REVIEW ARTICLE

Blood Science

OPEN

Immune therapy: a new therapy for acute myeloid leukemia

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Abstract

Although complete remission could be achieved in about 60%–70% of acute myeloid leukemia (AML) patients after conventional chemotherapy, relapse and the state of being refractory to treatment remain the main cause of death. In addition, there is a great need for less intensive regimens for all medically frail patients (both due to age/comorbidity and treatment-related). Immune therapy anticipates improved prognosis and reduced toxicities, which may offer novel therapeutic rationales. However, one of the major difficulties in developing immune therapies against AML is that the target antigens are also significantly expressed on healthy hematopoietic stem cells; B-cell malignancies are different because CD20/CD19/healthy B-cells are readily replaceable. Only the anti-CD33 antibody-drug conjugate gemtuzumab-ozogamicin is approved by the FDA for AML. Thus, drug development remains extremely active, although it is still in its infancy. This review summarizes the clinical results of immune therapeutic agents for AML, such as antibody-based drugs, chimeric antigen receptor therapy, checkpoint inhibitors, and vaccines.

Key Words: Acute myeloid leukemia; Antibody-based drugs; Checkpoint inhibitors; Chimeric antigen receptor therapy; Vaccine

1. INTRODUCTION

Acute myeloid leukemia (AML) is a genetically, epigenetically, and clinically heterogeneous disease. Responses occur in only 50%–60% of patients, with the rest in a state of relapse or refractory to conventional treatment; these die from progressive disease. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the best treatment option for high-risk AML patients; however, it is not appropriate for every patient, especially elderly individuals. Immunotherapy-based approaches have the promise of greater response rates and lower toxicity (allowing more patients to be more fit and eligible for transplant) and of lower rates of minimal residual disease (MRD) (factors associated with decreased rates of post-transplant relapse). The emergence of immune therapy greatly improves the prognosis of lymphoma and myeloma patients and even substitutes for chemotherapy in B-cell lymphoid malignancies.[1–4] Thus, many newly developed immune agents are in clinical trials expecting to achieve better response rates in AML.

There are 3 main difficulties in developing immune therapies against AML. First is lack of a specific AML surface antigen. It is still a challenge to find an antigen target that is essential to AML biology and selectively expressed on malignant cells. For example, chimeric antigen receptor (CAR)-T therapy has been successful in lymphatic hematological malignancies (such as acute lymphoblastic leukemia and diffuse large B-cell lymphoma) and multiple myeloma (eg, targeting CD19, CD22, and BCMA); owing to the lack of specific AML surface antigens to avoid killing normal hematopoietic cells, its application in AML is still limited. The second relates to tumor heterogeneity. The antigens could decrease or lose their expression during treatment, resulting in insensitivity to treatment. The third is that the bone marrow microenvironment is complex. Bone marrow stromal cells, macrophages, and myeloid-derived suppressor cells (MDSCs) can protect AML cells from toxic effects through various mechanisms. Therefore, until now, only the anti-CD33 antibody-drug conjugate (ADC) gemtuzumab-ozogamicin (GO) is approved by the FDA for AML.

Combinations of immunotherapy and chemotherapy are still in the early stages of clinical trials, and these may benefit AML patients. Immune agents induce AML cell death via antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity, and innate immune system activation. This study reviews the immune mechanisms and clinical trials regarding immunotherapy in AML, including antibody-based drugs, chimeric antigen receptor therapy, checkpoint inhibitors, and vaccines (**Fig. 1**).

2. CURRENT TREATMENT FOR AML

Briefly, the treatment of AML consists of remission induction and post-remission therapy. For induction therapy, a "7+3" therapy is recommended for newly diagnosed patients. For patients older than 60 years, the best chemotherapy remains to be identified. When it comes to post-remission treatment,

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Conflict of interest: The authors declare that they have no conflict of interest.

C.T. is supported by Grant ZC20171 from Tianjin Health Science and Technology Project and Natural Science Foundation of Xinjiang Autonomous Region (2022D01A09).

C.T. and Z.C. drafted the article. C.T. critically revised the article. All authors approved all versions including the final version and are responsible for the accuracy and integrity of all aspects of the article.

Blood Science (2023) 5, 15–24

Received August 21, 2022; Accepted October 18, 2022.

