Targeted Therapy with Biologicals and Small Molecules in Primary Immunodeficiencies

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Significance of the Study

- Several primary immune deficiencies are associated with immune dysregulation and hyperinflammation.
- A growing understanding of the pathophysiology of these disorders has led to the development of mechanism-based therapeutic strategies.
- Small molecules and biologics are effective in reversing clinical manifestations of primary immune deficiencies and as a bridge treatment to hematopoietic stem cell transplantation.

Keywords

Primary immunodeficiency diseases · Immunodysregulation · Combined immunodeficiency · Precision medicine · Monoclonal antibody · Small molecule inhibitor

Abstract

Primary immunodeficiencies are disorders resulting from mutations in genes involved in immune defense and immune regulation. These conditions are characterized by various combinations of recurrent infections, autoimmunity, lymphoproliferation, inflammatory manifestations, and malignancy. In the last 20 years, newborn screening programs and next generation sequencing techniques have increased the ability to diagnose primary immunodeficiencies. Furthermore, an advanced understanding of the molecular ba-

sis of these inherited disorders has led to the implementation of targeted therapies that utilize small molecules and biologics to modulate the activity of impaired intracellular pathways. This article will discuss selected primary immunodeficiencies, the genetic defects of which have been recently studied and are amenable to targeted therapy as a reflection of the potential of precision medicine in the future.

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Introduction

The broad availability of whole exome and whole genome sequencing analysis has made possible the discovery of an increasing number of genetic disorders of the immune system over the last 2 decades. The International Union of Immunological Societies has updated the classi-

Table 1. Targeted therapies used in PID

Molecular structure	Molecular target	Drug	Indication
Macrolide compound	mTOR	Sirolimus	NLCR4-GOF POMP deficiency CTLA-4 haploinsufficiency APDS
CTLA-4 IgG fusion protein	B7-1 (CD80), B7-2 (CD86)	Abatacept Belatacept	CTLA-4 haploinsufficiency LRBA deficiency CTLA-4 haploinsufficiency
Recombinant human IL-1R antagonist	IL-1R	Anakinra	Cryopyrin-associated periodic fever syndromes
Antihuman IL-1 IgG1 mAb IgG1 linked to IL-1R and IL-1R accessory protein	IL-1 β	Canakinumab Rilonacept	CAPS FCAS MWS DIRA
IgG1k recombinant humanized mAb	IL-6R	Tocilizumab	STAT3-GOF
Fusion protein Chimeric mAb Humanized mAb	TNF- α	Etanercept Infliximab Adalimumab	SAVI CANDLE syndrome POMP deficiency
Small molecule inhibitor	JAK1 and JAK 2	Ruxolitinib Baricitinib	STAT3-GOF ^a STAT1-GOF
	JAK 1 and JAK3 P110	Tofacitinib Leniolisib	CANDLE syndrome APDS
Recombinant IL-18 binding protein	IL-18-binding protein	Tadekinig- α	NLCR4-GOF

SAVI, STING-associated vasculopathy with onset in infancy. ^a Only ruxolitinib and tofacitinib.

fication of genetic defects associated with primary immune deficiencies (PID) to more than 350 disorders. Many of the newly discovered PID manifest with a broad phenotypical spectrum to include not only variable degrees of susceptibility to infections but also immune dysregulation manifesting as autoimmunity and/or hyperinflammation. The understanding of the immunobiology underpinning the pathophysiology of immune dysregulation has evolved significantly. Molecular studies have unraveled signaling pathways and metabolic requirements that are crucial to tightly control immune and inflammatory responses. As such, the capacity to precisely identify the molecular basis of immunological disorders has also largely increased, paving the path for the application of targeted therapeutic agents that replace, or can be combined with, traditional immunosuppressant treatments. These newly generated drugs aim to modulate the activity of intracellular pathways whose function is either increased or decreased as a result of a specific genetic defect. In this review, we illustrate various recently described PID, focusing in particular on selected disorders associated with immune dysregulation and hyperinflammation. We also present targeted therapeutic approaches based on the molecular mechanisms underpinning these diseases (Table 1).

Precision Medicine in Immune Dysregulation Disorders

Activated Phosphoinositide 3-Kinase δ Syndrome

Activated phosphoinositide 3-kinase δ syndromes 1 and 2 (APDS1 and APDS2) are combined immunodeficiency (CID) disorders due to mutations in either the *PIK3CD* or the *PIK3R1* gene [1–3]. These genes encode for the p110 δ catalytic subunit and the p85 α regulatory subunit of phosphoinositide 3-kinase (PI3K), respectively. PI3K phosphorylates phosphatidylinositol-4,5 biphosphate (PIP2) to phosphatidylinositol-3,4,5 trisphosphate (PIP3), an important mediator of PI3K downstream cellular pathways including mammalian target of rapamycin (mTOR).

