

Review

The Immune Microenvironment in Acute Myeloid Leukemia: Mechanisms of Immune Evasion and Emerging Therapeutic Strategies

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Acute Myeloid Leukemia (AML), a diverse type of blood cancer, is characterized by the unchecked multiplication of myeloid precursor cells within a disrupted bone marrow microenvironment (BMM). Leukemic blasts alter the BMM to create a leukemia niche, promoting immunological evasion and disease development. High relapse rates highlight the need for novel therapeutic approaches, even if chemotherapy is still the cornerstone of treatment. AML cells use many strategies to avoid immune identification, such as suppressing anti-leukemic immune responses and upregulating immune checkpoints. Immunotherapies like checkpoint inhibitors that target these pathways have shown encouraging promise. The intricate relationships between AML and the immunological milieu are examined in this review, with a focus on immune evasion, treatment resistance, and innovative immunotherapeutic strategies to improve anti-leukemic immunity.

1. INTRODUCTION

AML is a heterogeneous type of blood cancer that develops from transforming myeloid precursor cells within a disrupted BMM (1). Leukemogenesis is distinguished by the unregulated clonal expansion of malignant leukemic blasts, which shed their ability to differentiate correctly at numerous phases of maturation. Current insights indicate a two-way relationship among leukemic blasts and the BMM, with leukemic cells transforming the microenvironment into a niche that supports leukemia, while the modified

BMM promotes disease advancement (2,3). In adults, AML represents the most prevalent leukemia, while in children it ranks as the second most commonly diagnosed type (4). Various factors influence patient survival, such as age and the existence of cytogenetic or molecular genetic dysfunctions (5). Although chemotherapy is the standard treatment method for most cases of AML and can lead to complete remission, most patients eventually relapse, highlighting the urgent need for new therapeutic strategies (5,6).

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Consequently, a deeper comprehension of the immune regulatory mechanisms and tumor microenvironment has resulted in new treatment approaches for patients with AML. It is well known that in blood-related malignancies such as AML, the immune defense can recognize and eradicate tumor cells (7). Nonetheless, AML blasts utilize a variety of immune evasion tactics to avoid host immune reactions and escape destruction by the immune system (8). As a result, focusing on these co-inhibitory molecules—whether as a standalone treatment or in conjunction with other therapies—has shown considerable efficacy in AML by bolstering anti-leukemic immune responses (9,10).

This review aims to deliver a comprehensive examination of the immune microenvironment in AML, highlighting its impact on disease advancement, resistance to treatment, and new immunotherapeutic approaches. It also investigates the various approaches used by leukemia cells evading the immune response. This review aims to help develop more efficacious and personalized treatment methods for AML by elucidating these complex interactions.

2. Bone marrow microenvironment in blood cancers

The BMM is extremely crucial for controlling hematopoiesis. The self-renewing hematopoietic stem cells (HSCs) in the bone marrow (BM) are carried up by specialized niche cells, such as megakaryocytes, mesenchymal stromal cells, osteoblasts, endothelial cells, macrophages, sympathetic nerve fibers, and non-myelinating Schwann cells (Figure 1) (11). HSCs are mainly situated in perivascular niches in proximity to sinusoidal vessels, considering that prominent progenitor niches are found close to sinusoids, arterioles, or the endosteum (12). Thanks to progress in single-cell technologies, the gene expression profile of osteoblast, perivascular, and vascular populations in mouse BM has been thoroughly identified out, revealing significant cellular diversity within the niche under steady state conditions. Additionally, the origin of cells of pro-hematopoietic elements in the homeostatic BM environment have been recognized (11). Complementary studies in mice have further defined the various cellular subtypes within BM stroma, creating a detailed atlas of stromal cell populations (13). These investigations have pinpointed various subsets of stromal cells that express unique hematopoietic regulatory genes, such as fibroblastic and osteoblastic subgroups, and have delineated various pathways of osteoblast differentiation.

Under stress-inducing situations such as chemotherapy, the BM niche undergoes significant remodelling. Research

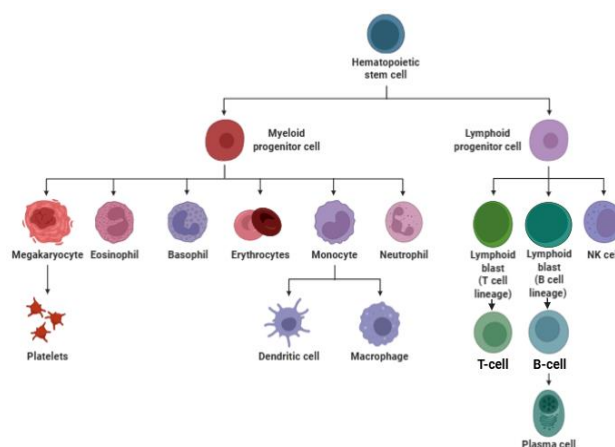


Figure 1. Illustration of mechanism of hematopoiesis.

with mice showed that perivascular mesenchymal cells exhibited a tendency to move toward adipogenic differentiation, as indicated by the transactivation of adipogenic genes and the inactivation of osteogenic genes. In addition, changes in the dissemination of pro-hematopoietic components were observed, along with a reduction in the magnitudes of vascular δ -like Notch ligands Dll4 and Dll1. It is worth mentioning that the lack of vascular Dll4 resulted in an early activation of a myeloid transcriptional program in HSCs. The results underscore the BM niche's dynamic qualities and its responsiveness to stress (11).

The BM niche is crucial to the onset, advancement, and treatment resistance of hematological malignancies, as it enables interactions between cancerous or potentially cancerous cells and their surrounding environment (14). Studies conducted with mice have demonstrated that modifications in cells environment correlate with myeloid neoplasms and changes in genetics which could lead to disorders bear a resemblance to myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPNs). For instance, the scarcity of Dicer1 in osteoprogenitors gives rise MDS, and studies with BM transplantation have exhibited that the environment of BM can influence disease progression by enhancing the expression of stress related response genes and inflammatory cytokines (15). Analogously, gene Rarg, which encodes retinoic acid receptor γ , can be knocked out to create a myeloproliferative-like disorder in mice. Signaling of tumor necrosis factor (TNF) within the BM niche activates this condition (16). Age-related changes result in a proinflammatory BMM, increasing the likelihood of dysplasia and myeloid neoplasms. The age-related revolutionization in the niche of HSC leads to an excess of proinflammatory cytokines, which further encourages the

excessive multiplication of myeloid cells and the expansion of progenitor cells with clonal hematopoiesis (CH) related changes, thereby commencing disease onset (17). When inflammation occurs in the BM niche, it disrupts hematopoiesis and requires HSCs to acclimatize to a high-stress environment marked by expanded adipogenic potential and dysfunctional sympathetic nerve activity (18,19). It is crucial to emphasize that research has demonstrated a significant reduction of sympathetic nerve fibers, nestin-expressing mesenchymal stem cells (MSCs), and Schwann cells in the BM of people with MPNs, as well as in mice with HSCs expressing the human JAK2-V617F mutation (20). It is important to note that sympathetic nerves undergo apoptosis due to the generation of interleukin-1 β (IL-1 β) by nestin-expressing niche cells when JAK2 mutations are present, which further disrupts BM homeostasis.

In MPNs, distinctive BM environment and HSC sequences have been recognized, impacting disease pathogenesis and resulting in diverse therapeutic responses, even in cases caused by the exact CH oncogenic mutation (21). Additionally, TET2 loss or mutation hinders MSC function in the BM, leading to a faster progression of myeloid malignancies (22). Comparable changes have been noted in the stem cell niche when ASXL1 mutations are present (23). Moreover, the age-related transformation of the BM stroma provides a selective benefit for DNMT3A-mutant clones, in part because of the guiding influence of TNF signaling (24,25).

Malignant cells and their released factors, such as exosomes, within the BM niche influence MSC function and alter their microenvironment to support leukemic cell survival. The atypical rise in myeloid cells leads to the excessive generation of proinflammatory cytokines by hematopoietic cells, whether they are mutated or non-mutated, adopting a proinflammatory phenotype (26). A downregulation of the chemokine CXCL12, which enables malignant HSCs to dominate their normal counterparts, is among the earliest inflammation-driven alterations in BM MSCs. This phenomenon is frequently seen in MPNs, acute lymphoid leukemia (ALL), AML, and chronic lymphocytic leukemia (CLL) (27,28).

Cytokines and growth elements, including bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF), thrombopoietin, and CCL3, influence the proinflammatory BMM. These are released by either genetically changed hematopoietic cells or reprogrammed environmental cells. These elements promote the increase of osteoblastic cells, MSCs, and fibroblastic cells in myeloid malignancies (29). Research by Schepers et al. indicates that

MPNs change the BM environment, as remodelled myeloid cells encourage MSCs to enhance the generation of osteoblastic lineage cells with altered functions. These cells accumulate in the BM cavity, contributing to both inflammation and myelofibrosis. In chronic myeloid leukemia (CML), the leukemic microenvironment produces elevated levels of BMP2 and BMP4, aiding in the persistence and self-renewal of leukemic progenitors (30). Within the MDS context, BM MSCs obtained from patients exhibit elevated apoptosis rates and a higher output of proinflammatory cytokines such as IL-6, IL-1 β , and TNF (31). According to Medyouf et al., human MDS cells can reconfigure BM MSCs, which may assist in the process of disease implantation and progression *in vivo*. Similarly, MSCs obtained from AML patients show genetic abnormalities and an improper regulation of cytokine secretion. Genetic fate-mapping studies have identified MSCs that express leptin receptors as crucial contributors to fibrosis in primary myelofibrosis. In the BM niche, these cells enlarge through PDGF receptor- α signaling (32). BM fibrosis, which characterizes myelofibrosis, is driven by GLI1+ MSCs that differentiate into myofibroblasts. In murine models, CXCL4 boosts the relocation and development of GLI1+ MSCs, and focusing on these cells has been shown to reduce fibrosis, culminating in their capacity as therapeutic targets (29).

Along with immune regulation, leukemias acclimate to and make the most of the vascular endothelial niches found in the BM. In murine models, factors such as CXCL12, Notch, and E-selectin signaling pathways drive this adaptation of the endothelium. These promote interactions between leukemia and vessels that play a role in vascular dysfunction (33,34). Furthermore, hypoxia and increased angiogenesis are important characteristics of hematologic malignancies (35). Hematopoietic cells that are malignant show a heightened rate of oxygen utilization and generate pro-angiogenic factors such as vascular endothelial growth factor A (VEGF-A) and different interleukins. These factors subsequently promote the production of placental growth component by BM MSCs. This process results in the creation of new blood vessels, which boosts the production of the generation of oxygen and nutrients and acts as a reservoir for elements that support the survival of malignant cells. Ultimately, the reprogramming of the niche of BM aids in the survival and multiplication of leukemia cells while contributing to their chemotherapy resistance.

3. AML: Overview and Clinical Characteristics

AML is a clonal disorder of the hematopoietic system that originates from genetic and molecular aberrations affecting

normal hematopoietic stem cells. As a consequence of the alterations, blasts—immature myeloid cells—are produced that multiply uncontrollably and accumulate in the BM (36). These abnormal cells outcompete and take the place of normal hematopoietic progenitors, leading to characteristic cytopenias and leukocytosis (37). According to recent epidemiological data, AML has an incidence rate of 4.3 cases per 100,000 people, with the median age at diagnosis being 68 years. In the United States, the estimated 5-year overall survival rate is around 24% (38).

