Review Article

# Immunotherapy in Acute Myeloid Leukemia

Dolores A. Grosso, DNP, CRNP; Rosemary C. Hess, MSN, CRNP; and Mark A. Weiss, MD

Despite the remarkable progress made in some leukemias such as CML and CLL, cytotoxic treatment for AML remains essentially unchanged over the last 4 decades. Several lines of evidence, including the graft versus leukemia effect associated with allogeneic hematopoietic stem cell transplantation (HSCT), suggest that immunotherapy is an active modality in AML. Given the lack of progress for chemotherapy in this disease, many novel immunologic treatment approaches have been explored. The goals of non-transplant-based immune approaches have largely consisted of the stimulation or restoration of endogenous immune responses or the targeting of specific tumor antigens by immune cells. These strategies have been associated with less toxicity than allogeneic HSCT but typically have inferior efficacy. Allogeneic HSCT exploits major and minor histocompatibility differences between the donor and recipient in order to recognize and eradicate malignancy. With the recognition that the immune system itself provides a basis for treating AML, immunotherapy continues to be an attractive modality to exploit in the treatment of this disease. ***Cancer*** **2015;121:2689-704**. © *2015 American Cancer Society*.

**KEYWORDS:** acute myeloid leukemia, hematopoietic stem cell transplantation, immunotherapy, monoclonal antibody therapy with or without toxins, vaccines.

## INTRODUCTION

Acute myeloid leukemia (AML) arises from leukemia stem cells, which have the ability to self-renew and sustain malignant populations and to produce subclones. Leukemia stem cells vary in genetic expression and transcription, immunophenotype, and potential for differentiation.[1] Aberrant activation of signal-transduction pathways promotes the proliferation and survival of leukemic stem cells, and extracellular cues from the microenvironment allow leukemic stem cells to usurp normal stem cell niches.[2]

Forty years ago, the successful treatment of AML was reported using 3 days of an anthracycline combined with 7 days of continuous-infusion cytarabine.[3] Since then, many variations in AML chemotherapy regimens have been developed with little success beyond this decades-old standard. This is not surprising given that any one chemotherapy approach is unlikely to address the multitude of biologic processes associated with the evolution of AML clones. Because AML alters normal immunologic function, strategies to restore immunologic control of the disease have been developed in an attempt to increase the frequency and quality of responses. Treatments using cytokine therapy, monoclonal antibodies (MoAbs) with or without conjugation, and AML vaccines have met with various levels of success. Some agents have been associated with a lack of efficacy when used in human clinical trials. Others have demonstrated activity against AML, but the inability to identify optimal treatment contexts or combinations with other therapies has abrogated successful use. Allogeneic hematopoietic stem cell transplantation (HSCT) is the most successful of the immune-based therapies for AML, especially with the advances made in the use of alternative donors over the past decade. However, given the toxicity of HSCT, investigations into the use of immunotherapies that have the activity of HSCT without the associated side effects are ongoing. The recent development of novel T-cell–based immune therapies, initially applied to the treatment of B-cell malignancies, represents an exciting new area of investigation in AML immune therapy. A review of these different immune-based approaches follows and is listed in Table 1.

### *Nontransplantation Approaches to AML Immunotherapy*

#### Cytokine therapy

In patients with AML, several tumor-induced processes that impair T-cell and natural killer (NK)-cell immunologic functions have been identified in the failure to control leukemia. The use of exogenous cytokines to restore T-cell and NK-cell

**Corresponding author:** Mark Weiss, MD, Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, 834 Chestnut Street, Suite 320, Philadelphia, PA 19107; Fax: (215) 955-9641; mark.weiss@jefferson.edu

Department of Medical Oncology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania

**DOI:** 10.1002/cncr.29378, **Received:** July 11, 2014; **Revised:** January 23, 2015; **Accepted:** February 9, 2015, **Published online** June 10, 2015 in Wiley Online Library (wileyonlinelibrary.com)

effector functions and, by extension, antileukemic effects represents immunologic strategies that have been attempted in leukemia treatment. Various cytokines have been used in the treatment of AML, but interleukin 2 (IL-2) and interferon-α have been the most widely investigated.

*IL-2.* Interest in the use of IL-2 for cancer therapy began with the recognition that this cytokine could stimulate tumor-infiltrating lymphocytes.[4] At higher doses, IL-2 provokes type 1 helper T-cell responses; whereas, at lower doses, it demonstrates both immune-enhancing and immune-suppressive activities.[5] More than 2 decades ago, it was recognized that IL-2 therapy may be associated with antileukemic effects in patients with minimal disease burdens,[6] and the majority of trials with IL-2 have occurred in the setting of AML postremission therapy. Unfortunately, a meta-analysis combining patient data from 6 randomized clinical trials failed to demonstrate any benefit from IL-2 monotherapy in extending leukemia-free survival (LFS) or overall survival (OS).[7] This lack of benefit may be caused by the down-regulation of IL-2–stimulated effector cells by normal negative-feedback mechanisms[8] or by the concomitant action of IL-2 in the generation and activation of regulatory T cells.[9] It has been demonstrated that increases in T-cell–regulatory responses impair antileukemic T-cell reactivity.[10-12] The inability to demonstrate the efficacy of IL-2 therapy in AML may also be a result of problems with experimental methodologies and an inability to deliver planned therapy. Trials testing the efficacy of IL-2 have been characterized by heterogeneous populations and drug schedules and, more recently, by the inability to conclusively assess outcomes because of patient attrition.[13] Such factors potentially affected the results from those trials, obscuring the possibility that IL-2 may be beneficial in certain contexts.

*Interferon-α.* The cytokine interferon-α is a type I interferon that has been used as an immune-stimulatory treatment for both solid tumors and hematologic malignancies.[5,14] It promotes the stimulation and cross-priming ability of dendritic cells (DCs),[15,16] the sensitization of T cells to IL-2,[17] and the enhancement of NK-cell cytotoxicity.[18] Interferon-α–activated monocytes inhibit malignant growth and induce apoptosis in tumor cells[19]; and, in addition to its immune-stimulating properties, interferon-α has been associated with direct inhibitory effects on leukemic cells.[20-22] Interferon-α has been used in the settings of AML induction therapy, postremission therapy and in the treatment of relapsed AML after HSCT.[22] Despite the strong theoretical basis for the use of interferon-α in the treatment of AML,[23] clinical data supporting a benefit for this therapy are lacking.[20,22] For example, there was no favorable impact on the risk of relapse, LFS, or OS with the receipt of interferon-α in AML postremission therapy in the Medical Research Council trial MRC-11, which was one of the largest analyses examining interferon-α in this setting.[24] The more recent finding that stable levels of interferon-α are important for its antileukemic effects[25] has brought into question the results from older trials in which intermittent dosing schedules may have accounted for heterogeneous results.[20] Although the use of pegylated interferon-α would be expected to provide more continuous levels of the cytokine, only a few case reports supporting its use in the treatment of AML have been published.[26,27]

Despite significant antileukemic effects in vitro, clinical trials using cytokine monotherapy for patients with AML have been largely disappointing. The many abnormalities associated with the development of AML clones likely render this form of immune stimulation alone ineffective, and combined approaches may be more successful. For example, sonic hedgehog (Shh) is

**TABLE 1.** Therapies Reviewed

- Nontransplantation-based immunotherapy
  - Cytokine therapy
    - Interleukin-2, interferon-α
    - Cytokine combination therapy
  - MoAbs
    - Unconjugated MoAb
      - Lintuzumab
    - Conjugated MoAbs
      - Gemtuzumab ozogamicin
      - SGN-CD33A
  - Radiolabeled MoAbs
    - β Particles
    - α Particles
  - AML vaccines
    - Peptide
    - GM-CSF
    - Dendritic cell
- Transplantation-based immunotherapy
  - Allogeneic HSCT
    - Matched related HSCT
    - Matched unrelated HSCT
    - NK effects in HSCT
    - Haploidentical HSCT
  - Cellular therapies with nonengraftment
    - Haploidentical donor lymphocyte infusions
- Future directions
  - Chimeric antigen receptors
  - Bifunctional antibodies

Abbreviations: AML, acute myeloid leukemia; GM-CSF, granulocyte-macrophage–colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; MoAbs, monoclonal antibodies; NK, natural killer cell; SGN-CD33A, anti-CD33 monoclonal antibody-drug conjugate.

expressed in AML cells, and Shh signaling has been implicated as a key pathway in tumor progression. The Shh inhibitor cyclopamine, in conjunction with immune stimulators like tumor necrosis factor-α, interferon-α, and interferon-γ, synergistically induced massive apoptosis in various AML cell lines.[28] In a murine study, infusion of CpG-signal transducer and activator of transcription 3 (CpG-STAT3) small-interfering RNA eradicated AML by silencing the oncogene STAT3 while simultaneously stimulating potent tumor-specific immune responses through several mechanisms, including the up-regulation of major histocompatibility complex (MHC) class II antigens, the stimulation of proinflammatory cytokines like IL-12, and an increase in the ratio of cluster of differentiation 8 (CD8; T-cell coreceptor)-positive effector cells to regulatory T cells.[29] Finally, the production of reactive oxygen species by malignant myeloid cells down-regulates T-cell and NK-cell functions through nicotinamide adenine dinucleotide phosphate oxidase 2 (NOX2). The histamine derivative histamine dihydrochloride inhibits oxygen radical formation by targeting NOX2 and restores the ability of IL-2 to activate T cells and NK cells.[30,31] In the setting of postremission therapy for patients with AML, a phase 3, multicenter, randomized trial of 320 patients demonstrated that the combination of IL-2 and histamine dihydrochloride significantly extended LFS compared with observation alone.[32]

### MoAbs

Hematopoietic cells express surface antigens that are critical for normal immune responses. In 1975, Kohler and Milstein[33] developed a technique for producing antigen-specific MoAbs that led to the characterization of cell type and maturational status based on the phenotypical expression of cell-surface antigens on both normal cells and malignant cells. The nomenclature of the antigens is based on a CD number determined by the reaction of MoAbs to the antigen.[34] The potential for MoAbs to bind malignant cells for targeted eradication with fewer side effects was quickly recognized. Unfortunately, it was determined that MoAb-mediated tumor elimination through antibody-dependent cellular toxicity was limited in the treatment of AML, especially in the presence of a high disease burden.[35] Recognizing the limited activity of *naked* MoAbs, some investigators modified these agents to deliver toxins or radiation.

