Thesis

Long-term results of the ALL-BFM 2000 trial in Austria

A retrospective analysis

submitted by

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I. ABBREVIATIONS

ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
B-ALL	B-cell derived acute lymphoblastic leukaemia
BCP ALL	B-cell precursor acute lymphoblastic leukaemia
BFM	Berlin-Frankfurt-Münster
BM	Bone marrow
cALL	common(-type) acute lymphoblastic leukaemia
CCG	Children's Cancer Group
CNS	Central nervous system
CR	Complete remission
CSF	Cerebrospinal fluid
DNA	Desoxyribonucleic acid
EFS	Event-free survival
EGIL	European Group for the Immunological Classification of Leukaemia
ESG-MRD-ALL	European Study Group for MRD detection in ALL
FAB	French-American-British
FISH	Fluorescence in situ hybridization
fTBI	fractioned Total Body Irradiation
GvHD	Graft-versus-Host-Disease
Gy	Gray
HLA	Human leukocyte antigen
HRG	High risk group
HSCT	Haematopoietic stem cell transplantation
Ig	Immunoglobulin
IR	Intermediate-risk
Mb	Morbus
MLL	Mixed lineage leukaemia
MRG	Middle risk group
MRD	Minimal residual disease
MPO	Myeloperoxidase

MTX	Methotrexate
LRG	Low risk group
N	Number
NCI	National Cancer Institute
NK	Natural killer(-cell)
OS	Overall Survival
P	P-value
pB-cell ALL	B-cell precursor acute lymphoblastic leukaemia
PB	Peripheral blood
PCR	Polymerase-chain-reaction
PEG-L-ASP	Polyethylenglycol-L-Asparaginase
RG	Risk group
RT-PCR	Realtime-polymerase-chain-reaction
SRG	Standard risk group
T-cell ALL	T-cell derived acute lymphoblastic leukaemia
TCR	T-cell receptor
WBC	White blood cell
WHO	World Health Organisation

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III. Zusammenfassung

Die akute lymphoblastische Leukämie (ALL) ist die häufigste maligne Erkrankung im Kindes- und Jugendalter und mit einem Anteil von 80% auch die häufigste aller Leukämieformen.

Seit 1979 folgt die Behandlung der ALL in Österreich den Studienprotokollen der Berlin-Frankfurt-Münster (BFM) Studiengruppe. Die Anpassung der Behandlungsintensität erfolgt im Rahmen dieser sogenannten Therapieoptimierungsstudien zum einen durch das anfänglich ermittelte Rückfallsrisiko aufgrund von klinischen und biologischen Parametern und zum anderen durch das Ansprechen auf die Therapie im Verlauf – wie Prednisone Response an Tag 8, Knochenmarksbefund an Tag 15 und Remissionsstatus an Tag 33.

Die ALL-BFM 2000 Studie, die in Österreich, Deutschland, Italien und der Schweiz durchgeführt worden ist, führte erstmals die sogenannte “minimal residual disease” (MRD) als unabhängigen Risikofaktor zur Patientenstratifizierung ein. Aufgrund des Nachweises von Leukämie-spezifischen Umlagerungen in Immunglobulin- und T-Zell-Rezeptor-kodierenden Genen mittels Polymerase-Kettenreaktion (PCR) wurden Patienten in drei Risikogruppen eingeteilt.

Ziel dieser Arbeit waren die Auswertung und Darstellung der Langzeitergebnisse [10-Jahres Werte für ein ereignisfreies Überleben (EFS) und Gesamtüberleben (OS)] von 608 an dieser Studie teilnehmenden Patienten in Österreich. Außerdem wurde der prognostische Wert der MRD als solches ermittelt und untersucht, ob durch MRD innerhalb von traditionellen Risikogruppen, die nach biologischen und klinischen Charakteristika und frühem morphologischem Therapieansprechen charakterisiert waren, Subgruppen mit unterschiedlicher Prognose zu finden sind.

In der ALL-BFM 2000 Studie konnten 521 pB-Zell-ALL und 87 T-Zell-ALL Patienten eingeschlossen werden, wobei die geschätzten 10-Jahres Werte für ein EFS bzw. OS bei 83±2% bzw. 92±1% für pB-Zell-ALL Patienten lagen und bei 79±6% bzw. 82±5% für T-Zell-ALL Patienten.

Mithilfe der PCR-Analysen konnten 495 pB-Zell ALL und 78 T-Zell ALL Patienten in nach MRD-definierte Risikogruppen eingeteilt werden: In 30% MRD Niedrigrisiko-Patienten wurden EFS Raten nach 10 Jahren von rund 90%, in 60% der Patienten mit mittlerem MRD Risiko EFS Raten von rund 80% und in 10% MRD Hochrisiko-Patienten EFS Raten von 65% beobachtet.

Weiters war es mittels MRD möglich in bedeutenden prognostischen biologischen und klinischen ALL Subgruppen drei distinkte Risikogruppen mit unterschiedlichem Rezidivrisiko zu definieren. In nahezu allen Subgruppen konnte eine Überlegenheit der MRD gegenüber traditionell verwendeten Risikofaktoren gezeigt werden, wodurch bisherige sogenannte prognostische Marker bei Kindern und Jugendlichen neu definiert wurden. Eine mögliche Therapieanpassung auf Grundlage der MRD-Ergebnisse der Patienten in frühen Therapiephasen stellt den größten Erfolg dieser Studie und Kernaussage dieser Arbeit dar.

III. Abstract

Acute lymphoblastic leukaemia (ALL) is the most common malignancy in childhood and adolescence, accounting for 80% of all leukaemias in children.

Since 1979 treatment of ALL in Austria follows the protocols of the Berlin-Frankfurt-Münster (BFM) Study Group. Treatment has been adapted and constantly amended through the assessment of initial risk of relapse, based on clinical and leukaemia-associated parameters on the one hand and through the evaluation of response to treatment, such as prednisone response on day 8, bone marrow evaluation on day 15 and remission status on day 33 on the other hand.

Minimal residual disease was primarily introduced as an independent risk factor for patient stratification in the ALL-BFM 2000 trial which was performed in Austria, Germany, Italy and Switzerland. MRD levels were monitored by detecting clone-specific rearrangements in immunoglobulin and T-cell receptor genes as semi-quantitative PCR targets and thereby patients were stratified into 3 subgroups.

The aim of this thesis was the evaluation and analysis of the long-term results - hence the calculation of event-free-survival (EFS) and overall survival (OS) rates after 10 years from diagnosis - of 608 participating patients in Austria. Further aims of this thesis were to evaluate the prognostic impact of MRD among the whole study cohort and among different subgroups of patients in order to show whether MRD was able to clearly define groups with a low, intermediate or high risk of relapse among them.

In the ALL-BFM 2000 trial, 521 pB-cell ALL patients and 87 T-cell ALL patients were identified and EFS and OS rates after 10 years were as follows: 83±2% and 92±1% in pB-cell ALL patients compared to 79±6% and 82±5% in T-cell ALL patients.

By using this PCR-based method of MRD assessment 495 pB-cell ALL patients and 78 T-cell ALL patients were eligible for MRD stratification and thus classified as either low-, intermediate- or high-risk due to their MRD results on day 33 and 78 of treatment.

Approximately 30% were considered as MRD low-risk and performed with EFS rates of about 90%, whereas about 60% were MRD intermediate-risk patients with EFS of approximately 80% and the remaining 10% were MRD high-risk patients presenting with EFS rates of 65%.

Furthermore, a distinct definition of three risk-groups facing different risks of relapse was possible in various biologically and clinically defined subgroups through the application of MRD. In almost all subgroups a superiority of MRD to traditionally used risk factors was observable, redefining prognostic factors in children and adolescents with ALL. The potential of adjustment of treatment based on patient's MRD results during the initial course of therapy is one of the main achievements of the ALL-BFM 2000 trial and the main message of this thesis.

1. ACUTE LYMPHOBLASTIC LEUKAEMIA

1.1. Introduction

Leukaemia is the most common malignant disease in childhood and adolescence. It accounts for approximately one third of all types of cancer.

Acute lymphoblastic leukaemia (ALL) comprises about 80-85% of all types of blood cancer, followed by acute myeloid leukaemia (AML) with 10-15% and the rarely occurring chronic myeloid leukaemia, which accounts for approximately 2-5% of childhood leukaemia. [1,2]

The annual incidence rate in Western Europe and North America is 3-4 cases per 100.000 children and young adults with a peak incidence occurring at 2-5 years of age. [3]

The female to male ratio is 1:1,2 for children who are older than one year, in T-cell ALL the male preponderance is even higher, whereas in infants a female preponderance can be observed. [4] Furthermore, substantial geographical variation exists in the incidence of ALL. [5] In North Africa and the Middle East the Epstein-Barr virus related Burkitt's lymphoma is rather more frequent than ALL. On the other hand, ALL is more frequent in Germany, Australia, Costa Rica and amongst the white population of the United States, whereas rates in India and amongst black children in the United States are lower. [6]

Due to the development of multiagent chemotherapy as well as the enrolment of almost all of the children in multi-centric studies in developed countries, cure rates have been permanently increasing since the 1970ies.

ALL has since become a disease that achieves a cure rate of nearly 85%, when treated in controlled clinical trials. [2]

1.2. Etiology

1.2.1. Genetics

Each lymphoid cell derives from a haematopoietic progenitor cell. Certain transcription factors are activated in order to initiate the process of lymphoid development; leading to every lymphoid cell taking its line to be either from B- or T- cell-type. [7]

Transformations within one of these haematopoietic progenitor cells lead to ALL. One single mutant haematopoietic progenitor cell gives rise to the proliferation of malignant, poorly differentiated precursors. [8]

The prevailing theory of the multiple step model of carcinogenesis suggests that not only primary oncogenic events but also secondary mutations consolidate a malignant transformation. [9,10]

Most genetic deviances are rather acquired than inherited, since the majority of cases derive from spontaneous somatic mutations. Nevertheless, 5% of acute leukaemias rise from inherited genetic syndromes. [5]

In children with Down syndrome for instance, a 20-fold increased risk of developing ALL can be observed. [3,11]

Moreover, ALL is associated with congenital immunodeficiency conditions, such as X-linked lymphoproliferative syndrome (XLP), Wiscott Aldrich Syndrome (WAS), ataxia telangiectasia, Nijmegen breakage syndrome and acquired immunodeficiency syndrome (AIDS).

Other genetic syndromes, like Bloom-Syndrome, neurofibromatosis type 1, Schwachmann Syndrome, Kostmann's Syndrome and Fanconi anaemia are also associated with an increased risk of ALL. [4,11,12]

Identical twin studies and analysis of Guthrie cards - that have been compared to blood samples at time of diagnosis - suggest that many cases of ALL originate in utero, since characteristic translocations that are seen in ALL have been detected in blood samples at time of birth, especially in patients with *TEL/AML1* and *MLL/AF4* fusion genes. These findings constituted a major success in ALL research. [13]

Inherited factors, however, have a rather small influence on the risk of developing ALL. [8]

This surveillance is also supported by the observation that siblings of ALL patients fail to show a higher incidence rate of leukaemia and other malignant diseases. [14,15]

Even the higher incidence rate of 30% of concordant leukaemia in identical twins seems rather to attribute to a transfer of pre-leukaemic cells through the placenta than to inherited factors. [5,14,15]

Furthermore, analysis of umbilical cord blood of healthy children revealed those same genetic aberrations, which, however, never developed to ALL. This discovery eliminates the possibility of performing a general screening - targeted on early detection of leukaemia - on children at time of birth. [16]

Last but not least, the sole occurrence of aberrant mutations is not sufficient to cause ALL. Consecutive genetic aberrations, which alter the highly vulnerable genome of lymphoid precursor cells, are necessary to develop the full disease pattern of ALL.

Moreover, environmental, dietary, maternal and other external factors are likely to interact with multiple subtle genetic polymorphisms to affect the development of ALL. [5,16,17]

1.2.2. Environmental factors

Several environmental factors, such as ionizing radiation, exposure to certain chemicals, non-ionizing radiation, tobacco smoking and alcohol consumption during pregnancy have been discussed to have an association with increased risk of ALL. [18]

Ionizing radiation, meaning diagnostic x-ray is probably the most established risk factor. Considering the atomic bombing of Japan in 1945, ionizing radiation has been proposed as a risk factor, since data provided from the survivors of the Japanese bombing showed that cases increased among those living closest to the affected area. [5,6]

Furthermore, the risk for children, exposed to in utero diagnostic x-ray marks 1.5 to 1.7 for ALL. An increase of risk for ALL due to postnatal exposure to diagnostic x-ray does not seem to exist.

Regarding non-ionizing radiation, there is inconsistent believe that electromagnetic fields may have leukaemogenic potential. Studies suggest that electromagnetic fields have adverse biological effects, recent findings, however, failed to prove any such association.[5]

Though some studies suggest an association between parental alcohol consumption as well as smoking and ALL, no consistent linkage has been reported. [5]

Maternal history of foetal loss as well as maternal age and weight and length at birth of the child indicate a higher risk for ALL. As for maternal age, an analysis of the Swedish Family-Cancer Database, excluding children with Down syndrome, revealed a risk of developing childhood leukaemia associated with a higher maternal age. [3,11,19]

1.2.3. Infections

Various epidemiologic surveys suggest an association between infections and the risk of developing ALL. [20,21]

Concordant ALL in identical twins – as described above – showed a time lag in occurrence, for example, which may support the assumption that multiple other factors, such as infections as well as an inadequate response of the immune system, play a significant role in developing ALL. [6,17,21]

1.3. Classification

Diagnosis and morphologic as well as genetic classification of ALL is absolutely essential for a successful treatment.

Currently, classification of leukaemia needs morphologic examination, immunophenotypic analysis, cytogenetics as well as molecular-genetic analyses.

1.3.1. Morphologic classification

Bone marrow (BM) sampling poses an essential remedy in diagnosing leukaemia, since 20% of patients with acute leukaemia lack blast cells that circulate in the peripheral blood at diagnosis. Furthermore the morphologic appearance of leukemic cells in peripheral blood can present itself very different from that in BM. [23]

In general, marrow samples are taken by aspiration from the posterior superior iliac crest; in obese children the anterior iliac crest can be taken alternatively. [22]

Morphologically classifying ALL basically consists of establishing the diagnosis of acute leukaemia and classifying the leukaemic process according to lineage and degree of differentiation. [22]

The replacement of BM by leukaemic blasts is characteristic for acute leukaemia. Hence a proportion of 25% of leukaemic lymphoid blasts in the BM attests for the diagnosis of ALL.

The French-American-British classification (FAB) system is based on describing three subtypes – L1, L2, L3. [24,25]

Although immunophenotypic, cytogenetic and molecular-genetic features of ALL have highly improved risk stratification, the FAB classification remains the “gold standard” in forming a diagnosis and distinguishing between ALL, AML and the leukaemic phase of Burkitt lymphoma, which constitutes a major impact on therapeutic schemes. [22]

The three subtypes of the FAB classification:

L1-type blast cells are prevailingly small with homogenous and dispersed-to-clumped chromatin, unobtrusive nucleoli and basophilic cytoplasm.

L2-type blast cells are larger, have a more heterogeneous size, the chromatin is variable from dispersed to condensed, with prominent nucleoli and profuse cytoplasm as well as variable degrees of basophilia.

L3-type blast cells (Burkitt subtype) are large with regular nuclei and dense but finely stippled chromatin, a fiercely basophilic cytoplasm and conspicuous vacuolization.

L3 ALL has a mature B-cell immunophenotype and various translocations that dysregulate the *MYC* locus. It presents with the same morphologic trait and chemosensitivity as Burkitt lymphoma and is therefore similarly treated. [22]

Vacuolization, however, is not a peculiar characteristic of L3-type ALL, since L1 and L2 types of blast cells may comprise cytoplasmatic vacuoles in almost 30% of ALL cases – its diagnosis is essential, though, due to the therapeutic consequences of L3- morphology. [22,24]

1.3.2. Immunophenotypic classification

Immunophenotypic classification is based on the fact that lymphoid cells harbour antigens on their surface, characterising their affiliation to a lineage and their stage of maturation.

In the mid 1970ies immunophenotypic classification has been established as a consequence of the observation that ALL cases that featured immunologic characteristics of T-cell precursors or mature B-cells faced a worse outcome. [22]

Presently, precursor B-cell and precursor T-cell lymphoblastic leukaemia are the two main immunophenotypic subtypes of ALL according to the WHO classification. [26,27]

Some types of ALL share lymphoid- as well as myeloid-associated markers, such as MPO (myeloperoxidase), CD13, CD33, CD15 or CDw65 and are called bi-phenotypic or acute mixed-lineage leukaemia. [31]

In the 1990ties the multicolour flow cytometry enabled the introduction of a classification system, by using specific antibodies directed against lymphoid- and myeloid-associated antigens, improving the immunologic detection of minimal residual disease (MRD) at the same time. [28,29,30,33,34]

In Europe the prevalent classification system is orientated by the guidelines of the European Group for the Immunological Classification of Leukaemia (EGIL), whose system has been adapted in 2008 and is shown in tables 1 and 2. [31]

Table 1 Immunophenotypic classification of ALL according to the EGIL criteria[32]

ALL subtype	Immunophenotypic profile
B-cell ALL	CD19+ and/or CD22+ and/or CD79α+ often TdT+, CD34+ and HLA-DR+ criterion: at least 2 of the three above
pro-B ALL (B-I)	no further B-cell differentiation marker
common ALL (B-II)	CD10+
pre-B ALL (B-III)	cytoplasmic IgM+
Mature B-AL (B-IV)/B-NHL	Ig+ (cytoplasmic or surface) or sIgκ+ or sIgλ+
T-cell ALL	cytoplasmic or surface CD3+ often TdT+, HLA-DR+ and CD34-
pro-T ALL (T-I)	CD7+
pre-T ALL (T-II)	CD2+ and/or CD5+ and/or CD8+
cortical T-ALL (T-III)	CD1a+
mature T-ALL (T-IV)	surface CD3+, CD1a-
α/β+ T-ALL	anti-TCRα/β+
γ/δ+ T-ALL	anti-TCRγ/δ+
ALL with myeloid markers (My+)	CD13+ and/or CD33+ and/or CDw65+ on >20% of lymphoblasts in ALL

Abbreviations table 1: Ig: Immunoglobulin; ALL: acute lymphoblastic leukaemia; TCR: T-cell receptor; NHL: Non-Hodgkin's Lymphoma; AL: acute leukemia

Table 2 Scoring system for bi-phenotypic leukemias according to the EGIL criteria[95]

Points	B-cell marker	T-cell marker	Myeloid marker
2	CD79a cyt IgM cyt CD22	cyt CD3 or sCD3 anti-TCRα/β anti-TCRγ/δ	anti-MPO
1	CD19, CD10, CD20	CD2, CD5, CD8, CD10	CD13, CD33, CDw65, CD117
0,5	TdT, CD24	TdT, CD7, CD1a	CD14, CD15, CD64

Abbreviations: cy: cytoplasmic; s: surface; TCR: T-cell receptor; MPO: myeloperoxidase
Bi-phenotypic leukemia: total scores must be >2 points for the myeloid and >1 point for the lymphoid markers

1.3.3. Genetic classification

Leukaemia arises from a single haematopoietic progenitor cell, sustaining specific genetic damage, which ultimately leads to malignant transformation and abnormal proliferation. [8]

Due to the introduction of molecular-genetic methods such as fluorescence in situ hybridization analysis (FISH) and reverse transcription-polymerase chain reaction (RT-PCR), chromosomal numeric and structural aberrations can be verified in 70-80% of ALL cases.

Microdeletions and point mutations cannot be detected with the conventional cytogenetic methods, however, which delimitates the possibilities of verifying the assumption that all leukaemic cells harbour chromosomal aberrations. [14,35]

These aberrations are of highly prognostic significance and are associated with certain immunophenotypes and clinical features. Thus cytogenetic (tumour-)classification has become an essential diagnostic instrument that diversifies the risk stratification of ALL in therapeutic studies. [36,40,41]

ALL can be broadly classified referring to the ploidy – the chromosomal number of leukaemic cells. [8]

Hypodiploidy, which is defined as less than 46 chromosomes and high-hyperdiploidy, defined as more than 50 chromosomes, are of significant clinical value.

The latter has a good prognosis, especially high-hyperdiploidy with 51-65 chromosomes (54-55 chromosomes are detectable in the majority of patients), due to an increased sensitivity to antimetabolite agents, a lower leukocyte count, a tendency to accumulate methotrexate polyglutamates, the tendency of occurrence at an age between 1 and 10 years and a propensity to undergo spontaneous apoptosis. [8]

Approximately 25% of ALL cases present with high-hyperdiploidy. [37,38,39]

Hypodiploidy, however, has a less favourable outcome and is associated with a high risk of relapse. [37,38,39]

Structural chromosomal aberrations, however, mostly balanced reciprocal translocations appear to be the most frequent karyotypic changes in ALL. [42]

The following translocations are of high prognostic and therapeutic value and mostly common in children with ALL: t(12;21)(p13;q22), t(4;11)(q21;23), t(9;22)(q34;q11), t(1;19)(q23;p13) and t(11;19)(q23;p13). [15]

Translocations t(4;11) and t(9;22), known as “Philadelphia chromosome” and featuring the *BCR/ABL* fusion transcript, are of specific prognostic relevance.

Patients carrying this unfavourable genetic attribute are immediately categorised as patients of high-risk of relapse and preferred candidates for allogeneic stem-cell transplantation. [14,36]

The t(12;21) with the *TEL/AML1* fusion transcript on the other hand, is the most frequent balanced chromosomal aberration in childhood ALL - appearing in 20-25% of children with ALL - and is associated with a favourable outcome. [14,16,36]

Table 3 provides an insight into the genetic abnormalities and their association with specific subtypes of ALL.

Table 3 Genetic abnormalities in childhood ALL and LBL (lymphoblastic lymphoma): incidence, association with immunophenotypes and prognosis[6]

Aberration	Molecular genetics	Immunophenotype	Incidence	Prognosis
t(1;19)(q23;p13)	E2A/PBX1	pre-B ALL	5%	good
t(4;11)(q21;23)	MLL/AF4	pro-B ALL und CD10-neg. pre-B ALL	4%	poor
t(11;19)(q23;p13)	MLL/ENL	pro-B ALL und CD10-neg. pre-B ALL	1%	intermediate
t(9;22)(q34;q11)	BCR/ABL	common ALL	3%	poor
t(12;21)(p13;q22)	TEL/AML1	common ALL	22%	good
t(8;14)(q24;q32)	MYC/IgH	mature B-AL, Burkitt's Lymphom	3%	good
t(8;22)(q24,q11)	MYC/Igλ	mature B-AL, Burkitt's Lymphom	3%	good
t(2;8)(p12;q24)	Igκ/MYC	mature B-AL, Burkitt's Lymphom	3%	good
High-hyperdiploid	/	common ALL	25%	good
Near-tetraploid	/	common ALL, T-ALL	1%	unclear
Near-triploid	/	pre-B ALL	<1%	unclear
Hypodiploid	/	common ALL	5%	poor
14q11-Aberration	TCRα/δ	T-ALL, T-LBL	5-10%	intermediate
t(11;14)(p13;q11)	TTG2			
t(10;14)(q24;q11)	HOX11			
t(8;14)(q24;q11)	MYC			
t(1;14)(p32;q11)	TAL1			

Abbreviations: Ig: immunoglobulin; AL: acute leukaemia; TCR: T-cell receptor; ALL: acute Lymphoblastic leukemia

1.4. Clinical presentation

The majority of patients with ALL present with an acute onset while in some insidious signs and symptoms appear and can persist for months.

Symptoms are the reflection of the expansion of the BM cavity by leukaemic blasts and its failure, as well as the extramedullary spread. [8,16]

Usually the anamnesis is very short, patients with two to three months histories of unspecific symptoms, such as undulating bone pain and replicating infections - leading to a delay of diagnosis - are, however, seen. Therefore, ALL is sometimes diagnosed during routine examinations. [6,14]

The infiltration of the BM and its subsequent expulsion by leukaemic blasts leads to neutropenia, anaemia and thrombocytopenia; thus, fever, recurrent infections, pallor, fatigue, lethargy, headache and bruises, petechiae and ecchymoses are commonly seen. [6,16]

Over one third of patients, especially young children, repine about bone pain, specifically in long bones, and arthralgia due to infiltration of the periosteum. Therefore, in children, who present with a limp or refusal to walk, ALL should be taken into consideration as a possible diagnosis. [8]

Signs of extramedullary involvement consist of hepatomegaly, splenomegaly and generalised or localised indolent swelling of the lymph nodes (especially in the cervical and nuchal region), which can be observed in more than a half of patients at diagnosis. [8]

Abdominal pain may be a sign of rapid enlargement of the liver or spleen, is, however seen only in approximately 20% of patients. [35]

Severe and mostly feared complications can occur in patients with T-cell lymphoblastic diseases. Those cases feature a thymic enlargement and/or mediastinal lymphadenopathy that compress the trachea and oesophagus. The clinical consequences are coughing, dyspnoea, pain, dysphagia, respiratory distress and a life-threatening superior vena cava syndrome. [6,8]

In case of central nervous system (CNS) involvement headache, impaired vision, cranial nerve palsies and nuchal rigidity are common symptoms. [6]

In male patients painless (unilateral) enlargement of the testis may be a sign of testicular involvement, which can be diagnosed in as many as 25% by testicular biopsy. [8]

Further symptoms are subcutaneous nodules, enlarged salivary glands (Mikulicz syndrome) and involvement of the orbita, gingiva, lungs, heart and the kidney, which are, however, more rarely observed. [8]

1.5. Diagnosis

Though precise diagnosis of ALL is the foundation of a successful treatment, avoiding and adequately responding to life-threatening complications at presentation have absolute priority. Certain subtypes of ALL asset a high tumour burden and feature grave complications due to tumor lysis syndrome or local tumour effects. [6]

Thus, an entire evaluation of the patient's physical condition and vital parameters is of importance. Signs of infection or bleeding, signs of anaemia or an increased cranial pressure and vital signs should be thoroughly examined before starting the diagnostic work-up of ALL.

