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

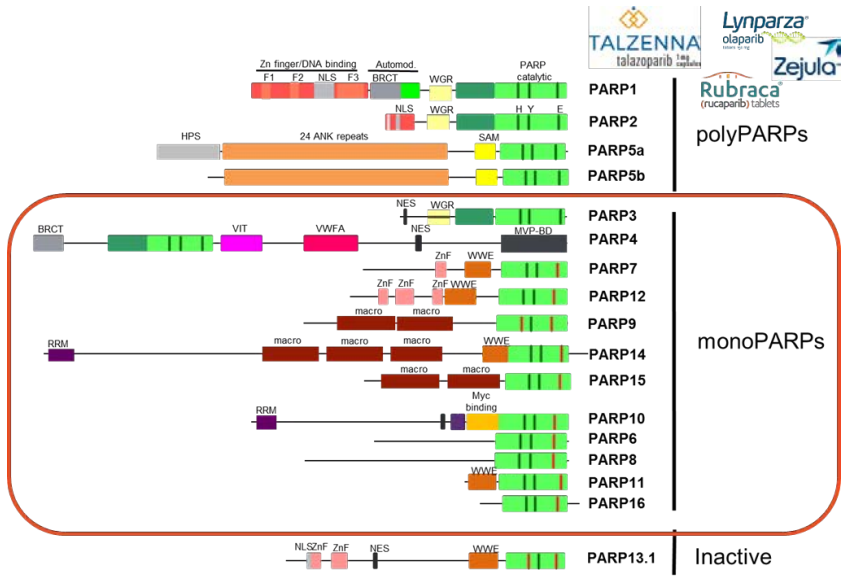
- Joe Gozgit
- Ribon Therapeutics

Disclosure Statement

- **I am an employee and shareholder of Ribon Therapeutics**

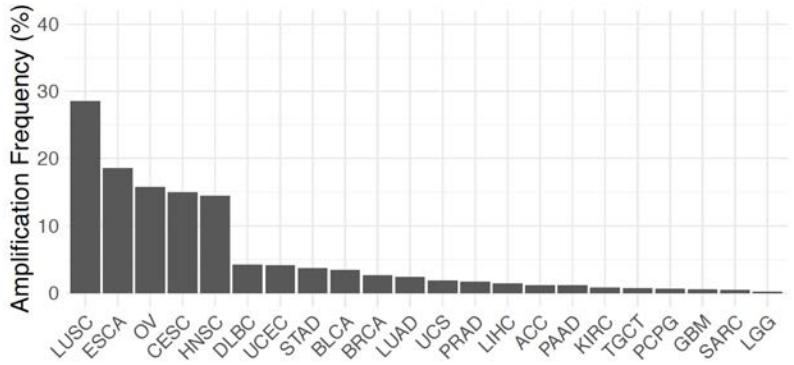
Not All PARPs Are Alike – Outside of PolyPARPs the PARP Family Is Unexplored for Therapeutic Development

- **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)



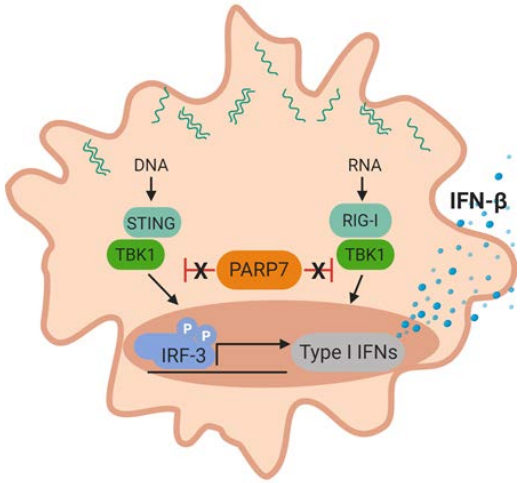
Vyas, Chang et. al. Nature Comm. 2013

PARP7 is frequently amplified in cancer



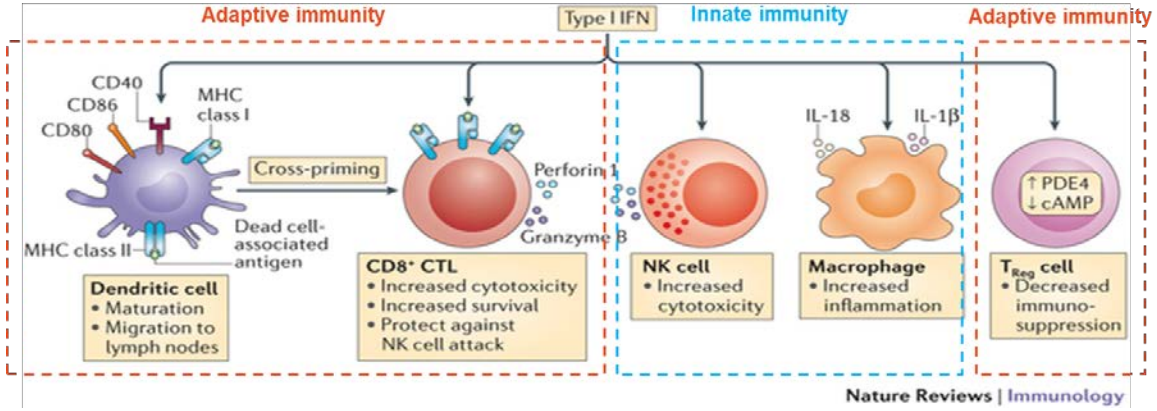
PARP7 Acts as a Brake in Cytosolic Nucleic Acid Sensing and the Type I Interferon (IFN) Response

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



PARP7 has been reported to negatively regulate the Type I response by interacting with TBK1 during viral infection (Yamada-Nat Immunol-2016)

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



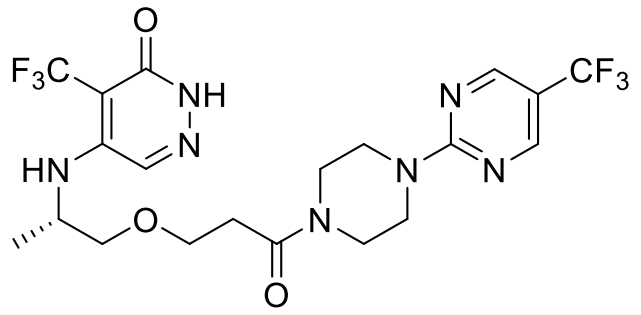
Zitvogel-Nature Rev Cancer-2015

- **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**

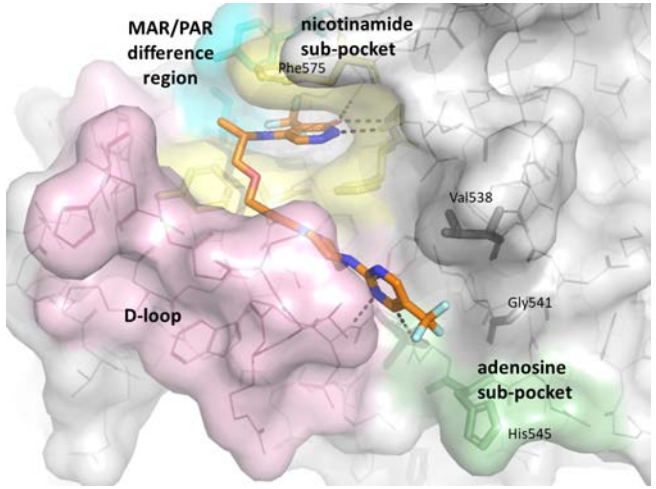
RBN-2397 is a Potent and Selective Inhibitor of PARP7