In 2014 Lucas et al. [1] and Angulo et. al. [2] identified heterozygous gain-of-function (GOF) mutations in *PIK3CD* in patients with a CID phenotype (APDS1) [1, 2]. Soon thereafter it was reported that heterozygous loss-of-function (LOF) mutations in *PIK3R1* result in a similar clinical phenotype (APDS2) [3]. Since then, different heterozygous mutations have been reported. However, the E1021K amino acid substitution is by far the most common in APDS1, while in APDS2 a heterozygous donor

splice site mutation causing skipping of exon 11 is the main disease-causing alteration [3–5]. The consequent loss of the p110 δ -binding site in exon 11 results in the loss of p85 α subunit-mediated inhibitory control on p110 δ , thus causing hyperactivation of the PI3K pathway [3]. The clinical phenotypes of APDS1 and APDS2 significantly overlap [6, 7]. Both diseases are characterized by the coexistence of immunodeficiency with a high susceptibility to infections and autoimmunity, lymphoproliferation, and an increased risk of lymphoma. The onset of the disease is typically in childhood, with sinopulmonary infections often leading to bronchiectasis over time. Respiratory infections are mainly due to *Streptococcus pneumoniae* and *Haemophilus influenza* [6]. Recurrent or persistent infections due to Herpesviridae, such as Epstein-Barr virus, cytomegalovirus, herpes simplex virus, and Varicella-Zoster virus, are also frequent [6]. Lymphadenopathy, splenomegaly, and/or hepatomegaly represent the clinical signs of lymphoproliferation and are present in the majority of the patients. Other clinical characteristics include autoimmunity, lymphoid hyperplasia of the airways and gut, developmental delay, and enteropathy [8]. Moreover, APDS patients are at a higher risk for lymphomas (particularly Epstein-Barr virus-driven B cell lymphoma) [6, 7].

Growth retardation has been reported in approximately 50% of APDS2 patients, but not in APDS1; this may be explained by the dysregulated activity of the p110 α and p110 β PI3K subunits [4, 5, 7]. The immunological phenotype of APDS includes both T-cell abnormalities with decreased naive T cells, increased T effector memory cells, and exhausted T effector memory reexpressing CD45RA (T_{EMRA}) cells and high numbers of T follicular helper (T_{fh}) cells. B-cell impairment is also present as indicated by variable degrees of hypogammaglobulinemia, elevated IgM levels and high numbers of transitional B cells, decreased switched memory cells, and an impaired response to vaccinations [1]. Increased AKT and S6 phosphorylation in T and B cells have been observed in APDS patients as a result of augmented mTOR signaling [1], thereby supporting the use of mTOR-targeted therapy to control the disease [9]. Standard treatments for APDS include antimicrobial prophylaxis and immunoglobulin replacement to prevent infectious complications [7]. Different combinations of immune suppressive regimens have been used to control lymphoproliferation and autoimmunity, with the best results being obtained with rituximab and mTOR inhibitors (such as rapamycin) [9]. Hematopoietic stem cell transplantation (HSCT) has been successful in reversing the clinical phenotype especially in

the context of optimal chimerism; however, elevated rates of post-transplant viral reactivation and engraftment failure at different time points have been reported [10, 11].

Selective PI3K δ inhibitors represent a targeted therapy based on characterization of the molecular mechanisms underpinning APDS. Two phase 2 trials are currently ongoing to establish the safety and efficacy of these drugs. The first trial is based on oral administration of leniolisib (NCT02435173), while the second one is based on inhaled nemiralisib (NCT02593539). The initial results of the first trial, with dose-escalating administration of leniolisib for 12 weeks, showed that the drug was well tolerated; reduction of lymphadenopathy and splenomegaly and improvement of cytopenias were reported following the therapy [12].

However, despite the promising results of these targeted therapy, in patients with treatment-refractory disease and those with drug-related adverse events, HSCT represent an option that must be taken into account. In addition, the long-term safety profile of mTOR and PI3K δ inhibitors in patients with APDS has yet to be fully defined, especially considering some evidence that P110 δ inhibitors may lead to genomic instability in B cells [13].

