

Novel Approaches to Acute Myeloid Leukemia Immunotherapy

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Abstract

Acute myeloid leukemia (AML) is a rapidly progressive, poor-prognosis malignancy arising from hematopoietic stem/progenitor cells. The long history of successful use of allogeneic hematopoietic cell transplantation (alloHCT) in AML indicates that this disease is immunoresponsive, leading to optimism that novel immunotherapies such as bispecific antibodies, chimeric antigen receptor T cells, and immune checkpoint inhibitors will generate meaningful disease control. However, emerging data on the immunoevasive tactics employed by AML blasts at

diagnosis and at relapse indicate that optimism must be tempered by an understanding of this essential paradox. Furthermore, AML has a low mutational burden, thus presenting few neoantigens for attack by autologous T cells, even after attempted reversal of inhibitory receptor/ligand interactions. In this review, we outline the known AML targets, explore immune evasion mechanisms, and describe recent data and current clinical trials of single and combination immunotherapies. *Clin Cancer Res*; 24(22); 5502–15. ©2018 AACR.

Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults with an annual incidence rate of 4.2 per 100,000, a median age at diagnosis of 68, and a 5-year survival of only 26.9% based on data collected from 2007 to 2013 in the United States (www.seer.cancer.gov, accessed March 22, 2018). Treatment and response vary based on patient's age, comorbidities, and disease genetics with intensive chemotherapy followed by consolidation chemotherapy or a stem cell transplant in younger patients, or low-dose chemotherapy (including demethylating agents) in older patients. Efforts over the last two decades to develop AML therapy that is targeted to specific genetic lesions [for e.g., constitutive activation of the tyrosine kinase fms-like tyrosine kinase-3 (FLT3), or neomorphic function of the metabolic enzymes isocitrate dehydrogenase (IDH1 or IDH2)] have led to the discovery, and in some cases regulatory approval, of well-tolerated small-molecule drugs that yield moderate overall response and low complete response rates. While exciting, targeted therapies such as these benefit only a subset of patients bearing the identified mutations and are rarely if ever curative as monotherapy (1, 2).

After achieving apparent chemotherapy-induced remission in AML, postremission therapy is necessary for relapse prevention. The most effective form of postremission therapy is allogeneic hematopoietic cell transplantation (alloHCT), which is the earliest form of immunotherapy still in widespread use (3). AlloHCT provides an opportunity for additional myeloablative

chemotherapy, a fresh source of healthy hematopoiesis and most importantly, T-cell-based antileukemic immunity. However, alloHCT is only reliably effective in patients in morphologic remission, because patients transplanted in active disease (>5% marrow blasts) or with measurable residual disease (MRD) have poor outcomes (4) and infusion of supplemental donor lymphocytes (DLI) is only effective in patients who have attained chemotherapy-induced remission (5). Stated otherwise, the antileukemic activity of polyclonal T cells is relatively weak. The aim of the current efforts in AML immunotherapy is to build upon and enhance the ability of immune effector cells to reject leukemia.

Immunotherapy ultimately works by killing tumor cells and relies for its success on the recognition of suitable tumor antigens by active immune effector cells in the absence of overwhelming counter-regulatory mechanisms. Suitable tumor antigens are those that are expressed selectively on the malignant cells or, if expressed on normal tissues, those normal tissues are at least partially dispensable. Membrane-expressed antigens such as CD33, CD123, CD44v6, CLEC12A (CLL1), FLT3, and others are suitable for targeting with antibody-based approaches including bispecific T-cell engagers or chimeric antigen receptor T (CART) cells but are rarely if ever cancer-specific. Intracellular antigens can be relatively [leukemia-associated antigens (LAA) such as Wilms tumor 1 (WT1) or PR1] or completely [cancer-driving oncoproteins such as BCR/ABL or nucleophosmin 1 (NPM1)] specific to malignant cells but the mutant peptides may or may not be capable of being presented to T cells in any given human leukocyte antigen (HLA) molecule. Although AML is commonly thought of as an immune-responsive disease, it is paradoxically also immunosuppressive. Emerging data indicate that AML blasts can employ multiple immunosuppressive mechanisms either directly or by recruiting a suppressive microenvironment thus indicating that even treatment-naïve patients may have dysfunctional immune effector cells (6, 7). Furthermore, the patient's immune effector cells such as T cells, natural killer (NK) cells or macrophages will have been exposed to cytotoxic chemotherapy during the primary treatment of the leukemia and thus may be functionally or numerically impaired.

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Here, we will review (i) the potential target antigens in AML, (ii) the evidence for dysfunction of the immune system in AML and potential avenues to circumvent it, and (iii) the classes of therapeutics that have been or are currently being trialed as novel approaches to AML immunotherapy. We will conclude by speculating on interesting combinatorial approaches to targeting AML in a specific and rationale manner.

Target Antigens in AML

The ideal target antigen in immunotherapy is critical for disease initiation or propagation, present on all malignant cells and absent from all normal cells. As will be apparent from the discussion below, there are no antigens that reliably satisfy these criteria in AML. Potential antigens for AML targeting are listed in Table 1.

Cell surface antigens

Cell surface antigens are usually lineage-associated. Thus, they are shared by at least some normal cells. In B-cell acute lymphoid leukemia (B-ALL), CD19 is a lineage antigen that is also expressed on normal immature and mature B cells. In this context, the success of CART cells directed against CD19 rests in part on the fact that humans can tolerate prolonged B-cell aplasia (8, 9). In contrast, prolonged myeloid lineage aplasia is not tolerable due to the complications of bone marrow failure such as neutropenic infections and transfusion requirements.

CD33, a transmembrane receptor belonging to the sialic acid-binding immunoglobulin-like lectin (SIGLEC) family, is expressed in more than 90% of AML and while its expression level is unlikely to be independently prognostic, low expression tends to be associated with both adverse karyotype (poor risk) and core binding factor (good risk) AML, and high expression is associated with the mutations in FLT3-ITD (10, 11). While its function in the normal immunohematopoietic system is poorly understood (12), CD33 is expressed on the myeloid series from

the progenitor to the most differentiated cells, including neutrophils, monocytes, and tissue resident macrophages (13). CD33 has been targeted clinically using antibodies and antibody–drug conjugates (ADC), and clinical trials using bispecific T-cell–engaging antibodies (BiTE) and CART cells are in progress (see later section).

