Immune Biology of Acute Myeloid Leukemia: Implications for Immunotherapy

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INTRODUCTION

Immune surveillance of incipient tumor cells is important for defense against cancer development. However, active immune evasion is a cancer hallmark.1 In acute myeloid leukemia (AML), a complex hematologic malignancy, AML blasts and leukemic stem cells (LSCs) evade and suppress host immune systems. Traditional AML therapies, such as allogeneic hematopoietic stem cell (HSC) transplantation (allo-HSCT) and donor lymphocyte infusions (DLIs), rely on T-cell-mediated effects, demonstrating AML cell sensitivity to functional immune cell cytotoxicity.2,3 These observations support immunotherapy to evoke anti-AML immunity.

Clinical translation and AML immunotherapy development have been relatively slow. Intrinsic AML features complicate translation from basic immunobiology to effective immunotherapy. First, AML is not a single disease, but a family of unique malignancies; thus, genetic/epigenetic heterogeneity4,5 and subclonality6 contribute to biologic variation. Second, AML has two main compartments: peripheral blood (PB) and bone marrow (BM). BM is composed of endothelial cells, stromal cells (eg, mesenchymal stromal/stem cells [MSCs]), and most immune cell types, including cytotoxic T lymphocytes (CTLs), regulatory T cells (Tregs), natural killer (NK) cells, and myeloid subsets (eg, myeloid-derived suppressor cells [MDSCs]).7 Global changes in BM immune cell profiles are associated with AML microenvironment immunosuppression.8 Furthermore, the microenvironment protects LSCs, potential drivers of relapse, from treatment- or immune-mediated destruction.9,10

Increased knowledge of AML immune escape is needed to translate immunotherapy from bench to bedside. To date, a systematic analysis of basic science addressing this fundamental gap is lacking. We conducted this review to identify knowledge gaps and opportunities for AML immunotherapy development.

IMMUNE EVASION/SUPPRESSION IN AML

Mechanisms by Which AML Cells Form the Basis of Defective Immune Responses

AML cells evade or suppress the immune system through five main mechanisms: reduced expression of major histocompatibility complex (MHC) molecules, enhanced inhibitory ligand expression, reduced activating ligand/receptor expression, ligand shedding, and manipulation of soluble factors within the microenvironment (Fig 1).2,7,11 Genetic mutation or immuno-editing reduces expression of HLA class II molecules and regulators required for T-cell AML recognition. Because endogenous or allogeneic immune cells eliminate high HLA/MHC-expressing cancerous cells, those with reduced or lost expression survive.2,12 Without immunostimulation, little evidence exists of endogenous T-cell responses against AML. Reduced or lost HLA/MHC expression is predominantly observed after allo-HSCT.13,14 In a study comparing samples from patients with AML obtained at diagnosis and relapse post–allo-HSCT, AML cells showed decreased expression of MHC class II proteins after transplantation in 17 of 34 patients. Interferon gamma (IFN-γ) administration reversed this downregulation, suggesting an epigenetic mechanism.14 Another study of AML posttransplantation relapse samples reported transcriptional silencing of HLA class II molecules.15 These findings suggest epigenetic mechanisms of immuno-editing may reduce HLA/MHC expression after allo-HSCT. Defective processing and loading of leukemia-associated antigens onto HLA class II proteins may also contribute to immune escape.16

Immune checkpoint pathways inhibit T- or NK-cell function. Enhanced expression of ligands for T-cell–regulating checkpoints, including cytotoxic T-lymphocyte antigen-4 (CTLA-4; surface protein expression),17 programmed cell death protein 1 (PD-1; RNA and surface protein expression),15,18 B7-H3 (surface protein expression),19 and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3; RNA and surface protein expression)20 were reported in AML and correlated with inferior outcomes.21-23 In AML, the most extensively studied immune checkpoint pathway is PD-1/programmed death-ligand 1 (PD-L1). Several studies found absent or restricted surface protein expression of PD-L1 in de novo AML, but increased expression at relapse.24-26 PD-L1 expression on AML blasts during disease progression could be adaptive for antitumor immunity and the associated inflamed microenvironment.7 Supporting this hypothesis, ex vivo addition of IFN-γ and interleukin-6,

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inflammatory cytokines associated with activated T cells, upregulated PD-L1 on AML blasts.24-26

Downregulation of NK-cell receptor DNAX accessory molecule-1 (DNAM-1)27 and NK group 2D ligand (NKG2DL), the ligand for the NK-cell immunostimulatory receptor NKG2D,11 are potential AML mechanisms of NK-cell evasion. Although DNAM-1 cross-linking with its ligands on AML cells (CD112 and CD155) results in NK-cell–mediated killing, chronic cross-linking downregulates DNAM-1. Additionally, AML cells lacking NKG2DL because of intrinsically low expression,28 ligand shedding,29 and/or epigenetic silencing30 escape NK cells.11,28,31 Along with expressing immune checkpoint ligands, AML cells secrete or shed soluble factors, receptors, and ligands into the microenvironment to create an immunosuppressive milieu as summarized in Table 1.