Detailed guidelines of study protocols, based on common agreements and requirements have to be followed when initially diagnosing ALL. [15]

A laboratory check-up is mandatory when specifically diagnosing ALL: anaemia, abnormal leukocyte counts and thrombocytopenia are common laboratory findings in ALL and usually present in 90% of cases at diagnosis. The remaining, however, present with normal haematologic cell counts, which sometimes leads to a delayed diagnosis. [19]

The leukocyte count can range from 0.1 to 1500 x 10^9/L and increased values are measured in more than a half of patients. Hyperleukocytosis (≥10x10^9/L) occurs in 15% of cases and 40% present with granulocytopenia at diagnosis, which is responsible for the high risk of infection. [8]

Two thirds of patients feature anaemia at initial diagnosis, which is typically normochromic and normocytic. They can present with low to normal reticulocyte counts though. [8]

Moreover, thrombocytopenia is usually present with fewer than 100.000/mm^3 in children; severe haemorrhage is, however, unusual. [8]

Further laboratory findings are elevated uric acid levels, especially in children with a large leukaemic cell burden; raised serum lactate dehydrogenase levels and increased creatinine, urea nitrogen and phosphorus levels in patients with renal involvement. [8,15]

An enlarged kidney has no prognostic impact, even though it is documented in 30-50% of children at diagnosis.

Diagnostic imaging, in terms of chest x-ray is needed to detect a possible mediastinal mass - present in 50-60% of patients with T-cell ALL - or enlarged mediastinal lymph nodes and pleural effusions. [8]

To confirm a definite diagnosis of ALL a BM aspirate and its following assessment due to its percentage of blasts and myeloid cells is of utmost importance, as already mentioned above. [22]

ALL is confirmed if 25% of the BM consists of leukaemic lymphoid blasts. A ratio below 25% argues for lymphoblastic lymphoma with BM involvement. [6,22]

If BM aspiration fails, a trephine biopsy can serve as an alternative.
Last but not least, cerebrospinal fluid (CSF) should be examined in all patients at diagnosis, since 1-3% of patients show leukaemic blasts in CSF. [8]
ALL with CNS involvement can be readily defined through 3 categories:
CNS 1-status, which means the absence of leukaemic blasts in CSF; CNS 2-status, defined as fewer than 5 WBCs/μl and blast cells and CNS 3-status with more than 5 WBCs/μl with unequivocally identifiable blast cells or the presence of a cerebral mass or cranial nerve palsy. [8,15]
Hereafter, treatment needs to be adapted in regard of the testing results.

1.6. Differential diagnosis

Several malignant as well as non-malignant diseases need to be distinguished from ALL. Osteomyelitis and juvenile rheumatoid arthritis for example may mimic symptoms of ALL, such as bone pain, bone tenderness and fever. A BM aspirate is therefore absolutely mandatory in order to get an exact diagnosis, since rapid use of corticosteroids may delay a diagnosis of ALL and convey further complications. [11,15,19]
Furthermore, fever, pallor, splenomegaly and generalised lymphadenopathy are common signs of infectious mononucleosis ("kissing disease") and other viral infections that need to be differentiated from ALL. [11,15,19]
Idiopathic thrombocytopenic purpura causes bleeding and petechiae. It can be distinguished from ALL by its normal leukocyte counts however. [11,15,19]
Aplastic anaemia, myelodysplastic syndrome, non-Hodgkin's lymphoma and solid tumours with involvement of the BM, such as neuroblastoma, rhabdomyosarcoma and retinoblastoma also need to be differentiated from ALL. [11,15,19]

1.7. Prognostic factors

Adjustement of treatment of ALL through eliciting prognostic factors both at initial diagnosis and during treatment has become indispensable in the design of therapeutic studies.
This adaption is achieved on the one hand through the initially investigated risk of relapse and assessment of early response to treatment on the other hand. [42,43,44]
Most studies aim at characterising a low risk group (LRG), who has a potential of nearly 100% cure rate and therefore receives a less toxic therapy that minimalises the chance of late sequelae and a high risk group (HRG) that will be in dire need of intensification of therapy, presumably. [45,46]

Though the dynamics of “response to treatment” are the most important prognostic factor, other features with potential of prognostic relevance have been delineated.

A conference held in Rome in 1985 for instance established the so-called National Cancer Institute (NCI)- or Rome-criteria of risk-stratification of pB-cell ALL, regarding age and leukocyte count at initial diagnosis. [47]

Moreover, clinical features at diagnosis, immunophenotype, cytogenetic subtypes, in-vitro resistance profiling as well as sex, race, organomegaly, CNS status and early response to treatment share prognostic value. [6]

1.7.1. Clinical parameters

Age and leukocyte count have been established as relevant prognostic factors in most clinical trials.

Infants have the worst outcome, being associated with a 6-year event-free survival (EFS) of fewer than 50%. [49,50] The unfavourable pro-B ALL immunophenotype, associated with adverse *MLL* rearrangements occurs in about 70-80 percent of cases in infants. [51,52]

Approximately two thirds, thus the majority of patients are 2-5 years old and have the best outlook. Furthermore, patients between 1-10 years have a highly favourable prognosis and feature EFS rates of nearly 90%. [53]

Since T-cell and pro-B ALL as well as the detrimental Philadelphia chromosome are increasingly represented in adolescents, this age group has a relatively poor prognosis with an EFS of 65-70%. [53]

Furthermore, the favourable *TEL/AML1* rearrangement or high-hyperdiploidy are rarely seen in adolescents.

Considering the initial white blood cell (WBC) count, a clear correlation between WBC count and outcome seems evident in most clinical trials. The combination of WBC count and size of liver and spleen at diagnosis, reflecting the leukaemic cell mass has been used as the only risk factor in ALL-Berlin-Frankfurt-Münster (BFM) studies of 1981 and 1983. [54]

In addition, as mentioned before, the NCI of the US has sponsored a conference held in Rome in 1985, where two defined risk groups for pB-cell ALL have been developed:

A standard risk group (SRG), defined by a WBC count of < 50.000/μl and children aged between 1-9 years a HRG with a WBC count ≥ 50.000/μl and/or children ≥ 10 years of age. [6]

Patients with T-cell ALL and its formidable consequence of a mediastinal mass do not face a worse prognosis as recent analyses suggest. Through considering the high risk of relapse of T-cell ALL patients already, therapeutic studies were able to achieve survival rates comparable to children with pB-cell ALL. [48,55,56,57]

Gender is of significant prognostic merit, since girls show a consistently better prognosis than boys in most studies. Though testicular relapses have dramatically decreased since the introduction of higher dosages of systemic methotrexate, the higher rate of poor response to prednisone treatment in male patients, which was observed in recent BFM-studies, may serve as an explanation. [48,56]

Initial CNS involvement is associated with an amiss prognosis without adequate treatment. The ALL-BFM 95 study illustrated that children with CNS 3-status comprise a numerous amount of unfavourable features such as a high WBC count, male gender, age $\geq$ 10 years, T-cell immunophenotypes and a poor prednisone response. [58]

Additionally, a traumatic lumbar puncture and CNS-3 status is a poor prognostic marker. Meanwhile, CNS-2 status and CNS-1 status have similarly good prognosis.

Originally, the extent of a mediastinal involvement has been of prognostic value, because of being associated with a high leukocyte count and the occurrence of T-cell ALL. If treated with adequate intensive therapy however, a mediastinal involvement fails to show a worse outcome in recent BFM-studies. [56]

The infiltration of the testicles with leukaemic blasts is associated with a slightly worse outcome. [35]

1,5% of patients were diagnosed with Down syndrome and ALL in BFM-studies and presented with increased risks of relapse and death due to therapeutic toxicity. [59]

1.7.2. Immunophenotype

The four most common immunophenotypes of ALL present with variable EFS rates.

T-cell ALL and pro-B ALL have the worst outcome, shown by high risks of relapse, compared to children with common ALL (cALL).

Association with *MLL* rearrangements, poor early response to treatment and the frequent occurrence of infant ALL cause a relatively poor prognosis in pro-B ALL. [48,60,61]

The poor prognosis of T- cell ALL is due to a higher incidence of poor prednisone responders, not, however, as initially believed, due to the additional finding of a mediastinal mass or high WBC counts at diagnosis. [56,62]

A steadily improving prognosis of T-cell ALL could be achieved, however, due to the use of higher dosages of methotrexate since the ALL-BFM 86 study. [6]

Most factors, which are associated with a favourable outcome, such as the *TEL/AML1* fusion gene or high-hyperdiploidy, are related to pre-B ALL and cALL and therefore account for the good prognosis of these ALL subtypes. [39,63,64] The latter also represents the most common ALL subtype in patients.

Last but not least, a heterogenous group of patients presenting with leukaemias that cannot be clearly assigned to one lineage is detectable and poses a diagnostic and clinical challenge for the ALL treatment scheme. Patients experiencing the phenomenon of “lineage switch”, however, face a dismal prognosis.[35]

1.7.3. Cytogenetic abnormalities

Due to their prognostic value and their correlation to certain ALL subtypes, cytogenetic abnormalities have become one of the most essential parts of risk-stratification in contemporary protocols.

High-hyperdiploidy is associated with a favourable prognosis, distinctly visible with EFS rates of more than 85%. [37,39]

A high sensitivity to chemotherapy, the predisposition to undergo spontaneous apoptosis and a high accumulation of methotrexate polyglutamates in blasts may explain the positive outcomes. [65]

Hypodiploidy on the other hand shows a worse outcome, although a recent inter-group study illustrated that patients with 44 chromosomes have better outcomes than patients with less chromosomes, suggesting that outcomes may not uniformly be poor. [66]

The translocation t(12;21) with the *TEL/AML1* rearrangement shows an extremely good outcome. It is associated with low initial WBC counts, a cALL phenotype with co-expression of myeloid markers, an improved early response to treatment and a favourable age. [64,67,68]

The Philadelphia chromosome positive ALL with the t(9;22) translocation and a *BCR/ABL* fusion transcript is associated with a highly unfavourable prognosis due to high WBC counts, CNS involvement and a poor prednisone response. Patients with this feature therefore often need an intensification of therapy and subsequent stem cell transplantation. [35,69,70]

11q23/MLL rearranged ALL with the t(4;11) translocation in infants seems to have the worst outcome. [61]

1.7.4. In-vitro resistance profile

The in-vitro resistance profiling of prednisone, vincristine and L-asparaginase, which tests the in-vitro drug sensitivity at primary diagnosis is used especially in the Cooperative ALL and the Dutch Childhood Leukaemia study group as a strong prognostic factor.[6]

1.7.5. Assessment of early response to treatment

Assessment of early response to treatment by a variety of methods, such as the so-called prednisone response (measured by the cell count in peripheral blood) as well as the reduction of blasts in the BM and the MRD are reliable predictors for treatment outcomes and amplified the possibilities of risk-stratification.

The most established and easiest method is the detection of the blast cell count in peripheral blood on day 8 of induction therapy, thus after a 7-day prednisone therapy and one intrathecal dose of methotrexate, given on day 1 – designated as the prednisone response. (6)

In the ALL-BFM 83 study patients with $\geq$ 1000 blasts/μl had a worse prognosis than patients with ≤ 1000 blasts/μl on day 8 of induction therapy, showing the prognostic value of prednisone response. (71)

Furthermore, trial ALL-BFM 90 reported that patients with $<$1000 blasts/μl at diagnosis do not have the edge over patients showing $>$1000 blasts/μl at initial diagnosis, being, however, good prednisone responders. (72)

Prednisone response has maintained to be a valuable prognostic marker and facilitated to divide patients in groups with good or rather adverse prognosis. It is, however, undefined whether patients with a very high initial WBC count, but a monumental cell reduction after 7 days, who show however still $\geq$ 1000 blasts on day 8, might be subjects of successive overtreatment. (6,35)

Additionally, prednisone response protruded as a significantly independent marker among unfavourable leukaemia subtypes, such as infant ALL and Philadelphia positive ALL, showing that genetic factors do not only determine individual prognosis, but response to chemotherapy also has a remarkable influence. (6,60,69)

Another auspicious method of assessment of early response to treatment includes the cytological analysis of the BM on day 7 and 14 of treatment. Three categories, defined as M1, M2 and M3, are being used to assess the BM. (73,74,75)

M1 is associated with signs of reviving haematopoesis and $<$ 5% of blasts persisting in the BM, whereas M2 shows 5-25% residual blasts and M3 a BM with $\geq$25% of residual blasts. M1 on day 7 and day 14 insinuates a good prognosis, whereas M2 and M3 on day 14 show a poorer outcome.

The BM-response-to-treatment-assessment on day 15 has become a fundamental part of ALL-BFM risk stratification. In earlier trials the BFM group was able to differ probable candidates for stem cell therapy by the usage of BM status on day 15. (6)

Overall, analysis of the prednisone response in PB and BM cytological evaluation have highly contributed in distinguishing a very high risk of relapse in patients, but are less suited to further subcategorise between high- and intermediate risk groups of relapse. [6]

Moreover, specificity (as well as sensitivity) of both methods is limited which becomes evident when considering that the majority of relapses occur in patients who are classified as good prednisone responders as well as equally likely in all three subtypes of BM risk stratification. [35,76]

Assessment of MRD was primarily used for risk-stratification in ALL-BFM 2000 and has emended the appraisal of early response to treatment on a submicroscopic level. [14,16]

Through semi-quantitative PCR, clone-specific rearrangements in T-cell receptor and immunoglobulin genes - which are considered leukaemia-specific - leukaemic cells that could lead to relapse are detected.

Especially the cost-efficient flow-cytometry enables a fast and relatively accurate detection of cells with a sensitivity of 10^{-3} to 10^{-4}. [6,45,46]

In the ALL-BFM 2000 study, MRD, next to prednisone response and genetic evaluation, was integrated as a valid and central item of risk-stratification and is ordained after 5 (day 33) and 12 weeks (day 78) of initiation of therapy. [6,35]

Thus, 3 different groups of patients that feature diverse risks of relapse could be identified through the thorough analysis of MRD at two quite early time points. [77]

Approximately one third of patients lack MRD at both instants and therefore are considered as standard-risk, encountering a chance of 90-95% to stay in permanent remission. [6]

On the other hand, patients with MRD$\geq 10^{-3}$ at time point two are categorised as high-risk patients with cure rates of 50-60%. [6]

The remaining two thirds of patients represent the intermediate-risk group, comprising MRD levels of $<10^{-3}$ at time point two and harbouring cure rates from 75-80%. [6]

Through enhancing the risk-evaluation of patients via MRD the ALL-BFM 2000 trial clearly showed that not only all three risk-groups could be definitely discriminated, but sub-groups of patients with highly favourable or severely inauspicious prognosis, being of clinical significance could also be defined. [45,46]

1.8. Treatment

Treatment of ALL in Austria follows the BFM-protocol since 1979, consisting of a four-component concept.[14]

The therapy approach is a multiagent polychemotherapy - solely intensified or alleviated, to meet the specific requirements of groups with high- or standard-risk of relapse.
The BFM-orientated approach comprises:
1. Remission induction and induction consolidation therapy, 2. treatment of the extracompartment, 3. late re-intensification/re-induction therapy and 4. continuation therapy. [16]

1.8.1. Remission induction therapy

The aim of remission induction therapy is to achieve remission, defined by the absence of blasts in the peripheral blood, the absence of clinical signs or symptoms of ALL and fewer than 5% of blasts in the BM. [6,14,16,36]
This is obtained by the usage of vincristine, L-asparaginase and a corticosteroid, as well as intrathecal methotrexate and daunorubicin and usually lasts 5 weeks. [6]
There is a controversial opinion about the choice of corticosteroid. Dexamethasone shows a possible stronger anti-leukaemic efficacy and a better infiltration of the CSF and therefore seems to achieve better EFS-rates than prednisone, however also has a higher toxicity in combination with the other remedies (vincristine, anthracyclines, L-ASP) used in induction therapy. [78,79,80]
A benefit in EFS rates and the prevention of CNS relapses when using prednisone instead of dexamethasone is however currently examined.[6] The divergent use of dosages (of prednisone and dexamethasone) as well as variable time points of appliance complicate a thorough assessment of possible advantages and disadvantages of the use of one or the other. [81]
The assessment of prednisone response takes place after a 7-day prephase, where prednisone and one intrathecal dose of methotrexate on day one is applied. This results in a “window”, which is adjuvant in avoiding early complications caused by tumour lysis syndrome. [6]
Through induction treatment over 95% of patients are able to achieve remission, whereas the remaining few succumb from disease- or treatment-related complications or are non-responders to therapy. [6]

1.8.2. Consolidation therapy

A subsequent 4-weeks early intensification phase, using cyclophosphamide, cytarabine, intrathecal methotrexate as well as oral 6-mercaptopurine follows the 33-days induction therapy regimen. [35]

The aim is to eradicate residual leukaemic blasts in order to improve the long-term outcome. Recent findings show a surprisingly beneficial effect due to the efficacy of this therapeutic part. [46]

1.8.3 Extracompartment therapy

Extracompartment therapy lasts 8 weeks and is especially targeted on CNS and the testicles, where remaining leukaemic cells are concealed and microscopically invisible. [6]
Hence, intrathecal and systemic methotrexate and oral 6-mercaptopurine are mainly responsible to further improve the quality of remission. Through the 1960ies and -70ies high-dosage prophylactic cranial radiotherapy was an essential element of extracompartment therapy due to its contribution to the major improvement of the overall results. [6]
However, the development of secondary malignancies, hormonal deficiencies and neurological as well as cognitive impairment as a sequela of cranial irradiation, made a replacement of cranial radiotherapy by a more CNS-directed chemotherapy inevitable. [82,83]
Omitting prophylactic cranial irradiation by intensified CNS-chemotherapy and intrathecal therapy was a tedious process:
In the ALL-BFM 81 trial an abolishment of remaining blasts in the testicles by intermediate-dosed methotrexate was indeed successful. It could not prevent CNS relapses, however. [84,85]

Through the introduction of four cycles high-dose methotrexate and a gradual reduction of cranial irradiation in trial ALL-BFM 86 and the following ALL-BFM studies, CNS relapses could be suitably avoided. [48,56,62]
In ALL-BFM 95 trial, patients with T-cell ALL and high-risk ALL, who were older than 12 months, were the remaining patients receiving prophylactic cranial radiotherapy. [86]
There seems to be a controversial discussion about the substitution of intensified intrathecal therapy for cranial irradiation in patients with T-cell ALL. An inter-group study, performed with intermediate-risk T-cell ALL patients, concluded that the replacement of irradiation by triple intrathecal chemotherapy (consisting of prednisone, cytarabine and methotrexate) did not show the expected results, but quite contrary was less successful in preventing CNS- and systemical relapses in patients, who were identical to the patients treated in trial ALL-BFM 90.[6]
On the other hand, a study conducted by the CCG suggests that a delayed intensification and an elaborated intrathecal treatment show an equal effectiveness in avoidance of CNS relapse.[6]

In summary, dose and duration of systemic chemotherapy are crucial to the effectiveness of the substitution of cranial irradiation in consolidation therapy. [6]

1.8.4. Re-intensification therapy

Re-induction therapy is basically a recapitulation of the induction therapy, using similar drugs as the latter and is applied 2-3 weeks after extracompartment therapy. [6]

Vincristine and L-asparaginase are given in equal dosages as in induction therapy, however, daunorubicin is substituted by doxorubicin and dexamethasone is preferred to prednisone. Introduction of re-induction-therapy has proven to be one of the most significant milestones of the ALL-treatment regimen, since it is of absolute value for a successful treatment and fundamentally improves EFS-rates. [6,35]

In ALL-BFM trial 1976, high-risk patients were treated with a delayed re-intensification therapy, showing 10-year event-free survival rates of 70%, whereas patients who were given intensification therapy immediately after induction showed an EFS of 60% and patients without any intensification element at all merely performed with EFS rates of 38%. [87]

The significant benefit of re-intensification treatment was particularly obvious when in an attempt to prevent treatment-related noxiousness, low-risk patients in ALL-BFM trial 83 were randomised in receiving re-intensification therapy or not. [6,71]

Both groups had a similarly low rate of isolated CNS relapses, patients who did not receive re-intensification therapy, however, suffered of a high number of combined CNS and BM relapses as well as isolated systemic relapses. [6,71]

Furthermore, ALL-BFM 86 trial suggested alike results when omitting delayed re-intensification therapy in standard-risk patients. [56]

This led to the conclusion of the eminent value of re-induction treatment in order to achieve high cure rates and decrease the number of relapses.

1.8.5. Maintenance therapy

Maintenance therapy helps to maintain remission by targeting at remaining leukaemic cells and meanwhile preserving the BM. The primarily used drugs are daily oral 6-mercaptopurine and weekly oral methotrexate.[6] An additional use of monthly vincristine pulses together with dexamethasone failed to show an increase in maintenance of remission, when attempted in a randomised study of the BFM-study group. [88]

Adequate dosages of 6-mercaptopurine and methotrexate, which might however increase liver-toxicity, are irrevocable to achieve a significant therapeutic effect. [89]

There is general agreement on the duration of maintenance treatment, which is up to two years, since more relapses occured when attempting to reduce the duration in several trials. [48]

1.8.6. Supportive care

A substantial contribution to the increasing cure rates of the last twenty years is also due to the continuous amendment of a risk-adapted prophylactic supportive care.[14] Supportive care during treatment is targeted at complications arising due to the treatment regimen, whose occurrence depend on diagnosis, phase of treatment and the patient's constitutional shape.[14,35]

Furthermore, supportive care intents to improve the patient's well-being and aims to adequately treat and prevent side-effects of the anti-leukaemic therapy (infection, nausea, pain etc.).

1.8.7. Haematopoietic stem cell transplantation (HSCT)

For high-risk ALL patients with poor response to treatment, stem cell transplantation is a generally approved option. There are clearly defined indications for HSCT that need to be respected in order to qualify for a successful transplantation. [35]

The donation should be made by an HLA-identical sibling preferably, however, improvements in HLA-typing, improvements of supportive care and the development of effective measures to select stem cells allowed the donation from HLA-matched unrelated donors, since nearly 75% of patients fail to find a matched sibling. [6,35]

According to a protocol, introduced by the BFM group in the mid-90ies, the following indications in newly diagnosed childhood ALL qualify as indications for stem cell transplantation from an HLA-identical sibling[35]:

- Philadelphia-chromosome positive ALL
- t(4;11)
- Failure of remission in the BM after induction-therapy
- Prednisone Poor Response (PPR) and one or more of the below:
    - T-cell ALL
    - Pro-B ALL
    - Leukocyte count > 100.000/µl

In the ALL-SCT BFM 2003 study patients with either the combination of poor prednisone response and the morphologic criteria M3 in the BM on day 15 of protocol I or a high level of MRD ($\geq 10^{-3}$) after 12 weeks of therapy were also considered as candidates for an allogenic HSCT. [35]

Considering the recipient of HSCT, a conditioning regimen, which is adapted to the patient's age, needs to be performed: Patients, who are older than 24 months receive fractioned total-body irradiation (fTBI) and etoposide. Patients younger than 24 months receive busulfan, cyclophosphamide and etoposide. [90,91]

In both cases antithymocyteglobuline (ATG) is given additionally if the donor is not related.[90]

Immunosuppression is generally applied through ciclosporin A and additional MTX, in case of unrelated donors.[35]

For relapsed ALL patients with a poor risk all available donors are suitable for transplantation, except autologous grafts, which are only suggested for treatment of patients with isolated CNS involvement.[6]

Ideally, HSCT is performed during the consolidation-therapy phase of treatment - approximately 3 months past induction therapy.[35]

Studies showed a definite improvement in preventing relapses due to HSCT. [92,93]

However, an improved outcome of high-risk ALL patients treated with chemotherapy and the achievement of being able to refine risk-stratification with MRD-monitoring resulted in a cutback of indications for HSCT in the ALL-BFM 2000 and 2009 trials.[46]

1.8.8. Treatment failure and relapses

Despite constant improvements in ALL treatment, 15-20 percent of patients suffer from a relapse, constituting one of the most common malignancies in childhood. [14,16] Furthermore, relapse after adequate chemotherapy is the most common factor of treatment failure in childhood ALL, whereas resistance to induction therapy, secondary neoplasia and severe adverse events account for the less frequent incidences. [6]

There are several features that influence the patient's prognosis and have an impact in relapse treatment, such as the duration of first remission, relapse site, age at diagnosis, immunophenotype and cytogenetics, gender and WBC or blast cell count.[94]

Since the acquisition of second remission depends distinctly on whether the patient relapses during or after frontline therapy, the BFM group differs between very early ($<$18months after initial diagnosis), early (18-30 months after initial diagnosis) and late ($\geq$6months after the end of frontline therapy) relapses.[94]

Moreover, site of relapse sustained as an important prognostic factor in relapse ALL.