CTLA4 Haploinsufficiency

Cytotoxic lymphocyte antigen-4 (CTLA4) haploinsufficiency is due to heterozygous germline mutations in the *CTLA4* gene. CTLA4 (also defined as CD152) is a receptor expressed by T cells that inhibits cell activation and immune response. CTLA4 binds to 2 different ligands on antigen presenting cells CD80 and CD86 [14]. Upon ligand binding, CTLA4 produces an inhibitory signal that limits the activation and proliferation of T cells. CD28, a T-cell costimulatory molecule, also binds to CD80/86 with an opposite effect [14]. CTLA4 expression is crucial for T regulatory cell function and immune tolerance as well. Two groups originally reported the presence of *CTLA4* mutations in patients with recurrent sinopulmonary and viral infections, associated with autoimmunity and lymphoproliferation [15, 16]. Clinical and laboratory findings were consistent with common variable immunodeficiency, but patients also suffered from T-cell infiltrates in the lungs, the gastrointestinal tract, and the nervous system, as well as significant autoimmune blood cytopenia. The disease is characterized by incomplete penetrance and variable expressivity [15, 16]. Functional studies showed diminished expression of CTLA4 associated with impaired suppressor function in FOXP3⁺ Treg cells [16]. Moreover, patients had a decreased expression

of CTLA4 on the surface of activated conventional T cells, suggesting that impaired expression of this molecule may cause a defective capacity to extinguish T-cell responses and to control self-reactive T cells that have escaped central deletion. Also the B cell compartment is defective in these patients, with a progressive loss of B cells and an increased proportion of autoreactive CD21^{low} B cells [15]. Recently, Schwab et al. [17] reported on a cohort of 133 patients with CTLA4, broadening the clinical and immunological spectrum associated with this disease. Clinical manifestations of this series included severe or refractory autoimmune blood cytopenias, nonmalignant lymphoproliferation, and respiratory and gastrointestinal disease. Respiratory tract findings included recurrent lower and upper respiratory tract infections, lymphocytic interstitial lung disease, bronchiectasis, and lung fibrosis. Gastrointestinal manifestations were present, with enteropathy and Crohn's-like colitis often being particularly severe. The immunological phenotype included CD4 T-cell lymphopenia, variable degrees of hypogammaglobulinemia, and impaired response to immunizations and B-cell maturational defects [17].

Identification of CTLA4 as a key immune regulator has led to the generation of abatacept and belatacept molecules derived from the fusion between the extracellular domain of CTLA4 and the Fc region of IgG1, functioning in vivo as inhibitors of T-cell activation [18]. Side effects of these drugs are related to their immune suppressive activity and include increased susceptibility to infections (especially viral) and to malignancy.

Initially, rapamycin was used as immunosuppressive therapy in CTLA4 haploinsufficiency; however, abatacept and belatacept have been shown to be an effective targeted treatment to control the immune dysregulation seen in this disorder [18].

Lee et al. [19] reported on a 14-year-old girl affected by CTLA4 haploinsufficiency with severe enteropathy, autoimmune hepatitis, and autoimmune blood cytopenia. Initiation of abatacept therapy diminished the diarrhea, controlled the autoimmune hemolytic anemia, and eliminated the need for additional immunosuppressive agents. In the cohort described by Schwab et al. [17], 11 patients received abatacept or belatacept and experienced improvement of autoimmune manifestations, lymphadenopathy, and lymphoproliferation in the lungs. Sirolimus was used in 13 patients with a good response in terms of reduced cytopenia, splenomegaly and lymphadenopathy [17].

Interestingly, another target therapy, i.e., vedolizumab, a humanized monoclonal antibody (mAb) that targets

T cells expressing the $\alpha 4 \beta 7$ integrin gut homing receptor, was successfully used to treat a CTLA4 patient with refractory autoimmune enterocolitis [20]. However, as expected, vedolizumab did not improve the hypogammaglobulinemia and pure red cell aplasia in the patient [20].

While the use of targeted therapy described for CTLA4 haploinsufficiency seems very effective in reversing some of the manifestations of immune dysregulation, the possibility of viral reactivation during the treatment may be a limitation to the use of this drug for prolonged periods of time. For this reason, HSCT should be considered as a possible definitive therapy in patients with CTLA4 haploinsufficiency especially in case a fully matched donor is available. Results of HSCT are limited to a small cohort of patients but have been encouraging. The best candidates for HSCT may be patients with severe clinical manifestations like autoimmune hepatitis with nodular regenerative hyperplasia and a limited response to treatment with immunomodulatory drugs [21].