CD33 (Siglec-3) antigen is expressed on the majority of myeloid leukemic cells and, as such, is an attractive target for MoAb therapy. The murine M195 MoAb is reactive to the CD33 on AML cells, which has led to intense study. Lintuzumab is an unconjugated, humanized version of the M195 MoAb constructed on a humanized immunoglobulin IgG1 framework combined with murine complement-determining regions.[36] This antibody has been extensively tested in humans; and, although it was well tolerated in clinical trials, it was not associated with meaningful disease response.[37-39] The development of lintuzumab was halted after a phase 3 trial comparing mitoxantrone, etoposide, and cytarabine with or without lintuzumab in the salvage setting and a phase 2B trial comparing low-dose cytarabine with or without lintuzumab both demonstrated no significant survival benefit in patients who could not tolerate more intensive chemotherapy.[36]

Somewhat more promising results were observed with the use of gemtuzumab ozogamicin (GO), a recombinant, humanized CD33 MoAb that differs from lintuzumab in the use of an immunoglobulin G4κ antibody portion, which is conjugated to the cytotoxic antibiotic calicheamicin. The drug was approved by the US Federal Drug Administration (FDA) in 2000 based in part on an analysis of 3 phase 2 trials of 142 collective patients with AML in first relapse. In those trials, the overall complete response (CR) rate was 30%, which included patients who achieved a CR and those who had blast clearance and neutrophil recovery without complete platelet recovery.[40] When larger groups of patients underwent treatment on phase 2 trials, transient grade 3 or 4 hyperbilirubinemia; elevated transaminases; and, in post-HSCT patients, veno-occlusive disease were recognized as toxicities associated with the drug.[41] The phase 3 Southwest Oncology Group trial SWOG S0106, which compared GO plus standard induction therapy versus induction therapy alone for patients with AML, not only failed to demonstrate an overall efficacy benefit for GO, but an interim analysis revealed a higher rate of fatal induction toxicity in the GO arm, resulting in study closure.[42] That closure ultimately led to a voluntary withdrawal of FDA approval in 2010. It is noteworthy that the comparatively increased induction mortality in the GO arm was a result of the lower than expected mortality rate in the control arm, and the failure to identify any efficacy benefit in the GO arm was potentially due to a lower dose of anthracycline used in that patient subset compared with the dose received in the control arm. Patients in the GO arm who had good-risk karyotypes experienced superior OS in the trial, although the difference was not statistically significant.

In Europe, lower dose or fractionated-dose GO (3 mg/m$^2$) has been tested in trials in which identical

AML therapy was received in each arm. In these trials, the drug has been better tolerated and a disease-free survival benefit for GO has been observed in patients with de novo AML having favorable[43] and in the case of the ALFA 0701 trial, favorable plus intermediate-risk cytogenetics.[44] In a recent meta-analysis of 5 prospective, randomized, phase 3 trials that included the latter 2 European trials, the use of GO in AML induction therapy significantly prolonged disease-free survival without excessive toxicity, but a subgroup analysis indicated that its efficacy may be restricted to patients who have favorable-risk cytogenetics.[45] In Europe, it has been demonstrated that the addition of GO to induction chemotherapy prolongs survival in patients with core binding factor AML.[46] The continued successful use of GO in Europe predicts the potential for renewed interest in the use of the drug in the United States, and some experts in the field have called for the FDA to reconsider their withdrawal of the drug.

The anti-CD33 MoAb-drug conjugate SGN-CD33A represents a more recent strategy to exploit CD33 expression on malignant cells. SGD-1882, a potent DNA cross-linker, is incorporated into the structure of SGN-CD33A by conjugation with cysteine residues. Upon binding of the MoAb portion to the CD33 surface antigen, the molecule is internalized, resulting in cell cycle arrest and induction of apoptosis. Promising results from a phase 1 interim analysis of 40 patients (median age, 75 years) with untreated or relapsed AML were presented at the 2014 American Society of Hematology meeting. Blast clearance was obtained in 47% of patients at a higher dosing level, and the maximum tolerated dose has not yet been identified.[47]

### Radiolabeled antibodies

Radioimmunotherapy uses MoAbs conjugated with radioisotopes to deliver radiation directly to malignant cells, thereby enhancing both the antitumor effect of the MoAb and the therapeutic index of the radionuclide. Determining the best isotope to use requires an understanding of the type of particle(s) emitted, the half-life of the radioactivity, the availability of a suitable chelator with which to attach the radionuclide to the antibody, an understanding of the potential for altered binding once the radionuclide is attached, and the purpose of the treatment. $\beta$-Particle emitters have a relatively long range (0.8-5.0 mm) and low linear energy transfer (approximately 0.2 kiloelectron volts/$\mu$m), which allows for the delivery of radiation to both the target cells and the surrounding cells. In theory, this "cross-fire" effect makes $\beta$-particle–emitting MoAbs useful for treating large tumor burdens and irradiating the entire bone marrow before HSCT. Alpha ($\alpha$)-particle emitters have a much shorter range (50-80 $\mu$m) and higher linear energy transfer (approximately 100 kiloelectron volts/$\mu$m), which is more effective for targeting specific tumor cells without damaging the surrounding cells; therefore, this modality may be more useful for targeting residual disease or smaller tumor burdens.[48,49] Because of the limited availability of both appropriate chelators and radionuclides, $\alpha$-particle therapy was not feasible until recently, so the initial work in this area was performed with $\beta$ emitters (with or without $\gamma$ emitters).

*$\beta$-Particle–labeled antibodies.* Iodine-131 ($^{131}$I), the most frequently used $\beta$-particle emitter, was initially combined with the anti-CD33 (mouse) MoAb M195 in a phase 1 trial of 24 patients with relapsed or refractory myeloid leukemia. Using a novel assessment of tumor burden, the investigators reported that 89% of patients demonstrated a decrease in leukemic burden in the bone marrow. Patients who received $>135$ millicuries/m$^2$ required bone marrow transplantation for count recovery.[50] After that study, $^{131}$I-M195 and $^{131}$I-HuM195 (a humanized version of M95) were used to intensify conditioning with busulfan and cyclophosphamide (CY) before bone marrow transplantation, but $>69\%$ of patients developed hyperbilirubinemia during the first month after transplantation. Three patients remained in remission at $\geq$59 months, and 2 of those 3 patients were children aged $<10$ years at the time of transplantation.[51] Yttrium-90 ($^{90}$Y), a $\beta$-particle emitter that has a shorter half-life than $^{131}$I and, unlike $^{131}$I, does not emit hazardous $\gamma$-rays, has also been conjugated with HuM195. In a phase 1 trial of patients with relapsed and refractory AML,[52] $^{90}$Y-HuM195 demonstrated decreased leukemia burden, but trials using $^{90}$Y-HuM195 as conditioning for bone marrow transplantation have not yet been published.[49]

CD45 (protein tyrosine phosphatase, receptor type, C) is a glycoprotein expressed on almost all leukocytes, including the majority of myeloid and lymphoid leukemic blasts. In an attempt to target radiation specifically to hematopoietic tissues, researchers at the Fred Hutchinson Cancer Research Center (FHCRC) conjugated $^{131}$I with an anti-CD45 antibody, BC8, in a phase 1 study, which produced increased radiation uptake in the bone marrow, spleen, and lymph nodes. Subsequent trials at FHCRC using $^{131}$I-BC8 as part of preparative regimens for HSCT have produced increased radiation to hematopoietic

tissues, minimal toxicity from $^{131}$I-BC8, and disease-free survival rates as high as 75% at 42 months after HSCT.[35,53,54] Despite these encouraging results, studies outside FHCRC have not been conducted, and $^{131}$I-BC8 remains an investigational agent.

CD66 is a glycoprotein typically expressed on myeloid or monocytic cells, but not on AML blasts. Therefore, anti-CD66 MoAbs are used to concentrate their conjugate radiation isotopes to the bone marrow and are not specific to malignant cells. At the Hannover Medical School in Germany, the $\beta$-emitting isotope rhenium-188 ($^{188}$Re) was conjugated to an anti-CD66 MoAb and combined with busulfan and high-dose CY as a conditioning regimen for 21 patients with AML or myelodysplastic syndrome (MDS), many of whom had refractory/relapsed disease and were undergoing allogeneic HSCT primarily from matched unrelated donors (URDs). Despite the high-risk status of those patients, the disease-free survival rate at 42 months was 43%, although toxicity accounted for 29% of deaths.[55] The use of anti-CD66 rhenium radioimmunotherapy as part of conditioning for allogeneic HSCT appeared to be feasible based on that trial and others, although veno-occlusive disease,[55] relapse,[56] and severe gut graft-versus-host disease (GvHD)[57] have been observed at higher than expected rates in some of those studies. These outcomes did not appear to be related to direct toxicity from the $^{188}$Re-labeled anti-CD66 MoAb itself but, rather, to the conditioning regimens used in the trials or an interaction between the conditioning regimen and the MoAb.

Currently, the most promising use of $\beta$-particle radioimmunotherapy may be the targeting of radiation preferentially to hematopoietic tissues in preparation for HSCT. Further evaluation, including a phase 3 trial, will likely be required for these agents to receive FDA approval.

*$\alpha$-Particle radioimmunotherapy.* The first human study with $\alpha$-particle radioimmunotherapy examined bismuth-213 ($^{213}$Bi) conjugated to HuM195 in patients with relapsed or refractory AML or accelerated-phase chronic myelomonocytic leukemia. Although no CRs were obtained, 14 of the 18 patients who received $^{213}$Bi-HuM195 had decreased leukemic blasts in the bone marrow.[58] Given the high tumor burdens of these patients and the targeted focus of $\alpha$-particle radioimmunotherapy, CRs were not anticipated. A follow-up study in which patients were pretreated with cytarabine before they received $^{213}$Bi-HuM195 indicated that $^{213}$Bi-HuM195 may increase the efficacy of cytarabine in AML induction therapy and is able to produce CRs in patients with previously untreated AML. However, this combination was unsuccessful in patients with primary refractory or multidrug resistance-expressing AML.[59] Actinium-225 ($^{225}$Ac), the more powerful parent isotope to $^{213}$Bi, has been conjugated to HuM195 and is being tested in patients with AML, with a recent abstract reporting its safety and antileukemic activity.[60] When the maximum tolerated dose of $^{225}$Ac-HuM195 is determined, the CR and OS rates can be more fully evaluated. Murine models with another $\alpha$-particle emitter, astatine-211 ($^{211}$At), conjugated to anti-CD45 antibody resulted in targeted radiation to the bone marrow and spleen with minimal toxicity, suggesting that $^{211}$At–anti-CD45 antibody may be a promising option as a preparative regimen for HSCT.[61] Despite more than 20 years of clinical research with radionuclide conjugates in AML, to date, none have been approved by the FDA for use outside the investigational setting.