Isolated BM relapse, for instants, only shows a 10-year EFS of 15% and combined BM relapse a 10-year EFS of 34%, when compared to extramedullary relapses with a 10-year EFS of 44%. The latter shows the best prognosis when not associated with a systemic relapse. [6]

Finally, T-cell phenotypes and translocations t(9;22) and t(4;11) present with less favourable outcomes in relapse as well. Good risk features imply the *TEL/AML1* rearrangement and low or undetectable MRD levels after relapse induction therapy. [6]

Treatment of relapses in Austria follows the ALL-REZ BFM guidelines, where patients are divided in individual risk groups regarding the stratification of risk features as seen in table 4 below.[6]

Re-induction, consolidation and maintenance chemotherapy, including prophylactic intrathecal chemotherapy with anti-leukaemic drugs that have already been used in first-line therapy are the main elements of treatment of relapses.

Additionally, cranial irradiation (18Gy) is performed on patients with CNS relapse.[6]

Patients with a peculiarly high risk for adjacent relapse are included in a myeloablative conditioning-regimen and ultimately receive stem cell transplantation.

40-50% of relapse patients stand the chance of permanent remission through this treatment regimen. [14,16,36]

Table 4 Risk groups for relapsed childhood ALL[6]

Therapy group	Patients	pEFS
S1	all patients with late extramedullary relapse	77%
S2	all patients with very early and early extramedullary relapse, all combined (early and late) non-T-ALL relapses, and all late non-T-ALL BM relapses	35%
S3	all patients with early isolated non-T-ALL BM relapses	4%
S4	all patients with very early combined and isolated BM relapses, and all patients with T-cell ALL BM relapses	0%

Abbreviations: pEFS: probability of event-free survival; BM: bone marrow

2. ALL-BFM 2000 trial in Austria

2.1. Introduction

Since the 1970ies the treatment of ALL has been constantly re-evaluated and subsequently improved, leading to remarkably high long-term remission rates of nearly 80%.

Nonetheless, the remaining 20% of patients ultimately relapse and face an inferior chance of second complete remission, namely (approximately) 25 to 40%. [14,16,36]

Through the constant amendment of risk-stratification of ALL treatment strategies, the methods currently in use were indeed able to provide information to subcategorise patients in groups of significantly high- or low-risk of relapse; however, failed to distinguish a group of patients that face an intermediate risk of relapse, who would be presently overtreated in the high-risk treatment regimen.[6] Overtreatment ultimately leads to a higher number of acute- and long-term side effects due to treatment toxicity.

By assessing the patient's response to treatment - which proofed to be one of the most significant prognostic factors - together with the development of various techniques to assess prognostic molecular markers, therapy stratification has been continually refined over the past few years.[45]

This led to the introduction of MRD, a concept that has been integrated in the ALL-BFM 2000 study as a primary factor for risk stratification for the first time.

PCR-Monitoring of MRD is based on detecting clone-specific rearrangements in T-cell-receptor and immunoglobulin genes as semi-quantitative PCR targets, that way persisting leukaemic cells that eventually lead to relapse can be detected.[6,35]

Groups collaborating in the International BFM-study group pioneered the assessment of MRD at two distinct time points: on day 33 and 78 of treatment by 2 clonal TCR/Ig-markers with a sensitivity of at least 10^{-4}. [6,35,45]

The introduction of MRD-adapted stratification has enhanced the in-vivo acquisition of treatment effectiveness and redefined and questioned the prognostic value of all former prognostic factors and the definition of "complete remission".[45,46]

2.2. Patients and Methods

2.2.1 Patients

Between June 1999 and December 2009, 610 children and adolescents up to the age of 18 years that have been diagnosed with either pB-cell or T-cell ALL, were enrolled in the ALL-BFM 2000 trial in Austria. 521 patients were diagnosed with pB-cell ALL and 86 patients were classified with T-cell ALL. In two patients substantial evidence of their immunological affiliation could not be detected and one was classified as NK-cell ALL.

The patient's age ranged from 1 to 18 years, since infants (children <1 year of age) were not included in this investigation.

Patients with *BCR/ABL1* or t(9;22) positive ALL, also known as Philadelphia Chromosome positive ALL have been excluded from this investigation.

Data of all patients has been provided by all attending study sites in Austria and has been recorded and reviewed by the National Study Center in Vienna, namely the St. Anna Children's Hospital.

ALL in patients has been diagnosed cytomorphologically and immunologically according to the international guidelines.

Informed consent has been procured by the patient's parents, the legal guardians or patients themselves and followed the guidelines of the Declaration of Helsinki, informing the patient's and their relatives about the treatment and possible adverse effects.

Stratification of patients followed the BFM study criteria; hence the applied treatment followed the guidelines determined in the ALL-BFM 2000 study protocol.

In the ALL-BFM 2000 protocol patients were primarily stratified due to their MRD levels on days 33 and 78 of treatment. Patients were classified as MRD low-risk, intermediate-risk or high-risk according to their MRD results, as described below.

Patients having been either prednisone poor responders, featuring the translocation t(4;11) or not having achieved complete remission (CR) on day 33 (as well as *BCR-ABL1* positive patients, who were excluded from this investigation, however) were treated in the high-risk arm impartial of their MRD results.

2.2.2. Diagnostic studies

ALL was diagnosed cytomorphologically according to the FAB-criteria and through the presence of more than 25% of lymhoblastic cells in the BM. Based on the guidelines of the European Group for Immunological Characterization of Leukemias, flow cytometric immunophenotyping was performed in order to further classify leukaemic patients as common ALL, pre-B ALL, pro-B ALL and T-cell ALL. The presence of genetic variants was screened based on the use of polymerase-chain-reaction and fluorescent in situ hybridization, targeted at rearrangements, such as translocations t(4;11) and t(9;22).
Furthermore, CSF samples of every patient were taken on the day of initial diagnosis as described above in order to figure CNS involvement.

2.2.3. MRD analysis

Monitoring of MRD was mainly performed through detecting clone-specific immunoglobulin (Ig) and T-cell-receptor gene rearrangements. DNA samples obtained at diagnosis were being screened for those rearrangements via PCR amplification using specifically designed primer sets.
Basically, patient-specific junctional region sequences, that had the potential to qualify as PCR-MRD targets were determined. Then, each target was provided with an allele-specific oligonucleotide primer that complemented its junctional region sequence. [45,46]
Those targets were then tested for specificity and sensitivity in order to be able to identify 2 targets with a sensitivity of at least 10^{-4} and were detected and analysed through quantitative reverse-transcription-PCR on as early as day 33 and 78 of the treatment regimen.
Guidelines, that provide assistance in interpreting MRD results, have been generated from the European Study Group for MRD detection in ALL (ESG-MRD ALL) and were followed in order to analyse the MRD levels.[45,46]

2.2.4. Risk group definitions

Patients were assigned to one of three risk-groups, comprising different risks of relapse and different EFS rates; according to criteria used in previous ALL-BFM study protocols on the one hand and to the newly introduced MRD risk-stratification on the other hand.
MRD standard-risk or low-risk was defined as being MRD negative at both time points (day 33 and 78 of treatment), intermediate-risk patients performed with positive MRD levels at both time points, however, having MRD results of less than 10^{-3} on day 78 and high-risk was considered as having MRD levels of 10^{-3} or more at both time points. [45,46]

Patients being either prednisone poor responders, thus showing ≥ 1000 blasts/µl in peripheral blood on day 8 of induction phase treatment or failed to achieve remission on day 33 of treatment – defined as > 5% of leukaemic cells in the BM or persistent extramedullary disease – were classified as high-risk and treated in the high-risk arm regardless of their MRD results.[45,46]

2.2.5 Statistical methods

The Kaplan-Meier method was used to attain EFS and overall survival (OS) rates

EFS was defined as the time from diagnosis to the first adverse event or to the date of last follow-up; whereas adverse events were defined as the failure to achieve remission (early death, refractory disease), a relapse at any site, death during CR or the development of a second malignancy. Patients who failed to achieve CR were assigned to a failure time of zero.

As for OS, which was defined as the time from diagnosis to death from any cause; and for surviving patients was censored at the date of last follow-up. A Cox model was used for multivariate analysis of prognostic markers for EFS.

Survival curves were compared by means of the log-rank test.

P-values < 0,05 were considered as statistically relevant.

2.3. Results

2.3.1. Initial parameters of all ALL-BFM 2000 trialed patients

In 608 patients of 610 patients, who were enrolled in the ALL-BFM 2000 trial in Austria, consolidated evidence of an immunophenotypic assignment could be detected. As mentioned above 521 patients were classified as pB-cell ALL and 86 patients were classified as T-cell ALL.

In patients with pB-cell ALL the highly preferable translocation t(12;21), also known as *TEL/AML 1* was detected in 144 (28%) patients and hyperdiploidy was found in 163 patients (31%).

On the other hand, the adverse translocation t(4;11) was detectable in 7 (1%) of pB-cell ALL patients, instantly qualifying them for the high-risk arm treatment regimen. The mean age in T- and pB-cell ALL patients was under 10 years. CNS involvement occurred in 9 (11%) T-cell ALL and 11 (2%) pB-cell ALL patients.

Considering response to treatment, the majority of patients – namely approximately two thirds of all T-cell ALL patients and about 94% of all pB-cell ALL patients - were good prednisone responders on day 8 of induction treatment.

81 (93%) of T-cell ALL patients achieved CR on day 33 and 505 (97%) patients in pB-cell ALL achieved CR on day 33. 7 deaths overall occurred before day 33 (pB-cell ALL: n=6; T-cell ALL: n=1).

Ultimately, patients were stratified to one of three definite risk groups - the majority of patients were assessed as intermediate-risk (60%, n=365), 25% (n=152) were considered as standard-risk patients and the remaining 13% (n=81) were stratified as patients with a high risk of relapse. 10 patients (representing about 2% of all patients) died before stratification (pB-cell ALL: n=8; T-cell ALL: n=2).

The patient, harbouring the NK-cell ALL was treated with the T-cell ALL patients in the intermediate risk arm and considered as a T-cell ALL patient in statistical processing.

Regarding the two patients without substantial evidence of an immunological attribution, one died a day after diagnosis and the other one was successfully treated in the intermediate-risk group.

Table 10 gives an overview of the initial clinical and laboratory characteristics of patients and table 11 illustrates the corresponding early response to treatment.

Table 5 Initial clinical and laboratory parameters of the 608 ALL patients of the ALL-BFM 2000 trial

Total	T-cell ALL (%)	pB-cell ALL
608	**87 (14)**	**521 (86)**
Gender		
Male	58 (67)	284 (55)
Female	29 (33)	237 (46)
Age (years)		
mean	**9,86**	**6,59**
range	**1,43-17,98**	**1,08-18,42**
< 10	44 (51)	405 (78)
≥ 10	43 (49)	116 (22)
WBC count/µl		
< 20.000	25 (29)	375 (72)
≥ 20.000	62 (71)	146 (28)
< 50.000	38 (44)	457 (88)
≥ 50.000	49 (56)	64 (12)
TEL/AML1	**0**	**144 (28)**
MLL/AF4	**0**	**7 (1)**
E2A/PBX1	**0**	**17 (3)**
High-Hyperdiploidy	**1 (1)**	**163 (31)**
FAB		
FAB L1	65 (75)	447 (86)
FAB L2	17 (20)	55 (11)
FAB L1/L2	1 (1)	3 (1)
Not available	4 (5)	16 (3)
CNS status		
Positive	9 (11)	11 (2)
Negative	73 (84)	503 (97)
Not available	5 (6)	7 (1)

Abbreviations: WBC: White blood cell; FAB: French-American-British classification of ALL; CNS: central nervous system

Table 6 Early response to treatment and risk group allocation of the 608 patients of the ALL-BFM 2000 trial

Total	T-cell ALL (%)	pB-cell ALL (%)
608	**87 (14)**	**521 (86)**
Prednisone Response		
Good PR	54 (62)	492 (94)
Poor PR	31 (36)	28 (5)
Not available	2 (2)	1 (0,2)
BM status on day 15		
M1 (< 5%)	34 (39)	309 (59)
M2 (5% ≥ and < 25%)	29 (33)	149 (29)
M3 (≥ 25%)	24 (28)	54 (10)
Not available	0 (0)	9 (2)
Remission on day 33	**81 (93)**	**505 (97)**
Not in remission	5 (6)	10 (2)
Death before remission	1 (1)	6 (1)
Definitive risk group		
SR	**13 (15)**	**139 (27)**
IR	**38 (44)**	**327 (63)**
HR	**34 (39)**	**47 (9)**
Death before stratification	2 (2)	8 (2)
MRD risk group		
LR	**14 (16)**	**142 (27)**
IR	**52 (60)**	**335 (64)**
HR	**12 (14)**	**18 (3)**
Not available	9 (11)	26 (5)

Abbreviations: PR: Prednisone Response; BM: Bone marrow; SR: Standard risk; IR: Intermediate risk; HR: High risk; MRD: minimal residual disease; LR: Low risk

2.3.3. Occurrence of adverse events in patients of the ALL-BFM 2000 study

Regarding the incidence and characteristics of relapses in pB-cell and T-cell ALL patients in the ALL-BFM 2000 study, 8 T-cell ALL patients (9%) and 56 pB-cell patients (11%) suffered from relapse.

When examining the onset of relapse, the majority, namely nearly two thirds (57%, n=32) of relapses in pB-cell ALL were late - defined by the occurrence beyond 6 months after cessation of frontline therapy, as mentioned before. Moreover, 27% of patients (n=15) experienced relapse with an early onset, which occurs beyond 18 months after initial diagnosis, up to 6 months after cessation of frontline therapy however.

Nine patients suffered from relapse with a very early onset - the appearance of relapse within 18 months of initial diagnosis by definition - representing 16% of all relapses in pB-cell ALL.

In T-cell ALL, 50% of patients (n=4) suffered from relapse with a very early onset and the remaining 50% (n=4) experienced late relapse.

The most frequent site of relapse was the BM, whereas 41 out of 56 relapses (73%) in pB-cell ALL patients and 6 out of 8 relapses (75%) in T-cell ALL patients were BM isolated. Furthermore, 8 pB-cell ALL patients featured a combined BM relapse, representing 14,3% of all relapses.

4 (7%) pB-cell ALL patients and 1 (13%) T-cell ALL patients suffered from extramedullary CNS relapse. Other extramedullary relapses occurred in 3 (5%) pB-cell ALL patients (testicles) and 1 (13%) T-cell ALL patient (bone).

Death occurred in 49 (8%) patients overall, whilst 36 (7%) of them happened in pB-cell ALL patients and 13 (15%) appeared in patients with T-cell ALL.

Table 7 Characteristics of adverse events of all 608 patients of the ALL-BFM 2000 trial

Total	T-cell ALL (%)	pB-cell ALL (%)
608	**87 (14)**	**521 (86)**
Relapses	**8 (9)**	**56 (11)**
Isolated BM	6 (75)	41 (73)
Combined BM		8 (14)
Isolated CNS	1 (13)	4 (7)
Isolated extramed. - others	1 (bone) (13)	3 (testicles) (5)
Very early	4 (50)	9 (16)
Early	0	15 (27)
Late	4 (50)	32 (57)
Death	**13 (15)**	**36 (7)**
Death without relapse or SMN	**6 (46)**	**14 (39)**
Before 1st CR	1 (17)	6 (43)
In 1st CR	5 (83)	8 (57)
After relapse	**7 (54)**	**20 (56)**
After SMN	**0 (0)**	**2 (6)**
SMN	**1 (1)**	**8 (2)**

Abbreviations: BM: Bone marrow; CNS: central nervous system; Extramed.: Extramedullary; CR: Complete remission; ALL: acute lymphoblastic leukaemia; SMN: Second malignant neoplasm

2.3.4. Outcome of all patients of the ALL-BFM 2000 trial

When evaluating the EFS and OS rates after 10 years of all patients being trialed in the ALL-BFM 2000 study, the findings were as follows: The EFS of 521 pB-cell ALL patients was 83±2% and their OS was 92±1%, respectively. The EFS of 87 T-cell ALL patients was 79±6% and their OS was 82±5%, respectively. The Figures 1, 2, 3 and 4 exhibit the EFS and OS of all patients of the ALL-BFM 2000 trial.

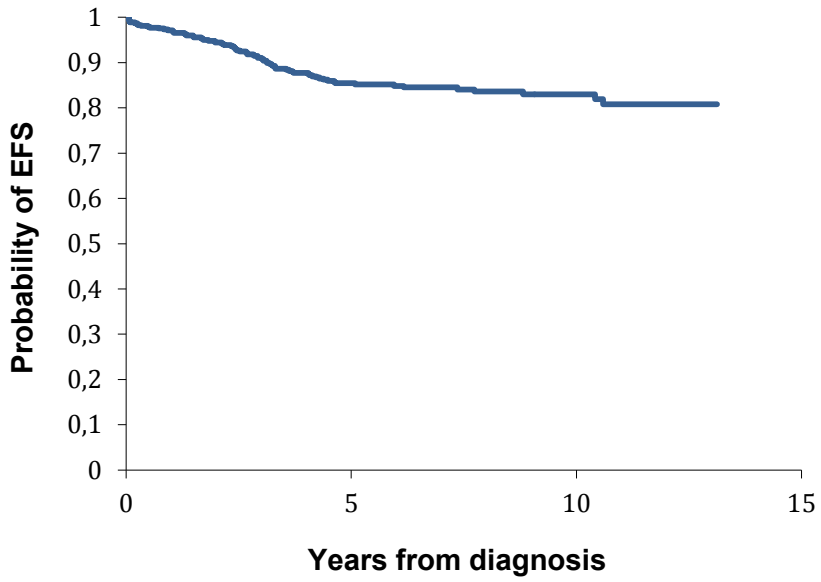

Figure 1 10-year EFS of all pB-cell ALL patients of the ALL-BFM 2000 trial

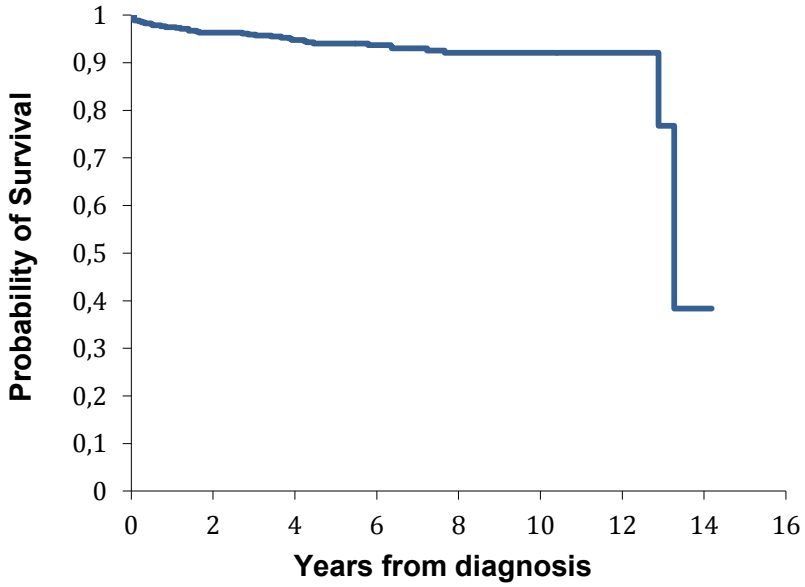

Figure 2 10-year OS of all pB-cell ALL patients of the ALL-BFM 2000 trial

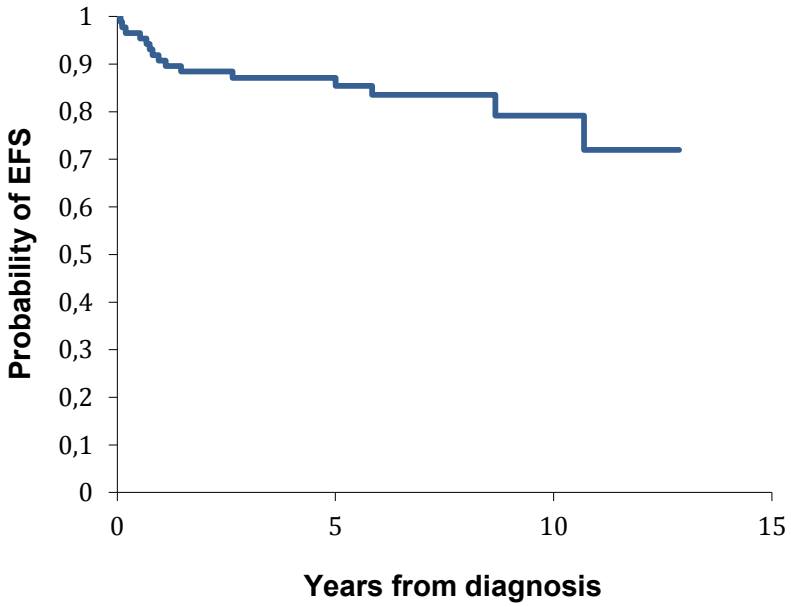

Figure 3 **10-year EFS of all T-cell ALL patients of the ALL-BFM 2000 trial**

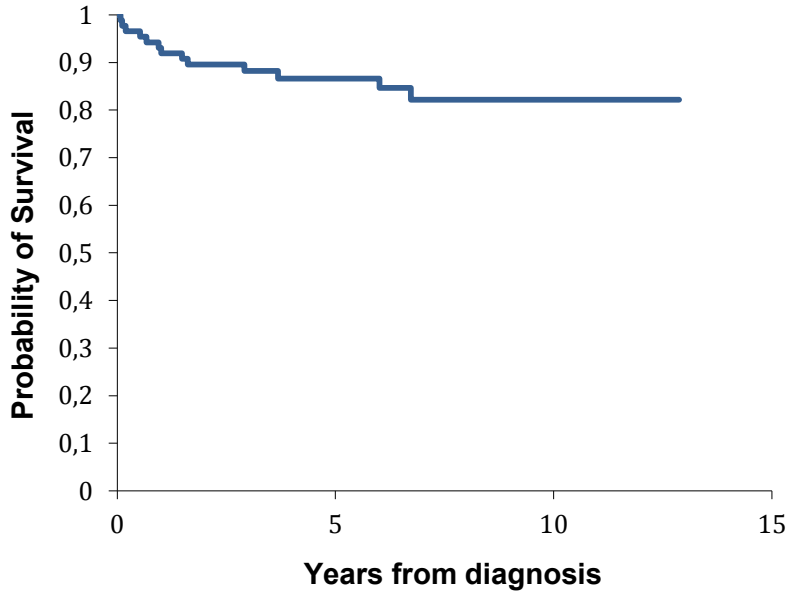

Figure 4 **10-year OS of all T-cell ALL patients of the ALL-BFM 2000 trial**

2.3.5. Outcome in patients according to gender

A significant difference in outcome according to gender could not be verified either in patients with pB- or in patients with T-cell ALL in this study. Male pB-cell ALL patients (n=284, 55%) performed with a 10-year EFS of 82±3% and female pB-cell ALL patients (n=237, 46%) performed with 10-year EFS rates of 84±3%. In T-cell ALL, male patients (n=58, 67%) performed with 10-year EFS of 78±8% and female patients (n=29, 33%) performed with 10-year EFS rates of 82±7%.

Figures 5 and 6 illustrate the EFS of patients according to gender.

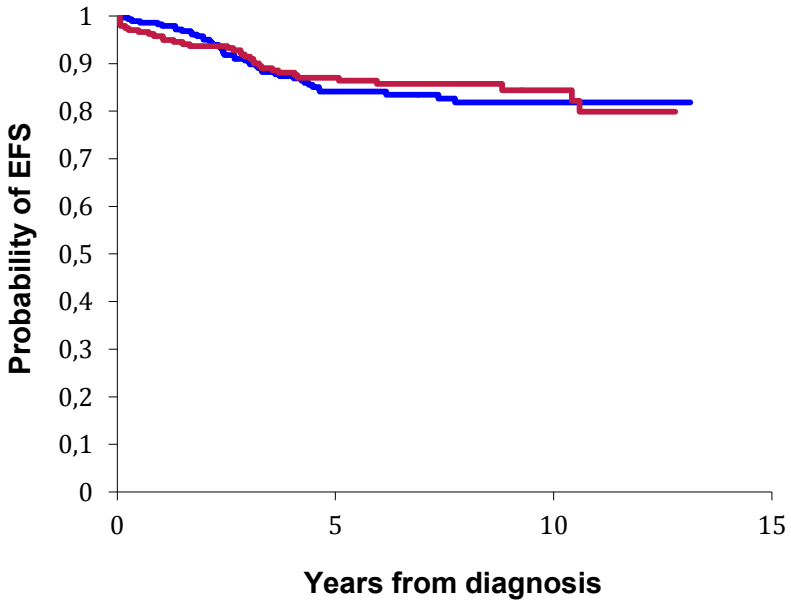

Figure 5 **10-year EFS according to gender in pB-cell ALL patients (P=0,692)**

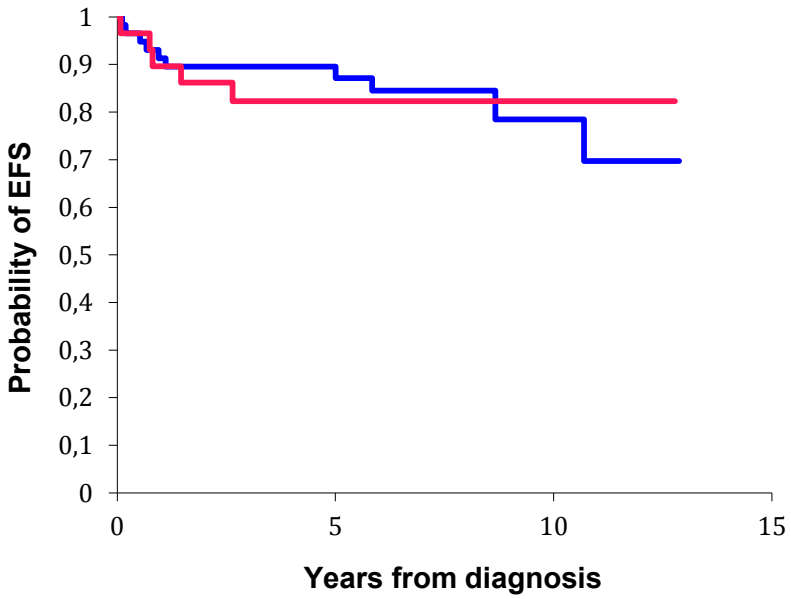

Figure 6 **10-year EFS according to gender in T-cell ALL patients (P=0,852)**
– males – females

2.3.6. Outcome in patients according to age

A statistical difference when observing outcome according to age at initial diagnosis was distinct. In pB-cell ALL patients, being between one and ten years of age a better outcome was observable; in T-cell ALL patients however, age at diagnosis failed to show a distinct statistical difference.