Lipopolysaccharide-Responsive and Beige-Like Anchor Deficiency

Lipopolysaccharide-responsive and beige-like anchor (LRBA) is a cytosolic protein that colocalizes with CTLA4 in endosomes; LRBA acts as a chaperone for CTLA4, enabling recycling of the molecule and the suppressive capacity of Treg cells. In the absence of LRBA, CTLA4 is targeted for lysosomal degradation, and its expression on Treg cells and activated conventional T cells is significantly diminished [22]. A deficiency of LRBA leads to an autosomal recessive form of CID that presents with hypogammaglobulinemia, a higher susceptibility to infections, and autoimmunity [23]. Since the original description, the clinical phenotype of the disease has broadened to include many more conditions of immune dysregulation like enteropathy, autoimmune blood cytopenias, and nonmalignant lymphoproliferation leading to splenomegaly and lymphadenopathy. Other autoimmune manifestations including autoimmune hepatitis, thyroid disease, diabetes type I, alopecia, myasthenia gravis, uveitis, and polyarthritides have also been described; Bratanic et al. [26] also reported multifocal gastric adenocarcinoma in a patient with LRBA deficiency [23–25, 27].

As enteropathy and polyendocrinopathy could coexist in the same patient, a phenotype that mimics the immune dysregulation, polyendocrinopathy, X-linked (IPEX) syndrome may be observed [28]. Respiratory complications including interstitial lung disease, granulomas, and bronchiectasis mainly related to viral and bacterial infections are also common [25, 27]. Involvement of the cen-

tral nervous system has been observed in one fourth of the patients and may manifest with demyelination but also with brain atrophy and granulomas [25].

The immunological phenotype of LRBA deficiency includes hypogammaglobulinemia, and a markedly reduced proportion of switched memory B cells along with an increase in double-negative T cells and circulating Tfh [23, 25, 27, 29]. Most of the patients present with reduced numbers and impaired function of Treg cells [28] that express decreased levels of FOXP3, CD25, and CTLA4 proteins [22, 28].

The phenotypical similarities with CTL4 haploinsufficiency may be explained by the functional interplay between LRBA and CTLA4 proteins and have offered a rational basis to attempt treatment with abatacept also for LRBA deficiency [22]. Abatacept was shown to be effective in reversing lymphocytic interstitial lung disease and blood cytopenias in 3 LRBA patients; however, the enteropathy was not as responsive, and sirolimus and other immunosuppressant drugs needed to be added to control the disease. Regarding the immunological phenotype, treatment with abatacept was associated with an increased naive:effector T-cell ratio and improved antibody responses to polysaccharide vaccines. Moreover, circulating Tfh frequencies and a soluble IL-2R α chain have been shown to be useful markers to assess the clinical response of LRBA- and CTLA-4-deficient patients to CTLA4-Ig therapy [29]. Prolonged treatment over several years resulted in minimal infectious or autoimmune complications [22]. Moreover, in vitro studies with chloroquine, an inhibitor of lysosomal degradation, showed efficacy in preventing the loss of CTLA4 in patients with an LRBA deficiency [22], suggesting that both chloroquine and hydroxychloroquine may be used as immunomodulatory drugs in this disease. However, as in CTLA4 haploinsufficiency, HSCT is the only potentially definitive treatment for LRBA deficiency; only few patients have been transplanted so far and further evidence is needed to assess its safety and efficacy [27, 30].

STAT1 and STAT3 GOF

Signal transducer and activator of transcription (STAT) molecules account for 7 different proteins that are expressed by immune and nonimmune cells and play a key role in immune and inflammatory responses. Several cytokines and growth factors (type I, type II, type III interferons [IFN], IL-6, EGF, PDGF, IL-21, and IL-23) bind to their own receptor on the cell surface and recruit 4 different Janus kinases (JAK) to the receptor intracytoplasmic tail. Upon activation and phosphorylation, JAK phos-

phorylate the intracellular tail of the cytokine receptor, offering a binding site for the Src homology 2 (SH2) domain of cytosolic STAT molecules that, upon phosphorylation, form homodimers or heterodimers and translocate to the nucleus to promote transcription of target genes that regulate various immune pathways and control cell proliferation, differentiation, survival, and death [31]. Although the protein functions of JAK and STAT proteins partially overlap in the transduction of cytokine-mediated signaling, mutations in individual *JAK* and *STAT* genes are associated with specific phenotypes [31].

