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Diagnosis, Prognostic Factors and Assessment of ALL in Adults: 2023 ELN Recommendations from a European Expert Panel

Tracking no: BLD-2023-020794R2

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Abstract:
Working groups of the European Leukemia Net (ELN) have published several important consensus guidelines. Acute lymphoblastic leukemia (ALL) has many different clinical and biological subgroups and the knowledge on disease biology and therapeutic options is increasing exponentially. The European Working Group for Adult ALL has therefore summarized the current state of the art and provided comprehensive consensus recommendations for diagnostic approaches, biologic and clinical characterization, prognostic factors and risk stratification as well as definitions of endpoints and outcomes. Aspects of treatment, management of subgroups and specific situations, aftercare and supportive care are covered in a separate publication. The present recommendation intends to provide guidance for the initial management of adult ALL patients and to define principles as a basis for future collaborative research. -

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: All authors contributed by writing parts of the recommendation. All authored participated in several rounds for agreement, reviewed and finally confirmed the whole manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement:

Clinical trial registration information (if any):

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11.1.2024

Word Count: 3847
Tables: 3
References: 70

Data Sharing Statement
The manuscript does not include any original data or protocols. Therefore, no data are available to be shared.

Abstract

Working groups of the European Leukemia Net (ELN) have published several important consensus guidelines. Acute lymphoblastic leukemia (ALL) has many different clinical and biological subgroups and the knowledge on disease biology and therapeutic options is increasing exponentially. The European Working Group for Adult ALL has therefore summarized the current state of the art and provided comprehensive consensus recommendations for diagnostic approaches, biologic and clinical characterization, prognostic factors and risk stratification as well as definitions of endpoints and outcomes. Aspects of treatment, management of subgroups and specific situations, aftercare and supportive care are covered in a separate publication. The present recommendation intends to provide guidance for the initial management of adult ALL patients and to define principles as a basis for future collaborative research.

Introduction

Scientific progress in acute lymphoblastic leukemia (ALL) has been driven by the activities of national academic multicenter study groups in Europe and US in the past decades. The European national study groups have founded a collaboration within the European Leukemia Net (ELN) named European Working Group for Adult ALL (EWALL). The group has published a Consensus Recommendation for the Management of Adult ALL[1] which was broadly distributed among the participating centers.

ALL is rare compared to other cancers of adulthood. It has many different clinical and biological subgroups and the knowledge on disease biology and therapeutic options is increasing exponentially. There is an unmet need for guidance for clinicians, regulatory agencies, and health care systems. Available guidelines from the National Comprehensive Cancer Center (NCCN)[2] or European Society of Medical Oncology[3] are relevant but need to be complemented by a broad and comprehensive expert consensus considering the different regulatory and socio-economic framework for ALL management in Europe. Therefore, the group decided to develop ELN Recommendations as published for other entities[4,5]. The focus of this article is on diagnostic approaches, biological and clinical characterization, risk stratification, definitions of endpoints and outcomes. Aspects of treatment, management of subgroups and specific situations, aftercare and supportive care are covered in a separate publication (cross-reference).

Methods

The panel includes 17 members representing national study groups. Members met in person and defined topics, tables, and responsibilities of co-authors (Table S1). Coauthors performed literature searches of PubMed database and considered relevant abstracts. The manuscript was reviewed by all co-authors. Formal corrections were performed by the corresponding author. Disagreements were summarized and discussed in the whole group. The whole group agreed on the final version of the manuscript. Due to rapid innovation and availability of new data together with a lack of randomized trials for many essential questions, most of the statements have an evidence level of 'expert recommendation' for clinical practice.

Diagnostic Procedures and Classification

The work-up should be comprehensive to allow a precise diagnostic work-up and an accurate stratification, as well as to set the basis for minimal residual disease (MRD) monitoring. Morphology, multicolor flow cytometry (MFC) and molecular genetics are obligatory (Table 1A). Additional genomic analyses are used to better define molecular subgroups. Diagnosis is usually based on a bone marrow (BM) aspirate which should be attempted even in cases with high peripheral blast (PB) counts. A BM biopsy is recommended in patients with a dry aspirate; in these cases, BM aspiration can be repeated after the pre-phase to obtain further material. Biobanking is recommended. It should include nucleic acid (DNA, RNA), viable cells and possibly germline material. Further work-up includes a lumbar puncture (LP) with classification and imaging procedures as needed to define potential extramedullary involvement (Table S2).

Morphology/Cytochemistry

BM morphology is necessary to evaluate the degree of infiltration and to differentiate ALL from mainly from acute myeloid leukemia (AML) and ALL from lymphoblastic lymphoma (LBL). There are no specific cytochemistry reactions to classify ALL. Myeloperoxidase is always negative with the exception of mixed-phenotype leukemia (MPAL), where low/dim/strong levels of expression have been described particularly in B-myeloid cases where isolated MPO expression (^{isoMPO}) may occur.

Immunophenotyping

MFC (at least eight colors) is central. It allows to: i) make a differential diagnosis with AML, ii) to establish lineage affiliation and differentiation, iii) define an aberrant phenotype for MRD monitoring and iv) to detect target antigens for immunotherapy. The EuroFlow consortium has

standardized MFC[6]. TdT is expressed by all B- and T-cell progenitors, except for mature B-ALL (Burkitt's Leukemia), while being negative in AML.