- **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)**

Structure of RBN-2397



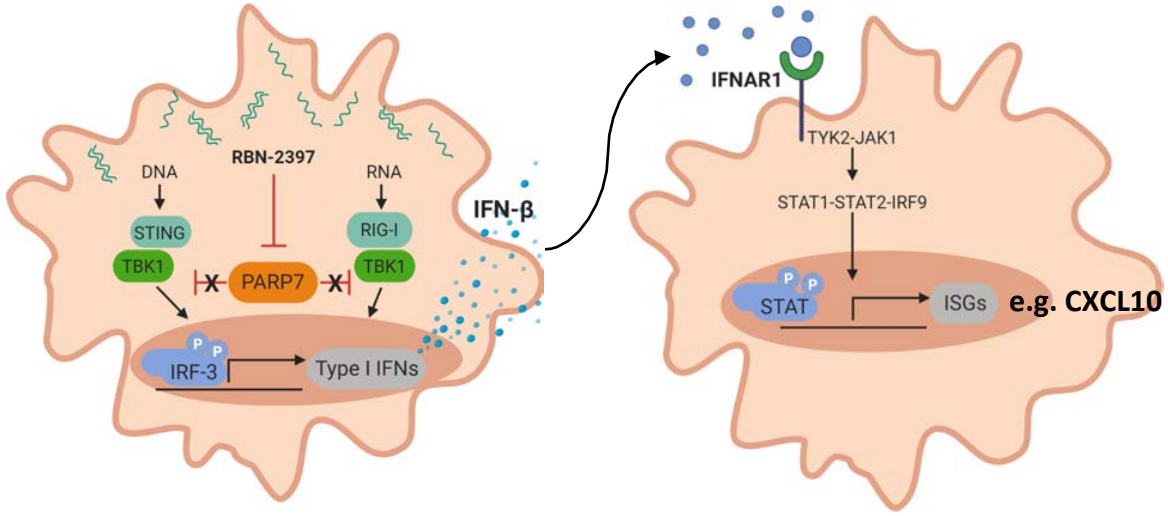
Co-crystal structure of RBN-2397 bound to PARP12/7



PARP12 was used as a surrogate for PARP7. Four labeled residues were mutated from PARP12 to match the PARP7 sequence.

RBN-2397 Restores Cytosolic Nucleic Acid Sensing in the Mouse CT26 Cancer Cell Line

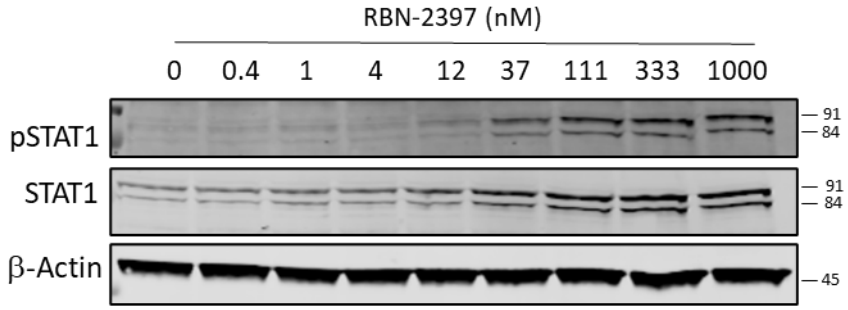
PARP7 inhibition "releases the brake" on cytosolic nucleic acid sensing and induces Type I IFNs



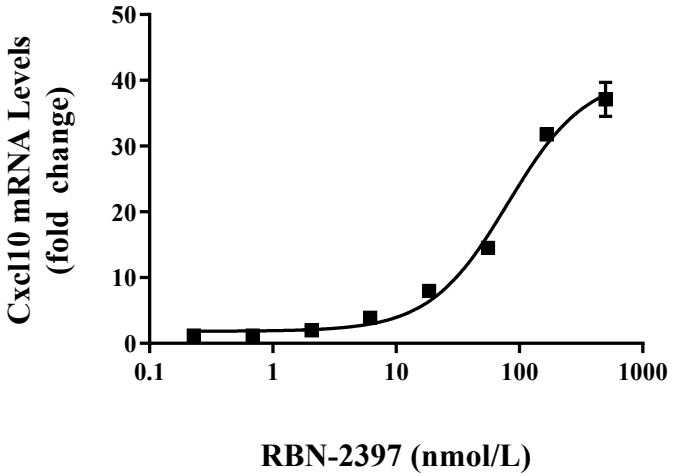
- **Restoration of Type I IFN response is measured by an increase in STAT1 phosphorylation and interferon stimulated genes (ISGs)**

