Registry-Based Studies in Adult Acute Lymphoblastic Leukemia in Sweden

Survival and Quality of Life

EMMA BERGFELT LENNMYR
Acute lymphoblastic leukemia (ALL), a common child malignancy, also constitutes a minor fraction of adult cancer with approximately 50 new cases per year in Sweden. While the five-year overall survival (OS) in pediatric ALL is more than 90%, the prognosis in adults is dismal. Using the Swedish ALL quality registry, this thesis investigates treatment and outcome of adult ALL according to national guidelines. In addition, the introduction of patient-reported outcome in the ALL and Acute Myeloid Leukemia registries is evaluated.

In Paper I, measurement of minimal residual disease by flow cytometry was found to be feasible but not consistently applied in the 35 patients with Philadelphia (Ph)-negative B-ALL investigated. In Paper II, treatment, toxicity and outcome of 155 patients, 55-85 years (y) with ALL diagnosis between 2005 and 2012 were studied in detail by patient charts review. An age-adopted protocol recommended from 2009 did not result in better outcome. In Paper III, disease recurrence in the same cohort as Paper II was studied. The median overall survival (OS) after ALL relapse was 3.6 months. In Paper IV, the whole ALL registry was studied and OS was estimated in 930 adult patients diagnosed in the periods 1997-2006 and 2007-2015. Five year OS improved in patients 18-45y from 50% to 65%, in patients 46-65y from 25% to 46%, and in patients >65y from 7% to 11%. This demonstrates that young patients have the best prognosis, in part due to the introduction of a dose-intense “pediatric-like” chemotherapy protocol. Compared to women, middle-aged men were found to have a worse outcome.

Historically, Philadelphia-positive (Ph-pos) ALL has a poor prognosis compared to Ph-negative ALL. In this material, the frequency of Ph-pos ALL was 34% of examined B-ALL. Analysis of the whole registry revealed that in 2007-2015, i.e. after the introduction of the tyrosine kinase inhibitor imatinib, Ph-pos ALL was no longer associated with inferior OS. In Paper V, ALL and Acute Myeloid Leukemia patients, six months after diagnosis, completed a web or paper questionnaire regarding quality of life, symptoms and experience with care. The response rate was 64%. Depression symptoms were frequent (18%), especially in young women who reported worrying about fertility.

In summary, although OS in adult ALL has improved, more effective and less toxic therapies in upfront treatment are highly warranted. Collection of patient-reported outcome in a national quality registry is feasible and can add important aspects of cancer care that are not usually addressed.

Keywords: Acute Lymphoblastic Leukemia, Philadelphia Chromosome, Overall Survival, Population-based, Quality Registry, Relapse, Patient-Reported Outcome, Health-Related Quality of Life, Depression

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“I do not wish by any means to infer that the disease in question [leukemia] is incurable; I hope on the contrary that for it too remedies will at length be discovered”

Rudolf Virchow (1821-1902)

"Utan tvivel är man inte riktigt klok”

Tage Danielsson (1928-1985)
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Contents

Introduction ...............................................................................................................................11

Background .............................................................................................................................12
    Epidemiology and Etiology .................................................................................................12
    Clinical Presentation and Diagnosis Classification ........................................................13
    Phenotypes and Genotypes in Acute Lymphoblastic Leukemia ......................................14
    Minimal Residual Disease ..............................................................................................17
    Other Prognostic Factors .................................................................................................19

Treatment and Outcome of Adult Acute Lymphoblastic Leukemia .....................................19
    A Glimpse Into History ...................................................................................................19
    Modern Therapy of Adult Acute Lymphoblastic Leukemia ...........................................20
    Supportive Care ................................................................................................................27

Swedish National Guidelines ................................................................................................28
    Diagnostic Recommendations .........................................................................................29
    Treatment Recommendations ........................................................................................29

Patient-Reported Outcome ....................................................................................................32

Aims ......................................................................................................................................33

Patients and Methods ..........................................................................................................34
    Patients in the Acute Leukemia Registries .....................................................................34
    Paper I – Minimal Residual Disease ...............................................................................34
    Paper II and III – Coverage, Comorbidities, Treatment, and Toxicity ..............................35
    Paper IV – Cytogenetic and Molecular Analysis ...............................................................35
    Paper V – Patient-Reported Outcome ..............................................................................36
    Ethical Permissions ............................................................................................................36
    Statistics ............................................................................................................................37

Results and Discussion ..........................................................................................................38
    Paper I .................................................................................................................................38
    Paper II ...............................................................................................................................39
    Paper III ..............................................................................................................................40
    Paper IV ..............................................................................................................................41
    Paper V ...............................................................................................................................43
    Limitations ..........................................................................................................................44

Conclusions ............................................................................................................................46
Future Perspectives...........................................................................................................47
Populärvetenskaplig sammanfattning på svenska .....................................................48
Acknowledgements ..................................................................................................51
References....................................................................................................................53
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Acute Leukemia</td>
</tr>
<tr>
<td>ALL</td>
<td>Acute Lymphoblastic Leukemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute Myeloid Leukemia</td>
</tr>
<tr>
<td>BCR</td>
<td>Blood Cancer Registry</td>
</tr>
<tr>
<td>BCR/ABL</td>
<td>Breakpoint cluster region-Abelson gene</td>
</tr>
<tr>
<td>BFM</td>
<td>Berlin-Frankfurt-Münster</td>
</tr>
<tr>
<td>BL</td>
<td>Burkitt Lymphoma</td>
</tr>
<tr>
<td>CAR-T</td>
<td>Chimeric Antigen Receptor T-cell</td>
</tr>
<tr>
<td>CC</td>
<td>Comorbidity Component</td>
</tr>
<tr>
<td>CCR</td>
<td>Continuous Complete Remission</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic Myeloid Leukemia</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CR</td>
<td>Complete Remission</td>
</tr>
<tr>
<td>CR1</td>
<td>First Complete Remission</td>
</tr>
<tr>
<td>CR2</td>
<td>Second Complete Remission</td>
</tr>
<tr>
<td>EFS</td>
<td>Event Free Survival</td>
</tr>
<tr>
<td>EORTC</td>
<td>European Organization for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>ETP</td>
<td>Early T-cell precursor</td>
</tr>
<tr>
<td>EWALL</td>
<td>European Working Group for Adult ALL</td>
</tr>
<tr>
<td>FAB</td>
<td>French-American-British</td>
</tr>
<tr>
<td>FCM</td>
<td>Flow Cytometry</td>
</tr>
<tr>
<td>GMALL</td>
<td>German Multicenter Study Group for Adult ALL</td>
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<tr>
<td>GvHD</td>
<td>Graft versus Host Disease</td>
</tr>
<tr>
<td>HD-Mtx</td>
<td>High Dose Methotrexate</td>
</tr>
<tr>
<td>HRQoL</td>
<td>Health Related Quality of life</td>
</tr>
<tr>
<td>HSCT</td>
<td>Hematopoietic Stem Cell Transplantation</td>
</tr>
<tr>
<td>LAIP</td>
<td>Leukemia Associated Immunophenotype</td>
</tr>
<tr>
<td>LBL</td>
<td>Lymphoblastic Lymphoma</td>
</tr>
<tr>
<td>MRD</td>
<td>Minimal/Measurable Residual Disease</td>
</tr>
<tr>
<td>MRD 1</td>
<td>Minimal/Measurable Residual Disease - first time point</td>
</tr>
<tr>
<td>Mtx</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-Hodgkin Lymphoma</td>
</tr>
<tr>
<td>NOPHO</td>
<td>Nordic Society of Paediatric Haematology and Oncology</td>
</tr>
<tr>
<td>NOS</td>
<td>Not Otherwise Specified</td>
</tr>
<tr>
<td>OS</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Ph</td>
<td>Philadelphia Chromosome</td>
</tr>
<tr>
<td>Ph-neg</td>
<td>Philadelphia Chromosome negative</td>
</tr>
<tr>
<td>Ph-pos</td>
<td>Philadelphia Chromosome positive</td>
</tr>
<tr>
<td>PRO</td>
<td>Patient Reported Outcome</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>qRT-PCR</td>
<td>Reverse Transcriptase Quantitative Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RIC</td>
<td>Reduced Intensity Conditioning</td>
</tr>
<tr>
<td>RQ-PCR</td>
<td>Real Time Quantitative Polymerase Chain Reaction</td>
</tr>
<tr>
<td>R/R</td>
<td>Primary Refractory or Relapsed Disease</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardized Mortality Ratio</td>
</tr>
<tr>
<td>SVALL</td>
<td>Swedish Adult ALL Group</td>
</tr>
<tr>
<td>TBI</td>
<td>Total Body Irradiation</td>
</tr>
<tr>
<td>TCR</td>
<td>T-cell Receptor</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine Kinase Inhibitor</td>
</tr>
<tr>
<td>TRM</td>
<td>Transplant Related Mortality</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>6-MP</td>
<td>6-Mercaptopurine</td>
</tr>
</tbody>
</table>
Introduction

With the development of magnifying lenses during the 17th century and hence the possibility to study biological material in a microscope, a new specialty in medicine – hematology – was born. Hematologists diagnose and treat blood and bone marrow disorders. In the middle of the 19th century, Rudolf Virchow (1821-1902) and John Hughes Bennett (1812-1875), independently described leukemia (white blood) as an anomalous blood disorder. In 1917, Gordon Ward published an epidemiological study that concluded that leukemia mostly affected children 0-5 years old and that there was little evidence that leukemia was indeed an infectious or hereditary disease. It was later discovered that leukemia was a malignant blood disordered sometimes curable with chemo-therapeutic agents\textsuperscript{1}.

Since the 1940s, treatment opportunities for acute leukemia (AL) have grown immensely, but has for decades been restricted to chemotherapy. Today, many old drugs are effectively used to treat leukemia, but killing fast-dividing cells comes with many side effects. Patients, clinicians and researchers have desired more precise and safe treatments, and only until recently has advances in immunological, biochemical, and genetic research rendered new possibilities to address this cancer conundrum.

This thesis evaluates treatment recommendations and outcome of adult Acute Lymphoblastic Leukemia (ALL) using the Swedish ALL Quality Registry to identify and study patients with a diagnosis since 1997.
Epidemiology and Etiology

ALL, a lethal but curable hematologic malignancy, affects children and adults with a two-tailed incidence maximum and a slight male predominance. Incidence varies between ethnic groups with being highest in Hispanics and lowest in African Americans. In children younger than 15 y, the average age-standardized incidence in Sweden is 4.1/100 000 children with a maximum at 2-4 years. In adults in Northern Europe the corresponding figure is 0.5-0.6/100 000 inhabitants reaching 1/100 000 in the seventh decade of life. In Sweden, approximately 50 new cases of adult ALL are diagnosed per year.

