The role of interferon-gamma and its signaling pathway in pediatric hematological disorders

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Abstract
Interferon-gamma (IFN-\(\gamma\)) plays a key role in the pathophysiology of hemophagocytic lymphohistiocytosis (HLH), and available evidence also points to a role in other conditions, including aplastic anemia (AA) and graft failure following allogeneic hematopoietic stem cell transplantation. Recently, the therapeutic potential of IFN-\(\gamma\) inhibition has been documented; emapalumab, an anti-IFN-\(\gamma\) monoclonal antibody, has been approved in the United States for treatment of primary HLH that is refractory, recurrent or progressive, or in patients with intolerance to conventional therapy. Moreover, ruxolitinib, an inhibitor of JAK/STAT intracellular signaling, is currently being investigated for treating HLH. In AA, IFN-\(\gamma\) inhibits hematopoiesis by disrupting the interaction between thrombopoietin and its receptor, c-MPL. Eltrombopag, a small-molecule agonist of c-MPL, acts at a different binding site to IFN-\(\gamma\) and is thus able to circumvent its inhibitory effects. Ongoing trials will elucidate the role of IFN-\(\gamma\) neutralization in secondary HLH and future studies could explore this strategy in controlling hyperinflammation due to CAR T cells.

KEYWORDS
aplastic anemia, graft failure, hematologic disorders, HLH, interferon-gamma, pediatric

1 INTRODUCTION

Interferon-gamma (IFN-\(\gamma\)) is a proinflammatory cytokine that activates effector immune cells and enhances antigen presentation in vivo.\textsuperscript{1} In pediatric medicine, IFN-\(\gamma\) has a central role in the pathogenesis of rare hematologic diseases, including hemophagocytic lymphohistiocytosis (HLH) and aplastic anemia (AA).\textsuperscript{1}

IFN-\(\gamma\) is secreted by natural killer (NK) cells as part of the innate immune response, and by CD4\(^+\) Th1 cells and CD8\(^+\) cytotoxic T lymphocytes (CTLs) once adaptive immunity has been triggered.\textsuperscript{1,2} In tissues, IFN-\(\gamma\) binds to and activates specific receptors on histiocytes and dendritic cells, inducing the synthesis and secretion of CXCL9 and CXCL10.\textsuperscript{2} In peripheral blood (PB), these chemokines recruit CD8\(^+\) CTLs, which migrate to the site of inflammation. Subsequent release of CTL-derived IFN-\(\gamma\) at tissue level amplifies the inflammatory response. IFN-\(\gamma\) acts on modulating transcription factor expression and perturbing cytokine signaling.\textsuperscript{3} Indeed, IFN-\(\gamma\) induces expression of SOCS proteins, such as SOCS1 and SOCS3, which, in turn, inhibit STAT protein signaling. This perturbs, for example, responses to thrombopoietin (TPO, which acts through STAT5 inducing hematopoietic stem cell [HSC] self-renewal) and G-CSF (which acts through STAT3 increasing the differentiation and maturation of neutrophil granulocytes).

As already mentioned, IFN-\(\gamma\) has a prominent role in regulation of hematopoiesis during acute or chronic inflammation, acting at multiple levels, from HSCs to differentiated progenitors:
1. **HSCs**: Several works demonstrated that IFN-γ has a strong impact on HSCs. Indeed, (a) it strongly diminishes expansion of HSCs in the primary liquid cultures; (b) it strongly inhibits long-term culture-initiating cell (LTC-IC), indicating functional impairment of HSCs; (c) it decreases the number of self-renewing cell divisions; (d) its overexpression in transgenic mice decreases the number of CFU-GEMM colonies, indicating a loss of functional multipotent HSCs. While the detrimental role on HSCs reconstitution capacities is clear, the effect of IFN-γ on HSCs proliferation is more disputed. In general, it seems that it depends, as for type I IFNs, on the stimulatory conditions.

