Routes and molecular mechanisms of central nervous system involvement in acute myeloid leukemia (Review)

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Abstract. Acute myeloid leukemia (AML) is a predominant form of leukemia. Central nervous system (CNS) involvement complicates its diagnosis due to limited diagnostic tools, as well as its treatment due to inadequate therapeutic methodologies and poor prognosis. Furthermore, its incidence rate is unclear. The mechanisms of AML cell mobilization from the bone marrow (BM) to the CNS are not fully elucidated, and the molecular factors contributing to CNS infiltration are insufficiently recognized. The present review aimed to enhance the understanding of CNS involvement of AML and its impact on CNS. The latest research on the pathways and mechanisms facilitating AML cells to escape the BM and infiltrate the CNS was reviewed. Additionally, novel therapeutic strategies targeting specific molecules and genes for treating CNS involvement in AML were examined.

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Abbreviations: AML, acute myeloid leukemia; CNS, central nervous system; BM, bone marrow; CSF, cerebrospinal fluid; LP, lumbar puncture; MBB, marrow-blood barrier; CAM, cell adhesion molecules; ECM, extracellular matrix; ACCR, adventitial cell coverage ratio; SDF-1, stromal cell-derived factor 1; CXCL12, C-X-C motif chemokine 12; CXCR-4, chemokine receptor 4; CD31, cluster of differentiation 31; CD38, cluster of differentiation 38; LFA-1, lymphocyte function associated antigen-1; ICAM-1, intercellular CAM-1; VCAM-1, vascular CAM-1; CD49d, the rate-limiting α-chain of the CD49d/CD29 integrin heterodimer very late antigen-4; MMP-9, matrix metalloproteinase-9; BBB, blood-brain barrier; BLMB, blood-leptomeningeal barrier; BCSFB, blood-CSF barrier; LILRB4, immunoglobulin-like receptor B4

Key words: AML, CNS, cell adhesion molecules, brain barrier, therapeutic strategies

Contents

1. Introduction

Acute myeloid leukemia (AML), a prevalent form of leukemia, poses significant treatment challenges and is frequently associated with specific chromosomal abnormalities. Symptoms predominantly include fever, bone pain and bleeding (1,2). Despite advancements in personalized therapy (3) improving diagnosis and treatment (4-6), central nervous system (CNS) involvement remains a significant contributor to severe complications and mortality in patients with AML, hindering effective long-term disease management (7-9). Although CNS infiltration and tumor formation are rare in adult patients with AML (10,11), they occur more commonly in pediatric patients (12). In adults, CNS

involvement incidence ranges from 0.6-3% (13), with a recurrence rate of ~2.9-4.1% (10,13,14).

However, the rate of CNS infiltration by AML cells in adults may be significantly underestimated due to detection challenges (7,10). Previous studies indicated that up to 32% of cerebrospinal fluid (CSF) samples and 46% of autopsy specimens from adult patients with AML reveal CNS involvement (15,16). Paul and Short (17) reported a CNS involvement rate of 3.2%, with 52% of patients with AML who underwent CSF screening testing positive for leukemia cells. In a recent study, flow cytometry detected AML cells in the CSF of 645 patients with AML, showing 41.7% positivity among those without neurological symptoms (18). This substantial disparity arises primarily because lumbar puncture (LP) assessments are typically performed only on patients with neurological symptoms (19). Although clinical symptoms and neuroimaging can indicate CNS involvement, their sensitivity and specificity vary, with LP remaining the diagnostic gold standard (20).

In patients with AML, 58% exhibit clinical symptoms such as headaches, vomiting, fatigue, cognitive changes, seizures and various cranial nerve palsies, which are often subtle and difficult to differentiate from drug side effects (21-23). Although CNS radiological imaging techniques, including magnetic resonance imaging and computer tomography, demonstrate high sensitivity, their specificity remains limited (20,24). Treatment for CNS infiltration involves systemic chemotherapy capable of reaching the CNS, augmented by intrathecal chemotherapy and radiotherapy (25-27).

Despite their potential efficacy, these approaches are associated with relatively high recurrence rates and adverse effects (28-30). Moreover, the unique CNS microenvironment serves as a reservoir for AML cells, influencing disease biology and chemotherapy resistance, which contributes to BM relapse and reduced survival rates (31,32). This emphasizes the necessity of deeper understanding of the specific pathways and mechanisms underlying CNS involvement in AML.

The infiltration of AML cells into the CNS involves two primarily phases: Escaping the BM and migrating to the CNS (1). AML cells must overcome two barriers-the marrow-blood barrier (MBB) and the brain barrier to reach the CNS. This process resembles leukocytes migrating from the BM into the vasculature and subsequently into the tissues, relying on interactions between migratory cells, vascular endothelial cells and stromal cells. However, the mechanisms regulating the entry of AML cells and leukocytes into the vasculature and CNS differ. In normal hematopoiesis, myeloid progenitor cells, which have not yet matured into myeloid cells, encounter obstacles crossing into the CNS, an immune-privileged organ. By contrast, AML cells, originating from a single myeloid progenitor cells with mutations, acquire malignant traits that facilitate invasion and migration (2). Additionally, changes in the vascular anatomy within the tumor microenvironment during AML development enable AML cells to escape from the BM (3,4).