Precursor B-cell ALL patients who were between 1 and 10 years of age (n=405, 78%) performed with a 10-year EFS of 85±2% and patients with ≥ 10 years of age (n=116, 22%) performed with a 10-year EFS of 75±4%.

T-cell ALL patients who were between 1 and 10 years of age (n=44, 51%) had a 10-year EFS of 80±7% and patients with ≥ 10 years of age (n=43, 49%) had a 10-year EFS of 72±14%.

Figures 7 and 8 illustrate the EFS of patients according to age.

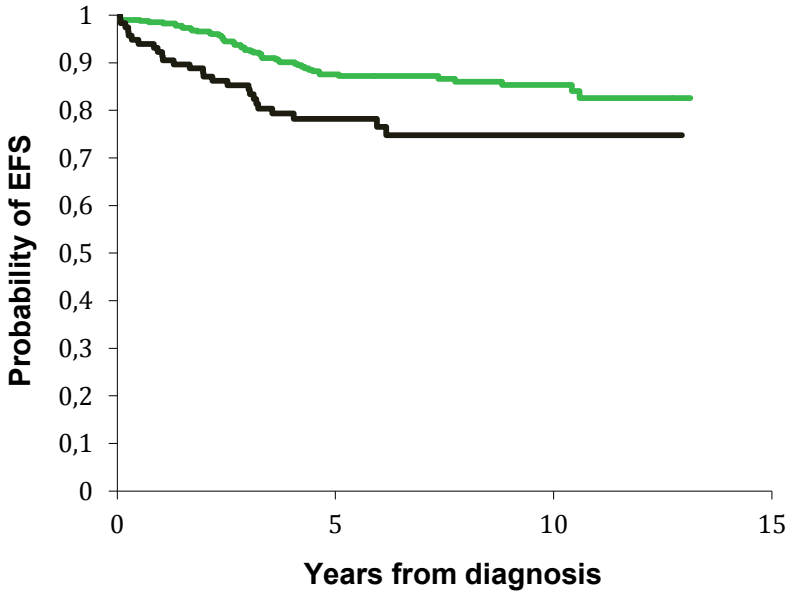

Figure 7 **10-year EFS of pB-cell ALL patients < vs. ≥ 10 years of age (P=0,004)**
≥ 1-10 years ≤ 10 years

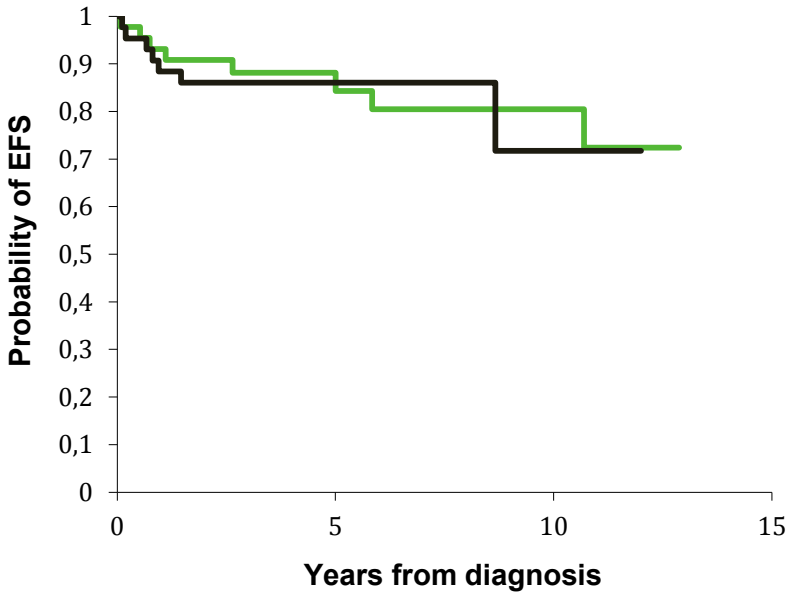

Figure 8 **10-year EFS of T-cell ALL patients < vs. ≥ 10 years of age (P=0,970)**
≤ 1-10 years ≥ 10 years

2.3.7. Outcome in patients according to leukocyte count

Patients have been categorised by their initial leukocyte count and divided in four groups, depending if they had WBC counts of either < or ≥ 20.000/µl and either < or ≥ 50.000/µl.

In pB-cell ALL, patients with a WBC count of < 20.000/µl (n=375, 72%) had a 10-year EFS of 85±2% and patients with a WBC count of ≥ 20.000/µl (n=146, 28%) had a 10-year EFS of 79±4%.

Patients with pB-cell ALL and a WBC coumt of < 50.000/µl (n=457, 88%) performed with a 10-year EFS of 84±2% and patients with a WBC count of ≥ 50.000/µl (n=64, 12%) performed with a 10-year EFS of 75±7%.

Regarding T-cell ALL, patients with a leukocyte count of < 20.000/µl (n=25, 29%) had a 10-year EFS of 79±9%, and patients with a leukocyte count of ≥ 20.000/µl (n=62, 71%) had a 10-year EFS of 81±7%.

Patients with T-cell ALL and a WBC count of < 50.000/µl (n=38, 44%) performed with a 10-year EFS rate of 79±7% and patients with a WBC count of ≥ 50.000/µl (n=49, 56%) performed with a 10-year EFS rate of 81±8%. A statistically relevant difference between leukocyte counts at initial diagnosis and outcome could not be consistently detected, however.

The figures 9, 10, 11 and 12 below show the EFS rates of patients with different leukocyte counts.

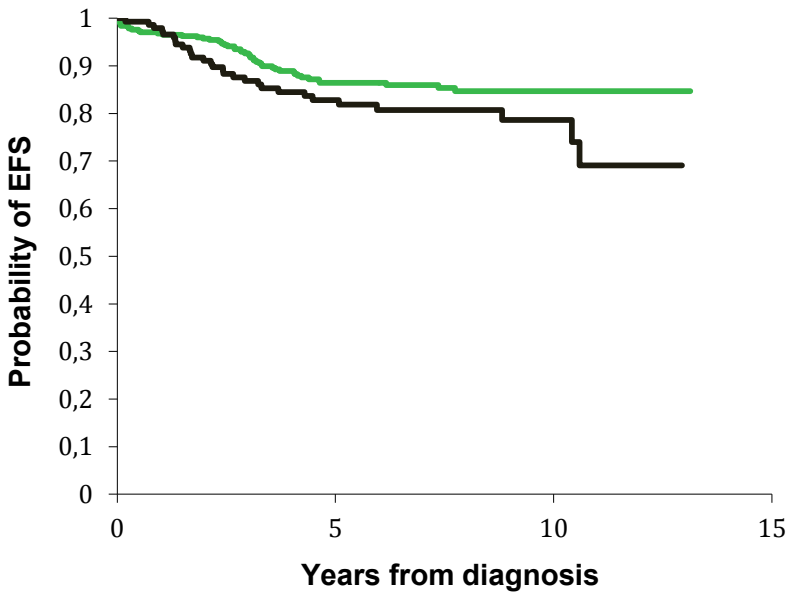

Figure 9 **10-year EFS of pB-cell ALL patients with an initial leukocyte count of < vs. ≥ 20.000/µl (P=0,055)**

< 20.000/µl ≥ 20.000/µl

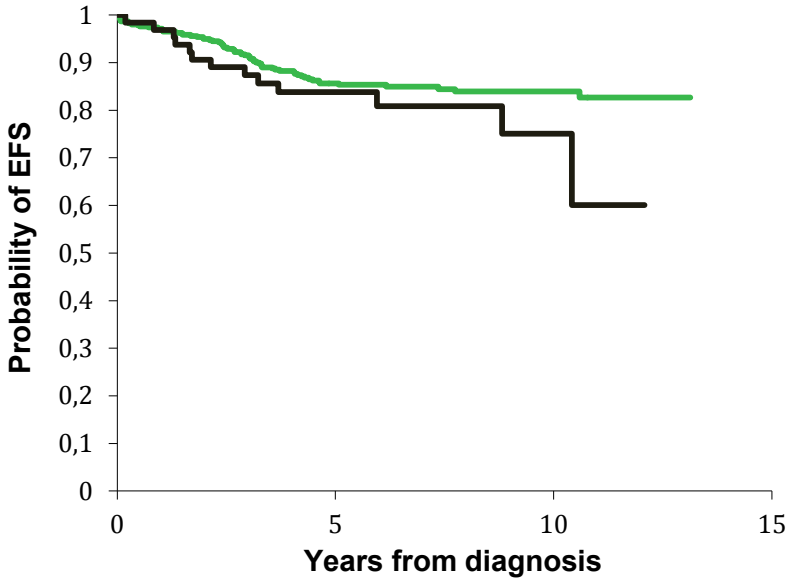

Figure 10 10-year EFS of pB-cell ALL patients with an initial leukocyte count of < vs. ≥ 50.000/µl (P=0,195)

– < 50.000/µl – ≥ 50.000/µl

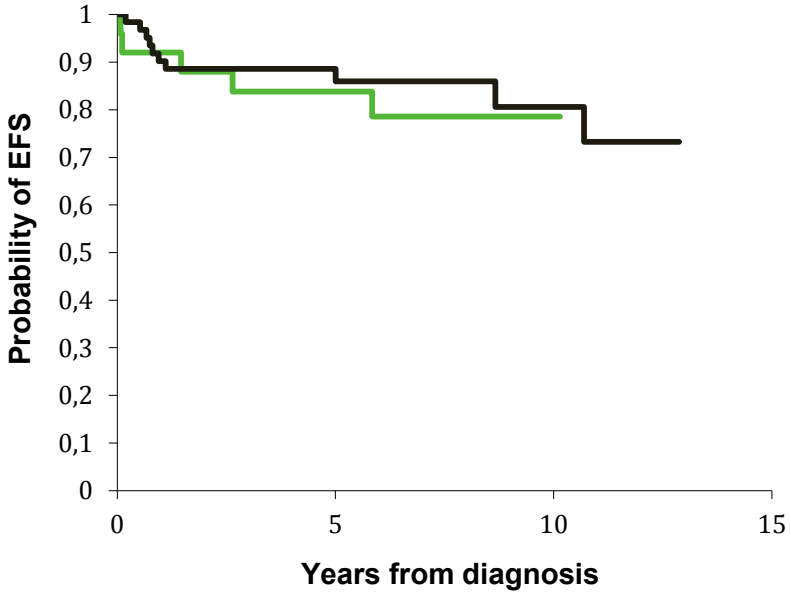

Figure 11 10-year EFS of T-cell ALL patients with an initial leukocyte count of < vs. ≥ 20.000/µl (P=0,554)

– < 20.000/µl – ≥ 20.000/µl

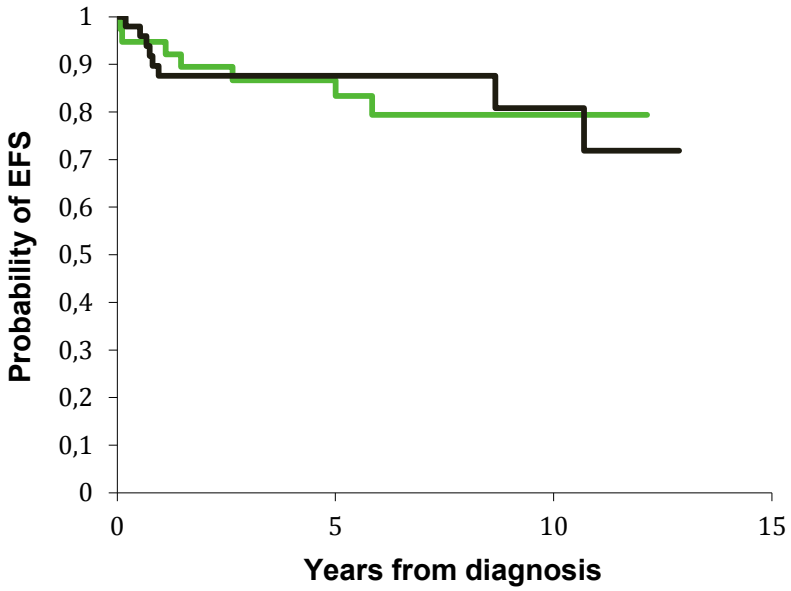

Figure 12 **10-year EFS of T-cell ALL patients with an initial leukocyte count of < vs. ≥ 50.000/µl (P=0,732)**

– < 50.000/µl – ≥ 50.000/µl

2.3.8 Outcome in patients according to the CNS status

11 (2%) pB-cell ALL patients and 9 (11%) T-cell ALL patients presented with a positive CNS status at initial diagnosis. In pB-cell ALL, patients with a positive CNS status had a 10-year EFS of 90±9% and patients with a negative CNS status (n=503, 97%) had a 10-year EFS of 83±2%.

In T-cell ALL patients, patients with a positive CNS status performed with a 10-year EFS of 89±10% and patients with a negative CNS (n=73, 84%) performed with a 10-year EFS of 82±5.

In 7 (1%) pB-cell ALL patients and 5 (6%) T-cell ALL patients the CNS status was not available.

No statistical relevance of CNS involvement on the patient's outcome could be observed.

Figures 13 and 14 reveal the association between CNS status and outcome.

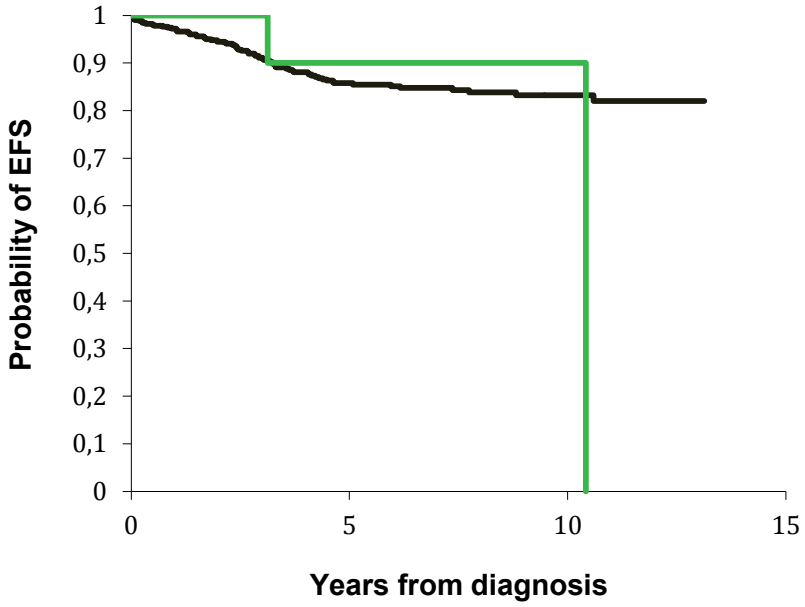

Figure 13 10-year EFS according to CNS status in pB-cell ALL patients (P=0,805)
CNS status: positive negative

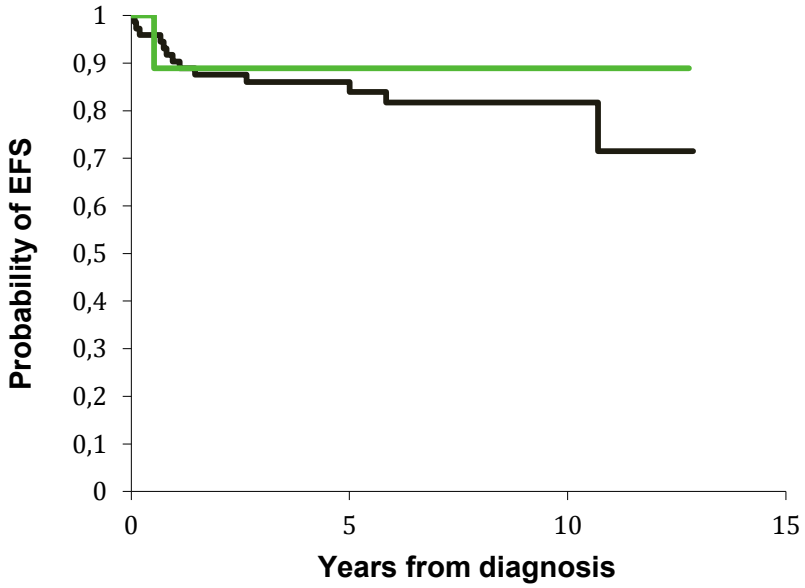

Figure 14 10-year EFS according to CNS status in T-cell ALL patients (P=0,600)
CNS status: positive negative

2.3.9. Outcome according to immunophenotypic characteristics

We evaluated the 10-year EFS of certain pB-cell subtypes and of T-cell ALL patients with different stages of maturity.

Patients with cALL (n=328, 63%) and pre-B ALL (n=148, 28%) were analysed according to their EFS rates. Patients with cALL performed with a 10-year EFS of 83±2% and patients with pre-B ALL performed with a 10-year EFS of 84±3%, respectively. A better outcome due to a certain subtype was not observable. The 21 (4%) pro-B ALL patients and the remaining 24 (5%) patients with other pB-cell ALL subtypes were not included in the outcome analysis.

T-cell ALL patients were divided into categories of early T-ALL (n=30, 34%), intermediate T-ALL (n=38, 44%) and mature T-ALL (n=15, 17%). Early T-cell ALL patients performed with a 10-year EFS of 71±12%, intermediate T-cell ALL patients with a 10-year EFS of 89±5% and mature T-ALL patients with a 10-year EFS of 86±9%, respectively. Four patients (T-cell ALL not further specified: n=3, 3%; NK-cell ALL: n=1, 1%) were not included in the outcome analysis.

The figures 15 and 16 show the outcome of patients according to immunophenotypic characteristics.

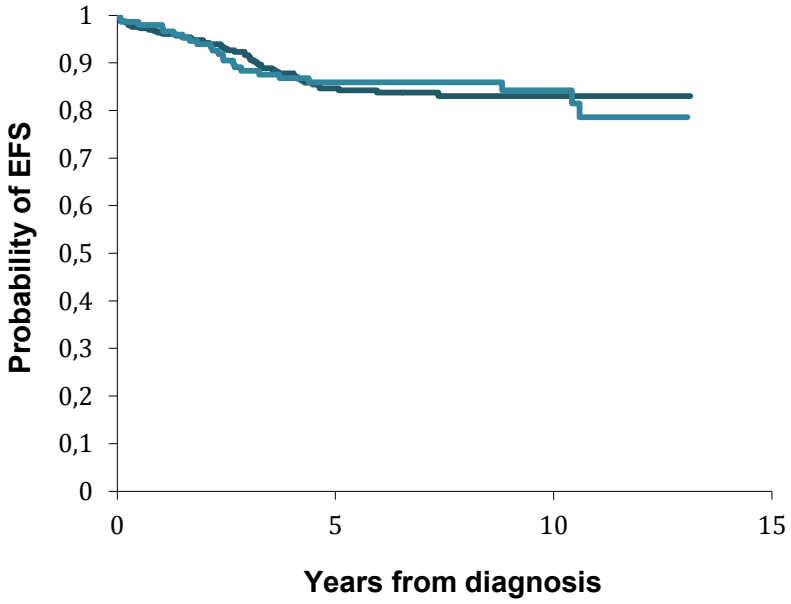

Figure 15 10-year EFS of patients with cALL and pre-B ALL (P=0,915)
cALL pre-B ALL

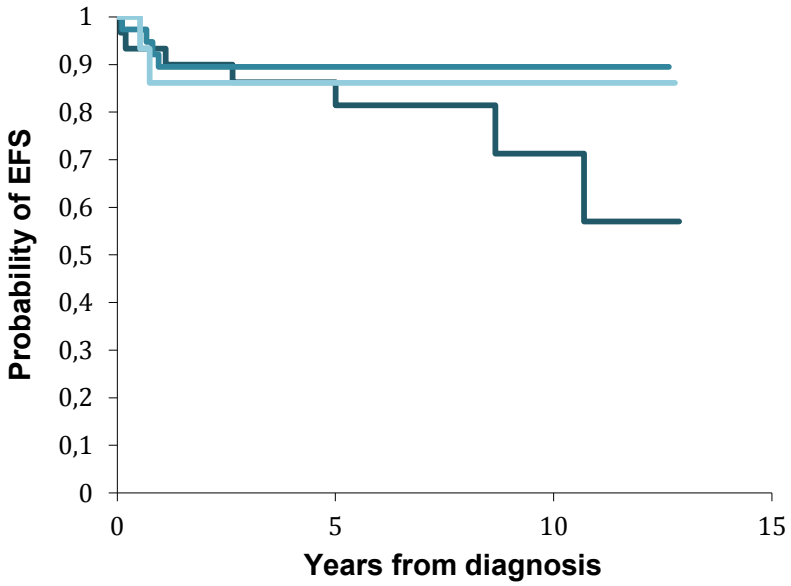

Figure 16 10-year EFS of patients with early, intermediate and mature T-ALL (P=0,473)

early T-ALL intermediate T-ALL mature T-ALL

2.3.10. Outcome in pB-cell ALL patients according to the *TEL/AML1* status

Comparing the outcome of pB-cell ALL patients featuring the fusion oncoprotein *TEL/AML1* and pB-cell ALL patients with *TEL/AML1* negativity, a statistically different outcome was observable.

The 10-year EFS of 144 (28%) *TEL/AML1* positive patients was 89±3%, whereas the 10-year EFS of 377 (72%) *TEL/AML1* negative patients was 81±2%.

Figure 17 illustrates the 10-year EFS of *TEL/AML1* positive and *TEL/AML1* negative patients.

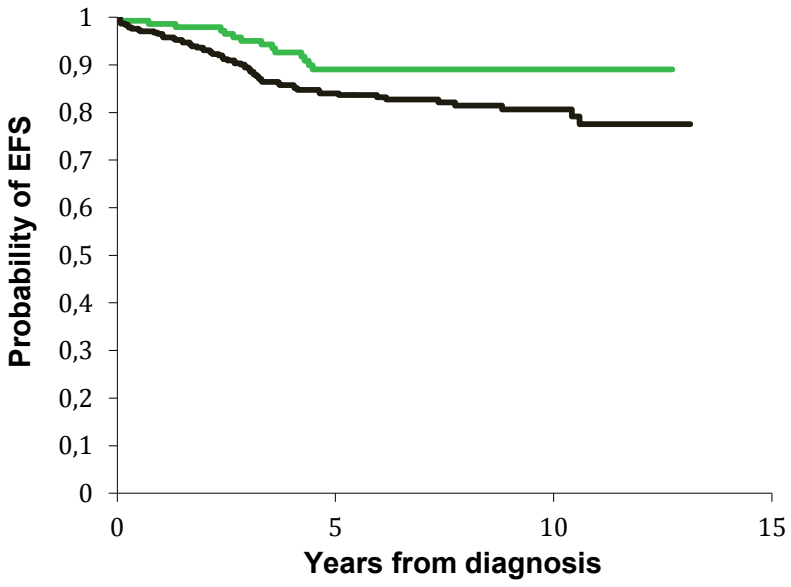

Figure 17 10-year EFS of *TEL/AML1* positive and *TEL/AML1* negative patients (P=0,031)

positive negative

2.3.11. Outcome in pB-cell ALL patients according to high-hyperdiploidy

When observing the association between outcome in patients with a high-hyperdiploid karyotype at initial diagnosis and patients without high-hyperdiploidy, no statistical difference was evident.

Patients with high-hyperdiploidy (n=163, 31%) performed with a 10-year EFS of 84±3% and patients without high-hyperdiploidy (n=358, 69%) at initial diagnosis had a 10-year EFS of 84±2%.

Figure 18 illustrates the 10-year EFS of patients with high-hyperdiploidy compared to patients without high-hyperdiploidy.

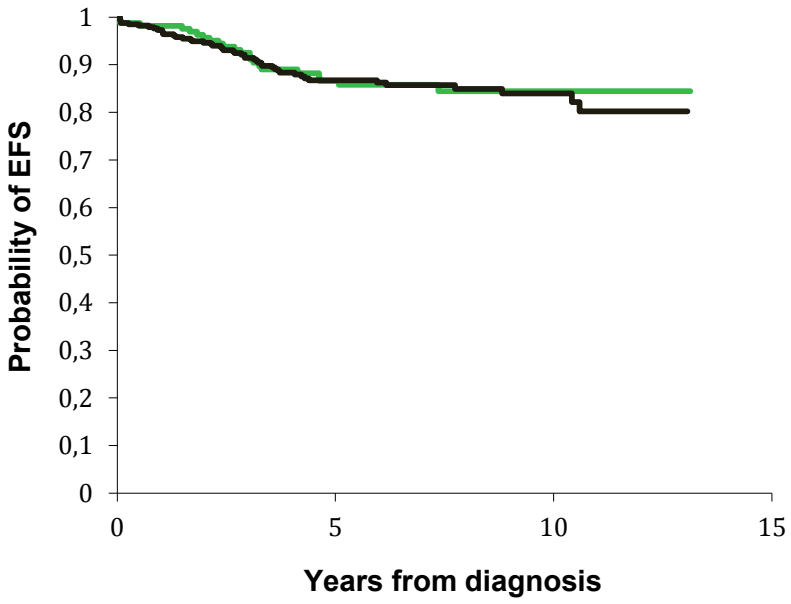

Figure 18 10-year EFS of patients with high-hyperdiploidy and patients without high-hyperdiploidy at initial diagnosis (P=0,807)
High-hyperdiploidy: yes no

2.3.12 Outcome in patients with ALL and Morbus Down

We evaluated the EFS of patients with Mb. Down at initial diagnosis and patients without Mb. Down in pB-cell ALL. Patients with Mb. Down and a diagnosis of ALL (n=21, 4%) performed with a 10-year EFS of 73±12% and patients without Mb. Down (n=500, 96%) performed with a 10-year EFS of 83±2%. A statistically relevant difference in outcome was not observable.

