Relapsing Hematologic Malignancies after Haploidentical Hematopoietic Stem Cell Transplantation

Yong-Xian Hu,1,* Qu Cui,2,3,* Bin Liang,4 He Huang1

Haploidentical hematopoietic stem cell transplantation (HSCT) is a potentially curative therapeutic regimen that could increase donor availability to nearly 100%. Rapid advances in medical technology and the application of novel drugs mean that most haploidentical HSCT-associated complications can now be prevented or remarkably well controlled, even cured. However, relapsing hematologic malignancy remains a major cause of death in haploidentical HSCT recipients. Haploidentical HSCT should theoretically trigger a more potent graft-versus-tumor effect compared with human leukocyte antigen-identical transplantation, due mainly to the major histocompatibility complex and minor histocompatibility antigen disparities on donors’ immune cells and recipients’ tumor cells. The underlying mechanisms of such relapsing hematologic malignancies remain elusive. In this review, we suggest correlating factors and potential mechanisms and examine feasible therapeutic and preventive strategies for relapsing hematologic malignancies after haploidentical HSCT.


KEY WORDS: Bone marrow, Graft-versus-tumor, Human leukocyte antigen compatibility, Targeted therapy

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative strategy for hematologic malignancies [1], solid malignancies [2], and other nonmalignant diseases [3]. This regimen has benefited patients since its emergence more than 50 years ago. In current clinical situations, a human leukocyte antigen (HLA)-matched HSCT is commonly the preferred type of transplantation, with HLA-matched sibling donors usually the first choice. For cases in which an HLA-matched related donor is not available, an HLA-matched unrelated donor is identified and selected through a donor registry. Clinical practice has demonstrated that only 50%–60% of HSCTs from HLA-matched donors are successful, with a much lower rate of success in patients of ethnic minorities [4]. With the aim of solving this conundrum and benefiting more patients, much effort has been expended in searching for feasible alternative approaches. Haploidentical HSCT appears to be a promising strategy, with a theoretically high donor availability of almost 100%. It also is less time-consuming than conventional HSCT, which requires a stringent matching process. Nevertheless, even after years of application, the high incidence of several critical complications, including severe graft-versus-host disease (GVHD), delayed engraftment, severe infection, and graft failure, still poses a barrier to the wider application of haploidentical HSCT to the benefit of more patients.

In recent years, based on surprising advances in transplantation and immunology, several attempts have been made to use T cell–depleted haploidentical bone marrow (BM) for the prevention of GVHD. The first ex vivo T cell–depleted haploidentical HSCTs using BM were performed in 4 children with immunodeficiency syndromes more than 20 years ago; all 4 patients were healthy at 12-15 months after discharge [5]. Since then, mega-doses of purified stem cells and T cell–depleted grafts have been used for haploidentical HSCT, and considerable progress has been made. Today, with 2 principle protocols—T cell...
depletion and T cell repletion—established worldwide, an encouraging survival rate of 20% has been achieved in patients with progressive hematologic malignancies [6]. Using this method, most complications can be prevented or remarkably well controlled, and in some cases even cured, due mainly to the application of advanced medical technologies and novel therapeutic drugs.

According to traditional immunobiological theory, haploidentical HSCT could be expected to trigger a more potent graft-versus-tumor (GVT) effect compared with HLA-identical transplants due to the major histocompatibility complex (MHC) and minor histocompatibility antigen (mHA) disparities on donor immune cells and recipient tumor cells, which might facilitate the repression of tumor relapse. In fact, a high risk of relapse after haploidentical HSCT could be expected to trigger a more potent graft-versus-tumor (GVT) effect compared with HLA-identical transplants due to the major histocompatibility complex (MHC) and minor histocompatibility antigen (mHA) disparities on donor immune cells and recipient tumor cells, which might facilitate the repression of tumor relapse. In fact, a high risk of relapse after haploidentical HSCT has been documented in various hematologic malignancies, including acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), myelodysplastic syndrome, multiple myeloma, chronic myelogenous leukemia, and lymphoma, under both myeloablative and nonmyeloablative (NMA) conditioning regimens, in both children and adults [7-13]. Studies performed within the past 5 years are summarized in Tables 1 and 2. These reports infer that relapsed hematologic malignancy after haploidentical HSCT is still the most common cause of death, although the specific incidence varies among reports, possibly due to the heterogeneity in patient series and diagnostic variations in the different cohorts. The mechanisms by which high-risk malignant cells survive under GVT effects remain elusive, however.

In our opinion, haploidentical HSCT has merit in improving survival probability, and haploidentical donors likely would be one of the main stem cell sources. In this review, we suggest correlating factors and potential mechanisms causing relapse of hematologic malignancies after haploidentical HSCT, then address the newly identified indications for relapse, as well as feasible therapeutic and preventive strategies.

POTENTIAL MECHANISMS FOR RELAPSING HEMATOLOGIC MALIGNANCIES AFTER HAPLOIDENTICAL HSCT

In ideal haploidentical HSCT, the initiating conditioning therapy, especially the myeloablative regimen, eradicates the majority of malignant hematologic cells. In addition, the posttransplantation GVT effects then eradicate any residual malignant cells remaining after conditioning therapy. Thus, in the case of in situ relapse, the residual malignant cells must survive ablation of the hematopoietic system, which includes malignant hematologic cells, and also survive the GVT reaction [38]. According to recent reports, malignant cell resistance to conditioning therapy demonstrated a similar process to that involved in drug resistance in chemotherapy [39-41], and certain factors might influence malignancy relapse, including, but not limited to, the cancerous microenvironment, cancer stem cells, gene polymorphisms, host ages, GVHD prevention strategies, disease status at transplantation, and gene mutations. Here we discuss corresponding factors and potential immune mechanisms involved in the emergence of relapsing malignancies after haploidentical HSCT (Figure 1).