B-lineage ALL (B-LIN) accounts for 75-80% of cases. The crucial markers are CD19, CD22 and cytoplasmic (cy) CD79a. The EGIL classification[7] defines four differentiation stages: pro-B, common, pre-B ALL and mature B-ALL (Table 1A). An aberrant coexpression of myeloid markers can be detected in about 40%[8] of cases. Pro-B ALL is CD10-negative and often associated with t(4;11)/*KMT2A* [9] while pre-B ALL often carries t(1;19)/*TCF3::PBX1* aberration. The identification of surface markers as potential targets for immunotherapy is essential for therapeutic approaches (cross-reference)

T-lineage ALL (T-LIN) represents 20-25% of adult ALL. Crucial markers are cyCD3 and sCD7. Furthermore, CD1a, CD2, sCD3, CD4, CD5 and CD8, as well as TCRα/β or γ/δ, may be variably expressed. T-ALL can also be classified into four subtypes: pro-T ALL, pre-T ALL, cortical (thymic) and mature T-ALL[7]. Pro-T ALL cases express only cyCD3 and CD7, cortical T ALL is CD1a positive, while mature T-ALL express sCD4 and/or CD8, and usually CD3. The so-called ETP-ALL (Early-T Precursor)[10], represents an entity within pro-T/pre-T ALL with weak CD5 expression which shows at least one myeloid and/or stem cell marker[10]. CD34 and myeloid markers are expressed in a proportion of T-ALL[8]. The maturation stage correlates with molecular aberrations such a *LMO2/HOXA* in immature, *TLX1/TLX3* in cortical, TAL1 in mature T-ALL. Beside EGIL there is no uniform international classification of immunophenotypes. To achieve comparability, it is recommended to report results including this classification.

Mixed-phenotype acute leukemia (MPAL) represents 1-5% of acute leukemias[11] and is characterized by blasts coexpressing antigens of more than one lineage on the same cells or that have separate populations of blasts of different lineages. The definition of MPAL has been refined in the WHO 2008 [12]and 2016 [13] classification, the latter discriminating B-myeloid from the other MPAL The combination of antigens used for MPAL recognition is summarized in Table 1A. Furthermore, the WHO 2016 classification takes into account also the presence of specific genetic aberrations, particularly the *BCR::ABL1* and KMT2A rearrangements.

Cytogenetics/Molecular Genetics

Karyotyping, fluorescence-in situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR) techniques are used for the genetic characterization.

B-LIN: t(9;22)/*BCR::ABL1* is the most frequent aberration. Its detection in the shortest possible time is essential[14]. Other subtypes include *KMT2A* rearrangements, monosomy 7, hypodiploidy/low hypodiploidy (and the related near-triploid), t(1;19)/*TCF3::PBX1* and t(17;19)/*TCF3::HLF*. t(8;14)/*MYC/IGH* is rarely detected; also t(12;21)/*ETV6::RUNX1* and high hyperdiploidy (51-65 chromosomes) are rare in adults[15].

T-LIN: Genetic aberrations often comprise the 14q11 and 7q34 breakpoints, leading to the juxtaposition of the TCR loci to transcription factors, namely *TAL1*, *TAL2*, *LYL1*, *LMO1*, *LMO2*, *TLX1 (HOX11), TLX3* (*HOX11L2*), *HOXA*, *MYC* and *MYB*[15]. In addition, cryptic ABL-1 rearrangements with different partner genes e.g. *NUP214*, *EML1* and *ETV6* have been identified.

MPAL: The list of genomic lesions detected in MPAL is rather large, and is likely to increase, given its heterogeneity. As mentioned, the most frequent rearrangements ,as already mentioned, are *BCR::ABL1* detected in about 15% of cases and *KMT2A* found in roughly 10% of patients; *ZNF384* rearrangements have also been reported in about 49% of B/ MPAL[16].

Extended Genomics

Gene expression profiling (GEP), copy number alteration analysis (CNA), genome-wide and next generation sequencing (NGS) techniques have identified new subgroups. These techniques are not part of the standard work-up but may be carried out for a refined classification in research laboratories.

The Philadelphia (Ph)-like subgroup is discussed separately (cross-reference). ETP-ALL[10] is genetically heterogeneous, with mutations of multiple pathways including hematopoietic and lymphoid development, Ras, cytokine receptor, and kinase signaling, and loss-of-function mutations targeting epigenetic regulators[17,18].

CNA allows to identify aberrations that affect cell cycle (*CDKN2A/B* and *RB1*), as well as lymphoid development (*IKZF1*, *PAX*5, *VPREB1* and *LEF1*). Among those, *IKZF1* deletion (Δ*IKZF1*)[19,20] can be found in about 80% of Ph+ and Ph-like cases[21]. The IKZF1-plus genotype (i.e. iKZF1 plus *CDKN2A/B* and/or *PAX5* deletions) is considered in some trials.
Recently, further subtypes have been identified in B-LIN. The *DUX4*- and *ERG*-rearrangement leads to the formation of an *ERG* isoform, which - in mice models - induces leukemic transformation. *MEF2D* rearrangements have transforming capability in vitro. Finally, *ZNF384* deregulation, which can rearrange with several partners (*EP300*, *CREBBP*, *TAF15*, *SYNRG*, *EWSR1*, *TCF3* and *ARID1B*) often displays a weak CD10 and CD13/CD33 expression[15].
NGS has identified novel mutations and rearrangements; the most frequent involve the RAS pathway (*N/K RAS, FLT3, PTPN11, NF1* and *BRAF* mutations) and can be detected in more than 40% of cases, are prevalent in the hyperdiploid and *KMT2A*-rearranged cases and tend to increase at relapse. More rare mutations affect the Janus kinase (JAK)/STAT pathway (*JAK1/2, JAK3, IL7R,* rarely *CRLF2, SH2B3* and *IL2RB).* RAS and JAK/STAT mutations are detected in both B- and T-LIN[22]. In T-ALL, *NOTCH1/FBXW7* lesions represent the most frequent event (60%) (Table 1B).

WHO (World Health Organization) and International Consensus Classification (ICC)
The updated classifications are largely overlapping; the ICC identifies more distinct molecular categories[15,23] (Table S3). A variety of methods would be required to establish the respective categories. This is not standard in most countries and beside the most relevant diagnostic characterizations summarized in Table 1A the new subcategories to date have limited clinical consequences. Nevertheless, their identification is of interest in a research context. Further WHO and ICC classifications with more detailed guidance on recommended and standardized methods for identification of subgroups are warranted.

