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**Allogeneic stem cell transplantation in the treatment of acute myeloid leukemia: an overview of its obstacles and opportunities from the perspective of clinical immunology**

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**Abstract**

As an important treatment for acute myeloid leukemia (AML), <sup>26</sup>allogeneic hematopoietic stem cell transplantation (allo-HSCT) plays an important role in reducing relapse and improving long-term survival. With rapid advancements in basic research in molecular biology and immunology and with deepening understanding of the biological characteristics of hematopoietic stem cells (HSCs), allo-HSCT has been widely applied in clinical practice. During allo-HSCT, preconditioning, the donor, and the source of stem cells can be tailored to the patient's conditions, greatly broadening the indications for HSCT, with clear survival benefits. However, the risks associated with allo-HSCT remain high, i.e., hematopoietic reconstitution failure, delayed immune reconstitution, graft-versus-host disease (GVHD), and post-transplant relapse, which are bottlenecks for further improvements in allo-HSCT efficacy and have become hot topics in the field of HSCT. <sup>14</sup>Other bottlenecks recognized in the current treatment of individuals diagnosed with AML and subjected to allo-HSCT include the selection of the most appropriate conditioning regimen and post-transplantation management. In this paper, we will review the progress of relevant research regarding these aspects.

<sup>14</sup>**INTRODUCTION**

Leukemia is a malignant disease caused by the abnormal proliferation and differentiation of hematopoietic stem cells (HSCs). Chemotherapy is still one of the main treatments for leukemia, with most patients achieving complete remission (CR) after induction and consolidation chemotherapy. However, some patients relapse in months or years despite CR followed by maintenance chemotherapy. To improve the prognosis of leukemia subjects, some researchers have tried to increase the dose of induction and consolidation chemotherapy to kill as many leukemia cells as possible before they become resistant to certain antineoplastic drugs. The results, however, are unsatisfactory. Certain malignant cells, such as leukemic stem cells (LSCs), hide in the bone marrow niche, resulting in minimal residual disease (MRD), which is difficult to clear and is an important cause of resistance and relapse <sup>[1]</sup>. In addition, high-dose chemotherapy drugs can easily damage HSCs and cause bone marrow suppression <sup>[2-5]</sup>. Therefore, an appropriate post-CR treatment plan is important for improving the disease-free survival (DFS) of leukemia patients.

Hematopoietic stem cell transplantation (HSCT) has been one of the most important breakthroughs in the therapy of malignant tumors over the last 5 decades. In 1957, Professor Thomas, a renowned hematologist, first used allogeneic bone marrow transplantation (allo-BMT) to successfully treat hematological malignancies. Since then, allo-HSCT technology has been improving and has been implemented worldwide. Allo-HSCT has completely transformed the treatment of hematological malignancies, with substantial survival benefits *via* the graft-versus-leukemia (GVL) effect. However, the risks of allo-HSCT include hematopoietic reconstitution failure, delayed immune reconstitution, graft-versus-host disease (GVHD), and posttransplant relapse, which are past and current challenges and research topics in the field of HSCT <sup>[1-5]</sup>.

According to the most recent guidelines of the National Comprehensive Cancer Network, allo-HSCT can be considered in patients diagnosed with AML in the following clinical contexts <sup>[6]</sup>:

subjects aged less than 60 years who display induction failure after induction with high-dose cytarabine, i.e., after at least two courses of intensive induction therapy, the patient does not achieve complete response or complete response with incomplete hematological recovery.

in the setting of post-induction therapy in subjects aged 60 years or more who achieve complete response after induction with standard dose cytarabine and are fit to be subjected to conventional consolidation or in those individuals who display induction failure in whom allo-HSCT preferably should be performed in the setting of a clinical trial.

in the setting of post-induction therapy in subjects aged 60 years or more who achieve response after being subjected to lower-intensity regimens.

in patients with relapsed AML after the use of targeted therapy or chemotherapy, depending on the genomic profile of the malignancy.

### HEMATOPOIETIC RECONSTITUTION AFTER BONE MARROW TRANSPLANTATION

During allo-HSCT, whether the transplanted donor hematopoietic stem and progenitor cells (HSPCs) can successfully home to the bone marrow (BM) niche, with successful hematopoietic reconstitution in an appropriate hematopoietic microenvironment, is key for the success of allo-HSCT. HSPCs homing and engraftment is a complex multistep process that involves complex interactions between HSPCs and a range of stromal cells in the hematopoietic microenvironment, as well as various molecules, *e.g.*, adhesion molecules and chemokines [7-8].

Primitive CD34<sup>+</sup> HSPCs express a wide range of cell adhesion molecules, some of which are closely related to HSPC homing, *e.g.*, P-selectin glycoprotein ligand 1 (PSGL-1), integrins such as <sup>1</sup>very late antigen-4 (VLA-4), lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1), lymphocyte function-associated antigen-1 (LFA-1), specific antigens such as CD44, and cadherins [7, 9]. Most of the adhesion molecules

on HSPCs have corresponding ligands on the BM mesenchymal stromal cells (MSCs) and the extracellular matrix. The adhesion molecules and their ligands recognize each other and mediate the adhesion of HSPCs (**Table 1**).

**Table 1.** Molecules that mediate HSPC adhesion and chemotaxis during transplantation.

**HSPC receptors**

**Bone marrow ligands**

**Effect**

**Reference**

PSGL-1/CD162

Selectins (P and E)

Promote HSPC homing

[10]

$\beta_1$  integrin

Osteopontin (Opn)

Contribute to HSC trans-marrow migration toward the endosteal region

[17, 18]

VLA-4/ $\alpha_4 \beta_1$

VCAM-1, fibronectin

Promote HSPC homing

[7, 11]

VLA-5/ $\alpha_5 \beta_1$

Fibronectin

Promote HSPC homing and proliferation

[19, 20]

LFA-1/ $\alpha_L \beta_2$

ICAM-1

Promote HSPC homing

[7, 11]

LPAM-1/ $\alpha_4\beta_7$

Mucosal addressin cell adhesion molecule-1 (MAdCAM-1)

Promote HSPC homing and engraftment

[21]

Cx43

Participate in the formation of intercellular transmembrane channels, facilitate the transportation of mitochondria or other substances, and promote bone marrow regeneration and HSPC engraftment

[22]

CXCR4

SDF-1

Promote HSPC homing and engraftment and participate in the regulation of HSPCs survival and proliferation

[7]

c-kit

Stem cell factor (SCF)

The transmembrane isoform of SCF is critical in the lodgment and detainment of HSCs within the bone marrow niche

[23]

c-MPL

TPO

TPO promotes the survival and proliferation of HSPCs and upregulates SDF-1 in bone marrow niche, thereby contributing to HSPC homing and engraftment

[24, 25]

CD44/Pgp-1

Selectins (P, E and L), hyaluronic acid (HA)

CD44 and HA <sup>12</sup> play a key role in SDF-1-dependent transendothelial migration of HSPCs and their final anchorage within the bone marrow niche

[26]

CD82/KAI1

CD82 modulates HSPC bone marrow maintenance, homing, and engraftment

[27]

Annexin II receptor/ Anxa2r

Annexin II/ Anxa2

Regulate stem cell adhesion, homing, and engraftment

[28]

