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Review Series

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Immune escape and immunotherapy of acute myeloid leukemia

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In spite of the recent approval of new promising targeted therapies, the clinical outcome of patients with acute myeloid leukemia (AML) remains suboptimal, prompting the search for additional and synergistic therapeutic rationales. It is increasingly evident that the bone marrow immune environment of AML patients is profoundly altered, contributing to the severity of the disease but also providing several windows of opportunity to prompt or rewire a proficient antitumor immune surveillance. In this Review, we present current evidence on immune defects in AML, discuss the challenges with selective targeting of AML cells, and summarize the clinical results and immunologic insights from studies that are testing the latest immunotherapy approaches to specifically target AML cells (antibodies, cellular therapies) or more broadly reactivate antileukemia immunity (vaccines, checkpoint blockade). Given the complex interactions between AML cells and the many components of their environment, it is reasonable to surmise that the future of immunotherapy in AML lies in the rational combination of complementary immunotherapeutic strategies with chemotherapeutics or other oncogenic pathway inhibitors. Identifying reliable biomarkers of response to improve patient selection and avoid toxicities will be critical in this process.

Introduction

Acute myeloid leukemia (AML) is a genetically, epigenetically, and clinically heterogeneous disease characterized by accumulation and expansion of immature myeloid cells in the bone marrow (BM) and peripheral blood (PB), with consequent failure of normal hematopoiesis (1). Advances in genomic and epigenomic characterization of AML have fostered better understanding of leukemogenesis, paving the way for development and approval of several novel targeted therapies. Still, the mainstay of treatment is chemotherapy, which has remained mostly unchanged over the past four decades. Fewer than one-third of adult AML patients enjoy durable remission, indicating a need for different therapeutic approaches. Immunotherapy carries a promise to eradicate chemoresistant clone(s) and provide long-term disease control.

Historically, AML has been considered an immunoresponsive malignancy and continues to represent the most common indication for which patients receive allogeneic hematopoietic stem cell transplantation (alloHSCT) (2, 3). However, modest association of graft-versus-host disease with relapse reduction in AML (4), and alloHSCT's inability to prevent relapse in most patients who undergo transplantation with active disease (5), underscore the limited effectiveness of alloreactive T cells and a need for more potent and specific targeting of AML.

In this Review we discuss the hurdles of finding suitable targets for AML immunotherapy, summarize the immune features of the leukemia microenvironment, review results obtained in clinical trials using novel strategies such as bispecific antibodies, cell therapies, and checkpoint blockade, and ultimately discuss how this information might translate into future developments.

Selecting antigen targets in AML

Finding an antigen target that is critical for AML biology and selectively expressed on malignant cells remains a challenge. Besides AML's heterogeneity, the complexity of its clonal composition and propensity to change with disease progression further complicate this quest.

Antigen targets in AML can generally be divided into lineage-restricted antigens (LRAs), leukemia-associated antigens (LAAs; wild-type proteins overexpressed in leukemia versus normal cells), and leukemia-specific antigens (LSAs; neoantigens originated by mutations in protein-coding genes). The LRAs (Table 1), which are expressed on the surface of leukemia cells, are suitable targets for approaches based on antibodies or chimeric antigen receptor (CAR) T cells, but are often shared with normal hematopoietic or even non-hematopoietic tissues, and their utility as immunotherapeutic targets is mostly countered by on-target toxicity. On the other hand, despite their higher leukemia cell specificity, LAAs (e.g., WT1 [ref. 6]) and LSAs (e.g., mutated NPM1 [ref. 7]) are often expressed intracellularly, and their potential as immunotherapeutic targets depends on their ability to be processed and presented to T cells within the HLA complex (8). CD33 and CD123 are the LRAs that have been most studied in AML, but myelosuppression has been of concern due to their expression on normal hematopoietic tissues (9–11). In fact, integrated transcriptomic and proteomic analysis of AML surfaceome failed to identify a single surface antigen that meets the favorable characteristics of CD19 in acute lymphoid leukemia (ALL) (12),

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Table 1. Select lineage-restricted surface AML antigen targets

Antigen	Description	AML blast expression	LSC expression	Normal tissue expression
CD33 (Siglec-3)	Transmembrane receptor	90%	Yes	HSCs; myeloid progenitors, monocytes, mast cells, Kupffer cells, microglial cells in the brain
CD123 (IL-3Rα)	IL-3 receptor-α	50%–100%	Yes	HSCs (little or no); myeloid progenitors, monocytes, basophils, dendritic cells, epithelial cells (respiratory, gastrointestinal)
CLL1 (CLEC2A)	Transmembrane receptor	77%–100%	Yes	Monocytes, granulocytes, tissue-resident lung macrophages
CD44v6	Transmembrane receptor/splice variant	64%	Yes	Monocytes, keratinocytes; different epithelial tissues (respiratory, gastrointestinal, genitourinary)
CD56 (neural cell adhesion molecule [NCAM])	Member of the immunoglobulin superfamily CAM	28%	Possibly	Dendritic, NK, and T cells, monocytes, neural and neuroendocrine tissues
Lewis Y (CD174)	Blood group carbohydrate antigen	50%	Likely	HSCs; intestinal epithelial cells
FLT3 (CD135)	Type III receptor tyrosine kinase	70%–100%	Yes	HSCs; myeloid progenitors, neurons
CD7	Transmembrane protein; member of the immunoglobulin superfamily	30%	Possibly	T cells
FOLR2 (folate receptor-β)	Folate-binding protein receptor	70%	Possibly	Myeloid cells, macrophages
CD25	IL-2 receptor-α	20%	Yes	Activated T cells

Others include IL1RAP (IL-1 receptor accessory protein), CD64 (Fc receptor that binds IgG), CD13 (type II membrane glycoprotein–aminopeptidase N), CD38 (cyclic ADP ribose hydroxylase), CD45 (tyrosine phosphatase), CD15 (adhesion molecule, carbohydrate antigen), and NKG2D/NKG2DL (C-type lectin–like transmembrane receptor protein). LSC, leukemia stem cell.

but identified several target pairings or combinations that could potentially enhance selective targeting of AML cells (13).