Pretransplantation: Donor Selection Serves as an Initial Stage for Preventing Future Relapse

Maternal Tolerance Reduces GVT Potency Induced by Donor Transplants

Siblings, parents, and offspring are all potential haploidentical donors. Maternal tolerance and/or immunization should make consideration of maternal transplants a priority. The immune systems of mother and child are in close contact during pregnancy and achieve a delicate equilibrium, which might exert an influence on haploidentical transplantation later in life.

The maternal immune system, unlike that of the fetus, is mature and usually functional and thus is capable of being immunized by paternal histocompatibility antigens transmitted from the fetus. Antibodies directed against paternal HLA antigens [42] and memory type T lymphocytes directed against paternal major and minor histocompatibility antigens [43,44] are frequently found in multiparous women. Under such circumstances, humoral and cellular immunity against HLA and mHA in the offspring might mediate enhanced GVT effects after maternal donor transplantation. For patients undergoing T cell–depleted haploidentical HSCT, even though the majority of T cells have been removed, the small population of contaminant memory T cells transferred with the graft could still spontaneously undergo unopposed proliferation and play a vital role in the GVT process by virtue of the absence of pharmacologic GVHD prophylaxis [33]. Stern et al. [33] found relapse rates of 22.7% with maternal donors and 46.5% with paternal donors after T cell depletion in vitro excluding the sex effect, supporting the notion that the use of immunized maternal donors is associated with reduced relapse mortality with both T cell–depleted and T cell–replete HSCT. In contrast, for maternal donors who were not immunized but tolerized during pregnancy, low immune reactivity would weaken the recipient’s GVT effect, contributing to the higher relapse rate than seen with immunized maternal donors.

The situation might be different when considering reciprocal transplantation, in which the mother is the recipient and the offspring is the donor. It is assumed
that the fetus has an immature immune system, incapable of initiating immunity against noninherited maternal alloantigens. As a result, no decreased risk of relapse has been reported with the use of offspring as haploidentical donors. The use of noninherited maternal alloantigens has been found to have no statistically significant effect on the outcome of relapse [33]. Unfortunately, currently no detailed information is available on donor humoral and cellular immune reactivity against mismatched HLA on recipient cells before transplantation, and we cannot yet exclude the possibility that some family members might be immunized by different HLA molecules expressed in patients through as-yet unclarified pathways. In any case, when assessing the maternal factors recorded during pregnancy, stem cell donor alloimmune status should be determined when selecting a haploidentical donor from among family members or other relatives, to achieve a potent GVT effect after haploidentical HSCT.

**HLA Genetic and Epigenetic Variations Affect the GVT Reaction**

The frequency of HLA-mismatched loci in haploidentical donors ranges from 1 to 5 in 10/10 alleles. HLA mismatches can involve different numbers of mismatched locus sites and different combinations of HLA mismatches can involve different numbers of mismatched locus sites and different combinations of HLA mismatches. HLA-mismatched donor recipients have a reduced risk of relapse post-HSCT, particularly with respect to HLA-DRB1 and HLA-Cw disparities [45-47]. To date, no