Although ALL has no known common etiological factor, patients with Downs’ syndrome have a 20-fold excess risk of ALL (the exact mechanism is unclear). Other familial diseases such as Li-Fraumeni syndrome (germ line mutations in the tumor suppressor gene TP53) also increase the risk of malignancies and in particular hypodiploid ALL. While ALL seldom runs in families, first-degree relatives to ALL patients have an increased risk of developing the disease (still a low life-time risk).

Experimental studies have shown that ionic radiation can cause chromosomal translocations in cells and that children exposed to irradiation in utero have an increased incidence of leukemia. In the 1950s, an excess risk for childhood leukemia was reported for survivors of atomic bombings. It was also hypothesized that irradiation caused leukemia in patients with an inherent susceptibility due to existing pre-leukemic clones. This hypothesis seems to be confirmed by recent research. Studies of twins have shown that in some cases initiating pre-leukemic genetic events can happen even in utero and then different “second hits” lead to the development of ALL. With the advances in techniques for genome, exome, and transcriptome sequencing, many genetic changes that are associated with ALL have been discovered. Some genes (e.g., ETV6, IKZF1, and PAX5 often with somatic mutations in ALL) are transcription factors involved in hematopoiesis or lymphopoiesis. In these genes, germ line alterations also seem to predispose to childhood ALL although penetrance is low and these changes only account for a small fraction of ALL cases.
Clinical Presentation and Diagnosis Classification

ALL is characterized by the rapid proliferation of monoclonal, immature blood cell precursors called blasts (Figure 1). They are under normal conditions present in a few percentages in the hematopoietic tissues of the body, predominantly the bone marrow. Because the expansion of blasts gives rise to symptoms related to the lack of a functional hematopoiesis and the insufficient production of normal blood cells, patients seek healthcare with symptoms of anemia, thrombocytopenia, leukopenia, and hyperviscosity symptoms due to leukostasis. In addition, constitutional symptoms such as night sweats, weight loss, and fever are common. ALL is also prone to extramedullary involvement of the central nervous system (CNS) (about 5% at diagnosis), lymph nodes/mediastinal mass (3-47% depending on age/phenotype), liver (15%), spleen (30%), and testis 19.

Figure 1. A bone marrow biopsy (Hematoxylin-Eosin staining) without normal architecture but almost maximal cellularity constituting of immature blast cells, further characterized with immunophenotyping as acute lymphoblastic leukemia. Courtesy of Rose-Marie Amini.

ALL diagnosis is defined by the presence of small to medium-sized blast cells with an immunophenotype typical for the disease, comprising more than 20% of the bone marrow hematopoiesis 20. The entity of bulky disease without prominent bone marrow engagement (<25%) is called lymphoblastic lymphoma (LBL) and is not discussed further in this thesis.
The first modern systematic classification of AL was the French-American-British (FAB) classification that divided ALL into three morphological subgroups. Subsequently, the introduction of monoclonal antibodies in immunohistochemistry refined the diagnostic possibilities. Using multicolor flow cytometry (FCM), hematopathologists could determine Leukemia Associated Immuno Phenotype (LAIP) to further improve diagnostic means as well as monitor minimal residual disease (MRD).

A summary of terms and phenotypes of ALL is presented in Table 1.

Table 1. Morphological and Immunophenotypic Subtypes of Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>WHO</th>
<th>FAB</th>
<th>Terms used in (older) literature</th>
<th>Overview of immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-ALL</td>
<td>L1</td>
<td>B-cell precursor ALL</td>
<td>CD19+, cCD79a+, cCD22+, TdT+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Often CD10+, sCD22+, CD24+, PAX5+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Variable CD20+ and CD34+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Co-expression myeloid ag CD13, CD33</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>Pro B-ALL</td>
<td>nTdT+</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>Common B-ALL</td>
<td>CD10+</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>Pre-B-ALL</td>
<td>c-µ chains+</td>
</tr>
<tr>
<td>T-ALL</td>
<td>L1</td>
<td>T-ALL</td>
<td>cCD3+, CD7+, TdT+,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Variable CD1a+, CD2+, CD3+, CD4+, CD5+, CD8+,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD10+, CD99+, CD34+,</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>Early including ETP-ALL</td>
<td>CD1a-, CD4-, CD8-, MPO-neg</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>Cortical T-ALL</td>
<td>Co-expression of stem cell and/or myeloid ag</td>
</tr>
<tr>
<td>Burkitt Leukemia</td>
<td>L3</td>
<td>(Mature) B-ALL Burkitt leukemia</td>
<td>TdT-, mIgM+, CD19+, CD20+, CD22+, CD20+, BCL6+,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD38+, CD77+, CD43+</td>
</tr>
</tbody>
</table>


In addition to morphology and immunophenotyping, the most frequent genetic alterations have their own cytogenetic diagnostic groups in the recently updated “WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues” 20,22. A summary of this classification is presented in Table 2.

Phenotypes and Genotypes in Acute Lymphoblastic Leukemia

The urge to divide ALL into different subgroups partly emerges from their different clinicobiological features and therefore the response to treatment leading to different long-term outcomes for different patients. Modern therapy
with risk-adapted and response-adapted protocols and targeted drugs have made correct diagnosis and classification even more important. As mentioned above, morphology was the initial classification followed by immunophenotype and then genotype. Much of the research in ALL is done on pediatric disease. Although the distribution of leukemia phenotype and genetic alterations differs between children and adults, unfavorable changes seem to be similar but more prevalent in adults\textsuperscript{23-26}. In recent years, the genetic landscape in ALL has grown immensely and full coverage is not within the scope of this thesis, but this has been reviewed by others\textsuperscript{18,27,28}.

Table 2. The WHO Classification of Acute Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>Frequency in adults</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-lymphoblastic leukemia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Otherwise Specified</td>
<td>70-85%</td>
<td>Age dependent</td>
</tr>
<tr>
<td>t(9;22)(q34.1;q11.2); \textit{BCR-ABL}</td>
<td>10-44%, Old \textsuperscript{↑↑}</td>
<td>Poor without TKI</td>
</tr>
<tr>
<td>t(v;11q23.3); \textit{KMT2A(MLL)} rearranged</td>
<td>8-15%</td>
<td>Poor in majority of cases</td>
</tr>
<tr>
<td>t(12;21)(p13.2;q22.1); \textit{ETV6-RUNXI}</td>
<td>1-3% &lt;35y</td>
<td>Good</td>
</tr>
<tr>
<td>Hyperdiploidy</td>
<td>7-13%</td>
<td>Good or depending on chromosomal gain</td>
</tr>
<tr>
<td>Hypodiploidy</td>
<td>6%</td>
<td>Poor or intermediate depending on chromosomal modal numbers</td>
</tr>
</tbody>
</table>

| Provisional entity: \textit{BCR-ABL}–like | 20-28%             | Poor                          |
| Provisional entity: iAMP21                | <1%                 | Poor if not intensively treated |
| **T-lymphoblastic leukemia**             | 5-25% Young \textsuperscript{↑↑} | Poor outcome in older         |

| Provisional entity: Early T-cell precursor | 20% of T-ALL        | Poor or intermediated depending on treatment |

| **Burkitt Leukemia**                      |                     |                               |
| (Pure) Burkitt Leukemia                   | 5-10% Old \textsuperscript{↑↑} | Good in young. Poor in old     |
| often with \textit{MYC}-rearrangement     |                     |                               |

TKI; Tyrosine Kinase Inhibitor

**B-cell Lymphoblastic Leukemia**

The majority of ALL is of B-cell origin and comprises 70-85% of cases depending on cohort\textsuperscript{4,6,25}. Immunophenotyping can determine the maturity of the leukemia. Historically, the B-ALL diagnosis was divided depending on maturity into pro B-, common B-, and pre-B ALL. The nomenclature has changed over the years and between countries, but the general term used today is B-ALL (previously B-cell precursor ALL [BCP-ALL]). The WHO classification does not separate diagnosis based on maturation but predominantly on genetic alterations (Table 2).

Philadelphia chromosome positive (Ph-pos) ALL is the most prevalent genetic subgroup in adults. The Philadelphia chromosome is a translocation of
chromosomal material between chromosome 9 and 22 and was first described in Chronic Myeloid Leukemia (CML) in the 1960s and in an adult ALL patient in 1970 29. The t(9:22) gives rise to the fusion gene BCR/ABL-1 and a constitutional active tyrosine kinase. The most often found gene transcripts are p190 and p210; p190 dominates in ALL 30,31. Additional chromosomal abnormalities are described in up to 70% of cases with inferior outcome compared to Ph-pos ALL only 32,33. Nevertheless, with the introduction of tyrosine kinase inhibitors (TKI) and allogeneic hematopoietic stem cell transplantation (HSCT) 34, the former status of Ph-pos ALL as a poor prognostic factor compared to Ph-neg disease is no longer valid 35.

The KMT2A (or mixed lineage leukemia; MLL) gene, located on the long arm on chromosome 11, has many different fusion partners described in ALL and acute myeloid leukemia (AML). The t(4;11) leading to KMT2A-AFF1, is the most common fusion partner. MLL-rearrangement is present in 75% of infant ALL (<1y), very rare in older children, and involves almost 13% of adult ALL 36. It is associated with a poor prognosis in both pediatric and adult ALL.

Although t(12;21) leading to the ETV6-RUNXI-fusion gene is prevalent in young children and associated with excellent outcome, it is extremely rare in adults and might be associated with late relapses 37.

Hyperdiploidy (i.e., chromosomal gain; modal number >50) is present in 30% of childhood ALL, in about 10% of adults, and associated with a favorable prognosis but depending on which chromosomal gain 38.

In both children and adults, hypodiploidy is associated with inferior outcome in an age-dependent manner and is associated with relapse 39. Misclassification due to doubling in chromosomal banding analysis is a diagnostic pitfall.

Another subgroup of pediatric patients with an extreme risk of relapse but rescuable with additional therapy was found to have an intrachromosomal amplification of chromosome 21 (iAMP21). This aberration is present in 3-5% of pediatric patients; although extremely rare in adults, iAMP21 is often included as part of risk stratification in childhood protocols 40.

A new, provisional entity with high prevalence (20-28%) in adolescents and adults is the BCR/ABL-like ALL with a gene expression profile similar to BCR-ABL-positive ALL but without its typical Philadelphia chromosome and fusion protein 41,42. In a recent study, 88% of adult patients with a BCR-ABL-like signature had different kinase-activating changes 43. Thus, these can be potential targetable alterations with different signal transduction inhibitors.

