Patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) generally require allogeneic hematopoietic cell transplantation (allo-HCT) for a cure, except for patients with favorable genetic genotypes such as those with core-binding factor AML. However, the use of intensive chemotherapy followed by prompt HCT does not fully prevent relapse or refractory disease. Despite improvements in transplant techniques and management of complications, further improvement of HCT outcomes is urgently needed. Moreover, careful patient counseling, donor selection, and choice of transplant type are essential to maximize the benefits of early allografting. Maintenance after HCT focusing on selective immunomodulation combined with targeted immunotherapies that control persisting or relapsed hematologic malignancies is currently under active investigation. To improve the balance between GVHD, relapse, and infection, the use of purified blood stem cell grafts in conjunction with ex vivo expanded T-cells from stem cell donors targeting common infectious and leukemic antigens has been explored. T cells against infectious agents might also be generated using partially HLA-matched third-party T cells from cryopreserved cell banks, and a series of studies confirmed the clinical value of donor-derived CMV- and EBV-specific T cells. This approach has also been applied to acute leukemia, and trials using donor-derived cytotoxic T-cells targeting multiple leukemic antigens such as WT1, PRAME, survivin, and NY-ESO, as well as donor-derived CAR19 T-cells after allo-HCT, are currently underway.

Key words hematopoietic cell transplantation, AML, MRD, GVHD, cytotoxic T-cell, CAR T-cell

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The concept of maintenance therapy after either allo-HCT or autologous hematopoietic cell transplantation (auto-HCT) mainly focused on selective immunomodulation using niche-refreshing agents, such as azacitidine and CXCR4 inhibitors combined with specific- or non-specific conventional immunotherapies, as a major promoting part of treatment against primary chemoresistant refractory or relapsed high-risk acute leukemias. Wilms’ tumor gene 1 (WT1) is a marker of non-specific leukemia-associated antigen (LAA) to monitor the minimal residual disease (MRD) status during and after treatment of AML or as a target for anti-leukemic therapy in patients with AML\(^6\)–\(^9\).

Adoptive immunotherapies using stratified WT1-specific vaccine, WT1-specific CTLs, CART/NK cells, bispecific killer cell engager T cells, gene transferred mesenchymal stromal cells (MSCs), and the combination of various immunomodulating novel agents or well-designed sequential approaches have been introduced. These adoptive immunotherapies should be applied to many high-risk patients with acute leukemias in need of treatment and to prevent relapse\(^6\)–\(^13\).

Many trials have shown that allo-HCT could compensate for the negative prognostic impact of the FLT3-ITD mutation. In a subgroup of patients with FLT3 mutated AML, a series of tyrosine kinase inhibitors (TKIs), such as gilteritinib, are currently being investigated to clarify their efficacy in preventing post-transplant relapse. These trials must shed light to enrich the benefits of TKIs post-HCT in very high-risk patients with AML. However, further investigations are warranted to develop a more potent and precise strategy as a post-transplant maintenance therapy combined with various novel drugs, small molecules, or advanced immunomodulating techniques and cells, especially vaccines.

In this short report, we introduce the background and clinical outcomes of our anti-leukemic CTL therapy to understand the mechanisms of anti-leukemic T-cell immunity in adult patients with AML and WT1 expression, as a foundation and a next step developmental strategy for conventional adoptive cellular therapy, which can be effectively utilized in therapeutic and preventive settings in refractory and relapse-prone patients with AML. Based on our previous reports of sustained clinical remission achieved by T cell responses after infusing the \textit{in vitro} cultured WT1-specific T cells, which were expanded \textit{in vivo} to elicit long-lasting anti-leukemic immunity in allo-HCT settings, it should still be one of the good ways of applying personalized care in patients with high-risk AML in real clinical settings\(^15\).

A patient was diagnosed with high-risk secondary AML transformed from myelodysplastic syndrome (RAEB-2) in 2005 and received two cycles of consecutive induction chemotherapies, but failed to achieve CR. He subsequently underwent the first allo-HCT from his sibling donor, but leukemia relapsed 10 months later without any acute or chronic graft-versus-host disease (GvHD). He received further salvage chemotherapy with FLAG-Ida followed by donor lymphocyte infusion.
and achieved complete remission approximately 2-3 weeks after the completion of the above therapies. Then, four consecutive doses of WT1-CTLs (2 × 10⁷/m²) with a 3-week interval were intravenously infused without any immediate and delayed toxicity, but severe acute hepatitis developed after the initial two doses of IL-2. After completion of CTL therapy, the patient developed an overlap syndrome at the skin, mouth, and eyes. The patient remained free of AML for 37 months after WT1-specific CTL immunotherapy. Unfortunately, AML relapsed in November 2008, and the patient died 2 months later.

