Thesis

Outcome of Adult Patients with Acute Lymphoblastic Leukemia Treated in Graz

submitted by

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Declaration of Academic Integrity

I hereby confirm that the present diploma thesis is the result of my own independent scholarly work. I also confirm that in all cases, where material from the work of others (in books, articles, essays, dissertations, and on the internet) is acknowledged, quotations and paraphrases are clearly indicated. No material other than that cited in the reference list has been used. I have read and understood the Medical University's regulations and procedures concerning plagiarism.

Graz, 25.05.2024 *Lukas Schloffer m.p.*

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Zusammenfassung

Einleitung und Ziel dieser Arbeit: Die akute lymphatische Leukämie (ALL) ist eine seltene und aggressive, maligne Erkrankung des blutbildenden Systems, die eine große biologische Heterogenität aufweist und eine lange und intensive Therapie erforderlich macht. ALL stellt 20% aller Fälle von akuten Leukämien im Erwachsenenalter dar und die Prognose ist trotz erheblicher Fortschritte in der Therapie nach wie vor schlecht.
Bis zum jetzigen Zeitpunkt existiert keine umfassende Untersuchung aller Patientinnen und Patienten, die an der Universitätsklinik für Innere Medizin, klinische Abteilung für Hämatologie am LKH-Universitätsklinikum Graz behandelt oder an diese Abteilung zur weiteren Behandlung transferiert wurden. Das Ziel dieser Studie ist deshalb das Outcome aller Patientinnen und Patienten, welche im Zeitraum von 2001 bis 2021 behandelt wurden, zu evaluieren und mit publizierten Daten aus Krebsregistern und Studien zu vergleichen.

Material und Methoden: In dieser retrospektiven Kohortenstudie wurden die Daten von 95 Patientinnen und Patienten (älter als 18 Jahre) erhoben, welche im Zeitraum von Jänner 2001 bis April 2021 in Graz wegen einer B-/T-Zell ALL oder eines lymphoblastischen Lymphoms (LBL) behandelt wurden. Diese Daten wurden statistisch ausgewertet und in weiterer Folge die komplette Remissionsrate (CR), das Gesamtüberleben (OS) und das krankheitsfreie Überleben (DFS) berechnet.

Ergebnisse: Das mediane Erkrankungsalter lag bei 40,3 Jahren (18 - 85 Jahre) und das mediane Follow-up bei 91,2 Monaten (95% Konfidenzintervall [CI]: 64,3 - 118,2). 71 Patientinnen und Patienten hatten eine B-ALL (75,8%) und 23 (24,2%) Patientinnen und Patienten eine T-ALL bzw. ein T-LBL. Insgesamt wiesen 32,6% der Patientinnen und Patienten die *BCR::ABL1* Translokation und 7,3% eine Aberration von *KMT2A* auf. 35 Patientinnen und Patienten (36,8%) wurden der Hoch-Risiko Gruppe nach Kriterien der deutschen ALL-Studiengruppe (GMALL) zugeordnet. 83 Patientinnen und Patienten erhielten ihre Induktionstherapie in Graz (Steiermark) und die komplette Remissionsrate lag hier bei 96,4%.

Insgesamt erhielten 89 Patientinnen und Patienten eine Behandlung im Rahmen des Therapieprotokolls der GMALL Studiengruppe, vier Patientinnen und Patienten wurden initial mit einem anderen Therapieprotokoll behandelt und zwei Patientinnen und Patienten erhielten eine palliative Therapie.
Das mediane Gesamtüberleben lag bei 30,8 Monaten (95% CI: 18,4 - 72,9) bei einem 1-, 3-, und 5-Jahres-Gesamtüberleben von 75,5%, 46,6% und 41,3%. Das mediane DFS in erster kompletter Remission (CR1) lag bei 16,4 Monaten (95 % CI: 10,4 - 29,7), bei einem 1-, 3-, und 5-Jahres DFS von 57,6%, 34,8% und 31,9%. 42 (44,2%) Patientinnen und Patienten erhielten eine allogene Stammzelltransplantation in CR1. Das mediane und 5-Jahres OS nach Transplantation in CR1 lag bei 20,7 Monaten (95% CI: 7,2 - 34,3), bzw. bei 31,3%. Neun Patientinnen und Patienten erlitten ein Rezidiv nach Transplantation in CR1. Alter über 30 Jahre (p = 0,024) und die Hochrisiko-Gruppe nach GMALL (p = 0,028) waren signifikante Risikofaktoren für schlechteres OS in der multivariaten Analyse.
Außerdem wurden auch Patientinnen und Patienten, welche im Zeitraum von 2001 bis 2010 und 2011 bis 2021 diagnostiziert wurden, miteinander verglichen. Die erste Gruppe zeigte ein medianes OS von 30,8 Monaten (95% CI: 12,2 - 127,2) und die zweite Gruppe 33,1 Monate (95% CI: 19,2 - 74,8). Das 5-Jahres-OS der ersten Gruppe war 38,3% und der zweiten Gruppe 45,7%. Diese Unterschiede waren jedoch statistisch nicht signifikant (p = 0,680).

Diskussion: Die Ergebnisse dieser retrospektiven Studie stimmen mit publizierten Daten aus Krebsregistern überein. Jedoch besteht nach wie vor ein erheblicher Bedarf an neuen Therapien in der Behandlung der ALL im Erwachsenenalter.

Abstract

Introduction and Aim: Acute lymphoblastic leukemia (ALL) is a rare and aggressive malignant disease of the hematopoietic system with great biological diversity, which requires intensive and long-term therapy. Additionally, it only accounts for approximately 20% of acute leukemia cases in the adulthood and patients' prognosis is, despite considerable progress in therapy, still unsatisfying. To date, there is no comprehensive evaluation of patients diagnosed with ALL, who have been treated at or transferred to the Division of Hematology, Department of Internal Medicine, of the Medical University of Graz. Therefore, the aim of this study is to evaluate the outcome of these patients treated from 2001 to 2021 and to compare these real-world data with published cancer registries and studies.

Material and Methods: In this retrospective cohort study, medical records of 95 patients (≥18 years) diagnosed with B-/T- ALL/ lymphoblastic lymphoma (LBL) and treated in Graz from January 2001 to April 2021, were reviewed and statistically analyzed regarding complete remission (CR) rate, overall survival (OS) and disease-free survival (DFS).

Results: Patients' median age at diagnosis was 40.3 (18 - 85) years and the median follow-up time was 91.2 months (95% CI: 64.3 – 118.2). Seventy-one patients (75.8%) had B-ALL and 23 patients (24.2%) T-ALL/LBL. Out of the entire cohort 32.6% were positive for the *BCR::ABL1* translocation and 7.3% presented with a *KMT2A* rearrangement. Thirty-five patients (36.8%) were classified as high-risk according to criteria of the German Multicenter Study Group for Adult ALL (GMALL). Eighty-three patients received their initial treatment in Graz (Styria) and the CR rate was 96.4%. Of the patients included in the study, 89 patients started treatment according to the GMALL protocols, four patients initially received different protocols, while two patients were treated palliatively.
The median OS of all patients was 30.8 months (95% CI: 18.4 – 72.9), with 1-, 3-, and 5-year OS rates of 75.5%, 46.6% and 41.3%, respectively. The median DFS of patients in first CR (CR1) was 16.4 months (95% CI: 10.4 – 29.7), with 1-, 3-

and 5-year DFS rates of 57.6%, 34.8% and 31.9%. Forty-two (44.2%) patients received an allogeneic hematopoietic stem cell transplantation (HSCT) in CR1. Posttransplant median OS was 20.7 months (95% CI: 7.2 - 34.3) and 5-year survival rate 31.3%. Nine patients relapsed after receiving HSCT in CR1.
Age >30 years (p = 0.024) and GMALL high-risk group (p = 0.028) were significant adverse risk factors for worse OS in multivariable analysis.
Patients diagnosed and treated from 2001-2010 had a median OS of 30.8 months (95% CI: 12.2 – 127.2) and a 5-year OS of 38.3%. In comparison, those diagnosed and treated from 2011-2021 showed a median OS of 33.1 months (95% CI: 19.2 – 74.8) and a 5-year OS of 45.7%. However, these differences were not statistically significant (p = 0.680).

Discussion: These real-world outcome data are consistent with published reports from national cancer registries that continue to indicate a significant need for improvement in treatment of adult patients with ALL.

Publications and presentations based on this thesis

The results of this thesis were presented at the OeGHO- & AHOP-Frühjahrstagung 2023 (30.03.2023 – 01.04.2023).

ePoster – title: Outcome of Adult Patients with Acute Lymphoblastic Leukemia Treated in Graz Between 2001 to 2021.
Lukas Schloffer, Peter Neumeister, Hildegard T. Greinix, Stefan Hatzl, Eduard Schulz

Table of content

List of abbreviations and definitions

ALL	*Acute lymphoblastic leukemia*
Allo-HSCT	*Allogeneic HSCT*
Allo-HSCT	*Allogeneic HSCT*
AML	*Acute myeloid leukemia*
AYA	*Adolescents and young adults*
BCR::ABL1	*Fusion gene of BCR (breakpoint cluster region) and ABL1*
BM	*Bone marrow*
CAR T-cell therapy	*Chimeric antigen receptor T-cell therapy*
CD	*Cluster of differentiation*
cDNA	*Complementary deoxyribonucleic acid*
CNS	*Central nervous system*
CR	*Complete hematological remission (BM blasts < 5 %)*
CRP	*C-reactive protein*
cyIgM	*Cytoplasmic Immunoglobulin M*
DFS	*Disease-free survival*
EBMT	*European Society for Blood and Marrow transplantation*
ECOG PS	*European Cooperative Oncology Group Performance Status*
EFS	*Event-free survival*
EGIL	*European Group for the Immunological Characterization of Leukemias*
FACS	*Fluorescence-Activated Cell Sorting*
FISH	*Fluorescence in situ hybridization*
GMALL	*German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia*
GRAALL	*Group for Research on Adult Acute Lymphoblastic Leukemia*
GvHD	*Graft versus host disease*
Gy	*Gray, the unit of ionizing radiation*
HCT-CI	*Hematopoietic Cell Transplantation specific Comorbidity Index*
HLA	*Human Leukocyte Antigen*
HLA-MMUD	*Mismatched unrelated donor*
HLA-MUD	*Matched unrelated donor*
HSC	*Human stem cell*
XI	

HSCT	*Hematopoietic stem cell transplantation*
ICC	*International Consensus Classification of Myeloid Neoplasms and Acute Leukemias 2022*
IgM	*Immunoglobulin M*
KMT2A/MLL	*Lysine methyltransferase 2A, mixed-lineage leukemia 1*
LBL	*Lymphoblastic lymphoma*
B-ALL/ B-LBL	*B-cell precursor ALL/LBL*
T-ALL/ T-LBL	*T-cell precursor ALL/LBL*
LP	*Lumbar puncture*
MAC	*Myeloablative conditioning*
MolCR	*Molecular remission*
MRD	*Minimal residual disease*
mRNA	*Messenger ribonucleic acid*
MSD	*Matched sibling donor*
NGS	*Next generation sequencing*
NRM	*Non-relapse mortality*
OS	*Overall survival*
PB	*Peripheral blood*
PCR	*Polymerase chain reaction*
Ph-negative ALL	*Philadelphia-chromosome negative ALL*
Ph-positive ALL	*Philadelphia-chromosome positive ALL*
RQ-PCR	*Real-time quantitative PCR*
RT-PCR	*Reverse-transcriptase PCR*
SEER	*Surveillance, Epidemiology and End Results Program of the National Cancer Institute*
sIgM	*Surface Immunoglobulin M*
TBI	*Total body irradiation*
TCR	*T-cell receptor*
Tdt	*Terminal deoxynucleotidyl transferase*
TKI	*Tyrosine kinase inhibitor*
WBC	*White blood cell count*
WHO	*World Health Organization*

WHO-HAEM4R	*Revised 4th edition of the WHO classification of haematolymphoid tumours*
WHO-HAEM5	*5th edition of the WHO classification of haematolymphoid tumours*

List of figures

List of tables

1. Introduction

1.1 Definition of Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma

Acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LBL) represent overlapping manifestations of one malignant process of the hematopoietic system, in which a wide range of specific chromosomal and genetic alterations lead to disturbed differentiation and clonal proliferation of immature lymphoid precursor cells, so called lymphoblasts. These lymphoblasts are able to infiltrate the bone marrow (BM), peripheral blood (PB), lymphoid organs and extranodal sites.(1,2)

Depending on their lineage respectively immunophenotype, B-cell precursor acute lymphoblastic leukemia/lymphoblastic lymphoma (B-ALL/B-LBL) and T-cell precursor acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/T-LBL) can be distinguished. (1,3)
ALL presents with massive infiltration of the bone marrow and presence of immature lymphoblasts in the peripheral blood. If there is no bone marrow infiltration or only an infiltration of less than 25% (or 20% by WHO criteria) and a predominant mass lesion involving lymphatic and extranodal sites, it is classified as LBL. (1,4)
In this thesis, the focus will be on adult ALL and the description of its epidemiology, pathogenesis, its immunophenotypically and genetically determined subgroups, its clinical presentation, treatment options as well as the outcome of this disease.

1.2 Epidemiology

ALL is a rare malignant disease in adults with estimated 6.660 new cases representing 0.3% of all new cancer cases in the United States in 2022. (5) In the literature the overall incidence is reported to be 1 – 1.7/100.000 per year and SEER, the Surveillance, Epidemiology and End Results Program of the National Cancer Institute, estimated the incidence to be 1.8 for 2022. (2,5,6) Similar numbers are not available for Austria because Statistics Austria computes

numbers for leukemias in general (C91-C95), whereas ALL forms a specific subgroup of all leukemia entities. (7)
Men are slightly more often affected by ALL with a male to female ratio ranging from 1.2:1 to 1.4:1. (2,8) SEER estimated 3740 male and 2920 female cases for 2022, which makes a ratio of 1.3:1, supporting these numbers. (5)
ALL accounts for approximately 20% of all cases of acute leukemia in adults (80% Acute Myeloid Leukemia, AML) (9), whereas ALL is the most common malignancy of the childhood (0-14 years), accounting for 80% of acute leukemias in children and 56% in adolescents (15-19 years). (10) Therefore, the age-specific incidence shows significant differences with its highest incidence in children between the ages 1-4 years (5.3/100.000), then decreasing to its lowest incidence in people between 25 and 45 years. A further increase can be seen in adults over 80 years (2.3/100.000). (2,8)

1.3 Risk Factors and Predisposing Conditions

Although somatic mutations are the main pathway of ALL development, some inherited or de novo mutations representing genetic susceptibility to this disease have been identified. These genes are mainly involved in proliferation and differentiation of blood cells. Common genetic polymorphisms involve for example *IKZF1*, *CKDN2A/2B*, *E2A/TCF3*, *GATA3* and *ERG*. Furthermore, inherited alterations of TP53 are known in children, suggesting a manifestation of Li-Fraumeni-Syndrome (see 1.6.1 Genetics of B-cell precursor ALL). (11–13)
Additionally, some congenital syndromes are associated with risk of developing ALL including Down Syndrome, Fanconi anemia and Ataxia telangiectasia. Environmental factors such as pesticide exposure and ionizing radiation are also mentioned in the literature to initiate ALL. (2,13)

1.4 Clinical Presentation

While symptoms of ALL commonly appear quickly, slower manifestations are also possible. Untreated acute leukemia, including ALL, generally progress rapidly leading to death if left untreated. (8,14)

Clinical features of ALL result from infiltration of lymphoblasts in the bone marrow leading to disturbed hematopoiesis as well as infiltration of lymphoid tissue and extranodal sites. Anemia, neutropenia and thrombocytopenia are characteristics of bone marrow failure, that can be seen in blood work, result in the symptoms listed below (8,9):

- Anemia: fatigue, dyspnea, tachycardia, pallor;
- Neutropenia: susceptibility to infections, especially infections of the skin and mucous membranes, common pathogens include candida albicans, herpes simplex virus, *Staphylococcus* spp.;
- Thrombocytopenia: spontaneous or easy bleedings like petechiae, bruising (ecchymosis), epistaxis, menorrhagia.

General symptoms of ALL include bone and joint pain, as well as B symptoms comprising fever, night sweats and unintentional weight loss. Thirty to 50% of patients present with swelling of lymph nodes, splenomegaly and or hepatomegaly.(14,15) Involvement of the central nervous system (CNS) at the time of diagnosis is present in 5 – 15 % of adult patients.(16) Symptoms of CNS involvement include meningeal symptoms, cranial neuropathies like cranial nerve palsy, headache, nausea and vomiting. Other immune-privileged sites such as retina, testes and ovaries are also more susceptible to ALL infiltration. However, lymphoblasts can potentially infiltrate any organ. A characteristic symptom of T-ALL are mediastinal tumors that can cause superior vena cava syndrome. Although the majority of patients present with elevated white blood cell (WBC) counts, WBC can be normal or even decreased and only the detection of blasts in the peripheral blood or bone marrow define ALL (see 1.8 Diagnosis). If leukocyte numbers are extremely elevated - a condition called hyperleukocytosis (>100.000/µl), leukostasis syndrome can evolve resulting in disturbance of microcirculation and hypoxia. Further findings in laboratory work up include elevated ESR (erythrocyte sedimentation rate), uric acid and LDH (lactate dehydrogenase). (9,14,15)

1.5 Basics of Hematopoiesis

Definition and different sites of hematopoiesis

The constant process of blood cell formation and development throughout life is called hematopoiesis. Hematopoiesis takes place in various places during embryonic and fetal development. The embryonic yolk sac represents the site of primitive hematopoiesis whereas definitive hematopoiesis is located in the fetal liver and spleen during early fetal period until shortly after birth and the bone marrow from the 6-7 month of pregnancy on. (17–19) During the first years of life, the entire bone marrow is hematopoietic, but later during childhood most parts of the bone marrow will be replaced by fat. In the healthy adult, hematopoiesis is restricted to the central skeleton and proximal femur and humerus. (17)

The hematopoietic stem cell

These cells, that migrate to the fetal liver, spleen and bone marrow and initiate definitive hematopoiesis at these sites derive from the hemogenic endothelium of the aorta-gonad-mesonephros, and ultimately represent the definitive hematopoietic stem cell (HSC). This process is called endothelial-to-hematopoietic transition. (19) HSC have the ability to renew themselves by cell division and to form all mature blood cells of the human body through several complex steps including hematopoietic progenitor cells (multipotency). (20)

The stem cell niche

The bone marrow (BM), with its heterogenous stromal cells and microvascular network, forms an optimal microenvironment for the HSCs and its progenitor cells, called the hematopoietic stem cell niche, which in its entirety forms the hematopoietic system. (15,19,21) This niche, first described by Schofield, influences and contributes to hematopoiesis and regulation of HSCs by various factors in a complex manner. Additionally, the stem cell niche is also thought to play a role in the development of hematological diseases, which could be shown in mice models. However, the influence of the stem cell niche on the development of malignant diseases in humans is still unclear. (19,21)

Furthermore, studies showed, that leukemic cells also have the ability to remodel the stem cell niche in various ways. This results in an environment that supports

survival of malignant cells and may lead to disease progression and resistance to chemotherapy. (21) Regarding ALL, Hawkins et al. were able to show the influence of T-ALL cells on the surrounding microenvironment in a mouse model. In this study, the malignant cells developed highly dynamic interactions with the environment, without preference for any specific sub-compartments in the BM. Therefore, this study suggests to target the potential of T-ALL cells to interact with the BM stroma as an option of treatment.(22)