**T-cell Lymphoblastic Leukemia**

T-ALL, comprising 15-25% of adult ALL, is most prevalent in young males. If bone marrow involvement is less than 20%, it is classified as T-LBL. T-ALL is heterogenous disease and at diagnosis often associated with a high leukocyte count (one-third having >100x10^9/L), mediastinal mass (almost
every second young patient), and CNS involvement that is more prevalent than in B-ALL\textsuperscript{44,45}.

The immunophenotype corresponds to its thymic maturity and differentiation and is sometimes risk stratifying. Early T-cell precursor ALL (ETP) has stem cell like properties and has been associated with an impaired outcome with a slower MRD response compared to other T-ALL\textsuperscript{45,46}. Data are conflicting whether the chemotherapeutic resistance might be abrogated by allogeneic HSCT in adults\textsuperscript{47-49}. In recent years, numerous genetic alterations in both ETP and other T-ALL have been described with potential therapeutic implications, and some are risk stratifying in current protocols\textsuperscript{50-53}. There are also rare cases with typical B-ALL alterations such as Ph-pos and \textit{MLL}-rearranged T-ALL.

**Burkitt Leukemia**

In the FAB classification, the L3 morphology describes medium-sized blasts with basophilic cytoplasm and numerous vacuoles\textsuperscript{21}. In older studies and literature, L3 morphology combined with a mature phenotype is called ALL-L3 or B-ALL (i.e., mature B ALL in contrast to pro-, common, or pre-B-ALL)\textsuperscript{54}. This combination was eventually classified as a leukemic variant of Burkitt Lymphoma (BL), an aggressive lymphoma of which the majority of cases are Epstein Barr Virus-driven endemic lymphomas. Sporadic cases of BL are rare. The leukemic variant with predominantly bone marrow involvement presents clinically as acute leukemia and represents about 5% of adult ALL cases. The majority of patients have a translocation of \textit{MYC}, most often to the \textit{IGH} gene, t(8;14). The disease is highly proliferative with an extreme risk of tumor lysis syndrome, but young patients receiving modern therapy now have a favorable prognosis\textsuperscript{20,55}. In the WHO classification, Burkitt Leukemia is categorized as a part of BL and not within the ALL spectra. Burkitt Leukemia is reported to the Swedish ALL registry, so it is reviewed here.

**Minimal Residual Disease**

Minimal or Measurable Residual Disease (MRD) refers to the level of remaining leukemic blasts not visualized by conventional microscopy and staining in a bone marrow sample in complete morphologic remission. MRD is a composite marker for the susceptibility of leukemic cells to treatment in the individual patient. Thus, MRD reflects not only disease biology but also pharmaco-kinetics and dynamics.

In 1981, Bradstock et al. demonstrated low levels of residual leukemia in bone marrow with immunofluorescence of terminal deoxynucleotidtransferase\textsuperscript{56}. In the early 1990s, prospective trials found MRD measured by FCM or real time quantitative (RQ) PCR of the immunoglobulin (Ig) or T-cell-receptor (TCR) genes to be a risk factor for relapse in pediatric ALL\textsuperscript{57,58}. Minimal residual disease was shown to be a risk factor in adult ALL as well\textsuperscript{59-61}. For
abundant genomic alterations with fusion gene transcripts, such as the BCR/ABL- and MLL-rearrangement, reverse transcriptase quantitative (qRT) PCR from RNA was developed and standardized 62-64.

Later studies confirmed MRD to be an independent risk factor for relapse and death in B-, T-, and Ph-pos ALL in children and in adults65-70. Fast MRD clearance as well as later responses (1-3 month) have been reported to predict outcome 60,61,71. In adult patients without other risk features, those with an MRD of >10⁻⁴ detected after 16 weeks of treatment had a 3y relapse rate of 94%, whereas none of the MRD-negative patients had relapsed 61. Comparable data have been shown by others 69.

MRD was primarily introduced for treatment escalation in standard risk/non-Ph-pos patients 69,72. However, in a Spanish trial, investigators refrained from consolidation with allogeneic HSCT in high-risk patients with low or unmeasurable MRD post induction with encouraging results 73. In children, most modern protocols have MRD-driven risk stratification with both de-escalation and intensification depending on treatment response.

The methods for MRD assessment depend on disease biology, laboratory facilities, and available biomarkers 74,75. In addition, the majority of evidence comes from research on childhood B-ALL. In 2008, a consensus document for MRD measurement was produced to harmonize the methods and use of MRD in clinical trials 76. As the techniques have developed, the differences in sensitivity, specificity, and applicability between FCM and PCR have diminished and are now suggested to be complementary 77. Six-color FCM and RQ-PCR used for childhood B- and T-ALL analysis have shown comparable results and were concordant in 95% of cases if a cut-off of 10⁻⁴ was applied 78,79. Prompt results and the possibility to detect clonal evolution are some of the advantages with FCM, but the need for large samples of bone marrow cells and the risk, although minor, of false positive results in a regenerating marrow are drawbacks. The EuroFlow consortium (www.euroflow.org) has combined multi-color flow, new antibodies, and advanced statistics to reach applicability in more than 90-95% of cases and with a sensitivity of 10⁻⁴ to 10⁻⁵ 80. Clinical validation and standardization between laboratories and quality control are now doable 74.

PCR methods are very labor intensive due to the design of individual primers (apart from PCR of recurrent fusion-gene transcripts) and are associated with false negative results in the case of clonal evolution. The development of high throughput PCR with different techniques is ongoing. Without the need for patient specific assays, high throughput PCR can also reach even higher sensitivity, down to 10⁻⁶. Although time-consuming and requiring complex bioinformatic processing, these methods may soon be a part of routine clinical practice 81-83.
Other Prognostic Factors

Many clinical prognostic factors are not predictive after the introduction of risk-directed and response-directed therapy with improved MRD measurements and genetic profiling. In addition to primary resistant disease/late CR, age is a negative prognostic factor in almost all studies. Age is a marker of more high-risk leukemia, intolerance to therapy, and probably other biological mechanisms. A high WHO performance status (WHO-PS) has been correlated to induction death and therefore the possibility to reach complete remission (CR) and are even with modern response directed therapy sometimes predictive. Thrombocytopenia has been associated with inferior OS. Phenotype (T-cell) is a high-risk feature in many protocols, sometimes depending on maturity. Historically, central nervous relapses have been a problem but with high doses of CNS-penetrating drugs and intrathecal prophylaxis diminished. With modern therapy, CNS recurrence can be prevented without cranial irradiation. However, CNS disease at diagnosis has been a negative prognostic factor associated with impaired OS and it is often treated with irradiation in addition to intensified intrathecal therapy.

Treatment and Outcome of Adult Acute Lymphoblastic Leukemia

A Glimpse Into History

During the second world war, both American and British researchers explored the effect of nitrogen mustard gases on hematopoiesis. Further research resulted in the development of alkylating agents such as busulphan, which are active in many hematological malignancies. In parallel, folic acid was found to be important for hematopoiesis. In 1948, Sidney Farber published the first paper from a clinical trial on 16 children with acute leukemia who received the folic antagonist aminopterin of which 10 reached a clinical, hematological, and pathological remission. Aminopterin was later replaced by methotrexate and during the 1950s methotrexate’s (Mtx), corticosteroids’ and 6-mercaptopurine’ (6-MP) effects on lymphocytes were discovered. Also, the Vinca alkaloid vincristine was found to have anti-tumor activity in lymphatic tumors. Combinations of these new drugs were subsequently used in treatment of childhood leukemia.

The initial course of chemotherapy, still called induction, aimed to eradicate most of the malignant cells and induce a first complete remission (CR1). Because most patients relapsed, consolidation therapy was needed to establish disease control. Sometimes the disease was resistant to initial therapy or
resistant at the time of relapse. The first reports of successful treatment of ALL in two patients with advanced disease using total body irradiation (TBI) and bone marrow transplantation, were published 1970\textsuperscript{98,99}. However, the two patients succumbed due to cytomegaly virus infection and due to relapse of disease, respectively.

It became obvious that young children reaching remission of ALL also had a high risk of CNS relapse, so prevention methods were needed. Later, it was discovered that craniospinal irradiation could be avoided provided that high-dose systemic chemotherapy was supplemented with intrathecal administration of Mtx and/or cytarabine and corticosteroids\textsuperscript{94,100,101}. Based on preclinical data, a randomized study also found that systemic high-dose dexamethasone compared to prednisolone decreased the risk of CNS relapse, but was also found more toxic\textsuperscript{102,103}. In a later study dexamethasone during induction was beneficial only in a subgroup of T-ALL\textsuperscript{104}.

Another important drug explored was the enzyme asparaginase as it depletes tumor cells on the amino acid asparagine, reducing the risk of relapse, especially during consolidation/intensification\textsuperscript{105}. Numerous studies have later focused on different types of asparaginase and routes of administration.

It was also discovered that maintenance with low-dose chemotherapy was a cornerstone in relapse prevention and many studies with various regimens were conducted. In the 1990s, a Childhood ALL Collaborative Group summarized 42 recent trials of ALL maintenance and reinduction. It was concluded that intensive reinduction therapy reduced the risk of relapse and that a couple of years of low-dose maintenance was required but sufficient\textsuperscript{106}. In pilot studies from the 1970s, the Berlin-Frankfurt-Münster study group (BFM) of childhood ALL reported Event Free Survival (EFS) of 55% that improved to 78% in studies 20 years later\textsuperscript{107}.

In adult ALL, one of the first, large prospective trials included patients 15-55y and reported a remission frequency of 78% and in the lowest risk group an overall survival (OS) at 3y of almost 60%\textsuperscript{108}. Treatment advances in childhood ALL were adopted for adults, and the large study groups in Europe, US, and other parts of the world have continued together towards more efficient and less toxic treatment of adult ALL.

Modern Therapy of Adult Acute Lymphoblastic Leukemia

Modern therapy for adult patients with ALL is not easily defined. Many of the efficient therapeutic drugs, described above and introduced in the 1950s, 1960s, and 1970s are still in use although in different combinations and doses. Today, remission rates of >90% are common, but disease recurrence has been, and still is the major cause of death. To reduce the risk of relapse, high-dose chemotherapy with autologous stem cell rescue or “immunological rescue” with allogeneic HSCT was introduced. Following the introduction of risk- and response-adapted protocols and in the last two decades with “pediatric
inspired” multiagent therapy followed by maintenance, the frequency of HSCT in CR1 has become very low in children and probably diminishing also in young adults. An overview of drugs commonly used in international treatment protocols is displayed in Table 3 including some new drugs currently on their way into the clinic.