Several LAAs are considered leukemia-restricted given their very low expression (hTERT, survivin) or tissue-specific expression (oncofetal antigen–immature laminin receptor antigen [OFA-ILRP] and the cancer/testis antigens PRAME and MAGE) in other compartments (8, 14, 15). Among different LAAs, WT1 protein has gathered substantial attention (6, 16): clinical trials with T cells genetically redirected against WT1 generated promising results without significant off-target tissue toxicity (17), and WT1-specific $CD8^+$ responses were detected in vaccine trials in AML patients (18, 19). Other LAAs such as PR1, hTERT, and PRAME were found to elicit $CD8^+$ T cell or humoral responses in vivo in leukemia patients after vaccination (20–26), while immune responses against a broader range of LAAs were detected in the post-transplant setting (reviewed in ref. 27).

AML is characterized by one of the lowest mutational burdens among diverse malignancies (28). Nonetheless, while the frequency of immunogenic mutations creating neoantigens (i.e., LSAs) is expected to be low, mutational quality rather than quantity may be relevant in eliciting antileukemia responses. Several common translocations (AML1-ETO, DEC-CAN, PML-RARα, BCR-ABL) and gain-of-function mutations (FLT3-ITD, NPM1, $IDH1^{R132H}$) produce AML-specific immunogenic proteins that drive the malignant phenotype and thus represent ideal antigen targets. In addition, unlike LAAs, neoantigens are not part of the "self" antigen repertoire, and thus are unlikely to be subject to central tolerance (29). As a consequence, ex vivo isolation or in vivo expansion of high-affinity T cells against LSAs from AML patients or healthy individuals appears more likely than use of historical LAAs, and is currently an object of intense investigation (30, 31). In particular, mutated cytoplasmic protein NPM1 (found in 30% of AML patients) can induce $CD4^+$ and $CD8^+$ T cell responses (7, 32), and the $IDH1^{R132H}$ mutation that is common to both AML and gliomas has been shown to generate an HLA-DR–restricted neoantigen recognized by $CD4^+$ T cells (33). Finally, several TP53 "hotspot" mutations have been shown to give rise to immunogenic neoepitopes (34), and studies have linked TP53 to the immunosuppressive BM environment in AML (35) and to response to checkpoint blockade in solid tumors (36, 37).

Several additional mechanisms may provide antigen targets in AML. Notably, AML is enriched for spliceosome mutations, and recent studies have identified aberrant splice variants for 29% of expressed genes genome-wide in AML compared with normal donor $CD34^+$ cells. The most commonly mis-spliced genes (>70% of AML patients) were *NOTCH2* and *FLT3*, encoding myeloid cell surface proteins (38). CD44v6, a splice variant of the ubiquitously expressed hyaluronate receptor CD44, is expressed on AML blasts but not on normal HSCs, rendering it an attractive target for CAR T cell therapy (39). Dysregulated posttranslational modification can also provide a source of antigens that can stimulate immune responses. A recent study identified 61 phosphopeptides specifically presented on the surface of hematologic tumors but not normal tissues and documented robust $CD8^+$ T cell responses against many of these phosphopeptides in healthy individuals (40).

Lastly, in addition to HLA class I and II proteins presenting peptide antigens, CD1-lineage proteins present lipids and glycolipids to T cells. In particular, C16 methyl-lysophosphatidic acid–specific (C16 mLPA–specific) CD1c-restricted T cells could kill CD1c-expressing primary AML blasts (41).

In the design of immunotherapeutic strategies it should, however, be considered that each of these antigens, when targeted individually, faces the issue of genetic loss, mutation, or downregulation and, in the case of peptide or lipid antigens, loss of the restriction element (42). The documented success of alloHSCT in controlling or eradicating AML might conversely relate to the fact that a wide repertoire of T cells is transferred from the donor to the patient (27, 43), recognizing not only LAAs and LSAs, but also

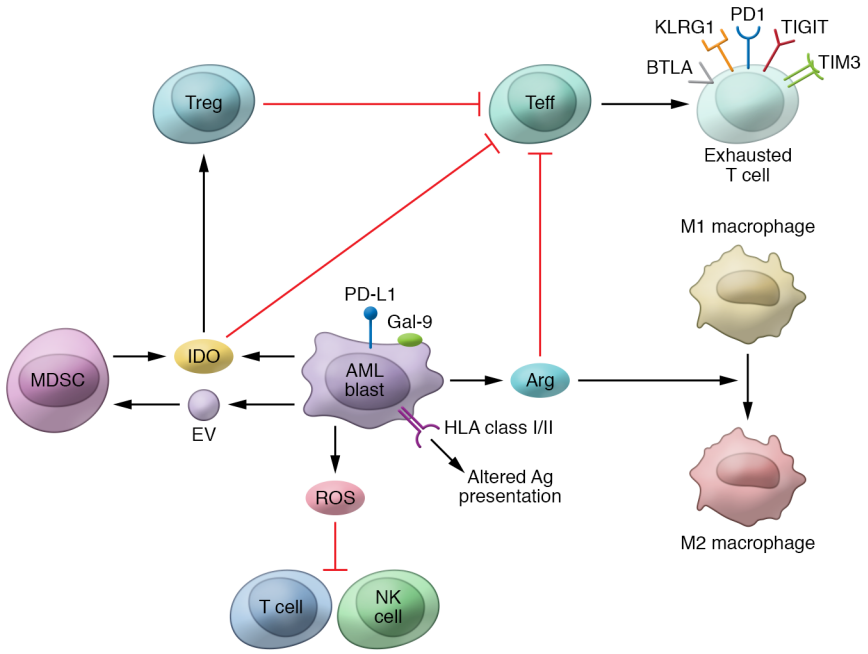

Figure 1. The pathologic immune microenvironment of acute myeloid leukemia. The illustration summarizes known leukemia-intrinsic and -extrinsic immune evasion mechanisms. As described in the main text, AML blasts can reduce their expression of antigen presentation molecules, overexpress inhibitory T cell ligands (including PD-L1, Gal-9, and others), and release in the bone marrow niche reactive oxygen species (ROS), indoleamine 2,3-dioxygenase (IDO), arginase (Arg), and extracellular vesicles (EVs). This, in turn, can inhibit the cytotoxic function of T and NK cells, drive effector T cell (Teff) exhaustion and apoptosis, induce regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and promote the switch of macrophages from M1 to suppressive M2 phenotype.

minor histocompatibility antigens (the number of which exceeds by several orders of magnitude those of tumor antigens [refs. 44, 45]) and incompatible HLA molecules (each recognized by multiple T cell clonotypes [ref. 46]).