The binding of type I and type II IFN, IL-10, IL-2, IL-21, and other cytokines to their cognate receptors activates STAT1. The signal is transmitted through JAK1, JAK2, and JAK3. These molecules act in different combinations [32]. Dupuis et al. [33] showed that biallelic LOF mutations of *STAT1* lead to a severe susceptibility to viral and mycobacterial infections due to an impaired response to both type I and type II IFN. Later, the same group showed that heterozygous mutations with a dominant negative effect cause an increased susceptibility to mycobacterial disease [34]. Furthermore, heterozygous GOF mutations in *STAT1* have been primarily associated with chronic mucocutaneous candidiasis (CMC) [35, 36]. Most of these mutations occur in the coiled-coil and the DNA-binding domains of the protein [35, 37] leading to increased *STAT1* expression, increased *STAT1* phosphorylation, or delayed dephosphorylation after stimulation with IFN- α , IFN- γ , and IL-27 [38, 39]. The identification of many more patients with *STAT1* GOF mutations broadened the phenotypical spectrum of the disease. Toubiana et al. [37] reported on a cohort of 274 patients collected from multiple centers. CMC was present in virtually all of the subjects in childhood, and 74% of the patients also suffered from bacterial infections, mainly affecting the respiratory tract and skin, with *S. aureus* being the most common bacterium isolated. Viral infections, especially due to Herpesviridae, and mycobacterial disease were also reported [37]. Severe and invasive fungal infection were due not only to *Candida* but included mucormycosis [40], coccidioidomycosis, histoplasmosis, and aspergillosis [41].

Autoimmunity and immune dysregulation were reported in 30% of the patients [38]. Hypothyroidism, blood cytopenias, juvenile diabetes, and systemic lupus erythematosus are especially common, but inflammatory bowel disease, arthritis, and multiple sclerosis have also been reported [37]. In *STAT1* GOF patients, all of these immune-related disorders are often refractory to conventional treatment and they are challenging to treat.

Moreover, cerebral aneurysms and vasculopathy are frequently observed and put patients with STAT1 GOF mutations at risk for intracranial bleeding if not diagnosed in a timely fashion [37, 38]. Malignancies (including squamous cell carcinoma) may also occur [37]. The immunological phenotype includes decreased numbers and function of T, B, and/or NK cells, and hypogammaglobulinemia [37]. In a small number of patients a loss of B cells over time has been reported. This immunological feature is associated with poor clinical conditions and worse outcomes (pers. obs.). Furthermore, patients have circulating Tfh cells that have an aberrant phenotype, with a reduced proportion of CCR6+ cells (effective B-helper Tfh cells) and an increased expression of IFN- γ and programmed death 1 (PD1) proteins [42].

HSCT is in the only curative treatment and it has been attempted in patients with complicated disease. However, low survival and increased rates of graft failure have been reported after HSCT in STAT1 GOF patients [37, 43, 44].

Conservative medical therapy is based on long-term use of systemic antifungal agents; however, this approach has not always been successful due to the side effects of medications and the onset of resistance. Antibacterial and antiviral medications, and the use of immunosuppressive drugs, are often needed as well. Despite these therapies, the rate of mortality by 60 years of age is more than 10% in patients without invasive infections, cancer, and/or symptomatic aneurysms, but it is around 70% in those with prominent immune dysregulation and autoimmunity.

The molecular understanding of the JAK-STAT pathway has paved the way for the use of targeted pharmacologic inhibitors in patients with STAT1 GOF [45–47]. Currently the following 5 different small molecule JAK inhibitors (Jakinibs) are available: tofacitinib (a JAK1 and JAK3 inhibitor), ruxolitinib (JAK1 and JAK2 inhibitor), baricitinib (JAK1 and JAK2 inhibitor), filgotinib (a more selective JAK1 inhibitor), and decernotinib (a selective JAK3 inhibitor) [48, 49]. The 5 different molecules display slightly different toxicity profiles and availabilities.

Recently, treatment with Jakinibs was assessed in 11 STAT1 GOF patients. Clinical features included severe fungal infections in 6 patients (including CMC in 5 patients and disseminated coccidiomycosis in 1 patient), cytopenias ($n = 6$), autoimmune enteropathy ($n = 5$), and hepatitis ($n = 5$). Polyendocrinopathy and interstitial lung disease were also present in some patients [45].

Therapy with Jakinibs led to improvement of cytopenias, interstitial lung disease, and total parenteral nutrition-dependent enteropathy. Moreover, improvements

have been observed regarding the ability to control infections. CMC was responsive to the treatment [45] while the disseminated coccidiomycosis progressively worsened and the patient eventually died. Another case was described in the same cohort with severe fungal infection and unfavorable outcomes despite therapy with a Jakinib, suggesting that the treatment may be insufficient to reverse systemic fungal infection [45]. Meesilpavikkai et al. [47] reported on the efficacy of baricitinib in 1 patient who had a reduction of the IFN signature and downstream IFN activation.

