Delivery of effective anti-leukemic agents to the central nervous system (CNS) is considered essential for cure of childhood acute lymphoblastic leukemia. Current CNS-directed therapy comprises systemic therapy with good CNS-penetration accompanied by repeated intrathecal treatments up to 26 times over 2–3 years. This approach prevents most CNS relapses, but is associated with significant short and long term neurotoxicity. Despite this burdensome therapy, there have been no new drugs licensed for CNS-leukemia since the 1960s, when very limited anti-leukemic agents were available and there was no mechanistic understanding of leukemia survival in the CNS. Another major barrier to improved treatment is that we cannot accurately identify children at risk of CNS relapse, or monitor response to treatment, due to a lack of sensitive biomarkers. A paradigm shift in treating the CNS is needed. The challenges are clear—we cannot measure CNS leukemic load, trials have been unable to establish the most effective CNS treatment regimens, and non-toxic approaches for relapsed, refractory, or intolerant patients are lacking. In this review we discuss these challenges and highlight research advances aiming to provide solutions. Unlocking the potential of risk-adapted non-toxic CNS-directed therapy requires: (1) discovery of robust diagnostic, prognostic and response biomarkers for CNS-leukemia, (2) identification of novel therapeutic targets combined with associated investment in drug development and early-phase trials and (3) engineering of immunotherapies to overcome the unique challenges of the CNS microenvironment. Fortunately, research into CNS-ALL is now making progress in addressing these unmet needs: biomarkers, such as CSF-flow cytometry, are now being tested in prospective trials, novel drugs are being tested in Phase I/II trials, and immunotherapies are increasingly available to patients with CNS relapses. The future is hopeful for improved management of the CNS over the next decade.

INTRODUCTION

Childhood acute lymphoblastic leukemia (ALL) is a curable disease with more than 90% of children achieving long term survival [1, 2]. This has led to a shift in focus from intensifying treatment to achieving cure with fewer side-effects. Progress is being made by use of minimal residual disease (MRD) monitoring to adjust treatment intensity according to clinical response, and more recently the introduction of targeted immunotherapies. However, major challenges remain. One particularly problematic area is how best to prevent and/or treat leukemic relapse involving the central nervous system (CNS). Detection of leukemic blasts in the CNS by cytology is commoner in patients with higher white blood cell count at diagnosis, T-lineage ALL, and high risk cytogenetics, but early studies in the 1960s and 1970s established that giving CNS-directed therapy to all patients, including those with negative CSF-cytology, is essential for cure [3, 4]. Initial protocols used craniospinal irradiation as CNS-directed treatment. Unfortunately, this caused high rates of neurocognitive impairment [5, 6] and secondary CNS malignancies [7], leading to a shift towards chemotherapy approaches [8]. However, significant toxicity can still occur. The balance between adequate treatment to prevent relapse, whilst minimizing chemotherapy exposure to reduce adverse effects, is especially important when it comes to a child’s developing brain [9]. Here we review the current clinical challenges in CNS-ALL and discuss possible solutions.

CHALLENGE 1 – INABILITY TO ACCURATELY MEASURE CNS INVOLVEMENT

CNS staging is usually performed by counting white blood cells in the cerebrospinal fluid (CSF) along with microscopy of a cytocentrifuged CSF sample to morphologically identify leukemic blasts. This is used to assign patients to CNS1, CNS2 or CNS3 status (Table 1). CNS3 status is also given to patients with clinical or...
different trial groups have different approaches to using CNS-ALL. Several observations challenge whether cytology accurately reflects the amount of leukemic infiltration in the CNS and the likelihood of CNS relapse. Firstly, rates of CNS2 status vary widely between individual centers, and between trial groups, as does their prognostic relevance (Table 2) – suggesting that this is an analytical, rather than a clinical, difference. Indeed, cytospin-based cytology has been shown to have low sensitivity, poor specificity, and low reproducibility between laboratories [10,11]. Leukocytes in CSF rapidly decay ex vivo [12–14], and if cytospin samples are not stabilized or processed immediately, this may lead to understimation of CNS leukemia. Furthermore, microscopy-based analysis has a low sensitivity for detection of rare events [15], and discrimination of leukemic cells and normal/reactive T-lymphocytes in cytospins may be difficult and can lead to false-positives [10]. Further doubt on the sensitivity of CNS cytology comes from clinical observations – only 2–5% of children are classified as CNS3 at initial diagnosis, but up to 75% used to relapse within a few weeks to months prior to institution of universal CNS-directed therapy [3] suggesting that leukemia is present in the CNS compartment from disease outset in most patients. This is also supported by animal models [16] and by evidence that patients often have significant CNS infiltration on post-mortem brain biopsies despite lack of cells in the CSF [17]. Moreover, even in patients with CNS3 status, the cells in CSF usually become undetectable after only 1–3 intrathecal treatments despite clinical knowledge that prolonged treatment is required to reduce risk of CNS relapse [4]. Thus, CSF cytology is unable to differentiate good from poor treatment responders. Finally, most CNS relapses occur in children who were CNS1 – therefore, the prognostic value of CNS1–3 status is quite poor. It is clear that better diagnostic, response and prognostic biomarkers are needed for CNS-ALL.

The timing of when to perform the diagnostic lumbar puncture is also controversial. Some reports suggest that delaying the first lumbar puncture is associated with lower traumatic lumbar puncture (TLP) rates and less CNS relapse [18,19]. However, no randomized comparisons have been performed, and some practitioners are concerned that delayed LP may interfere with CNS-staging. This is particularly important for protocols that use CNS status to stratify the CNS and systemic therapy intensity (see below). More sensitive biomarkers are required before this question can be properly answered [20].

In keeping with the large variability in CNS2/3 rates across different protocols, and conflicting data on CNS2 status as an independent prognostic factor (Table 2), it is unsurprising that different trial groups have different approaches to using CNS-staging to allocate treatment. In some protocols patients with CNS3 status are assigned to high-risk treatment arms (or at least excluded from low-risk arms), whilst in others they receive additional intrathecal therapies in induction, but no change to their overall risk-group allocation (Table 3). For CNS2 status there is even more controversy, with trials using additional intrathecal treatments for CNS2 patients reporting that CNS2 has no impact on prognosis [21,22], in contrast to trials which did not escalate therapy [23]. Traumatic lumbar punctures with blasts (TLP+) are associated with particularly poor outcomes and generally considered to require additional CNS-directed therapy [24]. The reason why TLP+ has such a poor outcome is currently unknown, it may reflect a correlation with high-risk disease characteristics, rather than the prevalent, but unlikely, hypothesis that it introduces blasts from the periphery into an otherwise leukemia-free CSF.

Most importantly, the inability to accurately quantify CNS leukemic load and response to treatment means that intensive CNS-directed therapy is given to all patients, even in those whose disease is judged to be at “ultra-low” risk of relapse based on karyotype and bone-marrow (BM) MRD quantification. Sensitive response biomarkers capable of accurately quantifying CSF MRD and thus the dynamics of CSF clearance are clearly needed.

**CHALLENGE 2 – WHAT IS THE MOST EFFECTIVE CNS-DIRECTED THERAPY REGIMEN FOR NEWLY DIAGNOSED PATIENTS?**

**Overview**

All modern ALL protocols employ a combination of systemic therapy and intrathecal (IT) therapy against CNS leukemia, with some trial groups also using radiotherapy for selected high-risk groups. This upfront CNS therapy is sometimes called “CNS prophylaxis”, which reflects the aim to prevent CNS relapse. However, it can be wrongly misconstrued as meaning it is preventing dissemination of leukemia to the CNS, although CNS leukemia is likely to be present at the time of diagnosis. Thus, “CNS-directed therapy” is a more appropriate term than “CNS prophylaxis”. The current CNS-directed therapy approaches taken by selected study groups are summarized in Table 3.

The main agents with significant CNS activity are intrathecal and systemic glucocorticoids (not least dexamethasone), intrathecal or intravenous (high-dose) methotrexate and cytarabine, and asparaginase. When choosing therapy, it is also important to consider potential neurotoxicity. Interested readers are referred to comprehensive reviews on this topic [25, 26]. Briefly, 4–12% of children suffer a neurotoxic serious adverse event (SAE) such as seizures, stroke-like syndrome, posterior reversible encephalopathy syndrome, and/or long-term neurocognitive deficits [27]. ALL patients score 6–8 IQ points lower than controls [6] and 15–35% of children have significantly impaired working memory, attention span and/or executive functioning [28–30]. In addition, there are concerns that CNS-directed therapy may result in reduced cognitive reserve, thus risking early-onset dementia [9, 31–33]. The major culprit is thought to be methotrexate, although other agents may also contribute. Table 4 outlines the common agents used for ALL treatment, along with their CNS penetration and any known neurotoxic side-effects.

