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New attempts for central nervous infiltration of pediatric acute lymphoblastic leukemia

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Abstract
The cure rate of acute lymphoblastic leukemia (ALL), the commonest childhood cancer, has been sharply improved and reached almost 90% ever since the central nervous system (CNS)-directed therapy proposed in the 1960s. However, relapse, particularly in the central nervous system (CNS), is still a common cause of treatment failure. Up to now, the classic CNS-directed treatment for CNS leukemia (CNSL) has been aslant from cranial radiation to high-dose system chemotherapy plus intrathecal (IT) chemotherapy for the serious side effects of cranial radiation. The neurotoxic effects of chemotherapy and IT chemotherapy have been reported in recent years as well. For better prevention and treatment of CNSL, plenty of studies have tried to improve the detection sensitivity for CNSL and prevent CNSL from happening by targeting cytokines and chemokines which could be key factors for the traveling of ALL cells into the CNS. Other studies also have aimed to completely kill ALL cells (including dormant cells) in the CNS by promoting the entering of chemotherapy drugs into the CNS or targeting the components of the CNS niche which could be in favor of the survival of ALL cells in CNS. The aim of this review is to discuss the imperfection of current diagnostic methods and treatments for CNSL, as well as new attempts which could be significant for better elimination of CNSL.

Keywords CNSL · Childhood ALL · Mechanisms · Treatments · Targeted therapy

1 Introduction

Acute lymphoblastic leukemia (ALL), the most common childhood cancer, is now a curable hematological malignancy as a result of the development of risk-stratified treatment based on cytogenetics, molecular, and immunophenotyping [1–3]. A recent study showed that the 5-year survival rate of children with ALL during 2010 to 2014 in 14 countries was elevated by 10% or more as compared with that in 1995 [4]. At the same time, about 3–8% of children with ALL still suffer from relapse in the central nervous system (CNS) [5] and children suffering from CNS leukemia (CNSL) are believed to have poorer outcomes in comparison to CNS-negative kids [6]. CNSL is caused by infiltration of leukemia cells into the CNS. The diagnosis of CNSL is dependent more on clinical manifestations, imaging manifestations, and primarily the cytological examination of cerebrospinal fluid (CSF) [7, 8]. However, the sensitivity of current diagnostic methods is considered to be inadequate in determining the risk-stratified CNS-directed treatment. Most CNS relapse cases occur in children with CNS-negative ALL without risk factors, and minimal residual disease (MRD) is not applicable to predict the risk of CNS relapse [7, 9]. Intensive CNS-directed treatments are applied to all children with ALL since detection sensitivity of CNS involvement is limited and there is still no explicit predictor for CNS relapse [7]. Plenty of studies have pointed out serious side effects (especially neurotoxicity) caused by cranial radiation and chemotherapy plus intrathecal (IT) chemotherapy [5]. The limitations of current diagnostic methods and CNS-directed therapy remind us that it is quite important to dig out the deep mechanisms by which leukemia cells enter the CNS, and pick out the key factors for CNS involvement in ALL to better eradicate CNSL with less toxicity. This review is meant to summarize the possible mechanisms by which leukemia cells enter and survive in the CNS, and figure out biomarkers which could be applied to adjust the risk-stratified treatments by improving the sensitivity of CNS

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diagnoses. Furthermore, this review also aims to characterize the optional targeted therapies available for preventing and curing children with CNSL.

2 The status of current diagnoses and treatments for CNSL

Due to the advanced risk-stratified treatment, the cure rate of childhood ALL is improved but accompanied by increased relapses in the CNS. It is quite difficult to eliminate ALL cells in the CNS since most chemotherapy drugs are prevented from reaching the brain from systemic circulation by the blood-brain barrier (BBB) [10]. CNS-directed treatment is proposed for better eradicating leukemia cells in the CNS, and the intensity of treatments is mostly based on cytological examination of CSF [11].

2.1 The limitations of current diagnostic measures for CNSL

Cytological examination of CSF, the most conventional examination for diagnosing CNSL, is limited in sensitivity compared to autopsy reports [11] and is obviously inadequate for risk stratification as the majority of CNS relapses occur in CNS-negative children [12, 13]. Therefore, more sensitive and noninvasive diagnostic methods or new biomarkers which could reflect treatment response and prognosis are urgently needed for CNSL [5]. So far, several new methods have been investigated for better CNSL diagnoses. For example, CSF proteomics [14–17], flow cytometry (FCM) and terminal deoxynucleotidyl transferase are believed to improve the detection sensitivity of CNSL [18–20]. ALL children with newly diagnosed CNSL, traumatic lumbar puncture, and hyperleukocytosis are considered to be at high risk for CNS relapses [13, 21]. Besides, ALL patients characterized by t(9;22)[*BCR-ABL1*] [22], *MLL* rearrangement, hypodiploidy (less than 45 chromosomes), or *TCF3-PBX1* fusion [23] are reported to be at increased risk for CNS relapses. However, not all of these factors are involved in the risk-stratified CNS-directed treatment. Whether these risk factors can be brought into diagnoses for CNSL and the necessity of improving detection sensitivity for diagnoses, treatments, and prognoses of CNSL remains to be verified.

