Decoding the Effects of Gain-of-Function Mutations in *STAT1* and *STAT3*: A Literature Review on Immune Dysregulation and Treatment Strategies

Abstract

Inborn errors of immunity (IEIs) are genetic conditions that disrupt the immune system, leading to susceptibility to infections, autoimmunity, inflammation, and other immune dysregulations. Under normal circumstances, the JAK-STAT pathway transduces signals from the cytokine receptors to the nucleus, where it regulates immune cell responses. Gain-of-function (GOF) mutations in the *STAT1* and *STAT3* genes are examples of IEIs that disrupt the JAK-STAT signaling pathway. *STAT1* GOF mutations often result in chronic mucocutaneous candidiasis (CMC), recurrent infections, and autoimmune diseases, while *STAT3* GOF mutations are associated with severe bacterial infections, systemic autoimmunity, and multi-organ effects, such as diabetes and enteropathy. Both *STAT* syndromes show significant heterogeneity in clinical manifestations and responses to treatments. This heterogeneity impacts the diagnosis and treatment strategies for *STAT1* or *STAT3* GOF diseases. Current therapies, such as JAK inhibitors, are not effective in all patients. Some *STAT3* GOF patients benefit from combination therapies, while *STAT1* GOF patients often respond to JAK inhibitors alone. Novel research strategies, such as patient-derived stem cells are being used to better understand the disease and improve treatment strategies. Further research is needed to investigate the molecular mechanisms underlying *STAT* GOF mutations and develop patient-specific therapeutic strategies. Insights from these *STAT* GOF syndromes may also help in understanding and treating other immune-related diseases such as cancer and autoimmune disorders.

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Layman Summary

The immune system is a complex system that protects humans from infections with viruses, bacteria, and other pathogens. However, for some people, the immune system does not work properly due to mutations known as "inborn errors of immunity" (IEIs). These mutations can cause proteins in immune cells to function abnormally, resulting in dysfunctions of the immune system that cause more frequent infections, autoimmune diseases, inflammation, or even cancer. One key part of the immune system is the JAK-STAT signaling pathway. This pathway transmits signals, such as cytokines, from outside of the immune cells to the inside. This signal transmission allows immune cells to divide, secrete cytokines, or activate other defense mechanisms to fight infections. Two important proteins in the JAK-STAT pathway, STAT1 and STAT3, activate genes that help regulate the immune response. When mutations are present in one of these genes, these proteins no longer function properly. In this review, we focus on the so-called "gain-of-function" (GOF) mutations. These GOF mutations lead to a constitutively activated STAT1 or STAT3 protein that disrupts the immune response.

People with *STAT1* GOF mutations often experience fungal infections, such as chronic mucocutaneous candidiasis (CMC), and autoimmune diseases that affect organs such as the lungs, liver, and salivary glands. On the other hand, people with *STAT3* GOF mutations often experience autoimmune diseases, severe bacterial infections, and complications involving multiple organs, including diabetes and intestinal issues. It is difficult to diagnose and treat patients with *STAT1* and *STAT3* GOF syndromes because they show a wide range of symptoms that can vary from person to person. Patients with one of these mutations are treated with drugs known as JAK inhibitors. These inhibitors block the JAK-STAT signaling and can slow down the overactive immune response. However, not all patients respond to these therapies, and some patients need combination treatments with other drugs that inhibit the effects of the *STAT* GOF mutation through different mechanisms. Researchers are now investigating other innovative techniques, such as using patient-derived stem cells, to better understand the effects of these mutations.

Despite the progress made in investigating these *STAT* GOF syndromes, many unresolved questions remain. For example, researchers are still trying to understand why the same mutation can cause varying symptoms in different patients. Additionally, they are working to develop patient-specific therapies based on the unique mutation and clinical manifestations of the patient themselves. Studying these rare syndromes not only helps patients with *STAT1* and *STAT3* GOF mutations, but also provides insights into other immune-related diseases, such as cancer and autoimmune disorders. By increasing our understanding of the JAK-STAT pathway, scientists aim to improve treatment strategies and achieve better outcomes for patients.

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Introduction

Patients with an inborn error of immunity (IEI) are more susceptible to infections, allergies, autoimmunity, autoinflammation, and malignancies (Mudde & Booth, 2023). Genetic variants that disrupt JAK-STAT signaling are found in an increasing number of IEIs (Tangye et al., 2020). Proteins that are affected often have critical functions in the development, function, and maintenance of immune system cells. Dysfunction of these proteins leads to aberrant immunity. Loss-of-function mutations often cause immune deficiencies, such as severe combined immunodeficiency (SCID), which is caused by mutations in *JAK3*, while gain-of-function (GOF) mutations lead to hyperactive immune pathways, driving autoimmune diseases (Maródi et al., 2012; Ott et al., 2023; Tangye et al., 2020). IEIs were initially considered to be rare diseases, affecting only 1 in 10,000 to 1 in 50,000 births. However, more and more IEIs are being discovered, and clinical phenotypes are better defined, indicating that there is a higher prevalence of IEIs. As more IEIs are described, this prevalence is now estimated to be at least 1 in 1,000 to 1 in 5,000 (Tangye et al., 2020; Zhang et al., 2017).

The JAK-STAT pathway is an evolutionarily conserved signal transduction pathway in which target genes are transcribed as a result of interaction between a specific cytokine and the target cell (Figure 1). The JAK family of proteins consists of four receptor-associated tyrosine kinases, the JAK1, JAK2, JAK3, and TYK2 kinases (Philips et al., 2022). Cytokines bind to their corresponding receptors and activate the signaling cascade. JAKs are activated after interaction with a cytokine and undergo autophosphorylation. Phosphorylated JAK proteins are then able to recruit proteins of the STAT family: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Phosphorylated STAT proteins dimerize and translocate to the nucleus, where they function as transcription factors that regulate target genes involved in biological processes such as the regulation of immune responses (Samra et al., 2024).

The STAT proteins consist of six protein domains: the N-terminal domain, coiled-coil domain (CCD), DNA-binding domain (DBD), linker domain, Src homology 2 (SH2) domain, and the transactivation domain. The N-terminal domain and the CCD facilitate the dimerization of two STAT proteins. Additionally, the DBD is essential for STAT proteins to interact with DNA and to function as transcription factors while the SH2 domain recruits STAT proteins to phosphorylated JAK proteins after activation by cytokines (Lei et al., 2024; Leiding et al., 2023; Samra et al., 2024; Staab et al., 2020). Recurrent GOF mutations in STAT1 and STAT3 are present in all the functional domains of the protein but are mostly found in the DBD and the CCD (Hijikata et al., 2017; Lei et al., 2024; Leiding et al., 2023; L. Liu et al., 2011; Maffucci et al., 2016; Russell et al., 2018; Staab et al., 2020; Zimmerman et al., 2019).

With this review, we aim to provide a comprehensive overview of the molecular mechanisms, the clinical manifestations, and the current treatment strategies for *STAT1* and *STAT3* GOF syndromes. Studies of patients with IEIs in *STAT1* and *STAT3* have provided insights into the immune system and the regulation of a controlled immune response. Patients with *STAT1* or *STAT3* GOF syndromes are characterized by a range of clinical manifestations, such as autoimmunity and high susceptibility to infections. By addressing existing knowledge gaps in these *STAT* GOF syndromes, we highlight the need for personalized therapeutic approaches and further research to understand the molecular mechanisms of disease in different *STAT* GOF variants. Furthermore, we highlight the importance of larger patient cohorts and innovative techniques for disease modeling, such as patient-derived stem cells, to improve

research in the field of IEIs. Finally, we provide suggestions for future research to bridge the knowledge gaps and improve outcomes for patients with *STAT1* and *STAT3* GOF syndromes.

