

# Acute graft-versus-host disease

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## Abstract

Acute graft-versus-host disease (GVHD) is a common immune complication that can occur after allogeneic haematopoietic cell transplantation (alloHCT). Acute GVHD is a major health problem in these patients, and is associated with high morbidity and mortality. Acute GVHD is caused by the recognition and the destruction of the recipient tissues and organs by the donor immune effector cells. This condition usually occurs within the first 3 months after alloHCT, but later onset is possible. Targeted organs include the skin, the lower and upper gastrointestinal tract and the liver. Diagnosis is mainly based on clinical examination, and complementary examinations are performed to exclude differential diagnoses. Preventive treatment for acute GVHD is administered to all patients who receive alloHCT, although it is not always effective. Steroids are used for first-line treatment, and the Janus kinase 2 (JAK2) inhibitor ruxolitinib is second-line treatment. No validated treatments are available for acute GVHD that is refractory to steroids and ruxolitinib, and therefore it remains an unmet medical need.

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Introduction

Epidemiology

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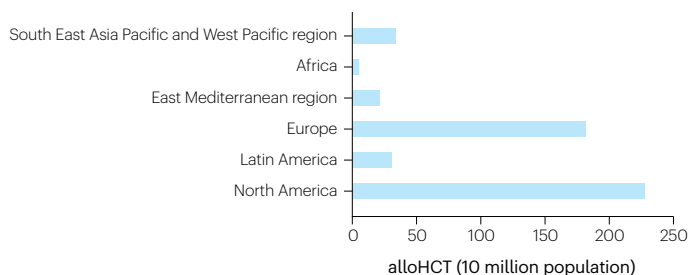
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## Introduction

Acute graft-versus-host disease (GVHD) was first described as a secondary disease that developed after recovery from conditioning-associated toxicity in murine models of bone marrow transplantation<sup>1</sup>. Mice developed inactivity, wasting syndrome, and skin, fur, and posture changes, and died from 'secondary disease'. The 1966 criteria of Billingham accurately characterize standards for GVHD: the donor graft must contain immunocompetent cells, the recipient must express tissue antigens which are different from the donor's, and the recipient must be unable to mount an adequate response to eliminate and reject the donor graft<sup>2</sup>. These criteria are applicable for both allogeneic haematopoietic cell transplantations (alloHCT)-associated GVHD and GVHD occurring after blood transfusion in individuals who are immunosuppressed.

The number of alloHCT performed annually continues to rise<sup>3</sup>, despite a transient decrease during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic<sup>4</sup>. In 2016, the last year for which data are available, 38,425 alloHCT were performed worldwide, compared with 20,333 in 2006, an increase of 89.0%<sup>5</sup>. Despite this increase, the incidence of alloHCT is higher in North America and Europe than in the rest of the world (Fig. 1). For haematological malignancies, the therapeutic efficacy of alloHCT relies on the cytotoxic effect of the conditioning regimen (Box 1) and on the immune-mediated graft-versus-tumour or graft-versus-leukaemia (GVL) effect. However, this effect is counterbalanced by the destruction of the tissue and organ by the donor immune effector cells, termed GVHD. Apart from the relapse of the underlying malignancy, GVHD remains the major complication after alloHCT and is associated with high morbidity and mortality<sup>6</sup>.

GVHD can be subclassified as acute or chronic disease. Acute and chronic GVHD were previously distinguished by the timing of their onset: acute GVHD was classified as symptom presentation before 100 days after alloHCT and chronic GVHD was classified as symptom presentation >100 days after alloHCT. This classification was refined by a National Institutes of Health (NIH) consensus conference in 2005, and the distinction between acute and chronic GVHD is now based on the features of the disease<sup>7</sup>. Chronic GVHD can affect any organ in the body (acute GVHD primarily affects the skin, liver and gastrointestinal (GI) tract), with no time limit on diagnosis. Disease that fulfils the NIH definition of chronic GVHD is classified as either classic chronic GVHD or overlap chronic GVHD if acute GVHD is also present, irrespective of the time of GVHD onset<sup>7,8</sup> (Fig. 2). GVHD with acute symptoms only is classified as classic acute GVHD (first episode within 100 days of transplantation),



**Fig. 1 | Worldwide incidence of alloHCT in 2016.** The number of alloHCT carried out per 10 million population is higher in North America and Europe than in other continents<sup>5</sup>. This difference is explained by an accessibility issue due to cost of the procedure and the low numbers of teams that perform alloHCT in Africa, the East Mediterranean region, South East Asia Pacific and West Pacific region and Latin America compared with the numbers in North America and Europe<sup>5</sup>. alloHCT, allogeneic haematopoietic cell transplantation.

late onset acute GVHD (first episode more than 100 days after transplantation), recurrent acute GVHD (new episode of acute GVHD more than 100 days after transplantation in a patient with a history of classic acute GVHD), or persistent acute GVHD (classic acute GVHD symptoms that persist for more than 100 days after transplantation).

Acute GVHD can also be graded based on severity as I (mild), II (moderate), III (severe) and IV (very severe), based on quantification of skin rash for skin acute GVHD, serum bilirubin level for liver acute GVHD, volume of diarrhoea for lower GI acute GVHD and persistent nausea for upper GI acute GVHD<sup>9</sup>. Grade I acute GVHD is usually not considered as clinically important given its lack of effect on patient outcome<sup>10</sup>; therefore, most studies focus on grade II–IV and severe grade III–IV acute GVHD. Several systems can be used for grading acute GVHD. The MAGIC grading system is not yet used in routine clinical practice; however, it is used in this Primer as it facilitates and helps standardize acute GVHD clinical data collection, as shown by the development and validation of the electronic eGVHD application to assist health-care professionals in the assessment of acute GVHD in clinical practice<sup>11,12</sup>.

This Primer discusses the epidemiology and pathophysiological mechanisms of acute GVHD. This Primer also discusses management, patient quality of life (QOL) and outstanding research questions including the need for more efficient prophylaxis.

## Epidemiology

### Incidence

In the absence of effective prophylaxis, most patients develop acute GVHD; for example, in one historical series, only 19 of 93 patients did not develop acute GVHD when no prophylaxis was administered<sup>13</sup>. Nevertheless, acute GVHD can still occur, despite the routine use of prophylaxis after alloHCT. Acute GVHD incidence varies considerably depending, predominantly, on the degree of mismatch between HLA protein and the GVHD prophylaxis administered (Supplementary Table 1). In the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1202 study<sup>14</sup>, the cumulative 100-day incidence was 62% for acute GVHD reported by centres and 49% after validation by an end point review committee<sup>14</sup>. Of note, the incidence of acute GVHD seems to be decreasing over time. In one study, the incidence of grade II–IV acute GVHD was 40% and the incidence of grade III–IV acute GVHD was 19% in patients who received a transplant during the period 1990–1995, and 28% and 11%, respectively, in those who received a transplant during the period 2011–2015 (ref. 15).

Evaluating the incidence of acute GVHD associated with specific countries or continents is not possible as it is directly dependent on the incidence of alloHCT, which is higher in Europe and North America than in other regions<sup>5</sup> (Fig. 1). Some studies have suggested that ethnicity affects the risk of developing acute GVHD. Indeed, a higher incidence of grade III–IV acute GVHD was reported in Black patients who received alloHCT from HLA-identical sibling donors than in white patients (37% versus 21%, respectively;  $P = 0.047$ ) and in those who received alloHCT from unrelated donors (61% versus 36%, respectively;  $P = 0.014$ )<sup>16</sup>. However, a higher incidence of acute GVHD in Black patients was not confirmed in more recent studies<sup>17,18</sup>. In another study, Asian patients had a significantly lower incidence of acute GVHD than white patients; the incidence of grade II–IV acute GVHD was 40.0% in Japanese patients, 42.1% in non-Japanese Asian patients and 56.5% in white patients, and the incidence of grade III–IV acute GVHD was 15.3% in Japanese patients, 15.7% in non-Japanese Asian patients and 22.6% in white patients ( $P < 0.001$ )<sup>19</sup>. Moreover, the incidence of grade III or IV acute GVHD was significantly lower in Japanese patients than in white patients irrespective of the stem cell source ( $n = 2,652$ ; HR 0.74, 95% CI 0.57–0.96)<sup>20</sup>.

## Survival

Despite the improvement in overall survival (OS) in patients with acute GVHD over time<sup>21,22</sup>, mortality remains high, with a 1-year OS of 70% in patients with grade II acute GVHD and 40% in patients with grade III–IV acute GVHD<sup>21</sup>. Both overall mortality and length of hospital stay were significantly increased in patients who developed acute GVHD during alloHCT admission compared with patients who did not develop acute GVHD (overall mortality 16.2% versus 5.3%;  $P < 0.01$ ; length of hospital stay 42.0 versus 26.0 days;  $P < 0.01$ ).

## Risk factors

Numerous risk factors for acute GVHD have been identified, including degree of HLA disparity (unrelated donor or HLA-mismatched donor)<sup>23,24</sup>, stem cell source (higher risk of acute GVHD with peripheral blood and bone marrow graft versus umbilical cord blood)<sup>25</sup>, donor and recipient sex disparity (female donor to male patient)<sup>26</sup>, higher intensity of alloHCT conditioning regimen<sup>25</sup> and type of GVHD prophylaxis. Other risk factors, such as cytomegalovirus (CMV) negativity in both donor and recipient<sup>25</sup> and older donor age<sup>27</sup>, have also been reported as acute GVHD risk factors. In the largest study to date, unrelated donor (HR 1.61, 95% CI 1.54–1.67;  $P < 0.001$ ), underlying malignancy not in complete remission at alloHCT (HR 1.25, 95% CI 1.2–1.3;  $P < 0.001$ ) or untreated (HR 1.11, 95% CI 1.02–1.2;  $P = 0.02$ ), bone marrow as the source of stem cells (HR 1.2, 95% CI 1.15–1.25;  $P < 0.001$ ) and a female donor for a male recipient (HR 1.16, 95% CI 1.11–1.21;  $P < 0.001$ ) were associated with increased risk of grade II–IV acute GVHD, whereas the use of antithymocyte globulin (ATG) or alemtuzumab prophylaxis was associated with a lower incidence (HR 0.79, 95% CI 0.74–0.84;  $P < 0.001$ )<sup>15</sup>.