2.2 The importance of CNS-directed treatments for ALL

In the absence of CNS-directed prophylactic treatment, infiltration of CNS during disease progression occurs in 30–70% of patients with ALL [24]. Due to the adoption of CNS-directed treatments, the 5-year event-free survival rate of ALL children has increased from 9% in 1967 to 36% in 1979 [25]. Childhood ALL is gradually becoming a curable cancer due to the continued improvement in treatments. Over the past 50 years, the mainstay of CNS-directed treatments has shifted from cranial radiation to intensive systemic chemotherapy combined with IT for avoiding the cognitive impairments caused by cranial radiation [26–28]. To eliminate leukemia cells in the CNS, several attempts have been taken, among which IT chemotherapy is considered as the most effective one by direct injection into CSF with maximized drug exposures in the CNS and lower systemic drug toxicities. In the USA, only limited drugs, such as methotrexate (MTX), cytarabine (Ara-C), liposomal-cytarabine, and thiotepa, are approved to be both safe and efficacious for IT chemotherapy [29, 30]. In order to reduce chemical arachnoiditis caused by IT and further increase the cytotoxicity towards ALL cells, corticosteroids are included in IT as well [31].

2.3 Further treatments for ALL patients with higher risk for CNSL

As only limited drugs can be involved in IT chemotherapy, CNSL remains a critical clinical problem with high risks for chemotherapy-resistant relapses [32]. Several targeted drugs have been tried for dealing with the CNS-relapsed ALL patients who are refractory to conventional therapy in recent years. Kondo et al. applied dasatinib for the treatment of Philadelphia (Ph) chromosome-positive leukemia patients (including ALL) with CNS involvement, and dasatinib worked despite its lower concentration in CSF than in blood [33]. The possible explanation for dasatinib to have worked at a lower concentration could be that the lower protein concentration in CSF contributed to more free drugs in CSF [33]. Ebadi et al. reported that the combination of oral ruxolitinib and chemotherapy successfully eradicated chemo-refractory CNSL in a 42-year-old woman [34]. Jaime-Perez et al. tested the efficacy of IT rituximab on seven pediatric patients with CNS-relapsed cluster of differentiation 20 positive (CD20+) B-ALL refractory to triple IT therapy. And Ceppi et al. tested the efficacy as well as the safety of IT rituximab in children with B cell lymphoid CD20+ malignancies [35, 36]. It is likely that oral administration of dasatinib/ruxolitinib and IT/intraventricular administration of ituximab are possible options for specific CNSL children.

The chimeric antigen receptor T cell (CAR-T) immunotherapy which binds CAR to the surface antigen of cancer is now a hot spot in target immunotherapy [37, 38]. CD19 CAR-T cells are reported to be remarkably efficient in extremely high-risk ALL patients, and the complete remission (CR) rate is about 67–90% [39]. In Nair's follow-up clinical study, an ALL patient who suffered from multiple CNS relapses finally achieved CR by receiving the CD19-specific CD28/interleukin 15 receptor alpha (IL-15Rα) co-signaling (153z) CART treatment [40]. The CR achieved in

Nair's study can favor the application of CAR-T cells for future cure of CNSL.

2.4 Side effects of standard treatments for CNSL

As the cure rate of pediatric ALL has noticeably improved and the 5-year survival rate has increased to around 90% [4], long-term health problems of childhood ALL survivals present a new clinical challenge. The early mainstay CNS-directed treatment, cranial radiation treatment (CRT), is successfully omitted in all patients in some recent clinical trials as CRT results in cognitive impairments, endocrinopathy, and development of secondary cancers [5]. In Krull's study, the cognitive impairments of CRT occurred in patients with younger age at first CRT and higher dose of CRT [41].

However, the omission of CRT fails to absolutely avoid development of cognitive impairments. Recently, several studies have reported the neurotoxicity of chemotherapy for ALL survivals, including acute and late adverse effects. Neurotoxic drugs, such as intravenous high-dose MTX (HD-MTX), IT MTX, and corticosteroids, are adopted in the contemporary chemotherapy protocols [42]. These drugs are associated with long-term health problems in memory, attention, processing speed, and executive function. In addition to the acute side effects which promote asymptomatic leukoencephalopathy, seizures and cerebrovascular injury can occur following a course of chemotherapy [42–45]. In addition, the glial dysregulation caused by MTX underlies the mechanism of chemotherapy-related cognitive impairment [45].

Though IT is considered to be indispensable for CNSL prevention and treatment, it is much likely to cause catastrophic consequences. Chemical arachnoiditis which is characterized by headache, vomiting, and even cauda equina syndrome, paraplegia, cranial nerve palsies, and seizures could possibly occur hours after IT [42, 46]. The incidence of IT chemotherapy-related neurotoxicity in children is around 3–11% [47]. Moreover, as an invasive treatment, IT injection is likely to bring leukemia cells from peripheral blood into the CNS, and is less accepted for the possible traumatic lumbar puncture (TLP) [48–50]. Besides, the CAR-T treatment is frequently complicated with fever, hypotension, vascular leak, and even neurotoxicity, such as the glial injury in nearly 40% of patients due to cytokine release syndrome (CRS) [51, 52]. And the BBB is supposed to be disrupted by systemic inflammatory signaling while CAR-T cell is expanding [53].

3 Mechanisms of CNS infiltration in childhood ALL

With awareness of the severe cognitive impairments involved in contemporary chemotherapy for childhood CNSL, clinicians are anxious to find more effective and less toxic alternatives for the prevention and treatment of CNSL. Meanwhile, CNS relapses still occur in 3–8% pediatric ALL patients [5, 54]. To reduce the CNS relapses with a more targeting ability and eliminate the neurotoxicity caused by classical CNS-directed treatments, it is vital for clinicians to find out the mechanisms by which CNS is involved in ALL.

3.1 The sites where ALL cells infiltrate CNS

Williams et al. proposed two possible models for the development of CNSL. One model assumed that only specific clones of leukemic cells were able to leave bone marrow (BM) and enter the CNS; the other one assumed that all ALL cells had the ability to go inside the CNS, but only specific subclones were able to survive [55]. With the help of clonal tracking, Williams et al. revealed the fact that CNS infiltration was the generic property of BCP-ALL without selection in an ALL mice model [55]. Jack et al. also declared that ALL cells in CNS and BM shared common characteristics and all clones were capable of entering the CNS in 12 newly diagnosed or relapsed CNSL patients [7]. Studies of Williams et al. reported that 79% (23/29) of diagnosed ALL samples contributed to CNS engraftment in xenograft mouse models, among which most of the samples (21/29) were collected from patients who were diagnosed to be CNS negative by lumbar puncture [55].

