IL-12 Induced Regulation of the JAK-STAT Pathway in the Tumor Microenvironment of Acute Myeloid Leukemia

Arjun Ganesan[1]

[1] *Centreville High School Class of 2027*

Abstract

Acute Myeloid Leukemia (AML) is a prevalent cancer worldwide, accounting for one-third of all leukemia diagnoses and only with a 5-year survival rate of 31. 9%. A significant factor within AML is the tumor microenvironment (TME), which engineers its surroundings to inhibit immune cells from accessing and suppressing the tumor. This is primarily the causative factor for the poor prognosis, symptoms, and treatment of cancer. Interleukin-12, an immune cytokine, is believed to promote the activity of IFN-γ and TNF-α, both of which associated with heightened immune responses and apoptosis. Though IL-12 is hypothesized to be an anticancer drug, tumor studies have not found a significant influence, a concept this study attempts to address. Upon binding, IL-12 activates the JAK-STAT pathway, which ultimately upregulates STAT4, a transcription factor further stimulating Th1, IFN-γ, and TNF-α activity. This study assesses the strength of association between IL-12 and the JAK-STAT pathway in the context of the AML tumor microenvironment. Utilizing the UCSC Xena in-silico modeling analysis software, IL-12 was found to decrease VEGFA, a growth factor associated with the TME adaptability. However, IL-12 contradictorily down-regulated STAT4 and therefore limited IFN-γ and TNF-α development, indicating that JAK-STAT signaling was compromised. This may be attributed to inflammation induced by IL-12 as its increase promoted CRP & IL-6 expression. Holistically, this study reveals the underlying biological mechanisms associated with IL-12 in AML, providing evidence that IL-12 can be a therapeutic treatment by decreasing inflammation and increasing JAK-STAT binding affinity, stagnating the tumor microenvironment and relieving the effects of AML.

***Keywords*:** Acute myeloid leukemia, interleukin-12, tumor microenvironment, STAT4, interferon-γ, tumor necrosis factor α, helper T cells, JAK-STAT pathway.

1 Introduction

Acute myeloid leukemia, or AML, is a prevalent cancer throughout the world. It is a subtype of leukemia, a hematologic cancer defined by the uncontrolled proliferation of leukocytes, also known as white blood cells. The primary site of this occurrence is within the myelogenous tissue of the bone marrow. In acute myeloid leukemia, a myeloblast, which is a precursor of myeloid leukocytes, experiences genetic alterations that inhibit maturation, promote proliferation, and suppress apoptotic functioning. This induces immense fatigue, shortness of breath, frequent bruising and bleeding, and increased susceptibility to infection. Acute myeloid leukemia accounts for 1/3 of all leukemia diagnoses with a 5-year survival rate of only 31.9%. Furthermore, in the past 30 years, there have not been many advances in the diagnosis and treatment of AML, and a major factor contributing to this is the tumor microenvironment.

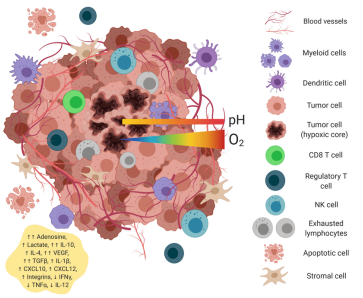

Figure 1: The Tumor Microenvironment (Wikipedia)

The **tumor microenvironment**, or TME, is composed of the tumor and the ecosystem surrounding it, consisting primarily of stromal tissue, cancerous cells, and the extracellular matrix. In particular, the extracellular matrix is instrumental in the tumor development due its presence of cancerous growth factors, exemplified by the vascular endothelial growth factor (VEGFA), that promote cancer growth and inhibit immune responses. This essentially allows the tumor to engineer its environment to inhibit im-

