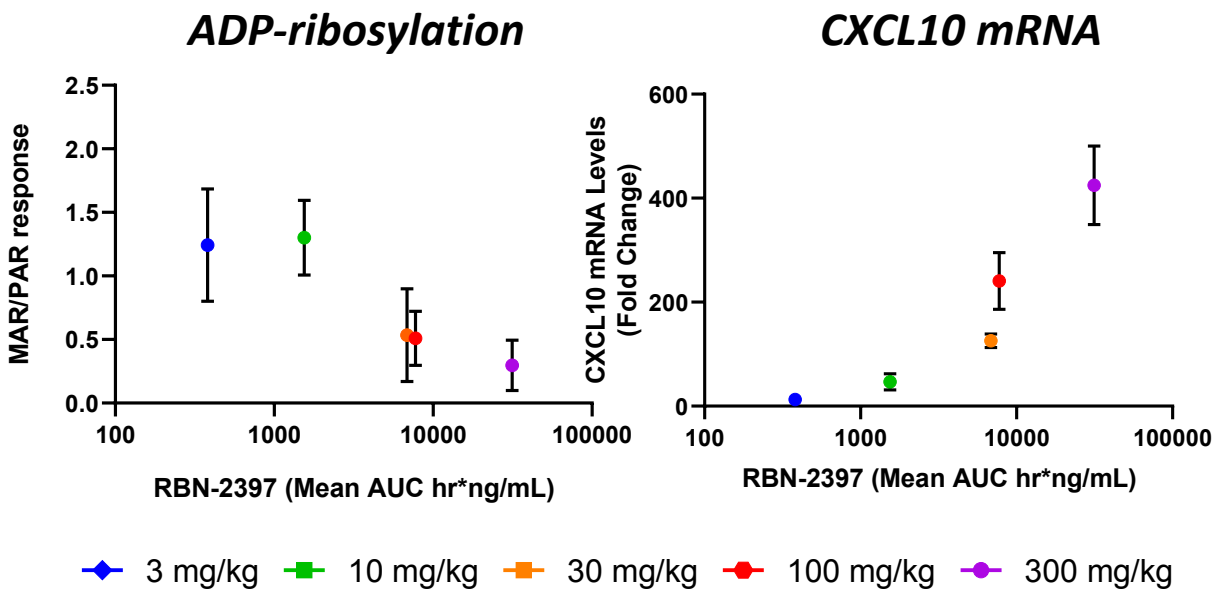
RBN-2397 Causes Complete Regressions in Human NSCLC NCI-H1373 Xenografts and Dose-Dependent Pharmacodynamic Effects