Table 3. Drugs common in treatment of acute lymphoblastic leukemia

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Use</th>
<th>Major side effects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparaginase</td>
<td>Enzyme cleaving asparagine leading to depletion in tumor cells</td>
<td>Induction and consolidation</td>
<td>Pancreatitis, Thrombosis, Dyslipidemia, Allergic reactions</td>
</tr>
<tr>
<td>Cytarabine</td>
<td>Antimetabolite, inhibit DNA elongation and repair</td>
<td>High systemic doses as CNS-prophylaxis. Intratechial</td>
<td>GI-tract, keratitis, Cerebellitis, “Ara-C syndrome”</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>Alkylation agent, cross links DNA</td>
<td>Induction and conditioning regimens</td>
<td>Hemorrhagic cystitis, SIADH, Secondary malignancies</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Broad Tyrosine Kinase Inhibitor</td>
<td>(R/R) Ph-pos disease continuously</td>
<td>Edema and pleural effusions, GI-tract</td>
</tr>
<tr>
<td>Doxo/Daunorubicin</td>
<td>Anthracyclines, disrupting DNA synthesis</td>
<td>Induction and consolidation</td>
<td>Heart Failure</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Topoisomerase II inhibitor, DNA cannot unwind</td>
<td>Consolidation, relapse and conditioning</td>
<td>Secondary malignancies</td>
</tr>
<tr>
<td>Fludarabine</td>
<td>Antimetabolite, purine analogue</td>
<td>Consolidation, Remission, conditioning</td>
<td>Long term T-cell depression</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Tyrosine Kinase Inhibitor</td>
<td>Ph-pos, BCR/ABL-like disease</td>
<td>Liver, Gl. Interaction with Asparaginase</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Antimetabolite, antifolate</td>
<td>HD in consolidation and po maintenance</td>
<td>Mucositis, Renal, CNS, liver</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Anthracycline and Topoisomerase II inhibitor</td>
<td>Consolidation or relapse</td>
<td>Less cardiotoxicity, Secondary leukemia</td>
</tr>
<tr>
<td>Nelarabine</td>
<td>Antimetabolite</td>
<td>R/R of T-ALL CD20 Mab</td>
<td>Neurotoxicity</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Chimeric anti CD-20 Mab</td>
<td>CD20-pos B-ALL</td>
<td>Decreased number of B-cell, Hypogammaglobulinemia,</td>
</tr>
<tr>
<td>Steroids</td>
<td>Glucocorticoid, lympholytic in high doses</td>
<td>Induction, consolidation and maintenance</td>
<td>Numerous; diabetes, osteoporosis etc.</td>
</tr>
<tr>
<td>Vincristine</td>
<td>Vinca alkaloid, disturbs microtubuli</td>
<td>Induction, consolidation and maintenance</td>
<td>Neuropathy, obstipation</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>Antimetabolite</td>
<td>Consolidation and maintenance</td>
<td>GI and liver. Depend-</td>
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### New therapies on their way into routine care

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Use</th>
<th>Major side effects*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blinatumomab</strong></td>
<td>CD19/CD3 bi-specific Mab</td>
<td>R/R B-ALL</td>
<td>TLS, CRS, neurotoxicity, hypogammaglobulinemia</td>
</tr>
<tr>
<td><strong>Inotuzumab-Ozogamicin</strong></td>
<td>CD22 Mab coupled toxin</td>
<td>R/R B-ALL</td>
<td>TLS, Liver toxicity including SOS after HSCT</td>
</tr>
<tr>
<td><strong>Ponatinib</strong></td>
<td>Broad tyrosine kinase inhibitor active also in T315I-mutated cells</td>
<td>R/R Ph-pos ALL</td>
<td>CVE, Pancreatitis</td>
</tr>
<tr>
<td><strong>Tisagenlecleucel</strong></td>
<td>CAR-T cell</td>
<td>R/R B-ALL &lt;25y</td>
<td>CRS, TLS, neurotoxicity</td>
</tr>
</tbody>
</table>

*Excluding myelosuppression.

ALL; Acute Lymphoblastic Leukemia, CAR-T; Chimeric Antigen Receptor T-cell, CNS; Central Nervous System, CRS; Cytokine Release Syndrome, CVE; Cardiovascular Events, HD; High Dose, Mab; Monoclonal antibody, Ph; Philadelphia, R/R; primary refractory or relapsed, TLS; tumor lysis syndrome, TPMT; 6-Tiupurine methyltransferase, SIADH; Syndrome of Inappropriate Secretion of Antidiuretic Hormone, SOS: Sinusoidal Obstructive Syndrome

### Population-based Outcome in Adult ALL

The great achievements in treatment outcome and long-term survival of childhood ALL are without parallel. A mortality ratio of 85-90% has been reversed to >90% long-term survival. However, most published data on adult ALL are from studies performed in referral centers and therefore population-based results are of great interest.

In a population-based cohort of Danish patients with ALL diagnosed between 1998 and 2008 (15-91y, median 47y), OS in the total cohort at 5y was 34%. In patients 15-35y and 36-65y with curative treatment, 5y OS was 51% and 39%, respectively. No patient older than 65y survived 5y after diagnosis. In a more recent population-based registry study from the Netherlands, OS had improved over the last decades in all age-groups except in patients over 70y. In patients diagnosed with B- and T-ALL between 2007 and 2012, OS at 5y in the age groups 18-24y, 25-39y, 40-59y, 60-69y, and ≥70y were 75%, 57%, 37%, 22%, and 5%, respectively. None of the patients over 70y were included in a clinical trial. Different registry analyses demonstrate equal trends with modest to great improvements in younger patients but almost none in older patients (>70-75y). In Ph-pos ALL, the introduction of TKI has change this and Ph-pos ALL now has similar or better prognosis than Ph-neg ALL, even in older patients.
B and T Acute Lymphoblastic Leukemia

As mentioned above, treatment of adult ALL has evolved from pediatric research and further developed in different parts of the world although no consensus has been established. Since the mid 1980s, in the US most adult ALL patients are treated according to the HyperCVAD protocol. This protocol is based on a total of eight courses of chemotherapy consisting of fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone, alternating with high dose cytarabine (3g/m²) and intermediate dose of Mtx (1g/m²), followed by delayed intensification and maintenance. Today, this treatment is modified according to age and subgroup of ALL diagnosis. In B-ALL, one modification includes the addition of rituximab in CD20+ ALL because of its previous adverse outcome.

In Europe and other parts of the world, different ALL groups have run their own studies with different protocols. In the 1980s, risk-adapted therapy was proposed. Simultaneously, autologous and allogeneic HSCT as consolidation therapies were introduced. Initially, allogeneic HSCT was offered to all patients with a sibling donor, but this strategy proved more efficient in high-risk patients at the time constituting predominantly of Ph-pos ALL. On the contrary, when Ph-neg ALL were randomized depending on donor availability, the advantage of HSCT was seen only in standard risk leukemia and that chemotherapy with maintenance was better than autologous HSCT. With the introduction of MRD assessment, it was noted that standard risk, Ph-neg ALL patients with a low MRD (<10⁻⁴) had a very favorable prognosis without allografting. On the other hand, if MRD persisted, patients took advantage of allogeneic HSCT. This was later shown also for high risk Ph-neg ALL.

Most T-ALL patients are initially treated as B-ALL patients. The CR rate and prognosis have been similar to B-ALL in recent years, although immature phenotype has been associated with adverse prognosis. The trend of poorer outcome in patients over 35y (or 50y) remains as well. In a recent publication of patients 1-45y treated according to NOPHO ALL 2008, ETP was associated with slower MRD response and therefore treated with more intensive chemotherapy but without inferior outcome compared to the total cohort, a finding confirmed by others. Most relapses of T-ALL disease occurred early within one year of diagnosis and the prognosis in case of relapse was dismal with isolated patients surviving and none surviving allogeneic HSCT in second CR (CR2). Nelarabine, a T-cell specific purine nucleoside analogue, has been introduced in relapsed T-ALL/LBL, but in upfront treatment this strategy has not yet proved to be more efficient than traditional treatment.
For both B- and T-ALL in Europe, the last two centuries of protocol development in younger patients has been intensification of treatment but with the goal to evade allogeneic HSCT. For older patients, protocol development has been adjustment of doses, reduced intensity conditioning regimens in case of allogeneic HSCT, and introduction of new compounds in upfront therapy.

**Pediatric-inspired Therapy**

One of the reasons for improved outcome in younger patients is thought to be the application of pediatric-inspired regimens. These protocols allocate patients to different treatment intensities depending on predefined risk factors and after response to initial treatment assessed by MRD monitoring. They also include high doses of conventional ALL drugs such as steroids and vincristine. The use of asparaginase, often already during induction, and intensification with high dose (HD) Mtx (3-5g/m²) are other key features. In low-risk groups, de-escalation is performed for myelotoxic and cardiotoxic drugs such as anthracyclines. Maintenance phase extends to more than two years from diagnosis and HSCT is rarely recommended in CR1.

Substantial improvement was seen when younger adults with Ph-neg B- and T-ALL were treated according to these protocols, especially up to the age of 25 but was also feasible up to 35y, 40y, 45y, 50y, 55y, or even 65y with dose reductions 110,128-138. However, optimal handling of toxicity and how to treat the increasing proportion of elderly patients with high-risk disease remains problematic 26. In adults, the incorporation of asparaginase during induction requires precautions, especially if concomitant imatinib is administered 139,140. In addition, some adult protocols have kept allogeneic HSCT in CR1 in case of different high-risk criteria such as MLL rearrangement 26.

**Philadelphia Positive Acute Lymphoblastic Leukemia**

Historically, Ph-pos ALL has been associated with a miserable prognosis because of disease recurrence leading to short median OS and few patients surviving three years 30,141. The only chance for long-term remission was with allogeneic HSCT 34,119,142,143. With the introduction of TKI, prognosis has improved 35,144-150.

Imatinib (first generation) and dasatinib (second generation) are the most commonly used TKIs in ALL. Imatinib is the only drug registered in upfront therapy of adult Ph-pos ALL in Sweden. Available TKIs have different mechanism of actions and therefore efficacy depending on mutation in the tyrosine kinase. They also have distinctive side effects depending on their respective off-target consequences, but imatinib has the narrowest kinase inhibitor spectrum. TKI has been used in monotherapy with steroids, on top of standard protocols or with reduced intensity chemotherapy in ALL 24,147,151-153. No head-to-head comparison of different TKI in upfront therapy has been published, but a recent review suggests that the third generation TKI, ponatinib, which is the only one effective in T315I mutations, might lead to improved long-term
outcome. ALL patients often harbor TKI-resistant sub clones at diagnosis and develop resistance during treatment more frequently than CML patients. Due to poor CNS penetration of imatinib, prophylaxis with intrathecal chemotherapy is essential to prevent relapses. Dasatinib has been effective in CNS relapses but failed to prevent it, at least in retrospective analysis. Outcome after autologous and allogeneic HSCT has been similar in Ph-pos ALL in the TKI era, but patients were few, data were analyzed retrospectively, and follow-up has been relatively short. Most study groups still recommend allogeneic HSCT in CR1 in eligible patients but in recent years, this approach has been questioned. Following the use of a TKI-based protocol, Ph-pos patients (n=51) in complete molecular remission (absence of detectable transcript <0.01%) at three months from diagnosis had an excellent outcome with a 4y OS of 66% without allografting. Post allografting, both prophylactic and MRD-driven TKI treatment have been used. One small randomized study found no difference in OS between the two options. As TKI has immunomodulatory effects, a lower risk of GvHD has been seen in patients receiving TKI post HSCT, and this is one of the reasons why many centers give TKI prophylactically for one or two years.