Like most cancers, AML develops from the accumulation of somatic driver mutations and secondary genetic alterations. Currently, cytogenetic analysis and next-generation sequencing (NGS) are used for diagnosing AML (39). Among the mutated genes linked to leukemogenesis, those involved in signaling pathways and protein kinase activation, such as FLT3, are essential for the abnormal activation and multiplication of leukemic blasts (40). FLT3 mutations are indispensable in the development of AML, principally because they frequently interact with other genetic changes, particularly NPM1 mutations. NPM1-mutated AML is usually linked to a positive prognosis; however, the presence of both FLT3 and NPM1 mutations changes risk stratification and correlates with early relapse and worse clinical outcomes (41,42). A further significant category of driver mutations encompasses genes that control epigenetic alterations, including DNA methylation and chromatin remodeling. Mutations in DNMT3A, for example, hinder the differentiation of HSCs, whereas mutations in TET2 disrupt normal myeloid differentiation (42). Moreover, significant cytogenetic abnormalities—such as chromosomal translocations, gene amplifications, insertions, and deletions—play essential role in the development of AML and are essential markers for risk classification and treatment strategy formulation (43).

AML is a heterogeneous cancer type that primarily affects the BM and PB, but can also impact extramedullary tissues in certain cases (44). Pancytopenia, characterized by anemia, thrombocytopenia, and neutropenia, arises from bone marrow failure due to the infiltration of leukemic blasts. Clinically, AML often presents with symptoms such as weakness, fatigue, recurrent infections, and hemostatic problems, including bruising, ecchymosis, and nosebleeds (45). In AML, fever can arise from two different mechanisms: it may be a neoplastic fever resulting from the leukemic proliferation itself, or it may occur due to neutropenia, which makes patients more susceptible to recurrent infections. Moreover, extramedullary manifestations can involve lymphadenopathy, hepatosplenomegaly, and bone lesions (46).

Coagulation abnormalities, whether hemorrhagic or thrombotic, rank among the most severe complications of AML and are responsible for approximately 7% of mortality in these cases (47). Coagulation dysfunction can arise in different AML subtypes, but it is most frequently linked to acute promyelocytic leukemia (APL) (48). Leukostasis, a medical emergency characterized by symptomatic hyperleukocytosis, is another important clinical manifestation that occurs in 10 to 20% of AML patients. Leukostasis can lead to pathological alterations in different organs; the central nervous system (CNS) and lungs, however, are impacted the most, with complications that can be deadly (49). The diagnosis of AML is made when blasts constitute at least 20% of the BM or PB, with the exception of certain cases where particular cytogenetic findings are sufficient for confirmation (50). Bone marrow aspiration is a crucial diagnostic procedure assessed through various methods, such as morphological evaluation, flow cytometry for classifying AML subtypes, cytogenetic analysis to find chromosomal abnormalities, and NGS for detecting gene mutations (51).

The main objective of AML treatment is to attain disease remission, which is defined by the lack of detectable blasts and molecular markers. The treatment method adheres to a proven protocol and is divided into two primary phases: induction and consolidation therapy (52). As the primary treatment, intensive induction chemotherapy aims to reduce leukemic blasts in the bone marrow to below 5%. The standard treatment for patients who qualify for intensive therapy is a regimen that combines Cytarabine for seven days with Daunorubicin or Idarubicin for three days. This is known as the "7 + 3 protocol." Since the 1970s, this treatment has led to disease remission in over half of patients across all age groups (53). To establish the suitable treatment plan, especially for elderly patients who might not withstand aggressive chemotherapy, a comprehensive initial evaluation is crucial. Other treatment options, including hypomethylating agents in combination with Venetoclax or low-dose Cytarabine, have demonstrated encouraging outcomes regarding overall survival and quality of life enhancement (54,55). Following induction therapy, consolidation treatment is administered to reinforce remission achieved during the initial phase. Consolidation treatment is given after induction therapy to bolster the remission achieved in the initial phase (56). Patients may acquire high-dose chemotherapy or pass through bone marrow transplantation with the aim of curing the disease (57).

Although remission can be achieved in AML, relapse is frequent due to drug-resistant leukemic clones, which leads

to complications and death related to the disease. Approximately 24% is the overall 5-year survival rate, which remains low (58). Regrettably, the addition of re-induction regimens to targeted therapies like tyrosine kinase inhibitors has not led to significant improvements in patients' long-term survival outcomes (59).

A unique and well-defined subtype of acute myeloid leukemia (AML), acknowledged by the WHO as AML with PML-RARA (60), is acute promyelocytic leukemia (APL), which is also mentioned to as AML-M3 in the French-American-British (FAB) classification system (61). APL is distinct from other AML subtypes due to its specific histological and clinical features, including the presence of Auer rods and a high propensity for disseminated intravascular coagulation (62). Therapeutic approaches for APL also differ from those used for standard AML. Most AML cases are treated using the 7 + 3 induction regimen (which involves cytarabine alongside daunorubicin or idarubicin), but for low-risk APL patients, induction therapy consists of arsenic trioxide and all-trans retinoic acid. This approach results in high remission rates and excellent overall survival outcomes (63). As a result, the primary research focus remains on improving therapeutic strategies for non-APL AML cases.

A considerable advancement in the comprehension of different facets of AML physiology has occurred in recent years, especially regarding the function of BM niches. A range of innovative treatment methods have been explored to target specific niche cell populations, components of the extracellular matrix, various adhesion molecules, and growth factors secreted by niche cells. These strategies aim to modify the BMM in order to disrupt the initiation and progression of leukemia, with the ultimate goal of improving clinical outcomes (2,64).

4. The immune microenvironment in AML

The BMM is crucial for the initiation and progression of AML, with recent research shedding light on its transcriptional changes during transformation (42,65). Changes in stroma have been observed in the leukemic bone marrow niche, characterized by impaired mesenchymal cell differentiation and reduced expression of genes involved in hematopoiesis (13). AML disrupts both osteogenic and adipogenic differentiation pathways within the stroma of BM, adversely affecting hematopoiesis and overall tissue function. A groundbreaking study utilizing single-cell RNA sequencing combined with co-detection by indexing (CODEX) generated a spatially resolved multi-omic map of human BM. This work revealed distinct spatial microenvironments and previously unrecognized

heterogeneity among human MSCs. Additionally, the study emphasized the spatial association between MSCs and leukemic blasts in AML patients (66,67).

Extensive investigations of the endothelial portion in the BM microenvironment of AML have uncovered a significant increase in vascular permeability, alongside molecular changes in endothelial cells (68). Patients with elevated nitric oxide levels who underwent induction chemotherapy exhibited vascular dysfunctions in the BM, which are linked to a poor prognosis. Furthermore, AML blasts alter the bone marrow niche by secreting exosomes that increase DKK1 expression, thereby undermining the ability of mesenchymal stromal progenitors to differentiate into osteoblasts. In addition, these explosions reduce the expression of HSC-supporting elements like IGF1, CXCL12, KITL and in stromal cells of BM (69). Research has shown that leukemic cells can modify the tumor microenvironment (TME) by generating a range of immunosuppressive enzymes, including arginase, CD73, CD39 and indoleamine-2,3-dioxygenase-1 (70-73). Additionally, it has been pointed out that AML cells residing in the BM niche rely on cytokines and soluble components generated by MSCs and AML cells as well, such as CXCL12, IL-10, IL-4, and IL-1 β , transforming growth factor- β (TGF β). This paracrine signaling pathway fosters immune tolerance and facilitates blast growth by involving neighboring cells in the TME (74,75).

Metabolic rewiring's role in the onset, progression, and relapse of AML has been thoroughly investigated (76). Hypoxia and competition for nutrients are crucial elements that promote immunosuppression in the TME. The growth of tumors and the activation of immune subsets cause an increase in glycolytic metabolism, which raises lactate production and leads to acidosis within the TME. The effector functions of various subsets of immune cells, comprising monocytes, regulatory T (Treg) cells, NK-cells, macrophages, dendritic cells, and effector T-cells, are directly impeded by this acidic environment (77). Numerous investigations have shown that individuals undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) display elevated oxidative markers and an imbalanced antioxidant status in their serum (78). Cells that have dysregulated reactive oxygen species (ROS) levels show significant oxidative DNA damage, which could jeopardize antileukemic immune surveillance (79).

Transformations in the immune microenvironment are closely linked to modifications in the BM niche. It is essential to comprehend the phenotypes, functions, and developmental trajectories of immune cells in the AML microenvironment in order to reveal mechanisms of

immune evasion and enhance immunotherapy responses. Changes at the junction of T-cells and leukemic blasts can result in decreased immune recognition, playing a role in post-transplant relapse in up to 40% of cases. After allo-HSCT, signals that are costimulatory and mandatory for T-cell switching on are consistently interrupted. Leukemic cells specifically downregulate activators like CD11A and B7-H3 while upregulating inhibitory ligands like PVRL2, PD-L1, and CD80 (80,81). The immunophenotypic changes of T-cells during relapse must be noted as being comparable to those seen in leukemic blasts (80,82). There is mounting evidence indicating that the shared articulation of several inhibitory checkpoint receptors on T-cells acquired from donors correlates with relapse after transplantation. Leukemia antigen-specific T-cells show an increased expression of TIM-3 and LAG-3 in HLA-matched transplants (83). In line with this, transplant recipients showed that PD-1hiTIM-3+ T-cells demonstrated indications of productive exhaustion and rose in frequency prior to clinical relapse, highlighting a robust correlation between the quantity of these cells and leukemia relapse (84). Furthermore, the bone marrow of relapsed AML patients exhibits elevated levels of CD8+PD-1+TIM-3+ and PD-1+LAG-3+ T-cell populations (85). Upon diagnosing relapsed AML, the BM exhibits an increased proportion of Treg cells and a greater incidence of PD-1+ and OX40+ T-cells (85). Single-cell analyses have revealed that myeloid cells resulting from AML-associated differentiation possess immunosuppressive characteristics (86). In addition, immune profiling in AML has identified various immunosuppressive subsets of dendritic cells and macrophages, accompanied by reduced and dysfunctional T-cell and NK-cell populations, along with regulatory T-cells exhibiting abnormal developmental pathways (87). While NK cells can contribute to the suppression of AML in its initial stages, disease advancement is linked to diminished perforin expression and the depletion of immature NK-cell subsets, driven by the dysregulation of a microRNA essential for NK-cell maturation (88). In the bone marrow immune microenvironment of both adult and pediatric AML patients, an increase in dysfunctional B-cells has been observed (notably atypical memory B cells), granzyme-producing CD8+ progenitors of depleted T-cells, and Treg cells, while T-cell clonal expansion is diminished, all occurring against a backdrop of heightened inflammation (89). Research into pediatric AML has uncovered unique gene expression patterns linked to relapse, diagnosis, and complete remission, which are associated with survival outcomes (90). At diagnosis, among a cohort of pediatric patients, those who later experienced relapse exhibited a

greater depletion of T-cells and a reduced presence of inflammatory macrophages compared to those who maintained sustained remission. These findings underscore the complexity of immune dysfunction in AML and its progression with the disease, highlighting the diverse immune cell populations present in the bone marrow. Understanding how immune populations are reprogrammed is crucial for interpreting the unique immune landscapes of patients, as these may influence the success of immunotherapies. In this regard, research on PD-1 blockade therapy has revealed significant variability in T-cell subsets, with responders showing an expansion of the CD8+ T-cell population, further supporting the potential of targeted immune therapies in AML treatment (91).