The traversal of AML cells through the MBB and brain barrier is driven by chemotactic factors, governed by cell adhesion molecules (CAM), and facilitated by proteolytic enzymes (33,34). Previous literature was analyzed to elucidate the pathways and mechanisms by which AML cells evade the BM and migrate to the CNS. Additionally, similarities and differences in escape mechanisms of AML cells and leukocytes from the BM are discussed. The present comprehensive overview aimed to assist clinicians and researchers in developing novel diagnostic and therapeutic strategies. To identify relevant studies, PubMed was searched for original articles and reviews up to May 1, 2024, using the search terms 'AML', 'CNS', 'peripheral blood' and 'therapeutic strategies'. Studies were eligible if they met the following criteria: i) Provided detailed descriptions of the pathways and molecular mechanisms through which AML cells escape from the BM and infiltration into the CNS; ii) therapeutic approaches targeting molecules associated with AML cells' egress from BM and entry into the CNS; iii) were written in English and published in peer-reviewed journals. Studies were excluded if they solely identified an association of a molecule with increased leukocyte count in patients with AML or CNS infiltration without validation *in vivo* or *in vitro*. Additionally, conference articles lacking original research and studies for which full-text access was unavailable were excluded.

2. The escape from the BM

Anatomical structures involved in AML cells' escape. AML cells originate within the extravascular niche, necessitating their traversal through the MBB to access the bloodstream. The hematopoietic blood supply in the BM primarily derives from nutrient arteries, which return via nutrient veins situated in the central trabecular bone of long bones (Fig. 1A) (35). Within the BM, nutrient arteries bifurcate into arterioles, which further subdivide into capillaries and sinusoidal capillaries, also referred to as blood sinuses (Fig. 1B) (36). These blood sinuses constitute the MBB, a barrier delineating the hematopoietic compartment from the bloodstream (37). The MBB is composed of endothelial cells, a basement membrane and adventitial cells, sequentially arranged from the interior outward (Fig. 1C and D) (38).

Endothelial cells form a monolayer lining the inner wall of blood sinuses essential for cellular trafficking within the MBB. They connect via tight junctions and exhibit transient fenestrations that permit cell passage (38). The subendothelial basement membrane comprises a network of laminin and type IV collagen interacting with other extracellular matrix (ECM) molecules such as collagen and fibronectin to facilitate vascular wall development (39,40). Unlike typical basement membranes, the blood sinus basement membrane is discontinuous and lacks a reticular structure. Additionally, it contains an exceptionally high concentration of sulfated glycosaminoglycans, facilitating communication and adhesion between hematopoietic cells and vascular endothelial cells (41). Adventitial cells form the outermost layer of the blood sinus (42), partially encircling the outer wall with an adventitial sheath covering ~2/3 of its surface.

The adventitial cell coverage ratio (ACCR) represents the proportion of adventitial cells enveloping endothelial cells. A reduced ACCR signifies an increased propensity for AML cells to enter the bloodstream. Advanced morphometric analysis demonstrated a substantial decline in ACCR, from 53 to 14%, in a transplantable monomyelocytic leukemia murine model, with no alterations in the endothelial cell area or perimeter (43). Transmission electron microscopy revealed that ACCR dropped to 40% among 24 untreated patients with various forms of acute

