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Review

Review

Immunotherapy in leukaemia

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Abstract

Leukaemia is the common name for a group of malignant diseases of the haematopoietic system with complex classifications and characteristics. Remarkable progress has been made in basic research and preclinical studies for acute leukaemia compared to that of the many other types/subtypes of leukaemia, especially the exploration of the biological basis and application of immunotherapy in acute myeloid leukaemia (AML) and B-cell acute lymphoblastic leukaemia (B-ALL). In this review, we summarize the basic approaches to immunotherapy for leukaemia and focus on the research progress made in immunotherapy development for AML and ALL. Importantly, despite the advances made to date, big challenges still exist in the effectiveness of leukaemia immunotherapy, especially in AML. Therefore, we use AML as an example and summarize the mechanisms of tumour cell immune evasion, describe recently reported data and known therapeutic targets, and discuss the obstacles in finding suitable treatment targets and the results obtained in recent clinical trials for several types of single and combination immunotherapies, such as bispecific antibodies, cell therapies (CAR-T-cell treatment), and checkpoint blockade. Finally, we summarize novel immunotherapy strategies for treating lymphocytic leukaemia and clinical trial results.

Introduction

Leukaemia constitutes a group of life-threatening malignant diseases of the haematopoietic system that are characterized by increased numbers of clonal leucocytic cells in the bone marrow and/or in the peripheral blood/tissue with subsequent failure of normal haematopoiesis and destruction of organs. Leukaemia is classified into four main types, namely, acute lymphocytic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphoblastic leukaemia (CLL), and chronic myeloid leukaemia (CML), as defined by cell lineage and the stage of maturation arrest. Leukaemia affects people of any age, from infants to elderly individuals [1,2]. Different forms of the disease are associated with different age distributions: ALL is most commonly seen in children and is rarely diagnosed in adults, while AML is less common in children but more frequently diagnosed in older adults. AML is the most common acute leukaemia in adults. CML is uncommon in young children, and CLL is almost exclusively diagnosed in people older than 40 years old, with the median age at diagnosis higher than 70 years [3,4]. Haematopoietic stem cell transplantation (HSCT), an immunotherapy, is considered the most effective method for treating leukaemia patients. However, several effects have limited HSCT application and success in leukaemia, such as HLA mismatches, graft-versus-host disease and relapse [5]. Recently, several types of leukaemia have been profoundly attenuated with targeted drugs; for example, 80%–90% of acute promyelocytic leukaemia (APL) patients were cured after the application of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) [6], almost all BCR-ABL1$^+$ CML patients benefited from tyrosine kinase inhibitors (TKIs) [7], and small-molecule drugs targeted to specific genetic lesions in AML (*e.g.*, constitutive activation of the tyrosine kinase FMS-like tyrosine kinase-3, FLT3 mutations [8], neomorphic function of the metabolic enzyme isocitrate dehydrogenase, and IDH1/2 mutations [9]) have benefitted only a subset of patients bearing specific mutations. In fact, the molecular heterogeneity in AML patients has complicated the successful development of new therapeutic agents. Nevertheless, the mainstay of treatment for other types/subtypes of leukaemia, especially AML types, is still chemotherapy, which has remained

mostly unchanged over the past four decades, and the specific treatments and responses vary based on patient age, comorbidities and the genetics associated with the disease. Most leukaemia patients do not seek only durable remission, indicating an urgent need for developing novel and synergistic therapies. Recently, several breakthroughs in immunotherapy have rekindled patient hopes and carry the promise to eradicate chemoresistant leukaemia stem cells and provide long-term disease remission. One of these outstanding breakthroughs is CAR-T immunotherapy, which targets the B-cell lineage antigen called cluster of differentiation 19 (CD19) or B-cell maturation antigen (BCMA) in B-cell malignancies, such as B-ALL, B-cell lymphoma and multiple myeloma, and has shown promising application prospects for other leukaemia types [10–12]. Is CAR-T therapy suitable for AML and other types of leukaemia? The answer is based on complex factors, and better biological knowledge is needed before CAR-T immunotherapy can be applied in AML and other types of leukaemia. Fortunately, basic and preclinical studies of AML immunotherapy are being intensively performed, and remarkable progress has been made. Therefore, we will take AML as an example and mainly introduce basic/preclinical research progress and identify bottlenecks in the immunotherapy field.

Immunotherapy is a potential treatment for leukaemia, and several forms of the treatment can be administered, including allogeneic bone marrow transplantation, therapeutic cancer vaccines, T-cell therapies, monoclonal antibody therapies and donor lymphocyte infusion.

In this review, we summarize the main strategies of immunotherapy for leukaemia and focus on the progress and challenges of research into immunotherapy use for AML. Finally, we further summarize the novel immunotherapy strategies for lymphocytic leukaemia and clinical trial results.

Several Types of Immunotherapies Used for Treating Leukaemia

Allogeneic bone marrow transplantation

By using HLA-matched bone marrow from donor or peripheral blood stem cells for transplantation, allogeneic stem cell transplantation (ASCT) helps reconstruct the immunity and haematopoietic function in patients with bone marrow failure or haematological malignancy. Because of the graft-versus-leukaemia (GVL) effect, the lymphocyte population, which is transplanted from a donor, can remove residual malignant clones. This outcome contributes a large part of the anticancer effect mediated by ASCT [13]. For patients with high-risk leukaemia, ASCT offers the best chance of a cure.

Despite its potential benefits, ASCT is not always a viable option, such as for patients with high-risk AML, and many of these patients do not reach the first remission (CR1) stage [14]. Moreover, ASCT carries a substantial risk of associated morbidity and mortality. Reduced-intensity conditioning regimens have broadened the pool of patients eligible for ASCT; however, elderly patients are still more likely to be excluded due to comorbidities. Relapse after allogeneic transplantation remains a significant challenge even in eligible patients with AML. Recent data also suggest that detectable pretransplantation AML or minimal residual disease (MRD indicates a poor outcome and a high rate of relapse after allogeneic transplantation [15].