Calcium-sensing receptor (CaR)

Ca<sup>2+</sup>

Enhance HSC lodgment and engraftment in the bone marrow niche

[29]

N-cadherin

N-cadherin

N-cadherin-mediated cell adhesion is <sup>18</sup> functionally required for the establishment of hematopoiesis in the bone marrow niche after bone marrow transplantation

[30]

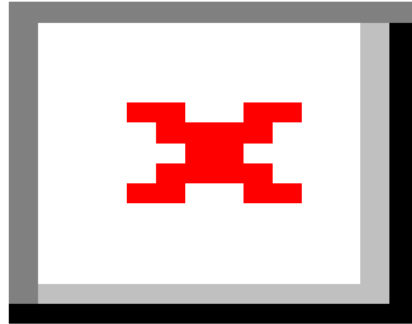
Upon entering the BM cavity from the blood circulation, the initial adherence of HSPCs to the BM sinusoidal endothelial cells (BMECs) requires PSGL-1, P-selectin, and E-selectin <sup>[10]</sup>. After this, the adhesion between HSPCs and BMECs becomes tighter, and the HSPCs enter the BM hematopoietic microenvironment by passing through BMECs. The process requires integrins and immunoglobulin superfamily members, especially <sup>1</sup> VLA-4/vascular cell adhesion molecule-1 (VCAM-1) and LFA-1/intercellular adhesion molecule-1 (ICAM-1) pathways <sup>[7, 11]</sup>. In the BM hematopoietic microenvironment, HSPCs adhere and interact with stromal cells and the extracellular

matrix and stimulate BM stromal cells to secrete hematopoietic cytokines to regulate the quiescence, self-renewal, proliferation, and differentiation of HSPCs [12-16].

A large body of evidence indicates that the axis composed of stromal derived factor-1 (SDF-1/CXCL12) secreted by osteoblasts and endothelial cells and the HSC surface receptor CXCR4 plays a critical role in HSC homing and subsequent engraftment. The SDF-1/CXCR4 signal induces HSPCs to pass through the endothelial layer to adhere to the BM matrix, promoting HSPC homing and engraftment *via* chemotaxis and participating in the regulation of HSPC survival and proliferation [7, 31]. Given the important role of SDF-1/CXCR4 in HSPC homing and engraftment, the regulation of this signal axis is also important for promoting posttransplant hematopoietic reconstitution. Studies have shown that mild heat treatment, prostaglandin E2 (PGE2), histone deacetylase (HDAC) inhibitors, and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) enhance the SDF-1/CXCR4 signal and promote HSPC homing and engraftment [11]. In addition to the SDF-1/CXCR4 axis, other chemokine axes and numerous molecules, *e.g.*, receptor tyrosine kinase (C-Kit), thrombopoietin (TPO), and matrix metalloproteinases (MMPs), are involved in HSPC maintenance, homing, and engraftment [23, 25, 32, 33] (**Table 1**).

Recent research has delineated that the adhesion molecule connexin-43 (Cx43) plays an important role in BM regeneration and HSPC engraftment after irradiation preconditioning. With Cx43-mediated cell-to-cell contact, donor HSPCs transfer mitochondria to postradiation recipient MSCs, promoting the metabolic recovery of radiation-damaged MSCs and improving the BM hematopoietic compartment reconstitution and donor HSPC engraftment [22]. The mechanism of HSPC homing and engraftment is depicted in **Figure 1**.





**Figure 1.** Mechanism underlying HSPC homing and engraftment. Numerous adhesion molecules and chemokines are involved in the regulation of HSPC homing and engraftment. The interaction between recipient bone marrow stromal cells (BMSCs) and donor HSPCs contributes to HSPC homing and engraftment. In addition, donor HSPCs improve the metabolism of recipient BMSCs *via* mitochondrial transfer, accelerating the recovery of damaged BMSCs. Copyright ©The Author(s) 2022.

MSCs are the main components of the BM hematopoietic microenvironment and play important roles in supporting, regulating, and protecting HSPCs [34, 35]. During HSCT preconditioning, chemotherapy/radiotherapy causes damage to MSCs, resulting in severely low numbers of MSCs, impaired cytokine production and adhesion molecule expression, and impaired function in supporting and regulating hematopoiesis [36].

Histocompatibility is another consideration for allogeneic transplantation, as transplantation failure may also occur not only due to immune rejection but also to major histocompatibility complex (MHC) restriction between donor HSCs and

recipient stromal cells and because recipient stromal cells do not support the proliferation and differentiation of donor HSCs. There is a complex interplay between MSCs and HSCs in HSCT, as MSCs are known to support HSCs and enhance their engraftment. Due to their properties, i.e., adherence to plastic and ability to be expanded *ex vivo*, as well as the lack of reported side effects after their administration, MSCs have been employed in clinical patient research and MSCs infusion have been co-administered with HSCs to enhance the engraftment of the latter, particularly in the setting of haploidentical alloHSCT with/without T-cell depletion. In addition, MSCs secrete soluble molecules (e.g., IFN- $\gamma$ , cytokines, chemokines, *etc.*) and exhibit immunomodulatory actions, having already been employed successfully in the prevention and treatment of GVHD in individuals who had been subjected to alloHSCT. Several of the processes in which MSCs are involved include decrease in inflammation and in the proliferation of B-cells and T-cells, as well as an increase in tissue repair [34, 37-38]. *In vitro* cell culture studies have demonstrated that, when primed with nitric oxide, MSCs can significantly boost the engraftment potential of HSCs *via* the intercellular transfer of microvesicles harboring mRNAs encoding HSC-supportive genes [39]. *In vitro* research has revealed that, under mild hypoxia (5% oxygen), MSCs promote CXCR4 expression in CD34<sup>+</sup> CD38<sup>-</sup> cells, thereby enhancing HSPC homing [40]. MSCs are investigated in phase I/II clinical trials of HSCT to promote HSC engraftment. In clinical applications, MSCs have been used to expand HSCs *in vitro* [34]. Previous assessments have reported that the engraftment success rate is related to several factors, *e.g.*, the number of stem cells, the stem cell source, donor-specific anti-HLA antibodies (DSAs) and the pretreatment protocol. In most cases, increasing the HSPC infusion dose contributes to successful HSPC engraftment and hematopoietic reconstitution. In addition, the quantity and quality of the grafts, as well as the age of donor, can also affect immune reconstruction after alloHSCT. For example, alloHSCT from donors aged >50 years has been linked with lower CD8<sup>+</sup>CD45RA<sup>+</sup> naïve T-cells and CD19<sup>+</sup> B-cells counts, reduced serum IgM and IgA concentrations, and higher EBV reactivation rates [12, 41-45].

