Diagnostic Work-up, Prognostic Factors and Assessment of Adult Acute Lymphoblastic Leukemia. 2024 ELN Recommendations from a European Expert Panel

Supplementary Materials

Table S1: Distribution of Chapter to Coauthors

Paragraph	Author
1. Introduction / Methods	Gökbuget
2. Diagnostic Procedures and Classification	Chiaretti, Foa
3. Prognostic factors	Bassan
4. Response criteria and survival outcomes	Gökbuget
5. Treatment	
5.1. Induction and consolidation therapy	Bassan
5.2. Maintenance therapy	Doubek
5.3. CNS-Prophylaxis	Fielding
5.4. Minimal residual disease based treatment	Gökbuget
5.5. Stem cell transplantation	Giebel
6. Treatment of specific subgroups	
6.1. Adolescent patients	Boissel
6.2. Ph/BCR-ABL positive ALL	Ottmann, Rousselot, Martinelli
6.3. Ph/BCR-ABl-like ALL	Rijneveld
6.4. Treatment of older patients	Gökbuget
6.5. T-ALL	Marks
6.6. Lymphoblastic lymphoma	Hoelzer
7. Relapsed ALL	Ribera
8. Novel therapies	Dombret
9. Late effects	Gökbuget
10. Management of specific situations	Fielding
11. General setting and supportive care	Hunault
12. Summary and outlook	Gökbuget

Table S2: Procedures for Initial Workup

Test/procedure	General practice	Clinical trial
Tests to establish the diagnosis		
Complete blood counts and manual differential count	Yes	Yes
BM aspirate	Yes	Yes
BM trephine biopsy	Optional[e]	Optional[e]
Immunophenotyping	Yes	Yes
Cytogenetics	Yes	Yes
MRD analysis	Yes	Yes
Molecular genetics	Yes	Yes
Additional tests/procedures at diagnosis		
Demographics and medical history[a]	Yes	Yes
Performance status (ECOG/WHO score)	Yes	Yes
Assessment of comorbidities	Yes	Yes
Biochemistry, coagulation tests, urine analysis[b]	Yes	Yes
Serum pregnancy test[c]	Yes	Yes
Information on fertility preservation	Yesl[f]	Yesl[f]
Eligibility assessment for allogeneic HSCT	Yes	Yes
Hepatitis A, B, C; HIV-1 testing; HPV; EBV	Yes	Yes
Chest x-ray, 12-lead ECG; echocardiography (on indication)	Yes	Yes
Lumbar puncture	Yes	Yes
Biobanking[d]	Optional	Yes

[a] Including family history, prior exposure to toxic agents, prior malignancy, therapy for prior malignancy, information on smoking.
[b] *Biochemistry*: glucose, sodium, potassium, calcium, creatinine, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase, lactate dehydrogenase, bilirubin, urea, total protein, albumin, uric acid, total cholesterol, total triglycerides, creatinine phosphokinase (CPK). *Coagulation tests*: prothrombin time (PTT), international normalized ratio (INR) where indicated, activated partial thromboplastin time (aPTT). *Urine analysis*: pH, glucose, erythrocytes, leukocytes, protein, nitrite.
[c] In women with childbearing potential.
[d] Pretreatment leukemic BM and blood sample.
[e] Mandatory in patients with a dry tap (punctio sicca).
[f] In patients with childbearing potential and respective disease condition; Cryopreservation to be done in accordance with the wish of the patient.

Table S3: WHO Classification and International Consensus Classification[1,2]