RBN-2397 restores Type I IFN response in CT26 cells

Induction of pSTAT1

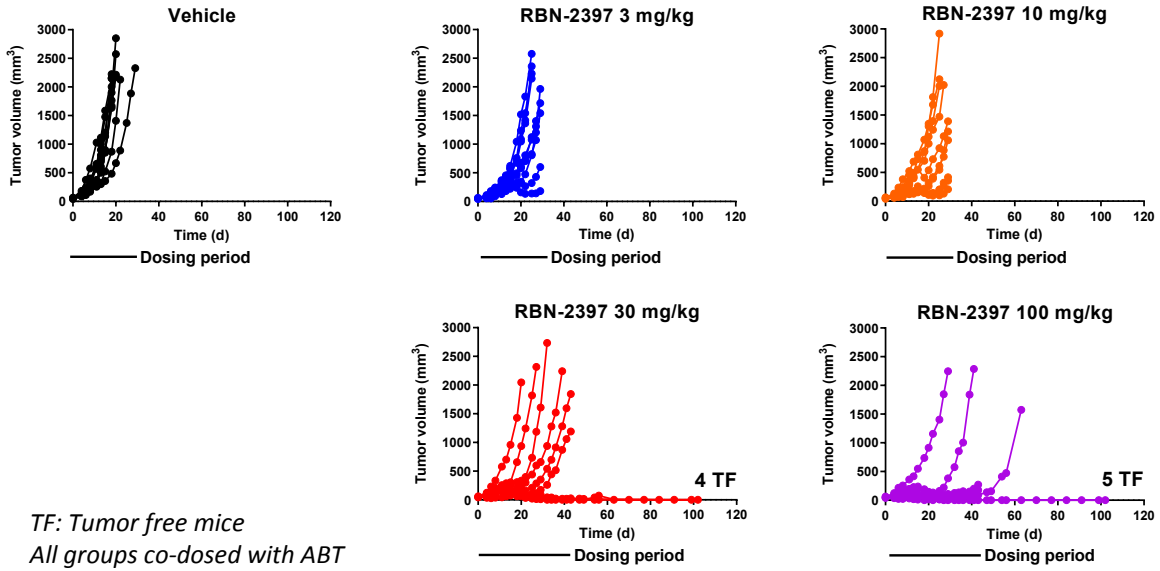


Induction of CXCL10 mRNA



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

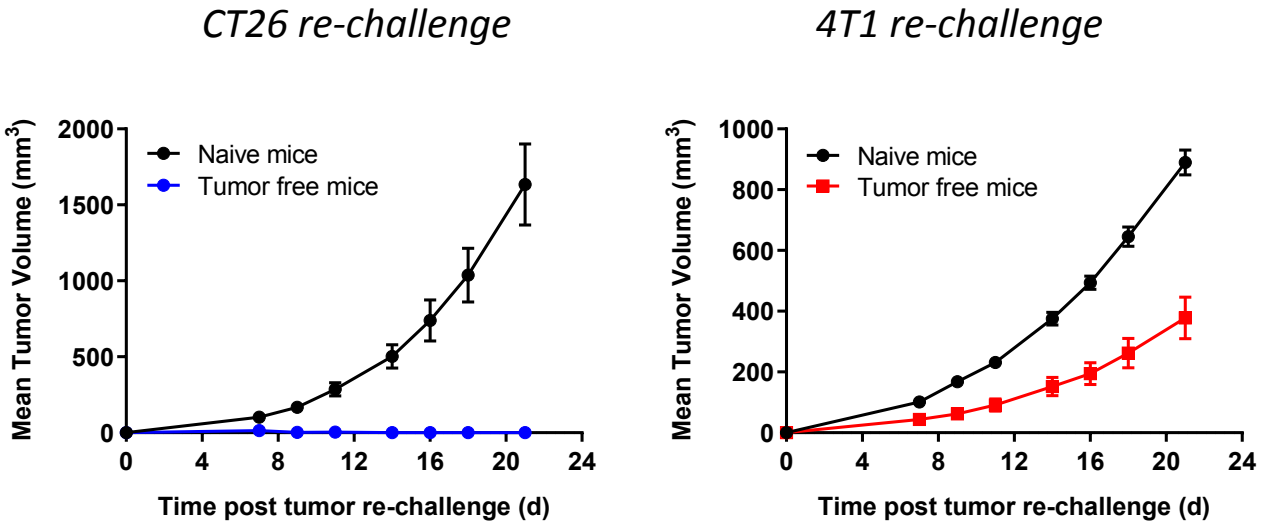
Primary Efficacy: RBN-2397 induces durable regressions



TF: Tumor free mice
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**

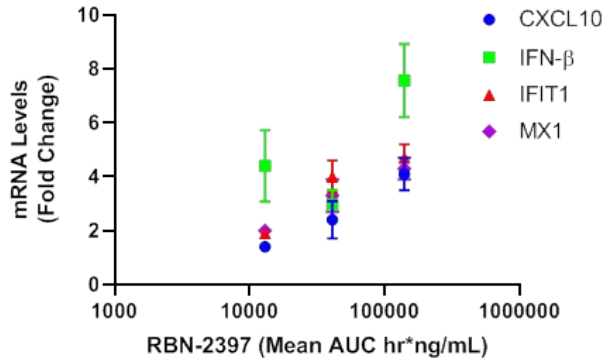
Re-challenge of tumor-free mice: Rejection of CT26 cells



- **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

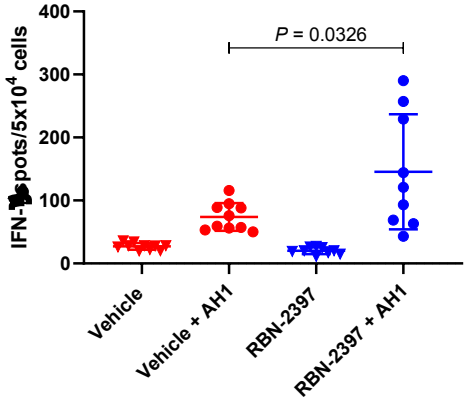
RBN-2397 shows exposure-dependent effects on PD markers in CT26 tumors



CT26 tumor-bearing mice administered as single oral dose.

RBN-2397 increases tumor antigen-specific T cells:

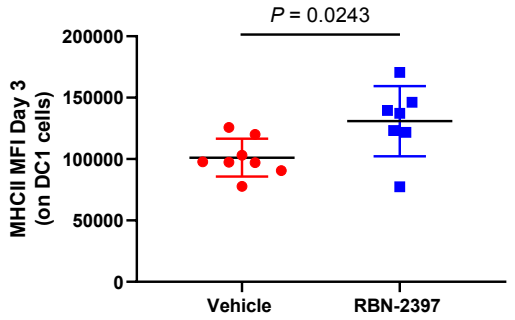
Increase in the number of splenic T cells producing IFN-γ in response to the CT26 antigen



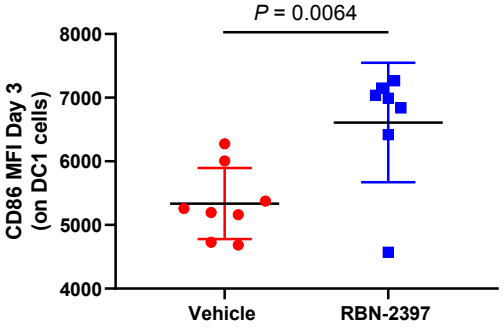
CT26 tumor-bearing mice dosed with RBN-2397 100 mg/kg+ABT for 15 Days.

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

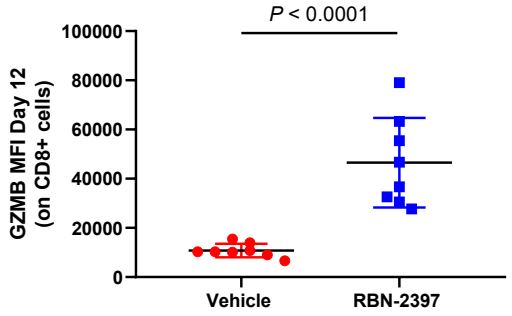
Increase in MHCII on DC1 cells



Increase in CD86 on DC1 cells



Increase in GrzB on CD8 T cells

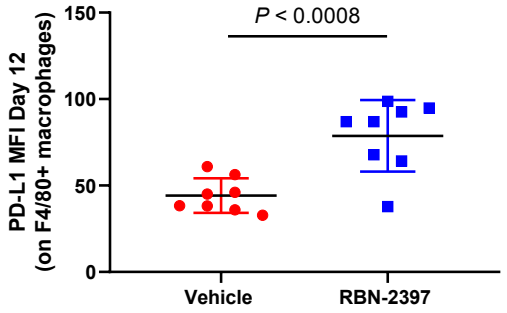


CT26 tumor-bearing mice dosed with RBN-2397 500 mg/kg. Tumors collected on days 3, 6 & 12.