## AML vaccines

To stimulate antileukemic responses and thereby control minimal residual disease (MRD), active immunization through vaccination has been explored for high-risk patients after HSCT as well as for non-HSCT candidates after traditional therapy. Three types of vaccines have been targeted at AML: peptide vaccines, granulocyte-macrophage–colony-stimulating factor (GM-CSF) vaccines, and DC vaccines.

*Peptide vaccines.* Peptide vaccines use leukemia-associated antigens to stimulate CD8-positive cytotoxic T lymphocytes (CTLs) to target antigen-expressing leukemia cells, but not normal tissues (which lack the targeted antigen). Wilms tumor 1 (WT1) antigen, an antigen overexpressed in AML that induces CTLs against WT1-expressing leukemia cells, has been used in several peptide vaccine studies. Twelve patients with de novo AML in hematologic CR after therapy were included in a phase 1 trial of a human leukocyte antigen (HLA)-A*0201–restricted WT1 peptide vaccine. Five patients responded to the vaccine, with either the disappearance of detectable MRD and/or a reduction in WT1 expression, as determined by polymerase chain reaction in peripheral blood analysis of mononuclear cells.[62] Three of those patients continued to receive the vaccination after completion of the initial treatment and remained in CR 8 years later, demonstrating that WT1 vaccination may eliminate and/or control MRD.

Proteinase 3 is a serine protease that is commonly overexpressed in myeloid leukemia, and PR1 is a peptide

derived from proteinase 3 that induces myeloid-targeted CTLs. Four patients with AML in CR who received a PR1 peptide vaccination remained in CR 4 years after vaccination. In 8 patients with MDS, chronic myeloid leukemia, and AML, combined PR1 and WT1 peptide vaccines increased PR1-positive and WT1-positive, CD8-positive CTLs 1 week after a single vaccination. The responses became undetectable after 4 weeks with a concomitant increase in WT1 transcripts, the marker used for MRD assessment in the majority of patients, suggesting that continued vaccination may be more likely to induce a long-term response. A follow-up study with repeat vaccinations was not able to demonstrate an increase in WT1-positive, CD8-positive CTLs after the first vaccination, and the immune response was lost after the sixth vaccination in all patients. The receptor for hyaluronic-acid–mediated motility (RHAMM) is another potential leukemia-associated antigen used in peptide vaccination that has demonstrated positive immune responses in AML patients.

*GM-CSF vaccines.* GM-CSF has also been used to stimulate the immune system in conjunction with AML vaccines. Seventeen patients with refractory disease or incomplete responses as well as patients who were not eligible for induction chemotherapy received a combined GM-CSF and HLA-A*0201–restricted WT1 peptide vaccine. Ten of those patients achieved stable disease levels and an increase in WT1-tetramer–positive T cells, but the therapy did not produce CRs.[63]

*DC vaccines.* DCs mediate the production of antigen-specific CTLs through their role as antigen-presenting cells. To date, DC vaccine trials have used leukemic cell-derived DCs or monocyte-derived DCs. Leukemic cell-derived DC vaccines, although safe, have demonstrated only a minimal, transient antileukemic effect that only developed in a small subset of patients.[64] Monocyte-derived DC vaccines combined with WT1 have demonstrated immune responses including molecular responses for some patients in partial remission. It is noteworthy that HLA-matched, allogeneic, monocyte-derived DCs may be able to stimulate more CTLs than autologous, leukemic cell-derived or monocyte-derived DCs, opening another avenue for DC vaccine trials.

## *Transplant-Based Approaches to AML Immunotherapy*

### Allogeneic HSCT

Allogeneic HSCT was initially developed as a method to reconstitute hematopoiesis after intensive chemoradiotherapy, and it evolved as a successful treatment only after the requirement for MHC matching was understood. Early in the history of this procedure, the development of GvHD in patients undergoing HSCT was correlated with decreased rates of leukemia recurrence, providing the first insights into the graft-versus-leukemia (GVL) effects of this immune-based therapy. Whereas other treatment modalities can temporarily control the disease, allogeneic HSCT appears to have the most potent antileukemic effect for patients with AML and has produced some superior survival rates compared with chemotherapy in patients with intermediate and adverse prognostic factors.[65,66] One caveat is that patients who are considered for HSCT are typically more favorable treatment candidates, enriching the pool of tested participants in HSCT trials compared with trials that use chemotherapy. Therefore, comparisons between allogeneic HSCT results and results from other therapies should be made with this bias in mind.

### Matched-related HSCT for AML

The largest experience with allogeneic HSCT in the treatment of AML has been with HLA-matched related donors. The Center for International Blood and Marrow Transplant Research (CIBMTR) reported 5-year OS rates in >7000 patients with AML who had early stage disease (first CR [CR1]) or intermediate-stage disease (≥CR2) of approximately 55% and 45%, respectively, in the last decade, with much poorer OS in patients who had advanced disease.[67] In addition to disease stage at HSCT, the primary influences on outcome included age[65,68,69] and cytogenetic subtype. The outcomes reported to the CIBMTR of 390 patients in CR1 or CR2 undergoing myeloablative HSCT with favorable, intermediate, or adverse cytogenetics revealed 5-year OS rates of approximately 65%, 55%, and 25%, respectively,[70] a finding that was corroborated in the multicenter Dutch-Belgian Hemato-Oncology Group/Swiss Group for Clinical Cancer Research (HOVON/SAKK) trial.[65]

In recent years, it has been demonstrated that molecular abnormalities associated with AML have prognostic implications for patients undergoing primary therapy. These abnormalities potentially also have a significant impact on post-HSCT outcomes, although only a few, such as the FMS-like-tyrosine kinase-3 internal tandem duplication (FLT3-ITD) mutation, have been examined in this context. In patients with normal cytogenetics, the presence of FLT3-ITD predicts for poorer rates of LFS,[71] although allogeneic HSCT appears to be associated with superior disease control compared with chemotherapy

alone in patients who have this mutation.[72,73] The effects of various molecular abnormalities on the biology of AML and the response of patients with AML to HSCT represent an important area for future research.

There does not appear to be a significant relapse penalty for T-cell depletion (TCD) in patients with early stage AML who undergo myeloablative HSCT compared with conventional HSCT approaches, and there is a lower incidence of GvHD in the former approach. The LFS rate in patients who underwent TCD HSCT in 2 multicenter trials was 58% at 3 years, which was not significantly different from that of patients who underwent conventional HSCT.[74,75]

With the recognition that allogeneic immune effects, not conditioning intensity, were responsible for long-term disease control after allogeneic HSCT, nonmyeloablative regimens were developed almost 20 years ago for patients who were unlikely to tolerate the rigors of an ablative regimen.[76,77] These approaches use immunosuppressive agents to facilitate donor lymphoid and stem cell engraftment, allowing the transition to donor chimerism and GVL effects. There is a spectrum of intensity between nonmyeloablative approaches, with more intensive conditioning regimens typically referred to as reduced-intensity conditioning (RIC). Unlike low-grade lymphomas, in which strong GVL effects allow the provision of a less intensive regimen, patients with AML undergoing nonmyeloablative HSCT primarily have been treated with the more intensive RIC approach. AML is considered to be intermediately sensitive to GVL effects[78]; therefore, the intensity of the conditioning regimen, in addition to a GVL effect, appears to be important in lowering relapse rates in AML,[79] especially in patients who have higher risk cytogenetics[66] or active disease at HSCT.[80] The need for a greater degree of regimen intensity is especially problematic in older patients with AML who have high-risk disease but may not be able to tolerate an aggressive regimen. Nonrelapse mortality increases with age and regimen intensity,[81,82] suggesting that risk-adapted therapy is especially critical in older patients to maximize OS rates.[83,84] Stratification of outcomes after RIC HSCT based on cytogenetic risk appears to be similar to that after myeloablative HSCT.[85]

Patients of all ages with relapsed[86] and refractory disease,[87] secondary AML,[88] and/or cytogenetically poor-risk disease typically have a long-term OS rate $<50\%$ after matched related donor HSCT.[85,89-91] Immunologic attempts to improve these results include post-HSCT use of prophylactic donor lymphocyte infusions and manipulation of immune suppression duration and intensity in these high-risk populations.[92-95]

### URD HSCT for AML

Over the past decade, high-resolution typing of prospective recipient/donor pairs has increased the precision of HLA matching in URD HSCT, and this may be a factor in the observed improvement in long-term survival.[96-98] Five-year OS rates for patients with AML in CR1 or $\geq$CR2 who underwent URD HSCT increased from approximately 30% between 1998 and 2008[99] to 40% between 2000 and 2010.[67] Many attribute this improvement to donor selection based on high-resolution HLA typing, which diminished the impact of donor source on OS between URD and matched related donor HSCT.[100-103] Rates of acute GvHD are higher after URD HSCT compared with matched related donor HSCT, but there are conflicting results regarding whether this translates into superior leukemic control.[104-108]

Recent recognition that specific donor-recipient mismatch combinations, some associated with previously unrecognized MHC polymorphisms,[109] have the potential to further optimize URD selection. Kawase et al[110] demonstrated that specific mismatch combinations at the allele level involving HLA-DP$\beta$1 (DPB1) or HLA-C mismatches due to amino acid substitutions in the HLA-C molecule were significantly associated with a decreased risk of relapse without GvHD.

