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Mini Review

Combination Strategies for Immunotherapy in Acute Myeloid Leukemia

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Abstract

Despite the emergence of new approaches in acute myeloid leukemia (AML) treatment in recent years, the overall prognosis remains poor. Particularly for elderly patients and relapsed/refractory cases, the five-year survival rate consistently remains below 30%. While traditional chemotherapy regimens can rapidly suppress tumor burden and alleviate clinical symptoms, they suffer from limitations such as insufficient targeting, prominent toxic side effects, and a tendency to induce drug resistance. Immunotherapy offers a novel therapeutic pathway for AML due to its advantages of precise targeting, long-lasting antitumor effects, and a controllable safety profile. However, single-agent immunotherapy demonstrates limited clinical response rates in AML and struggles to achieve complete tumor cell clearance. In this context, combination regimens of chemotherapy and immunotherapy are increasingly becoming the focus of research. This review aims to summarize the rationale and advances in the combination of immune checkpoint inhibitors, chimeric antigen receptor T-cell therapy, antibody-drug conjugates, bispecific antibodies, and cancer vaccines with chemotherapy for the treatment of AML. We have detailed the preclinical research and clinical trial progress of each combined regimen, analyzed the core challenges—including off-target toxicity, high tumor heterogeneity, and limited efficacy in specific AML subtypes—and further propose targeted solutions and future development directions, such as exploring novel specific antigens, developing multi-targeted drugs, and formulating precision individualized treatment plans. The clinical application of such combined strategies is attracting increasing attention. In conclusion, chemo-immunotherapy combinations represent a highly promising therapeutic paradigm for AML, harnessing the synergy of chemotherapy-mediated immune microenvironment remodeling and the specific antitumor activity of immunotherapies to overcome single-agent limitations and deliver meaningful survival benefits.

Introduction

AML is a highly heterogeneous hematological malignancy characterized by clonal proliferation of abnormal myeloid progenitor cells, which disrupts normal hematopoietic function and triggers life-threatening complications, including bone marrow failure, infection, bleeding, and organ infiltration. Conventional AML therapies are dominated by chemotherapy, targeted therapy, and hematopoietic stem cell transplantation. While these approaches achieve partial remission, they carry critical limitations: chemotherapy induces severe systemic toxicity and drug resistance, targeted agents are limited by genetic heterogeneity and high relapse rates, and transplantation is restricted by donor shortage and graft-versus-host disease. Given these clinical bottlenecks, immunotherapy has emerged as a promising alternative, and the combination of chemotherapy and immunotherapy has become a frontier strategy to break through monotherapy constraints. Recent studies have confirmed that chemotherapy drugs can reshape the tumor immune microenvironment through multiple pathways and

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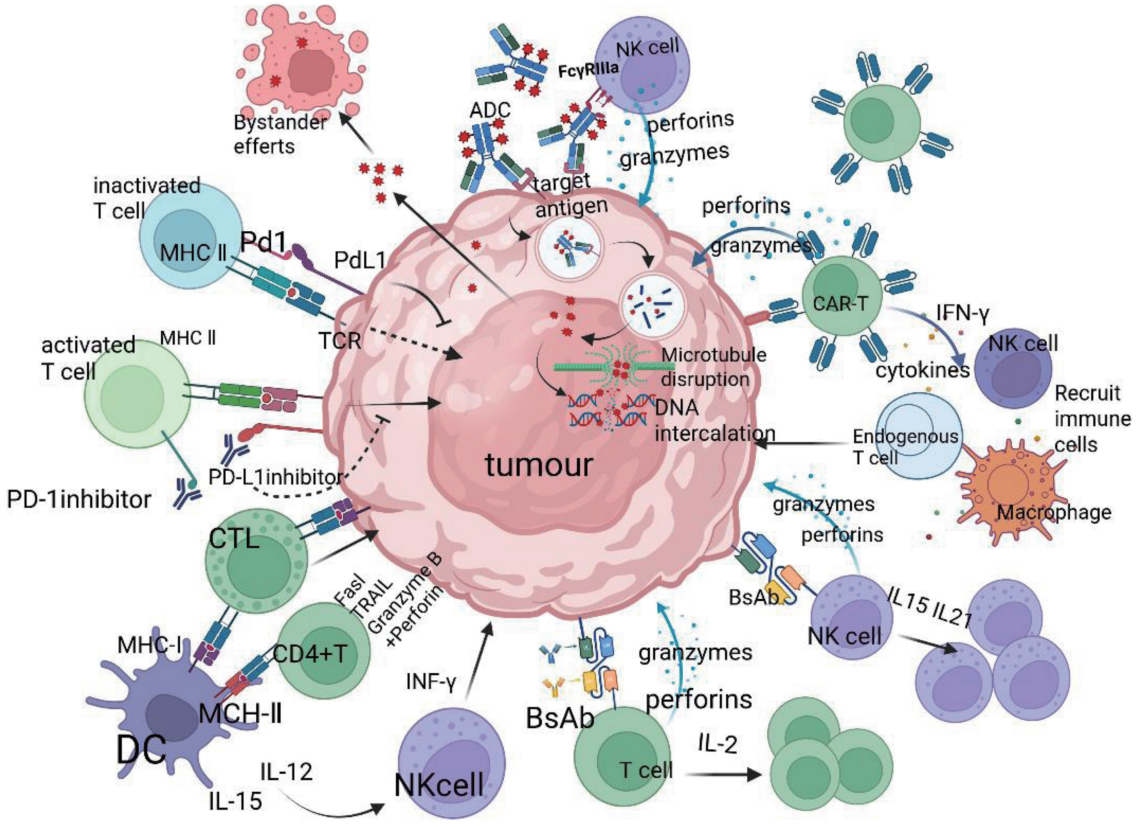