1.5.1 Cell differentiation in hematopoiesis

Hematopoiesis has long been portrayed as a strict hierarchical tree, but Laurenti and Göttgens clarify in their review, that due to research and new technologies strict separation between stem cells and progenitor cells and former routes of blood lineage relationships are reconsidered. (20) Notably, transcription factors play an essential part in the regulation of cell fate decisions and differentiation and maturation of cells during hematopoiesis. (11)

On the top of the hierarchy (**Figure 1**), there is a heterogenous pool of HSC and lineage biased multipotent progenitors (20,23) that differentiate into common myeloid progenitors (CMP) and lymphoid-primed multipotential progenitors/ multipotential lymphoid progenitors (LMPP/MLP), suggesting that the lymphoid and myeloid branch stay longer associated during hematopoiesis. This questioned the former concept of early segregation between lymphoid and myeloid fate in human hematopoiesis into common lymphoid progenitors (CLP) and common myeloid progenitors (CMP). (24) This means, that both CMPs and the LMPP/MLP population have the ability to form the heterogenous pool of GMP (progenitors of granulocytes and monocytes), then differentiating into mature eosinophile, basophile and neutrophile granulocytes and monocytes.(25)
Megakaryocytes and erythrocytes derive from a common progenitor called the megakaryocyte-erythrocyte progenitor (MEP). MEP emerge from the population of CMPs. (26) However, several groups were able to give evidence for megakaryocyte biased HSCs, suggesting, that there is direct differentiation from HSC to megakaryocytes without the precursor stages CMP and MEP. (27,28)
For dendritic cells, it could be shown that they originate from both myeloid- and lymphoid lineages and their precursors. (24)

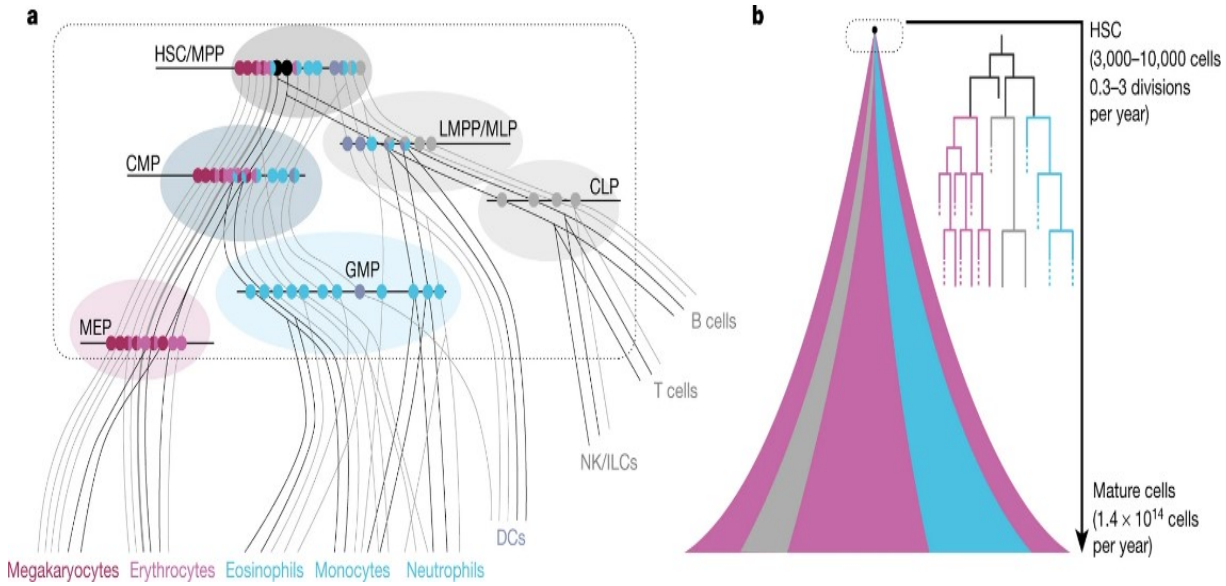


Figure 1. *"Trajectory-based visualization of the hematopoietic hierarchy. a) Two-dimensional visualization of early haematopoiesis. b) Three-dimensional visualization of the progeny of a single HSC." (19)*

1.5.2 Development of lymphocytes

All different cell types of the lymphoid lineage (B, T, NK, and innate lymphoid cells, subset of dendritic cells) derive from common progenitors in the bone marrow, the common lymphoid progenitor (CLP), which in turn derives from LMPP. LMPP have lymphoid and myeloid potential, however while differentiating into CLP, the potential to form cells of the myeloid lineage vanishes. (11)
Inlay, Bhattacharya et. al. were able to define two subtypes of CLP depending on their expression of the surface marker Ly6D. Populations that are negative for Ly6D are called all-lymphoid progenitors (ALP), having the potential to form all lymphoid cell types, and Ly6D positive cells, called B-cell biased lymphoid progenitors (BLP). (29) However, Ly6D positive cells can be subdivided into BLP 1–3, with BLP 1 and 2 still having the potential to differentiate into T and NK cells and BLP 3 forming CD 19+ pro-B cells. (11,30)

Regulators of lymphoid development include various transcription factors, which regulate gene expression of the lymphoid lineage. Important for early lymphopoiesis (MPP to LMPP and LMPP to CLP) are Ikaros, PU.1 and E2A. Thus, even in very early stages of hematopoiesis, such as the MPP stage, one recognizes a gradual differentiation to the lymphoid cell line. (31)

Under the influence of transcription factors such as E2A (TCF3), EBF1, FOXO-1 and PAX-5, B cells undergo different maturation stages (pro-B, pre-B, immature B-cell) in the bone marrow, which can be distinguished on the basis of surface markers and rearrangement of B-cell receptor genes causing the expression of pre-B-cell receptors. B cells that express IgM on their surface (sIgM) represent the last stage of maturation in the bone marrow (immature B cells), allowing them to migrate to the spleen (transitional B cells), where maturation is completed. After antigen-activation of naïve B cells via the B-cell receptor during immune response, they become either memory B cells or plasma cells, the latter producing immunoglobulins. (11,32)

Progenitors of T-cells migrate to the thymus and are called early thymic progenitors (ETP). ETP mark the first step of T-cell development, lacking CD4 and CD8 and are therefore called double negative thymocytes (DN). Furthermore, these cells do not present CD3 on their surface, which is a part of the T-cell receptor. There are four stages of maturation of double negative thymocytes called DN1 – DN4, that subsequently differentiate into γ:δ-cells and α:β-cells. γ:δ-cells leave the thymus as γ:δ+CD3+CD4-CD8- cells. However, α:β -cells pass through further maturation steps and become CD4+CD8+ (double positive) thymocytes that also express CD3. These cells undergo positive and negative selection, after which they express the T-cell receptor and either CD4 (helper cells) or CD8 (cytotoxic cells) on their surface. Notch signaling pathway is the initiator of T-cell development and induces important transcription factors such as TCF, GATA-3 and BCL11b. (33,34)

1.6 Pathogenesis and Genetics

Leukemias, as malignancies in general, arise from a precursor cell, that accumulated genetic mutations within critical genes involved in differentiation and proliferation. Besides changes in the structure of chromosomes and genes itself, epigenetic alterations contribute to the malignant transformation and uncontrolled growth of neoplastic cells. Hence, leukemia development is a multistep process involving various cellular pathways. This process is called leukemogenesis.(35,36)

Chromosomal alterations, especially translocations, are very common in malignant hematological diseases and play an essential role in disease development. (35) Other major structural chromosomal alterations include aneuploidy (gain and loss of complete chromosomes) as well as deletion and amplification. (13) Subtypes of ALL are defined primarily by these specific chromosomal alterations that result in deregulation of genes through juxtaposition with strong enhancers and formation of chimeric genes. Genes involved in the pathogenesis of ALL encode for various transcription factors, tyrosine kinases, cytokine receptors and epigenetic modifiers. (12,13) Structural chromosomal alterations represent primary lesions, with additional secondary alterations and mutations (copy number alterations, sequence mutations) contributing importantly to leukemogenesis.(13)
In children, a large proportion of chromosomal aberrations develop prenatally and leukemogenesis is driven by acquired secondary mutations after birth. Gene fusions that are proven to have a prenatal origin in children include for example ETV6::RUNX1, TCF3::PBX1 and hyperdiploidy. Importantly, only a small proportion of children with these aberrations will develop ALL, supporting the importance of secondary genetic hits in the development of leukemia. (37)

The development of ALL on the basis of clonal hematopoiesis is a special pathogenesis that occurs mainly with ageing or after previous cytotoxic therapy. Clonal hematopoiesis represents a precursor lesion of malignant hematological diseases associated with somatic mutations that frequently occur in the development of myeloid neoplasms (e.g. TP53). ALL with myeloid mutations, associated with clonal hematopoiesis, could be shown to have higher resistance to chemotherapy and adverse outcome. (38)

Distribution of these genetically defined subgroups vary among age groups. Moreover, cytogenetics is an important determinant of prognosis, besides other clinical and immunophenotypical factors, and identifying certain lesions is relevant for modern targeted therapies. (13)
These recurring chromosomal abnormalities will be discussed briefly in the following chapters.

1.6.1 Genetics of B-cell precursor ALL

BCR::ABL1

The translocation t(9::22)(q34;q11.2) (Philadelphia chromosome, Ph-positive ALL) leads to the *BCR::ABL1* fusion gene encoding a constitutively activated tyrosine kinase. This genetic alteration is present in 25-40% of adult patients. Deletion or mutation of the *IKZF1* gene, encoding the transcription factor Ikaros, can be frequently found in BCR::ABL1 positive ALL. Alteration of IKZF1 in B-ALL is associated with resistance to treatment and poor outcome. (2,39,40) This subtype can be targeted with tyrosine kinase inhibitors, which significantly improved the former poor outcome. (41) (see 1.10.2 Tyrosine kinase inhibitors)

***KMT2A* Rearrangements**

Rearrangements of *KMT2A* (Histone-lysine [K] MethylTransferase 2a gene), formerly known as MLL (mixed lineage leukemia gene) accounts for 10-15% of adult ALL. The gene is located on chromosome 11q23 and various partners of translocations are described as t(v;11q23). The presence of *KMT2A* rearrangements has been shown to be an independent risk factor, with high relapse rates, resistance to treatment, increased risk of CNS involvement, and generally aggressive behavior. (2,42)

ETV6::RUNX1

This genetic subtype derives from a translocation involving the *ETV6* gene, located on chromosome 12, and *RUNX1* on chromosome 21, thus the resulting fusion gene is referred to as t(12;21)(p13;q22). Approximately a third of pediatric ALL patients present with this translocation and it could be shown to be a favorable prognostic factor in children, however in adults it is present in less than

5% of ALL. Importantly, this translocation is not detectable by conventional cytogenetics, but by RT-PCR or FISH. (13,43)

TCF3::PBX1 and TCF3::HLF

TCF3 (synonym *E2A*) is an important gene (located on chromosome 19) encoding for transcription factors that play a crucial role in maturation of B cells. It is involved in two translocations. Translocation t(1;19)(q23;p13) creates the *TCF3::PBX1* fusion gene, which appears in 5% of adults and has variable prognostic impact. *TCF3::HLF* with t(17;19)(q23;p13) is rare and associated with poor prognosis. (2,43)

IGH::IL3

The translocation t(5;14)(q31;q32) leading to the fusion gene IGH::IL3 is extremely rare and there is only sparse description and data on this distinct subtype in the literature. However, it is classified as a distinct entity in the 5th WHO classification of hematolymphoid tumors and in the International Consensus Classification. (see 1.7 Classification) The only described specific clinical feature is eosinophilia, which results from juxtaposition of the IGH enhancer to IL3 gene, and therefore interleukin-3 overproduction. Interleukin-3 is responsible for maturation and release of eosinophils in further consequence. (44)

High Hyperdiploidy

High hyperdiploidy is defined as gain of at least five chromosomes in a nonrandom pattern involving chromosomes 4, 6, 10, 14, 17, 18, 21 and X. Accounting for 25-30% of pediatric patients and showing favorable prognosis, high hyperdiploidy is however rare in adults, affecting about 3% of all patients. Mutations of the RAS pathway and epigenetic modifiers are frequently found in patients with high-hyperdiploidy ALL. (2,45)

Hypodiploidy

Hypodiploidy is defined as less than 44 chromosomes and can be subdivided in near-haploid ALL (24-31 chromosomes) and low-hypodiploid ALL (32-39 chromosomes), representing two very distinct genetic subtypes. RAS pathway

mutations, and deletions of *IKZF3* are important genetic alterations in near-haploid ALL, which is a rare subtype (<1% in adults, 2% in children). (2,46)

Low-hypodiploid ALL is highly associated with mutation of TP53. In pediatric low-hypodiploid ALL patients, TP53 could be found also in nontumor hematopoietic cells, suggesting that this alteration is inherited and a manifestation of Li-Fraumeni-syndrome. However, low-hypodiploid ALL represents less than 1% of pediatric ALL cases. (2,46) In adults, this subtype is more common with frequencies of 10 to over 30 % in older patients.(2,47) Here it was shown, that the pathogenesis of low-hypodiploid ALL in adults is based on somatic TP53 mutated clonal hematopoiesis, leading to aneuploidy and B-ALL in further consequence. (47)

Further defined genetic abnormalities in B-ALL

Particularly in recent years, considerable progress has been made in identifying new genetical alterations through broad genomic analysis. These new subtypes comprise rearrangements of Myocyte enhancer factor 2D (*MEF2D*), zinc finger 384 (*ZNF384*), *PAX5* and subtypes that show gene expression profile similar to known genetic subtypes, but lacking the specific translocation. (12)
These are described here:

***BCR::ABL1*-like ALL (Ph-like ALL)**

This subtype shows similar gene expression to *BCR::ABL1* but lacks the Philadelphia chromosome. Alterations found in Ph-like ALL are heterogenous and involve ABL-class tyrosine kinase genes, activating mutations in the JAK-STAT pathway, rearrangements of *CRLF2* and others. Thus, *ABL1* and *JAK2* serve as targets for treatment with tyrosine kinase and JAK inhibitors. Incidence of *BCR::ABL1* like ALL rises from 10 % in children to over 20% in adults. (12)

***ETV6::RUNX1*-like ALL**

Comparably to *BCR::ABL1*-like ALL, this subtype shows gene expression and immunophenotype similar to *ETV6::RUNX1*, however the typical fusion is missing.

Alterations frequently found within this subtype involve *ETV6*, *TCF3* and *IKZF1*. *ETV6::RUNX1* like ALL is predominantly found in children. (12)

Roberts and Mullighan state that due to these advances in the discovery of new subtypes, more than 90 % of B-ALL cases can be classified by their specific genetic alterations. (12)

1.6.2 Genetics of T-cell precursor ALL

The genomic landscape of T-ALL is extremely heterogenous with alterations of various genes that are normally involved in proliferation, differentiation and survival during development of T cells (thymopoiesis). (2)

As already described (see 1.5.2 Development of lymphocytes), the Notch signaling pathway plays an essential role in the development and especially survival of T-cell progenitors. For this reason, constitutive activation of the Notch pathway is a potent driver of leukemogenesis. Activation can occur through mutations within the *NOTCH1* gene or deletions/loss of function mutations within *FBXW7*. Furthermore, T-ALL shows abnormalities of RAS (activating mutations of N-RAS/K-RAS) and PTEN (loss of function), two regulators in the pro-proliferative signal pathways Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR. Mutations of NOTCH1/FBXW7 show favorable outcome when there is no alteration of RAS or PTEN. In contrast, mutation of RAS or PTEN is associated with dismal prognosis in the absence of alterations in the Notch1 pathway. (48)

Another important factor in T-ALL are mutations affecting *CDKN2A*, which is involved in cell cycle regulation. Similar to B-ALL, in approximately half of the cases of T-ALL, one can find specific chromosomal translocations leading to deregulation and overexpression of oncogenic transcription factors (TAL1/2, LYL1, TLX1, TLX3 and others). Additionally, genes of epigenetic modifiers and regulators present mutations including *SUZ12*, *EZH2*, *PHF6* and *KDM6A*. (13)

1.6.3 Pathogenetic alterations in relapse

Relapse of ALL affects approximately 40-50% of adult patients and has a dismal prognosis with conventional chemotherapy. New treatment options were established in recent years and will be discussed in the chapter treatment of ALL. (1.10.5 Treatment of relapse and refractory disease).

In most cases, the primary leukemic clones at diagnosis can be eradicated by treatment. Relapse of ALL can develop from a minor clone present at the time of diagnosis that accumulated further alterations or, less frequently, cells of the primary clone surviving treatment acquire additional genetic lesions. Thus, development of relapse is based on genetic lesions persisting from diagnosis in combination with new alterations. Common additional genetic alterations involve the *CREBBP* (a transcriptional coactivator and acetyl transferase) gene and *NR3C1* (encoding for glucocorticoid receptors) leading to an abrogated response to treatment with glucocorticoids. Impaired response to mercaptopurine is caused by mutations within the *NT5C2* gene (5'-nucleotidase, cytosolic II) and loss of *MSH6* leads to resistance to thiopurines. Further genetic lesions in relapsed patients can be found in *TP53*, *NRAS* and *IKZF1* genes. (2,12,13)

1.7 Classification

There are several systems of classification, that categorize different subtypes of ALL/LBL according to their immunophenotype, cytogenetics and molecular alterations. Since 2022, there exist two distinct classifications, based on cytogenetical findings, that share common subtypes but also differences in the classification and categorization of ALL/LBL subgroups. In this chapter these two, the 5th edition of the WHO classification of hematolymphoid tumors (WHO-HAEM5) and the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias (ICC) will be presented and compared to each other and to the revised 4th edition of the WHO classification (WHO-HAEM4R) published in 2016. Unlike WHO/ICC cytogenetics-based classifications, the classification used by the GMALL study group aims to stratify patients by disease risk considering immunophenotype, associated cytogenetical findings, and WBC.
The GMALL risk stratification and treatment procedures are used at the Division of Hematology in Graz. (8)

1.7.1 The revised 4th edition of the WHO classification of the Tumors of Hematopoietic and Lymphoid Tissues

In 2008, the WHO published its 4th edition of the WHO classification of the Tumors of Hematopoietic and Lymphoid Tissues, which was revised in 2016. In WHO-HAEM4R (see **Table 1**), precursor lymphoid neoplasms comprise precursor B-cell neoplasms (B-ALL/LBL) and precursor T-cell neoplasms (T-ALL/LBL). Within the precursor B-cell neoplasms there are five subtypes with specific translocations and their resulting fusion genes (*BCR-ABL1*, *KMT2A* rearrangement, *ETV6-RUNX1*, *IL3-IGH*, *TCF3-PBX1*) as well as two subtypes presenting with aneuploidy (hyperdiploidy and hypodiploidy). B-ALL/LBL that does not show a recurrent genetic abnormality is listed as not otherwise specified (NOS). Additionally, there are two provisional entities: BCR-ABL1-like and B-ALL/LBL with iAMP21.
Precursor T-cell neoplasms include T-ALL and T-LBL, however there are no defined subtypes, but only a provisional entity: early T-cell precursor lymphoblastic leukemia. Another provisional entity, the NK-cell lymphoblastic leukemia/lymphoma, is included to the classification. (49)

Table 1. *The revised 4th edition of the WHO classification 2016, adapted from* (8)

Precursor lymphoid neoplasms
Precursor B-cell neoplasms
B-lymphoblastic leukemia/lymphoma NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); *BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.1); *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
Precursor T-cell neoplasms
T-lymphoblastic leukemia/lymphoma NOS
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural Killer cell lymphoblastic leukemia/lymphoma

1.7.2 The 5th edition of the WHO classification of Haematolymphoid Tumours

The most recent WHO classification, which can be seen in **Table 2**, was published in 2022 and includes the same division in precursor B- and T-cell neoplasms as its predecessor and also the classification of subtypes is based on cytogenetic findings. Regarding the nomenclature, there are two changes in the new classification of 2022. First, the recommendation of the Human Gene Nomenclature Committee (HGNC), to use a double colon (::) instead of a hyphen (-) was implemented (e.g. *BCR-ABL1* changed to *BCR::ABL1*) (50) and secondly, there is a focus on the molecular changes of every subtype and the cytogenetic alterations are not described (e.g. B-lymphoblastic leukemia/lymphoma with

BCR::ABL1 fusion instead of B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2); *BCR-ABL1*). (51)

B-ALL/LBL with *BCR::ABL1*-like features, which was previously classified as provisional entity, was added to the classification. (51) This is due to its frequency in all age groups (20% in adults, 25-30% in AYAs) and the importance of targeted therapy options like tyrosine kinase inhibitors. (12,51,52) Another provisional identity, B-ALL/LBL with iAMP21 was added to the new classification, as well as two new identities: *TCF3::HLF* and *ETV6::RUNX1*-like. Characteristics of these two subtypes are described in chapter 1.6.1 Genetics of B-cell precursor ALL.

