PARP7 Inhibitor RBN-2397 Induces Tumoral IFN Signaling and Intrinsic Effects Resulting in Regressions in Mouse Models

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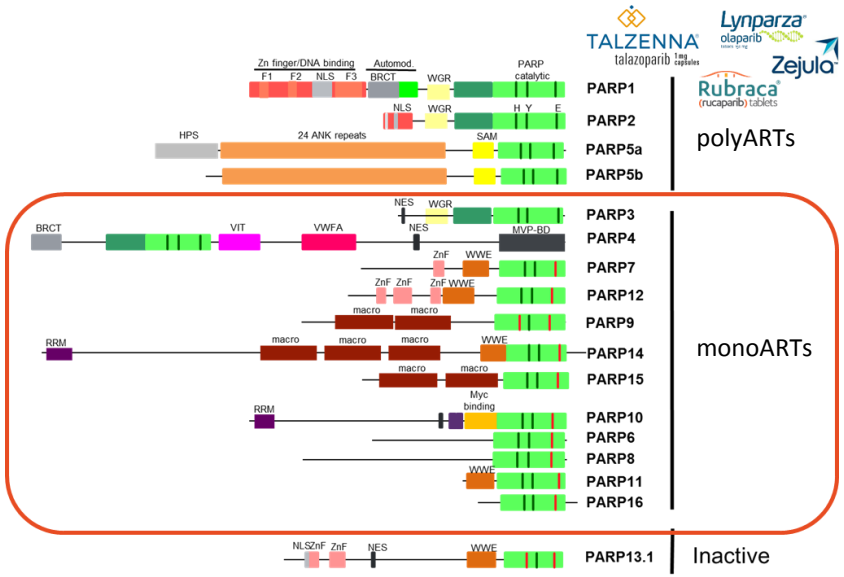
- Ribon Therapeutics

Disclosure Statement

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

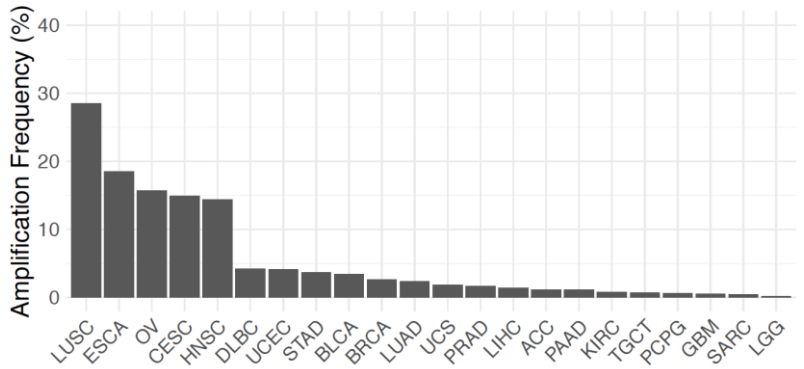
Not All PARPs Are Alike – Outside of PolyARTs the PARP Family Is Unexplored for Therapeutic Development

- **PARP family consists of 17 members**
  - Three subfamilies based on catalytic activity (polyARTs, monoARTs and inactive)
  - PARPs regulate cellular function by modifying target proteins with ADP-ribose
  - monoARTs transfer a single unit of ADP-ribose onto their substrates where polyARTs attach polymers of ADP-ribose units
- **PARP7 is a monoART**
  - 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)



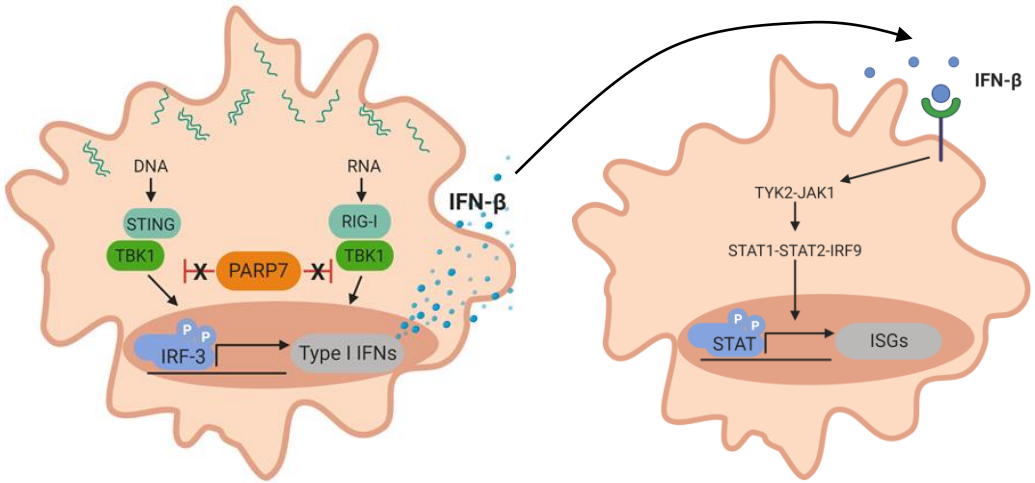
Vyas, Chang et. al. Nature Comm. 2013

PARP7 is frequently amplified in cancer



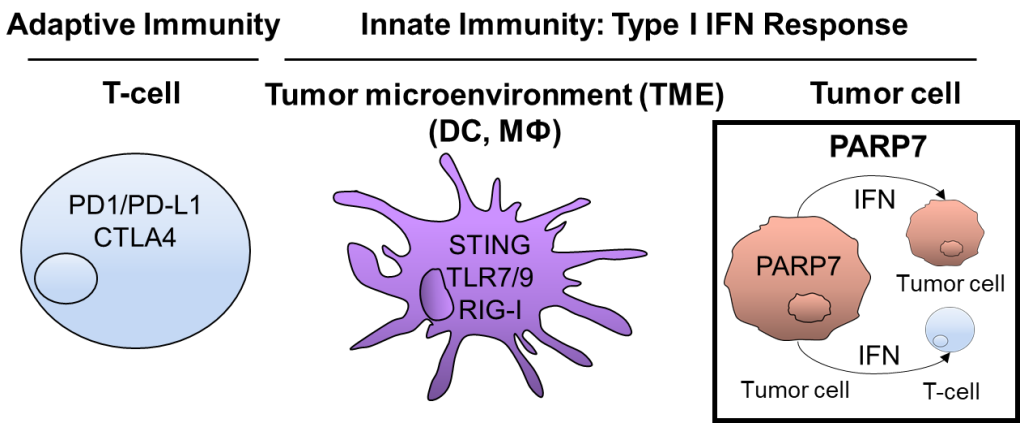
PARP7 Acts as a Brake in Cytosolic Nucleic Acid Sensing and the Type I Interferon (IFN) Response in Cancer Cells