**Table 1. Recent Studies of T Cell–Replete Haploidentical HSCT**

<table>
<thead>
<tr>
<th>Year, Reference</th>
<th>Disease (n)</th>
<th>Age, Years, Median (Range)</th>
<th>Conditioning Regimen</th>
<th>Relapse (Median Follow-Up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010 [9]</td>
<td>High-risk MDS (36)</td>
<td>34 (10-51)</td>
<td>Modified Bu/Cy2 + ATG (myeloablative)</td>
<td>18.2% in CR, 20.0% in NR (2 years)</td>
</tr>
<tr>
<td>2010 [12]</td>
<td>Leukemia, multiple myeloma, MDS (66)</td>
<td>54 (13-70)</td>
<td>TBI (or Flu) + Bu + Me-CCNU + ATG (NMA)</td>
<td>19.1% in high-risk group (9 months)</td>
</tr>
<tr>
<td>2010 [14]</td>
<td>Leukemia, multiple myeloma, lymphoma, MDS (86)</td>
<td>44 (21-69)</td>
<td>TBI + Cy + Flu (NMA)</td>
<td>58.0% (2 years)</td>
</tr>
<tr>
<td>2010 [15]</td>
<td>High-risk acute leukemia (14)</td>
<td>32 (8-51)</td>
<td>Modified Bu/Cy/ATG (myeloablative)</td>
<td>29.6% in granulocyte colony-stimulating factor peripheral blood stem cells (2 years)</td>
</tr>
<tr>
<td>2010 [15]</td>
<td>High-risk acute leukemia (109)</td>
<td>25 (3-56)</td>
<td>Modified Bu + Cy + ATG (myeloablative)</td>
<td>34.0% in granulocyte colony-stimulating factor peripheral blood stem cells/primed bone marrow (2 years)</td>
</tr>
<tr>
<td>2009 [16]</td>
<td>Refractory non-Hodgkin lymphoma (10)</td>
<td>19 (7-38)</td>
<td>TBI + Ara-c + Cy or Bu + Thio + Cy (myeloablative)</td>
<td>10.0% (60.7 months)</td>
</tr>
<tr>
<td>2009 [17]</td>
<td>Leukemia, non-Hodgkin lymphoma (45)</td>
<td>35 (31-39)</td>
<td>TBI + Ara-c + Cy + ATG or Bu + Ara-c + Cy (myeloablative)</td>
<td>24.4% (36 months)</td>
</tr>
<tr>
<td>2009 [18]</td>
<td>High-risk acute leukemia (33)</td>
<td>23 (7-43)</td>
<td>Flu + Ara-c + Cy + ATG + TBI (NMA)</td>
<td>18.2% (2-7.5 months)</td>
</tr>
<tr>
<td>2009 [7]</td>
<td>Leukemia (56)</td>
<td>35 (1-14)</td>
<td>TBI (or CCNU + Bu) + Cy + Ara-c + ATG (myeloablative)</td>
<td>22.0% (207 days)</td>
</tr>
<tr>
<td>2009 [19]</td>
<td>Leukemia, MDS (58)</td>
<td>N/A (3-14)</td>
<td>Bu/Cy2 + ATG + Ara-c + Me-CCNU (myeloablative)</td>
<td>44.6% in high-risk group, 11.3% in standard-risk group (3 years)</td>
</tr>
<tr>
<td>2009 [20]</td>
<td>Leukemia (46)</td>
<td>25 (5-54)</td>
<td>TBI (or Bu + CCNU) + Ara-c + Cy + ATG (myeloablative)</td>
<td>23.9% (2 years)</td>
</tr>
<tr>
<td>2009 [21]</td>
<td>Acute leukemia (250)</td>
<td>N/A</td>
<td>Ara-c + Bu/Cy + ATG + semustine (myeloablative)</td>
<td>11.9% and 24.3% for AML and ALL in standard-risk group, 20.2% and 48.5% for AML and ALL in high-risk group (3 years)</td>
</tr>
<tr>
<td>2008 [22]</td>
<td>Leukemia, MDS, lymphoma, paroxysmal nocturnal hemoglobinuria (68)</td>
<td>46 (1-71)</td>
<td>TBI + Cy + Flu (NMA)</td>
<td>58.0% (2 years)</td>
</tr>
<tr>
<td>2008 [23]</td>
<td>Acute and chronic leukemia (42)</td>
<td>N/A (3-14)</td>
<td>Ara-c + Bu/Cy + Me-CCNU + ATG (myeloablative)</td>
<td>37.0% in high-risk group ALL 0 in AML and CML (2 years)</td>
</tr>
<tr>
<td>2008 [24]</td>
<td>Leukemia, lymphoma, and MDS (30)</td>
<td>30 (16-42)</td>
<td>TBI + Flu + Cy + Ara-c (myeloablative)</td>
<td>20.9% (3 years)</td>
</tr>
<tr>
<td>2008 [25]</td>
<td>CML (93)</td>
<td>29 (9-54)</td>
<td>Bu + Cy + ATG (myeloablative)</td>
<td>3.29% in CML chronic phase, 31.45% in non-chronic phase (4 years)</td>
</tr>
<tr>
<td>2007 [26]</td>
<td>Hematologic malignancies (68); solid tumors (4)</td>
<td>7 (0.5-19)</td>
<td>TBI-based or no TBI (myeloablative or NMA)</td>
<td>0 in CR group, 37.0% in NR (26 months)</td>
</tr>
<tr>
<td>2007 [27]</td>
<td>Leukemia, MDS (157)</td>
<td>25 (6-50)</td>
<td>Ara-c + Bu/Cy + ATG (myeloablative)</td>
<td>18.0% (2 years)</td>
</tr>
<tr>
<td>2006 [28]</td>
<td>Leukemia, MDS (135)</td>
<td>37 (5-50)</td>
<td>Bu/Cy2 + ATG (myeloablative)</td>
<td>18.0% (2 years)</td>
</tr>
<tr>
<td>2006 [29]</td>
<td>Leukemia, MDS (171)</td>
<td>23 (2-56)</td>
<td>Me-CCNU + Bu/Cy + Ara-C + ATG (myeloablative)</td>
<td>12.2% in standard-risk group, 38.9% in high-risk group (2 years)</td>
</tr>
</tbody>
</table>

HSCT indicates hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; Bu, busulfan; Cy, cyclophosphamide; ATG, antithymocyte globulin; CR, complete remission; NR, no remission; TBI, total-body irradiation; Me-CCNU, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea; Flu, fludarabine; Ara-c, cytarabine; NMA, nonmyeloablative; Thio, thiotepa; N/A, not available; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia.
report has documented the different relapse rates among various mismatched loci.

