A Potent and Selective PARP14 Inhibitor Decreases Pro-Tumor Macrophage Function and Elicits Inflammatory Responses in Tumor Explants

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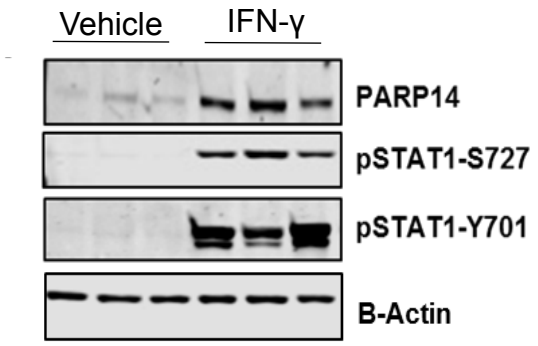
Disclosure Statement

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

PARP14 Is Overexpressed in Cancer and Implicated in Macrophage and T Cell Biology

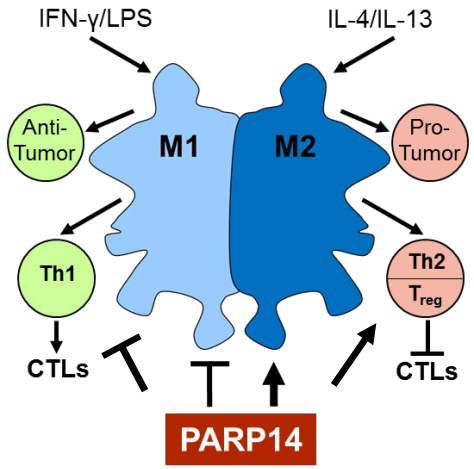
- **The PARP family consists of 17 proteins (16 catalytically active) that use NAD^+ to post-translationally ADP-ribosylate substrates**
  - PolyPARPs are well-studied, and PARP1/PARP2 inhibitors have been approved
  - MonoPARPs offer a mechanistically distinct and untapped opportunity
- **PARP14 is a member of the monoPARP sub-family**
  - Downstream regulator of IFN-γ and IL-4 signaling
  - Immuno-oncology functions including regulation of M1/M2 macrophage differentiation
  - High expression in multiple tumor types