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**

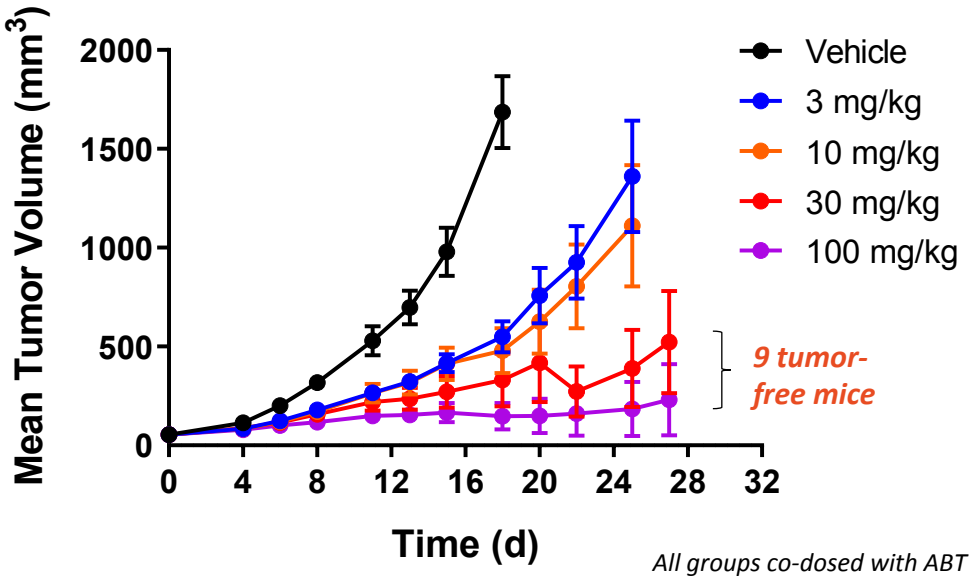
Exposure-PD relationship



- **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

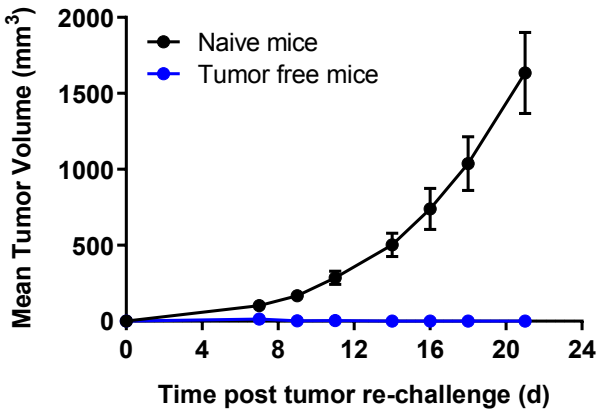


All groups co-dosed with ABT

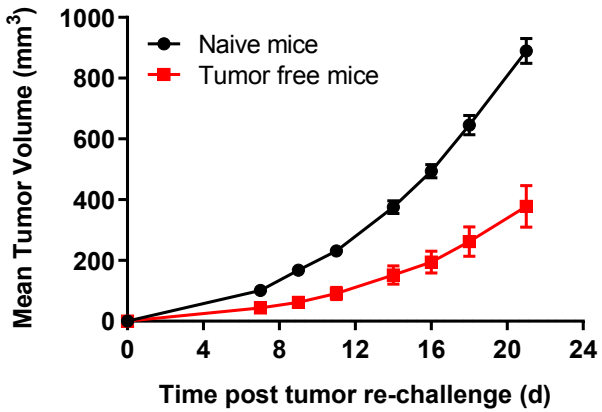
- **Once daily oral dosing of RBN-2397 in CT26 tumor-bearing BALB/c mice**
- **Tumor-free mice were monitored for 60 days**