CNS is relatively a special site with a unique immune tolerance and immune surveillance system since it is isolated from the blood circulation by the blood-brain barrier and the blood-CSF barrier. Frishman-Levy et al. reported that natural killer cells (NK cells) activated by interleukin 15 (IL-15) could reduce the leukemia burden in blood rather than in CNS since NK cells failed to enter the brain [56]. Though ALL cells are apt to metastasize towards the CNS, the metastatic sites are quite different from those of solid tumors. ALL cells have an affinity to infiltrate the leptomeninges instead of the parenchyma [55]. Williams et al. believed that leukemia cells initially transited and localized at the blood-CSF barrier [55, 57]. And the histological findings of Williams et al. demonstrated that ALL cells are located close to the dural sinus, where newly discovered CNS lymphatics are situated [55]. In addition, the *postmortem* examination of brains from 126 ALL children found that the first identifiable ALL cells were localized within the basement membrane of superficial arachnoid veins [58]. Price et al. declared that the rare infiltration of brain parenchyma only occurred on the circumstances that arachnoid and CSF were severely infiltrated by ALL cells and the pia-glial membrane was destroyed [58]. ALL cells were reported to penetrate meningeal space only one week after engraftment, and the invasion of brain parenchyma was not observed until the late stages in a mouse ALL model [59, 60].

Though the dangers of CNSL have drawn much attention from clinicians, it is still hard to absolutely eliminate the occurrence or progression of CNS involvement since the

mechanisms are still being explored till now. All subclones of ALL cells in BM were capable of travelling into the CNS without selectivity as Williams et al. and Jack et al. reported both in mice models and in patients [7, 55]. This reminds us that leukemia cells in CNS are characterized by heterogeneity, same as in BM. Recently, intra-tumor heterogeneity (ITH) was approved to influence the immune surveillance by mediating cancer growth and rejection in a melanoma mice model [61, 62]. Whether ITH of ALL cells in CNS is associated with their survival in CNS needs further study. These two diametric models approved by Williams et al. and Jack et al. need to be additionally verified since these two models can influence the destination of future treatments to cure CNSL and prevent it from relapse.

3.2 The pathways through which ALL cells enter the CNS

To date, the exact pathways through which ALL cells infiltrate the CNS are still unknown. There are several possible pathways demonstrating how ALL cells pass through the CNS (as shows in Fig. 1). ALL cells are likely to infiltrate the CNS by hematogenous seeding, by migrating along the surface of emissary vessels, or by being directly brought into the CNS by cerebral hemorrhage/traumatic lumbar puncture. Among these mentioned pathways, hematogenous seeding is thought to play a significant role in CNS invasion. For example, ALL cells could intrude into CSF through choroidal vessels, invade the subarachnoid space through meningeal vessels, or infiltrate brain parenchyma through the perivascular space of the parenchyma [63]. The infiltration of the CNS by ALL cells consists of several steps. Firstly, leukemia cells migrate from BM towards the brain by traveling in the circulation and adhering to BBB. Once adhered, ALL cells release active cytokines, chemokines, or other biomolecules to disrupt the extracellular matrix (ECM) and the tight junction (TJ) so as to cross the BBB [64]. Through extravasation, cells leave the circulation and enter the extramedullary tissues.

Si et al. found that when *in vitro* co-cultured with leukemia cells or leukemia sera, human brain microvascular endothelial cells would secrete more vascular endothelial growth factor A (VEGF-A) and matrix metalloprotease 9 (MMP-9) [64]. The basal membrane of the BBB was reported to be destroyed by MMP-9 and VEGF-A which thus contributed to the entry of ALL cells into the CNS [64]. Si et al. also suggested that MMP-9, C-C motif chemokine ligand 2 (CCL2), and vascular cell adhesion molecule 1 (VCAM-1) in CSF could be used for predicting the CNS involvement of ALL patients in another clinical study [65]. VCAM-1 could promote cell adhesion and diapedesis of leukocytes [65]. Kinjyo et al. reported that leukemia-derived exosomes played critical roles in the ALL infiltration of the CNS by directly modulating the integrity of the endothelial cell-cell junction *in vivo* and promoting blast cells to travel across the BBB into brain parenchyma [32]. The co-culture of purified leukemia cell-derived exosomes and astrocytes *in vitro* resulted in the production of VEGF-A by astrocytes and then promoted the transmigration of leukemia cells [32].