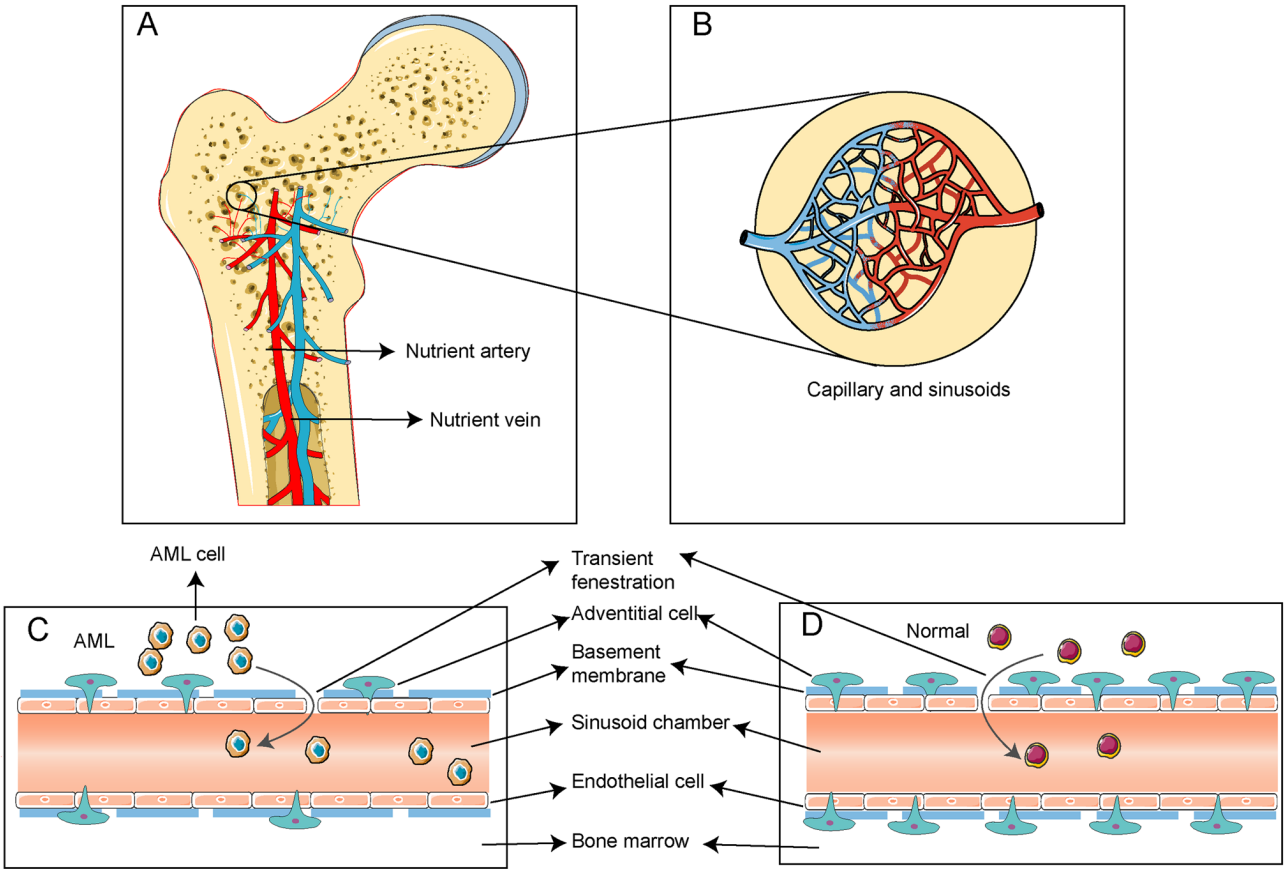

Figure 1. Anatomical structures of sinusoids. (A) Blood supply in the bone marrow. (B) Sinusoid's location. (C and D) The marrow-blood barriers of patients with AML and healthy individuals. The adventitial cell cover rate is reduced in patients with AML. AML, acute myeloid leukemia.

leukemia, compared with controls. Furthermore, no abnormal translocation of leukemia cells at intercellular junctions within blood sinuses was observed (44,45).

In the blood sinuses of patients with AML, leukemia cells coexist with normal hematopoietic cells. The marrow stroma also comprises stromal cells, soluble molecules and ECM components (46). Specific ECM proteins of leukemic cells can bind leukocyte-specific proteins, thereby limiting leukocyte migration from the BM (38).

Mechanisms involved in AML cells' escape. The traversal of the MBB by AML cells hinges on alterations surface chemokine receptor expression and adhesion molecules profiles. Endothelial cells critically mediate interaction between AML cells and the barrier. This section delves into intrinsic changes within AML cells and their interactions with the tumor microenvironment, particularly examining how these interactions facilitate BM escape.

Chemokines and their receptor, such as stromal cell-derived factor 1 (SDF-1), also known as C-X-C motif chemokine 12 (CXCL12) and chemokine receptor 4 (CXCR-4) (47), are fundamental for the AML cells' egress from the BM. A previous study by Möhle *et al* (48) demonstrated that CXCR-4 expression modulates AML cells' traversal of the MBB through the chemotactic response to SDF-1. Specifically, AML subtypes M4 and M5, marked by monocytic differentiation, show heightened CXCR4 expression, enabling SDF-1 to induce rapid intracellular calcium flux and significantly enhance extramedullary migration (49). Nonetheless, the mechanisms governing SDF-1 upregulation and its cellular sources remain to be elucidated.

Cluster of differentiation 31 (CD31), or platelet endothelial cell adhesion molecule 1, is a member of the immunoglobulin superfamily (50). Present in both endothelial and AML cells, CD31 significantly contributes to trans-endothelial migration via homophilic binding (51). Its expression is markedly elevated in the M4/M5 subgroups, which exhibit high metastatic potential (50). Cluster of differentiation 38 (CD38), a transmembrane glycoprotein, is also expressed on AML cells (52,53). The interactions of CD31 with endothelial cells and CD38 with hyaluronic acid in the ECM influence the equilibrium between AML cells release and retention. A CD31/CD38 ratio exceeding 1 facilitates homophilic interactions with the vasculature, enhancing trans-endothelial migration. Conversely, a ratio below 1 promotes retention of AML cells in the BM via interactions with hyaluronic acid (54).

Elevated levels of lymphocyte function-associated antigen-1 (LFA-1), an integrin superfamily member, can facilitate the migration of normal immature progenitor cells ($CD34^+$ precursor cells) into the bloodstream (55,56), likely by enhancing their trans-endothelial migration (57,58). LFA-1 acts as the primary receptor for intercellular CAM-1 (ICAM-1, CD54). During leukocytosis, AML cells induce endothelial cells to secrete ICAM-1, thereby enhancing adhesion via LFA-1 (59). Notably, LFA-1 expression is significantly elevated in patients with AML with extramedullary organ infiltration