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

CT26 re-challenge

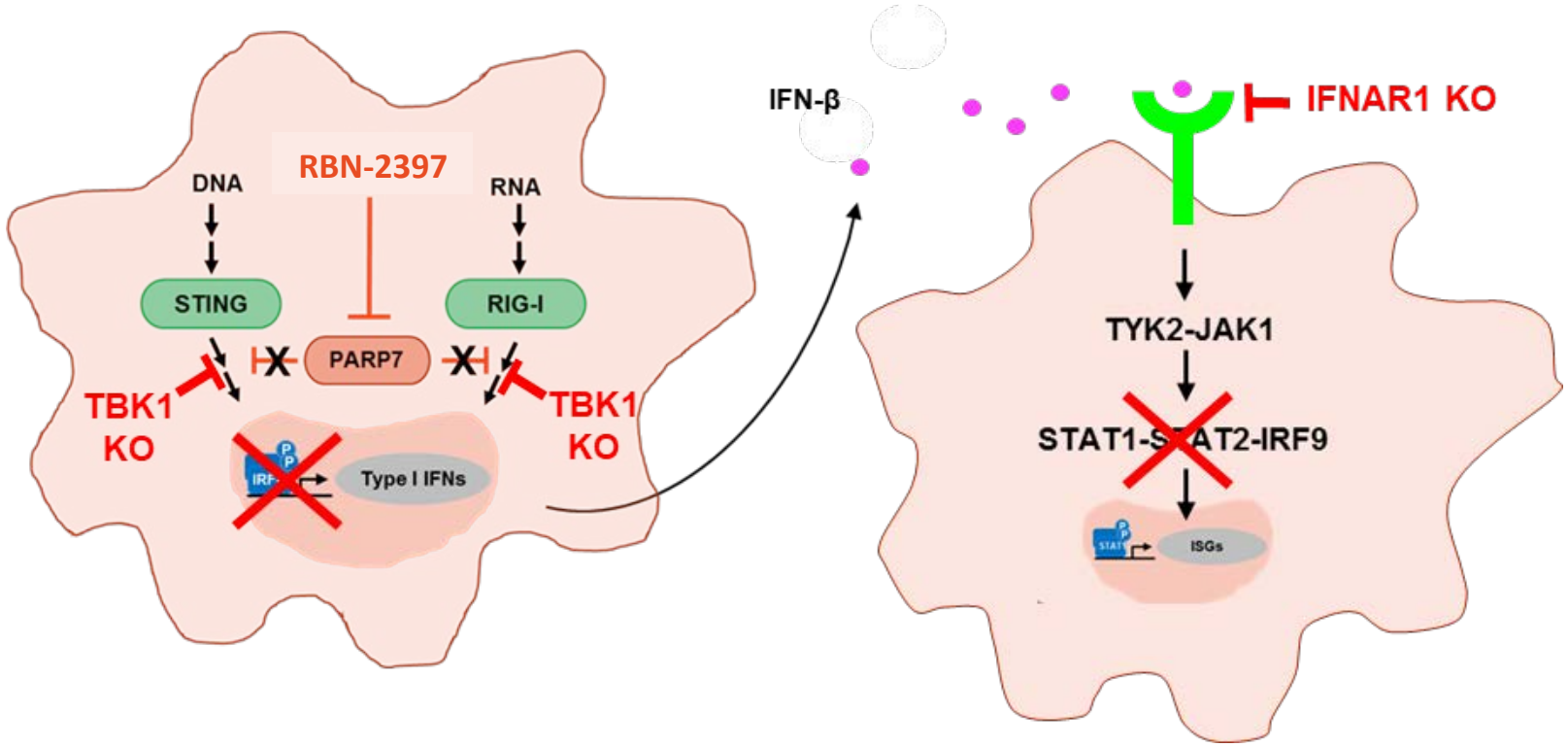


4T1 re-challenge



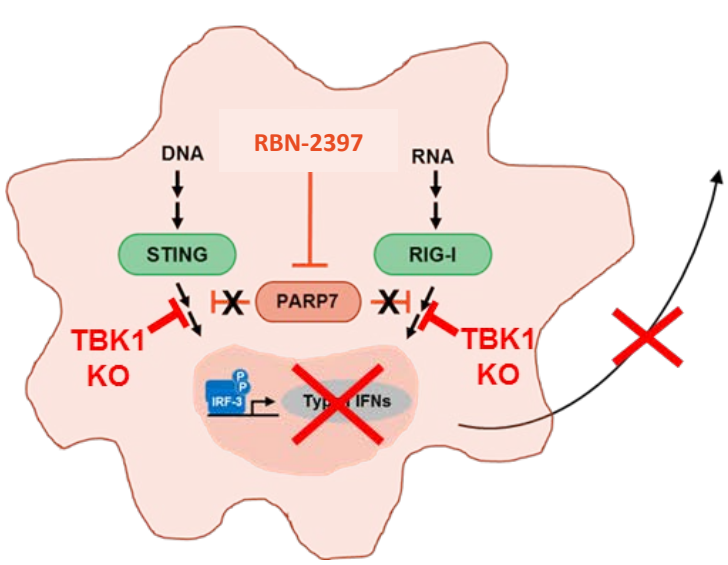
- **Tumor-free mice re-challenged with CT26 and subsequently 4T1 cells**
- **All tumor-free mice rejected CT26 cells but not 4T1, demonstrating induction of tumor-specific adaptive immune memory**

CRISPR-Cas9 Used to Ablate either TBK1 or IFNAR1 in CT26 Cells to Investigate the Mechanism of Action of RBN-2397