MRD Testing
MRD testing aims at detecting and quantifying residual blasts beyond the sensitivity of cytomorphology which is around 5%[24,25]. It is important to establish the target structures for MRD testing based on primary diagnostic material. The panel strongly advises to perform MRD evaluation in reference laboratories participating in standardization approaches.
The most common methods for MRD monitoring are MFC and real-time quantitative PCR (RQ-PCR). MFC can be applied to most cases (>90%), can reach a sensitivity of 0.1-0.01% (10^{-3}-10^{-4}) and results are promptly available. Disadvantages are: i) samples must be rapidly analyzed; ii) small number of evaluable cells due to BM hypocellularity; iii) misleading results due to phenotypic shift; iv) interpretation to some extent operator dependent; v) suboptimal sensitivity. The EuroFlow Consortium has standardized the procedures[26]. Further efforts are ongoing to implement the use of next generation flow mainly for better interoperability and sensitivity.
Molecular MRD monitoring of fusion genes (e.g. *BCR::ABL1*) has a sensitivity around 0.01%, is possible in about 40% of cases, is not patient-specific, relatively easy to perform and inexpensive. However, its accuracy is hampered by the variability in the number of RNA transcripts per leukemic cell. The EuroMRD Consortium has standardized MRD detection based on the *BCR::ABL1*[27].
In most patients, a clonal Immunoglobulin/T-cell receptor (IG/TR) rearrangement can be identified for MRD evaluation. The technology is applicable to over 90% of cases[28] and has been extensively standardized within the EuroMRD Consortium. Technical issues are: i) in the most immature forms, IG/TR gene rearrangements may not be found; ii) the designed probes may not be sufficiently sensitive; iii) clonal evolution may lead to false negative results; iv) the amount of diagnostic DNA may be too low.
Any RQ-PCR may fail to precisely define the amount of residual disease in cases with a very low burden, i.e. “positive-not-quantifiable” (PNQ); their identification is an unmet need in clinical practice. These cases are being investigated with novel techniques e.g. digital droplet PCR (ddPCR) and NGS. For NGS neither methodology nor reporting is standardized. In part similar technical issues apply as for IG/TR PCR and particularly the claimed higher sensitivity

depends on the amount of DNA. MRD-testing by NGS is so far not incorporated in the clinical practice[29]. The various techniques are summarized in Table S4 and therapeutic application of MRD is discussed separately (cross-reference).

Prognostic Factors (PF)

Different risk subsets according to disease- and host-related factors can be identified[30]. The individual prognosis must be refined by assessing MRD response[24,25] and its integration with other PFs. Patients without poor PF and/or with a favorable post-induction MRD course may represent 50-60% of all cases and are defined as standard-risk (SR, 5-year overall survival >50-60% and up to 70-80% in selected good-risk subsets) while those with any poor PF and/or poor MRD response are classified as high-risk (HR, 5-year overall survival <40%) (**Table 2A**). This distinction is crucial to develop an effective risk-oriented treatment strategy. One approach is to select HR patients for an allogeneic stem cell transplantation (SCT) and/or other novel therapies, while SR patients are treated with chemotherapy with good to very good results and low risk of therapy-related mortality (TRM). Risk models depend on treatment protocol, differ among clinical studies and are periodically updated (Table 2B).

Clinical Risk Factors: Age, White Blood Cell Count (WBC) and Immunophenotype

Older age and high WBC counts are universally recognized as predicting poorer outcome[30]. WBC is still a relevant and easily assessable PF. Cut-offs for HR B-ALL are often set >30 x10^9/L. A WBC based stratification in T-ALL (>100 x10^9/L) is not generally adopted. Other clinical PF are poor performance score (ECOG >1), CNS-positive ALL in some trials, and undue reductions or delay of post-induction therapy[31,32]. Pro-B-ALL is sometimes defined as HR. Some trials report a poorer prognosis in CD20-positive cases[33], unless associated with good MRD response[34] or treated with rituximab. In T-ALL the prognosis is worse for the pro/pre-T (or ETP as subentity of immature T-ALL) and mature T subsets whereas the cortical phenotype has a good prognosis[35]. Outcome of HR T-ALL may be improved using pediatric-based (p-b) chemotherapy in conjunction with SCT[36]. In newly diagnosed ALL, the bone marrow blast count differentiating ALL from LBL is not considered as a prognostic feature but as a feature identifying a clinically relevant subtype which has an impact on practical management as outlined below. In relapsed ALL bone marrow blast count can be predictive for response rates (cross-reference).

Cytogenetics

Recent cytogenetic classifications suffer from lack of information in up to 50% of the patients, low incidence of some aberrations and interactions with other PFs[37-39]. 'Good risk' karyotypes such as t(12;21) are rare in adults. Most adults fall within an intermediate-risk (IMR) group. According to one of the published classifications, IMR includes the normal diploid subset, hyperdiploidy and some other abnormalities. Monosomy 7, low hypodiploidy/near triploidy and complex karyotype with 3-5 simultaneous abnormalities are allocated to a HR cytogenetic category as the t(4;11)/*KMT2A* rearranged ALL by several groups[39,40]. For complex karyotype a uniform definition is recommended (Table 2A). There is no generally accepted cytogenetic classification for adult ALL and it remains open whether the prognostic impact of rare aberrations as outlined above is still valid in modern protocols.

