Abstract #1104

Targeting the Stress Response Kinase GCN2 to Restore Immunity in the Tumor Microenvironment

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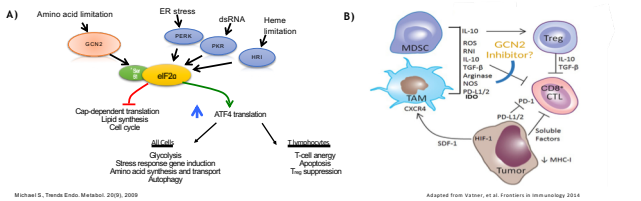
Abstract

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. Key suppressive cell types in the TME include tumor, stromal and myeloid-derived suppressor cells (MDSC) which create a nutrient-poor environment that supports tumor growth and limits immune surveillance. General control nonderepressible 2 (GCN2), a stress response kinase, plays a key role in sensing and modulating the cellular response to amino acid deprivation. GCN2 activation in T cells triggers the integrated stress response pathway and promotes T cell anergy and apoptosis. We have developed small molecule GCN2 inhibitors (GCN2i) that are highly potent and selective in vitro. Culturing primary mouse or human immune cells under low nutrient conditions activates the GCN2 pathway limiting T cell proliferation and function. Treatment of these nutrient-deprived T cells with GCN2i resulted in rescue of CD8⁺ T cell proliferation and effector functions. In addition, GCN2 inhibition in MDSC alone fully reversed CD33⁺MDSC-induced T cell suppression and effector functions. Our GCN2 inhibitors are orally bioavailable with drug like in vivo ADME properties. Our GCN2i is currently being evaluated in vivo, in murine syngeneic tumor models. Our results demonstrate that inhibition of GCN2 is an attractive approach for relieving T cell suppression and promoting effector function, demonstrating GCN2 as a promising therapeutic target for the treatment of cancer.

GCN2 is an integral part of the integrated stress response pathway



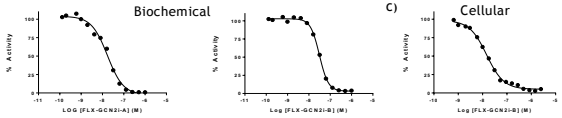
A) GCN2 is a stress response kinase detecting amino acid starvation. Activation of GCN2 leads to T cell anergy, apoptosis and enhanced T_{reg} suppression. B) MDSC/TAMs use multiple mechanisms such as amino-acid deprivation and oxidative stress to suppress CD8⁺ T effector cells. This may make GCN2 act as a critical convergence point downstream of tumor myeloid suppressor cells.

FLX-GCN2i potently reduces EIF2α phosphorylation

A)

ID	GCN2 Biochemical IC₅₀ (nM)	PERK Biochemical IC₅₀ (nM)	PKR Biochemical IC₅₀ (nM)	HRI Biochemical IC₅₀ (nM)	SKOV-3 Cellular (SF) IC₅₀ (nM)	SKOV-3 Cellular (25%HS) IC₅₀ (nM)	SKOV-3 Cellular Tox IC₅₀ (nM)	Bioavailability (Rodent)
FLX-GCN2i -A	27.5	>50000	390	440	16	608	>25000	22%
FLX-GCN2i -B	21.2	>50000	4400	>25000	24	1190	>25000	80%
Literature Compound*	47.0	84	3000	10	0.15	5500	>25000	48%