5. Mechanisms of Immune Evasion in AML

In AML, numerous mechanisms aid in immune evasion (Figure 2) (92). The exact contribution of each mechanism to the promotion of leukemia immune tolerance, their functioning in PB compared to BM (the primary tumor site), and the effects of AML treatment and genetics on them require further exploration.

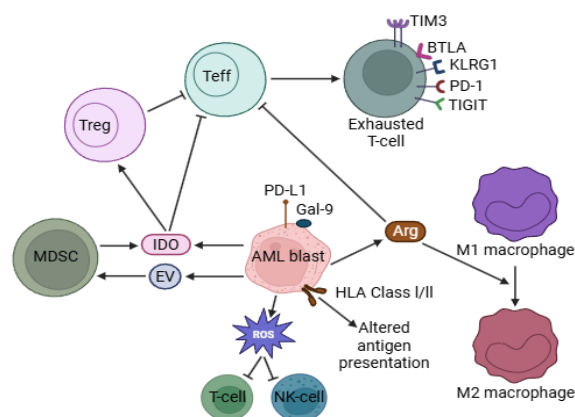


Figure 2. The dysfunctional immune landscape associated with acute myeloid leukemia.

5.1. Changes in Antigen Presentation

After allo-HSCT, leukemia blasts exhibit significant immunoediting in response to selective immune pressure (93). Additionally, the downregulation of HLA class II molecules through epigenetic mechanisms has been observed in various donor transplant scenarios (80,81). Mouse models have shown that leukemia antigen presentation by immature antigen-presenting cells or splenic CD8α+ dendritic cells can induce T-cell tolerance, including deletional and CD8+ T-cell tolerance (95). Furthermore,

recent research has linked impaired plasmacytoid dendritic cell differentiation to the persistence of measurable residual disease post-AML treatment, which is associated with poorer survival outcomes (96).

5.2. T and NK-cells

T-cell existence at the tumor site is crucial for immune identification and destruction of AML cells, as well as for the effectiveness of therapies that utilize this mechanism. Compared to healthy controls, AML patients show a similar number of T-cells in the BM and increased levels of total and CD8⁺ PB T-cells (85). Higher proportions of lymphocytes and T lymphocytes in the BM have been associated with enhanced treatment responses and better survival rates (97), while a robust recovery of lymphocytes after chemotherapy is linked to a reduced relapse risk (98). It has been shown that higher expressions of CD8⁺ and CD3⁺ T-cells in the BM, when combined with a hypomethylating agent (HMA), are predictive of positive responses to the checkpoint inhibitor nivolumab (99).

Research using mouse models of AML has shown that with disease progression, CD8⁺ T-cells expressing the PD-1 checkpoint inhibitory receptor (CIR) and Tregs increase at the tumor site, suggesting the potential of targeting the PD-L1/PD-1 pathway to reduce Treg levels (100). Additionally, a mouse model revealed that exhausted CD8⁺ T-cells co-expressing PD-1 and TIM3 could have their cytotoxic function restored through combined checkpoint inhibition (101). In AML patients, phenotypic and gene expression analyses of CD8⁺ and CD4⁺ T-cells at diagnosis showed an abnormal activation profile and alterations in genes crucial for immunologic synapse formation (102). Despite reduced IFN- γ production by CD4⁺ T-cells, AML T-cells remain functionally competent in cytokine generation and proliferation, with PD-1 upregulation indicating a shift towards effector memory cells (103). Multiple studies, including ours, have shown that the frequency of T-cells co-expressing CIRs in AML patients is higher than in healthy controls, with this frequency increasing as the disease progresses (85,92). Smaller-scale studies have suggested that CD8⁺ T-cells with CIR expression are functionally impaired and can predict relapse in AML patients (104). While the general cytokine expression in CD8⁺ T-cells of AML patients is similar to healthy controls, senescent and exhausted T-cells with distinct cytokine profiles have been identified, with reversible transcriptional signatures observed in patients responding to chemotherapy (92). A recent study found that in favorable-risk AML, BM CD8⁺ T-cells contribute to leukemia stem cell (LSC) expansion, while in adverse-risk AML, LSC expansion is independent

of CD8⁺ T-cells (105). The frequency of PD-L1⁺ AML cells varies across studies, ranging from 18% to over 50%, and PD-L1 expression increases upon relapse, especially in acute monocytic leukemia, which is associated with poor prognosis (85,106). Additionally, PD-L1 expression and other co-signaling ligands are upregulated on AML blasts during post-alloHSCT relapse, alongside corresponding T-cell alterations (80). Exposure to IFN- γ results in increased PD-L1 expression in leukemia blasts (107) and primary AML blasts rapidly upregulate PD-L1 as an adaptive resistance mechanism against immune therapies that activate T-cells (108).

Leukemic blasts are crucial for the modulation of T-cell responses. When T-cells are exposed to AML blasts *in vitro*, they experience apoptosis, reduced proliferation, and diminished costimulatory molecule expression. Cells of monocytic leukemia generate ROS, which lead to the death of T cells and NK cells *via* apoptosis that depends on poly(ADP-ribose) polymerase-1 (PARP-1) (109). When exposed to AML blasts *in vitro*, NK cell cytotoxicity and effector functions are diminished. At the time of diagnosis, patients with AML already exhibit functionally compromised NK-cells, marked by a decrease in activating receptors and an increase in the inhibitory receptor NKG2A. Upon remission, these phenotypic and functional variations in NK-cells can be partially reversed; however, they persist in patients who do not react to therapy (110). Furthermore, it has been shown that the leukemia-associated fusion proteins PML-RAR α and AML1-ETO specifically diminish the display of CD48 on AML cells. CD48 serves as the ligand for the NK cell receptor 2B4 (CD244), and this decrease impacts the cytotoxic function of NK cells (111).

5.3. Immunosuppressive microenvironment

Studies conducted on both mice and humans indicate that Tregs have a considerable impact on weakening immune responses in AML (112). In AML patients, Tregs in the PB and BM are elevated and have a stronger immunosuppressive effect on T effs compared to normal Tregs. Additionally, their levels show minimal changes with chemotherapy (85). Moreover, elevated Treg levels correlate with worse AML outcomes (113).

Myeloid-derived suppressor cells (MDSCs) are elevated in AML patients and contribute to T-cell tolerance through various mechanisms, including the expression of PD-L1 and VISTA, indoleamine 2,3-dioxygenase (IDO) activity, and the production of reactive oxygen species (ROS), peroxynitrate, arginase, and cytokines like TGF- β and IL-10 (9). AML cells promote MDSC development by releasing extracellular

vesicles (EVs) containing the oncoprotein MUC1, which increases c-myc expression and drives MDSC proliferation (114). In high-risk myelodysplastic syndromes (MDS), Tregs and MDSCs are positively correlated, suggesting their role in disease progression, a correlation not seen in low-risk MDS (115).

Enzymes produced by AML blasts contribute to the creation of a suppressive microenvironment. The expression of IDO in AML blasts, which catalyzes the rate-limiting step of tryptophan degradation via the kynurenine pathway, is linked to shorter relapse-free survival and overall survival times (116). The depletion of tryptophan and accumulation of its metabolites suppress T-effector (Teff) cell proliferation, promote T-cell apoptosis, and induce Tregs (117). Arginase II, elevated in AML patients, inhibits myeloid-monocytic differentiation, reduces T-cell proliferation, and drives monocyte polarization towards a suppressive M2-like phenotype (71). Studies in animal models of monocytic AML have shown that arginase I enhances leukemia aggressiveness by promoting tissue infiltration and T-cell suppression through the LILRB4 receptor signaling pathway (118).

6. Targeting the Immune Microenvironment in AML Therapy

Since leukemic blasts exploit the immune microenvironment to avoid detection by the immune system, numerous novel therapeutic approaches are being created to focus on these immune-evasive mechanisms and reinstate anti-leukemic immune responses.

6.1. Checkpoints inhibitors (CPIs)

In AML, immune evasion is mediated by checkpoint molecules such as CTLA-4 and PD-1 on immune cells, and Tim-3 and PD-L1 on leukemic blasts. Immune checkpoint inhibitors (CPIs) targeting PD-1 (e.g., Nivolumab, Pembrolizumab) and CTLA-4 (e.g., Ipilimumab) have shown efficacy in solid tumors (119). Currently, the safety and feasibility of these CPIs are being investigated in several ongoing phase I/II clinical trials (120).

In relapsed hematologic malignancies post-allogeneic HSCT, the CTLA-4 inhibitor Ipilimumab has shown limited clinical benefit, with Graft versus Malignancy (GvM) responses observed in under 10% of cases. While Ipilimumab did not induce Graft versus Host Disease (GvHD) in initial trials, immune-related adverse events occurred in 14% of responders (121). Another study reported immune-related toxicities in 10 of 28 patients, including one death and four dose-limiting GvHD cases,

with complete remission achieved in 4 out of 12 patients (122,123). Similarly, a retrospective analysis of clinical trials using PD-1/PD-L1 inhibitors in AML and high-risk MDS revealed dose-limiting immune toxicities (38). The efficacy and safety of CTLA-4 blockade appear dose-dependent and influenced by AML histological subtypes. Notably, durable remissions were seen in extramedullary AML, including leukemia cutis, alongside mild GvHD (122). In progressive AML, the anti-PD-1 antibody CT-011 showed limited anti-leukemic activity but was well tolerated as monotherapy (124). The varying response to checkpoint inhibitors in AML may reflect heterogeneous checkpoint expression profiles (125), with murine studies showing increased co-expression of checkpoint molecules and effector cell exhaustion as the disease progresses (101).

To address the limited efficacy of CPI monotherapy in AML, combination strategies are under investigation. Ongoing clinical trials (NCT03417154, NCT02464657, NCT02768792) are assessing the effectiveness of CPIs with chemotherapy. A Phase II study adding Nivolumab three weeks after standard chemotherapy (anthracycline and cytarabine) in newly diagnosed AML and high-risk MDS patients demonstrated feasibility and efficacy, though 6 out of 44 patients experienced grade 3 or 4 immune-related toxicities (126). Additionally, a Phase I/II trial showed that Nivolumab combined with the hypomethylating agent Azacitidine is both effective and well-tolerated in relapsed, high-risk AML cases (127).

In order for CPI therapy to work, it is crucial that effector lymphocytes react appropriately. In the context of AML, though, this reactivity diminishes in the immunosuppressive leukemic BMM, that probably obstructs treatment effectiveness. Furthermore, the usually low tumor mutation burden of AML, acknowledged as a predictive marker for CPI response, may impede T-cell recognition (128). Considering the highly immunosuppressive environment and the generally low baseline expression of immune checkpoints in AML (128,129), HMAs that boost anti-leukemic immune responses through increased cellular reactivity and upregulation of checkpoint molecule expression (130) could potentially enhance CPI treatment outcomes. Ongoing studies are examining the use of combined checkpoint blockade approaches aimed at PD-(L)1, CTLA-4, or Tim-3 in conjunction with HMA therapy (NCT03066648, NCT02530463). Among these, the Tim-3/Galectin-9 axis stands out as especially promising for targeting, given its involvement in LSC self-renewal (131) and its upregulation in AML therapy-resistant cases (104). Considering that various studies have identified distinct shared-expression sequences of immune checkpoints that

hold prognostic significance (85,131), combination or dual checkpoint inhibition may represent a promising strategy for tackling immune system avoidance in AML. In a pediatric patient with refractory AML, initial symptom improvement was observed without adverse effects after simultaneous obstruction of CTLA-4 and PD-L1 combined with HMA treatment. Nonetheless, despite this initial response, the treatment did not end up preventing the disease's progression. Further studies are necessary to evaluate the clinical effectiveness of this combined therapy strategy in pediatric AML (132).