2. **Myeloid progenitors**: Several studies demonstrated that IFN-γ reciprocally regulates the production of neutrophils and monocytes. Indeed, SOCS3-mediated inhibition of STAT3 reduces G-CSF stimulation; on the other hand, increased expression of PU.1 and IRF8 transcription factors promotes monocyte production. Moreover, IFN-γ reduces the differentiation of myeloid progenitor to eosinophils, which is mediated by interleukin (IL)-5. Notably, this finding is consistent with the fact that IFN-γ is produced mainly during immune responses against intracellular pathogens; thus, favoring monopoiesis at the cost of neutrophils and eosinophils (more active in controlling extracelluar pathogens) may be advantageous.

3. **Erythropoiesis and megakaryocytes**: Anemia is a common feature of chronic inflammation and IFN-γ is a well-known mediator of such a response. There are several responsible mechanisms: (a) alteration of iron metabolism with iron retention in the macrophages; (b) inhibition of early stages of erythroid proliferation and differentiation; (c) indirect inhibition of erythropoiesis via IL-15. For what concern megakaryopoiesis, IFN-γ has ambivalent effects; from one side it impairs TPO signaling (see below), from the other it stimulates platelet production through STAT1 and IRF1/IRF2.

The aims of this review are to summarize our understanding of the role of IFN-γ in the development of HLH, AA, and graft failure (GF) occurring after allogeneic hematopoietic stem cell transplantation (HSCT), and to highlight how recent evidence of the therapeutic potential of IFN-γ inhibition has been applied in the clinic. For this purpose, a brief overview on IFN-γ-driven pathophysiology mechanisms in each disease is given in the specific subsections.

### 2 | HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

HLH is a rare, severe, hyperinflammatory disease due to hyperactivation and accumulation of lymphocytes and macrophages. The disease principally affects the bone marrow (BM), liver, and spleen; it may also affect other organs, including the central nervous system and lungs. Inflammation in HLH is driven by the excessive activation of CD8+ CTLs. Activated CD8+ CTLs recruit and activate other immune effector cells, leading to a “cytokine storm,” translating into the clinical features of the disease. The most common clinical manifestations of HLH are fever, hepatomegaly, and cytopenias, which are frequently accompanied by hypertriglyceridemia, coagulopathy, hepatitis, and neurologic symptoms.

Several cytokines are involved in the inflammatory storm responsible for the clinical and laboratory features of hemophagocytic lymphohistiocytosis (HLH). Notably, there is no widely accepted definition of “cytokine storm”; moreover, there is uncertainty about the distinction between cytokine storm and physiologic inflammatory response. However, apart from IFN-γ, other potential HLH-promoting cytokines are:

- **IL-2**: It is produced by T cells and stimulates CTLs, as well as regulatory T cells (Tregs); it can be increased or even decreased (e.g., in perforin deficiency); moreover, IL-2 can be preferably consumed by hyperactivated CTLs, which upregulate IL-2 receptor (i.e., CD25) expression, thus increasing IL-2 consumption and depriving Tregs of this cytokine.
- **IL-18**: It is produced by monocytes, macrophages, and dendritic cells; high levels of IL-18 induce CTL and macrophage activation. Notably, IL-18 levels are particularly elevated in patients with activating lesions of the inflammasome (XIAP and NLRC4).
- **Tumor necrosis factor (TNF)-α**: The levels of this cytokine are commonly increased in many inflammatory processes, as HLH. Notably, TNF-α and IL-6 are secreted spontaneously by circulating monocytes of patients with HLH. Moreover, Billiau and colleagues documented in situ expression of IL-6 and TNF-α by hemophagocytic macrophages in the liver of patients with HLH.
- **IL-33**: It is an important amplifier of immune dysregulation in murine models of perforin-deficient HLH. In particular, signaling through the IL-33/ST2 axis promoted CTLs activation and production of IFN-γ.