Interferon-stimulated gene

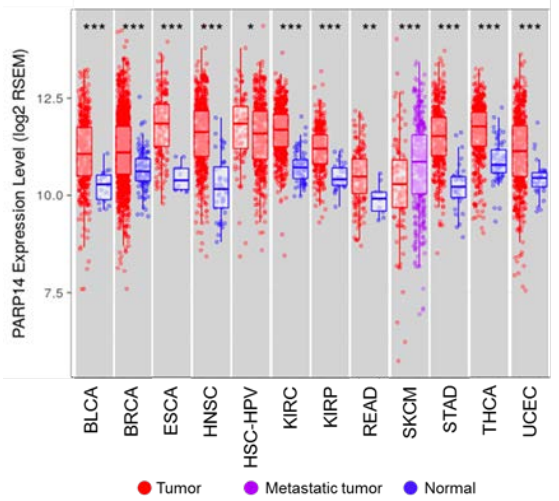


SU-DHL-8 lymphoma cells

Affects the differentiation of immune cell subsets associated with human disease

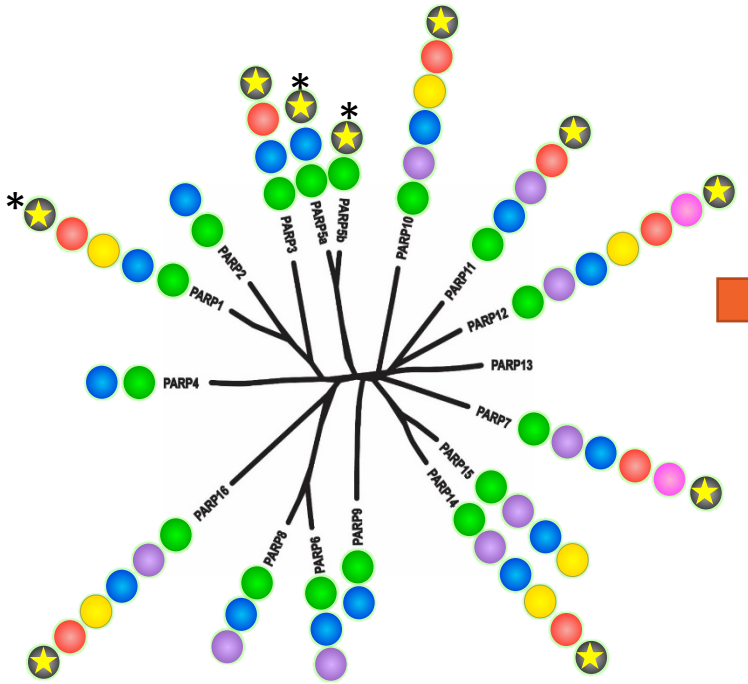


Overexpressed in several cancer types compared to normal tissue



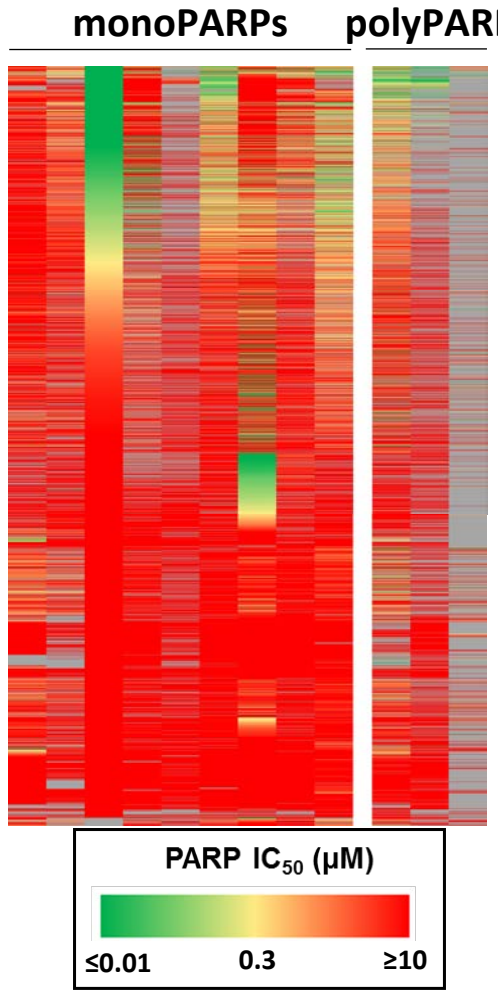
Screening Ribon's Compound Collection and Profiling Against a Panel of PARP Assays Identified a PARP14 Hit

Ribon Platform enabled the monoPARP family for drug discovery

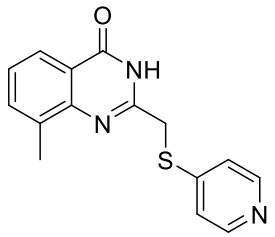


Wigle, et. al. SLAS Discovery, 2019

PARP14 was screened against Ribon's proprietary compound collection



A moderately potent hit was identified and profiled across the PARP enzymes



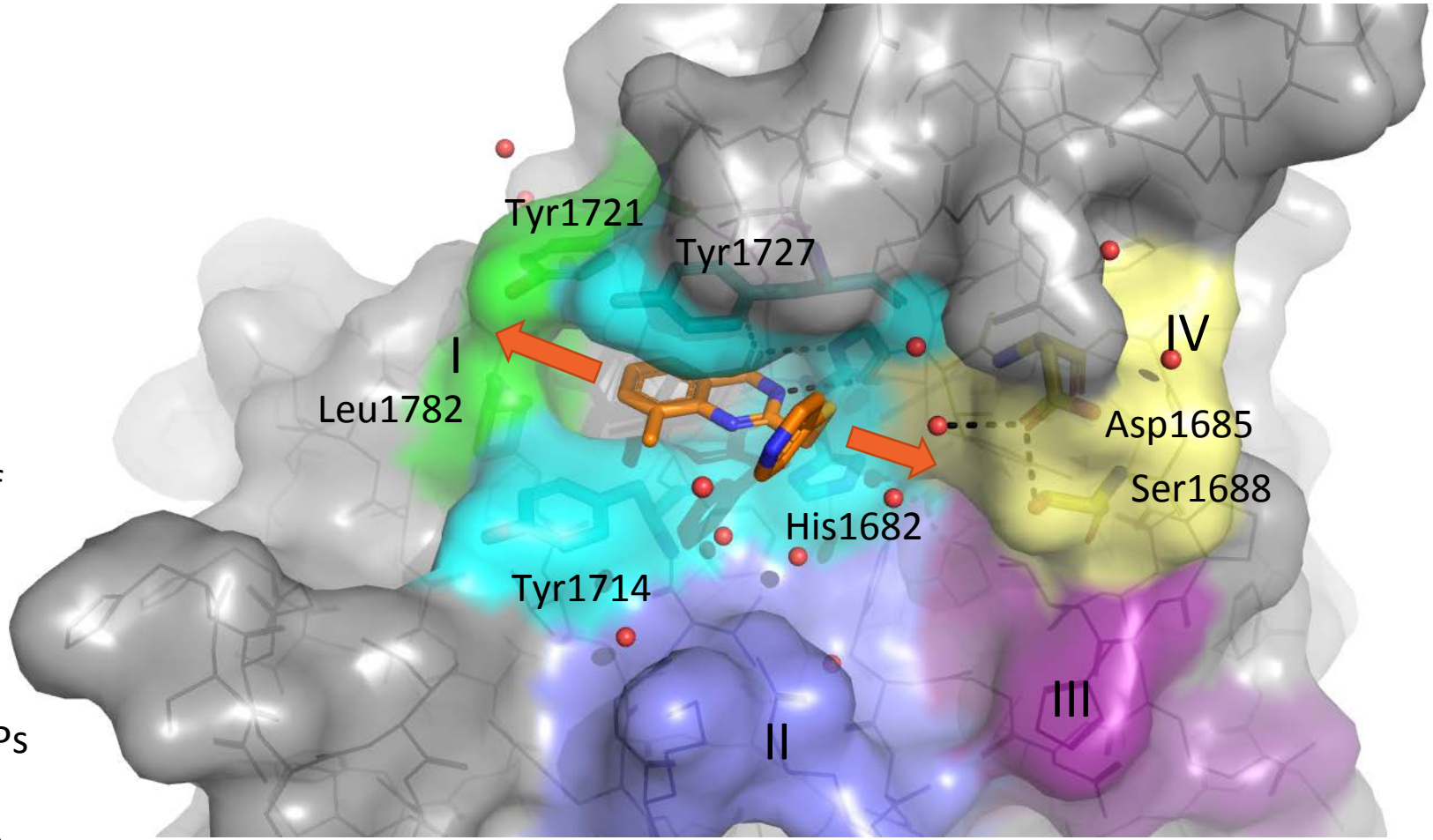
Compound 1

PARP Assay	IC_{50} (µM)
PARP14	1
PARP1	0.1
PARP2	0.3
PARP3	1
PARP4	1
PARP5a / 5b	0.09 / 0.03
PARP6	1
PARP7	10
PARP8	4
PARP9	>100
PARP10	1
PARP11	4
PARP12	4
PARP15	6
PARP16	7