mune cells from accessing the primary site of the tumor and suppressing, promoting tumor development. Furthermore, this is often the causative factor for the clinical severity of AML and the difficulty of diagnosis and treatment as the disease progresses. A significant element in the human immune system is the interleukin family. Interleukins are a type of cytokine, which are immune regulators secreted by numerous leukocytes to promote immune responses in a specified region. Interleukin-12 specifically has shown promising immune responses to cancerous tumors. A heterodimeric protein, IL-12 is encoded by two genes: IL-12A at p35 and IL-12B at p40. IL-12A primarily consists of a bundle of four alpha helices and IL-12β consists of three beta sheets. Interleukin-12 is primarily secreted by dendritic cells, macrophages, neutrophils, T-helper cells, B cells, and similar antigen-presenting cells. Furthermore, interleukin-12 is associated with the stimulation of interferon gamma, tumor necrosis factor alpha, and similar immune responses. Interferon gamma promotes the chemokine referred to as the inducible protein P-10 that is enhances anti-angiogenic effects, yielding IL-12 as an anti-angiogenic interleukin. On account of this, IL-12 has been hypothesized to be a plausible anti-cancer, but numerous tumor studies have not found a significant influence. This study essentially attempts to assess the efficacy of IL-12 in acute myeloid leukemia tumors and the underlying biological mechanisms for which these previous studies haven't found a significant influence. When interleukin binds to its IL-12Rβ1 and IL-12Rβ2 receptors, it activates a signal transduction pathway referred to as the **JAK-STAT Pathway**. A signal transduction pathway is a biological method of communication, primarily for the purpose of upregulating a specific gene, through sequential phosphorylation of molecules. The JAK-STAT Pathway is composed of three primary components: Janus kinases (JAKs) which are a type of non-receptor tyrosine kinase family and signal transducer and activator of transcription proteins (STATs). JAKs consist of a FERM domain, an SH2-related domain, a kinase domain which is critical to functioning as it allows for phosphorylation, and a pseudokinase domain. Upon IL-12 binding, the IL-12Rβ2 experiences tyrosine phosphorylation to introduce binding regions for non-receptor tyrosine kinase TYK2 and tyrosine protein kinase JAK2. This induces the receptors to dimerize to decrease the proximity of the receptor-associated JAKs in which they then phosphorylate each other on tyrosine residues on regions referred to as the activation loop. This process is referred to as transphosphorylation, increasing the activity of the JAK kinase domains. Following this, the activated JAKs then phosphorylate the tyrosine residues on the receptor, exposing binding sites for proteins consisting of SH2 domains. Then, STATs bind to the phosphorylated tyrosines on the receptor with their SH2 domains, which are then tyrosine phosphorylated by JAKs, inducing STATs to dissociate from its rececptor. These activated STATs then construct heterodimers or homodimers, in which the SH2 domains of each STAT binds the phosphorylated tyrosine of the corresponding STAT, influencing dimer translocation towards the cell nucleus for transcription. As a method of introduction to the nucleus, STAT dimers surpass the nuclear pore complexes through an amino acid sequence, referred to as the nuclear localization signals, or NLS, in which it experiences binding to proteins referred to as importins and progresses into the nucleus. Once in the nucleus, a protein referred to as Ran, which is associated with GTP, binds to the importins, releasing them from the STAT dimer and allowing the STAT dimmer to undergo transcription. **STAT4** is a transcription factor upregulated by the JAK-STAT axis in which it primarily expressed in myeloid cells, the thymus, and testis. The STAT4 structure consists of six functional domains: an N-terminal interaction domain, helical coiled coil domain, a central DNA-binding domamin, a linker domain, a Src homology 2 (SH2) domain, and a C-terminal transactivation. The N-terminal interaction domain is imperative for the dimerization of inactive STATs in addition to nuclear translocation. The helical coiled coil domain is associated with regulatory facts. A central DNA-binding domain binds to the enhancer region of IFN-γ activated sequences (GAS) family genes. The Src homology 2 (SH2) domain is critical for specific binding to the cytokine receptor following tyrosine phosohorylation. The C-terminal transactivation domain initiates the transcription of the STAT gene. STAT4 itself is instrumental in immune responses through activating natural killer cells, which are analogous to cytotoxic T cells in the adpative immune system of vertebrates in addition to the further promotion of IFN-γ, TNF-α and T cell differentiation. IFN-γ is a prominent immune signaling molecule secreted by host and somatic cells in response to an infection or a pathogen that consist of anti-pathogenic molecules, preventing other healthy cells from being infected. IFN-γ specifically is a type two interferon activated by IL-12, and its promotion by STAT4 has been found to be associated with antitumor immunity. Tumor necrosis factor alpha, or TNF-α is a protein superfamily of a type II transmembrane protein in which they essentially function as a cytokine and promote apoptosis and necroptosis.