Historically, HLH has been classified into two types. Primary HLH (pHLH), also known as familial HLH, is an inherited (autosomal recessive or X-linked) disorder with a reported incidence of approximately 1/50 000 live births. However, the true incidence is likely to be higher because of underdiagnosis. In patients with pHLH, the clinical features of the disease typically develop during the first years of life.
and median survival is <2 months without treatment. Secondary HLH (sHLH), which is similar in presentation to primary disease, is associated with, or triggered by, viruses or other infections, malignant disease, or rheumatologic disorders. Although genetic testing is used to differentiate between primary and sHLH, their classification as two distinct entities is being revisited because of recent evidence that, in at least some patients, sHLH may develop as a consequence of both acquired and genetic factors. What is clear is that HLH is essentially a spectrum of related disorders that share clinical and immunologic features, but have different (albeit related) etiologies. For this reason, the North American Consortium for Histiocytosis (NACHO) recommends to categorize HLH subtypes based on specific etiology (i.e., familial HLH, rheumatologic HLH, malignancy-associated HLH, HLH with immune compromise, iatrogenic HLH) instead of ambiguously classifying HLH as “primary” or “secondary.”

Diagnosis of HLH is confirmed when a patient either has a molecular diagnosis consistent with HLH, or meets five of the core clinical diagnostic criteria validated by the Histiocyte Society and used in the HLH-2004 protocol (see Table S1). Nine causative gene defects have been identified in patients with pHLH; however, HLH is a phenotype of many recently identified monogenic diseases. The common pathogenic consequence of these defects is impaired cytotoxic machinery of CTLs and NK cells.

The aims of treatment in HLH are: (a) to induce remission by suppressing the hyperinflammatory state related to the immune dysregulation that leads to organ damage and susceptibility to infection, and (b) for pHLH to control the patient disease until HSCT can be performed (it must be emphasized that most of the patients with sHLH do not need HSCT). The treatment protocols run by the Histiocyte Society (HLH-94 and HLH-2004) comprise an epipodophyllotoxin derivative (etoposide [VP-16]) in combination with glucocorticoids and cyclosporin. Dexamethasone is the preferred glucocorticoid because of its greater ability to cross the blood-brain barrier. In addition, intrathecal methotrexate and glucocorticoids are recommended for patients with evidence of central nervous system involvement.

Antithymocyte globulin (ATG) and alemtuzumab have also shown efficacy in frontline treatment or as salvage therapy in patients with resistant or relapsing disease. An initial 8-week treatment course is followed by a continuation phase that acts as a bridge to transplantation, maintaining disease control until a suitable donor is found. Allogeneic HSCT is the only treatment option that has the potential to eradicate pHLH. It should also be considered in relapsed or refractory sHLH.

In HLH-2004 study 5-year probability of survival among 396 patients enrolled was 61% overall and 59% among those with verified pHLH. These results did not represent a significant improvement compared with the previous protocol, HLH-94; this observation, coupled with the significant toxicities associated with HLH-2004, underlines the need for new treatments.

### 3 | IFN-γ IN HLH

Several groups have observed that IFN-γ is essential for the development of HLH and/or CXCL9 are markedly elevated in animal models and patients with HLH. Indeed, measurement of IFN-γ in PB using a commercial IFN-γ release assay (e.g., quantiferon-TB) may help to diagnose HLH. In a mouse model, IFN-γ was found to be essential for the development of HLH-like pathology. Indeed, the administration of neutralizing antibodies to IFN-γ was found to have a positive impact on survival, whereas antibodies neutralizing TNF-α, IL-12, IL-18, and colony-stimulating factors had no effect. Recent mouse model research has suggested that IFN-γ overproduction is responsible for the hematologic manifestations of HLH, whereas immunologic features are caused by excessive consumption of IL-2.

Additionally, blockade of IFN-γ using the anti-IFN-γ antibody XMG1.2 has been shown to significantly decrease levels of CXCL9, CXCL10, and proinflammatory cytokines in a mouse model of HLH secondary to rheumatic disease. Mice treated with XMG1.2 showed significantly improved clinical outcomes, with marked amelioration of ferritin, fibrinogen, and alanine aminotransferase levels, compared with untreated mice.