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)

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



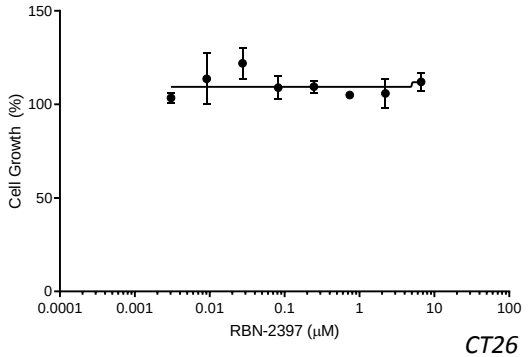
Most immunotherapies restore pre-existing immunity within immune cells while PARP7 inhibition aims to restore IFN-I signaling within tumor cells

- **High levels of PARP7 in tumors blocks interferon production resulting in an immunosuppressive environment leading to immune escape and tumor progression**
- **Targeting PARP7 restores the type I IFN signaling within tumor cells leading to induction of innate and adaptive immune mechanisms**
- **Targeting a negative regulator "brake" of Type I IFN signaling is a novel therapeutic strategy in cancer**

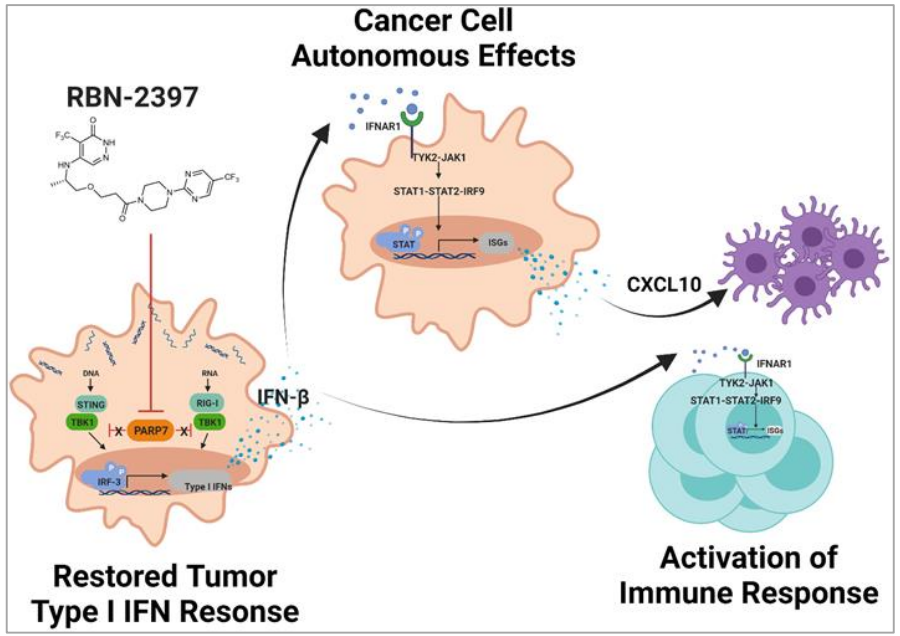
RBN-2397 Induces Tumor Growth Inhibition in CT26 Syngeneic Model with Durable Complete Responses

In vitro:

No effect on in vitro cell viability



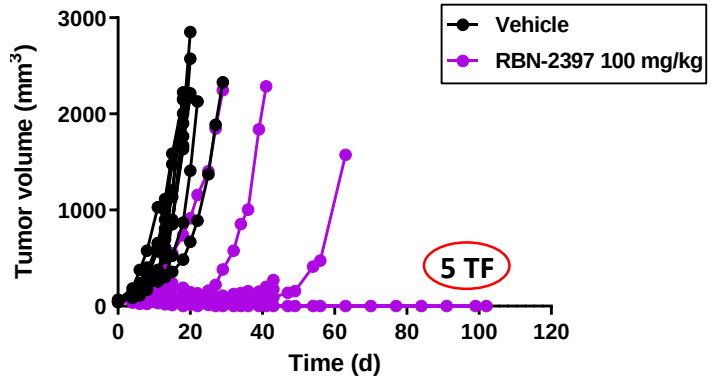
CT26
6-day assay



CT26 mouse tumor cell line

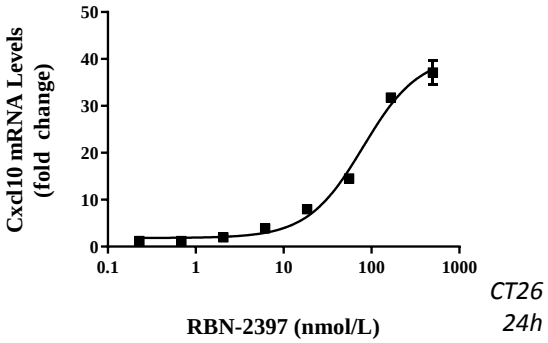
In vivo:

RBN-2397 induces durable regressions



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

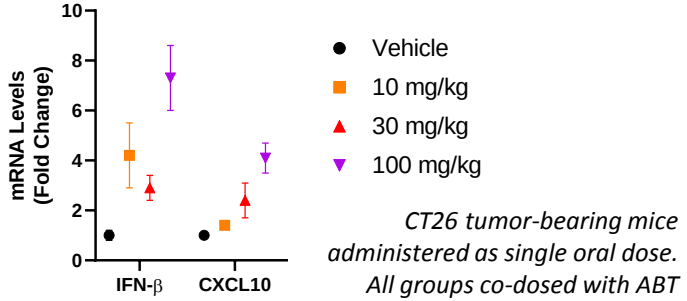
Induction of ISG CXCL10



CT26
24h

- **RBN-2397 leads to activation of the immune response and durable and complete regressions in the CT26 model**