Of note, some studies have suggested that the deleterious effect of HLA disparity on risk of acute GVHD can be overcome with the use of post-transplant cyclophosphamide (PTCy)<sup>28</sup>. In patients who received PTCy for GVHD prophylaxis after haploidentical alloHCT<sup>29</sup>, stem cell source or conditioning regimen had no effect on the incidences of grade II–IV acute GVHD, whereas older donor age (30 to 49 versus <29 years) was significantly associated with higher rates of grade II–IV acute GVHD (HR 1.53, 95% CI 1.11–2.12;  $P = 0.01$ )<sup>29</sup>. Although single-centre studies have identified many significant single nucleotide polymorphisms as genetic risk factors for GVHD, these findings have not been consistently reproduced in large multicentre trials<sup>30</sup>.

## Mechanisms/pathophysiology

The pathophysiology of acute GVHD occurs in three phases: initiation phase, T cell activation and the effector phase (Fig. 3).

### Initiation phase

During the initiation phase, the alloHCT conditioning regimen (Box 1) damages patients' tissues and causes release of inflammatory cytokines that lead to activation of host antigen-presenting cells (APCs).

**Intensity and type of conditioning.** While in the early years of alloHCT most of the regimens in patients used high-intensity myeloablative total body irradiation (TBI) to eliminate the recipient's haematopoiesis (blood cell production process), leukaemia and immune system, variations in the TBI dose and fractionation, and in the use of cytotoxic drugs, revealed that conditioning-induced damage itself contributed to the kinetics and severity of acute GVHD<sup>31</sup>. As an example, there were higher systemic levels of tumour necrosis factor (TNF) inducing inflammation and tissue damage itself after high-dose TBI, and overall TNF release correlated with the severity of GVHD<sup>32</sup>. The effect

## Box 1

### Allogeneic haematopoietic cell transplantation

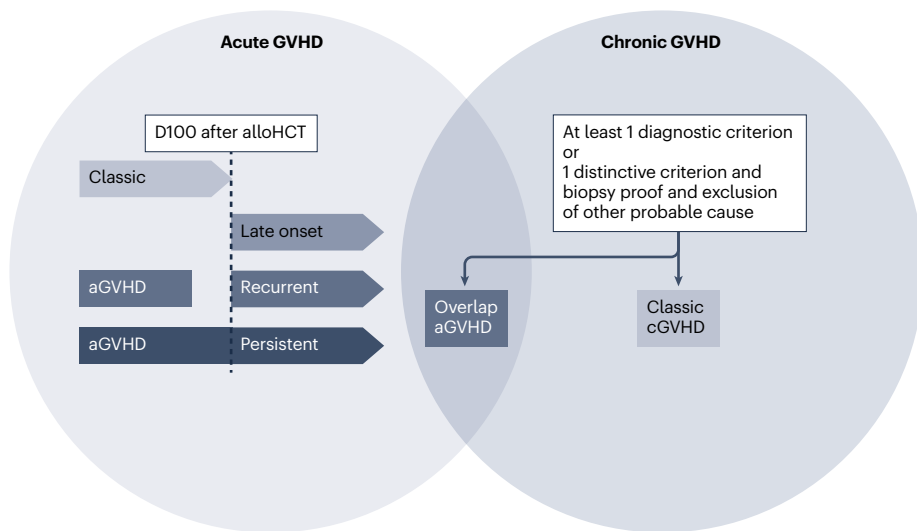
Allogeneic haematopoietic cell transplantation (alloHCT) is a curative therapy for some life-threatening malignant and non-malignant haematological diseases. Acute myeloid leukaemia is the most important indication for alloHCT, with 14,334 alloHCT performed worldwide in 2016 for this disorder<sup>5</sup>. Other common malignant indications for alloHCT include acute lymphoid leukaemia ( $n=6,895$ ), myelodysplastic syndrome and myeloproliferative neoplasm ( $n=5,616$ ), and Hodgkin and non-Hodgkin lymphoma ( $n=3,246$ )<sup>5</sup>. For non-malignant disorders, 5,427 alloHCT were performed worldwide in 2016, with bone marrow failure, haemoglobinopathies and immune deficiency as the most frequent indications<sup>5</sup>.

The therapeutic efficacy of alloHCT for haematological malignancies relies on the cytoreductive effect of the conditioning regimen (chemotherapy and/or radiotherapy) and the graft-versus-tumour effect mediated by the donor's immunocompetent cells and the presence of minor histocompatibility antigens on tumour cells<sup>233,234</sup>. By contrast, the efficacy of alloHCT for non-malignant disorders relies on the replacement of diseased bone marrow with healthy bone marrow. The aim of the conditioning regimen, in addition to cytoreduction specifically in those with haematological malignancies, is to induce immunosuppression that permits engraftment. By contrast, myelosuppression is not mandatory for alloHCT for both malignant and non-malignant indications, and a broad spectrum of conditioning regimens from fully myeloablative to non-myeloablative regimens are used<sup>235</sup>.

Bone marrow obtained by repeated aspiration of the posterior iliac crests (while the donor is under general or local anaesthesia) was initially used to source haematopoietic stem cells<sup>234</sup>. However, granulocyte colony-stimulating factor-mobilized peripheral blood stem cells (PBSCs) collected by leukapheresis is now used in most cases<sup>3</sup>.

An HLA-identical sibling is generally considered the best donor for alloHCT; however, fewer than one-third of patients have one<sup>236</sup>. Thus, unrelated donor registries have been developed and comprise >40 million volunteer donors (WMDA). The probability of finding a donor matched at the HLA-A, HLA-B, HLA-C HLA-DRB1 and HLA-DQB1 level is between 16% and 75% depending on ethnicity<sup>236</sup>, with the lowest and highest probabilities in patients of African and European descent, respectively. Accordingly, alternative donor types have been developed, initially using umbilical cord blood, although this approach has now been supplanted by haploidentical related donors, which has allowed almost all patients to have access to a donor as their parents and children can now be donors<sup>237</sup>.

of the conditioning regimen on kinetics and severity of acute GVHD became more evident with the introduction of non-myeloablative conditioning. Interestingly, the severity of neutropenia caused by non-myeloablative conditioning correlates with the severity of acute GVHD; in one study, grade III–IV acute GVHD occurred in only 3% of patients without neutropenia and in 12% of patients with severe neutropenia,



**Fig. 2 | Classification of acute and chronic GVHD.** Acute graft-versus-host disease (GVHD) is classified as classic acute GVHD with onset  $\leq 100$  days after allogeneic haematopoietic cell transplantation (alloHCT), as late onset acute GVHD with first onset  $>100$  days after alloHCT and as recurrent acute GVHD with onset  $>100$  days after previous classic acute GVHD. Acute GVHD that persists beyond 100 days after alloHCT is classified as persistent acute GVHD. aGVHD, acute GVHD; cGVHD, chronic GVHD; D100, day 100.

which was also reflected by an increase in non-relapse mortality (NRM) from 3% to 25%, respectively<sup>33</sup>. These data suggest that several parameters, including the release of inflammatory cytokines (TNF, IL-1 and IL-6), contribute to the effects of conditioning intensity on acute GVHD, not only conditioning-related damage itself, but also from the general susceptibility to inflammation. This susceptibility is influenced by several further factors, such as translocation of pathogen-associated molecular patterns (PAMPs) and the ability of the host to mount the inflammatory response (for example, by the presence of neutrophils).

**Pathogen-associated molecular patterns and damage-associated molecular patterns.** Molecules derived from the activation or destruction of bacteria are summarized as PAMPs, while molecules derived from the destruction of human cells (such as uric acids and others) are called damage-associated molecular patterns (DAMPs). The first PAMP associated with acute GVHD was lipopolysaccharide (LPS). The severity of acute GVHD was reduced in LPS-resistant mouse strains compared with LPS-sensitive mouse strains, and neutralization of LPS by antibodies contributed to the suppression of acute GVHD<sup>34</sup>. Other PAMPs and their receptors have also been evaluated as triggers of GVHD: TLR4 and NOD2 (also known as CARD15) were associated with acute GVHD in several studies<sup>35</sup>, although findings were not conclusive in other studies, suggesting that single pathways may not be sufficient to trigger disease. Of note, in one multicentre study, the association of acute GVHD with NOD2 was only observed in centres using specific types of GI decontamination which is widely used to reduce neutropenic infections and bacterial translocation across the damaged GI tract<sup>36</sup>.

PAMPs induce pro-inflammatory cytokines in myeloid and epithelial cells, specifically TLRs induce the myeloid differentiation factor 88 (MyD88) pathway and thus augment GVHD<sup>37</sup>. After stimulation by viral or bacterial DNA, which represents a further group of PAMPs, type I interferon (IFN) signalling via retinoic acid-inducible gene-1 (RIG-I) is induced: While induction of this pathway before alloHCT protects against GVHD by stabilizing epithelial damage, it can augment GVHD if induced at later time points by activating donor CD8 cells<sup>38,39</sup>.

Tissue damage induced by pretransplant conditioning causes release of intracellular molecules, which can act as danger signals (DAMPs). Among the DAMPs, high-mobility group box 1 (HMGB1), uric

acid, ATP, heat shock proteins and heparan sulfate proteoglycans have been described as triggers of acute GVHD<sup>40</sup>. Evaluation of inhibitors of these molecules such as purinergic P2X receptor 7 (P2X7R) antagonists in murine models<sup>41</sup> or  $\alpha 1$ -antitrypsin (AAT) in patients have been tested with some success. IL-33 is a DAMP produced by endothelial and epithelial cells on inflammation and binds to its receptor suppression of tumorigenicity 2 (ST2) to induce immunoregulation; soluble ST2 is now recognized as a general biomarker of acute GVHD<sup>42</sup>.