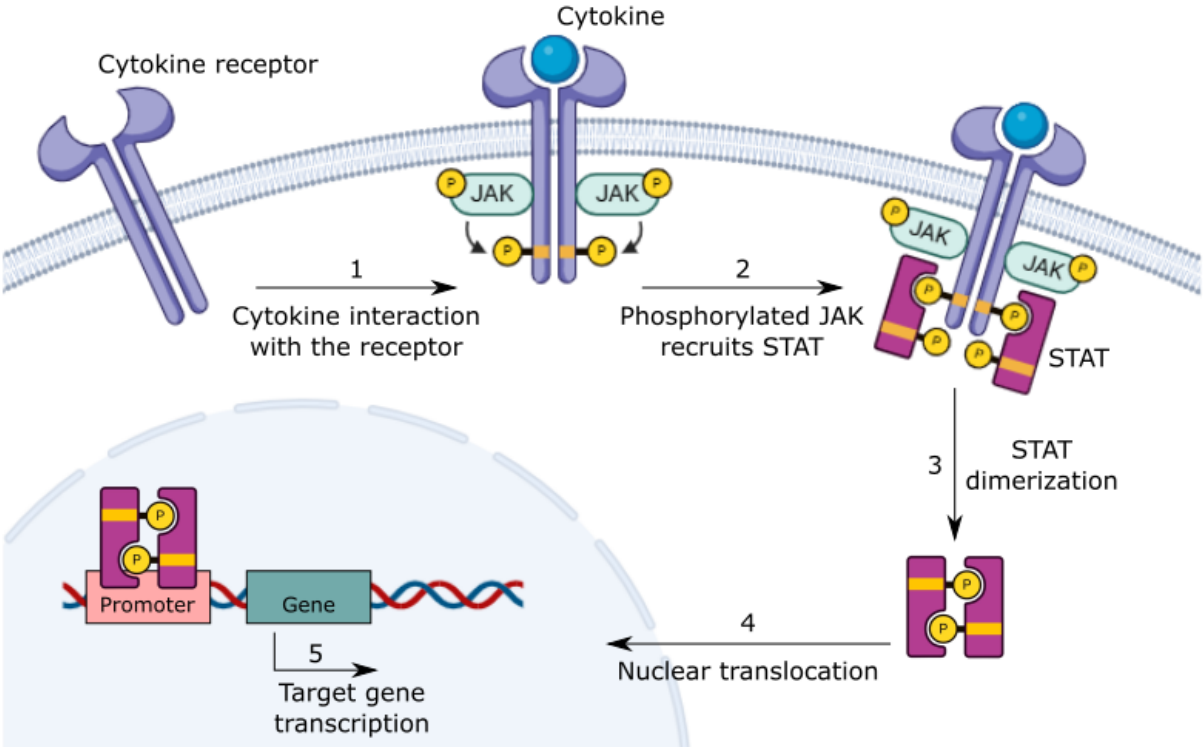

Figure 1: Overview of the JAK-STAT Pathway. (1) The cytokine binds to its receptor, recruiting JAK proteins that phosphorylate themselves. **(2)** Phosphorylated JAK proteins recruit STAT proteins, which are then phosphorylated. **(3)** Phosphorylated STAT proteins dimerize with one another. **(4)** The dimerized STAT proteins translocate into the nucleus where they **(5)** act as transcription factors to activate the transcription of target genes. The image was created using BioRender.

Mechanisms of Immune Dysregulation Due to *STAT1* and *STAT3* GOF Mutations

STAT1 and *STAT3* mutations are found in all functional domains of the genes and are mostly located in the DBD and CCD (Figure 2) (Hijikata et al., 2017; Lei et al., 2024; Leiding et al., 2023; L. Liu et al., 2011; Maffucci et al., 2016; Russell et al., 2018; Staab et al., 2020; Zimmerman et al., 2019). Over the past few years, several novel mutations in the *STAT1* and the *STAT3* genes have been identified (Weinacht et al., 2017; Zimmerman et al., 2019). These mutations are mostly missense mutations that create GOF proteins, enhance phosphorylation and activation, and increase STAT-mediated transcriptional activity. Nevertheless, mutations in different domains have varied impacts on STAT signaling, resulting in a heterogeneous response (Jägle et al., 2020).

Phosphorylation and Transcriptional Regulation Influenced by STAT1 and STAT3 Mutations

Mutations in the *STAT* genes affect key functions of the protein, including tyrosine (Tyr701) phosphorylation and transcriptional regulation. These mutations often lead to elevated levels of phosphorylated STAT1 or STAT3 and result in increased target gene transcription (Jägle et al., 2020; Russell et al., 2018; Staab et al., 2020). Even though elevated phosphorylated STAT levels are observed, the precise mechanisms of phosphorylation dynamics remain debated. Previous research has shown that nuclear dephosphorylation of phosphorylated STAT proteins is impaired when *STAT* is mutated. Mutated gene residues located in the CCD and DBD were originally thought to disrupt anti-parallel dimer formation, thereby preventing phosphatases from reaching the Tyr701 residue and, as a result, impeding protein dephosphorylation. (Fujiki et al., 2017; Hijikata et al., 2017; L. Liu et al., 2011).

Although researchers initially believed that mutations in the CCD and DBD impaired dephosphorylation, recent studies (Meesilpavikkai et al., 2017; Weinacht et al., 2017) suggest that mutations in the SH2 or the linker domain do not lead to this outcome. Additionally, previous research on *STAT* mutations in the CCD and DBD reported STAT1 GOF variants with normal dephosphorylation dynamics compared to control conditions. Some *STAT1* GOF patients even displayed enhanced dephosphorylation rates compared to control conditions (Bernasconi et al., 2018; Jägle et al., 2020; Largent et al., 2023; Meesilpavikkai et al., 2017; Weinacht et al., 2017; Zimmerman et al., 2019). It is hypothesized that these *STAT* GOF mutations enhance autotranscription, increasing STAT protein levels, leading to higher concentrations of phosphorylated STAT, rather than causing a defect in dephosphorylation (Largent et al., 2023; Zimmerman et al., 2019). This hypothesis suggests that increased STAT protein abundance, as well as delayed dephosphorylation contribute to the overall increase in STAT transcriptional activity (Bernasconi et al., 2018).

To determine the differential effects of *STAT3* mutations on the transcriptional activity and target gene transcription, Jägle et al. (2020) categorized mutations spread across the *STAT3* gene into subgroups based on their STAT3 reporter activity. While all mutant groups were associated with increased STAT3 target gene expression, varying DNA-binding patterns resulted in differential gene transcription, reflecting the heterogeneity of the disease (Jägle et al., 2020). Additionally, Liu et al. (2011) suggest that a GOF mutation in *STAT1* may not necessarily increase the transcription of all target genes and could even repress some genes (L. Liu et al., 2011). Mutations in the CCD or DBD may have variable functional impacts and

potentially reduce the specificity for the canonical GAS consensus, a short DNA sequence found in the promoter regions of target genes (Varinou et al., 2003). Each mutation can also upregulate a different subset of genes, thereby affecting distinct sets of target genes (Giovannozzi et al., 2021). The complexity and heterogeneity among patients with *STAT* GOF mutations highlights the importance of analyzing the transcriptional activity in these patients.