http://dx.doi.org/10.1097/BS9.0000000000000140

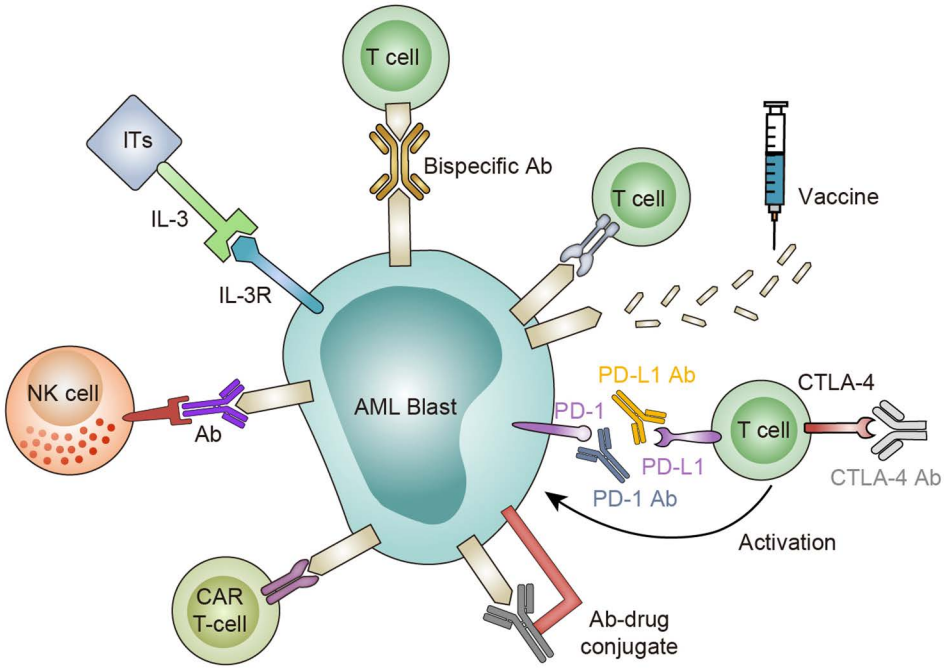

Figure 1. Schematic mechanism between immunotargeted drugs and AML cells. Ab = antibody, AML = acute myeloid leukemia, CAR = chimeric antigen receptor, CTLA-4 = cytotoxic T-lymphocyte-associated protein 4, IL-3 = interleukin-3, IL-3R = interleukin-3 receptor, NK = natural killer, PD-1 = programmed cell death 1, PD-L1 = programmed cell death ligand-1.

including consolidation and maintenance therapy, risk stratification should be taken into account. AML patients are categorized into high-risk, medium-risk or low-risk groups based on cytogenetic, molecular, and clinical characteristics. For younger patients, high-dose cytarabine is recommended for patients with favorable cytogenetics; while for those with an adverse prognosis, allo-HSCT should be performed.

3. SELECTED TARGETS IN AML

The ideal target in AML should be highly expressed on leukemic cells, but not expressed in normal tissues, resulting in more effective treatment with decreased morbidity and mortality. CD33 and CD123 are identified as suitable and feasible targets for immunotherapy in AML, since they are expressed on 90% of AML cells and are important growth and differentiation receptors for early leukemic stem cells (LSCs).[5] Human C-type lectin-like molecule-1 (CLL-1) is also an attractive target because of its overexpression in LSCs and AML blasts across different types of AML.[6]

4. METHODOLOGY

A comprehensive PubMed search was performed to identify clinical studies of AML and antibody-based drugs, vaccines, and checkpoint inhibitors. Articles were classified based on the general type of immune approach to treat AML. This is a systematic review with a methodological development to obtain the data (compilation of studies, specification of variables, cataloguing, and analysis). The inclusion criteria were patients older than 15 years, with newly diagnosed or relapsed AML. The sole exclusion criterion was pediatric AML.

5. ANTIBODY-BASED DRUGS

5.1. Monoclonal antibodies

5.1.1. CSL360 CSL360 is a recombinant, chimeric immunoglobulin G1 (IgG1), anti-CD123 monoclonal antibody (MoAb), which demonstrates anti-leukemic activity in vitro. However, a multicenter, dose-escalating, nonblind phase I clinical trial (NCT00401739) to assess the pharmacokinetics and safety among high-risk or refractory/relapsed (R/R) AML cases revealed that although CSL360 was well tolerated, it did not induce anti-leukemic activity in most patients, indicating that MoAb blockade of CD123 function was insufficient as a therapeutic strategy.[7]

5.1.2. CSL362 CSL362, a fully humanized CD123-neutralizing MoAb containing a modified Fc structure, has been found to activate donor-derived natural killer (NK) cells to effectively lyse leukemic cells through the ADCC pathway. It was reported that CSL362 could reduce leukemic cell growth in AML xenograft mouse models.[8] CSL362 extended the survival of cytarabine-/daunorubicin-treated mice with AML xenografts, while augmentation of NK-cell-deficient NSG mice with adoptively transferred human NK cells improved survival against a single xenograft.[9] Another in vitro study demonstrated that CSL362 potently induced ADCC of AML blasts including CD34+CD38–CD123+ LSCs by NK cells. Importantly, compared with healthy donor (HD) NKs, NKs drawn from AML patients in remission had a comparable ADCC activity against leukemic cells; of note, during remission, the number of immature NKs in AML patients was 5 time greater than in HDs.[10] However, there are no ongoing clinical trials of CSL360 or CSL362 because of toxicity.

5.1.3. Talacotuzumab Talacotuzumab (TAL, JNJ-56022473) is an anti-CD123 IgG1 MoAb, which has been shown to induce potent in vitro ADCC against AML blasts/LSCs and to reduce leukemic cell growth in murine xenograft models.[11] A multicenter, phase II/III study confirmed the recommended dose of talacotuzumab to be 9 mg/kg. However, talacotuzumab combined with decitabine showed no improvement in efficacy, resulting in the early termination of enrollment and discontinuation of talacotuzumab treatment.[12]

5.1.4. Cusatuzumab It has been reported that LSCs upregulate the tumor necrosis factor (TNF) family ligand CD70 in

response to treatment with hypomethylating agents (HMAs), resulting in CD70/CD27 activation. Blocking CD70/CD27 interaction with cusatuzumab, a human CD70 MoAb with enhanced ADCC activity, eliminates LSCs in vitro and in xenotransplantation models. A phase I/II trial in previously untreated older AML patients given cusatuzumab combined with azacitidine (NCT03030612) found that cusatuzumab substantially reduced LSCs and triggered gene signatures related to myeloid differentiation and apoptosis.[13]

5.1.5. Lintuzumab Lintuzumab (HuM195) is an unconjugated humanized murine MoAb directed against the cell surface myelomonocytic differentiation antigen, CD33. The addition of lintuzumab to salvage induction chemotherapy was safe but did not result in a statistically significant improvement in response rate or survival in patients with R/R AML.[14] Another study also revealed that HuM195 was well tolerated, except for infusion-related fevers and chills, which were the predominant side effects seen.[15]

5.2. Antibody-drug conjugates

ADCs are antibodies combined with chemotherapy agents or radioactive particles to specifically target tumor cells with the dual functions of efficiency and specificity. Currently, GO has been approved by the FDA, and many ADCs are ongoing in clinical trials, which are providing promising prospects as summarized in Table 1.