Immune state in AML and immune evasion mechanisms

Multiple mechanisms are implicated in AML's immune evasion, as depicted in Figure 1 (47, 48). Each mechanism's exact contributions to leukemia immune tolerance, how mechanisms operate in PB versus BM (primary tumor site) versus extramedullary tissues, and how they are affected by AML treatment or influenced by AML genetics require further understanding.

Altered antigen presentation. Leukemia blasts exhibit impressive immunoediting capabilities under the selective immune pressure that occurs after alloHSCT (49), as evidenced by loss of mismatched HLAs in haploidentical transplants (50–52) or epigenetic downregulation of HLA class II molecules in different donor transplant settings (53, 54). Deletional T cell tolerance and $CD8^+$ T cell tolerance induction due to leukemia antigen presentation by immature antigen-presenting cells or splenic $CD8\alpha^+$ dendritic cells (DCs), respectively, have both been reported in mouse models of AML (55, 56). Recently, loss of plasmacytoid DC differentiation was linked to persistence of measurable residual disease after AML treatment and inferior survival outcomes (57).

T and NK cells. The presence of T cells at the tumor site is the prerequisite for immune recognition and elimination of AML cells, and for any therapy leveraging on this axis. Relative to healthy controls (HCs), AML patients have a comparable number of T cells in BM and even higher levels of total and $CD8^+$ PB T cells (58, 59). Higher percentages of lymphocytes and T lymphocytes in BM correlated with improved response and survival (60), and robust lymphocyte recovery following chemotherapy was associated with reduced relapse risk (61). Recently, higher percentages of $CD3^+$ and $CD8^+$ T cells in BM were found to predict response to the checkpoint inhibitor (CPI) nivolumab in combination with hypomethylating agent (HMA) (62).

Regarding T cell function, a study in a mouse model of AML showed that the frequencies of $CD8^+$ T cells expressing the checkpoint inhibitory receptor (CIR) PD-1 and Tregs increase at the tumor site with disease progression, and highlighted the therapeutic potential of PD-L1/PD-1 blockade and Treg depletion (63). Similarly, increased frequency of functionally exhausted $CD8^+$ T cells coexpressing the CIRs PD-1 and TIM3 coincided with disease progression in a mouse model of AML; these cells' cytotoxic function was reinvigorated by combined checkpoint inhibition (64). Phenotypic and gene expression profiling of PB $CD8^+$ and $CD4^+$ T cells in AML patients at diagnosis identified an aberrant activation profile and alterations in genes important for immunologic synapse formation (58). Subsequently, it was suggested that T cells in AML are functionally intact in terms of cytokine production and proliferation, with the exception of reduced IFN-γ production by $CD4^+$ T cells, and that PD-1 upregulation reflects changes in T cell differentiation toward effector memory cells (65). Several groups, including ours, reported that the frequency of T cells coexpressing CIRs in AML patients is higher than that of T cells coexpressing CIRs in healthy controls, and that the frequency of T cells coexpressing CIRs (PD-1 and TIM3; PD-1 and LAG3) increases with disease progression (47, 59). Several smaller studies found that CIR-expressing $CD8^+$ T cells are functionally impaired and predictive of AML relapse (66–70). While general cytokine expression by $CD8^+$ T cells in AML patients may not substantially differ from that in healthy controls, senescent and exhausted T cells with markedly different cytokine expression profile have been identified. In-depth profiling of $CD8^+$ T cells, however, suggested reversibility of transcriptional T cell signatures in responders to induction chemotherapy (47). A recent

study also suggests that BM $CD8^+$ T cells promote expansion of leukemia stem cells (LSCs) in favorable-risk AML, whereas LSC expansion in adverse-risk AML is cell-intrinsic and independent of $CD8^+$ T cells (71). Lastly, the frequency of PD-$L1^+$ AML cells varied across different studies from 18% to more than 50%. PD-L1 expression appears to increase at the time of relapse, is more frequently noted on acute monocytic leukemia, and is associated with poor prognosis (59, 72–75). Additionally, upregulation of PD-L1 and other cosignaling ligands on AML blasts with corresponding changes in T cells was identified as an immune escape mechanism in post-alloHSCT relapse (54, 76). IFN-γ exposure strongly increases PD-L1 expression on leukemia blasts (77–79), and rapid PD-L1 upregulation by primary AML blasts as an adaptive immune response–driven resistance mechanism was observed upon treatment with immunotherapeutics that activate T cells (42, 80–82).

Leukemic blasts play a critical role in modulating T cell response, and T cell exposure to AML blasts in vitro leads to T cell apoptosis, inhibition of proliferation, and downregulation of costimulatory molecule expression. Monocytic leukemia cells produce ROS, which kills T cells and NK cells by triggering poly(ADP-ribose) polymerase-1–dependent (PARP-1–dependent) apoptosis (83). NK cytotoxicity and effector function were also reduced upon exposure to AML blasts in vitro. Functionally impaired NK cells with reduced expression of activating receptors and upregulation of the inhibitory receptor NKG2A are already present in AML patients at diagnosis. Like those in T cells, the phenotypic and functional changes in NK cells were partially reversible upon achievement of remission but persisted in nonresponders to therapy (84, 85). The aberrant leukemia-specific fusion proteins PML-RARα and AML1-ETO were shown to specifically downregulate CD48 (the ligand of the NK cell receptor 2B4, or CD244) on AML cells, resulting in impaired NK cytolytic activity (86).

Immunosuppressive microenvironment. Studies in mice and humans also implicate Tregs as a major contributor to defective immune responses in AML (87). Tregs are increased in PB and BM of AML patients, where they exhibit more potent immunosuppressive effects on effector T cells (Teffs) than their normal counterparts, and are minimally affected by chemotherapy (59, 88). Moreover, increased Treg levels appear to be associated with worse outcomes in AML (88, 89).

Myeloid-derived suppressor cells (MDSCs) also appear to be increased in AML patients, inducing T cell tolerance through multiple mechanisms, including VISTA, PD-L1, indoleamine 2,3-dioxygenase (IDO), arginase, excess ROS, peroxynitrate, and multiple cytokines (TGF-β and IL-10) (90–94). AML cells may induce MDSC expansion by releasing extracellular vesicles (EVs) containing the oncoprotein MUC1, which, in turn, increases c-myc expression in EVs, leading to MDSC proliferation (95). Positive correlation between the number of Tregs and that of MDSCs was found in high- but not in low-risk myelodysplasia (MDS), indicating Tregs' and MDSCs' potential role in disease progression (96).