STAT1 and STAT3 Mutations Drive T-cell Dysregulation

Alterations in STAT phosphorylation and transcriptional activity not only influence the regulation of target gene expression but also have downstream effects on immune cell differentiation. Patients with a mutation in *STAT1* often have a diminished proportion of T-helper (T_h) 17 cells compared to healthy individuals and show decreased IL-17A- and IL-22-producing T cells, even after stimulation with various cytokines. Consequently, IL-17A, IL-17F, and IL-22 cytokine levels are consistently low (Liu et al., 2011). Enhanced STAT1 phosphorylation upregulates expression of the transcription factor T-bet in T follicular helper cells and drives their differentiation into T_h1 cells, reducing the differentiation into T_h17 cells (Largent et al., 2023). The increased T_h1 differentiation may be stimulated by the elevated expression of SOCS3, a gene that inhibits STAT3 signaling, which is crucial for T_h17 differentiation. As a result, the T_h1 responses dominate with increased recruitment of T_h1 cells due to the upregulation of chemokines such as CXCL9 and CXCL10 (Fujiki et al., 2017; Koh et al., 2024; L. Liu et al., 2011).

Patients with *STAT3* GOF mutations also display disturbed T-cell differentiation, but they exhibit a more diverse outcome compared to *STAT1* GOF patients (Schmitt et al., 2022). Studies in mice and patient samples with a *STAT3*^{G421R} GOF mutation revealed defects in FOXP3+ regulatory T cells, together with an elevated IFN-γ production (Flanagan et al., 2014; Haapaniemi et al., 2015; Milner et al., 2015). *STAT3* GOF T cells showed a reduced ability to generate induced regulatory T cells both in vitro and in vivo. Despite the role of STAT3 in promoting T_h17 differentiation, T_h1 cell skewing was observed in the spleen of *Stat3* GOF mice, evidenced by an increase in IFN-γ-producing CD4+ T cells, rather than T_h17 cells. Patient samples with a *STAT3* GOF mutation exhibited enhanced levels of CD8+ effector memory cells and a transcriptomic profile in regulatory T cells, consistent with the findings from the mouse model (Schmitt et al., 2022). Interestingly, recent research by Toth et al. (2024) provided evidence for elevated levels of T_h17 cells in the same mouse model with a *Stat3* GOF mutation and in vitro cultures (Schmitt et al., 2022; Toth et al., 2024). These mice developed skin inflammation characterized by enhanced levels of local T_h17 cells and IL-17A- and IL-22-producing CD4+ T cells in the skin and the lymph nodes. These results suggest organ-specific immune dysregulation. Together, these findings highlight the complex and organ-specific disease that promotes immune dysregulation driven by *STAT3* GOF mutations (Schmitt et al., 2022; Toth et al., 2024).

B-cell Function Is Disrupted in STAT1 and STAT3 GOF Syndromes

T cells, as well as B cells display an increased response to IFN-γ stimulation. This response led to an enhanced STAT1 expression and phosphorylation, driving apoptosis and thereby reducing B-cell counts (Giardino et al., 2016; Largent et al., 2023; Romberg et al., 2013; Toubiana et al., 2016). Specifically, switched and unswitched B cells, memory B cells, and IgG2 or IgG4 levels are diminished. STAT1 hyperactivation promotes expansion of $CXCR3^+IgD^+IgM^+$ B cells that exhibit low CXCR5 expression in patients with *STAT1* GOF mutations. These findings indicate that B-cell activation predominantly occurs via the extrafollicular route, and

that this activation results in the production of short-lived plasma cells that secrete large amounts of antibodies. Elevated levels of IgM and IgD autoantibodies are detected in patient samples, supporting the hypothesis that dysregulated B-cell activation contributes to autoimmunity (Giardino et al., 2016; Largent et al., 2023). These findings highlight how excessive STAT1 signaling alters B-cell differentiation and antibody production, and how this STAT1 signaling links to autoimmune effects and susceptibility to infections.

Approximately half of the patients with a *STAT3* GOF mutation display hypogammaglobulinemia and reduced memory B-cell levels (Erdős et al., 2021; Leiding et al., 2023; Milner et al., 2015b). Although normal B-cell counts were observed in some cases, these patients often exhibit antibody-mediated autoimmunity, suggesting that B-cell tolerance is disturbed. Studies in EBV-transformed B-cell lines reveal that STAT3 target genes such as SOCS3 and BCL3, are upregulated upon IL-6 stimulation, and that these target genes may contribute to the autoimmune effects in these patients (Jägle et al., 2020; Milner et al., 2015b).

STAT1 and STAT3 GOF Syndromes Also Affect NK Cells and Innate Immunity

In addition to T- and B-cell abnormalities, *STAT1* and *STAT3* GOF mutations also affect innate immunity. Downregulation of NK-cell function and NK-cell cytotoxicity was observed in a patient with a *STAT1*^{T385M} GOF mutation (Kayaoglu et al., 2021). These effects were hypothesized to be the consequences of decreased STAT5 signaling, as reported in multiple patients (Tabellini et al., 2017; Vargas-Hernández et al., 2018). It is likely that *STAT1* GOF mutations result in the upregulation of SOCS1-mediated suppression of STAT5, leading to dysregulated STAT5 activity (Kayaoglu et al., 2021; Tabellini et al., 2017). In addition, studies report that *STAT3* GOF mutations also affect dendritic cell and monocyte activity, as well as NK-cell counts (Ott et al., 2023; Schmitt et al., 2022; Toth et al., 2024). These findings highlight the broad impacts of *STAT1* and *STAT3* mutations on both the adaptive and the innate immune systems.