Tumor PD: increased ISGs

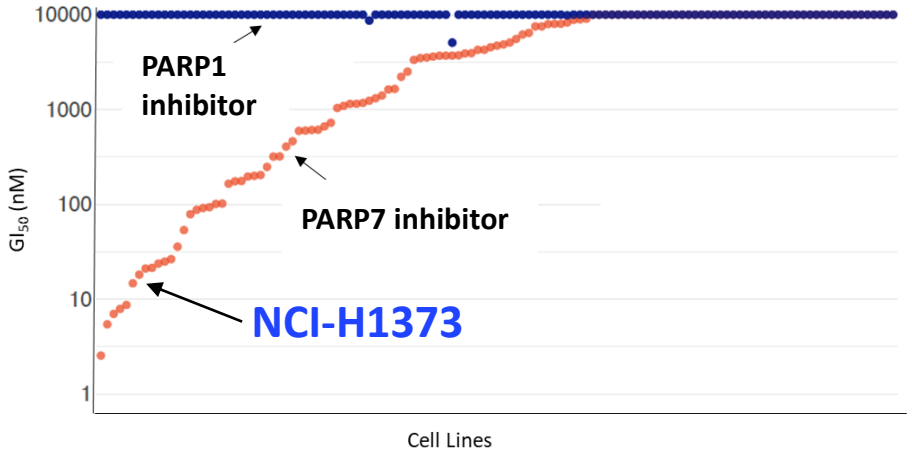


CT26 tumor-bearing mice administered as single oral dose.
All groups co-dosed with ABT

Gozgit 2021 Cancer Cell

In Addition to Restoring Type I IFN Signaling, RBN-2397 Leads to TBK1-Independent Proliferation Inhibition in a Subset of Cancer Cell Lines

Subset of human 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 PARP7 mRNA expressors
  - High expression of genes involved in Type I IFN response

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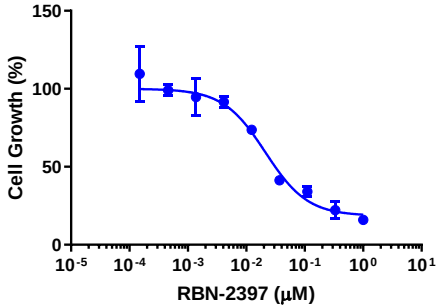
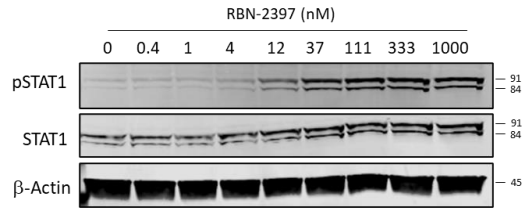
NCI-H1373 cells demonstrate dependency on PARP7 for proliferation and suppression of Type I IFN signaling

Proliferation: RBN-2397 GI_{50}

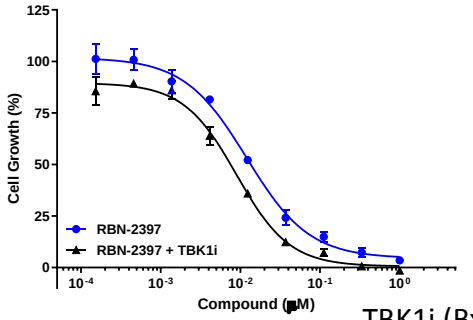


RBN-2397 induction of pSTAT1



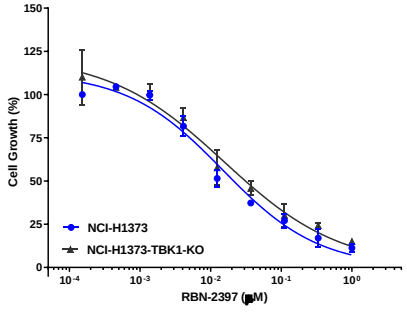
RBN-2397's antiproliferative effect is independent of the TBK1/STING pathway

RBN-2397 + TBK1i



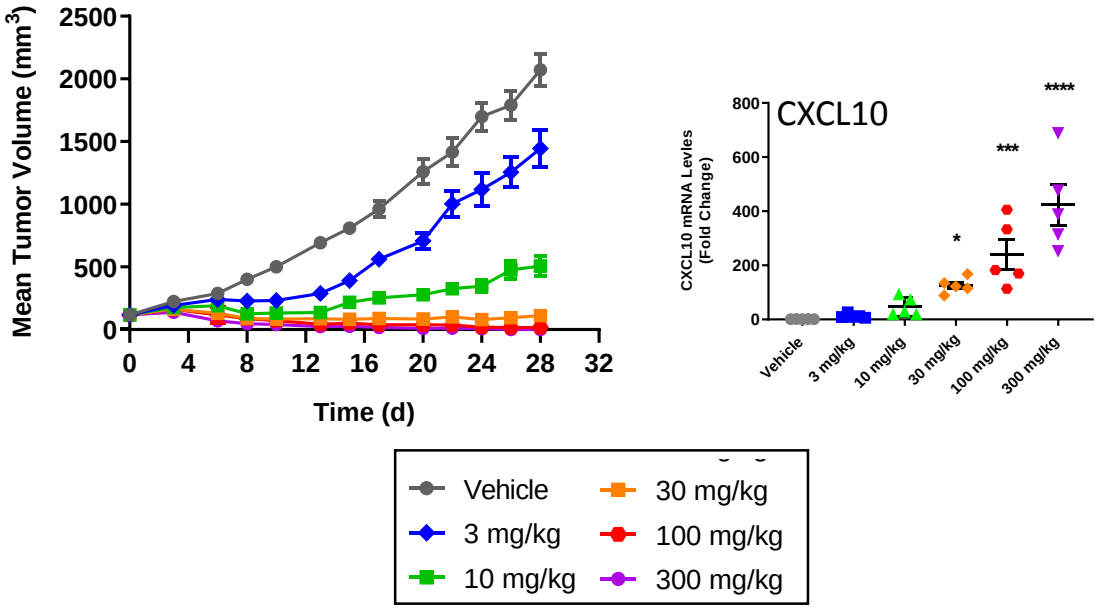
TBK1i (Bx795)

NCI-H1373 TBK1 KO with RBN-2397



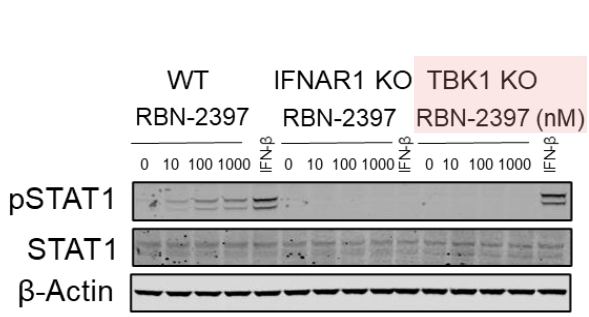
RBN-2397 Shows Antitumor Activity in NCI-H1373 Xenografts Despite Immunodeficient Host