5.2.1. Anti-CD33 ADC GO, an IgG4 ADC, is an immunoconjugate between CD33 and calicheamicin; it was approved by the FDA in 2000 for relapsed AML patients aged over 60 years but was forced to be withdrawn in 2010. In 2011, an open-label trial recruited 1113 patients younger than 60 years for treatment with GO (3 mg/m^2) on day 1 of an induction course. The trial revealed significant survival benefit for patients with favorable cytogenetics, no benefit for patients with high-risk disease, and a trend towards benefit in intermediate-risk patients, indicating that younger AML patients had improved survival with the addition of GO to induction chemotherapy with little additional toxicity.[16] Borthakur et al.[17] showed that the gemtuzumab-ozogamicin with fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG-GO) regimen resulted in a 95% remission rate with 5% induction deaths in patients with core-binding-factor (CBF) AML; this suggested that addition of GO to FLAG induction therapy improved the response rate. A randomized phase III trial suggested that GO significantly improved event-free survival (EFS) and disease-free survival (DFS) but not overall survival (OS) in children with newly diagnosed AML.[18] To compare single-agent GO with supportive care as first-line therapy in older AML patients unsuitable for intensive chemotherapy, a randomized phase III EORTC-GIMEMA AML-19 trial revealed that the median OS and 1-year OS rate was higher in the GO group compared with a supportive care group.[19] Owing to its benefit in AML, FDA reapproved GO as the first-line treatment for CBF AML patients in 2017. A phase III ALFA-0701 trial (NCT00927498) then evaluated the efficiency of GO combined with DA3 + 7 chemotherapy for newly diagnosed AML patients, showing that combination prolonged EFS.[25] A phase I/II trial found that an azacytidine + GO combination displayed significant tolerability and reached a complete remission rate (CR) of 24% for R/R AML.[20] A German trial revealed that GO was effective for bridging children with very advanced AML to HSCT.[21] In NPM1-mutated AML, a significant beneficial effect of GO in female, younger (≤70 years), and *FLT-3* internal tandem duplication-negative patients was

Table 1

ADCs clinical trials in AML.

Agent	Target	NCT number	Phase	Inclusion/exclusion criteria	Age	ORR	CR/CRi	References
GO	CD33			>15 y Untreated Not pregnancy Liver function within normal	0–69	85%	CR: 82% CRi: 3%	14
FLAG-GO	CD33	NCT00801489	II	≥18 y New diagnosis of AML t(8;21), Inv(16), or t(16;16)	19–76	95%	CR: 91% CRp: 4%	15
GO	CD33	NCT00372593	III	Newly diagnosed AML Except M3	1 mo to 30	88.3%	CR: 85.8%	16
GO	CD33	NCT00091234	III	Newly diagnosed AML ineligible for intensive chemotherapy	62–88		CR: 15.3% CRi: 11.7%	17
GO	CD33	NCT00927498	III	50–70 y Newly diagnosed AML ECOG 0–3 Cardiac function is normal	50–70	81.5%	CR: 70.4% CRp: 11.1%	18
Azacitidine and GO	CD33	NCT00766116	I/II	>18 y Relapsed or refractory AML, excluding M3	29–82	50%	CR/CRp: 24%	19
GO	CD33	NCT00893399	III	AML Secondary AML Therapy-related AML M3 CBF AML	18.4–82.3		CR/CRi: 85.3%	20
GO	CD33	NCT00893399	III	Patients ≥18 y Newly diagnosed NPM1mut AML	19.3–82.3			21
SGN-CD33A	CD33	NCT01902329	I	CD33 + AML	26–89		CR: 11% CRi: 17%	22
SGN-CD33A with HMA	CD33	NCT01902329	I	CD33 + AML	60–87		CR: 43% CRi: 26%	23
SGN-CD123A	CD123	NCT02848248	I	Relapsed/refractory AML CD123-detectable leukemia				24

ADC = antibody-drug conjugate, AML = acute myeloid leukemia, CBF = core-binding-factor, CR = complete remission, CRi = complete remission with incomplete blood count recovery, FLAG-GO = gemtuzumab-ozogamicin with fludarabine, cytarabine, and granulocyte colony-stimulating factor, GO = gemtuzumab-ozogamicin, HMA = hypomethylating agent, ORR = overall response rate.

observed.[22] A randomized phase III AMLSG 09-09 trial evaluated the effect of GO on the cumulative incidence of relapse (CIR) in patients with NPM1-mut AML and found that the addition of GO to intensive chemotherapy resulted in a significantly reduced NPM1-mut transcript levels, leading to a markedly lower relapse rate.[23]

Vadastuximab talirine (SGN-CD33A), a novel anti-CD33 ADC combined with pyrrolobenzodiazepine dimer (PBD), possesses superior activity. SGN-CD33A synergistically promotes AML cell death with HMAs, because HMAs upregulate CD33 expression, increase DNA incorporation and enhance cytotoxicity. A phase I clinical trial (NCT01902329) found that at the recommended monotherapy dose of 40 μg/kg, the CR + CRi (complete remission + complete remission with incomplete blood count recovery) rate was 28%; 50% of patients who responded had no MRD, demonstrating activity and a tolerable safety profile as a single agent in AML patients.[24] The same trial found that, compared with HMA monotherapy, the combination of vadastuximab talirine with HMAs produced a high remission rate but was accompanied by increased hematologic toxicity.[26] However, clinical trials of SGN-CD33A have been discontinued owing to increased death due to infections and prolonged neutrophil recovery.