Therapeutic cancer vaccines

A vaccine for AML aims to select and destroy AML cells by introducing tumour antigens that actively stimulate the patient's immune system [16]. A peptide vaccine binds firmly to AML cells that display an antigen but not with nonmalignant cells that do not exhibit the target antigen. Examples of cancer vaccines include Wilms tumour 1 (WT1) antigen vaccines, proteinase 3 vaccines, granulocyte-macrophage-colony stimulating factor (GM-CSF) vaccines, dendritic cell (DC) vaccines, and whole-tumour-cell vaccines. In summary, the vaccine stimulates the immune system to recognize and attack AML cells while leaving the healthy cells intact.

T-cell therapies

Genetically engineered T cells have allowed powerful new medicinal strategies to be employed for treating patients with cancer and have revitalized the field of adoptive cell therapy. Recently, several strategies have been developed to harness anti-leukaemia T-cell activity, including the recruitment of T cells, the actions of chimeric antigen receptor T cells and TCR-modified T cells, and the reactivation of endogenous T-cell responses. These strategies have shown promising results in the treatment of leukaemia. To date, CAR-T cells have been widely investigated for their ability to treat certain malignancies, in particular B-ALL leukaemia and lymphoma [17]. This therapy is discussed in detail in the final section of the review.

Monoclonal antibody therapies

Monoclonal antibodies, which are among of the most promising agents for treating leukaemia, especially ALL and AML, enhance treatment while inducing low levels of nonspecific toxicity. Specifically, these antibodies target specific antigens, such as CD20, CD52, CD19, and CD22 expressed on cells in ALL [18] and CD33, CD123, CD32, CD47, CD56, and LILRBs expressed on cells in AML [19,20]. By targeting these antigens, monoclonal antibodies enhance treatment efficacy while minimizing nonspecific toxicity. They have shown great potential in improving patient outcomes and are being extensively studied in clinical trials. In summary, monoclonal antibodies represent a promising therapeutic approach for leukaemia by selectively targeting cancer cells and sparing healthy cells.

Donor lymphocyte infusion

Immune exhaustion in donor cells after allogeneic stem cell transplantation (ASCT) is a common cause of relapse in leukaemia patients. Donor lymphocyte infusion (DLI) counters immune exhaustion after transplantation, thereby reducing the risk of relapse in leukaemia patients; however, this benefit is conferred at the expense of increased toxicity-induced complications, such as graft-versus-host disease and pancytopenia [21]. Patients with CML show the optimal response to DLI, with a nearly 80% response [22]. The use of DLI to treat CML has been diminished since the discovery of imatinib; therefore, recent investigations have been centred on enhancing the effects of DLI for treating post transplantation relapse in AML patients. Unfortunately, patients with acute leukaemia tend to have a relatively low response rate, likely due to the increased rate of cell proliferation and a slow response to donor lymphocytes [23,24].

Progress and Bottlenecks in AML Immunotherapy

Acute myeloid leukaemia (AML) is a malignant disorder characterized by uncontrolled proliferation of immature myeloid cells.

Immunotherapy has shown promise in the treatment of AML, but progress has been hampered by several bottlenecks.

Heterogeneity of AML

AML is a heterogeneous disease characterized by significant variability at the molecular level [25]. This heterogeneity poses a challenge for developing effective immunotherapy strategies, as it makes it difficult to identify uniform targets for therapeutic interventions [26]. The molecular and genetic differences among AML patients mean that an individualized approach may be required to achieve optimal outcomes. Consequently, identifying biomarkers for patient stratification and developing personalized immunotherapies tailored to each patient's specific disease characteristics represents a critical need in the field of AML immunotherapy.

Complexity of immune evasion

The immune escape of AML refers to the ability of AML cells to evade immune surveillance and attack by various mechanisms, which may lead to unlimited proliferation of AML cells in the body and consequently disease progression and relapse. There are several specific immune escape phenomena of AML, such as abnormal expression surface molecules, production and mediation of cytokines, secretion of immunosuppressive substances and inhibition of cell apoptosis. The mechanisms include the following: (1) Mutations, deletions or negative expression of cell surface molecules: AML cells can alter the expressions of surface molecules through mechanisms such as mutation or epigenetic changes, thereby avoiding recognition and attack by the immune system [27]. (2) Production and mediation of cytokines: AML cells can produce and release some immunosuppressive cytokines, such as IL-10 and TGF-β, to suppress the function of immune cells, thereby avoiding attack by the immune system. (3) Secretion of immunosuppressive substances: AML cells can secrete some immunosuppressive substances, such as IDO and PDL-1, to suppress the function of immune cells, thereby avoiding attack by the immune system [28]. (4) Inhibition of cell apoptosis: AML cells can activate some anti-apoptotic pathways, such as members of the Bcl-2 family, to resist cell apoptosis induced by immune cells, thereby avoiding clearance by the immune system.

These are some common phenomena of AML immune escape, which can act alone or in combination to help AML cells evade attack by the immune system [29]. Understanding the mechanisms of immune evasion in AML is crucial for developing effective immunotherapeutic strategies that can overcome these obstacles and improve patient outcomes.

Toxicity and safety concerns

Certain types of immunotherapies, including chimeric antigen receptor (CAR) T-cell therapy, can cause significant toxicity and adverse effects, such as cytokine release syndrome (CRS) and neurological toxicities [30]. CRS is a systemic inflammatory response that can lead to fever, hypotension, and organ dysfunction, while neurological toxicities can result in seizures or delirium. Therefore, mitigating these risks is essential for advancing the use of immunotherapy for AML [31]. Developing effective strategies to prevent or manage these toxicities is crucial for ensuring the safety and efficacy of these treatments.

Despite these challenges, there has been progress in AML immunotherapy. Several clinical trials have shown promising results for therapies such as CAR T-cell therapy, immune checkpoint inhibitors, and bispecific antibodies. Additionally, advances in gene editing technologies, such as CRISPR/Cas9, have enabled more precise targeting of AML cells. Further research is needed to address the bottlenecks and develop safe and effective immunotherapies for AML.

