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

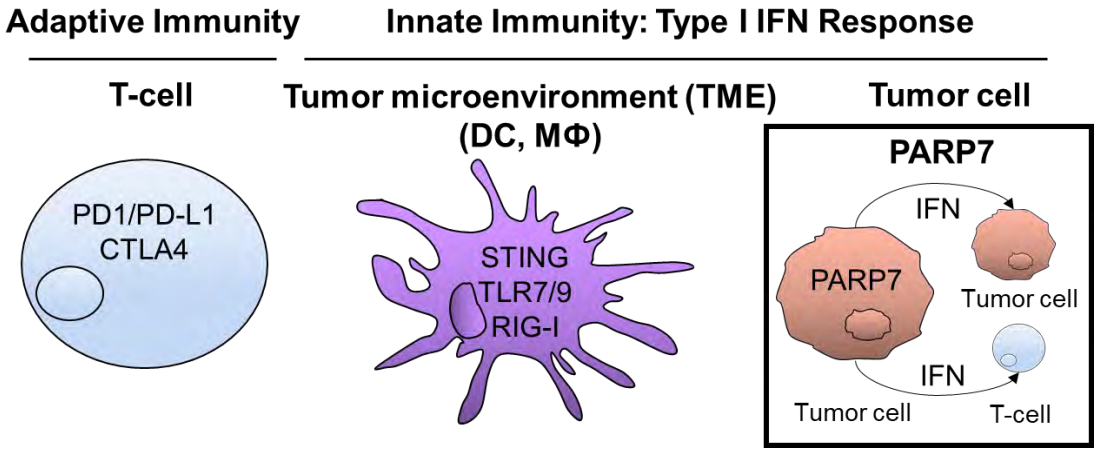
Disclosure Statement

- **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

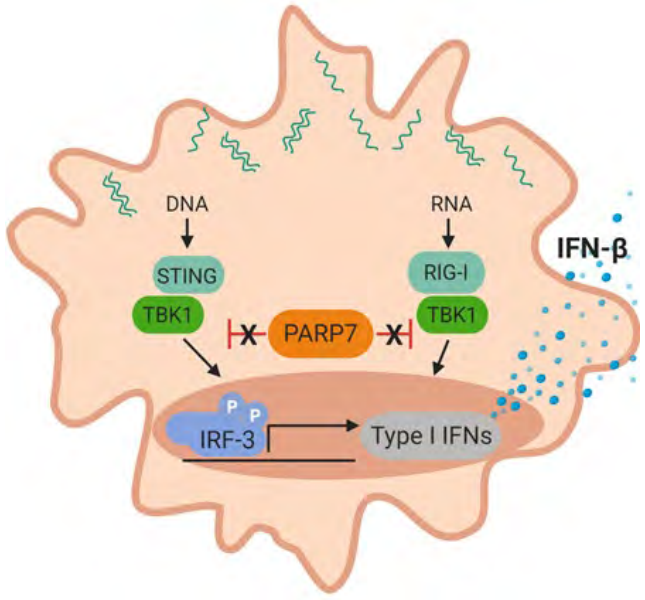
Targeting a negative regulator of tumor-produced Type I IFN is a novel therapeutic strategy



PARP7 acts as a fundamental regulator of intrinsic stress support pathways and is a novel tumor vulnerability in cancer cells

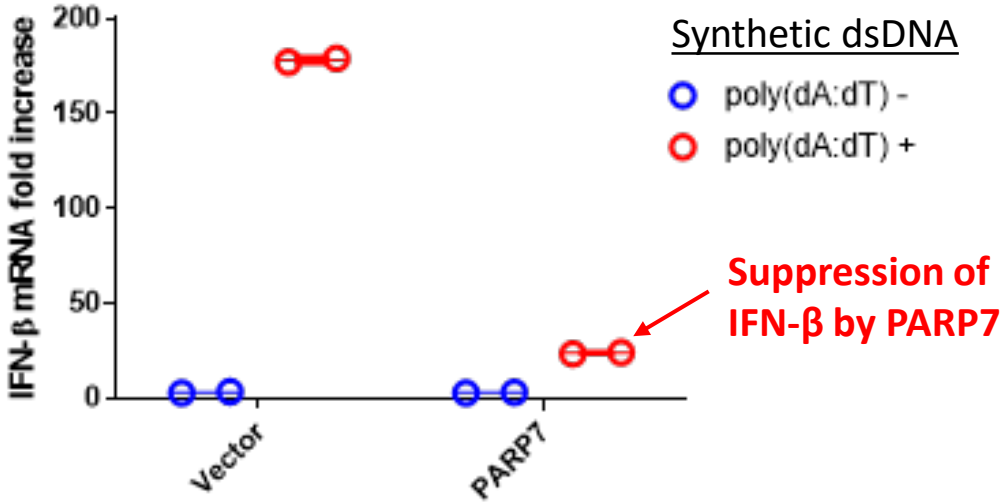
PARP7 Acts as a Brake on Cytosolic Nucleic Acid Sensing and the Type I IFN Response

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



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

Overexpression of PARP7 suppresses IFN-β response to dsDNA

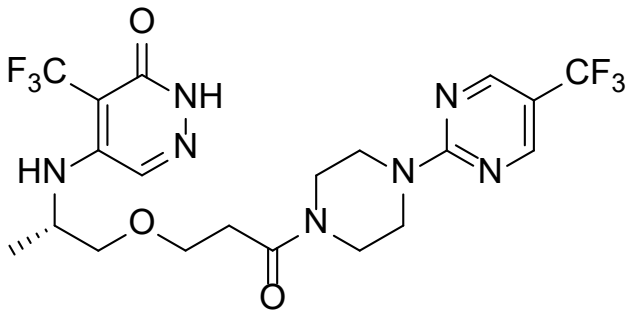


HEK293T cells transfected with PARP7 treated with synthetic double stranded (ds)-DNA for 24 hours

