Targeting the Stress Response Kinase GCN2 to Restore Immunity And Decrease Tumor Cell Survival

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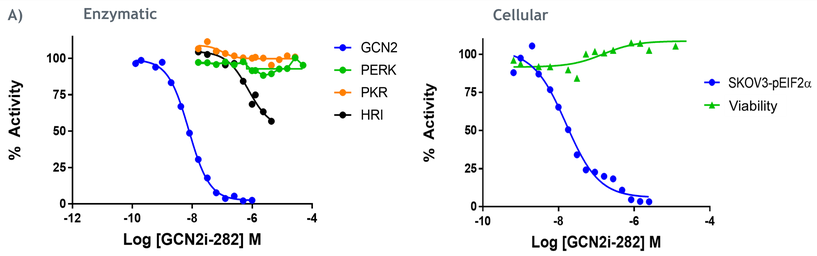
1. Abstract and Introduction

The tumor microenvironment (TME) is characterized by deficiencies in oxygen and key nutrients, such as glucose and amino acids, resulting in an overall immune suppressive environment. Stromal cells and myeloid-derived suppressor cells (MDSC) within the tumor create a nutrient-poor environment that inhibits immune function and supports tumor growth. GCN2 (general control nonderepressible 2), a stress response kinase, plays a key role in sensing and modulating the response to nutrient deprivation. GCN2 activation in T cells leads to an induction of the integrated stress response pathway and subsequently to T cell anergy and apoptosis, enhanced MDSC-dependent immune suppression and tumor growth survival.

Treatment of these nutrient-deprived T cells with an inhibitor of GCN2 (GCN2i) resulted in rescue of $CD4^+$ and $CD8^+$ T cell proliferation and effector functions as measured by flow cytometry. In addition, GCN2 inhibition in MDSC alone fully reversed $CD33^+$ MDSC-induced T cell suppression and effector functions. Using the CT26 colorectal syngeneic mouse tumor model we demonstrated that the pharmacologic inhibition of GCN2 in-vivo leads to an observed anti-tumor effect. Furthermore, GCN2 inhibition induced enhanced tumor specific $CD8^+$ T cell immunity. Our GCN2i is currently being evaluated to further elucidate the immune contribution in the tumor microenvironment.

The GCN2 pathway is activated in immune and tumor cells during nutrient deprivation, resulting in functional suppression of the immune response. Our results demonstrate that inhibition of GCN2 is an attractive approach for relieving T cell suppression and promoting anti-tumor activity, demonstrating GCN2 as a promising therapeutic target for the treatment of cancer.

2. GCN2i Potently Reduces EIF2α Phosphorylation

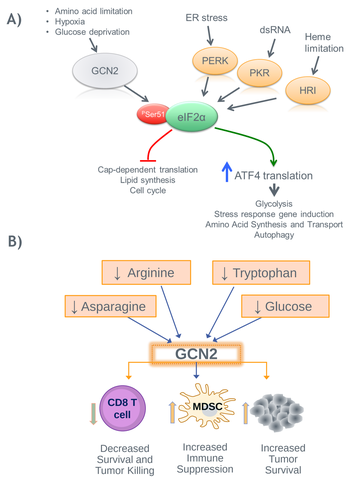


B)

ID	GCN2 Enzyme IC_{50} (nM)	PERK Enzyme IC_{50} (nM)	PKR Enzyme IC_{50} (nM)	HRI Enzyme IC_{50} (nM)	SKOV3 Cellular pEIF2a IC_{50} (nM)	SKOV3 Cellular Toxicity IC_{50} (nM)	Bioavailability (Rodent %F)
GCN2i - 490	4.5	>50000	390	440	16	>25000	22%
GCN2i - 282	7.6	>50000	4400	>25000	24	>25000	80%

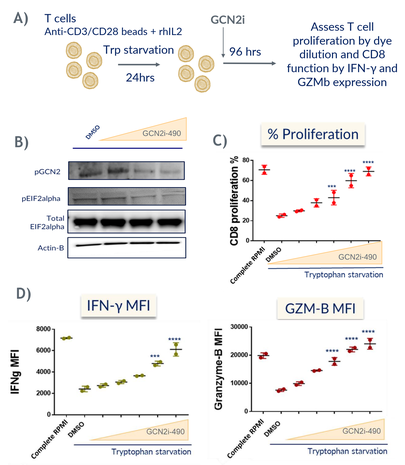
A) Enzymatic and cellular potency for GCN2i-282; B) Potency and selectivity parameters for GCN2i-490 and GCN2i-282. For enzymatic assays, compounds were incubated with recombinant human kinases and EIF2α-GFP substrate. Phosphorylation of EIF2α was measured by TR-FRET and used to calculate inhibition of kinase activity. For cell-based pEIF2α assay, SKOV-3 cells were incubated with compounds and then stimulated with halofuginone (1 hour) to activate GCN2 and then pEIF2α was measured by AlphaLisa. For toxicity assessment, SKOV3 cells were incubated with compounds for 72 hours and viability was assessed with CellTiter-Glo reagent.