6.2. Cell-based therapy

Cellular therapies for leukemia were initially inspired by the potent T-cell-mediated anti-leukemic effects seen following allogeneic HSCT (133). A 20-year single-centre study on haploidentical T-cell infusion in relapsed AML patients showed moderate feasibility, though immune-related toxicities, particularly severe GvHD, limited its clinical applicability (134).

Preclinical studies have demonstrated that CAR T-cells engineered to target CD123 or CD33 exhibit anti-leukemic activity against AML cells (135,136). Ongoing early-phase clinical trials are evaluating anti-CD33 CAR T-cells in pediatric and adolescent patients with relapsed or refractory AML (NCT03971799). Moreover, bispecific CAR T-cells targeting both CD123 and CD33 have shown effectiveness in eliminating leukemic blasts and LSCs *in vivo* (137). Despite these advances, CAR T-cell therapy remains challenged by significant T-cell-mediated toxicities, including cytokine release syndrome and an increased risk of GvHD (138). On the other hand, NK-cells present a safer option because of their MHC-independent activity, which leads to reduced cytokine release and a lower risk of GvHD. Consequently, research is being conducted into the viability of adoptive NK-cell therapy as a promising, inexpensive, and easily accessible off-the-shelf treatment option (139).

Various NK cell-based immunotherapies have been explored for AML. Preclinical models showed that CD123-targeting CAR-engineered NK cells demonstrated anti-leukemic activity and safety (140). In a pediatric case of relapsed AML post-HSCT, adoptive transfer of cytokine-induced allogeneic NK cells proved effective and well tolerated (141), with further evaluation underway in Phase 1/2 trials (NCT03068819, NCT01898793). However, a Phase I clinical trial of CD33-directed CAR-NK cells reported good tolerability but limited therapeutic efficacy (142).

Adoptive transfer of purified allogeneic haploidentical KIR-HLA mismatched NK cells following low-dose

immunosuppression and IL-2 administration has shown encouraging response rates with minimal toxicity and no GvHD, making it a potential consolidation therapy for AML across age groups (143,144). However, efficacy appears dose-dependent, as a higher dose ($29 \times 10^6/\text{kg}$) was effective, while a lower dose ($12.5 \times 10^6/\text{kg}$) was not in pediatric patients (143,145). In contrast, lower doses ($1-5 \times 10^6/\text{kg}$) have proven effective and feasible in elderly AML patients (146), suggesting that other factors like NK-cell purification and cytokine stimulation may influence therapeutic success (147).

6.3. Antibody-based therapy

While true tumor-specific antigens are rare, leukemia-associated markers such as CD33 and CD123—commonly expressed on AML blasts and LSCs—have been investigated as therapeutic targets, despite their presence on some healthy HSCs (148). Traditional monoclonal antibody therapies targeting these antigens have shown limited clinical efficacy (149). Consequently, recent efforts have shifted toward engineered antibodies, including antibody-drug conjugates (ADCs) and Fc-optimized antibodies designed to enhance CD16 binding and improve ADCC.

Initial clinical trials of the CD33-targeting ADC Gemtuzumab ozogamicin (GO), linked to the cytotoxic agent calicheamicin, demonstrated its feasibility and effectiveness in treating pediatric patients with relapsed or refractory (R/R) AML (150). The U.S. FDA approved GO for R/R AML in adults with CD33-positive AML and for children aged two years and above. Similarly, the European Medicines Agency (EMA) authorized its use in patients over 15 years with newly diagnosed AML, following positive results from the ALFA-0701 trial (NCT00927498) (151). Recently, the FDA expanded GO's indication to include children as young as one month with newly diagnosed CD33-positive AML, based on data from the AAML0531 study (NCT00372593) (152). Additionally, preliminary results from a phase I trial of a CD123-targeting ADC linked to an alkylating agent in relapsed or refractory AML showed both safety and clinical efficacy (153).

CLL-1 is predominantly expressed on AML blasts and leukemia stem cells (LSCs), but not on hematopoietic stem cells (HSCs), making it a promising therapeutic target that preserves hematopoietic regeneration (154,155). Both *in vitro* and *in vivo* models have shown strong anti-leukemic effects of CLL-1-targeting ADCs and the bispecific T-cell-engaging antibody CLL1-CD3. The latter demonstrated greater efficacy than CD33-targeted therapies by specifically sparing HSCs (156).

Phase I trial results of a dual-affinity anti-CD123-CD3 antibody in patients with refractory or relapsed AML and MDS showed T-cell-mediated anti-leukemic effects and an acceptable safety profile, with manageable cytokine release syndrome (mainly grades 1 and 2) as a persistent adverse effect (157). Animal studies using an AML-monkey model indicated that a bispecific T-cell engager with lower CD3 affinity resulted in reduced cytokine release compared to its high-affinity CD3 counterpart (158).

Preclinical studies showed that the Fc-engineered CD123 antibody CSL362, designed to enhance NK-cell affinity, effectively mediated NK-cell cytotoxicity against AML blasts and LSCs (159). However, CSL362 showed limited anti-leukemic efficacy in phase I/II trials, likely due to NK-cell depletion in heavily treated advanced AML patients (160). Proposals suggest that bi- and tri-specific NK-cell engagers targeting CD123 and CD33, such as CD33-CD16, CD123-CD33-CD16, and CD123-IL15-CD16, could enhance antibody-dependent cellular cytotoxicity (ADCC) (161). Additionally, HMAs may improve NK-mediated anti-leukemic responses by counteracting immune evasion and enhancing NK-receptor activation, boosting NKG2D-mediated NK activation (162). A novel strategy combining immune checkpoint inhibitors (CPIs) and bispecific T-cell engagers (BiTEs) aims to revitalize exhausted T- and NK-cells, indicating that combining immune therapies with NK-cell-engaging treatments could be a promising future direction, though further research is needed.

Tetra-specific antibodies have been developed for solid tumor treatment, incorporating CD16 linked to IL-15 to activate NK cells. These antibodies are fused with single-chain variable fragments (scFvs) targeting cancer-associated antigens (163). Due to the genetic and morphological diversity of AML blasts, this multi-specific approach could offer a promising antibody-based treatment for AML, allowing precise targeting of leukemic blasts based on their unique surface marker profiles.

6.4. Cytokines and Immune-Modulating Factors in Therapeutic Intervention

Researchers have looked into cytokine-based therapies, both as standalone treatments and alongside other immunogenic anti-leukemic methods, since various pro-inflammatory cytokines like IL-12, IL-15, IL-18, and IL-21 have been demonstrated to enhance leukemia-specific immunogenicity (164).

To enhance anti-leukemic immunogenicity, NK-cells can be modified into memory-like NK-cells *via ex vivo* stimulation using a combination of IL-12, IL-15, and IL-18. When contrasted to their native/non-activated counterparts, these

memory-like cells demonstrate increased leukemia-targeting activity (165). These cytokines enhance the sensitivity of IL-2 receptors on NK cells, thereby improving their functionality. Because of the elevated levels of the IL-2 receptor alpha-chain (IL-2R/CD25) on Treg cells, which sequesters IL-2 and restricts its availability for NK-cell stimulation, IL-2 mainly activates immunosuppressive Tregs instead of NK-cells (147). This led to the outcome that various studies evaluating IL-2 as a single treatment did not accomplish the expected anti-leukemic effectiveness (166). A meta-analysis examining the effects of IL-2 as a sole treatment found no significant advantage compared to no intervention in terms of overall or disease-free survival rates (167).

To improve the efficacy of allogeneic NK-cell-based therapy, an IL-2 diphtheria toxin fusion protein targeting Treg cells expressing IL-2R (CD25) was administered before NK-cell infusion and IL-2, successfully restoring NK-cell mediated anti-leukemic immunity (147). Phase I/II trials showed that substituting IL-2 with recombinant IL-15 enhanced NK-cell expansion *in vivo* and resulted in promising remission rates in patients with advanced AML (NCT01385423, NCT02395822) (168). These studies also revealed that subcutaneous administration was linked to cytokine release syndrome and neurotoxicity, while intravenous delivery was not (169). Additionally, a phase I trial is ongoing to evaluate the safety and feasibility of IL-21-expanded NK-cells *ex vivo* (170).

N-803, a super-agonist of IL-15/IL-15Ra, is designed to replicate the immune-stimulating effects of antigen-presenting cells. These cells convey a trans-presenting form of IL-15, which is bound to IL-15Ra, to NK-cells and cytotoxic T-cells via the common IL-2/IL-15 alpha/beta receptor (169). N-803 showed promise as a single-agent treatment in early clinical trials, demonstrating both tolerability and effectiveness in managing leukemic disease. In more recent times, studies have been evaluating the use of N-803 alongside NK-cell infusion (NCT01898793, NCT02782546). Type I interferons are believed to have both anti-leukemic and pro-immunogenic effects on cancers. However, their effectiveness as a standalone treatment is limited (171).

Due to the complex nature of the leukemic BMM, many immunosuppressive factors play a role in restricting anti-leukemic immune responses. Therapeutic agents that reduce lymphocyte suppression caused by leukemia may counteract these constraints. In this context, focusing on inhibitory-mediators like IDO 2,3 or TGF- β could provide promising strategies to address leukemia-associated immune evasion and improve the effectiveness of cell-based therapies

in AML. It has been demonstrated that blocking IDO 2,3 can improve immune regulation mediated by T and NK cells, while also averting the differentiation of Treg cells (172). In a similar vein, blocking PGE2 has shown to enhance anti-leukemic immune responses (173). Therefore, the integration of these immunoregulatory approaches could boost the efficacy of cell-based therapies.

Studies have underscored the role of TGF- β in dampening immune responses across different cancers, and inhibiting TGF- β has been demonstrated to bolster lymphocyte-mediated immune reactions against tumors, especially in non-hematologic cancers. Preclinical studies have shown that blocking TGF- β maintains NK-cell anti-leukemic activity in the presence of high pathological levels of TGF- β (174).

In certain subtypes of AML, phases of both cell bound and soluble HLA-G are heightened, which is thought to be an effective means of reestablishing immune responses due to HLA-G's regulatory influence on NK, T, and dendritic cells. Moreover, by binding to NKG2A, HLA-E inhibits the activity of NK cells, which makes it a potential target for therapy. Nonetheless, preclinical and clinical data assessing the practicality of these strategies are limited or have yet to be investigated.