Several enzymes expressed by AML blasts may contribute to the suppressive microenvironment. AML blast expression of IDO, an enzyme that catalyzes the rate-limiting step of tryptophan degradation along the kynurenine pathway, is associated with shortened relapse-free survival and overall survival (OS) (97–99). Depletion of tryptophan and accumulation of its metabolites result in inhibition of Teff proliferation, increased T cell apoptosis, and Treg induction (100). Arginase II, produced by AML blasts, is increased in patients with AML and suppresses T cell proliferation, inhibits myeloid-monocytic differentiation, and promotes monocyte polarization into a suppressive M2-like phenotype (101). In animal models of monocytic AML, arginase I was also associated with leukemia aggressiveness, driving tissue infiltration and T cell suppression through a signaling pathway involving the LILRB4 receptor (102).

Immunologic effects of common antileukemia therapies

Although they were primarily developed to be cytotoxic or to inhibit oncogenic pathways, it is increasingly recognized that most of the AML therapeutics have potent effects on the immune system and on leukemia immunogenicity, which may be further enhanced in specific settings, such as after alloHSCT.

For instance, exposing leukemia cells to cytarabine, even at low doses, can increase expression of HLA class II and the costimulatory molecule CD80, and downregulate PD-L1 expression (103). Anthracyclines, on the other hand, can induce immunogenic cell death, leading to cell surface translocation of calreticulin and extracellular release of ATP and HMGB1 and ultimately promoting DC-based cross-priming of antileukemia T cells (104). Notably, pretreatment calreticulin exposure on AML blasts significantly correlated with superior OS following treatment (105). However, anthracyclines can also elicit immunosuppressive pathways, such as induction of IDO1 in DCs (106).

In AML, and other myeloid malignancies, founder mutations occur frequently in genes that regulate DNA methylation (e.g., *DNMT3A*, *TET2*, and *IDH1/2*) or chromatin remodeling (e.g., *ASXL1*, *EZH2*, and *KMT2A*) (107). A number of drugs modulating the activities of these epigenetic regulators, including HMAs, have been approved for MDS and AML.

Interestingly, a sizable number of immune-related genes increase their expression upon HMA exposure, and second-generation HMA guadecitabine might have even more potent immune-related effects compared with azacitidine and decitabine (108). Genes known to be upregulated in AML cells upon exposure to HMAs include those encoding HLA class I and II molecules, their regulators, and many cancer/testis antigens or LAAs, including MAGE, PRAME, and WT1 (109). More recently, studies performed in epithelial malignancies demonstrated that HMAs can promote reactivation of dormant human endogenous retroviruses, which, upon transcription, elicit innate immune sensing and induction of adaptive responses (110, 111). In addition, azacitidine was shown to improve T cell repertoire in MDS (112), and its efficacy in combination with donor lymphocyte infusion in the post-alloHSCT setting supports azacitidine's ability to induce tumor-specific responses (113–115).

On the other hand, DNA methylation tightly regulates expression of several immune checkpoints, and exposing leukemia cells to HMAs was shown to elicit PD-L1 upregulation (116). For these reasons, HMAs have been combined with immune CPIs in clinical studies in AML, discussed in more detail below. However, HMAs were also shown to reduce methylation of the *FOXP3* promoter, promoting Treg expansion (117).

Finally, drugs that selectively block an oncogenic pathway can have considerable immunotherapeutic activity, as evidenced by a recent study on FLT3-mutated AML relapsed after alloHSCT and treated with the multi–tyrosine kinase inhibitor (TKI) sorafenib. In animal models and patient samples, FLT3 inhibition prompted leukemic blasts to release IL-15, which in turn potentiated responses mediated by early-differentiated T and NK cells (118). Other TKIs, including BCR-ABL inhibitors, can exert immune-related effects by inducing mature NK cells and leukemia antigen–specific T cells and decreasing the frequency of Tregs and PD-1$^+$ T cells (119). Moreover, studies in epithelial malignancies demonstrated that selective inhibition of genes commonly mutated or deregulated in AML, such as *RAS* and *MYC*, can indirectly boost antitumor responses (120, 121).

Antibody therapy in AML

The anti-AML activity of antibodies relies on their ability to (a) recruit immune effectors via their Fc domain, causing NK cell antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell phagocytosis, or complement-dependent cytotoxicity; (b) deliver toxic payload after receptor-mediated internalization of antibody-drug conjugates (ADCs) and radionuclide conjugates; or (c) enhance T or NK cell antileukemia efficacy by engaging these cells with the target leukemia cells using bispecific or polyspecific antibodies. Supplemental Table 1 reviews antibodies clinically tested in AML (supplemental material available online with this article; https://doi.org/10.1172/JCI129204DS1).

Clinical development of unmodified mAbs targeting CD33, CD123, and several other antigens in AML, alone or in combination with chemotherapy, demonstrated limited clinical activity (122–124). Ongoing clinical studies are investigating BI836858, a CD33-targeting antibody with an Fc domain engineered to optimize interaction with the Fc receptor (CD16) on NK cells, in combination with HMAs or the immunocytokine F16-IL2 (teleukin) (125). In vitro studies suggest that decitabine may potentiate BI836858-mediated ADCC through upregulation of NKG2D ligands on leukemia cells (126). The mAb ARGX-110 targets CD70, which serves as a ligand for CD27 (TNF receptor), both of which are highly expressed on AML blasts and LSCs, but not normal HSCs. In murine xenograft models, decitabine upregulated CD70 expression on LSCs but not normal HSCs, and showed synergism with CD70 blockade (127). ARGX-110 in combination with azacitidine produced a high overall response rate (ORR) with no dose-limiting toxicities in early clinical testing (128). Promising clinical activity and tolerability were recently reported for magrolimab, an anti-CD47 antibody that promotes macrophage-mediated phagocytosis, when given in combination with azacitidine to newly diagnosed MDS and AML patients, including those carrying TP53 mutations (129).