Fig. 1. Schematic of the distinct mechanisms of five classes of immunotherapeutic drugs: programmed death-1/programmed death-ligand 1 (PD-1/PD-L1) inhibitors specifically bind to PD-1 or PD-L1, blocking their interaction. This releases the inhibitory state of T cells, restoring their activity so they can recognize and attack tumor cells once again. After specifically recognizing and binding to antigens on the surface of tumor cells, the antibody-drug conjugate (ADC) is internalized into the tumor cell. Within the cell, the ADC is degraded, releasing the cytotoxic drug that damages the tumor cell's DNA or inhibits its division. Another portion can penetrate the cell membrane and diffuse into surrounding tumor cells, targeting those that do not express or express low levels of the target antigen. Chimeric antigen receptor T cells (CAR-T) can specifically recognize and bind to tumor antigens. Activated T cells can also release cytokines such as interferon-gamma (IFN-γ), thereby helping to activate a small number of innate immune cells and enhance immune responses. Bispecific antibodies (BsAbs) directly recruit immune cells to the vicinity of tumor cells, enabling more precise killing. Simultaneously, they activate immune cells such as natural killer (NK) cells to release cytokines, enhancing their cytotoxic function and promoting their proliferation. Dendritic cells (DCs) present tumor antigens to T cells, activating specific T cell immune responses and inducing the production of large numbers of cytotoxic T lymphocytes (CTLs) capable of recognizing tumors. These cells then kill tumor cells. IL, interleukin; MHC I, major histocompatibility complex class I; TCR, T-cell receptor; TRAIL, TNF-related apoptosis-inducing ligand.

enhance the body's own antitumor immune response. This process is termed immunogenic cell death.[1] Chemotherapy can also eliminate immunosuppressive cells, such as myeloid-derived suppressor cells and regulatory T cells, within the tumor microenvironment. Simultaneously, it can downregulate the expression levels of immune checkpoint molecules such as programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) and reduce the production of immunosuppressive factors, including interleukin (IL)-10 and transforming growth factor-β.[2,3] This disrupts the immune evasion mechanisms of tumor cells, creating more favorable conditions for the efficacy of immunotherapy drugs. The core advantage of immunotherapy lies in its ability to activate the body's own immune system to target and eliminate tumor cells specifically. This approach offers persistence and memory, enabling the effective recognition and destruction of leukemia stem cells (LSCs) while disrupting their immortal proliferative properties.[4,5] LSCs are considered the core factor driving acute myeloid leukemia (AML) relapse. Targeting and eliminating LSCs to prevent disease recurrence represents the most promising pathway toward achieving a permanent cure for AML, offering the potential for deep remission and long-term disease-free survival.[5,6]

In current AML treatment, immunotherapy is primarily categorized into five types based on distinct drug mechanisms (Fig. 1): immune checkpoint inhibitors, chimeric antigen receptors (CARs), antibody-drug conjugates (ADCs), bispecific antibodies (BsAbs), and tumor vaccines.[7] Multimodal immunotherapy combination treatment integrates immunotherapies with distinct mechanisms of action alongside chemotherapy, generating synergistic effects between the two. This represents the most promising development direction in current AML treatment. Accordingly, this review aims to elaborate the theoretical basis, core implementation strategies, and clinical research progress of chemotherapy–immunotherapy combination therapy for AML, analyze the existing challenges in clinical application, and explore future development directions, so as to provide theoretical references for optimizing AML combination regimens and developing individualized precise treatments, thereby improving therapeutic efficacy and survival outcomes of AML patients.