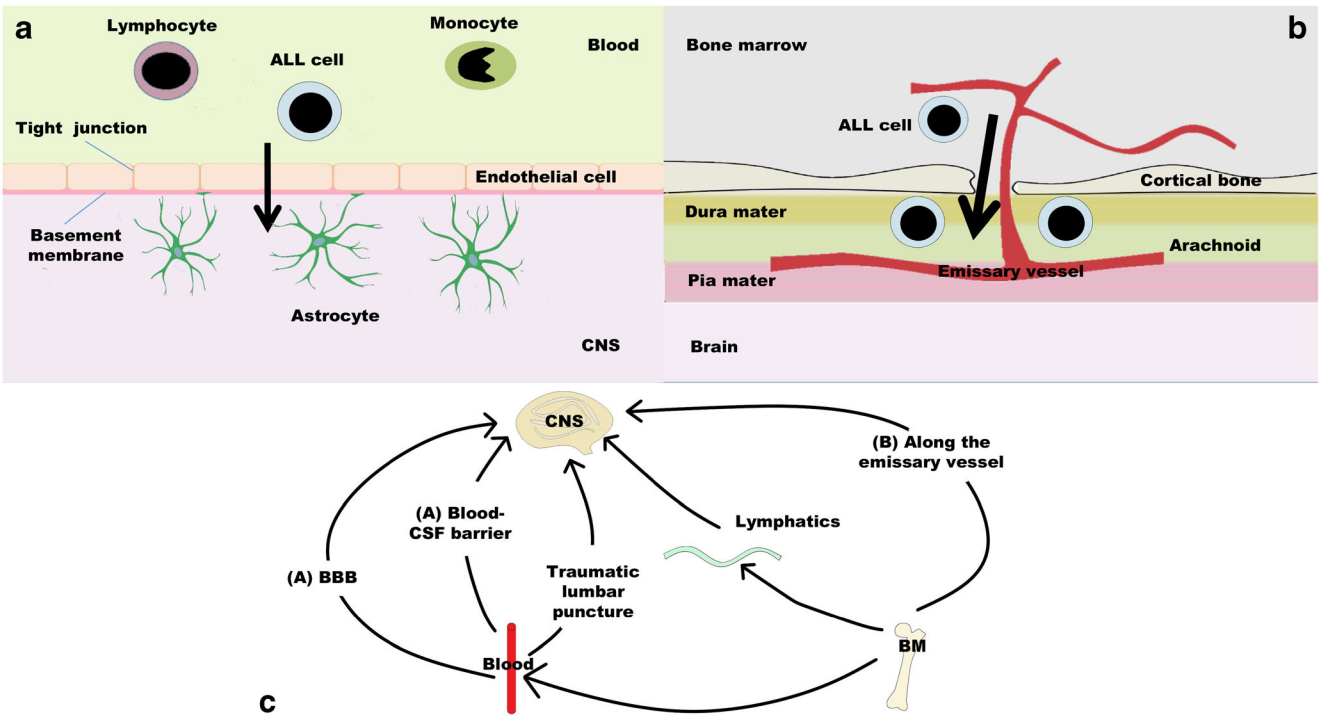

Fig. 1 **a**–**b** Possible pathways by which ALL cells enter the CNS

A recent study found that ALL cells in the circulation failed to cross the BBB, and instead, ALL cells invaded the CNS directly along the vessels that passed through the subarachnoid space and bone marrow of the vertebrae or calvarium [66]. By infiltrating along the external surface of vessels, ALL cells left the BM and traveled directly into the subarachnoid space without entering and exiting CNS vasculature. Furthermore, on the occasion that a lymphatic system was found to exist in the dura mater in mice, the CNS may no longer be an immune-privileged site [67]. The lymphatic system can drain macromolecules and immune cells from the brain; this phenomenon reminds us that there could be some other unrecognized pathways for the links between circulation and the CNS. And whether the lymphatic system pathway is involved in CNS immune surveillance or inflammatory processes remains to be identified.

3.3 The cytokines and chemokines that attract ALL cells into the CNS

Over the past few years, chemokine receptors such as C-X-C motif chemokine receptor 3 (CXCR3), CXCR4, C-C motif chemokine receptor 7 (CCR7), CCR6, and CXCR7 expressed on ALL cells have been found to have the role of mediating CNS infiltration [55, 68]. Gomez et al. reported that C-X-C motif chemokine ligand 10 (CXCL10) not only participated in the migration of ALL cells but also diminished chemotherapy-induced apoptosis on CXCR3-expressing ALL cells [69]. Alsadeq et al. found that the upregulation of chemokine receptors CCR7 and CXCR4 could promote the entry of ALL cells into the CNS towards CCL19 and CXCL12 gradients. Besides, the downregulation of a tyrosine kinase, zeta-chain-associated protein kinase 70 (ZAP70), could reduce the expression of CCR7/CXCR4 and impaired the infiltration of the CNS in NSG mice *via* the regulating extracellular signal regulated kinase (ERK) [68]. Irving et al. reported that ras-mutations activating mitogen-activated protein kinase (MAPK) pathways were also involved in the infiltration of the CNS in ALL [70]. Kothur et al. found that, though CXCL12 was one of the most highly concentrated chemokines in CSF [71], *in vitro* invasion of ALL cells towards human CSF was only partially inhibited by AMD3100, a blockade of the CXCL12 receptor CXCR4.

Munch et al. performed human genome-wide gene expression analysis for leukemia cells separated from CSF and BM, and a hypoxic adaptation and a higher VEGF expression were found in ALL cells from CSF than that from BM [72]. They also found that VEGF played an important role in trans-endothelial migration of ALL cells *in vitro* and participated in the CNS involvement in an ALL mice model [72]. The receptor of laminin, α6 integrin (ITGA-6), was reported to be expressed in most ALL cases, and the interactions between ITGA-6 and laminin mediated the migration of ALL cells towards CSF both *in vivo* and *in vitro* [66]. In recent years, plenty of other chemokines and cytokines have also been reported to promote CNS infiltration of leukemia cells to a certain degree. For example, IL-15 [8, 73], asparaginyl endopeptidase (AEP), intercellular adhesion molecule 1 (ICAM1) and ras-related C3 botulinum toxin substrate 2 (RAC2) [74], mitogen-activated protein kinase (MER) [3, 75], CCL19, vascular endothelial cadherin (VE-cadherin), platelet endothelial cell adhesion molecule 1 (PECAM-1) [76], secreted phosphoprotein 1 (SPP1) [77], pre-B cell leukemia transcription factor 1 (PBX1) [78], interleukin receptor 7 (IL-7R) [79], and myosin-IIA [80] were reported to be associated with CNS infiltration by affecting the invasion and adhesion properties of ALL cells (as shown in Table 1).