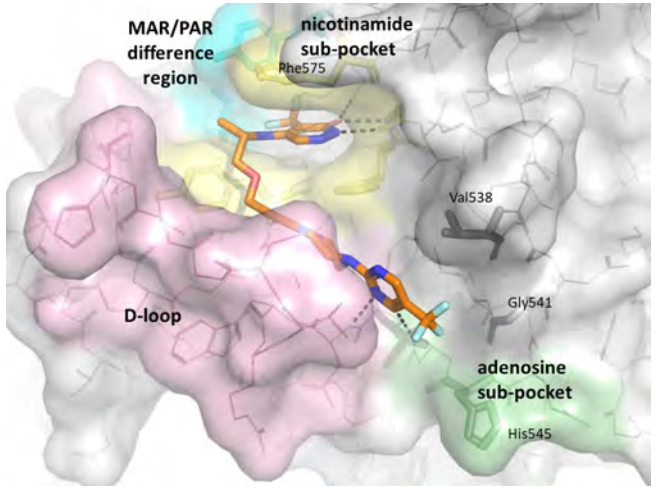
RBN-2397 is a Potent and Selective Inhibitor of PARP7

- **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)**

Structure of RBN-2397



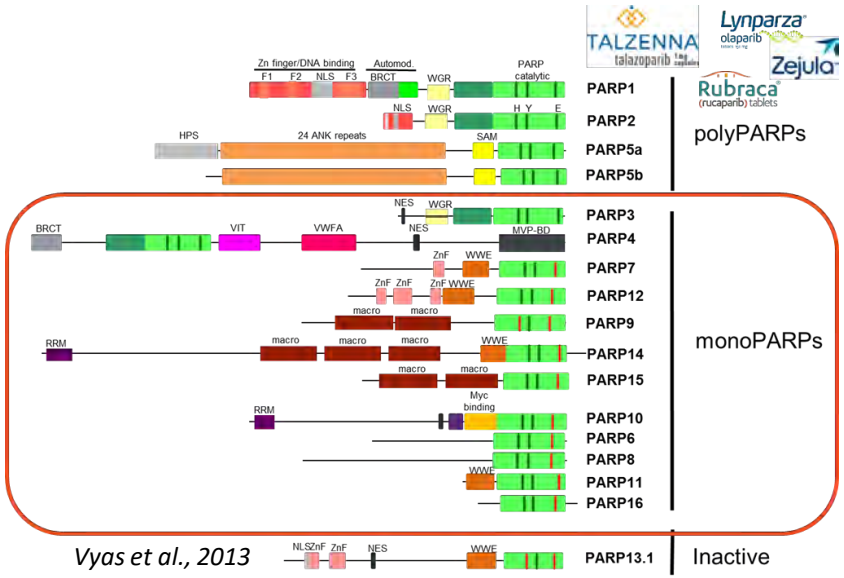
Co-crystal structure of RBN-2397 bound to PARP12/7



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

RBN-2397 Potently and Selectively Inhibits PARP7-Dependent Activity Compared to PARP1

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

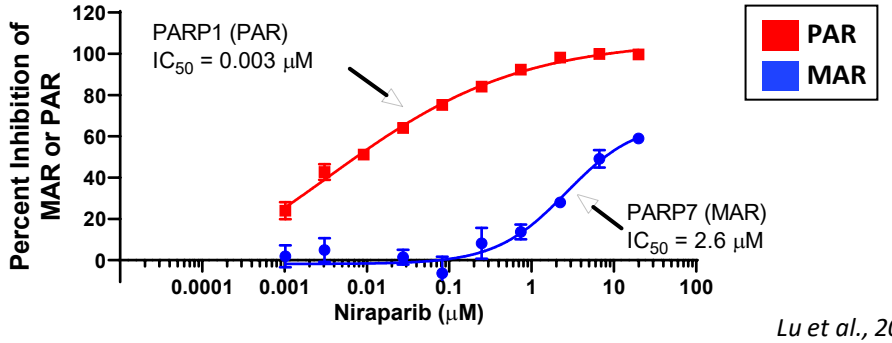


Vyas et al., 2013

- **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)

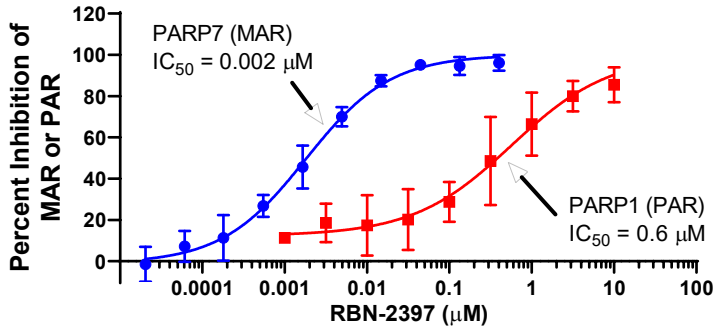
RBN-2397 potently inhibits MARylation with a 300-fold window over PARylation

PARP1 inhibitor: Niraparib



Lu et al., 2019

PARP7 inhibitor: RBN-2397

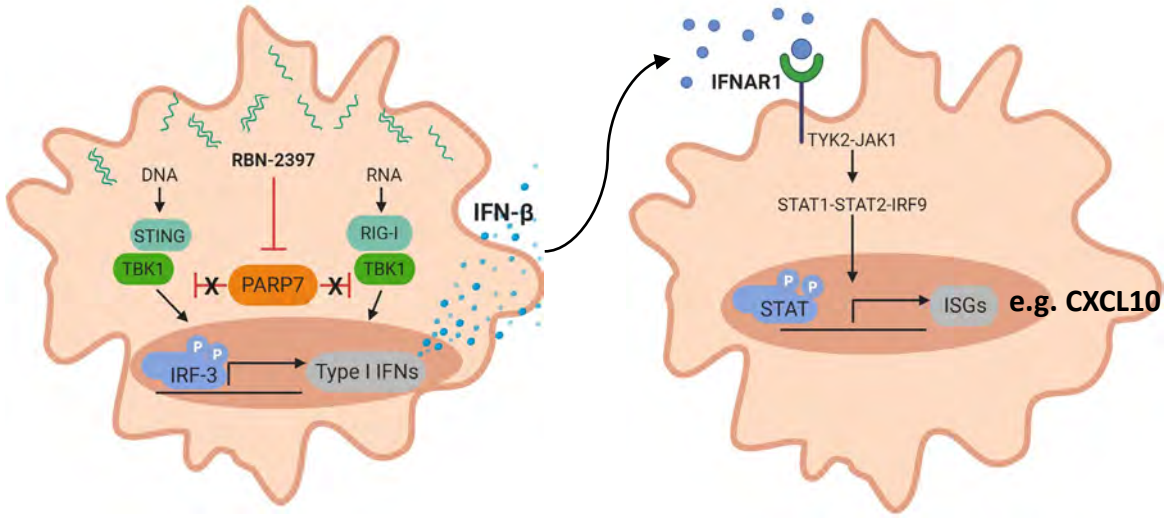


24-hour treatment

PARP1-H_2O_2 activated Hela cells (PAR)
PARP7-overexpressing SK-MES-1 cells (MAR)

