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

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Background

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 non-essential amino acid 2), a stress response kinase, plays a key role in sensing and modulating the response to amino acid deprivation. GCN2 activation in T cells leads to an induction of the integrated stress response pathway and subsequently to T cell anergy and apoptosis. Here, we demonstrate that the pharmacologic inhibition of GCN2 restores the T cell proliferation and effector function in amino-acid, glucose-deficient media and in MDSC-induced T cell suppression.

Mouse and human T cell viability, proliferation and function were assessed in vitro under amino-acid deprived conditions and in a co-culture with MDSCs. Pharmacodynamic markers including phospho-GCN2, phospho-EIF2alpha, and ATF4 were measured via western blot. Cell proliferation (CVT dye dilution) and effector markers (IFNγ and Granzyme B) were measured by flow cytometry. Our selective, sub-µM GCN2 inhibitor (GCN2i) was used to examine the role of GCN2 in T cell and MDSC function.

GCN2 is an integral part of the integrated stress response pathway

A) Amino acid limitation

B) GCN2 is a stress response kinase detecting amino acid starvation. Activation of GCN2 leads to T cell anergy, apoptosis and enhanced Treg 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 EIF2alpha phosphorylation

A) Biochemical

B) Cellular

A) Potency and selectivity parameters for FLX-GCN2i. B) FLX-GCN2i was preincubated with recombinant human GCN2 and pEIF2alpha substrate. Enzymatic activity was measured by TR-FRET and used to demonstrate dose-dependent inhibition of p-EIF2alpha. C) SKOV-3 cells were preincubated with FLX-GCN2i for 1hr and enzymatic activity was measured by AlphaLisa.

FLX-GCN2 inhibitor restores proliferation of mouse CD8+ T cell 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. C) Representative proliferation of CD8+ T cell (offset histogram) of the quantified data (B)

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

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

Results and Conclusions

- FLX Bio is developing potent and selective inhibitors of the stress response kinase GCN2 (GCN2i)
- FLX-GCN2i inhibited phosphorylation of GCN2 and EIF2alpha 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
- Treatment of human CD33+ MDSC alone with FLX-GCN2i, reverses the suppressive function of MDSC on CD8+ T cells
- Inhibition of GCN2 is an attractive approach for relieving immune mediated suppression and promotion of T effector activation