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Evaluation and management of measurable residual disease in acute lymphoblastic leukemia

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***Abstract*:** With standard chemotherapy regimens for adults with acute lymphoblastic leukemia, approximately 90% of patients achieve complete remission. However, up to half of patients have persistent minimal/measurable residual disease (MRD) not recognized by routine microscopy, which constitutes the leading determinant of relapse. Many studies in pediatric and adult populations have demonstrated that achievement of MRD negativity after induction chemotherapy or during consolidation is associated with significantly better long-term outcomes, and MRD status constitutes an independently prognostic marker, often superseding other conventional risk factors. Persistence of MRD after intensive chemotherapy is indicative of treatment refractoriness and warrants alternative therapeutic approaches including allogeneic stem cell transplantation, blinatumomab, or investigational therapies such as inotuzumab ozogamicin or chimeric antigen receptor T cells. Furthermore, the incorporation of novel monoclonal antibodies or potent BCR-ABL1 tyrosine kinase inhibitors, such as ponatinib into frontline treatment may have the advantage of achieving higher rates of MRD negativity while minimizing chemotherapy-related toxicities. Many studies are therefore ongoing to determine whether this strategy can improve cure rates without the need for allogeneic stem cell transplantation.

Keywords: acute lymphoblastic leukemia, minimal residual disease, risk stratification

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Introduction

The outcomes of adults with acute lymphoblastic leukemia (ALL) have dramatically improved with the use of multiagent chemotherapy. After standard multiagent chemotherapy, approximately 90% of patients achieve complete remission (CR), which is defined as the presence of less than 5% blasts on routine microscopy and adequate peripheral blood count recovery.[1,2] Despite these high remission rates, relapses still commonly occur. These relapses, which constitute the major cause of death in adults with ALL, are due to the persistence of leukemic blasts that generally exhibit resistance to cytotoxic chemotherapy and are present at low levels, making them undetectable by conventional pathologic assessment. With the use of sensitive technologies such as multiparameter flow cytometry (MFC) and polymerase chain reaction (PCR), persistent leukemia cells can be detected in approximately 30–50% of patients who achieve CR.[3,4] These persistent leukemia cells in the setting of CR are referred to as measurable (also called 'minimal') residual disease (MRD), which reflects the remaining disease burden after initial therapy, thus informing about chemosensitivity and treatment efficacy. MRD is highly prognostic in all ALL subtypes, including B- and T-cell lineages and in Philadelphia chromosome (Ph)-negative and Ph-positive disease. Across ALL subtypes, methods of MRD assessment, treatment regimens and other contexts, the detection of MRD after initial treatment nearly universally correlates with poorer relapse-free survival (RFS) and

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Table 1. Methods of measurable residual disease assessment.

Method	Advantages	Limitations	Sensitivity
Multiparameter flow cytometry • **Comparison of baseline and remission LAIPs** • **DfN method**	- Rapid - Sensitive - Relatively inexpensive - Ability to quantify antigen expression for targeted agents - Does not require access to pretreatment specimen (DfN method only)	- Lack of standardization - Need for significant technical expertise - Requires fresh cells - Risk of immunophenotypic shifts and false-negative results - Difficulty to differentiate malignant lymphoblasts from hematogones	10^{-4} (0.01%)
Quantitative PCR for IG/TCR rearrangements	- Sensitive - Standard guidelines for application and interpretation (Euro-MRD)	- Time-consuming - Labor intensive - Requires pretreatment sample - Expensive - May not be accurate for early T-cell precursor ALL	10^{-4} to 10^{-5} (0.01–0.001%)
Quantitative PCR for gene fusions (e.g. *BCR-ABL1*)	- Sensitive - Simple (uses same standard primers as used for diagnosis)	- Applicable to $<$ 50% of ALL cases	10^{-4} to 10^{-5} (0.01–0.001%)
NGS	- Ultrasensitive - Fast - Can detect multiple clones and track clonal evolution - Only US FDA-approved assay (ClonoSEQ)	- Lack of standardization - Requires pretreatment sample - Expensive - Minimal clinical validation	10^{-6}

ALL, acute lymphoblastic leukemia; DfN, different from normal; FDA, Food and Drug Administration; IG, immunoglobulin; LAIP, leukemia-associated immunophenotypes; NGS, next-generation sequencing; PCR, polymerase chain reaction; TCR, T-cell receptor.

overall survival (OS).[5,6] Besides its prognostic importance, knowledge of MRD status can influence treatment strategies, such as informing the switch to blinatumomab, with or without subsequent consolidative allogeneic stem cell transplantation (alloSCT).[7] Herein, we review the evaluation, prognostic impact, and management of MRD in adult patients with ALL.

Evaluation of MRD

Several different laboratory methods exist that are capable of detecting and quantifying MRD in ALL.[8,9] The most commonly used techniques are MFC by detection of immunophenotypic aberrancy on leukemia blasts and real-time quantitative polymerase chain reaction (RQ-PCR) by the analysis of rearranged immunoglobulin (IG) or T-cell receptor (TCR) genes or of recurrent gene fusions (e.g. *BCR-ABL1*).[10] Even more sensitive and accurate techniques have also been recently introduced, including 10-color flow cytometry, droplet digital PCR, and high-throughput next-generation sequencing (NGS).[11] The main advantages and disadvantages of the commonly used methods of MRD assessment are summarized in Table 1.