CD123 is the IL3 receptor α chain, which is expressed on virtually all AML samples, across various cytogenetic and molecular risk groups, and on putative AML stem cells (14, 15). CD123 expression is higher in AML with FLT3-ITD mutation or NPM1 mutation compared with AML cells with no such mutation. This high expression was not described in t(8,21) AML (14). Given the role of IL3 signaling in hematopoietic development, it is not surprising that CD123 is also present on hematopoietic stem/progenitor cells as well as more mature populations and has been detected on small-caliber blood vessel endothelial cells (13). CD123 has been targeted clinically using antibodies, and clinical trials using ADC, BiTE, and CART are in progress (see later section).

CLEC12A, also known as c-type lectin-like molecule-1 (CLL1), is expressed in AML blasts as well as in putative leukemia stem cells (16). However, it is also found on some myeloid progenitors, monocytes and dendritic cells where it plays a dual role in dampening responses to dead cells and in antigen presentation (17, 18).

CD44v6, a splice isoform of CD44, is present on approximately 65% of AML samples at varying levels and is also found on normal monocytes and keratinocytes (19).

IL1 receptor-accessory protein (IL1RAP) is expressed on the majority of AML samples and particularly on those with adverse karyotypes, and high expression is associated with a poor prognosis (20). IL1RAP plays a role in inflammatory responses and is expressed on some normal myeloid cells.

FLT3 is a receptor tyrosine kinase with surface expression on AML, normal myeloid progenitors, dendritic cells, and brain.

Table 1. Potential targets in AML and treatment approach

	Antigen	Function	Involves LSC	Expression on blasts	Treatment approach ^a	
Surface	CD33	Transmembrane receptor	Yes	90%	mAb (60), ADC (10), BiTE (68), CART (86)	
	CD123	IL3 receptor α chain	Yes	100%	mAb (110), TsAb (111), BiTE (73), CART (75)	
	CLL1	Transmembrane receptor	Yes	77%–100%	mAb (112), BiTE (76), CART (113)	
	CD44v6	Type I transmembrane glycoprotein	Yes	65%	mAb (114), CART (19)	
	IL1RAP	Receptor for IL1 and IL33	Yes	80%	mAb (115), CART (116), BiTE (117)	
	FLT3	Receptor tyrosine kinase	Unresolved	30%	mAb (118), CART (76), BiTE (119)	
	CD64	Receptor for IgG	No	Variable (mostly APL)	ADC (120), bsAb (121)	
	CD13	Metalloprotease	Yes	44%	mAb (122), bsAb (123)	
	CD38	Cyclic ADP ribose hydroxylase	Yes	40%	mAb (124), CART (77)	
	FOLR2	Mediate folate delivery	No	70%	CART (125)	
	CD15	Adhesion molecule	No	50%	bsAb (126)	
	CD45	Tyrosine phosphatase enzyme	Yes (low)	100%	bsAb (127)	
	Lewis Y	Blood group antigen	No	50%	CART (84)	
	CD7	Transmembrane protein	No	30%	CART (128)	
	CD133	Transmembrane glycoprotein	No	60%	mAb (129)	
	CD98	Membrane transport protein	No	Variable	mAb (130)	
	CD25	Interleukin-2 receptor α	Yes	25%		
	CD32	Fc receptor	Yes	34%		
	Intracellular	WT1	Transcription factor	No	90%	BiTE (131), vaccines (132)
		PR1	HLA	No	50%	mAb (133), CART (134), vaccines (135)
hTERT		Telomerase length	Unresolved	80%	Vaccines (136)	
Survivin		Antiapoptotic	Yes	60%		
RHAMM		Receptor for hyaluronan-mediated motility	No	70%	Vaccines (137)	
OFA-iLRP		Stabilizes binding of laminin	Unknown	ND	Vaccines (138)	

Abbreviations: ADC, antibody–drug conjugate; APL, acute promyelocytic leukemia; BiTE, bispecific T-cell engager; bsAb, bispecific antibody; CLL1, c-type lectin-like molecule-1; DC, dendritic cells; FLT3, Fms-like tyrosine kinase-3; FOLR2, folate receptor β ; LSC, leukemic stem cells; mAb, monoclonal antibody; ND, no data; RHAMM, receptor for hyaluronan-mediated motility; TsAb, trispecific antibody; WT1, Wilms tumor 1.

^aA representative reference is provided for every treatment approach.

Constitutive activation of FLT3 is driven by mutations in intracellular domains and therefore only the domains with homology to wild-type FLT3 are accessible to antibody-based therapeutics.

Thus, at present, there are no known leukemia-specific cell surface antigens. While target discovery using unbiased proteomic and transcriptomic analyses of AML specimens could yet yield candidates that satisfy the abovementioned strict criteria for leukemia-specific targets, a recent analysis failed to reveal single candidate antigens and prompted the conclusion that only combinatorial approaches could lead to the required degree of specificity (21). As discussed below, engineered cell therapies such as CART cells could conceivably be used to achieve this degree of specificity.

Intracellular targets

Intracellular targets are more likely than surface antigens to be leukemia-specific. These include LAA including cancer-testis or oncofetal antigens, and the products of mutated genes associated with leukemia pathogenesis (leukemia-specific antigens, LSA). Although the landscape of pathogenic (disease-associated) mutations in AML has been well described (22), whether any of the resultant aberrant peptide products are immunogenic remains largely unknown, with only rare examples of FLT3-ITD or NPM1-specific immunologic responses (23). Furthermore, because AML has among the lowest mutational burdens in human cancer, the likelihood of nonpathogenic immunogenic passenger mutations leading to neoantigens is low (24). Nonetheless, because AML is responsive to T-cell-based immune alloreactivity, there clearly are some MHC-presented peptides capable of stimulating a specific antileukemic response. Most of these, at least in the setting of MHC-matched alloHCT, are minor histocompatibility antigens (peptides that are self to the recipient but foreign to the donor, usually due to single-nucleotide polymorphisms; ref. 25). An interesting recent study outlined MHC class I-restricted phosphopeptides as a new class of potential leukemia antigens, demonstrating strong T-cell responses to such antigens in normal donors but absent responses in patients with AML (26).

Among LAA, WT1, PR1, hTERT (human telomerase reverse transcriptase), survivin, RHAMM (receptor for hyaluronan-mediated motility), BCL-2 (B-cell lymphoma-2), OFA-iLRP (oncofetal antigen immature laminin receptor protein), and G250 have been shown to be recognized by CD8⁺ T cells and/or humoral immune responses (reviewed in ref. 27).