By monitoring the kinetics of infused truncated WT1-CTLs, we found that WT1-specific IFN-γ-secreting T cells in the peripheral blood increased progressively during serial infusion of WT1-CTLs and the pattern persisted until 9 months, and specific T-cell responses against WT1 were maintained until the last examination at 2 years when WT1-specific IFN-gamma-secreting T cells were detected in both CD4+ and CD8+ T cells. IFN-γ-secreting CD8+ T cells specific for WT1-187 and WT1-235 were more frequently observed than those for WT1-126. These results were similar to the specificity of the generated CTLs, suggesting that WT1-specific T cells might have been derived from infused CTLs and had been maintained. Despite some limitations, this pilot study has several important clinical implications. This trial using a truncated gene was a new approach to trace transferred cells in a clinical setting.

A total of 13 newly diagnosed adult patients treated for AML between 2007 and 2008 in the Catholic Hematology Hospital (former Catholic Blood and Marrow Transplantation Center) were considered eligible for a prospective phase II/I study with very long-term follow-up if they had a human leukocyte antigen (HLA)-identical sibling donor. Despite the small number of patients enrolled in this refined prospective study, the exploratory trial of WT1-CTLs after allo-HCT for high-risk AML suggested that this well-coordinated therapeutic strategy may be used combined with elective allo- or even auto-HCT with WT1-CTL infusion. The generation of multi-antigen-specific T-cells that enhance the graft-versus-leukemia effect and prevent infection after allo-HCT, as well as the development of immunotherapy using T-cell receptor (TCR)-engineered T-cells, may be a promising treatment in the near future.

Furthermore, our group found that most adult patients with AML expressed at least one leukemia-associated antigen WT1, survivin, and TERT, as well as different combinations of the three LAAs that predicted poor clinical outcomes. Adopting monovalent WT1-specific CTL therapy performed in a pilot trial more than a decade ago provided positive results; therefore, we designed multi-tumor antigen-specific T-cells to maximize anti-leukemic effects in patients with high-risk AML. To generate three antigen-specific T-cells that recognize three LAAs, dendritic cells were transfected with three AML antigen-encoding RNAs. These DCs were used to stimulate CD8 and CD4 T cells and overcome the limitations of known HLA-restricted epitopes.

The three antigen-specific T-cells were more effective against leukemic blasts that expressed all three LAAs compared to those that expressed one or two LAAs. Engrafted leukemic blasts in the bone marrow of NSG mice significantly decreased in the presence of three antigen-specific T-cells. This technique is an effective immunotherapeutic strategy for AML. We are planning to further a phase 1/2 trial to evaluate tri-T cells against high-risk MRD-positive patients with adult AML in the near future.

**Timing of Allogeneic Hematopoietic Cell Transplantation (Allo-HCT): the Knowns and the Known Unknowns**

Treatment timing has a substantial impact on the success of allo-HCT (Figure 1). While it is generally recognized that overall survival (OS) after HCT is best if performed during CR1, those with a long initial remission and late (beyond 1.5-2 years) relapse may have similarly favorable post-HCT OS, even if performed during this late CR2. This is partly because those with a higher risk of relapse are excluded from reports of CR2 HCT. Some patients who relapsed from CR1 failed to achieve CR2, while others accumulated complications during reinduction that precluded the option of HCT in CR2. Those receiving CR2 HCT were in neither of those higher risk and excluded cohorts.

Conversely, the time to HCT when performed in CR1 reflects an indirect selection bias. In most series, the median time to AML CR1 HCT is > 6 months, thus reflecting either exclusion of those with early relapse or whose HCT could not be arranged and initiated more promptly. A longer time to HCT in CR1 generally results in better survival, reflecting this indirect but generally not quantified or reported selection bias.

Patient age, comorbidities, and complications acquired during induction directly influence the risk of non-relapse mortality (NRM). Organ dysfunction, infections, nutrition, and frailty measures summed into either performance status or detailed in the HCT-comorbidity index (-CI) have been reported to predict HCT outcomes, particularly NRM. Whether these influences are quantitatively similar across donor types and following ablative vs. reduced-intensity conditioning is uncertain.