*Whilst all modern protocols achieve low rates of CNS relapse, the optimal treatment regimen to maximize CNS control whilst minimizing toxicity is currently unknown. One consistent observation is that trials that intensify CNS-directed therapy often have reduced CNS relapse rates but an excess of later BM relapses. This results in no differences in EFS or OS, or sometimes worse OS (as bone marrow relapses may be more difficult to salvage). This has led to the concept of BM and CNS relapses being “competing events”. Poor risk ALL may relapse early (often “on treatment”) in the CNS, due to less chemotherapy exposure and immune*
Table 2. CNS status at diagnosis, use of prophylactic cranial radiotherapy and outcomes in selected childhood ALL trials [8, 22, 23, 34, 37, 132–145].

| Study | Recruitment period | Patients, n | CNS1 | CNS2 | CNS3 | TLP | CNS positive [definition where available] | Indication for prophylactic cranial irradiation | 5-year cumulative incidence of CNS relapse, % | S-5 year EFS, % |
|-------|--------------------|-------------|------|------|------|-----|------------------------------------------|-----------------------------------------------|---------------------------------|-------------|
| AIEOP-BFM ALL2000 [34, 132, 133] | 2000–2006 | 3720 | n/a | n/a | n/a | n/a | 2.5 | MRD levels ≥10 – 3 at day 78, no CR at day 33, t(4;11) translocation, and poor prednisolone response | 1.5 | 1.2 | 82.2 |
| CCG 1991 [134] | 2000–2005 | 1769 | 92.1 | 3.8 | 2.0 | 2.1 | No | 1.8 | 0.7 | 90.7 |
| COALL 07–03 [135] | 2003–2010 | 763 | n/a | n/a | n/a | n/a | 2.6 [non-traumatic LP with leukocytes >5/uL in CSF] | B-precursor ALL and an initial WBC count of >200/nL or 100 to 200/nL and >1 × 10⁹/L leukemic blasts in the peripheral blood at day 8, and patients with T-ALL with a WBC count of ≥50/nL | 1.4 | 2.1 | 84.3 |
| COG AALL 0232 [37] | 2004–2011 | 2914 | 85.9 | 14.1 | n/a | n/a | BM D15 M2 or M3, or D29 MRD > 0.1% | n/a | n/a | 75.3 |
| COG AALL 0331 [23, 136] | 2005–2010 | 5299 | 91.0 | 8.0 | 1.0 | n/a | No | 2.0 ‡ | 0.8 ‡ | 89.0 ‡ |
| DCOG ALL9 [137] | 1997–2004 | 859 | n/a | n/a | n/a | n/a | 2.4 [CNS3 (>5 cells/uL + blasts with <15 RBC/uL) or CT/MRI features] | No | 2.6 | 0.8 | 83.3 |
| DCOG ALL10 [138] | 2004–2012 | 778 | 42.4 | 42.2 | 1.0 | 10.3 | | Patients aged >3 years on non-transplant protocol with TP1 MRD level of ≥5 × 10−4 or unknown and TP2 MRD level of ≥5 × 10−4, MLL-AF4 rearrangements, poor prednisone response, or no CR day 33 | 1.4 | 0.9 | 88.7 |
| DFCI 00–01 [22] | 2000–2004 | 487 | 84.2 | 12.3 | 3.5 | n/a | WBC ≥ 50,000/µL, non-B-precursor phenotype, or presence of mediastinal mass | 1.5 | 2.7 | 80.0 |
| JACLS ALL-02 [139] | 2002–2008 | 1047 | 90.6 | 2.6 | 2.2 | 0.6 | No | 0.8 ‡ | 0.6 ‡ | 85.4 ‡ |
| NOPHO ALL2000 [140, 141] | 2002–2007 | 1020 | 97.1* | n/a | 2.9 | n/a | | Any age and WBC ≥ 200 × 10⁹ L−1, BM D29 M3, t(9;22)(q34;q11), t(4;11) (q21;q23), or low hypodiploidy. And ≥5 years WBC 100—200 × 10⁹ L−1 or T-ALL and mediastinal mass | 2.7 | 2.1 | 79.4 |
| SJCRH total therapy 15 [8] | 2000–2007 | 498 | 72.1 | 20.5 | 1.8 | 5.6 | No | 2.7 | 0.8 | 85.6 |
| SJCRH total therapy 16 [142] | 2007–2017 | 598 | 57.4 | 33.4 | 3.5 | 5.7 | No | 1.2 | 0.3 | 88.2 |
surveillance in this compartment. If CNS-therapy is intensified this risk is reduced, but the same poor risk features make the patient at higher risk of BM relapse once intensive systemic treatment has finished.

**Standard therapy**

The most intensively studied areas are around (i) choice of glucocorticoid, (ii) systemic methotrexate regimen, (iii) the use of single versus triple intrathecal therapy, and (iii) the safety of omitting radiotherapy.

**Glucocorticoids**

Dexamethasone is considered to be the most effective oral glucocorticoid to treat CNS ALL. This is based on several randomized clinical trials comparing prednisolone and dexamethasone, including the collaborative study group’s (AIEOP-BFM) ALL2000 [34] and the UK Medical Research Council ALL97 trials [35]. In both trials those in the dexamethasone arm experienced a significantly lower isolated CNS (ICNS) relapse rate, although without an improvement in overall survival (OS). This is likely to reflect dexamethasone achieving higher concentrations in the CSF with a longer half-life compared to prednisolone [36]. It is worth noting however that other trials have failed to show a benefit for dexamethasone [22, 37]. The outcome difference in part reflects the dose relationships [38]. In terms of toxicity, dexamethasone is associated with worse acute neurobehavioral side-effects as well as increased rates of systemic toxicities such as avascular necrosis compared to prednisolone. The neurobehavioral side effects can in some patients be ameliorated by co-administration of hydrocortisone at physiologic doses [39].

**Systemic methotrexate**

Intravenous methotrexate regimen, and glucocorticoid choice, were the subjects of the Children’s Oncology Group’s (COG) AALL0232 trial [37]. 3154 high risk B-ALL patients aged 1–30 years were randomized to receive high-dose (5000 mg/m²) or Capizzi regimen (100 mg/m²) then escalating intravenous methotrexate during interim maintenance. An improvement in 5-year EFS was reported with the high-dose regimen. This was associated with a reduced, but non-significant, CNS relapse rate without excess toxicity. Conversely when studied in T-ALL, in COG AALL0434, the Capizzi regimen was superior to high-dose methotrexate by 5-year EFS and OS [40].

**Intrathecal therapy**

Given the poor CSF penetration of many systemic anticancer agents (Table 4), direct injection of chemotherapy into CSF – so-called intrathecal (IT) therapy – is an essential part of CNS-directed therapy. Although IT drug administration involving methotrexate, cytarabine, and/or glucocorticoids has been used for many decades, recent clinical trials have continued to investigate the optimal protocol.

COG AALL1131 hypothesized that prophylactic triple intrathecal therapy (TIT) (methotrexate, cytarabine and hydrocortisone) would improve EFS, due to reduced CNS relapses, compared with single intrathecal therapy (methotrexate) (IT MTX) post-induction [41]. The trial enrolled 1734 high-risk B-ALL patients aged 1–31 years, but CNS3 patients were excluded. The trial was stopped early after a futility boundary was crossed showing that TIT could not be superior to IT MTX. There was a (non-significant) trend towards reduced CNS relapse rates in the TIT arm but this was balanced by an opposite (also non-significant) trend for increased isolated BM relapses in this arm. A similar observation was seen in earlier COG trials for standard-risk ALL [42]. In both trials, there was no significant difference in overall rates of neurological toxicity between IT MTX and TIT.

---

**Table 2.** continued

| Study | CNS status at diagnosis, % | 5-year cumulative CNS relapse, % | 5-year cumulative incidence of CNS status at diagnosis, % | CNS positive | CNS positive [definition when available] | CNS1 | CNS2 | CNS3 | Combined CNS1 and CNS2 | Isolated CNS1 | Isolated CNS2 | Isolated CNS3 | TLP | 5-year EFS, % | 5-year cumulative CNS relapse, % |
|-------|---------------------------|---------------------------------|--------------------------------------------------------|--------------|------------------------------------------|------|------|------|------------------------|---------------|---------------|---------------|-----|---------------|---------------------|
| TCGSG L99-15 | [148] | 1999–2003 | 754 | n/a | n/a | 2/13 (15%, CNS-3, TLP+ blasts) | n/a | n/a | n/a | n/a | 1.6 | 1.5 | 1.5 | 1.6 | 78.2 | 1.6 | 2.9 |
| UKALL 2003 | [144] | 2003–2011 | 3113 | 93.8 | 5.1 | n/a | 1.1 | n/a | n/a | n/a | 1.1 | n/a | n/a | n/a | 1.1 | n/a | n/a |

*Combined CNS1 and CNS2.
†4-year EFS.
‡6-year EFS.
§Derived from methotrexate randomization data.

Notes:
- **CNS1**: isolated CNS relapse.
- **CNS2**: CNS-2 relapse.
- **CNS3**: CNS-3 relapse.
- **TLP**: cranial irradiation.
- **WBC**: white blood cells.
- **EFS**: event-free survival.
- **BM**: bone marrow.
- **CNS**: central nervous system.
- **CR**: complete remission.
- **CNS-1**: first CNS relapse.
- **CNS-2**: second CNS relapse.
- **CNS-3**: third CNS relapse.
- **TLP**: cranial irradiation.
- **ALL**: acute lymphoblastic leukemia.
- **BM**: bone marrow.
- **CNS**: central nervous system.
- **CR**: complete remission.
- **CNS-1**: first CNS relapse.
- **CNS-2**: second CNS relapse.
- **CNS-3**: third CNS relapse.
- **TLP**: cranial irradiation.

**Glossary**

- **CNS1**: isolated CNS relapse.
- **CNS2**: CNS-2 relapse.
- **CNS3**: CNS-3 relapse.
- **TLP**: cranial irradiation.
- **WBC**: white blood cells.
- **EFS**: event-free survival.
- **BM**: bone marrow.
- **CNS**: central nervous system.
- **CR**: complete remission.
- **CNS-1**: first CNS relapse.
- **CNS-2**: second CNS relapse.
- **CNS-3**: third CNS relapse.
- **TLP**: cranial irradiation.