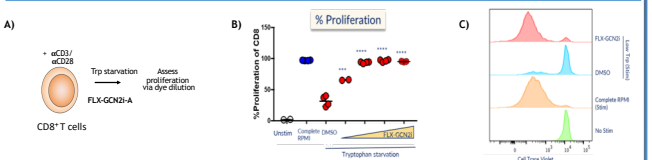
B) Biochemical



C) Cellular

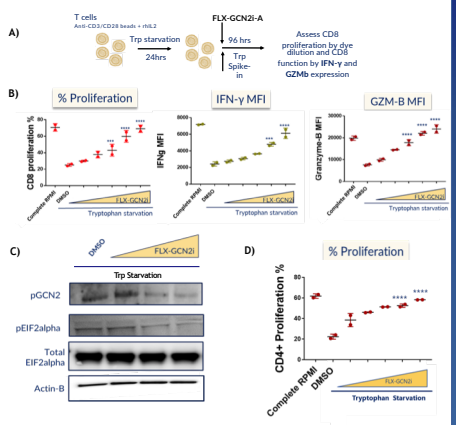
A) Potency and selectivity parameters for FLX-GCN2i-A, FLX-GCN2i-B and literature compound (* Represents Nakamura A., et al. Inhibition of GCN2 sensitizes ASNS-low cancer cells to asparaginase by disrupting the amino acid response. Proc. Natl Acad. Sci. USA. 2018;115:E7776–E7785). B) FLX-GCN2i-A and -B were preincubated with recombinant human GCN2 and pEIF2α substrate. Enzymatic activity was measured by TR-FRET and used to demonstrates dose-dependent inhibition of p-EIF2α C) SKOV-3 cells were preincubated with FLX-GCN2i-B for 1hr and enzymatic activity was measured by AlphaLisa.

FLX-GCN2i restores proliferation of mouse CD8⁺ T cells in amino acid starved conditions



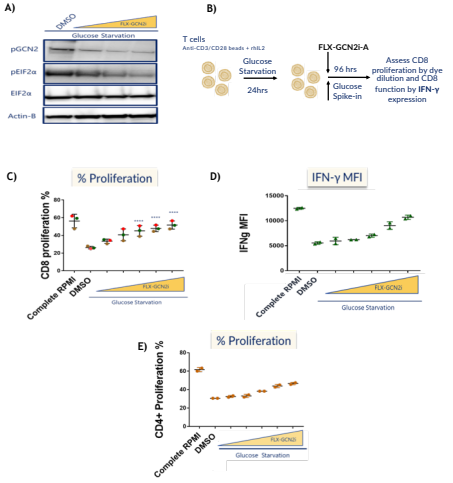
A) Activated mouse CD8⁺ T cells were starved under tryptophan limiting conditions. B) Assessed for proliferation via flow cytometry in the presence or absence of FLX-GCN2i-A. C) Representative proliferation of CD8⁺ T cell (offset histogram) of the quantified data (B)

FLX-GCN2i restores human T cell proliferation and function in tryptophan limited conditions



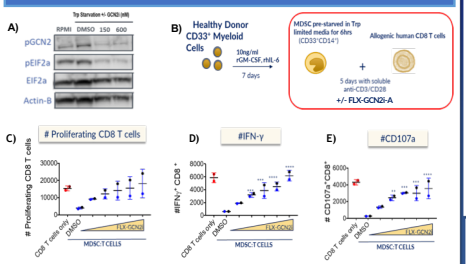
A) Activated human CD8⁺ T cells were starved for 24hrs in tryptophan-depleted media followed by the addition of FLX-GCN2i-A and scheduled spike-ins of low concentration of tryptophan for 96hrs.
B) CD8⁺ T cell proliferation was assessed by dye dilution and the effector functional markers were analyzed by flow cytometry.
C) Western blot analysis of whole cell lysates from activated T cells starved for 24hrs with the GCN2i showed decrease in pGCN2, pEIF2alpha when compared to actin
D) CD4⁺ T cell proliferation was assessed by dye dilution.

FLX-GCN2i restores human T cell proliferation and function in glucose-starved conditions



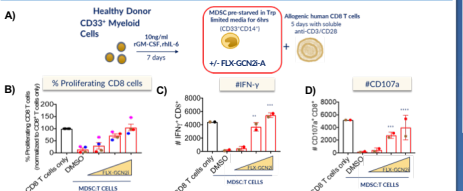
A) Western blot analysis of whole cell lysates from activated T cells starved for 24hrs with the FLX-GCN2i-A showed decrease in pGCN2, pEIF2alpha when compared to actin; B) Activated human CD8⁺ T cells were starved for 24hrs in glucose-depleted media followed by the addition of GCN2i and scheduled spike-ins of glucose for four days C) CD8⁺ T cell proliferation was assessed by dye dilution and D) effector functional marker IFN-γ was analyzed by flow cytometry. E) CD4⁺ T cell proliferation was assessed by dye dilution via flow cytometry

FLX-GCN2i reverses human MDSC suppressive function and increases effector functions of human CD8⁺ T cells



