



# Consensus opinion on immune-mediated cytopenias after hematopoietic cell transplant for inherited metabolic disorders

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## Abstract

Hematopoietic stem cell transplantation (HCT) has been increasingly used for patients with inherited metabolic disorders (IMD). Immune mediated cytopenias (IMCs) after HCT, manifesting as hemolytic anemia, thrombocytopenia, and/or neutropenia, are recognized as a significant complication in this patient population, yet our understanding of the incidence, risk factors, and pathophysiology is currently limited. Review of the published literature demonstrates a higher incidence in younger patients who undergo HCT for a nonmalignant disease indication. However, a few reports suggest that the incidence is even higher among those with IMD (incidence ranging from 10 to 56%). This review summarizes the literature, provides an approach to better understanding of the possible etiology of IMCs, and proposes a diagnostic and management plan for patients with IMD who develop single or multi-lineage cytopenias after HCT.

## Introduction

Over the past 30 years, use of hematopoietic stem cell transplantation (HCT) has expanded beyond the treatment of malignant disorders, with increasing numbers of transplants being performed for a wide spectrum of non-malignant conditions that include primary immunodeficiency diseases, marrow failure syndromes, and inherited metabolic disorders (IMD). Immune mediated cytopenias (IMC), often described as either allo- or auto-immune cytopenias, after HCT have been increasingly recognized as a significant complication in patients with both malignant and nonmalignant diseases, manifesting as hemolytic anemia, thrombocytopenia, and/or neutropenia

alone or in combination [1–3]. The very task of naming this cluster as autoimmune or alloimmune cytopenias is difficult as the underlying etiology is still largely unclear. We have referred to these as broadly IMCs owing to the lack of clarity in the pathophysiology of the condition in some cases. IMC includes both autoimmune and alloimmune etiology for the development of these immune cytopenias. For the purpose of this review, we have used the term IMC and the individual cytopenias labeled as immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (IMT), and immune-mediated neutropenia (IMN).

Reports from single centers and international registries have provided some guidance on the general incidence, risk factors, time of onset, treatment strategies, and outcomes of patients who develop IMC [4–9]. However, only limited information is available on the incidence of IMC in those with IMD, potentially a higher risk population. Furthermore, there are no clear guidelines on the diagnosis and management of IMC after transplantation. Since IMD itself appears to be an independent risk factor for IMC or has a relatively higher incidence of IMC [10–14], a multinational panel of physician experts in the treatment of IMD was convened, with the primary aim to identify the strategies for effective and timely diagnosis and develop a management strategy for IMC in this patient population. The panel included experts in HCT for IMDs from centers with experience in treating these disorders as well experts in

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nonmalignant disorders and transplant immunology. This report summarizes a review of the current literature and proposes a diagnostic algorithm, monitoring plan, and treatment strategy, that might inform future trials focused on IMD.

## Incidence

The literature review was performed using the key words “posttransplant cytopenias”, “HCT and autoimmune hemolytic anemia”, “HCT and autoimmune thrombocytopenia”, and “HCT and autoimmune neutropenia” investigating the databases such as Pubmed, Google Scholar, and Medline. Despite the recognized importance of IMC after transplantation, review of the literature demonstrated a lack of consistent rules for the diagnosis of IMC following HCT. Inconsistency in the criteria for diagnosis and management of IMC by individual investigators highlight the challenge with developing a uniform approach for this complex issue. Based on the review of available literature, seven publications were identified that examined the incidence of cytopenias in recipients of HCT for IMDs (Table 1). In these analyses, the diagnosis of IMC either required the presence of documented antibody directed against one or more hematopoietic lineages or used other laboratory markers in the setting of cytopenias consistent with such a diagnosis in the absence of documented antibody. While the presence of antibody is highly likely documented for the red blood cell lineage, it is less commonly detected in those with thrombocytopenia and neutropenia [10, 12]. For this reason, the physician’s working diagnosis of IMC was sufficient in some reports, despite the absence of a documented antibody [7, 15]. The overall incidence of IMC after allogeneic HCT for various indications ranged from 2.1 to 52.6% [7, 9–12, 16–18]. However, the studies focusing specifically on the IMD cohort, the incidence of IMCs were reported between 10 and 52.6% [6, 7, 10–14, 16], which seems to be substantially higher relative to other disease groups. The cumulative incidence in this patient population from various studies is noted to be 22%, which is higher than those with other nonmalignant disorders (9%) and malignant disorders (2%) (Table 1). Time to onset was typically within the first year after transplant with a median onset of about 4–6 months, though a later onset (median, 1 year) was observed in one study [10–12, 17, 18].