The leukemia phenotype directly influences the risk
of relapse, and difficulty or delay in achieving CR re- quiring additional induction treatments can also com- promise fitness for HCT\textsuperscript{26-34}. If transplantation is de- ferred for those in CR1, 20-40\% of those with favor- able molecular and cyto genetic risk can still face early progression with a median time to relapse of ~12 months. Intermediate and high-risk features generally favor early transplantation because CR1 can be brief, and relapse is nearly always expected. However, even for AML in CR1, a greater number of chemotherapy cycles to achieve CR1 and measurable residual disease (MRD) pre-HCT compromises the likelihood of HCT success, both from NRM and higher risks of post-HCT relapse\textsuperscript{28-33}. MDS patients have no alternative curative option despite the benefits of hypomethylating agents or other treatments. Progression beyond early-stage MDS greatly limits HCT success, mostly by augmenting relapse risks. IPSS-R beyond low- and intermediate-risk or WHO subgroups with excess blasts have poor survival without HCT, especially for those > 60 years old.

If the choice favors HCT, a suitable and promptly available allogeneic donor is essential. Donor searching, therefore, should be initiated soon after diagnosis to fa- cilitate donor identification and testing. While HLA- matched siblings and unrelated donor (URD) HCT are the standard first choice and yield similar HCT success, the time required for URD identification and graft arrangements can, even without unplanned delays, com- promise those needing an urgent transplant, and are sometimes confounded by leukemia recurrence while awaiting an HCT that was planned for CR1\textsuperscript{30-41}. Haplo- identical, related donor grafts (who can be parents, sib- lings, or children) have been revolutionized by post- HCT cyclophosphamide as GVHD prophylaxis. Haplo- identical grafts are immediately available, although the completeness of immune constitution, the hazards of in- fection, and risks of relapse remain uncertain. Umbilical cord blood (UCB), another graft that can overcome HLA disparity and thus serve ethnic and racial minori- ties, is limited by greater risks of graft failure and con- sequent NRM.

All these factors need to be discussed with patients to assess their willingness to assume risks in the hope of better outcomes. Older patients, even after successful induction therapy, may be debilitated or fatigued\textsuperscript{42-52}. They may be unwilling to assume greater risks and ac- cept the unknowns that accompany the decision for transplantation during CR1. While recent data support the survival advantage of HCT during CR1 for older patients with AML, it reflects the yield of patient and care team discussions balancing the risks and potential benefits of this aggressive approach. Thoughtful patient selection may inflate these benefits for those who have chosen HCT; however, results for older AML and MDS patients without HCT are disappointing. Thus, early do- nor searching, patient education about available options, and willingness to offer HCT for fit patients even in their 70s may increase those who elect this potentially curative option. Careful patient counseling along with risk-adapted and intensive supportive care is essential to minimize the hazards and maximize the benefit of early allografting for all high-risk illnesses. Withholding the decision-making option from patients yields self- fulfilling and expectedly poor outcomes.

Combining Infection and Malignancy Targeted T- cell Therapy with Stem Cell Isolation for a New Paradigm in Allo-HCT

Infection, GVHD, and relapse of malignancy remain the most difficult complications associated with allogeneic HCT. Over more than 50 years, the separation of GVHD and post-transplant relapse has been the holy grail of transplant but has remained elusive. Treatment of either complication promotes development of the other, and treatment of both results in increased infec- tion. Infection is the most common contributor to NRM, the largest cause of death in the first year fol- lowing allograft. Disease relapse is the dominant cause of death beyond 1 year after allo-HCT\textsuperscript{53}.

The early discovery that cloned T-cells could reconsti- tute cytomegalovirus immunity after transplantation was followed by seminal work by Brenner, Rooney, and Heslop, showing that ex vivo expanded Epstein-Barr virus (EBV)-specific T-cells manufactured from stem cell donors were not only free from complications, but were extremely effective both in the treatment and prophylaxis of EBV-driven diseases\textsuperscript{54}. Their work focused on post-transplant lymphoproliferative disease and was fol- lowed by investigation of CMV-specific T-cells in pa- tients with refractory CMV disease, particularly pneu- monitis, where very encouraging rates of response were observed\textsuperscript{55}.

T cell therapies were largely considered valuable ad- ditions to a relatively restricted therapeutic armamen- tarium against diseases caused by CMV and EBV. The idea was that they would be called upon when standard therapies failed due to lack of efficacy or intolerance. The concept of using T-cells for rapid reconstitution of broad immunity, though inherent in early EBV prophylaxis studies, grew with the observation that CMV- specific T-cells administered prophylactically reduced the peak of viremia and the requirement for CMV antiviral therapy\textsuperscript{56}. In addition, there was a growing realization that T cells could be manufactured with specificity for a wider range of viral infections (including ade- novirus, BK virus, influenza, HHV-6, varicella, and JC virus), and that T cells play a key role in antifungal im-
munity. The latter could also be expanded ex vivo. These findings suggest that infusion of broadly reactive T-cells might rapidly recreate immune competence after immune- ablative therapy such as HCT.