3. GCN2 Activation Leads to Enhanced Tumor Survival Through the ISR Pathway



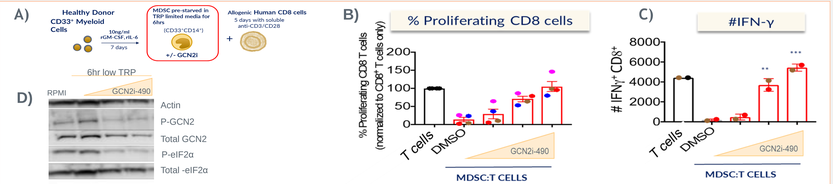
A) GCN2 is a stress response kinase detecting amino acid starvation in the tumor microenvironment. B) Activation of GCN2 leads to 1) T cell anergy, apoptosis and enhanced T_{reg} suppression and a decrease overall T cell function 2) increases myeloid-derived suppressor cell function and 3) increased tumor survival.

4. GCN2i Restores Human T Cell Proliferation And Function In Tryptophan Limited Conditions



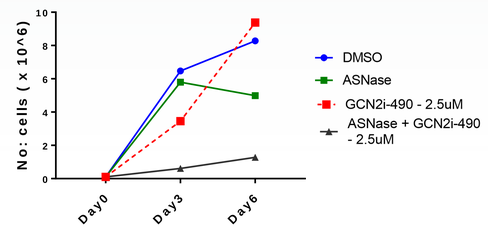
A) Experimental schematic
B) Western blot analysis of whole cell lysates from activated T cells starved for 24hrs with the GCN2i showed decrease in pGCN2, pEIF2alpha
C) $CD8^+$ T cell proliferation was assessed by dye dilution (CD4 proliferation is similarly increased – data not shown)
D) T cell functional markers were analyzed by flow cytometry.

5. Treatment Of Human $CD33^+$ MDSC With GCN2i Reverses Their Immuno-suppressive Function



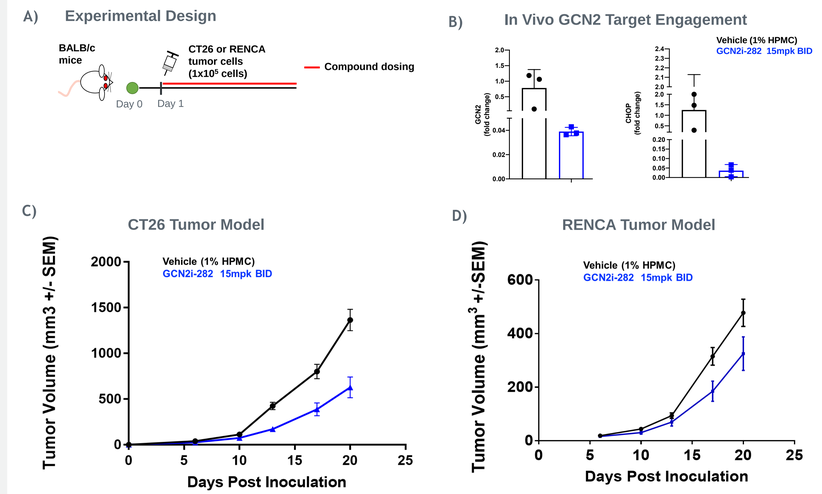
A) $CD33^+$ myeloid cells from healthy donor (isolated and expanded as described) were pre-starved in tryptophan-limited media and pretreated with GCN2i-490 for 6hrs and then washed. Pre-starved and pretreated MDSC were co-cultured in complete media with activated $CD8^+$ T cells. B) Proliferation (n=4) and C) IFNγ (n=2) were measured by flow cytometry. Similar increases in CD107a+CD8+ cells were also observed (data not shown). D) Western blot indicating pathway is activated at 6 hours under low tryptophan condition and GCN2i-490 leads to a decrease in pGCN2.

6. GCN2i Inhibits CT26 Growth in Low Amino Acid Conditions



CT26 cells cultured with vehicle (DMSO), Asparaginase (ASNase), or GCN2i alone, or the combination of ASNase and GCN2i. Cell number was determined after three and six days. Asparagine depletion by ASNase or GCN2i has minimal effects on cell growth alone, but in combination results in a dramatic reduction in cell number

7. GCN2i as a Single Agent Induces Modest Tumor Growth Inhibition in Multiple Models



A) Experimental schematic
B) CT26 Tumors from treated or untreated animals were harvested on day 12 and evaluated for levels of GCN2 or CHOP mRNA by qRT-PCR. GCN2i-282 resulted reduction of GCN2 and CHOP mRNA.
C) Twice daily dosing (BID) of GCN2i-282 results in modest inhibition of CT26 tumor growth compared to vehicle. Minimal effect on body weight is observed (inset graph).
D) Reduction in growth of RENCA tumors is observed with twice daily dosing of GCN2i-282 (compared to vehicle)

8. Conclusions

- RAPT Therapeutics is developing potent and selective inhibitors of the stress response kinase GCN2
- GCN2i inhibited phosphorylation of GCN2 and EIF2α in human $CD8^+$ T cells and human MDSC cultured under amino-acid starved conditions
- Inhibition of GCN2 increased human and mouse $CD8^+$ T cell proliferation and effector functions when cultured under amino-acid deprived conditions
- GCN2i reversed both human and mouse tumor-derived MDSC-mediated suppression and effector functions of $CD8^+$ T cells
- Treatment of human $CD33^+$ MDSC alone with RPT-GCN2i, reverses the suppressive function of MDSC on $CD8^+$ T cell
- RPT-GCN2i demonstrates moderate single-agent antitumor effect in CT26 and RENCA mouse prophylactic tumor models
- We are currently investigating various combination treatments
- Thus our data collectively demonstrates that GCN2 is a promising therapeutic target for the treatment of cancer