However, it should be noted that for allogeneic peripheral blood stem cell transplantation (allo-PBSCT), a high dose of CD34<sup>+</sup> cells increases the risk of extensive chronic graft-versus-host disease (cGVHD) [46]. The higher incidence of extensive cGVHD leads to adverse effects on the patient's prognosis and increases transplant-related mortality, particularly among subjects receiving T-cell depleted allogeneic transplantation with myeloablative conditioning. In contrast, individuals who are subjected to low-intensity preconditioning rather than myeloablative regimens may benefit from a higher dose of CD34<sup>+</sup> cells, as it has been shown that relapse and/or progression rates were significantly lower (9% vs 36%) in subjects who had received an elevated number of CD34<sup>+</sup> cells [46]. Most clinical studies have indicated that in HLA-identical sibling donor transplantation, the application of peripheral blood-derived stem cells accelerates platelet and neutrophil engraftment, which is related to the use of G-CSF during mobilization of peripheral blood-derived stem cells [47-49]. In addition to successfully mobilizing CD34<sup>+</sup> stem cells from the BM of healthy donors, G-CSF can induce changes in immune cell function, redirect T-cell polarization, and change the expression of adhesive molecules, resulting in rapid and long-lasting engraftment [50].

With the widespread development of HLA haploidentical stem cell transplantation in recent years, engraftment failure and poor engraftment are still an urgent problem to be solved in HLA haploidentical transplantation. DSAs are the most important factors causing engraftment failures of HLA haploidentical transplantation. Chang *et al* suggested that high DSA levels were associated with primary engraftment failure and poor primary engraftment in HLA haploidentical transplantation [51]. Ciurea *et al* used plasma exchange, rituximab, intravenous immunoglobulins, and irradiated donor buffy coat to intervene in patients with high DSA levels and found that the engraftment success rate was higher in patients with decreased DSAs and negative complement component 1q (C1q) [52]. Pretreatment protocols can also affect the engraftment success rate. The Beijing Protocol and the posttransplant cyclophosphamide (PTCy) protocol are

some of the most commonly used pretreatment protocols for HLA haploidentical transplantation worldwide. Recently, Tang *et al* conducted a retrospective study and found that the Beijing Protocol exhibited some advantages *vs* single-cell source + the PTCy transplant regimens in terms of 30-day neutrophil engraftment rate, 90-day platelet engraftment rate, median neutrophil engraftment time, and platelet engraftment time [53]. Other pretreatment protocols have been previously summarized by Baumeister *et al* elsewhere [54].

In recent years, researchers have paid more attention to alternative routes of stem cell administration to reduce the ineffective homing of donor cells [55-56]. Animal model studies have highlighted that compared with intravenous infusion, intra-bone marrow injections (IBM-Is) for HSPC transplantation are more effective in promoting hematopoietic reconstitution and reducing the incidence of GVHD [57-59]. At present, the clinical application of IBM-Is is still in its infancy and is mostly used for umbilical cord blood cell transplantation (UCBT).

### **POSTTRANSPLANT IMMUNE RECONSTITUTION**

HSCs have the capacity for self-renewal, to proliferate and to differentiate into hematopoietic cells and immune cells. Therefore, HSCT is essentially a dual transplantation of hematopoietic cells and immune cells. After allo-HSCT, the recipient's hematopoietic system and immune system are reconstituted simultaneously. The restoration of immune function helps patients fight pathogens and ensures successful HSCT. For allo-HSCT recipients, immune reconstitution is a highly dynamic process, including innate immune system reconstitution and adaptive immune system reconstitution. Posttransplant immune reconstitution takes time, and different immune cells follow different reconstitution patterns, having important implications for the outcome of allo-HSCT.

<sup>17</sup> The innate immune system is mainly composed of natural killer (NK) cells, neutrophils, monocytes, macrophages, and antigen presenting cells (APCs) [60], of which

NK cells are the first group of lymphocytes to recover after transplantation, taking only 1-4 mo to return to normal levels, independent of the stem cells source [61-64]. The function of NK cells is regulated by the interaction between killer immunoglobulin-like receptors (KIRs) and the ligand human leukocyte antigen (HLA) [65]. For haploidentical transplantation, HSCTs with alloreactive donor NK cells (KIR-HLA mismatched HCT) are shown to be associated with less relapse and better overall survival [66-69]. Such alloreactive NK cells may also have a beneficial effect on alleviating GVHD because they can eliminate host APCs that prime alloreactive T cells that cause GVHD [67, 70, 71]. As with NK cells, neutrophils and monocytes also recover in a short period of time after transplantation. Dendritic cells (DCs), shown to be the most potent APCs, take longer to restore. In adults, while donor DCs can be detected in the peripheral blood in the first few weeks after stem cell transplantation, the total number may not return to normal even after a year [60, 72, 73]. Furthermore, previous investigations have pinpointed that while peripheral blood DCs are mainly derived from donors (>80% by day 14), up to 70% of tissue DCs may still come from the host [60, 74-76], and these tissue DCs of host origin may persist for up to a year following HSCT [75]. Researchers have confirmed that host APCs, rather than donor APCs, play an important role in the post-allo-HSCT GVL effect and GVHD [77-79]. Therefore, proper regulation of host APCs may alleviate GVHD and enhance the GVL effect.

Adaptive immune reconstitution mainly includes the restoration of the number and function of B-cells and T-cells. Reconstitution of the B-cell compartment after HSCT occurs primarily through *de novo* regeneration from BM progenitors [80]. Generally, the proportion of total B-cells in most patients reaches normal levels by 3 mo, but the absolute number may not return to normal for 6-12 months [81-83]. During the first year of HSCT, most reconstituted B-cells are mainly composed of transitional and naive subsets, however, the restoration of memory B-cells takes much longer [83]. Consistent with this, immunoglobulin M (IgM) levels recover in 2 to 6 months after transplantation, and then IgG levels return to close to normal in 3 to 18 months after transplantation, whereas IgA reconstitution may be delayed for up to 3 years [60]. T-cell



immune reconstitution is markedly different from B-cell immune reconstitution. T-cells mainly include two subgroups, CD4<sup>+</sup> T-cells and CD8<sup>+</sup> T-cells, which are reconstituted through thymus-independent and thymus-dependent pathways. The early increase in blood T-lymphocyte numbers is related to the thymus-independent peripheral expansion of mature donor T-cells. The recovery of a broader T-cell repertoire, however, depends on the *de novo* generation of naive T-cells through the thymus after the engraftment and differentiation of hematopoietic stem cells in the BM [80, 84]. Preconditioning or GVHD impairs thymus function, resulting in decreased CD4<sup>+</sup> T cells after transplantation. Memory or effector CD8<sup>+</sup> T-cells can rapidly expand through a thymus-independent pathway and return to normal in 12 months. Therefore, an inverted CD4:CD8 ratio after transplantation is one of the earliest signs of T-cell reconstitution and may last for several years, depending on preconditioning and GVHD prevention regimens [83]. CD4<sup>+</sup> CD25<sup>+</sup> regulatory T-cells (Tregs), a subgroup of CD4<sup>+</sup> T-cells, play an important role in HSCs maintenance. Cytotoxic T-cell activation and decreased Treg counts are believed to be the etiology of idiopathic severe aplastic anemia [85]. Tregs reconstitute faster than do effector T cells after HSCT. They suppress the activation and proliferation of effector T-cells and downregulate the body's response to foreign antigens or autoantigens, thereby maintaining immune tolerance [86]. Numerous recent studies show that an imbalance between Tregs and effector T-cells may be an important link in the occurrence of GVHD [87, 88].