In recent years, new genetic alterations have been identified (*DUX4*, *MEF2D*, *ZNF384*, *NUTM1*, *IG::MYC* fusion, *PAX-5* alterations), and they are summarized as B-lymphoblastic leukemia/lymphoma with other defined genetic alterations. It is noted that although clinical and prognostic characteristics have already been described for these alterations, evidence is still lacking to classify them as distinct subtypes. (51)

Contrary to B-ALL/LBL, there is only one defined distinct subtype in T-ALL/LBL: early T-precursor lymphoblastic leukemia/lymphoma (ETP-ALL/LBL). Lacking subtypes of T-ALL/LBL is due to insufficient evidence of relevant clinical features of genetically defined subtypes. However, ETP-ALL/LBL is a subtype deriving from very early progenitors in thymopoiesis (see 1.5.2 Development of lymphocytes), presenting with similarities in gene expression and surface markers to HSCs and myeloid progenitors. (51) In a retrospective study by Jain et. al.,19 (17%) out of 111 T-ALL/LBL patients presented with ETP-ALL/LBL and showed worse OS (1.7 years vs. median not reached, p=0.008) and dismal clinical features (higher BM blast count, lower CR rate, higher CNS involvement rate). Therefore, ETP-ALL/LBL is stated to be a high-risk subtype of precursor T-cell neoplasms and ALL in general. (53)

Table 2. *The 5th edition of the WHO classification of haematolymphoid tumours, adapted from* (51)

Precursor B-cell neoplasms
B-lymphoblastic leukemia/lymphoma NOS
B-lymphoblastic leukemia/lymphoma with high hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with iAMP21
B-lymphoblastic leukemia/lymphoma with *BCR::ABL1* fusion
B-lymphoblastic leukemia/lymphoma with *BCR::ABL1*-like features
B-lymphoblastic leukemia/lymphoma with *KMT2A* rearrangement
B-lymphoblastic leukemia/lymphoma with *ETV6::RUNX1* fusion
B-lymphoblastic leukemia/lymphoma with *ETV6::RUNX1*-like features
B-lymphoblastic leukemia/lymphoma with *TCF3::PBX1* fusion
B-lymphoblastic leukemia/lymphoma with *IGH::IL3* fusion
B-lymphoblastic leukemia/lymphoma with *TCF3::HLF* fusion
B-lymphoblastic leukemia/lymphoma with other defined genetic abnormalities
Precursor T-cell neoplasms
T-lymphoblastic leukemia/lymphoma, NOS
Early T-precursor lymphoblastic leukemia/lymphoma
~~NK-lymphoblastic leukemia/lymphoma~~ *Entity deleted*

1.7.3 International Consensus Classification of Myeloid Neoplasms and Acute Leukemias

The International Consensus Classification (ICC) has similarities to the WHO-HAEM5, but defined further subcategories of known genetic subtypes and added subtypes that are summarized as B-ALL/LBL with other defined genetic abnormalities within the WHO classification. The ICC can be seen in **Table 3**. Importantly, the ICC subdivides B-ALL with *BCR::ABL1* in B-ALL with lymphoid only and multilineage involvement. B-ALL with *BCR::ABL1* and multilineage

involvement suggests blast crisis in chronic myeloid leukemia (CML), due to detection of the Philadelphia chromosome by FISH not only in lymphoblasts, but also in myeloid cells. Distinguishing these two entities is important, because treatment and outcome of B-ALL with *BCR::ABL1* and CML in lymphoid blast crisis differ (median OS for B-ALL 52 months vs. 20 months in CML with blast crisis, p=0.0012) (54,55)

Furthermore, ICC subdivides hypodiploid B-ALL in low hypodiploid and near haploid and *BCR::ABL1* like B-ALL in *ABL1* rearranged and JAK-STAT activated subtypes. The peculiarities of these distinct genetic alterations are described in 1.6.1 Genetics of B-cell precursor ALL. Two distinct subtypes that cannot be found in the WHO-HAEM5 arise from point mutations in two important transcription factors involved in B-cell development. B-ALL with mutated *IKZF1 (N159Y)* and *PAX5 (P80R)* both present with unique gene expression that is different to any other defined subtypes and for *PAX5 P80R* it has been shown to be a good risk factor in B-ALL. (55,56)

Considering T-ALL, the ICC added a novel subtype to ETP-ALL, defined by rearrangement of the *BCL11B* gene, which is present in approximately a third of ETP-ALL patients. (55)

Table 3. *International Consensus Classification of Myeloid Neoplasms and Acute Leukemias 2022, adapted from* (55)

Classification of ALL (lymphoblastic leukemia/lymphoma)
B-ALL
B-ALL with recurrent genetic abnormalities
B-ALL with t(9;22)(q34.1;q11.2) *BCR::ABL1* with lymphoid only involvement with multilineage involvement
B-ALL with t(v;11q23.3) *KMT2A* rearranged
B-ALL with t(12;21)(q13.2;q22.1) *ETV6::RUNX1*
B-ALL, hyperdiploid
B-ALL, low hypodiploid
B-ALL, near haploid
B-ALL with t(5;14)(q31.1;q32.3) *IL3::IGH*

B-ALL with t(1;19)(q23.3;p13.3) *TCF3::PBX1*
B-ALL, BCR::ABL1-like, ABL-1 class rearranged B-ALL, BCR::ABL1-like, JAK-STAT activated B-ALL, BCR::ABL1-like, NOS
B-ALL with *MYC* rearrangement
B-ALL with *DUX4* rearrangement
B-ALL with *MEF2D* rearrangement
B-ALL with *ZNF384(362)* rearrangement
B-ALL with *NUTM1* rearrangement
B-ALL with *HLF* rearrangement
B-ALL with *UBTF::ATXN7L3/PAN3*, *CDX2 ("CDX2/UBTF")*
B-ALL with mutated *IKZF1 N159Y*
B-ALL with mutated *PAX5 P80R*
Provisional entity: B-ALL, *ETV6::RUNX1*-like
Provisional entity: B-ALL, with *PAX5* alteration
Provisional entity: B-ALL, with mutated *ZEB2 (p.H1038R)/IGH::CEBPE)*
Provisional entity: B-ALL, *ZNF384* rearranged-like
Provisional entity: B-ALL, *KMT2A* rearranged-like
B-ALL, NOS
T-ALL
Early T-cell precursor ALL with *BCL11B* rearrangement
Early T-cell precursor ALL, NOS
T-ALL, NOS
Provisional entities
Provisional entity: natural killer cell ALL

1.7.4 Immunological classification of ALL

The immunological classification is based on the immunophenotype expressed by lymphoblasts, which is analyzed by fluorescence-activated cell sorting (FACS). Immunophenotyping allows lineage assessment (B- or T-immunophenotype) and, based on cell surface marker expression, determination of the degree of differentiation. The immunological classification of the German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia (GMALL) (see **Table 4**) is based on the EGIL (European Group for the Immunological Characterization of Leukemias) classification with slight modifications concerning subdivision of T-lineage ALL, whereas B-lineage ALL classification is the same in both systems. In the EGIL classification, there are four subtypes of T-lineage ALL: pro-, pre-, cortical- and mature T-ALL. Pro-T-ALL is defined as CD7+ and Pre-T-ALL as CD2a+ and/or CD5+ and/or CD8+, whereas cortical-T-ALL corresponds to thymic-T-ALL in the GMALL classification and mature T-ALL is defined the same way in both classifications. Importantly, the definition of early T-ALL is different compared to the ETP-ALL subtype in WHO-HAEM5 and the ICC. Furthermore, GMALL lists associated genetic alterations that are commonly found among the defined subtypes. (8,57)

Mature B-ALL, as it is classified by the GMALL study group (TdT-, sIg + and *MYC*-rearrangements) belongs to mature B-cell neoplasms, more precisely it is considered as Burkitt Leukemia/Lymphoma and is treated differently and not subject of this thesis. (8,51) B-ALL with *MYC* rearrangement, as it is cited in the ICC 2022 classification represents a precursor B-cell neoplasm and shows an immature phenotype (for example positivity for TdT) and is therefore distinct from Burkitt Leukemia/Lymphoma. (55) However, this distinct B-ALL subtype, in turn, cannot be found within the WHO-HAEM5.

Table 4. *GMALL classification, adapted from* (8)

Subtypes	**Immunophenotype**	**Associated alterations**
B-lineage ALL	TdT+/-, HLA-DR+, CD19+ and/or CD22+ and/or CD79alpha	
B-precursor ALL		
Pro-B-ALL	CD10-	t(v::11); *KMT2A*-rearrangements
Common ALL	CD10+	t(9;22); *BCR::ABL1* *IKZF1*
Pre-B-ALL	cyIgM+	t(1;19); *TCF3::PBX1* t(9;22); *BCR::ABL1* *IKZF1*
Mature B	TdT-, CD34-, sIg+	t(8;14); *MYC*-rearrangements
T-lineage ALL	Tdt +/-, cyCD3+, CD7+	
Early T-ALL	CD2-/+, sCD3-, CD1a-	*PTEN* *N/K-RAS*
Thymic T-ALL	sCD3+/-, CD1a+	*NOTCH1/FBXW7*
Mature T	sCD3+, CD1a-	

1.8 Diagnosis

In order to confirm ALL, it is necessary to perform a bone marrow examination, either as bone marrow aspiration or in case of a dry tap (punctio sicca), a biopsy of the bone marrow. Typically, the bone marrow shows hypercellularity and more than 25% blasts, which is required to distinguish ALL from LBL. Additionally, blasts can usually be detected in the peripheral blood. (4,14,15) **Figure 2** shows a bone marrow sample filled with lymphoblastic cells.

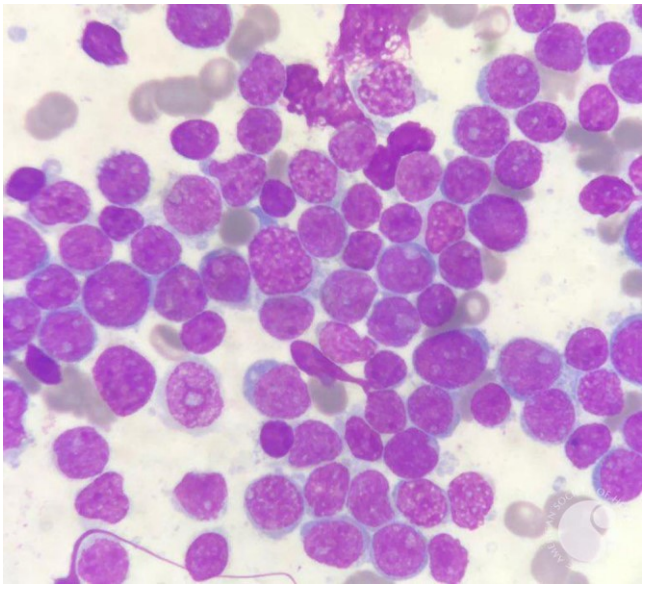


Figure 2. *"Bone marrow filled with blasts in acute lymphoblastic leukemia (ALL"). This image was originally published in ASH Image Bank.* (58)

Besides morphology and cytochemistry of blasts (lymphoblasts are myeloperoxidase negative), immunophenotyping of malignant cells is the most important examination in order to distinguish B- and T-cell phenotype as well as to define the degree of differentiation and the immunological subtype. (8,14) Immunophenotyping, using FACS and/or immunohistochemistry, is performed based on the following markers: (15,59)

- B-lineage: CD19, CD79a, cCD22, TdT, CD10, CD20, CD24, cIgM, sIg (kappa or lambda);
- T-lineage: cCD3, TdT, CD1a, CD2, CD5, CD7, CD4, CD8, TCR α:β or γ:δ;
- Myeloid or stem cell markers: CD34, CD117, CD13, CD33, HLADR (negative except in multilineage leukemia and ETP-ALL).

Furthermore, conventional chromosomal analysis, FISH and RT-PCR are important (cyto-) genetical tests to detect recurrent chromosomal alterations such as specific translocations and aneuploidies described in 1.6 (Pathogenesis and Genetics) and to identify the genetical subtype. Especially early detection of *BCR::ABL1* positive ALL is important due to the initiation of tyrosine kinase inhibitors in these cases, and should therefore be performed within the first days of the diagnostic work-up. (59)

Lumbar puncture is essential to examine cerebrospinal fluid for lymphoblasts and to exclude CNS involvement. Simultaneously, intrathecal chemotherapeutic agents are administered for therapy or CNS prophylaxis. (14,15)
Further basic laboratory investigations during the initial diagnosis include full blood count, coagulation panel, liver and kidney function, electrolytes, uric acid, LDH, urine analysis, blood group and pregnancy test for women. Comprehensive laboratory analysis also includes virus serology, especially for hepatitis A, B, C, HIV, CMV, HSV and VZV in order to exclude acute infections and to be prepared for virus reactivation. (14)
HLA (human leukocyte antigen) typing of the patient and family should be performed during the diagnostic work-up to identify possible donors if allogenic stem cell transplantation is needed. (59)
The basic examination also includes a thorough examination and investigation of comorbidities, as well as electrocardiogram (ECG) and echocardiography. Necessary imaging procedures include chest X-ray, abdominal ultrasound and, if necessary, further examinations such as CT/MRI of head and CT of chest/abdomen, as indicated for presenting symptoms. (8,16)

1.9 Prognostic Factors and Risk Stratification

Several risk factors have been established in ALL that comprise conventional clinical and biological features at the time of diagnosis but also factors based on the response to treatment in the further course of the disease. Besides complete (hematological) remission (CR), defined as BM blasts <5%, after induction therapy, measurable residual disease (MRD), measured at different stages of treatment, is a major single risk factor for the probability of relapse and overall survival, and influences treatment decisions in different trials. (60–62) Based on these risk factors, various ALL study groups defined risk groups that implicate different treatment options, e.g. hematopoietic stem cell transplantation (HSCT), and are relevant for prognosis. (6) (see chapter 1.10 Treatment)

1.9.1 Clinical and biological factors

The two most important clinical risk factors in ALL are age and white blood cell count (WBC) at the time of diagnosis. Especially adults older than 55 years show worse outcome in comparison to adolescents and young adults. Many factors, including comorbidities, higher prevalence of unfavorable genetic subtypes, and lower tolerance to intensive treatment contribute to the inferior outcome of older patients. (6,63) Furthermore, a white blood cell count greater than 30×10^9 per liter for B-ALL and 100×10^9 per liter for T-ALL is a high risk factor in most studies. (6)

Concerning immunophenotype, the CD10 negative pro-B ALL is defined as a high risk feature, which is commonly associated with unfavorable *KMT2A* rearrangement. (6) CD 20 positivity (defined as 20% positive blasts) is present in up to 50% of B-ALL cases and could be shown to be a poor risk factor by Thomas et al. In this study, the three-year complete remission duration for CD20 positive patients was 20 % versus 55 % in CD20 negative patients ($p < 0.001$) and three-year OS was 27% versus 40% ($p = 0.03$). However, this study was conducted in the pre rituximab era. (64) Adding rituximab, a monoclonal antibody against CD20, to standard chemotherapy showed improved event free survival (EFS) of adult patients in a randomized trial by the Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study group. (65)

In T-ALL, thymic T-ALL shows better prognosis than pro-, pre- (summarized as early T-ALL) and mature T-ALL. (6)
The prognosis of specific cytogenetical findings have already been described (1.6 Pathogenesis and Genetics). In summary, hyperdiploidy and the translocation *ETV6::RUNX1* show favorable outcome whereas *BCR::ABL1*, *KMT2A* rearrangements, hypodiploidy and a complex karyotype (defined as ≥ 5 chromosomal abnormalities) represent adverse risk factors. (2)
Besides the adverse prognostic impact of *KMT2A* rearrangements in B-ALL, the GRAALL study group indicated furthermore that *IKZF1* gene deletion in B-ALL and missing mutations of the *NOTCH1/FBXW7* and/or mutated *N/K-RAS* and/or *PTEN* in T-ALL, have a dismal impact on incidence of relapse and overall survival. (62).
Another important factor in ALL is CNS involvement at the time of diagnosis, acting as a prognostic marker for inferior outcome and CNS relapse probability. This underlines the importance of CNS prophylaxis and therapy. (66)

1.9.2 Response to treatment and MRD

CR is the primary objective of induction therapy, the first step of ALL treatment, and is defined as elimination of visible leukemic blasts (<5%) from the bone marrow by microscopic examination. Further criteria of CR include absence of extramedullary disease, absolute neutrophil count > $1x10^9$ per liter and platelet count > 100.000 x 10^9 per liter. (4) CR is essential for long time survival and late CR is defined as a risk factor within the GMALL study group. (8) However, the detection limit of microscopical-morphological review by experienced examiners is 1 to 5 in 100 cells, which is equivalent to a sensitivity of 1-5 × 10^{-2}. By applying MRD (minimal residual disease) techniques to detect low levels of disease burden, sensitivities of 10^{-4} to 10^{-6} (which means 1 blast in 10.000 to 1.000.000 normal cells) can be reached. (67,68) If no MRD can be detected by a technique with a sensitivity of at least 10^{-4}, it is referred to as molecular remission (MolCR). For this reason, MRD detection provides a much more accurate assessment of a patient's remission and early detection of impending relapse and is therefore the most important independent prognostic factor in ALL. (59,69)
MRD detection can be established by different methods, that try to identify a leukemia specific fingerprint of the malignant cells and to subsequently quantify

them at different time points within treatment. (70) Importantly, MRD levels of B-ALL cells are lower in peripheral blood than in bone marrow samples, implying that samples need to be taken from the bone marrow to receive informative results. For T-ALL, the difference between peripheral blood and bone marrow is described as not significant. (71)

1.9.2.1 Polymerase chain reaction

PCR based methods comprise two different approaches: real-time quantitative PCR (RQ-PCR) and reverse-transcriptase PCR (RT-PCR). (70) Targets of RQ-PCR are Immunoglobulin (Ig) and T-cell receptor (TCR) gene rearrangements or specific breakpoint fusion regions from chromosomal rearrangements such as *BCR::ABL1* p190, *BCR::ABL1* p210 and *KMT2A::AFF1*. Thus, this method is based on individual gene regions and therefore it is essential to examine a sample taken at the time of diagnosis, to find the unique rearrangement. Detection of patient specific Ig and TCR rearrangements is possible in more than 95% of ALL patients, whereas chromosomal rearrangement based PCR is applicable to approximately 40% of B-ALL and 20% of T-ALL cases. (67,70) A disadvantage of Ig/TCR based RQ-PCR is clonal evolution of these specific rearrangements, that can result in false-negative results and therefore different, independent regions have to be analyzed to receive diagnostically conclusive results. Contrary, specific chromosomal rearrangements are directly associated with the disease and are therefore stable components for analysis. (70)