The WT1 gene is of particular interest, ranked as the top cancer target for immunotherapy by an NCI-convened panel (28). WT1 is a transcription factor participating in leukemogenesis that is overexpressed by most human AML (29) and mutated in a minority of AML. While WT1 is also expressed in some normal CD34⁺ cells, it has been the target of several different immunotherapeutic efforts (see below).

Thus, several intracellular peptides have been described as LAA or LSA and efforts are underway to develop immunotherapeutic approaches to target them.

Evidence for Immune System Dysfunction in AML

Somewhat paradoxically for an apparently immune-responsive malignancy, there is mounting evidence that AML is an immunosuppressive, or at least immunoevasive disease. Understanding of the mechanisms by which AML induces immune dysfunction is

mostly based on preclinical and *in vitro* data. However, compelling clinical data from patients relapsing after MHC-mismatched alloHCT implicate loss of the mismatched HLA haplotype in approximately 30% of cases. In some patients, this may be due to acquired uniparental disomy of chromosome 6p (on which reside the HLA genes). Loss of the mismatched HLA allowed leukemia cells to evade recognition by donor T cells (30, 31). This is an example of immunoeediting, whereby an otherwise effective immune response is frustrated by loss of a target antigen. Other studies have shown that the immune synapse between T cells and AML blasts is defective, likely due to impaired actin cytoskeleton formation, and this leads to decreased recruitment of signaling molecules to the immune synapse (7). AML has also been shown to evade immune attack by (i) expressing ligands for inhibitory receptors (checkpoint ligands), (ii) inducing a suppressive cellular microenvironment, and (iii) inducing a metabolically unfavorable microenvironment. The most important mechanisms for immune system evasion in AML are presented in Table 2.

Checkpoint ligands and receptors in human AML

Despite the unbridled enthusiasm with which immune checkpoint inhibitors (ICI) are being tested in a variety of malignancies, mechanistic or observational data supporting a major role for single receptor/ligand-inhibitory receptors in AML immunoevasion are relatively scant. ICI are more effective in malignancies with a high mutational burden, presumably because there are more neoantigens to present to polyclonal T cells. As noted above, AML has among the lowest mutational burdens in human cancer. However, the relative paucity of neoantigenic targets could be compensated for by the presence of minor histocompatibility antigens that are not driven by mutations (as explained above). When autologous T cells are cultured with AML cells, CTL-associated antigen 4 (CTLA-4) blockade has been shown to enhance the expansion of functional AML-specific T cells (32). The role of programmed death-ligand 1 (PD-L1) and related ligands is likely dependent on the presence of an inflammatory stimulus: whereas some groups have found PD-L1, B7, B7-H2, and other ligands to be expressed on primary AML blasts, other groups have not been able to recapitulate these findings (33, 34). When present, it is likely that PD-L1 is induced by exposure to IFN γ or toll-like receptor (TLR) stimuli, and thus may contribute to functional impairment of T cells in patients who relapse after immunotherapy such as alloHCT or in blasts exposed to T cells via a bispecific T-cell-engaging antibody (35–37). Other checkpoint ligands studied in AML are:

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), a type I membrane glycoprotein regulating T-cell response as well as innate immune cells. TIM-3 is upregulated on T cells (CD4⁺ and CD8⁺) from newly diagnosed AML patients and was correlated with FLT-ITD mutation and NCCN high-risk group (38). Preclinical data show that leukemic cells as well as host immune cells express galectin-9, a TIM-3 receptor leading to an exhausted T-cell environment with coexpression of TIM-3 and programmed death-1 (PD-1) on CD8⁺ T cells (39). Blockade of TIM-3 resulted in modest T-cell response and a combined TIM-3/PD-1 blockade led to a synergistic antitumor effect supporting further clinical research in this direction (NCT03066648).

T-cell immunoglobulin and ITIM domain (TIGIT) is a recently identified inhibitory receptor on CD8 T cells. TIGIT⁺ CD8 T cells are functionally impaired and their presence correlates with post-alloHCT relapse (40). Overexpression of the inhibitory ligand

Table 2. Immune dysfunction mechanisms in AML

Evasion mechanism	Target	Description	Reference
Immunoediting	HLA	AML cells acquire uniparental disomy of chromosome 6p, resulting in loss of HLA mismatch haplotype and escaping T-cell response	30
Checkpoint ligand and receptors: Upregulation of inhibitory receptors (CD28/B7 family receptors)	CTLA-4, PD-L1, PD-L2, PD-1	Upregulation of inhibitory checkpoints resulting in suppression of T and NK cells	35, 36, 139-142
Upregulation of inhibitory receptors (non-CD28/B7 family receptors)	LAG-3, TIM-3, TIGIT, BTLA, CD200, GITRL	Upregulation of inhibitory checkpoints resulting in suppression of T/NK cells and macrophages	38-40, 44, 143-145
Downregulation of stimulatory receptors	CD226 (DNAM-1), CD48	Downregulation of stimulatory signals, leading to dysfunction of T and NK cells	40, 146, 147
Suppressive microenvironment	Not defined	Increased frequency of circulating CD4 ⁺ CD25 ^{high} T cells (Treg) and enhanced suppression effect	45
	c-myc	AML cells induce MDSCs expansion by releasing extracellular vesicles containing c-myc leading to MDSC proliferation	48
	PARP-1	AML cells produce extracellular reactive oxygen species, triggering PARP-1-dependent apoptosis of T and NK cells	49
	NKG2A-expressing NK cells	AML cells induce upregulation of NKG2A expression, impairing NK cell effector function and cytotoxicity	148
Metabolically deficient microenvironment	IDO (tryptophan metabolism)	AML cells expressing IDO result in increase in CD4 ⁺ CD25 ⁺ T cells and act as Tregs	149
	Arginine	AML cells secrete arginase II, resulting in suppression of T-cell proliferation polarizing monocytes to a suppressive M2-like phenotype	55

Abbreviations: BTL, B- and T-lymphocyte attenuator; CTLA-4, CTL-associated antigen 4; GITRL, glucocorticoid-induced TNF receptor ligand; IDO, indoleamine 2,3 dioxygenase; MDSC, myeloid-derived suppressor cell; PARP-1, poly(ADP-ribose) polymerase 1; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; PD-L2, programmed death-ligand 2; TIGIT, T-cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3, transmembrane immunoglobulin mucin-3; Treg, regulatory T cells.