Immune Cell Evasion Mechanisms of AML Cells

AML cells develop several mechanisms to escape immune surveillance, including but not limited to hiding from immune recognition proteins (mediated by the altered antigen presentation processing, upregulation of inhibitory ligands/receptors and upregulated release of immunosuppressive molecules) and AML-mediated modifications/regulations of effector T cells, natural killer (NK) cells, DCs, myeloid-derived DC cells, NK cells, effector T cells, myeloid-derived suppressor cells and tumour-associated macrophages, and immunoinhibitory soluble factors. Both basic and clinical studies are required to determine how each mechanism contributes to AML immune evasion.

It is crucial to conduct both basic and clinical studies to determine the exact contribution of each mechanism in AML immune evasion. These studies will aid in developing targeted therapeutic strategies that can effectively counteract AML-mediated immune suppression and improve clinical outcomes for AML patients.

Altered antigen presentation

MHC-I and MHC-II molecules continuously present antigenic peptides to $CD8^+$ and $CD4^+$ T cells. However, the number of MHC molecules is often reduced, or they are absent in cancer cells; the reduction in these molecules has long been considered a major stumbling block to T-cell recognition and prevents cancer cell elimination [32]. Leukaemia blasts show immunoediting capability. Mismatched MHC molecules are lost during haploidentical transplantation, and MHC II molecules are epigenetically downregulated in various contexts [33–35]. According to Vago *et al.* [35], 17 AML patients underwent relapse following HSCT and donor-derived T-cell therapy. The expression of the HLA haplotype in the AML cells was lost in 5 of these patients due to uniparental disomy on chromosome 6p. As a result, donor-derived T cells were incapable of recognizing and killing AML cells that did not express the restricted number of MHC molecules required for T-cell recognition [35].

Utilizing inhibitory ligands/receptors

Negative regulatory ligands/receptors modulate T-cell responses, thus limiting T-cell-mediated damage to self-tissues. Multiregulatory receptors are important mechanisms in immune cells to enable them to evade the immune system. Regulatory receptors include programmed cell death protein-1 (PD-1), T-cell immunoglobulin and mucin domain-3 (TIM-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and lymphocyte activating 3 (LAG3), as well as their ligands on AML leukaemia cells, such as programmed death-ligand 1 (PD-L1), PLC2, galectin-9, and poliovirus receptors. AML cells can upregulate the expressions of inhibitory ligands and engage in intracellular signalling events that stop activation cascades after recognizing and costimulating the antigen-carrying cells.

The PD-1/PD-L1 axis

To avoid immune surveillance, AML cells overexpress immune

checkpoint (IC) ligands, which inhibits the function of T and NK cells [28]. Among the AML ligands of ICs, PD-L1 binds to the PD-1 receptor on T cells, triggering T-cell exhaustion and their differentiation into regulatory T cells (Tregs) [36,37].

This process of T cell exhaustion and differentiation into Tregs can contribute to the immunosuppressive environment in AML, allowing for continued growth and survival of AML cells. Therefore, targeting PD-L1/PD-1 interaction has emerged as a promising strategy to overcome immune evasion in AML. Several clinical trials evaluating PD-1/PD-L1 inhibition as a therapeutic approach for AML are ongoing, and early results have shown promising clinical responses.

The TIM3/GAL-9 axis

TIM-3, another coinhibitory receptor, induces the activation of downstream pathways, including the PI3K/AKT/mTOR, β-catenin and NF-κB pathways, in AML blasts that express galectin-9 (GAL-9), which are crucial for cell survival and AML progression [38–40]. In addition, the TIM-3/GAL-9 axis promotes the NK cell-mediated production of IFN-γ, which is critical to indoleamine 2,3-dioxygenase 1 (IDO1) expression in AML blasts and ultimately causes NK cell dysfunction [41]. Several lines of evidence indicate that coexpressed TIM-3 and PD-1 T cells are closely associated with poor prognosis in patients with AML and in murine models [42–44].

TIGIT-CD155/CD112 axis

As a novel target for cancer immunotherapy, the TIGIT receptor is highly expressed on activated T cells, NK cells, and Tregs [45,46]. Notably, TIGIT shows much higher binding affinity for CD155 and CD112 than for CD226, and both CD155 and CD112 are expressed on AML blasts [47]. Similarly, TIGIT has also been implicated in AML cell immune evasion. Kong *et al.* [48] reported that TIGIT contributed to dysfunctional T-cells and cytokine production as well as high apoptosis susceptibility in AML. According to another clinical trial, patients with AML who express CD112 and CD155 have a poor prognosis [49].

Targeting the TIGIT pathway represents a promising approach to overcome immune evasion in AML. Several clinical studies evaluating TIGIT inhibition as a therapeutic strategy for AML are currently ongoing, and pre-clinical studies have demonstrated the potential synergy between TIGIT blockade and other immunotherapeutic agents such as PD-1/PD-L1 inhibitors. Further research is needed to elucidate the precise mechanisms of TIGIT-mediated immune suppression in AML and optimize the clinical efficacy of TIGIT-targeted therapies.

Others

Other inhibitory receptors, such as LAG-3, are involved in the dysfunction of T cells. Although LAG-3-expressing Tregs produce immunosuppressive cytokines (IL-10 and TNF-a), the mechanism by which LAG-3 regulates anti-immunity has not been elucidated [50]. Some recent reports have indicated that a higher frequency of $LAG3^+$ T cells was found in AML patients, and high mRNA expression predicted an unfavourable prognosis [51,52]. The expression of CTLA-4 on both activated T cells and regulatory T cells enabled T-cell attachment to CD80 and CD86 on antigen-presenting cells (APCs). The higher affinity of CTLA-4 for B7 molecules allows it to replace CD28 in an immune synapse and thus inhibits T-cell and PPC costimulation. Studies conducted with humans and mice have demonstrated that the interaction of CTLA-4 expressed on T cells with CD80 and CD86 expressed on AML cells results in the inhibition of T-cell activation [53]. CD47 is a macrophage checkpoint and LSC marker that conveys a "do not eat me" signal after binding the receptor signal regulatory protein alpha (SIRPα) and suppresses the engulfment of cells by macrophages, which are some of the common signals in a mechanism that enables a tumour cell to evade innate immunity [54,55].