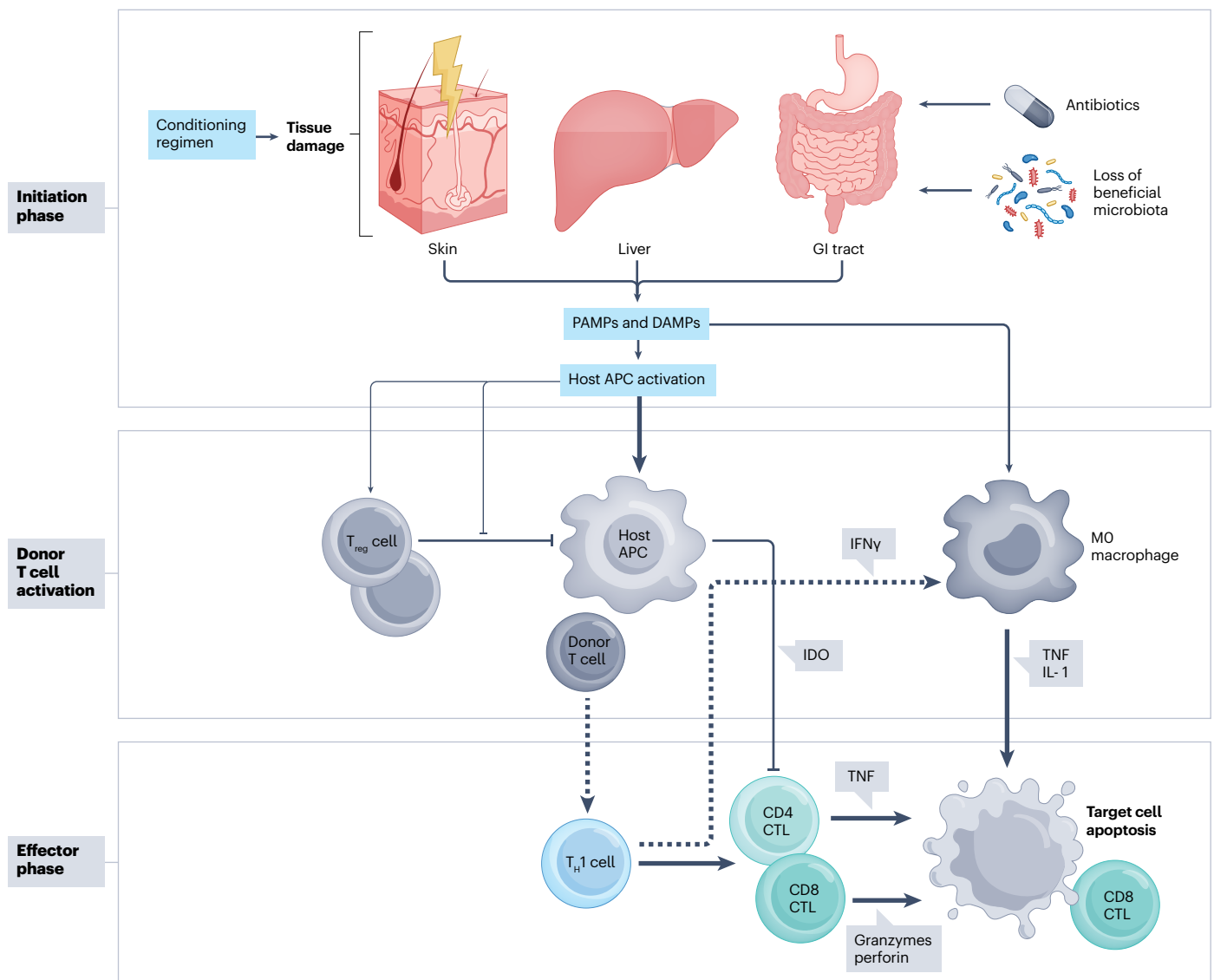
**Microbiota: more than multiple PAMPs.** As previously mentioned, the main organs affected in acute GVHD are the skin, liver and GI tract. These organs represent the main epithelial surfaces that interact with commensal and pathogenic bacteria (the organ-specific microbiota). The liver is connected to the GI tract via the portal vein. Early studies demonstrated the involvement of the intestinal microbiota in acute GVHD pathophysiology, such as one study that demonstrated the absence of acute GVHD in mice grown under germ-free conditions that received bone marrow grafts<sup>43</sup>. Based on these early findings, decontamination and isolation of patients was introduced in clinical practice, and reduced the incidence of GVHD<sup>44</sup>.

In the initiation phase of acute GVHD, bacteria translocated as a consequence of epithelial damage by the conditioning regimen contribute to initiation of acute GVHD in particular through immune cell activation by PAMPs<sup>45</sup>. Experiments in murine models have demonstrated the prevention of GVHD by administration of antibiotics initiated before alloHCT and subsequent reduction of neutrophil influx in epithelial tissues. However, a study in humans found the failure of gut decontamination in ~50% of patients in whom the incidence of acute GVHD was significantly higher than in patients who achieved successful gut decontamination<sup>46</sup>. Moreover, more recent studies using 16S rRNA sequencing, have demonstrated deleterious effects of antibiotic use, namely, long-term loss of microbiota diversity, which can accelerate and aggravate acute GVHD<sup>47</sup>. Conditioning regimens and the use of broad-spectrum antibiotics during alloHCT lead to low gut microbiota diversity and intestinal domination. Indeed, one study demonstrated intestinal domination in 65% of patients, mainly related to *Enterococcus faecium*, which was associated with poor outcomes due to increased GVHD-related mortality<sup>48</sup>.

By contrast, some bacteria have protective effects. For example, increased numbers of bacteria belonging to the genus *Blautia* (order Clostridiales) is associated with reduced GVHD lethality and improved OS<sup>49</sup>. Of note, loss of *Blautia* is associated with the use of broad-spectrum antibiotics. These protective effects are mediated at least in part through the production of metabolites, which are essential for epithelial nutrition (such as short-chain fatty acids (SCFAs))<sup>50</sup>, intestinal stem cell support (via IL-22)<sup>51</sup> and, most importantly, immunoregulation (as SCFAs have a major role in maintenance and support of regulatory T ( $T_{reg}$ ) cells<sup>52</sup>). In addition, these metabolites also contribute to barrier function, and their loss results in barrier damage, allowing increased

translocation of pathogenic bacteria and amplifying the inflammatory cascades in GVHD<sup>53</sup>.

**APC activation.** PAMPs and DAMPs activate classic APCs (dendritic cells, macrophages and B cells) and non-classic APCs (MHC class II-expressing cells that can activate CD4<sup>+</sup> T cells such as mast cells, basophils, endothelial cells and epithelial cells)<sup>54</sup>, which are then responsible for activation of donor T cells in acute GVHD. Classic APCs have a central role in acute GVHD induction, as they are sufficient to activate donor T cells. Donor T cells recognize host antigens on the surface of host APCs, leading to the initiation of CD4<sup>+</sup> and CD8<sup>+</sup> T cell-mediated acute



**Fig. 3 | Pathophysiology of acute GVHD.** In the first (initiation) phase of acute graft-versus-host disease (GVHD), the conditioning regimen of chemotherapy and/or radiotherapy damages host tissue. This damage leads to the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), such as tumour necrosis factor (TNF), type I interferon (IFN $\gamma$ ) and lipopolysaccharide, that activate host antigen-presenting cells (APC) in the initiation phase. In addition, loss of microbiota diversity

contributes to loss of epithelial and immune homeostasis. In the T cell activation phase, host APCs activate alloreactive donor CD4<sup>+</sup> and CD8<sup>+</sup> T cells. In the effector phase, effector T cells and pro-inflammatory cytokines damage epithelial cells of the gastrointestinal (GI) tract, skin and liver, leading to apoptosis and necroptosis, resulting in acute GVHD symptoms. CTL, cytotoxic T lymphocyte; IDO, indoleamine 2,3-dioxygenase;  $T_H$ 1 cell, T helper 1 cell;  $T_{reg}$  cell, regulatory T cell.

GVHD (see section 'T cell activation')<sup>55,56</sup>. Moreover, donor-derived APCs can augment CD8<sup>+</sup> T cell-mediated acute GVHD, presumably by acquiring and presenting host antigens by cross-priming (whereby certain professional APCs, mostly dendritic cells, take up, process and present extracellular antigens by MHC class I molecules to CD8<sup>+</sup> T cells)<sup>57</sup>. In addition, in mixed haematopoietic chimeras, non-classic APCs, such as fibroblasts and endothelial cells, have also been shown to present antigens and induce acute GVHD after irradiation-induced damage<sup>58,59</sup>.

## T cell activation

Acute GVHD occurs when donor T cells respond to HLA differences on a recipient's tissue (usually referred to as alloreactive T cells). The central role of alloreactive T cells in acute GVHD is supported by the effectiveness of T cell depletion (TCD) for acute GVHD prophylaxis (see section 'T cell depletion prophylaxis').

**T cell activation by minor and major HLA antigens.** Donor T cells recognize HLA on the surface of host APCs, and HLA mismatch between recipient and donor is one of the most prominent risk factors for both acute and chronic GVHD, if classic immunosuppressive or non-immunosuppressive prophylaxis is used<sup>30</sup>. This holds true for HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1, whereas HLA-C and HLA-DPB1 interactions are more complex, and also may contribute to activations in the context of GVL<sup>60</sup>. CD4<sup>+</sup> T cells respond to antigens presented on MHC class II molecules (HLA-DRB1, HLA-DQB1 and HLA-DPB1) while CD8<sup>+</sup> T cells respond to antigens presented by MHC class I molecules (HLA-A, HLA-B and HLA-C)<sup>61</sup>. By contrast, minor histocompatibility antigens are the only targets of T cell alloreactivity in HLA-matched or identical alloHCT transplants with sibling donors and can lead to acute GVHD. Indeed, one study defined minor HLA antigens such as HA-1, HA-2, HA-4 and HA-5, and demonstrated a 6.4-fold increased risk of grade II–IV acute GVHD in minor mismatched transplants<sup>62</sup>.

**Regulatory cells.** Donor-derived host-specific natural T<sub>reg</sub> cells have a key role in suppression of acute GVHD, and are suitable for selective isolation to treat and prevent acute GVHD<sup>63,64</sup>. Besides these thymus-derived T<sub>reg</sub> cells, induced T<sub>reg</sub> cells have additional GVHD suppressive activity in peripheral target organs, especially in the GI tract<sup>65</sup>. Recent data suggest that non-lymphoid tissue T<sub>reg</sub> cells not only exert immunoregulatory effects but also are important for maintaining epithelial homeostasis<sup>66</sup>. Further subpopulations, such as CD8<sup>+</sup> T<sub>reg</sub> cells which can be stimulated by mammalian target of rapamycin (mTOR) inhibitors<sup>67</sup> and regulatory B cells producing IL-10, have been reported to suppress the severity of acute GVHD in experimental models<sup>68</sup>.