CD200 in AML has been shown to suppress memory T-cell function and confers a poor prognosis in multivariate analyses (41-43).

Inhibitory ligands belonging to the TNF receptor family have been found in AML. Surface expression of glucocorticoid induced TNFR-related protein (GITR) and its ligand (GITRL) was noted in AML cell lines and patients (44). Furthermore, these ligands were demonstrated to suppress NK-cell cytotoxicity and production of IFN γ .

The complex costimulatory and coinhibitory balance between AML cells and immune cells is presented in Fig. 1.

Suppressive cellular microenvironment

Regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC) can be recruited by cancer. In AML, there is an increased number of Tregs in the peripheral blood and bone marrow, with increased Treg numbers correlating with a poorer prognosis. Treg numbers decrease in remission and increase at relapse. Mechanistically, Tregs exhibit increased suppression compared with control healthy donor Tregs and appear to be induced by direct contact with AML blasts (6, 45, 46). Leukemia cells may produce adenosine via expression of ectonucleotides CD39 and CD73 thus suppressing effector T cells and supporting Treg function (47). Furthermore, MDSCs are increased in peripheral blood from patients with AML. MDSC expansion may be induced by tumor-derived extracellular vesicle-driven by the expression of MUC-1 (48). AML blasts, particularly monocytic subtypes, may act as MDSC and have been shown to directly inhibit T-cell responses (49-51). One mechanism described is by induction of reactive oxygen species by these leukemia cells leading to PARP-dependent apoptosis of T and NK cells (49). Furthermore, direct exposure of NK cells to blasts can lead to NK-cell dysfunction

including downregulation of activating receptors (52, 53) and exposure to chemotherapy further reduces the function of immune effectors, particularly NK cells (54).

Metabolically unfavorable microenvironment

Production of arginase II by AML blasts has been shown to induce T-cell immunosuppression directly and by polarizing surrounding monocytes to an immunosuppressive cell population. In addition, production of arginase II impairs the differentiation and proliferation of myeloid progenitors in the bone marrow, thereby producing the pancytopenia that characterizes AML (55). Indoleamine 2,3 dioxygenase (IDO) is of great interest as an enzyme that is upregulated in a variety of cancer to deplete environmental tryptophan, thereby impairing effector T-cell proliferation (56). In AML, IDO overexpression results in effector T-cell dysfunction and conversion of T cells to Tregs, as well as poor survival in multivariate analyses (57).

Immunotherapies in AML

Antibody-based approaches

Antibodies can be used to recruit immune effector mechanisms via their Fc domain (antibody-dependent cellular cytotoxicity, ADCC; antibody-dependent cell phagocytosis, ADCP), via a bispecific targeting domain (bispecific or trispecific T-cell or NK-cell engagers); or by delivering a toxic payload into target cells after receptor-mediated internalization (ADCs or radio-nuclide conjugates). The latter group is not strictly speaking immunotherapies, have been reviewed elsewhere, and will not be discussed here (58).

While in ALL the addition of rituximab to chemotherapy has been shown to improve event-free survival (59), in AML, naked

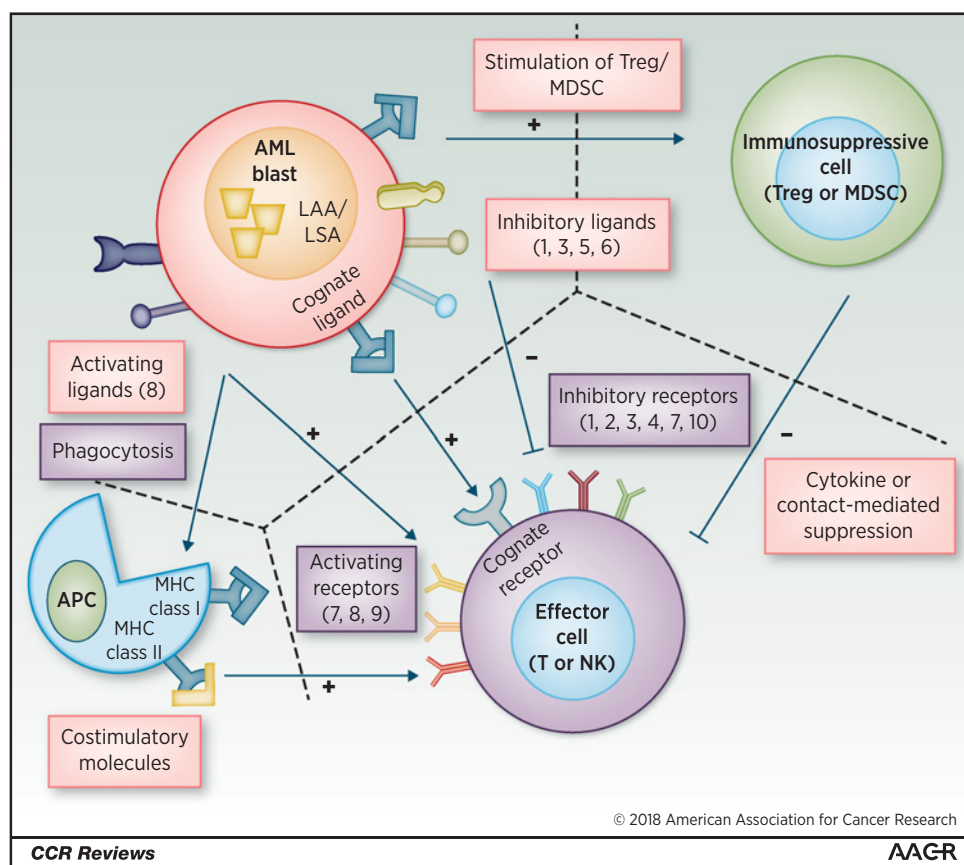


Figure 1.