Multiparametric flow cytometry

MFC is a fast and relatively inexpensive procedure that is broadly applicable to nearly all ALL cases. Standard MFC assays can detect residual leukemia cells in 1 out of 10,000 cells (10^{-4} or 0.01%) by relying on assessment of aberrant leukemia-associated immunophenotypes (LAIPs), which constitute either aberrant expression of myeloid antigens or increased or decreased density of antigens normally expressed on benign B-cell precursors. A higher sensitivity of 10^{-5} can be reached with the use of some $\geq$8-color flow cytometry assays, which can detect abnormal population at very low levels ($\leq$0.0002%) when a sufficient number of cells are acquired (2 to 5 million nucleated cells).[12–14] The precision in detecting LAIPs can be improved by using different combinations of fluorochromes that can simultaneously recognize multiple antigens for better sensitivity. A better combination of markers

may contribute to improved flow cytometric MRD detection in patients with B-cell ALL, particularly by differentiating leukemia cells from regenerative blasts. For example, one group has shown that a single eight-color tube consisting of CD9, CD10, CD19, CD20, CD34, CD38, CD45, and CD58 could provide as much diagnostic utility as compared with a previously used three-tube panel with 12 markers.[15]

By one MFC-based method, all LAIPs detected on leukemic blasts at time of diagnosis are assessed over the course of therapy and constitute MRD when still detectable in the remission sample. Another approach called the 'different-from-normal' (DfN) method relies on the difference in immunophenotypes present in the remission sample as compared with a highly stereotypical normal immunophenotype distribution.[16] This method has the advantage of not necessarily requiring an initial diagnostic sample and can also assess MRD regardless of any immunophenotypic changes that occur over the course of therapy.[10,16] However, there may be less certainty with this approach if information about diagnostic LAIPs is not available, and therefore even in laboratories were the DfN approach is used, baseline LAIPs are generally also used for comparison (when available). A combined 'LAIP-based DfN' approach has been advocated by some groups to evaluate MRD in acute myeloid leukemia, and this approach is also used in some laboratories when assessing ALL MRD.[17] Regardless of the specific method used, a major downside of MFC for MRD assessment is the challenge of standardization across laboratories and pathologists. Furthermore, significant expertise and knowledge of antigen expression patterns seen during differentiation and maturation of normal hematopoietic progenitors in both resting and regeneration states is needed to properly analyze the resultant data, particularly when the DfN method is used. Conversely, when MFC is used to compare LAIPs between diagnostic and remission samples, immunophenotypic shift that may occur as the result of therapy can also decrease the accuracy of this approach, potentially leading to false-negative results by MFC.

Real-time quantitative polymerase chain reaction

RQ-PCR is another standard method that is used for MRD detection and quantification. MRD targets for PCR involve rearranged immunoglobulin IG or TCR genes in Ph-negative B-cell ALL and T-cell ALL, while in Ph-positive ALL, *BCR-ABL1* mRNA transcripts are the preferred MRD marker. Other gene fusions involving *MLL* or *CRLF2* may also be used as targets in other subtypes of ALL, although there are few clinical data currently available to support their utility as reliable MRD markers. For patients with Ph-negative B-cell ALL or T-cell ALL, several studies have demonstrated a high concordance rate between MFC and PCR-based assays.[18–20] The choice between these two methods therefore largely depends on the level of expertise and availability in different laboratories.[18,19,21] MFC is widely used in hospitals and centers in the United States (US), as standardized allele specific oligonucleotide (ASO) PCR is generally not available. In contrast, there have been intense efforts to standardize ASO-based RQ-PCR in European countries, where the MRD assay is commonly used.[8]

In Ph-negative B-cell ALL and T-cell ALL, RQ-PCR analyzes unique sequences of the junctional regions of rearranged *IG* or *TCR* genes for which ASOs are specifically designed for each patient. Primers identified at diagnosis are then applied to subsequent post-therapy samples in order to quantify MRD.[22] This approach can be applied to 90–95% of patients with ALL.[8] In Europe, this process is standardized by international collaboration by the Euro-MRD group; however, there is no such standardization in the US, and therefore ASO-PCR is not used in clinical practice. Despite higher sensitivity compared with MFC (down to 10^{-5}), ASO-PCR is a time-consuming procedure, costly, and highly complex, requiring extensive knowledge and experience. Moreover, in early precursor T-ALL, it is difficult to monitor MRD by ASO-PCR, because the lymphoblasts are immature and often have not undergone *TCR* rearrangement.[23]

In Ph-positive ALL, the *BCR-ABL1* gene translocation is a reliable PCR target. Using reverse transcriptase PCR (RT–PCR), MRD is followed by quantification of *BCR-ABL1* mRNA transcripts with the same standard probes used for diagnostic purposes in Ph-positive leukemia.[24] This technique is simple, rapid, and broadly applicable. Droplet digital PCR is a relatively new technique that may have utility in Ph-positive ALL, with some early studies suggesting that it may be more sensitive than standard RQ-PCR.[25,26]

Next-generation sequencing

High-throughput NGS is a novel method in MRD detection in ALL that can overcome some of the limitations of standard methods. The targets are the same leukemia-specific rearranged *IG* and *TCR* genes analyzed by ASO-PCR. However, NGS has the capability of simultaneously amplifying multiple combinations of rearranged IG and TCR genes by multiplex PCR without the need of patient-specific probes. It can therefore identify and quantify multiple clones and subclones that can be tracked over the course of therapy, although the clinical utility of this theoretical advantage has yet to be robustly proven.[27,28] Another advantage of NGS is the achievement of very high levels of sensitivity based on dilution experiments, detecting as few as 1 leukemic cell in 1,000,000 nucleated cells (i.e. sensitivity of 10^{-6}), although only a few patients actually had MRD detectable at the 10^{-6} level in these studies.[29] NGS is relatively rapid (around 1 week for one sample) and reliable, with high concordance with standard MFC or PCR techniques.[29–31] Despite the higher sensitivity of NGS, the prognostic significance of MRD at very low levels is unclear. Whether these very low levels of MRD should prompt any changes in therapeutic decision is largely unknown, and to date, only a few relatively small clinical studies of NGS-based MRD in ALL have been published.[30,32,33] However, given the high sensitivity of this approach, the clonoSEQ NGS technology (Adaptive Biotechnologies, Seattle, WA, USA) was recently the first MRD assay to be approved by the US Food and Drug Administration (FDA).[34]