Compound 1 Provides Vectors to Access Areas of the NAD$^+$ Binding Pocket that Were Hypothesized to Increase PARP14 Potency and Selectivity

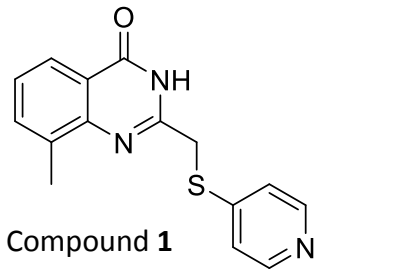
- Nicotinamide pocket
- I: MAR/PAR difference
- II: D-Loop
- III: Adenosine region
- IV: Asp/Ser region

- **Hit to lead efforts targeted interactions in key areas of the NAD$^+$ binding pocket**
  - Region IV: the Asp/Ser motif is unique to PARP14 and PARP15
  - Region I: the monoPARPs contain hydrophobic residues, while the polyPARPs contain polar residues that form a conserved salt bridge

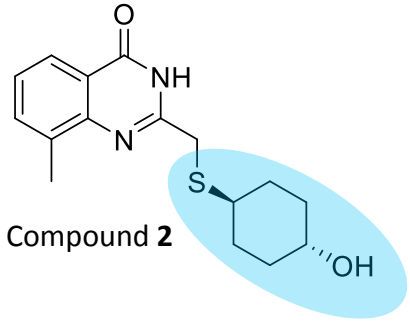


Modification of the Thioether Led to High Selectivity for PARP14 over the Other MonoPARPs

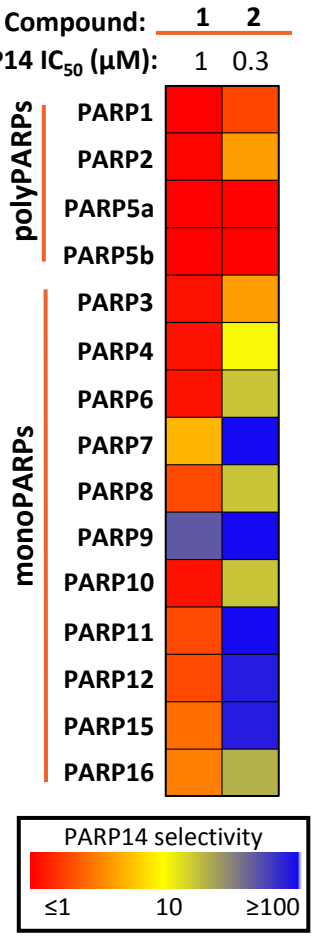
Optimization of the aromatic thioether improved PARP14 potency and selectivity over the monoPARPs