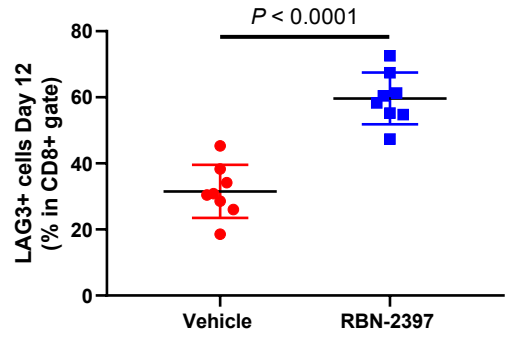
Combination of Anti-PD1 with RBN-2397 Increases the Number of Tumor-Free Mice in the CT26 Syngeneic Model

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

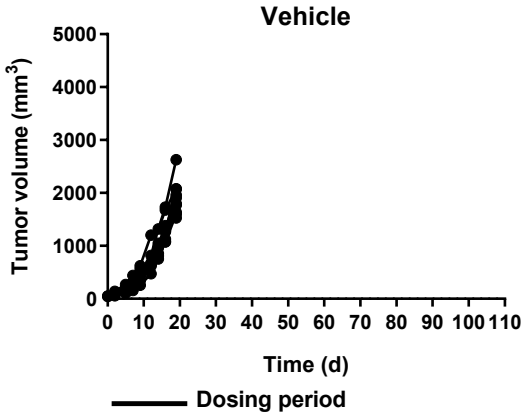
Increase in PD-L1 on macrophages

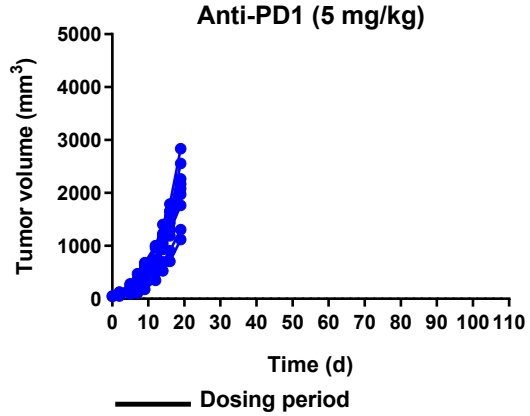


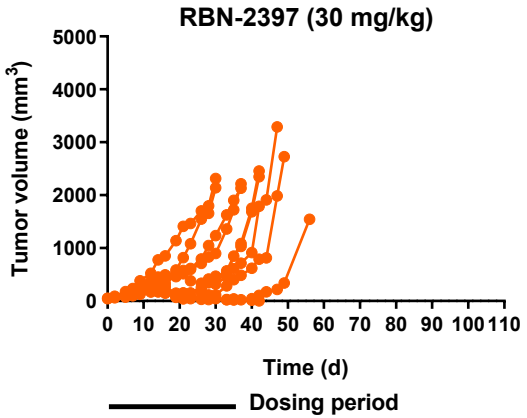
Increase in LAG3 on CD8 T cells

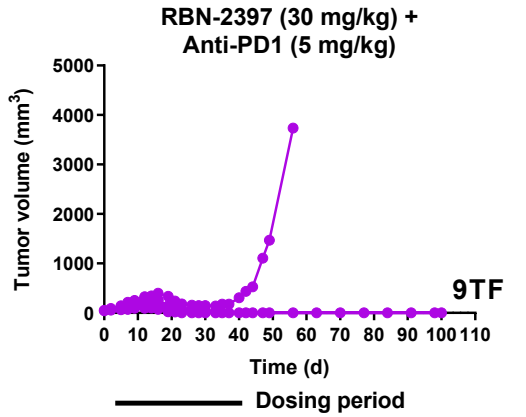


Enhanced antitumor immunity with combination with anti-PD1 in CT26



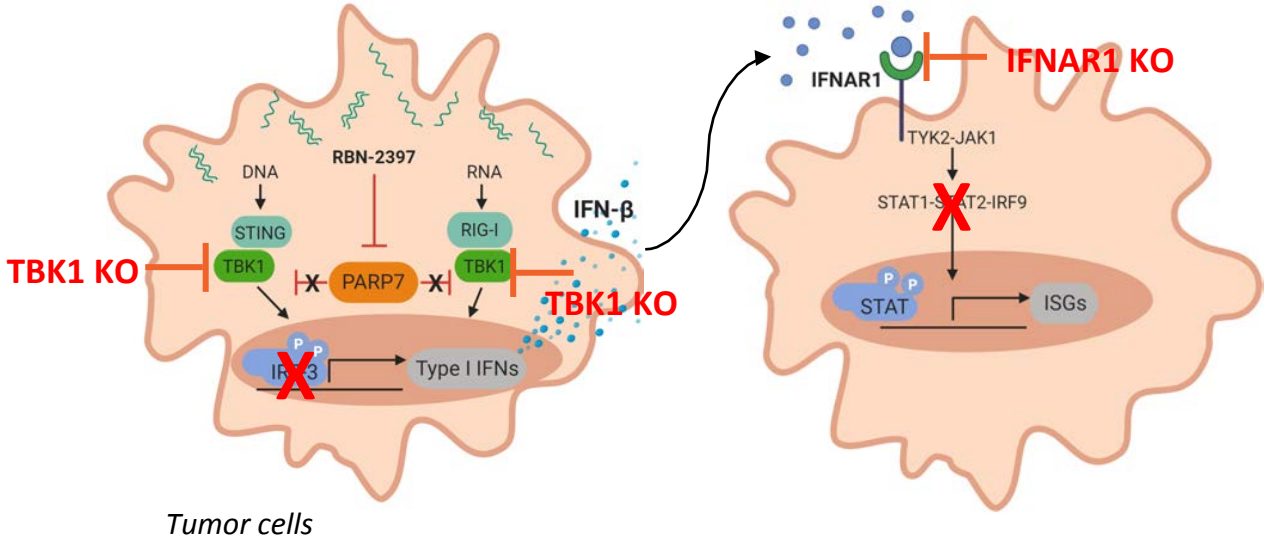




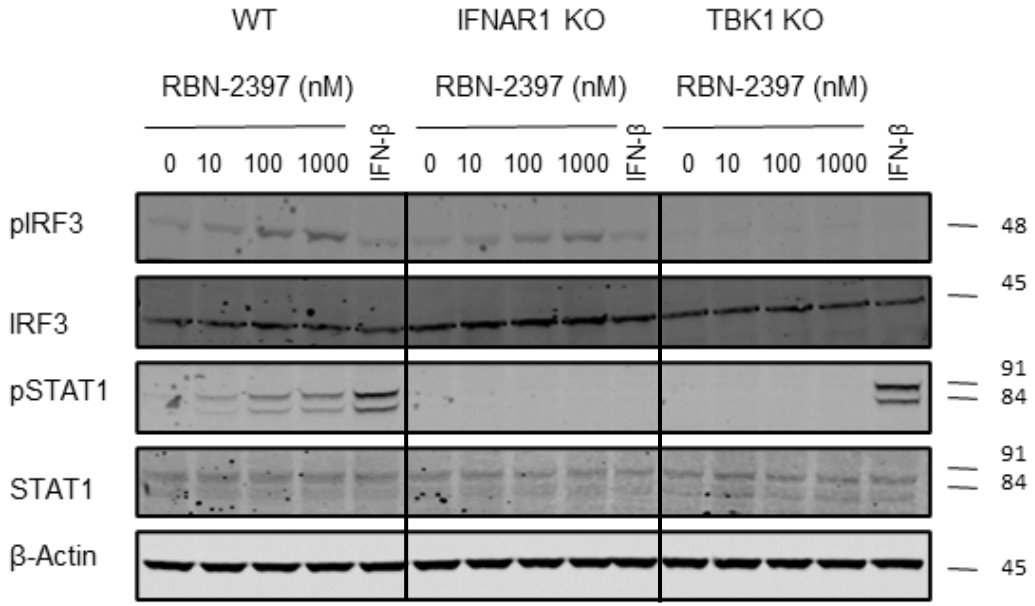


CRISPR-Cas9 Used to Ablate either TBK1 or IFNAR1 in CT26 Cells to Investigate the Mechanism of Action of RBN-2397