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

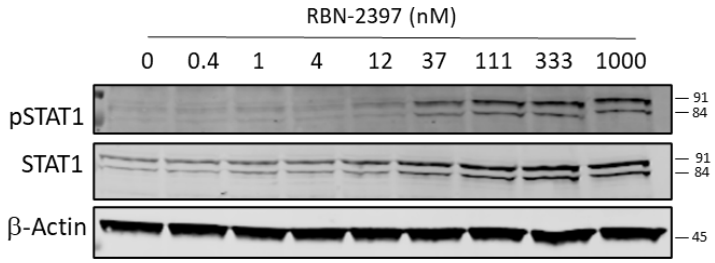
PARP7 inhibition "releases the brake" on cytosolic nucleic acid sensing and induces Type I IFNs



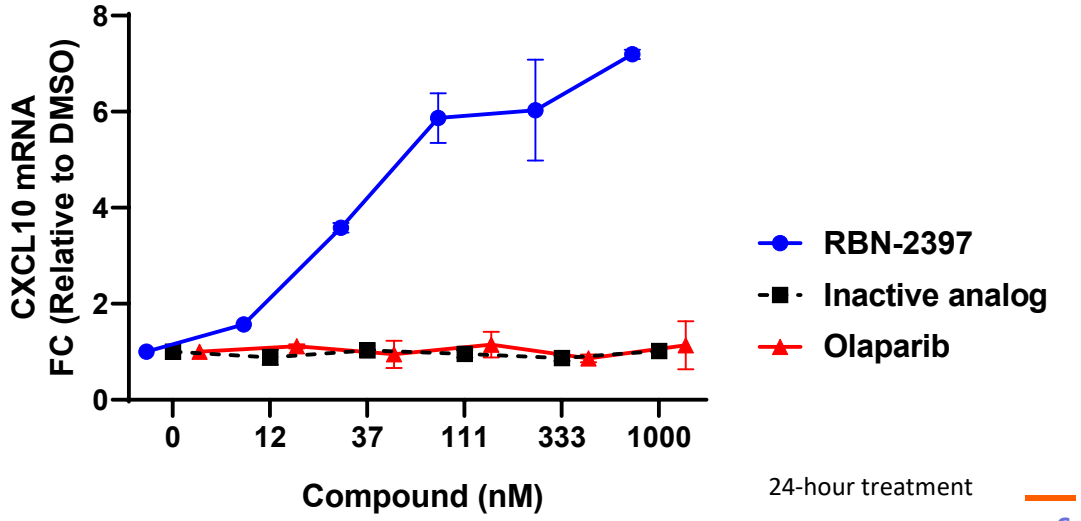
Restoration of Type I IFN response is measured by an increase in STAT1 phosphorylation and interferon stimulated genes (ISGs)