Combination therapy with immune checkpoint inhibitors

Immune checkpoints are based on interactions between inhibitory receptors and ligands on the surface of immune cells. By regulating signaling cascades, they can induce negative regulation of immune cells, thereby allowing tumor cells to evade immune surveillance. This ultimately leads to failure in eliminating tumor cells, promoting tumor progression.[8] The anticancer effects of immune checkpoint inhibitors stem from their ability to augment immune responses. To do this, they utilize monoclonal antibodies designed to bind to immune checkpoint proteins expressed on the surface of immune cells or tumor cells. By blocking key immune checkpoints such as PD-1, cytotoxic T-lymphocyte antigen 4 (CTLA-4), and PD-L1, these inhibitors suppress the activity of negative signaling receptors while promoting immune cell activation, ultimately driving the regression of cancer.[9–11] Currently, new immune checkpoints, including CD47, LAG-3, and TIM-3, are under investigation.[12,13]

PD-(L)1 and CTLA-4 inhibitors

Research findings from the Gojo team indicate that the combination therapy of pembrolizumab (a PD-1 inhibitor) with azacitidine (AZA) offers a new treatment option for elderly patients with AML. For newly diagnosed elderly patients, this regimen demonstrated superior outcomes across all metrics compared to monotherapy, while also exhibiting improved safety. Although trials demonstrate significantly superior efficacy in newly diagnosed elderly AML compared to relapsed/refractory (R/R) AML, traditional salvage therapies for R/R AML patients typically yield a median overall survival (OS) of less than 6 months. In this study, however, the combination regimen extended median OS to 10.8 months across the entire cohort. Among patients achieving complete response (CR), complete response with incomplete blood count recovery (CRi), or partial response (PR), the one-year OS rate reached 75%. This highlights the disease control efficacy of this combination regimen in refractory patient populations, indicating its salvage value for R/R AML and delivering clear survival benefits to patients.[14]

Another point worthy of attention is that immune checkpoint inhibitors may induce immune-related adverse events, representing a significant safety concern in the trial.[15] Within this study, individuals who experienced immune-related adverse events received effective control through the administration of steroid hormones.[14] This finding suggests that further exploration of approaches such as low-dose prophylactic steroid use may reduce the incidence of immune-related adverse events, thereby enhancing the safety profile of this combination therapy regimen. To improve outcomes for AML patients unsuitable for intensive induction therapy, Gojo et al. conducted a phase II trial (NCT04284787) evaluating pembrolizumab in combination with AZA plus venetoclax (VEN). However, current data indicate that adding pembrolizumab does not significantly improve patient survival or prognosis, and it may lead to worse treatment outcomes.[16] The team is currently performing immune-related and correlational analyses to further validate the aforementioned findings. Further studies are warranted to identify effective therapeutic strategies for this group of AML patients.

A Phase II study (NCT04214249) of pembrolizumab combined with intensive chemotherapy for AML is currently underway (Table 1), which will help us better understand its efficacy in patients with minimal residual disease status. To optimize therapeutic efficacy, a triple combination regimen of AZA plus nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) is being used for the treatment of R/R AML. Preliminary data indicate that this regimen demonstrates significantly superior efficacy in salvage phase I patients compared to subsequent phases, suggesting that early intervention with such regimens may substantially improve patient survival.[17] This provides a basis for selecting the optimal timing for clinical treatment, thereby preventing patients from missing the best window for intervention. Another study evaluated the combination of high-dose cytarabine (HiDAC) with pembrolizumab. Among the 28 patients receiving HiDAC plus pembrolizumab as their first salvage therapy, 13 (46%) achieved CR/CRi. This combination therapy significantly improved the CR rate compared to historical data for HiDAC monotherapy, yielding encouraging results.[18] A Phase II trial investigating the triple combination of AZA, nivolumab, and relatlimab for the treatment of AML is also underway (NCT04913922) (Table 1).

CD47 macrophage immune checkpoint

The immune checkpoint inhibitor CD47 targets macrophages, differing from traditional immune checkpoint inhibitors that act on T cells. Its primary mechanism involves modulating macrophage function. CD47, as a transmembrane protein, primarily transmits anti-phagocytosis or "don't eat me" signals, thereby inhibiting phagocytic clearance.[19] Anti-CD47 antibodies have demonstrated their potential to inhibit tumor immune escape.[20] Currently, extensive work has been conducted on CD47-SIRPα pathway inhibition therapies. The core intervention strategy primarily involves blocking CD47 with monoclonal antibodies.[21] Additionally, recombinant SIRPα proteins capable of binding and blocking CD47 are also considered potential intervention approaches.[22] In preclinical studies, anti-CD47 antibodies have demonstrated biological activity against multiple human malignancies, a conclusion validated through *in vitro* experiments and mouse xenograft models.[23] Researchers administered antibodies that specifically block the human CD47 molecule to mouse models implanted with human primary AML and acute lymphoblastic leukemia cells. They observed effective clearance of tumor lesions in the peripheral blood and bone marrow of the experimental animals, with some mice even achieving sustained disease remission.[23,24]