Model of PARP7 in suppressing Type I IFN signaling

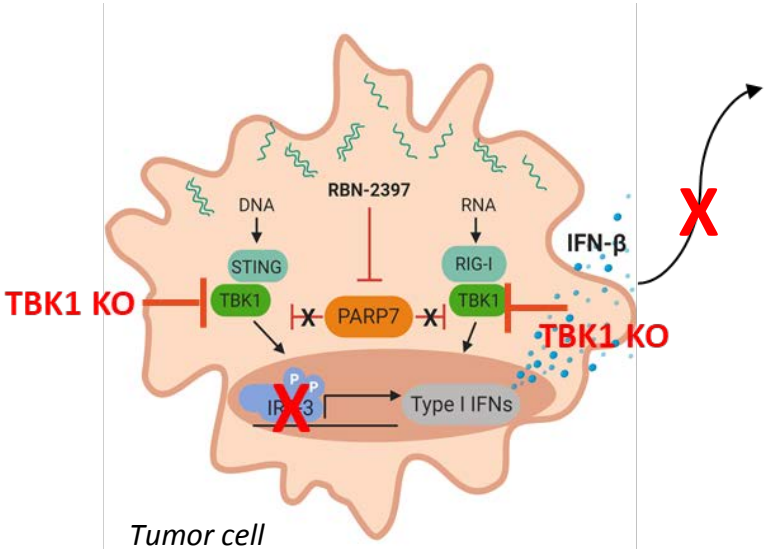


CT26 WT and KO cells treated with RBN-2397

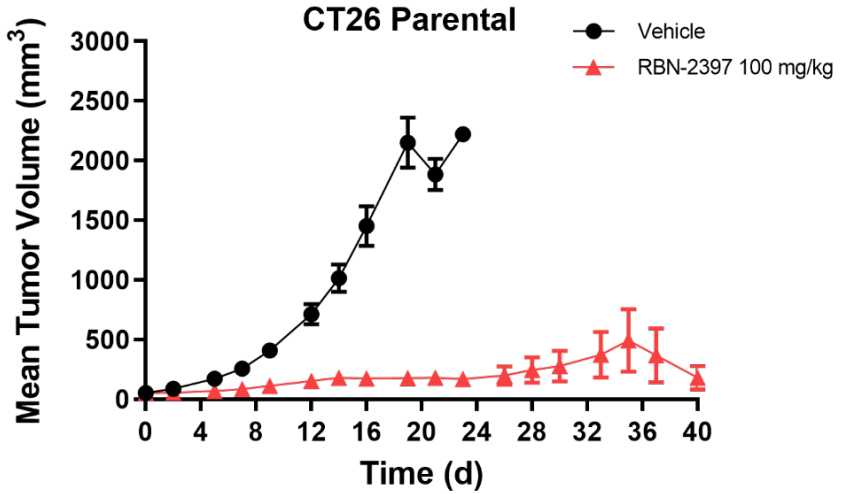


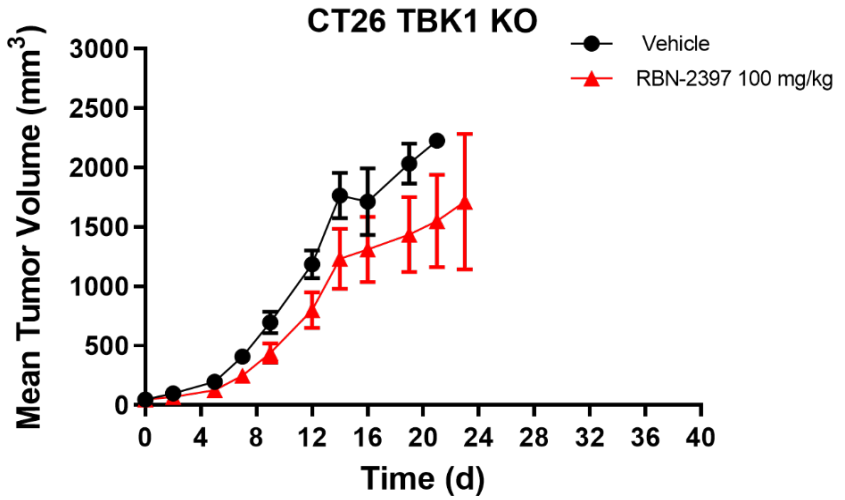
- **TBK1 knockout (KO) prevents both IRF3 & STAT1 phosphorylation by RBN-2397**
- **IFNAR1 KO prevents STAT1 phosphorylation by RBN-2397**

Tumor-derived Interferon Is Key for Antitumor Activity of RBN-2397



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

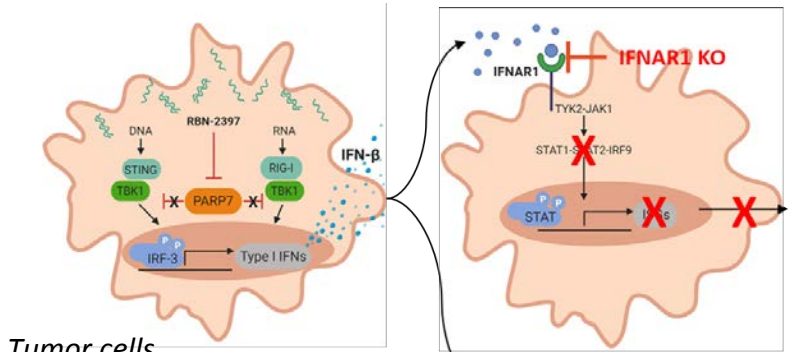




- **Ablation of tumor TBK1 nearly eliminates the antitumor activity of RBN-2397 in the CT26 tumor model**
- **Tumor-derived IFN-β is the source of the innate immune activation and crucial for RBN-2397 mediated antitumor response**