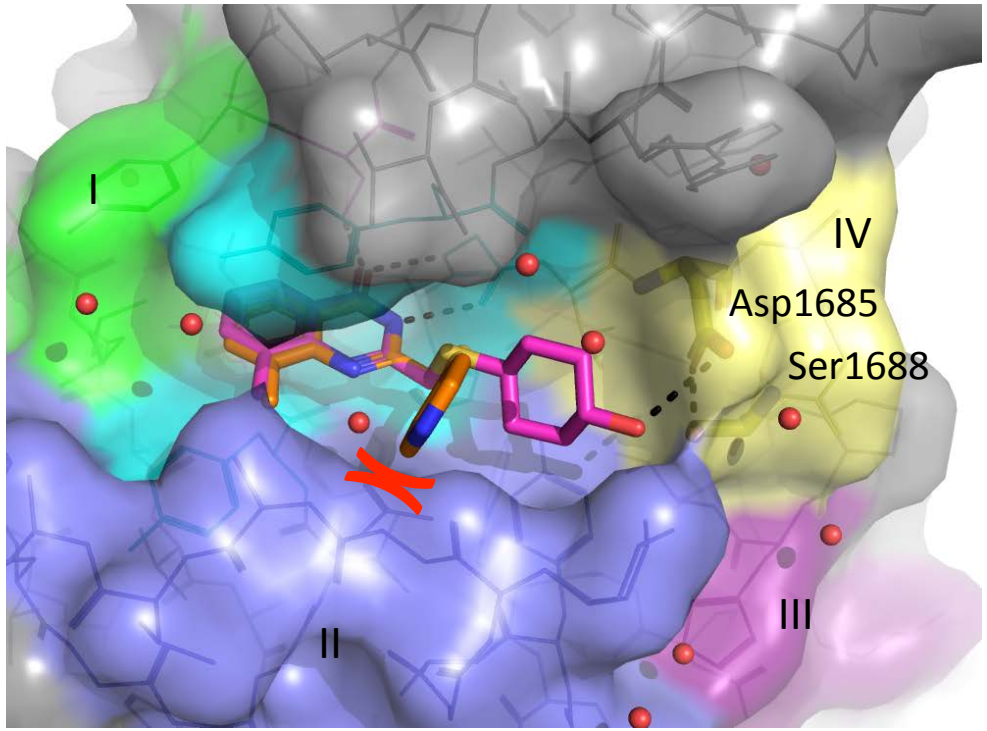
Compound **1**



Compound **2**

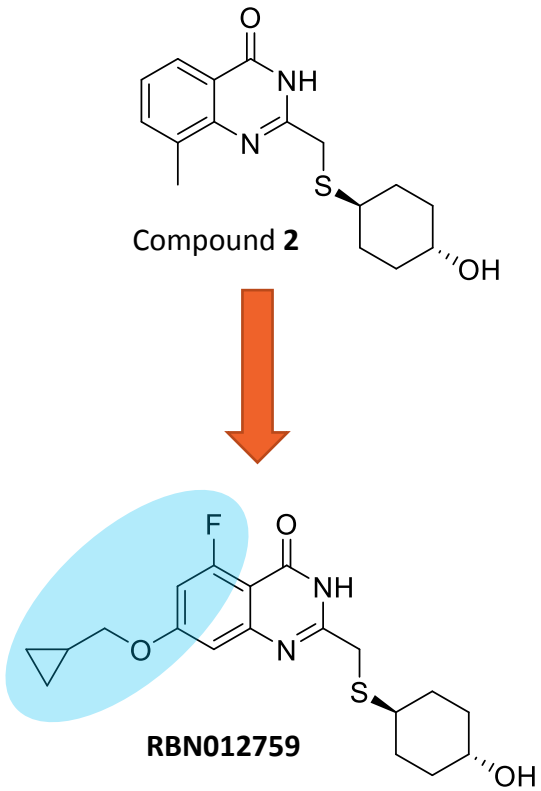


Changes in the binding conformation of the thioether led to the alcohol of compound 2 interacting with Asp1685 and the PARP14 D-loop adopting a "closed", ordered conformation

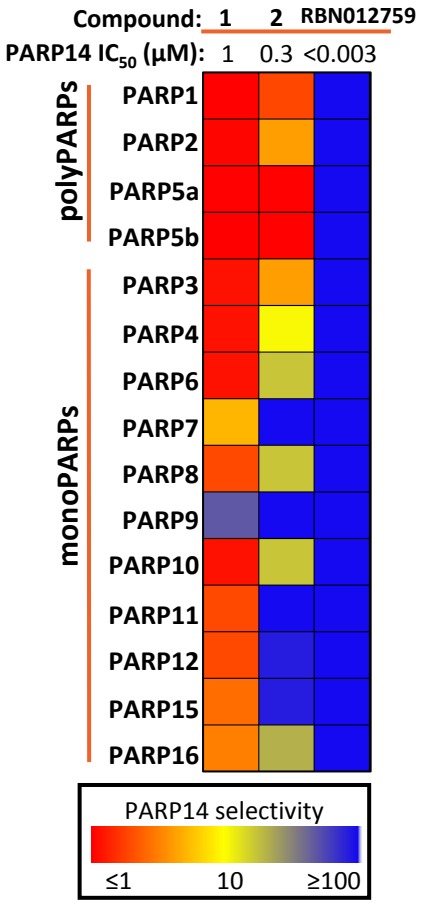


RBN012759 Is a Potent PARP14 Inhibitor that Is Highly Selective over All Mono- and PolyPARPs

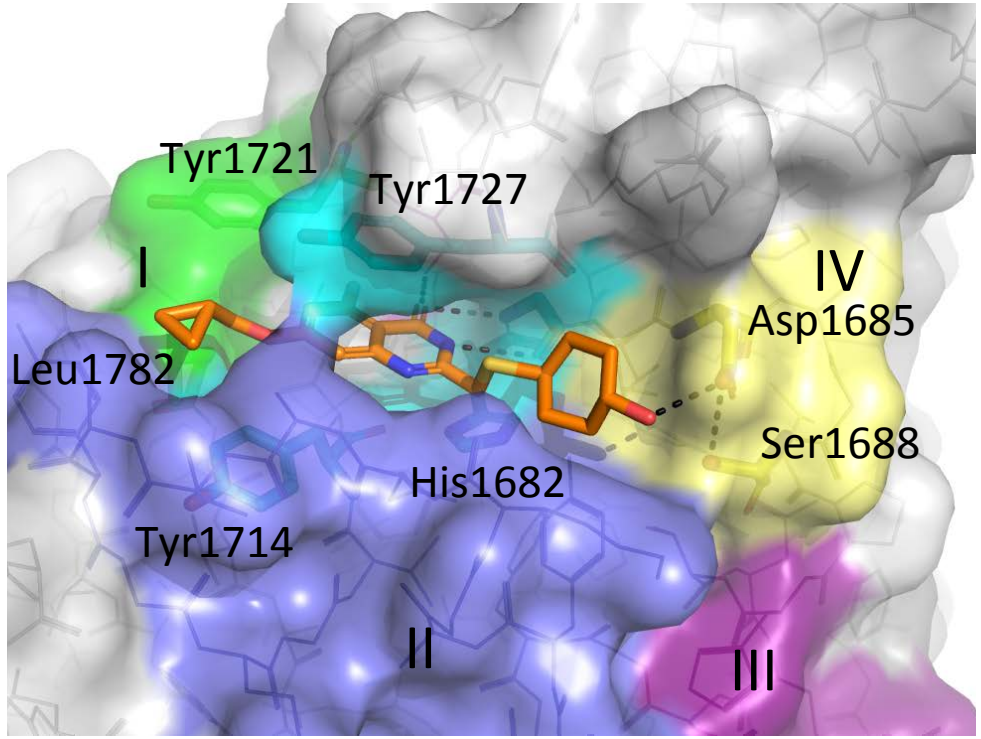
Optimization for PARP14 potency, selectivity and PK properties led to RBN012759