WHO	ICC
Precursor B-cell neoplasms	
B-ALL/LBL	**B-ALL/LBL**
NOS	NOS
High hyperdiploidy	Hyperdiploid
Hypodiploidy	Low hypodiploid
	Near haploid
iAMP21	iAMP21
BCR::ABL1 Fusion	t(9;22)(q34.1;q11.2)/BCR::ABL1 - With lymphoid only involvement - With multilineage involvement
BCR::ABL1 Like	BCR::ABL1-like, ABL-1 class rearranged BCR::ABL1-like, JAK-STAT activated BCR::ABL1-like, NOS
KMT2A Rearrangement	t(v;11q23.3)/KMT2A rearranged
ETV6::RUNX1 Fusion	t(12;21)(p13.2;q22.1)/ETV6::RUNX1
TCF3::PBX1 Fusion	t(1;19)(q23.3;p13.3)/TCF3::PBX1
IGH::IL3 Fusion	t(5;14)(q31.1;q32.3)/IL3::IGH
TCF3:HLF Fusion	HLF rearrangement
ETV6::RUNX1-like	Provisional: ETV6:: RUNX1-like
Other genetic abnormalities	
	MYC rearrangement
	DUX4 rearrangement
	MEF2D rearrangement
	ZNF384(362) rearrangement
	NUTM1 rearrangement
	UBTF::ATXN7L3/PAN3,CDX2('CDX2/UBTF')
	Mutated IKZF1 N159Y
	Mutated PAX5 P580R
	Provisional: - PAX5 alteration - Mutated ZEB2 - ZNF384 rearranged-like - KMT2A rearranged-like
Precursor T-cell neoplasms	
T-ALL/LBL	**T-ALL/LBL**
NOS	**NOS**
ETP	- **ETP with BCL11B rearrangement** - **ETP, NOS**
	Provisional: - HOXA dysregulated - SPI1 rearrangement - TLX1 rearrangement - TLX3 rearrangement - NKX2 rearrangement - TAL1-2 rearrangement - LMP1-2 rearrangement - BHLH, other

Table S4. Standard and Innovative Methods for MRD Evaluation

Standardized methods for MRD monitoring							
Method	**Target**	**Applica-bility**	**Material**	**Quantifi-cation**	**Sensitivity**	**Advantages**	**Disadvantages**
Multicolor Flow Cytometry	Leukemia-associated immune-phenotypes	>90%	Cell suspension (PB, BM, needle aspirates)	Absolute	3-4 colors: 0.1-0.01% 6-8 colors: 0.01%	• Fast • Widely applicable • Single cell analysis • Easy storage of data • Information about the whole cell population • Standardized in reference labs	• Relatively sensitive • Operator dependent • Relatively expensive • Cell number available
Real-time quantitative (RQ) PCR	IG/TR gene rearrange-ments	90-95%	Nucleic acid (DNA)	Related to diagnosis (on DNA)	0.01-0.001%	• High sensitivity • Good applicability • Well standardized: international guidelines for analysis and data interpretation	• Dependent on ASO-primer • Laborious and time consuming • Affected by clonal evolution • Large amount of diagnostic DNA • Relatively expensive
Real-time quantitative (RQ) PCR	Recurrent Fusion genes	30-40%	Nucleic acid (RNA or DNA)	Related to cell line or plasmid DNA (on RNA) Related to diagnosis (on DNA)	0.01-0.001%	• High sensitivity • Rapid • Relatively easy • Stable throughout treatment • Well standardized on DNA • Applicable for specific leukemia subgroups: BCR-ABL1 or KMT2A-AF4	• Limited applicability (target-negative in >50% of patients) • RNA instability • Risk of contamination • Limited standardization on RNA • Relatively expensive on DNA

Innovative methods for MRD monitoring							
NGF	Leukemia-Associated Immunophenotypes	> 95%	Cell suspension (PB, BM, needle aspirates of several tissues)	Absolute	0.01-0.0001%	• Potential high sensitivity • High applicability • Faster and reproducible • Accurate quantification • Highly standardized with possibilities for automated gating	• Education and training required • Many cells needed to reach the required sensitivity • Requires fresh material analysed within 24 h after sampling • Expensive
ddPCR	IG/TR and fusion genes	90-95%	Nucleic acid (DNA)	Absolute	0.01-0.001%	• Potential high sensitivity • Good applicability (90-95%) • No need of standard curve • Easy	• Dependent on ASO-primer • No standardized • No guidelines for analysis and data interpretation • Available in few labs • Relatively expensive
NGS	IG/TR gene rearrangments	>95%	Nucleic acid (DNA)	Absolute	0.01-0.0001% (depending on amounts of DNA analyzed)	• Potential high sensitivity • High applicability (>95%) • Potential to identify clonal evolution • Provides information on background repertoire of B and T cells • Non ASO-primer dependent	• Not standardized • No guidelinees for analysis and data interpretation • Available in few labs • Discrimination from normal clonal background • Need of a bioinformatic analysis • Expensive