RBN-2397 restores Type I IFN response in CT26 cells

Induction of pSTAT1



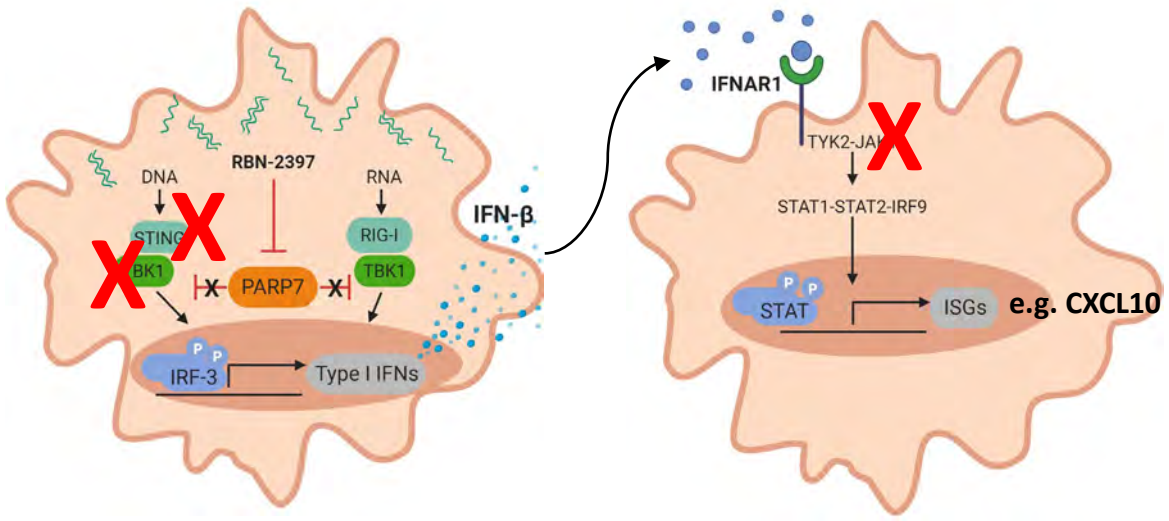
Selective induction of CXCL10



24-hour treatment

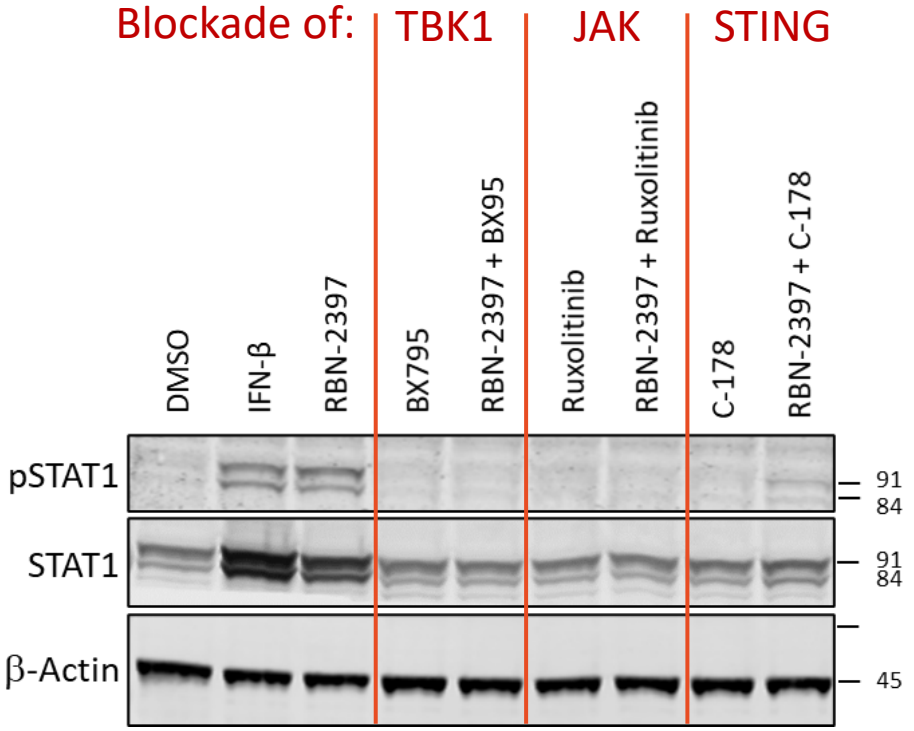
RBN-2397 Restores Cytosolic Nucleic Acid Sensing Dependent on Pattern Recognition Receptor Signaling

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



BX795: TBK1 inhibitor
Ruxolitinib: JAK inhibitor
C-178: STING inhibitor

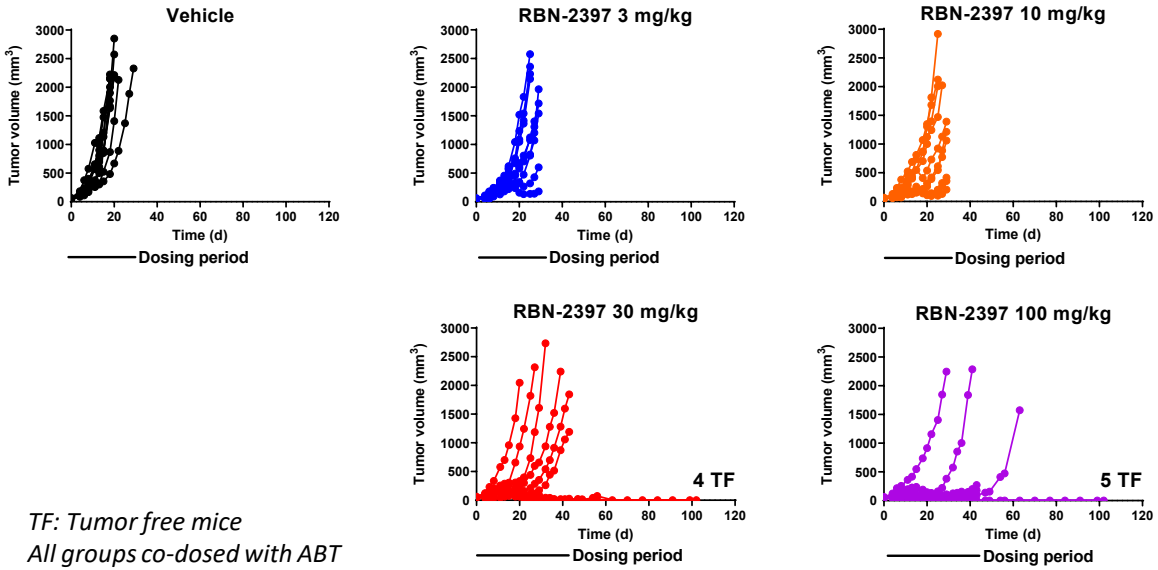
RBN-2397 restores Type I IFN signaling through pattern recognition receptor pathway



24-hour treatment

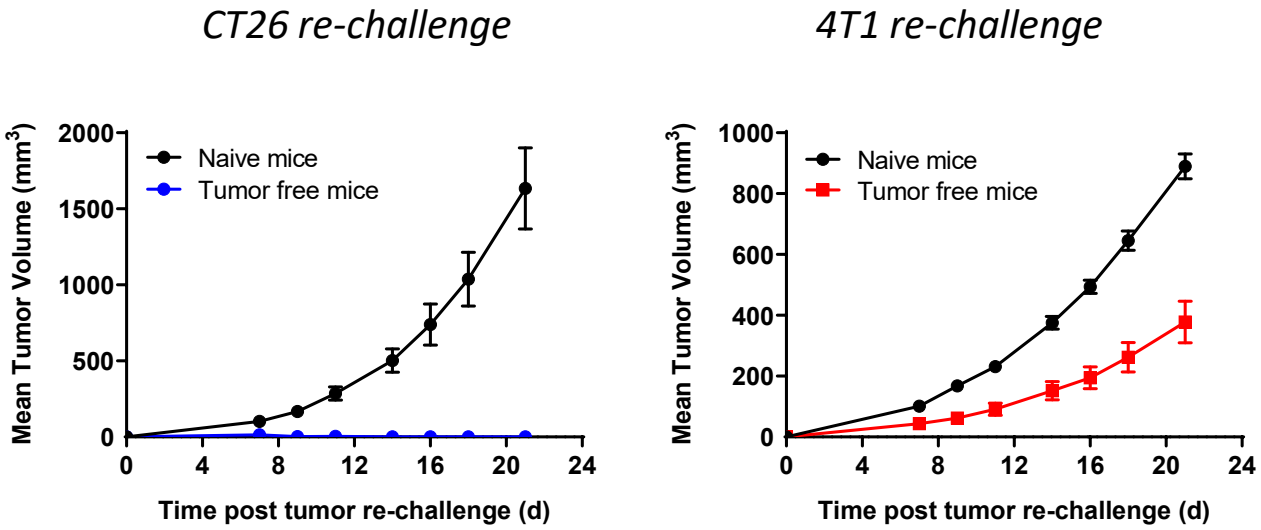
RBN-2397 Induces Tumor-Specific Adaptive Immune Memory in CT26 Syngeneic Model with Durable Complete Responses

Primary Efficacy: RBN-2397 induces durable regressions



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

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



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

- **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**

Adaptive Immune Response is Indispensable for RBN-2397 Antitumor Activity

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

CT26 mouse model		Innate immune cells		Adaptive immune cells		
Mouse Strain	RBN-2397 Efficacy	NK	Mac	B	T	DC
BALB/c 	Tumor regression	+	+	+	+	+
NOG 	~50% TGI	-	-[a]	-	-	-[a]