Inhibition of STAT1 phosphorylation and improved NK-cell cytotoxicity have been observed when peripheral blood mononuclear cells from patients with STAT1 GOF are treated with Jakinibs in vitro [50, 51]. Furthermore, normalization of the proportions of TH1, TH17, and Tfh cells has been associated with clinical improvement in patients undergoing treatment [46].

Considering the side effects, therapy with Jakinibs may cause elevation of liver enzymes and a mild decrease in the platelet count. Epstein-Barr virus, cytomegalovirus, BK, and JC viremia should always be monitored in patients on treatment, especially if more than one immunosuppressive agent is administered in combination with Jakinibs. Herpes zoster infections have been reported in 2 patients receiving this therapy, and prophylaxis with acyclovir or valacyclovir needs to be considered [45].

Type I, II, and III IFN and IL-6, IL-10, and IL-21 are the key molecules that activate STAT3 upon intracellular signaling. While dominant negative germline mutations in *STAT3* result in an autosomal dominant hyper IgE (AD-HIES) syndrome characterized by eczema, skin abscesses, recurrent pneumonias leading to pneumatoceles, and skeletal and connective tissue abnormalities, the original description of heterozygous *STAT3* GOF mutations included patients with early-onset autoimmunity, including type 1 diabetes in infancy [52]. Flanagan et al. [52] showed that transfection of mutant *STAT3* into HEK293T cells led to augmented transcriptional activity compared to transfection of wild-type *STAT3*. More patients with *STAT3* GOF mutations were identified shortly thereafter [53], expanding the clinical phenotype to nonmalignant lymphoproliferation including lymphadenopathy, splenomegaly and interstitial pneumonia and recurrent infections due to nontuberculous mycobacteria, fungi, and viruses. Acquired short stature is also a peculiar feature of the disease [53–55], while the most common autoimmune manifestations include enteropathy and cytopenias [53]. Studies on these families revealed that there are some genetically affected members with an

absent or very mild phenotype consistent with incomplete penetrance and variable expressivity [56].

Regarding the immunological phenotype, these patients may present with T-cell lymphopenia with a low number of Treg cells and skewing of CD4⁺ T cells to the TH1 phenotype, an increased proportion of double-negative TCRαβ⁺ T cells, hypogammaglobulinemia with terminal B cell maturation arrest, and a decreased number of circulating dendritic cells, eosinophils, TH17 cells, and NK cells [52, 53, 56]. All of these immunological abnormalities are caused by enhanced transcriptional activity of STAT3 or delayed kinetics of STAT3 dephosphorylation. The profound immune dysregulation associated with *STAT3* GOF mutations is consistent with the known role of STAT3 signaling in promoting inflammation and TH17 cell differentiation, and inhibiting Treg cells [57, 58].

Furthermore, *STAT3* GOF mutations lead to a decrease in STAT1 and STAT5 phosphorylation [56]. In particular, decreased levels of phosphorylated STAT5 have been proposed as a possible mechanism leading to diminished growth and stature postnatally, reflecting impaired signaling through the growth hormone receptor [59].

Characterization of the molecular abnormalities underlying the disease offered the basis to treat these patients with an IL-6-targeted therapy. Treatment with tocilizumab an anti-IL6 receptor (IL6R) mAb turned out to be effective in 1 patient with an *STAT3* GOF mutation who had severe arthritis and scleroderma-like disease that did not respond to conventional immunosuppressant therapies; significant improvement of contractures and inflammatory markers and normalization of the proportion of TH17 cells have been reported [56]. Three additional patients did benefit from administration of tocilizumab to control autoimmune manifestations, including hepatitis and enteropathy, lymphoproliferation, and interstitial lung disease. However, this treatment was not sufficient to completely reverse the immune dysregulation, and Jakinibs had to be added as well. In 3 other patients, tocilizumab and a Jakinib were started simultaneously, leading to complete resolution of manifestations of immune dysregulation. These data suggest that the combination of IL-6 blockade and Jakinib therapy is an effective treatment strategy, and both agents should be considered as combination therapy in the treatment of immune dysregulation in patients with *STAT3* GOF mutations [45]. Finally, 2 patients with refractory autoimmunity and severe clinical manifestations underwent HSCT; 1 of them died with disseminated adenovirus infection post-transplant, while the other was reported to be alive and in remission [56].

Precision Medicine in Autoinflammatory Disorders

Interferonopathies

Hyperactivation of the type I IFN response is the molecular signature of the interferonopathies, a group of disorders exacerbated by multiple triggers, including damaged nucleic acid in the cytosol. DNA sequences are recognized by cyclic guanosine monophosphate adenosine monophosphate synthase, a sensor molecule that in turn activates the stimulator of IFN genes (STING) [60–62]. STING promotes the inflammatory response by activating either nuclear factor-κB (NF-κB) or IFN regulatory factor 3 [63–65].