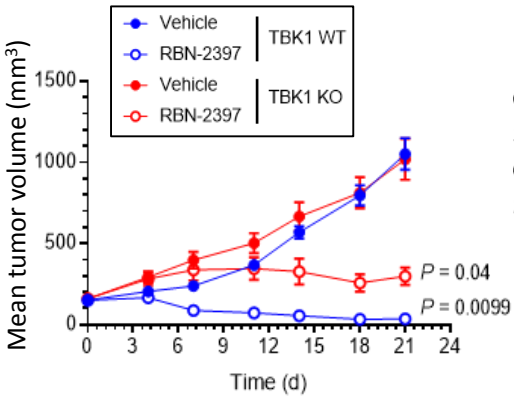
RBN-2397 causes complete regressions in NCI-H1373 xenografts



- 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

TBK1 depletion in tumor cells prevents regression but retains tumor growth inhibition by RBN-2397 in xenografts





- Robust tumor growth inhibition despite TBK1 KO
- However, TBK1 KO prevented tumor regression
- Suggests a tumor intrinsic mechanism leading to a cytostatic cellular state that synergizes with Type I IFN activation leading to complete regressions

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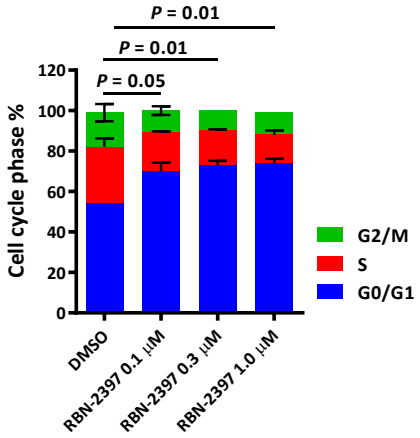
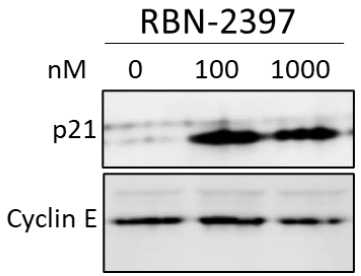
RBN-2397 Causes Cell Cycle Arrest and Senescence in NCI-H1373 Cells In Vitro and In Vivo

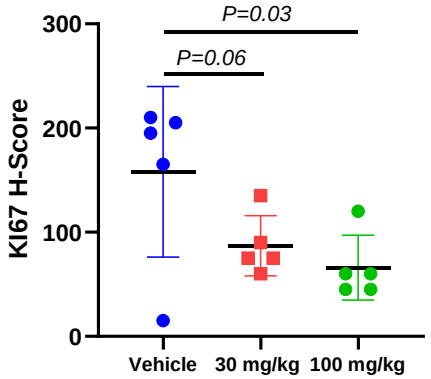
G1 cell cycle arrest and p21 accumulation in vitro

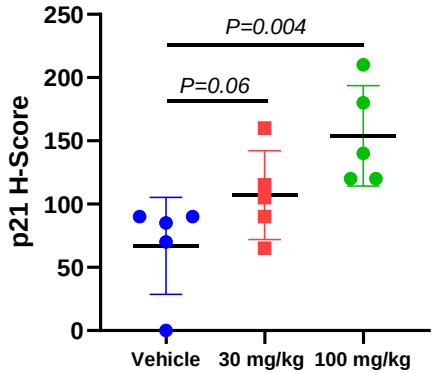




NCI-H1373
RBN-2397 1000 nM
72 hr

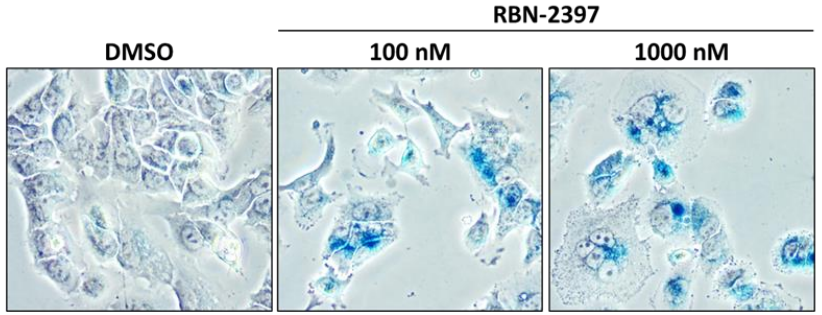
Decreased proliferation and p21 accumulation in vivo





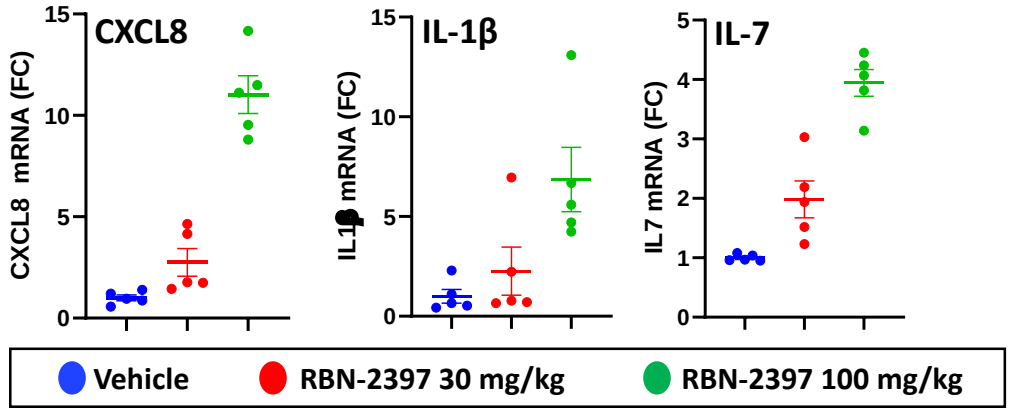
NCI-H1373 xenografts (CB17 SCID)
administered as single daily oral dose for 7 days

Senescence phenotype observed after 6 days of RBN-2397 treatment in vitro



NCI-H1373
β-gal staining
6 days

Senescence associated secretory phenotype (SASP) genes upregulated in vivo

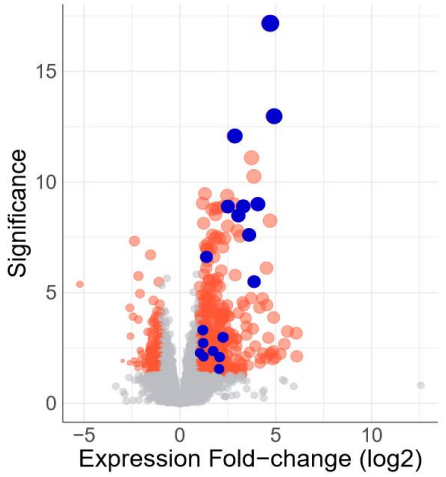


NCI-H1373 xenografts (CB17 SCID)
administered as single daily oral dose for 7 days