Helper T cells are a type of T cell in the adapative immune system that consist of a CD4 protein. They are critical in the immune system due to two primary processes: humoral immunity and cell-mediated responses. In humoral immunity, B cells emit a major histocompatibility complex II (MHC II) protein, inducing helper T cells to bind to and activate B cells through initiating proliferation into plasma or memory B cells. In cell-mediated immunity, helper T cells differentiate into T cells that secrete IFN-γ or those that secrete specified interleukins, in addition to activating macrophages.

2 Materials & Procedures

In this study, the following research question was assessed: what is the influence of increasing IL-12 concentrations on the immune responses in the tumor microenvironment? The independent variable in this study is the concentration of IL-12 in acute myeloid leukemia cancer cell lines, and the dependent variable is the correlating immune responses. The subsequent **hypothesis** was that: if IL-12 is implemented in myeloid cancer cell lines, its binding affinity to its receptor in myeloid cells will increase to amplify the expression of the JAK-STAT signaling transduction pathway and up-regulate the STAT4 gene, increasing interferon gamma responses and tumor suppression in its microenvironment and promoting immune responses as a therapeutic target for myeloid cancers. In this study, increasing IL-12 concentrations were mapped to six primary diagnostics: VEGFA, which is a prominent cancerous growth factor in the extracellular matrix, TNF, IFNG, STAT4, CD4, and CRP, which is a measure of inflammation. In order to undergo this experiment, the **UCSC Xena** tumor modeling software was used to assess tumor development with increasing IL-12 levels and cross-correlating immune variables to assess the causative immune response.