These and other observations have led to the development and investigation of novel therapeutic agents that block IFN-γ or its signaling pathway, JAK/STAT, as potential treatments for HLH.

Emapalumab is a novel anti-IFN-γ monoclonal antibody that is being investigated in clinical trials in children and adults with either primary or secondary HLH (Table 1). To date, two of these trials have reported results.

In an open-label, single-group, phase II/III trial, 34 children with primary HLH, 27 of whom had previously had conventional HLH treatment, received emapalumab in combination with dexamethasone. Emapalumab was given via intravenous infusion at an initial dosage of 1 mg/kg twice a week, increasing up to 10 mg/kg if clinically appropriate, for 8 weeks. Backbone dexamethasone was administered at a dosage of 5-10 mg/m².

Among previously treated patients, 63% had a response, 70% were able to proceed to HSCT, and 74% were alive at the data cutoff. For all emapalumab-treated patients, the percentages were similar (65%, 65%, and 71%, respectively). The primary efficacy endpoint was met, as the overall response rate was significantly greater than the prespecified null hypothesis (65% vs 40%, P = .005). Response was achieved at a median time of 8 days after drug start, and was associated with marked reductions in CXCL9 levels; 22 patients (including 19 previously treated patients) proceeded to HSCT. The estimated probability of survival at 12 months after transplantation was 89.5% among previously treated patients, and 90.2% overall.

Emapalumab was not associated with clinically relevant toxicities, although there were reports of severe infection (n = 10) and histoplasmosis (n = 1, leading to drug discontinuation). There were 10 deaths, but none was considered related to emapalumab treatment. The
### TABLE 1  Clinical trials of emapalumab (EMA) in the treatment of HLH

| NCT identifier | Phase | Age (years) | HLH subtype | Planned | Actual, total/previoulsy treated | Dosage and administration of EMA | Planned duration of treatment | Duration of follow-up |
|---------------|-------|-------------|-------------|---------|----------------------------------|--------------------------------|-----------------------------|----------------------|
| Ongoing/completed trials with data | | | | | | | | |
| NCT01818492/NCT02069899 | II/III | ≤18 | Primary | 45 | 34/27 | Initial dose 1 mg/kg IV every 3 days; could be increased up to 3, 6, or 10 mg/kg | 8 weeks\(a\) | 1 year\(b\) |
| NCT03311854 | II | ≤18\(c\) | Secondary | 10 | 6/6 | Initial dose 6 mg/kg IV, then 3 mg/kg twice weekly until day 28 | 4 weeks\(d\) | 4 weeks |
| Ongoing trials yet to report | | | | | | | | |
| NCT03312751 | III | ≤18 | Primary | 34 | NA | IV twice weekly; dosage not stated | 4-12 weeks; not more than 6 months | 1 year\(b\) |
| NCT03985423 | II/III | ≥18 | Secondary | 20 | NA | Initial dose 6 mg/kg IV, then 3 mg/kg twice weekly until day 28 | 4 weeks | 1 year\(e\) |

Abbreviations: HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; IV, intravenous infusion; NA, not available. 
\(a\)Duration could be shorter or longer, depending on response and HSCT plan. 
\(b\)Patients were followed up for 1 year after HSCT, or for 1 year after their final dose of EMA if HSCT was not performed. 
\(c\)Actual reported age range of participants was 2-25 years. 
\(d\)Treatment could be stopped earlier if a complete response was achieved. 
\(e\)Patients were followed up for 1 year after their final dose of EMA.

Toxicity profile was consistent with the clinical course of HLH. Only the case of histoplasmosis infection was considered related to emapalumab, because of the role played by IFN-\(\gamma\) in conferring immunity against this pathogen; however, the patient completely recovered with pathogen-specific treatment.

On the basis of these results, emapalumab was approved by the US Food and Drug Administration (FDA) in November 2018 for the treatment of pHLH in patients with refractory, recurrent or progressive disease, or intolerance to conventional HLH therapy.\(^{51}\) Emapalumab data are currently under review by other regulatory authorities.