Among ADCs, gemtuzumab ozogamicin (GO), a humanized anti-CD33 antibody conjugated to calicheamicin, was recently reapproved for treating newly diagnosed AML in adults, and relapsed/refractory (R/R) AML in adults and pediatric patients, after studies using lower or fractionated doses of GO in combination with chemotherapy demonstrated efficacy with limited toxicity (130–132). Patients with favorable-karyotype AML benefited the most from addition of GO to induction chemotherapy (133). Encouraging safety and clinical activity in R/R AML and blastic plasmacytoid DC neoplasm (BPDCN) have also been recently reported for IMGN632, a CD123-targeting antibody coupled to the novel indolinobenzodiazepine payload (134). Additional ADCs targeting CD33, CD123, and CD135 (FLT3) and antibody radioimmunoconjugates targeting CD33 and CD45 continue in clinical testing, the latter mostly in the pretransplant setting because of concern for myelosuppression (Supplemental Table 1).

Multispecific antibodies are engineered to combine specificities of two or more antibodies in order to bind tumor cell antigens, activate Teffs or NK cells, and bring two cell types into proximity, maximizing a tumoricidal interaction. Prompted by the success of blinatumomab, a CD3xCD19 bispecific T cell engager (BiTE) antibody in ALL, several variants of bispecific or even trispecific antibodies targeting CD33, CD123, or CLL1 are being explored in trials in AML (Supplemental Table 1 and Figure 2). So far, clinical studies of bispecific antibodies in AML have not reproduced the efficacy seen in ALL, as summarized in Table 2. Several studies are still in the dose-escalation phase, but cytokine release syndrome (CRS) has occurred with all the constructs, requiring careful dose escalation and symptom management. Deeper understanding of differential responses among primary refractory and relapsed patients, as seen with flotetuzumab, including the role of immune-enriched BM microenvironment, which is characterized by higher IFN-γ scores and tumor inflammation signature, will be critical to optimizing the activity of this approach and improving patient selection (135–138). Studies of bispecific antibodies in combination with PD-1 CPIs to overcome potential resistance mechanism are under way, and constructs integrating locally restricted blockade of the PD-1/PD-L1 checkpoint have been developed (139).

Genetically redirected T cells in AML

Investigators have been actively seeking strategies to reproduce in AML the successful results obtained with anti-CD19 CAR T cells in lymphoid malignancies. However, these efforts have been hampered by the difficulty in identifying suitable targets, as discussed previously. Alternative strategies formulated to bypass this problem include (a) CAR T cells activating only in response to a combination of targets (13, 140); (b) use of non-persisting cell types or safety switches to eliminate CAR-modified cells after disease eradication (141, 142); and (c) genome editing of HSCs to generate a CAR-resistant hematopoiesis (143, 144).

A number of anti-CD33 CAR T cell constructs have in fact demonstrated robust preclinical efficacy against primary AML blasts both in vitro and in humanized animal models, accompanied by evidence of toxicity against leukemic and nonleukemic myeloid cells with, in part, conflicting results in terms of their effects on HSCs (9, 10, 145, 146). Clinical trials using anti-CD33 CAR T cells are ongoing or just completed (NCT03126864, NCT03971799, NCT02799680, NCT01864902; ClinicalTrials.gov), but to date, only one report has been published on a single patient with refractory AML who developed CRS and experienced transient BM blast reduction following infusion of autologous anti-CD33 CAR T cells (147).

Regarding anti-CD123 CAR T cells, a number of studies documented promising preclinical antileukemia activity, but at least one study raised concerns about toxicity toward HSCs (9, 11,

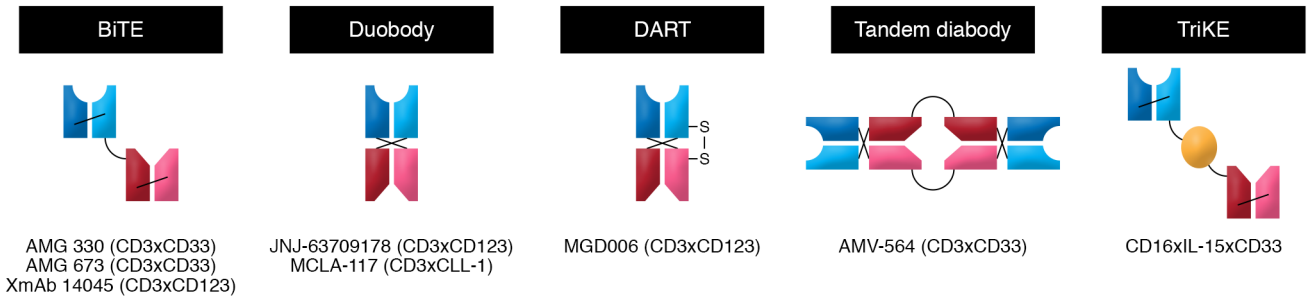

Figure 2. Bispecific antibody formats. Schematic summary of some of the strategies that are being tested to link antigen-binding fragments (Fabs) of two or more monoclonal antibodies with different specificities. Under each construct are provided some examples of bispecifics of that class in advanced clinical development (see also Table 2).

148). Recently, an anti-CD123 CAR, MB-102, received the FDA's orphan drug designation for both AML and BPDCN based on initial results from an ongoing phase I study (NCT02159495). In the study, patients with AML and BPDCN demonstrated complete remissions (CRs) at low MB-102 doses without dose-limiting toxicities. An expansion phase of the MB-102 study is ongoing, together with a number of other clinical trials testing different anti-CD123 CAR T cell products (NCT04106076, NCT03190278).

Besides CD33 and CD123, other targets are being tested for CAR T cell development, including folate receptor β (149), CLL1 (150), FLT3 (151), LeY (152), NKG2D ligands (153), CD70 (154), and CD44v6 (39). Clinical data regarding these approaches are scarce, limited to three AML patients treated with anti-LeY antigen CARs (155) and three early-phase studies on NKG2D-based CARs (156–158). No studies documented major toxicities, and some reported initial hints on efficacy.