5.2.2. Anti-CD123 ADC SGN-CD123A is composed of a humanized anti-CD123 MoAb with a PBD dimer, which can induce the activation of DNA damage, cell-cycle changes, and apoptosis. A preclinical study revealed that SGN-CD123A contributed to growth delay in AML cells, including cytotoxicity of $CD123^+$ AML cells with unfavorable cytogenetic profiles.[27] SGN-CD123A treatment In vivo led to AML eradication in a disseminated-disease model, remission in a subcutaneous xenograft model, and significant growth delay in a multidrug resistance xenograft model. When treated with FLT-3 inhibitor quizartinib, SGN-CD123A enhances the activity of quizartinib.

Kovtun et al.[28] demonstrated that IMGN632, composed of a humanized anti-CD123 MoAb and an indolinobenzodiazepine pseudodimer class of cytotoxic payload, exhibited potential anti-tumor activity against $CD123^+$ AML cell lines and prolonged the survival time of AML xenograft models. Several trials investigated the efficacy of IMGN632 in combination with other agents. For instance, IMGN632 with venetoclax showed anti-leukemic synergistic death of AML cells. However, no prospective clinical trials of SGN-CD123A and IMGN632 are reported.

5.2.3. Anti-CLL-1 ADC CLT030 is a humanized monoclonal ADC targeting CLL-1, linked covalently to a highly potent DNA-binding payload. Different from anti-CD33 ADC, anti-CLL-1 ADC does not affect normal hematopoietic cells. CLT030 is stable in the bloodstream and releases its DNA-binding payload only on internalization into the lysosomes of CLL-bearing AML cells. Jiang et al.[29] confirmed that CLT030 inhibited in vivo LSC colony formation and showed robust tumor-growth inhibition in an AML xenograft model.

5.3. Bispecific antibodies

Bispecific antibodies include bispecific T-cell engagers (BiTEs), bispecific killer-cell engagers (BiKEs), dual-affinity retargeting antibodies (DARTs), and tandem diabodies (TandAbs), which integrate 2 antigen recognition sites, redirecting tumor cells to immune cells. A list of clinical trials exploring bispecific antibodies in AML is summarized in Table 2.

5.3.1. Bispecific T-cell engagers AMG330 is a novel T-cell-engaging BiTE antibody construct, that is, bispecific for CD33 and CD3, which is highly effective in recruiting and activating T cells. Preclinical studies found that KG-1 and U937 cells were lysed in co-culture with healthy donor T cells at AMG330 concentrations at 0.1 ng/mL. AMG330 was able to activate and expand T cells in primary AML patient samples and effectively mediated the redirected lysis of AML blasts and normal myeloid cells.[30–34] It has been reported that AMG330 significantly prolonged survival of AML mice.[30] Krupka et al.[33] found that AMG330 induced T-cell-mediated pro-inflammatory conditions, favoring the upregulation of immune checkpoints on AML cells. Through blockade of the programmed cell death 1 (PD-1)/programmed cell death ligand-1 (PD-L1) interaction, AMG330-mediated lysis, T-cell proliferation, and IFN-γ secretion were significantly enhanced.[32] A first human phase I study (NCT02520427) of AMG330 is ongoing.

5.3.2. Bispecific killer-cell engagers BiKEs target specific antigens on tumor cells and CD16 on NK cells to induce potent NK-cytotoxicity against tumor cells. Wiernik et al.[35] generated a fully humanized BiKE that could trigger NK-cell activation and induce secretion of cytokines against $CD33^+$ AML cells. Because ADAM17 can promote or cause CD16 shedding from NK cells, the combination of ADAM17 inhibitor enhances NK-cell activation and cytotoxicity. Stimulation of primary NK cells from healthy volunteers with 16 × 33 BiKE led to increased

Table 2

Clinical trials of bispecific antibodies in AML.

Agent	Format	Trial ID	Target	Effector	Phase	Inclusion/exclusion criteria	Age
AMG330	BiTE	NCT02520427	CD33	CD3	I	Relapsed or refractory AML, excluding M3	≥18
		NCT04478695			I	Relapsed or refractory AML, excluding APL ECOG ≤ 1	≥18
AMG673	BiTE	NCT03224819	CD33	CD3	I	Relapsed or refractory AML, excluding APL ECOG ≤ 2	≥18
GEM333	BiTE	NCT03516760	CD33	CD3	I	CD33 + relapsed or refractory AML	≥18
AMV564	Tandem	NCT03144245	CD33	CD3	I	Relapsed/refractory AML	≥18
MGD006	DART	NCT02152956	CD123	CD3	I/II	Primary or secondary AML, excluding APL	27–82
		NCT04582864			II	Relapsed AML and MDS, excluding APL	≥18
		NCT04158739			I	Recurrent or refractory AML, excluding APL and t(15;17)	≤20
JNJ-63709178	DuoBody	NCT02715011	CD123	CD3	I	Relapsed or refractory AML excluding APL ECOG ≤ 1	≥18
MCLA-117	DuoBody	NCT03038230	CLL-1	CD3	I	Primary or secondary AML, excluding APL	≥18
GTB-3550	TriKE	NCT03214666	CD33	CD16	I/II	High-risk MDS Refractory or relapsed AML	≥18

AML = acute myeloid leukemia, BiTE = bispecific T-cell engager, CLL-1 = human C-type lectin-like molecule-1, DART = dual-affinity retargeting antibody, MDS = myelodysplastic syndrome, TriKE = trispecific killer engager.

cytotoxicity and IFN-γ and TNF-α production against CD33+ cell lines.[36] It was reported that the tri-specific killer engager (TriKE), which incorporated a novel modified human interleukin-15 (IL-15) crosslinker to BiKE, induced superior NK-cell cytotoxicity, degranulation, and cytokine production against HL-60.[37]

5.3.3. Dual-affinity retargeting antibodies MGD606 is a DART molecule generated to connect $CD123^+$ AML cells and $CD3^+$ T cells, which could redirect T cells against AML blasts. In a mouse model using continuous administration, MGD006 eliminated engrafted KG-1a cells in peripheral blood (PB) at doses as low as 0.5 μg/kg. MGD006 bound to human and Cynomolgus monkey antigens with similar affinities and redirects T cells from either species to kill CD123-expressing target cells. Depletion of circulating CD123-positive cells was observed as early as 72 hours after the start of treatment and persisted throughout the infusion period.[38,39] A phase I dose-escalation trial (NCT02152956) in R/R AML patients showed that the CR rates of MGD606 were 32.1% (9/28) with good tolerance.