## Risk factors

Review of the literature for the studies including IMD patients potential risk factors for IMC were identified (Table 1). For example, transplantation for a nonmalignant disease and younger recipient age are consistent between the reports. Other risk factors suggested include the type of

conditioning regimen, use of an anti T-cell serotherapy, recipient cytomegalovirus serostatus, use of umbilical cord blood stem cells, use of an unrelated donor source, and the development of graft versus host disease (GVHD) [6, 7, 10–12, 14, 16]. The use of serotherapy such as alemtuzumab based conditioning [9, 19] and minor ABO mismatch between recipient and the donor have also been reported [18]. Since the majority of patients in these reports were treated with myeloablative conditioning, a calcineurin inhibitor and unprocessed hematopoietic stem cells, the risk related to the conditioning intensity, the use of ex vivo lymphocyte depletion or GVHD prophylaxis without a calcineurin inhibitor remains unclear.

## Pathophysiology

The pathophysiology of IMC continues to evolve. The interaction of reconstituting donor immune system and depleted but persistent recipient immune system further questions allo- or autoreactivity in the setting of an immune cytopenia. Multiple factors can influence the immune reconstitution after HCT such as the underlying disease, conditioning regimen, age at transplant, cellular composition, and the type of donor graft used, immunoprophylaxis, complications such as microbial infections and GVHD. Immune status and the degree of immune reconstitution prior to development of IMC can help with better understanding of the immunobiology. A developing thymus in young children, where T cells undergo selection and differentiation, might also play an important role in the development of immune tolerance and autoreactivity [20, 21].

Kruizinga et al. examined the immune status of patients with and without IMC [9]. The absolute number of natural killer (NK), total T-cell numbers and B cells was noted to be similar in the two groups at the time of clinical diagnosis of IMC. However, the absolute number of CD8 + T-cell count was lower and the Th2-specific cytokines (IL-4, IL-5, and IL-13 but not IL-6) were elevated in patients with IMC. This suggests the difference in immune reconstitution and possible B-cell dysregulation [22]. Skewing toward a Th2 response and possible T-effector- regulatory T-cell (Treg) imbalance can result in poor Treg mediated autoimmune dysregulation [23]. However, the results from this limited dataset ( $n = 25$  for IMC patients;  $n = 6$  cases and controls each for cytokine analysis) needs further understanding to discern the underlying pathophysiology. In a recent study by Szanto et al. lower trend for NK and CD3 + CD8 + T cells was identified in patients prior to the diagnosis of IMC, but were not reported to be statistically significant [14]. The study also reported increase in IgM prior to development of IMC compared to patients who did not develop IMC, at similar time points after HCT, but the

**Table 1** Reported incidence of immune-mediated cytopenias in patients with inherited metabolic disorders.

Study	IMD	Other nonmalignant disorders	Malignant disorders	Risk factors
O'Brien et al. [10]	11/100 (IMHA-11)	5/68 (IMHA-5)	3/135 (IMHA-3)	<ul style="list-style-type: none"> <li>• Age &lt;10 years (incidence of IMC 8% in younger patients vs. 1%; <math>p = 0.04</math>)</li> <li>• Diagnosis of IMD (11% vs. 7% in other NMD, vs. 2% malignant)</li> <li>• Nonmalignant disease using CBT vs. bone marrow graft (HR 3.36; <math>p &lt; 0.001</math>)</li> <li>• Interval from diagnosis to CBT 11.4 months (median interval; HR 1.85; <math>p = 0.034</math>)</li> </ul>
Daikler et al. [6] <sup>a</sup>	12/30	14/155	26/570	Age <1 year (28% vs. 5% in those >1 year; $p < 0.01$ )
Page et al. [11]	10/18 (IMHA-4, IMT-1, ES-3, Pancytopenia-2)	NR	NR	<ul style="list-style-type: none"> <li>• Nonmalignant disease vs. malignant disease (OR 6.87; <math>p = 0.00017</math>)</li> <li>• Use of an alternate donor (MUD vs. MFD); (OR 3.73, <math>p = 0.019</math>)</li> </ul>
Faraci 2013 <sup>b</sup>	NE; six IMD patients with IMC (IMHA-2, IMT-1, ES-3)	22/355 (6/22 with IMD) (IMHA-7, IMT-6, ES-1)	11/1219 (IMHA-6, IMT-1, IMN-1 ES-1)	Unrelated donor status (0% MRD vs. 3.7% other donors; $p = 0.04$ )
Ahmed et al. [16]	1/10 (IMHA-1)	4/152 (IMHA-4)	7/326 (IMHA-7)	Pretransplant ALC (adjusted OR = 2.186; $p = 0.037$ )
Deambrosis et al. [12]	8/32	NR	NR	<ul style="list-style-type: none"> <li>• aGvHD grade II-IV (HR 2.45; <math>p = 0.0167</math>)</li> <li>• Chemo-naive prior to HCT (HR 2.36; <math>p = 0.0499</math>)</li> <li>• Serotherapy (HR 8.00; <math>p = 0.045</math>)</li> </ul>
Szanto et al. [14] <sup>b</sup>	NE; ten IMD patients with IMC (IMT-2, ES-3, pancytopenia-5)	21/184 (10 with IMD) (ES-1, pancytopenia-10)	9/196 IMT-1, ES-2, pancytopenia-6)	
<b>Incidence of IMC</b>	<b>42/190 (22%, range 10–56%)</b>	<b>45/730 (6%, range 2–10%)</b>	<b>60/2723 (2%; range 1–4%)</b>	