Immunosuppressive microenvironment

The immunosuppressive microenvironment is a pathological condition characterized by the presence of various immune evasion mechanisms employed by tumor cells and other factors within the tumor microenvironment (Figure 1).

AML and T cells

Leukaemia blasts are essential for the regulation of T-cell responses. *In vitro* studies have shown that exposure to AML blasts can inhibit T cell proliferation and downregulate costimulatory molecules, ultimately leading to T cell apoptosis. Furthermore, monocytic leukemia cells are capable of generating reactive oxygen species (ROS), which can induce poly (ADP-ribose) polymerase-1-dependent (PARP-1-dependent) apoptosis. This process can result in the death of both T cells and natural killer (NK) cells [56]. These findings highlight the ability of AML cells to manipulate the immune microenvironment and evade immune surveillance. Understanding the mechanisms underlying AML-mediated T cell dysfunction and apoptosis is crucial for developing effective immunotherapeutic strategies for AML. Targeting these mechanisms could potentially reverse T cell dysfunction and enhance anti-leukemia immune responses in AML patients.

AML and NK cells

In contrast to the adaptive immune system, NK cells interact with tumour cells through inhibitory and activating receptors in an MHC-II-dependent manner. However, after *in vitro* exposure to AML cells, NK cells show reduced cytotoxicity and effector function [57]. Dysregulated methylation of genes encoding NKG2D ligands was reported to contribute to AML cell immune evasion [58]. Moreover, decreased level of the activating receptor CD226 in AML blasts contributes to the degranulation of NK cells, leading to impaired cytotoxicity [59]. Additionally, the expression of CD48, a ligand of a receptor expressed on NK cells, was specifically downregulated after treatment with aberrant leukaemia-specific fusion proteins PML-RARα and AML1-ETO, which ultimately impaired NK cell-induced cytotoxicity [60].

AML and regulatory T cells (Tregs)

Tregs constitute an immunosuppressive $CD4^+$ T-cell population that plays a critical role in maintaining peripheral tolerance to self-antigens [61,62]. Tregs suppress immune responses in multiple ways, including competition for IL-2 [63], secretion of inhibitory cytokines such as IL-10 and IL-35 [64], generation of extracellular adenosine [65], and changes in the performance of antigen-presenting cells via their interaction with CTLA-4 and LAG-3 on Tregs [66–68]. Studies have shown that Tregs play a leading role in defective immune responses in AML [69]. In the PB and BM of AML patients, which appear to induce more potent immunosuppressive effects on effector T cells (Teffs), the number of Tregs was increased. Moreover, increased Treg level tends to correlate unfavourably with clinical outcomes [70,71].

MDSCs and tumour-associated macrophages (TAMs)

AML patients exhibit an increased abundance of myeloid-derived suppressor cells (MDSCs), which promote T-cell tolerance by expressing inhibitor ICs (*e.g.*, V-domain Ig suppressor of T-cell