Figure 19 shows the 10-year EFS of patients with Mb. Down and patients without Mb. Down at initial diagnosis.

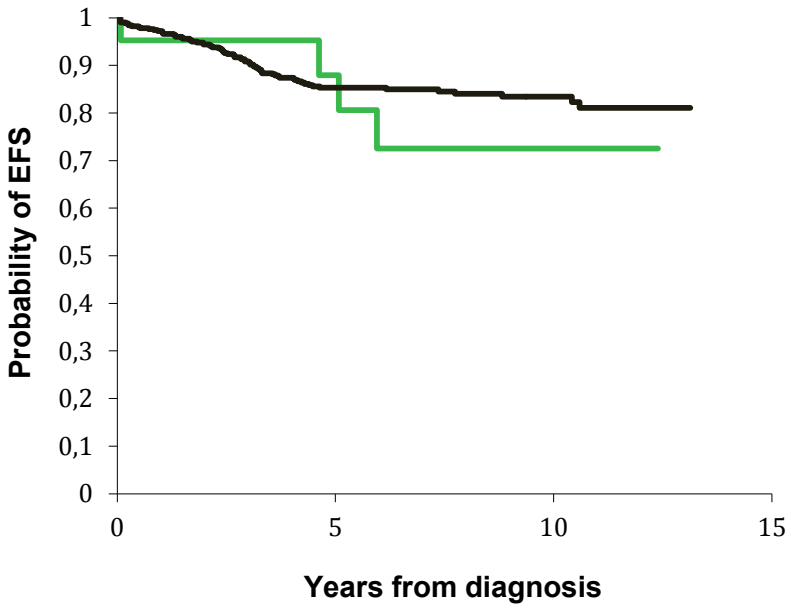

Figure 19 10-year EFS of patients with Mb. Down and patients without Mb. Down at initial diagnosis (P=0,562)

yes no

2.3.13. Outcome in patients according to prednisone response

The impact of prednisone response on the outcome of patients in pB-cell and T-cell ALL was analysed, no statistical difference was detected, however.

492 (94%) pB-cell ALL patients featured a good prednisone response and performed with a 10-year EFS of 83±2% and 28 (5%) patients with poor prednisone response had a 10-year EFS of 76±13%.

54 (62%) T-cell ALL patients with good prednisone response presented with a 10-year EFS of 85±5% and 31 (36%) patients with poor prednisone response showed a 10-year EFS of 66±14%.

Prednisone response on day 15 of induction treatment was not available in 1 (0,2%) pB-cell ALL patient and 2 (2%) T-cell ALL patients.

Figures 20 and 21 illustrate the EFS of pB-cell ALL and T-cell ALL patients according to prednisone response.

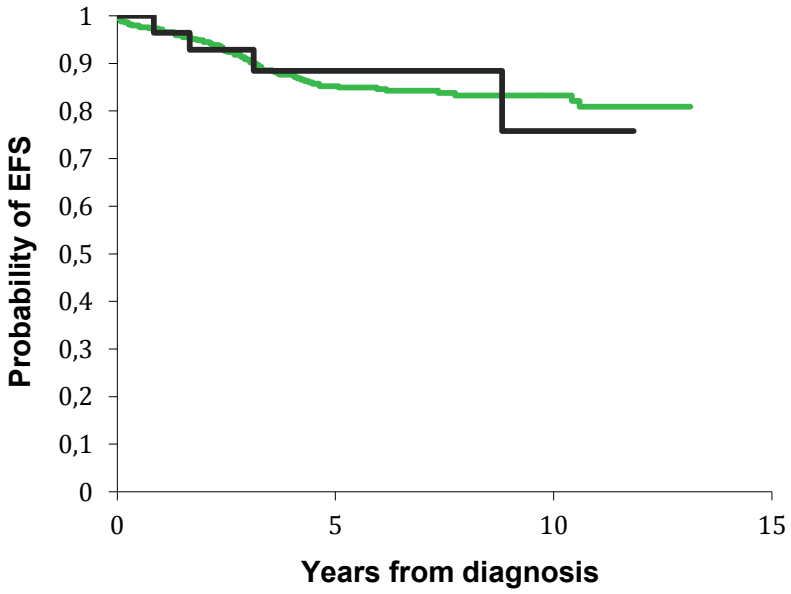

Figure 20 10-year EFS of pB-cell ALL patients with good vs. poor prednisone response (P=0,952)

good prednisone response poor prednisone response

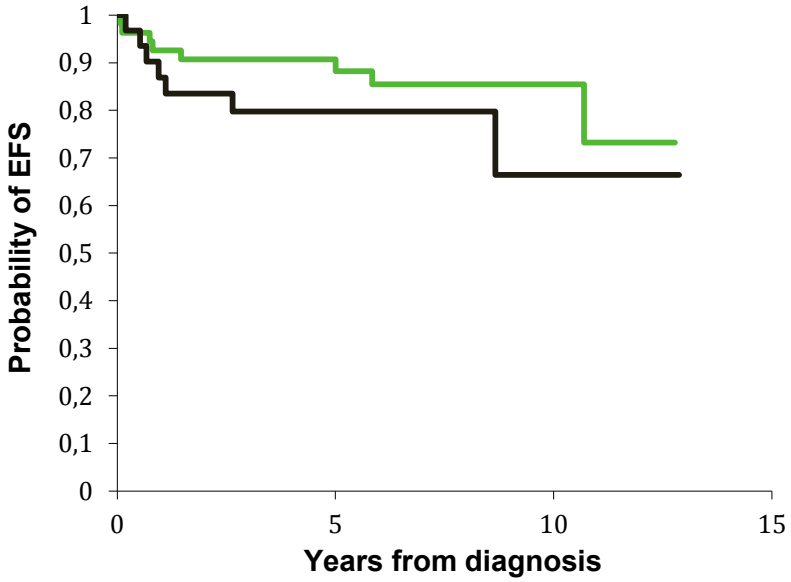

Figure 21 **10-year EFS of T-cell ALL patients with good vs. poor prednisone response (P=0,302)**
– good prednisone response ─ poor prednisone response

2.3.14. Outcome in patients according to the bone marrow status on day 15

We evaluated the EFS of pB-cell ALL and T-cell ALL patients according to the BM status on day 15.

Precursor B-cell ALL patients with < 5% of blasts in the BM on day 15 (n=309, 59%) performed with a 10-year EFS of 88±2%; patients who featured ≥ 5 and < 25% of blasts in the BM on day 15 (n=149, 29%) performed with a 10-year EFS of 80±4% and patients with ≥ 25% of blasts in the BM on day 15 (n=54, 10%) presented with a 10-year EFS of 59±9%. In 9 pB-cell ALL patients the BM status on day 15 of induction therapy was not available.

T-cell ALL patients with < 5% of blasts in the BM on day 15 of induction therapy (n=34, 39%) showed a 10-year EFS of 94±4%; patients with ≥ 5% and < 25% of blasts in the BM on day 15 (n=29, 33%) performed with a 10-year EFS of 59±12% and patients with ≥ 25% of blasts in the BM on day 15 (n=24, 28%) had a 10-year EFS rate of 87±7%. We observed a significant statistical difference of outcome in pB-cell ALL patients according to the BM status on day 15. Figures 22 and 23 below demonstrate the outcome of pB-cell ALL and T-cell ALL patients according to the BM status.

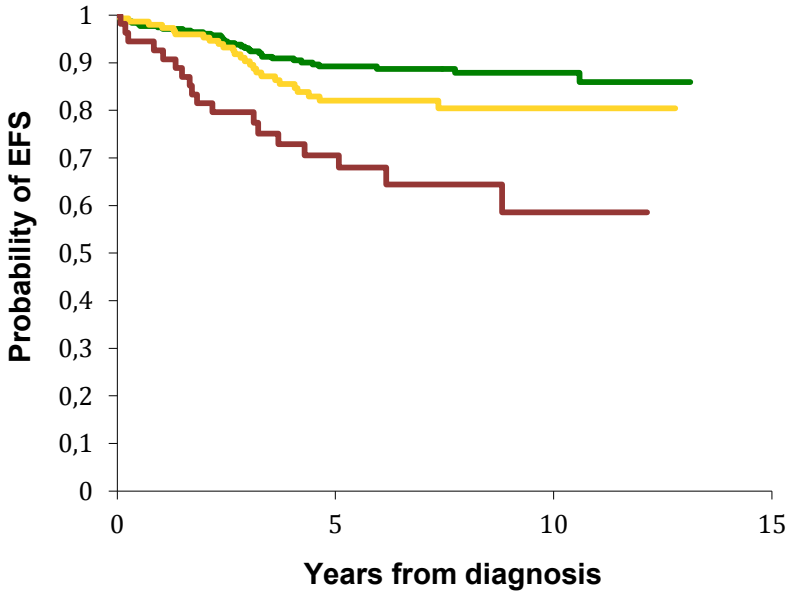

Figure 22 **10-year EFS of pB-cell ALL patients according to the bone marrow status on day 15 of induction therapy (P < 0,001)**

M1 M2 M3

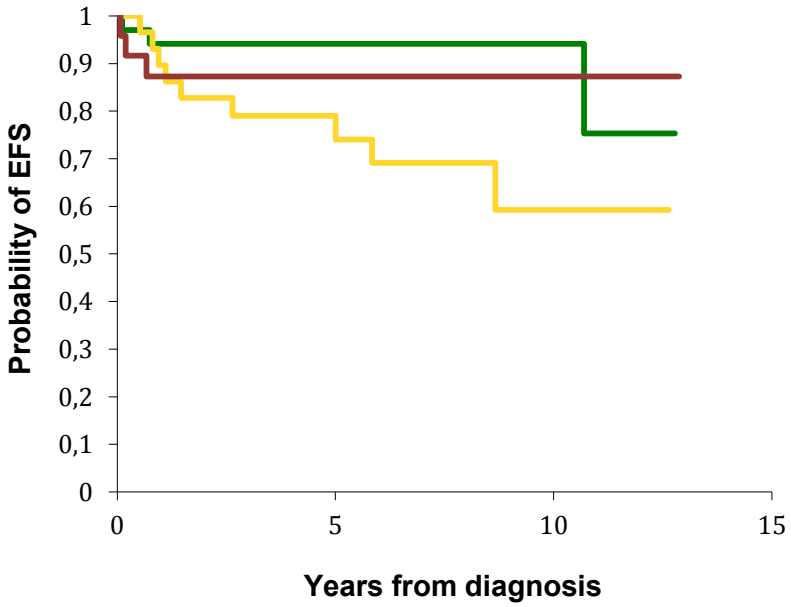

Figure 23 **10-year EFS of T-cell ALL patients according to the bone marrow status on day 15 of induction therapy (P=0,087)**

M1 M2 M3

2.3.15. Outcome in patients according to the status of remission on day 33

The association between EFS and remission status on day 33 in pB-cell ALL patients was analysed. 505 (97%) pB-cell ALL patients who were in CR on day 33 performed with a 10-year EFS of 85±2%, whereas 10 (2%) patients who were not in CR on day 33 had a 10-year EFS of 50±16%.

T-cell ALL patients who were in CR on day 33 (n=81, 93%) performed with a 10-year EFS of 80±6% and patients not in CR on day 33 (n=5, 6%) had a 10-year EFS of 80±18%.

6 (1%) pB-cell ALL patients and 1 (1%) T-cell ALL patient died before remission.

In pB-cell ALL patients, the difference in outcome according to the CR status on day 33 was statistically significant.

Figures 24 and 25 demonstrate the EFS according to the status of remission on day 33 in pB-cell ALL and T-cell ALL patients.

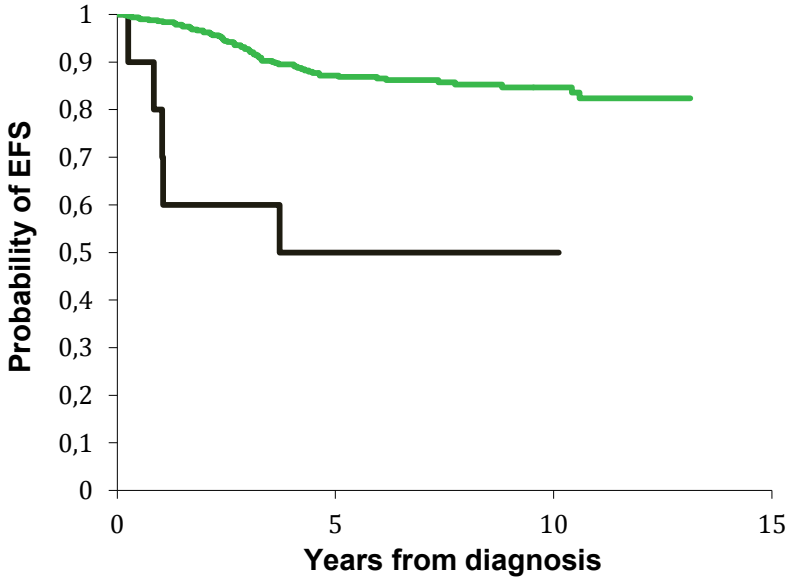

Figure 24 **10-year EFS of pB-cell ALL patients according to the status of remission on day 33 of induction therapy (P < 0,001)**

CR on day 33: yes no

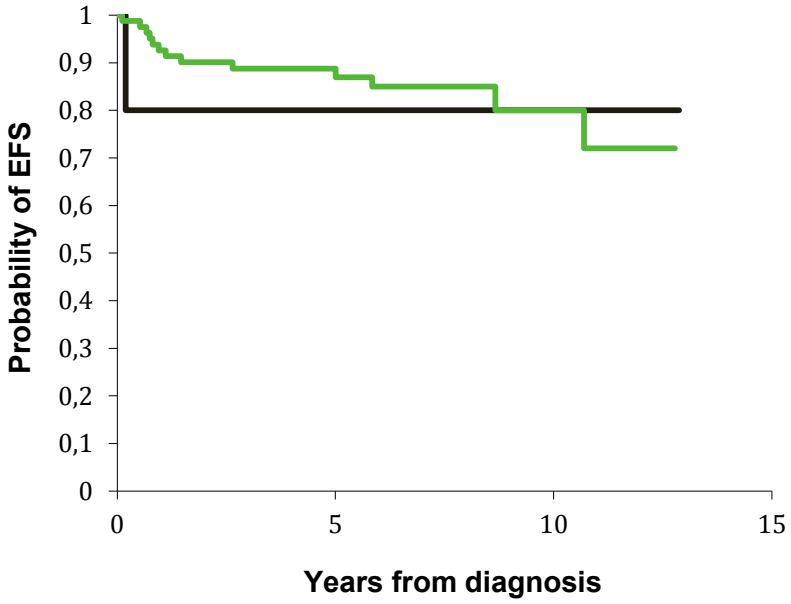

Figure 25 **10-year EFS of T-ALL patients according to the status of remission on day 33 of induction therapy (P=0,710)**

CR on day 33: yes no

2.3.16. Outcome in patients according to the definitive risk group

We analysed the outcome of pB-cell ALL patients and T-cell ALL patients according to the assignment of one of the three definite risk groups - standard-risk group (SRG), middle-risk group (MRG) and high-risk group (HRG).

139 (27%) pB-cell ALL patients were in the SRG, 327 (63%) were referred to the MRG and 47 (9%) were patients in the HRG. 8 (2%) pB-cell ALL patients died before stratification.

In T-cell ALL, 13 (15%) patients were in the SRG, 38 (44%) were patients in the MRG and 34 (39%) were in the HRG. 2 (2%) patients died before stratification.

10-year EFS rates of pB-cell ALL patients were the following: 92±2% in the SRG, 83±2% in the MRG and 68±10% in patients in the HRG.

Patients with T-cell ALL performed with EFS rates of 90±9% in the SRG, 88±6% in patients in the MRG and 70±12% in the HRG.

There was a significant statistical difference in outcome of pB-cell ALL patients according to the assignment to one of the three risk-groups.

Figures 26 and 27 illustrate the 10-year EFS rates of patients according to the assignment to one of the three definite risk-groups.

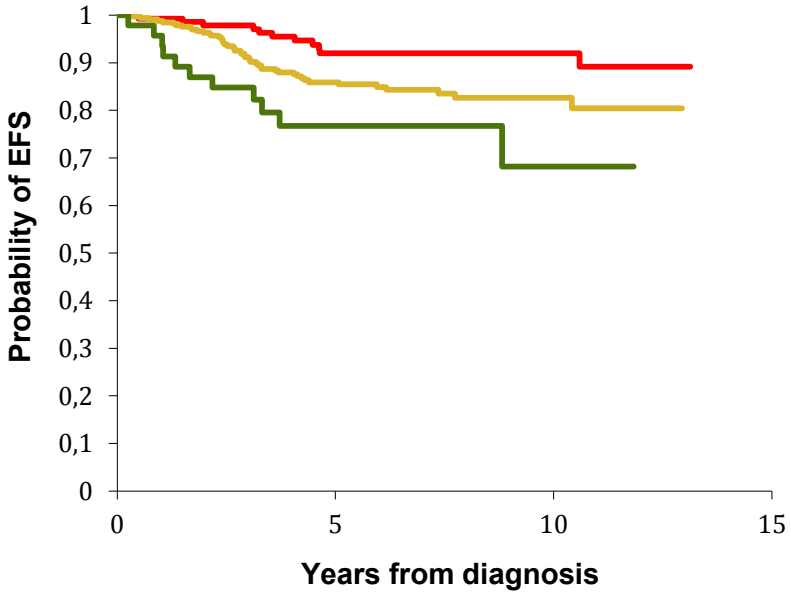

Figure 26 10-year EFS of pB-cell ALL patients according to the definitive risk group (P=0,005)

standard-risk group middle-risk group high-risk group

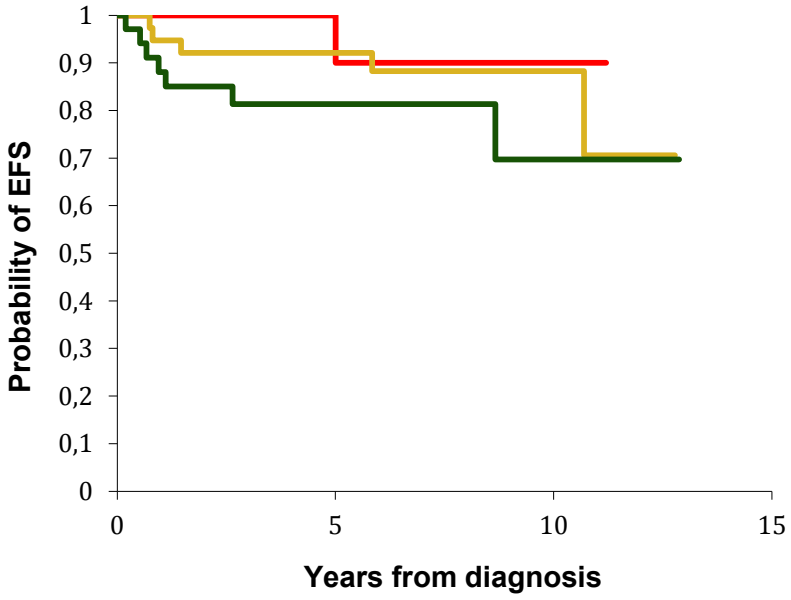

Figure 27 10-year EFS of T-cell ALL patients according to the definitive risk group (P=0,366)

standard-risk group middle-risk group high-risk group

2.3.17 Outcome in patients according to MRD group

We evaluated the outcome of pB-cell and T-cell ALL patients according to the MRD risk group. 495 pB-cell ALL patients and 78 T-cell ALL patients were eligible for MRD stratification.

In pB-cell ALL, 142 (29%) patients were considered as low-risk, 335 (68%) were patients with an intermediate risk of relapse and 18 (4%) were high-risk patients according to MRD. Their corresponding EFS rates were as follows: 93±2% in low-risk patients, 83±2% in patients with an intermediate risk and 44±19% in high-risk patients.

In T-cell ALL, 14 (18%) patients had a low risk of relapse, 52 (67%) were patients with an intermediate risk and 12 (15%) patients featured a high risk. EFS rates in T-cell ALL patients were: 73±18% in low-risk patients, 86±5% in intermediate-risk patients and 91±9% in high-risk patients, respectively.

A statistical difference in outcome according to MRD group in pB-cell ALL patients was observable.

Figures 28 and 29 illustrate the outcome in patients according to the MRD stratification.

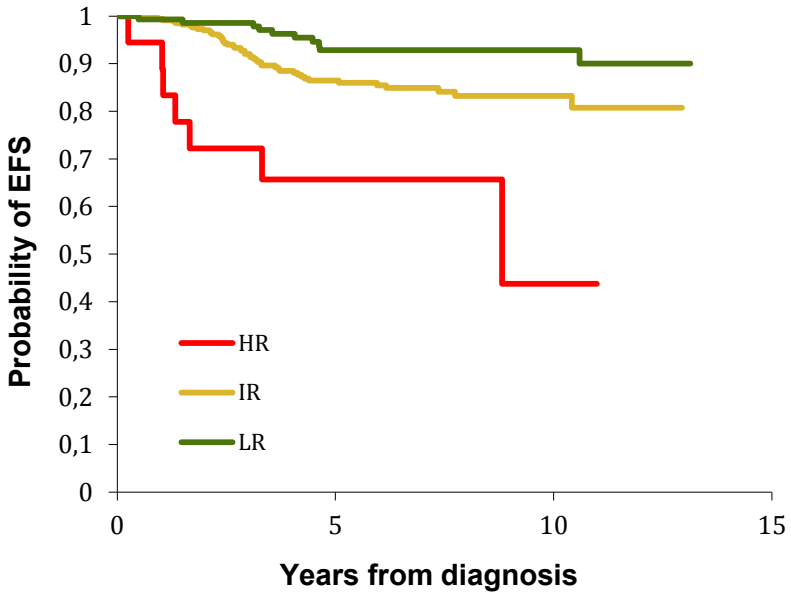

Figure 28 10-year EFS in 495 pB-cell ALL patients according to MRD group (P < 0,001)

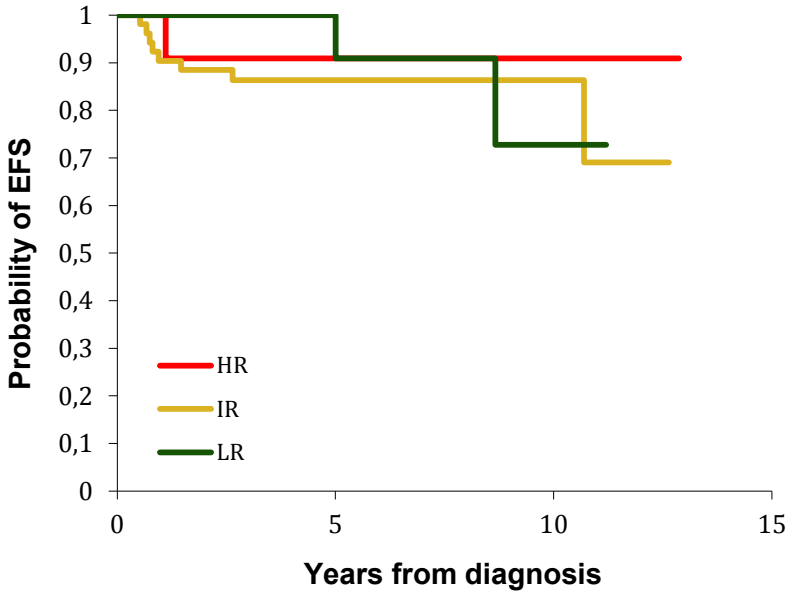

Figure 29 10-year EFS in 78 T-cell ALL patients according to MRD group (P=0,707)

2.4. Results according to MRD-Stratification

The introduction of MRD in order to be able to distinctly divide ALL patients in three groups of different prognosis and with different probabilities of EFS is one of the great achievements of the ALL-BFM 2000 trial, as mentioned above.

2.4.1. Initial parameters and early response to treatment of all MRD-stratified patients of the ALL-BFM 2000 trial

495 out of 521 pB-cell ALL patients were stratified by MRD at two different time points (day 33 and 78) of treatment; 26 patients were not eligible for MRD stratification.

In T-cell ALL, 78 out of 87 patients were stratified by MRD, however, 9 patients were not eligible for stratification.

Tables 11 and 12 illustrate the initial parameters of pB-cell and T-cell ALL patients according to the MRD results.

Tables 13 and 14 show the early response to treatment of all patients according to the MRD.

The characteristics of adverse events of MRD-stratifed pB-cell ALL and T-cell ALL patients are illustrated in tables 15 and 16.