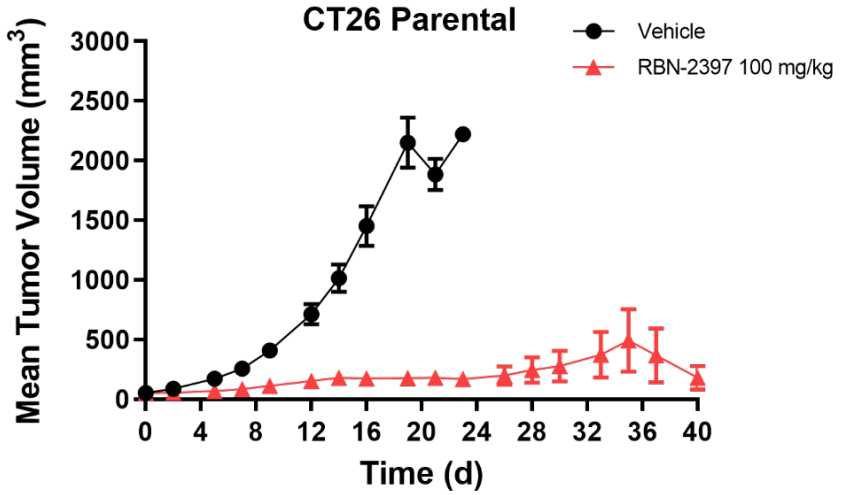


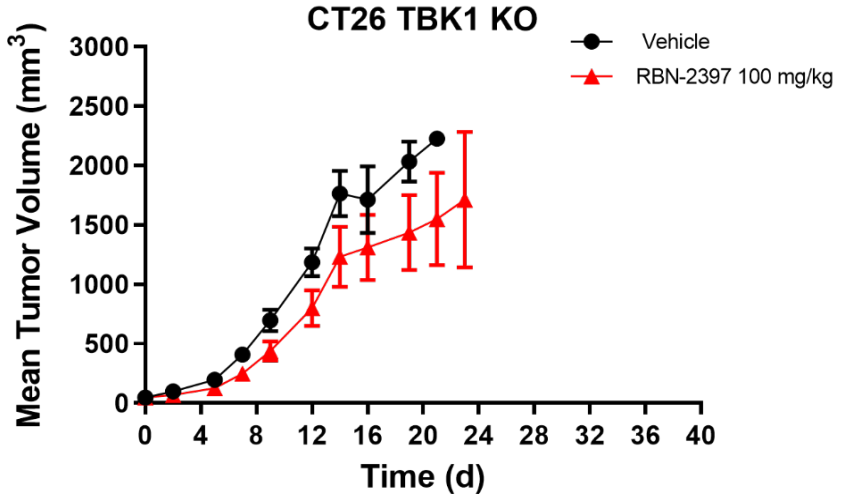
- **TBK1 knockout prevents both IRF3 & STAT1 phosphorylation by RBN-2397**
- **IFNAR1 knockout prevents STAT1 phosphorylation by RBN-2397**