Bold entries represent the column labels. The cumulative findings in the last row are marked in bold.

IMD indicates inherited metabolic disorders, IMC immune-mediated cytopenia, IMHA immune-mediated thrombocytopenia, IMN immune-mediated neutropenia, ES Evans syndrome, HR hazards ratio, OR odds ratio, MUD matched unrelated donor, MFD matched family donor, CBT cord blood transplant, HCT hematopoietic cell transplant, CMV cytomegalovirus, ALC absolute lymphocyte count, aGvHD acute graft versus host disease, NE not evaluated, NR not reported.

<sup>a</sup>The study does not report type of cytopenia based on the subgroups.

<sup>b</sup>The total number of IMD patients transplanted in the study duration not mentioned, excluded from final incidence calculation for IMD and other nonmalignant disorders.

underlying mechanism for this is unclear. Effectiveness of agents such as rituximab, that target CD20+ B cells, have shown efficacy in IMCs [4, 6–9], suggesting an important role of B cells. T cells on the other hand are important for B-cell activation and antibody production. Previous reports have demonstrated the expansion of autoreactive T- and B-lymphocytes in development of autoimmune disease [15, 24] and the crucial role of the Treg in immune restoration after HCT [25, 26]. With limited information on these variables, further studies are needed for in-depth exploration of the role and interaction of several T- and B-cell mediated factors.

In patients with IMD, several of these factors might predispose to development of IMCs. Unlike other indications for HCT, the underlying immune system in these patients is presumably intact with no prior exposure to chemotherapy or an inherent immune dysregulation. However, lysosomes play an important role in immune processes including antigen processing and presentation on MHCs, though the impact of lysosomal glycosaminoglycan accumulation on innate and adaptive immune system is little understood [27–29]. Impact of a developing thymus in IMD patients, who are relatively younger, along with a higher incidence of mixed T-cell chimerism in patients receiving reduced intensity or reduced toxicity conditioning in the setting of HLA disparity with mismatched UCBs, could potentially further increase the risk of IMC after HCT. However, no clear consensus could be derived from the limited available literature regarding the impact of mixed chimerism and HLA disparity [9, 14, 30]. Nevertheless, IMC could be mediated by recipient T- and B cells targeting donor-derived hematopoietic populations or by B-cell dysregulation secondary to T-cell B-cell imbalance due to delayed T-cell reconstitution leading to auto-antibody (donor anti-donor) production.

### Management and treatment response

Management of IMC after transplant is often challenging, as it can be refractory to multiple lines of treatment or recur as the immune suppression is tapered [10]. Administration of corticosteroids and/or intravenous immunoglobulin (IVIG) along with supportive therapy (e.g., use of granulocyte colony stimulating factor [G-CSF] and transfusion of packed red cells and/or platelets) appears to be the current mainstay of first-line therapy based on the literature [6, 7, 12, 31, 32]. Over the past few years rituximab has increasingly been used as an earlier line of therapy, but with variable response as shown in multiple studies [7, 9]. Kruijzinga et al. reported an ~13% complete remission rate and durable response with steroids alone [9] while Faraci et al. reported a complete response (CR) in ~36% with steroids alone while 87% sustained CR with anti-CD20

mAb (rituximab) [7]. Complete and sustainable response to anti-CD20 mAb as a second line agent was more likely in their autoimmune hemolytic anemia group (78%) compared to those with IMT (33%). For patients with Evans syndrome use of multiple agents resulted in CR in four out of five patients. Additionally, in patients treated with rituximab, CR was obtained after a median of 60 days (range, 30–180 days) from the start of rituximab treatment. In the study by Daikler et al. only partial response was noted in four patients to IVIG alone in single line cytopenias (IMHA and IMT), while additional agents were used in two other patients [6]. Bortezomib, a proteasome inhibitor, has also been used more recently as it targets T cells and plasma cells, which produce antibodies that can be directed against one or more hematopoietic lineages [33–35].