Posttransplant immune reconstitution is affected by many factors, such as the intensity of preconditioning, recipient thymus function, recipient age, graft source, and GVHD (Table 2). Delayed immune reconstitution makes HSCT recipients susceptible to various infections. In fact, despite the use of routine peritransplant prophylactic antibiotics, approximately 80-85% of HSCT recipients contract infections, which is one of the leading causes of nonrelapse death after allo-HSCT [89]. At present, there is no "standard-of-care" approach to enhance post-transplant immune reconstitution, however, several strategies such as protecting the thymic epithelium, stimulating thymopoiesis, or increasing the number of T-lymphoid precursors, are

being investigated in preclinical models as well as early clinical trials<sup>[83]</sup>, the effectiveness of these measures remains to be further verified and improved in practice.

**Table 2.** Main factors for posttransplant immune reconstitution.

**Factors**

**Effect**

**Reference**

Recipient age

Several studies show that immune reconstitution, especially the reconstitution of CD4<sup>+</sup> T-cells, is inversely related to age. However, some studies report that age has no effect on the reconstitution of any subgroup of lymphocytes.

[63, 90, 91]

Graft source

Immune reconstitution occurs faster after PBSCT than after BMT. This may be because PBSCT grafts are rich in mature lymphocytes. Delayed immune reconstitution after UCBT is related to low lymphocyte count and immature immune cells in umbilical cord blood.

[61, 92-95]

HLA matching between donor and recipient

HLA mismatch causes delayed reconstitution of neutrophils and T cells.

Intensity of preconditioning

Several studies show that compared with myeloablative stem cell transplantation (MA-SCT), reduced-intensity conditioning stem cell transplantation (RICSCT) reduces thymus damage and promotes immune reconstitution. However, some studies show no significant difference in recipient immune reconstitution between these 2 transplantation methods.

[60, 96-98]

#### GVHD

GVHD damages thymus structure and function and interferes with T cell differentiation at all stages, thereby affecting T-cell reconstitution. GVHD also affects the recovery of B cell number and function.

[84, 99]

#### GVHD prevention

Donor T-cell depletion (TCD) reduces the risk of GVHD; however, the lack of T cells increases the risk of infection and delayed immune reconstitution.

[100]

The use of antithymocyte globulin (ATG) or alemtuzumab has a negative effect on the reconstitution of T cells and B cells.

[101-103]

### **REGULATION OF GVL AND GVHD**

For allo-HSCT, an important mechanism for the treatment of leukemia is that donor immune cells recognize the surface antigens of recipient leukemia cells and trigger an immune response to attack and clear any residual leukemia cells, which is known as the GVL effect. GVL effect is closely related to GVHD, as both have similar pathways, effector cells, and cytokines. Therefore, during immune reconstitution after allo-HSCT, the precise regulation of GVL and GVHD (i.e., suppressing GVHD while preserving GVL) plays an important role in the final outcome of allo-HSCT [104, 105].

The mechanism of action of GVHD and GVL is very complex and not entirely clear. The interactions between many donor and recipient cells and cytokines make the mechanism even more challenging to understand. It is believed that donor T-cells play an important role in the occurrence of GVHD and GVL. Acute graft-versus-host disease (aGVHD) has 3 pathophysiological stages: (1) activation of APCs by the underlying disease and the HCT conditioning regimen: the damaged host tissue produces a large



amount of proinflammatory cytokines, *e.g.*, tumor necrosis factor alpha (TNF- $\alpha$ ) and chemokines, with elevated expression of adhesion molecules, MHC antigens and costimulators on host APCs; (2) donor T-cell activation: donor T-cells proliferate and differentiate in response to host APCs; activated donor T-cells secrete a large amount of Th1 cytokines, such as IFN- $\gamma$ , IL-2, and TNF- $\alpha$ , which trigger aGVHD; and (3) cellular and inflammatory effector phase: the complex cascade of cytotoxic T-lymphocytes (CTLs), NK cells, and soluble inflammatory mediators (*e.g.*, TNF- $\alpha$ , IFN- $\gamma$ , and IL-1) produces synergistic effects and causes further local tissue injury, inflammation, and target tissue damage<sup>[106]</sup>. The pathophysiology of cGVHD differs from that of aGVHD and is believed to be related to the following factors: (1) thymus damage and defective negative selection of T-cells, promoting the production of autoreactive T-cells; (2) decreased CD4<sup>+</sup> CD25<sup>+</sup> Tregs, affecting the suppressive effect of Tregs on effector T-cells; (3) abnormal activation of B-cells, promoting the production of autoantibodies and subsequently, an autoimmune response; and (4) the formation of profibrotic lesions<sup>[107]</sup>.

GVHD-related tissue damage, as well as GVL-linked tumor elimination, seem to share common immunological mechanisms<sup>[108]</sup>. Based on this understanding, mitigating the risk of GVHD while maximizing the GVL effect seems to be unrealistic. Clinically, clearing donor T-cells (TCD) effectively reduces the occurrence of and damage by GVHD; however, this approach also weakens the GVL effect, which results in a much higher risk of recurrent leukemia, especially chronic myeloid leukemia (CML). For recurrent cases, donor lymphocyte infusion (DLI) (containing primarily T-cells) enables long-term remission<sup>[109,110]</sup>. These data indicate that GVHD and the GVL effect are interdependent and that both are T-cell dependent. However, recent studies show that GVHD and the GVL effect may be mediated by different subgroups of T-cells. In the peripheral blood, the  $\alpha\beta$  T-cell receptor is expressed by 95% of T-cells, whereas the  $\gamma\delta$  T-cell receptor is expressed by the remaining T-cells. Since the primary mediators in GVHD are alloreactive  $\alpha\beta$  T-cells, their depletion from the graft is expected to decrease the chances of GVHD development. Contrastingly,  $\gamma\delta$  T-cell

receptor-expressing lymphocytes exert anti-infectious and anti-leukemia actions, are not marked by alloreactivity and are not involved in GVHD occurrence. Notwithstanding, the interest towards the use of  $\gamma\delta$  T-cells in allo-HSCT has rocketed and are currently investigated by the international scientific community [111-115]. Allo-HSCT studies in mice show <sup>2</sup> that naive T-cells consistently cause severe GVHD, whereas memory T-cells cause milder or no GVHD and have critical graft-versus-tumor functions [116-118]. Subsequent clinical trials have confirmed that the removal of donor naive T-cells effectively reduces the incidence of GVHD and opportunistic infections, without any significant increase in relapse [118-120]. In addition, GVL and GVHD effector T-cells have different target antigens. For GVHD effector T-cells, the target antigens are MHC antigens and minor histocompatibility antigens (MiHAs); for GVL effector T-cells, the target antigens are mainly MiHAs on recipient leukemia cells. Therefore, hematopoietic system-specific MiHAs <sup>13</sup> expressed on leukemic cells are considered important targets for leukemia-specific cellular immunotherapy with a low risk of GVHD [121].

Cytokines are critical drivers of both GVHD and GVL, and current evidence indicates that different cytokines may play different roles in GVHD *vs* the GVL effect [122]. In a recent study, Tugues et al. used an MHC-mismatched HSCT mouse model and found that donor T-cell-derived granulocyte-macrophage colony-stimulating factor (GM-CSF) can drive <sup>9</sup> GVHD pathology by licensing donor-derived phagocytes to produce inflammatory mediators such as interleukin-1 and reactive oxygen species (ROS). Moreover, anti-GM-CSF treatment improved the survival of recipient mice, without affecting the GVL effect of alloreactive T-cells, suggesting that GM-CSF may be an important target for GVHD-GVL uncoupling [108]. These data indicate that GVHD and the GVL effect are somewhat independent of each other and are not completely parallel, which makes it possible to target GVHD and the GVL effect separately in allo-HSCT recipients. In addition, alloreactive NK cells seem to play a role in the GVL effect especially in the early period that follows the execution of allo-HSCT (*via* interaction in the bone marrow of the recipient between the donor HLA

environment and the reconstitution of NK cells) without being involved in the development of GVHD [68].