Prognostic impact of MRD

While historically ALL was risk-stratified using baseline characteristics such as white blood cell count, immunophenotype, and cytogenetics, MRD information outweighs many of these traditional prognostic factors, and is often the strongest independent predictor of outcomes.[4,35–42] A meta-analysis involving 13,637 children and adults demonstrated the benefit of MRD negativity across disease subtypes (e.g. Ph-negative and Ph-positive, B-lineage and T-lineage), therapies, methods, timing of MRD assessment, and MRD cut-offs. In adults, the 10-year event-free survival (EFS) for patients who achieved MRD negativity was 64% compared with 21% for those with detectable MRD [hazard ratio (HR), 0.28; 95% confidence interval (CI): 0.24–0.33]. A significant OS benefit to achieving MRD negativity was also observed in children (HR, 0.28; 95% CI: 0.19–0.41) and adults (HR, 0.28; 95% CI: 0.20–0.39).[6] A subsequent meta-analysis of 23 published articles reporting on MRD in adults with B-cell ALL confirmed an overall improvement in both RFS and OS with random effects HRs of 2.44 (95% CI: 1.91–2.86) and 2.19 (95% CI: 1.63–2.94), respectively, for patients achieving MRD negativity.[5]

Different levels of detectable MRD may also have prognostic value, and risk of relapse is proportional to the quantity of MRD in several studies. For example, in one study, patients with lower detectable MRD (between 10^{-4} and 10^{-3}) by either ASO-PCR or MFC had significantly longer duration of remission, RFS and OS than those with very high MRD ($\geq 10^{-1}$).[43] While the consensus for what constitutes clinically relevant MRD response is generally defined as the achievement of a level below 10^{-4}, patients with detectable MRD have variable outcomes based on the quantity of MRD, with the best outcomes seen with early achievement of absence of any detectable residual disease.[7]

More recent reports have also suggested that coupling MRD information with different ALL molecular subtypes may improve prediction of relapse. In patients with Ph-negative ALL, high-risk genetics are independently associated with poorer outcomes and a high rate of MRD persistence after initial therapy.[44] In one study, the presence of *IZKF1* gene deletion or *MLL* gene rearrangement was associated with increased risk of relapse in B-cell ALL and a genetic profile defined as the absence of *NOTCH1*/*FBXW7* mutation or the presence of *NRAS*/*KRAS* mutation or *PTEN* alteration was also associated with worse outcomes in T-cell ALL.[39] Both molecular–genetic features and MRD status were independently associated with relapse and survival, suggesting that both should be incorporated into risk stratification. Other studies have similarly showed that cytogenetic features, such as complex karyotype (defined as ≥ 5 chromosomal abnormalities) or low hypodiploidy/near triploidy, are associated with poor outcomes regardless of the MRD status.[45] Thus, while MRD negativity is desirable in all cases, it does not appear to override the negative prognostic impact of these adverse-risk genomic alterations.

Future prospective studies are needed to determine how to fully incorporate genetic profiling, cytogenetics, and MRD status into risk stratification schemes that can inform therapeutic decision-making.

Ph-positive ALL

MRD is also highly prognostic in patients with Ph-positive ALL. In adults with Ph-positive ALL, detection of MRD measured by RT–PCR of *BCR-ABL1* transcripts is associated with worse outcomes.[46–49] In one study, patients who received chemotherapy and tyrosine kinase inhibitor (TKI) and achieved a complete molecular response (CMR; defined as absence of a quantifiable *BCR-ABL1* transcript by RT–PCR) after approximately 3 months of treatment had excellent long-term OS of 66% at 4 years in the absence of alloSCT.[50] Achievement of CMR was the only factor independently prognostic for OS. These data raise questions as to whether assessment of MRD can identify patients with Ph-positive ALL who do not require alloSCT in first remission. Integration of genomic features (e.g. *IKZF1* or *CDKN2A/B* deletions) into this assessment may further improve our prognostication.[49,51]

Pre- and post-transplant MRD

In patients undergoing alloSCT, both pre- and post-transplant MRD predict for higher risk of post-transplant relapse.[52–58] In one study, children achieving MRD negativity before alloSCT had better disease-free survival (DFS) and OS than those with persistent MRD (DFS of 83% *versus* 41%; OS of 92% *versus* 64%, respectively, $p < 0.0001$ for both).[53] In another prospective study of children with relapsed ALL, pre-alloSCT MRD was also prognostic in this context.[52] Similarly, in adults, pre-transplant MRD at a level $\geqslant 10^{-4}$ as measured by NGS was predictive of post-transplant relapse (HR 7.7, 95% CI: 2.0–30, $p = 0.003$).[55] Conversely, MRD reappearance after initial chemotherapy or alloSCT is also a sign of impending leukemia relapse.[54,55,59–61] After chemotherapy, 60–80% of patients with MRD recurrence experience hematologic relapse after a median of 3 months.[59,62] Among patients who received alloSCT in the NILG study, those with detectable MRD post-transplant (day +100) had a relapse risk of 80% compared with only 7% to those with undetectable MRD ($p = 0.0006$).[54]

Relapsed or refractory ALL

While there is evidence that MRD is highly prognostic in newly diagnosed ALL, its impact and how this information should guide therapy is less clear in the relapse/refractory (R/R) setting. In studies evaluating single novel agents (e.g. blinatumomab and inotuzumab ozogamicin) in R/R ALL, achievement of MRD responses was associated with lower rates of relapse.[63,64] In a single-arm phase II study of 36 patients with R/R B-cell ALL treated with blinatumomab, 69% of patients achieved MRD response defined as MRD $< 10^{-4}$ by ASO-PCR, which was associated with a 67% reduction in relapse risk.[63] In the INO-VATE trial comparing inotuzumab ozogamicin *versus* combination chemotherapy for patients with R/R B-cell ALL, MRD negativity (defined as $< 10^{-4}$ by MFC) was achieved in 63% of patients in the inotuzumab ozogamicin arm, and MRD response was associated with prolongation of both progression-free survival and OS compared with MRD nonresponders (median progression-free survival: 8.6 *versus* 5.4 months, $p < 0.0001$; median OS: 14.1 *versus* 7.2 months, $p = 0.009$).[64] Some data also suggest that the significance of MRD negativity may be more pronounced in first salvage than in later salvages. In a study involving 130 patients with R/R ALL, it was demonstrated that MRD negativity by MFC at the time of best response was associated with significant better EFS for patients treated in first salvage (median 18 *versus* 7 months, $p = 0.06$), but not in second salvage and beyond.[65] Patients who achieved MRD negativity after their first salvage and subsequently underwent alloSCT had the best outcomes, with a 2-year OS rate of 65%.[65]