Costimulatory and coinhibitory pathways described in AML. The regulation of T-cell response to antigens is mediated by ligand–receptor interactions between T cells and antigen-presenting cells (APC). These interactions require costimulatory signals and can induce inhibitory signals (both known as immune checkpoints) that modulate the extent of immune response and effector cell–mediated killing. T-cell recognition is based on the interaction between T-cell receptors (TCR) on T cells and MHC on tumor cells or APCs (cognate ligand–receptor interaction). Costimulatory signals as well as coinhibitory signals are then produced, leading to a net immunostimulatory or immunoevasive effect. Regulatory T cells (Treg) and/or myeloid-derived suppressor cells (MDSC) are induced in the AML microenvironment and lead to suppression of effector cells and APC in a contact-dependent or -independent approach (e.g., secretion of inhibitory cytokines such as IL4, IL10, or TGF β or by scavenging of activating cytokines such as IL2). AML cells can shift the immune balance via expression of immune checkpoint proteins, thus inducing immune resistance and tumor survival: 1, CD80 and CD86 (on AML cells) interact with CTLA-4 (on T cells), and PD-L1 and programmed death-ligand 2 (PD-L2; on AML cells) interact with PD-1 (on T cells). Furthermore, chemotherapy along with IFN γ can cause upregulation of PD-L1 expression on AML cells. 2, TIM-3 is highly expressed on T cells from patients with AML. AML cells expressing TIM-3 ligand (galectin-9) and PD-L1 lead to defective T-cell antitumor immunity. 3, TIGIT expression on CD8 $^{+}$ T cells interacts with CD155 on AML cells and is inversely associated with expression of the CD226 (DNAM1) costimulatory receptor on CD8 $^{+}$ T cells, leading to T-cell dysfunction. 4, B- and T-lymphocyte attenuator (BTLA) and PD-1 are highly expressed on CD8 $^{+}$ T cells following alloHCT. 5, CD200 is upregulated on AML cells and compromises T-cell response. 6, GITRL is highly expressed on AML cells and interacts with GITR on NK cells. 7, CD226 is downregulated on NK cells as well as T cells from patients with AML. 8, AML oncogenic fusion proteins can downregulate the expression of the NK-cell ligand CD48 and its receptor 2B4 on NK cells, resulting in reduced killing by NK cells. 9, NK cells from patients with AML demonstrate reduction in NKG2D (activating receptors) and increase in NKG2A (inhibitory receptors). 10, OX40 is expressed on AML cells and upon ligand interaction impairs NK-cell reactivity.

mAbs directed against CD33 or CD123 have not been particularly effective as monotherapies or when combined with chemotherapy against AML (60, 61, 62). The reasons for this disparity remain unclear, and could include the nature of the antigens targeted, characteristics of the Fc domain, or the immunoevasive mechanisms described above (63, 64).

The impressive results and nonoverlapping toxicity profile of the anti-CD19/CD3 BiTE blinatumomab in B-ALL have stimulated the development of anti-AML–bispecific constructs in a variety of formats (65). These constructs remain investigational and clinical results have not yet been published in the peer-reviewed literature. AMG-330 is an anti-CD33/CD3 T-cell engag-

ing construct with a similar format to blinatumomab. Preclinical studies indicate that the activity of AMG-330 is dependent more on drug concentration and T-cell numbers than on the level of expression of CD33, is particularly active in favorable-risk AML specimens, and is susceptible to immunoevasive resistance mechanisms such as Tregs and upregulation of PD-L1 on blasts (37, 66, 67). AMG-330 is currently under clinical investigation (NCT02520427). The low molecular weight of BiTEs leads to renal excretion and the need for continuous infusion, which can be logistically challenging. This could potentially be circumvented by the use of bispecific tandem diabodies that are larger thus exceeding the renal clearance threshold, and that have

increased valency and avidity. CD3/CD33 tandem diabodies have been tested preclinically (68). NK cells can be recruited using an anti-CD16/CD33 construct, and their function further enhanced by adding an IL15 crosslinker (69, 70).

Several CD123-targeting constructs are currently in clinical trials. Flotetuzumab is an anti-CD123/CD3 dual affinity retargeting molecule (DART) that induces cytokine release syndrome (CRS) and transient cytopenias at the highest dose tested in nonhuman primates (71) and is currently being tested in a phase I trial (NCT02152956). Patients receive 28 day cycles of a 4-day on/3-day off schedule or a continuous 7-day schedule with step-up dosing to mitigate CRS. According to a 2017 Meeting Abstract, antileukemic activity was documented in 8 of 14 evaluable patients [of whom 3 achieved a CR, 1 CRi, 1 morphologic leukemia free state (MLFS), and 1 PR]. Drug-related adverse events of grade 3 or higher were noted in 20 of 45 patients and the most common toxicity was CRS (72). Again, early correlative data implicate PD-L1 upregulation on blasts exposed to redirected T cells as a mechanism of treatment failure (73). Another construct, an anti-CD123/CD3 duobody, is currently being tested in a phase I clinical trial (NCT02715011).

T cells can also be redirected to CLEC12A, with nonhuman primate data showing transient depletion of late myeloid cells in the bone marrow after a single dose (74). A clinical trial of an anti-CLEC12A/CD3-bispecific construct is underway (NCT03038230).

CART cells

CAR-redirection T cells utilize antibody-like recognition domains along with in-built costimulation to redirect the effector functions of T cells to surface targets of choice. Anti-CD19 CART cells engender high response and survival in patients with relapsed/refractory ALL (8, 9). Because there are no AML-specific surface antigens, it is likely that CART cells that are powerful enough to eradicate malignant myeloid cells will also eradicate normal myeloid cells (75, 76). Preclinical studies have been published targeting CD33, CD123, CD44v6, CLEC12A, CD38, and FLT3 (75, 19, 77–80). Strategies that can selectively increase expression of the target molecule on malignant cells are of particular interest. In this context, small-molecule inhibition of FLT3 signaling in FLT-ITD-mutated AML appears to synergize with anti-FLT3 CART cells, partly by increasing surface expression of the target molecule (76). A possible approach for administering CD123 CART cells with acceptable toxicity is to deplete these T cells at an optimal time point after AML eradication followed by rescue stem cell transplantation or to use a CAR that could be switched on and off such as the universal-recognition CAR T cells (81, 82). A novel spin on CART-cell technology is genetic engineering of T cells to produce and secrete bispecific T-cell-engaging antibodies (83). In this article, T cells were generated to secrete CD123/CD3-bispecific engager molecules recognizing AML cells, resulting in regression of AML in mouse models. Furthermore, a CD20 suicide gene was introduced to allow elimination of these T cells upon infusion of rituximab. Although an interesting strategy, it is unlikely that this approach will significantly enhance the therapeutic window of CART cells.