Molecular Genetics and Genomics

Further genetic aberrations can exert significant prognostic effects (Table 1B). Cases with iAMP21, Ph-like ALL[21,41,42], and an altered CNA profile can be associated with a higher relapse risk (RR) in both Ph- ALL[43,44] and Ph/BCR::ABL-positive (Ph+) ALL[45,46]. An 8-gene CNA profile was validated in pediatric B-ALL[47]. The good-risk CNA classifier consisted of no deletion of *IKZF1*, *CDKN2A/B*, *PAR1*, *EBF1*, *RB1*; by an isolated deletion of *ETV6*, *PAX5*, *BTG1*; or by a deletion of *ETV6* with single additional deletion of either *BTG1*, *PAX5*, or *CDK2A/B*. Any other CNA combination exerted a negative prognostic effect. Adult studies identified *KMT2A-AFF1*, Ph-like, low hypodiploid/near haploid, *BCL-2/MYC*-rearranged, *PAX5*alt, *ZNF384*-like and *MEF2D*-rearranged as HR or IMR-HR genotypes[48,49]. CDX2 de-

regulated ALL with UBTF::ATXN7L3 has been described as a new unfavorable subtype [50-52] which may benefit from targeted therapies[53].
In T-ALL, overexpression of *HOX11L2* and *ERG*, lack of *NOTCH1* and *FBWX7* mutations, and presence of *RAS* or *PTEN* abnormalities yielded unfavorable outcomes[54,55]. Mutations/alterations of *TP53*, *JAK/STAT*, *BCL-2*, *MYC* are unrestricted to cell lineage and may confer poor prognosis. As for cytogenetics it is important to note that prognostic annotations are often based on small patient numbers and not always based on outcomes with current protocols.

Early Response Dynamics
Patients with poor blast cell clearance and late responders requiring more than one chemotherapy course to complete remission (CR) have an inferior outcome. The most useful prognostic information for RR and overall survival (OS) and individual risk stratification comes from MRD testing[2,24,56-58] . As a general rule, with RQ-PCR (sensitivity of 0.01%) good prognosis patients reach MRD levels <0.1% to <0.01% (better if negative) at end of induction (weeks 4-6) and <0.01% (better if negative) following 1-3 early consolidation courses around weeks 10-16. Therapeutic consequences are discussed separately (cross-reference).

Integrated Risk Models
In children, an independent genotype-specific effect on MRD-related outcomes improved the risk stratification[59]. In adults, one study demonstrated the usefulness of a mixed MRD and genetic risk classification, showing the independent prognostic effect of a 4-gene classifier (favorable: lack of *KMT2A* rearrangements and ∆*IKZF1* in B-ALL; mutated *NOTCH1/FWBX7* without *RAS/PTEN* abnormalities in T-ALL[54]. In addition a comprehensive risk model combined WBC, cytogenetics and MRD results[60]. Both models have not been uniformly adopted.

Recommendations for Analysis of PFs and Risk Stratification
MRD testing is recommended in all cases to optimize risk stratification[58,61]. In a European survey, MRD was the only PF shared by 11 national study groups to define HR ALL and to decide about SCT indication[62](**Table 2B**). No other PF achieved the same level of consensus, although adverse genetics rank high in HR definitions (8/11 groups). Altogether, it is strongly recommended to perform MRD analysis in all ALL patients[61]. New evidence may prove the independent prognostic power of several genetic abnormalities that in different combinations concur to define novel HR subsets. Appropriate diagnostic identification and clinical data in relevant patient numbers with modern protocols are a pre-requisite. New combined risk models are therefore recommended for research purposes to improve the diagnostic and prognostic platforms.

Response Criteria and Definition of Outcomes
Formal criteria for BM response assessment in ALL are based on AML[4,63] and those for extramedullary response on Non-Hodgkins'Lymphoma (NHL) [64]. Recently new standards for remission, treatment failure and relapse have been defined for pediatric ALL, which should be adopted for adult ALL[65].

Cytologic Response in BM
Whereas criteria for CR are uniformly used, CR with incomplete recovery (CRi) can be an endpoint in clinical trials. Failure or partial remission (PR) (Table 3) can be differentiated or combined. For PR cases a correlation with MRD testing is recommended. If technical criteria for MRD testing are met, PR or CRi with negative MRD status can be considered as CR. On the other hand, patients with MRD of 1% or higher can be considered as failure[65]. For future clinical trials a standardized terminology is requested (Table 3). Publications should also define time-point (TP) of response assessment and avoid reporting of cumulative response rates.

MRD Response

Response criteria are redefined by integrating MRD assessment. Usually, MRD assessment is only considered in patients with hematologic CR or CRi. In any case the reference population should be clearly stated and evaluable and non-evaluable MRD results should be named. Complete MRD response is defined as no detection of MRD with a minimum sensitivity of 0.01% at the respective TP[28]. MRD persistence is defined as quantifiable MRD, usually 0.01% or greater. Some groups consider any MRD positivity as prognostically relevant; others consider thresholds like 0.1% or 0.01% for different TP or different treatment consequences. Beyond these categories additional MRD results may be reported, including non-quantifiable MRD at different levels of sensitivity or negative MRD with insufficient sensitivity[66,67]. For TP with non-quantifiable MRD additional ddPCR or NGS testing may be used to separate true negative from false positive results[67]. Of note - any negative MRD result without the corresponding sensitivity level at a distinct TP does not fulfill technical requirements. The latter are different for MFC, PCR of IG/TR[28], PCR of fusion genes[27] and often not reported for NGS based methods. MRD results should include information on the material tested and specifics: PB versus BM, level, and status of MRD i.e. positive vs negative or non-evaluable. Two variants of MRD based response assessment are given in Table 3. The panel is in favor or variant 1 since the exact MRD level (not considered in variant 2) is relevant for any treatment decisions and for comparability of reported results.
For Ph+ ALL MRD response criteria are often derived from trials for chronic myeloid leukemia (CML). Whether these categories are meaningful for ALL remains open. Approaches with an ALL-specific definition are considered. Furthermore, discrepancies between BCR::ABL1 based MRD results and other methods may occur if two methods are applied in parallel. BCR::ABL1-based MRD may remain positive whereas IG/TR MR is negative. This is probably due to multilineage involvement with BCR::ABL1 also in myeloid precursors. Clinical consequences are not clear so far, but the ICC has now integrated two subgroups with BCR::ABL1 (lymphoid and multlineage)[15]. For MRD-based treatment decisions it is recommended to use in addition to BCR::ABL1-based MRD assessment one additional method i.e. MFC or IG/TR.