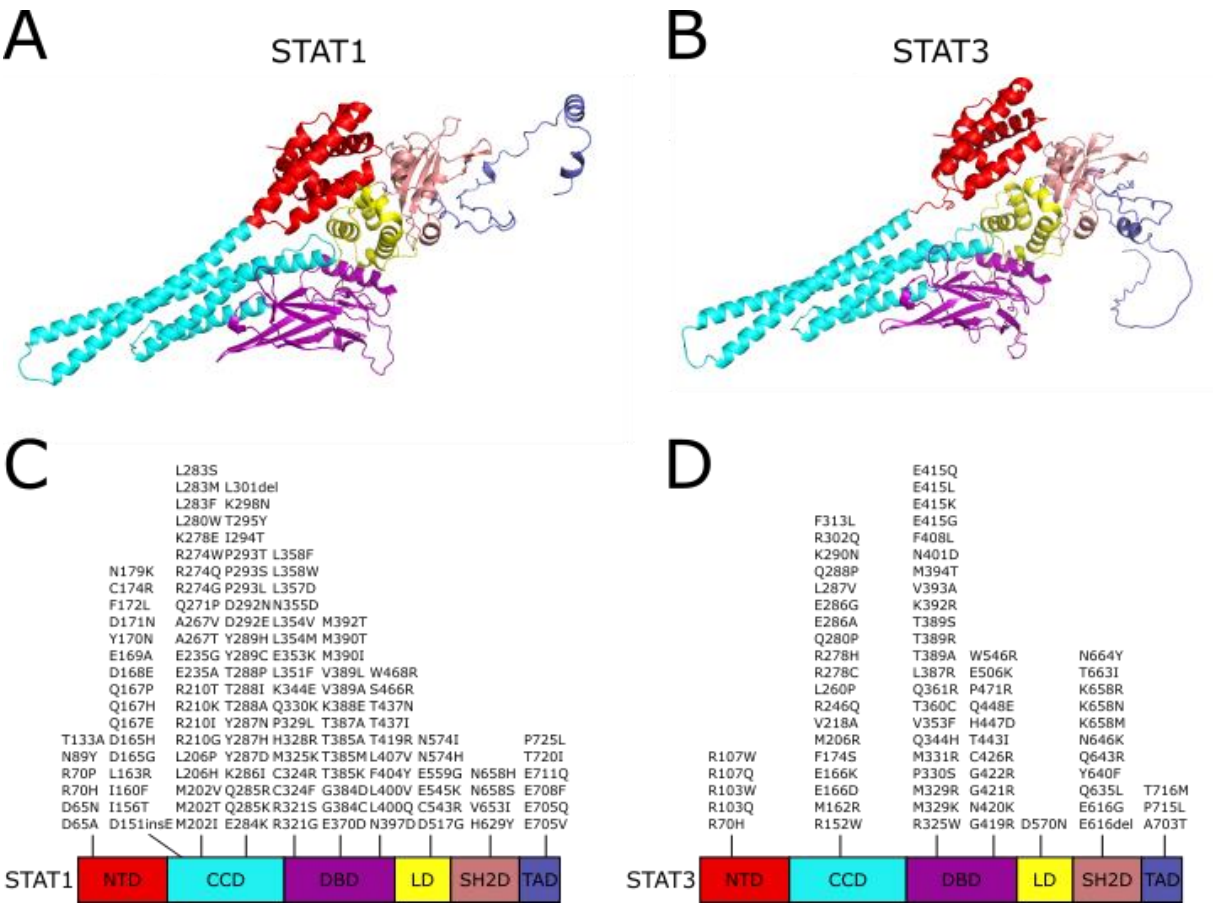

Figure 2: STAT1 and STAT3 Crystal Structure and GOF Mutations. (A-B) Predicted crystal structures of the STAT1 protein **(A)**, and the STAT3 protein **(B)** created via PyMOL and provided by AlphaFold. **(C-D)** Schematic representations of the STAT1 **(C)** and STAT3 **(D)** genes, showing six domains: the N-terminal domain (NTD) (red), the coiled-coil domain (CCD) (cyan), the DNA-binding domain (DBD) (purple), the linker-domain (LD) (yellow), the Src homology 2 domain (SH2D) (salmon), and the transactivation domain (TAD) (blue). Previously identified GOF mutations are shown above the genes (Lei et al., 2024; Leiding et al., 2023; Staab et al., 2020).

Clinical Manifestations of *STAT1* and *STAT3* Gain-of-Function Syndromes

Patients with STAT1 GOF Mutations Are More Susceptible to Infections

The JAK-STAT signal transduction pathway plays a critical role in the host defenses against bacterial and viral infections (Bernasconi et al., 2018). Mutations in the gene encoding STAT1 can disrupt this pathway, leading to impaired immune responses. Patients with *STAT1* GOF mutations often suffer from fungal infections, such as histoplasmosis and cryptococcosis, as well as mycobacterial diseases, tuberculosis, and recurrent respiratory tract bacterial infections (Bernasconi et al., 2018; Koh et al., 2024; Meesilpavikkai et al., 2017). Notably, a patient with the $STAT1^{T387A}$ GOF mutation suffered from eyelid infections, caused by *Staphylococcus* (Giardino et al., 2016; Maródi et al., 2012; Staab et al., 2020). This infection was a rare clinical manifestation, highlighting the complexity and heterogeneity of *STAT1* GOF disease, where different mutations can result in variable symptoms. Furthermore, cutaneous viral and herpetic infections are commonly observed, suggesting an altered immune response to diverse pathogens in patients with *STAT1* GOF syndrome (Giardino et al., 2016; Meesilpavikkai et al., 2017; Toubiana et al., 2016).

The most frequently observed clinical manifestation in patients with a *STAT1* GOF mutation is chronic mucocutaneous candidiasis (CMC) (Bernasconi et al., 2018; Giardino et al., 2016; L. Liu et al., 2011; Meesilpavikkai et al., 2017; Staab et al., 2020; van de Veerdonk et al., 2011). This fungal disease, caused by *Candida albicans* (Puel et al., 2010), can cause recurrent or persistent infections in the skin, esophagus, oral cavity, or genital mucosa. CMC is often observed in patients with a profound T-cell deficiency (L. Liu et al., 2011; Puel et al., 2010). Research by Puel et al. (2011) provided evidence that inborn errors of IL-17F or IL-17RA, which impair cytokine signaling, make patients more susceptible to *C. albicans*, indicating that IL-17F and IL-17A cytokine signaling is required for protective immunity to *C. albicans* (Puel et al., 2011). In *STAT1* GOF patients, low levels of IL-17- and IL-22-producing T cells, as well as T_h17 cells are observed, directly linking these low T-cell levels to CMC disease (Largent et al., 2023; L. Liu et al., 2011). Additionally, Largent and colleagues hypothesize that IFN-γ signaling induces defects in the skin barrier of patients with excessive STAT1 signaling, creating a favorable tissue niche for *C. albicans* and thereby contributing to the development of CMC disease (Break et al., 2021; Largent et al., 2023).

STAT1 GOF Mutations Drive Autoimmune Disease

STAT1 GOF mutations are also associated with autoimmune and auto-inflammatory disorders (Kayaoglu et al., 2021; Koh et al., 2024; Largent et al., 2023; Toubiana et al., 2016; Weinacht et al., 2017). In a cohort of 60 patients, over one-third exhibited autoimmune manifestations with variable severity, reflecting the heterogeneity of *STAT1* GOF disease. (Toubiana et al., 2016; Uzel et al., 2013). The upregulation of interferon-stimulated genes (ISGs), as well as increased antigen processing and dysregulated T- and B-cell functions, might contribute to the autoimmune manifestations in *STAT1* GOF patients (Kaleviste et al., 2019; Kayaoglu et al., 2021; Smyth et al., 2018; Weinacht et al., 2017); however, the exact mechanism is still unclear. Largent and colleagues suggest that IFN-γ signaling drives autoimmunity, supported by their mouse model experiments which showed increased CXCR3+ T follicular helper cells and CXCR3+ IgD+ IgM+ B cells. These cells promote extrafollicular B-cell activation and might cause STAT1 driven auto-immunity (Largent et al., 2023).

As a result, widespread inflammatory lesions in organs such as the lungs, liver, pancreas, and salivary glands are observed in *Stat1* GOF mice. These lesions are often composed of dense accumulations of plasma cells and neutrophils, creating an autoimmune response in these specific organs (Largent et al., 2023). Other clinical manifestations caused by the autoimmune effects are hypothyroidism, diabetes, autoimmune hepatitis, skin and gastrointestinal diseases, and autoimmune cytopenias (Koh et al., 2024; Toubiana et al., 2016).

Other Clinical Manifestations in STAT1 GOF Syndrome

Along with the more common clinical manifestations such as CMC and autoimmunity, a range of less common manifestations has been observed in some, but not all patients with *STAT1* GOF syndrome (Figure 3). These manifestations include growth hormone deficiency which may be a consequence of dysregulated STAT1 in endocrine signaling (Uzel et al., 2013). Cerebral aneurysms and tumors are reported in approximately 6% of patients (Okada et al., 2020; Toubiana et al., 2016). Additionally, researchers reported signs of vasculitis in a few patients (Okada et al., 2020). Together, these less common clinical manifestations indicate that *STAT1* GOF mutations can cause broad systemic dysregulation and highlight the variability in symptoms between patients.

STAT3 GOF Mutations Increase Susceptibility to Infections

Mutations in *STAT3* can also disrupt JAK-STAT pathway signaling, leading to an altered immune response. Patients with a *STAT3* GOF mutation exhibit a variety of clinical manifestations with most patients having lymphadenopathy, autoimmune disorders, growth failure, and susceptibility to infections (Fabre et al., 2019; Forbes et al., 2018; Leiding et al., 2023). In a study by Leiding et al. (2023), 72% of patients with a *STAT3* GOF mutation are more susceptible to infections. Most commonly, these were bacterial infections, followed by viral, fungal, opportunistic, and mycobacterial infections. *STAT3* GOF patients with hypogammaglobulinemia (e.g. low levels of IgG antibodies) were more susceptible to bacterial infections, since these IgG antibodies are crucial for neutralizing bacteria. On the other hand, patients with T-cell lymphopenia were at higher risk of viral and fungal infections due to the low numbers of T cells, which normally play a critical role in the immune response against these pathogens (Fabre et al., 2019; Leiding et al., 2023).