RBN012759 is >300-fold selective over the monoPARPs and >1000-fold selective over the polyPARPs

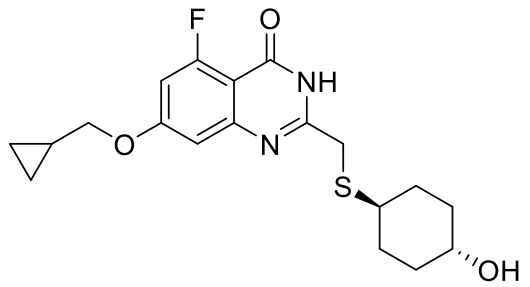


RBN012759 makes new interactions with PARP14 in the Asp/Ser (IV) and MAR/PAR difference (I) regions



The Profile of RBN012759 Supports Its Use as an In Vitro and In Vivo PARP14 Chemical Probe

PARP14 Activity/Affinity	
IC_{50} human catalytic domain (µM)	<0.003
IC_{50} mouse catalytic domain (µM)	0.005
IC_{50} human full length (µM)	<0.005
Cellular Target Engagement	
PARP14 NanoBRET cell probe displacement (µM)	0.003
PARP Family Activity	
PARP1 (µM)	>100
PARP2 (µM)	>100
PARP3 (µM)	>100
PARP4 (µM)	10
PARP5a (µM)	8
PARP5b (µM)	10
PARP6 (µM)	4
PARP7 (µM)	4
PARP8 (µM)	20
PARP9 (µM)	>100
PARP10 (µM)	1
PARP11 (µM)	1
PARP12 (µM)	5
PARP15 (µM)	3
PARP16 (µM)	6
In Vitro ADME and Physicochemical Properties	
Kinetic solubility at pH 7.4 (µM)	198
Caco-2 $P_{app(A-B)}$ (x 10^{-6} cm/s) / efflux ratio	19 / 1
Mouse microsomes Cl_{int} (mL/min/kg, scaled)	469
Mouse ppb (F_u)	0.13
MW / TPSA / XLogP	379 / 71 / 2.9
In Vivo PK	
Mouse Cl (mL/min/kg) / $t_{1/2}$ (h) / Vss (L/kg)	54 / 0.4 / 1.4
Mouse %F at 100 mg/kg	30