RT-PCR is based on transcripts (mRNA) of leukemia-specific fusion genes (e.g. *BCR::ABL1*) that are converted in cDNA through a reverse transcriptase, which can subsequently be amplified by PCR and quantified. (70) An advantage of this method is, that fusion transcripts are not unique and the same primers can be used for all patients with the same rearrangement. However, the transcription activity of malignant cells can be influenced by treatment and can vary for individual patients during the course of the disease, making it difficult to interpret. (69,70)

1.9.2.2 Multiparametric flow cytometry

Multiparametric flow cytometry is based on the specific surface marker expression of leukemic cells, that differ from normal cells of the bone marrow. Nowadays, there are standardized marker profiles and sensitivity of modern 8-12 color flow cytometers is between 10^{-4} to 10^{-5}, comparable to PCR based methods. Importantly, leukemic cells show shifts of the surface marker expression during disease and treatment. (69,70)

1.9.2.3 Next generation sequencing

Next generation sequencing (NGS) is a rather new technology in MRD detection consisting of amplifying specific rearrangements of genes (Ig or TCR) and subsequent sequencing of the DNA to search for clonal rearrangements. This technology is a fast method and shows high sensitivity up to 10^{-6}. (72)

1.10 Treatment

Modern therapy of ALL consists of a complex regimen including chemotherapy, CNS prophylaxis and treatment, HSCT for certain risk groups, targeted agents and newly developed antibodies and chimeric antigen receptor (CAR) T-cell therapy for relapsed and refractory disease. Basically, the treatment follows several steps consisting of induction, with the goal of CR, consolidation and maintenance, and should be performed according to treatment protocols such as the GMALL study protocol. Consolidation and maintenance are needed to maintain complete remission, which is the prerequisite for cure of ALL. (2,8)
In this chapter, general recommendations and therapy strategies as well as GMALL-specific treatment strategies will be explained, as therapy at the Division of Hematology is carried out according to this protocol.
GMALL defined in their consensus recommendation 2019 three risk groups (**Table 5**), which are of high importance, especially with regard to HSCT and the treatment with tyrosine kinase inhibitor in case of *BCR::ABL1* positivity. (73)

Table 5. *Risk stratification by GMALL, adapted from* (73)

Risk group	**Characteristics**
Standard risk group (SR)	**B-ALL** CR on day 22 (after induction I) WBC <30.000/µl No pro-B-ALL No *BCR::ABL1* positive ALL
	T-ALL thymic T-ALL
High risk group (HR)	**B-ALL** CR on day 44 (after induction II), respectively no CR after induction I WBC >30.000/µl Pro-B-ALL/MLL rearrangement No *BCR::ABL1* positive ALL
	T-ALL Early T- or mature T-ALL
Ph/BCR-ABL1 positive ALL group (Ph-positive ALL)	*BCR::ABL1* positive ALL

In children, intensive chemotherapy and intrathecal treatment of CNS involvement, lead to 5- and 10-year survival rates of approximately 90% and 84 %, respectively. (74,75) Outcome rates of adults are still below, but the use of pediatric inspired treatment regimens for adults up to the age of 50 years improved the outcome. In the GRAALL-2003 study the EFS at 42 months was 55% and the OS 60% in Ph-chromosome negative ALL patients. (76) In another study by DeAngelo et al. 92 patients between 18-50 years were treated on a pediatric inspired treatment and the 4-year OS was 67 %. (77)

1.10.1 Chemotherapy

In order to carefully reduce the leukemic cell burden, it is recommended to start with a five-day prephase treatment, mostly consisting of dexamethasone and cyclophosphamide, before initiating induction. This reduces the risk of tumor lysis

syndrome and allows for performing necessary examinations and collecting diagnostic results such as bone marrow infiltration, *BCR::ABL1* status and CD20 positivity. (8)

Prephase is followed by induction therapy, including vincristine, dexamethasone, and an anthracycline derivate (daunorubicin within the GMALL protocol) in induction phase I and cyclophosphamide, cytarabine and 6-mercaptopurin during induction phase II. In case of CD20 positivity, rituximab is added for induction. This procedure applies to Ph-negative ALL patients, induction therapy for Ph-positive ALL patients is different and described in the following chapter.(8)

Another important and very specific drug for ALL treatment, that is used during induction, is L-Asparaginase which nowadays derived from bacterial sources such as Escherichia coli and Erwinia chrysanthemi. L-asparaginase breaks down the amino acid L-asparagine into L-aspartate and ammonium. While normal, healthy cells are able to produce asparagine through asparagine synthetase, leukemia cells are lacking this enzyme and are dependent from circulating L-asparagine, making it an essential amino acid for malignant cells. Therefore, L-asparaginase cuts off the exogenous supply and acts as an antileukemic drug. (78)

Since L-asparaginase is a therapeutical enzyme of bacterial origin, it exhibits high immunogenicity resulting in the production of anti-drug antibodies. This often leads to hypersensitivity and allergic reactions or reduction of asparaginase activity through (silent) inactivation. Therefore, PEG-asparaginase, a pegylated form of E.coli derived L-asparaginase (addition of monomethoxypolyethylene glycol), was developed, which helps to extend the half-life of the native enzyme and reduces its immunogenicity. Furthermore, asparaginase activity should be measured at certain points, in order to identify silent inactivation and evaluate its therapeutical efficacy. (79) Additionally, L-asparaginase is associated with severe toxicities including hepatotoxicity, pancreatitis, vascular events and coagulation disorders, especially in older patients, obese patients, patients with fatty liver disease and those with Ph-positive ALL, possibly due to overlapping toxicities with imatinib, a frequently used TKI in case of *BCR::ABL1* positivity. Therefore, the use of L-asparaginase requires close monitoring of laboratory parameters, dose reduction if necessary and supportive therapy. (8,80)

If complete remission was achieved, consolidation and long-term maintenance are important treatment steps that follow. Besides chemotherapeutic drugs including high-dose methotrexate, asparaginase, cytarabine and glucocorticoids, HSCT is part of consolidation. Depending on the risk stratification before and during induction and especially MRD detection after consolidation I (in the GMALL protocol), decisions on further treatment pathways are made. Patients that are not admitted to HSCT receive cyclic consolidation therapy with reinforcements through repetition of induction.(2,6,8) This intensive treatment with chemotherapy applies within the framework of the GMALL protocol for young patients under 55 years of age; for older patients, there is a separate therapy recommendation that provides for reduced chemotherapy and also depends on the health condition of the individual person. (8)

Long-term maintenance represents the final stage of treatment for patients that did not receive HSCT and consists of weekly methotrexate and daily mercaptopurine with cyclic reinforcements over the next two years after induction. (8)

1.10.2 Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKI) are used for the treatment of Philadelphia chromosome positive ALL, as they target the constitutively activated tyrosine kinase, encoded by the *BCR::ABL1* translocation. Imatinib was the first TKI used in the treatment of this very common B-ALL subgroup in addition to chemotherapy and improved the poor long term survival rates of around 10-30% to over 50% and showed high CR rates. (41,81). Different treatment protocols including the GMALL study group combine imatinib with reduced intensity chemotherapy for the induction of Ph-positive ALL. (8,82)

In the case of the GMALL protocol daily imatinib is combined with vincristine, dexamethasone, L-asparaginase and a CD20 antibody in case of CD20 positivity. (8)

Dasatinib, a second generation TKI, was shown to have better penetration through the blood-brain barrier and has higher activity against kinase domain mutations than imatinib. (83,84) Furthermore, in a pediatric randomized clinical trial, outcome of children treated with dasatinib was better than with imatinib. Results of this study showed, that 4-year EFS (71% versus 48.9%, p = 0.005) and 4-year OS

(88.4% versus 69.2%) were higher and cumulative risks of relapse (19.8% versus 34.4 %) and isolated CNS relapse (2.7 % versus 8.4%) lower in patients who received dasatinib versus imatinib, supporting the use of dasatinib at least in pediatric patients. (85)

One specific mutation, T315I, in the ABL kinase domain represents a common cause of relapse in Ph-positive ALL. The third generation TKI ponatinib shows potent activity against the wild type ABL but also the ABL kinase mutation carrying T315I. Although completed randomized controlled trials of ponatinib compared with other TKIs are lacking, one propensity score matching analysis and a meta-analysis including 26 trials showed better outcomes, especially concerning the complete molecular response, for the third generation TKI ponatinib in combination with chemotherapy than TKIs of earlier generations. (86,87) The first results of one ongoing randomized controlled phase 3 study comparing ponatinib to imatinib confirms higher molecular response rates and a trend showing better EFS for ponatinib. (88)

1.10.3 Management of CNS involvement and prophylaxis

As already described in the chapter 1.8 Diagnosis, it is necessary to exclude CNS involvement during the diagnostic pathway because 5-15% of patients present with CNS disease at the time of diagnosis. Furthermore, about 5% of patients show an isolated CNS relapse after reaching complete remission. (16,89) Besides a neurological examination and neuroimaging (CT or MRI) in case of clinical symptoms, the evaluation of the cerebrospinal fluid by lumbar puncture (LP) is the standard diagnostic method that every patient should receive. CNS prophylaxis is standard of care and intrathecal drugs are commonly administered already during the first LP.(89) Methotrexate, cytarabine as well as corticosteroids are administered into the cerebrospinal fluid alone or in combination at different time points for prophylaxis, depending on the protocol. Intravenously applied high dose methotrexate and cytarabine also show good penetration through the blood-brain-barrier into immune-privileged sites.(16) Furthermore, the GMALL protocol still recommends cranial radiation as a component of CNS prophylaxis during induction II. (8)

Patients with CNS involvement at diagnosis receive a more intensive therapy. In the GMALL protocol, the initial treatment consists of methotrexate in combination with cytarabine and dexamethasone during induction until lymphoblasts are no longer detected. (8)

1.10.4 Allogeneic hematopoietic stem cell transplantation

Allogeneic HSCT (allo-HSCT) plays a crucial part in post-remission, respectively, consolidation in ALL. However, through the establishment of MRD detection and new, targeted therapies, the indications of allo-HSCT are critically reviewed. (90)

1.10.4.1 Indications for allo-HSCT

Allo-HSCT in first CR (CR1) is recommended for all patients with Ph-negative ALL of the HR group. However, the ongoing GMALL study 08/2013 is evaluating whether allo-HSCT can be omitted for patients initially classified as high-risk who achieve a good response to treatment and MRD negative status. Patients with standard risk features do not primarily undergo allo-HSCT unless they experience molecular failure. In case of poor response to therapy or MRD positive status (MRD level of ≥ 10-4 after early consolidation or after molecular CR), these patients receive targeted therapy to decrease MRD and then continue to allo-HSCT. (8)

A comparison with other European ALL study groups shows a similar risk-adapted indication for allo-HSCT in Ph-negative ALL patients in first complete remission, again primarily focusing on MRD status after induction or consolidation.(91)

Besides the treatment with TKIs and reduced intensity chemotherapy, Ph-positive ALL patients receive allo-HSCT in CR1 within the GMALL protocol, irrespectively of their MRD status. For patients who have relapsed, the goal is to achieve a second CR and then undergo allo-HSCT.(8) (see 1.10.5 Treatment of relapse and refractory disease)

1.10.4.2 Conditioning and donor

Before the administration of donor stem cells, conditioning therapy has to be performed, in order to eradicate remaining malignant cells, establish sufficient immunosuppression enabling engraftment and preventing rejection and graft-versus-host disease (GvHD). Myeloablative conditioning (MAC) is only applicable

for fit and young patients up to the age of 50 to 55 years due to its high toxicity. Non-myeloablative and reduced intensity conditioning (RIC) therapies have been developed in the last 20 years, that are suitable for older patients and patients with comorbidities. Primarily, these less intensive conditioning regimens aim for immunosuppression and graft-versus-leukemia effect and not maximal eradication of malignant cells. (92)
Regarding conditioning in ALL, the European Society for Blood and Marrow Transplantation (EBMT) recommends fractionated total body irradiation (TBI; cumulative dose of 12-13 Gy) combined with cyclophosphamide or etoposide (VP-16) or alternatively busulfan in combination with cyclophosphamide for myeloablative conditioning in patients younger than 45 years. For older patients or patients with contraindications for MAC, reduced intensity conditioning regimens include for example 8 Gy TBI combined with cyclophosphamide or fludarabine. (92)

As already mentioned, HLA typing of the patient and family is necessary to find a matched sibling donor (MSD). Further options, if an HLA identical sibling is not available, include HLA-MUD (matched unrelated donor), HLA-MMUD (mismatched unrelated donor) or haploidentical donors. (92) Stem cell donation from unrelated umbilical cord blood and autologous HSCT is another alternative if no matched sibling donor is available. (91)

1.10.4.3 Complications of allo-HSCT

Although allo-HSCT is an important treatment option, it is also associated with significant complications and considerable non-relapse mortality (NRM). The cumulative NRM for patients between 18-55 years treated within 2008-2012 was approximately 15% in case of MSD and 22% for patients receiving HSCT from unrelated donors. (93)
Common complications related to toxicities of conditioning therapy include mucositis, diarrhea, hemorrhagic cystitis, sinusoidal obstruction syndrome, cardiomyopathy and neuropathy. Furthermore, bacterial and fungal infections are very common especially shortly after transplantation due to immunosuppression and neutropenia. CMV and other opportunistic infections commonly occur later in

the post-transplantation period. Graft failure and graft rejection pose major early transplant specific complications. (9,94)

Acute and chronic graft-versus-host (acute GvHD and chronic GvHD) are specific complications of allo-HSCT, mediated by immunological processes. In case of acute GvHD, which classically develops within the first 100 days post-transplant, donor-lymphocytes recognize the recipient's tissue as foreign, leading to inflammatory tissue damage, mainly in skin, liver and lower gastrointestinal tract, whereas in chronic GvHD impaired tolerance mechanism, including allo- and autoreactive T and B cells, as well as mechanisms of chronic inflammation and fibrosis are leading pathophysiological features. (92,95)

Thirty to 60% of patients develop acute GvHD after allo-HSCT, with onset of classic acute GvHD until day 100 and late-onset referring to symptoms that occur after 100 days. Furthermore, there are persistent and recurring courses of acute GvHD. Clinical manifestations of acute GvHD include erythematous maculopapular rash of the skin, cholestasis and jaundice in involvement of the liver, as well as nausea, vomiting, anorexia in upper gastrointestinal disease and watery diarrhea in lower gastrointestinal disease usually associated with abdominal cramps and in severe case leading to paralytic ileus. The diagnosis is confirmed by biopsy and defined histological findings. To assess the overall severity of acute GvHD, a clinical assessment of the individual organ systems (gastrointestinal tract, liver, skin) is carried out. There are classifications according to Glucksberg or Harris, which both classify acute GvHD from grade I to IV. In order to prevent the development of acute GvHD, immunosuppressors are used prophylactically, depending on the age of the recipient, HLA compatibility and degree of remission of the disease. Commonly used agents include calcineurin inhibitors (cyclosporin A, tacrolimus) in combination with methotrexate or mycophenolate mofetil. Treatment consists of local immunosuppressors in case of limited skin disease (grade I) and systemic immunosuppressive treatment, mostly prednisolone, complemented by supportive therapy from grade II on. (95)

Chronic GvHD develops commonly after 2-3 months until 18 months to two years and affects approximately 50% of patients after receiving allo-HSCT. Clinical manifestations are similar to autoimmune disease such as Sjögren-syndrome, scleroderma and systemic lupus erythematodes. Most frequent affected tissues include skin, eyes, oral mucosa, salivary glands, genitalia, gastrointestinal tract, joints/fasciae and lungs. Classification of chronic GvHD is based on the National Institutes of Health (NIH)-consensus criteria assessing the number of affected organ systems and their severity, resulting in mild, moderate and severe chronic GvHD. First line treatment is based on systemic steroids in moderate or severe chronic GvHD and supportive measures depending on patient's individual symptoms. Several additional immunosuppressors besides steroids are recommended for second line treatment. (92,96)

1.10.5 Treatment of relapse and refractory disease

Gökbuket et al. showed in an international reference analysis that around 40-50% of patients with Ph-negative B-ALL experience relapse and conventional chemotherapy resulted in CR rates of 30% to 40% in first salvage, around 21% in second and 11% in third salvage therapy. Median OS in first salvage therapy was less than six months and one – and three-year survival rates were 26% and 11% in this study, respectively. Patients with early relapse showed worse outcome and response to therapy than patients in late relapse. (97)

The main strategy for treating relapsed ALL is to achieve CR, if possible, also molecular CR, and subsequent HSCT. However, the guidelines of the German, Austrian and Swiss hematologic societies state that a high NRM rate and an increased risk of relapse are to be expected in these patients with relapsed/refractory ALL who have undergone intensive pre-therapy. (8)
The use of newly developed antibody-targeted therapies and CAR T-cell therapies showed significantly better results than standard chemotherapy-based therapies in relapsed or refractory disease. For B-ALL, there are two specific antibodies, blinatumomab and inotuzumab ozogamicin, directed against CD19 and CD22, respectively. (98)

1.10.5.1 Blinatumomab

Blinatumomab is a bispecific antibody directed against CD19, which is expressed on almost all precursor and mature B cells, and CD3 on T cells. This results in an immunologic link between B cells and T cells, subsequent activation of T cells and induction of B-cell apoptosis through a cytolytic response. Important severe adverse events include cytokine release syndrome, which is associated with disease burden and neurological toxicities. Further side-effects are fever, anemia, neutropenia and hypogammaglobulinemia. Blinatumomab has been approved by the FDA (US Food and Drug administration) and EMA (European Medicines Agency) for treating adult patients with relapsed or refractory Ph-negative, CD19-positive ALL. Furthermore, this antibody is approved for the treatment of patients in first or second CR with continuous MRD. (2,98) The BLAST trial showed complete MRD response in 78% of patients with persistent MRD after treatment with blinatumomab and better OS (39 months versus 12.5 months, p = 0.002) for patients that achieved MRD negativity versus patients that did not. (99)

1.10.5.2 Inotuzumab ozogamicin

CD22, the target structure for the monoclonal antibody inotuzumab, is expressed on approximately 90% of B-ALL cells, and binding with an antibody leads to its internalization. Inotuzumab is linked to ozogamicin, a calicheamicin, causing double-strand breaks of DNA and subsequent apoptosis. (2,98) In a phase 3 trial (INO-VATE) comparing inotuzumab ozogamicin to standard of care chemotherapy in relapsed or refractory B-ALL, overall response rate was 81%, CR rate was 36% for inotuzumab ozogamicin versus 17 % for chemotherapy, and rate of MRD negativity was higher (for patients responding to treatment 78% versus 28%). Longer progression-free survival (PFS; 5.0 months versus 1.8 months) and better OS (7.7 months versus 6.7 months) could be shown for patients treated with inotzumab ozogamicin. Furthermore, more patients treated with inotuzumab ozogamicin were able to receive allo-HSCT (41% versus 11%). Important severe side effects include toxicities affecting the liver, especially veno-occlusive disease. (100)

1.10.5.3 Chimeric Antigen Receptor T-cell Therapy

For the production of CAR T-cells, T cells are taken from an individual patient and genetically modified to express an extracellular receptor that targets CD19 on leukemic cells. This receptor is linked to a costimulatory (mostly CD28 or 4-1BB) and an intracellular domain (CD3ζ) for enhanced T cell activation and signal transduction. When CAR-T cells encounter B cells, their activation and proliferation lead to a cytotoxic response and thus to the destruction of leukemic cells. An important potentially life-threatening adverse event of CAR-T cell therapy, that affects almost all patients, is cytokine release syndrome, that involves a systemic reaction with high fever, flu-like symptoms, hypotension, and in severe cases, changes in mental status. It is treated with tocilizumab – a monoclonal antibody that binds to the interleukin 6 receptor - and glucocorticoids. Further side effects include neurologic toxicities, infections, prolonged cytopenias and hypogammaglobulinemia.(98,101) A systematic review and meta-analysis analyzing 38 reports on anti-CD19 CAR T-cell therapy showed a high overall response rate of 76%. The median OS was 36.2 months and the median EFS was 13.3 months. 98% of all responding patients achieved complete MRD response. (102)

1.10.5.4 Treatment options for relapsed/refractory T-ALL

For relapsed/refractory T-ALL, specific antibodies and CAR-T-cell therapy as in B-ALL are not approved yet. However, several targets on T cells are evaluated including daratumumab (antibody binding to CD38) or venetoclax, which is a BCL-2 inhibitor. The only drug approved specifically for relapsed/refractory T-ALL is nelarabine, a purine nucleoside, which shows affinity for T cells and cytotoxic potential in these cells. (103) Nelarabine should be considered in molecular relapse (MRD) or in the event of treatment failure. (8) As in B-ALL, the goal is to achieve CR and subsequently proceed to allo-HSCT. (103)

1.10.6 Supportive care

Adverse events and toxicities pose a major challenge in the treatment of ALL. Therefore, supportive care measures are essential for successful outcomes. (104) The most important and frequent problem areas are highlighted within this section.