Unlike HLA-DQ$\beta$1 (DQB1), HLA-DPB1 is not in strong linkage disequilibrium with HLA-DR$\beta$1 (DRB1). Therefore, donor-recipient combinations considered to be highly matched, (ie, 8 of 8 alleles match at HLA-A, HLA-B, HLA-C, and HLA-DRB1) potentially have 1 or 2 allele mismatches at HLA-DPB1. HLA-DPB1 is a highly polymorphic molecule with variable T-cell epitope cross-reactivity patterns.[111] Various donor-recipient mismatching combinations at HLA-DPB1 can result in graft-versus-host or host-versus-graft responses, which can have clinically significant impacts on the severity of GvHD and mortality.[112] This information has led to the development of a donor-selection tool that can be used to avoid deleterious mismatches at this locus.[113]

### NK-cell effects in HSCT for AML

NK-cell functions are controlled by the net effect of inhibitory and activating signals received through cell surface receptors. One of the most studied NK receptors, the killer immunoglobulin-like receptor (KIR), mediates both inhibitory and activating stimuli. KIR inheritance is divided into A and B haplotypes, which are classified

based on gene content. Individuals with B haplotypes possess more activating KIR genes than those with A haplotypes.

To preserve tolerance, NK functions are suppressed by self-MHC through interaction with their cognate inhibitory KIR.[114] These NK/MHC interactions through inhibitory KIR are key in the development of functional responses in NK cells,[115] although it has been appreciated more recently that this functional "education" is also mediated through activating KIR.[116] After HSCT, NK cells are educated by the immune system with which they were transplanted.[117,118] Therefore, specific KIR ligand mismatches between HLA-mismatched donors and recipients result in the presence of donor NK cells capable of effector functions through education by the transplanted immune system, which are alloreactive against host leukemic cells that fail to express their inhibitory cognate MHC ligand.

The Perugia group was the first to recognize that, in TCD haploidentical HSCT, patients with AML who did not express a cognate MHC ligand for their donor's inhibitory KIR had lower relapse rates.[119-121] This finding was supported by strong in vitro and clinical trial data in which there was a 3% versus 41% frequency of relapse between patients with NK-alloreactive donors versus those without, respectively. The antileukemic effects of NK cells through KIR ligand mismatching are well supported for myeloid leukemia only and have been reproduced by other investigators in the setting of TCD haploidentical HSCT.[122,123]

In mismatched URD HSCT for myeloid malignancies, inhibitory KIR ligand mismatching is often associated with deleterious GvHD,[98,124-126] and although there are less data for haploidentical HSCT, the use of donors with a KIR B haplotype appears to confer a survival benefit after URD myeloablative HSCT.[127,128]

## Haploidentical HSCT

Haploidentical HSCT was initially developed as an alternate therapy for patients who required HSCT but lacked a matched related donor. To successfully cross the MHC barrier, ex vivo and in vivo removal of T cells from haplodisparate grafts was required to avoid lethal GvHD and secure engraftment. The consequence of this type of graft manipulation is protracted post-HSCT lymphopenia associated with a high risk of infectious mortality[129] and delayed GVL effects, whether from MHC, minor histocompatibility, or KIR ligand mismatches. Because AML is the leading indication for allogeneic HSCT,[66] improvements in the safety of haploidentical HSCT would be of significant benefit to patients with this disease.

Recent efforts[130-134] (Table 2) to avoid profound TCD from haplodisparate grafts have resulted in improved OS rates based on increases in post-HSCT immune reconstitution.[135] The rate of HSCT-related infectious death has been reduced to below 15% in most of these regimens compared with 35% to 40% after TCD haploidentical HSCT.[136] However, these newer approaches are characterized by large differences in the T-cell content of the grafts, making outcome comparisons difficult. Although severe GvHD is infrequent in haploidentical HSCT with T-cell doses $<5 \times 10^4$,[137] the optimal range of T-cell doses in terms of GVL effects is not known. Further advances in haploidentical HSCT may depend on the ability to define and optimize this range and correlate it with outcomes.

To address this issue, our group at Thomas Jefferson University developed a 2-step approach to haploidentical HSCT in which the myeloid and lymphoid portions of the graft are administered separately. This separation allows for fixed T-cell dosing, creating a consistent platform from which to compare outcomes. In the initial phase 1/2 trial,[134] 27 patients with various hematologic malignancies received 12 gray of total body irradiation followed immediately by a donor lymphocyte infusion containing $2 \times 10^8$/kg T cells (HSCT step 1). An alloreaction consisting of fever and, in some patients, rash and diarrhea developed after the donor lymphocyte infusion and resolved 2 days later in all patients with the administration of high-dose CY based on Johns Hopkins T-cell tolerization data.[133] One day after the completion of CY, patients received a CD34-selected stem cell graft. There were no reports of post-transplantation lymphoproliferative disorder, no deaths from GvHD, and no patient developed extensive chronic GvHD. At a median follow-up of 40 months (range, 28-56 months), the cumulative incidences of grade 3 and 4 GvHD and nonrelapse mortality were 7.4% and 22.2%, respectively. Patients who had controlled disease at HSCT had a disease-free survival rate of 75% at 3 years. A second-generation, 2-step trial for 28 patients who were in remission at the time of HSCT completed accrual in 2013. At a median follow-up of 28 months, their disease-free survival rate was 74%, confirming the efficacy of this approach for patients who have controlled disease at HSCT.[138] Immune reconstitution in both of these trials was robust. There were 3 infectious deaths in the initial trial, and no patient died of infection in the follow-up trial, highlighting the benefits of incorporating T cells into a haploidentical regimen.

**TABLE 2.** Non-T-Cell–Depleted Haploidentical Hematopoietic Stem Cell Transplantation Approaches

| Reference | No. of Patients | Stem Cell Source/Intensity | Method | Median [Range] | | Follow-Up | Infectious Mortality: No./Total No. (%) |
|---|---|---|---|---|---|---|---|
| | | | | CD3-Positive Dose/kg | CD34-Positive Dose/kg | | |
| Federmann 2011[130] | 28 | PBSCs/RIC | CD3/CD19 depletion | $2.9 \times 10^4$ [1.9–9.2] | $8.6 \times 10^6$ [4.3–18] | 748 d [611–1809 d] | 10/28 (36) |
| Di Bartolomeo 2013[131] | 80 | Primed bone marrow/RIC | No in vivo TCD; intense post-HSCT IS | $2.9 \times 10^7$ [1–9.8] | $2 \times 10^6$ [0.7–11] | Infectious follow-up: 76 d [6–369 d] | 11/80 (14) |
| Wang 2013[132] | 117 | Primed bone marrow and PBSCs/MA | No in vivo TCD; intense post-HSCT IS | $1.53 \times 10^8$ [0.1–8.29] | $2.21 \times 10^6$ [0.27–55.3] | 3 y [4–12 y] | 117/756 (15) |
| Luznik 2008[133] | 68 | Bone marrow/NM | Post-HSCT CY | Mean, $4.2 \times 10^7$ | Mean, $4.8 \times 10^6$ | 745 d [112–1483 d] | 4/68 (6) |
| Grosso 2011[134] | 27 | PBSCs/MA | 2 Step; pre-CD34 CY | $2.0 \times 10^8$ [no range] | $3.6 \times 10^6$ [1.3–7.4] | 40 mo [28–56 mo] | 3/27 (11) |

Abbreviations: ATG, antithymocyte globulin; CD, cluster of differentiation; CY, cyclophosphamide; HSCT, hematopoietic stem cell transplantation; IS, immunosuppression; MA, myeloablative; NM, nonmyeloablative; PBSCs, peripheral blood stem cells; RIC, reduced-intensity conditioning; TCD, T-cell depleted.

Recurrent disease was the most frequent cause of death in these 2-step trials, occurring primarily in patients who had resistant or cytogenetically high-risk disease. Strategies to improve outcomes for this patient subgroup are based on the further exploitation of allogeneic immune effects and include optimizing the dose and timing of the donor lymphocyte infusion. Second-generation myeloablative and RIC 2-step trials designed to further optimize the regimen are currently being conducted at our institution.[139]

Because of an expanded prospective donor pool for most patients, the focus of donor selection in haploidentical HSCT shifts from identifying a complete HLA match to selection based on KIR ligand mismatching, sex differences, and pregnancy-induced immunologic effects. Donor characteristics in addition to HLA match potentially strengthen the immunologic effects of haploidentical HSCT. Analysis of the effects of these donor characteristics will be possible as the population of patients who have received T-cell–containing haploidentical regimens expands.

### Cellular therapies with nonengraftment

Immunologic control of AML with haploidentical therapy has been explored outside the setting of engraftment. Here, efforts to control GvHD through graft manipulation or intensive postinfusion GvHD prophylaxis are minimal, highlighting the GVL effects of haploimmunotherapy. Colvin et al[140] reported the outcomes of 41 patients with refractory malignancies who received 100 centigray of total body irradiation followed by a G-CSF–primed, haploidentical product containing escalating doses of T cells. Only patients who received the highest doses of T cells (1 or $2 \times 10^8$/kg) had objective responses, including 10 of 13 patients with AML. In the setting of AML postremission therapy, Guo et al[141] reported the outcome of 100 patients in CR1 who received G-CSF–primed, haploidentical infusions after each of 3 cycles of high-dose cytarabine consolidation. In the multivariate analysis, patients who received $\geq 1.1 \times 10^8$ T cells in each course had higher LFS and OS rates compared with those who received lower T-cell doses. The 6-year LFS rate for the 81 patients who had intermediate-risk cytogenetics in that study was very high at 59.2%. These trials suggest that T-cell thresholds in haploimmunotherapy may be meaningful in terms of GVL effects.

Figure 1 summarizes the immune-based therapies that have been used to treat AML, including 2 promising new strategies, as discussed below.