6. CHIMERIC ANTIGEN RECEPTOR

6.1. CAR-T cells

CAR-T-cell therapy has produced potentially significant results in CD19+ B-cell malignancies and may overcome many of the limitations of conventional leukemia therapies. A barrier to widespread use of CAR-T-cell therapy is toxicity, primarily cytokine release syndrome (CRS) and neurologic toxicity. Currently, 4 anti-CD19 CAR-T products have been approved by the FDA. For AML, CAR-T products are still in clinical trials, which are summarized in Table 3.

6.1.1. Anti-CD33 CAR-T Preclinical results have revealed that CAR-T cells targeting CD33 exhibited significant effector functions in vitro and resulted in eradication of leukemia and prolonged survival in AML xenografts.[40] Modified CD33 CAR-cytokine-induced killer (CIK) cells exhibited significant anti-leukemic activity in vitro and In vivo in patient-derived AML xenograft models. CIK cells reduced AML development and delayed AML progression in mice and were effective toward chemotherapy-resistant/residual AML cells.[41] Moreover, CD33-specific CAR-T cells with different co-stimulators (CD28, 4-1BB, or both) have been developed; they have shown specific killing of AML cells and have prolonged survival of a xenograft mouse model.[42–44] Clinical trials numbered NCT03126864, NCT03971799, NCT02799680, and NCT01864902 are proceeding. So far, only 1 patient was reported to receive 1.12×10^9 autologous anti-CD33 CAR-T cells and although rapid degradation of AML cells was observed, the disease relapsed 9 weeks later.[45] As a result, the safety and efficacy of anti-CD33 CAR-T cells require further validation with more patients.

6.1.2. Anti-CD123 CAR-T Preclinical data indicated that CD123 CAR-T cells exhibited anti-leukemic activity In vivo.[46] Recently, it was reported that decitabine significantly enhanced the anti-leukemia activity of CD123 CAR-T cells in vitro and In vivo.[47] To reduce the risk of severe toxicity, a rapidly switchable universal CAR-T platform (UniCAR) has been developed. Here, CAR-T activity depends on the presence of a soluble adapter called "targeting module" (TM), and clinical proof-of-concept for targeting CD123 in AML with improved safety has been confirmed.[48,49] A clinical trial of anti-123 CAR-T cells (MB-102) in AML showed that 7 patients were treated with 50×10^6 MB-102 cells. Three of them achieved CR; the rest had stable disease (SD).

6.1.3. Anti-LeY CAR-T LeY, a difucosylated carbohydrate antigen, is highly expressed on AML cells and poorly expressed on normal hematopoietic stem cells (HSCs). In a phase I study, the safety and post-infusional durability of anti-LeY CAR-T were evaluated. Among the 5 recruited patients, one achieved a cytogenetic remission, whereas one other with active leukemia had a reduction in PB blasts; a third showed a protracted remission. Serial PCR of PB and BM for the LeY transgene demonstrated that infused CAR-T cells persisted for up to 10 months.[50]

Table 3

CAR clinical trials in AML.

Target	Trial ID	Phase	inclusion/exclusion criteria	age
Anti-CD33 CAR-T	NCT03126864	I	Relapsed or refractory CD33 + AML, excluding APL	1–80
Anti-CD33 CAR-T	NCT03971799	I/II	Relapsed or refractory CD33 + AML	1–35
Anti-CD33 CAR-T	NCT02799680	I	Relapsed or refractory CD33 + AML	≥50
Anti-CD33 CAR-T	NCT01864902	I/II	CD33 + relapsed or refractory AML	5–90
Anti-CD123 CAR-T	NCT03585517	I	CD123 + refractory or relapsed AML ECOG score ≤ 2	3–80
Anti-CD123 CAR-T	NCT03114670	I	CD123 + AML, excluding APL	≥18
Anti-CD123 CAR-T	NCT03556982	I/II	Relapsed or refractory CD123 + AML ECOG score ≤ 2	14–75
Anti-CD123 CAR-T	NCT02623582	I	Relapsed or refractory AML	≥18
Anti-CD123 CAR-T	NCT02159495	I	Relapsed or refractory CD123 + AML	≥12
Anti-CD123 CAR-T	NCT03672851	I	Relapsed or refractory CD123 + AML, excluding APL	
Anti-CD123 CAR-T	NCT03766126	I	Relapsed or refractory AML, excluding t(15:17)	≥18
Anti-CD123 CAR-T	NCT03796390	I	Recurrent or refractory CD123 + AML	2–65
CD123/CLL-1 CAR-T	NCT03631576	II/III	Relapsed or refractory AML	<70
UCAR TCD123 CAR-T	NCT04106076	I	Newly diagnosed CD123 + AML	18–65
UCAR TCD123 CAR-T	NCT03190278	I	Relapsed or refractory CD123+ AML excluding APL or CNS leukemia	18–65
Anti-LeY CAR-T	NCT01716364	I	Newly diagnosed AML/high-risk MDS with a poor prognosis or relapsed/refractory AML/high-risk MDS	≥18
Anti-CD33 CAR-NK	NCT02944162	I/II	Recurrent or refractory CD33 + AML	3–80
Anti-NKG2D CAR-NK	NCT04623944	I	Relapsed or refractory AML, MDS excluding APL	≥18
Anti-CD7 CAR-NK	NCT04033302	I/II	CD7+ T-ALL AML NK-cell lymphoma	6 mo to 75
	NCT02742727	I/II	CD7+ relapsed or refractory leukemia and lymphoma	≥ 18

AML = acute myeloid leukemia, CAR = chimeric antigen receptor, CLL-1 = human C-type lectin-like molecule-1, MDS = myelodysplastic syndrome, NK = natural killer.