Other agents that have been used for refractory IMC with variable responses including sirolimus [36], mycophenolate mofetil [37], vincristine [38, 39], cyclophosphamide [38], azathioprine [11], as well as physical removal of antibodies by plasma exchange [9]. More recently daratumumab, an anti-CD38 mAb that can eliminate CD38 high plasma cells has been used for hemolytic anemia or Evan's syndrome that developed after allogeneic HCT [40–42]. Though multiple agents are available, and used either alone or in combination depending on the severity of cytopenias, there has been no consistent treatment approach that identifies best practices.

Despite use of multiple agents, sometimes IMCs are refractory and difficult to treat. In a recent report in patients with Hurler syndrome, Deambrosio et al. [12] reported secondary graft failure in two patients who developed pancytopenia related to IMC, requiring second transplant after failing multiple immunosuppressive agents. Gupta et al. also reported two patients developing pancytopenia secondary to IMC in the IMD cohort using busulfan and fludarabine based conditioning following lack of response to multiple immunosuppressive agents [13]. The cumulative mortality in the reported data for those with IMHA (33/90), IMT (1/28), Evans syndrome (2/10), and pancytopenia (1/4) after HCT was summarized by Holbro et al. [8]. Despite the efficacy of initial therapies, IMC can result in graft failure and death from the associated profound immunosuppression [9, 10, 18]. A complete understanding of these risk factors for progression to graft failure and mortality is still largely unclear.

### Consensus recommendations

IMDs are a major group of nonmalignant diseases for which allogeneic HCT has been shown to stabilize disease progression. As the number of patients with IMD treated with HCT increases, IMC is increasingly recognized as a major cause of morbidity that limits the successful use of this

therapeutic approach. The first challenge is defining the diagnostic criteria for IMC. Testing for antibody to hematopoietic lineage cells, HLA, antigens, antibody bound to red cells (DAT, direct antiglobulin test) or autoantigens in the setting of a new onset cytopenias is often used to establish the diagnosis. The relatively low sensitivity of antiplatelet and anti-neutrophil antibody testing compared to that for IMHA, adds another hurdle to diagnose multiple lineage or non-IMHA single lineage restricted cytopenias. Earlier techniques, such as ELISA-based monoclonal antibody immobilization of platelet antigen assay and immunobead-based radioimmune assay for platelet antibody testing are less sensitive and specific (70%). The more recent assays (e.g., flow cytometric immunobead assays) are only marginally better with a sensitivity and specificity of about 80% [43, 44]. Similarly, neutrophil antibody testing based on indirect and direct immunofluorescence assays have low sensitivity and specificity (60–70%) [45]. Since these tests identify the presence of circulating IgG against neutrophils, prior treatment with recently administered IVIG can confound results. For these reasons, the panel felt that the diagnostic criteria could not be based on the presence of antibody alone. The initial workup should exclude other causes of cytopenias, such as use of myelosuppressive drugs, active infections, or other known etiologies of cytopenia, including acute and chronic GVHD, transplant associated thrombotic microangiopathy, viral associated graft suppression or failure, or splenic sequestration secondary to other etiologies. The consensus panel proposed a set of diagnostic criteria for IMC (Table 2), recognizing specific limitations as noted. This also presents a diagnostic framework for evaluation of patients suspected to have IMCs and exclude other possible causes of cytopenias post HCT.

Diagnosis of IMC involving multiple cell lines can be especially difficult to differentiate from other etiologies of graft suppression or failure. IMC, however, typically occurs after primary hematopoietic recovery with substantial, if not complete, chimerism in the myeloid compartment. It is commonly associated with an unexplained, rapid decline in hemoglobin with evidence of hemolysis (increased reticulocyte count, increased lactate dehydrogenase, low haptoglobin, indirect hyperbilirubinemia, and the presence of spherocytes on peripheral smear) and a positive DAT. However, in the setting of a negative DAT the diagnosis should still be considered if other evidence of hemolysis is present. IMT can present as a decrease in platelets without proven antiplatelet antibodies but with a poor response to platelet transfusions. While the marrow can demonstrate normal to decreased megakaryopoiesis, chimerism is often complete. Similarly, IMN can present as decrease in neutrophils without proven anti-neutrophil antibodies. Bone marrow evaluation often shows islands of neutrophil precursors and occasionally clear evidence of maturation arrest.

Szanto et al. tested for the presence of antibodies against all lineages even though there was evidence of cytopenias in only a single lineage and demonstrated the presence of lineage-reactive antibody even when that lineage was not yet affected.