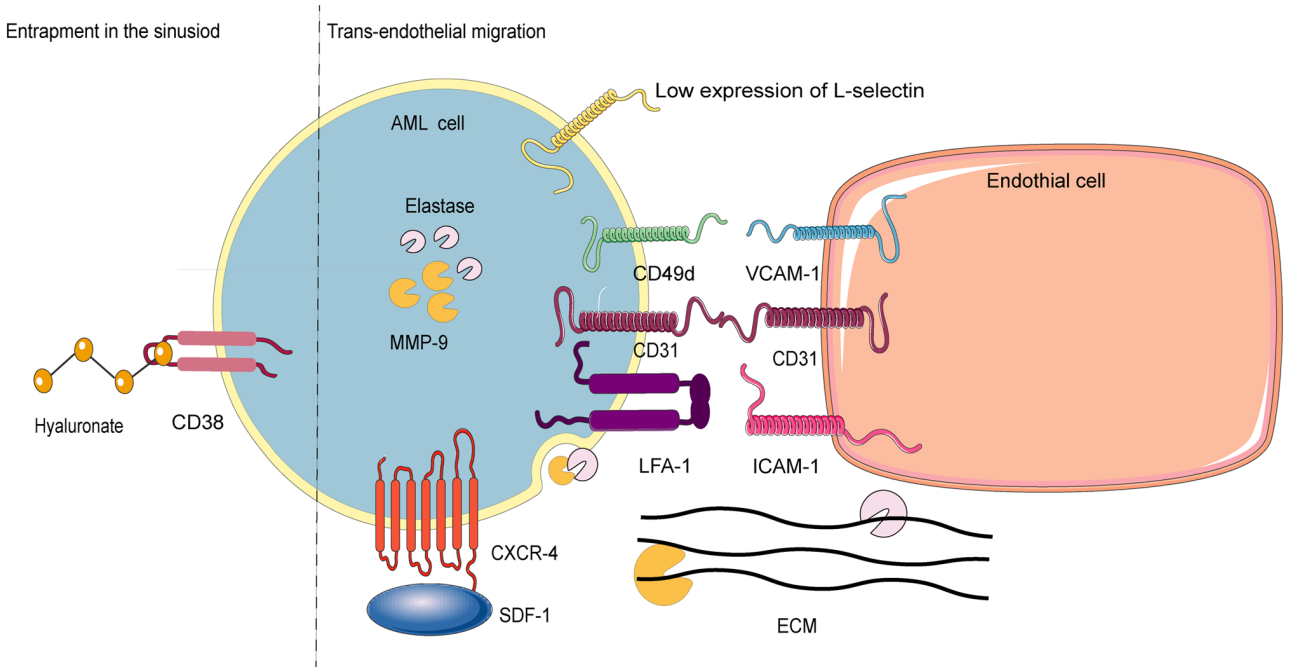

Figure 2. Escape mechanisms of AML cells. This figure illustrates two distinct processes: On the left side of the dotted lines-a mechanism underlying the capture of the AML cells within the sinusoid. On the right side of the dotted lines-a mechanism facilitating the transportation of AML cells into circulation. AML, acute myeloid leukemia; ECM, extracellular matrix; CD38, cluster of differentiation 38; CD49d, the rate-limiting α-chain of the CD49d/CD29 integrin heterodimer very late antigen-4; VCAM-1, vascular cell-adhesion molecule 1; MMP-9, matrix metalloproteinase-9; CD31, cluster of differentiation 31; LFA-1, lymphocyte function associated antigen-1; ICAM-1, intercellular cell adhesion molecule-1; SDF-1, stromal cell-derived factor 1; CXCR-4, chemokine receptor 4.

compared with those without, yet no substantial difference in LFA-1 expression is evident between AML cells in the BM microenvironment and those in the bloodstream. A previous study, however, suggested that LFA-1 may not be crucial for the release of AML cells into the bloodstream (60).

The heterodimeric integrin very late antigen 4, consisting of CD29 subunits, is another integrin family member. Vascular CAM-1 (VCAM-1), an immunoglobulin superfamily member, is primarily a membrane-bound transmembrane type I sialic acid glycoprotein with multiple Ig-like domains connected by disulfide bonds (61). Soluble VCAM-1, predominantly produced in response to pro-inflammatory cytokines in the vascular endothelium, facilitates the recruitment, adhesion and migration of monocytes, lymphocytes, eosinophils and basophils (62,63). Although AML cells can adhere to endothelial cells through the rate-limiting α-chain of the CD49d/CD29 integrin heterodimer very late antigen-4 (CD49d)/VCAM-1 adhesion mechanism, CD49d expression is not essential for AML cell release from the BM (60).

L-selectin (CD62L), a CAM in the selectin family, is highly expressed on $CD34^+$ precursor AML cells, contrasting with its low expression on normal $CD34^+$ cells. This expression contributes to aggregation of AML cells in peripheral blood (55,64).

Matrix metalloproteinases (MMPs), zinc (II)-dependent endopeptidases, are secreted by AML cells and the tumor microenvironment to remodel the ECM (65,66). By facilitating ECM degradation, MMPs enable the extensive release of AML cells into the bloodstream, resulting in widespread infiltration (67). The persistent expression of tumor necrosis factor-α in AML cells elevates MMP-9 expression, which is crucial for their early release into circulation (68). MMP-9 and/or MMP-2 mRNA were expressed in all samples from patients with AML and AML cell lines examined by Janowska-Wieczorek *et al* (69) but not in normal $CD34^+$ precursor cells. Interestingly, mature monocytes in the BM express and secrete MMP-9, suggesting its potential role in monocyte migration into the bloodstream (69). Consequently, AML and white blood cells may employ similar mechanisms for entering the bloodstream.

Elastase, a protease degrading elastin in the ECM, is excessively overexpressed on the cell surface of patients with AML and correlates with AML cell counts in the circulation. SDF-1 can upregulate the expression of cell surface elastase in AML cells. Inhibition of cell surface elastase diminishes migration and adhesion of AML cell lines *in vitro*, while administration of elastase inhibitors in AML mice significantly reduces circulating AML cell levels (70).