Table 2. Select reported studies with bispecific antibodies in AML

Antibody/study	Target/construct	Treatment plan/eligibility	Outcomes
AMG 330; phase I, NCT02520427 (192)	**CD3xCD33.** BiTE, scBsTaFv (low molecular weight, short $t_{1/2}$, rapid renal excretion). **Preclinical:** Cytotoxic against CD33$^+$ AML cells; adaptive resistance, upregulation of PD-L1 on AML cells (80, 193, 194).	**Administration:** CI 2–4 weeks on/1–4 weeks off. **Eligibility:** Relapsed/ refractory adult AML.	**Enrollment:** 40 Patients/12 cohorts (dose range, 0.5–480 μg/d). Tolerated (target) dose: 240 μg/d with lead-in dose escalation. **Toxicity:** Related SAE 43%, most commonly CRS (28%) and febrile neutropenia (18%). **Activity:** 2 CR and 2 CRi at the target dose of 120–240 μg/d.
AMG 673; phase I, NCT03224819 (195)	**CD3xCD33.** Half-life–extended BiTE (fused to the N-terminus of a single-chain IgG Fc region).	**Administration:** 2 Short i.v. infusions over 2 weeks. **Eligibility:** Relapsed/ refractory adult AML.	**Enrollment:** 30 Patients/10 cohorts (dose range, 0.05–72 μg i.v. per dose); dose escalation continues. **Toxicity:** Related SAE 50%, most commonly elevated hepatic enzymes (17%), CRS and leukopenia (13%). **Activity:** BM blast reduction in 44% of patients (22% of patients with >50% reduction); 1 CRi. **PD:** Upregulation of CD25 and CD69 on T cell subsets, cytokine release at higher doses.
AMV 564; phase I, NCT03144245 (196)	**CD3xCD33.** TandAbs. **Preclinical:** Cytotoxic against CD33$^+$ AML cells in vitro and in HL-60 xenograft mouse model in vivo.	**Administration:** CI over 2 weeks/4 weeks cycles. **Eligibility:** Relapsed/ refractory adult AML.	**Enrollment:** 36 Patients/10 cohorts (dose range, 0.5–300 μg/d); dose escalation continues. **Toxicity:** 11% ≥ Grade 3 anemia, no DLTs, no grade 3 CRS with lead-in dose escalation. **Activity:** BM blast reduction in 49% of patients; 1 CR; 1 CRi; 1 PR; 3 patients with neutrophil improvement. **PD:** T cell redistribution; increase in BM T cells with repeat cycles.
Flotetuzumab; phase I/II, NCT02152956 (136, 137, 197, 198)	**CD3xCD123.** DART.	**Administration:** 500 ng/kg/d CI weeks 2–4 with lead-in dose in week 1 (cycle 1), and a 4-day on/3-day off schedule for cycle 2+. **Eligibility:** Early relapsed/ refractory adult AML patients.	**Enrollment:** 50 Patients at RP2D. **Toxicity:** ≥ Grade 3 CRS in primary refractory and relapsed patients was 3% and 16%, respectively. **Activity:** 32% Response rate in 28 primary refractory patients (3 CR, 3 CRi, 3 CRh); no response in relapsed patients. **PD:** Higher CD123 receptor density in CR patients compared with nonresponders; baseline immune gene signatures, including significantly higher IFN-γ scores and tumor inflammation signature scores, correlated with flotetuzumab activity; increase in T cell infiltration and clustering around CD123 AML cells in the BM in responders; PD-L1 induction in nonresponders; baseline frequency of circulating CD4$^+$ cells correlated with CRS severity.
XmAb14045; phase I, NCT02730312 (199)	**CD3xCD123.** BiTE with a full-length Ig molecule.	**Administration:** Weekly administration, 4-week cycles. **Eligibility:** Relapsed/ refractory adult AML, B-ALL, CML-BC, and BPDCN.	**Enrollment:** 64 Patients (RP2D for flat weekly dosing 1.3 μg/kg); dose escalation with priming dose followed by increasing dose continues. **Toxicity:** 11% ≥ Grade 3 CRS; febrile neutropenia and peripheral edema (30%), elevated liver functions (19%), pneumonia, stomatitis, hyperglycemia, sepsis. **Activity:** 2 CR, 1 CRi at the highest doses (23%).

B-ALL, B cell acute lymphoblastic leukemia; BiTE, bispecific T cell engager; BPDCN, blastic plasmacytoid DC neoplasm; CI, continuous infusion; CML-BC, chronic myeloid leukemia–blast crisis; CR, complete remission; CRh, CR with partial hematologic recovery; CRi, CR with incomplete hematologic recovery; CRS, cytokine release syndrome; DART, dual-affinity retargeting molecule; DLT, dose-limiting toxicity; PD, parmacodynamic studies; PR, partial remission; RP2D, recommended phase II dose; SAE, serious adverse event(s).

An alternative approach is represented by genetic engineering of T cells with a high-affinity T cell receptor (TCR) specific for a leukemia antigen. Although limited by the antigen HLA restriction and the efficiency of processing and presentation, this strategy can theoretically target a much wider antigen repertoire, including LAAs and neoantigens. To date, a number of preclinical studies have focused on redirecting T cells against WT1 (159, 160), which rapidly translated into clinical studies demonstrating no major toxicities, persistence over time of the infused lymphocytes, and encouraging signs of activity (17, 161, 162). Alternative intracellular AML antigens for which TCR-based genetic redirection showed promising preclinical results include telomerase (163), RHAMM (164), and the mutated form of NPM1 (7).

Vaccine strategies in AML

Both peptide and DC-based vaccines against LAAs have been explored in AML to prime or boost leukemia-specific immune responses. WT1 peptide vaccination strategies appear to be safe with hints of clinical activity in terms of responses and survival outcomes in MDS/AML patients. Correlation between induction of WT1-specific T cells and reduction of *WT1* mRNA levels was observed in different studies (165). A recent phase II trial of a multivalent WT1 peptide vaccine, galinpepimut-S, given to 22 AML patients in first CR documented immunologic responses in 64% of patients, including increased $CD4^+$ T cell proliferation, $CD8^+$ T cell IFN-γ secretion, and frequency of WT1-specific $CD8^+$ T cells. Immunologic response but not baseline WT1 levels appeared to correlate with improved survival outcomes (166).