Extramedullary Response

Between 20-90% of ALL patients show extramedullary involvement at diagnosis. Type and extent of involvement should be documented. These localizations should have regressed in size at the TP of CR confirmation. Standard criteria for NHL are recommended for classification[64,68]. In case of persistent extramedullary involvement after induction and early consolidation, Positron Emission Tomography (PET) analysis can be considered. This approach raises challenges in dose dense treatment protocols for ALL due to the recommended treatment-free interval. For the purposes of clinical trials, patients with CRi or PR, PET negative may be considered as CR. Therapeutic consequences from positive PET remain to be defined.

Time-Point for Response Assessment

The TPs depend on protocol and treatment consequences. Usually, a CR should be achieved after the initial 1-3 phases of standard therapy depending on the protocol. Cases not achieving a CR after this period are considered as primary failure. This definition is important for the purpose of clinical trials. During the follow-up, BM response may be assessed every 2-3 months until end of maintenance. Further controls may rely on PB. More frequent assessments are recommended in patients with low level MRD of any level or PET positivity at any TP. Any treatment change based on MRD should be followed by MRD assessment of response. Further essential outcome parameters and major approaches for clinical trials are described in the supplement (Table S5).

Summary and Outlook

The management of ALL is often organized by national study groups and international consortia[69]. Their reference laboratories and/or associated biobanks together with clinical data bases are essential for research on disease biology and prognostication and are

sources of reliable real-world data. Reference laboratories are crucial to establish and maintain high standards for diagnostic procedures and biologic characterization as basis for risk stratification and optimal management. Cytomorphology, immunophenotyping and the identification of prognostically relevant molecular markers together with measurement of MRD are the basis for diagnosis and risk adapted therapy.

Further genetic characterization of samples from biobanks has identified many new subtypes of ALL. The prognostic and therapeutic impact however often remains open for current treatment regimens and diagnostic identification is not part of SoC in many countries. It will be essential to define reliable and cost-effective diagnostic procedures to improve classification of ALL. On this basis, future risk stratification may integrate molecular markers, potential treatment targets, risks for relapse and toxicities as well as the dynamics of MRD. Furthermore, improved understanding of disease biology can contribute to the development of new, targeted precision medicine approaches.

For comparability of data on an international level it will be essential to use uniform and standardized criteria for reporting of results. This applies particularly for the use of MRD as endpoint of clinical trials and for any MRD-based treatment decisions.

Whereas there is no standard risk model for adult ALL, all experts agree that depending on treatment protocols and clinical subgroup risk-adapted approaches for management are standard (cross-reference). Future risk models may also integrate pharmacogenomics in order to predict and potentially avoid toxicities and in-vitro-sensitivity testing for identification of potentially active drugs for later-line approaches in relapsed/refractory ALL.

Author Contribution Statement

All coauthors reviewed literature, wrote parts of the manuscript, reviewed the manuscript and approved the final manuscript.

Conflict of Interest Statement

NG has received institutional research funding from Amgen, Clinigen, Incyte, Jazz, Novartis, Pfizer and Servier, received speaker honoraria or fees for advisory board participation from Amgen, Astra Zeneca, Autolus, Celgene, Clinigen, Gilead, Incyte Jazz, Novartis, Pfizer and Servier. NB received honoraria from Amgen, Incyte, Jazz Pharma, Novartis, Pfizer, Servier and research grants from Amgen, Novartis, Incyte, Jazz Pharma and Sanofi. SC served in advisory boards of Incyte, Pfizer and Abbie and as consultant for Gilead and Amgen. HD received research funding from Amgen, Astellas, Celgene, Incyte, Jazz, Pfizer and Servier and honoraria from Daiichy Sankyo, Incyte, Jazz and Servier. MD received research support from AstraZeneca. Robin Foa received speaker honoraria from Amgen, Novartis and Astra Zeneca and served in advisory boardsfrom MSD. SG received speaker honoraria or joint advisory boards of Amgen, Novartis, Pfizer, Gilead received speaker honorariy from Angelini, travel grants from Gilead and served in advisory boards of Zentiva. MH received research support from Novartis, travel support from Abbvie and Beigene, speaker honoraria and joined advisory boards from Servier, and was part of advisory boards from Amgen and Clinigen. DM provided consulting for Pfizer, Novartis and Kite. OO received speaker honoraria, joined advisory boards and received research grants from Incyte, research support from Amgen and Celgene and received honoraria for advisory boards from Autolus. AR received research support from Servier. PR received research grants from Pfizer and Incyte. JR received speaker honoraria and joined advisory board from Amgen, Pfiizer, Shire, Ariad, Incyte, jointed advisory Boards from Takeda and received research support from Amgen. RB joined advisory boards from Novartis, Kite/Gilead, received honoraria or travel support from Amgen, Incyte, Servier, Jazz and Pfizer.