Inhibition of PARP7 with RBN-2397 Decreased Expression of Autophagy-Related Genes in NCI-H1373 Cells

Transcriptomic changes by RBN-2397 in NCI-H1373 cells implicate IFN and autophagy genes

Increase in multiple ISGs

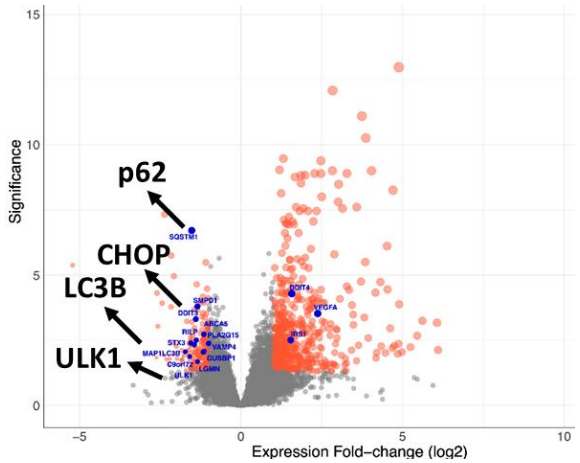


Nonsignificant

Padj<0.05, log2FCI>1

Genes enriched for Type I IFN & viral response

Decrease in autophagy genes

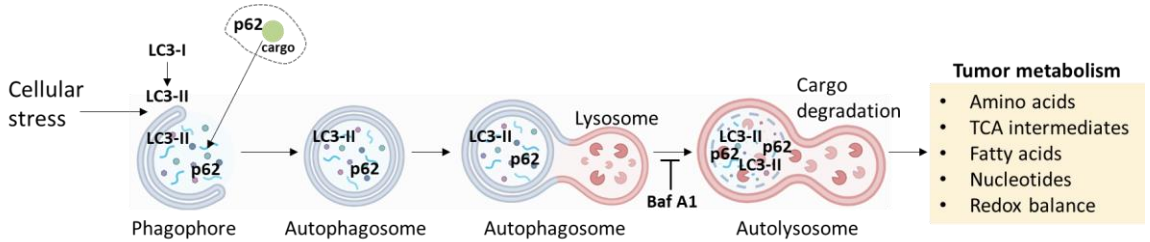


Padj<0.05, log2FCI>1

Autophagy genes

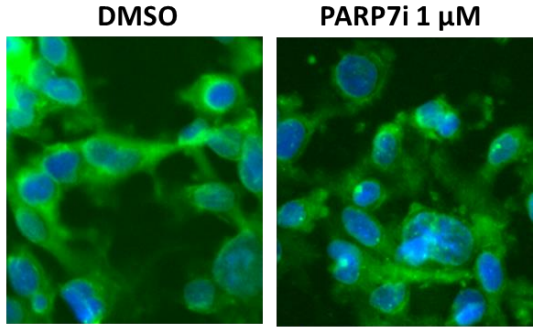
NCI-H1373
RBN-2397 1000 nM
24 hr

Autophagy, a catabolic pathway, degrades organelles and macromolecules to maintain homeostasis during cellular stress



- Autophagy supports metabolic plasticity and progression in some tumors

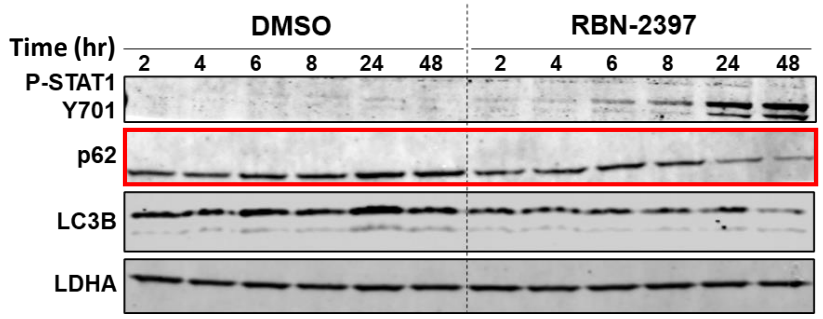
RBN-2397 leads to decreased expression of p62 and its localization to punctate structures



p62/SQSTM1 protein Hoechst
NCI-H1373

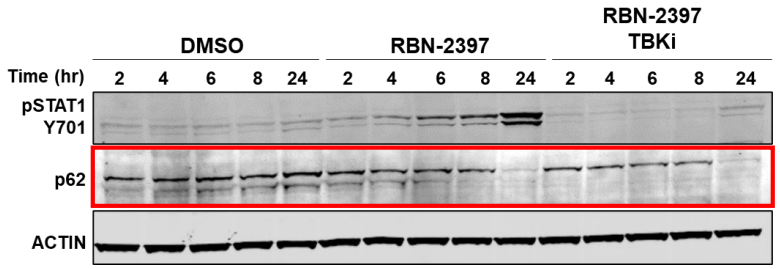
Autophagy-Related Protein p62 Decreases with RBN-2397 Treatment Independently of TBK1/STING Pathway Activation