Based on the evidence from literature and the recommendation of the expert panel, the approach to treatment should be based on risk stratification and response to first-line therapy (Fig. 1). The proposed treatment approach is based on the diagnosis and is irrespective of the detection of lineage specific antibody. Patients with an isolated lineage defect with a limited need for transfusion support or G-CSF and usually resolve without the need for further therapy. Moderate risk patients, however, are those with IMHA and/or IMT requiring transfusions not more than once a week to maintain a hemoglobin >7 g/dL and/or platelet count >10,000/ $\mu$ L, or frequent G-CSF support (more than twice a week) to maintain an absolute neutrophil count (ANC)  $\geq$ 200/ $\mu$ L. Overall, these patients usually respond well to a combination of steroids and IVIG as the first-line of therapy. Rituximab can be added to the therapy for those who fail to respond to first-line agents. Severe/high-risk patients are those with severe IMN (ANC <200/ $\mu$ L), IMHA requiring at least twice weekly transfusions (red cells and platelets) to maintain a hemoglobin >7 g/dL, and/or to maintain a platelet count >10,000/ $\mu$ L. These patients are at higher risk for needing prolonged courses of immune suppression and are more likely to require multiple agents to control the disease. In addition, these patients are at a higher risk of infection and graft failure. For these reasons, a more aggressive approach is recommended. The rationale for this systematic approach and the agents used to target different components of the immune system, such as plasma cells, activated T- and B cells, dendritic cells, macrophages, NK cells, and the complement system are shown in Fig. 2. As majority of patients develop IMCs while on post-HCT immunosuppression, another important consideration is switching the calcineurin inhibitors to sirolimus, which has shown improvement in recurrent and refractory IMCs [36]. Use of daratumumab has also now been favored for upfront therapy in moderate to severe IMCs [40–42]. These recommendations from the consensus panel are for consideration but the treating physicians should choose the best approach for their patients based on the individual patient scenario and the availability as well as experience with various agents.

## Future directions

Additional research is necessary to better understand the etiopathophysiology of IMC. Since patients with IMD who undergo HCT are typically young and naive to chemotherapy with an intact immune system, often receive reduced toxicity