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Table 1A: Specific Diagnostic Work-up in Adult ALL

	Recommendation	Outputs
Morphology	Mandatory	• Defines infiltration (>25% required for a diagnosis of ALL versus LBL) • Allows a differential diagnosis with AML (MPO- *vs* +) • Recognizes L3 (Burkitt-type) subsets
Flow cytometry	Mandatory	• Allows a differential diagnosis with AML • Permits to define the cell of origin • Permits to define the stage of differentiation
MPO*	Mandatory	• Allows a differential diagnosis with AML
B-lineage ALL*: CD19, cCD79a, c/sCD22 (minimal requirement), TdT, CD10, CD20, cIgM, sIg (kappa or lambda)	Mandatory	• Pro-B: CD19/CD79a/cCD22+/CD10- (B-I), NG2 • Common: CD10+/cIgM- (B-II) • Pre-B: cIgM+/sIg- (B-III) • Mature: sIg+ (B-IV), CD20+
T-lineage ALL*: c/sCD3, CD7 (minimal requirement), TdT, CD1a, CD2, CD5, CD4, CD8, TCR α/β or γ/δ	Mandatory	• Pro-T: cCD3/CD7+ (T-I) • Pre-T: CD2/CD5+ (T-II) • Cortical-T: CD1a+ (T-III) • Mature-T: sCD3+/CD1a- (T-IV)
Molecular genetics	Mandatory	• t(9;22)/ Ph+/ *BCR::ABL1* • t(4;11)+ / *KMT2Ar* • t(1;19)+ / *TCF3::PBX1* • Other high-risk cytogenetics
MRD (Molecular or MFC)	Mandatory	• Individual MRD assay for further follow-up
Extended genomics (GEP, CNA, WES, WGS, NGS)	In clinical trials and for research purposes	• Identification of novel subgroups with prognostic/biologic significance • Identification of molecular targets for targeted therapies

Abbreviations: MPO: myeloperoxidase; c: cytoplasmic; s: surface; GEP: gene expression profiling; WES: whole exome sequencing, WGS: whole genome sequencing, NGS: next generation sequencing

* MPAL: Myeloid: Myeloperoxidase or at least 2 of the following antigens: nonspecific esterase, CD11c, CD14, CD64, lysozyme); B-lineage: Strong CD19 expression plus at least 1 of the following strongly expressed antigens : CD79a, cyCD22, CD10 or weak CD19 with at least 2 of the following strongly expressed antigens: CD79a, cytoplasmic CD22, CD10 ; T-lineage: cCD3or rarely sCD3

Table 1B: Molecular Subgroups in Adult ALL: Incidence, Prognosis, and Molecular findings

ALL subset	Prevalence and prognosis*	Related aberration(s)
B-lineage ALL		
BCR::ABL1+/t(9;22)(q34;q11.2) (Ph+)	20-50%, increasing with age; improved by TKI therapy	*BCR::ABL1* rearrangement
Ph-like	25-27%; Unfavorable/Controversial for current regimens	Gene expression profile like *BCR::ABL1+* ALL except for lack of *BCR::ABL1* rearrangement
*TCF3::PBX1+/*t(1;19)(q23;p13)	10-15% Favorable with intensive therapy	*TCF3::PBX1* rearrangement
KMT2A(MLL)-AFF1+/ t(4;11)(q21;q23.3), *KMT2A*-rearranged/t(v;11q23.3)	~5% (*KMT2A-AF4+*) Unfavorable	*KMT2A::AFF1 or KMT2A*-other partner gene rearrangement
IGH::MYC+ t(8,14)(q24;q32)	1-5% Unfavorable	*IGH-MYC rearrangement*
TCF3::HLF t(17;19)(q22;p13.3)	<1% Unfavorable	*TCF3-HLF* rearrangement
iAMP21	~2% Intermediate/unfavorable	-
14q32 translocations	<5%, higher in adolescents Intermediate/unfavorable	*IGH* fusion with partner genes *CRLF2*, *ID4*, *CEBP*, *BCL2*, *EPOR*, *LHX4* and *IL-3*
9p13 deletions/translocations	~25% No impact on outcome	*PAX5* fusion with partner genes *ETV6*, *ELN*, *POM121*, *PML*, *FOXP1*, *MLLT3*, *JAK2*, *C20orf112*, *AUTS2*, *CHFR*, *SOX5*, *POM121C*
7p12.2 focal deletions/ mutations	50%; 80% in Ph+ and Ph-like Controversial prognosis	Deletions of *IKZF1*
DUX4-rearranged and *ERG*-deregulated	3-7% Favorable	*ERG* and *IKZF1* deletions
MEF2D-rearranged ALL	3-4% Poor	

ZNF384-rearranged ALL	6-7% Intermediate	*EP300, CREBBP, TAF15, SYNRG, EWSR1, TCF3,* and *ARID1B*
CDX2/UBTF	2.7-4% Poor	*High expression of CDX2 and gain (1q); UBTF::ATXN7L3; CDX2-cis-deregulation*
T-lineage ALL		
TAL and *LMO* rearrangements/ del(1)(p32), t(1;14)(p32;q11), t(1;7)(p32;q34), t(7;9)(q34;q32), t(11;14)(p15;q1), t(11;14)(p13;q1), t(7;11)(q35;p13)	30-40% Favorable, partly depending on additional lesions	*SIL-TAL1* rearrangement, TCR rearrangements with *TAL1, TAL2, LMO1, LMO2*
HOXA aberrations/ inv(7)(p15q34), t(7;7)(p15;q34), t(10;11)(p13;q14), t(v;11q23), del(9)(q34),	20-25% Outcome depending on additional lesions	*TCR-HOXA* rearrangement, *MLLT10 and MLL* rearrangements with various partners*, SET-NUP214* rearrangement
TLX1-10q24 rearrangements/ t(7;10)(q34;q24), t(10;14)(q24;q11)	20-30% No impact on prognosis	*TCR-TLX11* rearrangement
ETP ALL	10-15% Unfavorable/Controversial	Deregulation of myeloid transcription factors, of members of RAS pathway and of epigenetic regulators
TLX3-5q35 rearrangement/ t(5;14)(q35;q32)	10% No impact on prognosis	*TLX3-BCL11B* rearrangement
t(8;14)(q24;q11)	1% Unfavorable	*MYC* involvement
ABL1 rearrangements	~3% Potentially targetable by TKIs	*NUP214, EML1; ETV6*
LYL/MEF2C rearrangement and immature cluster/ t(7;19)(q34;p13), del(5)(q14)	3-17% Unfavorable; improved by intensive treatment	*TCR* with *LYL1* *MEF2C* rearrangements
NKX2-1/NKX2-2 rearrangements/ inv(14)(q11.2q13), t(7;14)(q34;q13), inv(14)(q13q32.33), t(14;20)(q11;p11)	6% No impact on prognosis	*TCR/IGH-NKX2- or NKX2-2* rearrangements

*Any prognostic statements should be considered carefully, since they depend on protocol, presence of other prognostic features and are often based on small patient numbers.