Chemokine (SDF-1), CAMs (CD31/CD38 ratio, CD62L), MMPs and elastase have been corroborated by *in vitro* and *in vivo* experiments for their roles in AML cells' egress from the bone marrow. However, the role of CAMs (LFA-1, CD49d) remains contentious and warrants further investigation. The release of AML cells into the bloodstream is influenced by their affinity for two blood sinuses components: Endothelial cells and the ECM. AML cells with higher endothelial affinity are more likely to enter the bloodstream, while those with a higher ECM affinity remain anchored within the blood sinus. Adhesion molecules (for example, CD31) and chemokines (for example, CXCL12) facilitate AML cells adhesion and migration, while elevated levels of proteases (such as MPP family) can degrade the ECM to enable cell movement (Fig. 2).

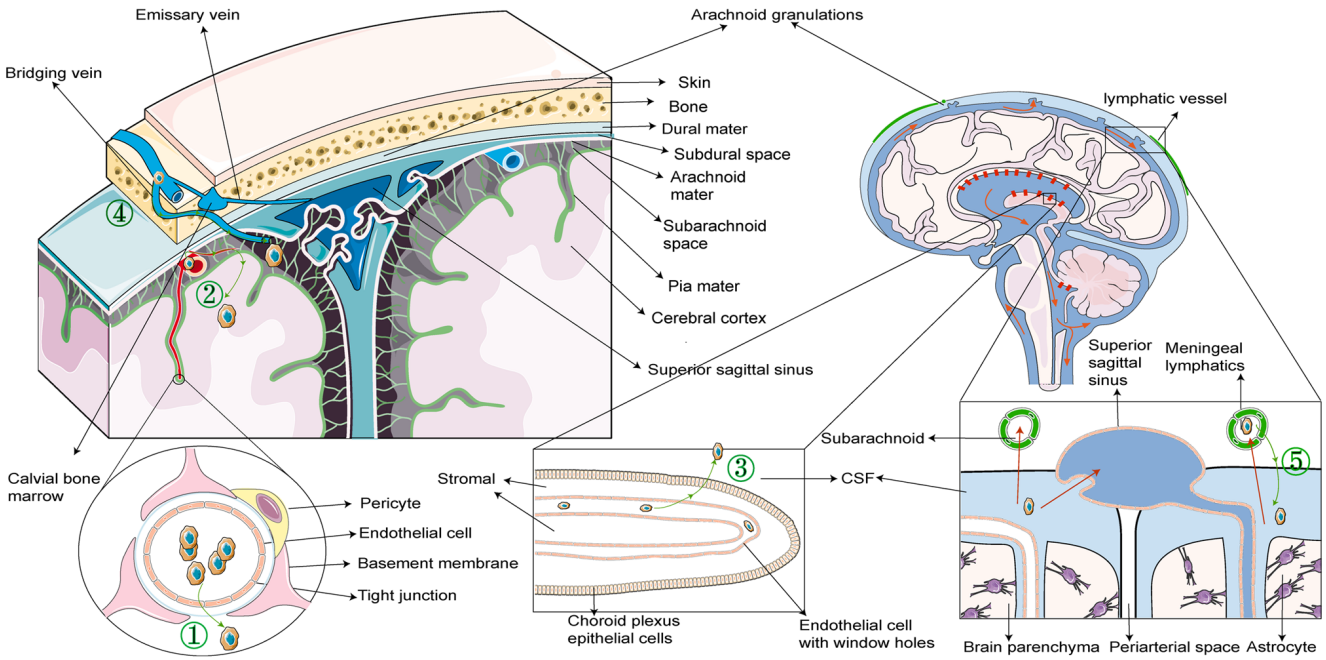

Figure 3. Routes and anatomical structures potentially involved in infiltration of the CNS. Green arrow: Routes of acute myeloid leukemia cell infiltration into the CNS. Orange arrow: Direction of the CSF influx. Route 1: Through the blood-brain barrier (1). Route 2: Through the blood-leptomeningeal barrier (2). Route 3: Through the blood-CSF barrier (3). Route 4: Through the bridging veins (4). Route 5: Through the dural lymphatic vessels (5). CNS, central nervous system; CSF, cerebrospinal fluid.

3. AML cell infiltration of the CNS

Potential routes and anatomical structures involved. Tumors metastasize primarily through four routes: Lymphatic metastasis, hematogenous metastasis, implantation metastasis and direct extension. Conventionally, AML cells are considered to enter the CNS predominantly through hematogenous metastasis, necessitating traversal of the brain barrier. However, a previous study suggested that AML cells may also infiltrate via the bridging veins (1). Although the CNS was once considered to possess immune privilege, recent insights into its lymphatic system challenge this view, potentially offering a more comprehensive understanding of how AML cells infiltrate into the CNS.

Hematogenous metastasis. The CNS receives its blood supply from the vertebral and internal carotid arteries. The interface between the vasculature and the structural components of the CNS forms the brain barrier, encompassing the blood-brain barrier (BBB), the blood-leptomeningeal barrier (BLMB) and the blood-CSF barrier (BCSFB) (71). This intricate system regulates the physiological entry of molecules and cells into the CNS, maintaining internal stability.