3 Data Results

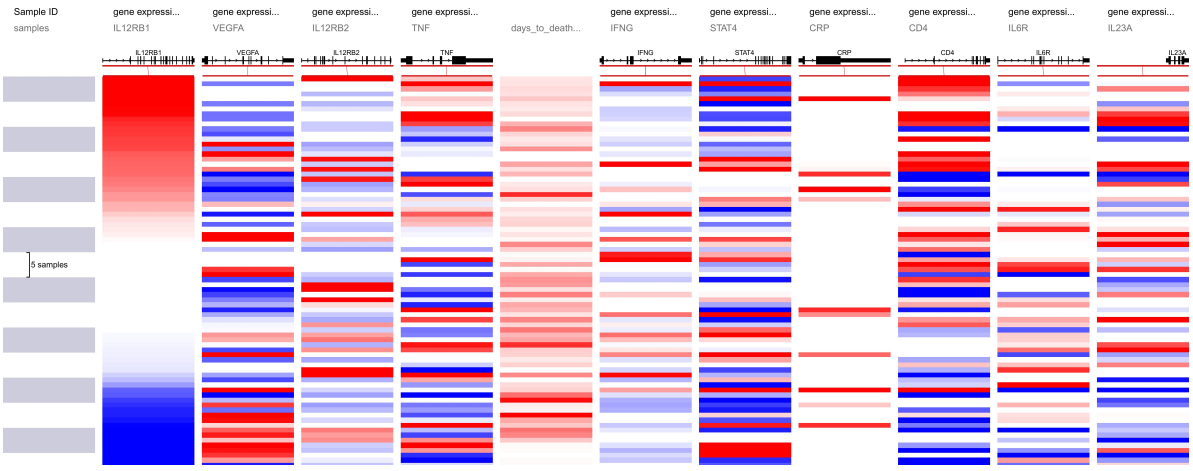

Figure 2: Holistic Biocomputational Heatmap

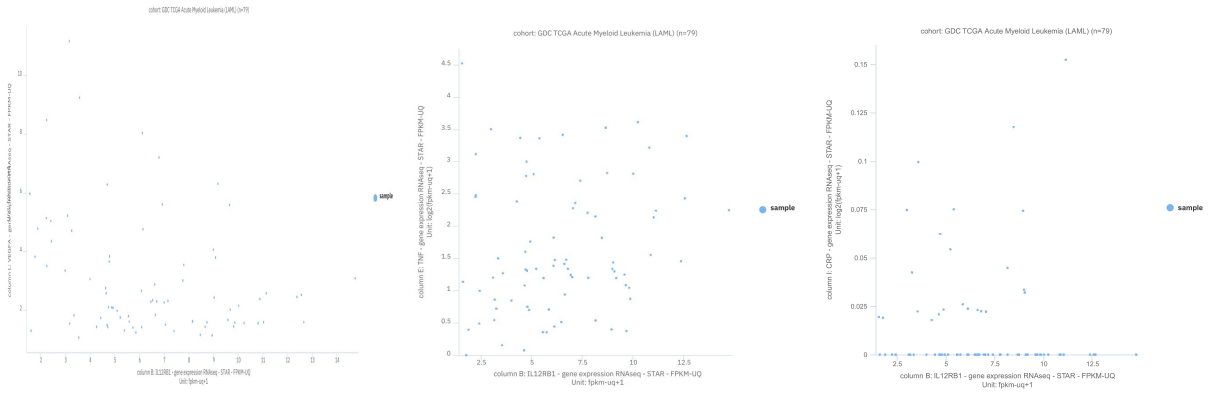

Figure 3: IL-12 vs VEGFA, TNF, & CRP, respectively

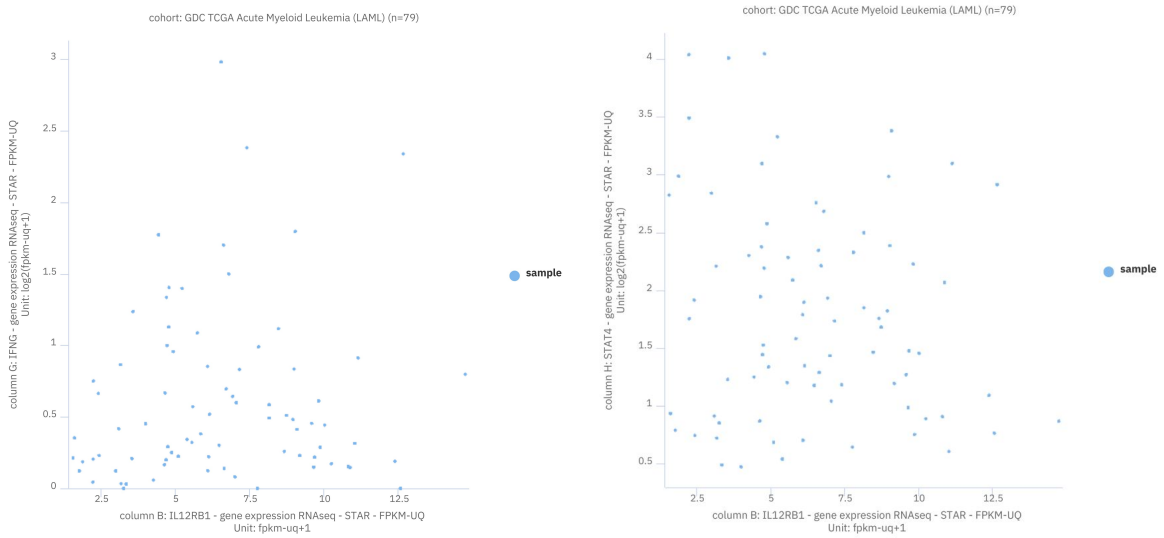

Figure 4: IL-12 vs IFNG & STAT4, respectively

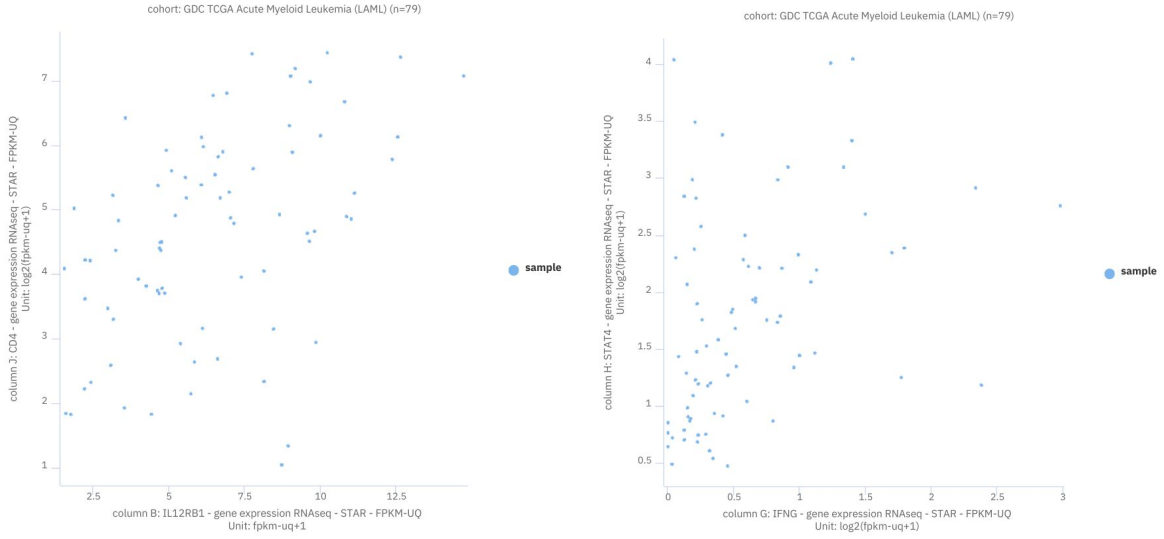

Figure 5: IL-12 vs CD4 & IFNG vs STAT4, respectively

4 Assessment & Interpretation of Data

As observed, when IL-12 was mapped to VEGFA, VEGFA expression decreased. This indicates that IL-12 is able to regulate and suppress the expression of cancerous growth factors in the tumor microenvironment of acute myeloid leukemia, stagnating it and thereby lengthening the five year survival rate. Furthermore, this implies that IL-12 can be a plausible therapeutic treatment to acute myeloid leukemia. However, when comparing IL-12 to IFN-γ and TNF-α, the distribution is relatively random, and this can be attributed to the influence of IL-12 in STAT4. When IL-12 was mapped to STAT4 as observed in the second image of Figure 4, the increase in IL-12 concentrations coincidentally decreased STAT4, which is somewhat counterintuitive as IL-12 should bind to its receptor, initiate the JAK-STAT pathway, and upregulate STAT4. This ambiguity is most likely due to two primary reasons: IL-12 upregulated suppressive genes around STAT4, exemplified by STAT3, which inhibited the expression of STAT4, and increasing IL-12 concentration induced inflammation to skyrocket, as observed in the third image in Figure 3. As inflammation is the hallmark of cancer, this most likely suppressed all the promoting immune responses driven by IL-12. These results were further corroborated with statistical analysis. The two statistical methods utilized were Pearson's rho and Spearman's rank rho correlation. Both of these methods utilized data to determine the ρ, or rho, which is the correlation between two variables. Due to data variation, both Pearson's and Spearman's rank correlations were used. Pearson's correlation