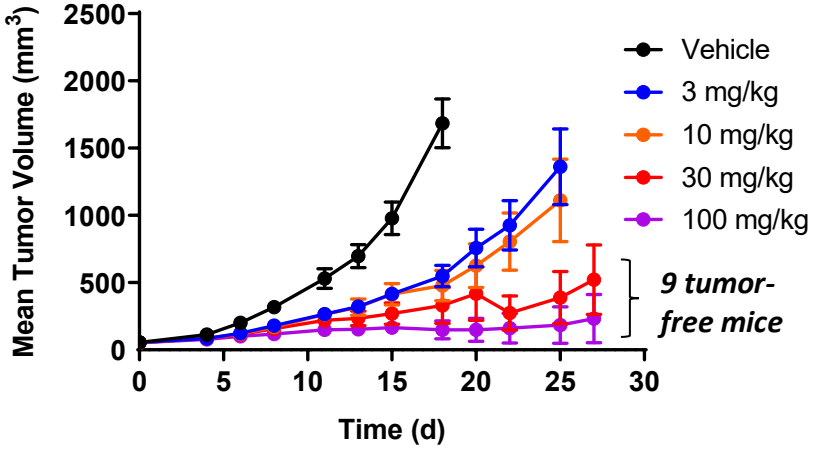
+: Present; -: Absent

a: Reduced macrophage and dendritic cell function

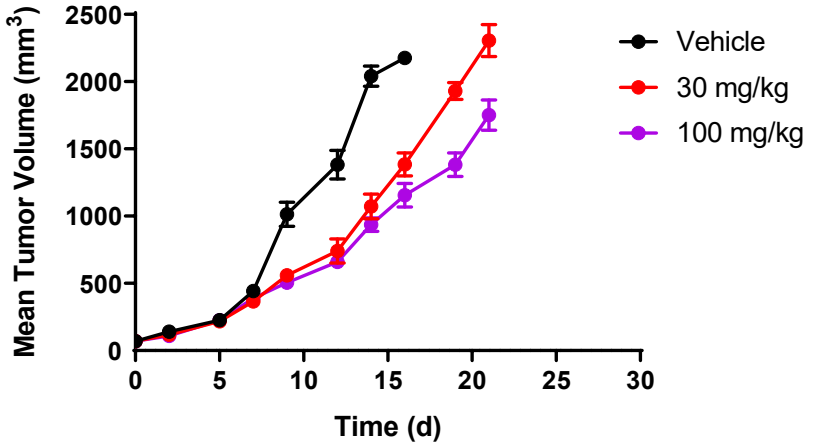
All groups co-dosed with ABT

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

BALB/c: Immunocompetent

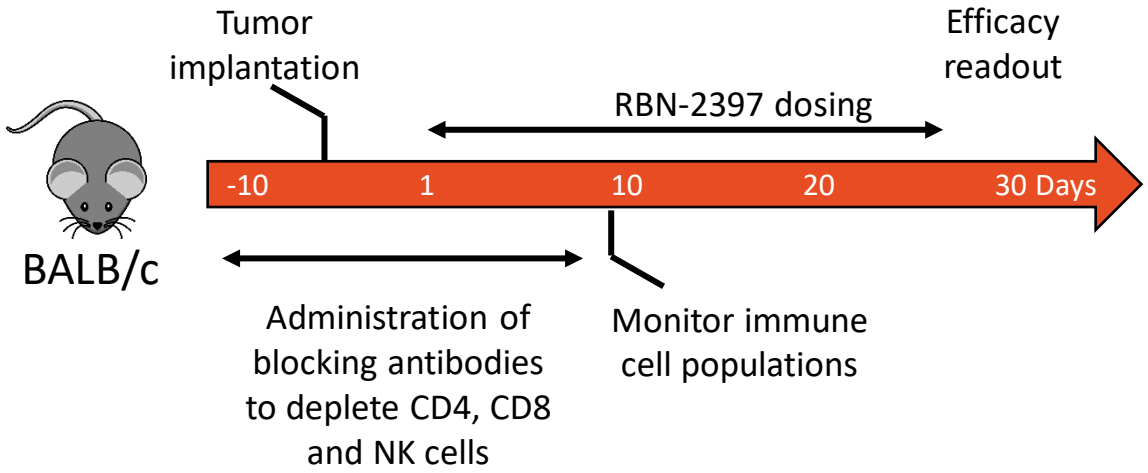


NOG: Immunodeficient



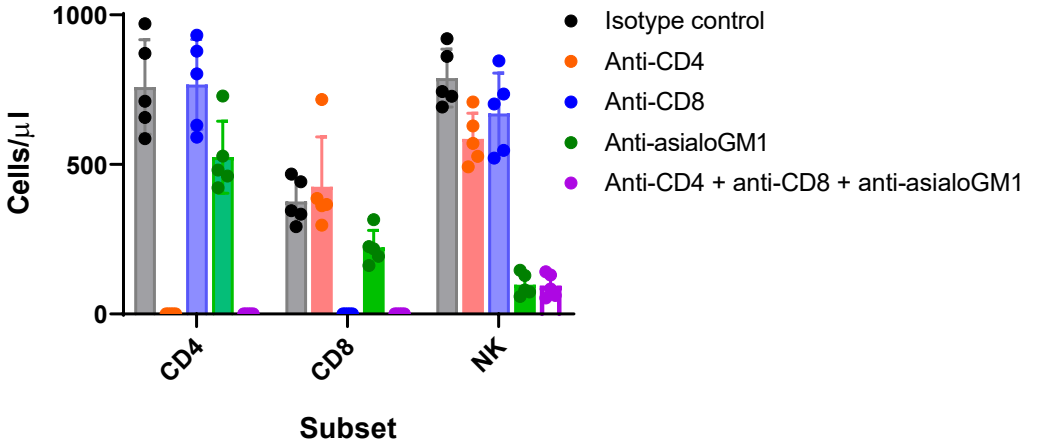
Robust Depletion of Immune Cell Populations in CT26 Tumor-Bearing Mice