To improve allo-HSCT efficacy and safety, researchers are making great progress in separating GVHD and the GVL effect, including the early prediction of GVHD risk, the modification of donor graft cells, and drug interventions (**Table 3**). However, the outcomes in clinical practice are still unsatisfactory. An important reason is an inadequate understanding about the mechanism of action of GVHD and the GVL effect. While it is known that GVHD and the GVL effect may involve different subgroups of T-cells, it is challenging to identify these T-cells. With the advent and application of new detection methods such as sequencing, a solution may be developed to address this issue. For example, T-cell receptor high-throughput sequencing (TCR HTS) can be used to analyze and identify the entire T-cell library involved in GVHD and the GVL effect, thus helping researchers learn more about relevant T-cells, clarify the targets and mechanisms of different effector cells, and better separate GVHD and GVL.

Table 3. Strategies to separate GVHD and GVL.	
Separation strategies	
Approaches	
Brief description	
Reference	
GVHD risk prediction	
GVHD biomarker testing	
Contributes to GVHD diagnosis and provides evidence for the early use of anti-GVHD drugs.	
[123]	
Cytokine gene polymorphism testing	

Helps to identify patients with a high risk of severe GVHD and take preventive measures.

[124]

Modification of donor graft cells

Donor T-cell depletion

Donor T-cell depletion reduces GVHD while increasing the risk of infections, graft rejection, and disease relapse.

[109]

Graft-specific cell population depletion

Removing specific cell populations such as naive T cells in the graft that consistently cause severe GVHD.

[118]

DLI to treat relapse

3

DLI is very effective in the treatment of relapsed slow-growing hematopoietic malignancies such as CML; however, the mechanism is unknown.

[122]

Application of chimeric antigen receptor (CAR) T-cell

The combination of single-chain variable fragment (scFv) that identifies leukemia-specific antigens and the activating domain of T cells enhances specific identification and killing of leukemia cells.

[125, 126]

Suicide gene transduced donor lymphocyte infusion

A genetically modified suicide gene is introduced. Donor lymphocytes expressing this gene are sensitive to prodrugs, a feature that can be used when needed to regulate GVHD through the drug clearance of transduced cells.

[127]

#### Selecting memory T cells

2

Memory T cells cause mild or no GVHD and have critical graft-versus-tumor functions.

[118]

#### Enhancing activated $\gamma\delta$ T cells

$\gamma\delta$  T cells have the ability to kill leukemic blasts, and allogeneic T-cell receptor (TCR)  $\gamma\delta$  T cells are not alloreactive and do not cause GVHD.

[128]

#### Selecting Tregs

Tregs suppress the activation and proliferation of effector T cells and downregulate the body's response to foreign antigens or autoantigens.

[Jiang, 2010]

15

#### Modifying/selecting other cells in the grafts

Selecting mesenchymal cells, NK cells, and manipulating dendritic cells and dendritic cell subsets.

[79, 122, 129]

#### Drug intervention

##### Application of immunosuppressants

Various immunosuppressants suppress T cells and reduce GVHD *via* different mechanisms.

[130]

##### Application of histone deacetylase inhibitors (HDACis)

HDACis, such as vorinostat, downregulate inflammatory cytokines and increase the number of Tregs, thereby reducing the occurrence of GVHD, without effecting the GVL effect of donor CTLs.

[131, 132]

#### Suppression of cytokines related to the occurrence of GVHD

Th1 cytokines such as TNF- $\alpha$ , IFN- $\gamma$ , and IL-6 are related to aGVHD; Th2 cytokines such as IL-4, IL-5, and IL-10 are related to cGVHD. Appropriate regulation of these cytokines facilitates GVHD management.

[122]

#### Enhancing cytokines that suppress GVHD

Various cytokines such as IL-11 and keratinocyte growth factor reduce GVHD while preserving the GVL effect.

[122]

#### Targeting MiHAs on hematopoietic cells

CTLs targeting <sup>25</sup> MiHAs such as HA-1 and HA-2 (expressed on hematopoietic cells only) promote the GVL effect.

[121]

#### Development and application of tumor vaccines

Vaccines targeting MiHAs on hematopoietic cells and leukemia-specific antigens improve GVL specificity.

[133]

### **MECHANISM OF POSTTRANSPLANT LEUKEMIA RELAPSE**

Over the past decades, the transplant-related mortality due to posttransplant complications such as GVHD and infections has decreased, thanks to continuous



improvements in stem cell transplantation technology. Post-allo-HSCT relapse has become the major cause of treatment failure and is associated with a dismal prognosis <sup>[134]</sup>. Post-allo-HSCT relapse may come from normal donor cells, known as donor cell leukemia (DCL; rare, 0.12% to 5%), or recipient cells (most cases) <sup>[135, 136, 137]</sup>. Despite the remarkable advancement in allo-HSCT technology in recent years, there has been little progress on how to reduce post-allo-HSCT relapse or improve the survival of relapsed patients. The main reason is a lack of information about the mechanism of post-allo-HSCT relapse.

DCL was first recognized in 1971. Since then, few DCL cases have been reported. The molecular mechanisms involved in DCL occurrence seem to involve cytogenetic abnormalities (chromosome 7 monosomy has been depicted in more than one fifth of DCL cases) or genetic aberrations that arise in RUNX1, ASXL1, DNMT3A, IDH1/2, EZH2, JAK2, CEBPA, GATA2 and other genes. In addition, it has been hypothesized that leukemia cells could have been transferred from the donor during the alloHSCT procedure. Moreover, several theories support the fact that DCL can arise due to reduced immune surveillance following alloHSCT, the donor cells' genomic instability or due to an aberrant stromal niche that exhibits a pro-leukemia potential <sup>[138, 139]</sup>.

For leukemia relapse derived from recipient cells, researchers had believed that MRD is the root cause of such relapse. However, a growing body of evidence indicates that this theory cannot fully explain the mechanism of leukemia relapse. With advancements in human whole-genome sequencing technology, several studies have demonstrated the presence of clonal evolution in leukemia relapse <sup>[140-142]</sup>. Mullighan et al. analyzed the genome-wide DNA copy number in the diagnosis and relapse samples of 61 children with acute lymphoblastic leukemia (ALL) and found concordance between the postchemotherapy relapse leukemia clone and the diagnosis clone in only 8% of the patients; in most cases, the relapse leukemia clone evolved from the diagnosis clone or normal ancestral clones <sup>[140]</sup>. <sup>4</sup> In an analysis of 92 cases of relapsed pediatric ALL, Waanders *et al* found that relapsed leukemic cells propagate primarily from clones already expanded at diagnosis and rarely from unexpanded dormant ancestral clones

[141], suggesting that the information gleaned through subclonal mutation analysis at diagnosis may help to predict relapse risk and select rational therapeutic measures with minimal relapse potential. In recent years, minor diagnosis subclones that initiate evolutionary trajectory toward relapse (termed diagnosis Relapse Initiating clones, dRI) had been identified in both ALL and AML [143, 144]. Compared with other diagnosis subclones, dRIs are drug tolerant with distinct engraftment and metabolic properties [143]. Genomic analysis of matched diagnosis and relapse samples shows that relapse often arises from dRIs [143], suggesting that the isolation and identification of dRIs and the elimination of dRIs by targeting the unique metabolic and transcription pathways may be novel approaches to prevent leukemia relapse.