Particular HLA antigens have varying immunogenicity in recipients. One or two amino acids might affect several determinants of alloreactivity; for example, Ser9C-Tyr9C and Phe99C-Tyr99C at variant positions are responsible for a decreased risk of relapse [48]. For a pregnant woman, alloantibodies are frequently induced by fetus-derived HLA-A2 or HLA-B5 mismatches, whereas a fetus bearing HLA-A30, -A31, or -A33 or HLA-A28 induced alloantibodies significantly less often than a fetus bearing other HLA class I mismatches [49]. Correspondingly, the donor immune system, recognizing different haploidentical recipient HLA antigens with different immunogenicity, might trigger the GVT effect and GVHD at different levels, and the GVT effect may correlate with the incidence of relapse posttransplantation. Moreover, the expression profiles of mismatched HLA and adhesion molecules on malignant cells and tentative malignant stem cells might be as varied as on normal hematopoietic cells and daughter cells, affecting GVT intensity in HLA-mismatched recipients [48]. These findings suggest that clarifying the HLA locus disparity and adhesion molecule expression profiles in haploidentical HSCT settings would be very helpful and could lead to improvements in therapy.

**Killer Immunoglobulin-Like Receptors Impede Natural Killer Cell–Mediated Cytotoxicity**

Natural killer (NK) cells are the first lymphoid cells to recover, appearing as early as 2–3 weeks posttransplantation by rapid differentiation from engrafted CD34+ cells. They express activating and inhibitory receptors, termed killer immunoglobulin-like receptors (KIRs), which stimulate or inhibit NK cell cytotoxicity, respectively. KIRs are characterized by 2 (KIR2D) or 3 (KIR3D) extracellular immunoglobulin domains specifically recognizing MHC class I molecules as their ligands [14,50]. In an allogeneic transplant recipient undergoing haploidentical HSCT with selected CD34+ cell or T cell depletion, alloreactive NK cells are characterized by the expression of KIRs that are not engaged by any of the HLA class I alleles expressed by the recipient—namely mismatched KIR ligands, which recognize and eradicate residual malignant cells through antibody-dependent cell-mediated cytoxicity, complement-dependent cell-mediated cytoxicity, Fas-dependent killing and perforin degranulation, and other methods. In the best case, T cells and NK cells would to some extent atone for their tumoricidal capacities by virtue of their differentially cytotoxic mechanisms.

There is considerable evidence indicating that alloreactive NK cells developing from haploidentical HSCT represent a group of the most potent effector cells that can successfully treat high-risk leukemia and reduce recurrence after both T cell-depleted and T cell–replete transplants [51-53]. In patients with

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**Table 2. Recent Studies of T Cell–Depleted Haploidentical HSCT**

<table>
<thead>
<tr>
<th>Year, Reference</th>
<th>Disease (n)</th>
<th>Age, Years, Median (Range)</th>
<th>Conditioning Regimen</th>
<th>Relapse Rate (Median Follow-Up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010 [30]</td>
<td>ALL (102)</td>
<td>8.7 (0.64-16)</td>
<td>TBI-based or not (unknown)</td>
<td>36.0% in CR (5 years)</td>
</tr>
<tr>
<td>2010 [31]</td>
<td>Leukemia, MDS, lymphoma (28)</td>
<td>36 (6-56)</td>
<td>Melphalan + Thio + Flu + ATG (NMA)</td>
<td>44.0% in advance, 0 in CR (72 days)</td>
</tr>
<tr>
<td>2010 [32]</td>
<td>Leukemia, lymphoma (28)</td>
<td>45 (19-65)</td>
<td>Flu (cladribine) + Thio + melphalan + OKT-3 (NMA)</td>
<td>28.6% (748 days)</td>
</tr>
<tr>
<td>2008 [33]</td>
<td>Leukemia (118)</td>
<td>18 (2-52)</td>
<td>TBI + Thio + Flu (or Cy) + ATG (myeloablative)</td>
<td>22.7% in mother donor, 46.5% in father donor (3-4 years)</td>
</tr>
<tr>
<td>2008 [34]</td>
<td>Leukemia (266)</td>
<td>31 (16-66)</td>
<td>TBI-based regimen (myeloablative)</td>
<td>AML: 16% in CR1, 23% in CR2, 27% in CR2, 49% in advance (2 years)</td>
</tr>
<tr>
<td>2007 [35]</td>
<td>AML (112)</td>
<td>N/A</td>
<td>TBI + Flu (methotrexate) + ATG (myeloablative)</td>
<td>25.0% (5.07 years)</td>
</tr>
<tr>
<td>2007 [36]</td>
<td>Hematologic malignancies or advanced myeloproliferative disorder (49)</td>
<td>48 (17-66)</td>
<td>Alectumuzumab + Flu + Cy (NMA)</td>
<td>49.0% (unknown)</td>
</tr>
<tr>
<td>2006 [37]</td>
<td>ALL, AML (34)</td>
<td>11 (1-16)</td>
<td>TBI + Cy-based (myeloablative)</td>
<td>13.0 in CR, 100.0 in NR (62 months)</td>
</tr>
</tbody>
</table>

HSCT indicates hematopoietic stem cell transplantation; ALL, acute lymphocytic leukemia; TBI, total-body irradiation; CR, complete remission; MDS, myelodysplastic syndrome; Thio, thiopeta; Flu, fludarabine; ATG, antithymocyte globulin; NMA, nonmyeloablative; Cy, cyclophosphamide; AML, acute myelogenous leukemia; N/A, not available.
AML who underwent T cell–depleted haploidentical HSCT, Ruggeri et al. [54] found lower relapse rates in recipients of KIR ligand-matched transplants compared with recipients of mismatched transplants (75% vs 0). Leung et al. [55] reported a reduced rate of relapse in patients with ALL. Recently, Symons et al. [14] reported that KIR haplotype AA recipients of BM from KIR Bx donors had a lower relapse rate in T cell–replete haploidentical HSCT with NMA conditioning [14]. Other clinical trials using adoptive transfer of allogeneic NK cells in combination with haploidentical transplantation have shown lower relapse rates compared with matched NK ligands, while donor NK cells can compensate for the paucity of T cell–specific alloreactivity depleted in the graft [51,56,57].