The BBB, located between the vasculature and the CNS, consists of three primary layers arranged from inner to outer: i) Capillary endothelial cells; ii) capillary basement membrane; and iii) astrocytes. The endothelial cells are tightly joined and lack fenestrations, limiting the passage of large molecules. However, in specific CNS regions, such as the circumventricular organs, pineal gland and neurohypophysis, the capillary endothelial cells have fenestrations and are connected by gap junctions, permitting the free movement of proteins and large molecules (Fig. 3, route 1). Histopathological evidence suggests that in advanced CNS involvement, AML cells may proliferate along perivascular spaces (or Virchow-Robin spaces) and infiltrate the brain parenchyma (72).

The BLMB comprises a layer of leptomeningeal cells encircling microvessels within the subarachnoid space (73). AML cells infiltrate the leptomeninges through trans-endothelial migration or direct vascular endothelium disruption (74). Once within, AML cells may infiltrate brain parenchyma by invading the perivascular spaces (74) (Fig. 3, route 2).

The BCSFB, situated in the choroid plexus of the brain ventricles, separates blood from the CSF. It is formed by choroid plexus epithelial cells interconnected by tight junctions and postcapillary venules of the meningeal microvasculature. However, choroid plexus capillary endothelial cells have fenestrations, providing some permeability. CSF is produced in the choroid plexuses of each brain ventricle, flowing from the lateral to the third ventricle, passes through the cerebral aqueduct of Sylvius into the fourth ventricle, and merging with the CSF generated by the third and fourth ventricles. It then enters the subarachnoid space through the median aperture (Magendie's foramen) of the fourth ventricle and is absorbed by the Pacchionian granulations (arachnoid granulations) into the dural venous sinuses, returning to the bloodstream. Leukemic cells can cross the BCSFB to enter the CSF (Fig. 3, Route 3).

Invasion through bridging veins, analogous to direct extension. The CNS is protected by four anatomical layers: Pia mater, arachnoid mater, dura mater and the cranial and vertebral bones, arranged from innermost to outermost. The BM within cranial and vertebral bones is hypothesized to serve as a crucial reservoir of marrow cells supporting the CNS. These reservoirs directly supply monocytes and neutrophils to the brain and spinal cord through dura mater-BM connections formed by vascular bridging veins (75), potentially serving

as routes for infiltration of AML cells into the CNS (Fig. 3, Route 4).

A study examining 31 cases of CNS involvement in acute leukemia reported frequent dural infiltration, whereas arachnoid infiltration without dural involvement was relatively rare, occurring in only 9% of cases. Anatomical evidence robustly supports the theory that AML cells infiltrate the CNS through veins connecting the dura mater and BM (76).

Lymphatic metastasis. The CNS was considered an immune-privileged site; however, previous murine studies have identified a lymphatic system within the dura mater that drains CSF from deep brain parenchyma, revealing new communication routes between the CNS and the circulatory system (77,78). This unique dural lymphatic vascular network extends along the dural sinuses, providing a unidirectional absorption and transport system (79). Additionally, the CNS contains the glymphatic system, which, unlike traditional lymphatic systems, comprises perivascular spaces between capillaries and astrocytes. CFS enters through perivascular spaces around arterial vessels, exits around venous vessels, and subsequently joins the dural lymphatic network near the dural sinuses (80).

Typically, CSF flow through the lymphatic system is unidirectional; however, retrograde flow can occur if the distal lymphatic system is obstructed, leading to the accumulation of cells, such as AML cells, in the lymphatic system and their subsequent entry into the CNS. This retrograde flow contributes to the increased risk of CNS infiltration in patients with high white blood cell counts, who are more susceptible to venous and lymphatic stasis, a common complication in leukocytosis (Fig. 3, Route 5).

Other routes. AML cells infiltrate the CNS through multiple routes. One involves traversing nerve roots through neural foramina into the epidural space. Another route is the infiltration of AML cells into the CNS during intracerebral hemorrhage. Additionally, CNS infiltration can occur iatrogenically when AML cells are introduced into the CFS during LP procedures (28).

Mechanisms of AML cells' infiltration. Current research on AML cells entry into the CNS focuses primarily on their traversal of the BBB. This discussion emphasizes this aspect. Interactions between AML and endothelial cells not only facilitate migration of AML cells but also induce endothelial cells necroptosis, thereby promoting AML cells' extravasation (81). Furthermore, astrocytes, as critical component of the BBB, significantly influence AML cell infiltration into the CNS.

Endothelial cells and astrocytes, components of the BBB, express ICAM-1 and VCAM-1, respectively, facilitating AML cell transmigration through interacting with LFA-1 and CD49d on AML cells (82,83). Inhibiting the VCAM1-CD49d pathway diminishes AML cells invasion into the brain and decelerates disease progression. Furthermore, the loss of interferon regulatory factor 7 promotes VCAM1-CD49d-mediated intracranial invasion by downregulating TG-interacting factor 1 (84). The expression of cluster of differentiation 56 on endothelial cells also plays a crucial role in AML cell passage through the BBB (74) and is associated with CNS relapse (85,86).

Immunoglobulin-like receptor B4 (LILRB4), part of the leukocyte Ig-like receptor B subfamily, is crucial for CNS infiltration by AML cells (87). Antibody blockade of LILRB4 can reduce CNS infiltration. The APOE protein (APOE-POPC) binds to LILRB4, suppressing immune responses in patients with AML and facilitating dissemination of AML cells (88). A study involving 56 patients with AML revealed that 91% of those with CNS infiltration exhibited high LILRB4 expression, compared with 38% without CNS involvement. Additionally, LILRB4 expression is elevated in M4 and M5 AML subtypes, which are more susceptible to CNS infiltration (89). The significant role of LILRB4 in CNS infiltration highlights its potential as a target for antibody-drug conjugates designed to eliminate AML cells and reduce CNS involvement (87).