Management of MRD

MRD status is increasingly used not only for risk classification and prediction, but also for post-remission treatment decision-making.[7] By tailoring treatment strategies based on MRD status, patients at higher risk of relapse may receive risk-adapted, novel therapies such as the CD3-CD19 bispecific T-cell engager blinatumomab or the anti-CD22 antibody–drug conjugate inotuzumab ozogamicin, with or without subsequent alloSCT. Conversely, patients at lower risk of relapse (e.g. those without baseline adverse-risk genomic features who rapidly achieve MRD negativity with standard therapy) may benefit from treatment de-escalation and not undergoing alloSCT in first

remission, thereby potentially sparing them from unnecessary treatment-related toxicities.[66,67]

Allogeneic stem cell transplantation

Several studies have suggested that alloSCT in first CR is associated with lower risk of relapse and longer survival in patients with ALL who achieve a suboptimal MRD response.[68,69] In the German Multicenter ALL Study Group (GMALL 07/03 trial), patients with persistent MRD ($\geq 10^{-4}$) measured by RQ-PCR of leukemia-specific IG and TCR gene rearrangements after first consolidation (week 16) were considered high risk for relapse and were offered alloSCT. Overall, 47% of patients with MRD persistence received alloSCT in first CR (alloSCT rates: 71% in the high-risk group and 39% in the standard-risk group). The 5-year continuous CR was significantly higher for patients who received alloSCT in first CR compared with those with chemotherapy alone (66% *versus* 12%, $p < 0.0001$), which also translated into better 5-year OS (54% *versus* 33%, respectively, $p = 0.06$).[4] In contrast, patients who achieved MRD negativity at week 16 had 5-year continuous CR and 5-year OS rates of 74% and 81%, respectively, in the absence of alloSCT. In the PETHEMA ALL-AR-03 prospective trial, adolescents or adults with high-risk Ph-negative ALL based on at least one high-risk disease feature (i.e. age between 30 and 60 years, white blood cells $> 30\times10^9$/l, or t(4;11) or other *MLL* rearrangements) were assigned to post-remission therapies based on early cytologic response (<10% blasts in bone marrow at day 14 of induction) and MRD status. Patient with favorable cytologic and MRD response continued to receive chemotherapy alone ($n = 108$) and those with poor cytologic response or suboptimal MRD response ($n = 71$) were assigned to receive alloSCT. The 5-year DFS and OS were 32% and 37%, respectively, for patients assigned to alloSCT, and 55% and 59% for those assigned to chemotherapy.[35] Together, these studies suggest that MRD assessment after initial chemotherapy can be used to identify patients most likely to benefit from alloSCT in first remission, even among patients who appear otherwise high-risk based on pretreatment characteristics. They also highlight the relatively poor outcomes for patients with persistent MRD positivity, even when alloSCT is performed.

Nontransplant MRD-directed therapies

Novel monoclonal antibodies such as blinatumomab or inotuzumab ozogamicin are capable of inducing remissions in R/R B-cell ALL.[70–72] Blinatumomab is generally more effective in patients with lower burden of disease, making a particularly promising agent for the treatment of MRD.[73,74] In the open-label, single-arm phase II BLAST study, adult patients with B-cell ALL in CR but with persistent or recurrent MRD at a level of $\geq 10^{-3}$ after intensive chemotherapy received up to four cycles of blinatumomab. Among 116 patients, 78% achieved complete MRD response after the first cycle. Despite the inclusion of higher-risk patients (35% of patients in second or later remission and 47% with MRD levels $\geq 10^{-2}$), the 18-month RFS rate was 54% and the median OS was 36.5 months. Patients who achieved complete MRD response had significantly longer RFS (23.6 months *versus* 5.7 months, $p = 0.002$) and OS (38.9 months *versus* 12.5 months, $p = 0.002$) compared with MRD nonresponders.[75] Based on these results, blinatumomab was approved by the US FDA in March 2018 for the treatment of patients with B-cell ALL in CR but with detectable MRD at a level of $\geq 0.1\%$.[76]

Whether alloSCT should be routinely offered to patients who achieve MRD negativity after blinatumomab is an open question. Interestingly, in the BLAST study, 33% of patients who achieved MRD negativity did not receive any additional treatment after blinatumomab, and 25% of them remained in continuous CR after a median follow up of 24 months (range, 2.8–41.6 months), suggesting that a proportion of patients with MRD-positive disease who respond to MRD-directed therapy can achieve prolonged remission duration, or possibly cure, without the need of alloSCT.[75] In a *post hoc* analysis, there was no statistical difference in OS between transplanted and nontransplanted patients (odds ratio = 1.83; 95% CI: 0.69–4.9, $p = 0.24$), in part because 27% of transplanted patients died from transplant-related mortality. Overall, the balance of evidence suggests that proceeding with alloSCT after blinatumomab for MRD-positive disease is associated with decreased risk of relapse but increased risk of transplant-related mortality, with similar long-term survival whether or not subsequent alloSCT is performed.

Inotuzumab ozogamicin was also associated with higher MRD response rates and improved OS compared with conventional chemotherapy in a randomized phase III study of patients with R/R B-cell ALL.[64,72] For patients with morphological relapse, inotuzumab ozogamicin appears to be more effective than blinatumomab in inducing remissions (CR rates with or without full hematologic recovery: 81% *versus* 44% comparing across two randomized phase III trials).[70,71] However, the role of inotuzumab ozogamicin in eradicating MRD in patients with MRD-positive remission is not currently known. An ongoing clinical trial using inotuzumab ozogamicin for patients with B-cell ALL and persistent or recurrent MRD is currently enrolling (ClinicalTrials.gov identifier: NCT03441061).