6.5. Vaccines

Vaccination is gaining attention as a potential treatment to improve anti-leukemic immune surveillance, with two main approaches: (i) targeting leukemia-associated antigens and (ii) using dendritic cells to present antigens. Peptide-based vaccines targeting the Wilms tumor gene (WT-1), created with a natural or modified 9-mer WT1 peptide and montanide ISA51 adjuvant, have shown good tolerability and induced specific cytotoxic T-cell responses (175). This led to significant reductions in leukemic burden and minimal residual disease (MRD) markers in 60% of AML patients after consolidation therapy. The WT-1 vaccine, administered intracutaneously, may serve as an effective second-line maintenance therapy to enhance the graft-versus-leukemia (GvL) effect after hematopoietic stem cell transplantation (HSCT) (176). In phase I/II trials, the multivalent WT-1 vaccine galinpepimut-S administered to AML patients in complete remission elicited a targeted immune response, improved survival, and showed no significant toxicity. A phase III trial (NCT04229979) has been initiated to further assess its efficacy and safety (177). Dendritic cells (DCs), as powerful antigen-presenting cells, are ideal for use as cellular adjuvants in therapeutic vaccination. A phase II trial (NCT00965224) showed that WT1-mRNA-loaded DCs could effectively stimulate

antigen-specific T-cell responses, potentially preventing or delaying relapse after chemotherapy (178). Additionally, autologous DCs electroporated with mRNA for human telomerase reverse transcriptase (hTERT) demonstrated safety and feasibility, contributing to extended recurrence-free survival in AML patients in complete remission (179). Another promising approach involves using a hybridoma of leukemic cells and DCs derived from the patient as a personalized vaccine, which has been shown to promote T-cell expansion and targeted anti-leukemic effects, reducing relapse risk in pre-treated AML patients (180).

A cryogel vaccine, combining immunostimulatory CpG-oligodeoxynucleotide and GM-CSF with leukemia-associated antigens, effectively triggered anti-leukemic immunity by activating dendritic cells (DCs). When used in combination with chemotherapy, this vaccine prevented leukemic engraftment and successfully eradicated established leukemia in mice with AML (181). Notably, the cryogel vaccine also cleared leukemia in the absence of predefined antigens, likely due to the accumulation of apoptotic AML blasts that provided sufficient antigens to enhance immune responses mediated by DCs (181).

Considering the difficulties associated with clinical trials of new immunotherapeutic strategies and the complex immunosuppressive characteristics of the leukemic microenvironment, combining cellular and non-cellular immunotherapies may provide a more powerful approach to effectively combat leukemic immune evasion.

7. CONCLUSION

Acute Myeloid Leukemia (AML) remains a formidable challenge due to its aggressive nature, high relapse rates, and complex interactions with the bone marrow microenvironment (BMM). Leukemic blasts actively reshape the BMM into a leukemia-supportive niche, fostering immune evasion and disease progression. While chemotherapy remains the standard treatment, its limitations highlight the urgent need for novel therapeutic strategies.

The immune microenvironment plays a crucial role in AML pathophysiology, with leukemic cells exploiting various immune evasion mechanisms, including immune checkpoint upregulation and suppression of anti-leukemic responses. Recent advances in immunotherapy, such as checkpoint inhibitors and other targeted approaches, offer promising avenues for overcoming immune resistance and enhancing treatment efficacy.

A deeper understanding of the intricate crosstalk between AML cells and the immune microenvironment is essential for developing more effective, personalized treatment

strategies. Future research should focus on refining immunotherapeutic interventions, identifying predictive biomarkers, and optimizing combination therapies to improve patient outcomes. Utilizing the capacities of the immune system can lead to more long-lasting and targeted treatments for managing AML.

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References

- Testa U, Castelli G, Pelosi E. Recent Developments in Differentiation Therapy of Acute Myeloid Leukemia. 2025;
- Ladikou EE, Sivaloganathan H, Pepper A, Chevassut T. Acute myeloid leukaemia in its niche: the bone marrow microenvironment in acute myeloid leukaemia. *Curr Oncol Rep*. 2020;22:1–9.
- Sendker S, Reinhardt D, Niktoreh N. Redirecting the immune microenvironment in acute myeloid leukemia. *Cancers (Basel)*. 2021;13(6):1423.
- Zhang M, Yang Y, Liu J, Guo L, Guo Q, Liu W. Bone marrow immune cells and drug resistance in acute myeloid leukemia. *Exp Biol Med*. 2025;250:10235.
- Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *New England Journal of Medicine*. 2015;373(12):1136–52.
- Luppi M, Fabbiano F, Visani G, Martinelli G, Venditti A. Novel agents for acute myeloid leukemia. *Cancers (Basel)*. 2018;10(11):429.
- Sun H, Li Y, Zhang Z fen, Ju Y, Li L, Zhang B chang, et al. Increase in myeloid-derived suppressor cells (MDSCs) associated with minimal residual disease (MRD) detection in adult acute myeloid leukemia. *Int J Hematol*. 2015;102:579–86.
- Teague RM, Kline J. Immune evasion in acute myeloid leukemia: current concepts and future directions. *J Immunother Cancer*. 2013;1:1–11.
- Wang A, Zhong H. Roles of the bone marrow niche in hematopoiesis, leukemogenesis, and chemotherapy resistance in acute myeloid leukemia. *Hematology*. 2018;23(10):729–39.
- Taghiloo S, Asgarian-Omran H. Current approaches of immune checkpoint therapy in chronic lymphocytic leukemia. *Curr Treat Options Oncol*. 2023;24(10):1408–38.
- Tikhonova AN, Dolgalev I, Hu H, Sivaraj KK, Hoxha E, Cuesta-Domínguez Á, et al. The bone marrow microenvironment at single-cell resolution. *Nature*. 2019;569(7755):222–8.
- Comazzetto S, Shen B, Morrison SJ. Niches that regulate stem cells and hematopoiesis in adult bone marrow. *Dev Cell*. 2021;56(13):1848–60.
- Baryawno N, Przybylski D, Kowalczyk MS, Kfoury Y, Severe N, Gustafsson K, et al. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. *Cell*. 2019;177(7):1915–32.
- Méndez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, Gribben JG, et al. Bone marrow niches in haematological malignancies. *Nat Rev Cancer*. 2020;20(5):285–98.
- Raaijmakers MHGP, Mukherjee S, Guo S, Zhang S, Kobayashi T, Schoonmaker JA, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*. 2010;464(7290):852–7.
- Walkley CR, Olsen GH, Dworkin S, Fabb SA, Swann J, McArthur GA, et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor γ deficiency. *Cell*. 2007;129(6):1097–110.
- Ho YH, Del Toro R, Rivera-Torres J, Rak J, Korn C, García-García A, et al. Remodeling of bone marrow hematopoietic stem cell niches promotes myeloid cell expansion during premature or physiological aging. *Cell Stem Cell*. 2019;25(3):407–18.
- Mendelson A, Frenette PS. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat Med*. 2014;20(8):833–46.
- Maryanovich M, Zahalka AH, Pierce H, Pinho S, Nakahara F, Asada N, et al. Adrenergic nerve degeneration in bone marrow drives aging of the hematopoietic stem cell niche. *Nat Med*. 2018;24(6):782–91.
- Arranz L, Sánchez-Aguilera A, Martín-Pérez D, Isern J, Langa X, Tzankov A, et al. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature*. 2014;512(7512):78–81.
- Grockowiak E, Korn C, Rak J, Lysenko V, Hallou A, Panvini FM, et al. Different niches for stem cells carrying the same oncogenic driver affect pathogenesis and therapy response in myeloproliferative neoplasms. *Nat Cancer*. 2023;4(8):1193–209.
- Li R, Zhou Y, Cao Z, Liu L, Wang J, Chen Z, et al. TET2 loss dysregulates the behavior of bone marrow mesenchymal stromal cells and accelerates Tet2 $^{-/-}$ -driven myeloid malignancy progression. *Stem Cell Reports*. 2018;10(1):166–79.
- Zhang P, Chen Z, Li R, Guo Y, Shi H, Bai J, et al. Loss of ASXL1 in the bone marrow niche dysregulates hematopoietic stem and progenitor cell fates. *Cell Discov*. 2018;4(1):4.
- SanMiguel JM, Eudy E, Loberg MA, Young KA, Mistry JJ, Mujica KD, et al. Distinct tumor necrosis factor alpha receptors dictate stem cell fitness versus lineage output in Dnmt3a-mutant clonal hematopoiesis. *Cancer Discov*. 2022;12(12):2763–73.
- Hanoun M, Zhang D, Mizoguchi T, Pinho S, Pierce H, Kunisaki Y, et al. Acute myelogenous leukemia-induced sympathetic neuropathy promotes malignancy in an altered hematopoietic stem cell niche. *Cell Stem Cell*. 2014;15(3):365–75.
- Rios de los Rios J, Enciso J, Vilchis-Ordoñez A, Vázquez-Ramírez R, Ramírez-Ramírez D, Balandrán JC, et al. Acute lymphoblastic leukemia-secreted miRNAs induce a proinflammatory microenvironment and promote the activation of hematopoietic progenitors. *J Leukoc Biol*. 2022;112(1):31–45.
- Balandrán JC, Purizaca J, Enciso J, Dozal D, Sandoval A, Jiménez-Hernández E, et al. Pro-inflammatory-related loss of CXCL12 niche promotes acute lymphoblastic leukemic progression at the expense of normal lymphopoiesis. *Front Immunol*. 2017;7:666.
- Agarwal P, Isringhausen S, Li H, Paterson AJ, He J, Gomariz Á, et al. Mesenchymal niche-specific expression of Cxcl12 controls quiescence of treatment-resistant leukemia stem cells. *Cell Stem Cell*. 2019;24(5):769–84.
- Schneider RK, Mullally A, Dugourd A, Peisker F, Hoogenboezem R, Van Strien PMH, et al. Gli1 $^{+}$ mesenchymal stromal cells are a key driver

of bone marrow fibrosis and an important cellular therapeutic target. *Cell Stem Cell*. 2017;20(6):785–800.

30. Zehtabcheh S, Yousefi AM, Salari S, Safa M, Momeny M, Ghaffari SH, Bashash D. Abrogation of histone deacetylases (HDACs) decreases survival of chronic myeloid leukemia cells: New insight into attenuating effects of the PI3K/c-Myc axis on panobinostat cytotoxicity. *Cell Biology International*. 2021 May;45(5):1111–21.

31. Medyouf H, Mossner M, Jann JC, Nolte F, Raffel S, Herrmann C, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell*. 2014;14(6):824–37.

32. Decker M, Martinez-Morentin L, Wang G, Lee Y, Liu Q, Leslie J, et al. Leptin-receptor-expressing bone marrow stromal cells are myofibroblasts in primary myelofibrosis. *Nat Cell Biol*. 2017;19(6):677–88.

33. Barbier V, Erban J, Fiveash C, Davies JM, Tay J, Tallack MR, et al. Endothelial E-selectin inhibition improves acute myeloid leukaemia therapy by disrupting vascular niche-mediated chemoresistance. *Nat Commun*. 2020;11(1):2042.

34. Duarte D, Hawkins ED, Akinduro O, Ang H, De Filippo K, Kong IY, et al. Inhibition of endosteal vascular niche remodeling rescues hematopoietic stem cell loss in AML. *Cell Stem Cell*. 2018;22(1):64–77.

35. Schmidt T, Masouleh BK, Loges S, Cauwenberghs S, Fraisl P, Maes C, et al. Loss or inhibition of stromal-derived PlGF prolongs survival of mice with imatinib-resistant Bcr-Abl1+ leukemia. *Cancer Cell*. 2011;19(6):740–53.

36. Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer: Interdisciplinary International Journal of the American Cancer Society*. 2006;107(9):2099–107.

37. Estey E, Döhner H. Acute myeloid leukaemia. *The Lancet*. 2006;368(9550):1894–907.

38. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. *Blood Rev*. 2019;36:70–87.

39. Patel JP, Gönen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *New England Journal of Medicine*. 2012;366(12):1079–89.