Table 2A: Potential Prognostic and Predictive Factors in Adult ALL

	Risk factors	Annotations
Patient-related		
Age (years)	>30-60 years (continuous variable) >55 years (older adults and elderly)	Independent PF, usually not affecting risk model (age-adapted protocols)
Performance (ECOG)	>1	Retrospective data; relevance in older patients
Disease-related		
WBC ($x10^9$/l)	>30 (B), >100 (T)	Variably considered
Immunophenotype	Pro-B, CD20+ (B), Pro/Pre-T, ETP, mature-T (T)	Variably considered
Cytogenetics and FISH	Ph+, t(4;11), hypodiploidy, complex*	Key prognostic elements; beside Ph+ variably considered
Genetics	*BCR::ABL1*+, *KMT2Ar*	Key prognostic elements
	Ph-like, mutated *CLRF2*/TP53/JAK-STAT, adverse CNA profile (B), unmutated *NOTCH1/FBWX7* and abnormal *RAS/PTEN* (T)	Variably considered
Miscellaneous	CNS involvement	Occasionally considered
	Poor treatment compliance, undue treatment reductions and delay	Retrospective data, of greater concern with pediatric-type protocols
	Pharmacogenomics (affecting antimetabolite disposition)	Data in children, not usually assessed in adults
	Immune marrow microenvironment	Investigational, for research purposes
	Drug response profiling	Investigational, for research purposes
Treatment response dynamics		
Corticosteroid sensitivity (pre-phase)	Poor prednisone response (peripheral blast ≥$1x10^9$/L at the end of prephase)	Historical relevance, occasionally considered
Early/incomplete blast cell clearance (BM morphology)	Day 8-15 or end of induction BM blasts ≥ 5%	Variably considered
Time to CR (no. of courses)	>1 cycle (late CR)	Variably considered
MRD (molecular/flow cytometry)	MRD positivity (from end of induction onwards): ≥ 0.1% / 0.01% after induction ≥ 0.01% / positive after/during consolidation and pre/post-allogeneic SCT	Key and unifying factor predicting outcome

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ETP, early thymic precursor; CNS, central nervous system; CR, complete remission
Definition of complex karyotype: 5 or more chromosomal abnormalities excluding those patients with an established translocation[37]

Table 2B: Risk Stratification Models for Allogeneic SCT in Adult Ph/BCR::ABL1-negative (Ph-) ALL (European Study Groups)

		Risk stratification criteria*			
National Study Group	**Patient age (years)**	**Post-induction MRD**	**Cytogenetics/ Genetics[§]**	**WBC (x10^9/L)**	**Miscellaneous**
GMALL (Germany)	<55	≥0.01% after consolidation I (week 16 onward)	*KMT2A*+	>30 (B)	Late CR, Pro-B, early/mature-T
GIMEMA (Italy)	<65	≥0.01% after early consolidation (week 10-16), any positivity (week 22)	Adverse, *KMT2A*+	>100	Early/mature-T
HOVON (The Netherlands)	<40	≥0.01% after consolidation (wk 14-16)	Adverse KMT2A, hypodiploidy, complex karyotype	>30 (B), >100 (T)	Late CR
PALG (Poland)	<55	≥0.1% after induction ≥0.01% during/after consolidation	*KMT2A*+	>30 (B), >100 (T)	CNS+
UK NCRI ALL Group (United Kingdom)	<40	≥0.1% after induction and consolidation (mathematical risk model integrating MRD, cytogenetics and WBC)	Adverse	High count	-
FALL (Finland)	<45	≥0.1% after consolidation block B	Abn11q23, hypodiploidy	>100	Late CR, d15 BM blasts >25%
RALL (Russia)	<55	Positive during/after consolidation	t(4;11), t(1;19), *KMT2A*+	-	Age >30
SVALL (Sweden)	<65	≥0.1% after consolidation	Hypodiploidy, *KMT2A*+	-	EOI BM blasts >5%
PETHEMA (Spain)	<55 (60 fit)	≥0.1% after induction ≥0.01% during/after consolidation	-	-	-
GRAALL (France/Belgium/ Switzerland)	<60	≥0.1% after induction at week 6 or ≥0.01% after consolidation at week 12	-	-	-
CELL (Czech Republic)	<65	≥0.1% after induction ≥0.01% after consolidation	*KMT2Ar*	>30 (B)	Early/mature-T

*independent risk factors adopted in clinical trials for the definition of HR ALL and the consequent allocation to SCT; adapted from[62]
[§]adverse cytogenetics/genetics (details to be found in single study protocols~~)~~

Abbreviations: CNS, central nervous system; CR, complete remission; EOI, end of induction

Table 3: Response Criteria for ALL (modified from[70])