Macrophage-secreted MMP-9 disrupts the vascular endothelial cells of the BBB, promoting secondary intracranial infections and brain lesions (90). Furthermore, MMP-mediated loss of endothelial integrity facilitates AML cells' extravasation into tissues (91,92). Given their ability to compromise the BBB and endothelial cells and their high expression in AML, MMPs likely play a significant role in CNS invasion.

Mutations in exon 18 of DNMT3A (D3Amut) enhance the monocyte invasiveness and migration of AML cells, leading to meningeal leukemia in mice (93). Additionally, D3Amut induces demethylation and upregulation of the *TWIST1* gene in AML cells, facilitating CNS infiltration (94). Overexpression of the human *TIMP-2* gene in mice results in increased tumor formation across various organs and severe CNS infiltration (95). These mutations are prevalent in the AML subtypes M4 or M5, which are more prone to CNS infiltration (96).

In conclusion, enhanced expression of adhesion molecules facilitates the interaction between AML cells and the BBB components. Additionally, enzyme and gene mutations significantly contribute to CNS infiltration in AML (Fig. 4).

4. Relevant molecules and genes for the treatment in AML

The absence of specific surface antigens on AML cells poses a significant challenge to developing targeted therapies and cell-based treatments for AML. While the aforementioned molecules and genes involved in AML cells' escape and CNS infiltration hold potential as therapeutic targets, their clinical application has been constrained by adverse effects. Recent advancements in drug synthesis and novel delivery systems have enabled more precise targeting of these drugs, mitigating side effects. Subsequently, the present discussion delved into the practical implications and current limitations of these therapeutic strategies.

The chemokine axis CXCR4/SDF-1 serves as a potential therapeutic target in AML, with the efficacy of related inhibitors validated in both *in vitro* and *in vivo* experiments. Some inhibitors have advanced to clinical trials. *In vitro* studies have shown promising results in AML treatment through reducing CXCR4 expression using lipid polymer/siRNA complexes (97). Currently, CXCR4 inhibitors such as AMD3100 are under investigation in Phase I/II clinical trials combined with chemotherapy for refractory/relapsed and newly diagnosed patients with AML (98,99). Although direct evidence is lacking, targeted CXCR4 therapy may potentially