Tumor lysis syndrome, which results from the elimination of large numbers of leukemia cell burden, is a major and common problem, especially in the beginning of treatment in ALL. Intracellular substances, most importantly potassium, phosphate and uric acid are released into the blood circulation, with the risk of acute kidney failure (AKI). In addition to the slow reduction of the leukemic cell count, which is achieved by prephase treatment, sufficient intravenous fluid administration must be ensured and further preventative measures include allopurinol and rasburicase. (59,104)

Concerning the use of asparaginase, possible toxicities and adverse events have already been addressed. For example, thrombosis prophylaxis and prevention and recognition of possible anaphylactic reactions. Another common issue in hematologic malignancies are anemia and thrombocytopenia, that have to be treated with leukoreduced or irradiated blood products. (104) Neutropenia, which is most commonly seen during induction period increases risk of severe infections and neutropenic fever. For this reason, preventive measures (e.g. granulocyte-colony-stimulating factor) are taken and infections consistently treated. (59,104)

1.11 Rational and Aims of this study

In summary, ALL is a disease with great biological diversity, particularly in terms of genetics and immunophenotype. In addition, it is an aggressive malignant disease of the hematopoietic system that requires a complex treatment concept. To date, there is no comprehensive evaluation of patients diagnosed with ALL, who have been treated at or transferred to the Division of Hematology, Department of Internal Medicine, of the Medical University of Graz. As this study analyzes an unselected cohort of patients, this work offers the possibility to evaluate and discuss the reality of care for adults affected by ALL, treated in Graz, in relation to their individual risk, ALL subtype etc. Therefore, patient characteristics will be analyzed and subsequently OS and DFS will be determined by survival statistics in order to obtain a statement about the outcome of the patients treated in Graz. Finally, these results shall be compared to published studies and register studies and further discussed in this context.

2. Material and Methods

2.1 Material and Data Collection

In this retrospective cohort study, clinical data from 95 patients with ALL treated from January 2001 to April 2021 at the Division of Hematology, Department of Internal Medicine, of the University Hospital of Graz were evaluated. These patients were first diagnosed in Graz or transferred for further treatment and/or HSCT. Thus, all adult patients older than 18 years diagnosed or treated for B-ALL, B-LBL, T-ALL, T-LBL were included in this study. There were no exclusion criteria. Patient records in the documentary system openMEDOCS were reviewed, their data was collected in Microsoft Excel (Microsoft Office Professional Plus 2016) and afterwards statistically analyzed with SPSS 25.0 (SPSS inc., Chicago, IL, USA) and MedCalc (Version 20.115; MedCalc Software Ltd, Ostend, Belgium).

If available, WBC, hemoglobin, platelets, LDH and CRP at the time of diagnosis were obtained. Furthermore, bone marrow infiltration, peripheral blast count as well as all records of extramedullary involvement were retrieved from medical records.

The European Cooperative Oncology Group performance status (ECOG PS) and the HCT-CI (Hematopoietic Cell Transplantation specific Comorbidity Index) was collected from the data or, in case of HCT-CI, calculated from previous findings and medical reports if not cited in the medical reports. The ECOG PS is a clinical score to measure the impact of a disease on patients' functional level in daily activity, ability to care for themselves and physical fitness. The score ranges from 0 (no limitations in physical ability) to 4 (fully bedridden) and 5 (dead), respectively. (103) For this reason, the ECOG was collected for every patient to describe their physical fitness at the time of diagnosis.

The HCT-CI score includes 17 categories of comorbidities, their degree and was developed to make a statement about risk and non-relapse mortality in stem cell transplantation. The score ranges from 0 to 29 and includes a wide range of comorbidities including cardiovascular, renal, pulmonary, psychiatric, hepatobiliary conditions and others. (90)

In this retrospective study, the HCT-CI was calculated or directly taken from medical records, independently from HSCT, to quantify comorbidities and to assess survival differences in patients with comorbidities or patients without previous health conditions.
Patient and disease related parameters that were collected within this study are summarized in **Table 6**.

Table 6. *Prespecified patient and disease related parameters*

Age at diagnosis
Hematopoietic Cell Transplantation specific Comorbidity Index (HCT-CI)
European Cooperative Oncology Group performance status (ECOG PS)
Immunologic subtype by GMALL classification (8)
Laboratory parameters at diagnosis: WBC, hemoglobin, platelets, LDH, CRP
Blasts in PB, BM infiltration
Karyotype and genetics • *BCR::ABL1* (Philadelphia chromosome) • *KMT2A* rearrangement
CNS involvement at diagnosis
Extranodal involvement at diagnosis
Treatment: GMALL, other protocol

For a chronological assessment and for further calculation of OS and DFS, further collected data included: date of diagnosis, date of first CR (CR1), date of relapse (if occurred) defined as reappearance of disease and day of last contact (alive or death). These parameters were used to calculate the OS and DFS for the entire cohort. OS was defined as time from day of diagnosis to death of any cause or lost to follow-up, DFS1 was defined as day of CR1 to day of first relapse (relapse 1), death of any cause or lost to follow-up.

Patients that proceeded to HSCT were evaluated for remission status before HSCT, conditioning therapy, acute GvHD, chronic GvHD and survival after HSCT. Survival after HSCT is defined as time from date of HSCT to death of any cause or lost to follow-up.

2.2 Methods

The outcome of the patients is determined by survival statistics. Primary end points of this retrospective cohort study are 5-year OS and 5-year DFS. OS and DFS were calculated with the Kaplan-Meier method, the log rank test was used for comparing survival estimates of different subgroups of ALL. The median follow-up time was calculated with the reverse Kaplan-Meier method. The univariate and multivariable Cox-regression were used to identify independent risk factors and hazard ratios of OS. Differences between groups were analyzed with chi-square and Kruskall-Wallis test. P-values <0.05 were regarded as statistically significant.

2.3 Institutional Review Board Approval

Approval for the study was granted by the Ethics Committee of the Medical University of Graz (No. 34-429 ex21/22 1233-2022).

3. Results

3.1 Patient Characteristics

In total, 95 patients were treated in Graz from 2001 to 2021, consisting of 57 (60%) male and 38 (40%) female patients (**Table 7**). Thus, the male to female ratio was 1.5. The median age at diagnosis was 40.3 and the mean age was 42.1 years with the youngest patient 18.3 years and the oldest 85.1 years old at the time of diagnosis. All patients were retrospectively assigned to three different age groups at the time of diagnosis: adolescents and young adults (AYA; 18-30 years), adults (31-55 years) and old adults (older than 55 years). Thirty-one (32.6%) patients were allocated to the AYA group, 43 (45.3%) patients to adults and 21 (22.1%) were in the old adult group.

Table 7. *Patient's characteristics*

Total number of patients	95
Sex - no. (%)	
Male	57 (60)
Female	38 (40)
Age at diagnosis, yr.	
Median age (range)	40.3 (18.3 – 85.1)
Mean age – yr. (SD)	42.1 (± 17)
Age groups, no. (%)	
18 – 30 (AYA)	31 (32.6)
31 – 55 (Adults)	43 (45.3)
>55 (Old Adults)	21 (22.1)
GMALL age groups, no. (%)	
≤55	74 (77.9)
>55	21 (22.1)
ECOG PS 0-4, no. (%)	
0	65 (68.4)
1	21 (22.1)
2	7 (7.4)
3	2 (2.1)
4	0
HCT-CI 0-29, no. (%)	
0	49 (51.6)
1-2	20 (21.1)
≥ 3	24 (25.3)

3.2 Immunologic Subtypes and Genetics

In total, there were 71 (75.8%) patients with B-cell precursor ALL and 23 (24.2%) patients with T-cell precursor ALL/LBL. One patient was not evaluable for immunologic subtype and there was no case of B-cell precursor LBL. The exact distribution of immunologic subtypes can be seen in **Table 8**.

Table 8. *Immunologic subtype*

Immunologic Subtype (GMALL classification)	**No. (%)**
B - cell precursor ALL	71 (75.8)
Pro-B-ALL	12 (12.6)
common B-ALL	48 (50.5)
Pre-B-ALL	9 (9.5)
Subtype unknown	2 (2.1)
T - cell precursor ALL / LBL	23 (24.2)
early T-ALL	10 (10.5)
T-ALL thymic-cortical	6 (6.3)
T-ALL subtype unknown	2 (2.1)
T-cell LBL	5 (5.3)
Immunologic subtype unknown	1 (1.1)

Thirty-one patients were tested positive for *BCR::ABL1* (Ph-positive ALL), which makes 43.7% of all 71 cases of B-ALL or 32.6% of all 95 patients in this study. Twenty-eight patients positive for *BCR::ABL1* showed the common B-ALL immunophenotype, 2 had B-ALL not further classified (subtype unknown) and 1 patient with this alteration had Pro-B-ALL.

Of the 7 (7.3%) patients positive for *KMT2A* rearrangement, 5 exhibited the Pro-B-ALL and 2 exhibited the Pre-B-ALL immunophenotype. However, 25 patients were not tested for this rearrangement. **Figure 3** shows a simplified overview of distribution of immunological subtypes and the *KMT2A* rearrangement as well as *BCR::ABL1*, representing important genetic alterations in adult ALL. It comprises the results of **Table 8** and demonstrates that almost a third of all patients in this study were positive for the Philadelphia chromosome.

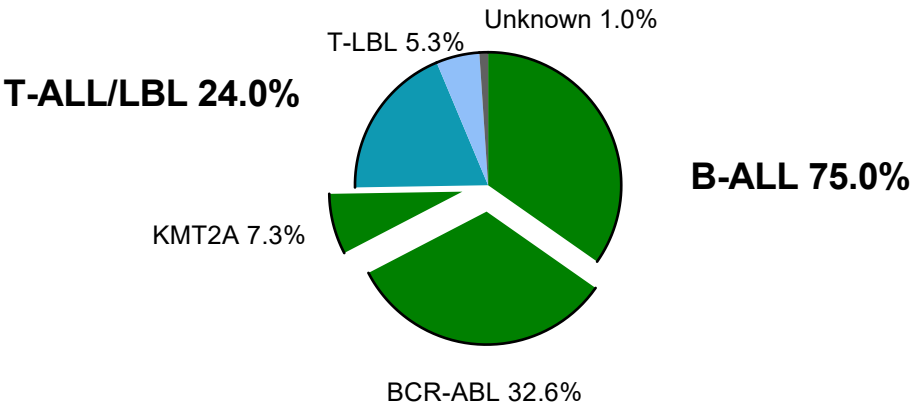


Figure 3. *Overview of immunophenotype and important genetic subtypes*

Data on karyotype was collected separately for B-ALL and T-ALL/LBL cases. Out of 71 patients with B-ALL, 29 (40.8%) had normal karyotype, 7 (9.9%) patients had hyperdiploidy, 4 (5.6%) patients high-hyperdiploidy and 8 (11.3%) patients hypodiploidy. In 23 (32.4%) cases, the karyotype was missing.
In T-ALL/LBL cases (23 patients), 12 (52.2%) patients had a normal karyotype and 3 (13%) showed hyperdiploidy. In 8 (34.8%) patients the karyotype was missing.

3.3 Laboratory Findings and Extramedullary Involvement

Twenty-nine patients (30.5%) showed extramedullary involvement and 5 (5.3%) patients had CNS involvement of whom 3 were of T-cell and 2 of B-cell origin. Mediastinal involvement was T-ALL/LBL specific, since all 10 patients that showed a mediastinal mass were positive for this immunophenotype. Sixty-six (69.5%) patients had no extramedullary involvement.

Table 9 provides a summary of laboratory findings and extramedullary involvement at the time of diagnosis.

Table 9. *Laboratory findings and extramedullary involvement at the time of diagnosis*

Parameters	
WBC, × 10^9/L [88/95]	
Median (range)	13.6 (0.86 – 809.5)
Median peripheral blasts, % (range)	42% (0-96)
BM infiltration at diagnosis [85/95]	
Median BM infiltration (range)	95% (0-100)
Mean BM infiltration (95% CI)	77.2% (70 – 84.5)
Hemoglobin, g/dl [88/95]	
Median (range)	10.8 (3.5 – 17.0)
Platelets, x10^9/L [88/95]	
Median (range)	55 (6.0 – 626.0)
LDH, U/L [86/95]	
Median (range)	535.5 (109 – 6050)
CRP, mg/L [86/95]	
Median (range)	18.5 (0.1 – 253.6)
Extramedullary involvement no. (%)	29 Patients (30.5)
CNS involvement	
yes	5 (5.3)
no	69 (72.6)
n/a	21 (22.1)
Lymphadenopathy/Spleen	8 (8.4)
Mediastinum bulky disease	10 (10.5)
Liver	5 (5.3)
Soft tissue	3 (3.2)
Bone	3 (3.2)
Testes	1 (1)
Thoracic and abdominal bulky disease	1 (1)
Others	4 (4.2)
No extramedullary involvement	66 Patients (69.5)

3.4 Initial Treatment

Eighty-nine (93.7%) patients received initial treatment according to the recommendations of the GMALL protocol. Rituximab was added in case of CD20 positivity and imatinib for Ph-positive ALL. Patients older than 55 years (21%) were treated with less intensive treatment in accordance with the recommendations of the GMALL elderly protocol. Two patients were initially treated in palliative setting and four patients received different treatment protocols, due to initial treatment in foreign countries.

3.5 Remission and MRD

The complete remission rate (CR) was calculated for all patients who received their induction therapy with curative intention in Graz within the framework of the GMALL treatment protocol. Eighty-three patients received their initial treatment in Graz and 80 patients (96.4%) achieved CR while one patient was not evaluable for remission evaluation.

Considering all patients, i.e. patients that received initial treatment and patients that were transferred to the University Hospital of Graz, 90 patients were in CR1. These 90 patients were evaluable for DFS1. The median time to CR1 was 34 days, ranging from 9 to 174 days. Of these 90 patients in CR1, 36 (40%) patients developed relapse. Subsequent CR (CR2+) and relapse rate (relapse 2+) were not evaluated within this study. The median interval between CR1 and relapse 1 was 230.5 days, ranging from 48 days to 1540 days (4.2 years).

Because MRD determination has only recently become part of standard diagnostics, MRD detection after induction therapy was performed in only 46 patients.

Out of these 46 patients, 24 were MRD-negative, resulting in a MRD negativity rate of 52.2%. This study did not include later MRD measurements.

3.6 Risk Groups by GMALL Criteria

As already discussed, the GMALL protocol distinguishes three different risk groups by clinical parameters (WBC), immunophenotype, *BCR::ABL1* and *KMT2A* rearrangement and time to CR resulting in standard risk (SR), high-risk (HR) and Ph-positive ALL (Ph+) groups (compare 1.10 Treatment). Twenty-eight (29.5%) patients were retrospectively assigned to the SR, 35 (36.8%) patients to the HR and 32 (33.7%) patients to the Ph-positive ALL risk group.

Table 10 provides an overview of the statistical distribution and composition of the respective risk group.

Table 10. *Risk groups by GMALL criteria (significant p-values are in bold letters)*

	SR	**HR**	**Ph-positive ALL**	**p - value**
No. of patients (%)	28 (29.5%)	35 (36.8%)	32 (33.7%)	
Age median (years)	30.6 (18.8 - 80.3)	42.9 (19.4 – 75.7)	47.9 (18.3 – 85.1)	**0.043**
male (no.)	19 (67.9%)	20 (57.1%)	18 (56.3%)	0.598
female (no.)	9 (32.1%)	15 (42.9%)	14 (43.7%)	
CR 1 (no.)	26 (92.3%)	35 (100%)	29 (90.6%)	0.199
Relapse (no.)	8 (28.6 %)	20 (57.1%)	8 (25.0%)	**0.035**
Median WBC (x 10^9/L)	7.6 (0.86 – 148.4)	40.2 (1.2 – 809.5)	18.7 (1.3 – 162.6)	**0.005**
Median peripheral blast, %	16% (0 – 79)	60% (0 – 96)	42% (0 – 88)	**0.035**

3.7 Survival Statistics

The median follow-up time, calculated with the reverse Kaplan-Meier method, was 91.2 months (95 % CI: 64.3 – 118.2). The minimum follow-up time was 0.92 months and the maximum follow-up time 212.7 months (17.7 years). Fifty-five (58%) of 95 patients died during the follow-up period and 40 (42%) patients were alive at the time of last contact. OS was calculated for the entire cohort, following survival statistics including only patients that were treated with curative intent.

3.7.1 Overall survival of the entire cohort

Figure 4 shows the Kaplan-Meier curve of the OS probability of the entire cohort. The median OS of the entire cohort was 30.8 months (95 % CI: 18.4 – 72.9). The 1-year OS was 75.5%, the 3–year OS 46.6% and the 5–year OS was 41.3%.