Other immunotherapeutic strategies include the use of CD19-directed chimeric antigen receptor (CAR) T cells for MRD eradication. In a phase I trial, 53 adult patients with R/R B-cell ALL received autologous CD19 CAR T cells and achieved a CR rate of 83%. Patients with low disease burden (defined as $<5\%$ bone marrow blasts) had significantly longer EFS and OS compared with patients with higher disease burden (defined as $\geqslant 5\%$ bone marrow blasts or presence of extramedullary disease; median EFS: 10.6 *versus* 5.3 months, $p=0.01$; median OS: 20.1 *versus* 12.4 months, $p=0.02$, respectively).[77] These findings suggest that CAR T cells may play a particularly important role in the management of MRD-positive disease, where such therapy may be curative for a subset of patients.

Frontline approaches to eradicating MRD

Ultimately, eradicating MRD with frontline therapy is likely to lead to the best outcomes. Incorporation of novel monoclonal agents into the frontline chemotherapy is a very exciting strategy under investigation, with the aim of increasing initial MRD responses and reducing chemotherapy-related toxicities and the need for alloSCT. This strategy was first evaluated in older patients where the use of intensive chemotherapy was historically associated with very poor outcomes, primarily due to treatment-related toxicities, including infections from myelosuppression.[78] In one large retrospective study of older patients (above 60 years) receiving hyper-CVAD chemotherapy without modification, the induction mortality rate was 10% and the death rate in CR was 34%, leading to a 5-year OS rate of only 20%.[79] In an attempt to reduce toxicity and improve survival, a phase II trial evaluated the combination of reduced intensity chemotherapy (the mini-hyper-CVD regimen) with inotuzumab ozogamicin in older patients with newly diagnosed Ph-negative B-cell ALL. The overall response rate was 98% and the rate of MRD negativity by MFC after one cycle of chemotherapy was 80%, with no early treatment-related deaths. The 3-year OS rate was 54%, which compares very favorably with historical outcomes with both intensive and low-intensity regimens.[80,81] Preliminary analysis of a phase II trial of older adults with ALL receiving frontline blinatumomab followed by POMP maintenance reported an overall response rate of 66%, with MRD negativity achieved in 92% of responders. The estimated 1-year OS was 65%, with no reported treatment-related mortality.[82] In younger adults, an ongoing study is investigating the addition of blinatumomab after four cycles of intensive chemotherapy with hyper-CVAD in the frontline setting. Preliminary results showed very high response rate (100%) with MRD negativity in 93%, and a 1-year OS of 90% with acceptable toxicity profile.[83]

Ph-positive ALL. In Ph-positive ALL, the achievement of CMR is highly predictive of longer survival.[50] When combined with intensive chemotherapy, the use of the third-generation pan-BCR-ABL TKI ponatinib achieves a CMR rate of 78%, translating into better OS compared with regimens using earlier-generation TKIs such as imatinib or dasatinib. In a recent update of a phase II trial using the hyper-CVAD regimen with ponatinib for adults with newly diagnosed Ph-positive ALL, the 3-year OS was 76%, with only three patients relapsing while on ponatinib.[84] Using a propensity score analysis comparing results from two phase II trials, hyper-CVAD plus ponatinib was associated with a superior MRD response rate, RFS and OS compared with hyper-CVAD plus dasatinib.[85] The benefit of ponatinib in this setting is likely driven by the higher rate of CMR (78%) compared with that achieved with other TKIs (30–50%), which has been shown to strongly correlate with survival.[86,87] In patients undergoing alloSCT for Ph-positive ALL, the use of post-transplant TKI has also been recommended to reduce risk of relapse post-transplant,

particularly in patients with detectable MRD.[88] Blinatumomab is also effective in Ph-positive ALL in both the R/R setting and for MRD eradication.[75,89] Several ongoing studies are therefore evaluating chemotherapy-free frontline regimens with the combination of blinatumomab with TKIs (ponatinib, ClinicalTrials.gov identifier: NCT03263572; dasatinib, ClinicalTrials.gov identifiers: NCT02143414 and NCT02744768) with the goal of increasing MRD responses with acceptable toxicity, and decreasing the need for alloSCT in these patients. Re-emergence of MRD disease in Ph-positive ALL is indicative of impending relapse, mostly due to acquired *ABL1* mutations, although these mutations may be difficult to detect a low levels of MRD using standard approaches.[90] The use of blinatumomab in addition to ponatinib in this setting may be reasonable in order to cover potential resistance mutations in the setting of MRD-only disease.

Conclusion

Assessment of MRD status is enormously important in the management of patients with ALL, not only in risk stratification, but also to inform subsequent treatment strategies. The development of more sensitive MRD assays, including NGS, may allow for even better risk stratification, although how very small amounts of residual leukemia detected at a level of $<10^{-4}$ should influence treatment (if at all) is largely unknown at the present time. With the availability of highly effective ALL therapies, particularly blinatumomab, inotuzumab ozogamicin and CD19 CAR T cells, these agents are likely to increasingly play a role in MRD eradication, both for patients with MRD-only disease and through their incorporation into frontline regimens in order to render patients MRD-negative early in their treatment. While long-term data are still eagerly awaited, this strategy holds promise in reducing the need for myelosuppressive chemotherapy and subsequent alloSCT for many patients. Chemotherapy-free regimens incorporating these active agents into the frontline setting may also be a possibility in the near future, particularly for older patients. Given its close association with better long-term outcomes, achievement of MRD negativity is already being used as a surrogate endpoint in several clinical trials, and this approach should ultimately allow for even more rapid approval of effective, novel regimens to patients with ALL.

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References

1. Kantarjian H, Thomas D, O'Brien S, *et al.* Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer* 2004; 101: 2788–2801.
2. Sive JI, Buck G, Fielding A, *et al.* Outcomes in older adults with acute lymphoblastic leukaemia (ALL): results from the international MRC UKALL XII/ECOG2993 trial. *Br J Haematol* 2012; 157: 463–471.
3. Bassan R, Spinelli O, Oldani E, *et al.* Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood* 2009; 113: 4153–4162.
4. Gokbuget N, Kneba M, Raff T, *et al.* Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood* 2012; 120: 1868–1876.
5. Bassan R, Bruggemann M, Radcliffe HS, *et al.* A systematic literature review and meta-analysis of

minimal residual disease as a prognostic indicator in adult B-cell acute lymphoblastic leukemia. *Haematologica* 2019; 104: 2028–2039.

