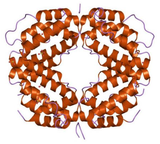
STAT4 Gene

- Gene within the family of STATs in which it is **transcription factor** associated with the JAK-STAT pathway as discussed above that is expressed within myeloid cells, the thymus, and testis
- Activated by Interleukin 12 through the JAK-STAT signal transduction
- Structure is beside the STAT1 gene locus and consists of six functional **domains**
  - **N-terminal interaction** domain imperative for dimerization of inactive STATs in addition to nuclear translocation
  - **Helical coiled coil domain**, which is associated with regulatory factors
  - A **central DNA-binding domain** regarding binding to the enhancer region of IFN-γ activated sequence (GAS) family genes
  - **Linker domain** that facilitates in the DNA binding procedure
  - **Src homology 2 (SH2)** domain that is significant for specific binding to the cytokine receptor following tyrosine phosphorylation
  - **C-terminal transactivation** domain that initiates the transcriptional procedure
- Critical in the promotion and activation of **natural killer cells**, analogous to cytotoxic T cells in the adaptive immune interface of vertebrates
- Imperative for the secretion and composition of interferon gamma, or IFN-γ, and the **differentiation** of helper T cells, otherwise referred to as Th1 cells from naïve Cd4+ cells
- STAT4 binds to numerous genomic loci, among others to the promoters of genes regarding cytokines, exemplified by **IFN-γ** and the Tumor Necrosis Factor (**TNF**) superfamily

Interferon-γ, TNF-α, Th1 Cells

Interferon Gamma | IFN-γ

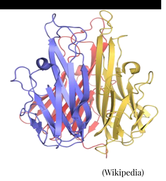


(Wikipedia)

- **Interferons** are signaling proteins composed and secreted by host cells in response to infection in which infected cells release these molecules to amplify anti-viral defenses
- Interferons are associated with **activation of immune cells**, promotion of antigen presentation, and similar ideals
- Interferon gamma is a type two interferon activated by IL-12
- Upregulated by STAT4 and associated with **antitumor immunity** through enhancing Th1 differentiation, cytotoxic T cell activity, and similar ideals that promoted apoptosis and necroptosis

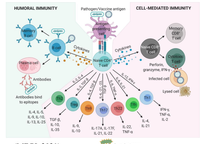
Tumor Necrosis Factor Alpha | TNF-α

- The **Tumor Necrosis Factor**, or TNF, family is a protein superfamily of type II transmembrane proteins that essentially function as a cytokine and are expressed in immune cells, associated with immune responses such as differentiation and apoptosis
- Promotion of TNF induces **leukocyte activation**, coagulation, **cytokine secretion**, and fever
- Stimulated and correlated to Interleukin-12
- Cell death response is expressed through apoptosis and necroptosis

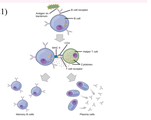


(Wikipedia)

Helper T Cells | Th1



(MDPI, 2021)



(Khan Academy, n.d.)

- Helper T cells are a type of T cell with a CD4 receptor that is associated with **humoral immunity** and **cell-mediated response**
- Differentiation and activations is promoted by STAT4 and therefore indirectly by IL-12
- Paramount in effective immune responses
- **Humoral immunity:** Refers to the secretion of antibodies to promote immune responses in which B cells emit the MHC II protein, inducing helper T cells to bind to the B cells and initiate proliferation into plasma or memory B cells
- **Cell-mediated immunity:** Not correlated to antibody secretion in which helper T cells differentiate into T cells that secrete IFN-γ or those that produce specified interleukins
  - Th1 cells activate macrophages as well

Related Research

Paper: Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation.