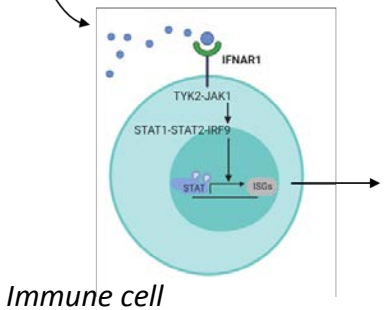
All groups co-dosed with ABT

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



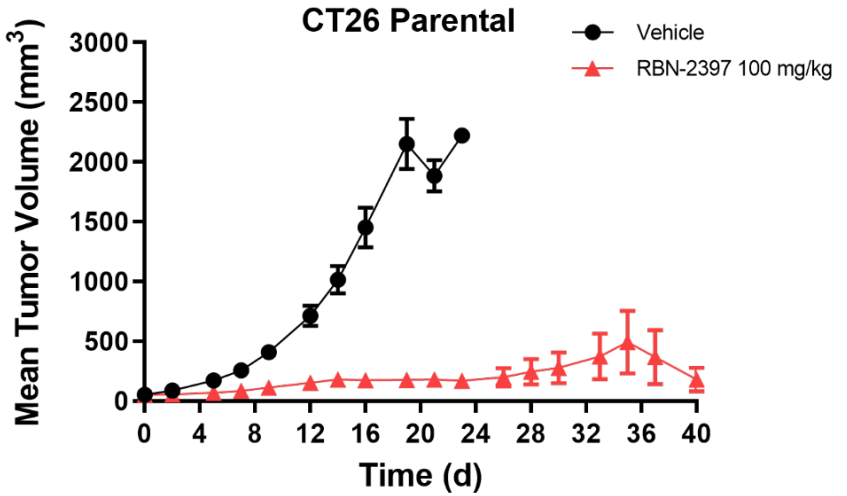
Tumor cells

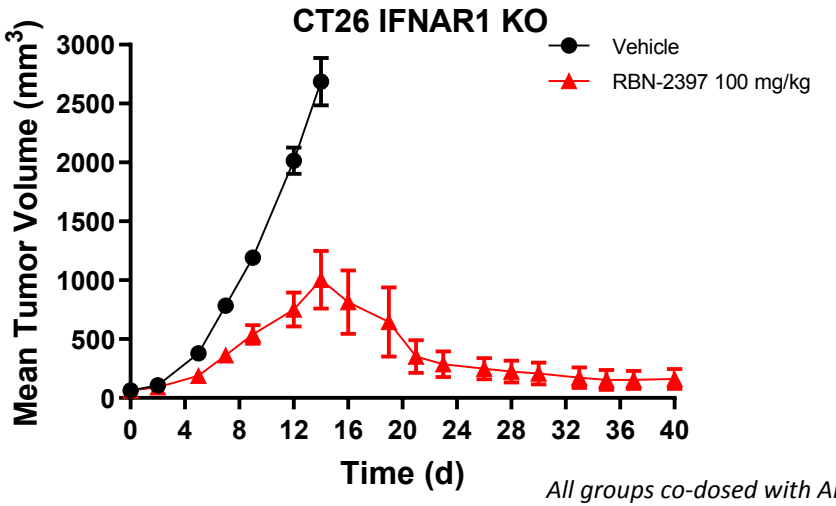
- No ISGs
- No tumor intrinsic effect



Immune cell

- Immune response

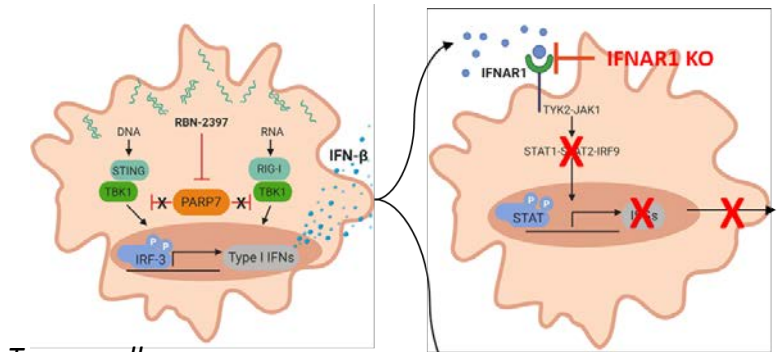




All groups co-dosed with ABT

- **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**

IFNAR1 Blockade on Tumor and Immune Cells Is Necessary to Prevent Antitumor Activity of RBN-2397 in the CT26 Tumor Model

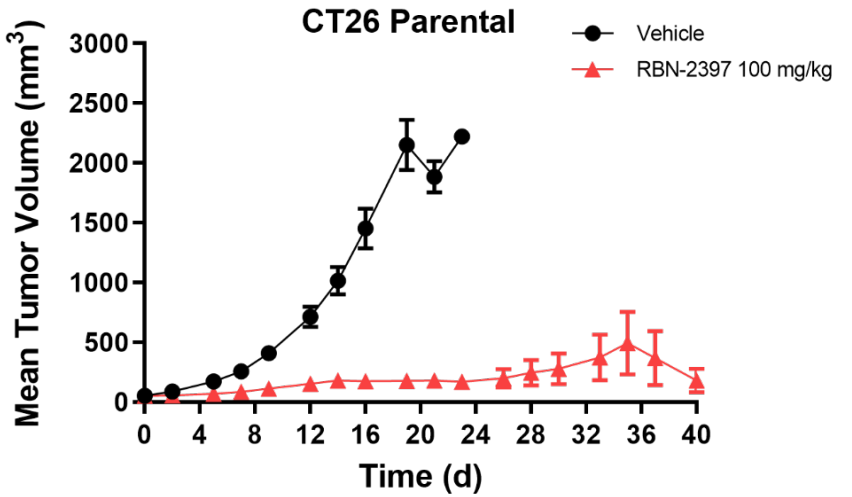


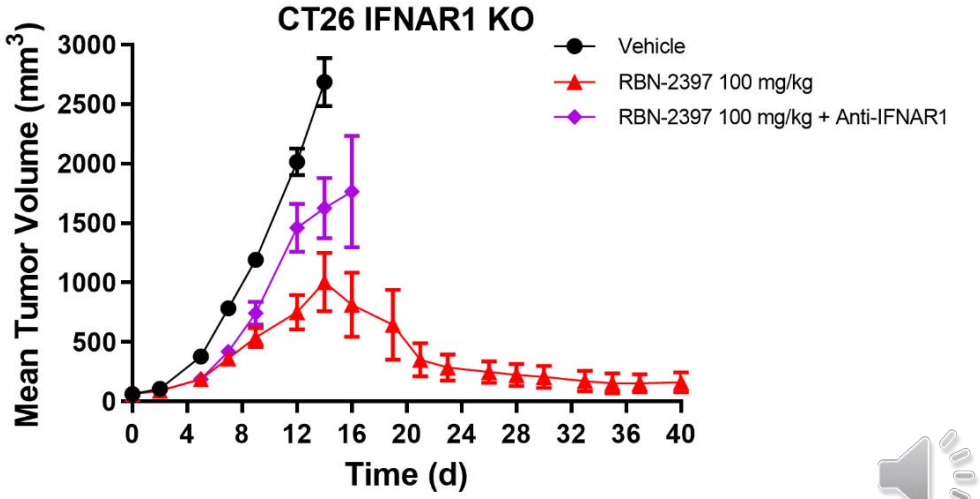
Tumor cells

Immune cell

- No ISGs
- No tumor intrinsic effect

- Immune response



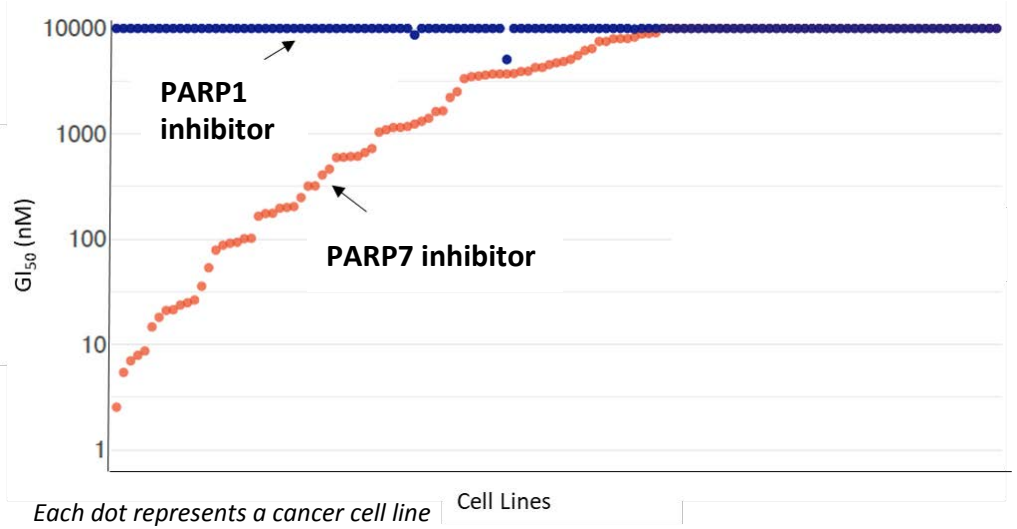


All groups co-dosed with ABT

- **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**

RBN-2397 Shows Cancer Cell Autonomous Effects and Restores Type I IFN Signaling in Human Cancer Cell Lines