**References**

1. [CNS-1s, CNS-2, CNS-3, TPL+ blasts](#).
2. [Berlin Frankfurt Münter, CCG](#).
3. [Children’s Cancer Group, COALL](#).
4. [Cooperative Acute Lymphoblastic Leukemia Study Group](#).
5. [Dutch Childhood Oncology Group, DCOG](#).
6. [DFCI Dana-Farber Cancer Institute](#).
7. [Japan Childhood Leukemia Study Group](#).
8. [NOPHO Nordic Society of Paediatric Haematology and Oncology](#).
9. [SJCRH St. Jude Children’s Research Hospital](#).
10. [TCCSG Tokyo Children’s Cancer Study Group](#).
11. [UKALL United Kingdom Medical Research Council Acute Lymphoblastic Leukaemia](#).
12. [M. Thastrup et al.](#).
13. [Dexamethasone is considered to be the most effective oral glucocorticoid to treat CNS ALL. This is based on several randomized clinical trials comparing prednisolone and dexamethasone](#).
Radiotherapy

In the last decade many ALL trials have explored reducing or omitting the use of cranial irradiation and, therefore, the associated toxicities without sacrificing event-free survival. The consistent finding of these trials is that in the frontline setting cranial irradiation can be safely excluded in conjunction with an appropriately augmented systemic regimen. A meta-analysis, published in 2016, included over 16,000 patients, aged 1–18 years, enrolled across 10 study groups, and concluded that cranial irradiation does not impact CNS relapse risk in modern protocols [43]. This meta-analysis also included subgroup analysis for CNS3 patients. In this subgroup cranial irradiation was found to reduce the risk of isolated and combined CNS relapses, but neither EFS nor OS. Thus, in the majority of contemporary ALL protocols radiotherapy is reserved for relapsed disease, although some trial groups still use radiotherapy upfront for selected high-risk groups as well as part of conditioning prior to hematopoietic stem cell transplantation [44] (Table 2).

Considerations for special groups

Philadelphia chromosome-positive ALL – choice of tyrosine kinase inhibitor. Following pre-clinical murine model data and case series data from Porkka et al. that demonstrated efficacy of dasatinib in CNS disease [45], COG AALL0622 [46] assessed the use of upfront dasatinib instead of cranial radiotherapy to prevent CNS relapse in Ph+ ALL. This single arm trial reported relatively high CNS relapse rates at 15% and made comparisons with historical controls. This strategy is complicated by differing rates of radiotherapy and hematopoietic stem cell transplant (HSCT) between the groups. Overall, the results suggest dasatinib alone (without cranial radiotherapy) may be insufficient to reduce CNS relapses rate in Ph+ ALL. A direct randomized comparison of imatinib versus dasatinib was performed by the Chinese Children’s Cancer Group study ALL-2015 in 189 pediatric patients with Ph+ ALL [47]. The number of patients with CNS disease at presentation was low (CNS3 n = 6) as was the total number of any CNS relapse events (n = 10). However, the investigators found a significantly reduced risk of CNS relapse in the dasatinib arm, but no difference in any CNS relapse. This difference could potentially reflect the higher dasatinib-imatinib dose relation used compared to dosing currently used in other protocols (80 mg/m² and 300 mg/m² used in ALL-2015 versus 60 mg/m² and 340 mg/m² in COG AALL0622 and COG AALL0031 [46, 48], respectively), and the ongoing EPhALL2017/COGAALL1631 protocol (ClinicalTrials.gov Identifier: NCT03007147).

T-ALL. T-ALL patients often receive intensified systemic and CNS-directed therapy. Many trial groups still routinely treat T-ALL patients with CNS radiotherapy, especially if they display other high-risk features such as hyperleukocytosis, although several groups have achieved good results despite omitting radiotherapy [49]. Nelarabine in frontline treatment was assessed in the randomized trial COG AALL0434; nelarabine versus no nelarabine in combination with intensive chemotherapy [50]. The nelarabine arm was found to significantly reduce the rates of isolated and combined CNS relapse although did not have a significant impact on OS. Other differences in systemic therapy may have also contributed to these results, such as less asparaginase in the non-nelarabine arm.

Conclusion

Similar cure rates are achieved using a variety of regimens combining systemic multi-agent chemotherapy with effective CNS-directed therapy – no one approach is clearly superior. It is hoped that better biomarkers will enable evaluation of the true impact of different agents on clearance of CNS-ALL, but until then choice of therapy should aim at ensuring adequate targeting of the CNS compartment while minimizing short- and long-term neurotoxicity.
relapse are usually categorized as ‘early’ or ‘late’. Two trials involving relapsed ALL, UK ALL R3 and IntReALL 2010, used the following definitions: very early relapse is those <18 months from diagnosis (and <6 months from completion of treatment in IntReALL 2010), early relapse as >18 months from diagnosis but <6 months from completion of treatment, and late relapse as those >6 months from completing treatment [52].

**Very early/Early CNS-relapses**

The optimal therapy of iCNS relapse remains controversial. Early iCNS relapses have poor outcomes with EFS/OS rates of 41%/52% on COG AALL0433 and very similar outcomes from other groups [53]. Randomized trials comparing HSCT to chemotherapy have not been feasible, but because survival is <50%, many groups treat early iCNS relapses with intensive systemic chemotherapy followed by HSCT [54]. With small patient numbers, non-significant trends favoring HSCT over chemotherapy and cranial radiation were reported on COGALL0433, UKALL R3, and single institution studies. A retrospective analysis of Italian children treated with HSCT for isolated extramedullary relapse from 1990 to 2015 showed improvements in 10-year survival rates; specifically survival in those with very early isolated extramedullary relapses was 56% with HSCT compared to historical rates of 20% to 30% with chemo-/radiotherapy only [55]. Early combined BM and CNS relapses also appear to benefit from HSCT. A trend towards a reduced rate of post-transplant CNS relapse but without OS benefit was found in a prospective trial involving cranial boost in the HSCT total body irradiation conditioning [56].

**Late CNS relapses**

Historically, late iCNS relapses have had an excellent outcome on protocols using high dose chemotherapy and cranial radiotherapy of 24 Gy. In the UKALL R3 trial this group had a 5 yr EFS of 81% and OS of 85% [52]. Unfortunately, attempts to reduce radiotherapy doses or delay treatment to minimize toxicity in this good prognosis group have led to inferior outcomes [57]. Moreover, recent data suggest that the prognosis of late isolated extramedullary relapses may be worse than previously reported [58]. This may reflect use of more intensive first-line therapy, especially dexamethasone, which may have changed the biology of late relapses as well as selected out the most resistant patients. Clearly efficacious and low toxicity approaches are needed.

**The role of immunotherapy**

Novel immunotherapies including bi-specific T cell engagers and antibody-drug conjugates have revolutionized the management of relapsed and refractory ALL in the last decade and are starting to be used for high-risk patients in frontline settings. Evaluation of the CNS activity of these agents has been difficult as patients with CNS relapses were often excluded from early trials due to concerns of enhanced risk of neurotoxicity and the difficulties in measuring disease response in the CNS. Recently prospective Phase 3 studies from Europe [59] and the US [60] have examined the efficacy of Blinatumomab in released ALL. Overall, post-reinduction consolidation with blinatumomab in COG AALL1331 gave equivalent or better outcomes than chemotherapy alone and a comparatively favorable side effect profile [59, 60]. However, on subgroup analysis, this approach did not appear to be effective in those with isolated extramedullary (including iCNS) relapses [58]. This may reflect reduced penetration of Blinatumomab into extramedullary sites [61]. CAR-T cell therapy for CNS relapses remains experimental and is discussed under “better drugs” below.

**Novel agents**

Despite use of all the agents described above there are a small but significant population of patients who present with recurrent or refractory CNS relapses. Clinical trials are hampered by the absence of sensitive biomarkers to monitor disease response similar to BM MRD. To this adds lack of access to precision medicine umbrella trials due to the challenges in obtaining material for genomic profiling and uncertainty regarding drug penetration into the CNS. As a result, iCNS relapses are excluded from current Phase I/II trials
for relapsed ALL, and no randomized trials have addressed the optimal approach for this group. There are however isolated case reports and small case-series indicating efficacy of some novel treatment approaches including intrathecal rituximab, thiopeta and liposomal cytarabine (Depcyte) [62–64]. In addition, intrathecal etoposide is used in brain tumor patients and has antileukemic activity and may thus be another option [65].

**Potential solutions**

Advances in treatment of CNS leukemia are hampered by two main issues. Firstly, the lack of sensitive and specific diagnostic, response, and prognostic biomarkers able to risk-stratify CNS-directed therapy and to measure the efficacy of novel treatment approaches. Secondly, the lack of non-toxic novel agents with activity against CNS leukemia. Recent progress in these two vital areas is discussed below.

**SOLUTION 1 – BETTER BIOMARKERS**

The ability to accurately diagnose and quantify CNS ALL and its response to treatment will unlock the door to risk-adapted therapy as well as facilitate Phase I/II trials for novel CNS-active agents. Options include more sensitive and reliable detection of leukemic cells in CSF, or measuring soluble biomarkers secreted from leukemic cells in situ. Figure 1 and Table 5 outlines the different classes of biomarkers under investigation, which are discussed below.