It is reasonable for us to believe that directional migration of ALL cells from BM to the CNS is caused by potent chemokines within CSF and factors that favor the infiltration of CNS by ALL cells. However, no specific causes among the mentioned chemokines and cytokines appear to play a permissive role in CNS invasion and survival for ALL cells, suggesting that these factors function additively in determining localization of leukemic blasts in the CNS compartment.

3.4 The interactions between ALL cells and CNS niche

In recent years, the importance of the microenvironment in the development of cancer has acquired more and more attention. Some cancer cells were reported to remodel the predisposed distant niches, which were in favor of cancer cell survival and proliferation [82]. As for leukemia, plenty of studies have explored interactions between leukemia cells and the BM niche. When it comes to the development of CNSL, the importance of the interactions between ALL cells and the CNS niche should not be ignored [57]. The interactions between ALL cells and the CNS niche can be discussed in three aspects: firstly, possible biomarkers on ALL cells or in CSF which could attract ALL cells into the CNS and promote survival of ALL cells; secondly, the direct cell-cell contact that promotes survival of ALL cells; and thirdly, other components in the CNS which favor survival of ALL cells.

Gaynes et al. found that the gene expression profile of ALL cells in CNS was quite different from that in BM, with 30 upregulated and 6 downregulated genes relative to that in BM in an ALL mice model. These differential expressed genes were reported to be involved in MAPK, RAS, and apoptosis pathways [57]. The differential expressed genes in the CNS imply that the CNS niche could influence the biology of leukemia. The upregulation of PBX1 was reported to partially protect leukemia cells from chemotherapy-induced apoptosis [57, 83]. Mark et al. declared that IL-15 promotes survival of ALL cells especially under low-serum conditions [73]. Known as stromal derived factor 1 alpha (SDF1α), CXCL 12 is one of the most important chemokines in the BM niche. Kothur et al.

Table 1 Biomarkers associated with CNSL

Biomarkers	Targeted drugs	Type of study		Main functions[a]			References
		Preclinical study	Clinical study	Survival	Migration	Undefined[b]	
AEP	/	*In vitro/in vivo*	No	/	/	Yes	[74]
CCL2	/	No	Yes	/	/	Yes	[65]
CCR7	/	*In vitro/in vivo*	Yes	/	Yes	–	[68]
CXCR4	/	*In vitro/in vivo*	Yes	/	Yes	–	[68]
	AMD3100	*In vivo*	No	/	/	Yes	[55]
CD99	/	*In vitro/in vivo*	No	Yes	/	–	[81]
ICAM1	/	*In vitro/in vivo*	No	/	/	Yes	[74]
IL-15	/	*In vivo*	No	/	Yes	–	[32]
	MEK/ERK, PI3K, and NF-kB inhibitors	*In vitro/in vivo*	No	Yes	Yes	–	[73]
	/	No	Yes	/	/	Yes	[8]
IL-7R	Anti-IL7R antibodies	*In vitro/in vivo*	Yes	/	/	Yes	[79]
ITGA6	PI3Kδ inhibitor/specific ITGA-6 neutralizing antibodies	*In vitro/in vivo*	No	/	Yes	–	[66]
Mer	UNC-569	*In vitro/in vivo*	Yes	Yes	/	–	[75]
MMP-9	/	*In vitro*	No	/	Yes	–	[64]
	/	No	Yes	/	/	Yes	[65]
Myosin-IIA	/	*In vivo*		/	Yes	–	[80]
PBX1	/	*In vitro/in vivo*	No	Yes	/	–	[78]
PECAM-1	/	*In vitro*	No	/	Yes	–	[76]
RAC2	/	*In vitro/in vivo*	No	/	/	Yes	[74]
SPP1	Agelastatin	No	Yes	/	/	Yes	[77]
SCD	Small-molecule SCD inhibitors	No	Yes	/	/	Yes	[77]
VCAM-1	/	No	Yes	/	/	Yes	[65]
	/	*In vitro/in vivo*	No	Yes	/	–	[81]
VE-cadherin	/	*In vitro*	No	/	Yes	–	[76]
VEGF-A	/	*In vitro*	No	/	Yes	–	[64]
	Bevacizumab	*In vitro/in vivo*	No	/	Yes	–	[72]
ZAP-70	MEK inhibitors	*In vitro/in vivo*	Yes	/	Yes	–	[68]

/ related targeted drugs or functions were not explored in this study

[a] The *main functions* of these biomarkers include promoting ALL cell survival in the CNS and promoting the migration of ALL cells towards the CNS

[b] *Undefined* means that some studies just report the association between biomarkers and CNSL without exploring the mechanisms

found that CXCL12 was one of the most highly concentrated chemokines in CSF [71]. CXCL12 favored hematopoietic stem cells (HSC) homing to BM and participated in the survival of HSC by binding to its receptor, CXCR4 [84]. Alsadeq et al. found that the upregulation of CXCR4 could promote the entry of ALL cells into the CNS towards CXCL12 gradients [68]. McCandless et al. showed that leukocytes were mostly located at the perivascular space due to the expression of CXCL12 on the basolateral side [85]. The overexpression of Mer was considered to promote survival of ALL cells when co-cultured with human glioma cells or normal rat astrocytes by maintaining quiescence and protecting from MTX-toxicity [75]. CD99 and VCAM-1 could participate in the adhering of ALL cells to meningeal cells. And CD99 could regulate other adhesion molecules/pathways as well [81].