is a statistical measure of the strength of a linear relationship between two variables. Conversely, Spearman's rank rho applies this same concept though it is nonparametric as it is more versatile, specializing in determining nonlinear relationships and therefore yielding more practicality as it is more suitable for original data that do not consist of a Normal distribution. Thus, both methods were utilized to account for both non-linear and linear relationships between the tests and procedures. In this statistical test, the null hypothesis H_0, was that there is no linear or nonlinear relationship between the two variables, with respect to the two given tests. Both tests were performed at the 0.05 significance level. When IL-12 was compared to VEGFA, both Pearson's and Spearman's rank correlation indicated that the negative association between IL-12 and VEGFA was statistically significant, validating IL-12 suppression in TME of acute myeloid leukemia. However, in IL-12 comparative tests between TNF, IFNG, and STAT4, no correlation was statistically determinant. This can be attributed to inflammation, as stated earlier. Further CRP analysis, however, indicates there was no statistical significance between increasing IL-12 levels in both Pearson's and Spearman's Rank correlation. Conversely, IL-12 proved to have a positive, statistically significant correlation with IL-23, another inflammatory gene. As CRP is known to vary significantly based on patient identity regarding their age, sex, ethnicity, and lifestyle, IL-23 may be a more viable diagnostic to measure inflammation as it is more biologically stable. Thus, this lack of statistical significance between IL-12 and TNF-α, IFN-γ, and STAT4, can be attributed to this increase in inflammation. Additionally, when IL-12 was compared to against CD4, a strong positive correlation was found, indicating that IL-12 is able to directly interact with t-helper cell differentiation to promote active immunity despite the lack of STAT4 amplification.