40. DiNardo CD, Cortes JE. Mutations in AML: prognostic and therapeutic implications. *Hematology 2014, the American Society of Hematology Education Program Book*. 2016;2016(1):348–55.

41. Martelli MP, Sportoletti P, Tiacci E, Martelli MF, Falini B. Mutational landscape of AML with normal cytogenetics: biological and clinical implications. *Blood Rev*. 2013;27(1):13–22.

42. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *New England Journal of Medicine*. 2016;374(23):2209–21.

43. Eisfeld AK, Kohlschmidt J, Mims A, Nicolet D, Walker CJ, Blachly JS, et al. Additional gene mutations may refine the 2017 European LeukemiaNet classification in adult patients with de novo acute myeloid leukemia aged < 60 years. *Leukemia*. 2020;34(12):3215–27.

44. Narayanan D, Weinberg OK. How I investigate acute myeloid leukemia. *Int J Lab Hematol*. 2020;42(1):3–15.

45. Weinberg OK, Hasserjian RP, Baraban E, Ok CY, Geyer JT, Philip JKSS, et al. Clinical, immunophenotypic, and genomic findings of acute undifferentiated leukemia and comparison to acute myeloid leukemia with minimal differentiation: a study from the bone marrow pathology group. *Modern Pathology*. 2019;32(9):1373–85.

46. Rose-Inman H, Kuehl D. Acute leukemia. *Hematology/Oncology Clinics*. 2017;31(6):1011–28.

47. Franchini M, Frattini F, Crestani S, Bonfanti C. Bleeding complications in patients with hematologic malignancies. In: *Seminars in thrombosis and hemostasis*. Thieme Medical Publishers; 2013. p. 94–100.

48. Naymagon L, Mascarenhas J. Hemorrhage in acute promyelocytic leukemia: Can it be predicted and prevented? *Leuk Res*. 2020;94:106356.

49. Stahl M, Shallis RM, Wei W, Montesinos P, Lengline E, Neukirchen J, et al. Management of hyperleukocytosis and impact of leukapheresis among patients with acute myeloid leukemia (AML) on short-and long-term clinical outcomes: a large, retrospective, multicenter, international study. *Leukemia*. 2020;34(12):3149–60.

50. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood, the journal of the american society of hematology*. 2016;127(20):2391–405.

51. Jongen-Lavrencic M, Grob T, Hanekamp D, Kavelaars FG, Al Hinai A, Zeilemaker A, et al. Molecular minimal residual disease in acute myeloid leukemia. *New England Journal of Medicine*. 2018;378(13):1189–99.

52. De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. *Blood Cancer J*. 2016;6(7):e441–e441.

53. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood, The Journal of the American Society of Hematology*. 2017;129(4):424–47.

54. DiNardo CD, Maiti A, Rausch CR, Pemmaraju N, Naqvi K, Daver NG, et al. 10-day decitabine with venetoclax for newly diagnosed intensive chemotherapy ineligible, and relapsed or refractory acute myeloid leukaemia: a single-centre, phase 2 trial. *Lancet Haematol*. 2020;7(10):e724–36.

55. Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Laribi K, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. *Blood, The Journal of the American Society of Hematology*. 2020;135(24):2137–45.

56. Datogui TS, Velloso EDRP, Helman R, Musacchio JG, Salvino MA, Soares RA, et al. Overall survival of Brazilian acute myeloid leukemia patients according to the European LeukemiaNet prognostic scoring system: a cross-sectional study. *Medical Oncology*. 2018;35:1–6.

57. Shimomura Y, Hara M, Konuma T, Itonaga H, Doki N, Ozawa Y, et al. Allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome in adolescent and young adult patients. *Bone Marrow Transplant*. 2021;56(10):2510–7.

58. Hirabayashi S, Uozumi R, Kondo T, Arai Y, Kawata T, Uchida N, et al. Personalized prediction of overall survival in patients with AML in non-complete remission undergoing allo-HCT. *Cancer Med*. 2021;10(13):4250–68.

59. Daver N, Wei AH, Pollyea DA, Fathi AT, Vyas P, DiNardo CD. New directions for emerging therapies in acute myeloid leukemia: the next chapter. *Blood Cancer J*. 2020;10(10):107.

60. O'Donnell MR, Tallman MS, Abboud CN, Altman JK, Appelbaum FR, Arber DA, et al. Acute myeloid leukemia, version 3.2017, NCCN clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network*. 2017;15(7):926–57.

61. Puccetti E, Ruthardt M. Acute promyelocytic leukemia: PML/RAR α and the leukemic stem cell. *Leukemia*. 2004;18(7):1169–75.

62. Mistry AR, Pedersen EW, Solomon E, Grimwade D. The molecular pathogenesis of acute promyelocytic leukaemia: implications for the clinical management of the disease. *Blood Rev*. 2003;17(2):71–97.

63. Burnett AK, Russell NH, Hills RK, Bowen D, Kell J, Knapper S, et al. Arsenic trioxide and all-trans retinoic acid treatment for acute promyelocytic leukaemia in all risk groups (AML17): results of a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2015;16(13):1295–305.
64. Zhang P, Zhang C, Li J, Han J, Liu X, Yang H. The physical microenvironment of hematopoietic stem cells and its emerging roles in engineering applications. *Stem Cell Res Ther*. 2019;10:1–13.
65. Brück O, Dufva O, Hohtari H, Blom S, Turkki R, Ilander M, et al. Immune profiles in acute myeloid leukemia bone marrow associate with patient age, T-cell receptor clonality, and survival. *Blood Adv*. 2020;4(2):274–86.
66. Black S, Phillips D, Hickey JW, Kennedy-Darling J, Venkataaraman VG, Samusik N, et al. CODEX multiplexed tissue imaging with DNA-conjugated antibodies. *Nat Protoc*. 2021;16(8):3802–35.
67. Bandyopadhyay S, Duffy MP, Ahn KJ, Sussman JH, Pang M, Smith D, et al. Mapping the cellular biogeography of human bone marrow niches using single-cell transcriptomics and proteomic imaging. *Cell*. 2024;187(12):3120–40.
68. Passaro D, Di Tullio A, Abarrategi A, Rouault-Pierre K, Foster K, Ariza-McNaughton L, et al. Increased vascular permeability in the bone marrow microenvironment contributes to disease progression and drug response in acute myeloid leukemia. *Cancer Cell*. 2017;32(3):324–41.
69. Kumar B, Garcia M, Weng L, Jung X, Murakami JL, Hu X, et al. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. *Leukemia*. 2018;32(3):575–87.
70. Munn DH, Mellor AL, Rossi M, Young JW. Dendritic cells have the option to express IDO-mediated suppression or not. *Blood*. 2005;105(6):2618.
71. Mussai F, De Santo C, Abu-Dayyeh I, Booth S, Quek L, McEwen-Smith RM, et al. Acute myeloid leukemia creates an arginase-dependent immunosuppressive microenvironment. *Blood, The Journal of the American Society of Hematology*. 2013;122(5):749–58.
72. Serra S, Horenstein AL, Vaisitti T, Brusa D, Rossi D, Laurenti L, et al. CD73-generated extracellular adenosine in chronic lymphocytic leukemia creates local conditions counteracting drug-induced cell death. *Blood, The Journal of the American Society of Hematology*. 2011;118(23):6141–52.
73. Dulphy N, Henry G, Hemon P, Khaznadar Z, Dombret H, Boissel N, et al. Contribution of CD39 to the immunosuppressive microenvironment of acute myeloid leukaemia at diagnosis. *Br J Haematol*. 2014;165(5).
74. de la Guardia RD, Lopez-Millan B, Lavoie JR, Bueno C, Castano J, Gómez-Casares M, et al. Detailed characterization of mesenchymal stem/stromal cells from a large cohort of AML patients demonstrates a definitive link to treatment outcomes. *Stem Cell Reports*. 2017;8(6):1573–86.
75. Carey A, Edwards DK, Eide CA, Newell L, Traer E, Medeiros BC, et al. Identification of interleukin-1 by functional screening as a key mediator of cellular expansion and disease progression in acute myeloid leukemia. *Cell Rep*. 2017;18(13):3204–18.
76. Mishra SK, Millman SE, Zhang L. Metabolism in acute myeloid leukemia: mechanistic insights and therapeutic targets. *Blood*. 2023;141(10):1119–35.
77. Wang ZH, Peng WB, Zhang P, Yang XP, Zhou Q. Lactate in the tumour microenvironment: From immune modulation to therapy. *EBioMedicine* 73, 103627. 2021.
78. Sabuncuoğlu S, Öztas Y, Uçkan Çetinkaya D, Özgüneş N, Özgüneş H. Oxidative protein damage with carbonyl levels and nitrotyrosine expression after chemotherapy in bone marrow transplantation patients. *Pharmacology*. 2012;89(5–6):283–6.
79. Kong H, Chandel NS. Regulation of redox balance in cancer and T cells. *Journal of Biological Chemistry*. 2018;293(20):7499–507.
80. Toffalori C, Zito L, Gambacorta V, Riba M, Oliveira G, Bucci G, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nat Med*. 2019;25(4):603–11.
81. Christopher MJ, Petti AA, Rettig MP, Miller CA, Chendamarai E, Duncavage EJ, et al. Immune escape of relapsed AML cells after allogeneic transplantation. *New England Journal of Medicine*. 2018;379(24):2330–41.
82. Novello M, Manfredi F, Ruggiero E, Perini T, Oliveira G, Cortesi F, et al. Bone marrow central memory and memory stem T-cell exhaustion in AML patients relapsing after HSCT. *Nat Commun*. 2019;10(1):1065.
83. Jain P, Tian X, Cordes S, Chen J, Cantilena CR, Bradley C, et al. Over-expression of PD-1 does not predict leukemic relapse after allogeneic stem cell transplantation. *Biology of Blood and Marrow Transplantation*. 2019;25(2):216–22.
84. Kong Y, Zhang J, Claxton DF, Ehmann WC, Rybka WB, Zhu L, et al. PD-1hiTIM-3+ T cells associate with and predict leukemia relapse in AML patients post allogeneic stem cell transplantation. *Blood Cancer J*. 2015;5(7):e330–e330.
85. Williams P, Basu S, Garcia-Manero G, Hourigan CS, Oetjen KA, Cortes JE, et al. The distribution of T-cell subsets and the expression of immune checkpoint receptors and ligands in patients with newly diagnosed and relapsed acute myeloid leukemia. *Cancer*. 2019;125(9):1470–81.
86. Yeaton A, Cayan G, Loghavi S, Dolgalev I, Leddin EM, Loo CE, et al. The impact of inflammation-induced tumor plasticity during myeloid transformation. *Cancer Discov*. 2022;12(10):2392–413.
87. Guo R, Lü M, Cao F, Wu G, Gao F, Pang H, et al. Single-cell map of diverse immune phenotypes in the acute myeloid leukemia microenvironment. *Biomark Res*. 2021;9:1–16.
88. Mundy-Bosse BL, Scoville SD, Chen L, McConnell K, Mao HC, Ahmed EH, et al. MicroRNA-29b mediates altered innate immune development in acute leukemia. *J Clin Invest*. 2016;126(12):4404–16.
89. Lasry A, Nadorp B, Fornerod M, Nicolet D, Wu H, Walker CJ, et al. An inflammatory state remodels the immune microenvironment and improves risk stratification in acute myeloid leukemia. *Nat Cancer*. 2023;4(1):27–42.
90. Mumme H, Thomas BE, Bhasin SS, Krishnan U, Dwivedi B, Perumalla P, et al. Single-cell analysis reveals altered tumor microenvironments of relapse- and remission-associated pediatric acute myeloid leukemia. *Nat Commun*. 2023;14(1):6209.
91. Abbas HA, Hao D, Tomczak K, Barrodia P, Im JS, Reville PK, et al. Single cell T cell landscape and T cell receptor repertoire profiling of AML in context of PD-1 blockade therapy. *Nat Commun*. 2021;12(1):6071.
92. Knaus HA, Berglund S, Hackl H, Blackford AL, Zeidner JF, Montiel-Esparza R, et al. Signatures of CD8+ T cell dysfunction in AML patients and their reversibility with response to chemotherapy. *JCI Insight*. 2018;3(21):e120974.
93. Zeiser R, Vago L. Mechanisms of immune escape after allogeneic hematopoietic cell transplantation. *Blood, The Journal of the American Society of Hematology*. 2019;133(12):1290–7.
94. McCurdy SR, Iglehart BS, Batista DA, Gocke CD, Ning Y, Knaus HA, et al. Loss of the mismatched human leukocyte antigen haplotype in two acute myelogenous leukemia relapses after haploidentical bone marrow transplantation with post-transplantation cyclophosphamide. *Leukemia*. 2016;30(10):2102–6.
95. Kline DE, MacNabb BW, Chen X, Chan WC, Fosco D, Kline J. CD8α+ dendritic cells dictate leukemia-specific CD8+ T cell fates. *The Journal of Immunology*. 2018;201(12):3759–69.