**Defective Immune Responses Created by AML Cells Foster an Immunosuppressive Microenvironment: Clinical Translation Opportunities**

AML cell interactions with the immune system create an immunosuppressive microenvironment with reductions in population and/or function of CTLs and NK cells and accumulation of Tregs, macrophages, and MDSCs. This is significant because AML LSCs preferentially reside in BM where they are protected from the immune system.

**LSC-specific mechanisms of immune escape.** AML LSCs, or leukemia-initiating cells, were discovered in xenotransplantation experiments.42,43 LSCs are capable of self-renewal and give rise to more differentiated bulk blast cells.44 Analyses of LSC populations show that they are generally quiescent,45 are chemotherapy resistant,46 and evade immunosurveillance.28,37 AML LSCs have elevated expression levels of CD47, a ligand for signal regulatory protein alpha located on macrophages and some dendritic cells (DCs). Activation via CD47 ligation inhibits phagocytosis. Thus, increased CD47 on LSCs enables evasion from macrophage–mediated phagocytosis.47 Promising preclinical data showed that an anti-CD47 monoclonal antibody (mAb) induced macrophage-mediated phagocytosis of AML cells.48 AML LSCs lack NKG2DL, resulting in escape from NK-cell–mediated lysis.28 Poly(ADP-ribose) polymerase 1 (PARP1) is enriched in NKG2DL-negative AML cells, providing a possible therapeutic target for this population.28 A bioinformatics approach identified two LSC-specific immunosuppressive targets, galectin-1 and CD200, with enhanced expression on LSCs compared with normal HSCs.49,50 CD200 positivity was associated with reduced immune-specific apoptosis and downregulation of inflammatory immune response-associated genes in AML cell lines.50

**Clinical translation.** These studies provide candidate targets for immune-mediated eradication of AML LSCs. Some LSC-directed agents are already under investigation. An anti-CD47 mAb is being evaluated in clinical trials for patients with relapsed/refractory AML (ClinicalTrials.gov identifiers: NCT02678338, NCT03248479).51 A CD47×CD33 bispecific antibody (bsAb) is also being evaluated preclinically.51 In patient xenograft models, PARP1 inhibition induced NKG2DL expression on LSCs, sensitizing them to NK-cell–mediated clearance.28 Another promising approach is disruption of AML-niche interactions (eg, E-selectin inhibition52) to free LSCs from the microenvironment and render them more vulnerable to immunosurveillance.

**AML microenvironment: T-cell repertoire.** Although spontaneous T-cell reactivity against defined AML antigens has been described,53 no consensus has emerged regarding number, distribution, and functional status of T cells in the AML microenvironment (Table 2).7 The contrasting findings may reflect underlying biologic differences between assessed AML compartments (PB or BM), disease heterogeneity, disease stage, prior therapy effects, limited patients evaluated, and/or patient differences.7,54-56

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**CONTEXT**

**Key Objective**

What are the current data and gaps in knowledge regarding the immune biology of AML, and what are the opportunities for translational research?

**Knowledge Generated**

Current data highlight the potential role for immunotherapy in AML. Gaps in knowledge include limited studies on the AML immune landscape and leukemic stem cell–specific mechanisms of immune escape, and lack of consensus in defining the AML tumor microenvironment.

**Relevance**

Addressing these knowledge gaps in AML immunobiology may translate to faster clinical development of immunotherapies for patients with AML.
FIG 1. Cellular and molecular mechanisms of immune evasion in acute myeloid leukemia (AML) in the vascular and bone marrow microenvironments.11,34 Cells that inhibit anti-AML immune function include regulatory T cells (Tregs), myeloid-derived suppressor cell (MDSCs), and mesenchymal stromal cells (MSCs). On the molecular level, activation of immune checkpoint pathways (eg, programmed death receptor-1 [PD-1], cytotoxic T-lymphocyte–associated protein 4 [CTLA-4]) and induction of immunosuppressive soluble factors (eg, kynurenine) by AML tumor microenvironment (TME) interactions fosters immune escape in AML. Additionally, under the pressure of allogeneic T cells, immuno-editing driven by epigenetic mechanisms may result in down-regulation of major histocompatibility complex (MHC) expression on AML cells, leading to immune escape and relapse in AML. ATP, adenosine triphosphate; CCR4, C-C chemokine receptor type 4; GAL9, galectin-9; IDO, indoleamine-2,3 dioxygenase; IFN-γ, interferon gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; LSC, leukemic stem cell; MPS, metalloproteinases; NK, natural killer; NOS-2, nitric oxide synthase 2; ROS, reactive oxygen species; SDF1, stromal cell-derived factor 1; SIRPα, signal regulatory protein alpha; TCR, T-cell receptor; TGFβ, transforming growth factor beta; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; VEGF, vascular endothelial growth factor; VISTA, V-domain immunoglobulin suppressor of T-cell activation.
### TABLE 1. Effects of Select Molecules Secreted or Shed by AML Cells on the Immune System