Abbreviations: PCR, polymerase chain reaction; BM, bone marrow; PB, peripheral blood; NGF, next generation flow; ddPCR, digital droplet PCR; NGS, next generation sequencing; IG, immunoglobulin receptor; TC, T-cell receptor; DNA, deoxyribonucleic acid; RNA, ribonucleid acid

Table S5: Clinical Trial Design and Outcome Criteria

As in AML[3], trial design is of increasing importance for future development of treatment protocols in ALL. The increasing number of smaller molecular subtypes and the number of compounds represents a major challenge. International academic trials may be one way to go. The EWALL has conducted two international academic trial at least in two countries[4,5], however the administrative efforts are tremendous and in the absence of large international grant programs and with increasing regulatory burden the future of this type of trial is uncertain. The Harmony project which is funded as a public-private partnership by the European Union aims to define standards for capture of big data in hematologic malignancies[6].
Another approach is the use of historical data sets for comparison of new compounds. The prospective collection of reasonable real-world data should be of utmost importance for health-care systems. In rare subtypes of ALL and in very poor prognostic subgroups randomized trials are not feasible. If well designed historic data sets are available, it is possible to put data with new compounds in a reasonable context[7,8]. These analyses should be performed according to a predefined analysis plan and - if possible - by independent statisticians. Another approach to make randomized trials in poor prognostic subsets more feasible, is to allow a cross-over to the treatment arm with a new compound. If the endpoint of the trial is defined as event-free survival (EFS) with non-response and cross-over as an endpoint. In the future new indications for international clinical trials may be considered. Thus, MRD identifies a subset of patients which is characterized by resistance to standard therapy. In the future, patients with positive MRD status after 2-3 blocks of standard therapy may become candidates for a clinical trial independent of the previously administered type of standard therapy.
Short and long-term endpoints of clinical trials in ALL are summarized in Table S5. As in AML[3], overall survival (OS) is used as the most relevant clinical endpoint. However, OS is only partly influenced by the effect of a given new compound; subsequent therapies are equally important. This includes SCT which is not standardized and may strongly impact survival. In addition, the rate of subsequent SCT may depend on health care systems. Other factors which interfer with OS are subsequent salvage therapies, which are increasingly available. Therefore, EFS appears to be a more reasonable endpoint.
Achievement of CR is a highly patient-relevant endpoint in ALL. Achievement of an MRD remission increases the value of response evaluation since it reflects the depth of the antileukemic effect of a new compound and should also be considered as a new endpoint for clinical trials and surrogate for OS.
The panel strongly recommends standards for reporting of clinical trials in ALL. This includes the CR rate, the rate of refractory disease and the rate of early death at distinct clearly identified timepoints e.g. ‘after induction’. The MRD response rate should be reported for the same time-points and refer to the number of analyzed patients and clearly state whether the rate refers to all patients or CR patients only. Furthermore, OS, EFS, relapse-free-survival (RFS) and remission duration (RD) should be reported as medians and as probabilities at 1, 3 and 5 years. The rate of SCT performed in ongoing remission should be reported as well (Table S5).
For comparability and applicability of clinical trial results standardized reporting of outcome is paramount (Table S5). Describing the outcome of initial induction therapy includes the definition of a time-point in protocol, categories of response and non-response and in addition early death in a protocol-specific predefined period. The panel decided to omit progression as an outcome parameter for ALL, since this is hardly to define in acute leukemia and there is no evidence that it has any clinical relevance.
The standardized analysis of outcomes after SCT is of increasing relevance. It is essential to report only SCT performed in the current treatment line i.e. separate patients with SCT after subsequent relapse.
Comparison of SCT outcomes with those of chemotherapy is challenging and direct comparison of transplanted vs non-transplanted patients is not a proper approach. Methods include comparisons with different landmarks, censoring versus non-censoring at the time-point of SCT, and considering SCT as a time-dependent in Simon-Makuch[9] or Mantel-Byar analyses or in a cox-model. Methods considering the immortal time-bias of transplanted