Tumor-derived Interferon Is Key for Antitumor Activity



No PARP7i-mediated IFN-β release
No effects on cancer or immune cells

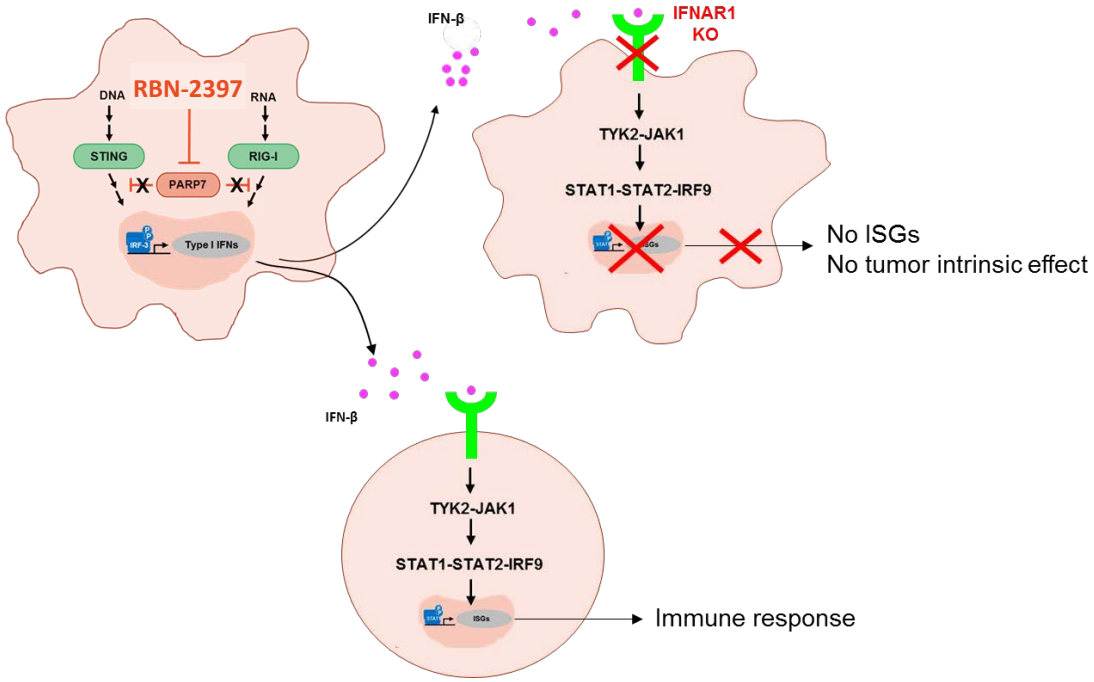


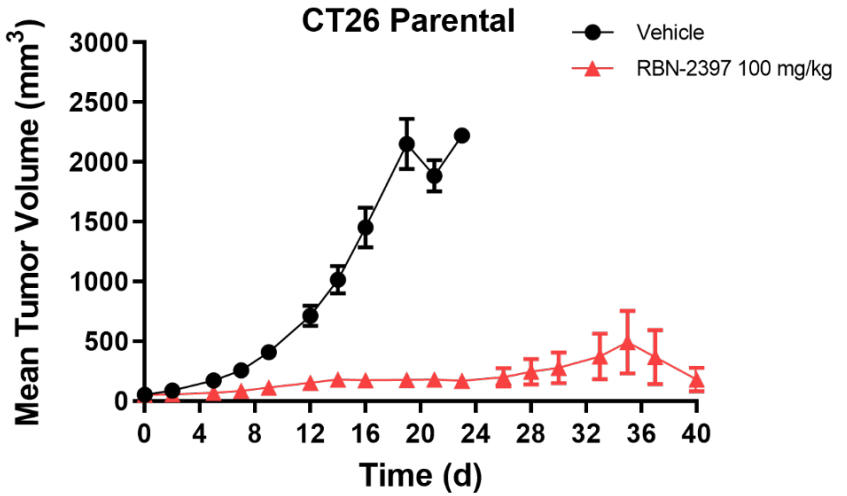


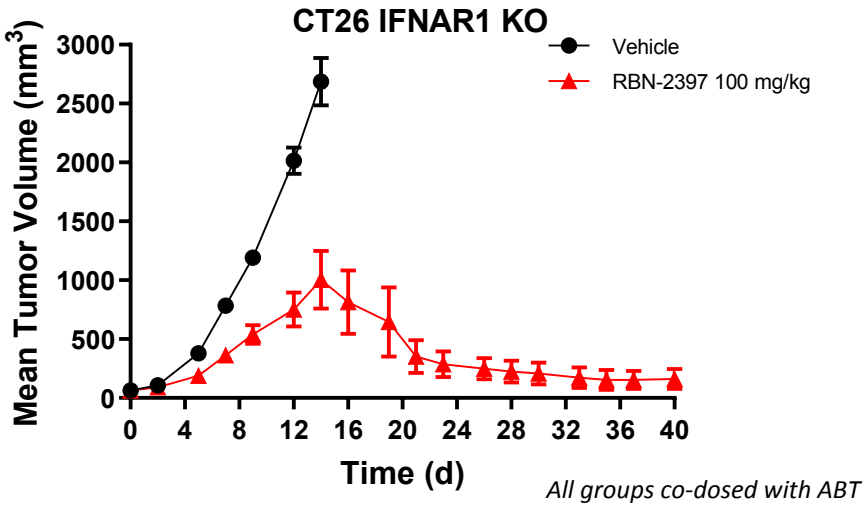
- **Ablation of tumor TBK1 prevents the antitumor activity of RBN-2397 in the CT26 tumor model**
- **IFN-β release by tumor cells is crucial for RBN-2397 mediated antitumor response**