Innate immune cells can also exert suppressive functions in GVHD. Mesenchymal stem cells<sup>69</sup> and myeloid-derived suppressor cells (MDSC)<sup>70</sup> both rely on the immunosuppressive function of indoleamine 2,3-dioxygenase which shifts the balance between alloreactive effector and T<sub>reg</sub> cells; polarized type 2 macrophages have also been reported to attenuate alloreactions including GVHD<sup>71</sup>. Moreover, innate lymphoid cells 2 (ILC2) and ILC3 contribute to regulation of epithelial homeostasis<sup>72</sup>. ILC2 are depleted after conditioning and can suppress T helper 1 (T<sub>H</sub>1) and T<sub>H</sub>17 cell responses, inducing MDSCs by IL-13 (ref. 72). ILC3 release protective IL-22 and suppress T cell activation by ATP via ectoenzymes<sup>72</sup>.

## Effector phase

**Cellular and cytokine effectors.** During the effector phase, activated alloreactive donor CD8<sup>+</sup> T cells kill target tissues via apoptosis<sup>73,74</sup>.

Apoptosis is most prominent in the stem cell niches of acute GVHD target tissues, which has been shown for keratinocytes, intestinal stem cells and associated basal Paneth cells, and neuroendocrine cells in GI crypts<sup>75</sup>. Inflammation-associated cell death, such as necroptosis<sup>76</sup>, and inhibition of inflammation-associated receptor-interacting serine-threonine kinase 1 (RIPK1) also contribute to tissue destruction, and could be targeted to develop future therapies.

Targeted cell damage by activated alloreactive CD4<sup>+</sup> T cells is mediated by pro-inflammatory cytokines such as IFN $\gamma$  and TNF; therapies targeting the latter are effective in the treatment of acute GVHD<sup>77</sup>. Of note, cytokines and other inflammatory pathways are important targets of corticosteroids and other drugs (such as JAK–signal transducer and activator of transcription (STAT) inhibitors) that are effective in the treatment of acute GVHD<sup>78</sup>.

**Other targets of GVHD.** An open question is the involvement of non-epithelial cells in acute GVHD. Endothelial cells are involved as endothelial cell sprouting precedes T cell infiltration in the initiation phase of GVHD, and endothelial apoptosis occurs in the effector phase<sup>79,80</sup>. Endothelial activation and damage may be the first steps not only in classic GVHD target organs but also in the less-recognized and less-characterized target tissues of acute GVHD, such as the central nervous system<sup>81</sup> or the lung<sup>82</sup>. Of note, lymphohaematopoietic tissues are the most sensitive targets of alloreaction, which contributes to GVL and the severe immunodeficiency of GVHD.

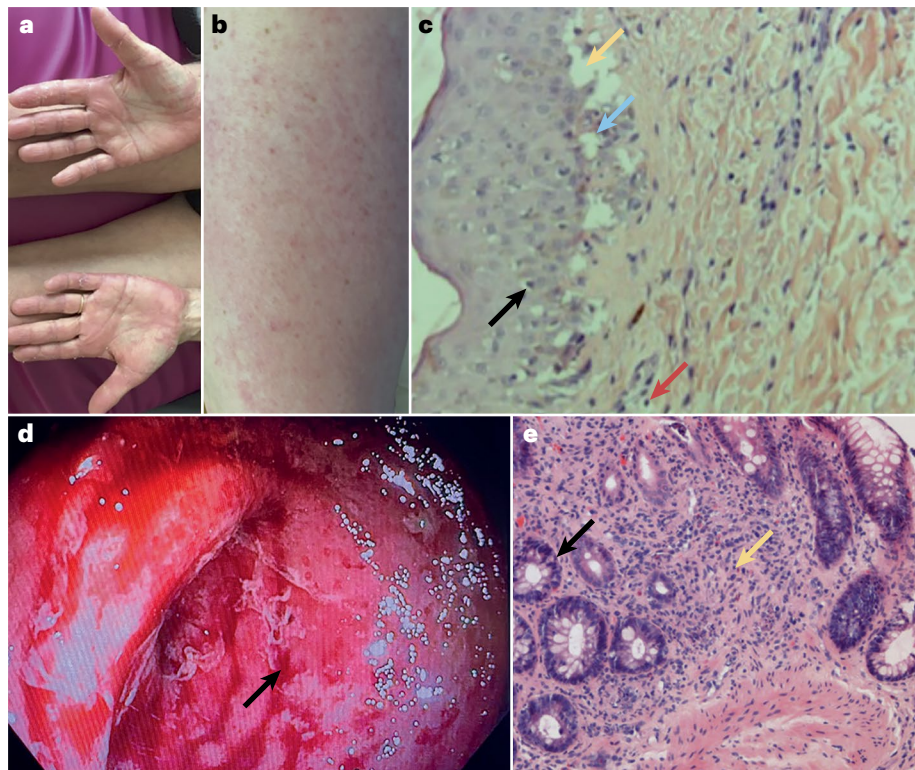
## Role of tissue homeostasis

Epithelial stem cell damage during the effector phase and the role of barrier damage in the pathophysiology of GVHD suggest that the susceptibility of the target tissue has a role in acute GVHD. Therefore, in 2017 the concept of tissue tolerance (the intrinsic, but variable, ability of tissues to tolerate or withstand damage from inflammatory immune activity during infection) was introduced in the setting of acute GVHD<sup>83</sup>. In addition to expression of inhibitors of apoptosis and epithelial protection, microbial-derived metabolites, such as SCFA metabolic pathways, have a crucial role in tissue tolerance. Moreover, mitochondrial complex II is a crucial regulator of epithelial damage in T cell-mediated diseases, including acute GVHD<sup>84</sup>. The list of metabolic changes, both in T cells and effector organs of GVHD, is continually increasing, providing further approaches for modulation of GVHD<sup>85</sup>.

## Diagnosis, screening and prevention

### Diagnosis

**Skin acute GVHD.** Skin acute GVHD is the first and most common clinical manifestation of acute GVHD in most patients<sup>14,86</sup>, and generally arises around white blood cell engraftment, which occurs 14–21 days after alloHCT<sup>87</sup>. Typically, patients with skin acute GVHD develop a maculopapular rash that initially involves sun-exposed areas such as the nape of the neck and the shoulders, or less frequently the palms of the hands and the soles of the feet (Fig. 4a,b). The rash can then spread throughout the body but does not affect the scalp. The rash is often pruritic (itchy) and can be painful. In stage 4 acute GVHD, the rash can form bullous lesions and can ulcerate. Pathological findings in the skin in acute GVHD include degeneration of the basal layer of the epidermis with apoptotic bodies, dyskeratosis (abnormal epidermal cell keratinization) with adjacent satellite lymphocytes, perivascular lymphocytic infiltration in the dermis and partial or total dermoepidermal disjunction (disjunction of the interface between the epidermal and the dermal layers of the skin)<sup>88</sup> (Fig. 4c).



**Fig. 4 | Clinical and histological findings of acute GVHD.** **a, b,** Clinical manifestation of stage 2 skin acute graft-versus-host disease (GVHD) with maculopapular rash of the skin. **c,** Skin biopsy sample from a patient with stage 3 skin acute GVHD, showing apoptotic bodies (blue arrow), dyskeratosis with adjacent satellite lymphocytes (black arrow), perivascular lymphocytic infiltration in the dermis (red arrow) and partial dermoepidermal disjunction (yellow arrow). **d,** Endoscopic finding of stage 3 lower gastrointestinal acute GVHD with diffuse erythema and scattered ulceration (black arrow). **e,** Rectal biopsy sample from a patient with stage 2 gastrointestinal acute GVHD, revealing with apoptotic epithelial cells (black arrow) and crypt loss (yellow arrow). Parts **c** and **e,** haematoxylin and eosin staining, original magnification  $\times 200$ .

**Gastrointestinal acute GVHD.** The lower and upper GI tract can also be involved in acute GVHD. Diarrhoea is the prominent symptom of lower GI acute GVHD, is secretory and is usually voluminous. Abdominal pain and ileus (non-mechanical decrease or stoppage of the flow of intestinal contents) occur in severe disease. In addition, haematochezia (passage of fresh blood via the anus) occurs in severe disease owing to mucosal ulceration. Anorexia, nausea and/or vomiting are symptoms of upper GI acute GVHD<sup>89</sup>. Although upper and lower GI acute GVHD are usually associated, they can also occur in isolation.

Diarrhoea is frequent after alloHCT and can be caused by toxicity of the conditioning regimen, drug toxicity, viral infection, *Clostridioides difficile* colitis or neutropenic enterocolitis<sup>90</sup>. Thus, exclusion of these differential diagnoses is important to confirm lower GI acute GVHD, particularly when diarrhoea occurs in isolation. Bacteriological, virological and parasitological stool culture are usually performed, and the presence of *C. difficile* toxin in stool and CMV in blood is searched for to exclude differential diagnoses.

Medical imaging is not diagnostic for acute GVHD but can be performed to rule out other diagnoses. Non-specific signs of lower GI acute GVHD on CT scan include bowel wall thickening, abnormal mucosal enhancement, bowel dilatation and air or fluid levels suggestive of ileus<sup>91</sup>. Endoscopic manifestations of acute GVHD include spotted or diffuse erythema, patchy erosion, scattered ulceration and active bleeding<sup>92–94</sup> (Fig. 4d). As these findings lack specificity for acute GVHD and as normal mucosa can be seen in this condition, biopsies are systematically performed for diagnosis. Histological findings include apoptotic epithelial cells, individual or multiple crypt loss and denudation (loss of the surface layer) of the epithelium<sup>95,96</sup> (Fig. 4e).

In addition to CT and endoscopy, other imaging techniques for the diagnosis of lower GI acute GVHD show promise. Contrast-enhanced

ultrasonography reveals transmural penetration of microbubbles into the bowel lumen in patients with acute GVHD, confirming bowel wall thickening and functional impairment<sup>97</sup>. However, despite a high specificity for diagnosis of GI acute GVHD in preliminary studies<sup>98,99</sup>, the use of contrast-enhanced ultrasonography is limited by the lack of prospective studies and by the fact that it can be carried out only by highly trained specialists who are not available in many hospitals. <sup>18</sup>F-Fluorodeoxyglucose (<sup>18</sup>F-FDG) PET-CT revealed a correlation between the localization of <sup>18</sup>F-FDG uptake in the gut and that of biopsy-proven GI acute GVHD<sup>100</sup>. Accordingly, <sup>18</sup>F-FDG PET-CT seems to be a non-invasive, sensitive and very specific biomarker for lower GI acute GVHD diagnosis in patients with diarrhoea<sup>100</sup>; however, the lack of availability of <sup>18</sup>F-FDG PET-CT in emergency settings strongly limits its use in these patients.