The characteristics of KIR and its ligands are complicated. The application of allosreactive NK cells to treat haploidentical HSCT, which potentiates the attenuation of NK cell–mediated cytotoxicity associated with the high risk of relapse after haploidentical HSCT is an area warranting further investigation.

**Peritransplantation: Graft Component Variability as a Precarious Factor that Hinders the GVT Effect**

The HSCT allograft consists mainly of CD34+ hematopoietic stem cells and allosreactive immune cells including T cells, B cells, and NK cells. Of these, allosreactive T cells are the most important effector cells in eliciting both GVHD and the GVT reaction, activated by the host's dendritic cells (DCs) through the presentation of mHA, MHC, or tumor-associated antigen. After activation, the T cells release cytokines, including interferon-γ and granulocyte macrophage colony-stimulating factor, resulting in a vicious cycle known as a “cytokine storm,” which leads to GVHD and the GVT reaction. NK cells (as described above) and B cells are considered to serve as effector cells with significant roles, but acting through different mechanisms [58].

The major allosreactive T cells in allografts are depleted in some haploidentical HSCTs. The GVT effect could be abrogated after T cell–depleted HSCT. This is considered a major cause of the high risk of relapse [59–61]. Despite this, previous studies have shown that the residual T cells in the allograft are sufficiently potent to trigger a GVT effect. Recently, several unmanipulated haploidentical blood and marrow transplantations elicited a potent GVT effect with no influence on GVHD severity [11,21]. Under such transplantation conditions, T cell subtype distribution might have some effect on relapse. The CD4/CD8 ratio is a conventional measure of immune function and response because CD4+ T cells are associated with helper/inducer function, whereas CD8+ T cells are associated with cytotoxic/suppressor activity [62]. Thus, the CD4/CD8 ratio in grafts can affect immune function and the GVT effect. Clinical experience shows that a low CD4/CD8 ratio is always associated with low risk of relapse and vice versa [62,63]. The number of CD3+ cells in the allograft is likely another important factor in determining the risk of relapse. It should be noted that these data were collected from a small number of patients; larger-scale clinical trials are needed to verify and clarify our results.

B cells are one of the main cell types involved in humoral immune responses. They act as antigen-presenting cells, create antibodies against foreign antigens, and develop into memory B cells or plasma cells. Few previous studies have examined whether antibodies produced by donor B cells also might contribute to tumor immunity after allogeneic HSCT (allo-HSCT). Interestingly, recent studies suggest that B cells also likely play an important role in the GVT effect [38,58]. In addition, the roles of the number of CD34+ cells, as well as other cell types transfused within grafts for haploidentical HSCT, merit further investigation. The reduced GVT effect in a variety of graft conditions may be attributed to the paucity of effector T cells, delayed immune reconstitution, incompletely activated B cells in the allograft due to lack of T cells, and impaired DC function due to the shortage of cytokines produced by effector T cells.

**Posttransplantation: Intensive Immunosuppression Is a Double-Edged Sword**

An intensive immunosuppressive regimen should be administered to patients undergoing haploidentical HSCT, especially those with T cell–replete transplantation, both during the conditioning regimen and after transplantation to prevent severe GVHD and graft failure. Traditional immunosuppressive agents include antithymocyte globulin, cyclosporine, methotrexate, glucocorticoids, and mycophenolate mofetil. Most of these agents inhibit the functions of various immune cells, including the immune cells initiating GVHD and the GVT effect, as well as other immune cells abrogating GVHD [64–66]. Accordingly, intensive use of immunosuppressive agents might weaken the immune function of donor cells (the main effector cells of the GVT reaction), resulting in primary disease relapse. There are plenty of clinical examples in which the administration of intensive immunosuppressive agents caused disease relapse [67,68]. After withdrawal of immunosuppressive agents, some relapsed patients achieved complete remission (CR) with no any further treatment [69]. Donor lymphocyte infusion (DLI) is another example of weak immune cell function contributing to disease recurrence. After DLI treatment, some patients can achieve CR [70].
Lymphocytes applied in DLI are isolated from donor blood, which is free of contact with immunosuppressive agents and thus is functionally competent to exert GVT effects through multiple pathways. What immunosuppressive agents to use, how many, and how long therapy should be applied to achieve equilibrium by reducing GVHD while maintaining the GVT effect to prevent relapse of primary malignancies remains a difficult problem.

A Potentially Neglected Factor: Loss of Mismatched HLA Haplotype

The HLA superlocus contains a large number of genes related to immune system function in humans. This group of genes resides on chromosome 6 and encodes cell-surface antigen-presenting proteins and many other genes. Genomic or phenotypic alterations of HLA and the antigen-presenting machinery are frequently seen in patients with solid tumors [71,72]. Loss of HLA class I surface antigens also has been described in patients with melanoma after a partial response to cellular immunotherapy [73]. In essence, a major contributor to the appearance of MHC class I-negative tumor clones is T cell immune selection. Malignant cells with total MHC class I loss are susceptible to NK cell lysis via repression of surface inhibitory receptors. However, partial loss of HLA class I antigens facilitates the escape of tumor cells from cytotoxic T lymphocyte (CTL)- and NK-mediated immune pressures. Further analysis has shown that a loss of HLA haplotype is associated with loss of heterozygosity, including 1 copy of chromosome 6 or a DNA fragment containing HLA-A, HLA-B, and HLA-C genes.