STAT3 GOF Mutations Drive Autoimmunity

Alternatively, patients with a *STAT3* GOF mutation often suffer from early-onset autoimmunity (Flanagan et al., 2014b), where multiple organs are affected, causing clinical manifestations such as diabetes, vasculitis, enteropathy, hypothyroidism, renal disease, growth hormone deficiency, and other systemic autoimmune manifestations (Flanagan et al., 2014b; Leiding et al., 2023; Toth et al., 2024; Zhou et al., 2024). Previous studies proposed a model in which $CD8^+$ T-cell dysregulation and an increase in T_h17 cells lead to an autoimmune attack on the pancreatic β-cells of a patient with a *STAT3* GOF mutation. The autoimmune attack leads to the destruction of these insulin-producing β-cells and causes diabetes in the patient (Toth et al., 2024; Zhou et al., 2024). A previously reported *STAT3* GOF mutant in the DBD was identified in a patient displaying clinical manifestations such as autoimmune cytopenia, lung disease, and lymphoproliferation (Milner,et al., 2015b). Later, research on this same *STAT3* GOF mutation in another patient showed that this patient had diverse clinical manifestations (Tanita et al., 2021), suggesting that this *STAT3* GOF disease exhibits clinical and

immunological heterogeneity. A molecular diagnosis, as well as long-term follow-up, is critical for these patients (Zhou et al., 2024).

STAT3 GOF and STAT1 GOF Mutations Display Overlapping Clinical Manifestations

In conclusion, both *STAT1* and *STAT3* GOF mutations are a cause of immune dysregulation. *STAT1* GOF mutations are associated with variable clinical phenotypes, including susceptibility to bacterial, fungal, and viral infections, CMC, and organ-specific autoimmunity (Toubiana et al., 2016; Vargas-Hernández et al., 2018). On the other hand, *STAT3* GOF mutations mainly cause lymphoproliferation, and multi-organ autoimmunity, including cytopenias, enteropathy, hypothyroidism, diabetes, and hepatitis (Figure 3) (Flanagan et al., 2014b; Milner et al., 2015b). While these *STAT1* and *STAT3* GOF syndromes share overlapping clinical manifestations, patients with these conditions also exhibit differences in their clinical manifestations. Furthermore, the severity of the disease varies significantly, even among patients with identical mutations in the same gene, suggesting that other genetic or environmental factors might contribute to disease progression (Fabre et al., 2019; Jägle et al., 2020).

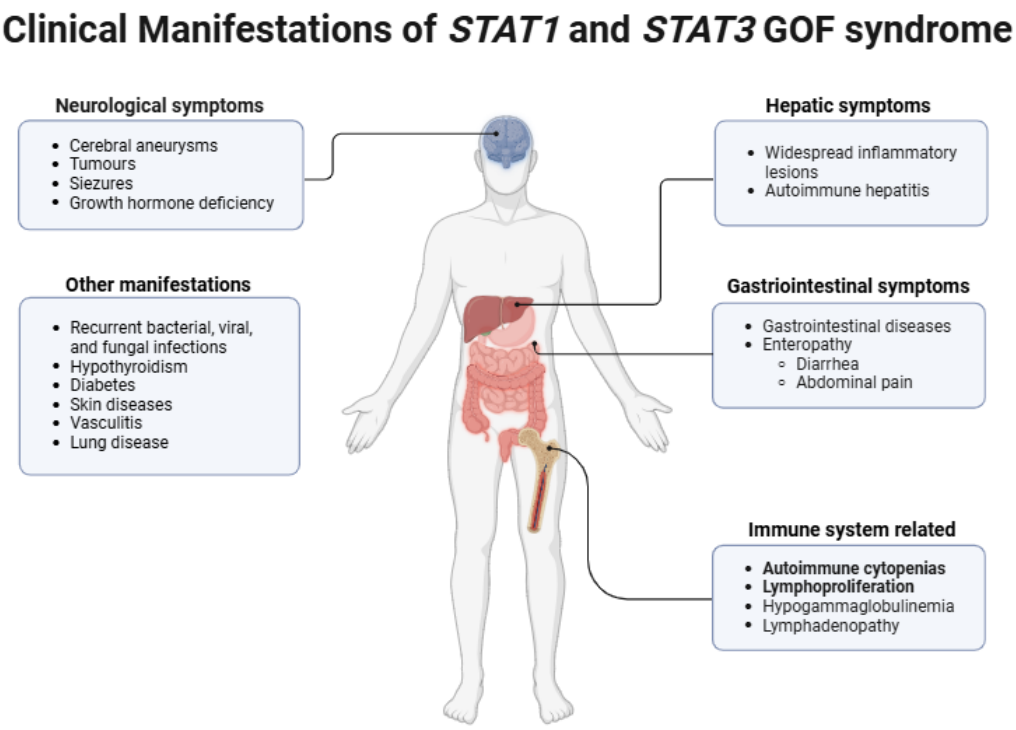

Figure 3: Clinical Manifestations of Patients with *STAT1* and *STAT3* GOF Syndromes. This figure displays the most common clinical manifestations of patients with *STAT1* and *STAT3* GOF syndromes. The image was created using BioRender.

Current and Emerging Therapeutic Approaches

JAK Inhibitors Can Downregulate Hyperactivated STAT Signaling

In patients with *STAT* GOF mutations, the JAK-STAT pathway becomes hyperactivated, which leads to increased STAT protein levels and excessive JAK-STAT signaling. To downregulate this hyperactivated STAT signaling, JAK inhibitors such as tofacitinib, baricitinib, and ruxolitinib, are prescribed (Forbes et al., 2018; X. Liu et al., 2024; Okada et al., 2020; Ott et al., 2023). These inhibitors target cytokine-induced JAK activation of JAK1/3 or JAK1/2 receptors. Previous research has shown clinical improvements after treatment with the JAK inhibitor tofacitinib in patients with a *STAT3* mutation (Forbes et al., 2018). However, research by Weinacht et al. (2017) revealed that suppression of STAT1 and STAT3 phosphorylation using tofacitinib in control and patient T cells was non-selective, which made this inhibitor less suitable for treating STAT1 hyperactivation (Weinacht et al., 2017).

Ruxolitinib, on the other hand, was more successful in reducing clinical manifestations in patients with either the *STAT1* or *STAT3* GOF syndrome. Long-term treatment with ruxolitinib led to the suppression of STAT hyperphosphorylation, reversed T_h1 and T follicular helper cell development, and enabled effective control of CMC and autoimmunity in several patients (Forbes et al., 2018; Koh et al., 2024; Weinacht et al., 2017). However, the effects on T_h17 responses and the associated cytokines, such as IL-17 were inconsistent between patients (Kayaoglu et al., 2021). While some patients showed improved T_h17 deficiencies (Weinacht et al., 2017), others experienced persistent T_h17 cell deficiency (Zimmerman et al., 2017). Additionally, ruxolitinib did not significantly ameliorate either the impaired neutrophil function or the increased interferon-stimulated gene expression (Kaleviste et al., 2019; Koh et al., 2024; Parackova et al., 2023).