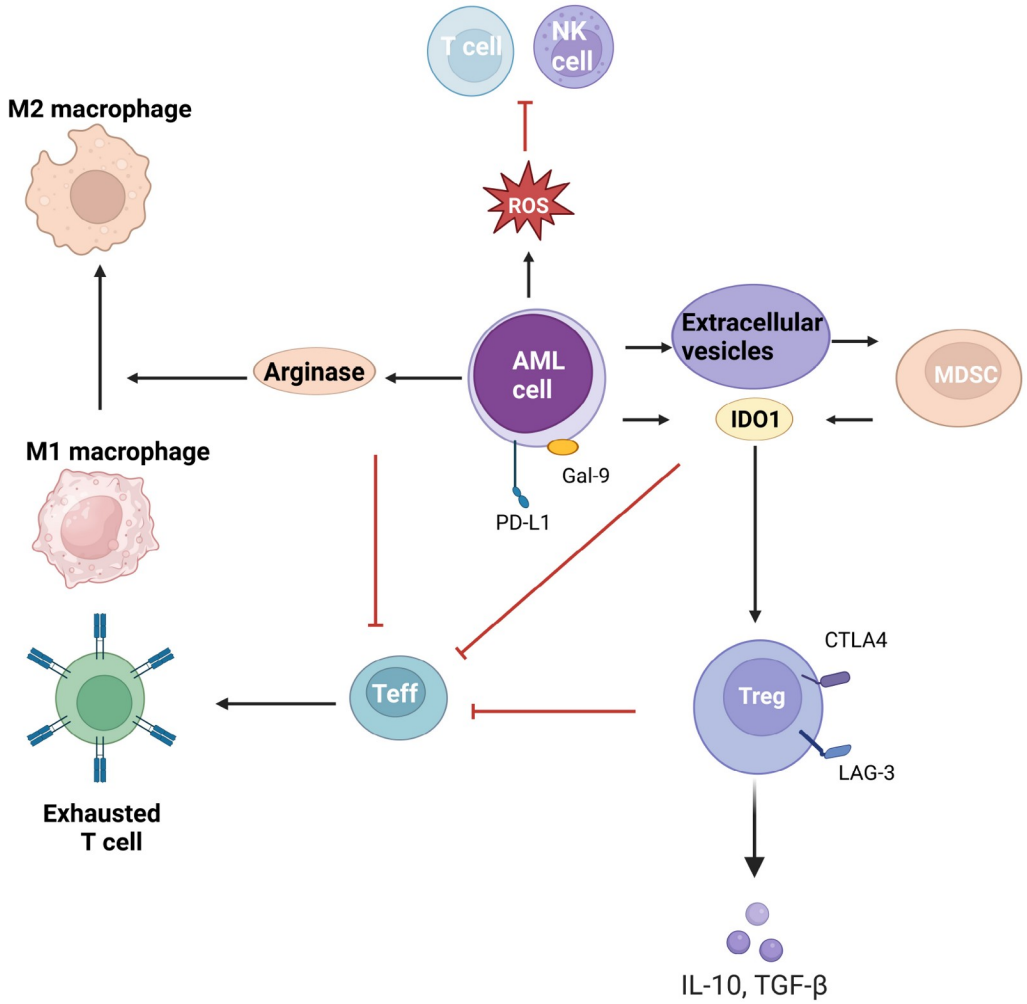

Figure 1. Pathological immune microenvironment of acute myeloid leukemia The immunosuppressive microenvironment is a pathological condition characterized by the presence of various immune evasion mechanisms employed by tumor cells and other factors within the tumor microenvironment. These mechanisms can include the downregulation of antigen presentation molecules, overexpression of inhibitory molecules such as PD-L1 and Gal-9, and the release of various immunosuppressive factors such as reactive oxygen species, indoleamine 2,3-dioxygenase, and myeloid-derived suppressor cells. These factors can inhibit the cytotoxic function of T and NK cells, induce T cell exhaustion and apoptosis, promote the generation of regulatory T cells, and switch macrophages from an M1 to suppressive M2 phenotype. The immunosuppressive microenvironment is a major challenge to effective cancer immunotherapy and represents a complex interplay between tumor cells and the immune system.

activation and PD-L1), immunoinhibitory factors such as indoleamine 2,3-dioxygenase 1 and arginase, and several cytokines, including TGF-β and IL-10. Additionally, MDSCs produce excessive amounts of reactive oxygen species (ROS) and peroxynitrate [72,73]. Pyzer *et al.* [74] reported that AML blasts released extracellular vesicles (EVs) containing the oncoprotein MUC1 to promote MDSC expansion; specifically, increased c-Myc expression in EVs led to MDSC proliferation. Several recent studies revealed that MDSCs were abundant in the BM of AML patients and correlated with poor prognosis [72,75]. Moreover, Tregs are main contributors to immune evasion in AML and exert greater immunosuppressive effects on effector T cells (Teffs) than is observed under normal conditions [70,76]. Recent data showed a strong relationship between the number of MDSCs and Tregs in myelodysplasia with high risk, indicating a potential role for MDSCs and Tregs in AML progression [77]. Within the tumour microenvironment, tumour-associated macrophages (TAMs) are mainly polarized into M2 macrophages, which promote the metastasis and progression of tumour cells by secreting arginase, metalloproteinases, TGF-β, IL-10, and other cytokines [78,79].

Immunoinhibitory soluble factors

Several enzymes expressed by AML cells or other cells with a disordered cytokine network may contribute to leukaemia progression. Immunoinhibitory factors secreted by AML blasts, such as IL-10, IL-35, TGF-β and IDO1, were reported to enhance T-cell polarization and promote T-cell tolerance [80]. For example, tryptophan N-formylkynurenine is catabolized by IDO1, a rate-limiting enzyme in the kynurenine pathway. Furthermore, the depletion of local tryptophan and the accumulation of harmful metabolites lead to a decrease in the proliferation of Teffs and an increase in T-cell apoptosis [81].

Another soluble factor, arginase II, has been shown to be involved in the regulation of the TME in AML. In AML patients, arginase II concentrations were found to be higher than those in

healthy controls, driving T-cell proliferation and monocyte differentiation towards an M2-like phenotype [82]. Studies conducted with animal models of monocytic AML revealed a connection between arginase I and the aggressiveness of leukaemia by facilitating cancer cell infiltration into tissues and T-cell suppression. These effects are thought to be mediated via a signalling pathway involving the LILRB4 receptor [20]. Other studies have shown that the metabolism of fatty acids and glucose and their metabolites plays a role in the AML process and provides targets to restore active immunity in AML, respectively [83].

Changes in metabolism in the AML niche

Deregulated energy metabolism in the BM niche and AML cells may be involved in the immune evasion process. In AML patients, mesenchymal stem cells (MSCs) tend to differentiate into adipocytes, which further interact with AML blasts in the BM niche to support the metabolic demands of leukaemia [84]. AML blasts activate lipolysis in adipocytes and promote fatty acid transport into leukaemia cells. Moreover, the accumulation of fatty acids disrupts the function of Teffs and contributes to Treg differentiation [85]. Increasing evidence links enhanced leukaemia cell apoptosis to the paracrine activity of leukaemia-initiating cells (LICs), which is mediated by the release of multiple metabolites in the bone marrow niche, supporting AML development. Recently, Zheng's team revealed that ATP concentrations were significantly increased in the BM microenvironments of AML mice and that LICs showed a tendency to reside in the endosteal niche. Their research indicated that ATP-P2X7-mediated signalling augments AML development through the CREB-PHGDH pathway [69].

Known AML Targets and Obstacles to Find Suitable Targets

Surface antigen targets in AML

Identifying antigen targets that are critical to AML biology and selectively expressed in malignant cells remains a challenge. In addition, the complexity of the clone composition in AML and the tendency for it to change with disease progression complicate the exploration of potential antigen targets. To date, antibody drugs have been developed for targeting cell surface markers in AML; these treatments include antibody–drug conjugates and chimeric antigen receptor T-cell therapy.

Antigen targets in AML can generally be classified on the basis of lineage-restricted antigens (LRAs), leukaemia-associated antigens (LAAs) and leukaemia-specific antigens (LSAs).

Several LRAs (Table 1) have been found on the surface of leukaemia cells and are considered proper targets for antibody or chimeric antigen receptor (CAR) T-cell-based therapeutic approaches. However, LRAs are also expressed in normal haematopoietic and even some nonhaematopoietic tissues, and thus their effectiveness as immunotherapeutic targets is largely offset by the reduction in targeted toxicity. Alternatively, LAAs and LSAs are immunotherapeutic targets when they are processed by the HLA complex and presented to T cells [86–88]. CD33 and CD123 are the most studied LRAs in AML, but myelosuppression has attracted considerable attention because the aforementioned targets are also expressed in normal haematopoietic tissues [89–93].