In certain preclinical xenograft models, the therapeutic efficacy of anti-CD47 antibodies is influenced to some extent by tumor burden. For example, in a mouse model of human AML, when the tumor burden in the bone marrow exceeded 70%, anti-CD47 antibody alone failed to completely eradicate the tumor tissue within the animals.[23,24] However, these residual tumor cells can be effectively phagocytosed by macrophages in *in vitro* experimental settings. This phenomenon suggests that the key reason for the difficulty in eradicating tumors in the aforementioned mouse models is likely the insufficient number of macrophage effector cells within the bone marrow cavity to cope with the high tumor burden. Currently, the combined use of macrophage stimulants has been proven to effectively increase the number of macrophages and enhance their function.[25] Moreover, when combined with antigen-specific therapeutic antibodies, these cytokines can generate stronger antitumor activity against multiple tumor types.[25]

While monotherapy targeting CD47 has demonstrated efficacy in various preclinical tumor models, combination strategies exhibit significantly greater therapeutic potential in comparison. Research combining anti-CD47 monoclonal antibodies with hypomethylating agents (HMAs) demonstrated favorable safety and tolerability in Phase I clinical trials.[26] Data from a Phase Ib clinical trial indicate that the combination of magrolimab and AZA significantly improves the complete remission rate in treatment-naive AML patients harboring TP53 gene mutations.[27] Based on these

Table 1. All data are obtained from ClinicalTrials.gov

Trial ID	Intervention	Phase	Target population	Primary endpoint	Status	Estimated study completion time	Location
NCT05199051	Gemtuzumab ozogamicin (GO) + cytarabine + gilteritinib	2	Acute myeloid leukemia (AML)	Event-free survival (EFS)	Recruiting	2027-03-15	France
NCT04168502	GO + daunorubicin + cytarabine	3	AML	Activity of minimal residual disease (MRD) negativity achievement	Recruiting	2027-04	Italy
NCT04214249	Chemotherapy + pembrolizumab (MK-3475)	2	AML	MRD/complete remission/ complete remission with incomplete recovery (CRi)	Active, not recruiting	2026-01-30	United States (US)
NCT04050280	Cladribine, cytarabine, granulocyte colony-stimulating factor (G-CSF), and GO (CLAG-GO)	2	AML	Response rate + adverse event (AE)	Recruiting	2027-02	US
NCT06034470	Pivekimab sunirine (PVEK) (IMGN632) + fludarabine + cytarabine + G-CSF + idarubicin (FLAG-Ida)	1	AML	AE	Recruiting	2027-12-31	US
NCT05904106	GO + standard chemotherapy	2	AML	Modified event-free survival (mEFS)	Recruiting	2028-09	Germany
NCT03679650	Dendritic cell (DC)/AML vaccine + decitabine	1	AML	The fold-increase in AML-specific T cells in the peripheral blood and bone marrow	Recruiting	2026-08-31	US
NCT06536959	Programmed cell death protein 1 (PD-1) inhibitor + venetoclax + decitabine + azacitidine	2	Relapsed/ refractory (R/R) AML	complete response rate and MRD response rate	Recruiting	2027-07-31	Beijing, China
NCT06384261	Cusatuzumab + venetoclax + azacitidine	2	AML	Overall survival	Recruiting	2027-06	US, Canada, Germany, Switzerland
NCT04086264	Azacitidine + IMGN632 + venetoclax	1,2	AML	Safety and tolerability	Active, not recruiting	2027-02	US, France, Germany, Italy, Spain, United Kingdom (UK)
NCT04913922	Azacitidine + nivolumab + relatlimab	2	AML	Maximum tolerated dose (MTD), dose-limiting toxicities (DLTs), objective response rate (ORR)	Recruiting	2026-03	Germany

positive early clinical results, multiple subsequent clinical studies were initiated, including a Phase III trial (NCT04778397) evaluating the efficacy of magrolimab in combination with AZA, and a Phase III study (NCT05079230) exploring a triple combination regimen of magrolimab, AZA, and VEN. However, data analysis revealed that this combination therapy not only failed to meet efficacy expectations but also resulted in an increased risk of death among patients in the treatment group.[28] Based on this, Gilead Sciences announced the termination of the trial. Following the successive discontinuation of these three pivotal Phase III trials, the U.S. Food and Drug Administration (FDA) also suspended all clinical studies of magrolimab for the treatment of AML and MDS. Gilead ultimately decided to cease further development of the drug in the field of hematologic malignancies, leading to the halt of subsequent research on magrolimab for myeloid malignancies.