STING-associated diseases include early-onset severe vasculopathy [66] and familial chilblain lupus [67]. Both diseases are caused by GOF mutations in *TMEM173*, the gene that encodes for the STING protein. Early-onset cold-induced blistering rash, elevated inflammatory markers, and fever are the most frequent clinical manifestations. Moreover, small vessel vasculopathy that can lead to necrosis of digits has been reported as a severe complication of this disease [66].

Augmented type I IFN signatures have also been associated with genetic defects in the components of the immunoproteasome, a complex proteolytic machinery derived from the constitutive proteasome and abundantly expressed in immune cells. The immunoproteasome plays a critical role in the immune system because it processes proteins for antigen presentation and regulates activation of the NF-κB pathway and management of oxidative stress [68, 69]. Mutations in multiple genes encoding for immunoproteasome subunits, such as PSMA3, PSMB4, PSMB8, and PSMB9, have been linked to autoinflammatory syndromes. The clinical phenotype in this group of diseases is summarized by the acronym CANDLE, which stands for early-onset Chronic Atypical Neutrophilic Dermatositis, Lipodystrophy, and Elevated temperature. In addition, a similar but not completely overlapping phenotype has been associated with proteasome maturation protein (*POMP*) mutations. This gene encodes for a protein that is crucial for immunoproteasome assembly [68, 70]. *POMP* mutations lead to a peculiar phenotype that is characterized by immune deficiency, neonatal-onset Sweet syndrome, and autoimmunity [68].

Several immunosuppressive therapies including corticosteroids, methotrexate, azathioprine, cyclophosphamide, 6-mercaptopurine, etanercept, infliximab, rituximab, and IL-1 antagonist have been used alone or in combination in patients with CANDLE and STING-associated diseases, but the response has been limited [66,

71, 72]. This is not surprising considering that stimulation with cGAMP of fibroblasts from patients with STING-associated diseases lead to increased transcription of *IFNB1* but not of the genes encoding IL-1 or IL-6 [66]. Because of the upregulation of expression of type I IFN and activation of the JAK/STAT pathway, targeted therapy with Jakinibs has been implemented in order to neutralize the dysregulated IFN signature. In particular, treatment with baricitinib, tofacitinib, and ruxolitinib has been shown to decrease the severity of clinical manifestations and inflammatory markers in CANDLE syndrome and STING-associated vasculopathy [66, 67, 71–73]. Moreover, small-molecule directly targeting STING have been studied in a mouse model with promising results and may represent an alternative therapeutic strategy in the future [74]. A more traditional immunosuppressive approach with steroids, rapamycin, and rituximab has been helpful in limiting some of the clinical manifestations in POMP disease; however, based on recent findings of increased IFN signature in this disease, a more targeted approach could be considered in the future [68].

Inflammasome Disorders

Mounting adequate inflammatory responses against pathogens and molecules resulting from cellular damage is a key feature of the innate immune system. In this regard, rapid availability of proinflammatory cytokines including IL-1 and IL-18 represents a fundamental mechanism. Mutations in genes coding for inflammasome molecules have been associated with dysregulation in IL-1 production and signaling. In particular, mutations in *NLRP3* and *IL1RN* genes lead to early-onset autoinflammatory diseases, cryopyrin-associated periodic syndromes (CAPS), and deficiency of IL-1 receptor antagonist.

NLRP3 is the most well-characterized cytoplasmic inflammasome sensor molecule. It is a protein of the NLR family and consists of a carboxy-terminal leucine-rich repeat (LRR) domain, a nucleotide-binding NACHT domain, and an amino-terminal PYRIN (PYD) domain. Upon stimulation, the *NLRP3* inflammasome activates proteolytic enzymes that cleave the inactive pro-IL-1 β and pro-IL-18 molecules into their active forms. In patients with CAPS, *NLRP3* mutations lead to constitutive hyperactivation of the inflammasome [75]. These mutations result in GOF, as demonstrated by spontaneous inflammasome formation in the absence of activating signals [76, 77]. More than 200 mutations in the *NLRP3* gene associated with CAPS have been reported [78].

In 2001, *NLRP3* mutations were first described as the cause of familial cold autoinflammatory syndrome [79] and Muckle-Wells syndrome [80]. Familial cold autoinflammatory syndrome is an early-onset disease with intermittent cold-induced neutrophilic urticaria, arthralgia, increased inflammatory markers, and fever. Muckle-Wells syndrome also manifests during infancy with painful and swollen joints, non-itchy rash, mild to moderate fever, and, in some cases, conjunctivitis. During adolescence, patients develop sensorineural hearing loss caused by progressive nerve damage. Progressive kidney disease due to amyloidosis is reported in about one third of patients affected by Muckle-Wells syndrome [80].