Differential diagnoses of upper GI acute GVHD include infection (oesophageal candidiasis and herpes simplex virus), conditioning regimen toxicity and peptic ulcers. Upper GI endoscopy is recommended, whenever possible, for biopsy confirmation, particularly in those with suspected isolated upper GI GVHD<sup>9</sup>. Symptom severity is also important for diagnosis of upper GI GVHD, as this condition should be considered in those with nausea for  $>3$  days, at least two vomiting episodes per day or anorexia with weight loss.

**Liver acute GVHD.** The liver is the least frequently involved organ in acute GVHD and liver acute GVHD is usually associated with skin and/or GI acute GVHD<sup>101</sup>. Liver acute GVHD is characterized by an increased total serum bilirubin level (hyperbilirubinaemia), which can lead to jaundice. Liver dysfunction and hyperbilirubinaemia after alloHCT has several causes, including sinusoidal obstruction syndrome, drug toxicity and infections; therefore, biopsy confirmation is required for

diagnosis of liver acute GVHD in those with no signs of acute GVHD in other organs. The most consistent histological feature of liver acute GVHD is bile duct damage<sup>102</sup>. Periportal and midzone hepatocellular necrosis and minimal lymphocytic infiltrates in the portal tract can also be observed. Liver biopsies are rarely performed early after transplantation owing to thrombocytopenia, making the diagnosis of liver acute GVHD one of exclusion.

**Non-classic manifestations of acute GVHD.** Other organs can be affected in acute GVHD, including lung, kidney, thymus and lymph nodes, bone marrow and the central nervous system, although acute damage is less apparent or is more difficult to distinguish from other alloHCT toxicities (drug or conditioning regimen toxicities, infectious complications). Symptoms of lung acute GVHD include fever, cough, dyspnoea (shortness of breath) and hypoxaemia (low blood oxygen levels), and are difficult to distinguish from those of other lung disease or injury<sup>103</sup>. Regarding the thymus and lymph nodes and the bone marrow, acute GVHD causes an impairment of thymic export of T cells<sup>104</sup>, a reduced haematopoiesis-supporting capacity of mesenchymal stem cells<sup>105</sup> and a delayed B cell reconstitution and impaired antibody responses in patients<sup>106</sup>, leading to an impaired immune reconstitution and haematopoiesis. Finally, neurological deficits and abnormal MRI findings in patients developing acute GVHD have been found in several studies<sup>107</sup>.

**Grading.** Once a diagnosis of acute GVHD is established, disease in each involved organ (skin, GI tract and liver) is staged from 0 to 4 (whereby 4 is the most severe) (Table 1) based quantification of skin rash for

**Table 1 | Acute GVHD organ staging (MAGIC criteria)**

Stage	Skin (active erythema only)	Liver (bilirubin (mg/dl))	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2	No or intermittent nausea, vomiting or anorexia	Adult: <500 ml/day or <3 episodes/day Child: <10 ml/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3	Persistent nausea, vomiting or anorexia	Adult: 500–999 ml/day or 3 or 4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25–50% BSA	3.1–6	NA	Adult: 1,000–1,500 ml/day or 5–7 episodes/day Child: 20–30 ml/kg/day or 7–10 episodes/day
3	Maculopapular rash >25% BSA	6.1–15	NA	Adult: >1,500 ml/day or >7 episodes/day Child: >30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation >5% BSA	>15	NA	Adult and child: severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

BSA, body surface area; GI, gastrointestinal; GVHD, graft-versus-host disease; NA, not applicable. Reprinted with permission from ref. 9, Elsevier.

skin acute GVHD, serum bilirubin level for liver acute GVHD, diarrhoea (number and/or volume of stool) for lower GI acute GVHD and persistent nausea for upper GI acute GVHD<sup>9</sup>. These stages are used to calculate the grade of acute GVHD based on the MAGIC criteria<sup>9</sup>: I (mild), II (moderate), III (severe) and IV (very severe) (Table 2). An electronic application has been developed and validated, the eGVHD App, to assist health-care professionals in the assessment of GVHD in clinical practice<sup>11,12</sup>. Other similar systems can be used for grading, including the Consensus (or modified Glucksberg) system<sup>108</sup>, the Minnesota system<sup>89,109</sup> and the Center for International Blood and Marrow Transplant Research (CIBMTR) system<sup>110</sup>.

**Biomarker screening.** Researchers have aimed to identify a biomarker panel for the diagnosis of acute GVHD. A composite biomarker panel of four proteins (IL-2R $\alpha$ , TNF receptor 1, IL-8, and hepatocyte growth factor) discriminates patients with and without acute GVHD, with an area under the receiver operating characteristic curve distinguishing these two groups of 0.91 (95% CI 0.87–0.94) in the training set and 0.86 (95% CI 0.79–0.92) in the validation set<sup>111</sup>. In addition, several proteins related to GVHD organ damage have also been identified as potential biomarkers. Elafin is overexpressed in skin biopsies from patients with GVHD, and plasma levels of elafin are significantly higher at the onset of skin acute GVHD compared with those in patients without skin acute GVHD<sup>112</sup>. Moreover, regenerating islet-derived protein 3 $\alpha$  (REG3A), hepatocyte growth factor and cytokeratin 18 (CK18) fragment levels are significantly increased in patients with lower GI acute GVHD compared with the levels in those with non-GVHD diarrhoea<sup>113,114</sup>. These three biomarkers are also elevated in patients with liver acute GVHD, but do not distinguish GVHD from other causes of hyperbilirubinaemia<sup>113</sup>.

The Ann Arbor (AA) biomarker risk uses serum concentrations of ST2 and REG3A at the onset of acute GVHD to generate a score from 1 to 3 to predict the risk of NRM and resistance to acute GVHD treatment<sup>115–117</sup>. The AA biomarker risk is used in large multicentre and multinational consortia (such as the MAGIC consortium) and as guidance for risk-adapted trials, but otherwise remains limited to a small number of centres. Overall, diagnostic or predictive biomarkers of acute GVHD are not used routinely in clinical practice owing to the lack of standardized commercially available assays.

## Prevention

**Pharmacological prophylaxis.** Pharmacological prophylaxis for acute GVHD is based on the inhibition of the cytoplasmic enzyme calcineurin, which is important for T cell activation. Calcineurin inhibitors are usually combined with the anti-metabolite drug methotrexate, based on early studies which established the superiority of the calcineurin inhibitor cyclosporine combined with methotrexate over treatment with cyclosporine alone<sup>118,119</sup>. Two subsequent phase III randomized studies demonstrated a significantly lower incidence of grade II–IV acute GVHD in patients who received the calcineurin inhibitor tacrolimus combined with methotrexate compared with patients who received cyclosporine combined with methotrexate (32% versus 44% in patients with a sibling donor ( $P = 0.01$ ) and 56% versus 74% in those with an unrelated donor ( $P = 0.0002$ ))<sup>120,121</sup> (Supplementary Table 1). Nevertheless, no difference in OS was found between groups. Thus, cyclosporine and tacrolimus are considered as roughly equivalent, and can be used according to centre practice. The toxic effects of cyclosporine and tacrolimus are similar and include nephrotoxicity, hypomagnesaemia, hyperkalaemia, hypertension and tremor<sup>122</sup>. Hypertrichosis (excessive hair growth) and gingival hyperplasia (overgrowth of gum tissue around the teeth) can also occur



with cyclosporine treatment, and tacrolimus can be associated with alopecia. The most severe adverse effect of calcineurin inhibitors is transplant-associated thrombotic microangiopathy (TA-TMA), which is caused by direct cytotoxic damage to the endothelial cells by the calcineurin inhibitor<sup>123</sup>. The kidney is the primary site of TA-TMA, leading to proteinuria, acute kidney injury and hypertension, although multiple organs can be involved leading to intestinal thrombotic microangiopathy, pulmonary hypertension and neurotoxicity (headache, seizures, confusion and hallucinations). Therapeutic plasma exchange is usually ineffective for TA-TMA, so treatment relies on calcineurin inhibitor withdrawal and, in patients with evidence of complement activation<sup>124</sup>, complement-directed therapy (eculizumab)<sup>123</sup>. In the absence of GVHD, calcineurin inhibitors are usually tapered over 3–6 months after alloHCT.

In some centres, methotrexate toxicities (mucositis and neutropenia) lead to replacement with mycophenolate mofetil (MMF). In a prospective randomized study, cyclosporine plus MMF was associated with a lower incidence of grade III–IV mucositis (21% versus 65%;  $P = 0.008$ ) and a quicker neutrophil engraftment (median day 11 versus 18;  $P < 0.001$ ) compared with cyclosporine plus methotrexate, with a similar incidence of grade II–IV acute GVHD in the two groups (48% versus 37%;  $P = 0.49$ )<sup>125</sup>. In a meta-analysis of retrospective studies, methotrexate was found to be associated with a lower incidence of grade III–IV acute GVHD than MMF, while the incidence of mucositis was lower with MMF, the time to engraftment was shorter with MMF, and the incidence of grade II–IV acute GVHD and OS were similar in the two groups<sup>126,127</sup>. In practice, MMF is usually given to patients who received an umbilical cord blood transplant or reduced intensity conditioning to achieve faster engraftment and avoid graft failure<sup>128–131</sup>.

Sirolimus, although not a calcineurin inhibitor, is an immunosuppressant that inhibits mTOR, thereby blocking B and T cell activation. In a phase III randomized study, no significant difference was found in the cumulative incidence of grade III–IV acute GVHD with cyclosporine and methotrexate ( $n = 106$ ) compared with tacrolimus and sirolimus (13% versus 7%;  $P = 0.09$ ), and no difference in OS (72% versus 71%;  $P = 0.71$ )<sup>132</sup>. However, another phase III randomized study demonstrated a significantly lower cumulative incidence of grade II–IV acute GVHD with sirolimus plus cyclosporine and MMF compared with cyclosporine and MMF (26% versus 52%;  $P = 0.0013$ ) in patients who received HLA-matched unrelated donor peripheral blood stem cell (PBSC) grafts following non-myeloablative conditioning<sup>133</sup>. Moreover, a concurrent multisite phase II study of triple immunosuppression (MMF, cyclosporine and sirolimus) in recipients of HLA-mismatched PBSC grafts demonstrated a cumulative incidence of 36% for grade II–IV acute GVHD at day 100 with only 1% of patients developing grade III and none developing grade IV acute GVHD<sup>134</sup>. Similar to calcineurin inhibitors, sirolimus damages endothelial cells and seems to be associated with TA-TMA<sup>132</sup>.