Another important factor for post-allo-HSCT relapse is the immune escape of leukemia cells. With immune escape, some leukemia cells avoid a potent GVL effect after transplantation and hide in the bone marrow niche to form MRD and eventually lead to leukemia relapse. Several studies show that the loss of HLA on the surface of leukemia cells prevents T-cells from recognizing leukemia cells, an important mechanism of immune escape [145, 146]. In addition, the changes in the number and function of T-cell subsets after allo-HSCT, as well as the high expression of the T-cell immune coinhibitory receptors programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), and T-cell immunoreceptor with Ig and ITIM domains (TIGIT), are closely related to the immune escape of leukemia cells [147, 148]. The mechanism of post-allo-HSCT relapse is very complex and multifactorial, and more extensive and in-depth research is needed to clarify the mechanism.

## **INTERVENTION AND TREATMENT STRATEGIES FOR POST-ALLO-HSCT RELAPSE**

Post-allo-HSCT relapse is a challenging issue for the treatment of leukemia. The overall incidence of post-allo-HSCT relapse is 20% to 30%. For refractory and high-risk leukemia, the relapse rate is 50% or higher [149, 150]. Posttransplant relapse has severe impacts on allo-HSCT outcomes because it affects long-term survival and it is a major



cause of death in leukemia patients after transplantation. Therefore, the identification of the risk factors for post-allo-HSCT relapse and posttransplant indicator monitoring are useful for preventing posttransplant relapse and for the timely identification of early relapse. Furthermore, optimizing treatment strategies with a personalized treatment plan will help to reduce posttransplant relapse and improve survival.

Many factors are related to posttransplant relapse, including disease type, pretransplant disease status, risk stratification, donor source, stem cell source, preconditioning, and GVHD (**Table 4**). Pretransplant disease status is the most important factor. The risk of relapse is high in nonremission patients and patients with a high level of residual leukemia cells before transplantation <sup>[151]</sup>. Studies have proven that pre-HSCT MRD may be an independent prognostic factor for relapse in AML patients after receiving myeloablative HSCT. The 2-year overall relapse rate is significantly higher for patients with MRD than for patients without MRD before transplantation (58% *vs* 14%). The 5-year overall survival rate is 26% and 79%, respectively, suggesting that the presence of pretransplant MRD is positively correlated with posttransplant relapse and mortality <sup>[151]</sup>. Kebriaei et al. retrospectively analyzed the data of 68 adult patients with AML/myelodysplastic syndrome (MDS), and found that the transplantation outcome was inversely related to the pretransplant tumor load, and that the mortality rate due to posttransplant relapse increased 1.21 times for every 10% increase in the percentage of leukemia blasts in the BM before transplantation <sup>[151]</sup>. These findings suggest that reducing the pretransplant tumor burden and achieving stable disease or remission before transplantation are critical for reducing posttransplant relapse. This requires preparatory regimens that maximize leukemia cell removal without increasing side effects. Clinical experience shows that the low selectivity of traditional chemotherapy drugs for leukemia cells is an important factor for pretransplant preconditioning. Therefore, improving selectivity with targeted drugs, such as inhibitors of BCR-ABL or FLT3, as well as targeting LSCs, may offer treatment breakthroughs [152, 153]. Over the last decade, the tumor-specific killing prodrug strategy based on the high level of ROS in tumor cells has provided a novel method for improving chemotherapy selectivity,

enhancing efficacy, and reducing side effects [154, 155]. Recently, several studies confirmed through *in vivo* and *in vitro* experiments that ROS-responsive anticancer prodrugs with ROS-sensitive linkers have precise killing effects on various types of leukemia cells and do not damage normal cells [156-160]. However, ROS-responsive anticancer prodrugs are ineffective in clearing MRD because the level of intracellular ROS in quiescent LSCs may be too low to activate the prodrug system, thus sparing these LSCs.

**Table 4.** Main factors for post-HSCT relapse.

**Factors**

**Brief description**

**Reference**

Disease type

The relapse rate is highest in ALL patients, followed by AML patients and CML patients.

[161]

Pretransplant disease status

The risk of relapse is significantly higher in nonremission patients and patients with a high level of residual leukemia cells before transplantation.

[151]

Risk stratification

The level of risk is positively correlated with the relapse rate and negatively correlated with the disease-free survival rate.

[162]

Stem cell source

Peripheral blood stem cells contain more lymphocytes with a more potent GVL effect; as a result, the relapse rate of BMT is higher than that of PBSCT.

[163, 164]

Preconditioning

Myeloablative preconditioning is more effective in reducing posttransplant relapse than is reduced intensity conditioning and nonmyeloablative preconditioning; T-cell depletion is associated with increased relapse rates in CML and AML.

[164, 165]

#### GVHD

Posttransplant GVHD, especially cGVHD, is associated with a significantly lower relapse rate and a higher survival rate.

[166, 167]

Another incriminated mechanism involved in post-alloHSCT relapse is HLA loss which has been reported in HSCT from both unrelated donors as well as sibling donors. Loss of HLA antigens reduces the efficacy of the GvL effect and favors the immune escape of AML cells. In haploidentical HSCT, as there is no incompatible target to stimulate alloreactivity, the GvL effect remains low [168, 169]. Wu *et al* analyzed nearly 800 cases of AML and ALL who were subjected, following an ATG T-cell-replete conditioning regimen, to haploidentical HSCT and delineated that relapse occurred faster in AML patients who experienced loss of HLA antigens *vs* those who did not (223 days *vs* 321 days,  $P = 0.03$ ). The factors linked with HLA loss in AML were acute GVHD (OR=4.84) and body mass index  $<18.5 \text{ kg/m}^2$  (OR=0.10) [169]. Similarly, Jan *et al* have evaluated HLA loss in the setting of haploidentical HSCT and concluded that minor HLA antigens might be involved in the process of immune recognition [170].