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

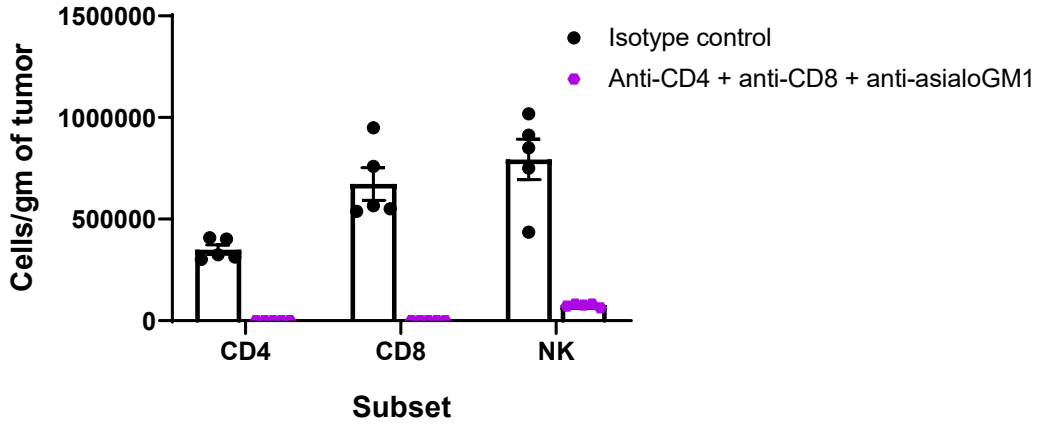


Specific depletion of immune cell populations

Blood absolute counts

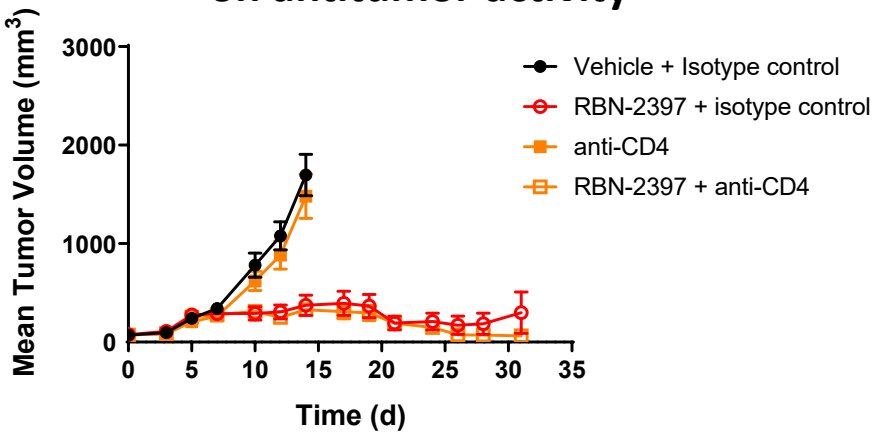


Tumor absolute counts

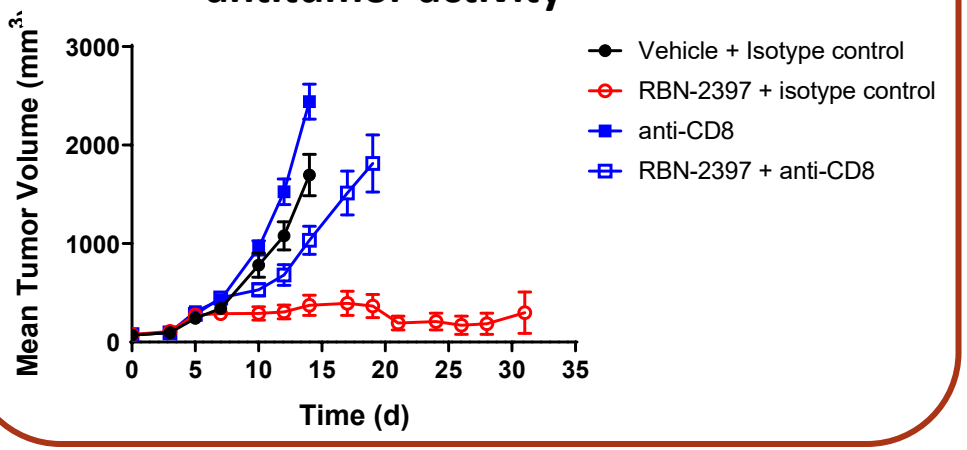


CD8 T Cells Are Essential for the Antitumor Immunity Induced by RBN-2397 in the CT26 Syngeneic Model

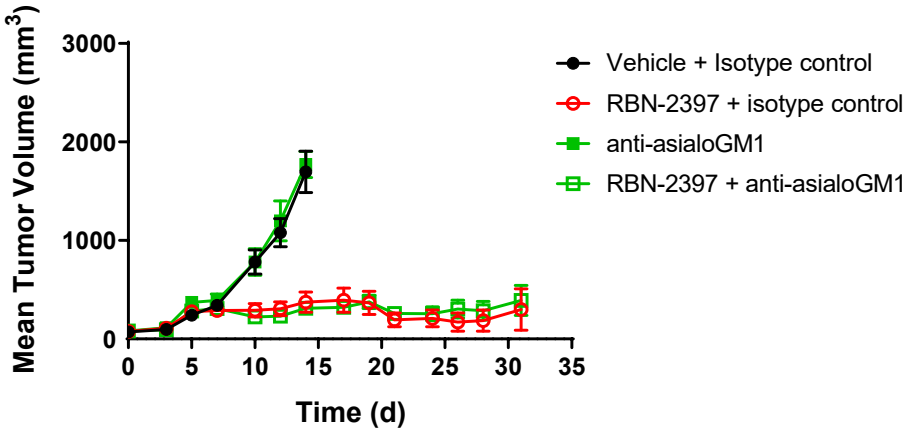
CD4 T cell depletion had no effect on antitumor activity