However, there was no response in 2 patients, while the other 2 patients achieved a short-term response but relapsed quickly. In conclusion, anti-LeY CAR-T cells can be a feasible and safe form of CAR-T-cell therapy in high-risk AML and demonstrate in vivo persistence.

6.1.4. Anti-CLL-1 CAR-T CLL-1 is a type II transmembrane glycoprotein, whose expression is restricted to myeloid cells and the majority of AML blasts. Moreover, CLL-1 is expressed in LSCs, but absent in HSCs, which may provide a potential therapeutic target for AML treatment. CLL-1 is not only a feasible target for bispecific antibody but also an ideal target for CAR-T cells. Wang et al.[50] developed a CAR containing a CLL1-specific single-chain variable fragment, in combination with CD28, 4-1BB costimulatory domains, and CD3-ζ signaling domain; a strong anti-leukemic effect was confirmed in vitro and in vivo.[51] Another preclinical study found that CLL-1 CAR-T cells selectively reduced leukemic colony formation compared with control T cells, and in a human xenograft mouse model, they mediated anti-leukemic activity against disseminated AML and significantly extended survival. Recently, optimized CLL-1 CAR with IL-15 was less terminally differentiated and had superior expansion compared with CAR-T cells without IL-15.[52] Further, anti-CLL-1 CAR-T cells may be an effective means for MRD eradication.[53] In order to test the safety and efficacy of CAR-T-cell therapy in R/R AML, 4 pediatric patients with R/R AML were enrolled in an ongoing phase I/II anti-CLL1 CAR-T-cell therapy trial, and 3 patients achieved CR while showing no MRD; this indicated that autologous anti-CLL1 CAR-T cell therapy had the potential to be a safe and efficient alternative treatment for children with R/R AML.[54] The activation of CAR-T cells can lead to persistently high levels of PD-1 antigen and eventually causes T-cell exhaustion. PD-1 silencing enhanced the killing ability of CLL-1 CAR-T cells.[55]

6.1.5. Anti-FLT-3 CAR-T While the identification of a truly AML-specific cell-surface antigen has remained elusive, FLT-3 appears to be promising for the development of AML immunotherapy. Expression of FLT-3 was reported to be restricted to hematopoietic lineage, including hematopoietic stem and progenitor cells (HSPCs) and plasmacytoid dendritic cells (DCs).[56] FLT-3 was highly expressed on the surface of AML blasts[57]; it is the most commonly mutated gene in AML and has been implicated in its pathogenesis and progression.[58] Cesar et al. developed an allogeneic CAR-T-cell therapy for the treatment of AML by engineering healthy donor T cells to express a high-performing fully human FLT-3 CAR-T and to eliminate endogenous T-cell receptor (TCR) expression, thereby minimizing the risk of alloreactivity. Allogeneic FLT-3 CAR-T cells exhibited target-dependent expansion and potent anti-AML activity in vitro and in vivo. Furthermore, incorporating a rituximab-inducible off-switch in the FLT-3 CAR-T had no effects on potency and provided a mechanism to deplete CAR-T cells after leukemia eradication, in order to limit hematopoietic toxicity and facilitate bone marrow recovery from residual HSPCs.[56]

6.1.6. Anti-CD44v6 CAR-T CD44 is a glycoprotein physiologically expressed on the surface of many mammalian cells, including endothelial and epithelial cells, fibroblasts, keratinocytes, and leukocytes. Human CD44 splice variants originate by alternative splicing of 9 variable exons in different combinations. Splice variants containing variable exon 6 (CD44v6) have been implicated in tumorigenesis, tumor cell invasion, and metastasis and are expressed in AML.[59] The CD44v6 CAR-T is currently under clinical development for the treatment of AML and multiple myeloma patients (NCT04097301).[60]

6.1.7. Bispecific CAR-T Because CAR-T-cell therapy may cause on-target/off-tumor side effects, it is ideal for reducing toxicity by increasing the specificity with multiple tumor markers. In this regard, novel bispecific CAR-T cells were developed to synergistically kill experimental tumor models by targeting tumor-associated antigen. CD13 is preferentially expressed in acute myeloid blast cells. TIM3, an immune-suppressing receptor, is highly expressed in the majority of human AML LSCs, but not in HSCs. A combinatory bispecific and split CAR (BissCAR) T-cell system was developed to effectively kill $CD13^{+}TIM3^{+}$ LSCs, while reducing the impact on normal cells that only express CD13.[50,61]

6.2. CAR-NK cells

NK cells mediate cytotoxicity against tumor cells, which can be genetically engineered with CAR. CAR-NK cells have a lesser toxicity profile compared with CAR-T cells owing to superior safety and lack of CRS and neurotoxicity.[62] Four clinical trials have been conducted to evaluate the safety of CAR-NK cells, including NCT02944162 (anti-CD33), NCT04623944 (anti-NKG2D), NCT04033302 (anti-CD7), and NCT02742727 (anti-CD7). Tang reported a phase I trial of CD33 CAR-NK cells in R/R AML patients and found that 5×10^9 NK cells resulted in no substantial adverse events (AEs), thus providing a new prospect for CAR-NK cells in AML.[63]

7. CHECKPOINT INHIBITORS

Immune checkpoint inhibitors (ICIs), targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the PD-1/PD-L1 pathways, have shown remarkable potential in several types of cancer including hematologic malignancies. These inhibitors reverse T-cell suppression and strengthen anti-tumor immunity after disrupting these pathways.

