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

Lisa Marshall, Buvana Ravishankar, Deepika Kaveri, Lavanya Adusumilli, Deepa Pookot, Thant Zaw, Raashi Sreenivasan, Mikhail Zibinsky, Jeffrey Jackson, Grant Shibuya, Paul Leger, Omar Robles, Anqi Ma, Andrew Ng, Anton Shahmin, Scott Jacobson, Steve Wong, Jerick Sanchez, Justy Guaguas, Martin Brovarney, Angola Wadsworth, Delia Bradford, Christophe Colas, Oezcan Talay, George Kaltbath, Gene Cutler, David Wustrow, Paul Kassner, Dirk Brockstedt, RAPT Therapeutics, Inc. South San Francisco, CA

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 EIF2a Phosphorylation

A) Enzymatic and cellular potency for GCN2-282. B) Potency and selectivity parameters for GCN2-490 and GCN2-282. For enzymatic assays, compounds were incubated with recombinant human kinases and EIF2a-GFP reporter. Phosphorylation of EIF2a was measured by TR-FRET and used to calculate inhibition of kinase activity. For cell-based pEIF2a assay, SKOV3-3 cells were incubated with compounds and then stimulated with hypoligmine (1 hour) to activate GCN2 and then pEIF2a was measured by AlphaScren. For toxicity assessment, SKOV3s were incubated with compounds for 72 hours and viability was assessed with CellTiter-Glo reagent.

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 cell suppression and a decrease overall T cell function 2) increased myeloid-derived suppressor cell function and 3) increased tumor survival.

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

A) GCN2i restores human T cell proliferation and effector function in Tryptophan Limited Conditions

4. GCN2 Restores Human T Cell Proliferation and Effector 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 pG27 and pG28a. 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+ -T cells from healthy donor (isolated and repurposed) were pre-stained in HypoLyse (2x) and pre-stained with GCN2i-490 or P85 alone and then replated. The stained cells were co-cultured with activated CD8+ T cells (B) Pretreatment w/ GCN2i-490 and C) P85 (1x) were measured by flow cytometry. Similar increases in GCN2i-490 alone cells were also observed (data not shown). Western blot analysis was conducted in those wells where HypoLyse condition and GCN2i treatment was a decrease in cell number.

6. GCN2i Inhibits CT26 Growth in Low Amino Acid Conditions

CT26 cells cultured with vehicle (DMSO), Asparagine (ASNase), or GCN2+ alone, or the combination of ASNase and GCN2i. Cell number was determined for three and six days. Asparagine depletion by ASNase or GCN2i lead to minimal effects on 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 (BD) of GCN2i-282 results in modulated inhibition of CT26 tumor growth compared to vehicle. Minimal effect on body weight observed (n=5). D) Reduction in growth of RENCA tumors is observed with twice daily dosing of GCN2i-382 (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 EIF2a 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 preclinical 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.