The cytotoxic T cell lymphocyte 4 (CTLA4) analogue abatacept, prevents APCs from delivering the costimulatory signal to T cells<sup>135</sup>. A randomized, double-blind, placebo-controlled phase II trial found no difference in the cumulative incidence of grade III–IV acute GVHD between patients who received abatacept plus a calcineurin inhibitor and methotrexate compared with those who received placebo plus a calcineurin inhibitor and methotrexate (6.8% versus 14.8%, HR 0.45;  $P = 0.13$ )<sup>135</sup>. Severe acute GVHD-free survival was 93.2% in the abatacept group versus 82% in the placebo group ( $P = 0.05$ ). These data are promising, and further investigations are expected.

**T cell depletion prophylaxis.** Given the important role of T cells in acute GVHD pathophysiology, TCD approaches for acute GVHD

**Table 2 | Overall acute GVHD grading (MAGIC criteria)**

Grade <sup>a</sup>	Stage			
	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)
0	0	0	0	0
I	1 or 2	0	0	0
II	3	1	1	1
III	– <sup>b</sup>	2 or 3	– <sup>b</sup>	2 or 3
IV	4	4	– <sup>b</sup>	4

GI, gastrointestinal; GVHD, graft-versus-host disease. <sup>a</sup>Overall grade based on the target organ with the most severe involvement. <sup>b</sup>These manifestations are not required for this grading. Reprinted with permission from ref. 9, Elsevier.

prophylaxis have been developed. We distinguish between ex vivo TCD (T cell-negative selection or CD34-positive selection) and in vivo TCD with antibodies.

Ex vivo TCD with either T cell-negative selection or CD34-positive selection is highly effective for GVHD prophylaxis; however, it is associated with a high rate of infectious complications and underlying disease relapse<sup>136,137</sup>. The most frequently used techniques to achieve TCD of the graft are based on CD34-positive selection using electromagnetic methods, which allow up to a 5-log reduction in T cells<sup>138</sup>. Contemporary studies have demonstrated reduced incidence of acute GVHD and have revealed a similar relapse risk with ex vivo TCD compared with that in patients who received an unmodified graft<sup>139,140</sup>. However, the risk of infectious complications, particularly of viral origin, remains higher with ex vivo TCD alloHCT compared with unmodified graft, owing to delays in immune recovery of CD4<sup>+</sup> T cells.

Other ex vivo TCD approaches include  $\alpha\beta^+$  T cell receptor (TCR)/CD19 depletion ( $\alpha\beta^+$  TCR and CD19-negative selection). This approach seems to be associated with a better immune response against viral infections and a low risk of acute GVHD, by enhancing  $\gamma\delta$  T cell reconstitution and lowering  $\alpha\beta^+$  TCR counts after transplantation<sup>141,142</sup>. Of note,  $\alpha\beta^+$  TCR/CD19 depletion has been mainly investigated in children, adolescents and young adults with haploidentical donors<sup>141,142</sup>. Another TCD approach involves naive TCD of PBSC grafts. In this approach, patients receive CD34-selected PBSC grafts with a defined dose of memory T cells and depleted naive T cells. Studies investigating this protocol revealed infrequent grade III–IV acute GVHD and chronic GVHD (4% and 7%, respectively) without excess risks of relapse or NRM<sup>143</sup>. Another study evaluated co-infusion of T<sub>reg</sub> cells and conventional T cells with CD34-selected PBSC haploidentical grafts in 43 adults with high-risk acute leukaemia<sup>144</sup>, and found grade II–IV acute GVHD in 15% of patients and a cumulative incidence of relapse of 5%.

Polyclonal ATGs are the most widely used antibodies for in vivo TCD. ATGs are obtained by immunizing rabbits either with fresh human thymocytes or with the Jurkat T lymphoblastoid cell line<sup>145</sup>. Several phase III randomized studies have evaluated the addition of ATG to a calcineurin inhibitor and methotrexate or MMF in patients with an unrelated donor or a matched related donor<sup>146–150</sup> (Supplementary Table 1). These studies found a lower incidence of acute and chronic GVHD and improved GVHD-free, relapse-free survival (GRFS) in patients who received ATG, despite no improvement in OS<sup>146–150</sup>. ATG administration can be complicated by several infusion reactions including fever, chills, erythema, oxygen desaturation, headache, hepatic cytolysis, serum sickness (5–15 days after infusion) and, exceptionally, systemic

anaphylaxis<sup>151</sup>. ATG also delays immune reconstitution, and is associated with increased risk of infections, especially of viral origin<sup>145,152</sup>.

The use of PTCy for GVHD prophylaxis was developed based on pioneering work of the Baltimore group in patients with a haploidentical alloHCT donor<sup>153</sup>, and it is now a well-established GVHD prophylaxis in this setting<sup>154</sup>. PTCy acts through induction of functional impairment of alloreactive T cells without toxic effects on haematopoietic stem cells<sup>155</sup>. One trial evaluated PTCy in patients with a 9/10 mismatched unrelated donor, and found a significantly lower cumulative incidence of severe grade III–IV acute GVHD in patients who received PTCy than in those who received ATG (9% versus 19%, respectively;  $P = 0.04$ )<sup>156</sup>. Similarly, a phase II study evaluated PTCy plus sirolimus and MMF after alloHCT with a mismatched unrelated donor ( $n = 80$ )<sup>157</sup>. This trial found grade II–IV and III–IV acute GVHD rates at day 100 of 43% and 18%, respectively, in patients who received a myeloablative conditioning regimen and grade II–IV and III–IV acute GVHD rates at day 100 of 33% and 0%, respectively, in patients who received a reduced intensity conditioning regimen. Another study (a randomized phase II study) compared ATG and PTCy in patients with a matched related or unrelated donor<sup>158</sup> and found no difference in cumulative incidence of grade II–IV acute GVHD at 6 months (36.4% for PTCy versus 24.3% for ATG;  $P = 0.34$ )<sup>158</sup>. The BMT CTN1703 phase III study compared tacrolimus plus MMF and PTCy ( $n = 214$ ) with tacrolimus plus methotrexate ( $n = 217$ ) in patients with a matched related, a matched unrelated or mismatched unrelated donor<sup>159</sup> and found a lower cumulative incidence of grade III–IV acute GVHD at day 100 in the PTCy group versus the tacrolimus plus methotrexate group (6.3% versus 14.7%;  $P = 0.001$ ). Moreover, in the multivariate Cox regression model, the PTCy group had a significantly lower hazard of GFRs than the tacrolimus and methotrexate group (HR 0.641, 95% CI 0.492–0.835;  $P = 0.001$ ). In another phase III study (BMT CTN1301 (ref. 160)), PTCy monotherapy was compared with CD34-selected PBSC grafts for GVHD prophylaxis after HLA-matched alloHCT. This study found higher cumulative incidences at 100 days of grade II–IV acute GVHD (37.6% for PTCy and 16.3% for CD34 selection;  $P = 0.002$ ) and grade III–IV acute GVHD (10.1% for PTCy and 2.9% for CD34 selection;  $P = 0.05$ ). Nevertheless, OS was significantly higher in the PTCy group (76.2%) than in the CD34-selected group (60.1%;  $P = 0.019$ ).

Similar to ATG, PTCy is associated with delayed immune reconstitution which leads to an increased incidence of viral infection<sup>161</sup>. Furthermore, particular attention must be paid to patients with a history of cardiac problems, as a higher incidence of early cardiac events (within the first 100 days after alloHCT) has been reported with PTCy treatment<sup>162</sup>. The addition of low-dose ATG to PTCy for GVHD prophylaxis in

patients with a haploidentical donor shows promise, with a lower incidence of acute GVHD in those who received ATG and PTCy than in those who received PTCy alone (22% versus 12%, respectively;  $P = 0.029$ )<sup>163</sup>.

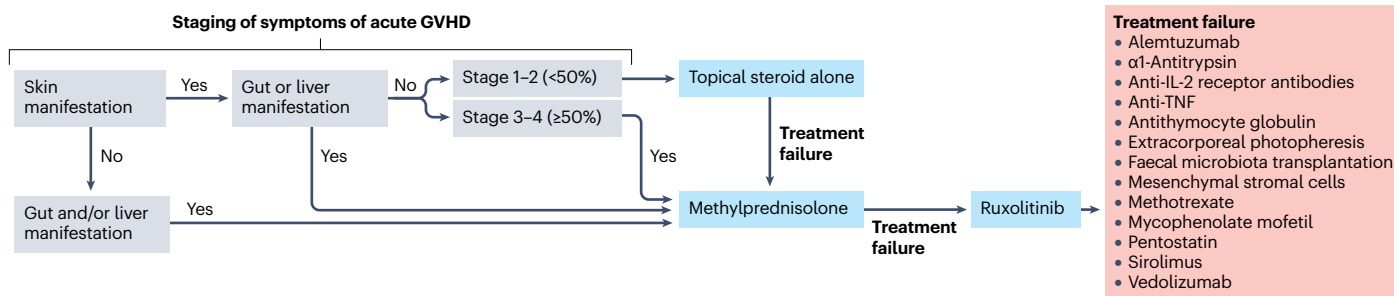
The monoclonal antibody alemtuzumab has also been evaluated for in vivo TCD. Alemtuzumab is an anti-CD52 monoclonal antibody that targets T and B cells, dendritic cells, natural killer cells, monocytes and macrophages. Although alemtuzumab was associated with a low incidence of acute GVHD in non-randomized studies<sup>164–166</sup>, results were not confirmed in prospective randomized studies. Furthermore, alemtuzumab can remain in the blood for up to 1–2 months after transplantation, which can substantially delay immune reconstitution, leading to a high incidence of viral infection and relapse, and no OS benefit<sup>167,168</sup>.

Finally, vedolizumab, a monoclonal antibody that selectively antagonizes  $\alpha 4\beta 7$  GI integrin receptors, preventing lymphocyte trafficking to the gut, has also been evaluated. A phase III randomized placebo-controlled study demonstrated improved lower GI acute GVHD-free survival at 180 days with vedolizumab prophylaxis than with placebo (85.5% versus 70.9%;  $P < 0.001$ ), with no significant difference in serious adverse events between groups (69% versus 71%, respectively)<sup>169</sup>. This is the first positive phase III study for specific prevention of lower GI acute GVHD.