Table 8 Initial parameters of 495 pB-cell ALL patients according to the MRD stratification

Parameter	MRD-LR No	%	MRD-IR No	%	MRD-HR No	%	Total No.
	142	**29**	**335**	**68**	**18**	**4**	**495**
Gender							
Male	72	27	187	69	11	4	270
Female	70	31	148	66	7	3	225
Age (years)							
mean	5,37	/	6,25	/	6,38	/	**6,2**
range	1,47-14,98	/	1,08-18,42	/	1,1-17,34	/	**1,08-18,42**
< 10	128	33	255	65	8	2	391
≥ 10	14	14	80	77	10	10	104
WBC count/µl							
< 20.000	109	31	238	68	5	1	352
≥ 20.000	33	23	97	68	13	9	143
< 50.000	132	31	293	68	8	2	433
≥ 50.000	10	16	42	68	10	16	62
TEL/AML1	55	39	84	60	1	1	140
MLL/AF4	0	/	4	57	3	43	7
E2A/PBX1	7	41	10	59	0	/	17
Hyperd.	42	27	114	73	1	1	157
FAB							
L1	127	30	290	68	12	3	429
L2	12	25	32	65	5	10	49
L1/2	/	/	2	100	/	/	2
Not available	3	20	11	73	1	7	15
CNS status							
Positive	2	18	7	64	2	18	11
Negative	137	29	325	68	16	3	478
Not available	3	50	3	50	0	0	6

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; WBC: white blood cell; Hyperd.: hyperdiploidy; FAB: French-American-British classification; CNS: central nervous system

Table 9 Initial parameters of 78 T-cell ALL patients according to the MRD stratification

Parameter	MRD-LR No (%)	MRD-IR No (%)	MRD-HR No (%)	Total No.
	14 (18)	**52 (67)**	**12 (15)**	**78**
Gender				
Male	7 (14)	35 (67)	10 (19)	52
Female	7 (27)	17 (65)	2 (8)	26
Age (years)				
mean	9,34	10,51	8,02	
range	2,22-15,42	2,35-17,98	1,43-17,47	
< 10	6 (15)	24 (62)	9 (23)	39
≥ 10	8 (21)	28 (72)	3 (8)	39
WBC count/µl				
< 20.000	2 (11)	15 (79)	2 (11)	19
≥ 20.000	12 (20)	37 (63)	10 (17)	59
< 50.000	7 (22)	20 (63)	5 (16)	32
≥ 50.000	7 (15)	32 (70)	7 (15)	46
Hyperd.	0	2 (100)	0	2
FAB				
L1	11 (18)	41 (68)	8 (13)	60
L2	3 (19)	11 (69)	2 (13)	16
L1/2	/	/	1 (100)	1
Not available	/	/	1 (100)	1
CNS status				
Positive	1 (13)	6 (75)	1 (13)	8
Negative	12 (19)	43 (66)	10 (15)	65
Not available	1 (20)	3 (60)	1 (20)	5

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; WBC: white blood cell; Hyperd.: hyperdiploidy; FAB: French-American-British classification; CNS: central nervous system

Table 10 Early response to treatment of 495 pB-cell ALL patients according to MRD stratification

Response evaluation	MRD-LR No (%)	MRD-IR No (%)	MRD-HR No (%)	Total
	142 (29)	**335 (68)**	**18 (4)**	**495**
Prednisone Response				
Good PR	137 (29)	319 (68)	11 (2)	467
Poor PR	5 (19)	16 (59)	6 (22)	27
Not available	/	/	1 (100)	1
BM day 15				
M1 (<5%)	108 (36)	187 (62)	5 (2)	300
M2 (≥ 5% and < 25%)	30 (21)	111 (77)	3 (2)	144
M3 (≥ 25%)	4 (8)	37 (73)	10 (20)	51
Remission on day 33	**142 (29)**	**331 (68)**	**13 (3)**	**486**
Not in remission	0 (0)	4 (44)	5 (56)	9
Definitive risk group				
SR	137 (100)	0	0	137
IR	0	312 (100)	0	312
HR	5 (11)	23 (50)	18 (39)	46

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; PR: prednisone response; BM: bone marrow; CR: complete remission

Table 11 Early response to treatment of 78 T-cell ALL patients according to MRD stratification

Response evaluation	MRD-LR No (%)	MRD-IR No (%)	MRD-HR No (%)	Total
	14 (18)	**52 (67)**	**12 (15)**	**78**
Prednisone Response				
Good PR	13 (27)	34 (71)	1 (2)	48
Poor PR	1 (4)	17 (61)	10 (36)	28
Not available	/	1 (50)	1 (50)	2
BM day 15				
M1 (< 5%)	9 (28)	23 (72)	0	32
M2 (≥ 5% and < 25%)	5 (18)	19 (68)	4 (14)	28
M3 (≥ 25%)	0	10 (56)	8 (44)	18
Remission on day 33	**14 (19)**	**52 (70)**	**8 (11)**	**74**
Not in remission	0 (0)	0 (0)	4 (100)	4
Definitive risk group				
SR	13 (100)	0	0	13
IR	0	35 (100)	0	35
HR	1 (3)	17 (57)	12 (40)	30

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; PR: prednisone response; BM: bone marrow; CR: complete remission

2.4.2. Occurrence of adverse events in MRD-stratified patients of the ALL-BFM 2000 study

The vast majority of relapses occurred in the intermediate-risk group: 41 out of 53 (77,4%) overall relapses occurred in intermediate-risk pB-cell ALL patients and 5 out of 7 (71,4%) overall relapses occurred in intermediate-risk T-cell ALL patients.

7 pB-cell ALL patients (13,2%) and 1 T-cell ALL patient (14,3%) of the low-risk group according to MRD suffered from relapse.

Five pB-cell ALL patients (9,4%) and 1 T-cell ALL patient (14,3%) of the high-risk group according to MRD experienced relapse.

32 deaths happened overall, whereas 23 of them (5%) were pB-cell ALL patients and 9 of them (12%) were T-cell ALL patients. Once more, the majority of deaths happened in the intermediate-risk group: 17 pB-cell ALL patients (74%) and 7 T-cell ALL patients (77,7%) of the intermediate-risk group died. In the low-risk group, 3 pB-cell ALL patients (13%) and 1 T-cell ALL patient (11,1%) suffered from death and 3 pB-cell ALL patients (13%) and 1 T-cell ALL patient (14,3%) suffered from death in the high-risk group.

Considering second malignant neoplasms, 8 (2%) occurred in pB-cell ALL patients and 1 (1%) happened in T-cell ALL patients.

Tables 15 and 16 demonstrate the characteristics of adverse events in pB-cell ALL and T-cell ALL patients.

Table 12 Characteristics of adverse events of 495 pB-cell ALL patients according to MRD stratification

Characteristics	MRD-LR No (%)	MRD-IR No (%)	MRD-HR No (%)	Total
Total Pat. Nr.	**142 (29)**	**335 (68)**	**18 (4)**	**495**
Relapses	**7 (13)**	**41 (77)**	**5 (9)**	**53**
Isolated BM	6 (15)	28 (72)	5 (13)	39
Combined BM	0	7 (100)	0	7
Isolated CNS	1 (25)	3 (75)	0	4
Isolated extramed. - others	0	3 (testicles) (100)	0	3
Very early	0	5 (56)	4 (44)	9
Early	1 (7)	13 (93)	0	14
Late	6 (20)	23 (77)	1 (3)	30
Death	**3 (13)**	**17 (74)**	**3 (13)**	**23**
Death without relapse or SMN	**1 (33)**	**1 (33)**	**1 (33)**	**3**
Before 1st CR	0 (0)	0 (0)	0 (0)	0
In 1st CR	1 (33)	1 (33)	1 (33)	3
After relapse	**1 (6)**	**15 (83)**	**2 (11)**	**18**
After SMN	**1 (50)**	**1 (50)**	**0 (0)**	**2**
SMN	**2 (25)**	**5 (63)**	**1 (13)**	**8**

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; No: number; BM: Bone marrow; CNS: central nervous system; Extramed.: Extramedullary; CR: complete remission; ALL: acute lymphoblastic leukaemia; SMN: Second malignant neoplasm

Table 13 Characteristics of adverse events of 78 T-cell ALL patients according to MRD stratification

Adverse events	MRD-LR No (%)	MRD-IR No (%)	MRD-HR No (%)	Total
	14 (18)	**52 (67)**	**12 (15)**	**78**
Relapses	**1 (14)**	**5 (71)**	**1 (14)**	**7**
Isolated BM	1 (20)	3 (60)	1 (20)	5
Combined BM	/	/	/	/
Isolated CNS	/	1 (100)	/	1
Isolated extramed. - others	/	1 (bone)	/	1
Very early	/	3 (75)	1 (25)	4
Early	/	/	/	/
Late	1 (33)	2 (67)	/	3
Death	**1 (11)**	**7 (78)**	**1 (11)**	**9**
Death without relapse or SMN	**0 (0)**	**3 (100)**	**0 (0)**	**3**
Before 1st CR	0 (0)	0 (0)	0 (0)	0
In 1st CR	0 (0)	3 (100)	0 (0)	3
After relapse	**1 (17)**	**4 (67)**	**1 (17)**	**6**
After SMN	**0 (0)**	**0 (0)**	**0 (0)**	**0**
SMN	**1 (100)**	**0 (0)**	**0**	**1**

Abbreviations: MRD: minimal residual disease; MRD-LR: low-risk group according to minimal residual disease; MRD-IR: intermediate-risk group according to minimal residual disease; MRD-HR: high-risk group according to minimal residual disease; No: number; CNS: central nervous system; Extramed.: Extramedullary; CR: complete remission; ALL: acute lymphoblastic leukaemia; SMN: Second malignant neoplasm

2.4.3. Outcome in patients according to MRD stratification

EFS rates were eligible in all groups of pB-cell ALL, however the falling number of T-cell ALL patients, who were eligible for MRD stratification, was too marginal for the validation of EFS rates according to individual parameters.

Therefore, the outcome of MRD-stratified patients according to clinical and laboratory parameters, as well as early response to treatment will only be reported of pB-cell ALL patients in the following chapters.

2.4.4. Outcome in MRD-stratified pB-cell ALL patients according to gender

In male pB-cell ALL patients, 72 (26,6%) were considered as low-risk, 187 (69,3%) were intermediate-risk patients and 11 (4,1%) were patients with a high risk of relapse according to MRD.

In female patients on the other hand, 70 patients (31,1%) were considered as low-risk, 148 (65,7%) were intermediate-risk patients and in 7 (3,1%) were patients with a high risk of relapse according to MRD.

EFS rates in male patients were as follows: 95±3% in low-risk patients, 79±4% in intermediate-risk patients and 61±16% in patients with a high-risk according to MRD. Female patients had EFS rates after 10 years of: 91±3% in low-risk patients, 88±3% in intermediate-risk patients and 36±27% in high-risk patients according to MRD.

A statistical difference in male and female patients and their affiliation to one of the three risk-groups was evident.

Figures 30 and 31 illustrate the 10-year EFS of male and female patients of the ALL-BFM 2000 trial according to the MRD.

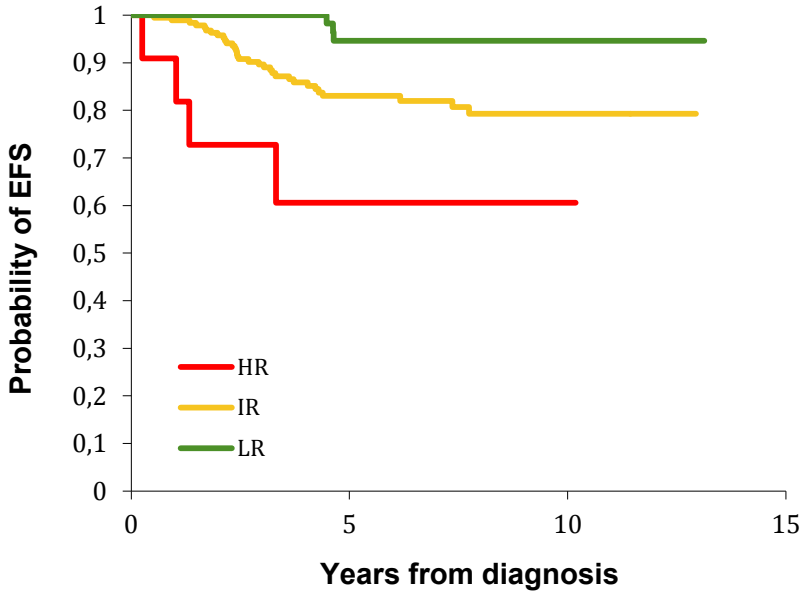

Figure 30 10-year EFS of male pB-cell ALL patients according to the MRD results (P=0,001)

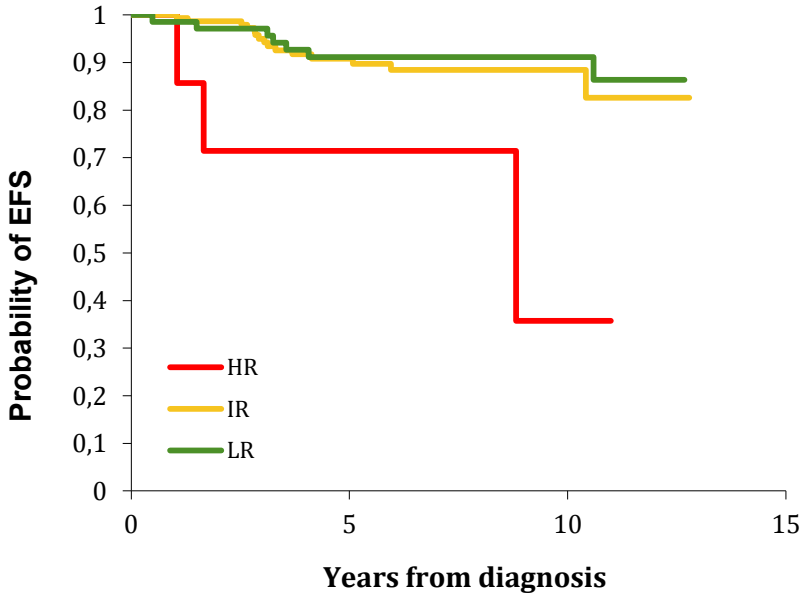

Figure 31 10-year EFS of female pB-cell ALL patients according to the MRD results (P=0,018)

2.4.5. Outcome in MRD-stratified pB-cell ALL patients according to leukocyte count

In patients with an initial WBC count of < 20.000/µl, 109 (31%) were considered as low-risk, 238 (67,6%) were intermediate-risk patients and 5 (1,4%) were patients with a high risk according to MRD.

Their corresponding EFS rates were as follows: 93±3% in low-risk patients, 84±3% in intermediate-risk patients and 60±22% in high-risk patients.

In patients with leukocyte counts of ≥ 20.000/µl at time of diagnosis, 33 (23,1%) were considered as low-risk, 97 (67,8%) were intermediate-risk patients and 13 (9,1%) were patients with a high risk of relapse according to MRD.

Their EFS rates were: 93±5% in low-risk patients, 80±4% in intermediate-risk patients and 35±25% in high-risk patients.

In patients with a WBC count of < 50.000/µl at time of diagnosis, 132 (30,5%) were low-risk patients, 293 (67,7%) were considered as intermediate-risk and 8 (1,8%) were patients with a high risk according to MRD. Their corresponding 10-year EFS rates were: 92±2% in low-risk patients, 84±3% in intermediate-risk patients and 50±17% in patients with a high risk.

In patients with WBC counts of ≥ 50.000/µl, 10 (16,1%) were considered as low-risk patients, 42 (67,7%) were intermediate-risk patients and 10 (16,1%) were patients with a high risk according to MRD.

Their 10-year EFS rates were as follows: low-risk patients showed an EFS of 100%, intermediate-risk patients showed an EFS of 80±7% and patients with a high risk had an EFS of 40±29%.

In patients with WBC counts of < and ≥ 20.000/µl, as well as WBC counts of < 50.000/µl a statistical difference in outcome according to the MRD group was evident.

Figures 32, 33, 34 and 35 illustrate the EFS of patients with different leukocyte counts according to MRD stratification.

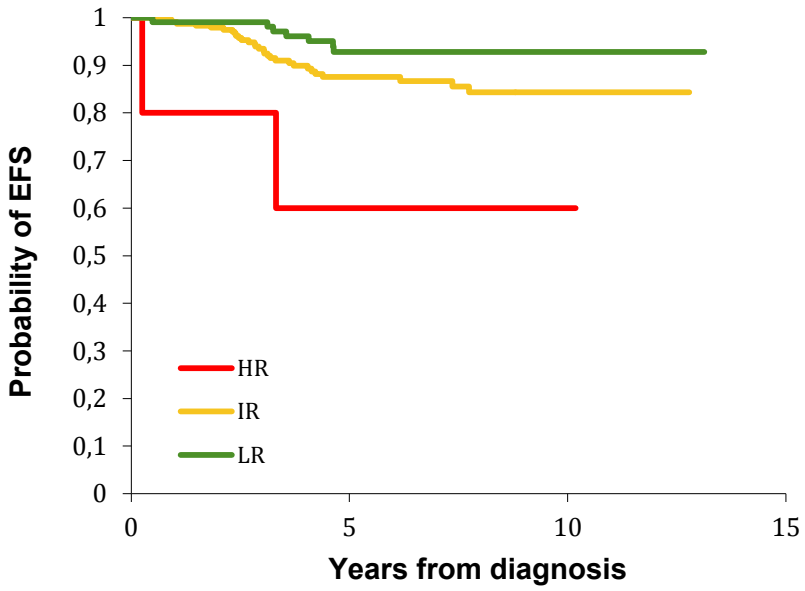

Figure 32 **10-year EFS in pB-cell ALL patients with an initial leukocyte count of < 20.000/µl according to the MRD results (P=0,013)**

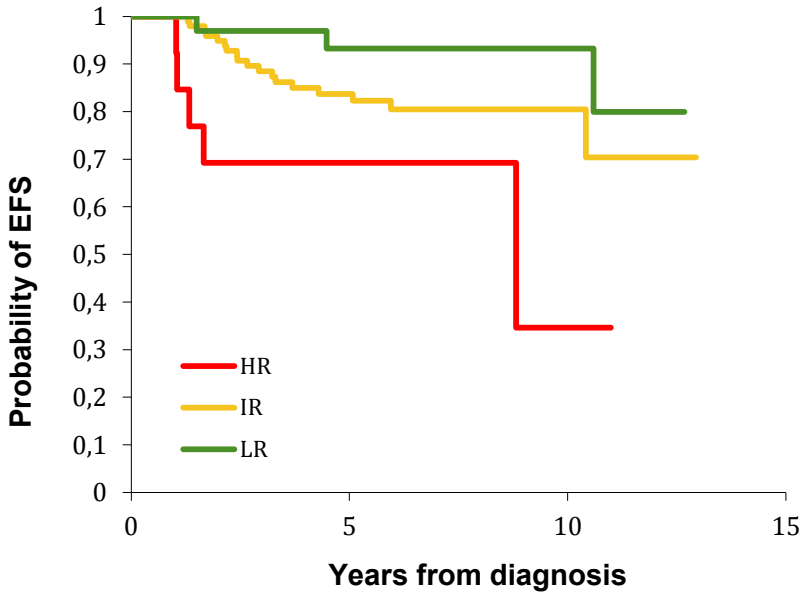

Figure 33 **10-year EFS in pB-cell ALL patients with an initial leukocyte count of ≥ 20.000/µl according to the MRD results (P=0,018)**

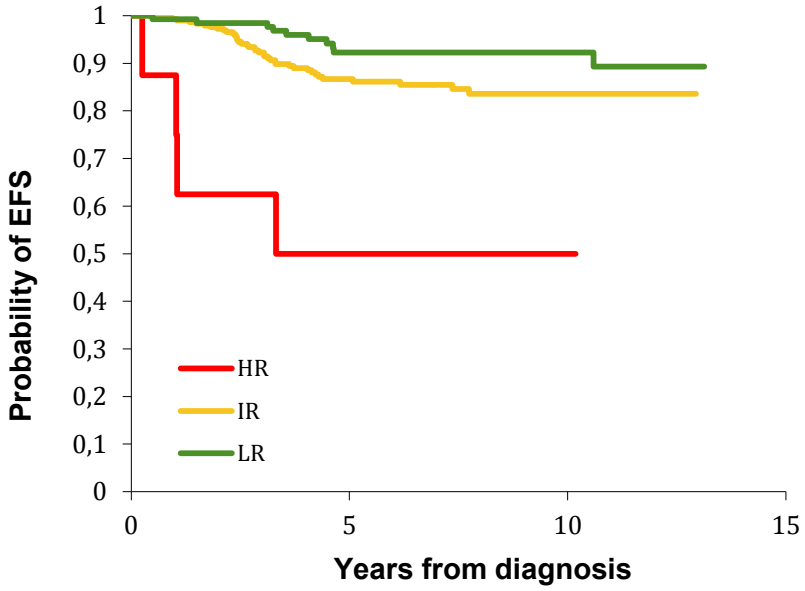

Figure 34 **10-year EFS in pB-cell ALL patients with an initial leukocyte count of < 50.000/µl according to the MRD results (P < 0,001)**

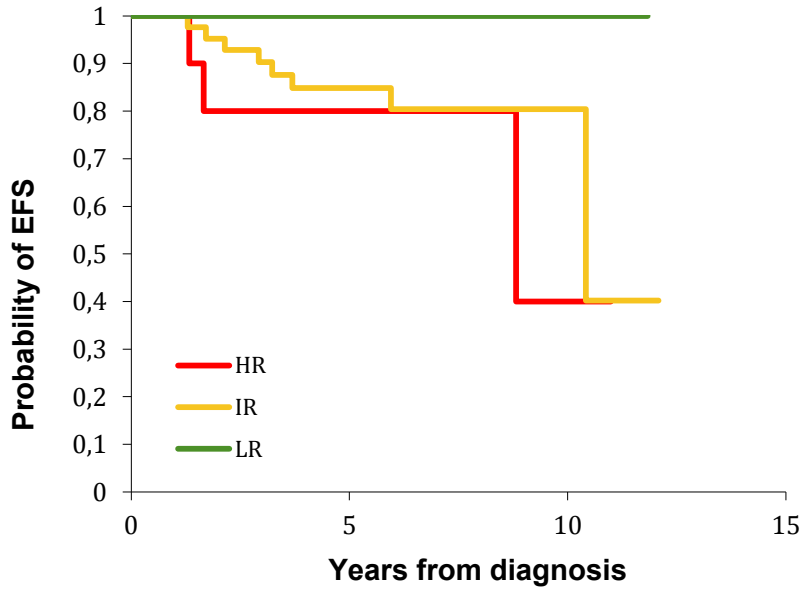

Figure 35 **10-year EFS in pB-cell ALL patients with an initial leukocyte count of ≥ 50.000/µl according to the MRD results (P=0,168)**

2.4.6. Outcome in MRD-stratified pB-cell ALL patients according to age

When examining pB-cell ALL patients with an initial age of < 10 years, 128 (32,7%) were considered as low-risk, 255 (65,2%) were intermediate-risk patients and 8 (2%) patients were high-risk according to MRD.

Their corresponding EFS rates were: 94±2% in low-risk patients, 84±3% in intermediate-risk patients and 30±23% in patients with a high risk of relapse.

In patients with ≥ 10 years of age at time of diagnosis, 14 (13,4%) were low-risk patients, 80 (77%) were intermediate-risk patients and 10 (9,6%) patients had a high risk of relapse. Their 10-year EFS rates were: 84±11% in low-risk patients, 80±5% in patients with an intermediate risk and 70±14% in high-risk patients.

There was clear evidence of a statistical difference in pB-cell ALL patients with 1 to 10 years of age, who were assigned to either of the three risk-groups defined by MRD and their outcome. Figures 36 and 37 disclose the 10-year EFS rates of patients with < or ≥ than 10 years of age according to the MRD results.

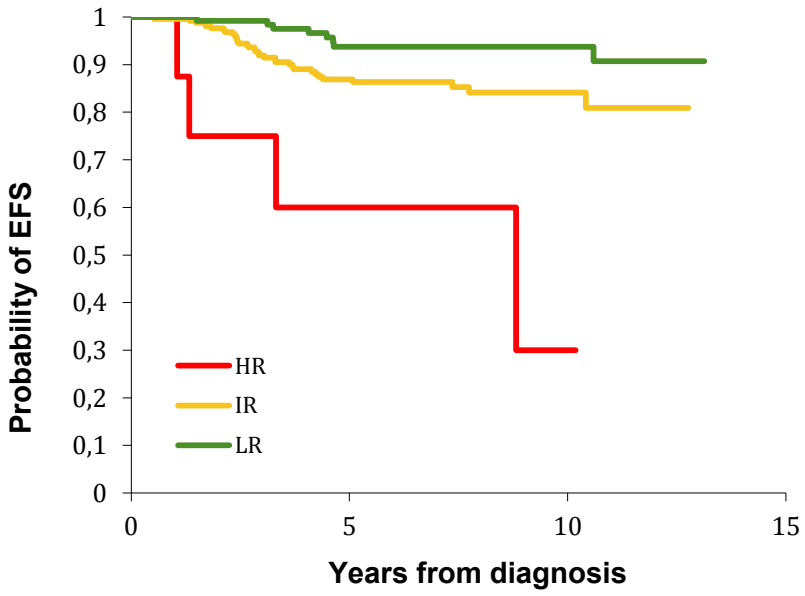

Figure 36 **10-year EFS of pB-cell ALL patients < 10 years of age at initial diagnosis according to the MRD results (P < 0,001)**

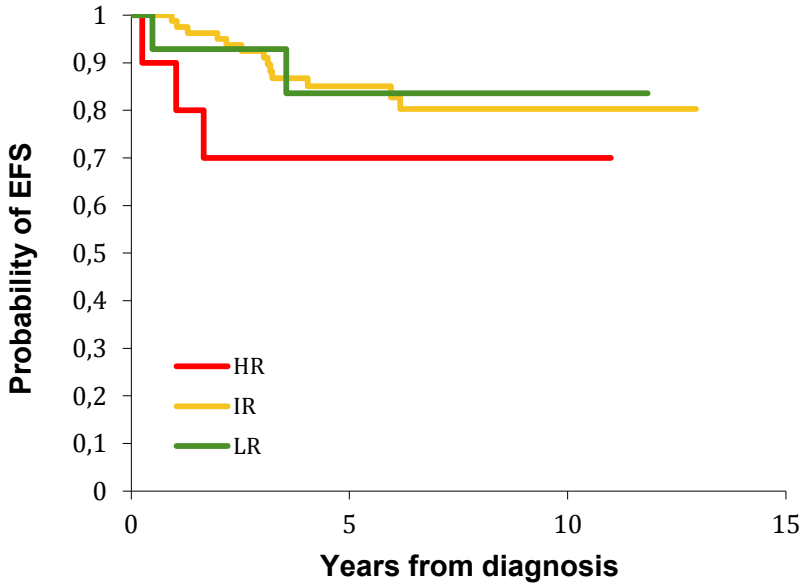

Figure 37 10-year EFS of pB-cell ALL patients ≥ 10 years of age at intial diagnosis according to the MRD results (P=0,446)

2.4.7. Outcome in MRD-stratified pB-cell ALL patients according to the CNS status

We analysed the performances of patients with a positive CNS status and patients with a negative CNS status at time of diagnosis according to the MRD results.