Table 1: IL-12 Mapping & Linear Statistical Analysis

Test	**Pearson's** ρ	$p - value$	H_0
IL-12 vs VEGFA	$\rho = -0.3133$	$p = 0.004955$	Reject H_0
IL-12 vs TNF	$\rho = 0.1620$	$p = 0.1539$	Fail to Reject H_0
IL-12 vs CRP	$\rho = 0.009467$	$p = 0.9340$	Fail to Reject H_0
IL-12 vs IFNG	$\rho = 0.1064$	$p = 0.3509$	Fail to Reject H_0
IL-12 vs STAT4	$\rho = -0.1439$	$p = 0.2059$	Fail Reject H_0
IL-12 vs CD4	$\rho = 0.4626$	$p = 0.00001799$	Reject H_0
IL-12 vs IL-23	$\rho = 0.2304$	$p = 0.04108$	Reject H_0
IFNG vs STAT4	$\rho = 0.3846$	$p = 0.0004701$	Reject H_0

Table 2: IL-12 Mapping & Non-linear Statistical Analysis

Test	**Spearman's Rank** ρ	$p - value$	H_0
IL-12 vs VEGFA	$\rho = -0.2508$	$p = 0.02580$	Reject H_0
IL-12 vs TNF	$\rho = 0.1940$	$p = 0.08678$	Fail to Reject H_0
IL-12 vs CRP	$\rho = -0.09697$	$p = 0.3953$	Fail to Reject H_0
IL-12 vs IFNG	$\rho = 0.1245$	$p = 0.2745$	Fail to Reject H_0 h
IL-12 vs STAT4	$\rho = -0.08625$	$p = 0.4498$	Fail to Reject H_0
IL-12 vs CD4	$\rho = 0.4905$	$p = 0.000004553$	Reject H_0
IL-12 vs IL-23	$\rho = 0.2309$	$p = 0.04068$	Reject H_0
IFNG vs STAT4	$\rho = 0.4348$	$p = 0.00006286$	Reject H_0

5 Conclusion

Through data analysis, four main conclusions were able to be drawn: 1. IL-12 is capable of decreasing the expression of cancerous growth factors, particularly VEGFA, in patients with AML, stagnating the tumor microenvironment, and decreasing clinical severity; 2. IL-12 is capable of eliciting immune responses in itself, exemplified by the promotion of helper T cell differentiation; 3. IL-12-induced JAK-STAT signaling is compromised in acute myeloid leukemia, which explains the main reason for which previous IL-12 studies have not observed any antitumor mediation; 4. Inflammation driven by IL-12 may have overcome its associated immune responses, further limiting anticancer activity. The limitations of this study may have been the restriction of the data set, as the composition of various data sets can account for the variables overlooked or substantiate the data. Similarly, the comparison of IL-12 to only seven genes may have introduced high specificity, thereby neglecting to account for other variables explanatory for the results. Future directions include in vitro validation to ensure that these interactions are occurring and further quantify the study. In addition, numerous studies have determined a synergistic interleukin influence with IL-12 and IL-18 or IL-23. Thus, conducting a dual interleukin analysis may pose higher yielding results in addition to higher potency with regard to anticancer immunity. Ultimately, this study elucidates a significant defect in the JAK-STAT pathway that occurs in acute myeloid leukemia that, if reregulated, can promote IL-12-induced anticancer immunity with several additional implications.

6 References

References

[Academy,] Academy, K. The immune system review (article).

[Brooks and Putoczki, 2020] Brooks, A. J. and Putoczki, T. (2020). Jak-stat signalling pathway in cancer. *Cancers*, 12:1971.

[Contributors, 2019] Contributors, W. (2019). Tumor microenvironment.

[Contributors, 2022] Contributors, W. (2022). Interferon-alpha/beta receptor.

[Duenas and Jupe, 2017] Duenas, C. and Jupe, S. (2017). Reactome — interleukin-12 signaling.

[Fenton et al., 2021] Fenton, S. E., Saleiro, D., and Platanias, L. C. (2021). Type i and ii interferons in the anti-tumor immune response. *Cancers*, 13:1037.