CAR-engineered immune cells combination therapy

CAR-T cell therapy

Since the FDA approved the first CAR-T therapy in 2017, it has ushered in a new era in hematological treatment, demonstrating remarkable efficacy in the management of blood cancers. In adoptive cell transfer therapy, CAR-T targeting CD19 or BCMA has demonstrated high response rates and durable responses in certain types of B-cell malignancies.[29] No similar breakthroughs have yet emerged for AML, but they encourage us to turn our attention to potential treatments for AML. The greatest challenge in CAR-T therapy for AML is identifying specific target antigens, as antigens expressed on AML cells are often also expressed on healthy hematopoietic stem cells. This lack of exhaustive antigens means that targeting myeloid antigens can lead to severe side effects, such as clinically intolerable long-term bone marrow ablation, or the target antigen may not be expressed on the majority of AML cells.[30]

Patients with AML harboring DNMT3A gene mutations exhibit poor treatment outcomes and prognosis.[31,32] However, it is noteworthy that AML cells harboring DNMT3A mutations exhibit significantly elevated CD44v6 expression on their surfaces.[33] CD44v6 CAR-T cells can precisely target and kill such mutated AML cells. Low-dose decitabine pretreatment enhances this killing effect and further upregulates CD44v6 expression on the surface of mutated cells.[34,35] Therefore, the combination of CD44v6 CAR-T therapy with decitabine boosts the recognition and eradication of malignant cells, broadening treatment options for AML with DNMT3A gene mutations. This target has demonstrated significant potential in trials (NCT04097301) treating AML and multiple myeloma.

A study found that combining anti-CD123 CAR-T therapy with HMA drugs may enhance anti-leukemia efficacy and survival rates. Aza increases cellular immunogenicity and boosts CD123 surface expression on AML cells, thereby enhancing the ability of anti-CD123 CAR-T cells to discern and eradicate malignant cells.[36] Compared to monotherapy, it significantly improves patient survival rates. Further studies are anticipated to evaluate the efficacy of this combination therapy. Research has revealed that abnormal activation of mTORC1 signaling in CAR-T cells reduces bone marrow infiltration, preventing CAR-T cells from effectively eliminating AML cells within the bone marrow.[37] However, pretreatment with rapamycin effectively resolves this issue, significantly enhancing the ability of CAR-T cells to infiltrate the bone marrow.[38] As research progresses, mounting evidence indicates that chemotherapy not only eradicates leukemia progenitor cells but also enhances sensitivity to immune-mediated elimination when combined with immunotherapies such as CAR-T cell therapy or checkpoint inhibitors, thereby boosting cytotoxic effects.[34,39]

CAR-natural killer (NK) cell therapy

NK cells are a key component of the human innate immune system. Not only do they exhibit a broader spectrum of anticancer activity, but they also do not induce severe graft-versus-host disease.[40,41] Compared to T cells, NK cells possess numerous unique advantages. Without requiring prior antigen sensitization, they can directly recognize and lyse cancer cells by releasing perforin and granzyme B.[42] Additionally, they secrete cytokines such as interferon-gamma to activate surrounding immune cells and achieve systemic immune regulation, thereby amplifying the overall immune response and enhancing antitumor efficacy.[43,44] The development of CAR-NK targets has achieved breakthroughs across multiple dimensions. CD33 and CD123, as classic targets for CAR-NK therapy, have demonstrated significant potential in multiple trials.[45–47] Wang *et al*.[48] designed a novel CD33-MSLN Loop CAR that further enhances the clearance efficiency of heterogeneous AML cells. They also discovered that gene-edited knockout of CD33 prevents self-killing among Loop CAR-NK and Loop CAR-iNK cells, significantly promoting their expansion capacity and antitumor efficacy.[48] Chen *et al*.[49] identified a novel target for endoplasmic reticulum chaperone proteins. Their engineered HSP90B1 CAR-NK cells demonstrated precise killing with excellent safety profiles in both *in vitro* and animal models.[49] CAR-NK therapy is gradually emerging as a new approach to cancer cell therapy.

Multiple clinical trials are underway to evaluate the safety and efficacy of combining CAR-NK therapy with chemotherapy. For example, a study (NCT05987696) is currently exploring the use of CD33/CLL1 dual CAR-NK cells, CD33 CAR-NK cells, and super NK cells in combination with chemotherapy for the treatment of AML. Another Phase I study (NCT05008575) evaluated the safety and efficacy of CD33 CAR-NK cells in R/R AML following chemotherapy pretreatment. The published data showed that 6 out of 10 patients achieved minimal residual disease negativity within 28 days, with no serious adverse events occurring.[50] Preliminary reports indicate that the combination therapy is well tolerated. However, in one trial, significant safety concerns arose in AML patients who underwent multiple pretreatments and subsequently received CD123 CAR-NK cell therapy due to excessive proliferation of the CAR-NK cells. Thorough investigations and expanded sample monitoring of its efficacy will still be required in the future.[51]