Jonart et al. and Akers et al. declared that it was the direct cell-cell contact between ALL cells and CNS-derived cells rather than the soluble factors which resulted in enhanced chemoresistance by affecting apoptosis balance and quiescence [81, 83]. The co-culture of ALL cells (accompanied with higher Mer) and CNS-derived cells converted ALL cells to a dormant/quiescent state and promoted chemoresistance of ALL cells [75]. The similar circumstance was observed in BM [86]. Jonart found that chemoresistance induced by adhering to meninges could be disrupted by separating leukemia cells from meningeal cells [81]. Dormancy/quiescence was believed to contribute to chemoresistance and stem cell property, and result in relapse [87, 88]. The large extracellular vesicles (EVs), which are released by budding from plasma membrane, are reported to be important for communications

between ALL cells and the surrounding microenvironment [89, 90]. Johnson et al. showed that ALL-derived EVs of varying sizes could result in phenotypic changes of recipient cells by transferring to other ALL cells, as well as BM stromal cells [90]. Membrane-bound carriers released by cancer cells with varying diameters, named EVs or exosomes, can deliver various cancer cell contents, such as proteins, mRNAs, and microRNAs. Evidences have declared that these carriers can participate in cell-cell communication and remodel predisposed distant niche [91, 92]. Studies have reported that tunneling nanotubes could promote leukemic cell survival and chemoresistance by mediating the exchange of cell content between leukemia cells and surrounding cells as well as to other leukemia cells [93]. In addition, gap junctions have also been reported to participate in the cell-cell interaction of ALL cells and niche [94]. Recently, Ombrato et al. proposed that surrounding cells in tumor microenvironment could influence the behavior of cancer cells on the circumstances that cancer-associated parenchymal cells (CAPs) exhibit stem-cell-like features, multi-lineage differentiation potential, and self-renewal activity in a breast cancer lung metastasis mice model [95]. Whether surrounding cells of CNSL cells exhibit similar features remains to be explored.

A hypoxia gene set profile was reported in leukemia cells retrieved from the CNS of xenografted mice, and VEGF-A, as the principal target of the hypoxia master transcription factor-hypoxia inducible factor 1 (HIF1), expressed at a particularly high level in ALL cells retrieved from the CNS [72]. Moreover, Munch et al. found that VEGF played an important role in trans-endothelial migration for ALL cells *in vitro* and participated in CNS involvement in an ALL mice model [72]. In addition, hypoxia could induce the expression of CXCL12 and CXCR4 [96]. It was reported that hypoxia participated in the promotion of radio- and chemoresistance, as well as metastasis for cancer cells [97]. A hypoxic microenvironment was discovered to cause mitochondrial fragmentation in NK cells. Zheng et al. believed that a hypoxic tumor niche could reduce the survival and antitumor capacity of NK cells in the tumor niche by disrupting mitochondrial metabolism [98]. As for ALL cells, hypoxia has also been reported to promote chemoresistance, confer stem-cell properties (such as maintain quiescence) [99, 100], and take part in CNS infiltration [72, 101]. Whether the dysfunction of NK cells caused by a hypoxic CNS niche could promote immune surveillance of ALL cells in the CNS needs more studies.

4 New attempts for curing CNSL

As the curing rate of pediatric ALL continues to increase due to the involvement of CNS-directed treatments and risk-stratified treatments over the past 50 years, treatments of CNS infiltration remain a challenge to clinicians. Owing to the limited drugs applied in IT chemotherapy, seeking optional treatments for CNSL children who are chemotherapy-resistant and refractory is rather important [32]. As plenty of factors are identified to be meaningful for ALL cells invading and surviving in CNS, future treatments for CNSL can be proposed from three aspects (as shown in Fig. 2): firstly, to prevent the progression of CNSL by targeting the vital pathways that ALL cells pass through into the CNS; secondly, to adopt better drug-delivering systems to kill more ALL cells in the CNS with less neurotoxicity and reduce the usage of traumatic IT chemotherapy; and thirdly, to target the CNS niche to limit ALL cell survival in the CNS.

4.1 Blocking the pathways by which ALL cells enter the CNS

Despite the fact that the entrance into the CNS was identified as a universal property for ALL cells in Williams' study [55], no single factor was found to be the determinant of CNS involvement. Plenty of biomolecules have been reported to be associated with CNSL development, and inhibition of some biomolecules has been believed to reduce CNS involvement. It could be a promising measure for clinicians to deal with refractory or chemoresistant CNS relapse in ALL children by targeting those specific biomolecules.

Gomez et al. reported that the inhibition of CXCR3 with AMG487 resulted in reduced leukemia burden simultaneously in the BM, spleen, and CNS in an ALL mice model [69]. Selumetinib (AZD6244, ARRY-142886) was applied to reduce the RAS-mutated CNSL in preclinical xenograft models by inhibiting the mitogen-activated protein kinase 1/2 (MEK 1/2) [70]. Antibody of CCR7 was reported to reduce the migration of ALL cells towards CCL19, while MEK inhibitor was reported to reduce the migration towards CCL19 and CXCL12 by inhibiting ZAP70 [68], which suggested that CCR7 and ZAP70 could be the future therapeutic targets. Besides, CXCR4 inhibitors, such as plerixafor, could be used for reducing the migration of ALL cells towards CXCL12 in the CNS [68, 102, 103]. Munch et al. found that trans-endothelial migration of ALL cells was regulated by VEGF, and capture of VEGF by bevacizumab significantly reduced leukemia burden in the CNS but not in the BM or spleen of an ALL mice model [72].