**Improving detection of cells in CSF**

Recently, multicolor flow cytometric analysis of CSF has been applied as a more sensitive method for detection of leukemic blasts in the CSF compared to cytopsin. In flow cytometry, blasts are identified based on their aberrant expression of leukemia-associated immunophenotype markers, making this technique highly specific [66]. Furthermore, flow cytometry can rapidly and precisely quantify the expression of multiple cell surface molecules even when the cell count is 1000 fold lower than the upper normal limit of leukocytes in CSF (5 × 10⁹/L) leading to a much higher sensitivity than conventional cytology [66]. Furthermore, the cells can be fixed prior to flow cytometry, e.g. in specialized CSF Transfix® tubes, which preserves the cells and allows for delaying analysis of CSF for 48–72 h, thus facilitating centralized analysis [67].

At diagnosis, CNS involvement has been detected by CSF flow cytometry in 17–41% of cases compared to only 3–10% of cases classified as CNS2 and CNS3 by cytopsin in children with ALL [24, 68–70]. One study also investigated the clearance of leukemic blasts during induction therapy and found that 7.5% of flow positive patients at diagnosis remained positive at day 15 [24]. It was recently shown in a large study (n = 673) by the NOPHO group that CSF flow positivity at diagnosis was an independent risk factor for relapse among children and adolescents with ALL [24]. This association was confirmed in a study by COACG group in patients with low level CNS disease (classified as CNS2 by cytopsin) [10]. Two other studies also showed that relapse occurred more frequently among patients who were CSF flow positive at diagnosis, but results did not reach statistical significance, likely due to the small study cohorts [68, 70].

TLP with blasts (TLP+) has previously been associated with increased risk of CNS relapse in childhood ALL [8, 71–73]. In the aforementioned NOPHO study, TLP at diagnosis was only associated with a higher risk of relapse in patients, where presence of blasts were confirmed by flow cytometry [24]. Accordingly, the current European ALLTogether1 treatment protocol (NCT04307576) requires flow cytometry confirmation of CNS involvement in case of TLP. The prognostic significance of the patient’s blast level at diagnosis and rate of clearance of leukemic blasts during treatment is also being tested in the ALLTogether1 trial to determine if these parameters can be used to assign a CNS-relapse score on which to base a future randomized trial of risk-adapted CNS-directed therapy to balance treatment efficacy and toxicity.

Another technique that has been proposed for detection of submicroscopic levels of CNS involvement is PCR on cell-free DNA in CSF. PCR on CSF DNA is typically performed with the patient-specific primers against variable regions in immunoglobulins and the T-cell receptor generated for bone marrow MRD. In pediatric ALL patients, PCR detected CNS dissemination in 20–47% of cases compared to 5–17% by cytopsin [74–77]. The rates of CNS involvement at diagnosis by PCR are equivalent to the rates obtained by CSF flow cytometry. However, in several studies it was not possible to analyze a large fraction of the CSF samples by PCR due to poor quality of the DNA or lack of suitable primers [75–77]. In studies sampling CSF during treatment, patients quickly became negative by PCR [74, 75, 77], which suggest that more sensitive techniques, such as flow cytometry or next generation sequencing, are needed to assess treatment response in the CNS.

**Soluble biomarkers for CNS-ALL**

Given that leukemic cells in the CNS are often adherent to stroma rather than free-floating in CSF it is hypothesized that measurement of a CSF biomarker that is released or taken-up by leukemic...
| Biomarker(s)                                                                 | Detection method                                                                 | Study design                    | Patient cohort                                                                                           | Results                                                                                                                                                                                                 |
|----------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Leukemic cells                                                             |                                                                                    |                                 |                                                                                                         |                                                                                                                                                                                                       |
| Biomarkers related to hypoxic adaption [85]                                | Immunohistochemical analysis, gene set enrichment analysis, mass spectrometry and quantification of VEGFA transcripts with microarray | Experimental                    | CNS- and bone marrow-derived leukemic cells from xenografts recipients of pediatric ALL patients (n = 4) | B-ALL cells that infiltrate the CNS are more quiescent with less oxygen consumption but with greater glycolytic activity compared to those residing in bone marrow |
| Biomarkers related to metabolic plasticity [86]                            | Mass spectrometry (gas and liquid gas chromatography, qPCR, RNA sequencing)       | Experimental                    | ALL live cells, CSF from normal controls and CSF from ALL patients                                      | CNS leukemic blasts adapt metabolically to the CNS environment dependent on Stearoyl-CoA desaturase (SCD1). SCD1 overexpression in vivo increased CNS disease, whilst genetic or pharmacological inhibition of SCD1 decreased CNS load |
| Circulating leukemic blasts in CSF [151]                                  | Cytospin with TdT immunostaining                                                  | Prospective non-intervention study | TdT+ ALL (n = 100), TdT+ acute nonlymphoblastic leukemia (ANLL) (n = 8) or TdT+ non-Hodgkin's lymphoma (NHL) (n = 5). 1643 CSF samples were collected at diagnosis and during follow-up | CNS involvement in diagnostic and follow-up samples: 9.1% by TdT and 3.9% by cytospin. 74% of TdT+ samples were followed by overt CNS leukemia and two consecutive findings of TdT+ cells were always followed by overt CNS leukemia |
| Circulating leukemic blasts in CSF [68]                                   | Flow cytometry (6-color)                                                          | Prospective non-intervention study | Newly diagnosed pediatric ALL (n = 108)                                                                  | CNS involvement at diagnosis: 28% by flow cytometry vs 3% by cytospin. 3-year cumulative incidence of relapse: 10.7% for flow positive group vs 6.9% for flow negative group (P = 0.648) |
| Circulating leukemic blasts in CSF [69]                                   | Flow cytometry (3–8 color)                                                        | Prospective non-intervention study | Newly diagnosed pediatric ALL (n = 214)                                                                  | CNS involvement at diagnosis: 17% by flow cytometry vs 10% by cytospin. 5-year cumulative incidence of relapse: 29% for flow positive group vs 7% for flow negative group (P = 0.028) |
| Circulating leukemic blasts in CSF [70]                                   | Flow cytometry (8-color)                                                          | Prospective non-intervention study | Newly diagnosed pediatric ALL (n = 87)                                                                  | CNS involvement at diagnosis: 41% by flow cytometry vs 8% by cytospin. Relapse rate: 11.8% for flow positive group vs 4.0% for flow negative group (P = 0.15) |
| Circulating leukemic blasts in CSF [24]                                   | Flow cytometry (6-color)                                                          | Prospective non-intervention study | Newly diagnosed pediatric ALL (n = 673)                                                                  | CNS involvement at diagnosis: 25% by flow cytometry vs 13% by cytospin. 4-year cumulative incidence of relapse: 16.5% for flow positive group vs 5.6% for flow negative group (P = 0.0013). Cytospin and/or flow positivity was an independent risk factor for relapse in multivariate analysis (HR: 2.2, P = 0.042) |
| Biomarker(s)                                      | Detection method                      | Study design                                | Patient cohort                          | Results                                                                                                                                 |
|--------------------------------------------------|---------------------------------------|---------------------------------------------|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Circulating leukemic blasts in CSF [10]          | Flow cytometry (6-color)               | Prospective non-intervention study          | Newly diagnosed pediatric ALL (n = 255) | CNS involvement at diagnosis: 23% by flow cytometry vs 51% by cytospin. High CNS2 rate potentially due to false-positives (cytospin evaluated by four observers in two different laboratories and defined as positive if one of the observers identifies 1 leukemic blast). 5-year RFS for CNS2 patients (n = 107): 87.9% for flow positive group vs 100% for flow negative group (P = 0.003) |
| DNA from CSF cells [76]                          | PCR for VDJ rearrangements             | Prospective non-intervention study          | Newly diagnosed pediatric ALL without TLP (n = 37) | CNS involvement at diagnosis: 46% by PCR vs 5% by cytospin. 4-year EFS: 41% for PCR positive group and 84% for PCR negative group (P = 0.01) |
| DNA from CSF cells [74]                          | PCR for VDJ rearrangements             | Prospective non-intervention study          | Newly diagnosed pediatric ALL (n = 30)   | CNS involvement at diagnosis: 20% by PCR vs 17% by cytospin. PCR confirmed confirmed cytospin results in 2 patients. Cytospin results could not be confirmed by PCR in 3 patients |
| DNA from CSF cells [77]                          | PCR for VDJ rearrangements             | Prospective non-intervention study          | Newly diagnosed pediatric ALL without TLP (n = 63) | CNS involvement at diagnosis: 47% by PCR vs 5% by cytospin. 5-year EFS: 54.8% for PCR and/or cytospin positive group vs 77.7% for PCR and cytospin negative group (P = 0.03), PCR and/or cytospin positivity not independent prognostic factor in multivariate analysis (P = 0.15) |
| DNA from CSF cells [75]                          | PCR for VDJ rearrangements             | Prospective non-intervention study          | Newly diagnosed pediatric ALL (n = 38)   | CNS involvement at diagnosis: 40% by PCR vs 13% by cytospin. PCR confirmed cytospin results in 4 patients. Cytospin results could not be confirmed by PCR in 1 patient |
| Soluble biomarkers                               |                                       |                                             |                                         |                                                                                                                                        |
| Cerebrospinal fluid proteome during PEG-         | Quantitative label-free LC-MS/MS (tryptic digest) | Cross-sectional observation                 | Newly diagnosed B- and T-ALL (n = 4) and lymphoblastic lymphoma (n = 1) | Proteome profiling of CSF alterations during PEG-asparaginase treatment. 635 proteins (406 proteins with 2 or more peptides), here 35 proteins had significantly altered intensities throughout day 0, day 8 and day 29 (P < 0.05 following Benjamini-Hochberg correction) |
| asparaginase treatment [78]                      |                                       |                                             |                                         |                                                                                                                                 |
| Cerebrospinal fluid proteome alterations         | Quantitative label-free LC-MS/MS (tryptic digest) | Cross-sectional observation                 | Pediatric patients with confirmed CNS B-ALL (n = 6) | 428 proteins identified comparing pre vs post induction chemotherapy, here with 10 proteins being significantly altered (P < 0.05 in a paired t-test without correction of multiple comparisons) |
In total, 47 candidate microRNAs were selected for profiling. Based on the discovery cohort, miRNA-181a was overexpressed 52-fold in CNS-ALL patients in the validation cohort.  