## Management

### First-line therapies

For acute GVHD treatment, consensus recommendations were published by the European Society for Blood and Marrow Transplantation<sup>131</sup> and more specific recommendations for management of acute GVHD after umbilical cord blood alloHCT were issued by the American Society for Transplantation and Cellular Therapy<sup>170</sup>. Systemic steroids remain the standard first-line treatment for acute GVHD. However, a randomized phase III controlled trial in patients with grade I acute GVHD showed more frequent infection and no advantage regarding the development of grade III–IV acute GVHD in patients treated with 6-methylprednisolone compared with no treatment<sup>171</sup>. Accordingly, systemic treatment is recommended only in patients with acute GVHD of grade II or higher, and topical steroids alone are used in patients with grade I disease (Fig. 5). One randomized phase III study compared the use of low-dose versus standard-dose prednisone in patients with grade II acute GVHD with stage I GI acute GVHD, no hepatic dysfunction and stage 1 or 2 skin acute GVHD<sup>172,173</sup>. In this trial, low-dose prednisone seemed as effective as standard-dose treatment, as the low dose did not increase the risk in patients requiring secondary immunosuppressive therapy<sup>173</sup>. By contrast, in patients with grade II acute GVHD with liver or



**Fig. 5 | Algorithm for acute GVHD treatment.** Patients with acute graft-versus-host disease (GVHD) should be assessed first for skin manifestations. Isolated stage 1–2 skin acute GVHD should be treated with topical steroid alone, whereas isolated stage 3–4 skin acute GVHD should be treated with

methylprednisolone. Gastrointestinal and liver acute GVHD can also be treated with methylprednisolone. If treatment fails, the dose of methylprednisolone can be increased in patients who received a low initial dose, or ruxolitinib therapy can be initiated. Third-line treatments can be used if ruxolitinib fails.

extensive skin involvement (rash  $\geq 50\%$  body surface area) or grade III–IV acute GVHD, the use of low-dose prednisone was associated with an increased risk of requiring secondary immunosuppressive therapy<sup>173</sup>.

For GI involvement, two randomized trials compared prednisone plus beclomethasone (a non-absorbable oral steroid) and prednisone plus placebo<sup>174,175</sup>. Beclomethasone was associated with a reduced risk of GVHD treatment failure and improved survival. Based on these data, non-absorbable oral steroids (such as oral beclomethasone) are recommended in patients with acute GVHD with GI involvement<sup>131</sup>. Budesonide can be used if oral beclomethasone is not available. Similarly, in patients with skin acute GVHD who received systemic steroids, the use of topical steroids in addition to systemic steroids is recommended until the skin rash disappears.

Formal assessment of acute GVHD is performed 3 and 7 days after diagnosis. Steroid-refractory GVHD is defined as disease that progresses by day 3 or disease that fails to improve by day 7 (ref. 176). The response rates to first-line treatment with prednisone are low (~50%)<sup>177</sup>; therefore, there is an important need for addition of other therapies to improve response rates.

One four-arm randomized phase II study aimed to identify the most promising agent for initial treatment of acute GVHD by evaluating methylprednisolone plus etanercept, MMF, denileukin diftitox or pentostatin<sup>178</sup>. Complete response (CR) rates at day 28 were 26% for methylprednisolone and etanercept, 60% for methylprednisolone and MMF, 53% for methylprednisolone and denileukin diftitox, and 38% for methylprednisolone and pentostatin<sup>178</sup>. The corresponding OS at 9 months was 47% for methylprednisolone and etanercept, 64% for methylprednisolone and MMF, 49% for methylprednisolone and denileukin diftitox, and 47% for methylprednisolone and pentostatin. As MMF was the most promising agent in this trial, a phase III randomized double-blind trial compared methylprednisolone plus placebo and methylprednisolone plus MMF<sup>179</sup>, and found similar CR rates at day 28 (46.6% in the methylprednisolone and MMF group versus 44.5% in the methylprednisolone and placebo group;  $P = 0.76$ ), with no difference in GVHD-free survival at day 56 in patients with grade III–IV acute GVHD (54.1% in the MMF group and 51.2% in the placebo group;  $P = 0.8$ ). Another randomized phase III trial found similar CR rates at day 28 for acute GVHD in patients who received infliximab plus methylprednisolone or methylprednisolone alone (62% versus 58%, respectively;  $P = 0.7$ )<sup>180</sup>.

More recently, another phase III study compared corticosteroids plus either the JAK1 inhibitor itacitinib or placebo for the initial treatment of acute GVHD ( $n = 439$ )<sup>181</sup>. This study found no difference in overall response rate (ORR) at day 28 between groups (OR 1.45, 95% CI 0.96–2.20;  $P = 0.078$ ). In addition, another phase III randomized study compared sirolimus and prednisone for the initial treatment of standard risk acute GVHD (defined by the Minnesota GVHD Risk Score and Ann Arbor biomarker status)<sup>182</sup>. This study found similar CR and partial response (PR) rates at day 28 for sirolimus (64.8%, 90% CI 54.1–75.5%) versus prednisone (73%, 90% CI 63.8–82.2%), with no difference in patients' outcomes. Moreover, sirolimus treatment was associated with reduced steroid exposure and hyperglycaemia, reduced number of grade II–III infections, improvement in immune suppression discontinuation, and patient-reported QOL, and increased risk of TA-TMA.

## Second-line and third-line therapies

Until recently, no standard second-line treatments were available for steroid-resistant or steroid-dependent acute GVHD. Different treatments were used for this purpose, including ATG, AAT, anti-TNF, MMF,

anti-IL-2R, alemtuzumab, sirolimus, extracorporeal photopheresis, methotrexate, mesenchymal stem cells, decidual stromal cells and faecal microbiota transplantation<sup>183–185</sup>. These therapies are now administered as third-line options.

Based on promising retrospective data and a phase II clinical trial<sup>186,187</sup>, the JAK2 inhibitor ruxolitinib was evaluated in a large phase III randomized clinical trial comparing the best available treatment for steroid-refractory or steroid-dependent acute GVHD ( $n = 309$ )<sup>188</sup>. This trial found a higher overall response at day 28 in the ruxolitinib group than in the control group (62% versus 39%; OR 2.64, 95% CI 1.65–4.22;  $P < 0.001$ ). The results of this study led to the approval of ruxolitinib as a second-line treatment for steroid-refractory or steroid-dependent acute GVHD by the FDA and the EMA. However, despite these positive results, in the phase III randomized clinical trial, 38% of patients who received ruxolitinib did not achieve a CR or PR by day 28, and the durable response rate at day 56 was 39.6% (indicating that 60.4% of the patients required a third-line immunosuppressive therapy or had died)<sup>188</sup>. Ruxolitinib-refractory acute GVHD can be defined as disease that progresses after 5 to 10 days of treatment with ruxolitinib, fails to improve (PR or better) after at least 14 days of treatment with ruxolitinib, or shows a loss of response at any time after initial improvement<sup>176</sup>. Management of patients with steroid-resistant and ruxolitinib-resistant acute GVHD is an unmet need, and it is recommended that such patients be included in clinical trials<sup>131</sup>. If trial enrolment is not possible, third-line treatment should be chosen according to centre practice<sup>183,184</sup> (Fig. 5; Table 3).

## Clinical trials

As treatment of acute GVHD remains associated with a high failure rate, inclusion in clinical trials of new therapies is recommended. In particular, attempts have been made to produce a risk adapted approach using the Ann Arbor (AA) biomarker risk score or the Minnesota risk score<sup>189</sup>, which predict response to steroid treatment, survival and transplant-related mortality more accurately than usual acute GVHD grading criteria<sup>89,109,110</sup>. The overall clinical response rate (CR and PR) 28 days after initiation of treatment is a validated surrogate for OS, and has been widely adopted as the primary end point in trials of treatments for acute GVHD. Secondary end points usually include the durable overall clinical response rate at day 56, OS, NRM and effect on underlying malignancy relapse (cumulative incidence of relapse), but also safety, exposure to steroids and QOL.

## Treatment complications

Use of high-dose steroids and other immunosuppressive drugs increases the risk of infectious complications and other non-infectious adverse effects. Accordingly, supportive care is indispensable in patients with acute GVHD.

Close monitoring of suspected invasive fungal infections is recommended using the serum biomarker antigen galactomannan and directed CT scans<sup>190,191</sup>. In a randomized phase III study, prophylactic posaconazole was as effective as fluconazole in preventing all invasive fungal infections (incidence 5.3% and 9.0%; OR 0.56, 95% CI 0.30–1.07;  $P = 0.07$ ) and was superior to fluconazole in preventing proven or probable invasive aspergillosis (2.3% versus 7.0%; OR 0.31, 95% CI 0.13–0.75;  $P = 0.006$ ) in patients with severe acute GVHD<sup>192</sup>. Therefore, invasive fungal infection prophylaxis with posaconazole is recommended in patients with acute GVHD. Patients should also receive prophylaxis for *Pneumocystis jirovecii* pneumonia with sulfamethoxazole trimethoprim<sup>193</sup>.

Close monitoring for CMV infection is recommended in patients with acute GVHD using CMV PCR or pp65 antigen assay<sup>194</sup>. Ganciclovir or foscarnet must be initiated early in those with CMV reactivation to avoid clinical manifestations and disease. Letermovir is approved for CMV prophylaxis for up to 100 days after alloHCT<sup>195</sup>, although letermovir prophylaxis can be used after day 100 in patients at high risk of late CMV infection, including those with acute GVHD who are receiving immunosuppressive treatment<sup>196</sup>. Importantly, letermovir is not effective for prevention of varicella zoster virus infection, and patients must continue to receive valacyclovir to prevent shingles and chicken pox<sup>197,198</sup>. Close monitoring for Epstein–Barr virus infection is essential to detect early viraemia, and to permit early treatment with an anti-CD20 monoclonal antibody to prevent post-transplantation lymphoproliferative disorder<sup>199</sup>.

Patients and their family and caregivers should also receive vaccination against influenza<sup>200,201</sup> and SARS-CoV-2 (ref. 202). Patients with influenza infection should receive neuraminidase inhibitors<sup>203</sup>. Nirmatrelvir and ritonavir are recommended in patients with COVID-19 who do not require supplemental oxygen and who have increased risk of severe disease<sup>204</sup>, and this recommendation is therefore highly relevant in patients with acute GVHD. However, ritonavir is a strong inhibitor of cytochrome P450–3A4, and interacts with several drugs, including calcineurin inhibitors, steroids and ruxolitinib; therefore, the use of nirmatrelvir plus ritonavir must be carefully assessed in patients as it is associated with withholding or reduction in the dose of some immunosuppressants<sup>205</sup>.

