Small Molecule Inhibitor of CD38 Modulates Its Intra- And Extracellular Functions Leading to Antitumor Activity

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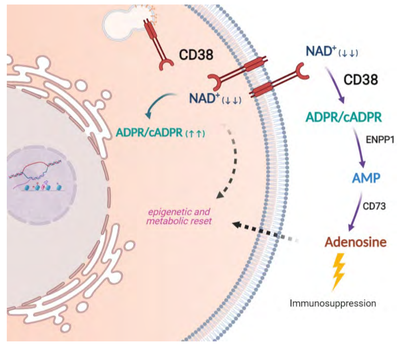
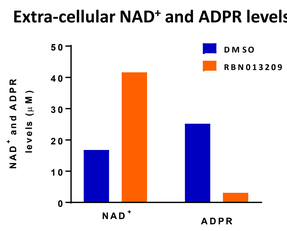
CD38 is a Multi-functional Enzyme and is an NADase

- ADP-ribosyl cyclase that converts NAD^+ to ADP-ribose (ADPR) or cyclic ADPR (cADPR) and NAADP
- Present in either an ecto- or endo-catalytic orientation with different sub-cellular localization
- Regulates internal and external pools of NAD^+ and its metabolites
- Drives non-canonical generation of adenosine
- Upregulated in various disease conditions and associated with immune dysfunction
- Therapeutic target for cancer, autoimmune and metabolic disorders

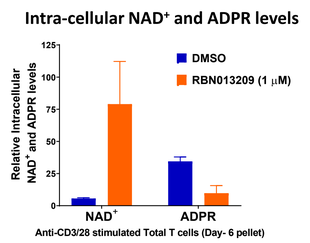


Extra-cellular and Intra-cellular Modulation of NAD^+ and Metabolites in Immune Cells

Inhibition of CD38 with RBN013209 affects both intra- and extra-cellular CD38 activity and modulates NAD^+ and ADPR levels



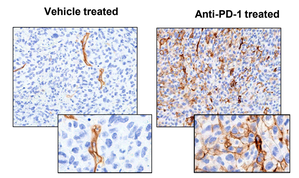
PBMCs were incubated with 50 μM NAD^+ for 2 hours
Supernatant was analyzed via LCMS for NAD^+, ADPR, and cADPR



LCMS analysis of T cell pellets obtained on day 6 of stimulation
Normalized to Day 0 NAD^+ or ADPR levels in pellets
Representative of 2 donors (+/- SEM)

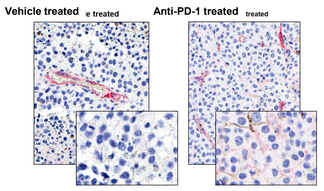
ICI treatment Drives CD38 Upregulation in the Tumor Microenvironment

MC38: colon cancer model



mCD38 IHC staining analysis from FFPE tumors
N=5 ; anti-PD-1 treatment for ~ 2 weeks

B16-F10: melanoma model

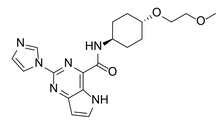


mCD38 IHC staining analysis from FFPE tumors
N=4 ; anti-PD-1 treatment for ~ 3 weeks

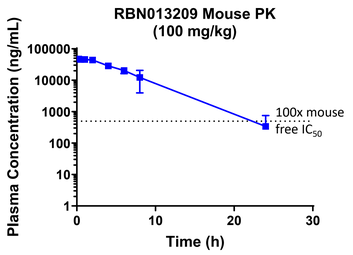
ICI – immune checkpoint inhibition

Increases in CD38 expression on tumor cells and infiltrating immune cells in MC38 colon cancer and B16-F10 melanoma, upon immune checkpoint (ICI) blockade inhibitor treatment

RBN013209 Potent and Selective Inhibitor of CD38



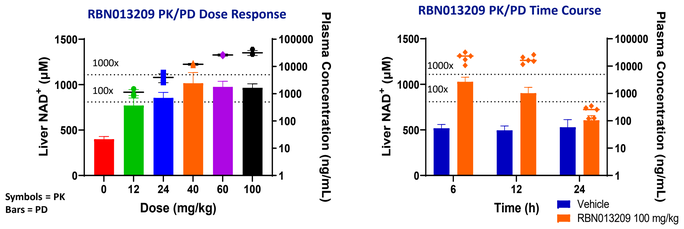
	RBN013209
IC_{50} human CD38 Biochemical (μM)	0.017
IC_{50} mouse CD38 Biochemical (μM)	0.017
IC_{50} CD38 Cellular NAD^+ Utilization Assay (μM)	0.003
PARPs, SARM1, ART1, CD157, CD73 (μM)	>10



- RBN013209 is a potent and selective inhibitor of human and mouse CD38
- High target coverage achieved with oral dosing

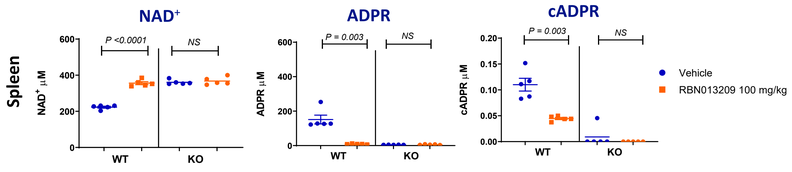
Pharmacodynamic Modulation of NAD^+ and Metabolites In Vivo

Oral Dosing of RBN013209 in Mice Modulates Liver NAD^+ at Multiple Doses and Increased NAD^+ for 12 Hours



LCMS based detection of NAD in homogenates from acid preserved tissue. Plasma and tissue collected after 6 hours for dose response study and 6, 12, and 24 hours for the time course study. Similar results observed in spleen.