**Results**  
Leukemia-derived exosomes may facilitate CNS invasion of ALL cell lines across the blood-CSF-barrier without destruction of the barrier integrity. Based on the continued observation of patients, biomarker(s) detection methods are needed. Multiple biomarkers and/or genetic/demographic features may be needed.

Recently, blast-derived extracellular vesicles have been hypothesized to foster malignant transformation of leukemic cells and facilitate transmigration across the blood-CSF-barrier by priming of choroid plexus cells. Technological progress has enabled fluorescent labeling of extracellular vesicles and also provide future biomarkers for CNS invasion and risk of CNS relapse.

Metabolic plasticity may be pivotal for adapting to the low nutrient microenvironment in CSF, and metabolites and metabolic regulators are potential biomarkers for CNS-ALL. Distinct biological mechanisms have been described, including hypoxic adaptation by upregulation of vascular endothelial growth factor A (VEGFA) and metabolic adaption by Stearoyl CoA desaturase (SCD1) dependent fatty-acid synthesis. Although rather non-specific, lactate dehydrogenase (LDH) has been used a biomarker for CNS lymphoma diagnostics, and elevated LDH levels may also indicate CNS involvement in ALL.

Circulating microRNA (miRNA) in CSF have been linked to CNS involvement. Many miRNAs have been reported, yet not validated, but may include high expression of the miRNA-181-family, miR-34a, miR-128a, miR-128b, and miR-146a, in CSF positive relative to CSF negative patients.

Finally, cfDNA is released from cells into the surrounding body fluids by a variety of mechanisms including active release and secondary to apoptosis and cell turnover. Measurement of cfDNA in plasma (and/or CSF for brain tumors) detects solid tumors at early stages and dynamically tracks treatment responses with levels rising prior to overt disease recurrence. ALL is a fast-growing malignancy with rapid cell turnover, and CNS blasts reside directly within the CSF compartment. Therefore, CSF cfDNA would be expected to provide a sensitive and specific biomarker for detecting and tracking CNS involvement. However, evidence for this is currently lacking.

**SOLUTION 2 – BETTER DRUGS**

Real advances in treating CNS leukemia will require new therapeutics. Replacement of conventional agents such as methotrexate with less neurotoxic alternatives will benefit all patients. Novel agents are also needed to unlock the potential of biomarker driven approaches to identify high-risk patients and for those with refractory disease. An overview of agents in preclinical development and clinical testing are shown in Fig. 2 and Table 6.
Novel immunotherapies

Chimeric antigen receptor T (CAR-T) cells targeting CD19 on B-ALL have demonstrated convincing evidence of activity in the CNS. In early-stage clinical trials, investigators have reported the presence of CAR-T cells within the CSF of treated ALL patients [93]. Subsequently, multiple case reports and case series have demonstrated clearance of CNS leukemia with CD19-targeted CAR-T cell treatment including those with iCNS disease [94, 95]. Post-hoc analysis from five CAR-T cell clinical trials with 195 patients with relapsed or refractory B-ALL, of whom 54% had evidence of CNS disease (usually as part of combined CNS and BM relapses), found similar rates of complete response and relapse-free survival irrespective of CNS status at relapse [96]. This conclusion was echoed by the Pediatric Real World CAR Consortium where they reported CD19 CAR-T therapy outcomes and toxicities for patients with CNS disease were similar to those with BM only involvement [97]. There are, however, reports of less favourable outcomes; a recently published international retrospective analysis reported a high rate of subsequent CNS relapse in those with iCNS disease (6 of 8 patients) following CD19 CAR-T therapy [61]. Although CAR-T cell therapy holds significant promise, the outcome data is somewhat inconsistent and longer follow-up is needed to determine the longevity of responses [61]. Emerging data from B-cell lymphoma patients suggests that CAR-T cells may undergo some degree of exhaustion or anergy in the leptomeninges the leukemic cells are in direct contact with the CSF that has low oxygen and nutrient levels compared to the brain [101]. Recent evidence supports that B-cell receptor signaling through the Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) pathway promotes survival of leukemic cells in the CNS [102–104]. In a phase II study, treatment with the Raf inhibitor sorafenib showed efficacy in patients with refractory CNS leukemia [105]. Preclinical xenograft studies have demonstrated inhibition of CNS leukemic load of the MEK inhibitor selumetinib alone [106] or in combination with dexamethasone [107]. In the SeluDex trial (NCT03705507), efficacy of selumetinib in combination with dexamethasone is being evaluated separately in the bone marrow and CNS in pediatric and adult patients with relapsed or refractory ALL, but no results have been reported yet. In T-cell ALL, inhibition of lymphocyte specific cell-kinase (LCK) (acting downstream of the T-cell receptor) by dasatinib has been shown to reduce cell proliferation in vitro and reduce leukemic burden CNS leukemia, however recent reports indicate that these toxicities are usually reversible with intensive supportive care [99, 100].

Novel drugs that target cell survival mechanisms

One strategy is to target the leukemic cells that have already entered the CNS by disrupting the molecular mechanisms that support their survival in the CNS microenvironment. In the leptomeninges the leukemic cells are in direct contact with the CSF that has low oxygen and nutrient levels compared to the blood [101]. Recent evidence supports that B-cell receptor signaling through the Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) pathway promotes survival of leukemic cells in the CNS [102–104]. In a phase II study, treatment with the Raf inhibitor sorafenib showed efficacy in patients with refractory CNS leukemia [105]. Preclinical xenograft studies have demonstrated inhibition of CNS leukemic load of the MEK inhibitor selumetinib alone [106] or in combination with dexamethasone [107]. In the SeluDex trial (NCT03705507), efficacy of selumetinib in combination with dexamethasone is being evaluated separately in the bone marrow and CNS in pediatric and adult patients with relapsed or refractory ALL, but no results have been reported yet. In T-cell ALL, inhibition of lymphocyte specific cell-kinase (LCK) (acting downstream of the T-cell receptor) by dasatinib has been shown to reduce cell proliferation in vitro and reduce leukemic burden CNS leukemia, however recent reports indicate that these toxicities are usually reversible with intensive supportive care [99, 100].

Novel drugs that target cell survival mechanisms

One strategy is to target the leukemic cells that have already entered the CNS by disrupting the molecular mechanisms that support their survival in the CNS microenvironment. In the leptomeninges the leukemic cells are in direct contact with the CSF that has low oxygen and nutrient levels compared to the blood [101]. Recent evidence supports that B-cell receptor signaling through the Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) pathway promotes survival of leukemic cells in the CNS [102–104]. In a phase II study, treatment with the Raf inhibitor sorafenib showed efficacy in patients with refractory CNS leukemia [105]. Preclinical xenograft studies have demonstrated inhibition of CNS leukemic load of the MEK inhibitor selumetinib alone [106] or in combination with dexamethasone [107]. In the SeluDex trial (NCT03705507), efficacy of selumetinib in combination with dexamethasone is being evaluated separately in the bone marrow and CNS in pediatric and adult patients with relapsed or refractory ALL, but no results have been reported yet. In T-cell ALL, inhibition of lymphocyte specific cell-kinase (LCK) (acting downstream of the T-cell receptor) by dasatinib has been shown to reduce cell proliferation in vitro and reduce leukemic burden CNS leukemia, however recent reports indicate that these toxicities are usually reversible with intensive supportive care [99, 100].