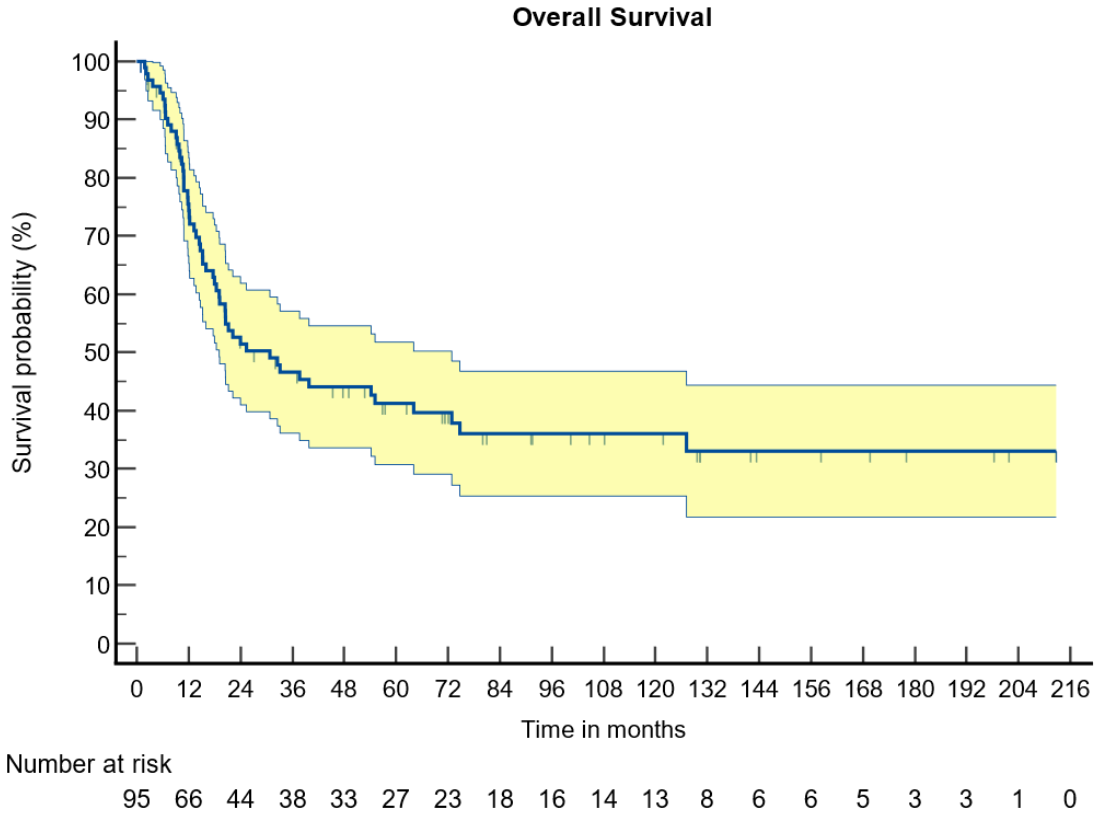


Figure 4. *OS of all 95 patients*

3.7.2 Overall survival by immunophenotype

Figure 5 compares OS for B-ALL and T-ALL/LBL which was not statistically different (p = 0.974). B-ALL patients had a median survival of 33.1 months, while those with T-ALL/LBL had a median survival of 32.5 months. The 5-year OS rates were 43.2% for B-ALL and 39.4% for T-ALL/LBL.

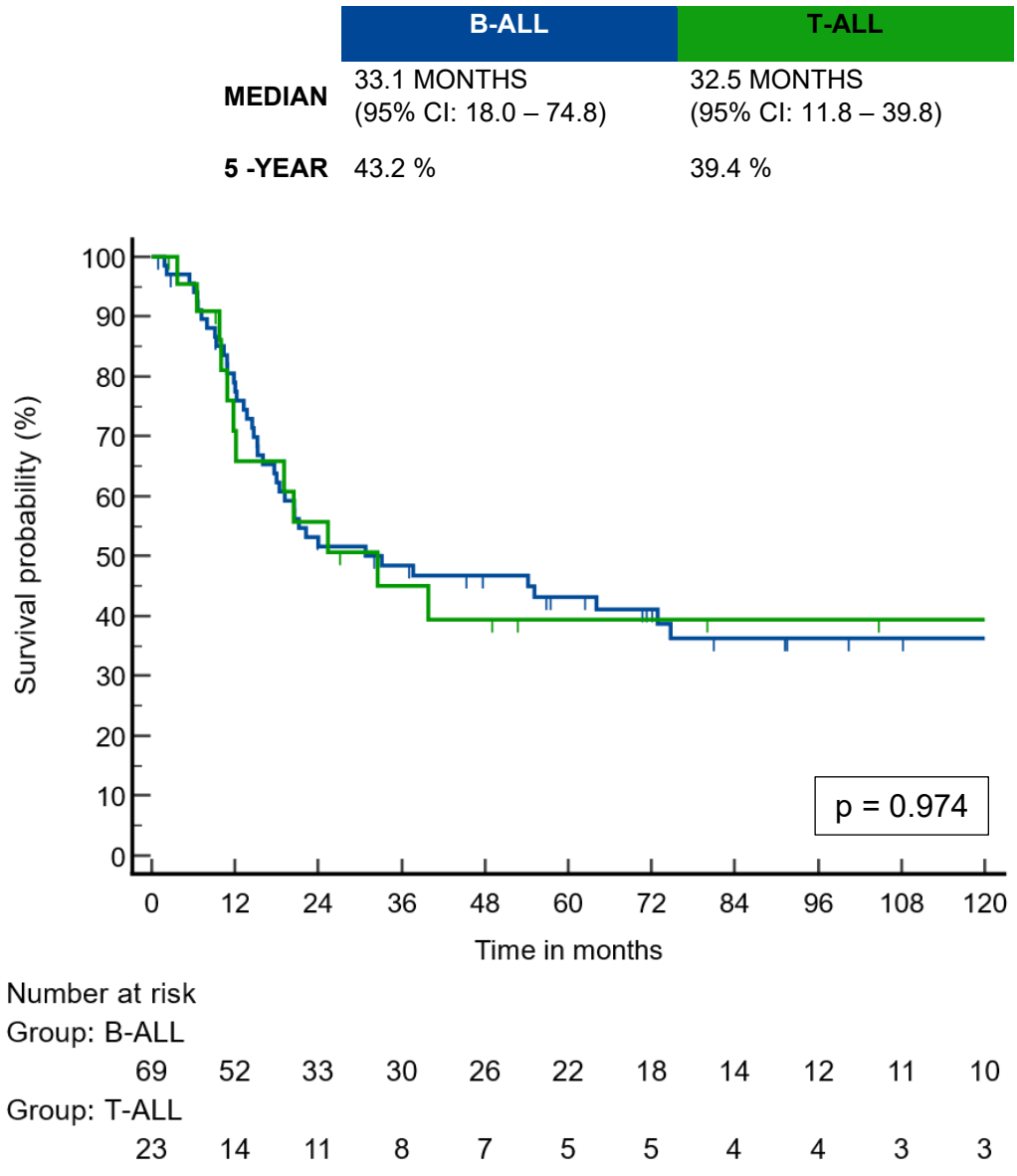


Figure 5. *OS for T-ALL/LBL vs B-ALL*

3.7.3 Overall survival for the GMALL risk groups

In order to compare different risk groups, the patients were retrospectively assigned to a risk group by the recommendation of GMALL definition (see chapter 1.10 Treatment). The results are shown in **Figure 6**. The median OS was not reached within the standard-risk group. For Ph-positive ALL patients, the median OS was 33.1 months, and 15.1 months for patients with high-risk ALL. The difference in OS was statistically significant (p = 0.023).

	SR	Ph +	HR
MEDIAN	NOT REACHED	33.1 MONTHS (95% CI: 18.0 – 127.2)	15.2 MONTHS (95 % CI 10.9 – 32.5)
1-YEAR	96.2 %	78.6 %	61.1 %
3-YEAR	65.2 %	46.1 %	33.3 %
5-YEAR	57.0 %	43.7 %	29.6 %

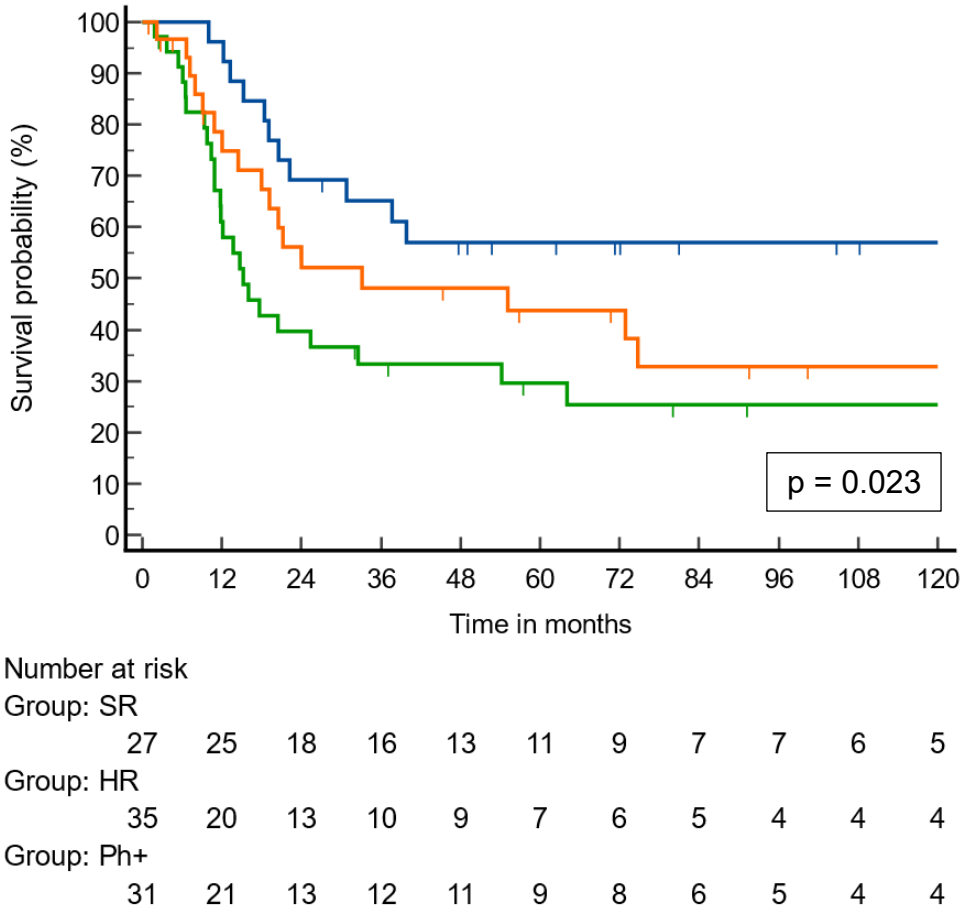


Figure 6. *OS for risk groups by GMALL definition*

3.7.4 Overall survival for age groups AYA versus Adults

Figure 7 shows the OS for young patients (AYA, 18 – 30 years old) and adults (older than 30 years). The median OS was not reached within the AYA group and 5-year OS was 55.2% versus a median OS of 20.5 months in older patients and a 5-year OS of 35.4%. The difference is statistically significant (p = 0.018).

	AYA	ADULTS
MEDIAN	NOT REACHED	20.5 MONTHS (95% CI: 14.5 – 55.1)
5 -YEAR	55.2 %	35.4 %

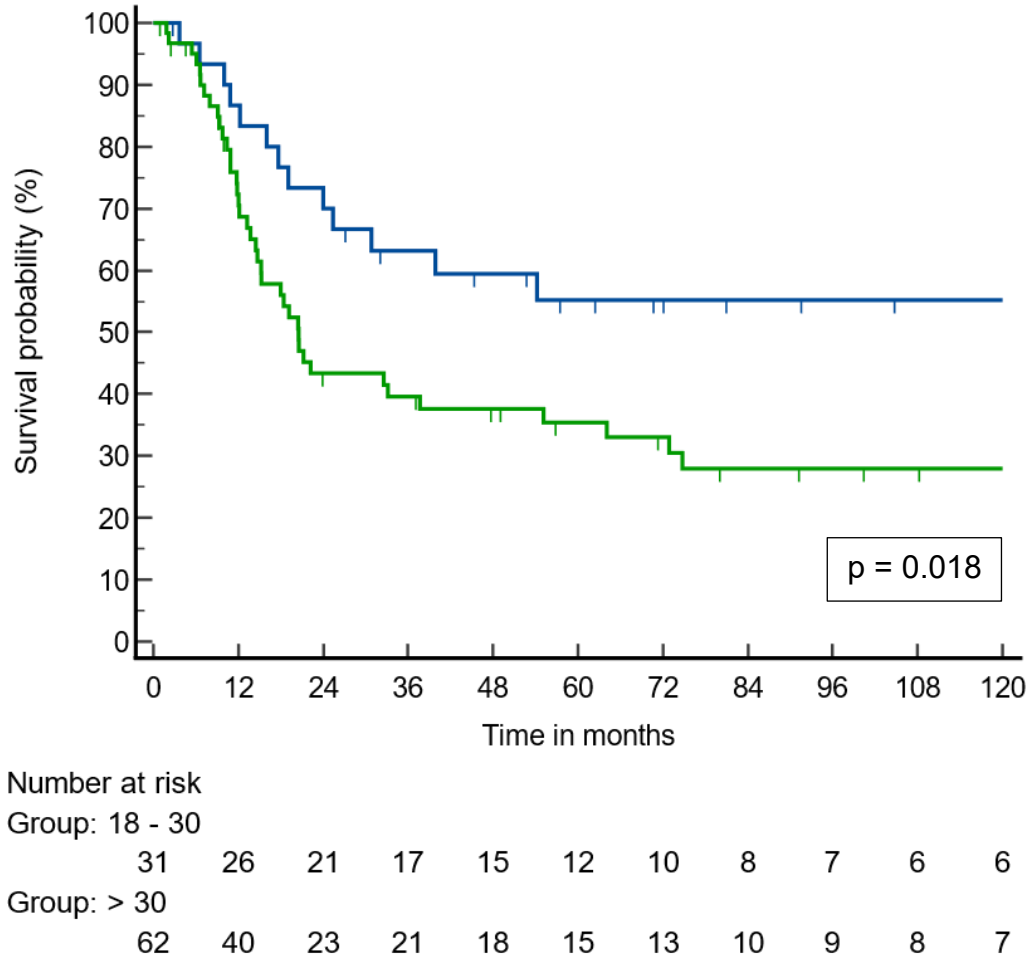


Figure 7. *OS for age groups AYA (18-30) vs Adults (> 30)*

3.7.5 Overall survival for HCT-CI and ECOG performance status

The calculation of survival data relating to the presence of comorbidities and ECOG performance status is intended to assess the extent to which these factors influence OS. **Figure 8** shows the comparison of patients that had no comorbidities (HCT-CI = 0) and patients that had at least one chronic comorbidity (HCT-CI ≥ 1). There is a trend towards better survival in patients without any concomitant diseases, however this difference is not statistically significant (p = 0.087).

	HCT-CI 0	HCT-CI ≥ 1
MEDIAN	64.1 MONTHS (95% CI: 24.0 – 127.2)	20.5 MONTHS (95% CI: 15.2 – 72.9)
5 -YEAR	50.1 %	35.2 %

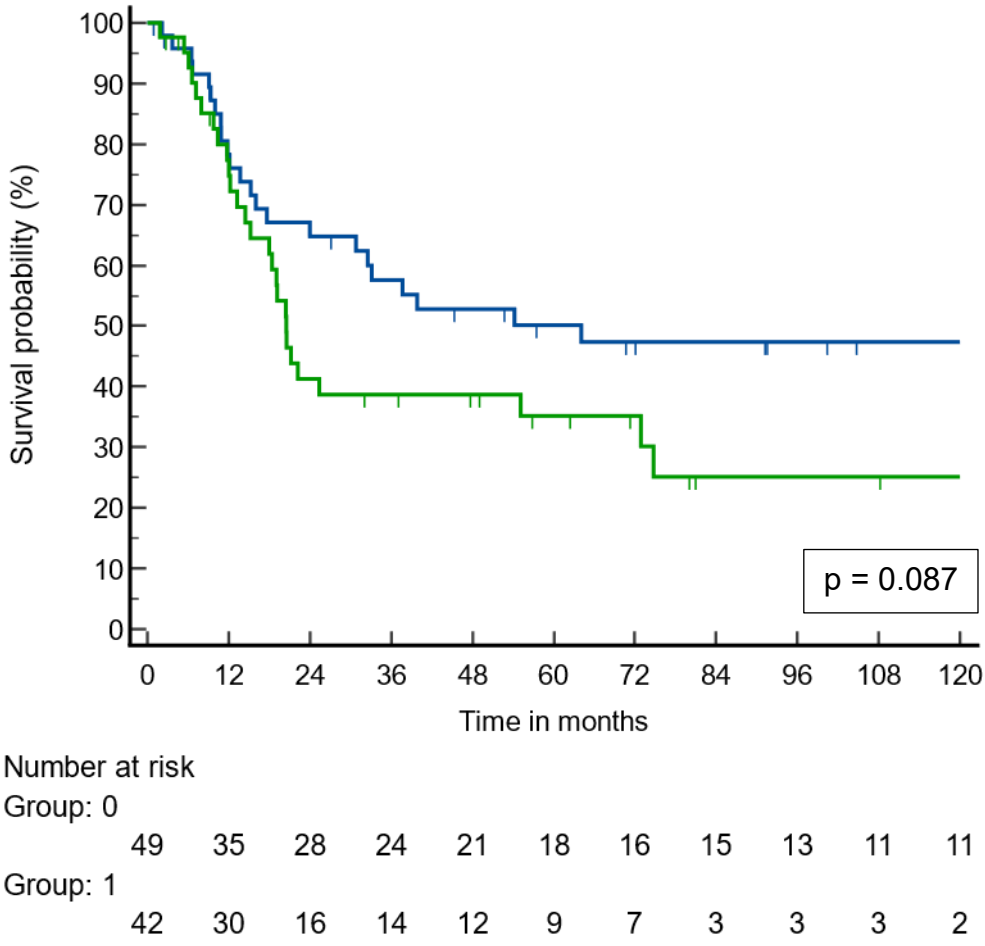
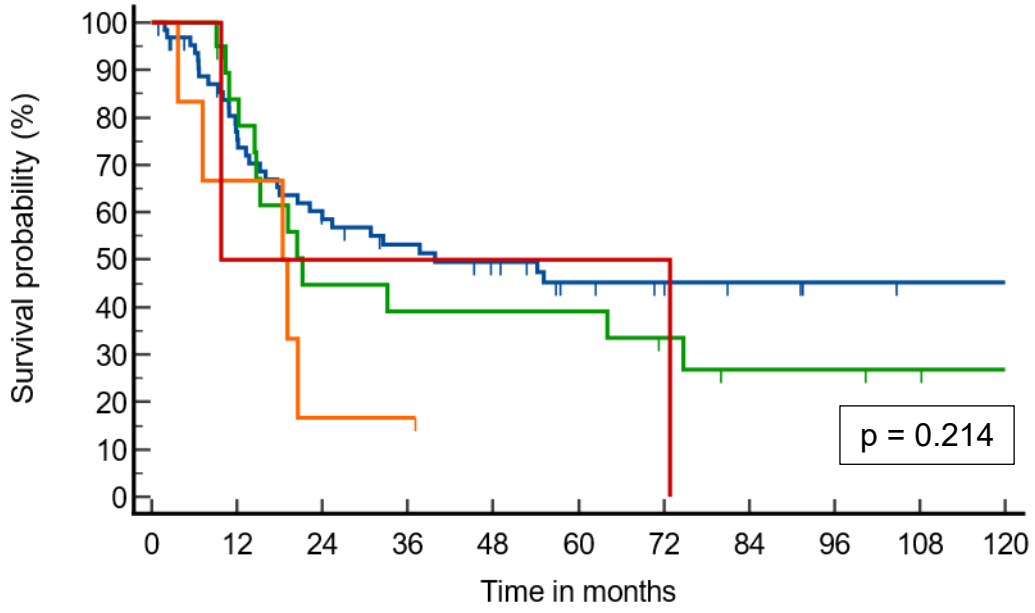


Figure 8. *OS for HCT-CI*

Figure 9 represents the results for the different ECOG performance status subgroups. No clear trend is recognizable here and the p-value of 0.214 is not statistically significant.

	ECOG 0	ECOG 1	ECOG 2	ECOG 3
MEDIAN	39.8 MONTHS (95% CI: 20.5 – 127.2 %)	21.2 MONTHS (95% CI: 14.5 – 74.8)	18.4 MONTHS (95 % CI: 3.6 – 20.5)	9.8 MONTHS (9.8 – 72.9)
5 -YEAR	45.2 %	39.1 %	n.e.	0 %



Number at risk

Group: 0	65	46	34	29	25	19	17	15	13	12	12
Group: 1	20	15	8	7	7	7	5	3	3	2	1
Group: 2	6	4	1	1	0	0	0	0	0	0	0
Group: 3	2	1	1	1	1	1	1	0	0	0	0

Figure 9. *OS for ECOG performance status*

3.7.6 Overall survival for patients treated in 2001-2010 vs 2011–2021

Two groups were formed including patients diagnosed and treated from 2001 – 2010 (36 patients) and 2011 – 2021 (57 patients). The median OS, 33.1 months, was slightly higher for the second group compared to 30.8 months in the first group as well as 5-year OS of 45.7% versus 38.3% (**Figure 10**). However, these results were not statistically significant (p = 0.680).