6. Berry DA, Zhou S, Higley H, *et al*. Association of minimal residual disease with clinical outcome in pediatric and adult acute lymphoblastic leukemia: a meta-analysis. *JAMA Oncol* 2017; 3: e170580.
7. Short NJ, Jabbour E, Albitar M, *et al*. Recommendations for the assessment and management of measurable residual disease in adults with acute lymphoblastic leukemia: a consensus of North American experts. *Am J Hematol* 2019; 94: 257–265.
8. van Dongen JJ, van der Velden VH, Brüggemann M, *et al*. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood* 2015; 125: 3996–4009.
9. Short NJ and Jabbour E. Minimal residual disease in acute lymphoblastic leukemia: how to recognize and treat it. *Curr Oncol Rep* 2017; 19: 6.
10. Chen X and Wood BL. How do we measure MRD in ALL and how should measurements affect decisions. Re: Treatment and prognosis? *Best Pract Res Clin Haematol* 2017; 30: 237–248.
11. Ladetto M, Bruggemann M, Monitillo L, *et al*. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia* 2014; 28: 1299–1307.
12. Wood B. 9-color and 10-color flow cytometry in the clinical laboratory. *Arch Pathol Lab Med* 2006; 130: 680–690.
13. Tembhare PR, Subramanian Pg PG, Ghogale S, *et al*. A high-sensitivity 10-color flow cytometric minimal residual disease assay in B-lymphoblastic leukemia/lymphoma can easily achieve the sensitivity of 2-in-10(6) and is superior to standard minimal residual disease assay: a study of 622 patients. *Cytometry B Clin Cytom* 2020; 98: 57–67.
14. Theunissen P, Mejstrikova E, Sedek L, *et al*. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood* 2017; 129: 347–357.
15. Shaver AC, Greig BW, Mosse CA, *et al*. B-ALL minimal residual disease flow cytometry: an application of a novel method for optimization of a single-tube model. *Am J Clin Pathol* 2015; 143: 716–724.
16. Wood BL. Principles of minimal residual disease detection for hematopoietic neoplasms by flow cytometry. *Cytometry B Clin Cytom* 2016; 90: 47–53.
17. Schuurhuis GJ, Heuser M, Freeman S, *et al*. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* 2018; 131: 1275–1291.
18. Gaipa G, Cazzaniga G, Valsecchi MG, *et al*. Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia. *Haematologica* 2012; 97: 1582–1593.
19. Ryan J, Quinn F, Meunier A, *et al*. Minimal residual disease detection in childhood acute lymphoblastic leukaemia patients at multiple time-points reveals high levels of concordance between molecular and immunophenotypic approaches. *Br J Haematol* 2009; 144: 107–115.
20. Thorn I, Forestier E, Botling J, *et al*. Minimal residual disease assessment in childhood acute lymphoblastic leukaemia: a Swedish multi-centre study comparing real-time polymerase chain reaction and multicolour flow cytometry. *Br J Haematol* 2011; 152: 743–753.
21. Malec M, van der Velden VH, Bjorklund E, *et al*. Analysis of minimal residual disease in childhood acute lymphoblastic leukemia: comparison between RQ-PCR analysis of Ig/TcR gene rearrangements and multicolor flow cytometric immunophenotyping. *Leukemia* 2004; 18: 1630–1636.
22. van der Velden VH, Cazzaniga G, Schrauder A, *et al*. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia* 2007; 21: 604–611.
23. Wu D, Sherwood A, Fromm JR, *et al*. High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. *Sci Transl Med* 2012; 4: 134ra63.
24. Pfeifer H, Cazzaniga G, van der Velden VHJ, *et al*. Standardisation and consensus guidelines for minimal residual disease assessment in Philadelphia-positive acute lymphoblastic leukemia (Ph + ALL) by real-time quantitative reverse transcriptase PCR of e1a2 BCR-ABL1. *Leukemia* 2019; 33: 1910–1922.
25. Coccaro N, Anelli L, Zagaria A, *et al*. Droplet digital PCR is a robust tool for monitoring minimal residual disease in adult Philadelphia-positive acute lymphoblastic leukemia. *J Mol Diagn* 2018; 20: 474–482.

26. Della Starza I, De Novi LA, Santoro A, *et al.* Digital droplet PCR and next-generation sequencing refine minimal residual disease monitoring in acute lymphoblastic leukemia. *Leuk Lymphoma* 2019: 1–3.

27. Theunissen PMJ, van Zessen D, Stubbs AP, *et al.* Antigen receptor sequencing of paired bone marrow samples shows homogeneous distribution of acute lymphoblastic leukemia subclones. *Haematologica* 2017; 102: 1869–1877.

28. Theunissen PMJ, de Bie M, van Zessen D, *et al.* Next-generation antigen receptor sequencing of paired diagnosis and relapse samples of B-cell acute lymphoblastic leukemia: clonal evolution and implications for minimal residual disease target selection. *Leuk Res* 2019; 76: 98–104.

29. Faham M, Zheng J, Moorhead M, *et al.* Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood* 2012; 120: 5173–5180.

30. Sala Torra O, Othus M, Williamson DW, *et al.* Next-generation sequencing in adult B cell acute lymphoblastic leukemia patients. *Biol Blood Marrow Transplant* 2017; 23: 691–696.

31. Brüggemann M, Kotrová M, Knecht H, *et al.* Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. *Leukemia* 2019; 33: 2241–2253.

32. Wood B, Wu D, Crossley B, *et al.* Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. *Blood* 2018; 131: 1350–1359.

33. Wu D, Emerson RO, Sherwood A, *et al.* Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. *Clin Cancer Res* 2014; 20: 4540–4548.

34. Monter A and Nomdedeu JF. ClonoSEQ assay for the detection of lymphoid malignancies. *Expert Rev Mol Diagn* 2019; 19: 571–578.

35. Ribera JM, Oriol A, Morgades M, *et al.* Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. *J Clin Oncol* 2014; 32: 1595–1604.

36. Bruggemann M, Raff T and Kneba M. Has MRD monitoring superseded other prognostic factors in adult ALL? *Blood* 2012; 120: 4470–4481.