In another ongoing trial,\(^{48}\) six patients with sHLH associated with juvenile idiopathic arthritis, all unresponsive to prior glucocorticoid-containing therapy, received emapalumab twice weekly. Pharmacodynamic studies showed marked reductions in both CXCL9 and soluble IL-2R levels, indicating neutralization of IFN-\(\gamma\) and deactivation of T cells, respectively. Complete response (Table S2) was achieved in all patients at week 8. Treatment was well tolerated, with no discontinuations due to adverse events. So far, the drug is not yet approved for secondary HLH.

Two other trials of emapalumab in the treatment of HLH are under way (see Table 1 for details).\(^{46,47}\)

Pharmacologic inhibition of the JAK/STAT signaling pathway may offer an alternative to emapalumab as a means of blocking the activity of IFN-\(\gamma\). Encouraging results with ruxolitinib, an oral JAK1/JAK2 inhibitor, have been reported in individual patients,\(^{52-57}\) in small case series\(^{58}\) and in a phase I pilot study in five adults,\(^{59}\) with sHLH. In this study, all patients experienced at least partial resolution of symptoms and laboratory abnormalities, allowing transfusion independence, discontinuation of glucocorticoid, and hospital discharge. Concern has been expressed, however, about lymphoma progression in patients receiving ruxolitinib for the treatment of refractory lymphoma-associated HLH.\(^{60}\)

The efficacy and safety of ruxolitinib (2.5-20 mg twice daily, depending on age/bodyweight) is currently being investigated in four clinical trials in patients with treatment-naïve or relapsed/refractory HLH (see Table 2).\(^{61-64}\) A recent paper reports the outcomes of 12 children affected by secondary HLH treated with ruxolitinib. No major adverse event was recorded. Eight out of 12 patients (66.7%) achieved complete response by day 28. With a median follow-up of 8.2 months, five of 12 patients had an event, leading to an estimated 6-month event-free survival of 58.3%.\(^{65}\) Finally, Meyer and coauthors showed that ruxolitinib treatment sensitizes CD8\(+\) T cells to dexamethasone-induced apoptosis;\(^{66}\) they found that IL-2 and IL-12 induce resistance to dexamethasone in this cell subset, via STAT5, by altering cellular apoptotic potential and that this can be reverted by the administration of ruxolitinib.

It is important to recognize that our understanding of HLH, its pathophysiology, and its relationship to similar diseases is still to be...
TABLE 2  Clinical trials on the use of ruxolitinib (RUXO) for treatment of HLH

| NCT identifier | Phase | Age     | HLH subtype | Planned enrollment | Dosage and administration of RUXO | Other drugs in combination | Planned duration of treatment |
|----------------|-------|---------|-------------|--------------------|----------------------------------|---------------------------|-----------------------------|
| Ongoing/completed trials with data |       |         |             |                    |                                  |                           |                             |
| NCT02400463    | II    | ≥18 years | Secondary   | 6                  | 15 mg twice daily on a continuous 28-day cycle | -                         | To be continued indefinitely<sup>a</sup> |

Ongoing trials yet to report

| NCT03795909    | I-II  | 1-18 years | Secondary    | 50                 | 2.5 mg twice daily or 5 mg twice daily or 10 mg twice daily<sup>b</sup> | Dexamethasone        | 8 weeks?                     |
| NCT04551131    | Ib/II | 6 weeks-22 years | Primary<sup>c</sup> | 62                 | 25 mg/m<sup>2</sup> per dose twice daily | Dexamethasone and etoposide | 8 weeks                     |
| NCT04120090    | 3     | 1-75 years | Any R/R HLH  | 80                 | Low-dose 10 mg twice daily<sup>d</sup> | NA                      | NA                           |
| NCT03533790    | 3     | 1-70 years | Any R/R HLH  | 80                 | 0.3 mg/kg/day for 2 weeks for 2 cycles | Doxorubicin 25 mg/m<sup>2</sup> day 1; etoposide 100 mg/m<sup>2</sup> on day 1; methylprednisolone 2 mg/kg days 1-5, then gradual tapering | 4 weeks?                     |

Abbreviations: HLH, hemophagocytic lymphohistiocytosis; NA, not available; NYR, not yet recruiting; R/R, refractory/relapsed.