96. Xiao W, Goldberg AD, Famulare CA, Devlin SM, Nguyen NT, Sim S, et al. Loss of plasmacytoid dendritic cell differentiation is highly predictive for post-induction measurable residual disease and inferior outcomes in acute myeloid leukemia. *Haematologica*. 2018;104(7):1378.
97. Ismail MM, Abdulateef NAB. Bone marrow T-cell percentage: A novel prognostic indicator in acute myeloid leukemia. *Int J Hematol*. 2017;105:453–64.
98. Behl D, Porrata LF, Markovic SN, Letendre L, Pruthi RK, Hook CC, et al. Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia*. 2006;20(1):29–34.
99. Daver N, Garcia-Manero G, Basu S, Boddur PC, Alfayez M, Cortes JE, et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II study. *Cancer Discov*. 2019;9(3):370–83.
100. Zhou Q, Munger ME, Highfill SL, Tolar J, Weigel BJ, Riddle M, et al. Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology*. 2010;116(14):2484–93.
101. Zhou Q, Munger ME, Veenstra RG, Weigel BJ, Hirashima M, Munn DH, et al. Coexpression of Tim-3 and PD-1 identifies a CD8⁺ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. *Blood, The Journal of the American Society of Hematology*. 2011;117(17):4501–10.
102. Le Dieu R, Taussig DC, Ramsay AG, Mitter R, Miraki-Moud F, Fatah R, et al. Peripheral blood T cells in acute myeloid leukemia (AML) patients at diagnosis have abnormal phenotype and genotype and form defective immune synapses with AML blasts. *Blood, The Journal of the American Society of Hematology*. 2009;114(18):3909–16.
103. Schnorfeil FM, Lichtenegger FS, Emmerig K, Schlueter M, Neitz JS, Draenert R, et al. T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. *J Hematol Oncol*. 2015;8:1–12.
104. Dama P, Tang M, Fulton N, Kline J, Liu H. Gal9/Tim-3 expression level is higher in AML patients who fail chemotherapy. *J Immunother Cancer*. 2019;7:1–7.
105. Radpour R, Riether C, Simillion C, Höpner S, Bruggmann R, Ochsenbein AF. CD8⁺ T cells expand stem and progenitor cells in favorable but not adverse risk acute myeloid leukemia. *Leukemia*. 2019;33(10):2379–92.
106. Brodská B, Otevřelová P, Šálek C, Fuchs O, Gašová Z, Kuželová K. High PD-L1 expression predicts for worse outcome of leukemia patients with concomitant NPM1 and FLT3 mutations. *Int J Mol Sci*. 2019;20(11):2823.
107. Yoyen-Ermis D, Tunalı G, Tavukcuoglu E, Horzum U, Ozkazanc D, Sutlu T, et al. Myeloid maturation potentiates STAT3-mediated atypical IFN- γ signaling and upregulation of PD-1 ligands in AML and MDS. *Sci Rep*. 2019;9(1):11697.
108. Rettig MP, Godwin J, Vey N, Fox B, Ballesteros-Merino C, Bifulco CB, et al. Preliminary translational results from an ongoing phase 1 study of flotetuzumab, a CD123 $\times$ CD3 DART[®], in AML/MDS: rationale for combining flotetuzumab and anti-PD-1/PD-L1 immunotherapies. *Blood*. 2017;130:1365.
109. Aurelius J, Thorén FB, Akhiani AA, Brune M, Palmqvist L, Hansson M, et al. Monocytic AML cells inactivate antileukemic lymphocytes: role of NADPH oxidase/gp91phox expression and the PARP-1/PAR pathway of apoptosis. *Blood, The Journal of the American Society of Hematology*. 2012;119(24):5832–7.
110. Stringaris K, Sekine T, Khoder A, Alsuliman A, Razzaghi B, Sargeant R, et al. Leukemia-induced phenotypic and functional defects in natural killer cells predict failure to achieve remission in acute myeloid leukemia. *Haematologica*. 2014;99(5):836.
111. Elias S, Yamin R, Golomb L, Tsukerman P, Stanitsky-Kaynan N, Ben-Yehuda D, et al. Immune evasion by oncogenic proteins of acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology*. 2014;123(10):1535–43.
112. Ustun C, Miller JS, Munn DH, Weisdorf DJ, Blazar BR. Regulatory T cells in acute myelogenous leukemia: is it time for immunomodulation? *Blood, The Journal of the American Society of Hematology*. 2011;118(19):5084–95.
113. Delia M, Carluccio P, Mestice A, Brunetti C, Albano F, Specchia G. Impact of bone marrow aspirate tregs on the response rate of younger newly diagnosed acute myeloid leukemia patients. *J Immunol Res*. 2018;2018(1):9325261.
114. Pyzer AR, Stroopinsky D, Rajabi H, Washington A, Tagde A, Coll M, et al. MUC1-mediated induction of myeloid-derived suppressor cells in patients with acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology*. 2017;129(13):1791–801.
115. Kittang AO, Kordasti S, Sand KE, Costantini B, Kramer AM, Perezabellan P, et al. Expansion of myeloid derived suppressor cells correlates with number of T regulatory cells and disease progression in myelodysplastic syndrome. *Oncoimmunology*. 2016;5(2):e1062208.
116. Mangaonkar A, Mondal AK, Fulzule S, Pundkar C, Park EJ, Jillella A, et al. A novel immunohistochemical score to predict early mortality in acute myeloid leukemia patients based on indoleamine 2, 3 dioxygenase expression. *Sci Rep*. 2017;7(1):12892.
117. Curti A, Pandolfi S, Valzasina B, Aluigi M, Isidori A, Ferri E, et al. Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25[–] into CD25⁺ T regulatory cells. *Blood*. 2007;109(7):2871–7.
118. Deng M, Gui X, Kim J, Xie L, Chen W, Li Z, et al. LILRB4 signalling in leukaemia cells mediates T cell suppression and tumour infiltration. *Nature*. 2018;562(7728):605–9.
119. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. *Int Immunopharmacol*. 2018;62:29–39.
120. Liao D, Wang M, Liao Y, Li J, Niu T. A review of efficacy and safety of checkpoint inhibitor for the treatment of acute myeloid leukemia. *Front Pharmacol*. 2019;10:609.
121. Bashey A, Medina B, Corringham S, Pasek M, Carrier E, Vrooman L, et al. CTLA4 blockade with ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. *Blood, The Journal of the American Society of Hematology*. 2009;113(7):1581–8.
122. Davids MS, Kim HT, Bachireddy P, Costello C, Liguori R, Savell A, et al. Ipilimumab for patients with relapse after allogeneic transplantation. *New England Journal of Medicine*. 2016;375(2):143–53.
123. Davids MS, Kim HT, Costello C, Herrera AF, Locke FL, Maegawa RO, et al. A multicenter phase 1 study of nivolumab for relapsed hematologic malignancies after allogeneic transplantation. *Blood, The Journal of the American Society of Hematology*. 2020;135(24):2182–91.
124. Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, et al. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clinical Cancer Research*. 2008;14(10):3044–51.
125. Chen C, Liang C, Wang S, Chio CL, Zhang Y, Zeng C, et al. Expression patterns of immune checkpoints in acute myeloid leukemia. *J Hematol Oncol*. 2020;13:1–5.