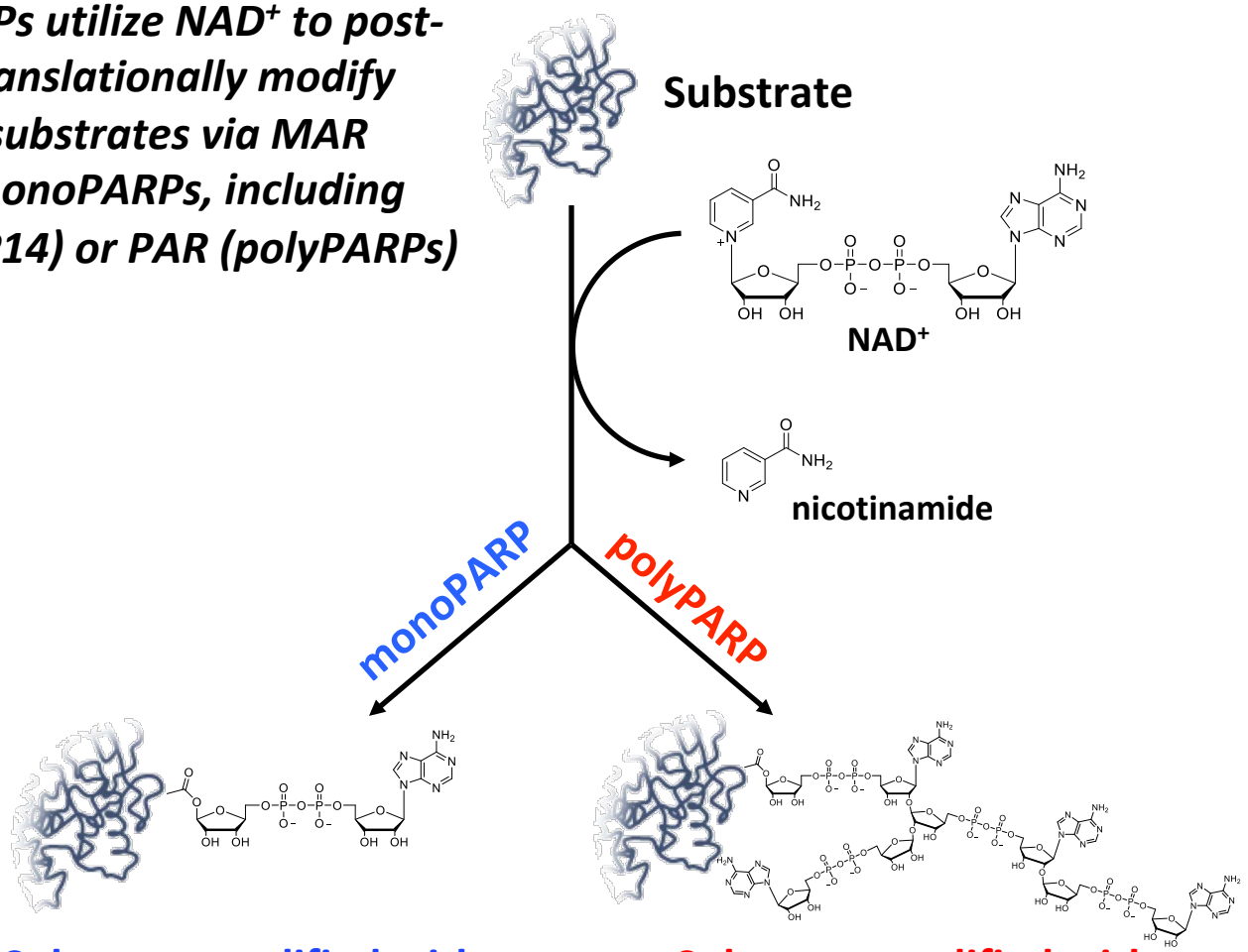


The chemical probe RBN012759:

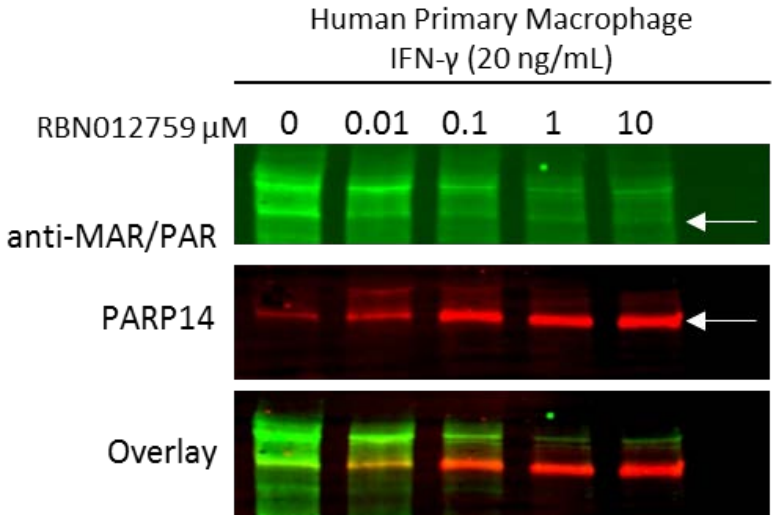
- Is a potent inhibitor of human and mouse PARP14 with high PARP family selectivity
- Is sufficiently soluble, highly permeable, low efflux
- Has moderate clearance and oral bioavailability in mice
- Is well-tolerated in mice with repeat dosing up to 500 mg/kg BID
  - 75-fold coverage of the PARP14 mouse free EC_{50} observed at C_{trough}

RBN012759 Demonstrates Intracellular Engagement of PARP14

PARPs utilize NAD⁺ to post-translationally modify substrates via MAR (monoPARPs, including PARP14) or PAR (polyPARPs)

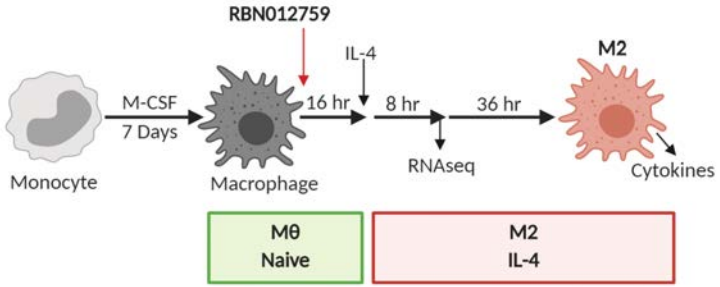


RBN012759 treatment of human primary macrophages decreased the MAR/PAR signal corresponding to PARP14 self-MARylation and stabilized PARP14 protein in a dose-dependent manner