Prevention and pre-emptive treatment of post-alloHSCT relapse remain major challenges for hematologists who manage individuals diagnosed with AML. The choice of therapy is dictated by measurable residual disease (MRD) levels. If MRD is undetectable, subjects should undergo maintenance therapy, whereas detectable MRD requires pre-emptive management strategies, *e.g.*, DLIs [171]. A recently published meta-analysis highlighted that FLT3 inhibitors are a safe and tolerable therapy option for individuals who have undergone alloHSCT for FLT3-mutated AML. The use of these

pharmacological agents as maintenance therapy post-alloHSCT was associated with prolonged overall and relapse-free survival, with no significant differences between the treatment and control groups in terms of non-relapse mortality, GVHD or adverse events [172]. Moreover, sorafenib maintenance therapy following alloHSCT for FLT3-mutated AML was linked with increased overall survival and reduced cumulative incidence of relapse in AML patients who were subjected to alloHSCT in the 1<sup>st</sup> complete remission [173]. Similarly, Fathi *et al* explored, in the setting of a clinical trial, the benefits of 100 mg/day enasidenib maintenance post-alloHSCT for IDH2-mutated AML. In their investigation, 2-year progression free survival was 69%, overall survival was 74%, and the cumulative incidence of moderate/severe GVHD and of relapse were 42% and 16%, respectively, with only one patient experiencing AML relapse while on enasidenib maintenance [174]. Another attractive option for post-alloHSCT maintenance in the management of AML is represented by hypomethylating agents, namely azacitidine and decitabine. A meta-analysis of 14 studies delineated that the use of hypomethylating agents in this setting was correlated with reduced rates of cumulative incidence of relapse and GVHD, as well as higher rates of overall and relapse-free survival *vs* observation only [175]. Similarly, the combination of low-dose decitabine and venetoclax, i.e., a BCL-2 inhibitor, was associated with lower rates of relapse in high-risk AML patients who received this combination as maintenance therapy post-alloHSCT [171, 175].

Many strategies have been developed for post-allo-HSCT relapse, including withdrawal of immunosuppressants, immunotherapy, DLI, radiotherapy and chemotherapy, molecular targeted drugs, and second transplantation. At present, DLI is the most used and most effective in clinical practice. However, the efficacy of DLI varies greatly for different types of hematological malignancies. Clinical data show that DLI enables most patients with relapsed CML to achieve CR in the early stage of relapse, while the remission rate is lower than 30% for patients with relapsed acute leukemia [169]. The main side effects of DLI are GVHD and pancytopenia. Data have indicated that, after

DLI, acute and/or chronic GVHD will be diagnosed in approximately one-third of the subjects. Moreover, 5-20% of these individuals will experience treatment-related mortality following DLI [176, 177]. To reduce DLI-related side effects, transplant specialists are modifying traditional DLI. Clinical experience shows that several modification measures, such as selective deletion of CD8<sup>+</sup> cells and escalating cell dosage regimens, have decreased GVHD-related morbidity without any impact on the DLI-mediated effect of GVL [178]. However, these methods cannot completely eliminate the risk of GVHD. Currently, researchers are developing conditional suicide protocols utilizing the HSV-tk or fas receptor-derived genes to achieve selective killing at will of the transduced cells if uncontrollable GVHD develops [178].

Apart from DLIs, other cell-based therapies, such as a second allo-HSCT, as well as CAR-T and CAR-NK cell-based treatments, have been developed. A second allo-HSCT can be tempted in younger patients, in whom relapse occurs at least 6 mo after the first allo-HSCT and who already have a matched related donor following the first allo-HSCT. However, there is a current need to conduct prospective studies to assess the benefits and risk of a second allo-HSCT, as most data have been derived from retrospective investigations. Impressive overviews of a second allo-HSCT in the setting of relapsed AML post-allo-HSCT has been published elsewhere [171, 179].

Hypomethylating agents, i.e., azacitidine and decitabine, IDH1/2 inhibitors and venetoclax have been recognized as members of the therapeutic *armamentarium* in the setting of post-allo-HSCT AML relapse as well. In addition, immune checkpoint inhibitors (e.g., ipilimumab, nivolumab), monoclonal antibodies (gemtuzumab, ozogamicin and the anti-IL3 agent CLS360) and vaccines are displaying promising results. In addition, several novel targeted agents are currently being developed and/or investigated [171, 179];

small-molecule inhibitors (apart from FLT3 inhibitors and the BCL-2 inhibitor venetoclax): trametinib (anti-MEK agent), glasdegib (a molecule that interacts with the Hedgehog pathway), uproleselan (anti-E-selectin agent)

histone-deacetylase inhibitors: panobinostat

IDH1/2 inhibitors: ivosidenib, enasidenib

In addition, the survival of individuals who have undergone alloHSCT is also affected by the compatibility of specific HLA loci of the donor and recipient. A recent publication has pointed out that HLA matching and the age of the recipient are simple factors that can accurately stratify subjects into prognostic groups, as well as predict overall survival and non-relapse mortality in alloHSCT [180]. However, a meta-analysis of 19 investigations with a patient sample of 3336 individuals concluded that mismatched alloHSCT from unrelated donors remains a safe procedure which is linked with favorable outcomes [181].

Furthermore, several predictors of relapse in haploidentical HSCT have been identified. For example, as compared to intermediate cytogenetic risk, higher relapse rates and shorter overall survival were noted in patients diagnosed with AML with adverse risk cytogenetic abnormalities who were subjected to haploidentical HSCT without T-cell depletion [182]. Pre-alloHSCT MRD levels are also implicated in the outcome of haploidentical HSCT. Zhang *et al* have demonstrated that AML subjects with undetectable MRD pre-alloHSCT registered elevated overall survival and disease-free survival *vs* MRD-positive cases. In addition, cumulative incidence of relapse was similar between MRD-positive and MRD-negative cases in the setting of haploidentical alloHSCT which was also linked with a better prognosis *vs* HLA-matched alloHSCT for individuals who remained MRD-positive pre-alloHSCT [183]. Similarly, Al Hamed *et al* identified predictors of relapse in NPM1-mutated AML individuals who were subjected to haploidentical HSCT. Detectable MRD pre-alloHSCT, presence of FLT3 mutations and alloHSCT in the 2<sup>nd</sup> complete remission negatively impacted on leukemia-free



survival and were linked with elevated percentages of relapsed cases. Overall survival was shorter in cases with concomitant detectable MRD pre-alloHSCT, presence of FLT3 mutations and older age, whereas haploidentical HSCT was correlated with elevated overall survival rates [184]. Similarly, Canaani *et al* confirmed that MRD status pre-alloHSCT was a predictor of relapse following haploidentical HSCT. Undetectable MRD was correlated with elevated percentages of leukemia-free survival and reduced relapse rates, whereas haploidentical HSCT in MRD-positive AML cases was linked with better outcomes when the donor had anti-CMV antibodies [185].

In recent years, with continuous advancements in immunology, novel cellular immunotherapies such as chimeric antigen receptor T-cell (CAR-T) have emerged and are being investigated in clinical trials, generating certain effects, such as significantly enhancing the capacity of immune cells to specifically recognize and kill leukemia cells. However, there are some obstacles for the clinical application of CAR-T therapy. For example, CAR-T targets only cover certain types of leukemia, with a risk of attacking normal tissues and cells due to off-target effects and an inflammatory storm. Moreover, more research is needed to validate the long-term effects of CAR-T therapy [186-189]. The implementation of CAR-T therapy in AML is extremely challenging as the targeted antigen needs to be primarily expressed by AML blasts and not by hematopoietic cells, by activated T-cells or by other cells in the body. In addition, the targeted antigen should be involved in or be a driver of the proliferation of AML blasts, as well as be present solely on AML blasts and LSCs. So far, the following antigens have been studied as potential targets of CAR-T therapy in AML: CD33, CD123, CD38, FLT3, Lewis Y, NKG2D ligand, CD116, CD117, CD70, CD93, CD44v6, CD276, CLL1, ILT3, TIM-3, Siglec-6, FR $\beta$ , h8F4, and the PR1/HLA-2 complex. Moreover, antigen pairs have also been studied by molecular biology techniques, with several research teams identifying CD33+ADGRE2, CLEC12A+CCR1, CD33+CD70, CD33+TIM3, CLL1+TIM3, CLL1+CD123 and CLL1+CD33 as potential candidates for the “ideal antigen” for CAR-T therapy. Furthermore, as the manufacture of autologous CAR-T cells can require

several weeks, the development of allogeneic CAR-T and allogeneic CAR NKs therapies has also been taken into consideration, but has failed to produce satisfactory results in the management of AML due to toxicity. Another potential future strategy is to target AML-associated, *e.g.*, WT1 or PR1, rather than AML-specific antigens using peptide vaccines [137, 190, 191]. However, at present, allo-HSCT remains the standard of care in for individuals diagnosed with AML and who display evidence of intermediate or unfavorable risk, and the potential benefits of CAR-T therapy in conjunction with pharmacological agents and/or allo-HSCT in the management of AML remains to be decided in future studies [192].