p62 protein decreases with RBN-2397 treatment in full nutrient conditions



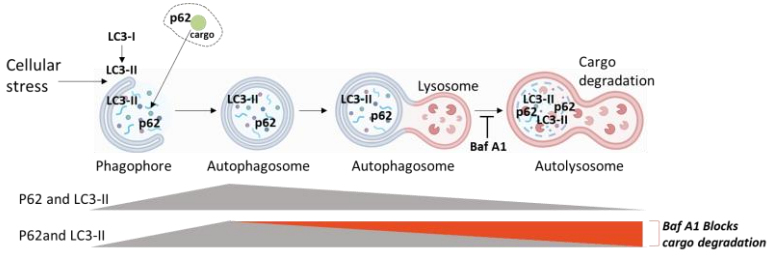
RBN-2397 300 nM

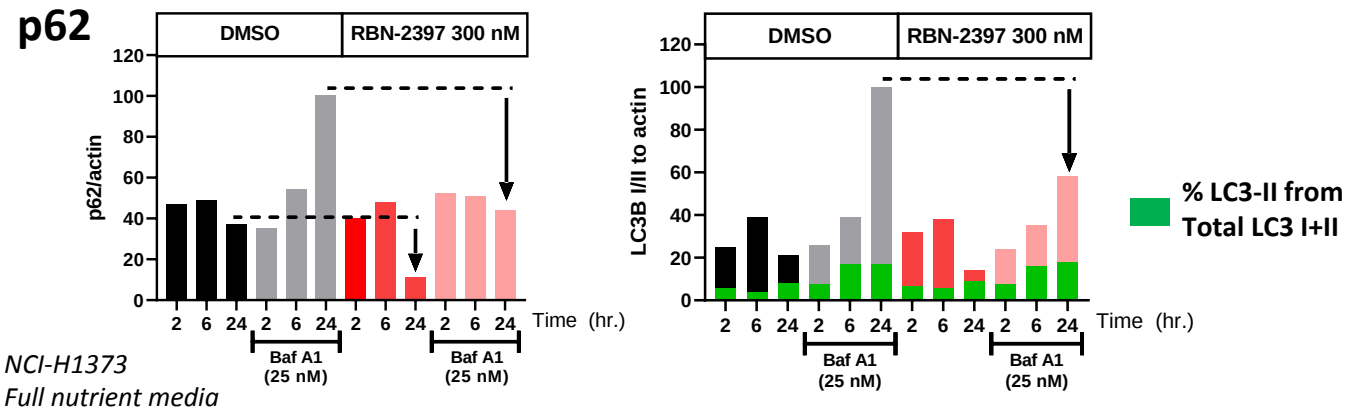
RBN-2397-induced p62 depletion persists despite inhibition of TBK1 activation



TBK1i (Bx795) 1 μ M

Bafilomycin-driven accumulation of p62 and LC3B (I/II) is reduced with RBN-2397 treatment in NCI-H1373



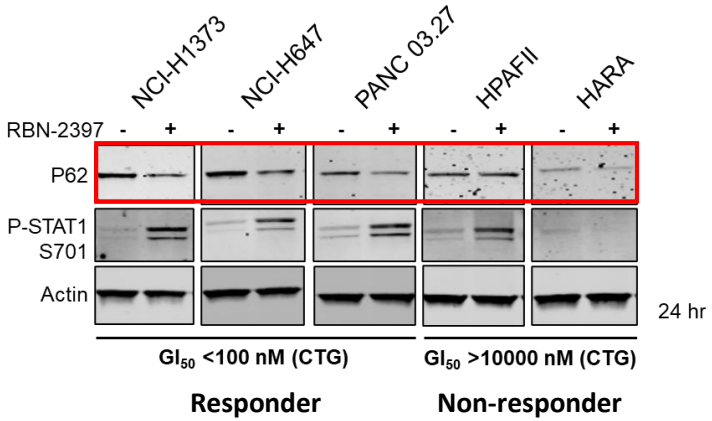


NCI-H1373
Full nutrient media
RBN-2397 300 nM
Bafilomycin A1 25 nM

- Active autophagy in NCI-H1373 cells under full nutrient conditions
- The amount of p62 and LC3-I/II protein accumulation observed with Baf-A1 treatment is reduced with RBN-2397 suggesting decreased flux thru the autophagy pathway

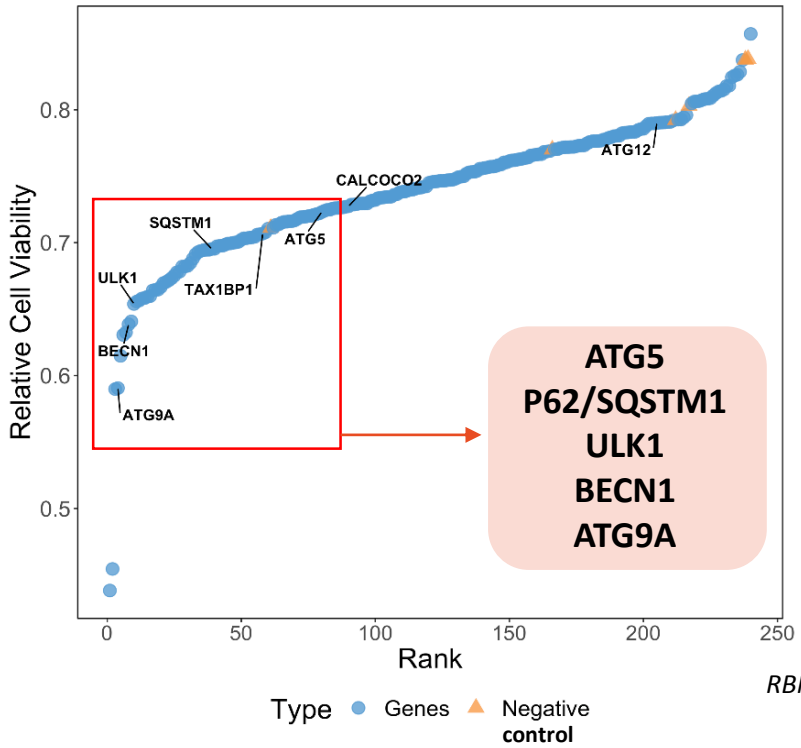
RBN-2397 Decreases Autophagy Flux in Responders, and Interference with Autophagy Sensitizes Non-Responders to RBN-2397

p62 is depleted with RBN-2397 in responder cell lines but retained or lowly expressed in non-responders



- RBN-2397 decreased p62 protein level in NCI-H647 and PANC.03.27 lines which also respond to RBN-2397 in the CTG assay
- No effect on p62 with RBN-2397 in the non-responder by CTG lines HPAF-II