Different DC-based strategies, including AML-DCs, monocytic DCs, or hybridomas, have been explored. While AML-DCs have the advantage of expressing a full spectrum of antigens, monocytic DCs were more effective in activating autologous antileukemia T cell responses (reviewed in ref. 167). The allogeneic DC vaccine DCP-001, developed from an AML-derived cell line, given to 12 AML patients resulted in median OS of 36 months from the start of vaccination in patients lacking circulating blasts. Long-term survival correlated with maintained T cell levels and induction of multifunctional immune responses (168). In a phase II study, DCs electroporated with *WT1* mRNA were given as a post-remission treatment to 30 patients with AML at high risk of relapse; 9 patients achieved molecular remission (5 still in remission after median follow-up of 9 years), and 4 patients achieved disease stabilization. Long-term clinical response correlated with the induction of WT1-specific $CD8^+$ T cell response (18). This approach is being tested in a randomized clinical study in the post-remission setting (WIDEA; NCT01686334). Interesting data have been published on the use of personalized vaccine with a hybridoma of AML cells and autologous patient-derived DCs. Among 17 patients vaccinated in CR after chemotherapy, 12 (71%) remain in remission at a median follow-up of 57 months. Vaccination was associated with expansion of leukemia-specific T cells against MUC1, WT1, and NY-EOS antigens that lasted more than 6 months (19, 169). The multicenter study exploring this approach is ongoing (NCT01096602). Research efforts to optimize immunostimulatory properties of DCs, such as use of IL-15–differentiated DCs, and combining DCs with CPIs or HMA continue. A more personalized approach using multi-epitope neoantigen vaccination was proven feasible not only in tumors with high mutational load such as melanoma (170), but also in glioblastoma (171), which typically has a low mutation load, raising the possibility of exploring a similar approach in AML. A clinical trial based on ex vivo enrichment and subsequent reinfusion of T cells specific for patient neoantigens recently started accrual in high-risk MDS (172).

Checkpoint inhibitors in AML

Studies of CPIs in AML are listed in Supplemental Table 2, and select reported studies are summarized in Table 3. With the hope that CTLA4 blockade would reactivate alloreactive T cells, early studies explored ipilimumab in hematologic malignancies in the setting of post-alloHSCT disease progression (173, 174). Ipilimumab at 10 mg/kg dose produced promising responses in 5 of 12 AML patients, 4 with extramedullary leukemia, with 3 responses lasting more than 1 year. Responses were accompanied by in situ infiltration of cytotoxic $CD8^+$ T cells, decreased systemic Treg activation, and expanded Teff subpopulations in PB (174). In contrast, nontransplanted MDS patients who failed HMAs only tolerated ipilimumab at 3 mg/kg, a dose displaying limited clinical activity. Patients benefiting from therapy had a higher frequency of T cells expressing ICOS after treatment compared with nonresponders (175). Ipilimumab in combination with decitabine is being explored in an ongoing phase I study, in both pre- and post-alloHSCT settings, with early hints of clinical activity, particularly in nontransplanted R/R AML patients (176).

Given the multiple immune effects of HMAs, their clinical testing in combination with PD-1/PD-L1 pathway inhibition has been pursued in several studies in AML (Table 3). Nivolumab given in combination with azacitidine to 70 older, heavily pretreated AML patients yielded an ORR of 33%, with a higher ORR of 58% in HMA-naive patients. The median OS of 6.3 months compared favorably with historical controls treated with azacitidine alone at the same institution. Higher percentages of $CD3^+$ and $CD8^+$ cells in pretherapy BM and $CD3^+$ cells in PB were predictive of response, whereas upregulation of CTLA4 on $CD4^+$ Teffs after treatment was observed in nonresponders, indicating a potential resistance mechanism (62). On the basis of this observation, azacitidine and nivolumab were combined with ipilimumab in a subsequent cohort of 31 patients, and produced an improved ORR of 44% and median OS of 10.5 months, but at the expense of more frequent immune-related adverse events (177).

Combinations of another PD-1–blocking antibody, pembrolizumab, with decitabine or azacitidine produced similar response and survival data to those observed with azacitidine and nivolumab in R/R AML patients (178, 179). Early reports from both azacitidine/nivolumab and azacitidine/pembrolizumab studies suggested encouraging ORR and survival among small cohorts of newly diagnosed and unfit older AML patients (179, 180). However, a randomized phase II study of azacitidine plus durvalumab (anti–PD-L1) versus azacitidine alone in 214 untreated high-risk MDS and AML patients reported safety but no additional efficacy for the combination (181). Whether upregulation of PD-L2 on leukemia cells following PD-L1 inhibition may influence activity of PD-L1 versus PD-1 inhibition in this setting requires further studies (182).

PD-1/PD-L1 pathway inhibition in combination with chemotherapy was also found to be feasible in both newly diagnosed

Table 3. Select reported studies of checkpoint inhibitors in combination with HMAs and chemotherapy in AML