- This paper assesses the correlation of STAT4 on interferon gamma signaling within the JAK-STAT pathway. Interferon gamma regulation occurs on account of the tyrosine phosphorylation and activation of the DNA binding activity within the STAT1 and STAT2 proteins
- However, through polymerase chain reaction, STAT4 was morphologically determined to consist of 52% identical composition to STAT1. STAT4 is restricted to myeloid, helper T cells, and spermatogonia
- This validates the association of STAT4 on interferon gamma regulation, proposing its integration of therapeutic treatments that increase immune signaling within the intricate and engineered tumor microenvironment

Paper: IL-12 and IL-23 and the immunoregulatory roles of STAT4

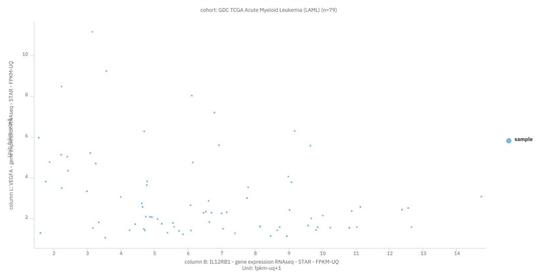
- This study assessed the immunoregulatory signaling initiated by interleukins 12 and 23
- These interleukins are cytokines correlated to both the innate and adaptive immune response; dimeric interleukins consist of a commonality subunit referred to as p40 and bind to resembling receptor chain: IL-12Rβ1
- These interleukins then activate Janus kinases TYK2 and JAK2, transcription factor signal transducer and activator of STAT4
- Furthermore, in addition to upregulation of STAT4, IL-12 has expressed increased differentiation of immature CD4+ T cells into helper T cells that secrete interferon gamma, aiding in further immune signaling in the tumor microenvironment of myeloid-associated cancers.

Paper: Intratumoral injection of IL-12-encoding mRNA targeted to CSFR1 and PD-L1 exerts potent anti-tumor effects without substantial systemic exposure

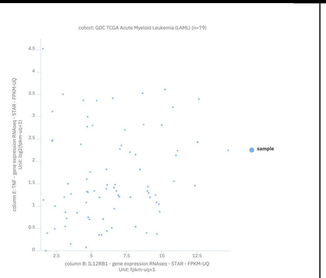
- Study that implemented IL-12 encoding mRNA within the mouse model
- Found that doses as low as 0.5 micrograms revealed potent antitumor effects and increased IFN-γ activity