Although patients with humoral immune deficiency (hypogammaglobulinaemia) after alloHCT and acute GVHD treatment are more susceptible to infection with encapsulated bacteria, prophylactic use of intravenous immunoglobulin is not supported by data<sup>206</sup>. Patients should receive routine prophylaxis with penicillin or equivalent antibiotics and receive vaccinations for *Streptococcus pneumoniae* and *Haemophilus influenzae*<sup>207,208</sup>. Patients who have received steroids have a high risk of bacteraemia and septic shock; therefore, blood culture should be performed regularly in these patients even in the absence of fever. The presence of fever should prompt an infectious work-up, including blood culture, without delaying the initiation of a broad-spectrum antibiotic<sup>209</sup>.

The use of high-dose steroids is also frequently associated with diabetes mellitus, osteoporosis, aseptic osteonecrosis, amyotrophy (progressive muscle wasting) and other symptoms of iatrogenic Cushing syndrome. Close monitoring of for corticoid-induced toxicity is therefore recommended to initiate early preventive or curative treatment that can include insulin, calcium, vitamin D or bisphosphonates<sup>210,211</sup>.

## Quality of life

QOL is evaluated using patient-reported outcome questionnaires. No specific QOL questionnaire is available for patients with acute GVHD; therefore, QOL in these patients is usually evaluated with a questionnaire for cancer, such as the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire–Core 30 (EORTC QLQ-C30)<sup>212</sup> or for alloHCT, such as the Functional Assessment of Cancer

**Table 3 | Available third-line treatment for acute GVHD**

Treatment	Mechanism of action	ClinicalTrials.gov identifier
Alemtuzumab	Anti-CD52 monoclonal antibody, which targets T cells, B cells, natural killer cells, monocytes and macrophages	NA
α1 Antitrypsin	Downmodulates inflammation and increases the ratio of T <sub>reg</sub> cells to T <sub>eff</sub> cells	NA
Daclizumab, basiliximab and inolimomab (anti-IL-2 receptor antibodies)	Anti-IL-2R monoclonal antibody, which inhibits activated T cells	NCT05021276, NCT04289103 and NCT05047328
Infliximab and etanercept (anti-TNF)	Anti-TNF monoclonal antibody	NA
Antithymocyte globulin	T cell depletion, induction of apoptosis in B cell lineages, and induction of T <sub>reg</sub> cells and natural killer T cells	NA
Decidual stromal cells	Immunomodulatory activity	NCT05132166 and NCT04118556
Extracorporeal photopheresis	Apheresis-based immunomodulator that has immunosuppressive effects against T cells	NCT05333029
Faecal microbiota transplantation	Re-establishes the gut microbiota	NCT04711967, NCT04280471, NCT03819803, NCT05094765, NCT03812705 and NCT04769895
Mesenchymal stromal cells	Immunomodulatory activity	NCT05333029, NCT04744116 and NCT04629833
Methotrexate	Inhibits dihydrofolate reductase; inhibits production of thymidylate and purines; suppresses T cell responses, proliferation and expression of adhesion molecules	NA
Mycophenolate mofetil	Prodrug of mycophenolic acid, an inhibitor of inosine-5'-monophosphate dehydrogenase, inhibits proliferation of T cells and B cells	NA
Pentostatin	Purine analogue, adenosine deaminase inhibitor and T cell suppression	NA
Sirolimus	mTOR inhibitor, inhibits activation of T cells and B cells by reducing their sensitivity to IL-2	NA
Vedolizumab	Anti-α4β7 integrin monoclonal antibody, which inhibits the relocation of circulating lymphocytes to the gastrointestinal tract	NA

GVHD, graft-versus-host disease; mTOR, mammalian target of rapamycin; NA, not available; T<sub>eff</sub> cells, effector T cells; T<sub>reg</sub> cells, regulatory T cells.

Therapy–Bone Marrow Transplant (FACT-BMT)<sup>213</sup>. The FACT-BMT is based on Functional Assessment of Cancer Therapy–General (FACT-G), a 27-item cancer-specific questionnaire<sup>214</sup>, which contains supplementary questions to assess additional concerns including overall treatment effects and regret related to transplantation. The 12-item Short-Form Health Survey (SF-12) can also be used to evaluate QOL and is more practical than all other available questionnaires given its short format<sup>215</sup>.

In a cohort of 96 patients, development of grade II–IV acute GVHD was associated with a measurable decline in QOL at 6 months after alloHCT, when assessed using the FACT-BMT, compared with patients who had not developed acute GVHD<sup>216</sup>. Moreover, in patients who survived at least 1 year after alloHCT, the SF-12 Physical Component Score (PCS) and Mental Component Score (MCS) were worse in survivors of grade III–IV acute GVHD compared with those with a history of grade 0–I acute GVHD<sup>217</sup>. Furthermore, patients with a history of grade III–IV acute GVHD had significantly higher rates of late medical comorbidities than those with a history of grade 0–I acute GVHD. In particular, the cumulative incidence of major late effects (acute respiratory distress syndrome, heart failure and renal failure requiring dialysis) at 10 years after alloHCT was 42% in the grade 0–I cohort and 61% in the grade III–IV acute GVHD cohort ( $P < 0.001$ ). In addition, patients who survived for >1 year after transplantation and had prior grade III–IV acute GVHD also had a worse 5-year OS (77.5% versus 83.6%;  $P = 0.006$ ) and higher NRM (19.2% versus 10.6%;  $P < 0.001$ ) compared with those with a history of grade 0–I acute GVHD.

Importantly, acute GVHD is a risk factor for chronic GVHD<sup>23,218</sup>, which is leading cause of late NRM, morbidity<sup>219</sup> and impaired QOL<sup>220,221</sup>. Patients also frequently experience psychosocial distress after alloHCT, with some studies finding depression in 25–35% of patients within the first year after alloHCT<sup>222,223</sup>. Overall, patients who survive for >1 year after alloHCT, and particularly those with a history of acute GVHD, are a high-risk population that must be monitored for long-term transplant complications, including chronic GVHD, multiorgan dysfunction and secondary malignancies<sup>224</sup>. Recommendations for long-term patient monitoring are provided in Table 4. Finally, available QOL questionnaires, developed for use in patients with cancer or more specifically those who undergo alloHCT, effectively evaluate QOL in patients with acute GVHD and should be used to improve patient monitoring without the need to create a specific QOL questionnaire for acute GVHD.

## Outlook

Prophylaxis and treatment of acute GVHD has significantly progressed in the past few years. Indeed, development of PTcY for GVHD prophylaxis has allowed the important development of haploidentical alloHCT worldwide<sup>3</sup> and a standard second-line treatment for steroid-refractory acute GVHD is now available<sup>188</sup>. Despite these achievements, there remain some important unmet medical needs in acute GVHD.

The most effective GVHD prophylaxis remains to be established. Further studies are needed to identify the best combinations of therapies and optimal doses to prevent severe acute GVHD without increasing the risk of infectious complications and relapse of the underlying disease. Similarly, the most effective first-line treatments for acute GVHD are still not defined, and, owing to their high failure rate and associated toxicity, steroid-sparing approaches are urgently needed. Biomarkers that are predictive of the risk of NRM and resistance to treatment in patients with acute GVHD<sup>115–117</sup> have been developed and efforts must be made to implement their use in routine practice to evaluate first-line treatments for acute GVHD in patients with a high risk of resistance to steroids. This is particularly important

**Table 4 | Long-term patient monitoring**

Organ/function	Clinical assessment
Pulmonary	Pulmonary function tests at baseline, 6 months, 1 year and annually after alloHCT
Cardiovascular	Routine screening for modifiable cardiovascular risk factors
	Blood pressure measurement at regular intervals
	Lifestyle modification counselling in people who are obese and sedentary
	Lipid and glucose profiles at baseline, 1 year and annually after alloHCT
Liver	Echocardiography at baseline, 3 months, 1 year and annually after alloHCT
	Liver function at regular intervals, 3–6 months after transplant, and on an annual basis  In case of abnormal hepatic values: hepatology consultation, imaging (MRI and ultrasound elastography) to measure the hardness of liver tissue, or a liver biopsy must be discussed
Endocrine	Thyroid-stimulating hormone and free T4 levels at the time of transplant, 3–6 months, 1 year and annually after alloHCT
	Screening for dyslipidaemia should begin at least at 6 months, then at 1 year and annually after alloHCT
Oral cavity	Routine oral examination at each clinic visit
	Regular dental appointments are encouraged  Any new, non-healing ulcers or raised, indurated lesions should be considered for biopsy
Ophthalmological	Evaluation of visual acuity at 6 months, 1 year and annually after alloHCT
Psychosocial	Screening and appropriate assessment of psychological health should be performed at regular intervals, and at least yearly after transplant

alloHCT, allogeneic haematopoietic cell transplantation.

given the irreversible tissue damage in refractory acute GVHD caused by epithelial stem suppression by steroids<sup>225</sup> or by GVHD through reduced patient-derived IL-22 levels<sup>226,227</sup> and increased telomere shortening<sup>228</sup>. Moreover, treatment of patients with steroid-resistant and ruxolitinib-resistant acute GVHD is an unmet medical need, and innovative approaches are urgently required. One of the most promising approaches is the development of gut microbiota manipulation, particularly faecal microbiota transplantation<sup>229–231</sup>.

Over the past few years, alloHCT has become more accessible across the world, partially owing to the development of haploidentical alloHCT with PTcY, which offers a cost-effective platform for low-income and middle-income countries<sup>5</sup>. However, management of severe acute GVHD will remain a challenge in these countries, particularly steroid-refractory acute GVHD, owing to the cost and lack of availability of ruxolitinib and third-line treatment. Similarly, effective prophylaxis for infectious diseases that is required during treatment of acute GVHD may be inaccessible in these countries. Accordingly, strategies developed to facilitate access to newly developed anticancer drugs in these regions<sup>232</sup> should also include treatments for complications such as acute GVHD and anti-infection prophylaxis.

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## Author contributions

Introduction (F.M., M.M.); Epidemiology (F.M. and M.M.); Mechanisms/pathophysiology (F.M., E.H. and M.M.); Diagnosis, screening and prevention (F.M., B.M.S. and M.M.);

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## Competing interests

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## Informed consent

The authors affirm that human research participants provided informed consent for publication of the images in Fig. 4.

## Additional information

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