All groups co-dosed with ABT

IFNAR1 Knockout in CT26 Tumor Cells Partially Attenuates Antitumor Activity of RBN-2397 in the CT26 Tumor Model



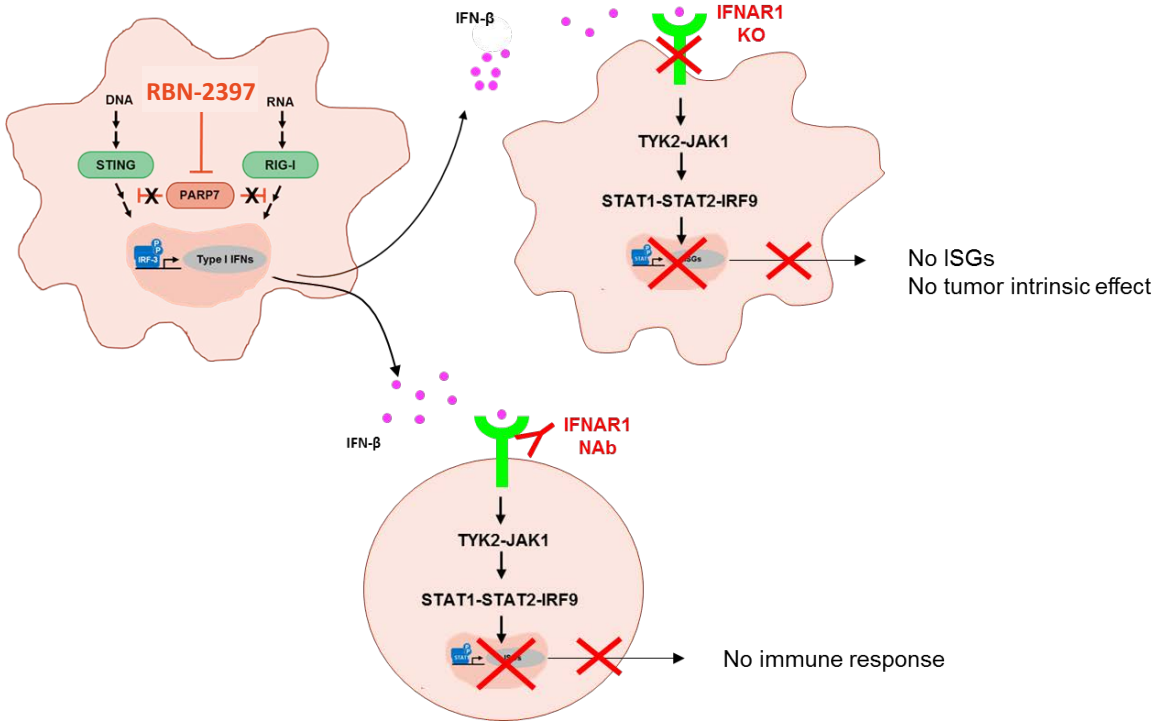


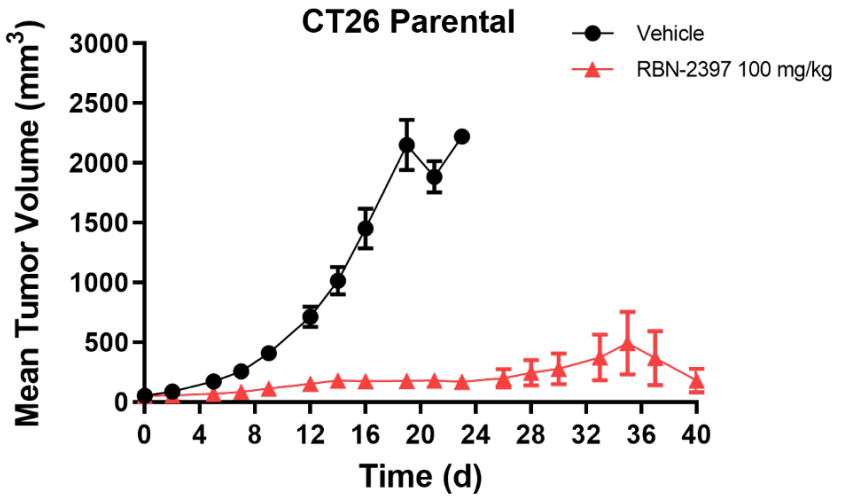


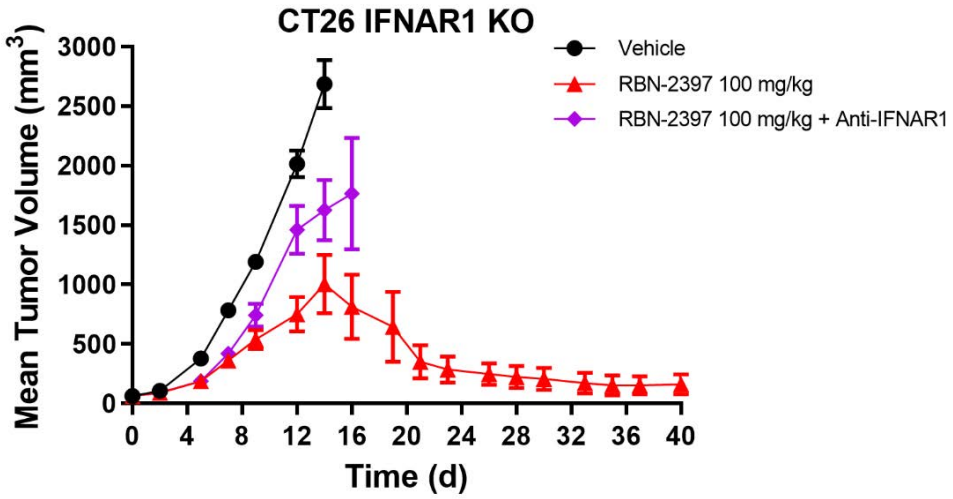
- **IFNAR1 knockout initially attenuates antitumor activity of RBN-2397, but a subset of tumors start responding after Day 12**
- **Suggests onset of antitumor immunity around Day 12, induced by effects of tumor-derived IFN-β on immune cells**