| Name of Molecule | Effect of Molecule on the Immune System |
|------------------|-----------------------------------------|
| Galectin-9/TIM-3  | Myeloid cell lines and primary human AML cells (including LSCs) overexpress immune checkpoint TIM-3 and its ligand galectin-9 compared with normal hematopoietic stem and progenitor cells, resulting in increased blood plasma levels of galectin-9 and soluble TIM-3. Galectin-9 inhibited NK-cell–mediated killing of AML cells by binding to TIM-3 on NK cells and blocking granzyme B transfer to AML cells. Soluble TIM-3 reduced the production and release of interleukin-2 from T cells, thereby preventing NK-cell and CTL activation. |
| Arginase II      | AML cells secreted high levels of enzymatically active arginase II in culture medium, which mediated the inhibition of T-cell proliferation. The plasma of patients with newly diagnosed AML was found to have higher levels of arginase II compared with healthy donors. AML plasma inhibited T-cell proliferation, and this inhibition was reversed with arginase inhibition or arginine replacement. AML blasts promoted monocyte polarization into a T-cell suppressive M2 phenotype in an arginase-dependent manner. |
| IDO1             | Tryptophan depletion and kynurenine production associated with IDO1 have been found to reduce proliferation and differentiation of T cells, increase levels of Tregs, and disrupt NK-cell activity. Constitutive expression of IDO protein in AML cells but not normal hematopoietic BM cells has been reported, with higher expression levels correlating with poorer patient outcomes. |
| SDF1             | SDF1 secretion by AML cells induced CTL migration away from AML cells on coculture. In vivo, this mechanism may hamper CTL infiltration into the BM, fostering AML immune evasion. |
| ATP              | In vitro, ATP release from chemotherapy-treated dying AML cells was associated with the generation of PD-1– and IDO1-overexpressing Tregs and dendritic cells, respectively. |

**Abbreviations:** AML, acute myeloid leukemia; ATP, adenosine triphosphate; BM, bone marrow; CTL, cytotoxic T cell; IDO, indoleamine 2,3-dioxygenase; LSC, leukemic stem cell; NK, natural killer; PD-1, programmed cell death protein 1; SDF1, stromal-derived factor 1; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; Tregs, regulatory T cells.

Increased inhibitory checkpoint molecule expression is consistently found on BM T cells, but the timing of increase (diagnosis or relapse) is unclear. It is also unclear whether this reflects T-cell exhaustion or a population shift to differentiated effector T cells.

In AML, Tregs dampen effector cell activity via secretion of cytokines and adenosine and increased adenosine triphosphate hydrolysis. Higher PB and BM Treg frequencies were reported in patients with AML versus healthy controls, possibly because of increased indoleamine 2,3-dioxygenase 1 (IDO1) expression. In AML, Tregs may preferentially accumulate in BM because of CXCL12/CXCR4 signaling. Higher BM Treg frequencies were observed at diagnosis, with additional increases at relapse, suggesting Treg induction is an early event that is further modulated by the immunosuppressive AML microenvironment.

**Clinical translation.** Functional T cells appear to infiltrate the AML microenvironment, but their efficacy is limited by immunosuppressive factors, including Tregs, impaired antigen recognition, and upregulation of immune checkpoints. Some therapies target immunosuppressive factors (eg, IDO1), whereas others are T-cell directed. Several immunotherapies have been developed to overcome AML immune evasion of T cells (Table 3).

Peptide- and cell-based vaccines induce expansion of AML-specific T cells via tumor-associated antigen recognition. In a phase II study of patients with AML in first complete remission (CR) treated with a Wilms tumor 1 peptide vaccine (n = 22), 64% had an immunologic response, and median disease-free survival since first CR was 16.9 months; the vaccine was well tolerated. Promising data were also reported in trials of DC-based vaccines and patient-derived AML cell vaccines, with some patients achieving prolonged remission.

Although checkpoint inhibitors (CPIs) targeting PD-1 and CTLA-4 have been approved for various solid tumors, the activity of these inhibitors in AML seems to be relatively less potent based on available data. In a phase Ib study of ipilimumab (anti-CTLA-4) 10 mg/kg in patients with hematologic malignancies relapsing post–allo-HSCT (n = 22), the CR rate was 23% (AML, n = 4; myelodysplastic syndrome [MDS], n = 1). Immune-related adverse events and graft-versus-host disease were reported. In a phase II study, patients with relapsed/refractory AML (n = 70) were treated with nivolumab (anti-PD-1) plus azacitidine. The rate of CR/CR with incomplete hematologic recovery (CRi) was 22%. Overall, the combination was safe, although immune-related toxicities were reported. CPIs targeting other molecules are also under investigation.

An antibody platform recently developed is the bsAb/antibody construct, which includes bispecific T-cell engager (BiTE) molecules and dual-affinity retargeting antibodies. BsAbs bind CD3 receptors on an endogenous T cell and a target antigen on a malignant cell. Simultaneous binding results in a cytolytic synapse with consequent lysis of the target cell.
TABLE 2. Studies of the T-Cell Repertoire and Function in AML