## *Future Directions*

### Chimeric antigen receptors

Chimeric antigen receptors (CARs) link the binding portion of a high-affinity antibody fragment that is specific to

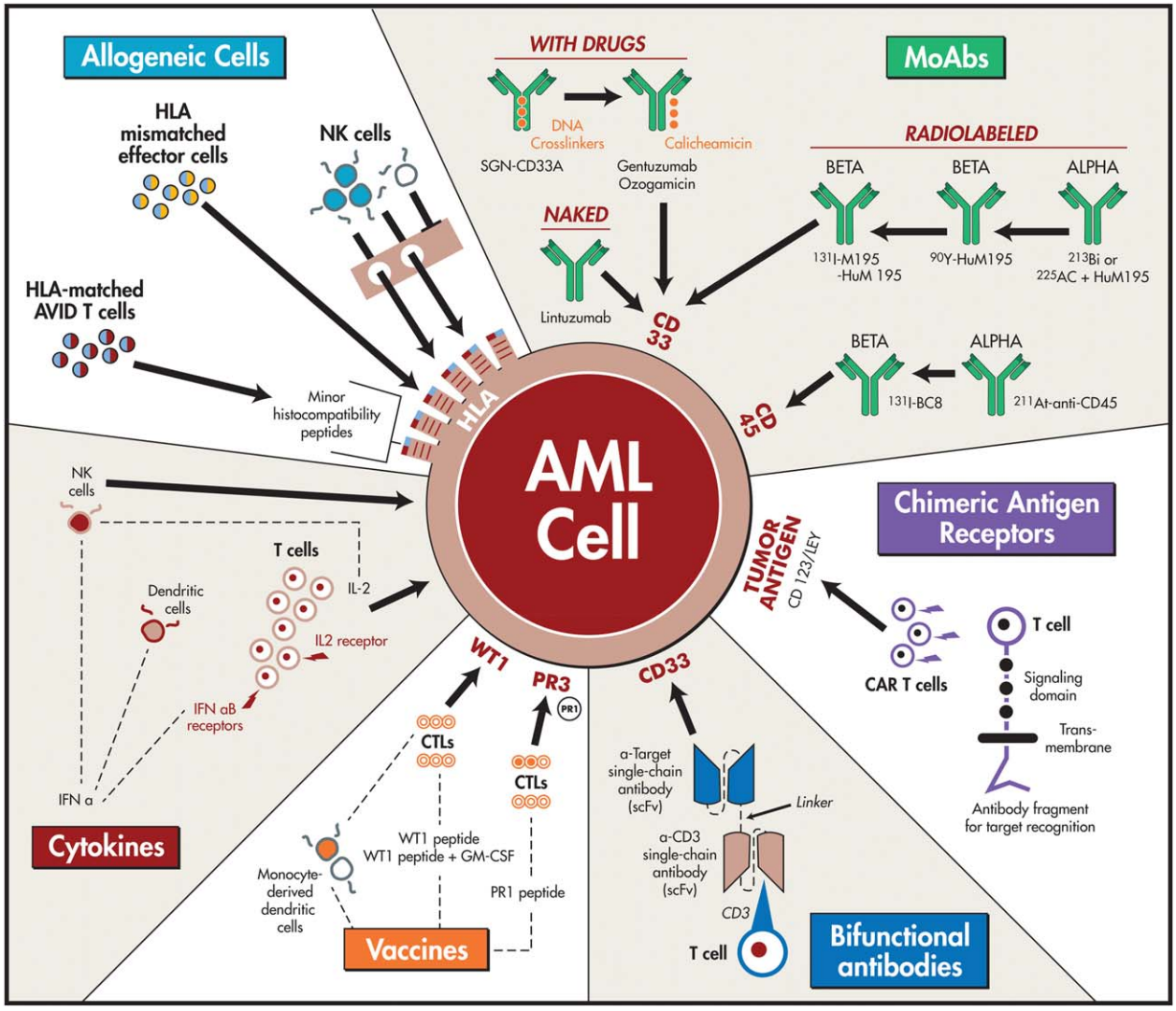


**Figure 1.** Immunotherapeutic approaches to acute myelogenous leukemia (AML) are illustrated. Different immune-based approaches to AML treatment are depicted from past to present. $^{131}$I indicates iodine-131; $^{211}$At, astatine-211; $^{213}$Bi, bismuth-213; $^{225}$AC, actinium-225; $^{90}$Y, yttrium-90; BC8, anti-CD45 antibody; CAR, chimeric antigen receptor; CD33, cluster of differentiation 33 (Siglec-3); CD45, cluster of differentiation 45 (protein tyrosine phosphatase, receptor type, C); CTL, cytotoxic lymphocytes; GM-CSF, granulocyte-macrophage–colony-stimulating factor; HLA, human leucocyte antigen; HuM 195, humanized monoclonal antibody 195; IFN, interferon; IL2, interleukin-2; LEY, Lewis antigen; M195, murine monoclonal antibody 195; MoAbs, monoclonal antibodies; NK, natural killer; PR, proteinase; scFV, single-chain variable fragment; SGN-CD33A, anti-CD33 monoclonal antibody-drug conjugate; WT1, Wilms tumor 1.

a tumor antigen with a T-cell signaling moiety, such as CD 3-ζ. The combined molecule is grafted to recipient T cells by retroviral transfection, resulting in a subset of genetically modified T cells capable of MHC-unrestricted activation through their signaling moiety against the target cells bound by the antibody fragment. A hinge region is usually included in the construct for optimal distancing between the binding and signaling regions to enhance T-cell activation. Second-generation and third-generation CARs incorporate additional signaling motifs, such as T-cell–costimulatory receptors or cytoplasmic signaling domains from costimulatory receptors, to enhance responsiveness. CAR therapy has been used primarily to treat B-cell malignancies, and antitumor activity has been demonstrated in chronic lymphoblastic leukemia and B-cell acute lymphoblastic leukemia (ALL).[142-145] The response of disease to CAR therapy has varied based on differences in the construct of the CAR model among different research groups as well as various responses of hematopoietic diseases to this type of therapy. The

expression of CAR on the T-cell surface and persistent engraftment of CAR T cells are associated with the efficacy of a particular construct. Toxicities of the therapy include a significant cytokine-release syndrome and permanent B-cell aplasia in patients who receive it as treatment for B-cell malignancies.[146]

Similar targeting of AML cells with CAR therapy is constrained by the expression of CAR target antigens on hematopoietic stem cells, although in vitro and in vivo studies testing the efficacy of CARs in this disease are ongoing. The IL-3 receptor α chain CD123 represents an attractive antigen for CAR therapy in AML, because it is overexpressed in AML cells compared with normal hematopoietic cells. In vitro studies of CARs targeting CD123 epitopes in AML cell lines exhibited potent effector activity without the elimination of nonmalignant granulocyte colony formation.[147,148] In mice engrafted with a human AML cell line, there was a significant reduction in leukemic blasts after treatment with a CD123 CAR regardless of the level of CD123 expression in the AML cell line. However, treatment with the CD123 CAR resulted in near complete eradication of normal bone marrow cells in mice engrafted with human CD34-positive cells,[149] highlighting the difficulty of developing CAR therapy in AML. More specific targeting of a tumor antigen was performed in a phase 1 trial in which 5 patients with resistant AML received a CAR targeting the Lewis antigen, which is expressed on many tumors, including some AML cells; however, its expression on normal cells is limited. Three of the 5 patients in that study responded to the therapy, including 1 patient who had a CR that lasted 23 months.[150]

In vivo and in vitro studies of CARs in AML therapy suggest potent antileukemic activity. However, deleterious effects on normal progenitor cells or narrow therapeutic targeting to avoid widespread toxicity represent major barriers to the implementation of CAR therapy in AML.

### Bifunctional antibodies

Bispecific T-cell engagers (BiTEs) combine single-chain antibody fragments, with 1 end targeting a tumor-associated antigen and the other end targeting a T-cell antigen. BiTE antibodies bind to tumor cells while simultaneously engaging T-cells to kill tumor cells. Blinatumomab, a CD19/CD3 BiTE antibody, has demonstrated promise in B-cell malignancies, including non-Hodgkin lymphoma and ALL, and has recently been approved by the FDA for relapsed/refractory B-lineage ALL.[151,152] Because CD33 is expressed on the majority of AML blasts and AML stem cells, it has been used in early BiTE antibody therapy in AML. Ex vivo CD33/CD3 BiTE antibody therapy (AMG330) demonstrated redirected, targeted lysis of AML blasts and stem cells, whereas an in vivo murine experiment produced decreased tumor growth.[153] Ex vivo primary human AML samples treated with AMG330 exhibited lysis of AML blasts with increased T-cell activation.[154,155] Another AML BiTE antibody, which combines CD3 with WT1 and HLA-0201 in a murine model, demonstrated undetectable leukemic growth by day 14 with MRD by day 18.[156] This early preclinical work supports the continued development of BiTE antibody therapy as a potentially useful and novel approach to the treatment of AML.

### *Summary*

Immune-based therapies for AML have had various levels of success, but work in this area continues to progress and to demonstrate promise. Because AML is a heterogeneous disease, it is unlikely that any single treatment modality will be universally effective. The capabilities to successfully combine various immune approaches with each other and with chemoradiotherapy and to determine the optimal timing of these therapies during the course of disease treatment represent major challenges ahead in the treatment of AML.

## FUNDING SUPPORT

No specific funding was disclosed.

## CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

## REFERENCES

1. Wiseman DH, Greystoke BF, Somervaille TC. The variety of leukemic stem cells in myeloid malignancy. *Oncogene.* 2014;33:3091-3098.
2. Vaidya A. Can systems biology approach help in finding more effective treatment for acute myeloid leukemia? *Syst Synth Biol.* 2014;8:165-167.
3. Yates JW, Wallace HJ Jr, Ellison RR, Holland JF. Cytosine arabinoside (NSC 63878) and daunorubicin (NSC 83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep.* 1973;57:485-488.
4. Ettinghausen SE, Lipford III EH, Mule JJ, Rosenberg SA. Recombinant interleukin-2 stimulates in vivo proliferation of adoptively transferred lymphokine-activated killer (LAK) cells. *J Immunol.* 1985;135:3623-3635.
5. Alatrash G, Jakher H, Stafford PD, Mittendorf EA. Cancer immunotherapies, their safety and toxicity. *Expert Opin Drug Saf.* 2013;12:631-645.
6. Foa R, Guarini A, Gansbacher B. IL-2 treatment for cancer: from biology to gene therapy. *Br J Cancer.* 1992;66:992-998.
7. Buyse M, Squifflet P, Lange BJ, et al. Individual patient data meta-analysis of randomized trials evaluating IL-2 monotherapy as remission maintenance therapy in acute myeloid leukemia. *Blood.* 2011;117:7007-7013.
8. Acuto O, Bartolo VD, Michel F. Tailoring T-cell receptor signals by proximal negative feedback mechanisms. *Nature Rev Immunol.* 2008;8:699-712.