7.1. PD-1/PD-L1 inhibitors

PD-1, also known as CD279, is an inhibitory checkpoint receptor, which selectively binds to PD-L1 and deactivates T-cell function, leading to immune escape. PD-1 inhibitors, including nivolumab, pembrolizumab, and pidilizumab, block the interaction between PD-1 and PD-L1, contributing to T-lymphocyte activation and increased cytotoxic effect.

Nivolumab, a humanized IgG4 PD-1 MoAb, has been approved by the FDA for relapsed or progressed classical Hodgkin's lymphoma. A combination of nivolumab with HMAs gave a good curative effect for R/R and elderly AML patients. A single-arm phase II trial (NCT02397720) treated 70 R/R AML patients with nivolumab and azacytidine; it resulted in 33% overall response rate (ORR), including 22% CR/CRi patients, 1.4% PR patients, and 9% SD patients. In terms of toxicity, pneumonitis, skin rash, and transaminitis were observed.[64] A phase I/II single-arm trial tested the combination of nivolumab with DA3+7 in 44 newly diagnosed AML or high-risk myelodysplastic syndrome (MDS) patients, showing that the CR/CRi rate was 78% (34/44), with 53% (18/34) having no MRD. This demonstrated the feasibility of combining nivolumab with cytarabine and idarubicin for AML.[65]

Pembrolizumab is also a humanized IgG4 PD-1 MoAb. A phase II clinical trial (NCT02768792) enrolled 26 patients with R/R AML to evaluate the outcome of pembrolizumab treatment after high-dose cytarabine.[66] The results reinforced the safety and feasibility of ICI therapy prior to allo-SCT in patients with AML and suggest that post-transplantation cyclophosphamide may abrogate GVHD risk and severity in these patients. Another clinical trial (NCT02981914) enrolled 11 patients treated with pembrolizumab following allo-HSCT; 8 patients exhibited modest response to pembrolizumab and 2 experienced progressive disease along with 1 patient who achieved SD. In addition, immune-related AEs were observed in 63% patients but were resolved after pembrolizumab withdrawal and corticosteroid treatment.[67]

Pidilizumab is a humanized IgG1 PD-1 MoAb. A phase I clinical trial evaluated its safety and efficiency for AML; only 1 out of 8 patients finally experienced disease progression.

7.2. Cytotoxic T-lymphocyte antigen-4 inhibitors

CTLA-4, also referred to as CD152, mainly competes with CD28 for binding to CD80 or CD86 to downregulate TCR activation. Inhibiting CTLA-4 enables cytotoxic T cells to mediate an anti-tumor immune response.[68,69] Preclinical trials showed that CTLA-4 ligand expression was upregulated in AML cell lines.[70] The anti-CTLA-4 MoAb, ipilimumab, exerts a positive effect on relapsed AML. A phase I/Ib trial (NCT01822509) of ipilimumab enrolled 28 relapsed patients and found that immune-related AEs were seen in 21% of patients (6/28) and GVHD in 14% (4/28). In total, 22 patients showed response to therapy with 23% CR and 9% PR.[71]

8. VACCINES

Tumor vaccines are an emerging approach based on antigens or DCs presenting neo-antigens to T cells, which can be used to stimulate the patients' auto-immune system to selectively remove tumor cells while sparing normal cells and tissues.

8.1. WT1 peptide vaccines

WT1 is a feasible target antigen for AML or MDS owing to its overexpression in leukemic cells. WT1 peptide-based vaccines can decrease leukemic burden through specific cytotoxic T-cell activity. A phase II study investigating WT1 peptide vaccine (galinpepimut-S) in adults with AML in first complete remission revealed that the vaccine was well tolerated.[72] OCV-501 is a helper peptide derived from the WT1 protein, which induced OCV-501-specific type 1 T-helper (T_H1) responses dose-dependently and stimulated helper activity of the specific T_H1 cells in the PB mononuclear cell population from healthy donors. OCV-501 also increased the number of WT1-killer peptide-specific cytotoxic T lymphocytes. A phase I clinical trial suggested that the subcutaneous administration of OCV-501 once weekly for 4 weeks at doses of 0.3, 1, and 3 mg in older patients with AML during complete remission was safe and well tolerated.[73]

8.2. DC vaccines

DCs are recognized as cells with strong antigen-presenting characteristics. DC-based vaccination can harness the potential of a patients' own immune system to destroy tumor cells. In an AML murine model, the cytotoxic anti-leukemic immune response induced by vaccination with DCs pulsed with eluted peptides in vitro and In vivo was mainly mediated by CD4+ T cells.[74] In a phase II study (NCT00965224) of 30 R/R AML patients to evaluate mRNA-electroporated DC vaccines, only 43% (13/30) of patients developed an anti-leukemic response while 30% (9/30) achieved molecular remission. The 5-year OS of patients responding to DCs was higher than nonresponders (53.8% vs 25%), suggesting that DC vaccines prevented or delayed recurrence and improved OS effectively.[75]

A phase I/II study tested the feasibility of a vaccine by autologous leukemic apoptotic corpse-pulsed DCs in elderly AML patients in first or second CR.[75] Pulsed DC were administered at doses of 9×10^6 cells subcutaneously (1 mL) and 1×10^6 intradermally (0.1 mL). Five doses of vaccine were planned on days +1, +7, +14, +21, and +35. Five DC vaccines were produced and injected into all 5 patients included in the study. No severe AE was documented. Larger phase II studies are now required to investigate more precisely the role of DC vaccines with leukemic apoptotic bodies in older as well as younger AML populations (NCT01146262). A novel allogeneic DC vaccine, DCP-001, was developed from an AML-derived cell line. A phase I study in 12 advanced-stage elderly AML patients concluded that DCP-001 in these patients was safe, feasible and generates both cellular and humoral immune responses.[76]