In patients with a negative CNS status, 137 (28,7%) were considered as low-risk, 325 (68%) were intermediate-risk patients and 16 (3,3%) were patients with a high risk.

Their corresponding EFS rates were: 93±2% in patients with a low risk of relapse, 84±2% in patients with an intermediate risk and 41±19% in high-risk patients, respectively.

In patients with a positive CNS status at initial diagnosis, 2 (18,2%) were considered as low-risk, 7 (63,6%) were intermediate-risk patients and 2 (18,2%) were patients with a high risk of relapse. The 10-year EFS of low- and high-risk patients was 100% and patients with an intermediate risk had an EFS of 83±15%.

A statistical difference in patients with a negative CNS status at initial diagnosis and the assignment to one of the three MRD risk groups was observable. The EFS rates of pB-cell ALL patients with positive and negative CNS status according to MRD are illustrated in figures 38 and 39.

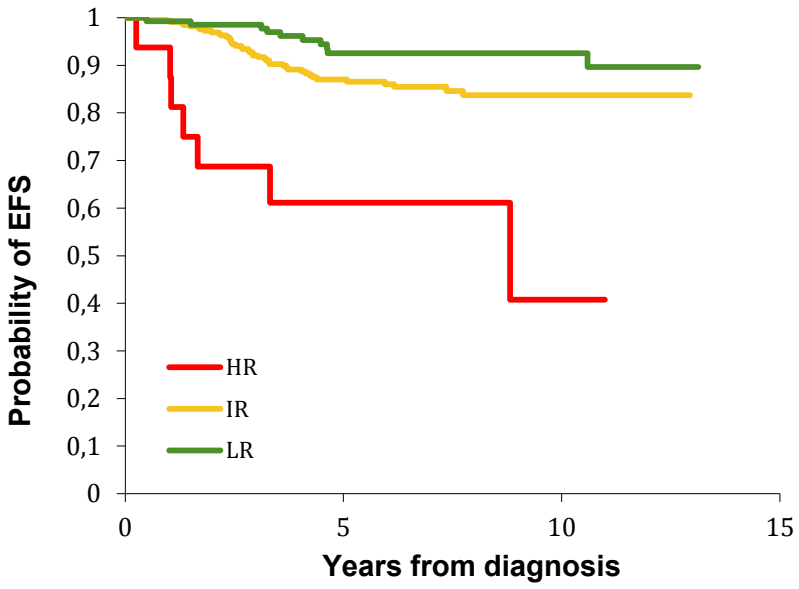

Figure 38 **10-year EFS of pB-cell ALL patients with a negative CNS status at initial diagnosis according to the MRD results (P < 0,001)**

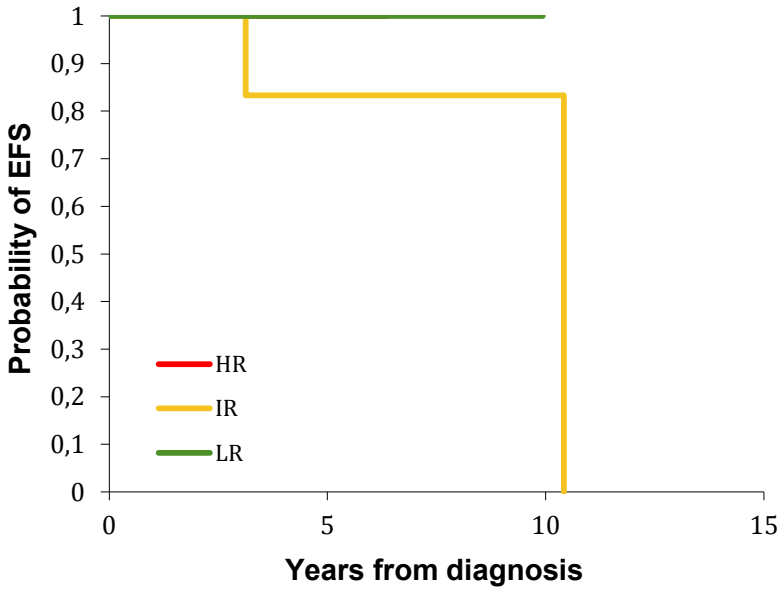

Figure 39 **10-year EFS of pB-cell ALL patients with a positive CNS status at diagnosis according to the MRD results (P=0,717)**

2.4.8. Outcome in MRD-stratified pB-cell ALL patients according to the immunophenotype

We evaluated the EFS of the most common pB-cell ALL subtypes – namely cALL and pre-B ALL – according to their MRD results.

In cALL, 87 patients (28,3%) were low-risk patients, 215 (70%) were considered as intermediate-risk and 5 (1,6%) were patients with a high risk.

The EFS rates of cALL patients were as follows: 92±3% in the low-risk category, 83±3% in intermediate-risk patients and 80±18% in patients with a high risk.

In pre-B ALL, 47 (33%) patients were considered low-risk, 92 (63,8%) were intermediate-risk patients and 5 (3,5%) were considered high-risk patients.

Their corresponding EFS rates were: 93±4% in low-risk patients and 86±4% in intermediate-risk patients. In pre-B ALL patients with a high risk of relapse the EFS rate after 10 years from diagnosis was 0%.

In pre-B ALL patients, a significant statistical difference of their assignment to the MRD risk-groups and the outcome could be observed.

The EFS rates after 10 years of cALL and pre-B ALL patients are demonstrated in figures 40 and 41 below.

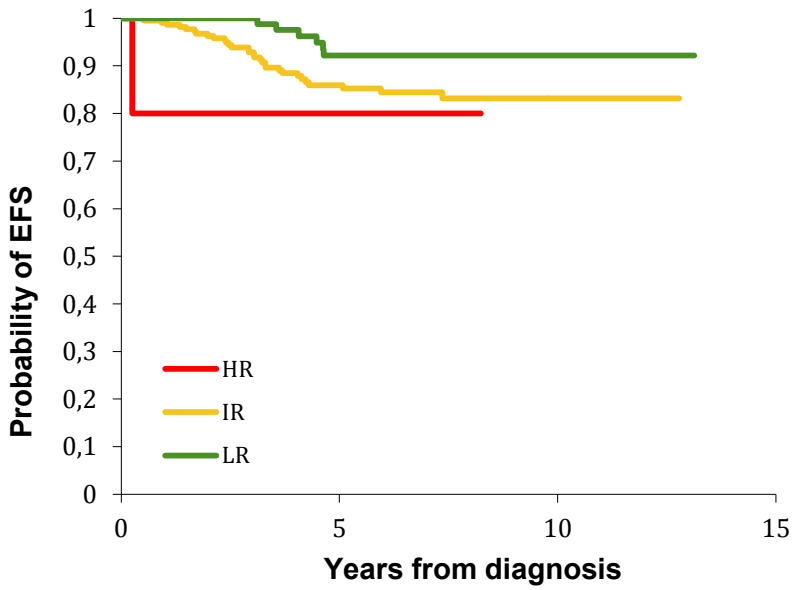

Figure 40 10-year EFS of cALL patients according to the MRD results (P=0,125)

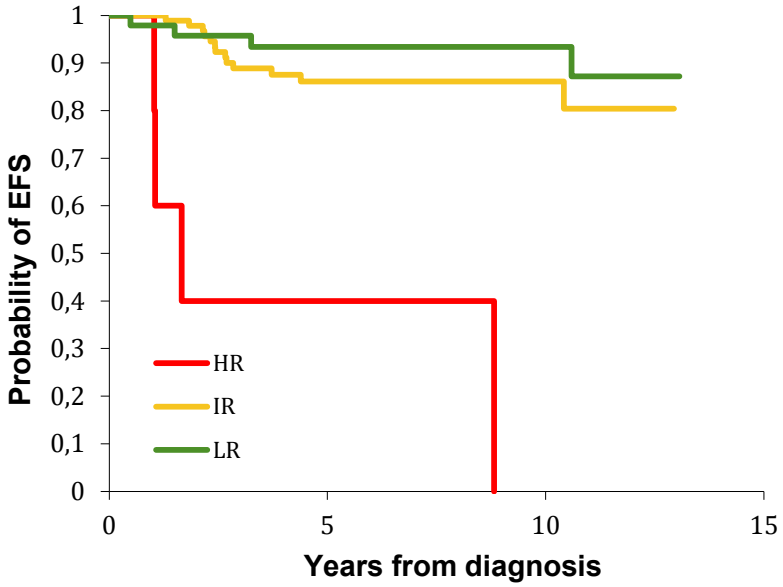

Figure 41 10-year EFS of pre-B ALL patients according to the MRD results (P < 0,001)

2.4.9. Outcome of MRD-stratified pB-cell ALL patients according to the *TEL/AML1* status

We evaluated the EFS of patients who featured the genetic fusion oncogene *TEL/AML1* and patients without *TEL/AML1* positivity according to the MRD results.

140 MRD-stratified patients were *TEL/AML1* positive in the ALL-BFM 2000 trial. 55 (39,3%) were considered as low-risk, 84 (60%) were intermediate-risk patients and 1 patient (0,7%) had a high risk of relapse. Their 10-year EFS rates were as follows: 96±3% in low-risk patients and 86±4% in intermediate-risk patients. In the very one patient with a high risk of relapse a 10-year EFS was not assessable.

In 355 patients the *TEL/AML1* oncoprotein was not detectable. 87 (24,5%) of them were considered as low-risk, 251 (70,7%) were intermediate-risk patients and 17 (4,8%) were high-risk patients according to MRD. Their corresponding EFS rates were: 91±3% in low-risk patients, 82±3% in intermediate-risk patients and 42±19% in patients with a high risk of relapse. There was a statistical difference in outcome of *TEL/AML1* negative patients according to MRD. Figures 42 and 43 reveal the 10-year EFS of *TEL/AML1* positive and *TEL/AML1* negative patients according to the MRD results.

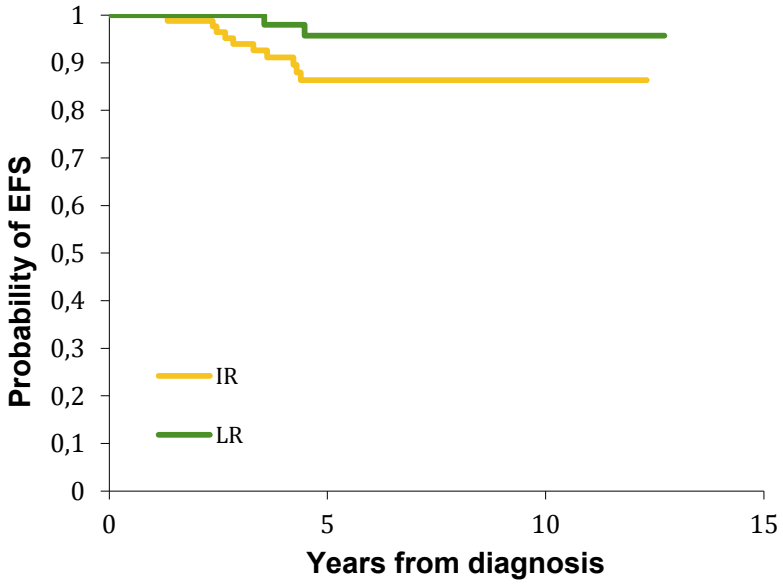

Figure 42 10-year EFS of *TEL/AML1* positive patients according to the MRD results (P=0,076)

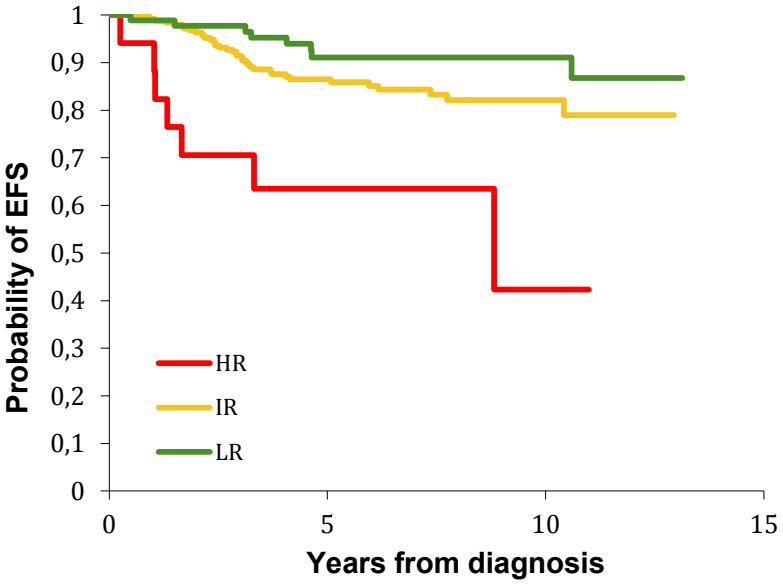

Figure 43 10-year EFS of *TEL/AML1* negative patients according to the MRD results (P=0,00028)

2.4.10. Outcome in MRD-stratified pB-cell ALL patients according to high-hyperdiploidy or non-high-hyperdiploidy

When examining MRD-stratified patients with hyperdiploidy at initial diagnosis and MRD-stratified patients without hyperdiploidy, the findings were the following:

In 157 patients with hyperdiploidy, 42 (26,8%) were referred to the low-risk group, 114 (72,6%) were considered intermediate-risk and 1 patient (0,6%) emerged as high-risk.

EFS rates of patients with hyperdiploidy at time of diagnosis were: 90±5% in low-risk patients and 86±4% for patients with an intermediate risk. Analysis of EFS was not possible in the patient with a high risk of relapse.

In 319 non-hyperdiploid patients, 96 (30,1%) were considered as low-risk patients, 208 (65,2%) were intermediate-risk patients and 15 (4,7%) were patients with a high risk of relapse.

Non-hyperdiploid patients performed with EFS rates of: 95±2% in the low-risk category, 82±3% in intermediate-risk patients and 53±23% in the high-risk group, respectively.

A statistical difference in outcome of non-hyperdiploid patients due to their stratification to one of the three risk-groups defined by MRD was evident.

Figures 44 and 45 illustrate the 10-year EFS of hyperdiploid and non-hyperdiploid patients according to the MRD results.

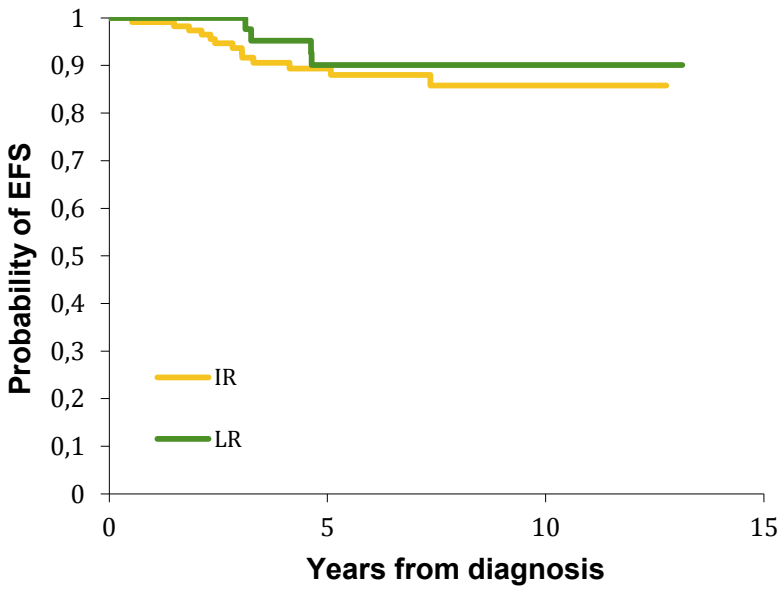

Figure 44 10-year EFS of hyperdiploid patients according to the MRD results (P=0,496)

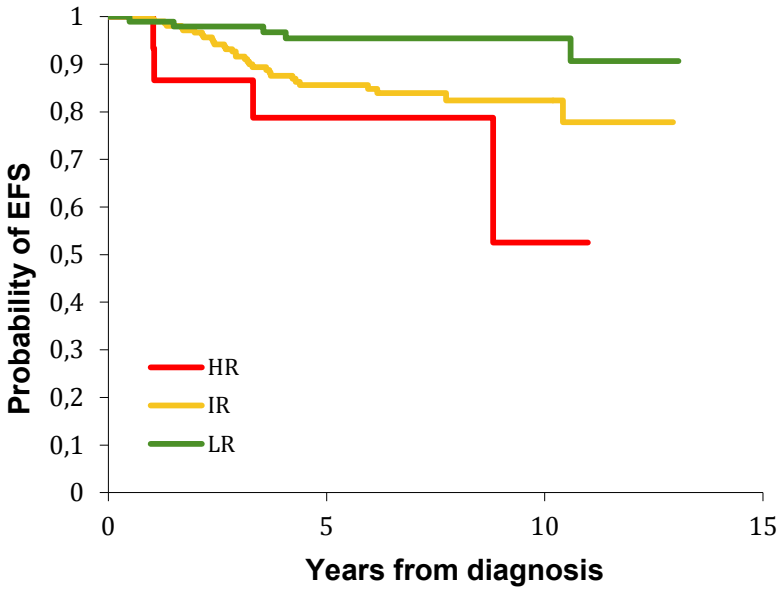

Figure 45 10-year EFS of non-hyperdiploid patients according to the MRD results (P=0,007)

2.4.11. Outcome in MRD-stratified pB-cell ALL patients with and without Mb. Down

19 patients suffering from Mb. Down were diagnosed with pB-cell ALL in the ALL-BFM 2000 trial and eligible for MRD stratification. 9 (47,4%) were considered as low-risk and the remaining 10 (52,6%) were patients with an intermediate risk. Their corresponding EFS rates were: 83±15% in the low-risk category and 71±17% in patients with an intermediate risk.

In patients without Mb. Down, 133 (27,9%) emerged as low-risk patients, 325 (68,3%) were intermediate-risk patients and 18 patients (3,8%) had a high risk of relapse.

Patients without Mb. Down performed with EFS rates of: 93±2% in low-risk patients, 84±2% in patients with an intermediate risk and 66±11% in patients with a high risk of relapse.

There was a significant statistical difference in outcome in patients without Mb. Down according to their affiliation to one of the three risk-groups defined by MRD.

Figures 46 and 47 illustrate the 10-year EFS of patients with and without Mb. Down according to the MRD results.

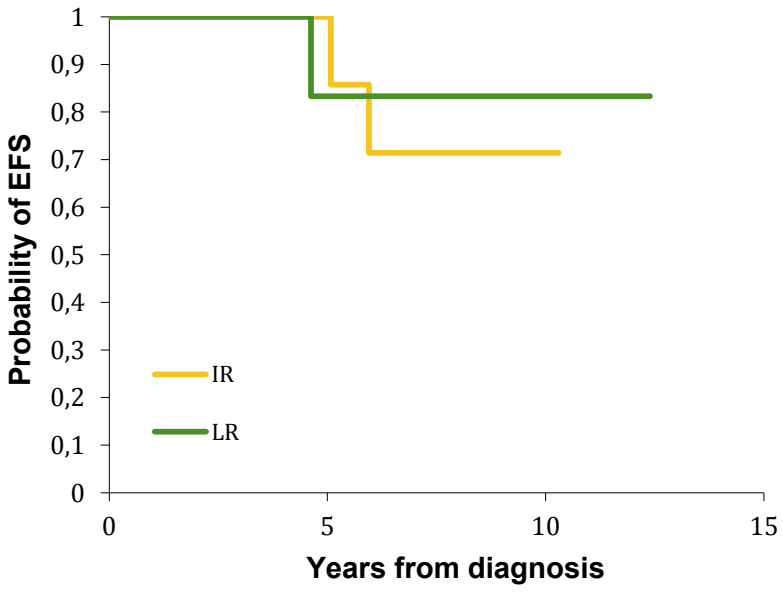

Figure 46 10-year EFS of patients with Mb. Down according to the MRD results (P=0,745)

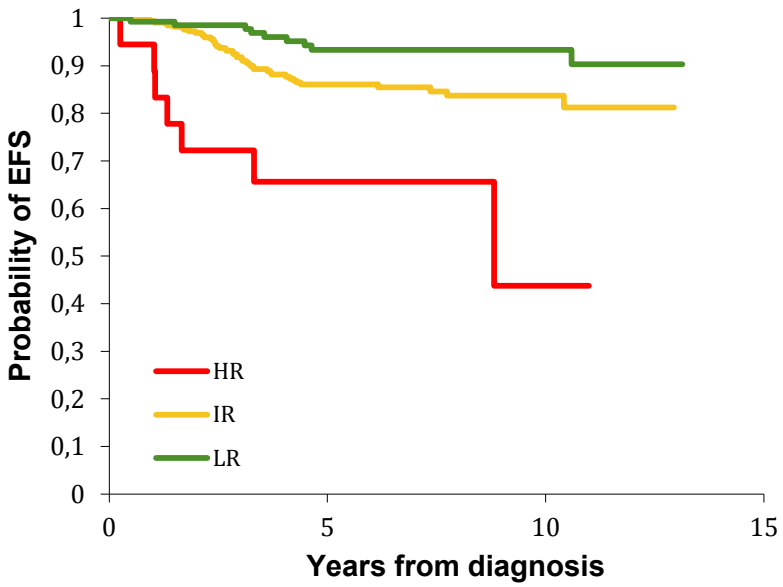

Figure 47 10-year EFS of patients without Mb. Down according to the MRD results (P < 0,001)

2.4.12. Outcome in MRD-stratified pB-cell ALL patients according to the prednisone response

467 patients were good prednisone responders, of whom 137 (29,4%) were patients with a low risk of relapse, 319 (68,3%) were considered intermediate-risk and 11 (0,3%) were patients with a high risk. Their corresponding EFS rates were: 93±2% in patients with a low risk, 83±2% in intermediate-risk patients and 53±15% in the high-risk category.

In 27 poor prednisone responders, 5 (18,5%) were considered as low-risk patients, 16 (59,3%) were patients with an intermediate risk and 6 (22,2%) patients were considered high-risk. The EFS rates of poor prednisone responders were: 100% in patients with a low risk of relapse, 91±9% in intermediate-risk patients and 42±30% in patients with a high risk of relapse.

A statistically relevant difference in outcome in good prednisone responders according to their assignment to one of the three risk-groups defined by MRD was evident.

Figures 48 and 49 illustrate the 10-year EFS rate of patients with a good and poor prednisone response according to the MRD results.