Data Results

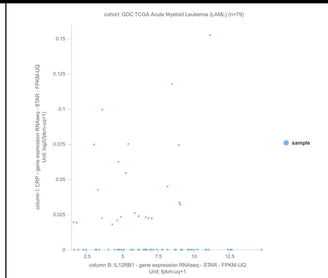
IL12Rβ1 & VEGFA



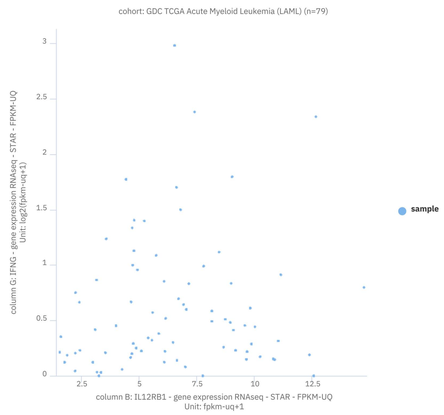
IL12Rβ1 & TNF



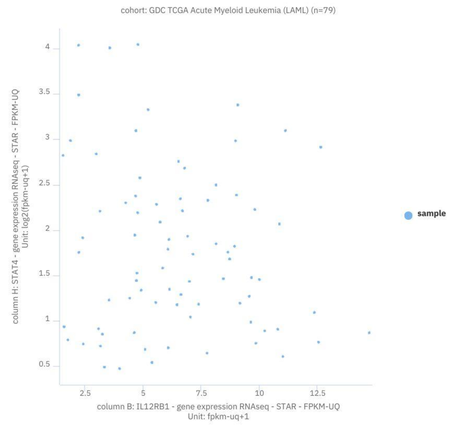
IL12Rβ1 & CRP



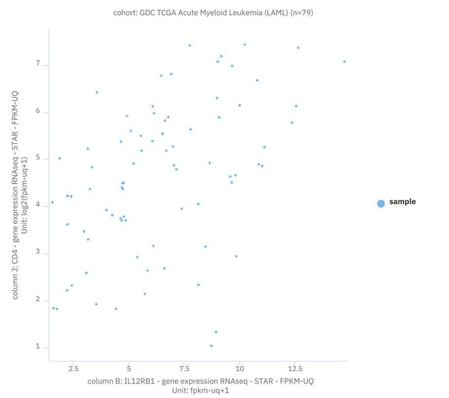
IL12Rβ1 & IFNG



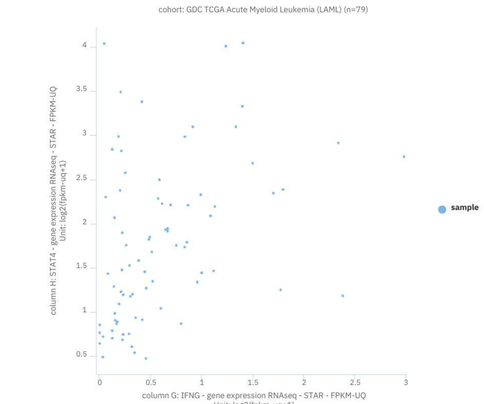
IL12Rβ1 & STAT4



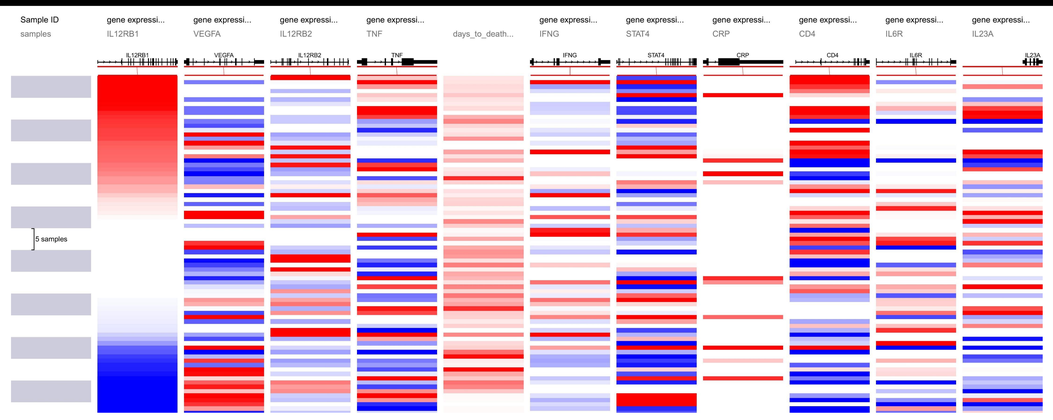
IL12Rβ1 & CD4



IFNG & STAT4



Holistic Biocomputational Heatmap of IL-12 Associated Mediation



Limitations

Complex Bio-interactions

- Regardless of the evidence of IL-12 tumor microenvironment modulation, numerous external factors associated with complex bio-interactions and pathways most likely limited the influence of this mediation
- The dual role of TNF alpha as a tumor suppressor and promoter may have undermined the tumor suppression by decreased VEGFA and rather promoted its response
- IL-12 inflammation seen by C-reactive protein may have been utilized by the tumor as a method of environmental modification and this inflammation may have ultimately decreased the influence of immune responses

In-Silico Plausibility

- In-silico studies often associate numerous variables that may induce confounding or skew the data
- Lacks true empirical evidence and therefore the results may often be accurate though not reveal critical concepts and insights of the experimental design and the study itself

Future Advancements

In-Vitro Validation

- In-silico analyses may be a potential source of error due to instances of **lurking or confounding variables** that may skew the results and data of the study
- In-vitro studies provide **empirical evidence** and allows for greater specialization in addition to the utilization of genomic methods to quantify and substantiate the study

CRISPR-Cas9 → STAT4 Isolation

- Initiation of the JAK-STAT Pathway may not only upregulate the STAT4 gene but promote proximal genes as well
- This may have been a potential inconsistency in the study as external genes may have been opposing or modulating the expression of STAT4, such as STAT3 whose stimulation is associated with cell proliferation and anti-apoptotic properties
- Utilizing genetic analysis technologies such as **CRISPSR-Cas9** can pinpoint STAT4 and mitigate external influences

Synergistic Interleukin Analysis

- Numerous studies have found **synergistic** upregulation of STAT4, IFN-γ, and similar immune responses
- Nakahira et al. finds that IL-12 and **IL-18** synergistically promote IFN-γ secretion and expression rather than IL-12 itself
- Studies such as that from Teng et al. reveal that IL-12 & IL-23 modulation of the immune interface is interconnected as they share a subunit, implying that there may be **collaborative interactions** between the two interleukins

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