In recent years, an increasing number of immune targets have been developed for the treatment of AML; these targets include CD33, CD123, CLL-1, CD44, FLT-3, CD47, CD70 and TIM-3. For example, the *CD33* gene encodes a sialic acid-bound Ig-like lectin that is expressed on different cell types, such as unipotent colony-forming cells, pluripotent myeloid precursor cells, monocytes and mature granulocytes. Additionally, it has been found on macrophages, dendritic cells, and in subsets of B cells, activated T cells, and natural killer cells. Although one study showed that CD33 was expressed on brain microglia, CD33 is generally thought to be expressed only in haematopoietic systems [94]. More than 80% of leukaemia cells isolated from AML patients express CD33, on which they also show very high density. Compared to that in HSCs, in malignant AML blasts, CD33 is differentially expressed, which makes it an ideal immunotherapy target. Another candidate antigen target called CD123 is highly expressed in various haematologic malignancies, such as AML, B-cell acute lymphoblastic leukaemia, Hodgkin lymphoma and, particularly, blastic plasmacytoid dendritic neoplasm. In addition to its expression in LSCs, CD123 is expressed in other differentiated leukaemia blasts, conferring an advantage to CD123 as a therapeutic target. In recent studies, CD123 CAR-T cells have been explored for potential immunotherapy in patients with refractory/recurrent AML or BPDCN. Together, the recent studies strongly support the choice of CD123 as a potentially effective therapeutic target in BPDCN treatment [95]. Early in 2004, type c lectin-like molecule-1 (CLL-1) was discovered by scientists using phage display technology. Since then, 92% of AML, but not granulocyte macrophage progenitor cells (GMPs), have been found to express CLL-1 [96]. Importantly, CLL-1 is also expressed on LSCs, which show unlimited self-renewal ability and generate a large number of daughter cells from mother cells with specific phenotypes that promote leukaemia recurrence. Therefore, due to its unique characteristics, CLL-1 is considered an ideal drug target for AML.

FLT3 (FMS-like tyrosine kinase 3) is a receptor tyrosine kinase that is commonly mutated in AML, particularly in cases with a normal karyotype. There are two main types of FLT3 inhibitors: multikinase inhibitors and selective FLT3 inhibitors. Multikinase inhibitors such as midostaurin and sorafenib target multiple kinases, including FLT3, and have been shown to improve overall survival when used in combination with chemotherapy [107]. Selective FLT3 inhibitors, quizartinib has also demonstrated significant activity in relapsed or refractory FLT3-mutated AML [108]. Another surface antigen has been explored as a potential therapeutic target in AML treatment due to its overexpression in leukemic cells and association with a poor prognosis. Several studies has applied monoclonal antibodies, small molecule inhibitors, and chimeric antigen receptor (CAR) T cells to intervene AML and displayed a promising outcome [109,110].

More recently, many surface antigen targets have been identified, such as CD44, CD96, CD90, CD32, CD25, TIM-3, JAM3, CD244, CD70, LILRB4, Siglec-6, CD117, colony-stimulating factor 1 receptor (CSF1R) and CD86 [20,104–106,111–116]. Some of these targets exhibit clear for targeting AML cells. However, the heterogeneity of their expression levels in individuals with AML has inhibited their large-scale application. Much more effort is needed to identify widely usable surface antigen targets.

Intracellular antigens

Although lineage-restricted antigens (LRAs) are considered suitable therapeutic targets in leukaemia, their low specificity and utility as immunotherapeutic targets lead to their low efficiency. The LAA Wilms' tumour protein 1 (WT1), which is highly expressed in AML, is an intracellular antigen in leukaemia immunotherapy [117]. More

Table1. Summary of target antigen in AML

Name of target antigen	Explanation	Expression in AML blast	Expression in LSC	Expression in normal tissue	Reference
CD33	A sialic acid-bound Ig-like lectin	90%	Yes	HSCs; myeloid progenitors; monocytes; mast cells	[94]
CD123	The interleukin receptor 3 alpha subunit	50%–100%	Yes	Myeloid progenitors; monocytes; basophils; dendritic cells; epithelial cells	[95]
CLL1	C-type lectin-like molecule-1	80%–100%	Yes	Monocytes; granulocytes	[96]
CD56	The neural cell adhesion molecule (NCAM)	30%	Maybe	Dendritic, NK, and T cells; monocytes; neural and neuroendocrine tissues	[19]
CD135	FMS-like tyrosine kinase receptor-3	70%–100%	Yes	HSCs; myeloid progenitors; neurons	[97]
CD174	Blood group carbohydrate antigen	50%	Probable	HSCs	[98]
CD7	A transmembrane glycoprotein	30%	Maybe	Mature T cells	[99]
CD25	α chain of interleukin-2 receptor	10%–20%	Yes	Activated T cells	[97,100]
CD44	Phagocytic glycoprotein-1	30%	Yes	Leukocytes; mesenchymal stem cells; embryonic stem cells	[101,102]
CD44v6	CD44 isoform 6 (CD44v6)	64%	Yes	Monocytes; keratinocytes; different epithelial tissues	[101]
Flt3	FMS-like tyrosine kinase 3	30%	Yes	HSCs; myeloid progenitors; CMP; GMP; DCPs; lymphoid progenitor cells	[103]
CD70	Tumor necrosis factor ligand superfamily member 7	80%	Maybe	B-cell; T-cell	[104]
CD117	A receptor tyrosine kinase protein encoded by the KIT gene	60%–80%	Maybe	HSCs; HPC; early germ cells; melanocytes	[105]
Siglec-6	Sialic acid-binding Ig-like lectin-6	Not sure	Maybe	AML	[106]

importantly, no toxicity against the haematopoietic system has been observed in WT1-mediated immunotherapy, indicating the possibility that WT1 is a candidate therapeutic target [118,119]. Moreover, mutated nucleophosmin 1 (NPM1) has emerged as a candidate target for treating AML. Dyantha *et al.* [87] revealed that AML-expressed NPM1-mutant-derived peptides and mutant NPM1 targeted antigens after TCR gene transfer. Additionally, mutant tumour proteins, such as proteins encoded by internal tandem duplications (ITDs) in the FMS-like tyrosine kinase 3 gene (Flt3) and NPM1 mutant gene, can function as tumour-specific antigens that can be recognized by the immune system [120]. However, the determination of the expression of these candidate antigens in AML in addition to their leukaemia cell specificity needs to be considered. For example, PML-RARa, Flt3-ITD and mut-NPM1 antigens are expressed only in certain subgroups of AML patients, and therefore, they cannot be widely used for immunotherapy in AML [88,121,122].