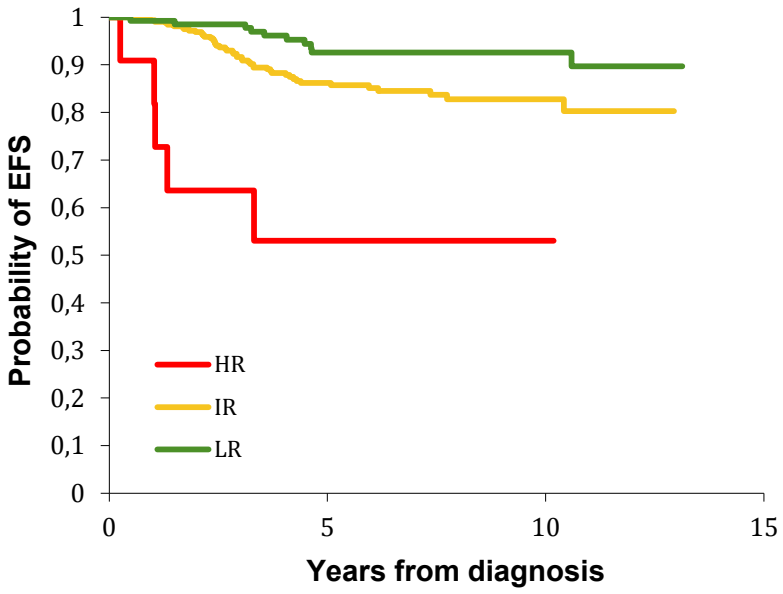

Figure 48 **10-year EFS of pB-cell ALL patients with prednisone good response according to the MRD results (P < 0,001)**

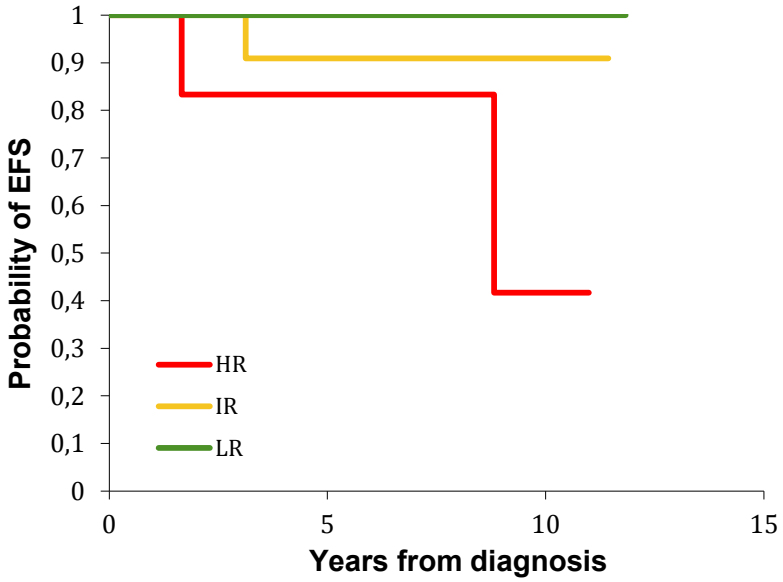

Figure 49 10-year EFS of pB-cell ALL patients with prednisone poor response according to the MRD results (P=0,222)

2.4.13. Outcome in MRD-stratified pB-cell ALL patients according to BM status on day 15 of induction therapy

In patients with a BM status of < 5% of blasts, 107 (36,5%) were low-risk patients, 182 (62,1%) were intermediate-risk patients and 4 (1,4%) were patients with a high risk of relapse. Their corresponding EFS rates were: 94±2% in low-risk patients, 87±3% in intermediate-risk patients and 67±27% in patients with a high risk of relapse.

In patients with a BM status of ≥ 5% and < 25% of blasts, 23 (17%) were considered as low-risk patients, 104 (77%) were intermediate-risk patients and 8 (6%) patients featured a high risk. Their EFS rates were as follows: 84±9% in low-risk patients, 69±8% in intermediate-risk patients and 38±28% in high-risk patients, respectively.

In patients with a BM status of ≥ 25% of blasts, 30 (20,8%) were low-risk patients, 111 (77,1%) were considered intermediate-risk and 3 (2,1%) were patients with a high risk. Their corresponding EFS rates were: 88±6% in low-risk patients, 82±4% in intermediate-risk patients and 34±27% in high-risk patients. There was a statistical difference in outcome of patients with a M3 BM on day 15 of induction therapy and their assignment to one of the three risk-groups of MRD. Figures 50, 51 and 52 illustrate the EFS rates of patients with either of the three BM categories according to the MRD results.

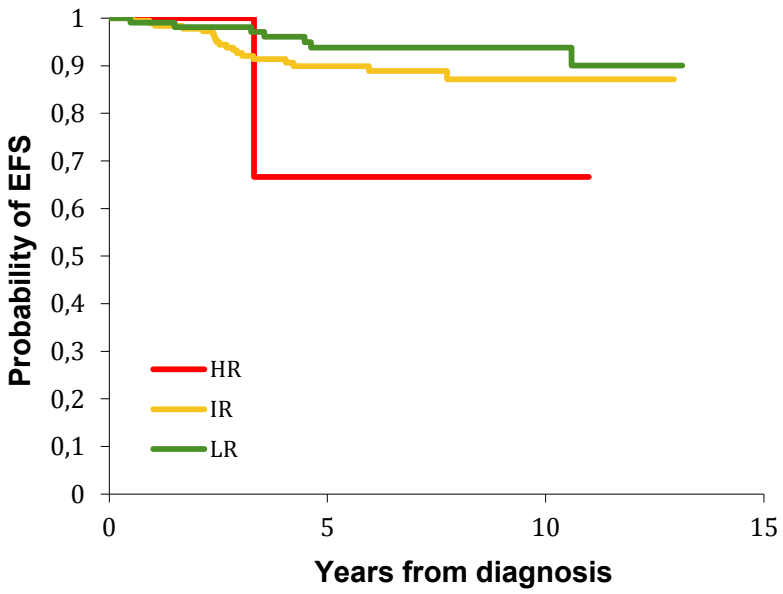

Figure 50 **10-year EFS of pB-cell ALL patients with a M1 BM on day 15 of induction therapy according to the MRD results (P=0,204)**

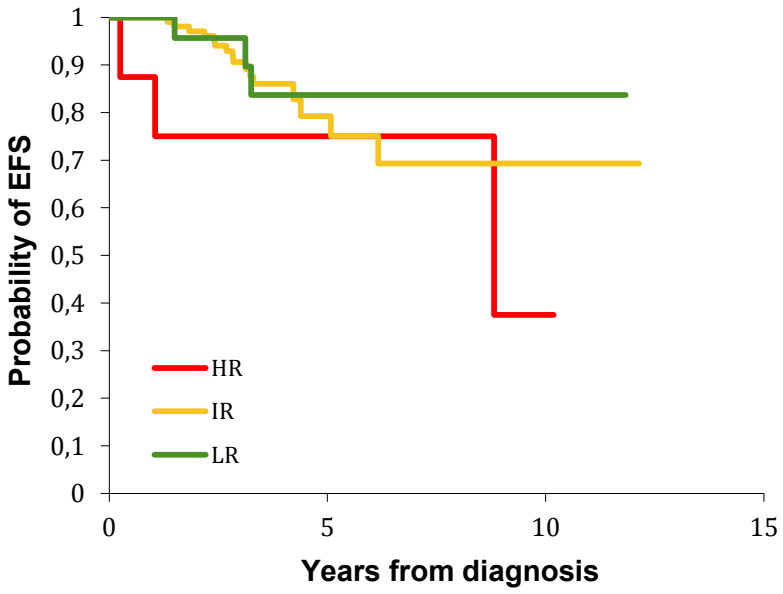

Figure 51 **10-year of pB-cell ALL patients with a M2 BM on day 15 of induction therapy according to the MRD results (P=0,590)**

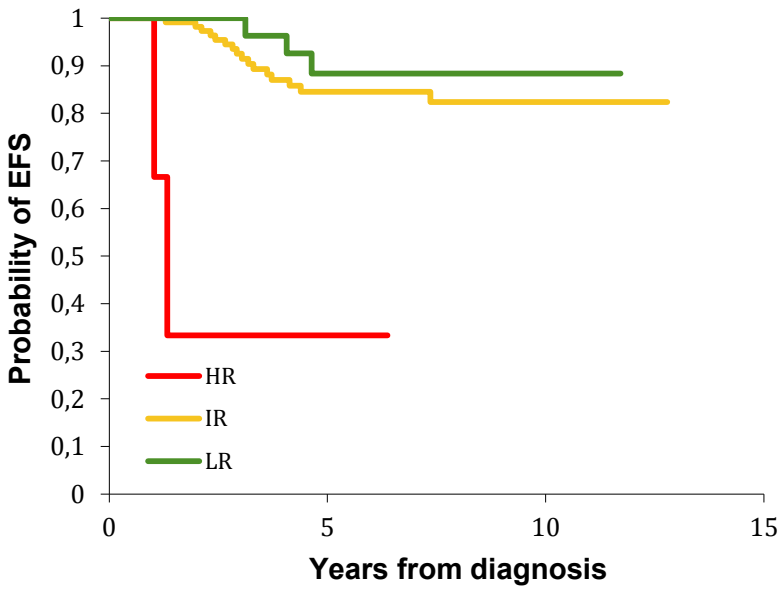

Figure 52 **10-year EFS of pB-cell ALL patients with a M3 BM on day 15 of induction therapy according to the MRD results (P=0,0004)**

2.4.14. Outcome of MRD-stratified pB-cell ALL patients according to the status of remission on day 33 of induction treatment

486 patients were in CR on day 33 of induction treatment, whereas 9 failed to achieve CR. In patients in CR, 142 (29,2%) were low-risk patients, 331 (68,1%) were intermediate-risk patients and 13 (2,7%) were patients with a high risk of relapse. The EFS rates of patients in CR were: 93±2% in low-risk patients, 83±2% in intermediate-risk patients and 50±22% in patients with a high risk.

In patients not in CR on day 33 of induction therapy, 4 (44,4%) were intermediate-risk patients and 5 (55,6%) were considered as high-risk patients. Their corresponding EFS rates were as follows: 75±22% in patients with an intermediate risk of relapse and 40±22% in patients with a high risk. A statistically relevant difference in outcome in patients in CR on day 33 of induction treatment according to their affiliation to one of the three risk-groups defined by MRD was observable. Figures 53 and 54 illustrate the 10-year EFS of patients with different status of remission on day 33 of induction therapy according to the MRD results.

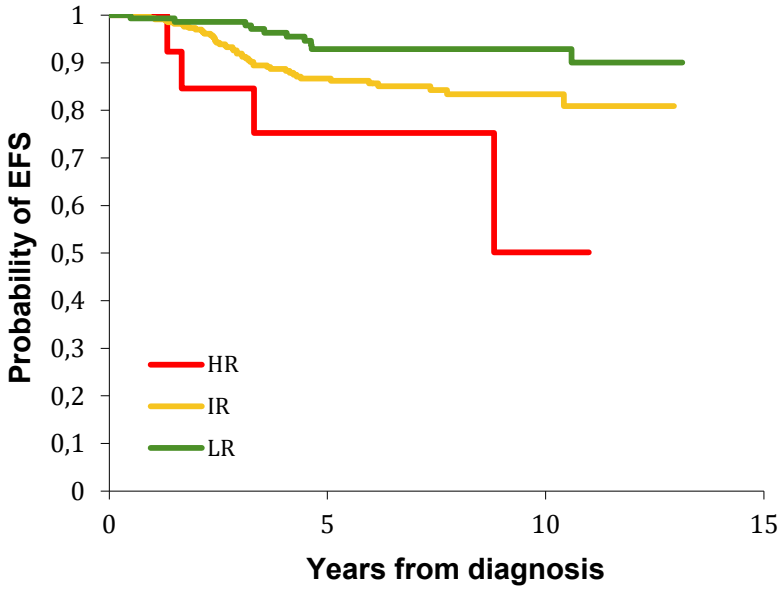

Figure 53 **10-year EFS of pB-cell ALL patients in CR on day 33 of induction therapy according to the MRD results (P=0,004)**

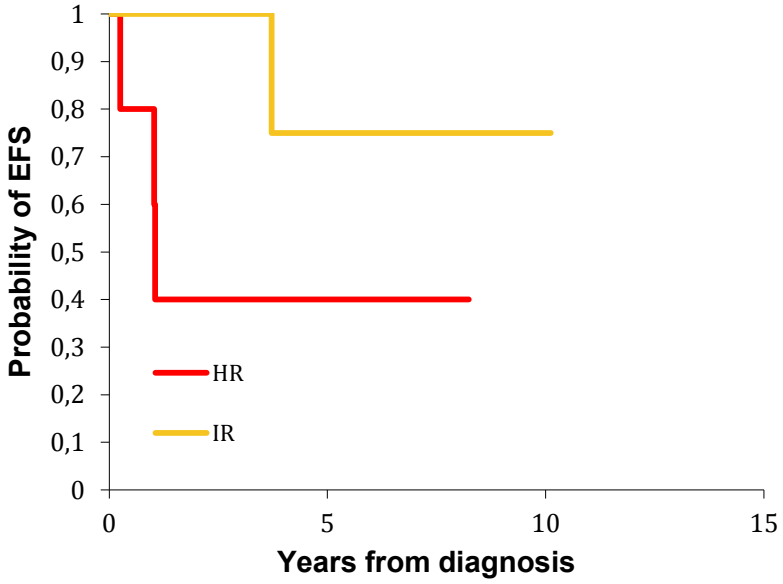

Figure 54 **10-year EFS of pB-cell ALL patients not in CR on day 33 of induction therapy according to the MRD results (P=0,228)**

2.4.15. Outcome of ALL BFM-95 patients according to the MRD-results

We evaluated the outcome of patients, who would have been in the standard-, middle- or high-risk group according to BFM-95 criteria (age, WBC count, response to prednisone or induction phase IA and t(9;22) or t(4;11) translocations)[45], when assessed by MRD. In 200 ALL BFM-95 SRG patients, 71 (35,5%) were low-risk patients, 127 (63,5%) were intermediate-risk patients and the remaining 2 (1%) were considered high-risk according to MRD. Their corresponding EFS rates were as follows: 95±3% in low-risk patients, 86±4% in intermediate-risk patients and 50±35% in patients with a high risk of relapse, respectively. A statistical difference in outcome and MRD stratification was evident in this group (P=0,023). In 255 ALL-BFM 95 MRG patients, 66 (25,9%) were low-risk patients, 185 (72,5%) were intermediate-risk patients and 4 (1,6%) were considered as high-risk patients according to MRD. Their EFS rates were: 90±4% in low-risk patients, 81±3% in intermediate-risk patients and 75±22% in high-risk patients, respectively. In 40 ALL-BFM 95 HRG patients, 5 (12,5%) were low-risk, 23 (57,5%) were considered as intermediate-risk and 12 (30%) were patients with a high risk according to MRD. Their EFS rates were as follows: 100% in low-risk patients, 84±9% in intermediate-risk patients and 33±25% in patients with a high risk. Figures 55, 56 and 57 illustrate the 10-year EFS rates of ALL-BFM 95 patients according to the MRD results.

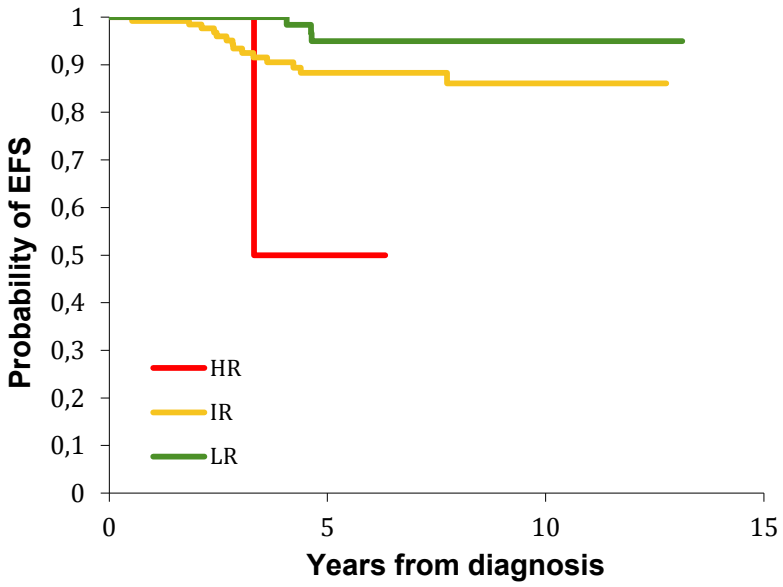

Figure 55 10-year EFS of ALL-BFM 95 SRG patients according to the MRD results (P=0,023)

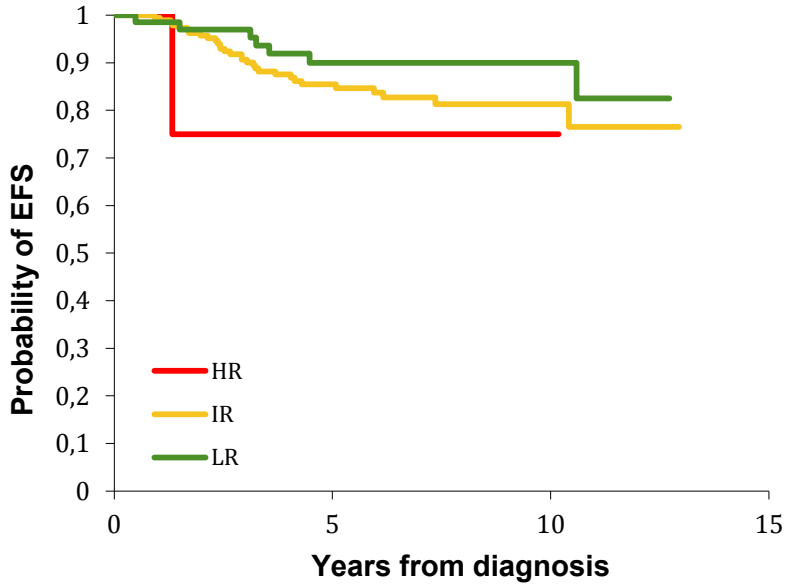

Figure 56 10-year EFS of ALL-BFM 95 MRG patients according to the MRD results (P=0,310)

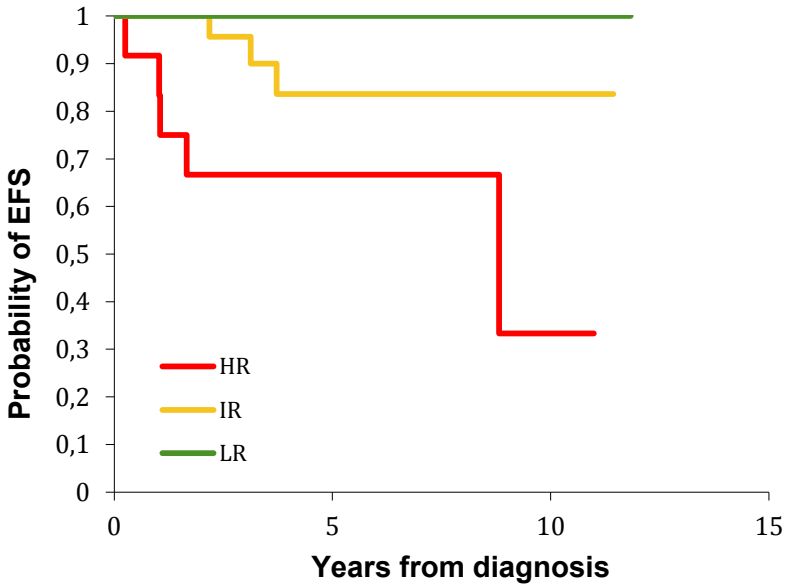

Figure 57 10-year EFS of ALL-BFM 95 HRG patients according to the MRD results (P=0,053)

2.5. Multivariate risk-factor analysis

We performed multivariate analysis to reveal whether parameters that showed a statistically significant difference in outcome in univariate analysis, have an independent influence on EFS including only the 488 patients for whom cell parameters were available.

Multivariate analysis of pB-cell ALL patients adjusting for age, *TEL/AML1* status, prednisone response, BM response on day 15 of induction treatment, remission status on day 33 of induction treatment and MRD-stratification demonstrated that BM response on day 15 and the evaluation of MRD were independent risk factors for EFS.

M3 BM on day 15 and MRD-HR emerged as statistically significant independent factors for an inferior EFS.

Due to the minor cases of events in T-cell ALL patients we were not able to perform multivariate analysis in that cohort of patients.

Table 14: Multivariate analysis for EFS of 488 pB-cell ALL patients

Parameter	P-value	Hazard-ratio	95% CI
Age			
1-10 vs. ≥ 10yrs.	0,58	1,18	0,66-2,11
TEL/AML1			
yes vs. no	0,318	1,4	0,73-2,69
Prednisone Response			
good vs. poor	0,084	0,34	0,10-1,16
BM on day 15 (vs. < 5%)	0,036		
5-25%	0,175	1,5	0,84-2,69
≥ 25%	0,011	2,63	1,25-5,54
CR on day 33			
yes vs. no	0,623	1,34	0,42-4,31
MRD (vs. LR)	0,023		
IR	0,094	1,83	0,90-3,71
HR	0,006	5,1	1,60-16,28

Abbreviations: CI: confidence interval; BM: bone marrow; CR: complete remission; MRD: minimal residual disease; LR: low-risk; IR: intermediate-risk; HR: high-risk

2.5. Discussion

Risk-directed therapy has become mandatory in the management of ALL. As response assessment by PCR proved to improve the dectection of subgroups with distinct prognosis, it has been primarly used as the main stratification factor in the ALL-BFM 2000 trial.

The ALL-BFM 2000 trial in Austria included 608 patients and we evaluated overall EFS rates after 10 years from diagnosis and in groups defined by traditionally used risk factors such as: gender, age, leukocyte count at initial diagnosis, *TEL/AML1* status, high-hyperdiploid karyotype, CNS status at initial diagnosis, pre-existing Mb. Down, immunophenotype, prednisone response on day 8, BM status on day 15 of induction therapy, status of CR on day 33 of induction treatment, MRD stratification and assignment to one of the three definitive risk groups.

In pB-cell ALL patients, the following clinical and laboratory parameters and early response features had a statistically relevant importance for outcome: age < vs. ≥ 10 years (P=0,004) with patients ≥10 years of age having an inferior prognosis, *TEL/AML1* fusion gene ± (P=0,031) with the *TEL/AML1* positive patients having a superior prognosis, BM status on day 15 (P < 0,001) with a M3 BM having a poorer outcome, status of remission on day 33 (P < 0,001) with the lack of CR being prognostically unfavorable, and assignement to the MRD group (P < 0,001) and definitve risk group (P=0,005) with a poorer outcome in the HRG.

MRD was introduced in the ALL-BFM 2000 trial as a primarily applied risk factor in order to be able to constitute three groups of patients facing a different risk of relapse, hence a different prognosis, and post-induction/early re-intensification was accordingly modified. [6,45,46] Patients being either prednisone poor responders on day 8 of induction treatment, harbouring a chromosomal translocation such as t(4;11) or t(9;22) or not being in CR on day 33 of induction therapy were treated in the high-risk group, regardless of their MRD results on day 33 and 78 of treatment.

Due to the minor falling number of MRD-stratified T-cell ALL patients (n=78) who were enrolled in the ALL-BFM 2000 trial in Austria, we were not able to analyse outcome according to the MRD levels within the respective prognostic subgroups.

In the group of pB-cell ALL patients, we evaluated the outcome according to the MRD stratification in each of the following subgroups: male and female patients; patients with initial WBC counts of either < and ≥ 20.000/µl or < and ≥ 50.000/µl; patients with < and ≥ 10 years of age; patients with a positive and negative CNS status; cALL patients, pre-B ALL patients; *TEL/AML1* positive and *TEL/AML1* negative patients; high-hyperdiploid and non-high-hyperdiploid patients; patients with and without Mb. Down; good and poor prednisone responders; patients with a M1, M2 and M3 BM on day 15; patients in CR and not in CR on

day 33 and patients stratified by ALL-BFM 95 criteria to the SRG, MRG and HRG of the previous ALL-BFM trial.

A statistically relevant difference in outcome according to the MRD results was observable in the following pB-cell ALL patients: male (P=0,001) and female (P=0,018) patients; initial WBC counts of < 20.000/µl (P=0,013), ≥ 20.000/µl (P=0,018) and < 50.000/µl (P < 0,001); patients < 10 years of age (P < 0,001); patients with a negative CNS status at diagnosis (P < 0,001); pre-B ALL patients (P < 0,001); *TEL/AML1* negative patients (P=0,0003); non-high-hyperdiploid patients (P=0,007); patients without Mb. Down (P < 0,001); good prednisone responders (P < 0,001); patients with a M3 BM on day 15 (P=0,0004); patients in CR on day 33 (P=0,004) and ALL-BFM 95 SRG patients (P=0,023). In the few remaining groups lack of statistical importance may have been due to the low number of patients and events, respectively.

The findings described above prospectively confirmed that the introduction of MRD as the sole stratification factor (at least for the SRG and MRG) in the ALL-BFM 2000 trial and the adaption of post-induction/consolidation treatment through it was successful - which marks the major achievement of the trial and the main message of this thesis.

Age and leukocyte count, i.e., are cardinal factors for risk-amendment in pB-cell ALL patients. Age is of significant prognostic merit, since patients with < 10 years of age faced a significantly better outcome (EFS-rate: 85±2%, P=0,004). MRD, however, was able to distinguish between three groups in patients < 10 years of age, varying in their risks (EFS of pB-cell ALL patients < 10 years: MRD-LR: 94±2%; MRD-IR: 84±3% and MRD-HR: 30±23%; P < 0,001). In patients with initial leukocyte counts of < 20.000/µl, ≥ 20.000/µl and < 50.000/µl MRD stratification was also able to distinguish between three groups of different risks once more, suggesting a superior value of MRD than traditional risk factors such as age and leukocyte counts.

In pB-cell ALL patients with a good prednisone response (MRD-LR: 93±2%, MRD-IR: 83±2%, MRD-HR: 53±15%; P < 0,001) and in patients with CR on day 33 (MRD-LR: 93±2%, MRD-IR: 83±2%, MRD-HR: 50±2%; P=0,004) the prognostic value and benefit of MRD crystallised again. This suggests that MRD stratification in patients with favourable parameters enables to further enhance patient's survival by subcategorising them in three distinct risk-groups.

In the 488 pB-cell ALL patients for whom all unvariately prognostically relevant parameters were available, we performed a multivariate analysis in order to reveal whether they have an independent influence on EFS and MRD distinctly emerged as a significant independent risk factor which underlines the observation that the introduction of MRD as an independent risk factor is the main achievement of the ALL-BFM 2000 study. Moreover, a distinct definition of three risk-groups facing different risks of relapse was possible in various

bioloically and clinically defined subgroups through the application of MRD. In almost all subgroups a superiority of MRD to traditionally used risk factors was observable, redefining prognostic factors in children and adolescents with ALL.

III. References

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