<sup>a</sup>Per protocol, treatment is to be continued indefinitely until disease progression, unacceptable toxicity, or meeting any other condition(s) for treatment discontinuation.

<sup>b</sup>2.5 mg twice daily for patients if age < 14 years and weight < 25 kg, 5 mg twice daily for patients if age < 14 years and weight ≥ 25 kg, 10 mg twice daily for patients if age ≥ 14 and < 18 years.

<sup>c</sup>Treatment-naïve or refractory/relapsed.

<sup>d</sup>For children of age < 14 years and weight ≥ 25 kg, the dose was generally 10 mg twice daily. For children of age < 14 years and weight < 25 kg, the dose was generally 2.5 mg twice daily.

In view of this changing treatment scenario, with several biological treatments becoming available (especially for secondary forms of the disease), it remains to be determined which agents fit better in different clinical settings.

4  | IFN-γ IN GRAFT FAILURE FOLLOWING HSCT

GF can occur in up to 30% of patients undergoing HSCT; its occurrence is correlated with type of disease, conditioning regimen used, and type of donor employed, and is associated with significant mortality. Risk factors for GF include human leukocyte antigen (HLA) and blood group mismatching in the donor-recipient pair, use of reduced intensity conditioning or myelosuppressive drugs, and viral infection.
Evidence from animal studies suggests that IFN-γ may have an important pathogenic role in GF.\(^7\) Via direct and indirect mechanisms on hematopoiesis (Figure 2). Studies in mice have shown that IFN-γ directly impairs the ability of hematopoietic stem and progenitor cells (HSPCs) to self-renew, proliferate, and differentiate.\(^6,7\) Additionally, IFN-γ induces the expression of Fas (CD95) on HSPCs, leading to an increase in cytotoxic T-lymphocyte-mediated apoptosis.\(^7,7\) Moreover, IFN-γ impairs in vitro maintenance of mesenchymal stromal cells (MSCs), altering their ability to support hematopoiesis.\(^7\) These mechanisms may also be important in the pathogenesis of AA (see below).

However, clinical data on the role of IFN-γ in GF are lacking, except for the indirect evidence provided by the observation that patients with IFN-γ-receptor 1 deficiency experience very high rates of primary and secondary rejection after HLA-identical HSCT.\(^8\) Thus, with the goal of exploring the clinical relationship between IFN-γ and GF, levels of several cytokines/chemokines were measured in 15 children with GF following HSCT and compared with those of 15 controls (with sustained donor cell engraftment).\(^8\) Serum levels of IFN-γ, CXCL9, IL-10, and TNF-α were higher in children with GF than in controls during the first 30 days after transplantation. The difference between groups was significant already at day 3 for all factors, and at days 7 and 14 for IFN-γ, CXCL9, and IL-10. Cell-surface markers of activation and exhaustion on both CD4+ and CD8+ cells were consistent with prolonged T-cell activation in patients with GF.\(^8\) Experiments in Ifngr1−/− mice also demonstrated that sole neutralizing IFN-γ by administering XMG1.2 before and after HSCT increased the proportion of engrafted donor cells compared with controls.\(^8\)

Three of the 15 reported children experiencing GF were affected by HLH; they received emapalumab (1-6 mg/kg by IV infusion every 3 days) on a compassionate-use basis in order to control HLH flare and, possibly, favor engraftment of the second allograft.\(^8\) Engraftment was successful in two patients, both of whom had CXCL9 levels <102 pg/mL, while the third patient, who had higher CXCL9 levels, experienced a new episode of GF after the second allograft. Taken together, these data suggest that CXCL9 serum levels may be used to predict GF in HSCT recipients, and that IFN-γ may be a potential therapeutic target for its prevention and/or treatment.