Genetic depletion of autophagy-related genes sensitizes HPAFII cell to RBN-2397

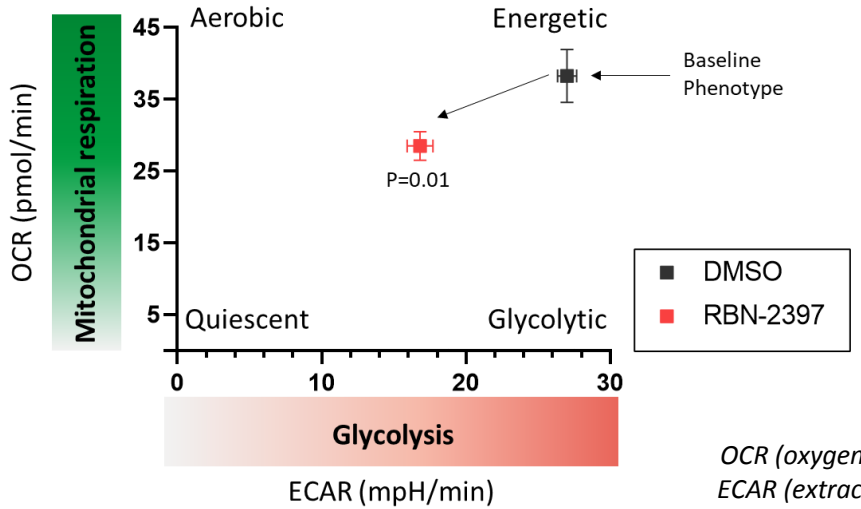


HPAFII
RBN-2397 500 nM
5 days

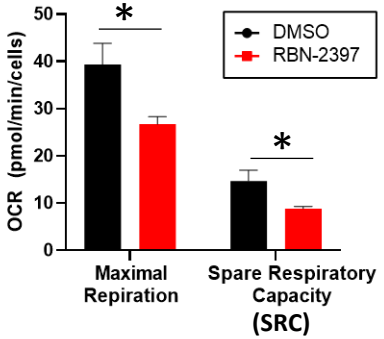
- Arrayed CRISPR KO screen measuring proliferation
- Genetic depletion of several autophagy related genes sensitizes HPAFII cells to RBN-2397

RBN-2397 Leads to Changes in Cellular Metabolism and Shift Toward a Quiescent Metabolic State

RBN-2397 decreases oxidative and glycolytic function in NCI-H1373 cells leading to a quiescent metabolic state

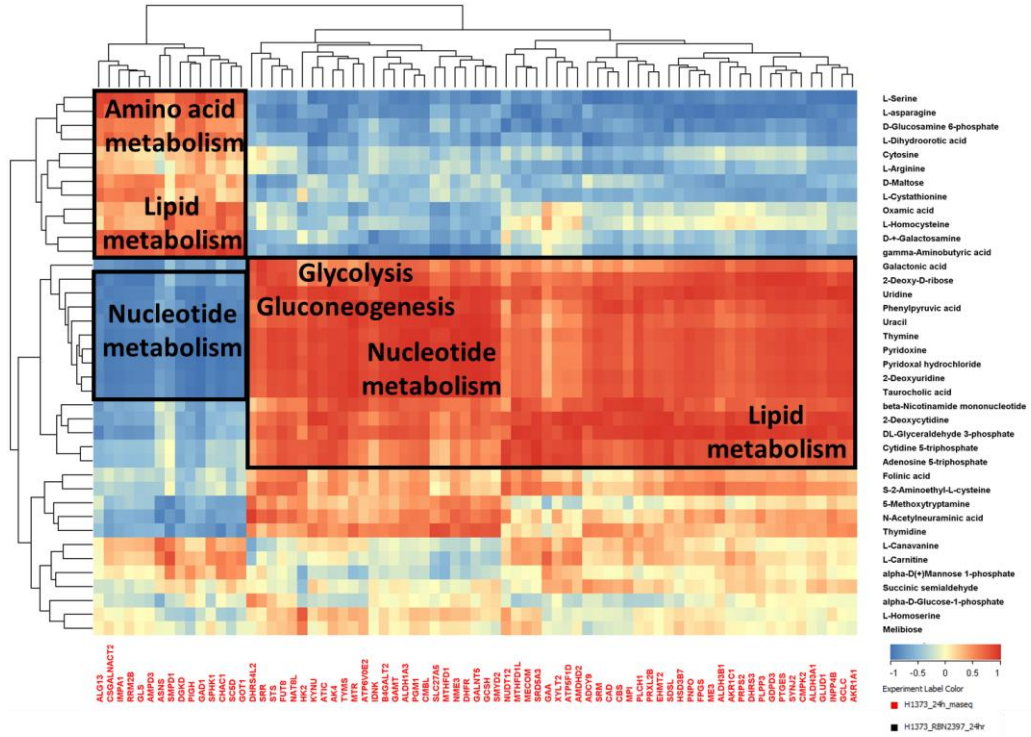


NCI-H1373
RBN-2397 300 nM
24 hr
OCR (oxygen consumption rate)
ECAR (extracellular acidification rate)



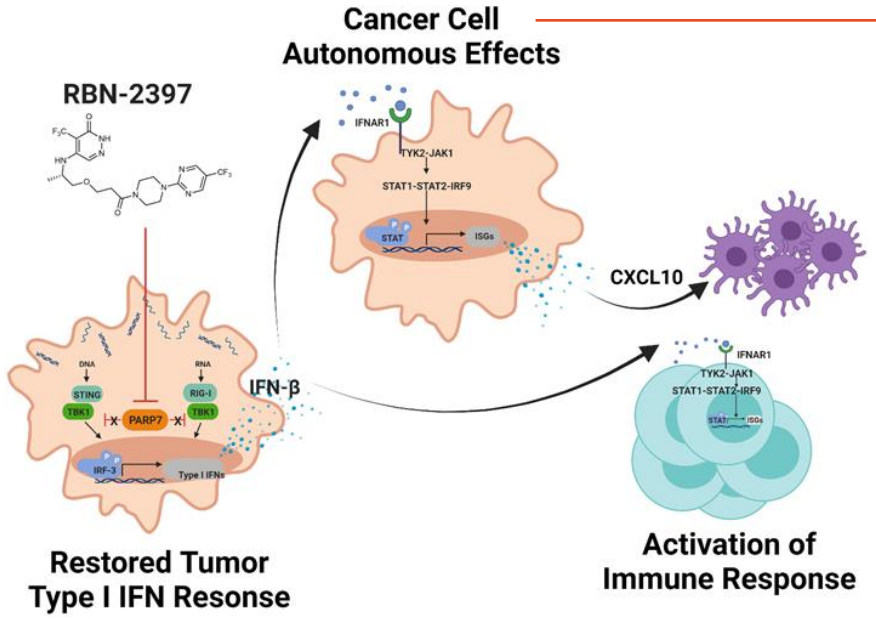
- Maximal respiration and SRC measures a cell's ability to respond to increased energy demand under stress
- RBN-2397 decreased both after 24h of treatment