Even though several patients were responsive to ruxolitinib treatment, other patients failed to respond (Forbes et al., 2018; Zimmerman et al., 2017). In particular, *STAT1* GOF patients exhibited active chromatin enriched in areas associated with ISGs, which were not altered upon ruxolitinib treatment. This allowed ISG expression to increase again after termination of the treatment (Kaleviste et al., 2019; Koh et al., 2024). Kaleviste and colleagues suggested that prolonged binding of *STAT1* proteins to the DNA may recruit chromatin-remodeling enzymes, enhancing active chromatin formation at these ISGs. This mechanism might explain why some patients fail to respond to the ruxolitinib treatment.

Patients with a *STAT1* or *STAT3* mutation that responded to ruxolitinib treatment suffered from adverse effects, including elevated transaminase and bilirubin levels, transient thrombocytopenia, concurrent infections with different viruses, and *Herpes zoster*. Researchers observed a trend of increased viral infections upon JAK inhibitor treatments, therefore, it is necessary to regularly monitor patients on JAK inhibitor therapy for invasive viral infections. Additionally, patients can be prescribed acyclovir prophylaxis, an antiviral medication, to reduce the incidence of *herpes zoster (Forbes et al., 2018)*.

IL-6 Antagonists Induce Remission when Combined with Ruxolitinib

Despite the limitations, ruxolitinib treatment provided clinical benefits for patients with *STAT* GOF mutations. To optimize therapeutic outcomes, a combination therapy of a JAK inhibitor together with an IL-6 antagonist was suggested for patients with *STAT3* GOF disease (Forbes et al., 2018). Tocilizumab, a humanized anti-IL-6 receptor antibody, blocks IL-6 signaling, which

normally activates the STAT3 pathway. Treatment with this IL-6 antagonist increased regulatory T-cell levels and improved clinical manifestations in patients (Forbes et al., 2018; Khoury et al., 2017; Milner et al., 2015). However, patients treated with tocilizumab alone were unable to achieve complete disease control, highlighting the need for the addition of another therapy. The combination therapy of tocilizumab together with ruxolitinib improves features of immune dysregulation and induces remission, indicating that this therapy may be an effective treatment strategy for *STAT3* GOF disease (Forbes et al., 2018).

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) is performed on patients with a *STAT1* or *STAT3* GOF mutation as a curative treatment (Kayaoglu et al., 2021; Largent et al., 2023; Leiding et al., 2023; Okada et al., 2020). However, the survival rate post-transplantation is only 40% due to complications such as graft-versus-host disease and infections. Therefore, HSCT as a treatment option reserved for patients at risk of severe disease progression (Kayaoglu et al., 2021; Okada et al., 2020). Research by Kayaoglu et al. (2021) suggests the use of ruxolitinib as a bridging therapy in *STAT1* GOF patients to reduce the risk of adverse outcomes following HSCT. They showed that T_h17 deficiency, as well as the dysregulated gene expression associated with the *STAT1*^{T385M} GOF mutation, were normalized when patients were treated with ruxolitinib before HSCT. Even though this combination therapy appears to be a promising treatment strategy to battle *STAT1* and *STAT3* GOF disease, this bridging therapy has been tested in only one case, suggesting that further research is needed to validate its efficacy (Kayaoglu et al., 2021).

Repairing GOF Mutations with Gene Editing

While HSCT appears to be a promising treatment to cure patients with *STAT* GOF disease, this therapy comes with significant risks, as described in the previous paragraph. An alternative therapeutic approach, which is particularly relevant for primary immunodeficiencies that are monogenic diseases, is gene editing (Orf, 2024). Clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein-9 (Cas9) is a gene editing tool that is used to either disrupt a mutated gene to create haploinsufficiency or repair a specific gene of interest (Ran et al., 2013). The CRISPR/Cas9 system consists of a single guide RNA (sgRNA) and a DNA endonuclease Cas9 protein. The sgRNA identifies the sequence of interest, binds complementarily to this sequence, and guides the Cas9 to the target sequence. After the target sequence is identified, the Cas9 endonuclease creates site-specific double-strand breaks. Following the double-strand break, the cell repair machinery repairs the DNA break through either non-homologous end joining or homologous recombination (Jensen, 2024; Orf, 2024; Ran et al., 2013).

Researchers are able to modify the gene of interest by transfecting target cells with Cas9, an sgRNA, and a repair template using electroporation (Orf, 2024). CRISPR/Cas9 is a suitable and innovative therapy to treat patients with monogenic diseases, including STAT GOF syndromes, since these single causative mutations can be corrected with CRISPR/Cas9. Correction of these mutations can lead to improved function of the STAT protein and overall cell function (Jensen, 2024). To check whether correction of the mutation improved STAT protein functionality, the amount of phosphorylated STAT can be measured. A reduction in total levels of phosphorylated STAT indicates that both STAT protein function and functionality of the target cells have improved. Although gene editing in primary immunodeficiencies is still in its early stages, gene therapy using CRISPR/Cas9 is already being used in clinical trials for

cancer therapeutics, sickle cell disease, and β-thalassemia, and might be a promising technique for treating primary immunodeficiencies such as *STAT* GOF syndromes (Frangoul et al., 2021; Jensen, 2024; Orf, 2024).

Future Directions and Unresolved Questions

Even though a lot of research has been done on *STAT1* and *STAT3* GOF diseases, these diseases exhibit a diverse clinical phenotype. Even among family members that harbor the same germline mutation, the mechanism of disease can be different. The heterogeneity of these diseases may be caused by undiscovered regulatory elements, which make it complicated to determine the clinical manifestations caused by one specific disease-causing mutation (X. Liu et al., 2024).

Research Gaps and Unresolved Mechanisms of Disease

One remaining question in understanding the *STAT1* and *STAT3* GOF diseases is the mechanism that leads to increased phosphorylated STAT levels and eventually to an increased transcription of target genes. Studies suggest that increased STAT protein levels, as well as impaired dephosphorylation rates, are associated with IFN-γ stimulation, but the underlying causes remain unknown. Advanced molecular tools, such as ChIP-sequencing and single-cell RNA sequencing, can be used to determine the exact binding sites for the mutated STAT variant, as well as the expression of certain genes. Combining the results of these two methods may provide new insights into the mechanism of action of different STAT GOF variants (Zimmerman et al., 2019).

Differences in the molecular mechanisms of disease and clinical manifestations, even within families with the same mutation, indicate that additional potential contributors, such as epigenetic alterations, environmental factors (e.g., infections), as well as germline or somatic mutations, play a role in causing heterogeneity. Therefore, we need an improved understanding of the molecular mechanisms of disease to better understand the genotype-to-phenotype correlations that lead to the variability in the disease progression of patients (Jägle et al., 2020; Zimmerman et al., 2019). Research performed by Jägle et al. (2020) suggested grouping *STAT3* mutations based on their luciferase activity. Researchers made three groups of mutations and identified associations between different *STAT3* mutations and immunological and clinical alterations (Jägle et al., 2020). Although some grouping of mutations was possible, it was not possible to explain the heterogeneity of the disease, and clinical diversity appears to be more complex than initially thought (Jägle et al., 2020; Tanita et al., 2021). To address this knowledge gap, studies examining patients with *STAT1* or *STAT3* GOF mutations over longer periods of time are needed to identify potential contributors and their impact on disease progression (Jägle et al., 2020; X. Liu et al., 2024; Tanita et al., 2021).

Furthermore, infections and other environmental factors may influence the mechanism of disease in patients with a *STAT1* or *STAT3* GOF mutation in ways that are not yet understood (Jägle et al., 2020; Schmitt et al., 2022). The use of animal models and patient-derived cells or organoid models could help unravel how these factors influence the mechanisms of disease and drive autoimmune disorders. With patient-derived organoid models, researchers could study the differential effects of STAT mutations on specific organs and cells. In addition, these organoids can be used to screen for therapies targeting different disease manifestations, creating a better association between disease manifestations and personalized medicine (X.