A recent case report on a child affected by ADA-SCID further corroborates this hypothesis: the child experienced two episodes of GF...
and was successfully treated with emapalumab to control GF-related HLH, as well as to prevent GF during the third HSCT. Notably, the child had several infections (including disseminated BCGitis, adenoviral infection and invasive aspergillosis) at the time of emapalumab treatment; nonetheless, these infections did not worsen with IFN-γ blockade.

5 | IFN-γ IN APLASTIC ANEMIA

The pathophysiology of acquired AA, a disease characterized by BM aplasia and PB pancytopenia, is complex and not fully understood. However, the observation that most patients with acquired AA respond to immunosuppressive therapy (IST) strongly points out to an immune basis for the disease. Available evidence now suggests that acquired AA is caused by a loss of HSPCs, secondary to T-cell attack and cytokine-mediated immune dysfunction.

In 1985, Zoumbos et al proposed a role for IFN-γ in the pathogenesis of AA based on observations that both production of IFN-γ in PB mononuclear cells and IFN-γ levels in BM sera were increased in patients with AA. In the same study, the authors showed that in vitro inhibition of IFN-γ through anti-interferon antisera resulted in an increase in hematopoietic colony formation in BM cells from AA patients. Another work implicated IFN-γ production by activated suppressor T lymphocytes in the pathogenesis of BM failure. In 1990, Marsh et al demonstrated the utility of the long-term BM culture (LTBMC) system for studying the hematopoietic defect in patients with AA. Several years later, LTBMC, in conjunction with LTC-IC assay, was used to demonstrate the profound suppression of hematopoiesis in vitro by human stromal cells retrovirally transduced to secrete IFN-γ. These findings supported an earlier work, which showed that IFN-γ potently suppressed growth of HSPCs at concentrations of 750-1000 U/mL, and triggered apoptosis of BM total and CD34+ cells.

Clinical studies have shown that approximately half of patients with severe AA have increased levels of IFN-γ in circulating T cells. Conversely, IFN-γ was not detected in PB lymphocytes from patients with other hematologic disorders, or from healthy volunteers. Additionally, the presence of IFN-γ in circulating T cells and BM was found to be predictive of response to IST. As noted above, IFN-γ increases the susceptibility of HSPCs to apoptosis; this, in combination with increased levels of IFN-γ in BM, could be sufficient to cause AA. Moreover, it has been shown that polymorphism VNDR1349 in IFNG, which causes an increased production of IFN-γ in vitro, is associated with an increased risk of AA. Together with TNF-α, increased levels of IFN-γ have been found in BM of patients with Fanconi anemia, an inherited BM failure syndrome. However, despite the growing evidence on the possible role of IFN-γ in AA pathogenesis, no data on the effects of in vivo inhibition of this cytokine are available.

Recently, it has been proposed that IFN-γ may exert its inhibitory effects on hematopoiesis also via steric hindrance of the interaction between thrombopoietin and its receptor, c-MPL, on HSPCs. Eltrombopag, a small-molecule nonpeptide agonist of c-MPL, is able to bypass this inhibition because its binding site is distinct to that of thrombopoietin. This explains its efficacy in the treatment of BM failure, even in the presence of high endogenous thrombopoietin levels.

Eltrombopag was originally shown to have efficacy in the treatment of AA refractory to IST. In a phase II trial of eltrombopag (up to 150 mg/day; NCT00922883), 11 (44%) of 25 patients had a hematologic response in at least one lineage, and nine were able to stop platelet transfusions. Encouragingly, response was associated with normalization of BM cellularity in three of four patients. Treatment was well tolerated; notably, there was no evidence of increased BM fibrosis in trephine specimens from patients treated for up to 30 months. Subsequent expansion of the study population provided additional support to the utility of eltrombopag in this setting, and showed that stable response could be maintained after treatment discontinuation in at least some responders.