**Burkitt Leukemia**

Previously, patients with “mature B-ALL”, which is now classified as Burkitt Leukemia, were included in ALL studies. In the 1980s, however, patients who were treated as if their diagnoses were Burkitt Lymphoma exhibited improved results compared to those treated with traditional ALL protocols. Long-term follow-up in a recently published study with intensive courses of chemotherapy with rituximab for Burkitt Leukemia and Lymphoma showed an excellent CR rate of 88%. In patients 26-55y and ≥55y, OS at 5y was 84% and 62%, respectively, and the treatment (dose-reduced) was well tolerated even in older patients. Similar results were reported from an Italian group.

**Autologous and Allogeneic HSCT**

The above section discusses indications for HSCT in relation to the different subgroups of ALL. In younger patients, the overall principle is to avoid relapse by eradicating residual disease with high doses of chemotherapy, total body irradiation (TBI), and stem cell rescue. Earlier studies could not show that autologous HSCT was more effective than chemotherapy with maintenance, although its use in Ph-pos ALL is still being investigated.

Allogeneic HSCT was the first (chemo-radio) immunotherapy for ALL. The graft versus leukemia effect is potentially curative, whereas its counterpart graft versus host disease (GvHD) can be lethal. The reduced risk of relapse and the transplant related mortality (TRM) often counterbalance each other with respect to OS.
Regarding donors, all available options are applicable in ALL. A recent retrospective analysis found that patients with haploidentical donors had similar outcome to matched unrelated donors\(^\text{167}\). In numerous retrospective analyses, TBI regimens have been superior to those based on chemotherapy alone\(^\text{159,168}\). Etoposide has been more effective in reducing the risk of relapse than cyclophosphamide, but its toxicity means it is only recommended in younger adults\(^\text{169,170}\). For older patients, reduced intensity conditioning (RIC) is an option\(^\text{166,171,172}\). Prophylaxis of GvHD and post-HSCT immunomodulation are usually given according to local routines and/or international guidelines.

**Refractory or Relapsed Acute Lymphoblastic Leukemia**

Primary refractory/relapsed (R/R) ALL in adults has a dismal prognosis. In studies from the late 1990s, 5y OS after relapse was as low as 7\%\(^\text{173,174}\). In later cohorts of Ph-neg ALL patients treated more intensively, OS improved to 25\% at 3y, and even higher in younger patients reaching CR2 after first salvage\(^\text{175,176}\). In a retrospective analysis from the Swedish ALL registry, similar figures for best outcome were found for young patients (<35y) allografted in CR2\(^\text{177}\). Relapse regimens have varied from single drugs to multidrug re-induction with none accepted as standard of care.

Suddenly, there seems to be light at the end of the tunnel, at least for B-ALL. The toxin coupled inotuzumab ozogamicin has entered the arena for CD22-positive leukemia\(^\text{178}\), and the bispecific antibody blinatumomab activates T-cells for lysis of CD19-positive cells, which are the majority of B-cells\(^\text{179}\). In monotherapy or together with chemotherapy or TKI, they have rendered impressive CR rates as salvage regimens in young patients and as upfront therapy in older patients\(^\text{180-182}\).

In addition to the third generation TKI in Ph-pos ALL\(^\text{149}\), the chimeric antigen receptor (CAR) T-cells were the major break-through of the last decade, at least for patients <25y\(^\text{183,184}\). In desolate cases CAR-T cell therapy has led to long-term remission in CD-19 positive B-ALL. Because toxicity, above all cytokine release syndrome, is an unsolved problem, CAR-T cells are hitherto only registered for children and young adults. Their role in up-front therapy is now being explored in clinical trials.

In T-cell ALL, nelarabine has been an effective treatment for R/R disease but is associated with dose-limiting neurotoxicity\(^\text{125,126}\). Compounds effective in multiple myeloma like bortezomib and daratumumab are also studied\(^\text{185,186}\).

**Elderly Patients**

Patients not eligible for intensive chemotherapy or allogeneic HSCT are often classified as elderly\(^\text{187}\); however, different studies use different ages for defining the cut-off age for classification of elderly. Historically, older/elderly patients with ALL are rarely included in randomized studies as co-morbidities and intolerance to chemotherapy make study design a challenge\(^\text{188}\). Efforts
have been made to adjust treatment protocol for elderly ALL\textsuperscript{146,189-191}. In 2002, the European Working Group for Adult ALL (EWALL) was established and a chemotherapy backbone protocol for adult ALL was suggested\textsuperscript{192}. To date, the most promising results of this backbone have been seen in Ph-pos ALL. The first study with the addition of dasatinib to the backbone in 71 patients with a median age of 69y led to a CR rate of 96\% and 5y OS of 36\%\textsuperscript{152}.

With the introduction of immunotherapies upfront, better survival is expected also in older patients with Ph-neg disease. The first report from reduced chemotherapy together with inotuzumab ozagamicin are promising. In 52 patients with Ph-neg B-ALL with a median age of 68y, 2y and 3y OS was 66\% and 56\%, respectively\textsuperscript{180}. For elderly patients with T-ALL, opportunities are still few although new compounds such as gamma secretase inhibitors are in clinical studies\textsuperscript{193}.

\textit{Comorbidity Assessment}

In addition to age-adapted therapy, different methods of comorbidity assessment have been used to evaluate elderly patients. Yet, none of this methods have been found to have a major impact on clinical decision making or outcome. In part, this lack of confirmation can be explained by the high mortality in leukemia itself in older patients. The Charlson Comorbidity Index, weighted for chronic disease and age\textsuperscript{194}, has been used in studies of older ALL patients but has not been associated with outcome in multivariable analysis\textsuperscript{88,195}. In an AML registry study, comorbidities were not associated with short term mortality\textsuperscript{196}. However, in another AML study, diabetes, if investigated separately, was associated with increased 30-day mortality\textsuperscript{197}.

In addition to comorbidity scores, geriatric assessment is sometimes suggested\textsuperscript{187} and this predicted OS in one AML trial\textsuperscript{198}. In lung cancer, for example, geriatric assessment spared frail patients toxicity as the clinician withdrew chemotherapy in some patients\textsuperscript{199}.

Nevertheless, the use of geriatric assessment in leukemia care is not well defined but in the transplantation setting, it is valuable to predict non-relapse mortality. Sorror \textit{et al}. developed a comorbidity index that can help clinicians and patients in the discussion and risk assessment of allogeneic HSCT when comparing other options\textsuperscript{200,201}.

\textbf{Supportive Care}

A cornerstone in the treatment of ALL is supportive care. Although outside the scope of this thesis, some remarks can be made.

At diagnosis, tumor lysis syndrome has been associated with impaired outcome and can now be prevented with allopurinol or rasburicase in high-risk patients – i.e., patients with large tumor burden or highly proliferative disease\textsuperscript{202}.  

27
Many of the drugs delivered when treating ALL have different side effects (Table 3). These side effects increase as patients age or have comorbid conditions. Challenges include HD-Mtx and impaired renal function, steroids and diabetes, vincristine in the case of polyneuropathy and anthracyclines that can lead to cardiotoxicity. Pegylated asparaginase is associated with specific early side effects such as coagulopathy and therefore thrombosis and bleeding, pancreatitis, hypertriglyceridemia, and hepatotoxicity accentuated in adults. Thrombosis prophylaxis has not been extensively studied in adults. In a large, randomized, trial, low molecular weight heparin prophylaxis or antithrombin replacement during induction in patients 7-18y were associated with a lower incidence of venous thromboembolism compared to a low-dose heparin-control arm. However, replacement of antithrombin did not result in fewer thromboembolic events in a small study of adult ALL. In summary, results indicate that thrombosis prophylaxis is safe and probably effective and thus could be suggested in adult patients, at least during induction.

Although patients still die from infections from bacteria, viruses, or invasive fungal diseases, therapeutic opportunities have expanded, except for patients with multi-resistant bacteria. During ALL treatment, antiviral prophylaxis is given to prevent reactivation of varicella and herpes viruses. Broad spectrum antibiotics with *Pseudomonas* activity in case of neutropenic fever are essential for preventing septicemia, and many centers provide oral prophylaxis during neutropenic phases. Prophylaxis against fungal infections is under debate and has been rarely studied, but is often recommended in block-based intensive induction regimens for high-risk disease. The interaction of azoles with vincristine is a pitfall that needs further attention. Prophylaxis against *Pneumocystis jirovecii* is generally recommended in ALL patients.

Supportive care of ALL patients after allogeneic HSCT does not diverge from other indications.

**Swedish National Guidelines**

Sweden is a large country with a small population (10 million inhabitants). Because Sweden is very rural, patients with ALL are treated at numerous centers across the country. To harmonize treatment, the Swedish Adult ALL group (SVALL) was created in 1984. The group consists of at least one member from each of the six health care regions in Sweden. The group gives recommendations about how to diagnose and treat adult ALL patients of different ages with B-, T-, or Burkitt Leukemia and with different performance status. The first guidelines were published in 1987 and are updated approximately every three years. The guidelines also include recommendations about investigation at diagnosis and follow-up and advice about palliative care.
Diagnostic Recommendations

Investigation at diagnosis has naturally improved since the first guidelines in 1987. A summary of the recommendations over the studied period in this thesis (1997-2015) is presented here.

Initially, hematopathological analyses were decentralized. With the introduction of the NOPHO ALL 2008 protocol for adult ALL in Sweden, university hospital laboratories analyzed most diagnostic samples from patients treated with curative intention. Diagnostic recommendations included bone marrow morphology with immunophenotyping including LAIP for MRD monitoring and PCR in case of T-cell disease or specific transcripts. Recommended cytogenetics included conventional karyotyping, fluorescent in situ hybridization for at least $KMT2A/MLL$ rearrangement, and $BCR-ABL1$ translocation, and PCR for $BCR-ABL1$ for all patients with remission inducing intention. After the introduction of imatinib, Ph-status was recommended in all patients regardless of age and treatment intention. In recent years, more advanced cytogenetic techniques have been used (micro arrays etc.).