CAR-macrophage (CAR-M) cell therapy

Research and development of CAR-M cell therapy is currently in its early stages, with the number of preclinical and clinical studies on malignant hematologic disorders gradually increasing. Morrissey and colleagues developed the first CAR-M cell in 2018, initially referred to as CAR-phagocyte. They discovered in a mouse phagocytosis receptor library that the Megf10 and FcRγ intracellular domains possess potent capabilities to trigger phagocytosis. Their research confirms that modifying the CAR domain can significantly enhance the phagocytic function and antitumor efficacy of CAR-M.[52] Jiang *et al*. successfully constructed CD26 CAR-M, which demonstrated specific phagocytosis in CD26-positive chronic myeloid leukemia (CML) cells both *in vitro* and *in vivo*. It is expected to eradicate CML-LSCs.[53] Zhang *et al*. designed the CD19 chimeric antigen receptor-engineered induced macrophages (CAR-iMacs). Subsequently, in co-culture with CD19-expressing K562 leukemia cells, CAR-iMacs demonstrated antigen-dependent phagocytosis and anti-cancer cell activity.[54] These groundbreaking

research achievements provide crucial theoretical references and practical approaches for developing multi-target CAR-M therapies and applying them in the treatment of malignant hematological diseases. Although CAR-M therapy is currently primarily confined to preclinical research, it is expected to emerge as a significant treatment option in the field of malignant hematological disorders as research progresses.

Combination therapy with ADCs

CD33, CD123, and C-type lectin-like molecule-1 (CLL1, also known as CLEC12A) are specific antigens highly expressed on the surface of AML cells. They represent the most extensively studied targets for immunotherapy, exhibiting extremely low or undetectable expression in normal hematopoietic stem cells and somatic cells.[55] CD33 is expressed in approximately 90% of AML cases, and its expression intensity and pattern in AML progenitor cells differ significantly from those in normal progenitor cells.[56]

Gemtuzumab ozogamicin (GO) has been approved by the FDA for the treatment of R/R AML. GO is an ADC targeting the CD33 antigen that is linked to a DNA-damaging cytotoxic drug. It has demonstrated excellent therapeutic efficacy in the treatment of AML.[57] Adding GO to induction chemotherapy significantly improved OS, delivering a clear survival benefit for patients without adverse cytogenetics.[58] Singh *et al*. demonstrated through a retrospective study that the use of intensified daunorubicin and GO, combined with FLT3 inhibitors, is safe for treating high-risk AML patients and significantly improves remission rates.[59] For R/R AML, the combination therapy of Lintuzumab-Ac225 targeting CD33 with CLAG-M (NCT03441048) achieved a CR/CRi rate of 67%. The combination demonstrated excellent tolerability and manageable toxicity. The study also revealed that as the dose increased, remarkably impressive results were demonstrated in R/R AML patients, exerting a profound impact on high-risk R/R AML patients.[60,61] Multiple studies investigating GO in combination with chemotherapy for AML are currently underway (Table 1).

IMGN632 is a novel CD123-targeted ADC featuring a humanized IgG1 antibody and a DNA alkylating agent that disrupts cancer cell DNA. It (NCT03386513) has demonstrated encouraging single-agent clinical activity in patients with R/R AML and BPDCN.[62] Kuruvilla *et al*. discovered that when IMGN632 is combined with VEN, cancer cells' DNA damage response is impaired, preventing them from repairing DNA damage induced by IMGN632. This ultimately increases cancer cell death, highlighting the synergistic cytotoxic effect of IMGN632 and VEN. Their findings demonstrate that VEN's inhibition of DNA damage response enhances the efficacy of IMGN632. AZA further assists in disrupting cancer cell DNA, while VEN simultaneously inhibits BCL-2. The combined action of these three agents achieves highly efficient killing of AML cells.[63] Notably, IMGN632 in combination with VEN and AZA demonstrates significant efficacy in CD123-positive AML, particularly in the FLT3-ITD mutation subtype. However, its efficacy diminishes in AML cells with TP53 loss-of-function mutations, providing a basis for subsequent precision patient selection.[64] A Phase Ib/II study (NCT04086264) evaluating IMGN632 as monotherapy or in combination with VEN and/or AZA in CD123-positive AML is currently ongoing (Table 1). To determine the optimal dose for combining IMGN632 with chemotherapy, a Phase I trial (NCT06034470) is currently underway evaluating a regimen of FLAG-Ida plus PVEK (IMGN632) in newly diagnosed AML patients with adverse-risk factors (Table 1).

Combination therapy with BsAbs

BsAbs represent a promising cancer immunotherapy approach. By combining the antigen-binding sites of two antibodies, they enable simultaneous binding to two distinct epitopes on the same or different antigens.[65] Multiple forms of BsAbs can be bridged to enable specific binding between tumor antigens and the T-cell co-receptor molecule CD3, redirecting polyclonal T cells to target tumor cells and activating the cytotoxic function of immune effector cells.[66] When combined with chemotherapy, they aim to eliminate minimal residual disease and reduce recurrence rates.