BM microenvironment targets in AML

Located inside bone cavities, bone marrow is a viscous, soft tissue with a highly complex and dynamic microenvironment consisting of heterogeneous cell populations, blood vessels, and a variety of molecules that are distributed throughout niches. Significant advances have been made in treating AML in recent years, but treatment failure is still associated with poor prognosis, highlighting the need for new, innovative treatments. A broad understanding of leukaemia occurrence and the complex interactions between leukaemia cells and their microenvironment is needed to address major obstacles to treatment.

Bone marrow microenvironments play major roles in the development, progression, and recurrence of leukaemia. Notably, interactions between leukaemia cells and surrounding cells, as well as noncellular components, are critical during leukaemia development. A malignant tumour is characterized by invasion and metastasis, which is realized through various mechanisms, including chemotaxis and adhesion. The candidate targets in the bone marrow microenvironment have been demonstrated to interact with the LSC niche and to allow tumour cell migration mediated through chemokines, adhesion molecules, and integrins [102,123]. Moreover, several studies have indicated that leukocyte counts in peripheral blood are increased and leukaemic mother cells are mobilized by CXCR4 antagonists, and these leukocytes may be subjected to the cytotoxic effects of chemotherapy drugs [124,125]. Nevertheless, research and analysis of the relationship between AML cells and their microenvironment are needed to develop an effective and durable response. A summary of the signalling axes in the AML microenvironment is shown in Table 2.

Recent Clinical Trials of Single and Combination Immunotherapies for AML

Checkpoint blockade

As anti-PD1 antibodies have limited efficacy in treating AML, combination immunotherapies have been applied to enhance the antitumour effect of PD-1 inhibitors. In a clinical study, 51 AML patients for whom prior therapy had failed were treated with a combination that included the PD1 inhibitor nivolumab, and 18% of the patients achieved complete remission (CR), and 15% of the patients showed an improved haematologic profile [135]. The results of another Phase II study that included relapsed/refractory AML patients revealed that combining PD1 inhibitors (azacitidine and nivolumab) and an anti-CTLA-4 antibody (ipilimumab) exerted a profound positive effect, with 43% of the patients achieving CR and a one-year overall survival reaching 58% [136]. Two studies showed that anti-CTLA-4 antibodies also exerted a profound

Table 2. AML microenvironment signalling axis

Receptor	Ligand	Cell	Function	Rreference
CXCR4	SDF-1/ CXCL12	Immune cells/AML leukemic cells	Migration/pro-survival	[126]
IL-1R1	IL-1β	Most hematopoietic and nonhematopoietic cells/ AML leukemic cell	Haematopoiesis/immune response/inflammation	[127,128]
VCAM1-1	VLA-4	Stromal cells	Adhesion/pro-survival/proliferation	[129]
LFA	ICAM-1	Immune cells/AML leukemic cells	Leukocyte adhesion	[130]
CD110	TPO	HSC/AML leukemic cells	HSC quiescence	[131]
RANK	TNF-R/ RANKL	NK cells	Bone remodeling	[132]
VEGFR	VEGF/ PIGF	HSC/MQ/VEC	Angiogenesis/GM-CSF/proliferation	[133]
CTLA-4	B7	T cells/AML leukemic cells	T-cell inhibitory/tolerance induction	[134]

response in AML patients who had relapsed after allo-HSCT; specifically, 23% (5/28) and 6.9% (2/29) CR rates were obtained [137,138]. Blockade of inhibitor receptor TIM3 function is an effective strategy for an antitumour response in AML. A recent study showed that 9 of 31 AML patients who received decitabine and the anti-TIM-3 antibody MBG453 achieved a better response, and five additional patients showed a bone marrow blast reduction ≥50% [139].

Cell therapy (CAR-T)

Despite the success of CAR-T-cell therapy targeting CD19 or B cell maturation antigen (BCMA) in B cell malignancies, such as B-ALL and B cell lymphoma, further CAR-T treatment of AML has been delayed because of the lack of suitable surface antigens.

The first Phase I clinical trial for CAR-T-cell therapy based on the Lewis Y antigen was reported in patients with relapsed/refractory acute myeloid leukaemia (RR-AML), and it displayed the persistence of CAR-T cells in body (up to 10 months) and the acceptable safety profiles, although the clinical results were not clear [140]. Recently, CD33, CD123 and CLL-1-targeting CAR-T therapy have been eagerly studied for use in AML patients. Wang *et al.* [141] found that the AML blasts in a patient were substantially decreased after CD33-directed autologous CAR-T-cell therapy. Tang *et al.* [142] reported that in RR-AML patients received CD33-targeted CAR-NK therapy, and the serum IL-6 and IL-10 levels were elevated in one patient on the 6th day, while the MRD level and WT1 copy number were decreased. Another Phase I clinical study identified a new CAR-T compound that targeted CD33 and CLL-1. This study included a 6-year-old female patient with FLT3-Ltd mutation and Fanconi anaemia-associated juvenile myelomonocytic leukaemia who had received many treatments, including FLT3 inhibitor treatments. After lymphocyte clearance treatment, the CAR-T cells were reinfused. The patient reached CR on the 19th day, and the MRD was negative; therefore, the patient was eligible for allo-SCT [143]. To date, there have been few clinical studies on CAR-T-cell therapy with AML patients, but the results obtained to date are encouraging and indicate that CAR-T-cell therapy for AML patients is worthy of further study.