	2001 -2010	2011 - 2021
MEDIAN	30.8 MONTHS (95% CI: 12.2 – 127.2)	33.1 MONTHS (95% CI: 19.2 – 74.8)
5 -YEAR	38.3 %	45.7 %

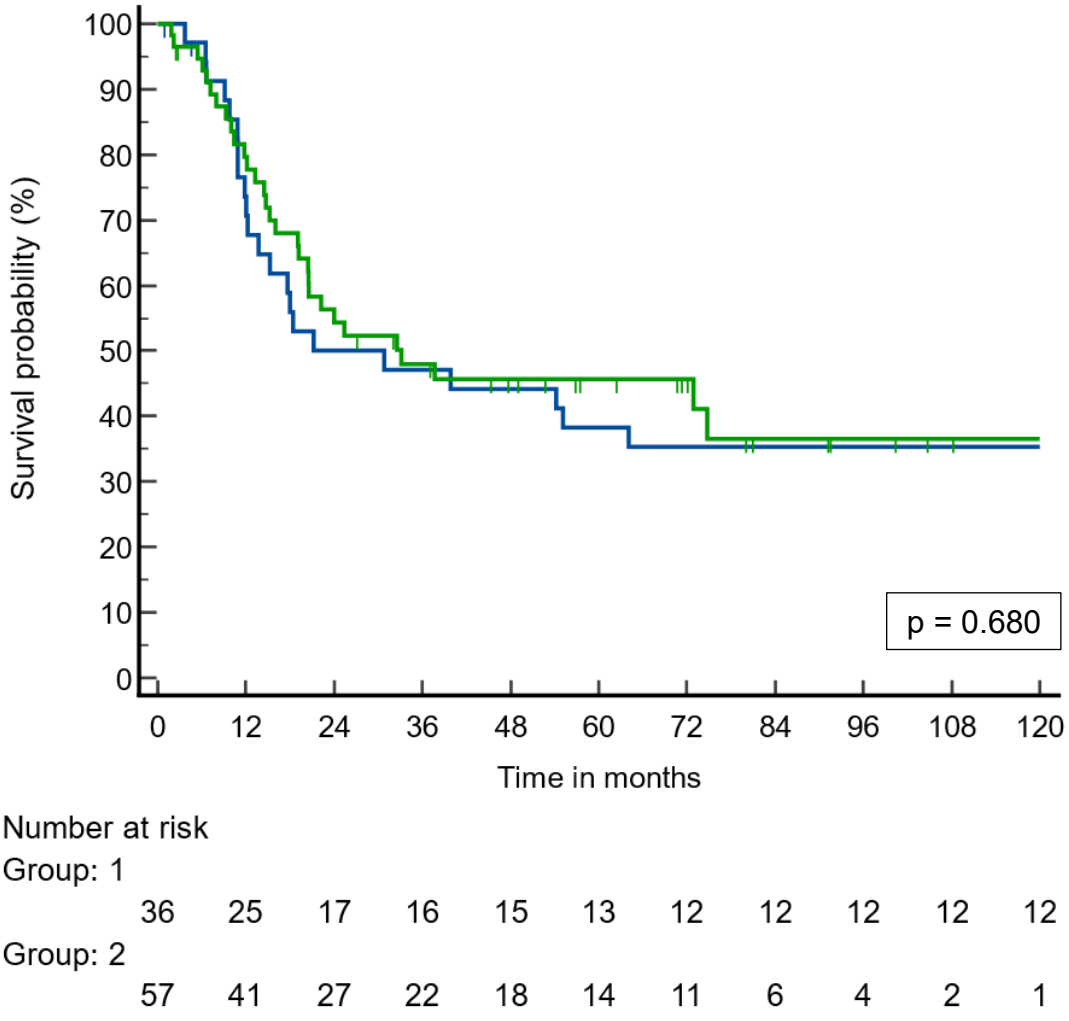


Figure 10. *OS for patients diagnosed and treated 2001 - 2010 vs 2011 - 2021*

3.7.7 Disease-free survival in first complete remission

There were 90 patients treated in Graz that achieved CR1. **Figure 11** shows the Kaplan-Meier curve and the DFS curve of these 90 patients. The median DFS was 16.4 months (95 % CI. 10.4 – 29.7). The 1-year DFS was 57.6 %, the 3-year DFS 34.8 % and the 5-year DFS was 31.9 %.

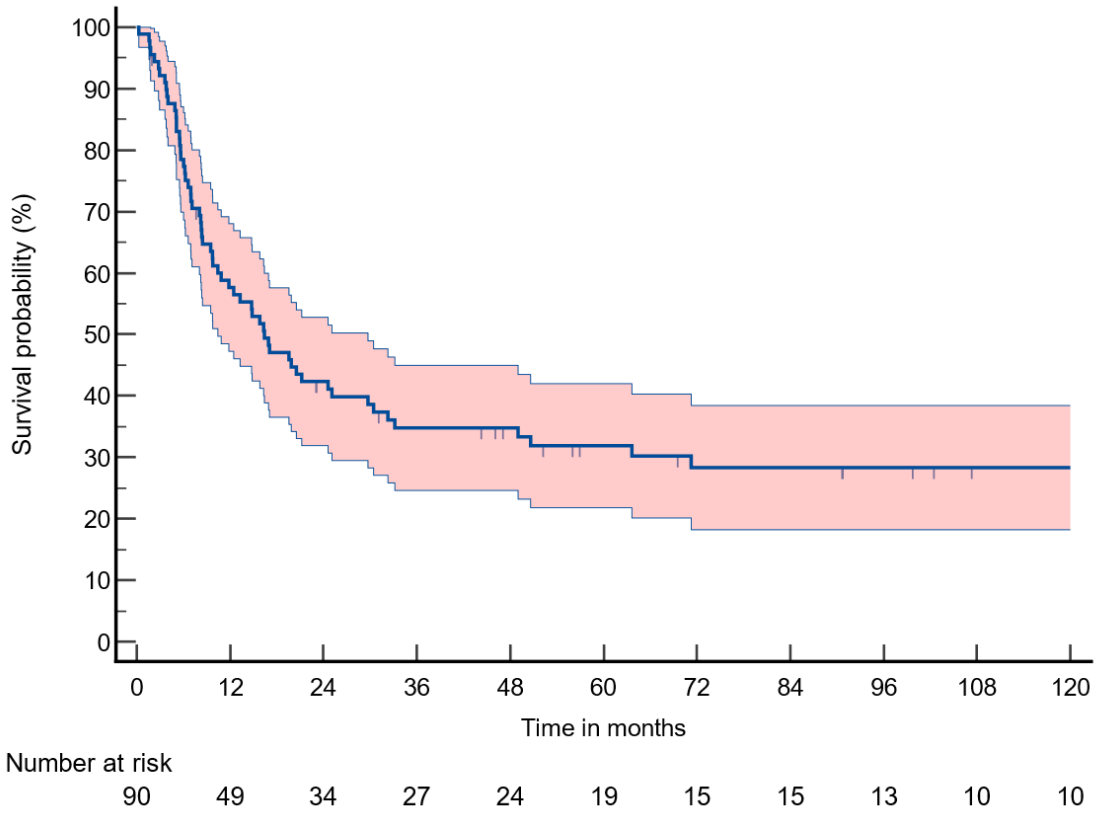
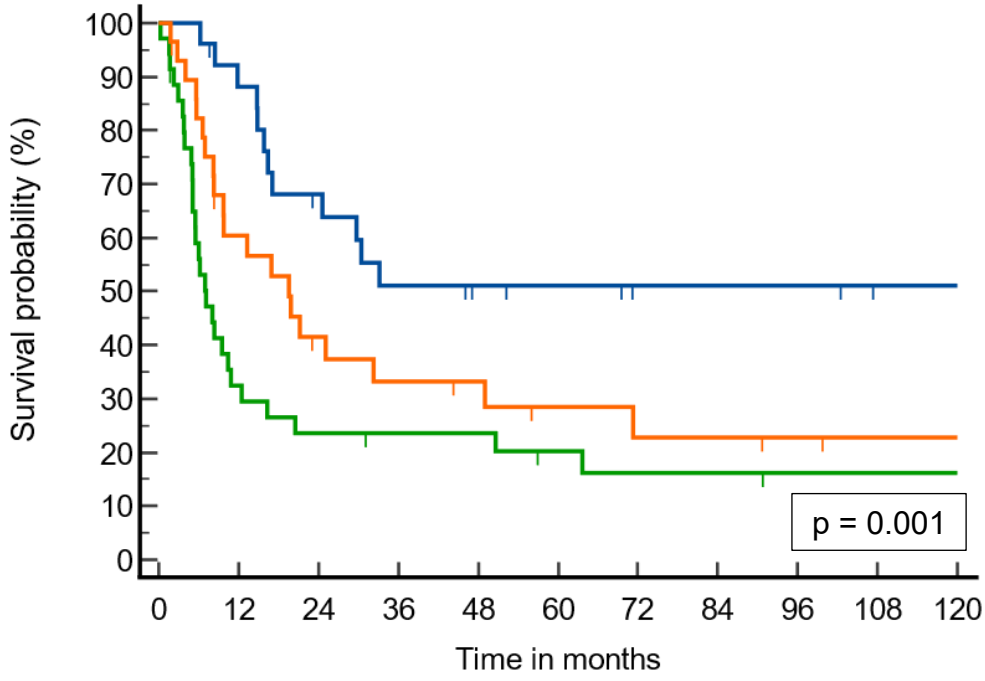


Figure 11. *DFS in CR 1*

The DFS was also estimated for the risk groups according to GMALL definition. **Figure 12** shows that the median DFS was not reached within the standard risk group, whereas the median DFS in Ph-positive ALL patients was 19.6 months and 7.1 months in the high-risk group, respectively. One-, 3- and 5- year DFS rates are shown in the figure. The results are highly significant at a p-value of 0.001.

	SR	Ph +	HR
MEDIAN	NOT REACHED	19.6 MONTHS (95% CI: 24.3 – 78.8)	7.1 MONTHS (95 % CI 5.1 – 10.8)
1-YEAR	88.1 %	60.4 %	32.4 %
3-YEAR	51.1 %	33.2 %	23.6 %
5-YEAR	51.1 %	28.5 %	20.2 %



Number at risk

Group: SR										
26	22	16	12	10	9	7	7	7	5	5
Group: HR										
35	11	8	7	7	5	4	4	3	3	3
Group: Ph+										
29	16	10	8	7	5	4	4	3	2	2

Figure 12. *DFS for the GMALL risk groups*

3.7.8 Overall survival for relapsed versus not relapsed patients

In order to evaluate if there is a difference in survival probability in patients that relapsed versus patients that did not, the OS was calculated and compared. **Figure 13** shows that the median OS in patients with sustained remission is 127.2 months, whereas the median OS in patients experiencing relapse is 20.4 months. The p-value is 0.027 and therefore statistically significant.

	NOT RELAPSED	RELAPSED
MEDIAN	127.2 MONTHS (95% CI: 20.5 – 127.2)	20.4 MONTHS (95% CI: 12.2 – 37.7)
5 -YEAR	57.4 %	24.4 %

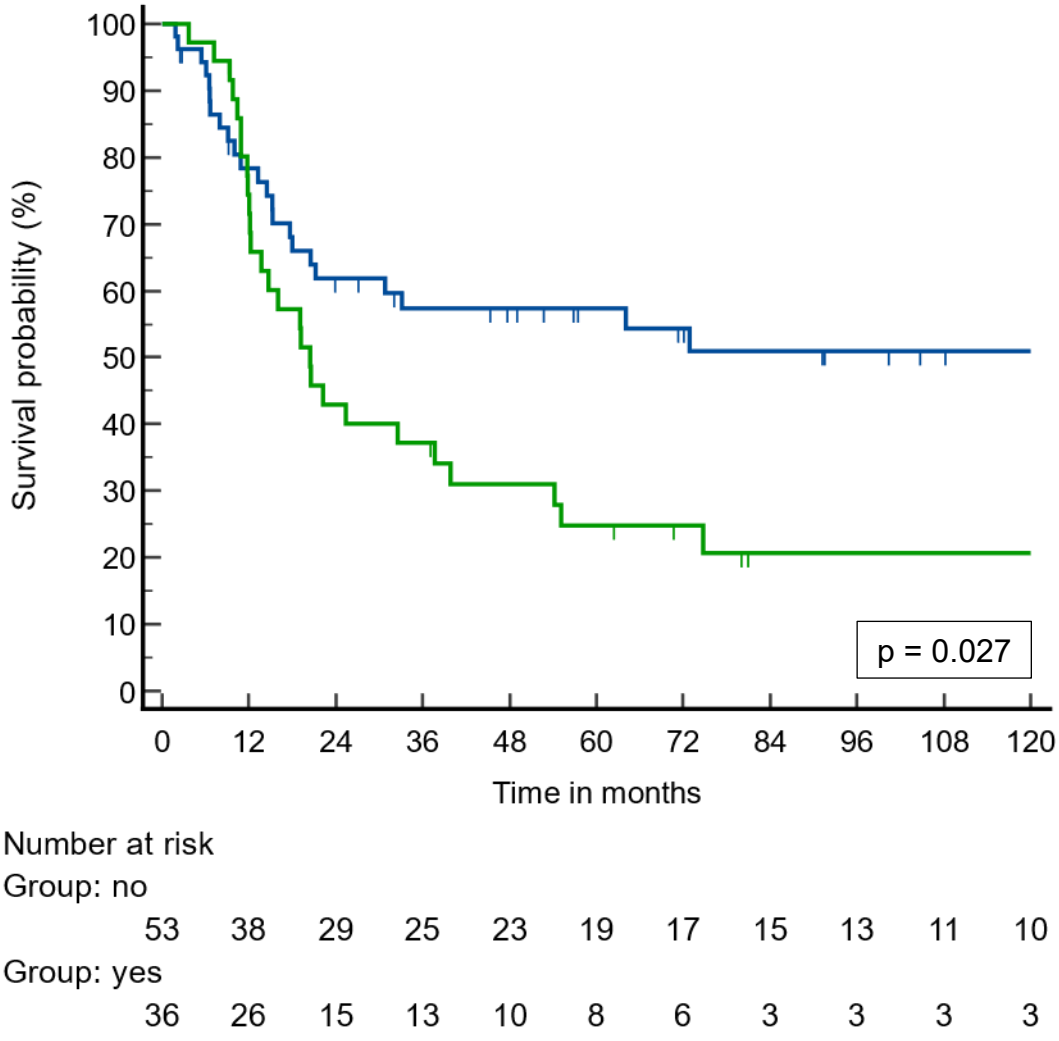


Figure 13. *OS for relapsed vs not relapsed patients*

3.8 Patients Treated with HSCT

3.8.1 Characteristics

Fifty-eight patients (61.1%) received their first HSCT in Graz. The great majority of patients (n=54; 93.1 %) received a related or unrelated donor graft, 3 (5.7%) patients received a cord blood graft and 1 (1.7%) patient was treated with autologous HSCT. There were 34 (58.6%) male patients and 24 (41.4%) female patients.
Forty-five (77.6%) patients were treated with myeloablative conditioning prior to HSCT and their median age was 33.2 years, ranging from 18.3 to 64.9 years. The other 13 (22.4%) patients received reduced intensity conditioning. In this cohort, the median age was 55.1 years with age ranging from 43.0 to 65.0 years.

Regarding HCT-CI prior to transplantation, 32 patients, slightly more than half of the patients (55.2%), had no known comorbidities, 15 (25.9%) patients had 1–2 points and 11 (19.0%) patients had 3 or more points.

With regard to the GMALL risk groups, 11 (29.3%) patients were transplanted within the SR group (in total 28 patients), 25 (71.4%) patients out of 35 in HR and 22 (68.8%) out of 32 patients in the Ph-positive ALL group.

Another important factor in HSCT is remission status before transplantation. The majority of patients, 42 (72.4%) were transplanted in CR1, 11 (19.0%) patients in CR2. Three (5.2%) patients were transplanted in partial remission and in two (3.4%) patients it was not possible to determine whether they were in CR1 or CR2.

Nineteen (54.3%) out of 35 patients in HR and 18 (56.3%) of 32 patients in the Ph+ risk group were transplanted in CR1. Another six patients in the HR group received HSCT in PR (partial remission) or CR2 and three Ph-positive ALL patients in CR2.

3.8.2 Outcome for patients treated with HSCT

Out of the 58 patients that were treated with HSCT, 36 (62%) patients died during follow-up and 22 (38%) patients were alive at the time of last contact. The median follow-up after HSCT was 95.3 months (95% CI 42.5 – 204.9). **Figure 14** shows the Kaplan-Meier curve for all patients from the time of HSCT. The median OS after HSCT was 16.7 months (95% CI 8.6 – 48.8), and 1-, 3- and 5- year OS after HSCT was 58.4 %, 41.7 % and 34.2 %, respectively.

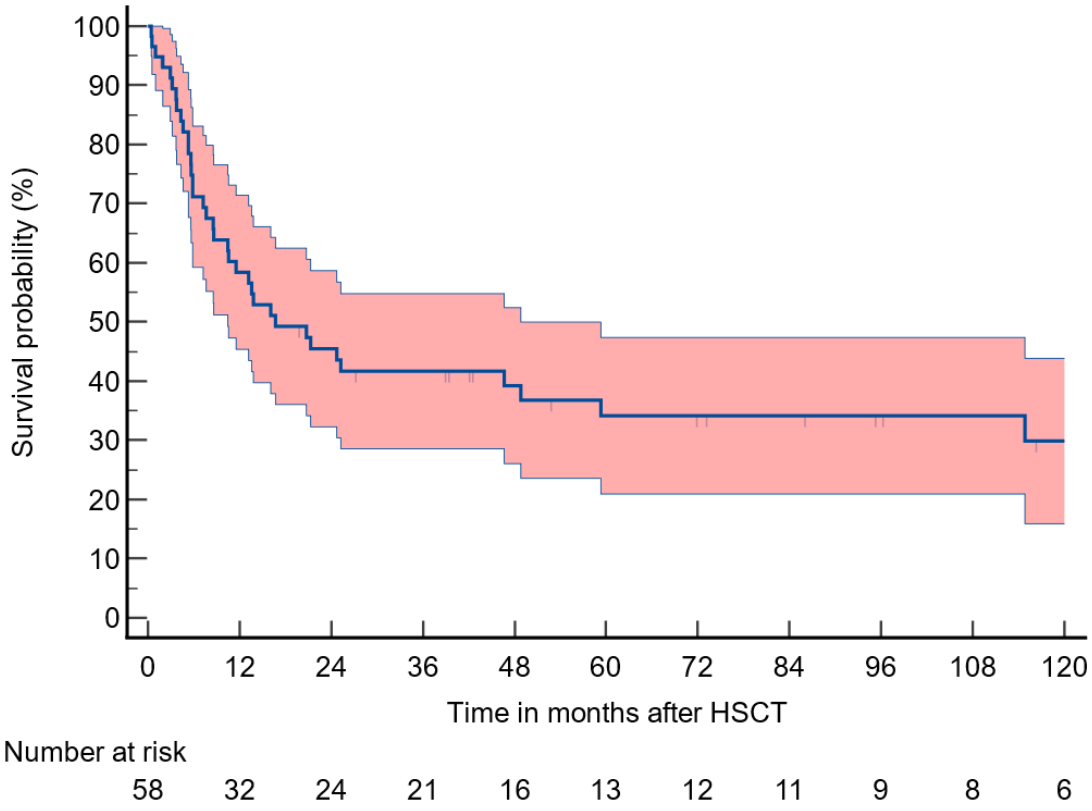


Figure 14. *OS after HSCT*

For patients transplanted in CR1, posttransplant median OS and 5-year survival rate were 20.7 months (95% CI: 7.2 - 34.3) and 31.3%. Nine (21.4%) patients relapsed after HSCT in CR 1.