Two interesting therapeutic strategies that have not yet progressed to clinical testing, include disruption of metabolic
### Overview of clinical and preclinical evidence for anti-CNS leukemia effects of novel therapeutic agents [85, 86, 93, 95, 96, 100, 103–108, 111, 113, 115, 119, 121, 124].

| Drug name | Target | Study design | Patient cohort/preclinical model | Results |
|-----------|--------|--------------|---------------------------------|---------|
| **Immunotherapies** | | | | |
| Tisagenlecleucel (CTL019) [93] | CD19 | Phase I–II clinical trial (case report of 2 enrolled patients) | Pediatric relapsed or refractory BCP-ALL (n = 2) | Complete response achieved in both patients. 1 patient relapsed after 2 months, 1 patient still relapse-free 11 months after treatment. CAR-T cells observed in bone marrow and CSF. |
| Anti-CD19 CAR-T cells [100] | CD19 | Phase I–II clinical trial (case report of 3 enrolled patients) | Adult relapsed or refractory B-ALL with CNS involvement (n = 3) | Complete response achieved in all patients with relapse-free survival of 2–5 months. CAR-T cells observed in CSF, but dynamics varied between patients. |
| Anti-CD19 CAR-T cells containing co-stimulator CD28 or 4-1BB [95] | CD19 | Phase I–II clinical trial (case report of 1 enrolled patient) | Pediatric or adult relapsed or refractory B-ALL (n = 1) | Complete response achieved with 36 months of relapse-free survival. |
| Tisagenlecleucel (CTL019) & huCART19 [96] | CD19 | Post-hoc analysis of 5 clinical trials | Pediatric or adult relapsed or refractory B-ALL (n = 195) | Patients categorised as CNS pos or neg based on CNS status at relapse or within the 12 months preceding CAR T-cell infusion. No significant difference in complete response rates (64% vs 66%) or 2-year overall survival (83% vs 71%) for CNS pos vs CNS neg patients. |
| **Survival** | | | | |
| Sorafenib [105] | Raf | Phase II clinical trial | Adult refractory CNS ALL (n = 26) | Complete response achieved in 17 patients and a partial response achieved in two patients. 2-year event free survival of 75% and 2-year overall survival of 76.9%. |
| Selumetinib(NCT03705507) | MEK | Phase I–II clinical trial | Pediatric and adult relapsed or refractory B- or T-ALL with RAS-pathway activating mutations (n = 42) | Ongoing clinical trial - no reported results yet. The aim is to investigate safety and efficacy of selumetinib in combination with dexamethasone. |
| U0126 [103] | MEK | Preclinical | Cell line ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
| Selumetinib [106] | MEK | Preclinical | Patient-derived BCP-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
| Selumetinib [107] | MEK | Preclinical | Patient-derived BCP-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. Combination therapy with dexamethasone resulted in no detectable CNS leukemia in 2 of 3 xenografts. |
| Dasatinib [108] | LCK | Preclinical | Patient-derived T-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS in 4 of 8 of xenografts. Combination therapy with dexamethasone resulted in no detectable CNS leukemia in more than 5 of 9 xenografts and significant reduction in leukemic load in CNS in remaining 4 xenografts. |
| Bevacizumab [85] | VEGF | Preclinical | Patient-derived BCP-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
| Bevacizumab [111] | VEGF | Preclinical | Patient-derived BCP-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
| SW203668 [86] | SCD | Preclinical | Patient-derived and cell line ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
| Me6TREN [115] | Meningeal adhesion of leukemic cells | Preclinical | Patient-derived and cell line B-ALL xenografts | Combination therapy with cytarabine significantly reduced leukemic load in CNS compared to monotherapy with cytarabine. |
| Omacetaxine mepesuccinate [113] | Ribosome | Preclinical | Patient-derived B-ALL xenografts | Monotherapy significantly reduced leukemic load in CNS. |
Table 6. continued

| Drug name | Target | Study design | Patient cohort/clinical model | Results |
|-----------|--------|--------------|-----------------------------|---------|
| Copanlisib (NCT04803123) | PI3K | Phases I clinical trial | Adult relapsed or refractory B-ALL (n=18) | Ongoing clinical trial - no reported results yet. | Monotherapy significantly reduced leukemic load in CNS. |
| GS-649443 & Copanlisib (NCT02763384) | PI3K | Phases II clinical trial | Adult relapsed and cell line T-ALL and T-LBL (n=20) | Patient-derived cell line T-ALL xenografts | Combination therapy with GS-649443 and copanlisib significantly reduced leukemic burden in CNS. |
| BL-8040 (NCT02763384) | CXCR4 | Preclinical | Cell line BCP-ALL xenografts | Preclinical | Monotherapy significantly reduced leukemic load in CNS. |
| Plerixafor (AMD-3100) & Copanlisib | CXCR4 | Preclinical | Cell line BCP-ALL xenografts | Preclinical | Monotherapy significantly reduced leukemic load in CNS. |
| BL-8040 & Me6TREN | CXCR4 | Preclinical | Cell line BCP-ALL xenografts | Preclinical | Monotherapy significantly reduced leukemic load in CNS. |

Novel drugs that target CNS invasion

Leukemic cells predominantly reside within the meninges that cover the surface of the brain and spinal cord and only invade the brain parenchyma in late-stage disease [116]. Due to the localization of leukemic cells, the leading hypothesis has been that the leukemic cells enter the CNS by crossing the blood-leptomeningeal-barrier in the meningeal microvessels or the blood-CSF-barrier at the choroid plexuses [117, 118]. However, recently leukemic cells were observed to migrate directly from the bone marrow and into the leptomeninges along the outer surface of vessels passing through fenestrations in the vertebral or calvarial bones [119]. Integrins α6 and α9 play a role in CNS involvement [120]. Treatment with PI3K inhibitors reduced expression of integrin α9 by cultured BCP-ALL cells, and reduced CNS involvement and prolonged survival in BCP-ALL xenografts [119, 121]. A phase I clinical trial with the pan-PI3K inhibitor copanlisib is currently ongoing to study the effect on α6 expression and lymphoblast proliferation in adult patients with refractory or relapsed B-ALL (NCT04803123). Various other integrins and adhesion molecules has been associated with CNS involvement in childhood ALL [117, 118, 122], but no drugs targeting these integrins or downstream signaling pathways have progressed to clinical testing yet.

Normal lymphocytes express numerous chemokine receptors, including CXCR3, CXCR4 and CCR7, which promote migration into the CNS through expression of the corresponding ligands in the brain endothelium and choroid plexus epithelium [123]. In T-ALL, CXCR4 expression in diagnostic bone marrow samples have been associated with CNS involvement [103] and treatment with the CXCR4 inhibitor plerixafor (AMD-3100) reduced CNS infiltration in T-ALL xenografts [124]. A phase II clinical trial is currently ongoing where the CXCR4 antagonist BL-8040 is added to nelarabine treatment in adult patients with refractory or relapsed T-ALL (NCT02763384). The results are awaited.

Since a large proportion of children with ALL already have CNS involvement at diagnosis, drugs blocking the entry of leukemic cells into the CNS may have limited effect in the clinical setting. Currently, it is not known if continuous trafficking of leukemic cells adapt to the CNS microenvironment and contact-induced quiescence. Accumulating evidence supports that the low oxygen and nutrient availability in the CSF induce metabolic adaptations in the leukemic cells that promote their survival in the CNS microenvironment [110]. Several studies have shown that hypoxia-gene expression signatures are upregulated in leukemic cells located in the CNS, and that treatment with the anti-VEGF antibody bevacizumab significantly reduced CNS leukemia in ALL xenograft models [85, 111]. Fatty acid synthesis is also upregulated in ALL cells isolated from the CNS [86, 112], and pharmacological inhibition of the enzyme stearoyl-CoA desaturase (SCD) (converting saturated fatty acids to mono-unsaturated fatty acids) significantly reduced CNS involvement in a recent study [86]. A recent xenograft study not only confirmed the altered metabolic state of ALL cells within the CNS, but also identified mRNA translation as potential therapeutic target [113]. Treatment with the ribosome A inhibitor omacetaxine mepesuccinate reduced mRNA translation in patient-derived xenografts and reduced leukemic burden in the CNS [113]. Adhesion of leukemic cells to meningeal cells induces a dormant phenotype and increased resistance to chemotherapy in animal and cell line studies [114, 115]. The small molecule inhibitor Tris[2-(dimethylamino)ethyl]amine (Me6TREN) in combination with cytarabine significantly inhibited CNS dissemination compared to monotherapy with cytarabine in xenograft models [115]. These studies highlight SCD, VEGF, mRNA translation and contact-induced quiescence as potential targets for CNS-directed therapy, but this needs to be explored in clinical trials.
from the blood or bone marrow into the CNS occurs after initial CNS seeding. The therapeutic benefit of targeting CNS invasion in childhood ALL thus needs to be confirmed in clinical studies and carefully designed xenograft studies where treatment is initiated only after CNS involvement has been established.

Finally, one other barrier to effective new therapies is whether the drug can enter the CSF compartment. Intrathecal delivery circumvents the need to cross the blood brain/blood-CSF barrier, but it is onerous for families and repeated general anesthesia in children may provide an added neurocognitive burden [125]. Oral small-molecule inhibitors vary in their ability to enter the CNS compartment and drug-engineering may be needed to enhance CSF-penetration of promising compounds [126, 127]. Alternatively, implanted intrathecal drug delivery devices may also be appropriate – especially in the CNS-relapse setting [128].

**SUMMARY/CONCLUSIONS**

Optimizing treatment of the CNS remains a challenge in childhood ALL. Current approaches are intensive, non-discriminative, and cause significant morbidity, whilst treatment options for patients with relapsed/refractory CNS-ALL are limited. Solutions lie in new drugs and better biomarkers. The lack of accurate biomarkers is most critical. Without a means to measure treatment response the testing of novel agents becomes difficult. Moreover, biomarkers will help identify patients at low or high risk for CNS relapse. For high-risk children who currently receive large amounts of CNS-directed therapy, reducing this treatment burden would have significant health and economic benefits. For high-risk children novel approaches are needed, facilitated by new drugs. Fortunately, research into CNS-ALL has increased over the last decade and is starting to provide a better understanding of disease biology as well as putative drug targets and biomarkers. Some biomarkers, such as CSF-flow cytometry, are now being tested in prospective trials. Novel drugs are also being tested in Phase II/III trials, although wider access for ICNS relapse patients is needed. The future is hopeful for improved management of the CNS over the next decade.