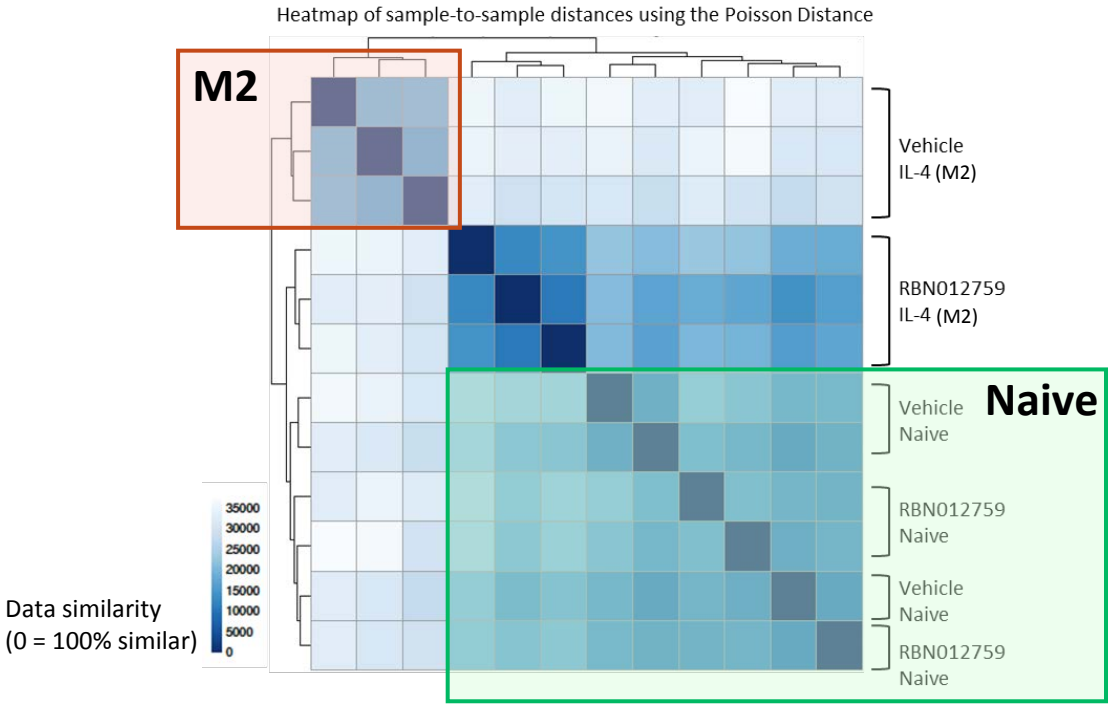


PARP14 Inhibition Reverses IL-4-Driven Gene Expression in Primary Human Macrophages

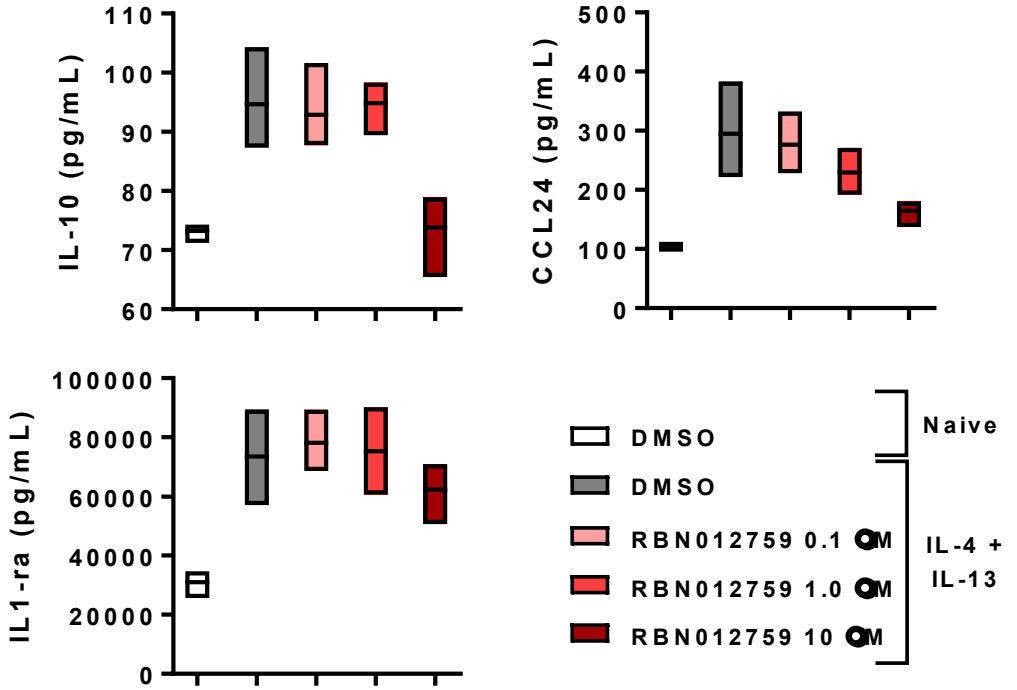
Experimental design



PARP14i reverses IL-4 stimulated gene expression



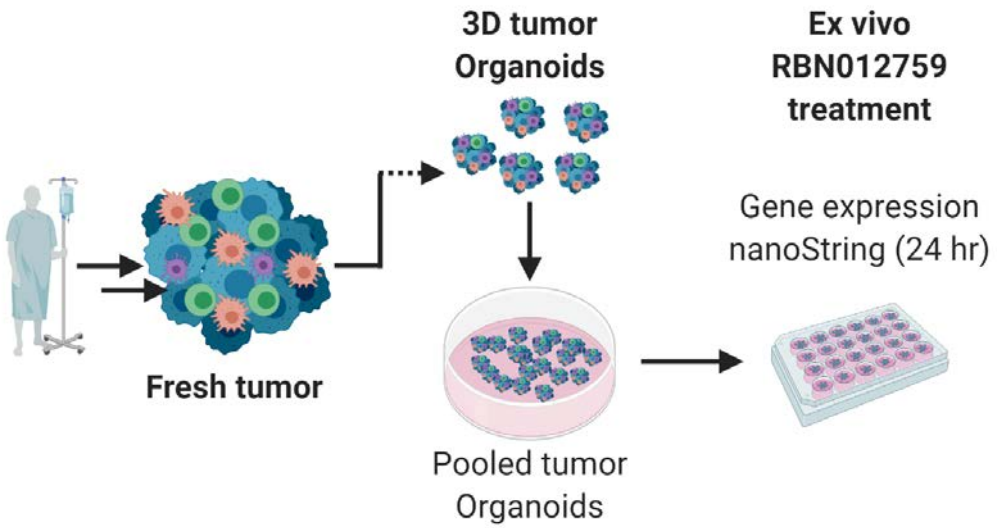
PARP14i reduces IL-4 stimulated cytokine secretion