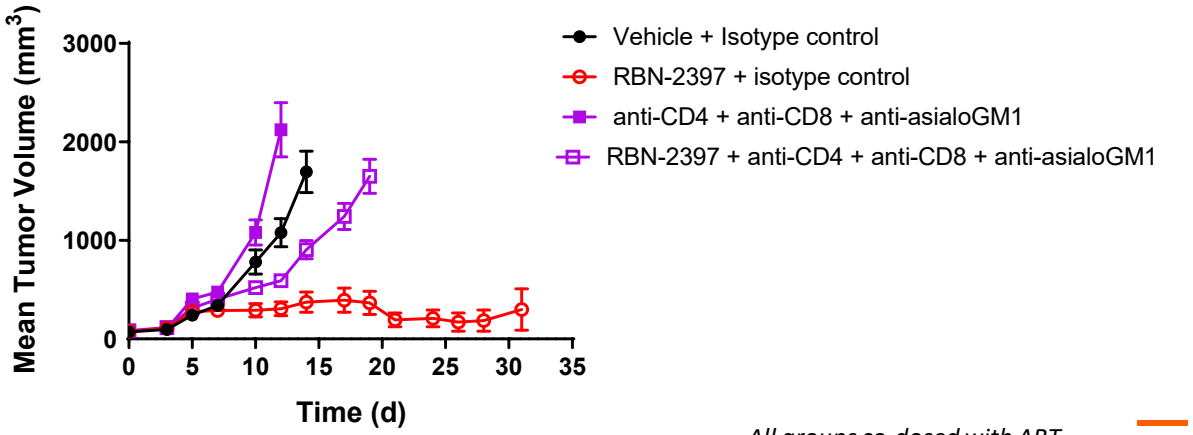
CD8 T cell depletion attenuated antitumor activity



NK cell depletion had no effect on antitumor activity



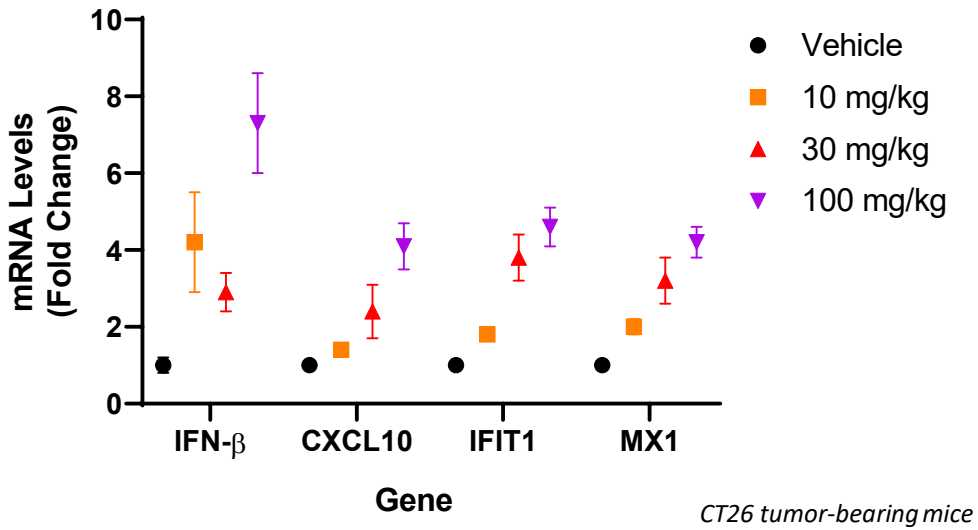
Triple depletion was not different than CD8 T cell single depletion



All groups co-dosed with ABT

RBN-2397 Induces Type I IFN Signaling and Enhances Immune Markers in CT26 Tumors

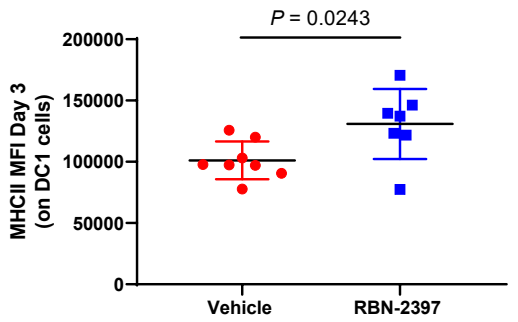
RBN-2397 shows dose-dependent effects on PD markers in CT26 tumors



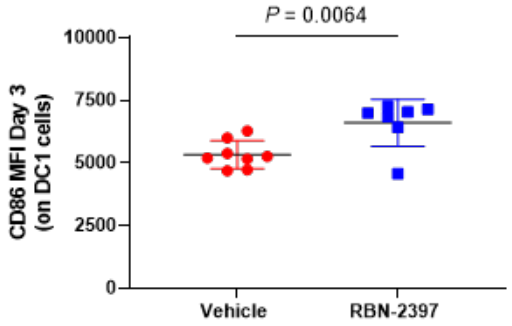
CT26 tumor-bearing mice administered as single oral dose. All groups co-dosed with ABT