In haploidentical HSCT, alloreactive T cell responses could be directed against epitopes on HLA molecules or against peptide–HLA complexes expressed on malignant cells via GVT effects. This would exert immunologic pressure on malignant cells at the same time. For relapsing malignancies after HSCT or reduced-intensity conditioning transplantation, DLI is usually applied repeatedly, which might cause potential immune selection and the loss of certain HLA haplotypes, leading to impaired specific GVT effects. Recently, Vago et al. [74] reported mutant leukemic cells in 5 of 17 patients with leukemia relapse after haploidentical transplantation and infusion of donor T cells, in which the HLA haplotype that differed from the donor’s haplotype had been lost because of acquired uniparental disomy of chromosome 6p. A recent independent report found that 2 out of 3 patients who relapsed after HLA-haploidentical HSCT demonstrated loss of HLA alleles in leukemic cells at relapse and a copy number–neutral loss of heterozygosity; that is, they acquired uniparental disomy on the short arm of chromosome 6, resulting in the total loss of the mismatched HLA haplotype [75]. Due to this loss of mismatched HLA haplotype, both T cells and B cells targeting the mismatched HLA molecules failed to eradicate the residual tumor cells, leading to malignancy relapse (Figure 2). These reports revealed a previously neglected phenomenon in the process of haploidentical HSCT relapse, in which leukemic cells

Figure 2. Schematic representation of loss of mismatched haplotype (A) and loss of heterozygosity (B) in hematologic malignancies after haploidentical HSCT leading to relapse. Patients without mutations in malignant cells maintain complete remission because of a potent GVT effect (C).
occasionally escape from immune surveillance through the loss of mismatched HLA haplotypes by uniparental disomy, under the influence of immunologic pressures.

The mechanism underlying the acquired uniparental disomy of chromosome 6p after haploidentical HSCT remains a mystery. It is also not known whether there are any alterations in gene expression or gene mutants of mHAs after HLA-identical or HLA-mismatched HSCT that ultimately result in hematologic malignancy relapse. To fully understand these points, note should be taken of HLA haplotype expression profiles, which have been previously neglected.

THERAPEUTIC AND PREVENTIVE STRATEGIES FOR RELAPSING HEMATOLOGIC MALIGNANCIES

Because relapsing malignancy is a nascent branch of HSCT treatment, the prognosis of relapsing hematologic malignancies after allo-HSCT is extremely limited, although various treatments have been applied in the clinic. However, as proof of principle, these strategies should be differentially adopted to cope with specific relapsing factors and mechanisms. Unfortunately, currently there is no effective detection system for distinguishing corresponding factors and mechanisms individually. Conventionally, the first intervention for relapsing hematologic malignancy with a low tumor burden is to enhance the GVT reaction, because controlling the malignancy depends almost entirely on the GVT effect. In patients with a high tumor burden or who show no improvement after the first intervention, reinduction of chemotherapy with or without enhancement of the GVT effect to eradicate relapsing malignancies should be considered (Figure 3). However, some generally acknowledged criteria should be established to identify the scale of the tumor burden. To treat these patients, a second allo-HSCT may be an endpoint choice; in addition, novel technologies and other pipeline drugs with different mechanisms may offer alternatives in the near future.

Enhancement of the GVT Effect

Donor immune cells are always at lower levels of immunity in recipients of haploidentical HSCT compared with recipients of other types of HSCT, probably due to the prolonged and intense treatment with immunosuppressive agents, delayed immune reconstitution, and T cell depletion in the allograft. Thus, haploidentical HSCT recipients are likely to benefit more from strategies that enhance recipient immunity. More investigations are needed to validate this finding.

Withdrawal of Immunosuppressive Agents

Withdrawal of immunosuppressive agents (WIA) is generally the first option in relapsing hematologic malignancies with either low or high tumor burden after allo-HSCT. WIA, especially for cyclosporine as immunotherapy for leukemia relapse, showed some effects in HLA-matched and HLA-mismatched cases, including haploidentical HSCT [76,77], but detailed response rates were not analyzed. In contrast, other independent investigations showed that WIA had a limited effect on relapse treatment [78], which

Figure 3. Summary of current clinical interventions after relapse post-haploidentical HSCT.
complicates the WIA strategy. In our opinion, WIA is suitable for patients in relapse without GVHD manifestations. In patients in relapse with complications of GVHD, WIA might not be an effective choice. For patients who relapse or show no improvement after WIA, alternative strategies to improve GVT effects should be considered. Notably, for these patients, the presence of GVHD should be carefully considered before opting for WIA.

**Donor Immune Cell Infusion**

Donor immune cell infusion is a developing technique aimed at eradicating relapsing tumor cells. DLI was first identified as an effective therapeutic and preventive strategy for relapsing hematologic malignancies after allo-HSCT [79]. Immunosuppressive agents should be administered during DLI to prevent GVHD and pancytopenia, which greatly reduces the GVT effect. Clinical trials on the use of modified DLI, such as adaptive transfer of donor-derived leukemic reactive T cells and mHA-specific CTLs without administration of immunosuppressive agents, aiming to achieve the strongest GVT effect with the least side effects, are currently under way [80]. Cell fate control gene therapy, another type of modified DLI, also has been proposed [81]. “Suicide” gene therapy targeting donor T cells can provide a molecular switch to control in vivo survival efficacy, which can enable maximal GVT responses to achieve the greatest attainable antitumor effects.