CNS disease was evaluated with lumbar puncture (with intrathecal Mtx) and radiology in case of overt symptoms. Chest X-ray was also recommended in all patients and further radiology in case of T-ALL or clinical suspicion of extramedullary disease.

Minimal Residual Disease in the National Guidelines

In 2003, the SVALL group introduced MRD monitoring in the guidelines. Because of the NOPHO ALL 2008 study, six laboratories in Sweden used an FCM-accredited method with four and, from 2008, six-color FCM. MRD by multicolor FCM was recommended for B-ALL, PCR of the TCR for T-ALL, and qRT-PCR for Ph-pos ALL. MRD monitoring was recommended after remission induction and after each consolidation as well as before allogeneic HSCT and during follow-up.

Treatment Recommendations

As the ALL incidence in Sweden is not high enough to enable randomized trials within a reasonable timeline, the SVALL group has collaborated with international groups to cover the spectrum of ALL diseases. Such collaborations were formed with the Nordic Society of Paediatric Haematology and Oncology (NOPHO) group, the EWALL group, and with the German Multicenter Study Group for Adult ALL (GMALL).
### Table 4. Overview of treatment recommendations, adapted from Paper IV

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<tbody>
<tr>
<td><strong>Age group →</strong></td>
<td>Remission inducing therapy intended regardless of age</td>
<td>18-45y</td>
<td>45-60/65y</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td><strong>Treatment protocol</strong></td>
<td><strong>B-ALL, Ph neg</strong></td>
<td><strong>B-ALL, Ph, pos</strong></td>
</tr>
<tr>
<td>ABCDV</td>
<td>ABCDV</td>
<td>NOPHO 08</td>
<td>ABCDV</td>
</tr>
<tr>
<td>ABCDV</td>
<td>ABCDV + imatinib*</td>
<td>ABCDV + imatinib</td>
<td>ABCDV + imatinib</td>
</tr>
<tr>
<td>ABCDV</td>
<td>HCVAD</td>
<td>NOPHO 08</td>
<td>ABCDV</td>
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<tr>
<td>***</td>
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</tbody>
</table>

ALL; Acute Lymphoblastic Leukemia, CHOP; Cyclophosphamide, Hydroxydaunorubicin, Oncovin, Prednisone, Ph; Philadelphia chromosome, Vcr; Vincristine, GL; Guidelines, The protocols NOPHO, EWALL, GMALL, HCVAD, NHL-BFM, NOPHO 08; See main text.

* Initially, only given to patients with resistant disease or persistent minimal residual disease after allogeneic stem cell transplantation. Gradually introduced upfront and recommended in all Philadelphia positive patients from 2007.

** Reduced courses of CHOP (75%), VAD, or Vincristine+steroids

*** Six injections of intrathecal methotrexate as CNS prophylaxis. No prophylactic irradiation. Patient treated according to NOPHO-2008/NHL-BFM90 or GMALL-B-ALL/NHL2002 followed the protocol.

References in main text.

The Swedish chemotherapy regimen ABCDV/VABA/BCDE was the main remission inducing protocol for B-ALL and T-ALL between 1997 and 2015 \(^{214}\). This regimen is an intensive block-based protocol with betamethasone as steroid backbone and high doses (3g/m\(^2\)) of cytosine arabinoside for central nervous system penetration. In addition, the regimen includes treating all patients with amsacrine and etoposide but does not include asparaginase or HD-Mtx in induction or early consolidation. Accordingly, it differs from international protocols; however, it resulted in similar results as international protocols at the time \(^{214}\). High-dose methotrexate and asparaginase were added in a high-risk consolidation arm and since 2012, rituximab was added in case of CD20-pos B-ALL. In patients not allocated to allogeneic HSCT, reinductions and
two years of maintenance are provided. No prophylactic irradiation is included in the regimen.

Between 2003 and 2009, the Hyper-CVAD protocol was recommended for T-ALL, but due to poor results this suggestion was withdrawn in 2009. For younger adults 18-45y with Ph-neg ALL, the pediatric NOPHO ALL 2008 protocol was, after a pilot study, introduced in the guidelines as standard of care from 2009. For clinically unfit or elderly patients, the EWALL backbone protocol was introduced. Since October 2009, this protocol was recommended for patients older than 55-60y or not eligible for allogeneic HSCT in the case of high-risk disease. For patients older than 75y, intensive palliation with different low-dose chemotherapy courses such as CHOP or VAD was suggested.

Since 1995, Ph-pos disease has been regarded as high-risk leukemia and allogeneic HSCT has been recommended in CR1. As imatinib became available for Ph-pos ALL, it was initially introduced in resistant disease or at relapse and then gradually introduced upfront and officially included in the guidelines from 2007. Imatinib was added to the chemotherapy backbone of choice or given in addition to steroids and low dose chemotherapy in elderly patients.

Since 2007, the Non-Hodgkin Lymphoma (NHL)/BFM-90 protocol for Burkitt Leukemia was replaced by the GMALL-B-ALL/NHL protocol. In 2009, this protocol was modified with the addition of rituximab and adjustment of doses for elderly.

High-risk Criteria and Allogeneic Stem Cell Transplantation

In patients treated according to ABCDV or Hyper-CVAD protocol, high risk criteria were: Ph-pos ALL, t(4;11)/MLL-rearrangement, leukocyte count of >30x10⁹/L (B-ALL) >100x10⁹/L (T-ALL), and CR after more than one course of chemotherapy. Patients with a high-risk criterion or in later guidelines MRD of ≥1.0% after remission induction and/or not reaching <0.1% after consolidation were recommended allogeneic HSCT in first CR. Since 2009, T-ALL was considered a high-risk disease as well. Allogeneic HSCT was done according to local routines including condition regimens (although suggested to be myeloablative and TBI-based in young patients) and GvHD prophylaxis. If no donor was found, autologous stem cell transplantation was optional although seldom performed.

Recommendations at Relapse

During the study period, different regimens at relapse were advocated including reinduction with previously used protocol in case of late relapse. Type of chemotherapy used was studied in a previous publication. Mutational analyses and change of TKI were suggested for cases with resistant or relapsed Ph-pos ALL.
Patient-Reported Outcome

When leukemia care goes from surviving to survivors, the cost of treatment needs to be addressed, and not only by health economists. Patient-reported outcome (PRO) of quality of life (QoL), physical and psychical function, symptom burden, and even adverse events are questions that need to be further studied. The European Organization for Research and Treatment of Cancer (EORTC) tries to standardize PRO in clinical trials and the Federal Drug Administration recommends how to use PRO in study design. For acute leukemia since the 1990s, Health-related QoL (HRQoL) has been measured in different studies, especially after allogeneic HSCT. To my knowledge, PRO has not been included in a population-based quality registry.

The Swedish Blood Cancer Registry (BCR) decided in 2012 to incorporate PRO in the registry and started with a pilot project in the AML and ALL registries (two of eight diagnose-specific registries in the BCR). The background to this introduction is described here.

PRO can be collected by different means, but the typical method is to use questionnaires or telephone interviews. For health economic analysis, generic instruments such as the Short Form 36 (SF-36) and The EuroQol-group five-dimension questionnaire (EQ-5D™) can be used, but these are not suited for evaluation of PRO in the individual patient. Therefore, disease-specific instruments as well as procedure-designed questionnaires have been developed. In the BCR, different diseases are included and therefore a generic but malignancy-specific instrument for HRQoL was sought. In clinical oncology research, the EORTC Cancer Quality of Life Questionnaire Core 30-item (EORTC QLQ-C30) or the questionnaire Functional Assessment of Cancer Therapy-General version are generally recommended. These two instruments were also the most frequently used in hematological research.

As depression has been associated with impaired outcome after allogeneic HSCT, we were also interested in assessing depressive symptoms in addition to HRQoL. The Patient Health Questionnaire scale (PHQ-8) has ≥80% sensitivity and specificity to identify patients with major depression and has been evaluated in cancer patients.

Despite all the knowledge about PRO in hematological care, interventional studies are uncommon and how they can improve given care remains to be established.
Aims

The aim of this thesis was to study clinical characteristics, outcomes, and treatment recommendations for adult ALL in a population-based setting using the Swedish ALL Registry. In addition, the introduction of patient-reported outcome in the ALL and AML registries was evaluated.

The specific aims were

- to investigate the use of MRD in clinical practice in patients with Ph-neg B-ALL treated according to the Swedish ALL protocol with respect to feasibility, cut-off adequacy, and correlation to treatment outcome (Paper I).

- to evaluate the introduction of an age-adapted protocol in the national guidelines in patients 55-85y and to describe disease and patients’ characteristics in relation to age, treatment, and outcome (Paper II).

- to study treatment and outcome after first relapse of ALL in patients 55-85y thus contributing to reference material for future studies of new therapies (Paper III).

- to investigate disease characteristics, especially the frequency of Ph positive disease in an unselected population-based cohort of adult ALL diagnosed between 1997 and 2015 and to study outcome including after allogeneic HSCT (Paper IV).

- to introduce patient reported outcome in the Swedish ALL and AML Registries and prospectively assess HRQoL, symptoms of depression, and patient experiences with cancer care (Paper V).
Patients and Methods

Patients in the Acute Leukemia Registries

Since 1958, patients diagnosed with cancer are reported to the Swedish Cancer Registry. Pathologists and clinicians are obliged to report every newly diagnosed cancer to the registry. Dual reporting improves coverage and accuracy. Through the Swedish citizen social security identification number, cancer incidence can be vigorously monitored. From the Cancer Registry, diagnose-specific quality registries evolved. Since 1997, patients with acute leukemia have been monitored in the Acute Leukemia (AL) Registry. In 2007, the AL Registry was incorporated in a new web-based registry, the Blood Cancer Registry (BCR). The AL Registry was then separated into the AML and ALL-sub registries. The coverage of these two new registries compared with the Cancer Registry has been 98%.

Patients should be informed by their treating physician that their diagnosis is reported to the respective quality registry if they do not opt-out, which is their right. Clinical and laboratory data are reported in a semi-prospectively way by the respective clinicians/hospitals. The initial report includes baseline data such as patient and disease characteristics, investigations, and treatment intention. A treatment report is then requested. This report includes information about given therapy, achievement of CR, and intention of allogeneic HSCT. If the patient is allografted, a separate report is written. Follow-up reports are regularly requested, but relapses and cause of death are preferred to be reported as soon as possible. By means of the social security identification number, vital status is continuously updated. In this thesis, all included patients were identified by the registry and in Paper II also via the Cause of Death Registry. Baseline data were obtained from the registries and were confirmed and supplemented as specified below.