| Reference       | No. of Patients With AML Assessed | Technique(s) for Assessing T Cells | Compartement (PB or BM) | Patient Population | AML Treatment(s) Received (if any) | Main Finding(s)                                                                 |
|-----------------|----------------------------------|-----------------------------------|-------------------------|--------------------|------------------------------------|---------------------------------------------------------------------------------|
| Van Galen⁵⁴     | 15                               | IHC                               | BM                      | AML                | Not reported                       | Fewer T cells and CTLs for patients with AML v normal individuals (n = 15)     |
|                 |                                  |                                   |                         |                    |                                    | Reduced CTL:T-cell ratio for patients with AML v normal individuals             |
|                 |                                  |                                   |                         |                    |                                    | Increased proportion of Tregs for patients with AML v normal individuals        |
| Williams⁵⁵      | 107                              | IHC                               | BM                      | Newly diagnosed and relapsed/refractory AML | MFC (on BMAs)                     | Comparable CD3⁺ T-cell infiltration in BM of relapsed/refractory AML (n = 13) v age-matched HD (n = 14) |
|                 |                                  |                                   |                         | Patients received different modalities of treatment, such as HMA-based, cytotoxic, targeted therapies, and investigational therapies |                      | Comparable CD8⁺ T-cell subset (HD v newly diagnosed AML [n = 39] v relapsed AML [n = 68]: 19.1% v 27.9% v 26.4%; P = .3) |
|                 |                                  |                                   |                         |                    |                                    | Increased proportion of Tregs (HD v newly diagnosed AML [n = 39] v relapsed AML [n = 68]: 1.7% v 2.1% v 3%; P = .02) |
| Le Dieu⁶⁶       | 10-36                            | Flow cytometry (cell quantification) | PB                      | Newly diagnosed AML | N/A                                | Increase in the absolute number of PB (but not BM) T cells in AML (n = 36) compared with age-matched healthy controls (n = 17) |
|                 |                                  |                                   |                         |                    |                                    | Both AML blasts and T cells from patients with AML exhibited impaired immune synapse formation in 10 independent experiments |
|                 |                                  |                                   |                         |                    |                                    | Aberrant gene expression profile in AML (n = 10) v healthy control (n = 10); pathway analysis did not reveal specific affected pathways |
| Schnorfeil⁶⁰    | 15-22                            | Immunophenotyping                 | PB and BM               | Newly diagnosed and relapsed AML | Proliferation and cytokine production assays | Similar expression of inhibitory molecules on PB T cells for newly diagnosed AML v age-matched healthy controls |
|                 |                                  |                                   |                         | Intensive chemotherapy or allogeneic SCT |                      | Increased PD-1 expression on PB and BM T cells at posttransplantation relapse compared with diagnosis, which correlated with an increased proportion of effector memory T cells |
|                 |                                  |                                   |                         |                    |                                    | No proliferation defect in PB T cells for any group of patients with AML (n = 15) compared with healthy controls (n = 8) |
|                 |                                  |                                   |                         |                    |                                    | No cytokine (IFN-γ, TNF-α, IL-2) secretion impairment in PB T cells from any group of patients with AML (n = 22) compared with healthy controls (n = 20), except for reduced IFN-γ production of CD4⁺ T cells in newly diagnosed AML |

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cancer cell. BsAb treatment was clinically validated for patients with B-cell precursor acute lymphoblastic leukemia (ALL).\textsuperscript{85} In AML, bsAbs demonstrated promising antitumoral activity (including CRs) in phase I studies, supporting their potential role in this disease.\textsuperscript{86-88} Their targets are primarily CD123, CD33, CLL1 (CLEC12A), and FLT3.

Adaptive transfer of T cells is another method of T-cell–mediated immunotherapy. Chimeric antigen receptor (CAR) T cells are modified T cells that express receptors engineered to engage target antigens on malignant cells\textsuperscript{89} in an MHC-independent manner. CAR T-cell therapy has revolutionized treatment of hematologic malignancies such as ALL and non-Hodgkin lymphoma. Translocation to AML has been slower, with only early-phase data in small populations.\textsuperscript{51,68} Slow uptake of CAR T-cell therapy reflects several challenges associated with this strategy in AML (Table 3). Alternative approaches, such as CAR NK cells and T-cell receptor (TCR) gene therapy, are being evaluated.\textsuperscript{51,88} Activation of immunoregulatory checkpoints can hamper the efficacy of T-cell adoptive immunotherapy, and lack of costimulation is a TCR gene therapy limitation.\textsuperscript{90} Studies to improve clinical translation of T-cell adoptive immunotherapy and TCR gene therapy are ongoing.\textsuperscript{90} A phase I/II study of patients with high-risk relapsed AML, MDS, or chronic myelogenous leukemia previously treated with allo-HSCT is underway (ClinicalTrials.gov identifier: NCT01640301).

**AML Microenvironment: NK Cells.** NK cells from patients with AML often present with an unfavorable phenotype, including downregulation of natural cytotoxicity receptors, reduced capacity to produce and secrete IFN-γ, and inhibited activity via Tregs and soluble factors in the microenvironment.

**Clinical translation.** Although AML cells are sensitive to NK-cell–mediated cytotoxicity, the immunosuppressive microenvironment fosters immune escape. Several strategies have been developed to overcome immune evasion of NK-cell surveillance.