Despite this, several logistic issues continue to stand in the way of a broad introduction of this approach to allo-HCT. The cost and complexity of manufacturing remained as obstructions, and the use of the stem cell donor as a source of anti-viral/fungal T-cells meant that the approach remained highly personalized. With long cell manufacturing times, there was little interest in providing complex and expensive services. Facilities and expertise were insufficient for such an approach to be widely implemented. However, improvements in manufacturing techniques, including shortening of the culture period from 7 to 10 days and the use of gas-permeable culture vessels have resulted in reassessment of personalized cell therapy.

Furthermore, the introduction of genetically modified chimeric antigen receptor (CAR)-bearing T-cell therapy has resulted in a thorough assessment of issues such as T-cell fitness, standardization of apheresis, product chain of custody, and cold chain supply. Once thought insurmountable, these challenges have been successfully addressed, albeit at some expense. The result has been broad acknowledgement of the logistic ability to introduce cellular therapies into routine clinical care and their acceptance by hematologists and transplant physicians with little prior experience in the field of cell therapies.

In contrast, the creation of individual T-cell products for all or the majority of stem cell transplant recipients remains a daunting prospect for routine transplant programs with no experience in cell manufacture. An important advance that might wholly or partially circumvent this problem was the recognition that T-cells from third-party donors (that is neither the patient nor the stem cell donor) can mediate important therapeutic benefits even when not fully HLA matched with the patient. However, some important differences exist between the use of donor-derived and third-party T-cell products.

Donor-derived T-cells persist for years after infusion, expanding, and contracting according to the antigen load, thus providing long-term immune competence. In contrast, most studies suggest that partially HLA-matched T cells persist for periods of weeks to a few months only, and even then, at very low levels, at least in the blood. The mechanism of their effects is unclear. Moreover, short- and long-term studies have demonstrated virological response rates of approximately 75% and over 90% for CMV, EBV, and adenovirus infections treated with partially HLA-matched T-cells. Because of the need for only partial HLA matching with the recipient, a relatively small bank of T-cell products (15-20 in total) is sufficient to cover more than 95% of infections in transplant patients if donors with HLA molecules common in the transplant cohort are selected for creation of bank products.

Multiple methods for the reduction of GVHD after allogeneic HCT have been proposed, including CD34 stem cell selection or its variants such as CD3/CD19 depletion or CD3/CD19 depletion, photodepletion of alloreactive donor T cells, and naive T-cell depletion. All such maneuvers can be combined with post-transplant addition of T cells targeting specific infections and malignancy. T cells targeting leukemia include those stimulated with peptides from tumor antigens such as WT-1, PRAME, survivin, NY-ESO, and MAGE proteins, but also potentially include the use of genetically modified T cells expressing either transgenic or CARs recognizing tumor antigens appropriate to the patient’s disease. The uncoupling of GVHD and disease relapse will rely on the removal of non-specific donor immune responses against major and minor HLA antigens expressed by the recipient and their replacement with known and desired immune responses to pathogens and tumor antigens that have little or no pathogenic effect on the recipient. Such trials are already in existence, and early results bode well for a fundamental change in the approach to allo-HCT.

Conclusion

Consideration of the best treatment for high-risk patients with acute leukemia and their suitability as candidates for allo-HCT requires careful attention through the process of early donor searching, patient education about the options and willingness to accept risk, and careful selection of transplant options. This is especially true in older patients. Careful patient counseling along with risk-adapted and intensive supportive care is essential to minimize hazards and maximize the benefit of early allografting for those with high-risk parameters. Although serious infections, high-grade acute and chronic GVHD, and relapse of underlying malignancy remain the most challenging complications after allo-HCT, the uncoupling of GVHD and disease relapse with reduction of non-specific donor immune responses against recipient HLA antigens and focused immune responses directed towards pathogens and tumor antigens is developing, and early results are promising. Allo-HCT may become safer and more available to a broader patient population still needing better options for ongoing leukemia management.
Author Contributions

HJK, DW, and DJG conceived and wrote the article.

Conflict of interest

The authors declare no conflicts of interest associated with this article. Disclosure forms provided by the authors are available on the website.

Hee-Je Kim is one of the Editors of Blood Cell Therapy. He was not involved in the editorial evaluation or decision to accept this article for publication.

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