Paper I – Minimal Residual Disease

Patients >45y with Ph-neg B-ALL diagnosed between 2007 and 2011 and treated according to the ABCDV protocol were identified and informed about inclusion in the study with informed consent implied by an opt-out option. All co-authors confirmed and collected missing data from patient charts. MRD levels were then retrieved by E Lennmyr from original hematopathological
reports at each laboratory that had performed the analysis. Original FCM plots were not scrutinized. Since 2008, all laboratories have measured MRD using six-color FCM according to the NOPHO ALL 2008 standards.

Paper II and III – Coverage, Comorbidities, Treatment, and Toxicity

From the ALL Registry, all patients 55-85y registered between 2005 and 2012 were included. Patients alive were informed about the study per mail and informed consent was implied by an opt-out option. A registry report from the Swedish Cause of Death Registry was collected for the same period to assess missing cases in the Swedish Cancer Registry. ALL registry data were confirmed by EL and PK from patient charts. The study database was then supplemented with retrospectively collected data of comorbidities, given treatment including dose reductions, and toxicity. Comorbidities were summarized as the comorbidity component (CC) from the Charlson Comorbidity Index excluding ALL as diagnosis in the score 194. Toxicity was graded according to the Common Terminology Criteria for Adverse Events v 4.0 238.

Paper IV – Cytogenetic and Molecular Analysis

All patients in the registry with ALL diagnosis between 1997 and 2015 were included. A study database was created to merge patients with ALL from the “old” regionally-based Acute Leukemia Registries (1997-2006) and the “new” central ALL registry (2007-2015). Some variables had to be recoded to enable overall analysis and some were studied only in the “new” registry cohort. Patients were reclassified into B-ALL, T-ALL, Burkitt Leukemia, or ALL Not otherwise specified (NOS). In the old registry, ALL phenotype by immunohistochemistry or flow cytometry was reported according to the old terminology: pro B-ALL, pre pre-B-ALL, pre-B-ALL, mature B-ALL, T-ALL, and ALL NOS. In addition, one French-American-British (FAB) classification variable divided ALL morphology into L3 or other ALL. Patients with a mature phenotype (former B-ALL) and L3 morphology were considered Burkitt Leukemia, and all other combinations were considered ALL NOS. From 2007, the precision and accuracy in diagnostics was assumed to be better with the introduction of the Systematized Nomenclature of Medicine – Clinical Terms (SNOMED-CT), and the registry included the numbers 98363, 98123, 98133, 98143, 98153, 98163, 98173, 98183, 98373, 98353, 98263, 983536, and 983535.
By means of the social security identification number, the database could be supplemented with cytogenetic information from the six central genetic facilities performing the analysis. A medical student collected the original reports, EL scrutinized the coded paper copies and if borderline results were noted HH was consulted. Since 2007, Ph-pos disease was reported in the registry and thus validated, but in the “old” registry no such information was present although cytogenetic analysis had been done. A pragmatic, clinical classification approach was used. To be classified as Ph-pos disease, a confirmatory test with only one diagnostic method was required. To be classified as true Ph-negative disease, patients were judged adequately examined if either a normal karyotype with ≥ 20 metaphases, another karyotype with clonal abnormality, a negative FISH, or a negative PCR for major and minor transcript were documented (see Paper IV for details).

Paper V – Patient-Reported Outcome

In a prospective study, all patients with ALL and AML alive and registered in the AL registries six months after a diagnosis from April 2014 were invited to participate in the PRO study. This first evaluation included patients diagnosed up to the end of December 2016. Patients received an invitation letter sent by regular mail with a web site address, an identification code and an electronic consent form. If no reply was registered, a reminder was sent after approximately one month that included a paper version of the questionnaire and a prepaid return envelope. The data were coupled to the AL registry but not available to clinicians. A study database was then created without the social security identification number. To assess HRQoL, the EORTC-QLQ-C30 questionnaire was chosen and to assess depressive symptoms, the PHQ-8 was chosen. Details on additional questions posed are described in Paper V. The study stopped inclusion the last of December 2019 and follow-up at two and four years from diagnosis is on-going.

Ethical Permissions

All studies were done with permission from the Regional Ethics Committee and in accordance with the Declaration of Helsinki. In Paper I-III, an information letter was sent to all patients alive (unfortunately not so many) with informed consent implied by an opt-out option (as they would have been informed about inclusion in the registry at diagnosis). In Paper IV, no informed consent was sought as it was judged as completion of genetic data to the ALL registry and no access to patient charts was required. In Paper V, patients were informed that completing questionnaires digitally or on paper were regarded
as informed consent. No verbal information was given as it would have jeopardized study design.

Statistics
Comparisons between groups were performed using the Chi² or Fischer’s Exact test for categorical data and the Mann Whitney U test or Student’s T-test for continuous variables. OS was defined as time from date of diagnosis to last follow-up or death. Continuous Complete Remission (CCR) was calculated from the date of morphologic remission to relapse or last follow-up, censoring for death in remission. Event Free Survival (EFS) was measured from date of diagnosis to relapse or death, and primary refractory or patients not evaluated were considered as an event on day one. OS, CCR, and EFS were estimated with the Kaplan-Meier survival method. The log rank test was used for comparison between groups. Multivariable analyses were calculated with the Binary Regression Model for Odds Ratios of variables with binary outcome and Cox proportional hazard model for Hazard Ratios of variables for survival.

Standardized mortality ratio (SMR) in Paper IV was calculated by a professional statistician using indirect standardization. Patients who had survived five years from ALL diagnosis were compared with the expected number of deaths in the Swedish population between 2002 and 2018. Patients were matched based on age, gender, and calendar year at risk. When needed, the expected number of deaths was adjusted to account for incomplete follow-up time at calendar year.

In Paper V, a professional statistician linearly transformed the item scores in the EORTC QLQ-C30 and the summary score was calculated according to the scoring manual (the summary score calculated if all of the required 13 scale scores were available provided that at least 50% of the items in that scale had been completed) 239. Mean values with standard deviations were calculated and compared to the values for a sample from the general Swedish population (40-79 y) 240. Cronbach’s alpha was used to assess internal consistency for all scales consisting of two or more items.

All tests were two-tailed, confidence intervals (CI) were 95%, and a P-value <0.05 was considered significant; however, tests should be interpreted as explorative because no adjustment for multiplicity testing was done. Statistical analyses were performed with SPSS (IBM) v21-v26, SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and R Studio 3.5.0-2 (R Studio, Inc.).
Results and Discussion

Selected results of this thesis are presented and discussed on the following pages. Details can be found in the respective Paper at the end of the book. An overview of included patients and study periods is presented in Table 5.

Table 5. Patients in the final cohorts

<table>
<thead>
<tr>
<th>Paper</th>
<th>Final cohort</th>
<th>n</th>
<th>Median age in years (range)</th>
<th>Diagnosis period in years</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Ph neg B-ALL</td>
<td>35</td>
<td>61 (47-79)</td>
<td>2007</td>
</tr>
<tr>
<td>II</td>
<td>B- and T- ALL</td>
<td>155</td>
<td>67 (55-85)</td>
<td>2005</td>
</tr>
<tr>
<td>III</td>
<td>Relapses from Paper II</td>
<td>63</td>
<td>66 (55-82)</td>
<td>2005</td>
</tr>
<tr>
<td>IV</td>
<td>All patients in ALL registry</td>
<td>933</td>
<td>53 (18-95)</td>
<td>1997</td>
</tr>
<tr>
<td>V</td>
<td>ALL and AML patients</td>
<td>255</td>
<td>64 (18-90)</td>
<td>2014</td>
</tr>
</tbody>
</table>

Paper I

The introduction of MRD monitoring in the Swedish National Guidelines was evaluated for 35 patients diagnosed with Ph-neg B-ALL between 2007 and 2011 and treated with the ABCDV protocol. Their median age was 61 y (range 46-79 y), the CR rate was 91%, and the probability of OS at 5 y was 47%. These figures are impressive in such an old cohort although patients were selected for intensive therapy \[135,187,188,241\]. The majority of patients were female (23 women and 12 men), and we speculated that a selection of healthy B-ALL patients might have contributed to the 47% surviving 5 y. Allogeneic HSCT was performed in five patients in CR1 and five in CR2, and no one in the latter group became a long-term survivor. In multivariable analysis, age >65 y and WHO PS \[≥2\] negatively influenced OS. MRD<0.1% after remission induction was associated with a better CCR compared to >0.1% \(P = 0.05\).

Concerning the introduction of MRD assessment, all patients had an LAIP to follow. MRD was measured in all patients when the result could lead to treatment escalation. A low MRD was associated with good CCR; however, because there were few patients and samples, robust conclusions about the predictive potential of MRD in our cohort were not possible. As described earlier, several groups have demonstrated that MRD-directed treatment escalation is feasible \[69,72,73,242\].

In 2017, the Swedish National Guidelines were changed, supported by the trends in this study and the overwhelming international data. The clinical cut-
off for high-risk MRD at the end of consolidation and therefore recommenda-
tion of allogeneic HSCT was reduced from 0.1 to 0.01%. Even if HSCT is
associated with significant morbidity and mortality, the extreme risk of relapse
in case of MRD persistence still means there is a chance, although small, for
long-term cure with allogeneic HSCT.

Paper II
To evaluate treatment and outcome for older patients with ALL, 183 patients
diagnosed between 2005 and 2012 were identified from the ALL registry.
Eleven patients were excluded and two patients were identified through the
Swedish Cause of Death Registry, resulting in a high coverage of ALL diag-
nosis.

In the 174 included patients, the median age was 68y (range 55-85y), and
82% had B-ALL, 7% T-ALL, and 11% had Burkitt Leukemia (excluding from
further analyses due to their different treatment). In the final cohort of 155
patients, 35% had Ph-pos disease, which is in line with one previous study.
The distribution of men and women was equal (48% and 52%), corroborating
previous findings in elderly ALL. The majority of patients were intensively
treated (80%), a proportion higher than recently published data from the US,
a finding that might illustrate the differences between health care insurance
systems.

Comorbidities were prevalent: 15% had diabetes; 10% a history of myo-
cardial infarction; 66% had one comorbidity or more; and 75% had a CC of
0-1. Similar proportions were found in a retrospectively evaluated cohort of
French elderly patients (>60y). We and others were unable to prove CC to
be an independent factor for OS.