Fragment crystallizable (Fc)-optimized antibodies are mAbs where the Fc region has been engineered to optimize therapeutic activity. In a recent phase Ib trial, an anti-CD33 Fc-optimized mAb (BI 836858) with azacitidine was evaluated in patients with previously untreated AML (n = 31). The combination demonstrated acceptable tolerability and was active, with five of 28 evaluable patients having CR/CRi.\textsuperscript{91} Coengagement of AML target cells via CD33 and NK cells via CD16 through bispecific/trispecific killer cell engager antibodies are also promising strategies to enhance AML-specific targeting by NK cells.\textsuperscript{51,92} Bispecific fusion proteins targeting NKG2DL on AML cells to activate NK cells are under investigation.\textsuperscript{93} NK-cell–mediated killing of cancer cells is not MHC presentation dependent. NK cells can be donor-derived haploidentical and transferred in conjunction with haploidentical transplantation. Promising efficacy and safety from phase I AML trials have been observed.\textsuperscript{51}

**AML microenvironment: Immunosuppressive MDSCs and macrophages.** MDSCs, heterogeneous CD33+ immature myeloid cells, act as a major immunosuppressive factor, and MDSC expansion is linked with poor outcomes.\textsuperscript{94,95} MDSCs exert their immunosuppressive activity via arginase-1, inducible nitric oxide synthase expression, and NOX2-derived reactive oxygen species (ROS) production.\textsuperscript{96,97} Although the AML MDSC immunobiology is not well understood,\textsuperscript{7} evidence suggests MDSCs accumulate in the AML microenvironment, contribute to immunosuppression, and could be immunotherapy targets.

### TABLE 2. Studies of the T-Cell Repertoire and Function in AML (continued)

| Reference | No. of Patients With AML Assessed | Technique(s) for Assessing T Cells | Compartiment (PB or BM) | Patient Population | AML Treatment(s) Received (if any) | Main Finding(s) |
|-----------|---------------------------------|-----------------------------------|-------------------------|-------------------|-----------------------------------|----------------|
| Craddock\textsuperscript{59} | AML (n = 24); MDS (n = 5) | Flow cytometry | PB | Relapsed AML or MDS after allo-SCT | Salvage therapy with lenalidomide and azacitidine at relapse after allo-SCT | Before lenalidomide/azacitidine therapy and compared with T cells from healthy donors, patient T cells were reduced in number, had an exhausted phenotype, and released fewer cytokines |

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; BMA, bone marrow aspirate; CTL, cytotoxic T cell; HD, healthy donor; HMA, hypomethylating agent; IFN, interferon; IHC, immunohistochemistry; IL, interleukin; MDS, myelodysplastic syndrome; MFC, multiparameter flow cytometry; N/A, not applicable; PB, peripheral blood; PD-1, programmed cell death protein 1; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; SCT, stem cell transplantation; TNF, tumor necrosis factor; Tregs, regulatory T cells.
One study found increased BM MDSC frequencies in patients with active AML versus normal controls; engraftment of mice with AML led to MDSC expansion in BM and spleen.\(^7\) Two other studies found increased BM and PB MDSC frequencies in newly diagnosed AML versus controls,\(^8\)\(^9\)\(^8\) suggesting MDSC expansion occurs early. Interactions between AML cells and the microenvironment likely play a role. In one study, AML cells released c-myc–containing extracellular vesicles that trafficked to myeloid accessory cells in the BM microenvironment, resulting in MUC1-mediated upregulation of proproliferative cyclins E1 and D2 in MDSCs.\(^7\) MDSCs inhibit T-cell proliferation.\(^7\)\(^8\)\(^9\) One study found this inhibition was partially mediated by immune checkpoint V-domain immunoglobulin suppressor of T-cell activation (VISTA). VISTA expression was greater on MDSCs from patients with AML versus healthy controls; VISTA knockdown reduced MDSC-associated T-cell inhibition. Furthermore, the proportion of VISTA-expressing MDSCs correlated with the proportion of PD-1–expressing T cells.\(^8\)

One study reported increased frequency of immunosuppressive M2-like macrophages in BM and spleen of patients with AML versus healthy patients.\(^10\) AML cells also polarized nonleukemic macrophages into an

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**TABLE 3. T-Cell–Directed Treatment Strategies in AML: Advantages and Limitations**