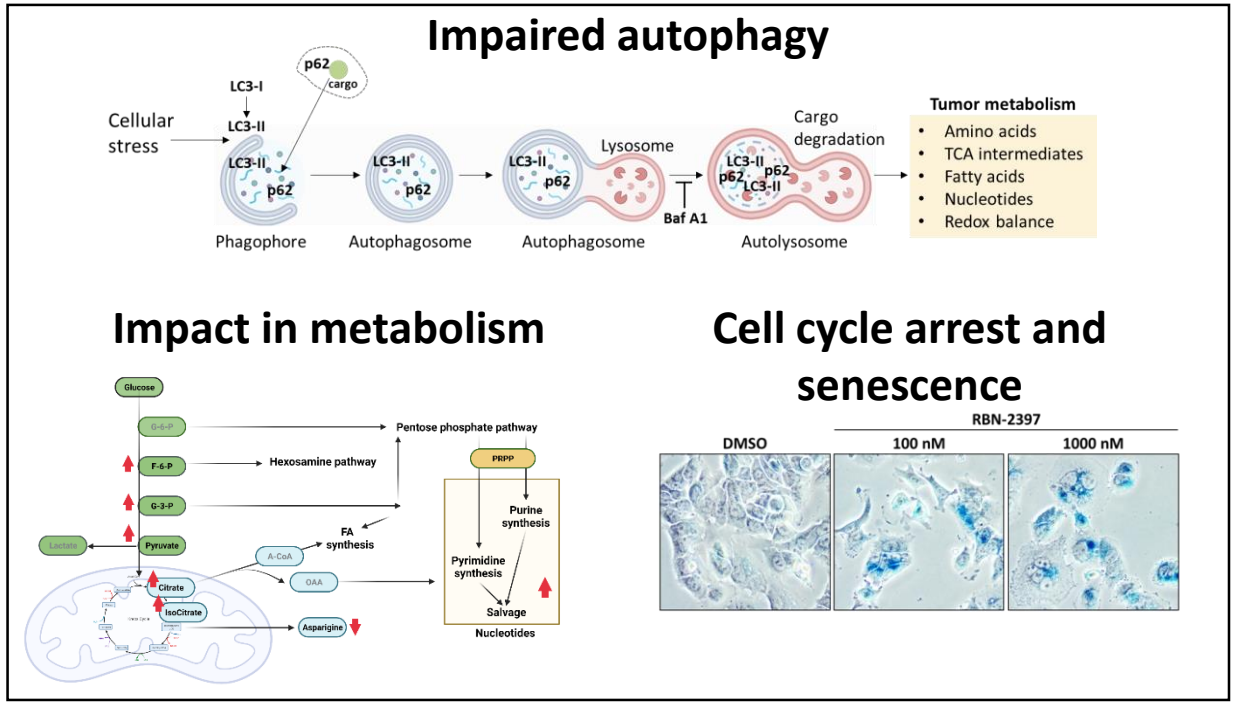
Targeted metabolomics analysis highlights metabolites that changed with RBN-2397 treatment that correlate with transcriptomic changes in metabolism genes



- RBN-2397 led to metabolic changes in several central carbon metabolism pathways

RBN-2397 Induces Tumor Intrinsic Effects that Synergize with Its Ability to Activate the Type I IFN Response, Leading to Tumor Regressions



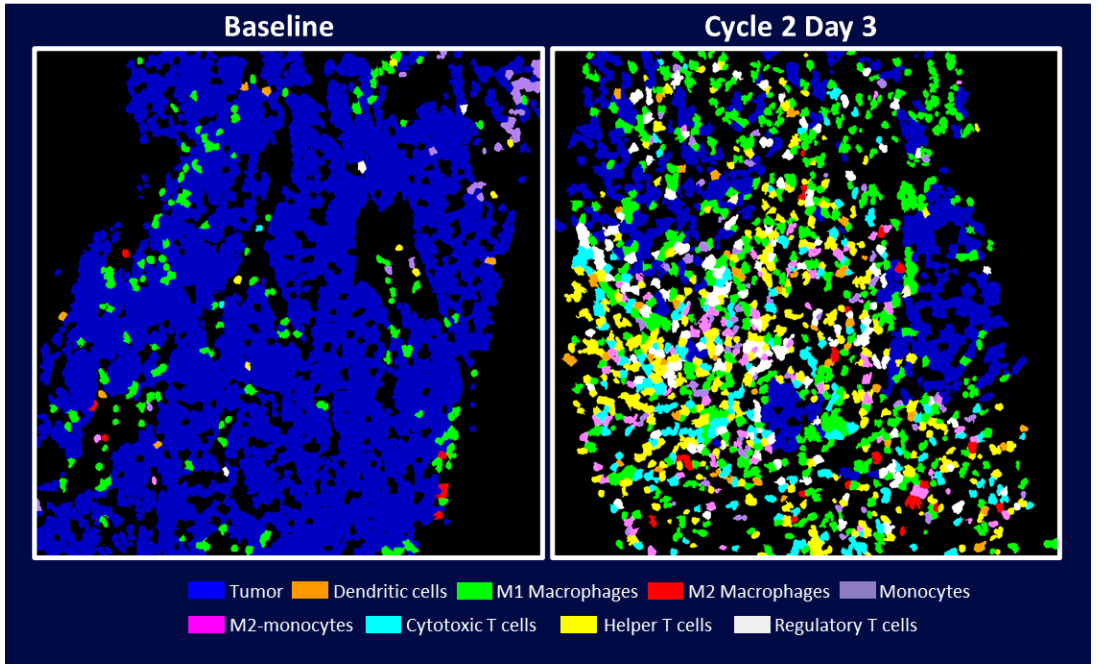


Combined mechanisms lead to tumor regression

Summary: RBN-2397 Leads to Tumor Regression by Immune and Intrinsic Mechanisms and Has Demonstrated Proof of Mechanism in Patients

- **Ribon's first-in-class PARP7 inhibitor, RBN-2397, targets a novel tumor dependency pathway**
- **RBN-2397 leads to tumor regression in cancer models by:**
  - *Activation of tumor interferon signaling*
  - *T cell-dependent antitumor immunity*
  - Impairment of tumor cell autophagy
  - Change of tumor cell metabolism
  - Induction of tumor cell senescence and SASP
- **RBN-2397 is currently being tested clinically as a single agent in multiple tumor types (SCCL, HNSCC, HR+ BrCa, PARP7-amplified tumors; NCT04053673) and in combination with pembrolizumab in SCCL (NCT05127590)**

RBN-2397-induced immune cell infiltration into patient tumors in the clinic



Paired tumor biopsies: NSCLC patient from single agent study dosed at 200 mg
Analysis by MIBI-SCOPE technology

For more information check out:

Kristy Kuplast-Barr

Poster 1836 / 8 - RBN-2397, a novel, potent, and selective PARP7 inhibitor, induces tumor-intrinsic type I interferon responses and adaptive immunity in patient tumors

Session PO.ET02.01 - Mechanisms of Drug Action 1 (Monday 1:30 – 5 pm)

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