Flotetuzumab (MGD006) is an investigational CD123 dual-affinity redirection toxin molecule targeting both CD123 and CD3. It simultaneously binds to CD123 on the surface of AML cells and to CD3 on the surface of effector T cells.[67] *In vitro* experiments and *in vivo* animal models demonstrate that activated T cells can exert targeted cytotoxic effects on AML blast cells by secreting granzyme and perforin, ultimately inducing AML blast cell death.[68] In a trial, flotetuzumab demonstrated synergistic enhancement of efficacy when combined with cytarabine for the treatment of pediatric AML.[69] Combination therapy did not interfere with the anti-AML activity of MGD006, confirming the feasibility of combining chemotherapy (cytarabine) with a BsAb (flotetuzumab). Results from two patient-derived xenograft models (high/moderate CD123 expression) demonstrated that the combination regimen adapts to pediatric AML with varying CD123 expression levels, showing potential for application across a broader range of patient subtypes. For the high CD123 expression group, the combination regimen not only achieved durable responses comparable to the T-cell + flotetuzumab group but also further reduced residual lesions. For the moderate CD123 expression group, the combination regimen demonstrated consistent efficacy.[69] This suggests that even in models with moderate CD123 expression levels, where flotetuzumab monotherapy demonstrates limited efficacy, the addition of cytarabine can sustain therapeutic effects. This opens up the possibility of combination therapy for pediatric AML patients with moderate CD123 expression in future clinical practice, expanding the potential patient population for flotetuzumab.

Multiple studies have now confirmed that immune suppression mechanisms play a crucial role in BsAb therapy. Research by Krupka et al. revealed that persistent PD-L1 expression was not detected in most samples from newly diagnosed AML patients. However, the BsAb AMG330 can trigger cytokine-dependent upregulation of PD-L1 on the surface of primary AML cells by inducing potent T-cell activation and cytokine release, ultimately mediating immune escape of tumor cells.[70] Research by Zhou *et al*. indicates that following suppression of regulatory T cells, PD-1/PD-L1 pathway blockade therapy significantly enhances the proliferative activity and function of locally resident cytotoxic T cells within tumors. This approach reduces tumor burden and further improves treatment efficacy.[71] Breakthroughs in combination therapy research in this area are anticipated, promising to inject new vitality into the advancement of immunotherapy for AML.

Combination of tumor vaccines and HMAs

Recent studies have confirmed that chemotherapy not only directly kills tumor cells but also enhances the cytotoxic effects of immune cells. Chemotherapy drugs can induce immunogenic cell death in leukemia cells.[72] For instance, decitabine has been demonstrated to induce the expression of tumor-associated antigens, enhance T-cell function, and generate cytotoxic immune effects.[73,74] Chemothera-

py promotes the release of tumor-associated antigens such as WT1 and CD19, providing an abundant antigen source for vaccines and enhancing immune recognition.[74]

One early clinical trial (NCT01834248) combined decitabine with the NY-ESO-1 vaccine, yielding exciting results that may increase the likelihood of leukemia cells being recognized and destroyed by the immune system. Another early-phase clinical trial (NCT03358719) investigated the combination of decitabine with the NY-ESO-1 vaccine and nivolumab in patients with AML and MDS. Notably, all patients exhibited CD4 T-cell responses capable of precisely recognizing the NY-ESO-1 marker, and this response was associated with increased immune gene activity related to nivolumab. This suggests that the immune system has indeed been activated against specific markers on cancer cells. This finding also paves the way for subsequent research into the NY-ESO-1 vaccine and anti-PD-1 monoclonal antibody regimen.

Challenges, limitations, and future directions

AML cells lack absolutely specific surface antigen markers such as CD33 and CD123, which are commonly used clinical targets. These markers are also expressed to varying degrees on the surface of normal hematopoietic progenitor cells. This makes it highly likely for off-target toxicity reactions to occur when immunotherapy is combined with chemotherapy. Taking the CD33-targeted ADC GO as an example, when used in combination with chemotherapy regimens, it effectively eliminates AML tumor cells but simultaneously causes severe damage to normal hematopoietic stem cells. This leads to persistent suppression of bone marrow hematopoietic function, inducing life-threatening complications such as infections and bleeding.[75] Moreover, there are significant differences in the driver gene profiles and target antigen expression levels among different patient groups. Clinical data indicate that over 97% of AML patients harbor somatic gene mutations.[76] This high degree of heterogeneity results in variable responses to personalized treatment. Currently, researchers are actively exploring novel tumor-associated antigens such as CD70, ILT3, and LAIR1. These antigens exhibit high specificity on the surface of AML cells while being expressed at extremely low levels on normal hematopoietic stem cells. Immunotherapy drugs developed based on these targets hold promise for fundamentally reducing treatment-related toxicity.[77,78] Multi-generational technological innovations in CAR-T cell engineering are advancing and aiming to create safer immunotherapies.[79] Significant differences exist in the molecular subtyping and tumor microenvironment of AML patients, leading to marked variations in the efficacy of immunotherapy combined with chemotherapy. For example, TP53-mutated AML patients exhibit reduced tumor cell immunogenicity and impaired DNA damage repair capacity, thereby decreasing sensitivity to standard chemotherapy and ultimately developing drug resistance.[80] Additionally, mutations in the PD-1/PD-L1 signaling pathway and major histocompatibility complex dysfunction lead to defects in antigen presentation and reduced T-cell activation, resulting in minimal efficacy of immune checkpoint inhibitors in TP53-mutated AML.[81] Therefore, there is an urgent need to develop other combination therapies to improve this situation.