| Therapy Type/Strategy | Advantages | Challenges/Limitations | Strategies to Overcome Challenges/Limitations |
|-----------------------|------------|------------------------|---------------------------------------------|
| Vaccine               | Target antigen specificity | Depends on endogenous T-cell functionality, which may be impaired because of immunosuppressive microenvironments\(^1\) | Combination approaches: vaccine + epigenetic agent\(^4\) or CPI\(^1\) |
| CPI                   | More widely studied compared with other cancer immunotherapies | Potentially serious immune-related toxicities\(^6\) | Combination approaches: CPI plus epigenetic agent\(^6\) or triplet combination with CXCR4 inhibition and chemotherapy (preclinical)\(^6\) |
| CAR T cells           | Demonstrated success in other hematologic malignancies (eg, ALL, NHL) | Suitable antigen selection: candidate AML antigens also expressed on normal hematopoietic stem and progenitor cells with risk for myeloblation\(^1\) | Identification of optimal antigen(s) and strategies to mitigate toxicity\(^6\) |
| MHC independent       | Safety: CRS, neurotoxicity\(^3\) | Conditional targeting and dual targeting\(^6\) | Preferential targeting of AML cells through the use of nanobodies\(^2\) |
| CD3 bispecific antibodies/antibody constructs | Not affected by T-cell receptor activity, MHC presentation, or costimulation\(^4\),\(^7\) | Depends on endogenous T-cell functionality | Combination approaches: bsAb + CPI,\(^7\)\(^8\)\(^7\) epigenetic therapy (preclinical)\(^7\) |
|                       | Use endogenous T cells (ie, overcome logistic challenges associated with ex vivo T-cell modification); off-the-shelf product\(^4\) | Convenience: some current constructs require continuous infusion of the agent because of rapid clearance | CITE molecule development\(^3\) |
|                       | May be less sensitive than other therapies (eg, mAbs) to mechanisms of AML drug resistance\(^7\) | Safety: CRS\(^7\) | Preferential targeting of AML cells through the use of nanobodies\(^2\) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; bsAb, bispecific antibody; CAR, chimeric antigen receptor; CITE, checkpoint inhibitory T-cell engaging; CPI, checkpoint inhibitor; CRS, cytokine release syndrome; HSPC, hematopoietic stem and progenitor cells; mAb, monoclonal antibody; MHC, major histocompatibility complex; NHL, non-Hodgkin lymphoma.
AML-supporting state and induced their proliferation and infiltration into BM of human AML mouse models. Preclinical evidence shows that immunosuppressive MDSC populations are expanded in AML, supporting evaluation of MDSC-targeted therapies. CD33-directed therapies hold promise for MDSC elimination. In ex vivo studies, CD33-directed bispecific molecules engaged T cells to MDSCs to achieve antileukemic effects and restore immune homeostasis in AML and MDS. Immunodepletion of MDSCs was accompanied by T-cell expansion and activation and improved hematopoiesis. Gemtuzumab ozogamicin, a CD33-directed antibody-drug conjugate, restored T-cell and CAR T-cell immunity against various cancers via MDSC targeting.

Clinical translation. Phase 1 studies of the BiTE AMG 330 in AML (ClinicalTrials.gov identifier: NCT02520427) and the bispecific T-cell engager AMV564 in AML (ClinicalTrials.gov identifier: NCT03144245) and MDS (ClinicalTrials.gov identifier: NCT03516591) are currently underway, and multiple trials investigating gemtuzumab ozogamicin in AML are recruiting or completed. Another strategy relies on pharmacologic blockade of ROS production, an important mechanism of MDSC immunosuppression and differentiation into macrophages and DCs. In a phase III study, postconsolidation treatment with the NOX2 inhibitor histamine dihydrochloride in combination with interleukin-2 improved leukemia-free survival versus no treatment of patients with AML in CR. Histamine dihydrochloride significantly reduced PB MDSCs in patients with AML, with strong reductions associated with longer leukemia-free survival. Other strategies for selective targeting of MDSCs in AML include MUC1 inhibition, granulocye colony-stimulating factor therapy, and HO-1 protein inhibition; these treatments may synergize with CPIs. Association of MDSC levels with clinical outcomes is unclear, because two small studies (N = 27 and N = 6) reported a correlation with minimal residual disease (MRD) or relapse, respectively, whereas one large study (N = 341) did not find a statistically significant relationship with MRD.

AML microenvironment: Immunosuppressive MSCs. MSCs are fundamental BM regulators, with potent immunosuppressive functions that affect innate and adaptive cellular immunity; however, relatively little is known about their effects in hematologic malignancies. This is likely because of a lack of standardized methods for isolating and characterizing MSCs, and the heterogeneity of AML and MSCs. Preclinical data show a dynamic interplay among MSCs, AML cells, and the immunosuppressive microenvironment (Table 4).

Clinical translation. MSCs are critical BM components, supporting long-term maintenance and quiescence of LSCs and protecting them from anti-AML therapy. Future immunotherapy research should consider MSC-to-AML cell cross talk in shaping the immune microenvironment. Targeting individual pathways to reduce MSC-associated immunosuppression or interfering with MSC-to-AML cell cross talk may be effective strategies.

Defining AML immune microenvironments. Tumor immune microenvironments vary substantially between patients. In solid tumors, immunologically hot versus cold tumor microenvironments may have prognostic implications for CPI therapy. However, little is known about AML microenvironment heterogeneity. There have been several attempts to describe the AML immune microenvironment. Using the Cancer Genome Atlas, a pan-cancer Tumor Inflammation Signature (TIS) was developed to characterize the immune microenvironment in a variety of cancers. TIS includes 18 genes related to abundance of antigen-presenting cells, T- and NK-cell levels, IFN activity, and T-cell exhaustion. Higher scores indicate inflamed or hot tumors that may be more recognizable by the immune system. The TIS score for patients with AML was approximately 4—lower than the median of 5.5 observed in the entire cancer dataset, indicating AML tumor microenvironments are generally cold. Another AML study investigated T-cell repertoires at diagnosis and relapse and identified a dual checkpoint-positive T-cell population (PD-1+ and TIM-3+ or LAG3+).