Patients in relapse after haploidentical HSCT also would benefit from other modified DLI modalities. Mismatched HLA loci are thought to be good GVT targets. Currently, various HLA epitopic peptides, as well as dissociated hematopoietic-specific antigens, including CD45, CD3, and CD20, are available, allowing the development of novel immunotherapeutic trials. Based on recent reports, haploidentical donor T cells could be cocultured with donor DCs engineered to present the recipient HLA locus and conjugated with a peptide derived from a recipient hematopoietic antigen. Such HLA locus/hematopoietic antigen pentamer-reactive cytotoxic CD8+ T cells could be readily obtained from haploidentical donors. These allorestricted T cells would provide GVT effects in the absence of GVHD even without the use of immunosuppressive agents. Abrahamsen et al. [82] obtained highly specific allogeneic T cells targeting B cell leukemia with high CD20 specificity using an HLA-A*0201/CD20p pentamer in vitro. Through this approach, potent efficacy has been achieved in haploidentical adoptive CTL therapy for Epstein-Barr virus–associated lymphoma after HSCT [83], as well as in vaccination for patients with metastatic melanoma using a gp100-derived epitopic peptide restricted to HLA-A*2402 [84]. Based on these achievements, immunotherapeutic strategies targeting the mismatched HLA locus of relapsing hematologic malignancies after haploidentical HSCT could have wide applicability in the clinic, especially taking into account their synergistic effects in combination with standard therapeutic modalities.

In addition to T cell–based strategies, NK cells as innate immune cells with non–HLA restricted cytotoxicity are also an attractive population. In haploidentical HSCT settings, differentiation of KIR+ alloreactive NK cells from hematopoietic stem cell precursors may require 6-8 weeks, and thus their antileukemia effect may occur only after this time period. In cases of high residual tumor burden and/or rapidly proliferating leukemia blasts, especially after T cell–depleted haploidentical HSCT, as mentioned earlier, this delay may be a major limitation, leading to leukemia relapse. With the aim of reducing this risk, mature alloreactive NK cells isolated from the haploidentical donor could be infused shortly after HSCT. These mature donor NK cells could be properly mobilized with specifically activating cytokines, such as interleukin-15, which is believed to cause a strong GVT reaction but no GVHD. Ruggeri et al. [85] suggested that the alloreactivity of donor-versus-recipient NK cells in human transplants could eliminate both leukemia relapse and graft rejection, and protect patients against GVHD.

For other immune cell subpopulations, such as γδ T cells and NK T cells, which have demonstrated tumoricidal effects in in vitro and in vivo studies [86-88], their effects on treatment of relapsing malignancies after HSCT requires further investigation.

In general, to achieve synergistic effects in combination with standard therapeutic modalities, we would incorporate the newly available modified infusion technologies, sensitizing relapsing hematologic malignancies to donor immune cell infusion. Donor immune cell infusion is likely to play an increasingly important role in treating and preventing relapsing hematologic malignancies after haploidentical HSCT in the future, due to the decreased immunity as well as the limited availability and feasibility of obtaining such cells from haploidentical donors, unlike from unrelated donors.

**Targeted Therapy**

Targeted therapy is often combined with donor immune cell infusion or reinduction chemotherapy in specific diseases. Currently, targeted drugs for treating hematologic malignancy include monoclonal antibodies and tyrosine kinase inhibitors (TKIs). Monoclonal antibodies contain tumor antigen–specific agents (e.g., anti-CD20, anti-CD33, anti-CD52), which might enhance the GVT effect through complement-dependent cell-mediated cytotoxicity as well as
antibody-dependent cell-mediated cytotoxicity, and anti-CTLA4. CTLA4 is induced on T cells on activation and is an important mediator of peripheral self-tolerance and tolerance to tumor antigens. Anti-CTLA4 (ipilimumab) is a fully human IgG1 monoclonal antibody that antagonizes CTLA4, thus leading to persistent T cell activation, and has recently been licensed as a drug for GVT enhancement. Bashey et al. [89] demonstrated that anti-CTLA4 was a potential therapy for relapsing hematologic malignancies after allo-HSCT without causing GVHD and graft rejection. More clinical trials are needed to clarify this finding.

Although TKIs have not been found to enhance donor cell immunity, they are the most effective agents for eradicating Ph+ leukemia including chronic myelogenous leukemia and Ph+ ALL. Because of mutations in the BCR/ABL gene, some TKIs are ineffective. New generations of TKIs have different kinetics of tyrosine inhibition. Some patients with mutations in the BCR/ABL gene relapse after allo-HSCT but achieve CR after TKI treatment. Recently developed FLT3-TKIs target the high levels of FLT3 expressed in AML blasts (70%-100%) and ALL blasts [90].

The use of novel monoclonal antibodies against mismatched MHC antigens specifically expressed on hematopoietic cells is another potentially targeted future therapy for relapse after haploidentical HSCT. The use of a bispecific antibody (diabody) targeting 2 antigens is another promising strategy for tumor therapy [91]; this could be designed to target both mismatched MHC and leukemic antigens to achieve a perfect antileukemia effect without causing GVHD in patients who relapse after haploidentical HSCT.