ALL cells were reported to directly enter the CNS along the emissary vessels that pass through subarachnoid space and bone marrow of the vertebra or cranium. And this pathway was considered to be blocked by PI3Kδ inhibitors, GS-649443, or idelalisib *in vitro* as well as in a mouse xenograft model [66]. Yao et al. found that GS-649443 could inhibit the progression of ALL in the CNS without affecting leukemia burden in the circulation or BM [66]. It is likely that PI3Kδ inhibitors could be applied to prevent CNS involvement in ALL children. The ECM molecule (laminin) was known to

be specifically bound by ITGA-6 [104]. Yao et al. tested the effects of specific ITGA-6-neutralizing antibodies which were found to stop ALL cells from invading the CNS in a mice model [66].

4.2 Targeting ALL cells in the CNS by efficient delivery of drugs into the CNS

Over the past few years, great progresses have been made in treatments for childhood ALL. Targeted drugs have been involved in the treatments for specific ALL [105, 106]. For example, tyrosine kinase inhibitors (TKI), such as dasatinib and imatinib, have already been used in Ph chromosome-positive ALL patients [105–108]. However, the targeted treatments of CNS involvement in ALL are still an unmet medical need due to the poor delivery of effective drugs into the CNS [33]. The limited BBB permeability stopped most of the targeted drugs from entering the CNS, making it quite difficult to cure CNSL. Nearly 100% of large-molecule drugs and more than 98% of small-molecule drugs are blocked outside the CNS [109]. A high drug dose has been adopted to achieve the adequate drug concentration in the CNS, which resulted in increasing toxicity risks [110, 111]. Thus, a novel drug delivery system by which chemotherapy drugs and targeted drugs are able to pass through the BBB and form an effective CSF-drug concentration is necessary. Efficient drug delivery systems to target cells and tissues are critical for improving the efficacy of cancer therapy [112].

Ultrasound exposure combined with microbubble was reported to target and open BBB reversibly and noninvasively. Besides, this combination could deliver drugs and antibodies into the CNS selectively [111, 113]. Tan et al. confirmed that ultrasound exposure combined with microbubble could carry drugs, antibodies, and genes to local brain tissue by opening BBB reversibly and noninvasively [113]. Kinjyo et al. reported the role of Nalm-6-derived exosomes in CNS involvement. By intravenous injection into the mouse, exosomes were found to be located along the wall of blood vessels in the brain [32]. As a type of EV, cellular microparticles (MPs) act as intercellular messengers. MPs could regulate many disease processes (such as cancers) by carrying different bioactive molecules from donor cells to target recipient cells [114, 115]. Guo et al. assessed the feasibility, safety, and efficiency of MPs in a group of lung adenocarcinoma patients [116]. As compared with normal tissue cells or antitumor cells, tumor cells have a tendency to uptake more MPs. Once going inside the tumor cells, MPs could not only deliver drugs into the nucleus but also stop the drug efflux by interfering with the ATP-binding cassette transporter system, and then contribute to the death of tumor-repopulating cells [116]. Moral et al. proved that tumor-derived EVs could successfully breach the integrated BBB by transcytosis [117]. The targeted nanoscale immunoconjugates (NICs) of a-CTLA-4 or a-PD-1 were successfully delivered across the BBB as a potential treatment for brain glioma in a mice model as well [118].

Since recent CNS-directed treatments are troubled with chemotherapy-resistant and refractory CNSL, and a large number of targeted drugs are blocked out of the CNS by BBB, it is of great importance for seeking adequate delivery of "active, free drug" to the target ALL cells in the CNS. ALL-derived exosomes were reported to participate in CNS involvement of ALL by predisposed in CNS [32], and the tumor-derived EVs with a larger diameter could be used for delivering drugs to cancer cells and act as chemo-