patients should be applied such as landmark analyses, analyses of SCT as time-dependent covariate, or combined methodologies.
Quality-of-Life is considered as a patient-relevant endpoint and is often requested by healthcare providers to assess the additional benefit of a new compound. Although theoretically of interest, in clinical practice assessment is often problematic. Severely ill patients may not be interested to fill questionnaires and there may be also socioeconomic hurdles. Therefore, the return rate of QoL questionnaires may be low and this return rate can only partly be influenced by physicians. Therefore, QoL should not be assessed as one of the main patient-relevant outcomes. More patient involvement including documentation of patient-reported adverse events should be the goal of future clinical trials in ALL.
Documentation of adverse events (AE) usually follows the CTCAE classification, which is not always helpful for definition of clinically relevant AEs in ALL. A pediatric collaboration specified 14 relevant AEs (hypersensitivity to asparaginase, hyperlipidaemia, osteonecrosis, asparaginase-associated pancreatitis, arterial hypertension, posterior reversible encephalopathy syndrome, seizures, depressed level of consciousness, methotrexate-related stroke-like syndrome, peripheral neuropathy, high-dose methotrexate-related nephrotoxicity, sinusoidal obstructive syndrome, thromboembolism, and Pneumocystis jirovecii pneumonia)[9] . This classification should be adopted for adult ALL trials. Furthermore, it is essential to clearly report AE in relation to defined treatment blocks. Reporting of AEs over a whole treatment trial with undefined number of cycles is not helpful to assess the expected toxicity of individual cycles in clinical practice.

Category	Definition
Outcome Measures	
Early death	Death during induction treatment, which is a pre-defined treatment interval; independent of response of non-response
Death in CR	Death after achievement of CR and after end of induction therapy
Second malignancy	Malignancy other than ALL occurring after ALL diagnosis; time-point and type should be recorded
Withdrawal	Stop of treatment due to patient's wish or physicians decision; time-point and reason should be recorded.
Relapse	Detection of more than 5% blast cells in the BM after prior achievement of CR or unequivocal demonstration of extramedullary leukemia involvement. In case of 5-20% blasts cells during the intensive treatment phase and/or during regeneration the BM assessment should be repeated one week later to distinguish BM relapse from regeneration phenomenons If available: MRD >= 1%[10]
- Hematologic relapse	Relapse in bone marrow
- Extramedullary relapse	Differentiate localization of relapse CNS relapse: CNS3 (cytomorphology, or imaging or biopsy) CNS2 (cytomorphology + 1 week apart 1 additonal consistent diagnostic[10])
MRD relapse	Re-occurrence of MRD after prior MRD complete response either according to variant 1 (>10-4) or variant 2 (any positivity)
Outcome analysis	
Overall Survival	Defined for all patients of a trial; measured from the date of entry into a study to the date of death from any cause; patients not known to have died at last follow-up are censored on the date they were last known to be alive
Relapse-free survival/ Leukemia-free survival*	Defined only for patients achieving CR or CRi [b], measured from the date of achievement of a CR until the date of relapse or death from any cause; patients not known to have

	relapsed or died at last follow-up are censored on the date they were last examined
Remission duration	Defined only for patients achieving CR or CRi [b], measured from the date of achievement of a CR until the date of relapse; patients not known to have relapsed at last follow-up are censored on the date they were last examined
Event-free survival (EFS)	Defined for all patients of a trial; measured from the date of entry into a study to the date of induction treatment failure, or relapse from CR or CRi [b], or death from any cause or occurrence of a secondary malignancy; patients not known to have any of these events are censored on the date they were last examined
Cumulative incidence of relapse (CIR)[a]	Defined for all patients achieving CR or CRi [b] measured from the date of achievement of a remission until the date of relapse patients not known to have relapsed are censored on the date they were last examined; patients who died without relapse are counted as a competing cause of failure
Cumulative incidence of death in CR (CID)	Defined for all patients achieving CR or CRi [b] measured from the date of achievement of a remission until the date of death without prior relapse independent of cause; patients not known to have died in CR are censored on the date they were last examined; patients who relapsed are counted as a competing cause of failure

[*] Relapse-free, leukemia-free and disease-free survival have been used with the same definition
[a] It is important to provide estimates of cumulative incidence of death (CID) as well, since just considering the results of CIR may be misleading if for instance CIR is lower for one group but CID is actually higher for that same group
[b] In studies where the criterion CRi is used, relapse-free survival should be defined for all patients achieving CR or CRi; for event-free survival, relapse should be considered from CR and CRi.

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