Study	Agents	Population	Outcomes	Pharmacodynamics/notes
Phase I/Ib, NCT01822509 (174)	**Ipilimumab** (3 mg/kg or 10 mg/kg every 3 weeks × 4 then every 12 weeks × 4)	28 patients (12 AML) relapsed hematologic malignancies after alloHSCT	**Toxicity:** 21% irAEs, including 1 death; 14% GVHD precluding further ipilimumab administration. **Activity:** No response at 3 mg/kg; 59% patients responded, with 23% CR at 10 mg/kg; 5/12 AML patients with CR, 4 having EMD.	Responses associated with in situ $CD8^+$ T cell infiltration, expansion of PB Teff subpopulations, decreased Treg activation
Phase II, NCT02397720: Cohort 1 (62)	**Nivolumab** (3 mg/kg every 2 weeks) **plus azacitidine**	70 R/R AML patients (median age, 70 yr)	**Toxicity:** 11% ≥ Grade 3 irAEs; 2 (3%) irAE-related deaths, 11% 8-week mortality. **Activity:** ORR 33% with 22% CR/CRi; ORR higher in HMA-naive vs. HMA-pretreated patients (58% vs. 22%); median OS, 6.3 months.	Predictors of response: Pretherapy BM $CD3^+$ and $CD8^+$ cells with optimal cutoffs of >13.2% and >4.01%, respectively; and PB $CD3^+$ with cutoff >20.5%
Cohort 2 (177)	**Nivolumab plus ipilimumab** (1 mg/kg every 6 weeks) **plus azacitidine**	31 R/R AML patients (median age, 71 yr)	**Toxicity:** 25% ≥ Grade 3 irAEs; no irAE-related death; 8% 8-week mortality. **Activity:** ORR 44% with 36% CR/CRi in 24 evaluable patients; median OS 10.5 months.	Survival compares favorably with nivolumab/azacitidine (6.3 months) and contemporary historical HMA-based trials at MD Anderson Cancer Center (4.6 months)
Phase I/II, NCT02996474 (178)	**Pembrolizumab** (200 mg every 3 weeks) **plus decitabine**	10 R/R AML patients (median age, 62 yr)	**Toxicity:** No grade 5 AEs. **Activity:** ORR 20%; median OS 7 months.	
Phase II, NCT02845297 (179)	**Pembrolizumab** (200 mg every 3 weeks) **plus azacitidine**	37 R/R (Cohort 1); 22 newly diagnosed, old, unfit (Cohort 2) AML patients	**Toxicity: Cohort 1** (median age, 65 yr): 24% ≥ Grade 3 irAEs; 13% 8-week mortality; **Cohort 2**: 14% ≥ Grade 3 irAEs; 9% 8-week mortality. **Activity: Cohort 1** (median age, 75 yr): ORR 32% with 14% CR/CRi among 29 evaluable patients; median OS 10.8 months; **Cohort 2**: ORR 71% with 47% CR/CRi among 17 evaluable patients; median OS 13.1 months.	
Phase II, NCT02464657 (183)	**Nivolumab** (3 mg/kg every 2 weeks) **plus cytarabine/idarubicin**	44 Newly diagnosed AML, high-risk MDS patients (median age, 54 yr)	**Toxicity:** 14% ≥ Grade 3 irAEs; grade 3–4 GVHD occurred in 26% of patients who proceeded to alloHSCT; no nivolumab-related deaths. **Activity:** ORR 80% with 78% CR/CRi; 53% MRD-negative CR after induction, 79% with subsequent therapy; median follow-up 17.25 months, EFS not reached, median RFS and OS both 18.54 months.	At baseline, BM of nonresponders had significantly higher percentages of $CD4^+$ Teffs coexpressing PD-1 and TIM3
Phase II, NCT02768792 (185)	**Pembrolizumab** (200 mg every 3 weeks) **plus HiDAC**	37 R/R AML patients (median age, 54 yr)	**Toxicity:** 14% > Grade 3 irAEs; 3% 8-week mortality; 24% of patients received subsequent alloHSCT without significant GVHD. **Activity:** ORR 46% with 38% CR/CRi; median follow-up 7.8 months, median OS 8.9 months, and median DFS 5.7 months.	Increased expression of innate immune genes by blasts and of cell cycle genes by the nonblast fraction before treatment correlated with response to therapy; CR was associated with increased measures of B and T cell diversity
Phase II, randomized, NCT02775903 (181)	**Durvalumab** (1000 mg every 4 weeks) **plus azacitidine (Arm A) versus azacitidine (Arm B)**	Untreated 214 patients: MDS (84) and AML (129) old, unfit patients	**Toxicity:** No safety signal; 17% and 27% irAEs in MDS and AML cohort, respectively, all resolved. **Activity: MDS: Arm A:** ORR 62%, CR 7%, median OS 11.6 months, versus **Arm B:** ORR 48%, CR 9.5%, median OS 16.7 months; **AML: Arm A:** ORR 31%, CR 17%, median OS 13 months, versus **Arm B:** ORR 35%, CR 21.5%, median OS 14.4 months.	
Phase IB, NCT03066648 (200)	**MBG453, anti-TIM3 Ab** (240–800 mg every 2 weeks) **plus decitabine**	HR-MDS (17), CMML (4), and untreated or R/R AML (38) patients (median age, 70 yr)	**Toxicity:** No MTD, 7% > grade 3 irAEs, no treatment-related deaths. **Activity:** HR-MDS: 50% mCR/CR; newly diagnosed AML: 29% CRi; R/R AML: 29% PR/CR; all patients with HR-MDS maintained response with exposure duration from 3.4 to 18.6 months.	

AE, adverse event; CMML, chronic myelomonocytic leukemia; CR, complete remission; CRi, CR with incomplete hematologic recovery; DFS, disease free survival; EFS, event free survival; EMD, extramedullary disease; GVHD, graft-versus-host disease; HiDAC, high dose cytarabine; HMA, hypomethylating agent; HR-MDS, high-risk myelodysplasia; irAE, immune-related adverse event; mCR, marrow CR; MDS, myelodysplasia; MRD, measurable residual disease; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PR, partial remission; RFS, relapse free survival; R/R, relapsed/refractory.

and R/R AML. Nivolumab in combination with induction cytarabine plus idarubicin produced an ORR of 80% and median OS of 18.54 months in untreated, younger AML patients, but 26% of patients who proceeded to alloHSCT developed grade 3–4 graft-versus-host disease. Higher percentages of $CD4^+$ Teffs coexpressing PD-1 and TIM3 in pretreatment BM were observed in nonresponders to therapy (183). In R/R AML patients, pembrolizumab with high-dose cytarabine yielded an ORR of 46% and median OS of 8.9 months, without significant graft-versus-host disease in patients subsequently receiving alloHSCT (184, 185). Randomized studies with CPIs are ongoing (NCT03092674; SWOG 1612) as well as intense search for biomarkers of response. Pretreatment BM T cell infiltration and measurements of T cell functionality appear promising in predicting response to CPIs and HMAs, and should be examined prospectively to improve patient selection and limit toxicities (177).

Single-agent PD-1 inhibition was also explored in the setting of minimal residual disease following chemotherapy or alloHSCT (186, 187). In the post-alloHSCT relapse setting, reports from several small studies suggest limited single-agent PD-1 inhibitor activity and safety concerns (188–190). Recent reports suggest that different transplant platforms, such as post-transplant cyclophosphamide, may be associated with less toxicity, and additional studies are required (191). Moreover, recent recognition that AML cells may exploit HLA class II downregulation versus immune checkpoint upregulation as alternative modalities to avoid recognition by donor-derived T cells could provide an opportunity to differentiate patients with post-transplant relapse who are more or less likely to benefit from CPIs (54).

Conclusion

While impressive progress has been made in the molecular understanding of tumor immunology and clinical application of immunomodulatory agents in recent years, we still must learn how to effectively incorporate different immunotherapeutic strategies in AML. In general, the efforts to improve delivery of diverse immune strategies with the goal of harnessing the full power of the immune system against AML cells have been hampered by host and disease heterogeneity, and complicated by the immunoevasive capabilities of AML blasts. The inherent genetic, epigenetic, and clonal complexity of this disease also continues to challenge identification of the best predictors of response to different immunotherapeutic interventions. Novel strategies need to be carefully integrated with standard chemotherapy as well as with alloHSCT and moved to less advanced or upfront therapeutic settings. Early identification of resistance mechanisms, both extrinsic and intrinsic, and development of strategies to overcome them will be crucial endeavors for improving the immunotherapeutic armamentarium against AML. Ultimately, the successful application of immunotherapy will depend on thorough mechanistic understanding of the immunologic, genomic, and microenvironmental complexity of AML, informing data-driven design of rational combination approaches.

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