### TABLE 3

Response rates assessed at 6 months in a phase I/II clinical trial of eltrombopag in the treatment of severe aplastic anemia (NCT01623167) [96–98,108]

| Parameter                  | Eltrombopag * | Historical controls |
|----------------------------|---------------|---------------------|
|                            | Cohort 1 (n = 30) | Cohort 2 (n = 31) | Cohort 3 (n = 31) | Totalâ |              |
| Complete response, n (%)   | 10 (33)       | 8 (26)              | 18 (58)           | 36 (39) | -             |
| Partial response, n (%)    | 14 (47)       | 19 (61)             | 11 (35)           | 44 (48) | -             |
| Overall response, n (%)    | 24 (80)       | 27 (87)             | 29 (94)           | 80 (87) | 67 (66)      |
| Patients ≤ 18 years 108     | -             | -                   | -                 | 28 (72) | 64 (74)      |

Note: Eltrombopag was added to standard immunosuppressive therapy with horse antithymocyte globulin (ATG) and cyclosporin.

*Patients received eltrombopag daily from day 14 to 6 months (Cohort 1), day 14 to 3 months (Cohort 2), or day 1 to 6 months (Cohort 3).

**All patients: n = 92; patients <18 years: n = 19.**

†Data for patients with AA (n = 102) who received horse ATG and cyclosporin for 6 months in two clinical trials conducted between 2003 and 2010.**7,97-98**

‡Data for patients with AA (n = 87), aged <18 years, who received horse ATG and cyclosporin, with or without additional immunosuppression, for 6 months between 1989 and 2010.**108**

*P < .001 versus historical controls.
In a phase I/II trial (NCT01623167) in previously untreated patients with severe AA (n = 92, 19 aged <18 years), eltrombopag was added to standard IST with horse ATG and cyclosporin and continued for up to 6 months. Eltrombopag was associated with improvements in overall hematologic response, in all cohorts, compared with historical controls who received only IST (Table 3). On this basis, eltrombopag has received approval from both the FDA and European Medicines Agency for the treatment of adults with severe AA refractory to IST. This is considered a major advance, because in the last 20 years clinical trials challenging the standard of care have failed to demonstrate any further improvement in outcomes for AA patients. Although eltrombopag is approved for use in children ≥1 year with idiopathic thrombocytopenic purpura, it is not currently approved or recommended for pediatric patients with AA. Dedicated pediatric clinical trials investigating the pharmacokinetics, efficacy, and safety of eltrombopag in acquired AA are ongoing. In the meantime, a subgroup analysis of pediatric patients enrolled in NCT01623167 has been performed. In contrast to adult patients, the addition of eltrombopag to IST did not improve overall response rates at 6 months, suggesting that there may be age-related differences in the pathophysiology of acquired AA that have implications for treatment.

6 | SUMMARY

There is now substantial evidence that increased/excessive levels of IFN-γ and/or its induced chemokines play an important role in the development of HLH, AA, and GF, and these findings have created new opportunities to improve outcomes in these rare disorders. Emapalumab, a specific monoclonal antibody neutralizing IFN-γ, has shown clinical utility in the treatment of HLH, and has been approved by the FDA for use in children and adults with primary disease in whom standard therapy has failed or was poorly tolerated. Clinical trials of emapalumab in secondary HLH are ongoing. In addition, interest in JAK/STAT pathway inhibition as a means of blocking IFN-γ has led to the initiation of clinical trials of ruxolitinib in relapsed/refractory HLH.

In the treatment of severe AA, the c-MPL agonist eltrombopag circumvents IFN-γ-mediated hematopoietic suppression, and has been shown to induce both hematologic responses and cellular regeneration of BM. As multiple cytokines (including IFN-γ) have been found to play a role in the pathogenesis of acute GvHD, it cannot be excluded that the beneficial role of ruxolitinib in this complication be partly due to the blockade of IFN-γ-pathway signaling.

Finally, since IFN-γ has been identified as one of the cytokines of immunotherapy-related cytokine-release syndrome and can play a role in CAR T-cell-related HLH, future studies will investigate if its inhibition can be exploited to control this complication, without jeopardizing the antitumor effect displayed by CAR T cells.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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