9. Ustun C, Miller JS, Munn DH, Weisdorf DJ, Blazar BR. Regulatory T cells in acute myelogenous leukemia: is it time for immunomodulation? *Blood.* 2011;118:5084-5095.
10. Memarian A, Nourizadeh M, Masoumi F, et al. Upregulation of CD200 is associated with Foxp3+ regulatory T cell expansion and disease progression in acute myeloid leukemia. *Tumor Biol.* 2013;34: 531-542.
11. Schick J, Vogt V, Zerwes M, et al. Antileukemic T-cell responses can be predicted by the composition of specific regulatory T-cell subpopulations. *J Immunother.* 2013;36:223-237.
12. Shenghui Z, Yixiang H, Jianbo W, et al. Elevated frequencies of CD4+CD25+CD127lo regulatory T cells is associated to poor prognosis in patients with acute myeloid leukemia. *Int J Cancer.* 2011;129:1373-1381.
13. Kolitz JE, George SL, Benson DM, et al. Recombinant interleukin-2 in patients aged younger than 60 years with acute myeloid leukemia in first complete remission: results from Cancer and Leukemia Group B 19808. *Cancer.* 2014;120:1010-1017.
14. Platanias LC. Interferons and their antitumor properties. *J Interferon Cytokine Res.* 2013;33:143-144.
15. Le Bon A, Tough DF. Type I interferon as a stimulus for cross-priming. *Cytokine Growth Factor Rev.* 2008;19:33-40.
16. Papewalis C, Jacobs B, Wuttke M, et al. IFN-α skews monocytes into CD56+-expressing dendritic cells with potent functional activities in vitro and in vivo. *J Immunol.* 2008;180:1462-1470.
17. Matikainen S, Sareneva T, Ronni T, Lehtonen A, Koskinen PJ, Julkunen I. Interferon-α activates multiple STAT proteins and upregulates proliferation-associated IL-2Rα, c-myc, and pim-1 genes in human T cells. *Blood.* 1999;93:1980-1991.
18. Liang S, Wei H, Sun R, Tian Z. IFNα regulates NK cell cytotoxicity through STAT1 pathway. *Cytokine.* 2003;23:190-199.
19. Bekisz J, Sato Y, Johnson C, Husain SR, Puri RK, Zoon KC. Immunomodulatory effects of interferons in malignancies. *J Interferon Cytokine Res.* 2013;33:154-161.
20. Anguille S, Lion E, Willemen Y, Van Tendeloo VF, Berneman ZN, Smits EL. Interferon-α in acute myeloid leukemia: an old drug revisited. *Leukemia.* 2011;25:739-748.
21. Colamonici OR, Domanski P, Platanias LC, Diaz MO. Correlation between interferon (IFN) a resistance and deletion of the IFN α/β genes in acute leukemia cell lines suggests selection against the IFN system. *Blood.* 1992;80:744-749.
22. Smits EL, Anguille S, Berneman ZN. Interferon α may be back on track to treat acute myeloid leukemia [serial online]. *Oncoimmunology.* 2013;2:e23619.
23. Robb RJ, Kreijveld E, Kuns RD, et al. Type I-IFNs control GVHD and GVL responses after transplantation. *Blood.* 2011;118:3399-3409.
24. Goldstone AH, Burnett AK, Wheatley K, et al; Medical Research Council Adult Leukemia Working Party. Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. *Blood.* 2001;98:1302-1311.
25. Benjamin R, Khwaja A, Singh N, et al. Continuous delivery of human type I interferons (α/β) has significant activity against acute myeloid leukemia cells in vitro and in a xenograft model. *Blood.* 2007;109:1244-1247.
26. Berneman ZN, Anguille S, Van Marck V, Schroyens WA, Van Tendeloo VF. Induction of complete remission of acute myeloid leukaemia by pegylated interferon-α-2a in a patient with transformed primary myelofibrosis. *Br J Haematol.* 2010;149:152-155.
27. Dagorne A, Douet-Guilbert N, Quintin-Roue I, et al. Pegylated interferon α2a induces complete remission of acute myeloid leukemia in a postessential thrombocythemia myelofibrosis permitting allogenic stem cell transplantation. *Ann Hematol.* 2013;92:407-409.
28. Lu FL, Yu CC, Chiu HH, et al. Sonic hedgehog antagonists induce cell death in acute myeloid leukemia cells with the presence of lipopolysaccharides, tumor necrosis factor-α, or interferons. *Invest New Drugs.* 2013;31:823-832.
29. Hossain DM, Dos Santos C, Zhang Q, et al. Leukemia cell-targeted STAT3 silencing and TLR9 triggering generate systemic antitumor immunity. *Blood.* 2014;123:15-25.
30. Martner A, Thorén FB, Aurelius J, Hellstrand K. Immunotherapeutic strategies for relapse control in acute myeloid leukemia. *Blood Rev.* 2013;27:209-216.
31. Romero AI, Thorén FB, Aurelius J, Askarieh G, Brune M, Hellstrand K. Post-consolidation immunotherapy with histamine dihydrochloride and interleukin-2 in AML. *Scand J Immunol.* 2009; 70:194-205.
32. Brune M, Castaigne S, Catalano J, et al. Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. *Blood.* 2006;108:88-96.
33. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature.* 1975;256:495-497.
34. Roitt IM. Transplantation. In:Roitt IM, Delves PJ, eds. Roitt's Essential Immunology. 10th ed. Oxford, UK: Blackwell Science Ltd; 2001:108-128.
35. Matthews DC. Immunotherapy in acute myelogenous leukemia and myelodysplastic syndrome. *Leukemia.* 1998;12:S33-S36.
36. Feldman EJ, Brandwein J, Stone R, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. *J Clin Oncol.* 2005;23:4110-4116.
37. Caron PC, Jurcic JG, Scott AM, et al. A phase 1B trial of humanized monoclonal antibody M195 (anti-CD33) in myeloid leukemia: specific targeting without immunogenicity. *Blood.* 1994;83:1760-1768.
38. Caron PC, Dumont L, Scheinberg DA. Supersaturating infusional humanized anti-CD33 monoclonal antibody HuM195 in myelogenous leukemia. *Clin Cancer Res.* 1998;4:1421-1428.
39. Feldman EJ, Kalaycio M, Weiner G, et al. Treatment of relapsed or refractory acute myeloid leukemia with humanized anti-CD33 monoclonal antibody HuM195. *Leukemia.* 2003;17:314-318.
40. Bross PF, Beitz J, Chen G, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin Cancer Res.* 2001;7:1490-1496.
41. Larson RA, Sievers EL, Stadtmauer EA, et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer.* 2005;104:1442-1452.
42. Petersdorf SH, Kopecky KJ, Slovak M, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood.* 2013;121:4854-4860.
43. Burnett AK, Hills RK, Milligan D, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. *J Clin Oncol.* 2011;29:369-377.
44. Castaigne S, Pautas C, Terré C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet.* 2012;379:1508-1516.
45. Li X, Xu SN, Qin DB, Tan Y, Gong Q, Chen JP. Effect of adding gemtuzumab ozogamicin to induction chemotherapy for newly diagnosed acute myeloid leukemia: a meta-analysis of prospective randomized phase III trials. *Ann Oncol.* 2014;25:455-461.
46. Burnett AK, Hills RK, Russell N, et al. Reasons for survival improvement in core binding factor AML: a 25 year analysis of the UK MRC/NCRI AML trials. *Blood.* 2013;122:358-358.
47. Stein EM, Stein A, Walter RB, et al. Interim analysis of a phase 1 trial of SGN-CD33A in patients with CD33-positive acute myeloid leukemia (AML) [abstract]. *Blood.* 2014;124. Abstract 623.
48. Caligiuri MA, Velardi A, Scheinberg DA, Borrello IM. Immunotherapeutic approaches for hematologic malignancies. *Hematology Am J Hematol Educ Program.* 2004:337-353.
49. Jurcic JG. What happened to anti-CD33 therapy for acute myeloid leukemia? *Curr Hematol Malig Rep.* 2012;7:65-73.
50. Schwartz MA, Lovett DR, Redner A, et al. Dose-escalation trial of M195 labeled with iodine 131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. *J Clin Oncol.* 1993; 11:294-303.