Figure 15 represents a comparison of OS after HSCT for patients 18 to 30 years old (AYA; n=19) and patients older than 30 years (adults; n=39). Within the AYA group the median OS after HSCT was 48.8 months, whereas the adults group showed a median OS after HSCT of 10.5 months. With a p value of 0.034, the results are statistically significant.

	AYA	ADULTS
MEDIAN	48.8 MONTHS (95 % CI 13.2 – 48.8)	10.5 MONTHS (95 % CI 5.6 – 24.7)
1-YEAR	78.9 (9.4 %)	47.5 % (8.3 %)
3-YEAR	57.9 % (11.3 %)	33.1 % (7.9 %)
5-YEAR	49.6 % (12.4 %)	26.5 % (34.2 %)

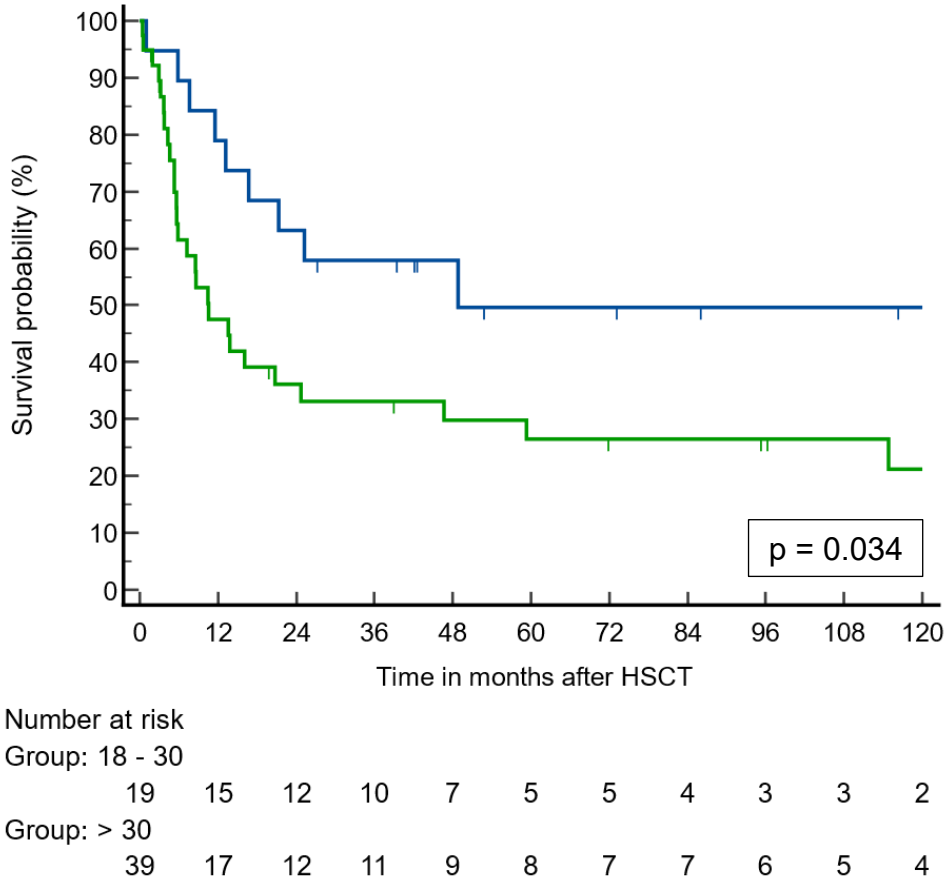


Figure 15. *OS after HSCT for age groups*

Furthermore, 14 (24%) patients experienced relapse of ALL after HSCT. Nine patients that were transplanted in CR1 relapsed during follow-up. Out of the 11 patients that were transplanted in CR2, 4 patients relapsed.

The development of acute or chronic GvHD is another important outcome parameter in HSCT. Considering acute GvHD, 35 (60.4%) patients developed the condition while 22 (37.9%) did not. One (1.7 %) patient was not evaluable for acute GvHD assessment. Regarding chronic GvHD, 38 (65.5%) had no signs of symptoms, 14 (24.1%) patients developed chronic GvHD. Six (10.3%) patients were not evaluable for chronic GvHD. GvHD rates are reported irrespective of prior relapse and cessation of immunosuppression.
Table 11 shows a detailed overview for grading of acute and chronic GvHD in this cohort of 58 patients.

Table 11. *GvHD overview*

Acute GvHD overall grading	no. (%)
0	22 (37.9)
1	11 (19.0)
2	12 (20.7)
3	8 (13.8)
4	4 (6.9)
unknown – n/a	1 (1.7)
Chronic GvHD	
no chronic GvHD	38 (65.5)
mild	5 (8.6)
moderate	3 (5.2)
severe	6 (10.3)
unknown – n/a	6 (10.3)

3.9 Cox-Regression

The univariate and multivariable Cox regression of OS is shown in **Table 12.**

Table 12. *Univariate and multivariable Cox regression for overall survival (significant p-values are in bold letters)*

	Univariate		**Multivariable**	
	Hazard ratio (95% CI)	p - value	Hazard ratio (95% CI)	p - value
Age				
AYA	Reference		Reference	
Adult	2.10 (1.21 – 3.93)	**0.021**	2.06 (1.10 – 3.86)	**0.024**
Risk group				
SR	Reference		Reference	
Ph+	1.88 (0.89 – 3.98)	0.100		
HR	2.65 (1.30 – 5.43)	**0.008**	1.84 (1.07 – 3.18)	**0.028**
WBC				
< 30 G/L	Reference			
≥ 30 G/L	1.48 (0.85 – 2.59	0.166		
Comorbidities				
HCT-CI 0	Reference			
HCT-CI 1-2	1.57 (0.80 – 3.07)	0.190		
HCT-CI ≥ 3	1.47 (0.76 – 2.83)	0.250		
Performance status				
ECOG 0	Reference			
ECOG 1	1.32 (0.69 – 2.51)	0.310		
ECOG 2	2.44 (0.94 – 6.33)	0.068		
ECOG 3	2.11 (0.50 – 8.84)	0.306		

The univariate Cox regression analysis showed that age over 30 years and assignment to the high-risk group by GMALL definition are significant factors for worse OS. White blood cell count ≥ 30 G/L, HCT-CI (comorbidities) and ECOG performance status were not associated with OS. Parameters that were associated with worse OS (age over 30, high-risk group by GMALL) in univariate Cox regression were put in a multivariable Cox proportional hazards model. It could be shown that age over 30 years (HR=2.06, p = 0.024) and high-risk group (HR=1.84, p = 0.028) were independent prognostic factors of OS.

4. Discussion

ALL is an aggressive malignant disease of the hematopoietic system with great biological diversity, which requires intensive and long-term therapy. Additionally, it only accounts for approximately 20% of acute leukemia cases in the adulthood, making ALL a rare disease. Although chemotherapy represents the backbone of ALL treatment and many patients still need HSCT in a curative approach, specific antibodies and CAR T-cell therapy complement and improve the treatment, especially in relapsed patients. For these reasons, ALL is treated at specialized centers offering extensive treatment options such as HSCT and inclusion in clinical trials. At the Division of Hematology, University Hospital of Graz, 95 patients were treated from 2001 to 2021 and this retrospective study aimed to evaluate the clinical outcome of these patients.

Descriptive statistics

In the literature a predominance of male sex is described and is within the scope of 1.2:1 to 1.4:1 (2,8). In this cohort, there is even a ratio of 1.5:1 or 60% male and 40% female patients. Considering incidence rates of the cancer statistics in the USA for AML, CML and CLL, and for Austria all forms of leukemias in total, in all groups men were more affected than women (7,105). However, there is no detailed explanation for male predominance in the literature.

With regard to classification of age groups, it has to be said that the definition of AYA is inconsistent within the literature and published studies. Malard and Mohty define AYAs as 15-39 years (2), whereas in a published multicenter – study conducted in Israel, AYAs were defined as patients from 18 to 35 years (106).

In this retrospective study, the definition of AYAs was set to 18 to 30 years, in order to evaluate younger age as a parameter on outcome more precisely. Furthermore, patients younger than 18 years are treated in the Department of Pediatric Hemato-Oncology and were not considered in this study. This way, around a third of all patients fell into the AYA cohort. Approximately 21% were older than 55 years and received less intensive treatment in accordance with the recommendations of the GMALL elderly protocol. With regard to physical performance and comorbidities it can be stated that the majority of patients (68%) had an ECOG PS 0 (no limitations to physical ability), 52% had no comorbidities

according to HCT-CI and 21% had 1-2 points. This can be explained by a relatively young cohort, where 78% of all patients were younger than 55 years at the time of diagnosis and the median age was 40.3 years.
The distribution of immunophenotypes, considering B- and T-immunophenotype and their immunological subtypes, is identical to published registry data from GMALL.(8) Seventy-six percent of patients within our study presented with B-cell precursor ALL and 24% with T-cell precursor ALL/LBL, compared to 76% and 24% reported by GMALL, respectively. The frequency of *BCR::ABL1* positive patients of 32.6% was also within the range reported in the literature. (8) Interestingly, in a retrospective analysis from the GMALL study group, where 2544 cell samples were tested for *BCR::ABL1* positivity, the frequency was even at the exact same number of 32.6 % and they showed an association with increasing age. The highest incidence was found in patients older than 44 years, where the frequency was around 40%. (107)

Regarding laboratory findings and extramedullary involvement, the median bone marrow infiltration was 95%, which reflects the markedly accelerated cell division and the aggressive behavior of this disease. Approximately a third of all patients, showed symptoms or diagnostic findings of extramedullary involvement, where lymphadenopathy and mediastinal bulky disease were leading manifestation sites. All cases of mediastinal mass were associated with the T– cell immunophenotype. Approximately 60% of T-ALL cases show a mediastinal tumor (8), in our study 10 (43.5%) out of 23 patients had mediastinal involvement.

Complete remission rates up to 90% and more are widely described in the literature, especially for patients treated with pediatric-inspired regimens. (76,98) In our study, the CR rate was 96.4%, however the duration, until CR is reached, also plays an important role as a risk factor. Within the GMALL risk stratification (see 1.10 Treatment), CR on day 44 (after induction II) or no CR after induction I displays a high-risk factor. (73) In this study, the median time to CR was 34 days, ranging from 9 to 174 days. Patients that did not reach CR after induction I were consequently allocated to the HR group and treated accordingly to the GMALL protocol.

The importance of MRD detection has already been described, however at the Department of Hematology, MRD was only established in recent years and became a routine diagnostic tool. In this study, the MRD status was not recorded at defined points in time, but only whether an MRD negativity had ever existed. MRD detection was established for 46 patients, out of these 52.2% showed MRD negativity by cut off of 10^{-4}. Due to the lack of data, MRD based therapy and the course of MRD values was not evaluated within this study.

Overall survival and disease - free survival

Discussing the results of the survival statistics, it has to be stated, that the median time of follow-up was 91.2 months (7.6 years) and therefore rather long, the maximum follow-up was 17.7 years. The median OS for the entire cohort was 30.8 months and the 5–year OS 41.3%. These results correspond to survival rates in published studies and cancer registries.

Data from in total 8 German cancer registries calculated a 5-year relative survival by period analysis of 42.9 % for patients diagnosed from 2008 – 2010. In this publication, patients from the age of 15 years were included. Furthermore, there are age-specific data given with 5-year relative survival of 60.8% for patients 15 to 49, 29.8% for patients between 50 and 69 years and 20.2% for patients older than 70 years. (108)

In another publication by Pulte et al., 5–year relative survival was calculated by period analysis for Germany and the United States and were compared to each other. Data was taken from SEER for the US and 11 cancer registries in Germany in the period of 2002 to 2006. The overall 5–year relative survival in this study was 43.4% for Germany and 35.5% for the US and here too, younger patients showed better survival than patients of higher age. (109)

In a retrospective study on ALL conducted in Israel and published in 2014, 106 patients treated between 1984 to 2009 with the GMALL protocol were analyzed. They were separated in two groups, the first group (40 patients) was treated with the GMALL 89/93 protocol and the second group (66 patients) was treated on the GMALL 99/2003 protocol from 2000 on. Therefore, the second group is comparable to our study group, especially for patients treated from 2001 to 2010 in

Graz due to size of the group but also the protocol that was used at that time. (see 3.7.6 Overall survival for patients treated in 2001-2010 vs 2011–2021).
In Israel, the 5-year OS of the first group was 29% versus 35% of the second treatment group. (110) In our study, the 5-years OS for patients treated between 2001 – 2010 was 38.3%, and thus shows a very similar survival rate to the study mentioned above. Furthermore, the OS increased over time, showing a 5-year OS of 45.7% for patients treated from 2011 – 2021. However, these results were statistically not significant and validation by a larger sample size is needed. Thus, it can be shown that the survival rates are comparable not only in comparison with registry studies, but also with retrospective studies that used GMALL as a treatment protocol.

We investigated different parameters on outcome and overall survival. Contrary to results published in the literature where patients with T-ALL showed better outcome (106), in our study, B-ALL was linked to a slightly better outcome than T-ALL, although this difference was not statistically significant. Two factors might explain this finding. First, the study group was relatively small. Second, 10 out of these 23 T-ALL/LBL patients had early T-ALL, which represents a high-risk feature according to GMALL risk stratification.
Regarding GMALL risk groups, Ph-positive ALL patients were assigned to the “very high risk” group in the GMALL 07/2003 treatment protocol. (111) However, as already mentioned (see 1.10.2) the outcome of Ph-positive ALL improved significantly due to the use of tyrosine kinase inhibitors. This could be shown in our results, too. As was to be expected, patients in SR reached the best outcome with 5-year OS of 57.0% and patients with Ph-positive ALL had a 5-year OS rate of 43.7% in contrast to 29.6% in HR patients. Additionally, comparison of these three risk groups showed that, median WBC, median peripheral blast count and frequency of relapse was higher in the high-risk group. This confirms the risk stratification of GMALL and its influence on overall survival for our study cohort.

In the literature, age is uniformly reported as a risk parameter and it has been demonstrated in several trials and registry studies that younger patients (AYAs) show significantly better survival than older patients. (2,106,108,109,111)

In our study, this could be confirmed by 5-year OS for AYAs at 55.2% versus 35.4% for patients older than 30 years.
Regarding the influence of comorbidities at the time of diagnosis, we hypothesized that HCT-CI ≥ 1 would lead to worse outcome. This could not be confirmed statistically in this study. When looking at the ECOG performance status, a trend towards better survival for patients in ECOG 1 and 2 was observed. However, the number of patients in groups ECOG 3 and 4 was too small in order to make a clear statement.

Another endpoint of this retrospective study was 5-year DFS in CR1. The relapse rate was 40% within the whole period of observation, which is consistent with the figures in the literature ranging from 40 to 50%. (98) Overall, the 5-year DFS rate was 31.9% with a median DFS of 16.4 months. Analysis according to GMALL risk groups showed clear disparities. Patients in SR showed a 5-year DFS rate of 51.1% in contrast to only 28.5% in Ph-positive ALL patients and 20.2% in HR patients underlining and confirming the importance of risk stratification and the influence of risk factors on relapse probability. By comparing OS for patients that relapsed to patients that did not, we could show that relapse represents a dismal event resulting in median OS of 127.2 months and 5-year OS of 57.4% for patients never experiencing relapse versus 20.4 months and 5-year OS of 24.4% for relapsed patients.

Hematopoietic stem cell transplantation

The GMALL protocol recommends allogeneic HSCT in CR1 for patients in HR and Ph-positive ALL risk groups. In SR patients, HSCT is performed when there is molecular relapse or treatment failure. (8) HSCT in CR1 was feasible in 19 (54.3%) of 35 HR patients and 18 (56.3%) of 32 Ph-positive ALL patients. In total, HSCT was feasible in 55.2% of patients that had the indication to receive HSCT in CR1. Another six HR patients received HSCT in PR or CR2, as well as three Ph-positive patients in CR2. In total, 68.5% of patients in the HR or Ph-positive group were treated with HSCT. In about a third of patients, HSCT was not feasible. This is due to disease progression, advanced age or inability to receive conditioning therapy.

Gökbuget et. al. state that especially patients with early and mature T-ALL and the pro-B-ALL immunophenotype benefit from this risk-adapted strategy. (111)
In our study, 1-, 3- and 5-year OS after HSCT was 58.4%, 41.7% and 34.2%, respectively. AYAs again reached better results than older adults with 3- and 5-year OS rates after HSCT of 57.9% and 49.6% versus 33.1% and 26.5%, respectively.
The outcome of patients receiving related versus unrelated allo-HSCT including standard risk, high-risk and very high-risk patients by DFS at 5-years was investigated in a study of 264 adult patients with ALL, published in 2004. In this study, there was no advantage of related allo-HSCT, but younger age was found to be a strong factor for better outcome. The 5-year DFS rate was 35% for patients between 17 to 26 years and 22% and 24% for patients aged 27 to 40 years and 41 years and older, respectively. (112)
In a German study including 180 adult patients treated with allo-HSCT between 1995 to 2018, the median OS after transplantation was 23.0 months and 5-year OS was 37.6%. The incidence of grade 0-I acute GvHD was 69% and for grade II and higher 31%. For chronic GvHD, the incidence of no or mild GvHD was 73% versus 27% severe GvHD. (113)
In our study, the frequency of grade ≤I acute GvHD was 57% and 41% for grade ≥II. No or mild chronic GvHD was observed in 74% and moderate or severe cases added up to 16%. We did not calculate competing risk cumulative incidences of GvHD. However, the OS and frequencies of GvHD in our cohort is similar to other studies, having in mind, that direct comparison of two retrospective cohort studies is not possible.

Risk factors of overall survival

Cox proportional hazard regression was intended to analyze the association of selected risk factors with OS. The risk factors included age, GMALL risk group, WBC, HCT-CI and ECOG performance status. The univariate Kaplan Meier estimates showed that age over 30 years at the time of diagnosis was associated with worse OS. Cox regression revealed that age over 30 years was an independent risk factor. With regards to risk groups, only the high-risk group but

not the presence of the *BCR::ABL1* alteration (Ph-positive ALL) was found to be independently associated with worse OS. This also tends to indicate that the historically poor prognosis of patients with *BCR::ABL1* alteration has improved since the introduction of imatinib. The high-risk group represents a decisive risk factor in the context of the GMALL definition including immunophenotypes such as pro-B-ALL—which is mostly associated with the prognostically adverse *KMT2A* alteration, early- and mature T-ALL, late CR and high WBC at the time of diagnosis. (8)
WBC >30 G/L in case of B-ALL, HCT-CI and ECOG performance status were other risk factors that we examined and that were found not to be associated with outcome in our cohort. This might be explained by the small sample size.

Limitations

This retrospective cohort study has some limitations. First, ten (10.5%) patients received their initial treatment in another hospital and four of these patients were treated on a protocol different to GMALL. This might have influenced the outcome statistics. Additionally, data from transferred patients was incomplete and not all relevant parameters could be collected. In addition, there is probably a selection bias present because high-risk and seriously ill patients are more likely to be transferred to University Hospital in Graz or further treatment.

Conclusion

This retrospective cohort study reports real-world ALL patient data from Graz, Austria, that are consistent with other published trial and registry data. The findings highlight an ongoing significant need for improved treatment strategies to optimize outcomes for this patient population.

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