Bispecific antibody

Small artificial molecules that recognize two different antigens are called bispecific antibodies, and they can connect with different cell types to maximize tumoricidal interactions [144]. Using the CD33-targeted bispecific T-cell engager (BiTE) AMG330 for relapsed/refractory AML, Farhad *et al.* [145] provided evidence that BiTE has tolerability and anti-leukaemic activity as an AML therapy. Another clinical trial organized by Westervelt *et al.* [146] demonstrated that the CD33-targeted BiTE AMV564 reduced BM blasts in 49% of the treated AML patients and reduced the neutrophil count. In addition, other bispecific antibodies, such as the anti-CD33/anti-CD3 BiTE AMG673 and CD33-directed BiTE XmAb14045, led to effective outcomes in AML treatment [147,148]. Moreover, flotetuzumab, another type of bispecific antibody called a DART (a dual-affinity retargeting molecule), led to a 32% response rate in 28 patients with primary and refractory AML [149].

Novel Immunotherapy Strategies for Lymphocytic Leukaemia and Clinical Trial Results.

Lymphocytic leukaemia can be classified into acute lymphocytic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL). Acute lymphoblastic leukaemia can be further classified into B-cell acute lymphoblastic leukaemia (B-ALL) and T-cell acute lymphoblastic leukaemia (T-ALL). Immunotherapy for patients with acute lymphocytic leukaemia involves (CAR) T-cell therapy, bispecific T-cell engager (BiTE) antibody (helps T-cells target and kill cancer cells), checkpoint inhibitors and immune-modulating agents.

Bispecific T-cell–engaging (BiTE) antibodies

Blinatumomab is a bispecific T-cell–engaging (BiTE) antibody that consists of two different single-chain Fv fragments and mediates the binding with CD19 and CD3. Specifically, one site targets the CD3 antigen on human T cells, which induces cytotoxicity of T cells; the other site binds to CD19, which occurs in most B-ALL cells. [150]. Thus, it facilitates the formation of a cell junction between $CD3^+$ T cells and $CD19^+$ cells, leading to the enhancement of cell adhesion molecules and higher production of cytolytic proteins and inflammatory cytokines, ultimately promoting the lysis of target cells [151].

Inotuzumab ozogamicin (Besponsa) is an antibody–drug conjugate (ADC) targeted for CD22 in haematological malignancy therapy [152]. The complex forms lysosomal vesicles upon binding between inotuzumab and CD22. After that, the acidic pH environment in the lysozyme assures the effect of calicheamicin and releases it. By binding to the minor groove of DNA, calicheamicin breaks double-stranded DNA, arrests the cell cycle, and eventually induces cell death [152].

CAR-T-cell therapy for B-ALL and T-ALL

In CAR-T-cell therapy, patient-derived peripheral T cells are modified *in vitro* by genetic engineering and express antigen-

specific, non-MHC-restricted receptors. Then, the modified CAR-T cells are injected into the donors, where they are recognized and kill tumour cells to exert a therapeutic effect on patients [153]. These engineered CAR-T cells are the most frequently used to target CD19, which displays only a high expression level on leukaemia cells, and is not expressed on other cell lines or nonhaematopoietic cells. However, it is estimated that 45% of patients in the adult population with B-ALL undergo relapse [10]. Thus, it is possible that adding CD19 and CD22 to CAR-T cells may help overcome relapse.

Despite the success of CAR-T cell therapy for B-cell acute lymphoblastic leukemia (B-ALL), effective treatment for T-cell ALL (T-ALL) remains challenging. One major obstacle is that cytotoxic cells also express antigens similar to those found on malignant cells, resulting in potential damage to healthy cells. To overcome this issue, researchers have identified CD7 and CD5 as attractive targets, since they are expressed in 95% and 80% of T-ALL malignant cells, respectively. Additionally, CCR9 is another potential target, since it is only expressed by a limited number of normal circulating T and B cells but is widely expressed in gut intraepithelial γδT cells, plasmacytoid dendritic cells, and double-positive thymocytes. These specific targeting strategies hold promise for developing more effective treatments for T-ALL [154–156].

Checkpoint inhibitors

Checkpoint inhibitors are a type of immunotherapy that block certain proteins on cancer cells or immune cells, allowing the immune system to recognize and attack cancer cells more effectively. Checkpoint inhibitors have shown efficacy in the treatment of CLL, with the FDA approval of the checkpoint inhibitor ibrutinib for the treatment of CLL [157].

Immune-modulating agents

Immune-modulating agents are a type of immunotherapy that modulate the immune system to enhance its ability to recognize and attack cancer cells. For example, the immunomodulatory drug lenalidomide has shown efficacy in the treatment of CLL [158].

Immunotherapy for patients with CLL (chemotherapy with an anti-CD-20 antibody)

Rituximab, ofatumumab and obinutuzumab are three generations of anti-CD20 monoclonal antibodies that activate the immune response of normal and malignant human B-cells, causing their lysis and increasing the sensitivity of malignant B cells to chemotherapy cytotoxicity. Rituximab is a human mouse chimeric mAb, while ofatumumab and obinutuzumab are humanized, and the FC segment of obinutuzumab has been modified.

Compared with that in other B-cell lymphomas, the expression level of CD20 is relatively low in patients with CLL [159]. Therefore, rituximab treatment is not as beneficial in these patients as it is in those with another lymphoma type, and generally, CD20 is targeted in combination with chemotherapy.

Immunochemotherapy is the first-line treatment for young patients with CLL. Based on the effectiveness of rituximab found in several clinical trials, fludarabine, cyclophosphamide, and rituximab (FCR) are recommended as initial treatments [160].

Conclusion

In conclusion, immunotherapy has emerged as a promising treatment option for leukaemia, with several novel strategies being developed and tested in clinical trials. CAR T-cell therapy, BiTEs, checkpoint inhibitors, and immune-modulating agents have all shown efficacy in preclinical and clinical studies, and some have already received FDA approval for the treatment of leukaemia. Further research is needed to optimize immunotherapy strategies and determine the best treatment approaches for patients with leukaemia.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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