**Table 2** Proposed diagnostic approach and criteria.

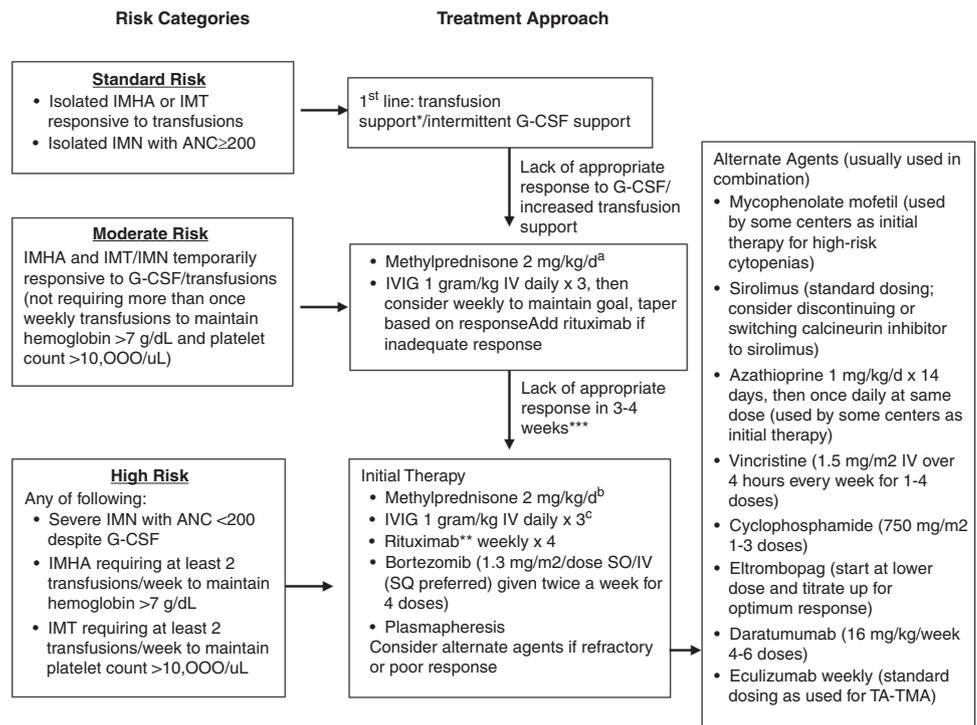
Lineage	Diagnostic approach	Diagnostic criteria for IMC	Limitations
Red cells	<ul style="list-style-type: none"> <li>• Rule out other attributable causes of severe anemia post HCT: bone marrow suppression due to infections (CMV, EBV, Adenovirus, HSV, and other related infections) or medications, graft insufficiency or graft failure based on clinical evaluation and diagnostic testing</li> <li>• Rule out other identifiable cause of hemolytic anemia after transplant: TA-TMA, GVHD, drug induced hemolysis, transfusion reaction and ABO or minor blood group incompatibility based on clinical evaluation and the diagnostic criteria for each</li> </ul>	<ul style="list-style-type: none"> <li>• Hemoglobin <math>\leq 7</math> g/dL (or 70 g/L) or a drop in previously stable hemoglobin of more than 2 g/dL within 5 days (or 20 g/L) without any other attributable cause AND</li> <li>• Presence of red cell directed antibody, DAT positive; if negative the diagnosis should still be considered if there is evidence of hemolysis as suggested by: <ul style="list-style-type: none"> <li>• Presence of spherocytosis and/or other evidence of hemolysis on the peripheral smear such as RBC clumping or agglutination</li> <li>• Suggestive findings: elevated LDH, elevated reticulocyte count, indirect hyperbilirubinemia, low serum haptoglobin</li> </ul> </li> <li>• Platelet count <math>&lt; 20,000</math> cells/mm<sup>3</sup> (<math>20 \times 10^9</math> cells/L) after initial platelet engraftment<sup>a</sup> or a significant drop (<math>&gt; 50\%</math> drop from previous stable platelet count) in platelet count from previously stable levels without any other attributable cause, AND either <ul style="list-style-type: none"> <li>• Presence of platelet directed antibody <ul style="list-style-type: none"> <li>• Anti-GPIIb/IIIa</li> <li>• Donor specific anti-HLA, OR</li> </ul> </li> <li>• If antibody negative, <ul style="list-style-type: none"> <li>• Pattern of a transient response/refractory to platelet transfusions</li> <li>• Other suggestive finding: bone marrow shows normal megakaryopoiesis</li> </ul> </li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• DAT may not detect IgA and other low affinity antibodies</li> <li>• Haptoglobin not always interpretable</li> <li>• LDH is a non-specific marker</li> <li>• Reticulocytopenia can be seen in early IMC (recommend bone marrow evaluation to rule out red cell aplasia)</li> </ul>
Platelets	<ul style="list-style-type: none"> <li>• Rule out other attributable causes of severe thrombocytopenia post HCT: etiologies causing increased platelet destruction (TA-TMA, GVHD, and TTP), splenic sequestration; bone marrow suppression due to infections and/or medications, graft insufficiency or graft failure based on clinical evaluation and diagnostic testing</li> </ul>	<ul style="list-style-type: none"> <li>• Platelet count <math>&lt; 20,000</math> cells/mm<sup>3</sup> (<math>20 \times 10^9</math> cells/L) after initial platelet engraftment<sup>a</sup> or a significant drop (<math>&gt; 50\%</math> drop from previous stable platelet count) in platelet count from previously stable levels without any other attributable cause, AND either <ul style="list-style-type: none"> <li>• Presence of platelet directed antibody <ul style="list-style-type: none"> <li>• Anti-GPIIb/IIIa</li> <li>• Donor specific anti-HLA, OR</li> </ul> </li> <li>• If antibody negative, <ul style="list-style-type: none"> <li>• Pattern of a transient response/refractory to platelet transfusions</li> <li>• Other suggestive finding: bone marrow shows normal megakaryopoiesis</li> </ul> </li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Poor sensitivity and specificity of antibody testing</li> </ul>
Neutrophils	<ul style="list-style-type: none"> <li>• Rule out other attributable causes of severe neutropenia post HCT: bone marrow suppression due to infections and/or medications, graft insufficiency or graft failure based on clinical criteria and diagnostic testing</li> </ul>	<ul style="list-style-type: none"> <li>• Absolute neutrophil count <math>&lt; 500</math> cells/mm<sup>3</sup> (<math>0.5 \times 10^9</math> cells/L) after initial neutrophil engraftment<sup>b</sup>, or a significant drop (<math>&gt; 50\%</math> drop from previous stable neutrophil count) AND either <ul style="list-style-type: none"> <li>• Presence of neutrophil directed antibody <ul style="list-style-type: none"> <li>• Anti-HNA</li> <li>• Donor specific anti-HLA, OR</li> </ul> </li> <li>• If antibody negative, despite <math>&gt; 95\%</math> donor myeloid chimerism <ul style="list-style-type: none"> <li>• Marrow myeloid arrest or absence with recovery of red cells and platelets</li> <li>• Poor or no response to G-CSF</li> </ul> </li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Poor sensitivity and specificity of antibody testing</li> <li>• False positive neutrophil antibody testing in the setting of IVIG use</li> </ul>

**C3 complement 3, DAT direct antibody test, G-CSF granulocyte colony stimulating factor, GPIIb/IIIa glycoprotein IIb/IIIa, GVHD graft versus host disease, HLA human leukocyte antigen, HNA human neutrophil antigen, IgG immunoglobulin G, IMC immune-mediated cytopenia, IVIG intravenous immunoglobulin, LDH lactate dehydrogenase, MMF mycophenolate mofetil, RBC red blood cell, TA-TMA transplant associated thrombotic microangiopathy, TTP thrombotic thrombocytopenic purpura.**

<sup>a</sup>Platelet engraftment defined as platelet count of  $> 50,000/\text{mm}^3$  for at least 7 consecutive days after transplant not supported by transfusion.