Liu et al., 2024). However, more research is needed to uncover the molecular mechanisms by which the immune system is dysregulated and how autoimmunity arises in patients with *STAT1* or *STAT3* GOF diseases (Jägle et al., 2020; Kaleviste et al., 2019; Schmitt et al., 2022; Toth et al., 2024).

One way to study these molecular mechanisms of disease through the model developed by Liu et al. (2024). These researchers provided a model in which they reprogrammed erythroid progenitor cells to create expanded potential stem cells (EPSCs), which are totipotent stem cells that possess developmental potency for embryonic and extra-embryonic cell lineages (Gao et al., 2019; X. Liu et al., 2024). Liu and colleagues used these cells for disease modelling and gene editing, and showed that correction of the *STAT1* GOF mutation using CRISPR/Cas9 reduced phosphorylated STAT1 levels and STAT1 target gene transcription. Additionally, they used this patient-specific model to test the effects of different JAK inhibitors on the phosphorylated STAT1 levels and target gene expression. In this way, patient-specific responses can be determined, supporting development of personalized treatment plans. This approach highlights the potential of CRISPR/Cas9 gene editing in EPSCs as a model to improve disease modelling and develop patient-specific targeted therapies (X. Liu et al., 2024).

Novel Treatment Targets

Existing therapies for *STAT1* and *STAT3* GOF diseases, such as JAK inhibitors, often reduce clinical manifestations by inhibiting secondary effects rather than addressing the underlying cause of the disease. Therefore, new therapeutic targets are needed. One promising therapeutic target is the binding affinity of the STAT molecules to DNA, which is strongly increased in one group of *STAT3* GOF mutants (Jägle et al., 2020). The small-molecule inhibitor inS3-54 targets the DNA binding domain of STAT3 and selectively inhibits the interaction of STAT3 with DNA, thereby inhibiting expression of downstream target genes in vitro and in vivo (Huang et al., 2014, 2016).

Another promising therapeutic strategy may be epigenetic modulation. Studies suggest that *STAT1* GOF mutations lead to epigenetic changes that contribute to increased transcription of ISGs (Kaleviste et al., 2019; Koh et al., 2024). These findings indicate that it is important to investigate potential treatment options involving histone deacetylase activators or DNA methyltransferase inhibitors to reduce chromatin accessibility, thereby inhibiting gene transcription of STAT1 target genes and improving clinical outcomes (Ott et al., 2023). Finally, targeting the JAK-STAT pathway using SOCS mimetics might be another interesting therapeutic strategy. SOCS mimetics are peptides that correspond to the KIR of a SOCS protein, which normally plays a role in the inhibition of the JAK2 kinase. The SOCS mimetic binds JAK2 and inhibits tyrosine phosphorylation of STAT1 (Ott et al., 2023). Currently, there are no ongoing clinical trials with this mimetic, but this SOCS mimetic could play a role as a therapeutic strategy to reduce clinical manifestations in *STAT* GOF patients (Ahmed et al., 2015).

Recommendations for Clinical Practice

Establishing a diagnosis of *STAT* GOF disease is complicated because of its clinical heterogeneity. *STAT* GOF syndromes can manifest already in early childhood, and early diagnosis is crucial for selecting the right treatment and preventing more severe complications. Symptoms of *STAT* GOF disease include autoimmunity, hypogammaglobulinemia, lymphoproliferation, enteropathy, growth delay, CMC, or recurrent infections. Research

proposes that individuals with more than one of these symptoms should be screened for *STAT* GOF variants (Erdős et al., 2021; Leiding et al., 2023). A diagnosis of *STAT* GOF disease can be achieved by genetic sequencing of the *STAT* gene, while whole exome sequencing can help identify unknown variants (Maffucci et al., 2016). Other diagnostic tools, such as flow cytometry, can be used to screen for STAT hyperactivity and are more cost effective (Zimmerman et al., 2019).

After the identification of a *STAT* GOF variant, patients should be treated using personalized treatment strategies based on the severity and specific manifestations of the disease. HSCT is a potential treatment, mostly for patients with severe *STAT* GOF disease progression, and requires bridging therapy with ruxolitinib (Kayaoglu et al., 2021; Okada et al., 2020). However, patients with less severe clinical symptoms can often be treated with only one of the JAK inhibitors. Personalized treatment strategies can be created using the EPSCs from Liu et al. (2024), where drug screening with a limited number of JAK inhibitors revealed that patients respond differently to specific JAK inhibitors. Using this cell model, the right JAK inhibitor can be identified to treat a specific patient. For example, treatment with either ruxolitinib or baricitinib led to a significant improvement in STAT1 hyperactivation and target gene expression, while this was not the case for tofacitinib treatment (Jägle et al., 2020; X. Liu et al., 2024). Therefore, the selection of specific, personalized therapies is needed to reduce *STAT* GOF syndromes and improve clinical outcomes.

Even though bridging therapy for HSCT with JAK inhibitors appears to be a promising treatment for *STAT1* and *STAT*3 GOF patients, long-term effects of these JAK inhibitors have not been sufficiently investigated (Kayaoglu et al., 2021; Ott et al., 2023). For HSCT, a potential curative treatment, the long-term effects also remain uncertain. Conditioning therapy before HSCT can cause severe and potentially irreversible damage to vulnerable organs, such as the liver, kidney, brain, and lungs. Additionally, a large proportion of patients suffer from graft-versus-host disease after HSCT, and the long-term efficacy of HSCT is mostly unknown. It is possible that if only a few *STAT* mutated stem cells are left in the patient, these stem cells will outcompete the *STAT* wildtype stem cells after some time. These uncertainties highlight that long-term monitoring is essential for patients receiving any of these treatments (Ott et al., 2023).

Given these challenges, it is important to find alternative treatment strategies. As shown previously, one emerging treatment strategy is gene editing using CRISPR/Cas9, which may be promising in correcting monogenic mutations present in these *STAT* GOF syndromes (Figure 4). Although more research is needed on this strategy, CRISPR/Cas9 gene editing could be a targeted therapeutic solution that directly repairs the disease-causing mutation in the genome. Gene editing as a treatment may enhance treatment outcomes and reduce long-term complications for patients with a *STAT* GOF syndrome.