The first clinical trial of CART cells in AML targeted the carbohydrate antigen Lewis-Y (Le-Y; ref. 84). Four patients received CART cells at a median dose of 1.1×10^9 . The best response was stable disease ($n = 2$), transient blast reduction ($n = 1$), and transient cytogenetic response ($n = 1$). The infusion was well tolerated and T cells persisted up to 10 months. Single patient case

reports targeting CD33 or CD123 have been published, showing transient activity with tolerable toxicity (85). We performed a pilot study using serial infusions of "biodegradable" T cells electroporated with anti-CD123 CAR mRNA in 5 patients with refractory/relapsed AML. These patients experienced fever and cytokine release syndrome, but no antileukemia effect was demonstrated. While there could be several reasons for this observation, it is likely that this was related to poor T-cell quality and lack of persistence (86). Of great interest is a current clinical trial that has been reported in abstract form. Six patients with relapsed AML post alloHCT received $50\text{--}200 \times 10^6$ CD123 CAR T cells manufactured from their donor. The toxicities reported were reversible and manageable, with no treatment-related deaths. CRS was seen in 5 patients. The patients attained an MRD-positive CR at day 28 ($n = 1$), MRD-negative CRi at day 14 ($n = 1$), MLFS ($n = 1$), SD ($n = 2$), or PD ($n = 1$). An additional patient was treated for blastic plasmacytoid dendritic cell neoplasm (BPDCN), a disease with high expression of CD123. This patient received autologous CD123 CART cells without toxicity and remained in CR 60 days after infusion (87). In another study, 6 patients with MDS/AML were treated with a single infusion of escalating doses of NKG2D-bearing autologous T cells with poor persistence, no CRS, and without antitumor activity (88). However, in a recent study, a 52-year-old male with relapsed AML was treated with NKG2D-bearing autologous T cells (CYAD-01) infusions at initial dose level of 3×10^8 cells/injection every 2 weeks for 3 administrations with no lymphodepleting chemotherapy (89). Treatment was well tolerated and resulted in MLFS at day 28 postinfusion, resolution of symptoms and following alloHCT is currently in CR 6 months posttransplant and 9 months postinfusion of CYAD-01.

As discussed, CART cells can only recognize surface-expressed targets, which limits the ability to target LAA or LSA. Recently, the intracellular oncoprotein WT1 was shown to be targetable using an scFv that recognizes its immunodominant peptide RMPNAPYL in the context of the HLA-A2 molecule. This scFv could be used as a mAb or as a CAR targeting domain to preclinically target WT1-overexpressing HLA-A2-expressing AML in a specific manner (90, 91).

Vaccines

Cancer vaccines have the potential to induce host immune effector cells to recognize tumor antigens to which they were previously tolerized by providing an appropriate antigen presentation signal. AML vaccine strategies have included single antigen-based approaches, dendritic cells loaded with curated LAA, or AML blasts that were fused with or differentiated into antigen-presenting cells (APC). Most published studies have shown immune responses and in a few cases there was evidence of a clinical effect (92–95). However, vaccines are likely to be more effective in patients who achieve a complete remission after prior chemotherapy.

A personalized leukemia vaccine in which patients' AML blasts were fused with autologous dendritic cells was serially injected into 17 patients who had achieved remission to chemotherapy. T cells were demonstrated to infiltrate the site of vaccination, there was an increase in circulating T cells with a detectable immunologic response to both whole AML cells and to LSA, and these cells persisted for at least 6 months. Of the 17 patients, 12 remained disease-free at a median follow up of 57 months, suggesting that this approach could protect against relapse (96).

Table 3. Current immunotherapy clinical trials in AML

Single agents	Identifier	Phase	Condition	Target	Treatment	Status	Comments
Vaccines	NCT03083054	I/II	High-risk MDS and AML with no blasts in peripheral blood	WT1	Vaccination with autologous WT1 mRNA-electroporated DCs	Recruiting	
	NCT01686334	II	AML in remission at high risk for relapse	WT1	Vaccination with autologous WT1 mRNA-electroporated DCs	Recruiting	Randomized
	NCT02405338	I/II	AML in remission	WT1/PRAVE	DC vaccination	Active, not recruiting	Active, not recruiting
	NCT01842139	I	AML in remission	WT1	WT1 vaccine	Active, not recruiting	Randomized
	NCT01734304	I/II	AML in remission	LAA	Vaccination with autologous DCs transfected with RNA encoding LAA	Recruiting	
	NCT00809250	I	High-risk MDS or AML after HSCT	LAA	Vaccination with leukemia cell vaccines (GM-K562)	Active, not recruiting	
	NCT01773395	II	High-risk MDS or AML after HSCT	LAA	Vaccination with leukemia cell vaccines (GVAX)	Recruiting	Randomized
	NCT01146262	I/II	AML in remission	Leukemic apoptotic corpse	Vaccination with leukemic apoptotic corpse autologous pulsed DCs	Active, not recruiting	
	NCT02715011	I	R/R AML	CD123+CD3	JNJ-63709178 (CD123 × CD3 duobody)	Recruiting	
	NCT03038230	I	AML	CLEC12A+CD3	MCLA-117 (CLEC12A × CD3 bispecific antibody)	Recruiting	
Antibodies (nonconjugated)	NCT02730312	I	R/R CD123-expressing hematologic malignancies	CD123+CD3	XmAb14045 (CD123 × CD3 bispecific antibody).	Recruiting	
	NCT02152956	I	R/R AML or intermediate-2/high-risk AML	CD123+CD3	MGD006 (CD123 × CD3 bispecific antibody)	Recruiting	
CART cells	NCT02520427	I	R/R AML	CD33+CD3	AMG330 (CD33 × CD3 bispecific antibody)	Recruiting	
	NCT02159495	I	AML	CD123	CART-123 (costimulatory domain-CD28, target-CD123)	Recruiting	
	NCT03114670	I	Relapsed AML post-alloHSCT	CD123	CART-123 (costimulatory domain-4-1BB, target-CD123)	Recruiting	
	NCT03190278	I	AML	CD123	UCART-123 (target-CD123)	Recruiting	
	NCT02799680	I	R/R AML	CD33	CART-33	Recruiting	
	NCT01864902	I-II	R/R AML	CD33	CART-33 (costimulatory domain-4-1BB, target-CD33)	Unknown	
	NCT03126864	I	R/R AML	CD33	CART-33	Recruiting	
	NCT02203825	I	AML/MDS/MM	NKG2D-ligand	GM-CSF T cells (costimulatory domain-DAP-10, target-NKG2D-ligand)	Active, not recruiting	
	NCT03222674	I/II	AML	Muc1/CD33/CD38/CD56/CD117/CD123	CART targeting: Muc1/CD33/CD38/CD56/CD117/CD123	Recruiting	
	NCT03291444	I	AML/ALL/MDS	CD33/CD38/CD56/CD117/CD123/CD34/Muc1	CAR T CELLS (CD33, CD38 CD56, CD117, CD123, CD34 and Muc1) combined with Eps8 peptide-specific DC	Recruiting	
	NCT02742727	I/II	CD7 ⁺ leukemia and lymphoma	CD7	Anti-CD7 CAR-pNK cells	Recruiting	
Checkpoint inhibitors	NCT02532331	II	AML in remission at high risk for relapse	PD-1	Nivolumab	Recruiting	
	NCT02275533	II	AML in remission, non-transplant candidates	PD-1	Nivolumab	Recruiting	Randomized
	NCT01757639	I	R/R high-risk MDS and AML with MRD	CTLA-4	Ipilimumab	Active, not recruiting	