**REFERENCES**

1. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med. 2015;373:1541–52.
2. Toft N, Birgens H, Abrahamsson J, Griskevičius L, Hallböök H, Heyman M, et al. Results of NOPHO ALL2008 treatment for patients aged 1–45 years with acute lymphoblastic leukemia. Leukemia. 2018;32:606–15.
3. Aur RJ, Simone J, Hustu OD, Walters T, Borella L, Pratt C, et al. Central nervous system therapy and combination chemotherapy of childhood lymphoblastic leukemia. Blood. 1971;37:272–81.
4. Pui CH, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol. 2008;9:257–68.
5. Halsey C, Buck G, Richards S, Vargha-Khadem F, Hill F, Gibson B. The impact of chemotherapy on IQ in patients with cancer. Cancer. 1998;81:733–9.
6. Halsey C, Escherich GA. “Goldilocks” approach to CNS leukemia is needed. Blood. 2021;138:288–9.
7. Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dördelmann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood. 2008;111:4477–89.
8. Vrooman LM, Stevenson KE, Supko JG, O’Brien J, Dahlberg SE, Asselin BL, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study. Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol. 2013;31:1202–10.
9. Winick N, Devidas M, Chen S, Maloney K, Larsen E, Mattano L, et al. Impact of initial CSF findings on outcome among patients with National Cancer Institute Standard- and high-risk B-cell acute lymphoblastic leukemia: a report from the Children’s Oncology Group. J Clin Oncol. 2017;35:2527–34.
10. Thastrup M, Marquart HV, Levinsen M, Grell K, Abrahamsson J, Albertsen BK, et al. Flow cytometric detection of leukemic blasts in cerebrospinal fluid predicts risk of relapse in childhood acute lymphoblastic leukemia: a Nordic Society of Pediatric Hematology and Oncology study. Leukemia. 2020;34:336–46.
11. Krull KR, Hardy KD, Kahalley LS, Schuitema I, Kesler SR. Neurocognitive outcomes and interventions in long-term survivors of childhood cancer. J Clin Oncol; Off J Am Soc Clin Oncol. 2018;36:2181–9.
12. Magge RS, DeAngelis LM. The double-edged sword: neurotoxicity of chemotherapy. Blood Rev. 2011;25:93–100.
13. Schmiegelow K, Attarbaschi A, Barzilai S, Escherich G, Frandsen TL, Halsey C, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17:e231–9.
14. Cheung YT, Krull KR. Neurocognitive outcomes in long-term survivors of childhood acute lymphoblastic leukemia treated on contemporary treatment protocols: a systematic review. Neurosci Biobehav Rev. 2015;53:108–20.
15. Cheung YT, Sabin ND, Reddick WE, Bhojwani D, Liu W, Brinkman TM, et al. Leukoencephalopathy and long-term neurobehavioural, neurocognitive, and brain imaging outcomes in survivors of childhood acute lymphoblastic leukemia treated with chemotherapy: a longitudinal analysis. Lancet Haematol. 2016;3:e456–66.
16. Krull KR, Brinkman TM, Li C, Armstrong GT, Ness KK, Sivastava DK, et al. Neurocognitive outcomes decades after treatment for childhood acute lymphoblastic leukemia: a report from the St Jude lifetime cohort study. J Clin Oncol; Off J Am Soc Clin Oncol. 2013;31:4407–15.
17. Zeller B, Tannes CK, Kanellopoulos A, Amilien IK, Andersson S, Due-Tønnessen P, et al. Reduced neuroanatomical volumes in long-term survivors of childhood acute lymphoblastic leukemia. J Clin Oncol. 2013;31:2078–85.
18. Armstrong FD. Implications of 25-year follow-up of white matter integrity and neurocognitive function of childhood leukemia survivors: a wake-up call. J Clin Oncol; Off J Am Soc Clin Oncol. 2013;31:3309–11.
19. Williams AM, Cheung YT, Hyun G, Liu W, Ness KK, Ehrhardt MJ, et al. Childhood neurotoxicity and brain resilience to adverse events during adulthood. Ann Neurol. 2021;89:534–45.
34. Morice A, Zimmermann M, Valsecchi MG, Stanulla M, Biondi A, Mann G, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood. 2016;127:2101–12.

35. Mitchell CD, Richards SM, Kinsey SE, Lilleymam J, Vora A, Eden TO, et al. Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL97 randomized trial. J Haematol. 2005;129:73–45.

36. Ballis FM, Lester CM, Chrousos GP, Heideman RL, Poplack DG. Differences in cerebrospinal fluid penetration of corticosteroids: possible relationship to the prevention of meningeal leukemia. J Clin Oncol. 1987;5:202–7.

37. Larsen EC, Devidas M, Chen S, Salzer WL, Raetz EA, Loh ML, et al. Dexamethasone and high-dose methotrexate improve outcome for children and younger adults with high-risk B-acute lymphoblastic leukemia: a report from Children’s Oncology Group Study AALL0322. J Clin Oncol. 2016;34:2380–8.

38. Feuvell O, Kuster SP, Hunger SP, Conter V, Hitzer J, Ethier MC, et al. Dexamethasone versus prednisone for induction therapy in childhood acute lymphoblastic leukemia: a systematic review and meta-analysis. Leukemia. 2011;25:1232–6.

39. van Hult AM, Verwaaijen EJ, Fiocco MF, Pluijm S, Grootenhuys MA, Pieters R, et al. Study protocol: DexaDays-2, hydrocortisone for treatment of dexamethasone-induced neurobehavioral side effects in pediatric leukemia patients: a double-blind placebo controlled randomized intervention study with cross-over design. BMC Pediatrics. 2021;21:427.

40. Winter SS, Dunsmore KP, Devidas M, Wood BL, Elashvili N, Chen Z, et al. Improved survival for children and young adults with T-Lineage acute lymphoblastic leukemia: results from the Children’s Oncology Group Group AALL0434 methotrexate randomization. J Clin Oncol. 2018;36:2926–34.

41. Salzer WL, Burke MJ, Devidas M, Dai Y, Hardy KK, Kairalla JA, et al. Impact of Intrathecal Triple Therapy Versus Intrathecal Methotrexate on disease-free survival for high-risk B-lymphoblastic leukemia: Children's Oncology Group Study AALL1131. J Clin Oncol. 2020;38:2629–38.

42. Mattlough Y. Intrathecal triple therapy decreases central nervous system relapse but fails to improve event-free survival when compared with intrathecal methotrexate: results of the Children’s Cancer Group (CCG) 1952 study for standard-risk acute lymphoblastic leukemia. Blood. 2006;108:1165–73.

43. Vora A, Andreano A, Pui CH, Hunger SP, Schrappe M, Moericke A, et al. Influence of Cranial radiotherapy on outcome in children with acute lymphoblastic leukemia treated with contemporary therapy. J Clin Oncol. 2016;34:919–26.

44. Peters C, Dalle J-H, Locatelli F, Poetschger U, Sedlack P, Buechner J, et al. Total body irradiation or chemotherapy conditioning in childhood ALL: a multinational, randomized, noninferiority phase III study. J Clin Oncol. 2011;29:395–307.

45. Pernka K, Koskenvesa P, Lundan T, Rimpiainen J, Mustojoki S, Smyka R, et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood. 2008;112:1005–12.

46. Sloatyn WB, Schultz KR, Kairalla JA, Devidas M, Mi X, Pulsipher MA, et al. Dasatinib plus intensive chemotherapy in children, adolescents, and young adults with Philadelphia chromosome-positive acute lymphoblastic leukemia: results of Children’s Oncology Group Trial AALL0622. J Clin Oncol. 2018;36:2306–14.

47. Shen S, Chen X, Cai J, Yu J, Gao J, Hu S, et al. Effect of dasatinib vs imatinib in the treatment of pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: a Randomized Clinical Trial. JAMA Oncol. 2020;6:358.

48. Schultz KR, Carroll A, Heerema NA, Bowman WP, Aledo A, Sloatyn WB, et al. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children’s Oncology Group Study AALL0031. Leukemia. 2014;28:1467–71.

49. Kelly MJ, Trikalinos TA, Dahabreh IJ, Gianferante M, Parsons SK. Cranial radiation for pediatric T-lineage acute lymphoblastic leukemia: a systematic review and meta-analysis. Am J Hematol. 2014;89:992–997.

50. Matloub Y. Intrathecal triple therapy decreases central nervous system relapse in childhood B-cell acute lymphoblastic leukemia treated with intensified systemic therapy and delayed reduced dose cranial radiation: a report from the Children’s Oncology Group study AALL02P2. Pediatr Blood Cancer. 2021;68:12.

51. Brown PA, Ji L, Xu X, Devidas M, Hogan L, Bhatia T, et al. A randomized phase 3 trial of blinatumomab vs chemotherapy as post-reinduction therapy in low risk (LR) first relapse of B-acute lymphoblastic leukemia (B-ALL) in children and adolescents/young adults (AYAs): a report from Children’s Oncology Group Study. Blood. 2021;138:363.

52. Locatelli F, Zugmaier G, Rizzi C, Morris JD, Grunh G, Klingebiel T, et al. Effect of blinatumomab vs chemotherapy on event-free survival among children with high-risk first-relapse B-cell acute lymphoblastic leukemia: a Randomized Clinical Trial. JAMA. 2021;325:843–54.

53. Gersheim E, Kalathil S, Gharbio M. Prolonged survival of acute lymphoblastic leukemia with intrathecal treatments for isolated central nervous system relapse. Case Rep Hematol. 2018;2018:8765285.

54. Jaime-Pérez JC, Rodríguez-Romo LN, Gómez-Llano O, Chapa-Rodriguez A, Gómez-Almagra D. Effectiveness of intrathecal rituximab in patients with acute lymphoblastic leukaemia relapsed to the CNS and resistant to conventional therapy. Br J Haematol. 2009;144:794–5.