37. Holowiecki J, Krawczyk-Kulis M, Giebel S, *et al.* Status of minimal residual disease after induction predicts outcome in both standard and high-risk Ph-negative adult acute lymphoblastic leukaemia. The Polish Adult Leukemia Group ALL 4-2002 MRD Study. *Br J Haematol* 2008; 142: 227–237.

38. Bruggemann M, Raff T, Flohr T, *et al.* Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood* 2006; 107: 1116–1123.

39. Beldjord K, Chevret S, Asnafi V, *et al.* Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. *Blood* 2014; 123: 3739–3749.

40. Mortuza FY, Papaioannou M, Moreira IM, *et al.* Minimal residual disease tests provide an independent predictor of clinical outcome in adult acute lymphoblastic leukemia. *J Clin Oncol* 2002; 20: 1094–1104.

41. Vidriales MB, Perez JJ, Lopez-Berges MC, *et al.* Minimal residual disease in adolescent (older than 14 years) and adult acute lymphoblastic leukemias: early immunophenotypic evaluation has high clinical value. *Blood* 2003; 101: 4695–4700.

42. Ravandi F, Jorgensen JL, O'Brien SM, *et al.* Minimal residual disease assessed by multi-parameter flow cytometry is highly prognostic in adult patients with acute lymphoblastic leukaemia. *Br J Haematol* 2016; 172: 392–400.

43. Gokbuget N, Dombret H, Giebel S, *et al.* Minimal residual disease level predicts outcome in adults with Ph-negative B-precursor acute lymphoblastic leukemia. *Hematology* 2019; 24: 337–348.

44. O'Connor D, Enshaei A, Bartram J, *et al.* Genotype-specific minimal residual disease interpretation improves stratification in pediatric acute lymphoblastic leukemia. *J Clin Oncol* 2018; 36: 34–43.

45. Issa GC, Kantarjian HM, Yin CC, *et al.* Prognostic impact of pretreatment cytogenetics in adult Philadelphia chromosome-negative acute lymphoblastic leukemia in the era of minimal residual disease. *Cancer* 2017; 123: 459–467.

46. Ravandi F, Jorgensen JL, Thomas DA, *et al.* Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. *Blood* 2013; 122: 1214–1221.

47. Xue YJ, Cheng YF, Lu AD, *et al.* Allogeneic hematopoietic stem cell transplantation, especially haploidentical, may improve long-term survival for high-risk pediatric patients with Philadelphia chromosome-positive acute lymphoblastic leukemia in the tyrosine kinase inhibitor era. *Biol Blood Marrow Transplant* 2019; 25: 1611–1620.

48. Wang L, Du J, Huang A, , *et al.*Chemotherapy vs. allogeneic transplantation as post molecular remission therapy in patients aged less than 60 years with Philadelphia-positive ALL. *Bone Marrow Transplant* 2020; 55: 245–248.

49. Li H, Zhang W, Kuang P, *et al.* Combination of IKZF1 deletion and early molecular response show significant roles on prognostic stratification in Philadelphia chromosome-positive acute lymphoblastic leukemia patients. *Leuk Lymphoma* 2018; 59: 1890–1898.

50. Short NJ, Jabbour E, Sasaki K, *et al.* Impact of complete molecular response on survival in patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood* 2016; 128: 504–507.

51. Pfeifer H, Raum K, Markovic S, *et al.* Genomic CDKN2A/2B deletions in adult Ph^+ ALL are adverse despite allogeneic stem cell transplantation. *Blood* 2018; 131: 1464–1475.

52. Bader P, Kreyenberg H, Henze GH, *et al.* Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol* 2009; 27: 377–384.

53. Sutton R, Shaw PJ, Venn NC, *et al.* Persistent MRD before and after allogeneic BMT predicts relapse in children with acute lymphoblastic leukaemia. *Br J Haematol* 2015; 168: 395–404.

54. Spinelli O, Peruta B, Tosi M, *et al.* Clearance of minimal residual disease after allogeneic stem cell transplantation and the prediction of the clinical outcome of adult patients with high-risk acute lymphoblastic leukemia. *Haematologica* 2007; 92: 612–618.

55. Logan AC, Vashi N, Faham M, *et al.* Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. *Biol Blood Marrow Transplant* 2014; 20: 1307–1313.

56. Bachanova V, Burke MJ, Yohe S, *et al.* Unrelated cord blood transplantation in adult and pediatric acute lymphoblastic leukemia: effect of minimal residual disease on relapse and survival. *Biol Blood Marrow Transplant* 2012; 18: 963–968.

57. Sanchez-Garcia J, Serrano J, Serrano-Lopez J, *et al.* Quantification of minimal residual disease levels by flow cytometry at time of transplant predicts outcome after myeloablative allogeneic transplantation in ALL. *Bone Marrow Transplant* 2013; 48: 396–402.

58. Zhao XS, Liu YR, Xu LP, *et al.* Minimal residual disease status determined by multiparametric flow cytometry pretransplantation predicts the outcome of patients with ALL receiving unmanipulated haploidentical allografts. *Am J Hematol* 2019; 94: 512–521.

59. Raff T, Gokbuget N, Luschen S, *et al.* Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. *Blood* 2007; 109: 910–915.

60. Sanchez J, Serrano J, Gomez P, *et al.* Clinical value of immunological monitoring of minimal residual disease in acute lymphoblastic leukaemia after allogeneic transplantation. *Br J Haematol* 2002; 116: 686–694.

61. Zhao XS, Liu YR, Zhu HH, *et al.* Monitoring MRD with flow cytometry: an effective method to predict relapse for ALL patients after allogeneic hematopoietic stem cell transplantation. *Ann Hematol* 2012; 91: 183–192.

62. Pemmaraju N, Kantarjian H, Jorgensen JL, *et al.* Significance of recurrence of minimal residual disease detected by multi-parameter flow cytometry in patients with acute lymphoblastic leukemia in morphological remission. *Am J Hematol* 2017; 92: 279–285.

63. Zugmaier G, Gokbuget N, Klinger M, *et al.* Long-term survival and T-cell kinetics in relapsed/refractory ALL patients who achieved MRD response after blinatumomab treatment. *Blood* 2015; 126: 2578–2584.