(Continued on the following page)

Table 3. Current immunotherapy clinical trials in AML (Cont'd)

Identifier	Phase	Condition	Target	Treatment	Status	Comments
NCT01822509	I	Relapsed hematologic malignancies after HSCT	CTLA-4/PD-1	Ipilimumab or nivolumab	Recruiting	
NCT02708641	II	AML in remission, non-transplant candidates	PD-1	Pembrolizumab	Recruiting	
NCT01096602	II	AML in remission	PD-1	Blockade of PD-1 combined with the DC/AML vaccine	Active, not recruiting	
NCT03059485	II	AML in remission	PD-L1	DC/AML fusion cell vaccine and durvalumab	Recruiting	Randomized
NCT02750995	I	High-risk MDS and AML initially responding to azacitidine	-	Peptide vaccination in combination with azacitidine	Recruiting	
NCT03066648	I	AML or high-risk MDS	PD-1/TIM-3	PDR001 and/or MBG453 combined with decitabine	Recruiting	Randomized
NCT03390296	II	AML	OX40/41BB/PD-L1/CD33	OX40 agonist mAb, anti-41BB mAb, anti-PD-L1 mAb, smoothened inhibitor, anti-CD33 mAb, and azacitidine as single agents and/or combinations	Recruiting	
NCT02892318	I	AML	PD-L1	Guadecitabine (DNA methyltransferase inhibitor) combined with atezolizumab	Recruiting	
NCT02771197	II	AML in remission	PD-1	Autologous HSCT followed by pembrolizumab	Recruiting	
NCT02768792	II	R/R AML	PD-1	Pembrolizumab and high-dose cytarabine	Recruiting	
NCT02845297	II	AML	PD-1	Pembrolizumab and 5-azacitidine	Recruiting	
NCT02996474	I/II	R/R AML	PD-1	Pembrolizumab and decitabine	Recruiting	
NCT02846376	I	AML/MDS with high risk of relapse	PD-1/CTLA-4	AlloHSCT followed by nivolumab, ipilimumab, or the combination of nivolumab with ipilimumab	Recruiting	
NCT02890329	I	R/R MDS or AML	CTLA-4	Ipilimumab in combination with decitabine	Recruiting	
NCT02397720	II	R/R AML or newly diagnosed older AML patients	PD-1/CTLA-4	Nivolumab and 5-azacitidine or nivolumab and 5-azacitidine and ipilimumab	Recruiting	
NCT02464657	I/II	High-risk MDS and AML	PD-1	Nivolumab and idarubicin and cytarabine	Recruiting	Randomized
NCT02775903	II	High-risk MDS and AML	PD-L1	Durvalumab and 5-azacitidine	Active, not recruiting	
NCT02953561	I/II	R/R AML	PD-L1	Avelumab and 5-azacitidine	Recruiting	

Abbreviations: ALL, acute lymphoblastic leukemia; DC, dendritic cell; MDS, myelodysplastic syndrome; MM, multiple myeloma; R/R, relapsed/refractory.

In a different approach, patients in remission received a multi-valent WT1 peptide vaccine designed to stimulate T-cell response to a variety of potential MHC class I and class II epitopes. Because WT1 is a self-antigen, the vaccine contained a single amino acid substitution designed to circumvent existent immune tolerance towards the native WT1 sequence (97). Nine of 14 tested patients had an immune response, and the median disease-free survival from the time of achieving a chemotherapy remission was 16.9 months.

T cells with known (engineered or expanded) T-cell receptor specificity

T cells with specificity toward a known LSA or LAA can be (i) enriched by repetitive stimulation against the target antigen (the "TIL" approach) or (ii) transduced with a construct encoding the alpha and beta chain of a known, high-affinity T-cell receptor (TCR) against the target antigen (98, 99). As mentioned earlier, the ideal TCR target would be a LSA required for oncogenesis. However, such neoantigens are rare (100, 101). In a pilot trial, 11 patients with or at high risk of posttransplant relapse received HLA-A*0201-restricted WT1-specific CD8 T-cell clones from their alloHCT donor. There was no graft versus host disease (GVHD) or evidence of other on-target toxicity. Among the first 7 patients, there was some indication of modest leukemia control but poor T-cell persistence. The latter four patients on this trial received WT1-specific T cells that had been expanded in IL21 and therefore had a more favorable memory phenotype. These T cells showed long-term persistence, and the patients remained in remission 8–30 months after the first T-cell infusion (22–38 months after alloHCT; ref. 102). T cells enriched for WT1 recognition (alone or in combination with other LAA such as PRAME, surviving and NY-ESO-1) are in clinical trials (NCT00620633, NCT02494167).

T cells engineered to express a high avidity WT1-specific TCR have been shown to have antileukemic activity preclinically, and clinical trials are underway (NCT01621724, NCT02770820; ref. 103).

Inhibitory receptor ("immune checkpoint") blockade

A recent phase I study assessed the CTLA-4 antibody ipilimumab in relapsed hematologic malignancies after allogeneic trans-

plantation. Among 12 patients with AML, CR was achieved in 4 patients with extramedullary disease and in one patient with secondary AML, and as expected this immune response was accompanied by GVHD (104). There are several phase I studies assessing Ipilimumab in relapsed AML and high-risk MDS with or without demethylating agents (NCT02890329, NCT01757639).