51. Burke JM, Caron PC, Papadopoulos EB, et al. Cytoreduction with iodine-131-anti-CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. *Bone Marrow Transplant.* 2003;32:549-556.
52. Jurcic JG, Divgi CR, Mcdevitt MR, et al. Phase I trial of yttrium-90-HuM195 in myeloid leukemia potential for myeloablation. *Cancer Biother Radiopharm.* abstract 39, 2000;15:402–404.
53. Matthews DC, Appelbaum FR, Eary JF, et al. Development of a marrow transplant regimen for acute leukemia using targeted hematopoietic irradiation delivered by 131I-labeled anti-CD45 antibody, combined with cyclophosphamide and total body irradiation. *Blood.* 1995;85:1122-1131.
54. Pagel JM, Gooley TA, Rajendran J, et al. Allogeneic hematopoietic cell transplantation after conditioning with 131I-anti-CD45 antibody plus fludarabine and low-dose total body irradiation for elderly patients with advanced acute myeloid leukemia or high-risk myelodysplastic syndrome. *Blood.* 2009;114:5444-5453.
55. Koenecke C, Hofmann M, Bolte O, et al. Radioimmunotherapy with [188Re]-labelled anti-CD66 antibody in the conditioning for allogeneic stem cell transplantation for high-risk acute myeloid leukemia. *Int J Hematol.* 2008;87:414-421.
56. Lauter A, Strumpf A, Platzbecker U, et al. 188Re anti-CD66 radioimmunotherapy combined with reduced-intensity conditioning and in-vivo T cell depletion in elderly patients undergoing allogeneic haematopoietic cell transplantation. *Br J Haematol.* 2010;148:910-917.
57. Klein SA, Hermann S, Dietrich CF, Hoelzer D, Martin H. Transplantation-related toxicity and acute intestinal graft-versus-host disease after conditioning regimens intensified with rhenium 188-labeled anti-CD66 monoclonal antibodies. *Blood.* 2002;99:2270-2271.
58. Jurcic JG, Larson SM, Sgouros G, et al. Targeted a particle immunotherapy for myeloid leukemia. *Blood.* 2002;100:1233-1239.
59. Rosenblat TL, McDevitt MR, Mulford DA, et al. Sequential cytarabine and α-particle immunotherapy with bismuth-213-lintuzumab (HuM195) for acute myeloid leukemia. *Clin Cancer Res.* 2010;16:5303-5311.
60. Jurcic JG, Ravandi F, Pagel JM, et al. Phase I trial of the targeted alpha-particle nano-generator actinium-225 (225Ac)-lintuzumab (anti-CD33) in combination with low-dose cytarabine (LDAC) for older patients with untreated acute myeloid leukemia (AML). *Blood.* 2013;122:1460-1460.
61. Orozco JJ, Bäck T, Kenoyer A, et al. Anti-CD45 radioimmunotherapy using At with bone marrow transplantation prolongs survival in a disseminated murine leukemia model. *Blood.* 2013;121:3759-3767.
62. Oka Y, Tsuboi A, Taguchi T, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci U S A.* 2004;101:13885-13890.
63. Keilholz U, Letsch A, Busse A, et al. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. *Blood.* 2009;113:6541-6548.
64. Roddie H, Klammer M, Thomas C, et al. Phase I/II study of vaccination with dendritic-like leukaemia cells for the immunotherapy of acute myeloid leukaemia. *Br J Haematol.* 2006;133:152-157.
65. Cornelissen JJ, Van Putten WL, Verdonck LF, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? *Blood.* 2007;109:3658-3666.
66. Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. *JAMA.* 2009;301:2349-2361.
67. Pasquini MC, Wang Z. Current use and outcome of hematopoietic stem cell transplantation: Center for International Blood and Marrow Transplant Research (CIBMTR) summary slides, 2012. Milwaukee, WI: CIBMTR; 2012. http://www.cibmtr.org.2012. Accessed December 28, 2014.
68. De Witte T, Hermans J, Vossen J, et al. Haematopoietic stem cell transplantation for patients with myelodysplastic syndromes and secondary acute myeloid leukaemias: a report on behalf of the Chronic Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). *Br J Haematol.* 2000;110:620-630.
69. Giebel S, Labopin M, Holowiecki J, et al. Outcome of HLA-matched related allogeneic hematopoietic stem cell transplantation for patients with acute leukemia in first complete remission treated in Eastern European centers. Better results in recent years. *Ann Hematol.* 2009;88:1005-1013.
70. Armand P, Kim HT, Zhang MJ, et al. Classifying cytogenetics in patients with acute myelogenous leukemia in complete remission undergoing allogeneic transplantation: a Center for International Blood and Marrow Transplant Research study. *Biol Blood Marrow Transplant.* 2012;18:280-288.
71. Sengsayadeth SM, Jagasia M, Engelhardt BG, et al. Allo-SCT for high-risk AML-CR1 in the molecular era: impact of FLT3/ITD outweighs the conventional markers. *Bone Marrow Transplant.* 2012;47:1535-1537.
72. Labouré G, Dulucq S, Labopin M, et al. Potent graft-versus-leukemia effect after reduced-intensity allogeneic SCT for intermediate-risk AML with FLT3-ITD or wild-type NPM1 and CEBPA without FLT3-ITD. *Biol Blood Marrow Transplant.* 2012;18:1845-1850.
73. Schlenk RF, Döhner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med.* 2008;358:1909-1918.
74. Bayraktar UD, de Lima M, Saliba RM, et al. Ex vivo T cell-depleted versus unmodified allografts in patients with acute myeloid leukemia in first complete remission. *Biol Blood Marrow Transplant.* 2013;19:898-903.
75. Devine SM, Carter S, Soiffer RJ, et al. Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the Blood and Marrow Transplant Clinical Trials Network protocol 0303. *Biol Blood Marrow Transplant.* 2011;17:1343-1351.
76. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood.* 1997;89:4531-4536.
77. Slavin S, Nagler A, Naparstek E, et al. Immunotherapy of leukemia in conjunction with reduced intensity conditioning: engraftment of blood stem cells and eradication of host leukemia with reduced intensity conditioning based on fludarabine and anti-thymocyte globulin (ATG) [abstract]. *Blood.* 1996;88(suppl 1). Abstract 614a.
78. William BM, DeLima M. Advances in conditioning regimens for older adults undergoing allogeneic stem cell transplantation to treat hematologic malignancies. *Drugs Aging.* 2013;30:373-381.
79. Shimoni A, Nagler A. Optimizing the conditioning regimen for allogeneic stem-cell transplantation in acute myeloid leukemia; dose intensity is still in need. *Best Pract Res Clin Haematol.* 2011;24:369-379.
80. Shimoni A, Hardan I, Shem-Tov N, et al. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. *Leukemia.* 2006;20:322-328.
81. Aoudjhane M, Labopin M, Gorin NC, et al. Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European Group for Blood and Marrow Transplantation (EBMT). *Leukemia.* 2005;19:2304-2312.
82. Martino R, Valcárcel D, Brunet S, Sureda A, Sierra J. Comparable non-relapse mortality and survival after HLA-identical sibling blood stem cell transplantation with reduced or conventional-intensity preparative regimens for high-risk myelodysplasia or acute myeloid leukemia in first remission. *Bone Marrow Transplant.* 2008;41:33-38.

83. Yoon JH, Cho BS, Kim HJ, et al. Outcomes of elderly de novo acute myeloid leukemia treated by a risk-adapted approach based on age, comorbidity, and performance status. *Am J Hematol.* 2013;88: 1074-1081.
84. Sorror ML, Appelbaum FR. Risk assessment before allogeneic hematopoietic cell transplantation for older adults with acute myeloid leukemia. *Expert Rev Hematol.* 2013;6:547-562.
85. Chevallier P, Labopin M, Milpied N, et al. Impact of cytogenetics risk on outcome after reduced intensity conditioning allo-SCT from an HLA-identical sibling for patients with AML in first CR: a report from the Acute Leukemia Working Party of EBMT. *Bone Marrow Transplant.* 2012;47:1442-1447.
86. Duval M, Klein JP, He W, et al. Hematopoietic stem-cell transplantation for acute leukemia in relapse or primary induction failure. *J Clin Oncol.* 2010;28:3730-3738.
87. Michallet M, Thomas X, Vernant JP, et al. Long-term outcome after allogeneic hematopoietic stem cell transplantation for advanced stage acute myeloblastic leukemia: a retrospective study of 379 patients reported to the Societe Francaise de Greffe de Moelle (SFGM). *Bone Marrow Transplant.* 2000;26:1157-1163.
88. Litzow MR, Tarima S, Pérez WS, et al. Allogeneic transplantation for therapy-related myelodysplastic syndrome and acute myeloid leukemia. *Blood.* 2010;115:1850-1857.
89. Gupta V, Tallman MS, He W, et al. Comparable survival after HLA-well-matched unrelated or matched sibling donor transplantation for acute myeloid leukemia in first remission with unfavorable cytogenetics at diagnosis. *Blood.* 2010;116:1839-1848.
90. Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group study. *Blood.* 2000;96:4075-4083.
91. van Gelder M, de Wreede LC, Schetelig J, et al. Monosomal karyotype predicts poor survival after allogeneic stem cell transplantation in chromosome 7 abnormal myelodysplastic syndrome and secondary acute myeloid leukemia. *Leukemia.* 2013;27:879-888.
92. Krejci M, Doubek M, Dusek J, et al. Combination of fludarabine, amsacrine, and cytarabine followed by reduced-intensity conditioning and allogeneic hematopoietic stem cell transplantation in patients with high-risk acute myeloid leukemia. *Ann Hematol.* 2013;92:1397-1403.
93. Liga M, Triantafyllou E, Tiniakou M, et al. High alloreactivity of low-dose prophylactic donor lymphocyte infusion in patients with acute leukemia undergoing allogeneic hematopoietic cell transplantation with an alemtuzumab-containing conditioning regimen. *Biol Blood Marrow Transplant.* 2013;19:75-81.
94. Craddock C. Pharmacological methods to reduce disease recurrence. In: Anderson K, Bauer K, Tallman M, Croether M, eds. American Society of Hematology Education Program. Washington, DC: American Society of Hematology; 2013:63.
95. Zhang WP, Yang D, Song XM, et al. Allogeneic peripheral blood stem cell transplantation is a promising and safe choice for the treatment of refractory/relapsed acute myelogenous leukemia, even with a higher leukemia burden. *Biol Blood Marrow Transplant.* 2013;19: 653-660.
96. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood.* 2004;104:1923-1930.
97. Lee SJ, Klein J, Haagenson M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood.* 2007;110:4576-4583.
98. Woolfrey A, Klein JP, Haagenson M, et al. HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant.* 2011;17:885-892.
99. Pasquini MC, Wang Z. Current use and outcomes of hematopoietic stem cell transplantation: Center for International Blood and Marrow Transplant Research (CIBMTR) summary slides 2010. Milwaukee, WI: CIBMTR; 2010. http://www.cibmtr.orgslides. Accessed December 28, 2014.
100. Robin M, Porcher R, Adès L, et al. Matched unrelated or matched sibling donors result in comparable outcomes after non-myeloablative HSCT in patients with AML or MDS. *Bone Marrow Transplant.* 2013;48:1296-1301.
101. Peffault de Latour R, Brunstein CG, Porcher R, et al. Similar overall survival using sibling, unrelated donor, and cord blood grafts after reduced-intensity conditioning for older patients with acute myelogenous leukemia. *Biol Blood Marrow Transplant.* 2013;19: 1355-1360.
102. Valcárcel D, Sierra J, Wang T, et al. One-antigen mismatched related versus HLA-matched unrelated donor hematopoietic stem cell transplantation in adults with acute leukemia: Center for International Blood and Marrow Transplant Research results in the era of molecular HLA typing. *Biol Blood Marrow Transplant.* 2011;17: 640-648.
103. Yakoub-Agha I, Mesnil F, Kuentz M, et al. Allogeneic marrow stem-cell transplantation from human leukocyte antigen-identical siblings versus human leukocyte antigen-allelic-matched unrelated donors (10/10) in patients with standard-risk hematologic malignancy: a prospective study from the French Society of Bone Marrow Transplantation and Cell Therapy. *J Clin Oncol.* 2006;24: 5695-5702.
104. Saber W, Opie S, Rizzo JD, Zhang MJ, Horowitz MM, Schriber J. Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia. *Blood.* 2012;119:3908-3916.
105. Horowitz MM. Does matched unrelated donor transplantation have the same outcome as matched sibling transplantation in unselected patients? *Best Pract Res Clin Haematol.* 2012;25:483-486.
106. Lee SJ, Kang BW, Moon JH, et al. Comparable analysis of outcomes for allogeneic peripheral blood stem cell transplantation from matched related and matched unrelated donors in acute myeloid leukemia. *Acta Haematol.* 2012;127:81-89.
107. Storb R, Gyurkocza B, Storer BE, et al. Graft-versus-host disease and graft-versus-tumor effects after allogeneic hematopoietic cell transplantation. *J Clin Oncol.* 2013;31:1530-1538.
108. Woolfrey A, Lee SJ, Gooley TA, et al. HLA-allele matched unrelated donors compared to HLA-matched sibling donors: role of cell source and disease risk category. *Biol Blood Marrow Transplant.* 2010;16:1382-1387.
109. Petersdorf EW. The major histocompatibility complex: a model for understanding graft-versus-host disease. *Blood.* 2013;122: 1863-1872.
110. Kawase T, Matsuo K, Kashiwase K, et al. HLA mismatch combinations associated with decreased risk of relapse: implications for the molecular mechanism. *Blood.* 2009;113:2851-2858.
111. Zino E, Frumento G, Marktel S, et al. A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. *Blood.* 2004;103: 1417-1424.
112. Fleischhauer K, Shaw BE, Gooley T, et al. Effect of T-cell-epitope matching at HLA-DPB1 in recipients of unrelated-donor haemopoietic-cell transplantation: a retrospective study. *Lancet Oncol.* 2012;13:366-374.
113. Shaw BE, Robinson J, Fleischhauer K, Madrigal JA, Marsh SG. Translating the HLA-DPB1 T-cell epitope-matching algorithm into clinical practice. *Bone Marrow Transplant.* 2013;48: 1510-1512.
114. Ciccone E, Pende D, Viale O, et al. Involvement of HLA class I alleles in natural killer (NK) cell-specific functions: expression of HLA-Cw3 confers selective protection from lysis by alloreactive NK clones displaying a defined specificity (specificity 2). *J Exp Med.* 1992;176:963-971.
115. Kim S, Poursine-Laurent J, Truscott SM, et al. Licensing of natural killer cells by host major histocompatibility complex class I molecules. *Nature.* 2005;436:709-713.
116. David G, Djaoud Z, Willem C, et al. Large spectrum of HLA-C recognition by killer Ig-like receptor (KIR)2DL2 and KIR2DL3 and restricted C1 specificity of KIR2DS2: dominant impact of KIR2DL2/KIR2DS2 on KIR2D NK cell repertoire formation. *J Immunol.* 2013;191:4778-4788.