64. Jabbour E, Gökbuget N, Advani AS, *et al.* Impact of minimal residual disease (MRD) status in clinical outcomes of patients with relapsed/refractory (R/R) acute lymphoblastic leukemia (ALL) treated with inotuzumab ozogamicin (InO) in the phase 3 INO-VATE trial. *J Clin Oncol* 2018; 36: 7013–7013.

65. Jabbour E, Short NJ, Jorgensen JL, *et al.* Differential impact of minimal residual disease negativity according to the salvage status in patients with relapsed/refractory B-cell acute

lymphoblastic leukemia. *Cancer* 2017; 123: 294–302.

66. Borowitz MJ, Devidas M, Hunger SP, *et al*. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood* 2008; 111: 5477–5485.

67. Vora A, Goulden N, Wade R, *et al*. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. *Lancet Oncol* 2013; 14: 199–209.

68. Dhedin N, Huynh A, Maury S, *et al*. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. *Blood* 2015; 125: 2486–2496; quiz 586.

69. Giebel S, Labopin M, Socie G, *et al*. Improving results of allogeneic hematopoietic cell transplantation for adults with acute lymphoblastic leukemia in first complete remission: an analysis from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Haematologica* 2017; 102: 139–149.

70. Kantarjian H, Stein A, Gokbuget N, *et al*. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med* 2017; 376: 836–847.

71. Kantarjian HM, DeAngelo DJ, Stelljes M, *et al*. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med* 2016; 375: 740–753.

72. Kantarjian HM, DeAngelo DJ, Stelljes M, *et al*. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. *Cancer* 2019; 125: 2474–2487.

73. Topp MS, Kufer P, Gokbuget N, *et al*. Targeted therapy with the T-cell-engaging antibody blinatumomab of chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. *J Clin Oncol* 2011; 29: 2493–2498.

74. Topp MS, Gokbuget N, Zugmaier G, *et al*. Long-term follow-up of hematologic relapse-free survival in a phase 2 study of blinatumomab in patients with MRD in B-lineage ALL. *Blood* 2012; 120: 5185–5187.

75. Gokbuget N, Dombret H, Bonifacio M, *et al*. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. *Blood* 2018; 131: 1522–1531.

76. Hilal T and Prasad V. Eliminating MRD - FDA approval of blinatumomab for B-ALL in complete remission. *Nat Rev Clin Oncol* 2018; 15: 727–728.

77. Park JH, Rivière I, Gonen M, *et al*. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* 2018; 378: 449–459.

78. Short NJ, Kantarjian H, Jabbour E, *et al*. Novel therapies for older adults with acute lymphoblastic leukemia. *Curr Hematol Malig Rep* 2018; 13: 91–99.

79. O'Brien S, Thomas DA, Ravandi F, *et al*. Results of the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone regimen in elderly patients with acute lymphocytic leukemia. *Cancer* 2008; 113: 2097–2101.

80. Jabbour EJ, Sasaki K, Ravandi F, *et al*. Inotuzumab ozogamicin in combination with low-intensity chemotherapy (mini-HCVD) with or without blinatumomab versus standard intensive chemotherapy (HCVAD) as frontline therapy for older patients with Philadelphia chromosome-negative acute lymphoblastic leukemia: a propensity score analysis. *Cancer* 2019; 125: 2579–2586.

81. Kantarjian H, Ravandi F, Short NJ, *et al*. Inotuzumab ozogamicin in combination with low-intensity chemotherapy for older patients with Philadelphia chromosome-negative acute lymphoblastic leukaemia: a single-arm, phase 2 study. *Lancet Oncol* 2018; 19: 240–248.

82. Advani AS, Moseley A, O'Dwyer KM, *et al*. Results of SWOG 1318: a phase 2 trial of blinatumomab followed by pomp (Prednisone, Vincristine, Methotrexate, 6-Mercaptopurine) maintenance in elderly patients with newly diagnosed Philadelphia chromosome negative B-cell acute lymphoblastic leukemia. *Blood* 2018; 132: 33.

83. Richard-Carpentier G, Kantarjian HM, Short NJ, *et al*. A Phase II study of the hyper-CVAD regimen in sequential combination with blinatumomab as frontline therapy for adults with B-cell acute lymphoblastic leukemia (B-ALL). *Blood* 2018; 132: 32.

84. Jabbour E, Short NJ, Ravandi F, *et al*. Combination of hyper-CVAD with ponatinib as

first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: long-term follow-up of a single-centre, phase 2 study. *Lancet Haematol* 2018; 5: e618–e627.

85. Sasaki K, Jabbour EJ, Ravandi F, *et al.* Hyper-CVAD plus ponatinib versus hyper-CVAD plus dasatinib as frontline therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: a propensity score analysis. *Cancer* 2016; 122: 3650–3656.
86. Jabbour E, DerSarkissian M, Duh MS, *et al.* Efficacy of ponatinib versus earlier generation tyrosine kinase inhibitors for front-line treatment of newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. *Clin Lymphoma Myeloma Leuk* 2018; 18: 257–265.
87. Short NJ, Kantarjian H, Jabbour E, *et al.* Which tyrosine kinase inhibitor should we use to treat Philadelphia chromosome-positive acute lymphoblastic leukemia? *Best Pract Res Clin Haematol* 2017; 30: 193–200.
88. Giebel S, Czyz A, Ottmann O, *et al.* Use of tyrosine kinase inhibitors to prevent relapse after allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: a position statement of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Cancer* 2016; 122: 2941–2951.
89. Martinelli G, Boissel N, Chevallier P, *et al.* Complete hematologic and molecular response in adult patients with relapsed/refractory Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: results from a phase II, single-arm, multicenter study. *J Clin Oncol* 2017; 35: 1795–802.
90. Soverini S, De Benedittis C, Papayannidis C, *et al.* Drug resistance and BCR-ABL kinase domain mutations in Philadelphia chromosome-positive acute lymphoblastic leukemia from the imatinib to the second-generation tyrosine kinase inhibitor era: the main changes are in the type of mutations, but not in the frequency of mutation involvement. *Cancer* 2014; 120: 1002–1009.