A small phase I study demonstrated that use of pidilizumab (at the time thought to block PD-1) led to a reduction of circulating blasts from 50% to 5% in one of 8 AML patients treated (105). Pembrolizumab and nivolumab are being tested in patients relapsing after alloHCT or at high risk of relapse after chemotherapy (NCT02981914, NCT02532231). The true potential of ICI may be achieved in rational combination with therapies that can induce upregulation of LAA.

Current immunotherapy clinical trials in AML are listed in Table 3, and a breakdown of the single and multimodality approaches is illustrated in Fig. 2, based on trials listed on www.clinicaltrials.gov.

Future directions: toward rational combination therapies

Compared with cytotoxic chemotherapy, epigenetically active agents and targeted small-molecule inhibitors, immunotherapy approaches have nonoverlapping toxicity and efficacy profiles. Thus, future directions should combine the various tools that have been developed against AML in the last few years.

Hypomethylating agents (HMA) are being combined with ICI in clinical trials. Epigenetic modifying agents have been shown to enhance LAA expression, antigen presentation and perhaps T-cell cytotoxicity (106), and patients treated with HMA demonstrate upregulation of PD-L1, PD-L2, PD-1, and CTLA-4 (107). A phase II trial presented recently in abstract form combined 5-azacytidine and nivolumab (108). Among the 53 patients treated, 11 (21%) achieved CR/CRi, and 7 (14%) had hematologic improvement with overall response rate of 35%. The responses were durable with 82% of patients alive at 1 year after censoring for alloHCT. Other combinations of HMA with ICI are underway (NCT02397720, NCT03092674, NCT02890329, NCT02953561,

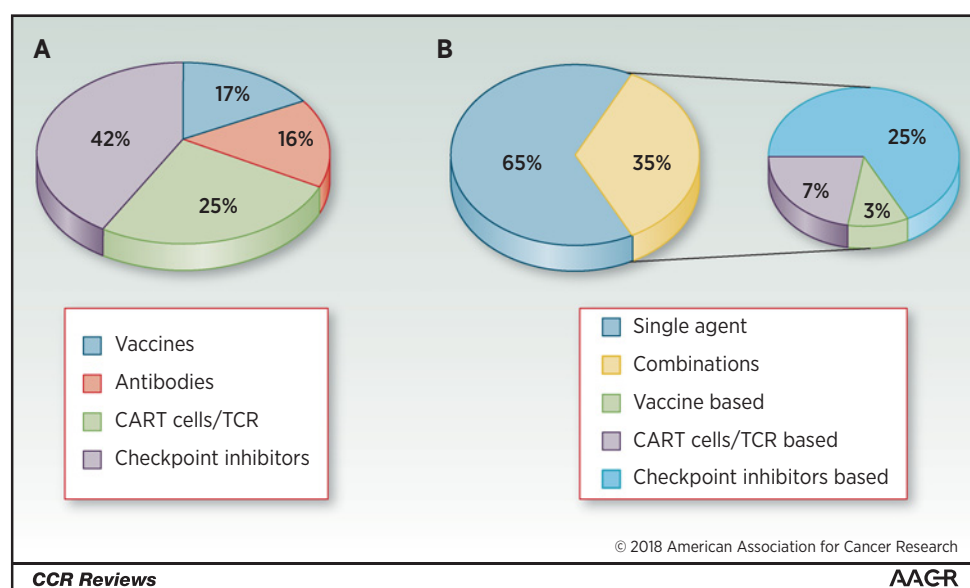


Figure 2. Presentation of the immunotherapy approaches based on the clinical trials. The clinicaltrials.gov website was reviewed for immunotherapy trials ongoing in AML treatment. **A**, Breakdown of the most widely studied immune approach is presented as pie chart. Checkpoint inhibitors are the most widely studied followed by CART/TCR-based vaccines and antibodies. **B**, Single and multi-modality approaches are illustrated. Single agents are the most commonly studied compared with combination treatment. Furthermore, combination treatments are mostly based on checkpoint inhibitors.

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NCT02775903), as is a trial investigating LAA peptide vaccines in combination with azacytidine (NCT02750995).

As noted above, T-cell-mediated inflammation can lead to upregulation of PD-1 ligands on blasts and thereby mediate adaptive resistance. In this context, a preclinical study showed that blockade of the PD-1/PD-L1 interaction augmented killing of AML blasts by AMG-330 (37), suggesting that BiTE or even CART should be tested in combination with PD-1 blockade (as is already being done in B-ALL). However, these studies will have to be done carefully due to the risk of increased toxicity.

Conclusions

AML is an aggressive and devastating disease that shows initial response to chemotherapy but if not eradicated in the first attempt becomes increasingly resistant to treatment. The growing immunotherapeutic armamentarium markedly expands options but should be employed judiciously, cautiously, and in the right setting. Chemotherapy induces functional and numeric changes in immune effector cells and may impede the collection and manufacturing of high-quality T cells for adoptive transfer (109), thus consideration should be given to the optimal timing and sequence of immunotherapy *vis a vis* chemotherapy. Vaccines are well tolerated but likely to be effective only in patients in apparent remission, as they do not induce a potent T-cell response. Antibody-based T-cell redirection strategies such as bispecific anti-

bodies or CART cells can generate powerful responses in B-ALL that will likely be reproducible in AML. However, these approaches suffer from the lack of truly AML-specific surface antigens and therefore will have to be carefully deployed. TCR-based approaches have the potential for exquisite antigen specificity but are hampered by the need for HLA restriction, limiting widespread applicability to patients of diverse HLA haplotypes and ethnic backgrounds. Furthermore, the above approaches remain potentially susceptible to adaptive resistance by AML blasts using a variety of escape strategies that include recruitment of immunosuppressive Tregs or MDSC, creation of a metabolically unfavorable microenvironment, or upregulation of inhibitory ligands. Thus, the potential for eradicating AML lies in rational combinations of immunotherapies with strategies to mitigate adaptive resistance by AML.

Disclosure of Potential Conflicts of Interest

S. Gill reports receiving commercial research grants from Novartis, is listed as inventor on intellectual property related to chimeric antigen receptor T-cell therapy that is licensed to Novartis and Tmunity, holds ownership interest (including patents) in CARMA Therapeutics, and is a consultant/advisory board member for Extellia. No potential conflicts of interest were disclosed by the other author.

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