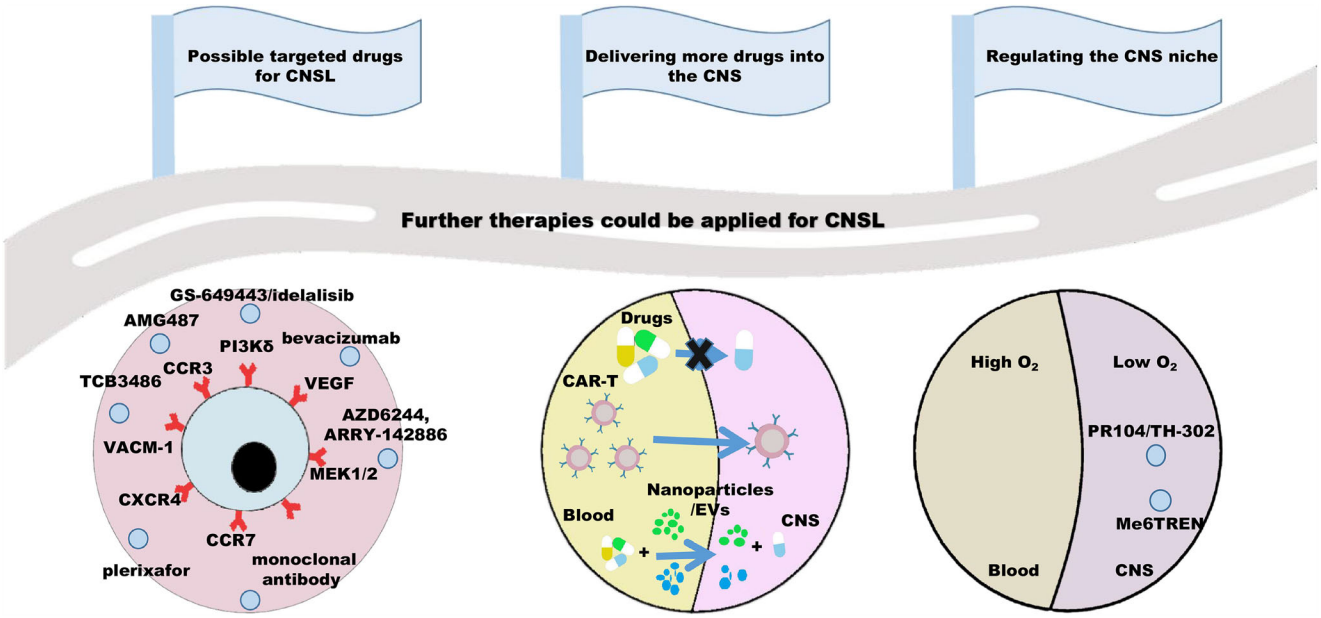

Fig. 2 Possible methods for preventing/treating CNSL

immunotherapeutic agents [116]. Whether ALL-derived EVs could be an optimal delivery system for CNS-directed therapy in ALL children needs further exploration. Besides, measures should be taken to avoid the possible anaphylaxis-like side effects which could be caused by systemic administration of EVs. Galstyan et al. eliminated the anaphylaxis-like side effects caused after repeated administrations by introducing antihistamine, triprolidine, and platelet activating factor (PAF) antagonist-CV6209 into the prevention of anaphylaxis [118].

4.3 Targeting the CNS niche to reduce leukemia burden

The associations between ALL cells and CNS niche gradually come into the researcher's view. Descot et al. reported that many cancer cells could remodel the predisposed niche [82] and *in vitro* co-culture of purified leukemia-derived exosomes and astrocytes resulted in the production of VEGF-A by astrocytes as Kinjyo et al. reported [32]. Besides, Akers et al. believed that the direct cell-cell contact between ALL cells and choroid plexus cells was the determinant of upregulated PBX1 in leukemia cells [57, 83]. In order to remodel the CNS niche to be not suitable for the survival of ALL cells, new attempts can be made as follows. Measures could be taken to target at biomarkers which are upregulated or downregulated in CNSL or factors which could favor the survival of ALL cells in CNS as well as attract ALL cells into the CNS. For example, inhibiting the expression of Mer, PBX1, IL-15, and VEGF in ALL cells [57, 75, 83]. As the CXCL12/CXCR4 axis disrupted by CXCR4 inhibitors (such as plerixafor), traveling of ALL cells into CNS might be reduced [68, 102, 103]. Targeting at ITGA-4 (the ligand of VCAM-1) by using inhibitors such as TCB3486 might be effective for reducing CNSL [103, 119]. A novel hematopoietic stem cell-mobilizing compound, Me6TREN (Tris [2-(dimethylamino) ethyl] amine), was reported to reverse the chemoresistance of ALL cells in CNS by disrupting leukemia-meningeal adhesion in a mice model [81]. Besides, regulating the CNS niche by the hypoxia-activated prodrugs, for example PR104 and TH-302, could possibly influence the survival of ALL cells [120, 121].

5 Conclusions

Despite the fact that pediatric ALL is now a curable disease, current risk-stratified treatment seems to be inadequate for CNSL treatment on the circumstances that the detection sensitivity and current CNS-directed treatments are limited. It is of great significance to adjust the risk-stratified treatment on the basis of a novel detection method with higher sensitivity. Novel developing detection methods, such as CSF proteomics, FCM, and terminal deoxynucleotidyl transferase, could be applied for improving the detection sensitivity of CNSL. Immunoglobin gene rearrangement analysis by the next generation test can also be applied to monitor MRD in CNSL. Besides, a great number of biomarkers (cytokines and chemokines) could participate in CNS involvement and some of these biomarkers, such as IL-15, have been tested for the diagnosis of CNSL in childhood ALL as well as predicting CNS relapse. Among these methods, flow cytometry of the CSF is thought to be the most affordable and applicable at present, and probably will be included in the next protocol for childhood ALL. Cheung et al. believed that neurotoxicity was associated with IT chemotherapy, and pediatric ALL survivors with high risk for cognitive impairment could be identified by monitoring CSF markers such as t-tau, phosphorylated tau (p-tau), and nerve growth factor (NGF) [122]. Whether monitoring these CSF markers could direct the adjustment of CNS-directed treatments needs further exploration. Nevertheless, a more effective and less toxic measure to eliminate the risk of CNSL is under exploration. As no single biomarker has been proved as the key factor for CNS involvement in childhood ALL, it seems like the invasion of CNS is multifactorial. More evidences are needed to identify whether it is the universal property of leukemia cells entering the CNS.

Leukemia cells are believed to infiltrate the CNS by crossing the BBB and blood-CSF barrier, but mechanisms of how they cross the BBB are still unclear. Recently, Strillic et al. highlighted the role of necroptosis in tumor metastasis, and whether leukemia cells could migrate into the CNS through necroptosis of BBB deserves further investigation [123]. A novel pathway is also proposed that leukemia cells travel into the CNS along the surface of emissary vessels. Besides, the lymphatic system that exists in the dura mater could possibly be the way for ALL cells traveling into the CNS. As for the cure of CNSL, measures could be taken in three aspects. Firstly, stop ALL cells from entering into the CNS by targeting molecules that are involved in the progression of CNSL. Lin et al. tested the efficacy of several cancer drugs and drug targets that were adopted in clinical testing, and proposed that some cancer drugs kill cells by off-target effects [124]. So, the seeking for CNS-directed targeted drugs should be taken seriously and carefully. This way could be partially effective since more than half of the newly diagnosed ALL patients suffer from CNS infiltration. Secondly, more chemotherapy drugs or targeted drugs should be delivered into the CNS to kill more leukemia cells with reduced complications. Thirdly, the CNS microenvironment should be targeted to reduce the leukemia burden in CNS. In all, with the deeper understanding of the mechanism of CNSL, we believe more and more ALL children suffering from CNSL will benefit from the development of novel targeted therapies and better drug delivery systems in the near future.

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Compliance with ethical standards

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

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