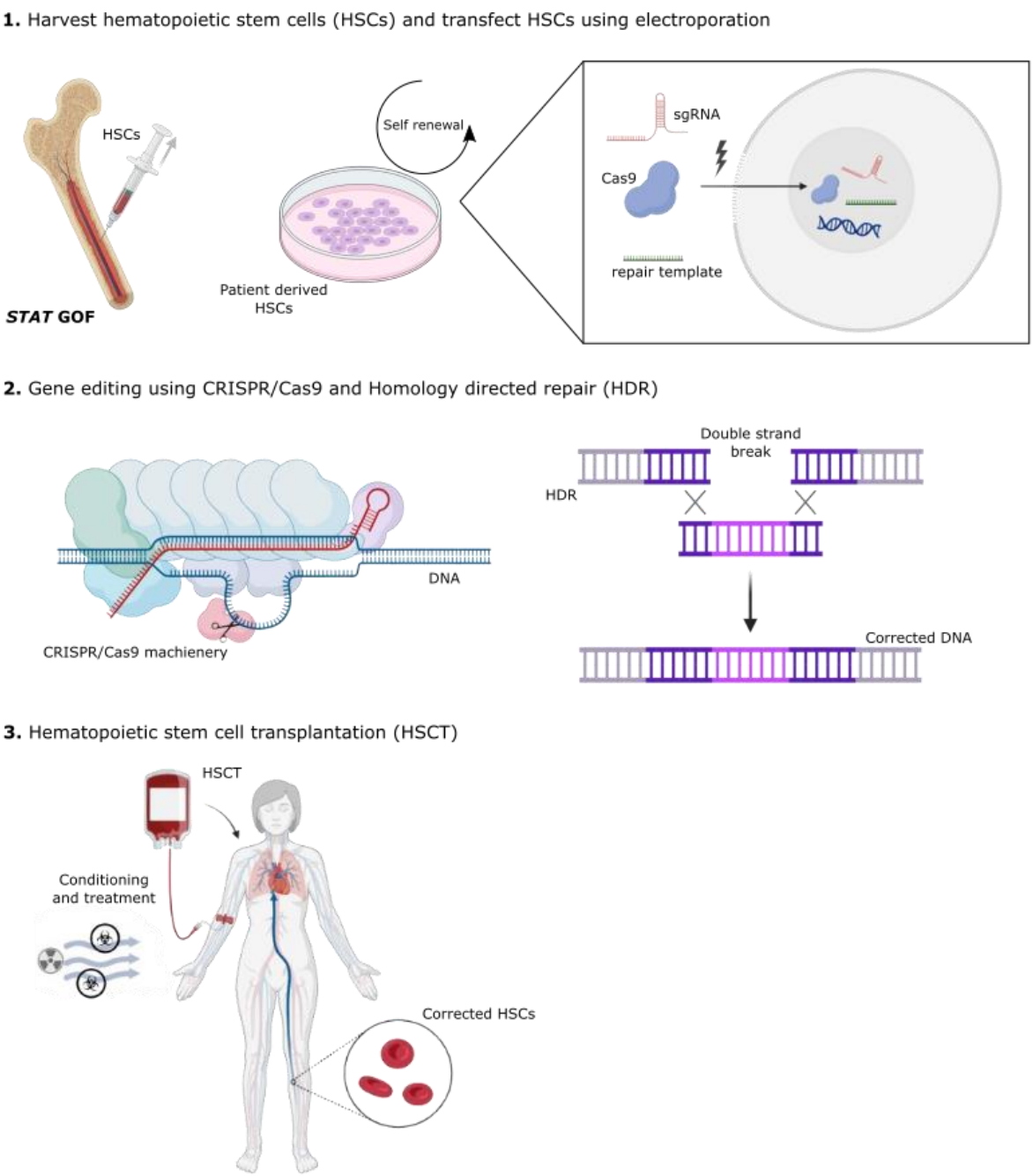

Figure 4: Workflow for Treatment of Patients with a *STAT* GOF Syndrome. (1) Patient-derived hematopoietic stem cells (HSCs) are collected, as these cells are capable of self-renewal. The HSCs are electroporated with an sgRNA, Cas9, and a repair template. **(2)** Cas9 and the sgRNA create a double-strand break at the site of the *STAT* GOF mutation, and the repair template corrects the mutated *STAT* sequence into a wildtype *STAT* sequence. **(3)** Pre-treatment, including bridging therapy and conditioning of the patient, is administered. The corrected HSCs with the wildtype *STAT* sequence are then transplanted into the patient. The image was created using BioRender.

Discussion and Conclusion

Recent research has identified novel GOF mutations in *STAT1* and *STAT3* that disrupt JAK-STAT signaling, causing severe immune dysregulation. These mutations present a diverse range of clinical manifestations. *STAT1* GOF mutations are frequently associated with CMC, driven by impaired IL-17 signaling and T-cell dysfunction, as well as recurrent infections and autoimmune conditions that affect multiple organs, such as the liver, lungs, and salivary glands. On the other hand, *STAT3* GOF mutations are linked to systemic autoimmunity, lymphoproliferation, hypogammaglobulinemia, and severe bacterial infections, and result in multi-organ effects, including diabetes and enteropathy. Both syndromes demonstrate significant heterogeneity in genotype-to-phenotype correlations, complicating the diagnosis and treatment of patients with these syndromes.

Early diagnosis of *STAT* GOF disease is necessary for optimal treatment outcomes, and precision therapies are needed to control the disease. As previously described, treatment of *STAT* GOF patients with JAK inhibitors provides clinical benefits for some, but not all, patients. Patients respond differently to various types of JAK inhibitors, including ruxolitinib, tofacitinib, or baricitinib (Forbes et al., 2018; X. Liu et al., 2024; Okada et al., 2020; Ott et al., 2023). Patients with a *STAT3* GOF mutation often respond better to combination therapy with a JAK inhibitor and an IL-6 antagonist. In contrast, patients with a *STAT1* GOF mutation usually respond to treatment with a JAK inhibitor alone (Forbes et al., 2018). A possible explanation for the lack of response to therapies in some patients is the significant heterogeneity of *STAT1* and *STAT3* GOF syndromes, where genotype-to-phenotype correlations are not well understood. Researchers suggest that germline or somatic mutations, epigenetic alterations, and environmental factors may contribute to the mechanism of disease and lead to heterogeneous responses (Jägle et al., 2020; Zimmerman et al., 2019).

With more research and larger patient cohorts, we can better investigate disease mechanisms and genotype-to-phenotype correlations. To optimize treatment outcomes, patients should be treated with patient-specific therapeutics. In addition, novel treatments, such as the small molecule inhibitor inS3-54, SOCS mimetics, or gene editing can expand treatment options for patients. These therapies could be tested in clinical trials to evaluate their efficacy and explore new combination therapies. For example, the EPC reprogramming approach to generate patient-derived EPSC lines is a promising approach for developing patient-specific therapeutic strategies (X. Liu et al., 2024). These EPSCs can be used for personalized disease modeling and to predict patients' responses to treatments.

Even though a significant amount of progress has been made in the field of IEI, more research is needed on the molecular genetics, cellular immunology, and clinical manifestations of patients with a *STAT1* or *STAT3* GOF mutation. For example, the exact molecular mechanisms underlying increased STAT phosphorylation and transcriptional activity are still under debate. Future research should aim to address how specific mutations drive differential STAT1 and STAT3 signaling, using techniques such as single-cell RNA sequencing and proteomics. These approaches may help to unravel the effects of single *STAT* GOF mutations.

Interestingly, dysregulated STATs are also found in cancer and other autoimmune diseases. This suggests that understanding these *STAT1* and *STAT3* GOF syndromes can also contribute to the understanding of other diseases, particularly since the same mechanisms of STAT dysfunction are present in these diseases. For example, abnormal STAT3 activation plays a key role in autoimmunity as well as in oncogenesis, where it can drive tumor growth

and immune evasion (Tolomeo & Cascio, 2021). Therefore, STAT3-targeting drugs that are being investigated in cancer clinical trials might be of interest as a treatment for patients with *STAT3* GOF disease. Additionally, gene editing using CRISPR/Cas9 has been successfully used in clinical trials for cancer therapeutics, sickle cell disease, and β-thalassemia, and might be a potential treatment approach for patients with a *STAT* GOF syndrome (Jensen, 2024; Orf, 2024).

In this review, we explored the clinical, molecular, and therapeutic aspects of *STAT1* and *STAT3* GOF syndromes. We highlighted the challenges in understanding genotype-to-phenotype correlations and the significant heterogeneity in clinical manifestations and responses to treatment. Further research into the molecular mechanisms underlying *STAT1* and *STAT3* GOF syndromes is needed to develop patient-specific therapies. Additionally, as dysregulated STAT signaling is shown in both cancer and other autoimmune diseases, new findings from studying *STAT1* and *STAT3* GOF mutations could also help in developing therapeutics for other diseases. Finally, more research, larger patient cohorts, and more clinical trials are needed to improve current therapies or develop new therapeutic approaches that enhance patient outcomes.

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AI statement: Generative AI was used for brainstorming purposes only.