126. Assi HI, Kamphorst AO, Moukalled NM, Ramalingam SS. Immune checkpoint inhibitors in advanced non-small cell lung cancer. *Cancer*. 2018;124(2):248–61.
127. Daver N, Kantarjian H, Ravandi F, Estey E, Wang X, Garcia-Manero G, et al. A phase II study of decitabine and gemtuzumab ozogamicin in newly diagnosed and relapsed acute myeloid leukemia and high-risk myelodysplastic syndrome. *Leukemia*. 2016;30(2):268–73.
128. Ghosh A, Barba P, Perales M. Checkpoint inhibitors in AML: are we there yet? *Br J Haematol*. 2020;188(1):159–67.
129. Karim LA, Wang P, Guzman J de, Higgins B, Chahine J, Sheehan C, et al. PDL1 protein expression and tumor mutation burden in hematologic malignancies: correlation with Hodgkin and high grade lymphoma. *Cancer Res*. 2017;77(13_Supplement):3724.
130. Lindblad KE, Goswami M, Hourigan CS, Oetjen KA. Immunological effects of hypomethylating agents. *Expert Rev Hematol*. 2017;10(8):745–52.
131. Kikushige Y, Miyamoto T, Yuda J, Jabbarzadeh-Tabrizi S, Shima T, Takayanagi S ichiro, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. *Cell Stem Cell*. 2015;17(3):341–52.
132. Broglie L, Gershan J, Burke MJ. Checkpoint inhibition of PD-L1 and CTLA-4 in a child with refractory acute leukemia. *Int J Hematol Oncol*. 2019;8(1):IJH10.
133. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. 1990;
134. Sakellari I, Konstantinou V, Gavrilaki E, Mallouri D, Batsis I, Kalaitzidou V, et al. Efficacy and Toxicity of Donor Lymphocyte Infusions in Relapsed Acute Myeloid Leukemia Post Allogeneic Hematopoietic Cell Transplantation: Single-Center Experience. American Society of Hematology Washington, DC; 2016.
135. Mardiros A, Dos Santos C, McDonald T, Brown CE, Wang X, Budde LE, et al. T cells expressing CD123-specific chimeric antigen receptors exhibit specific cytolytic effector functions and antitumor effects against human acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology*. 2013;122(18):3138–48.
136. Kenderian SS, Ruella M, Shestova O, Klichinsky M, Aikawa V, Morrisette JJD, et al. CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. *Leukemia*. 2015;29(8):1637–47.
137. Petrov JC, Wada M, Pinz KG, Yan LE, Chen KH, Shuai X, et al. Compound CAR T-cells as a double-pronged approach for treating acute myeloid leukemia. *Leukemia*. 2018;32(6):1317–26.
138. Vajari MK, Moradinasab S, Yousefi AM, Bashash D. Noncoding RNAs in diagnosis and prognosis of graft-versus-host disease (GVHD). *Journal of Cellular Physiology*. 2022 Sep;237(9):3480–95.
139. Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK cells: A promising cellular immunotherapy for cancer. *EBioMedicine*. 2020;59.
140. Tettamanti S, Marin V, Pizzitola I, Magnani CF, Attianese GMG, Cribioli E, et al. Targeting of acute myeloid leukemia (AML) by cytokine-induced killer (CIK) cells redirected with a novel CD123-specific chimeric antigen receptor (CAR). *Cytotherapy*. 2013;15(4):S37.
141. Bednarski JJ, Zimmerman C, Cashen AF, Desai S, Foster M, Schappe T, et al. Adoptively transferred donor-derived cytokine induced memory-like NK cells persist and induce remission in pediatric patient with relapsed acute myeloid leukemia after hematopoietic cell transplantation. *Blood*. 2019;134:3307.
142. Tang X, Yang L, Li Z, Nalin AP, Dai H, Xu T, et al. First-in-man clinical trial of CAR NK-92 cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. *Am J Cancer Res*. 2018;8(6):1083.
143. Rubnitz JE, Inaba H, Ribeiro RC, Pounds S, Rooney B, Bell T, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *Journal of clinical oncology*. 2010;28(6):955–9.
144. Curti A, Ruggeri L, D'Addio A, Bontadini A, Dan E, Motta MR, et al. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients. *Blood, The Journal of the American Society of Hematology*. 2011;118(12):3273–9.
145. Nguyen R, Wu H, Pounds S, Inaba H, Ribeiro RC, Cullins D, et al. A phase II clinical trial of adoptive transfer of haploidentical natural killer cells for consolidation therapy of pediatric acute myeloid leukemia. *J Immunother Cancer*. 2019;7:1–7.
146. Curti A, Ruggeri L, Parisi S, Bontadini A, Dan E, Motta MR, et al. Larger size of donor alloreactive NK cell repertoire correlates with better response to NK cell immunotherapy in elderly acute myeloid leukemia patients. *Clinical Cancer Research*. 2016;22(8):1914–21.
147. Bachanova V, Cooley S, Defor TE, Verneris MR, Zhang B, McKenna DH, et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood, The Journal of the American Society of Hematology*. 2014;123(25):3855–63.
148. Bras AE, de Haas V, van Stigt A, Jongen-Lavrencic M, Beverloo HB, Te Marvelde JG, et al. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. *Cytometry B Clin Cytom*. 2019;96(2):134–42.
149. Morsink LM, Walter RB. Novel monoclonal antibody-based therapies for acute myeloid leukemia. *Best Pract Res Clin Haematol*. 2019;32(2):116–26.
150. Niktoresh N, Lerijs B, Zimmermann M, Gruhn B, Escherich G, Bourquin JP, et al. Gemtuzumab ozogamicin in children with relapsed or refractory acute myeloid leukemia: a report by Berlin-Frankfurt-Münster study group. *Haematologica*. 2018;104(1):120.
151. Renneville A, Abdelali R Ben, Chevret S, Nibourel O, Cheok M, Pautas C, et al. Clinical impact of gene mutations and lesions detected by SNP-array karyotyping in acute myeloid leukemia patients in the context of gemtuzumab ozogamicin treatment: Results of the ALFA-0701 trial. *Oncotarget*. 2014;5(4):916.
152. Gamis AS, Alonzo TA, Meshinchi S, Sung L, Gerbing RB, Raimondi SC, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. *Journal of Clinical Oncology*. 2014;32(27):3021–32.
153. Daver NG, Montesinos P, DeAngelo DJ, Wang ES, Papadantonakis N, Deconinck E, et al. Clinical profile of IMG632, a novel CD123-targeting antibody-drug conjugate (ADC), in patients with relapsed/refractory (R/R) acute myeloid leukemia (AML) or blastic plasmacytoid dendritic cell neoplasm (BPDCN). *Blood*. 2019;134:734.
154. Bakker ABH, van den Oudenrijn S, Bakker AQ, Feller N, van Meijer M, Bia JA, et al. C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. *Cancer Res*. 2004;64(22):8443–50.
155. Van Rhenen A, Van Dongen GAMS, Kelder A, Rombouts EJ, Feller N, Moshaver B, et al. The novel AML stem cell-associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. *Blood, The Journal of the American Society of Hematology*. 2007;110(7):2659–66.

156. Jiang YP, Liu BY, Zheng Q, Panuganti S, Chen R, Zhu J, et al. CLT030, a leukemic stem cell-targeting CLL1 antibody-drug conjugate for treatment of acute myeloid leukemia. *Blood Adv.* 2018;2(14):1738–49.
157. Uy GL, Godwin J, Rettig MP, Vey N, Foster M, Arellano ML, et al. Preliminary results of a phase 1 study of flotetuzumab, a CD123 x CD3 bispecific Dart® protein, in patients with relapsed/refractory acute myeloid leukemia and myelodysplastic syndrome. *Blood.* 2017;130:637.
158. Leong SR, Sukumaran S, Hristopoulos M, Totpal K, Stainton S, Lu E, et al. An anti-CD3/anti-CLL-1 bispecific antibody for the treatment of acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology.* 2017;129(5):609–18.
159. Lee EM, Yee D, Busfield SJ, McManus JF, Cummings N, Vairo G, et al. Efficacy of an Fc-modified anti-CD123 antibody (CSL362) combined with chemotherapy in xenograft models of acute myelogenous leukemia in immunodeficient mice. *Haematologica.* 2015;100(7):914.
160. Kubasch AS, Platzbecker U. Beyond the edge of hypomethylating agents: novel combination strategies for older adults with advanced MDS and AML. *Cancers (Basel).* 2018;10(6):158.
161. Vallera DA, Felices M, McElmurry R, McCullar V, Zhou X, Schmohl JU, et al. IL15 trispecific killer engagers (TriKE) make natural killer cells specific to CD33+ targets while also inducing persistence, in vivo expansion, and enhanced function. *Clinical Cancer Research.* 2016;22(14):3440–50.
162. Raneros AB, Puras AM, Rodriguez RM, Colado E, Bernal T, Anguita E, et al. Increasing TIMP3 expression by hypomethylating agents diminishes soluble MICA, MICB and ULBP2 shedding in acute myeloid leukemia, facilitating NK cell-mediated immune recognition. *Oncotarget.* 2017;8(19):31959.
163. Schmohl JU, Felices M, Todhunter D, Taras E, Miller JS, Vallera DA. Tetraspecific scFv construct provides NK cell mediated ADCC and self-sustaining stimuli via insertion of IL-15 as a cross-linker. *Oncotarget.* 2016;7(45):73830.
164. Ojo EO, Sharma AA, Liu R, Moreton S, Checkley-Luttge MA, Gupta K, et al. Membrane bound IL-21 based NK cell feeder cells drive robust expansion and metabolic activation of NK cells. *Sci Rep.* 2019; 9 (1): 14916. Epub 2019/10/19. doi: 10.1038/s41598-019-51287-6. PubMed PMID: 31624330;
165. Leong JW, Chase JM, Romee R, Schneider SE, Sullivan RP, Cooper MA, et al. Preactivation with IL-12, IL-15, and IL-18 induces CD25 and a functional high-affinity IL-2 receptor on human cytokine-induced memory-like natural killer cells. *Biology of Blood and Marrow Transplantation.* 2014;20(4):463–73.
166. Buyse M, Squifflet P, Lange BJ, Alonzo TA, Larson RA, Kolitz JE, et al. Individual patient data meta-analysis of randomized trials evaluating IL-2 monotherapy as remission maintenance therapy in acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology.* 2011;117(26):7007–13.
167. Mao C, Fu X, Yuan J, Yang Z, Huang Y, YE Q, et al. Interleukin-2 as maintenance therapy for children and adults with acute myeloid leukaemia in first complete remission. *Cochrane Database of Systematic Reviews.* 2015;(11).
168. Cooley S, He F, Bachanova V, Vercellotti GM, DeFor TE, Curtisinger JM, et al. First-in-human trial of rhIL-15 and haploidentical natural killer cell therapy for advanced acute myeloid leukemia. *Blood Adv.* 2019;3(13):1970–80.
169. Soiffer RJ. Can IL-15 superagonist ALTer GVL? *Blood, The Journal of the American Society of Hematology.* 2018;131(23):2511–2.
170. Vasu S, Sharma N, Odonnell L, Bosse K, Lee DA. A phase I clinical trial testing the safety of IL-21-expanded, off-the-shelf, natural killer cells for relapsed/refractory acute myeloid leukemia and myelodysplastic syndrome. *American Society of Clinical Oncology;* 2020.
171. Smits ELJM, Anguille S, Berneman ZN. Interferon α may be back on track to treat acute myeloid leukemia. *Oncoimmunology.* 2013;2(4):e23619.
172. Sobash PT, Kolhe R, Karim NA, Guddati AK, Jillella A, Kota V. Role of indoleamine 2, 3-dioxygenase in acute myeloid leukemia. *Future Oncology.* 2020;16(36):3085–94.
173. Joshi M, Pathak S, Sharma S, Patravale V. Design and in vivo pharmacodynamic evaluation of nanostructured lipid carriers for parenteral delivery of artemether: Nanoject. *Int J Pharm.* 2008;364(1):119–26.
174. Otegbeye F, Ojo E, Moreton S, Mackowski N, Lee DA, De Lima M, et al. Inhibiting TGF-beta signaling preserves the function of highly activated, in vitro expanded natural killer cells in AML and colon cancer models. *PLoS One.* 2018;13(1):e0191358.
175. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, et al. Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. *Proceedings of the National Academy of Sciences.* 2004;101(38):13885–90.
176. Maeda T, Hosen N, Fukushima K, Tsuboi A, Morimoto S, Matsui T, et al. Maintenance of complete remission after allogeneic stem cell transplantation in leukemia patients treated with Wilms tumor 1 peptide vaccine. *Blood Cancer J.* 2013;3(8):e130–e130.
177. Maslak PG, Dao T, Bernal Y, Chaneil SM, Zhang R, Frattini M, et al. Phase 2 trial of a multivalent WT1 peptide vaccine (galinpepimut-S) in acute myeloid leukemia. *Blood Adv.* 2018;2(3):224–34.
178. Anguille S, Van de Velde AL, Smits EL, Van Tendeloo VF, Juliusson G, Cools N, et al. Dendritic cell vaccination as postremission treatment to prevent or delay relapse in acute myeloid leukemia. *Blood, The Journal of the American Society of Hematology.* 2017;130(15):1713–21.
179. Khoury HJ, Collins Jr RH, Blum W, Stiff PS, Elias L, Lebkowski JS, et al. Immune responses and long-term disease recurrence status after telomerase-based dendritic cell immunotherapy in patients with acute myeloid leukemia. *Cancer.* 2017;123(16):3061–72.
180. Rosenblatt J, Stone RM, Uhl L, Neuberg D, Joyce R, Levine JD, et al. Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remissions. *Sci Transl Med.* 2016;8(368):368ra171–368ra171.
181. Shah NJ, Najibi AJ, Shih TY, Mao AS, Sharda A, Scadden DT, et al. A biomaterial-based vaccine eliciting durable tumour-specific responses against acute myeloid leukaemia. *Nat Biomed Eng.* 2020;4(1):40–51.