### Table 4. Preclinical Studies Assessing the Role of Immunosuppressive MSCs in AML

| Reference | No. of Samples From | Patient Population | Main Finding(s) |
|-----------|--------------------|--------------------|-----------------|
| Mansour   | 12                 | De novo AML        | BM-derived MSCs from patients with AML showed higher levels of IDO expression compared with control patients, which positively correlated with Treg frequency |
| Ciciarello| 61                 | AML                | AML cells induced an upregulation of IDO1, PD-1, and NOS-2 on cocultured MSCs via secretion of IFN-γ |
| Wu        | Not reported       | FA patients with AML | MSCs derived from patients with FA secreted high levels of prostaglandins, which resulted in upregulated NR4A-WNT/β-catenin signaling, Treg induction, and CTL inhibition |
| Vasold    | 5                  | AML                | Cell-to-cell contact between MSCs and AML blasts significantly reduced NK-cell-mediated lysis of AML cells compared with no MSCs in culture |

**Abbreviations:** AML, acute myeloid leukemia; BM, bone marrow; CTL, cytotoxic T lymphocyte; FA, Fanconi anemia; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; MSCs, mesenchymal stromal/stem cells; NK, natural killer; NOS-2, nitric oxide synthase 2; NR4A, nuclear hormone receptor 4A; PD-1, programmed cell death protein 1; Treg, regulatory T cell.
authors proposed this population demarcated two types of patients with AML: those with and those without an exhausted immune microenvironment. The proportion of double-positive T cells increased from diagnosis to relapse, indicating greater immune system exhaustion at later stages.\(^{56}\) Finally, transcriptomic and proteomic profiling identified two types of immune microenvironments in AML BM samples: an immune-enriched and IFN-γ dominant type (elevated expression of lymphocyte-associated genes, IFN-γ, and immune checkpoint molecules) and an immune-depleted type (elevated expression of mast cell function– and T-cell exhaustion–associated genes, low expression of T-cell and B-cell genes).\(^{118}\) The immune-enriched profile was observed in approximately 30% of patients, indicating that most AML tumors are cold.\(^{119}\)

**Clinical translation.** Future studies evaluating AML BM heterogeneity are important for personalized immunotherapy. Agent (eg, CPIs) efficacy may be compromised by immune-depleted microenvironments, whereas hot tumors may be more susceptible to CPIs. Combination therapies incorporating CPIs may thus be more effective for tumors with immune-enriched microenvironments. Understanding how immunotherapies can modulate the microenvironment immune signature represents an additional research avenue. A preliminary study reported that treatment with a bsAb shifted the AML BM microenvironment signature to a more inflamed type as evidenced by increased immune cell infiltration, a higher TIS score, and enhanced IFN-γ signaling.\(^{120}\)

**Effects of standard AML therapies on the immune landscape.** To deliver optimal immunotherapy to patients relapsing after allo-HSCT or chemotherapy, it is important to understand immune-related effects of these treatments. Allo-HSCT efficacy depends on an immune-mediated graft-versus-leukemia (GVL) effect to confer anti-AML allograft immunity. The GVL effect is mediated primarily by alloreactive T cells, although there is increasing recognition of alloreactive NK-cell responses and tumor-specific T-cell and antibody responses.\(^{121}\) Understanding immune escape mechanisms during post–allo-HSCT relapse can inform immunotherapeutic strategies. A comprehensive study of immune signatures from patients relapsing after allo-HSCT found that significant downregulation of HLA class II transcripts commonly occurred posttransplantation and may be driven by epigenetic mechanisms.\(^{15}\) The same study described another modality of relapse after allo-HSCT, which was characterized by impaired T-cell costimulation by AML blasts.\(^{15}\) These effects were not observed at relapse after chemotherapy only.\(^{15}\) Another study showed that compared with healthy donors, patients with AML relapsing after allo-HSCT had reduced T-cell frequencies characterized by an exhausted phenotype and altered cytokine profile.\(^{59}\) These studies assessed circulating T cells but not BM-infiltrating T cells. A recent study reported early-differentiated T memory stem cells and central memory T cells from the BM of patients relapsing after allo-HSCT had an exhausted phenotype characterized by expression of multiple inhibitory receptors.\(^{122}\) These findings show systemic T-cell impairment and exhaustion present at relapse after allo-HSCT.

The percentage of donor-derived T lymphocytes (chimerism) after allo-HSCT is correlated with disease outcomes, with mixed chimerism predictive of relapse and shorter survival.\(^{123,124}\) Mixed chimerism via residual host Tregs and DCs may inhibit activation of donor-derived DCs and alloreactive T-cell induction.\(^{125}\)

Profound immunodeficiency occurs after T-cell–depleted HLA-haploidentical allo-HSCT (hHSCT).\(^{126}\) Reconstitution kinetics of various immune cell populations may be related to post-hHSCT relapse. NK cells reconstitute early posttransplantation, and their rapid recovery is associated with lower relapse risk in certain hematologic malignancies.\(^{127}\) Furthermore, transplantation from NK alloreactive donors has been associated with better outcomes.\(^{128}\) Another immune cell population of interest is invariant NK T cells (iNKT) cells. One study found that patients with hematologic malignancies who maintained remission after hHSCT had full reconstitution of iNKT cells, whereas patients who relapsed did not.\(^{126}\) IFN-γ production\(^{126}\) or direct killing of AML blasts in a CD1d-dependent manner\(^{129}\) may explain the role of iNKT cells in maintaining antitumor immunity after allo-HSCT.