117. Haas P, Loiseau P, Tamouza R, et al. NK-cell education is shaped by donor HLA genotype after unrelated allogeneic hematopoietic stem cell transplantation. *Blood.* 2011;117:1021-1029.
118. Shilling HG, McQueen KL, Cheng NW, Shizuru JA, Negrin RS, Parham P. Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation. *Blood.* 2003;101: 3730-3740.
119. Ruggeri L, Mancusi A, Capanni M, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood.* 2007;110:433-440.
120. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. *Blood.* 1999;94:333-339.
121. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science.* 2002;295:2097-2100.
122. Chen DF, Prasad VK, Broadwater G, et al. Differential impact of inhibitory and activating killer Ig-like receptors (KIR) on high-risk patients with myeloid and lymphoid malignancies undergoing reduced intensity transplantation from haploidentical related donors. *Bone Marrow Transplant.* 2012;47:817-823.
123. Pende D, Marcenaro S, Falco M, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. *Blood.* 2009;113:3119-3129.
124. Farag SS, Bacigalupo A, Eapen M, et al. The effect of KIR ligand incompatibility on the outcome of unrelated donor transplantation: a report from the Center for International Blood and Marrow Transplant Research, the European Blood and Marrow Transplant Registry, and the Dutch Registry. *Biol Blood Marrow Transplant.* 2006;12:876-884.
125. Miller JS, Cooley S, Parham P, et al. Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. *Blood.* 2007; 109:5058-5061.
126. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. *Biol Blood Marrow Transplant.* 2007;13:315-328.
127. Cooley S, Trachtenberg E, Bergemann TL, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood.* 2009;113:726-732.
128. Cooley S, Weisdorf DJ, Guethlein LA, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. *Blood.* 2010;116:2411-2419.
129. Oevermann L, Lang P, Feuchtinger T, et al. Immune reconstitution and strategies for rebuilding the immune system after haploidentical stem cell transplantation. *Ann N Y Acad Sci.* 2012;1266: 161-170.
130. Federmann B, Hägele M, Pfeiffer M, et al. Immune reconstitution after haploidentical hematopoietic cell transplantation: impact of reduced intensity conditioning and CD3/CD19 depleted grafts. *Leukemia.* 2011;25:121-129.
131. Di Bartolomeo PD, Santarone S, De Angelis G, et al. Haploidentical, unmanipulated, G-CSF–primed bone marrow transplantation for patients with high-risk hematologic malignancies. *Blood.* 2013; 121:849-857.
132. Wang Y, Liu DH, Liu KY, et al. Long-term follow-up of haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for the treatment of leukemia: 9 years of experience at a single center. *Cancer.* 2013;119:978-985.
133. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, post-transplantation cyclophosphamide. *Biol Blood Marrow Transplant.* 2008;14: 641-650.
134. Grosso D, Carabasi M, Filicko-O'Hara J, et al. A 2-step approach to myeloablative haploidentical stem cell transplantation: a phase 1/ 2 trial performed with optimized T-cell dosing. *Blood.* 2011;118: 4732-4739.
135. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2012;18:1835-1844.
136. Velardi A, Ruggeri L, Mancusi A, et al. Immunobiology Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Clinical impact of natural killer cell reconstitution after allogeneic hematopoietic transplantation. *Semin Immunopathol.* 2008;30:489-503.
137. Muller S, Schulz A, Reiss U, et al. Definition of a critical T cell threshold for prevention of GVHD after HLA non-identical PBPC transplantation in children. *Bone Marrow Transplant.* 1999;24: 575-581.
138. Grosso D, Gaballa S, Alpdogan O, et al. A 2-step approach to myeloablative haploidentical transplantation: low nonrelapse mortality and high survival confirmed in patients with early stage disease. *Biol Blood Marrow Transplant.* 2015;21:646-652.
139. Grosso D, Flomenberg N. A 2-step approach to allogeneic haploidentical hematopoietic stem cell transplantation. *Semin Oncol.* 2012;39:694-706.
140. Colvin GA, Berz D, Ramanathan M, et al. Nonengraftment haploidentical cellular immunotherapy for refractory malignancies: tumor responses without chimerism. *Biol Blood Marrow Transplant.* 2009; 15:421-431.
141. Guo M, Hu KX, Liu GX, et al. HLA-mismatched stem-cell microtransplantation as postremission therapy for acute myeloid leukemia: long-term follow-up. *J Clin Oncol.* 2012;30: 4084-4090.
142. Kalos M, Nazimuddin F, Finkelstein JM, et al. Long-term functional persistence, B cell aplasia and anti-leukemia efficacy in refractory B cell malignancies following T cell immunotherapy using CAR-redirected T cells targeting CD19 [abstract]. *Blood.* 2013;122. Abstract 163.
143. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med.* 2011;365:725-733.
144. Porter DL, Kalos M, Frey NV, et al. Randomized, phase II dose optimization study of chimeric antigen receptor modified T cells directed against CD19 (CTL019) in patients with relapsed, refractory CLL. *Blood.* 2013;122:873-873.
145. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med.* 2013;368:1509-1518.
146. Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. *Blood.* 2014;123:2625-2635.
147. Mardiros A, Dos Santos C, McDonald T, et al. T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. *Blood.* 2013;122:3138-3148.
148. Tettamanti S, Marin V, Pizzitola I, et al. Targeting of acute myeloid leukemia by cytokine-induced killer cells redirected with a novel CD123-specific chimeric antigen receptor [abstract]. *Blood.* 2012;120. Abstract 3010.
149. Gill S, Tasian SK, Ruella M, et al. Anti-CD123 chimeric antigen receptor T cells (CART-123) provide a novel myeloablative conditioning regimen that eradicates human acute myeloid leukemia in preclinical models. *Blood.* 2013;122:143-143.
150. Ritchie DS, Neeson PJ, Khot A, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. *Mol Ther.* 2013;21:2122-2129.
151. d'Argouges S, Wissing S, Brandl C, et al. Combination of rituximab with blinatumomab (MT103/MEDI-538), a T cell-engaging CD19-/CD3-bispecific antibody, for highly efficient lysis of human B lymphoma cells. *Leuk Res.* 2009;33:465-473.
152. Topp MS, Kufer P, Gökbuget N, et al. Targeted therapy with the T-cell–engaging antibody blinatumomab of chemotherapy-

refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J Clin Oncol.* 2011;29:2493-2498.

153. Aigner M, Feulner J, Schaffer S, et al. T lymphocytes can be effectively recruited for ex vivo and in vivo lysis of AML blasts by a novel CD33/CD3-bispecific BiTE antibody construct. *Leukemia.* 2013;27:1107-1115.
154. Arndt C, Von Bonin M, Cartellieri M, et al. Redirection of T cells with a first fully humanized bispecific CD33-CD3 antibody efficiently eliminates AML blasts without harming hematopoietic stem cells. *Leukemia.* 2013;27:964-967.
155. Krupka C, Kufer P, Kischel R, et al. Evaluation of CD33 expression and functional analysis of the CD33/CD3 bispecific BiTE antibody AMG 330 in primary AML samples. *Blood.* 2013;122:239-239.
156. Pankov D, Dao T, Wang Y, et al. A bi-specific T cell engaging monoclonal antibody (mAb) derived from a TCR-like mAb specific for WT1/HLA-A0201 (ESK-BiTE) shows a potent activity against human AML and Ph+ ALL in mouse models. *Blood.* 2013;122:2521-2521.