The immunosuppressive microenvironment of AML exacerbates chemotherapy resistance, while immunotherapy can overcome this microenvironmental constraint. For example, bispecific T-cell engagers establish a connection between T cells and leukemia cells, activating the cytotoxic activity of T cells. This immune-activating effect synergizes with chemotherapy, effectively improving treatment response rates in drug-resistant patients.[82] To address antigen heterogeneity, novel leukemia-specific antigen screening, structural modification of immunotherapeutic agents, and multi-target combination strategies can be developed to overcome challenges posed by antigen heterogeneity and off-target toxicity. Personalized treatment plans can be developed based on factors such as the patient's genetic mutations, antigen expression profile, and age. For elderly high-risk patients, low-intensity chemotherapy combined with the less toxic CD123-targeted ADC IMGN632 is selected to balance efficacy and safety.[83] For patients with T-cell depletion following chemotherapy, incorporating cytokines such as IL-7 and IL-12 into combination regimens can enhance T-cell function and improve the efficacy of immunotherapy.[84]

The development of AML immunotherapy combined with chemotherapy is currently at a critical juncture in its transition toward precision medicine. Despite facing multiple challenges such as treatment heterogeneity, dual drug resistance, and cumulative toxicity, continuous exploration and the ongoing refinement of immunotherapy approaches have now revealed clear pathways for breakthroughs. In the future, we should focus on screening and validating highly specific surface antigens of AML, ensuring that such antigens are not significantly expressed in normal hematopoietic cells, thereby reducing the risk of off-target damage. We should continue to advance the research, development, and upgrading of next-generation CAR-T cells, BsAbs, and ADCs, enhance the targeting selectivity of formulations, and attenuate associated immunosuppressive effects. To address the challenges posed by tumor heterogeneity and therapeutic resistance, we should further explore multi-target intervention strategies and optimize the theoretical basis for combination therapy. Meanwhile, an individualized diagnosis and treatment system should be established based on antigen expression profiles, clinical risk stratification, and other factors to achieve precision medicine. With continuous advances, long-term survival and quality of life for patients with AML are expected to improve, and more effective and safer immunotherapeutic strategies are anticipated to emerge.

Conclusions

Based on the systematic analysis of chemotherapy-immunotherapy combination therapies for AML in this review, it is concluded that such synergistic regimens effectively break through the limitations of single chemotherapy and monotherapy immunotherapy, demonstrating remarkable anti-leukemic efficacy in preclinical research and clinical practice. Representative combinations including PD-(L)1 inhibitors combined with HMAs, CD33/CD123-targeted ADCs plus chemotherapy, CAR-NK cell therapy merged with chemotherapy, and tumor vaccines combined with HMAs have achieved favorable clinical outcomes in heterogeneous AML populations: prolonging survival in elderly patients, elevating remission rates in R/R and high-risk patients, and showing reliable clinical tolerability.

Nevertheless, this combination strategy still faces critical challenges, including off-target toxicity caused by insufficient antigen specificity, tumor heterogeneity leading to variable therapeutic responses, drug resistance, and immunosuppressive microenvironment constraints. Notably, therapeutic benefits vary significantly among AML subtypes and disease stages, while early intervention and optimized treatment timing can further enhance clinical efficacy, and antigen-targeted precision therapy offers a feasible solution for genetically mutated AML patients. Moving forward, the development of novel specific antigens, upgraded immunothera-

peutic agents, multi-target intervention strategies, and individualized treatment regimens will help overcome existing bottlenecks, ultimately boosting the long-term survival and quality of life of AML patients.

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Conflict of interest

The authors have no conflicts of interest associated with this publication.

Author contributions

Manuscript drafting (HJ), language revision, data collection and table preparation (YB, SF), conceptual discussion of the manuscript (TY), revision and conceptualization of the manuscript (ZL). All authors have approved the final version and publication of the manuscript.

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