[Goldman et al., 2020] Goldman, M. J., Craft, B., Hastie, M., Repečka, K., McDade, F., Kamath, A., Banerjee, A., Luo, Y., Rogers, D., Brooks, A. N., Zhu, J., and Haussler, D. (2020). Visualizing and interpreting cancer genomics data via the xena platform. *Nature Biotechnology*, 38:675–678.

[Groner and von Manstein, 2017] Groner, B. and von Manstein, V. (2017). Jak stat signaling and cancer: Opportunities, benefits and side effects of targeted inhibition.

[Ikeda et al., 2002] Ikeda, H., Old, L. J., and Schreiber, R. D. (2002). The roles of ifn in protection against tumor development and cancer immunoediting.

[Institute, 2018] Institute, N. C. (2018). Acute myeloid leukemia - cancer stat facts.

[MDPI, 2021] MDPI (2021). Figure 1. humoral and cell-mediated adaptive immune responses. adaptive...

[Nakahira et al., 2002] Nakahira, M., Ahn, H.-J., Park, W.-R., Gao, P., Tomura, M., Park, C.-S., Hamaoka, T., Ohta, T., Kurimoto, M., and Fujiwara, H. (2002). Synergy of il-12 and il-18 for ifn-

gene expression: Il-12-induced stat4 contributes to ifn- promoter activation by up-regulating the binding activity of il-18-induced activator protein 1. *The Journal of Immunology*, 168:1146–1153.

[of California Santa Cruz, 2024] of California Santa Cruz, U. (2024). Welcome to the help pages for ucsc xena — user help pages.

[Society, 2024] Society, A. C. (2024). Key statistics for acute myeloid leukemia (aml).

[Teng et al., 2015] Teng, M. W. L., Bowman, E. P., McElwee, J. J., Smyth, M. J., Casanova, J.-L., Cooper, A. M., and Cua, D. J. (2015). Il-12 and il-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nature Medicine*, 21:719–729.

[Thomas et al., 2015] Thomas, S. J., Snowden, J. A., Zeidler, M. P., and Danson, S. J. (2015). The role of jak/stat signalling in the pathogenesis, prognosis and treatment of solid tumours. *British Journal of Cancer*, 113:365–371.

[University, 2024] University, G. (2024). G-doc.

[Vaddi, 2017] Vaddi, K. (2017). The role of the jak/stat signalling pathway in immunoregulation of gastrointestinal cancers. *Springer eBooks*, pages 147–186.

[Watford et al., 2004] Watford, W. T., Hissong, B. D., Bream, J. H., Kanno, Y., Muul, L., and O'Shea, J. J. (2004). Signaling by il12 and il23 and the immunoregulatory roles of stat4. *Immunological Reviews*, 202:139–156.

[Watford et al., 2003] Watford, W. T., Moriguchi, M., Morinobu, A., and O'Shea, J. J. (2003). The biology of il-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Reviews*, 14:361–368.

[Xiong et al., 2008] Xiong, H., Zhang, Z.-G., Tian, X.-Q., Sun, D.-F., Liang, Q.-C., Zhang, Y.-J., Lu, R., Chen, Y.-X., and Fang, J.-Y. (2008). Inhibition of jak1, 2/stat3 signaling induces apoptosis, cell cycle arrest, and reduces tumor cell invasion in colorectal cancer cells. *Neoplasia*, 10:287–297.

[Xue et al., 2023] Xue, C., Yao, Q., Gu, X., Shi, Q., Yuan, X., Chu, Q., Bao, Z., Lu, J., and Li, L. (2023). Evolving cognition of the jak-stat signaling pathway: autoimmune disorders and cancer. *Signal Transduction and Targeted Therapy*, 8.

[Yamamoto et al., 1994] Yamamoto, K., Quelle, F. W., Thierfelder, W. E., Kreider, B. L., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., Silvennoinen, O., and Ihle, J. N. (1994). Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation. *Molecular and Cellular Biology*, 14:4342–4349.

[Zheng et al., 2016] Zheng, H., Ban, Y., Wei, F., and Ma, X. (2016). Regulation of interleukin-12 production in antigen-presenting cells. *Advances in Experimental Medicine and Biology*, 941:117–138.