<sup>b</sup>Neutrophil engraftment defined as absolute neutrophil count  $> 500$  cells/mm<sup>3</sup> for 3 consecutive days after HCT.

**Fig. 1** Proposed risk categories and treatment algorithm for immune cytopenias.



\*Transfusion Goal: Maintain Hemoglobin  $\geq 7$  g/dL, Platelets  $\geq 10,000$ /uL

a- Once counts stabilize, taper when transfusion needed  $<7$  days. Start tapering at 10% every 5-7 days over 8-10 weeks. Consider slower taper with longer treatment at 0.5mg/kg/day for recurrent or refractory cytopenias

b- Taper when ANC  $\geq 200$ , transfusions needed  $<7$  days (IMHA) and  $<3$  days (IMT). Start tapering at 10% every 5-7 days over 8-10 weeks.

c- Consider replacing IgG for levels below 400, especially when used with rituximab. When using along with plasmapheresis recommended to do it after completion of plasmapheresis cycles.

\*\*Rituximab- 375 mg/m<sup>2</sup> dose weekly, up to 4 doses, use of rituximab can be [MOU1] associated with neutropenia and thrombocytopenia ~8-10 weeks post treatment.

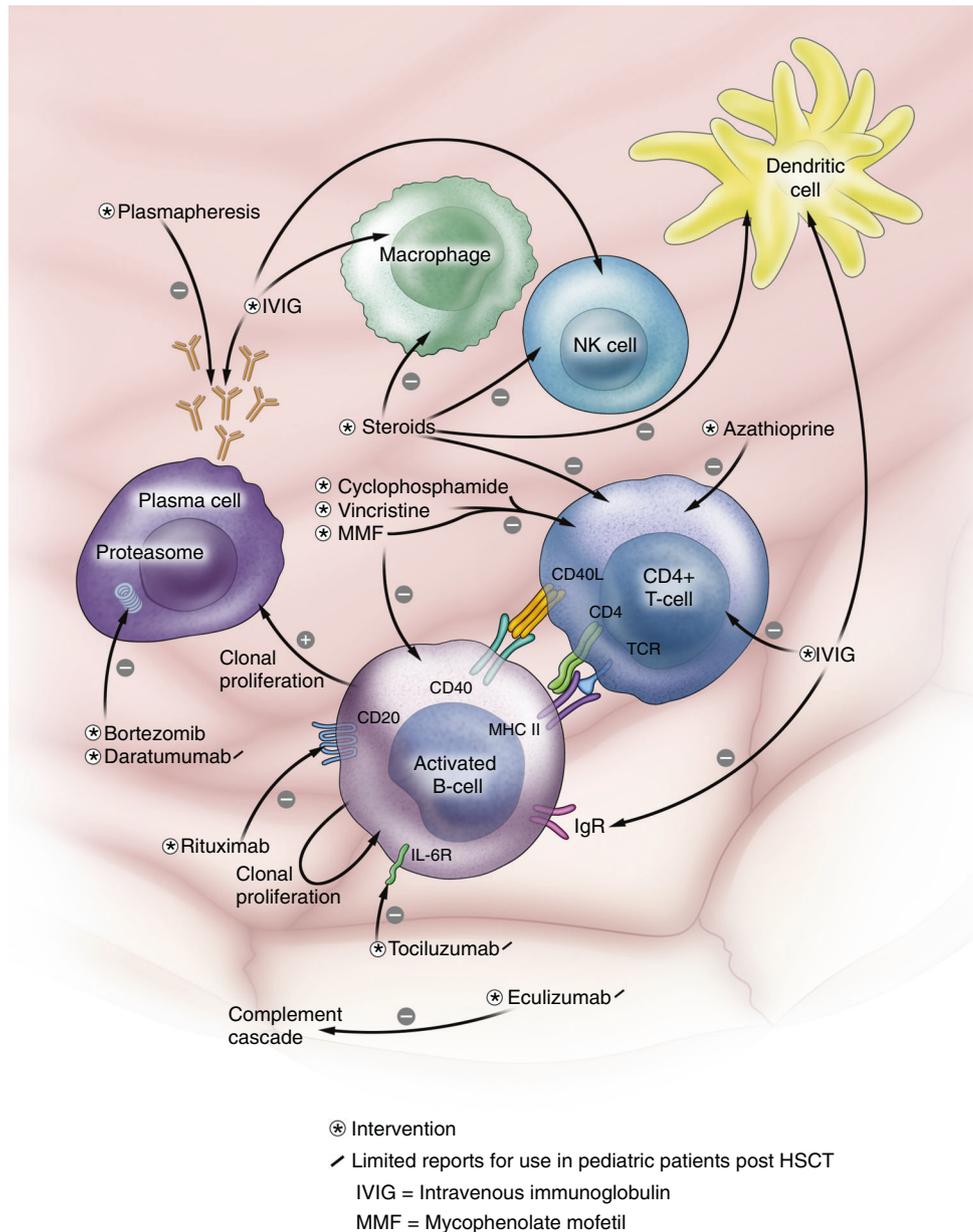
\*\*\*includes reduced transfusion requirement and/or G-CSF support along with stable counts. Add bortezomib and plasmapheresis, if refractory consider alternate agents.