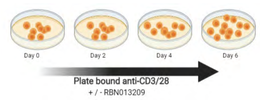
Oral Dosing of RBN013209 in WT and CD38 KO Mice Modulates Spleen NAD^+ and Metabolites in WT Mice to the Same Extent as Untreated KO Mice



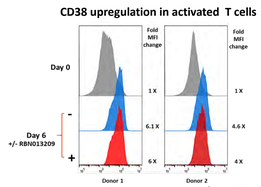
LCMS based detection of metabolites in homogenates from acid preserved tissue collected after 6 hours. Similar results observed in liver.

CD38 Inhibition Supports T cell Fitness Enabling Effector Functions

CD38 is upregulated upon T cell activation

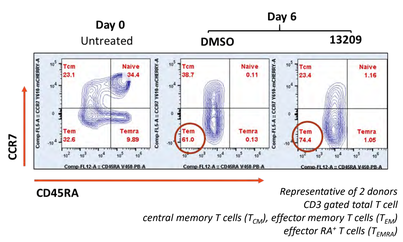


Total T cells stimulated and expanded using plate bound anti-CD3/28 for 6 days
Skewing to memory/effector T cell subsets and cytokine production followed



Surface CD38 staining
CD3 gated total T cells

CD38 inhibition enriches memory effector cells



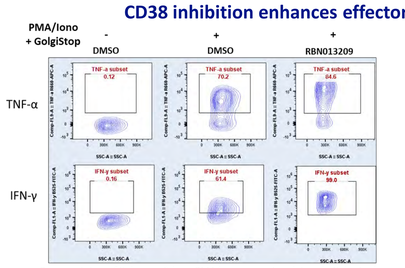
Representative of 2 donors
CD3 gated total T cell
central memory T cells (T_{CM}), effector memory T cells (T_{EM})
effector RA^+ T cells (T_{EMRA})

Changes in Effector Memory T cell (T_{EM}) Population (Day 6)

Donor / treatment	DMSO	RBN013209	↑ with RBN013209
Donor 1	61	74	13
Donor 2	84	92	8

CD3 gated total T cells
similar results with CD4 or CD8 gated T cells

CD38 inhibition enhances effector cytokine production



Representative of 2 donors; 6-hour PMA/Ionomycin stimulation (+ Golgistop)
Showing CD8 gated total T cells

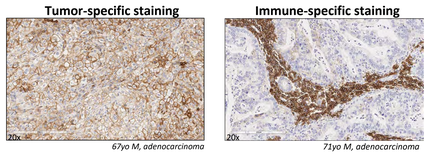
Changes in cytokine producing populations (Day 6)

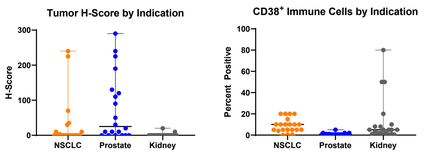
Cytokine	Donor / treatment	DMSO	RBN013209	↑ with RBN013209
TNF-α	Donor A	70	84	14
	Donor B	26	37	11
IFN-γ	Donor A	61	99	38
	Donor B	35	45	10

CD8 gated total T cells
similar results with CD4 gated T cells

CD38 Is Highly Expressed in Subsets of Lung and Prostate Cancer

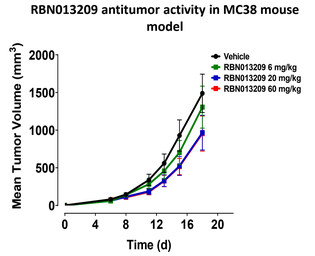
Confirmed CD38 expression in cancer patient samples

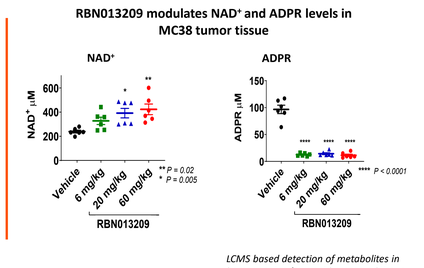




Samples exhibit varying levels of tumor and immune-specific staining patterns

RBN013209 Exhibits Antitumor Activity as Single Agent and Modulates Tumor NAD^+/ADPR

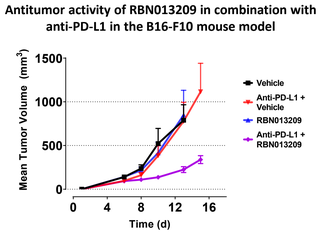


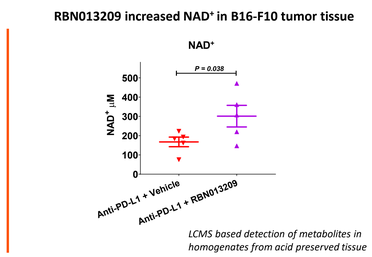


LCMS based detection of metabolites in homogenates from acid preserved tissue

- Single agent activity (~ 40 %) observed in MC38 syngeneic mouse model with oral BID dosing of RBN013209
- Tumor PD observed in terms of modulation of NAD^+ and ADPR levels

RBN013209 in Combination with ICI Treatment Shows Significant Tumor Growth Inhibition in B16-F10





LCMS based detection of metabolites in homogenates from acid preserved tissue

- Oral treatment with BID dosing of RBN013209 overcomes ICI resistance in B16-F10 tumor model
- Tumor PD observed in terms of modulation of NAD^+ levels

Conclusions

- RBN013209 is a potent and selective CD38 inhibitor with good PKPD properties
- Inhibition of CD38 with a small molecule affects both intra- and extra-cellular CD38 activity and modulates key metabolites playing an important role in immunomodulation
- CD38 is increased by ICI treatment and inhibition of CD38 can lead to antitumor activity