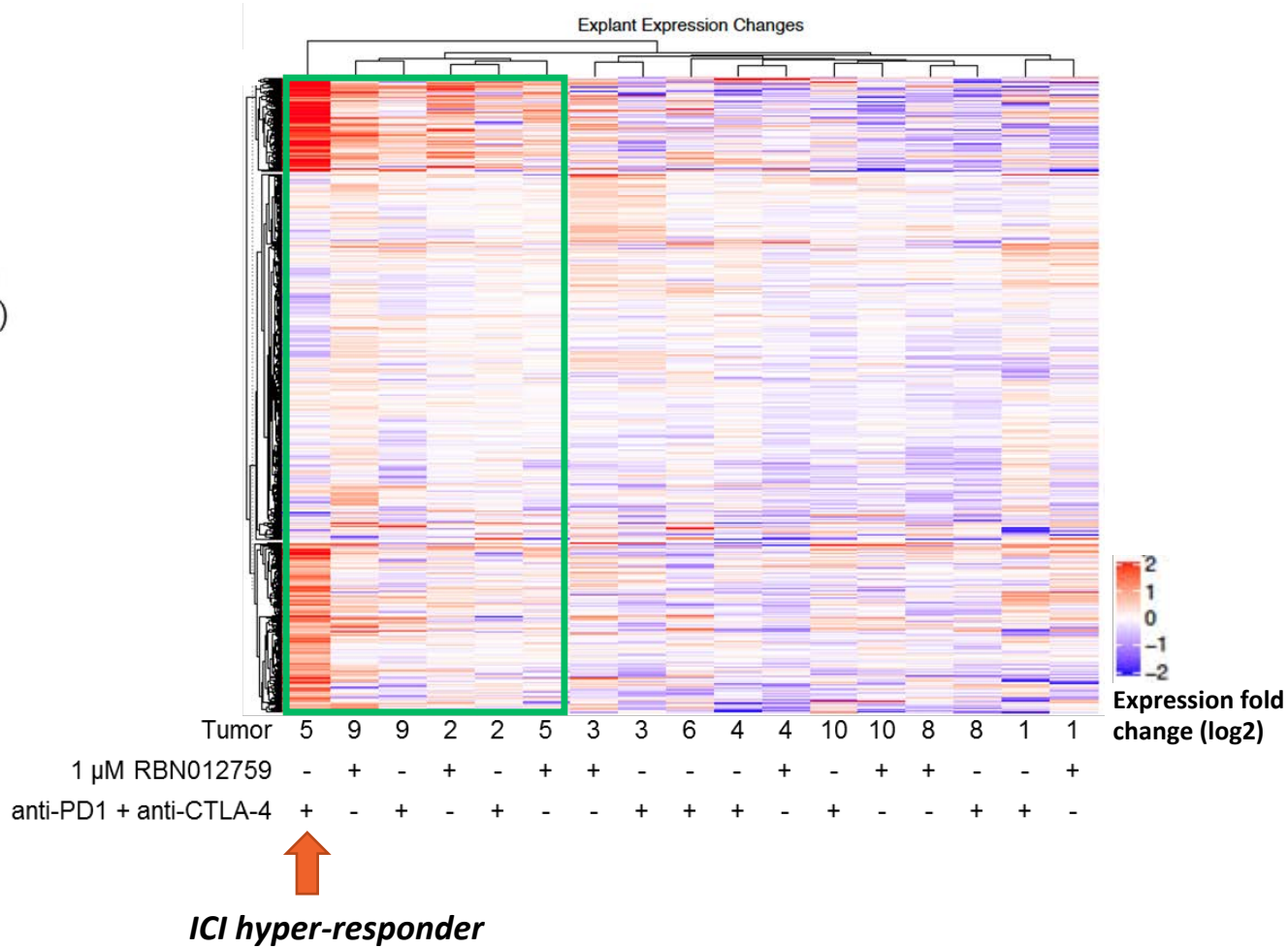
- **Treatment of primary human macrophages with RBN012759 led to decreased IL-4 driven M2-like gene expression, suggesting that PARP14 inhibition results in a less immunosuppressive phenotype**

PARP14 Inhibition Induces Inflammatory Gene Expression in an Unbiased Set of Kidney Cancer Tumor Explants

Experimental design: kidney renal clear cell carcinoma (KIRC) explants



RBN012759 induces gene expression changes similar to immune checkpoint inhibitor combination in some tumors



- **RBN012759 treatment induced gene expression changes in tumors 5, 9 and 2 that cluster with immune checkpoint inhibitor (ICI) hyper-responder tumor 5**

Conclusions

- **Detailed knowledge of the PARP family NAD^+ binding pockets enabled structure-based optimization of moderately potent, unselective PARP14 hit Compound 1 into RBN012759**
- **RBN012759 (<0.003 µM PARP14, >300-fold selective over monoPARPs, >1000-fold selective over polyPARPs) is the most potent and selective inhibitor of PARP14 reported to date**
- **The properties of RBN012759 enable its use as an in vitro and in vivo PARP14 chemical probe**
- **Data generated in human primary macrophages and KIRC human tumor explants with RBN012759 treatment links PARP14 with suppression of the anti-tumor immune response**
- **RBN012759 can serve as a useful tool for further exploration of PARP14 functions in cell biology and as a drug target**

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