In addition, other cell-based therapies, such as CAR-NK therapies, have emerged from the drug pipeline landscape. Ureña-Bailén and collaborators have reported that NK-92 cells transduced with CD276-CAR constructs triple knocked-out for CBLB, NKG2A and TIGIT (inhibitory checkpoints of NK cells), CD276-CAR-NK-92 with CBLB knock-out, as well as CD276-CAR-NK-92 with TIGIT knock-out, exerted significant cytotoxicity against cellular models of AML [193]. Similarly, CD123-CAR-NK constructs exhibited antileukemic potential and a satisfactory safety profile in a cellular model of CD123+ AML [194]. Similarly, NPM1-mutation-specific TCR-like CAR cytokine-induced memory-like NK constructs displayed significant antileukemic potential against a cellular model and patient-derived NPM1-mutated AML samples [195]. Thus, we may hypothesize that NK-CAR constructs might emerge as future therapies of AML.

Monitoring is critical for the prevention and treatment of post-allo-HSCT relapse. It also plays an important role in the long-term survival of leukemia patients. Flow-cytometry is useful for identifying leukemia-related abnormal phenotypes; real-time quantitative polymerase chain reaction (PCR) can be used to detect leukemia-specific fusion genes; and fluorescence *in situ* hybridization (FISH) can detect specific chromosomal translocations or deletions. These methods can be used to monitor MRD to facilitate the early detection of posttransplant relapse [196, 197]. In recent years,



next-generation sequencing technology (NGS) has been widely used in clinic. Because NGS has the advantages of high throughput, accurate quantification and high sensitivity, it is of great significance for evaluating curative effect, guiding treatment and predicting relapse [198]. In addition, graft mosaicism is a highly sensitive measure for predicting relapse and guiding immune intervention [199]. MRD monitoring may be combined with graft mosaicism monitoring. In case of elevated MRD or decreased donor mosaicism, immunosuppressants may be reduced or stopped, and targeted drugs or DLI may be used for timely intervention [200-202]. Until now, no consensus has been established for the frequency of and cut-off values for MRD and graft mosaicism monitoring, and the technologies and methods must be further standardized. Moreover, further research is needed to investigate the timing of monitoring, how fast to reduce or stop immunosuppressants, the timing of DLI, and the number of cells infused.

#### **OTHER COMPLICATIONS OF ALLO-HSCT**

Although only briefly discussed in this narrative review, allo-HSCT can also be associated with various complications. A serious complication of allo-HSCT is graft failure. Graft failure can be either primary, i.e., HSCs from the donor fail to engraft at all, or secondary, i.e., HSCs from the donor engraft successfully but a loss of donor cells occurs at some time point [203, 204]. In addition, poor graft function has also been identified as a complication of allo-HSCT, yet it must be differentiated from graft failure. In both graft failure and poor graft function, cytopenias are present, the bone marrow is hypocellular and there is no evidence of relapse. In terms of chimerism, poor graft function is associated with full-donor chimerism, whereas in graft failure it is either full-recipient or mixed. Initial donor engraftment is noted in both primary and secondary poor graft function, and also in secondary graft failure, but not in primary graft failure. However, initial hematological recovery only occurs in secondary graft failure and secondary poor graft function, whereas it is absent in both primary graft failure and primary poor graft function. Risk factors for graft failure include major ABO incompatibility, HLA mismatch, pre-transplantation MRD and disease type, stem cell

source and dose, conditioning regimen, and others, whereas poor graft function seems to be influenced more by the presence of BM fibrosis, damage to HSCs or stromal cells caused by the selected conditioning regimen or other pharmacological agents, infections or GVHD, as well as a low infusion dose of HSCs. Graft failure, poor graft function and their management have been reviewed elsewhere [203, 204].

In addition, apart from chronic GVHD, allo-HSCT poses the threat and several late onset complications which can develop in the context of GVHD or accompany it. Late-onset complications of allo-HSCT can affect the skin and mucosa, eyes, gastrointestinal tract, lungs (e.g., bronchiolitis obliterans syndrome), muscles and connective tissue, endocrine system and the metabolism (hypogonadism, thyroid dysfunction, osteoporosis, diabetes), kidneys, nervous system, and/or the heart. In addition, infections (e.g., with viruses such as varicella-zoster virus, Epstein-Barr virus or cytomegalovirus reactivation, fungi or encapsulated bacteria) and the development of secondary malignancies in allo-HSCT recipients have emerged as “swords of Damocles” in the survival of AML patients in the post-allo-HSCT setting. These complications have been discussed in detail elsewhere [205, 206]. A recent investigation of over 40000 Leukemia patients who were subjected to allo-HSCT revealed that the most frequent late-onset complications of this therapeutic procedure were azoospermia (~71%), chronic GVHD (5-year post-alloHSCT prevalence at ~43%), secondary malignancies (20-year post-alloHSCT prevalence at ~21%), depression (post-alloHSCT prevalence at ~18%), hypothyroidism (15-year post-alloHSCT prevalence at ~11%), bronchiolitis obliterans syndrome (4-month post-alloHSCT prevalence at ~10%), cardiovascular disease (15-year post-alloHSCT prevalence at ~7.5%) and avascular necrosis (10-year post-alloHSCT prevalence at ~5%) [207]. However, future prospective studies are needed to clarify the exact epidemiology of late complications of allo-HSCT.

What is more, future directions of research in the field of allo-HSCT should also focused on potential opportunities in this expanding field, such as the combination of allo-HSCT

with CAR-T-cell based therapies, the application of novel drugs in conditioning regimens, the use of ATG in combination with PTCY, and others.

## **CONCLUSION**

With continuous developments in immunology, molecular biology, and related disciplines, allo-HSCT is advancing rapidly with proven results and has emerged as a key actor in the management of AML. In recent years, with improved preconditioning regimens, optimized donor selection strategies, novel targeted drugs, and monoclonal antibodies, the incidence and severity of transplant-related complications have been greatly reduced, and the long-term survival of leukemia patients after allo-HSCT has significantly improved. In-depth research on the molecular mechanisms that drive AML will ensure the development of better treatments to further improve remission, prevent relapse, manage early and late onset complications of allo-HSCT, and improve patient survival.

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