Chemotherapy affects humoral immunity to a greater degree than cellular immunity. In one study, T-cell frequency and function recovered to normal levels after consolidation chemotherapy, whereas B-cell immunity was impaired.\(^{130}\)

**Clinical translation.** The role of chimerism is well recognized, and chimerism analysis remains an important tool to predict relapse post–allo-HSCT.\(^{131}\) Several ongoing clinical trials focus on chimerism in AML post–allo-HSCT, including early detection and treatment of mixed chimerism (eg, ClinicalTrials.gov identifiers: NCT03850418, NCT03128034, NCT02724163). MRD measurements in the posttransplantation period can be performed; however, MRD monitoring remains challenging because suitable molecular markers are not available for all patients with AML.\(^{132}\)

AML immune evasion mechanisms post–allo-HSCT suggest epigenetic therapies that reverse HLA expression loss may be effective for posttransplantation relapse. In the phase II RELAZA trial, azacitidine (hypomethylator) treatment of MRD post–allo-HSCT led to long-term responses in patients with MDS or AML.\(^{133}\) Azacitidine combined with immune therapy (DLIs or lenalidomide) was also effective for patients with AML relapsing post–allo-HSCT.\(^{59,134}\) Given impaired T-cell costimulation observed at relapse post–allo-HSCT, CPIs may be beneficial.\(^{15}\) The preserved T-cell population and function after chemotherapy suggests T-cell–directed immunotherapies, such as bsAbs and CPIs, will not be compromised by prior chemotherapy.
In conclusion, AML is a complex, heterogeneous disease, and fundamental understanding of its immunobiology is critical for immunotherapy. Immune dysfunction is important for AML pathogenesis, supporting the role of immunotherapies to restore local immunity to eradicate the disease. An improved understanding of how LSCs evade the immune system and how they can be eradicated is needed. Moreover, cellular components of the AML immune microenvironment, such as MSCs and B cells, have not been well characterized. These understudied populations may have important roles to play; for example, B cells from high-risk patients with durable GVL responses produce anti-AML antibodies. Standardized methods for characterizing the tumor microenvironment are important for translational development of patient-specific immunotherapies.

Allo-HSCT remains a cornerstone of immune-mediated AML therapy. Development of optimal strategies should be informed by lessons learned from allo-HSCT and the mechanisms of AML immune dysfunction. Allo-HSCT findings suggest combination therapies targeting multiple pathways and cellular effectors (including T cells and NK cells) will likely be optimal for developing alternative immunotherapeutic strategies. Post–allo-HSCT, the GVL effect enables donor immune cells to eliminate host leukemic cells by engaging a multicellular (T cells, NK cells, antibodies, antigen-presenting cells) response against multiple antigens, including leukemia-associated antigens and nonleukemia-specific antigens overexpressed in leukemia. This broad immune response is likely important in overcoming the multiple immunosuppressive mechanisms and clonal heterogeneity observed in AML.

The implications of allo-HSCT for target identification and cellular effectors are important. Allo-HSCT can target multiple intracellular (presented by HLA) and/or surface antigens, whereas current AML therapies can only target limited surface antigens. Furthermore, although minor histocompatibility antigen-specific T cells can induce a potent GVL effect, translating this observation into AML clinical trials is difficult. Prognosis for patients with relapsed/refractory AML remains suboptimal even with allo-HSCT, an important consideration when investigating combination therapies in relapsed patients.

Being cognizant of optimal early- and late-phase trial design with regard to endpoints and molecular inclusion criteria is critical. The allo-HSCT experience provides rationale for personalized immunotherapy based on immunologic profiles. For example, the modes of immune evasion after allo-HSCT (genomic loss of HLA v loss of HLA class II expression v T-cell exhaustion) could be targeted by distinct salvage immunotherapies. Early-phase trial endpoints can also include dose-dependent changes in immunity and stratification of responders versus nonresponders using immune readouts. For early- and late-phase trials, biomarkers should be collected and analyzed using modern platforms (eg, immunology panels), and retrospective stratifications should be made and published regardless of outcome. Given the prognostic significance of MRD in AML and the potential for immunotherapy to eliminate LSCs, MRD-negative CR could be a useful endpoint in late-stage anti-AML immunotherapy trials. Late-stage trials can implement findings from earlier phases to better identify responder populations and develop inclusion/exclusion criteria. Additional understanding of the optimal sequencing of immunotherapies—specifically, which immunotherapies can be reserved for later lines of therapy and retain activity in patients refractory to other agents—is also important.

Overall, the gaps and opportunities highlighted in this review reiterate the importance of a strong foundation in basic immune biology that can be harnessed for clinical translation. We expect current and future advances in our knowledge of immune escape mechanisms will translate to powerful immunotherapies for patients with AML.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Immune Biology of Acute Myeloid Leukemia: Implications for Immunotherapy

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