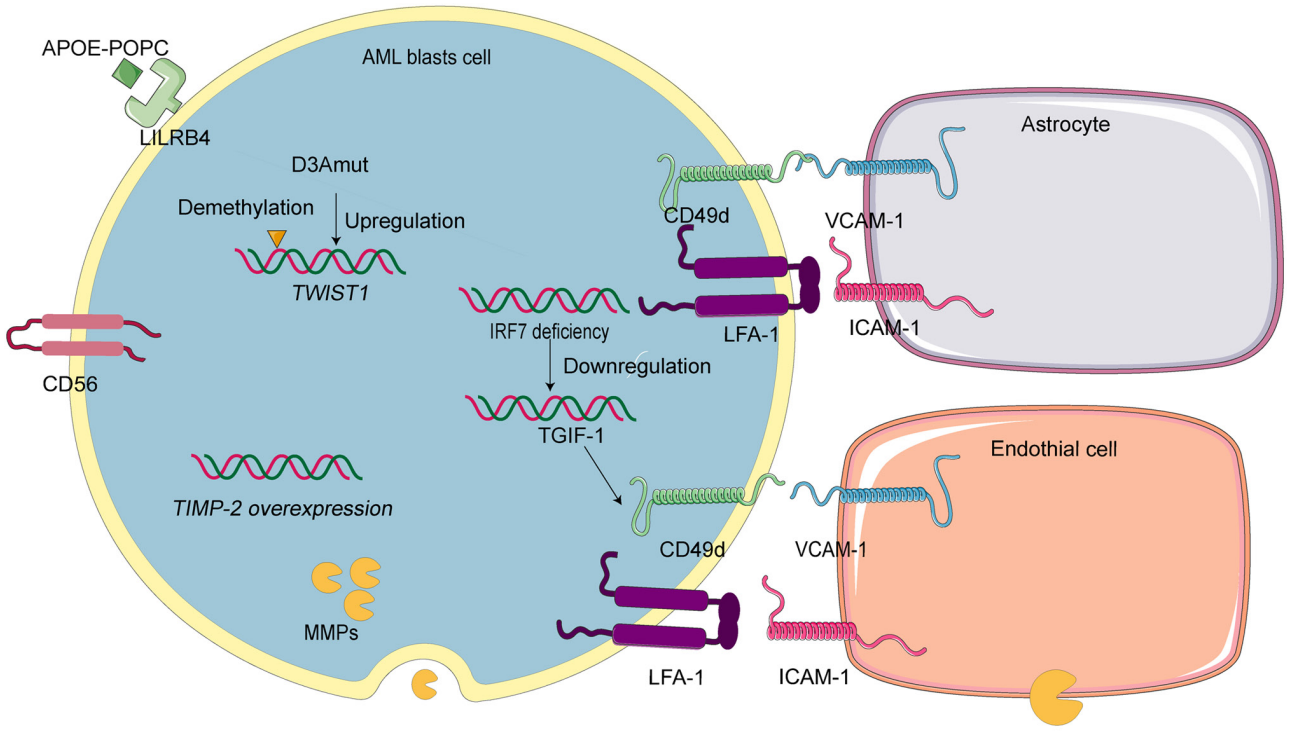

Figure 4. Mechanisms of the central nervous system exploited by AML cells. AML, acute myeloid leukemia; APOE-POPC, liposome-reconstituted APOE protein; LILRB4, immunoglobulin-like receptor B4; D3Amut, genetic mutation in exon 18 of DNMT3A; TWIST1, TWIST1 gene; TIMP-2, TIMP-2 gene; IRF7, interferon regulatory factor 7; TGIF-1, TG-interacting factor 1; CD56, cluster of differentiation 56. MMPs, matrix metalloproteinases; CD49d, rate-limiting α-chain of the CD49d/CD29 integrin heterodimer very late antigen-4; VCAM-1, vascular cell-adhesion molecule 1; LFA-1, lymphocyte function-associated antigen-1; ICAM-1, intercellular cell adhesion molecule-1.

reduce CNS involvement in AML. *In vivo* experiments have shown that the chemically synthesized CXCR4 antagonistic peptide E5, formulated as micelles (M-E5), significantly inhibits AML cells engraftment in the spleen, thereby reducing organ burden (100). Due to their widespread presence across various cells types, CAMs have been limited as targets for AML therapy. However, recent studies have opened new avenues for preclinical investigations. For instance, combining all-trans retinoic acid with daratumumab, an anti-CD38 antibody-conjugated polymer sulfate deoxycholic acid, effectively eliminated circulating leukemia cells and reduced organ invasion in AML models with low CD38 expression (101,102). Additionally, low and non-toxic doses of the microtubule destabilizer combretastatin-A4-phosphate (CA4P) downregulated CAM (VCAM-1), targeting circulating leukemia cells without inducing hematologic toxicity, providing an effective approach for treating refractory organ-infiltrative leukemia (103).

LILRB4, extensively expressed on monocyte AML cells, represents a promising tumor-associated antigen for immunotherapeutic targeting. A novel anti-LILRB4 CAR-T cell, exhibiting high affinity and specificity, has shown potent cytotoxicity against LILRB4-positive AML cells in both *in vitro* and *in vivo* models (104).

MMPs, as potential therapeutic targets for various diseases, have attracted substantial research interest. These enzymes are pivotal in extracellular matrix degradation and are regarded as primary drivers of cancer invasion and metastasis. Consequently, MMPs have become significant targets in anticancer drug development (105-108). Studies have explored the *in vitro* anti-AML activity of METVAN [bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxovanadium(IV); VO(SO(4))(Me(2)-Phen)(2)], which inhibits the expression and gelatinolytic activity of MMP-2 and MMP-9 proteins, thereby inducing apoptosis in AML cells (109).

DNMT3A, frequently mutated in AML, functions as a DNA cytosine methyltransferase and a key epigenetic driver of transcriptional silencing, commonly dysregulated in cancer. Hypomethylating agents, such as the cytidine analogs decitabine and azacitidine, have demonstrated clinical benefits in hematologic malignancies. However, their significant toxicity to normal blood cells restricts clinical dosing (110). A targeted delivery system using endogenous anti-CD33 antibody-fused protein conjugates to deliver DNMT3A-targeting siRNA into AML cells has been recently investigated, showing therapeutic efficacy both *in vitro* and *in vivo* (111).

Advancements in drug synthesis and delivery technologies have validated the therapeutic roles of relevant molecules and genes in AML through *in vitro* and *in vivo* experiments, and preclinical studies. While existing research primarily addresses extramedullary infiltration, specific data on inhibiting CNS involvement in AML remains scarce.

5. Conclusion and future considerations

The objective of the present review was to enhance the understanding of AML cells' invasion into the CNS, a significant concern due to its association with relapse and

mortality in patients with AML. Preventing CNS relapse is a key component of AML treatment, yet it remains a formidable issue. Elucidating the molecular mechanisms of CNS infiltration in AML can pave the way for innovative diagnostic and therapeutic strategies. AML cells migrate to the bloodstream through sinusoids under the influence of chemotactic factors, CAMs, MMPs and elastase. They subsequently cross the BBB into the CNS due to gene mutations, CAMs, MMPs and LILRB, or infiltrate the CNS via bridging veins and lymphatics. The present review highlights the role of CAMs and gene mutations in CNS infiltration, suggesting that immunotherapy and targeted therapy could offer effective therapeutic options. However, the precise pathways and mechanisms underlying AML cells' escape from the BM and CNS invasion remain partially understood. Current therapeutic approaches for CNS involvement in AML primarily rely on systemic chemotherapy, intrathecal chemotherapy and radiotherapy, which continue to exhibit high recurrence rates and side effects due to the lack of molecularly targeted agents and advanced cellular therapies. Further research is imperative to clarify the routes and regulatory mechanisms of AML cells' egress from the BM and CNS invasion. Additionally, detecting gene mutations in AML cells within the CSF could provide a novel and more effective diagnostic method. Future advances in drug synthesis and delivery systems hold promise for developing therapies targeting CAMs, MMPs, elastase and gene mutations, thereby offering new treatment options for patients with AML with CNS involvement.

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Availability of data and materials

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Authors' contributions

LC wrote the original draft. PZ wrote and reviewed the manuscript. HT performed investigation. GC conducted validation. JX performed visualization. XY supervised the study. XL conceptualized the study. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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