Category	Definition
Hematologic response criteria	
Complete remission (CR)*	BM blasts < 5% Absence of extramedullary disease Absolute neutrophil count > $1x10^9$/L Platelet count > $100x10^9$/L (Independence of red cell transfusions) *If available: MRD <1%*[65]
CR with incomplete recovery (CRi)[a]	All CR criteria except for residual thrombocytopenia <$100x10^9$/L or neutropenia <$1x10^9$/L *If available: MRD <1%*[65]
Morphologic leukemia-free state[b]	BM blasts < 5%; Absence of extramedullary disease; No hematologic recovery required If available: MRD <1%[65]
Partial remission (PR)[e]	Relevant in the setting of phase 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of BM blast percentage to 5% to 25%; and decrease of pretreatment BM blast percentage by at least 50% *If available: CR if MRD <1%*[65]
Failure (F)	None of the above *If available: MRD ≥1%*[65]
MRD response criteria (Variant 1)[c]	
Complete MRD response	No detectable MRD[d]
MRD Failure	MRD above 0.01% (i.e. 10^{-4})
MRD Other Negative Positive/Intermediate	 MRD negative with insufficient sensitivity MRD positive below 0.01%, quantifiable MRD positive below 0.01%, non-quantifiable MRD positive, non-quantifiable
MRD response criteria (Variant 2)[c]	
MRD complete response	No detectable MRD[d]
MRD persistence	Any quantifiable MRD
Criteria for extramedullary response assessment	
Published criteria for NHL[64] PET in case of CRu/PR according to published criteria for NHL[68]	

* All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5 to 7 days; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[a] CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved during the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients.

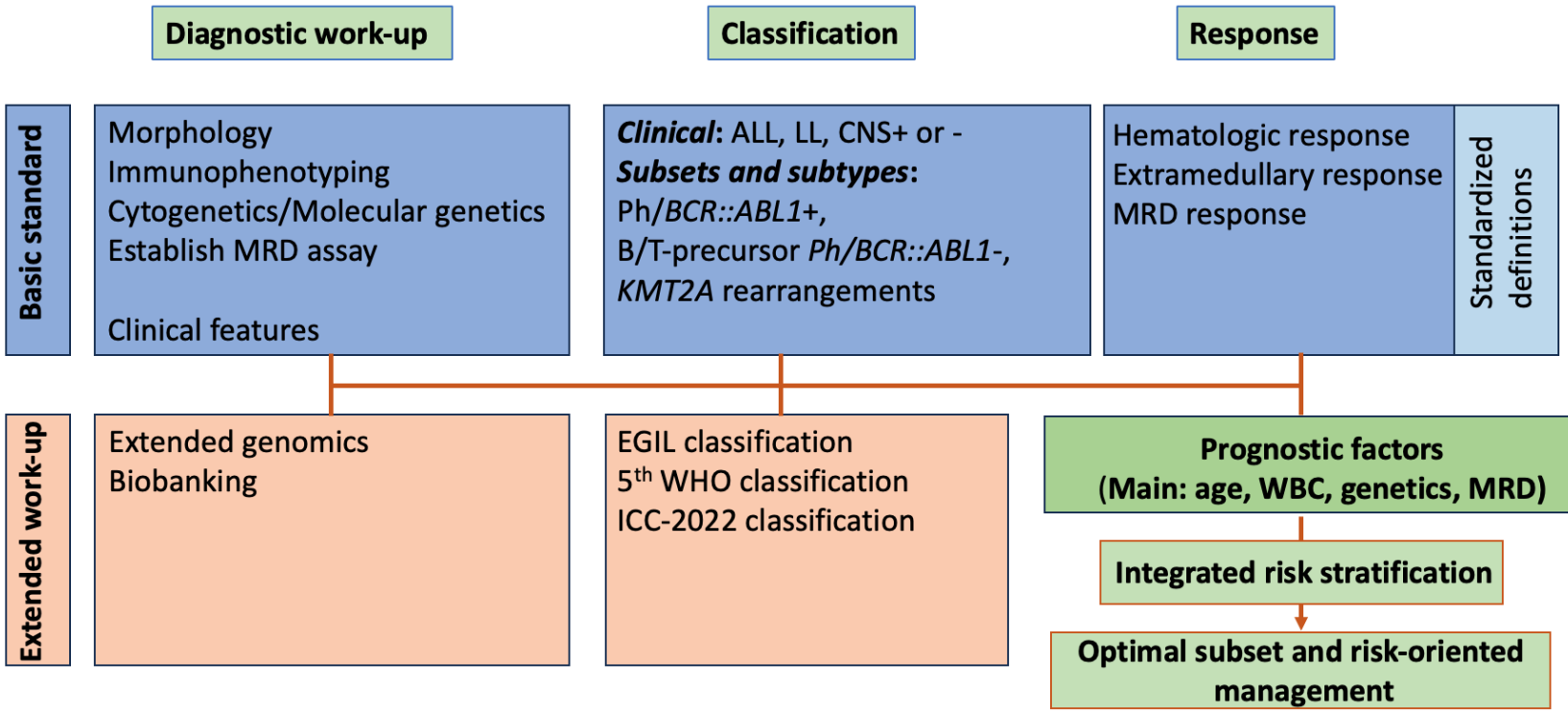
[b] This category may be useful in the clinical development of novel agents within phase 1 clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment. [e] Marrow should not merely be "aplastic"; at least 200 cells should be enumerated, or cellularity should be at least 10% [70]

[c] Confirmation of any MRD response requires the application of standardized methods with minimum technical requirements [28] in reference laboratories

[d] Confirmation of negative MRD requires that technical requirements for establishment of sensitivity of each individual TP are fulfilled

[e] Any PR should be confirmed or falsified by parallel MRD assessment.

Diagnosis, Prognostic Factors, and Risk Stratification of Acute Lymphoblastic Leukemia (ALL) in Adults: 2023 ELN Recommendations From a European Expert Panel



Conclusions: • **Basic diagnostic work-up should be performed in patients of all age groups in experienced reference laboratories** • **MRD assays should be established at diagnosis and followed in all patients** • **Risk stratification depends on protocols and should be pre-defined.**

*Gökbuget et al. DOI: 10.xxxx/**blood**.2024xxxxxx*

Blood Visual Abstract