Subset of cancer cell lines exhibit dependency on PARP7 for proliferation

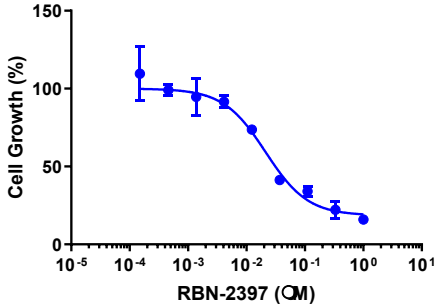


Each dot represents a cancer cell line
6 day assay

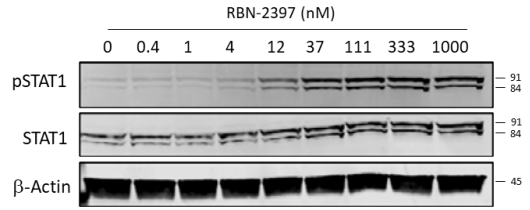
- **Responder cell lines are enriched for high expression of genes involved in Type I IFN response**

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

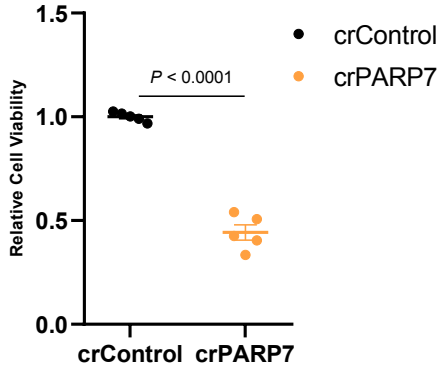
Proliferation: RBN-2397 GI_{50}



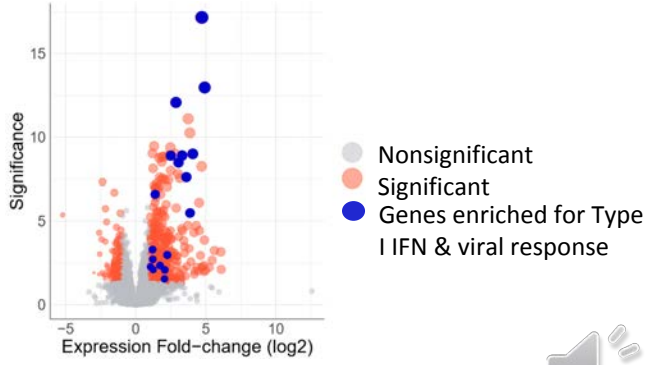
RBN-2397 induction of pSTAT1



Proliferation: PARP7 KO



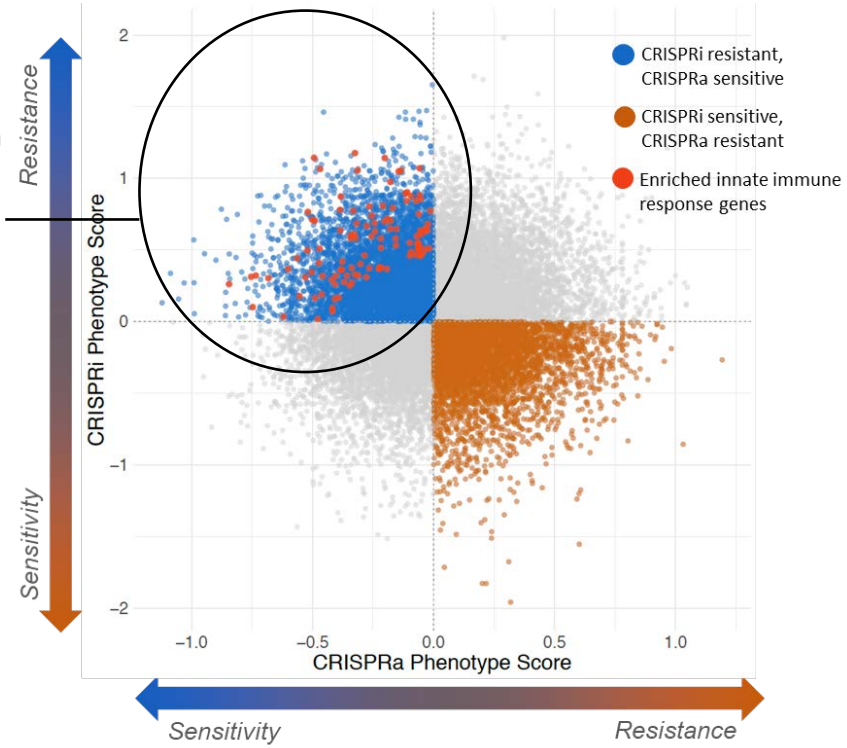
Transcriptional changes by RBN-2397: Increase in multiple ISGs



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

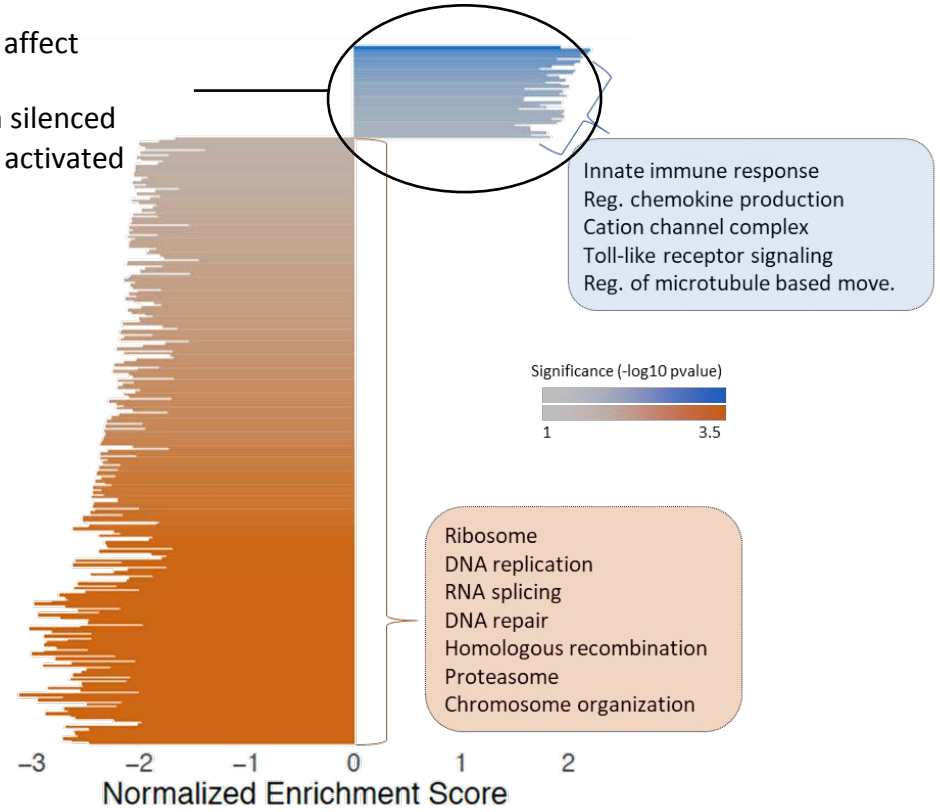
Effects of individual genes on RBN-2397 activity
- Resistance when silenced
- Sensitivity when activated