conditioning leading to mixed chimerism, and frequently transplanted with HLA mismatched unrelated donor HSCs, they may be particularly high risk for this complication. One of the major challenges is the lack of a consistent definition for IMC after HCT. Various studies have defined it differently and there is a need for consensus on the definition in order to identify the true incidence and assess the risk factors. Larger registry based (such as CIBMTR, Eurocord) and multicenter studies are required to further delineate populations at high risk and develop strategies for diagnosis and management in high-risk populations.

Another challenge is to identify the underlying pathophysiology of this process. Several biomarkers can be used to demarcate the immune biology with T-cell markers for Tregs (CD4/25br/127lo/FoxP3+), T-follicular helper cells, lymphocyte subsets including NK cells, B cells, T cells with activated CD4 and CD8 subsets, cytokine such as plasma B-cell activating factor, chemokines like CXCL13, CXCR4, immunoglobulins IgG, A, M, D, and their subclasses, B-cell activation markers and memory B-cell markers, plasmablasts (CD19LO CD27-), interleukins (soluble IL-6), and complement activation markers (C5a, sC5b9).

Newer agents and treatment strategies should be considered for multi-lineage immune cytopenias and those refractory to first or second line agents. Daratumumab has been used in refractory immune cytopenias and more reports are encouraging earlier use in immune cytopenias [40-42, 46]. Carfilzomib is a newer proteasome inhibitor that irreversibly binds and permanently inhibits activity. More effective B-cell depletion can be achieved with newer agents such as obinutuzumab, a humanized, type II, immunoglobulin- G1 anti-CD20 mAb, which acts through antibody-dependent cellular cytotoxicity and leads to direct apoptosis of mature B cells [47]. B-cell development and proliferation can also be targeted by agents such as ibrutinib that inhibit bruton tyrosine kinase, which is an important signaling protein for B-cell receptor. Costimulatory blocking of IgG Fc fusion protein containing cytotoxic T lymphocyte-associated protein-4 (CTLA4-Ig) has shown to modulate humoral immunity and B-cell function at the level of B cell-T follicular helper cell interaction in renal transplant patients [48] and could be another agent of potential interest. Antibodies targeting complement system (eculizumab) and IL-6/IL-6R (clazakizumab, tocilizumab) are other

**Fig. 2** Current and future targets for intervention in immune mediated cytopenias.



agents that could play important role in blocking the immune interaction.

IMC is a known complication in patients undergoing allogeneic HCT. It is a major complication with substantial morbidity in young children who receive HCT for IMDs. Greater consistency in defining the immune cytopenias, the diagnostic workup and treatment may help identify best practices for reducing the morbidity and mortality associate with this complication. Serial investigation of immune reconstitution including enumeration of lymphocyte and immunoglobulin subsets and cytokine profiles may yield new surveillance strategies for identifying those at highest risk.

Based on a 1-day, focused panel discussion of experts in the treatment of patients with IMD by HCT, a consensus opinion was developed on the diagnostic criteria and management of this increasingly important complication. This work represents a starting point for optimizing the treatment of IMC and possibly better understanding its etiology and pathophysiology. Further analysis of multicenter data is ongoing to better understand the risk factors and outcomes of IMC after HCT in children with IMDs.

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## Compliance with ethical standards

**Conflict of interest** This expert consensus was based on information developed for and during a meeting supported by Magenta Therapeutics and subsequent data and literature review along with discussions. The content of this paper is solely that of the authors.

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