NLRP3 mutations were shown to be associated with neonatal onset multisystem inflammatory disease [76, 77]. Together with many features present in the other relatively milder forms of CAPS, patients with neonatal onset multisystem inflammatory disease often manifest central nervous system involvement, with presence of cerebral calcifications.

Biallelic LOF mutations of the *IL1RN* gene are responsible for the deficiency of IL-1 receptor antagonist (DIRA). These mutations lead to a reduced expression of an antagonist of IL-1 signaling and a consequent elevated production of IL-1 β . The disease shares some similarities with CAPS. The onset is typically neonatal, and patients suffer from fevers, rash, joint swelling, oral mucosal lesions, and bone abnormalities, including wide clavicles and ribs, periosteal elevation along the long bones, and osteolytic lesions [35, 81].

Considering the specific molecular pathway, trials with IL-1 antagonists has been performed. Anakinra, a recombinant IL-1 receptor antagonist with a short half-life, binds to the IL-1 receptor and thus impairs the binding of IL-1 α and IL-1 β . Rilonacept is a fusion protein in which IL-1R1 and IL-1RACp are complexed to the Fc portion of IgG1 that binds IL-1 α and IL-1 β . Finally, canakinumab is a humanized IgG mAb that selectively binds IL-1 β . Both rilonacept and canakinumab have longer half-lives compared to Anakinra.

Therapy with these newly discovered drugs was demonstrated to be effective in improving clinical manifestations, inflammatory markers, and the frequency of disease exacerbations in CAPS and DIRA [79, 82–84].

Another disease that has been successfully treated with a precision medicine approach using mAb against interleukins is the autoinflammatory syndrome due to *NLR4* (NLR family CARD domain-containing protein 4) GOF mutations. Macrophage activation syndrome and enterocolitis that present in the first few weeks of life represent

the most frequent clinical manifestations [85–87]. The activation of NLRC4 protein causes the activation of both caspase-1 and caspase-8 and the consequent overproduction of proinflammatory cytokines, in particular IL-18 and IL-1 β [85, 86, 88, 89]. While administration of IL-1 inhibitory molecules alone has been able to only partially modulate the clinical manifestations of the disease, treatment with a recombinant IL-18-binding protein (tadekinig- α) was successful in a patient affected by NLRC4 GOF disease, and a phase 3 clinical trial (Clinical Trials No. NCT03113760) is currently enrolling patients [88]. In addition, a combination of rapamycin and IL-1 inhibition has been attempted with the aim of reducing caspase-1 activation and consequent overproduction of IL-1 and IL-18. Targeting of multiple disease-determining molecules was able to partially reverse clinical manifestations and should be considered in NLRC4 GOF patients whenever IL-18-binding protein is not easily available [88, 90].

Other Examples of Precision Medicine in PID

Escalating doses of IFN- α have been effective in patients with AR IFN- γ R deficiency while IFN- γ supplementation can be used in AD IFN- γ R deficiency to decrease the burden of mycobacterial disease [91]. IFN- γ may also be administered to patients with chronic granulomatous disease to aid the clearance of infectious agents [91]. Emapalumab, a human mAb inhibitor of IFN- γ , is an important option for the treatment of hemophagocytic lymphohistiocytosis in adult and pediatric patients. Alemtuzumab (anti-CD52) has also been effective in improving outcomes of hemophagocytic lymphohistiocytosis [91].

Ustekinumab, an mAb that binds to the p40 subunit common to IL-12 and IL-23, inhibiting IL-12 and IL-23 signaling and therefore downstream IL-17 response, has been effective in treating inflammatory complications of leukocyte adhesion deficiency type 1 including IBD and periodontitis [91]. Plerixafor is an inhibitor of the binding of CXCR4 to CXCL12 used as a therapeutic agent to counterbalance the increased cellular response to CXCL12 that characterizes WHIM syndrome [92]. TNF- α inhibition improved arthritis in patients with Blau syndrome due to mutations in the *NOD2* gene [91].

Conclusion

The recent identification of many new PID and the characterization of their underlying molecular mechanisms has given clinical immunologists the opportunity to use novel therapeutic tools to modulate the immune system. Small molecules and biologics have shown to be particularly useful in patients with clinical manifestations of immune dysregulation and hyperinflammation. This precision medicine approach is effective in directly reversing disease-related symptoms and also in ameliorating the clinical condition of patients prior to HSCT.

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