55. Levinsen M, Harila-Saari A, Grell K, Jonsson OG, Taskinen M, Abrahamsson J, et al. Submicroscopic bone marrow involvement in isolated extramedullary disease: results of the Children’s Oncology Group Study AALL02P2. J Cancer. 2015;62:951–5.

56. Martinez-Laperche C, Gómez-García AM, Lassaletta Á, Moscardó C, Vivanco JL, Gómez-Almaguer D. Effect of intrathecal liposomal cytarabine in children with acute lymphoblastic leukaemia relapsed to the CNS and resistant to conventional therapy. Br J Haematol. 2014;164:1252–307.

57. Levinsen M, Marquart HV, Groth-Pedersen L, Abrahamsson J, Albanek BS, Andersen MK, et al. Leukemic blasts are present at low levels in spinal fluid in one-third of childhood acute lymphoblastic leukaemia cases. Pediatr Blood Cancer. 2016;63:1935–42.

58. Martínez-Laperche C, Gómez-García AM, Lassaletta Á, Moscardó C, Vivanco JL, Molina J, et al. Detection of occult cerebrospinal fluid involvement during maintenance therapy identifies a group of children with acute lymphoblastic leukemia at high risk for relapse. Am J Hematol. 2013;88:359–64.

59. Ranta S, Nilsson F, Harila-Saari A, Saft L, Tani E, Söderhäll S, et al. Detection of central nervous system involvement in childhood acute lymphoblastic leukemia by cytomorphology and flow cytometry of the cerebrospinal fluid. Pediatr Blood Cancer. 2015;62:951–6.

60. Gabeli M, Disarò S, Scarpa P, Francescato S, Zangrando A, Valsecchi MG, et al. Cerebrospinal fluid analysis by B-color flow cytometry in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2019;60:2825–8.

61. Bürger B, Zimmermann M, Mann G, Kühl J, Löning L, Reinh M, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. J Clin Oncol. 2005;23:1848–54.
2766

88. Zhang H, Luo X-Q, Zhang P, Huang L-B, Zheng Y-S, Wu J, et al. MicroRNA

80. Mo F, Ma X, Liu X, Zhou R, Zhao Y, Zhou H. Altered CSF proteomic pro

76. Scrideli C, De Paula Queiroz R, Takayanagui O, Bernardes E, Melo E, Tone L. Moleculare detection of leukemic cerebrospinal fluid cells in children with newly diagnosed acute lymphoblastic leukemia. Haematologica. 2004;89:1035–103.

75. Yousafzai YM, Smith L, Smith A, Bhatti S, Gardiner M, Cousins A, et al. Use of quantitative polymerase chain reaction (qPCR) for the diagnosis and monitoring of CNS leukaemia. Leuk Res. 2019;87:102632.

81. Ghosh MC, Grass L, Soosaipillai A, Sotiropoulou G, Diamandis EP. Human kal-10. Wang Y, Springer S, Zhang M, McMahon KW, Kinde I, Dobbyn L, et al. Detection of tumor-derived DNA in cerebrospinal fluid of children with acute lymphoblastic leukemia by real-time polymerase chain reaction. J Mol Diagnostics. 2005;7:127–32.

Yousafzai YM, Smith L, Smith A, Bhatti S, Gardner M, Cousins A, et al. Use of quantitative polymerase chain reaction (qPCR) for the diagnosis and monitoring of CNS leukaemia. Leuk Res. 2019;87:102632.

Biojone E, Queronz RDP, Valera ET, Odashima NS, Takayanagui OM, Viana MB, et al. Minimal residual disease in cerebrospinal fluid at diagnosis: a more intense treatment protocol was able to eliminate the adverse prognosis in children with acute lymphoblastic leukemia. Leuk Lymphoma. 2012;53:89–95.

Prilda GM, Foster MW, Deal AM, Richardson BM, Thompson JW, Blatt J. Cere-}

90. Li, Zhao L, Zhang Y, Xiao Y, Wang D, Huang L, et al. CAR T-cell therapy is effective but not long-lasting in B-cell lymphoma of the brain. Front Oncol. 2020;10:1306.

91. Tan Y, Pan J, Deng B, Ling Z, Song W, Xu J, et al. Toxicity and effectiveness of CD19 CAR T therapy in children with high-burden central nervous system relapse. Br J Haematol. 2021;70:1979–93.

92. Hu K, Wang Y, Teng X, Hu Y, Huang C. Cell subsets and cytokine dynamics in cerebrospinal fluid after CAR-T cell therapy for B-cell acute lymphoblastic leu-

93. Leukemia (2022) 36:2751 – 2768

94. Leukemia (2022) 36:2751 – 2768

95. Lenk L, Carlet M, Vogiatzi F, Spory L, Winterberg D, Cousins A, et al. CD79a enhances CNS-infiltration and leukemia engraftment in pediatric B-cell pre-

96. Leahy AB, Newman H, Li Y, Liu H, Myers R, D’Inofia A, et al. CD19-targeted chimeric antigen receptor T-cell therapy for CNS relapsed or refractory acute lymphocytic leukemia: a post-hoc analysis of pooled data from five clinical trials. Lancet Haematol. 2021;8:e711–e22.

97. Fabrizio VA, Phillips CL, Lane A, Baggott C, Prabhu S, Egerle E, et al. Tisagenle-}

98. Pfeiffer C, Strube S, Alsadeq A, Fedders H, Vokuhl C, et al. Mer tyrosine kinase promotes the survival of t(1;19)-positive acute lymphoblastic leukemia. Cancer. 2019;125:1660–69.

99. Krause S, Pfeiffer C, Strube S, Alsadeq A, Fedders H, Vokuhl C, et al. Involvement of the central nervous system in relapsed/refractory extramedullary ALL: a Pediatric Real World CAR Consortium Report. Blood Adv. 2022;6:100–0.

100. Hu K, Wang Y, Teng X, Hu Y, Huang C. Cell subsets and cytokine dynamics in cerebrospinal fluid after CAR-T cell therapy for B-cell acute lymphoblastic leu-

101. Spectro R, Robert Snodgrass S, Johanson CE. A balanced view of the cerebro-

102. Williams MTS, Yousafzai Y, Cox C, Blair A, Camody R, Sal M, et al. Interleukin-15 enhances cellular proliferation and upregulates CNS homing molecules in pre-B acute lymphoblastic leukemia. Blood. 2014;123:3116–27.

103. Alsadeq A, Fedders H, Vokuhl C, Belau NM, Zimmermann M, Wirbelauer T, et al. The role of ZAP70 kinase in acute lymphoblastic leukemia infiltration into the central nervous system. Cancer Metastasis Rev. 2017;36:346–55.

104. Lenk L, Matheson EC, Minto L, Blair H, Case M, Halsey C, et al. Ras pathway mutations are prevalent in relapsed childhood acute lymphoblastic leukemia and confer sensitivity to MEK inhibition. Blood. 2014;124:3420–30.

105. Matheson EC, Thomas H, Case M, Blair H, Jackson RK, Masic D, et al. Gluco-

106. Gocho Y, Liu J, Hu J, Yang W, Dharia NV, Zhang J, et al. Network-based systems pharmacology reveals heterogeneity in LCK and BCL2 signaling and therapeutic sensitivity of T-cell acute lymphoblastic leukemia. Nat Cancer. 2021;2:284–99.

107. Matheson EC, Thomas H, Case M, Blair H, Jackson RK, Masic D, et al. Gluco-

108. Yuzhe S, Melanie CB, Helen JB, Ricky T, Sinrintra N, Amir E, et al. Phase II-like murine trial identifies synergy between dexamethasone and dasatinib in T-cell acute lymphoblastic leukemia. Haematologica. 2020;106:1056–66.

109. Sharma ND, Keewan EA, Matlawiska-Wasowska K. Metabolic reprogramming and cell adhesion in acute leukemia adaptation to the CNS niche. Front Cell Develop Biol. 2021;9:78510.

110. Vannier RJ, Dobson SM, Gan OI, McLeod J, Schoof EM, Grandal I, et al. Multimolecular profiling of central nervous system leukemia identifies mRNA translation as a therapeutic target. Blood Cancer Dis. 2022;3:16–31.

111. Krause S, Pfeiffer C, Strube S, Alsadeq A, Fedders H, Vokuhl C, et al. Mer tyrosine kinase promotes the survival of t(1;19)-positive acute lymphoblastic leukemia. Cancer. 2019;125:1660–69.

112. Van Der Velden VHJ, De Launaij D, De Vries JF, De Haas V, Sonneveld E, Voerman E. Treatment of pediatric acute lymphoblastic leukemia with central nervous system involvement. Bone Marrow Transplant. 2021;56:3088–90.

113. Vanner RJ, Dobson SM, Gan OI, McLeod J, Schoof EM, Grandal I, et al. Multimolecular profiling of central nervous system leukemia identifies mRNA translation as a therapeutic target. Blood Cancer Dis. 2022;3:16–31.

114. Krause S, Pfeiffer C, Strube S, Alsadeq A, Fedders H, Vokuhl C, et al. Mer tyrosine kinase promotes the survival of t(1;19)-positive acute lymphoblastic leukemia. Cancer. 2019;125:1660–69.

115. Vannier RJ, Dobson SM, Gan OI, McLeod J, Schoof EM, Grandal I, et al. Multimolecular profiling of central nervous system leukemia identifies mRNA translation as a therapeutic target. Blood Cancer Dis. 2022;3:16–31.