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

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

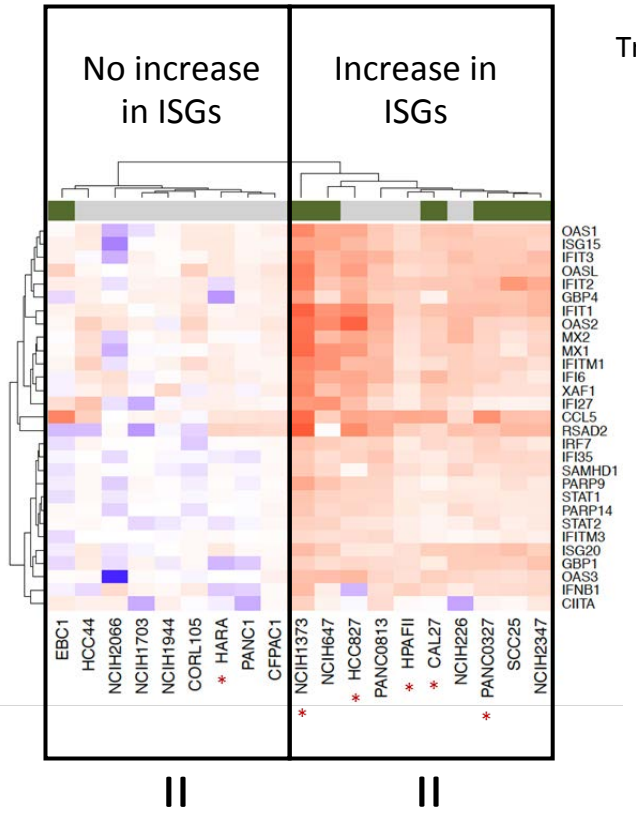
GSEA of genes that affect RBN-2397 activity
- Resistance when silenced
- Sensitivity when activated



GSEA: Gene set enrichment analysis

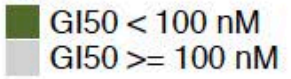
RBN-2397 Shows Antitumor Activity in Human Cancer Cell Lines that Show Induction of ISGs

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



Transcriptomic changes by RBN-2397 mRNA levels (log2 fold change)

RBN-2397 cell proliferation



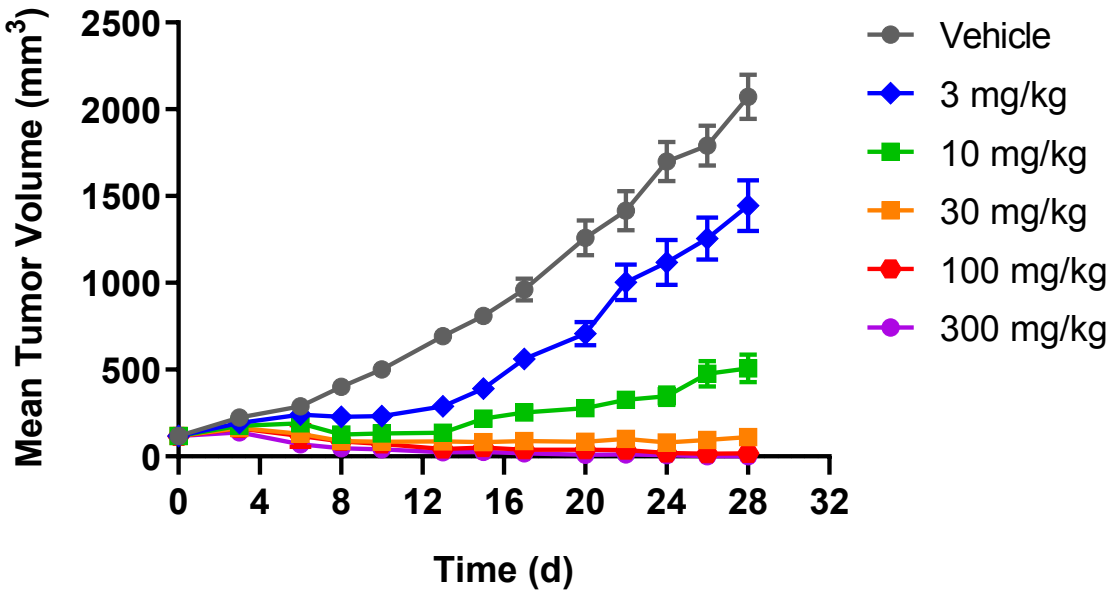
RBN-2397 efficacy in CB17 SCID xenograft mouse model (*)

No antitumor activity

Antitumor activity (50-100% TGI)

- Lung cancer
- Pancreatic cancer
- Oral squamous cancer

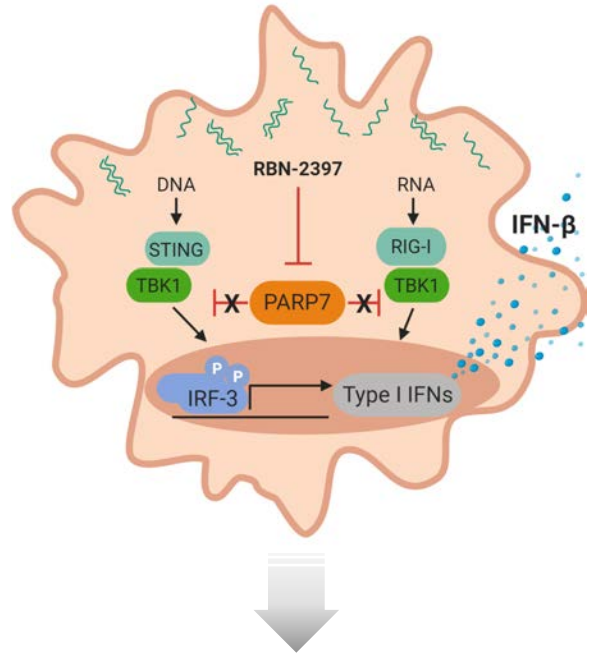
RBN-2397 causes complete regressions in NCI-H1373 xenografts



- **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**

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)**



IFN
Uncloaks the tumor cells and recruits immune cells

Complete regressions and antitumor immunity as a single agent

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Peter Kim
Danielle Knight
Kaiko Kunii
Kevin Kuntz
Kristy Kuplast-Barr
Jenkins Lemera
Chang Liu
Alvin Lu
Ahmed Mady
Christina Majer
Kristen McEachern
Maegan Mikula
Elena Minissale
Jason Mo
Jennifer Molina
Sunaina Nayak
Mario Niepel
Sudha Parasuraman
Nicholas Perl
Yue Ren
Victoria Richon
Andy Santospago
Laurie Schenkel
Richard Schroeder
Prashant Shambharkar
Jeff Song
Tad Stewart
Kerren Swinger
Luke Utley
Zacharenia Varsamis
Melissa Vasbinder
Tim Wigle
Jodie Wong

Founders and Advisors:

Paul Chang
Lee Kraus
Timothy Mitchison
James Audia
Larry Lasky
Patricia Rao