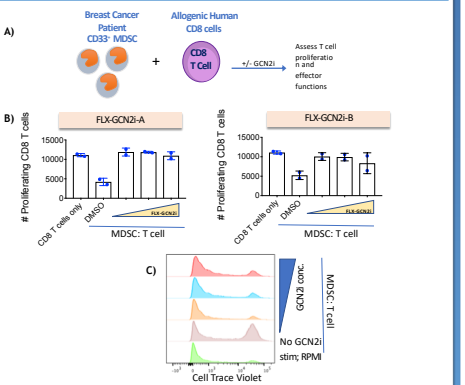
A) CD33⁺ myeloid cells isolated from healthy donors (n=2) were expanded in the presence of rGM-CSF and rIL-6 for 7 days. MDSC's were pre-starved in tryptophan-limited media and pretreated with FLX-GCN2i-A for 6hrs leading to reduction in pGCN2 and pEIF2α. B) Pre-starved MDSC's were co-cultured with activated CD8⁺ T cells and the FLX-GCN2i. C) Proliferation and D-E) effector functions were measured by flow cytometry.

Treatment of human CD33⁺ MDSC with FLX-GCN2i alone reverses their immuno-suppressive function



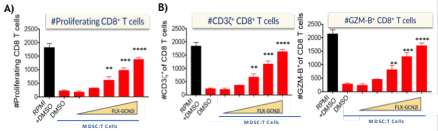
A) CD33⁺ myeloid cells from healthy donor (isolated and expanded as previously described) were pre-starved in tryptophan-limited media and pretreated with FLX-GCN2i-A for 6hrs and then washed. Pre-starved and pretreated MDSC were co-cultured in complete media with activated CD8⁺ T cells.
B) Proliferation (n=4) and C-D) effector functions (n=2) were measured by flow cytometry.

FLX-GCN2i reverses suppressive function of cancer patient-derived CD33⁺ MDSC



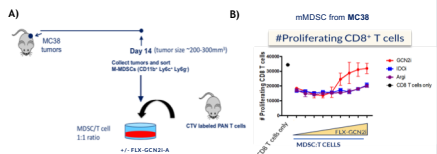
A) Schematic of the experimental design. B) CD33⁺ myeloid cells from breast cancer patient blood. MDSC were co-cultured in complete media with activated CD8⁺ T cells. Proliferation (n=1) was measured by flow cytometry. C) Representative proliferation of CD8⁺ T cell (offset histogram) of the quantified data (B)

FLX-GCN2i reverses suppressive function of mouse tumor-derived mMDSC and restores CD8⁺ T cell effector functions



A) mMDSC were isolated from CT26 tumors; co-cultured 1:1 with labelled T cells (isolated from naïve BALB/C mice) with DMSO or FLX-GCN2i-A. CD8⁺ T cell proliferation and effector functions were measured by flow cytometry at 72hrs.

Differentiation of FLX-GCN2i from IDOi and ARGi on tumor-derived mouse mMDSC



A) Schematic of the experimental design. B) FLX-GCN2i-A mediates complete reversal of CD8⁺ T cells following activation with anti-CD3/CD28 and cocultured in a ratio of 1:1 with tumor-derived mMDSC in the presence or absence of GCN2i/IDOi/Argi. Flow Cytometry was used to assess proliferation on CD8⁺T cells.

Results and Conclusions

- FLX-GCN2i inhibited phosphorylation of GCN2 and EIF2α in human CD8⁺ T cells and human MDSC cultured in amino-acid starved conditions
- Inhibition of GCN2 increased human and mouse CD8⁺ T cell proliferation and effector functions when cultured in amino-acid and glucose deprived conditions
- FLX-GCN2i reversed both human and mouse tumor-derived MDSC-mediated suppression and effector functions of CD8⁺ T cells
- FLX-GCN2i reverses mouse MDSC- mediated suppression more effectively than Argi and IDOi.
- Treatment of human CD33⁺ MDSC alone with FLX-GCN2i, reverses the suppressive function of MDSC on CD8⁺ T cells
- Thus our data collectively demonstrates that GCN2 is a promising therapeutic target for the treatment of cancer.

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Unless otherwise indicated, significance tests and p-values refer to test compared to vehicle control group. P values represented as follows: 0.05 > ** > 0.01 *** > 0.001 > **** > 0.0001