9. COMPARISON OF DIFFERENT APPROACHES

Harnessing the immune system against leukemic cells can be a powerful treatment strategy for AML patients. Immune-based therapies, such as CAR-T cell and BiTE therapy, have proven to be effective means of targeting chemotherapy-resistant AML. The individualized combination of different immune approaches along with chemotherapy and autologous or allogeneic SCT to achieve the highest efficacy may be a promising future trend, which will improve overall outcomes for patients. There is currently active development in all of these avenues of immunotherapy with multiple trials either showing early results or currently recruiting patients. This review summarizes the latest immunotherapy options for AML and research progress in clinical trials, and aims to update our understanding of immunotherapy and provide more options for treatment.

Immunotherapy is a highly promising strategy in AML, particularly for transplant-ineligible patients and individuals in MRD states. Accompanying these opportunities, there are also many challenges. Barriers to successful implementation of immunotherapeutic approaches include: the risk of possible off-target toxicities to normal myeloid progenitor and HSCs as well as life-threatening CRS and neurological events. Other challenges include technical aspects, nor are they free from economic considerations. Here, we summarize the comparison between several immunotherapies.

Table 4 lists the probable or anticipated advantages and disadvantages of different approaches that may help to emphasize a particular approach.

As a foundation for the molecular basis of cancer therapeutics, MoAbs have several major advantages. Given their long half-lives and effective biodistribution, the therapeutic systemic levels of MoAbs could last for weeks to months, long enough to mediate a prolonged anti-cancer response. ADCs combine the cytotoxic potential of drugs with the specificity of MoAbs, and theoretically can overcome the limitations of both nonspecific cytotoxic drugs and specific but often ineffective MoAbs. Bispecific antibodies have one arm that binds to the target cell, and another that binds to activating receptors on cytotoxic cells. Early studies demonstrated that bispecific antibodies with intact Fc activate T cells nonspecifically and result in unacceptable toxicity. Smaller bispecific molecules lacking Fc have short half-lives and need to be given by continuous infusion.

CAR-T cells are genetically modified to respond to target cells expressing a given antigen. Indeed, the long-lived CAR-T memory cells that have been observed in a number of patients represent both an advantage as well as a challenge. On the one hand, it likely contributes to the desirable long-term clinical responses in patients treated with CAR-T cells. On the other hand, such long-term immune memory can result in long-lasting, perhaps even permanent, depletion of any cells that express the target antigen. CAR-T cells need to be produced individually for each patient; this involves complex challenges related to cell engineering and considerable expense.

ICIs are a promising therapy widely used in solid tumors. However, ICIs are not effective in the treatment of AML because of their low tumor-mutational burden.

Somatic mutations can generate cancer-specific neo-epitopes that are recognized by autologous cells as foreign and constitute ideal cancer vaccine targets. The vaccine can target tumor stem cells or residual tumor cells to exert an anti-tumor immune effect. However, there are still important obstacles such as tumor cell immunosuppressive factor, which suppresses vaccine-mediated immune responses. Therefore,

Table 4

Comparison of different approaches.

	Antibody-based drugs	CART	Vaccines	Checkpoint inhibitor
Types	Monoclonal antibodies ADCs Bispecific antibodies	Anti-CD33 CART Anti-CD123 CART Anti-LeY CART Anti-CLL-1 CART	WT1 peptide vaccines DC vaccines	PD-1/PD-L1 inhibitors CTLA-4 inhibitors
Applications	Widely used and can be used in combination with chemotherapy drugs	Clinical trials are mainly used in patients with relapsed or refractory AML	Prevent recurrence	Combination with HMAs
Current challenges	1. Searching for AML-specific targets	1. Searching for optimal antigens	1. Tumor cell immunosuppressive factors suppress vaccine-mediated immune responses	1. Low AML mutation burden leads to poor efficacy
	2. Limited effectiveness	2. Not enough T cells from the patient	2. The effect is not obvious	2. Immune-mediated toxicity
	3. Lead to myelosuppression, CRS, and other treatment-related adverse reactions	3. Lack of memory T cells leads to nondurable response 4. Price is very expensive 5. The most common AEs include CRS, neurological toxicities, and hypogammaglobulinemia	3. Need to be customized, complex and expensive	
Advantage	No need for customization to the patient, avoiding the challenges associated with cell engineering	Individualized treatment, with greater specificity	Target tumor stem cells or residual tumor cells to exert anti-tumor immune effect	A promising therapy for some elderly patients and those who relapse after transplantation
Reference				

ADCs = antibody-drug conjugates, AEs = adverse events, AML = acute myeloid leukemia, CART = chimeric antigen receptor T, CLL-1 = human C-type lectin-like molecule-1, CRS = cytokine release syndrome, CTLA-4 = cytotoxic T-lymphocyte antigen-4, DCs = dendritic cells, HMAs = hypomethylating drugs, PD-1 = programmed cell death 1, PD-L1 = programmed cell death ligand-1.

its clinical effect is not yet clear. Finally, it is necessary to face its high cost and complex technology.

10. CONCLUSIONS

Immunotherapy has changed the therapeutic landscape in AML, especially for R/R and allo-HSCT-ineligible patients. Promising data from preclinical and clinical trials have elaborated their therapeutic value. Owing to the overlap of antigen expression between AML cells and normal hematopoietic cells, it is of the utmost importance to identify the antigen candidates, which minimize immunotoxicity.

ACKNOWLEDGMENTS

C.T. is supported by Grant ZC20171 from the Tianjin Health Science and Technology Project and Natural Science Foundation of Xinjiang Autonomous Region (2022D01A09).

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