**Epigenetic Modifiers**

Epigenetic modifiers are often combined with other immunity-enhancing strategies. The hypomethylating agent 5-azacytidine (Aza) has been shown to be an effective epigenetic modifier for GVT enhancement. Aza restores the expression of GVT-targeted antigens including mismatched mHA, HLA, and tumor-associated antigens in leukemic cells, effectively facilitating restoration of the ability of donor immune cells to eradicate malignant cells. Dubovsky et al. [92] reported that treating chronic lymphocytic leukemia (CLL) cells with Aza unleashed target antigen expression. These investigators also found that a combination of 2 epigenetic modifiers, Aza and the histone deacetylase inhibitor LAQ824, effectively restored the immunogenicity of CLL cell lines and primary cells from patients with CLL. Indeed, such a combination induces the expression of novel and highly antigenic cancer/testis antigens and costimulatory molecules [92]. These changes further facilitate the formation of robust supramolecular activation complexes between malignant cells and responder T cells, leading to intracellular signaling, lytic granule mobilization, and polarization of functional and relevant T cell responses. The cascades of T cell–activating events triggered by malignant cells indicate that combined epigenetic modifier treatment is a potential immunotherapeutic strategy for some relapsed patients after allo-HSCT [92]. Regarding haploidentical HSCT, epigenetic modifiers can restore mismatched HLA-locus expression in leukemic cells, thus a robust GVT effect could also be initiated.

**Reinduction Chemotherapy with or without an Enhanced GVT Effect**

Reinduction chemotherapy in specific regimens without initial administration is effective for some chemosensitive diseases, although the long-term efficacy is far lower than expected. According to some reports, intensified chemotherapy should be performed to achieve CR or cytoreduction on the premise of being tolerated [93, 94]. GVT-enhancing strategies are often combined to eradicate tumor cells resistant to reinduction chemotherapy. Even if patients achieve CR again after reinduction chemotherapy with or without an enhanced GVT effect, they are vulnerable to sudden relapse in the absence of further management, whereas the subsequent treatment of patients achieving CR or no remission (NR) remains a challenging issue. Despite this dilemma, reinduction intervention provides a new opportunity for a second allo-HSCT, which would provide new hope for such special populations.

**Second Allo-HSCT**

Whether or not patients with relapsing hematologic malignancy benefit from a second allo-HSCT remains controversial. Today only a minority of patients undergoing second allo-HSCT have been reported to have high morbidity and mortality. Outcome is associated with such factors as recipient age, underlying disease, disease status, and second donor type. Kurosawa et al. [95] reported significantly higher 1-year overall survival after second allo-HSCT compared with other interventions (58% vs 14%). Second allo-HSCT involving different donors would reconstitute immunity and induce GVT effects against different antigens. In cases involving loss of mismatched HLA in relapsing malignant cells after haploidentical HSCT, second allo-HSCT using the same donor would be ineffective. NMA HSCT has been demonstrated to reduce nonrelapse mortality but to increase relapse rate; however, whether this technique can benefit patients cannot be determined without more data from clinical trials.

Regarding other preventive strategies, conducting regular follow-up checks to detect donor immune status in recipient, monitoring minimal residual disease, and adjusting the quantity of immunosuppressive agents are most important. Some recent clinical trials
have demonstrated that the kinetics of mixed chimerism of donor and recipient is an important predictor of relapse post-HSCT in patients with hematologic malignancy [96]. The role of chimerism monitoring is elusive. Donor selection and allograft components are other important factors, although consistent worldwide standards are needed. Progress in the use of allografts with T cell subset depletion, selective allodepletion, or T cell anergization would be equally beneficial.

CONCLUSION

Haploidentical HSCT is a potentially curative therapy for hematologic malignancies. As novel medical technologies and drugs emerge, severe GVHD and graft failure and infection can be prevented or controlled while malignancy relapse remains unresolved, causing high morbidity and mortality. In this review, we have summarized tentative immune factors and mechanisms associated with a high risk of relapse after haploidentical HSCT and have discussed pertinent strategies to prevent or treat relapsing hematologic malignancies. Nevertheless, how to reduce the relapse rate remains a difficult question for both hematologists and researchers.

To achieve better treatment for haploidentical recipients, several crucial topics require further clarification. More clinical trials on donor selection should be considered, as well as the establishment of worldwide standards for haploidentical donor selection. Large-scale clinical trials and underlying research concerning graft components, such as the distribution of T cell subtypes, dose of CD34+ hematopoietic stem cells, CD4/CD8 ratio, and numbers of NK and B cells, should be performed. Specific immunosuppressive agents should be developed to specifically inhibit T cell subsets causing GVHD while ideally improving the GVT effect. Novel approaches for evaluating immune cell function should be developed to allow clinicians to readily adjust the optimal dose of immunosuppressive agents in a timely manner according to the recipient’s status, as well as the donor immune status. Novel factors and mechanisms affecting the high risk of relapse require further elucidation at both the cellular and molecular levels, as well as detecting methods affecting these factors and mechanisms, which would undoubtedly contribute to the prevention and treatment of individual primary malignancies. Proposed future therapeutic regimens are illustrated in Figure 4. Finally, new strategies targeting mismatched hematopoietic MHC antigens, such as monoclonal antibodies and diabodies to eradicate malignancies, should be developed.

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REFERENCES