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

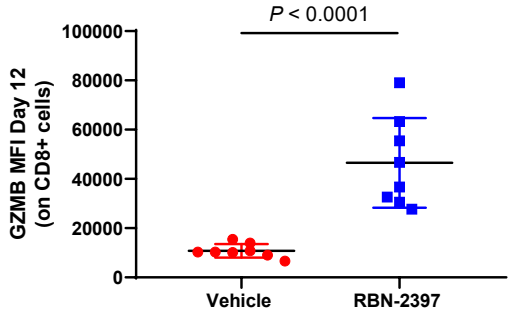
Increase in MHCII on DC1 cells



Increase in CD86 on DC1 cells

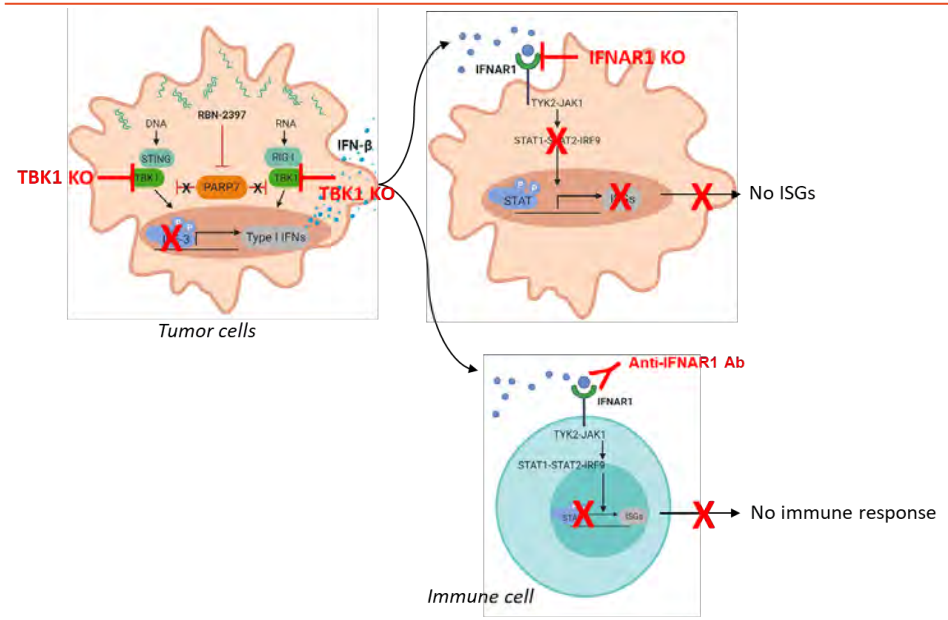


Increase in GrzB on CD8 T cells



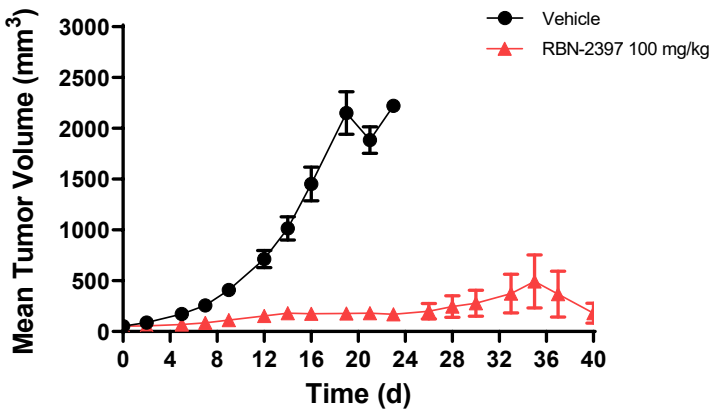
CT26 tumor-bearing mice dosed with RBN-2397 500 mg/kg. Tumors collected on days 3, 6 & 12.

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

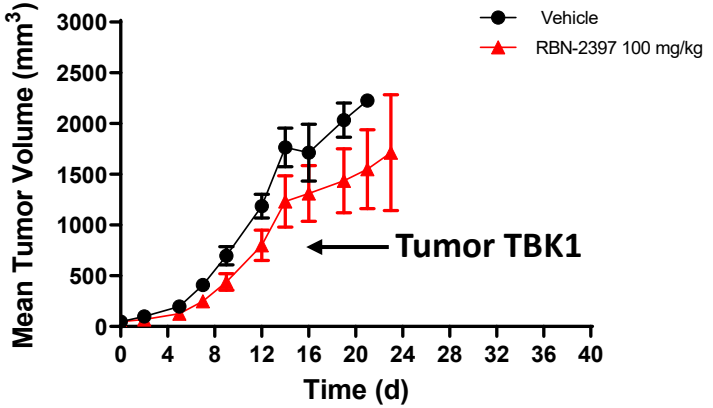


- **Ablation of tumor TBK1 nearly eliminates the antitumor activity of RBN-2397**
  - Tumor-produced IFN-β is the source of the innate immune activation and crucial for antitumor activity
- **Blockade of tumor and host IFNAR1 signaling prevents the antitumor activity of RBN-2397**
  - Suggests contribution of immune system through activation of IFN signaling in immune cells by tumor-produced IFN-β

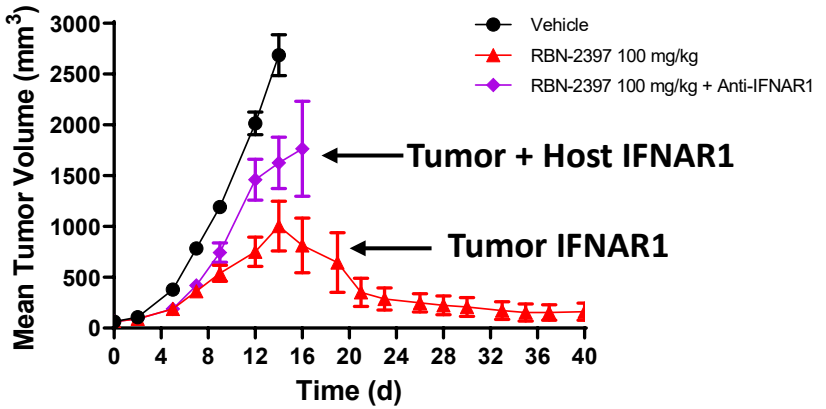
CT26 wild type



CT26 TBK1 KO



CT26 IFNAR1 KO + anti-IFNAR1



All groups co-dosed with ABT

RBN-2397 is the First Potent and Selective PARP7 Inhibitor to Enter Clinical Development

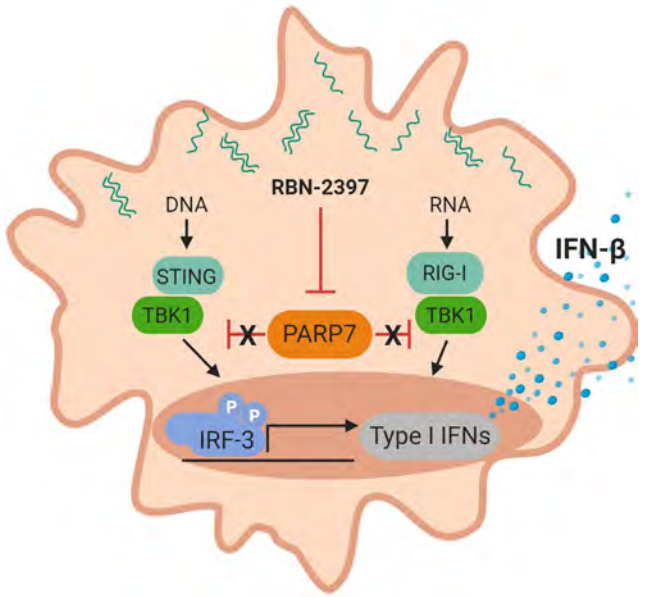
- **Targeting PARP7 to restore tumor-derived Type I signaling is a novel therapeutic strategy in cancer**
- **Inhibition of PARP7 induces antitumor immunity dependent on tumor-produced Type I IFN and CD8 T cells**
- **RBN-2397 is the first agent targeting this cancer vulnerability to enter clinical development**

Ribon PARP7 abstracts at AACR 2021

#381: PARP7 expression in cancer

#1021: PARP7 inhibitor mechanism of action studies

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



IFN
Uncloaks the tumor cells and recruits immune cells

Complete regressions and antitumor immunity as a single agent

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