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



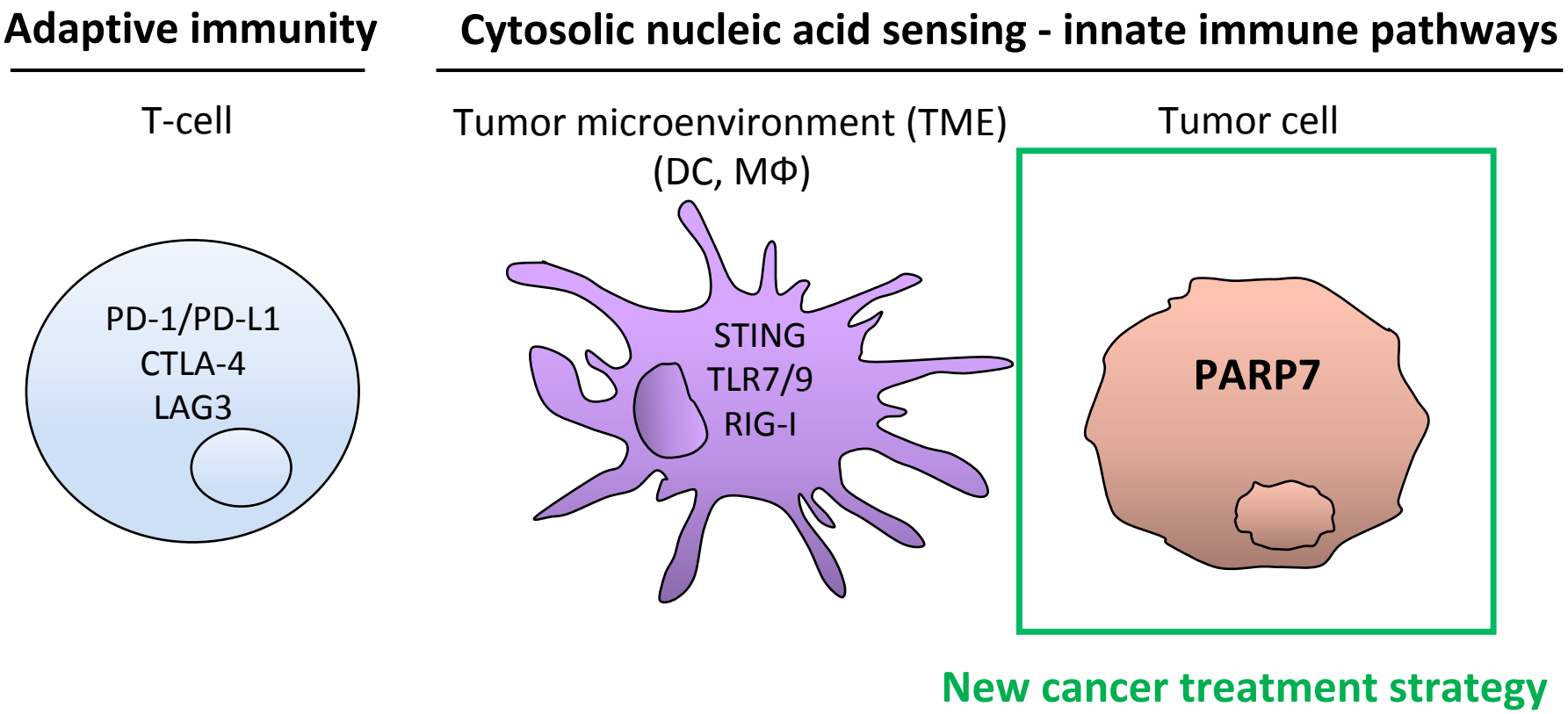




- **Dosing of anti-IFNAR1 neutralizing antibodies on the background of tumoral IFNAR1 KO prevents antitumor activity of RBN-2397**
- **Suggests contribution of immune system through activation of IFN-β signaling in immune cells**

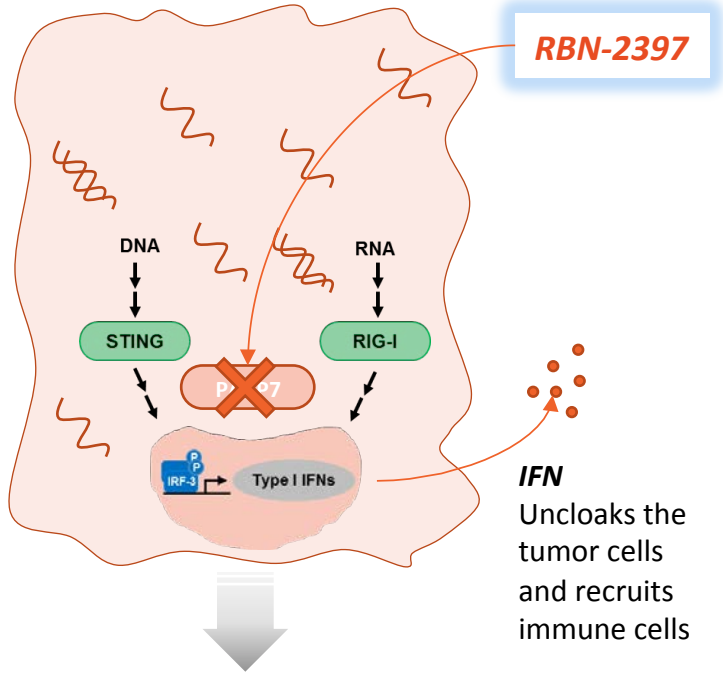
All groups co-dosed with ABT

Engaging Cytosolic Nucleic Acid Sensing in the Tumor Cell as an Emerging Therapeutic Strategy



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)**



Complete regressions and antitumor immunity as a single agent

Acknowledgements

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W. David Church
Lisa Cleary
David Cordo
Bryan Dorsey
Jennifer Downing
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