Outcome was evaluated for the whole cohort as well as separately for the
previously recommended ABCDV protocol and the age adopted EWALL pro-
tocol introduced in 2009. A first (morphological) CR was achieved in 83% of
intensively treated patients. The ABCDV protocol was applied in 64% and the
EWALL backbone in 28%. The latter group had a higher median age (63y vs.
69y). All Ph-pos patients received TKI in addition to chemotherapy. The treat-
mant protocols were modified/adjusted in 30% of cases. Early death rate
(within 60 days) was 15%, in line with previously published studies, and did not differ between the two protocols in patients aged 65-75y.

We found considerable toxicity regarding septicemia (65%), pneumonia
(23%), and invasive fungal infections (18%) during remission induction and
consolidation. Interestingly, the proportion of fungal infections (probable or
proven) was higher in patients with diabetes (7/16, 44%) compared to non-
diabetic (15/106, 14%) (P<0.05), a finding heretofore undescribed. Fungal
prophylaxis is now recommended in the Swedish National Guidelines for in-
tensively treated patients together with the caveats of interaction with
vincristine and aggravated neurotoxicity\textsuperscript{212}. The proportion of patients affected by toxicity did not differ between age or comorbidity groups.

As expected, in intensively treated patients (median age 65y), outcome was superior with 3y OS of 32\% in contrast to 3\% in the palliative cohort (median age 79y). These results were in line with a recent publication from the Mayo Clinic of 124 patients (median age 67y) and a 3y OS of 37.3\% and 0\%, respectively\textsuperscript{88}.

Twenty patients were allografted in CR1, the majority (N=14) because of Ph-pos ALL. OS at 3y was 40\%. OS did not differ between 14 Ph-pos patients allografted and 35 patients not allografted in CR1, including those treated with a palliative approach. This finding leads to the question if Ph-pos patients above the age of about 55y should be offered allogeneic HSCT in CR1 in the era of TKI-directed therapy\textsuperscript{168}? In younger patients, life-time cure is warranted; however, when life expectancy is shorter, the drawbacks of allogeneic HSCT can be so profound that the possibilities with different generations of TKI out weight the potential suffering from HSCT side effects\textsuperscript{71}. With better prediction of TRM and good quality MRD monitoring, we might improve the selection of patients who will benefit from allogeneic HSCT.

The outcome in T-ALL was discouraging as no patient survived two years. The reasons for these poor results are unknown. In the UKALL XII study, adults 50-59y with T-ALL had a 5y OS of 27\% (11-43)\textsuperscript{44}. The high median age, the absence of asparaginase and high-dose methotrexate in ABCDV (and HyperCVAD) might have contributed to our poor results. There was also a noteworthy difference in overall survival between men and women in the whole cohort, becoming statistically significant in the age cohort 55-65y, and not explained by conventional risk factors or T-ALL. This finding was further explored in\textit{Paper IV}.

No patients ≥75y survived 3y. In this group, even the EWALL backbone protocol seemed be too intensive. In a previous study using the Swedish AML registry, regions with different approaches to remission-inducing therapy in elderly patients were compared. A higher rate of intensively treated patients versus less intensively resulted in a similar eight-week mortality but longer survival for those given more intensive therapy\textsuperscript{246}. These facts demonstrate the importance of registry-based studies and the selection bias often seen in clinical trials\textsuperscript{247}. Because of the limited number of patients, we could not perform similar analyses within our study. Nevertheless, dose-reduced chemotherapy and in case of Ph-pos ALL treatment with TKI can provide older patients with disease control and result in less time in hospital\textsuperscript{146}.

**Paper III**

Because of the lack of knowledge concerning outcome after relapse in elderly ALL, patients in\textit{Paper II} who reached CR1 (n=106) and those who eventually
relapsed (n=63) were studied further. Seven patients relapsed after allogeneic HSCT. Median time to relapse was nine months (range 1-93). TKI was changed in most Ph-pos patients (n=20) and TKI was combined with intensive chemotherapy in five patients. Intensive chemotherapy was given to 58% (23 of 43) of Ph-neg patients and 44% (11/25) reached a second CR. A higher proportion of patients attained a second CR if late (>1y) versus early (<1y) relapse (11/27 vs. 5/36; \( P = 0.02 \)). Median OS after relapse was 3.6 months and the estimated 2y OS was 14%.

Surprisingly, these results were in line with a large compiled study cohort of R/R Ph-neg B-ALL. A median OS of 5.8 months was reported and 1 and 3y OS were 25% and 11%, respectively. Time to relapse was also associated with OS.

We concluded that older patients’ fit for intensive chemotherapy and treated with the intention to reach a second CR had a chance similar to younger patients to reach this. At ALL relapse, participation in clinical trials evaluating new drugs should be encouraged whenever possible.

**Paper IV**

To obtain population-based data of adult ALL, we studied the characteristics and treatment outcome of all patients diagnosed and reported to the ALL registry between 1997 and 2015. To enable meaningful comparisons of results from international trials, information about Ph-status was necessary and therefore supplemented for the “old” registry (1997-2006) and verified in the “new” registry (2007-2015).

We identified 937 patients in the registry of which three were excluded due blast crisis of CML and one due to relapse of childhood leukemia. Of 933 patients in the final analysis, B-ALL comprised 68%, T-ALL 15% (most prevalent in young males; median age 37y), and Burkitt Leukemia 4%. The ALL NOS group diminished from 22% in the old to 5% in the new registry, reflecting better diagnostic methods. These figures were in line with previously reported data but are one of the largest population-based cohorts including patients from 18 to 95y. This led to a relative lower incidence of T-ALL as it diminishes with age.

Testing for Ph-pos disease increased over time and the frequency of Ph-pos disease was 34% of tested B-ALL. The highest incidence was 47% in patients 50-59y and did not increase further with age as was previously suggested. Of the 111 patients reported as Ph-positive in the new registry, we verified 106 (95%) and found four Ph-pos patients not (yet) reported.

Treatment intention did not change between the “old” and “new” registries and was remission inducing in 89% (99% in young patients). Death within 60 days decreased from 11% to 6% between the two registry periods (\( P < 0.05 \)).
Overall Survival in the total cohort (n=930) is presented in Figure 2.

**Figure 2.** OS in ALL patients in Sweden (n=930) in 1997-2006 and 2007-2015. Five-year OS improved in patients 18-45y from 50% (95% CI 43-57) to 65% (95% CI 58-72), in patients 46-65y from 25% (95% CI 18-32) to 46% (95% CI 37-55), and in patients >65y from 7% (95% CI 2.6-11) to 11% (95% CI 5.9-16) (P < 0.05, log-rank for pairwise comparison).


Allogeneic HSCT in CR1 was applied in 24% of patients and increased in middle aged patients between the two periods. Forty-six percent of patients allografted had Ph-pos ALL and OS did not differ from Ph-neg ALL. The corresponding 5y OS improved for patients 18-45y from 50% to 66% (P=0.01, log-rank) and for patients 46-65y from 30% to 60% (P=0.05) log-rank).

In multivariable analysis, age and WHO-PS were associated with increased risk of death, but sex and phenotype were not. Ph-pos disease was associated with improved outcome between 2007 and 2015, reflecting the TKI era as described by others.

However, in view of the results in Paper II, we analyzed the subgroup of patients 46-65y and found that males had inferior OS than woman in both periods. In the Ph-neg B-ALL cohort (n=130), men had a 5y OS of 20% compared to 52% for women (P < 0.01). The addition of rituximab, better individualized allografting, and supportive care could have contributed to improved survival seen in the age cohort but does not explain the difference between sexes. Frequency of CR and treatment with allogeneic HSCT were similar in men and women. The reason for this large difference in outcome remains unclear and has not been reported in larger studies. In a study of patients >60y in Poland, between 1992 and 2002 male sex negatively affected the probability of reaching CR, but outcome was generally very poor for both sexes. A
retrospective analysis of patients 55-65y failed to demonstrate that sex was an independent factor for OS in multivariable analysis as it compared outcome in 67 patients (42% males) treated intensively to 44 patients (68% males) treated with an age-adopted protocol\textsuperscript{136}. Overall survival was low, 27% at 5y, and therefore in parity with the men in our cohort.

Nevertheless, in view of these results and data indicating that dose-adjusted pediatric therapy and HD-Mtx can be delivered to patients up to the age of 60-65y\textsuperscript{136,250}, the ABCDV protocol was removed from the 2017 Swedish National Guidelines in Ph-neg B- and T-ALL and replaced by a dose-adjusted NOPHO ALL 2008 protocol. The results in Ph-pos ALL with the strategy of ABCDV induction and allogeneic HSCT in CR1 yielded international comparable results and is therefore still recommended.

To assess the impact of ALL diagnosis (and treatment) on survival, SMR was calculated as the number of deaths compared to the age-adjusted expected deaths in the general population. The SMR decreased from 6.5 (95% CI 5.8-7.8) to 3.8 (95% CI 2.8-5.2) between 1997 and 2006 and 2007 and 2015, respectively. This indicates that adults surviving five years after ALL diagnosis still have an almost four-fold excess risk of death compared to the general population, a finding that highlights the importance of developing less toxic therapies for ALL patients of all ages.

**Paper V**

The feasibility and results of PRO reported into the AL registries were evaluated in this first interim analysis. An invitation letter was sent to 398 patients alive six months from AL diagnosis. The response rate was 64% (n=255); 60% responded digitally and 40% answered a paper-based questionnaire. Responders were older than non-responders and more AML than ALL patients participated. The OS in patients responding and not responding were equal so extreme selection bias was unlikely. The response frequency was as expected and in line with clinical trials\textsuperscript{231}. The number of patients preferring a paper form was surprisingly high and must be considered when planning future registry studies. The time to response from letter sent was diverse with a median of 58 days (range 10-316). This illustrates one of the difficulties with data collection out of hospital and makes comparison of data between patients and with data from other studies more difficult.

As expected, HRQoL measured with the EORCT-QLQC30 was lower and symptom burden higher in AL patients requested six months from diagnosis than in the general Swedish population. Role, emotional functioning, and social functioning were lower in patients <65y compared to older and this finding is believed to reflect the larger impact malignant disease and associate treatment has on life\textsuperscript{224}. Compared to older patients, younger patients also had more symptoms and financial difficulties.
Depression was assessed with the PHQ-8 screening formula and a score of \(\geq 10\) indicated depression \(^{234}\). A score of \(\geq 10\) was reported by 18\% of the cohort: 28\% (12 of 43) of ALL and 16\% (33 of 210) of AML patients \((P = 0.08)\) and without significant differences in median age or sex. Symptoms of depression were about twice the prevalence in a Swedish normal population \(^{251}\). Factors associated with depressive symptoms were difficulties understanding information, not having a close friend, and the experience of being offended/mistreated repeatedly. Notably, prescription of antidepressants was reported in 33\% (14 of 42) of patients with a score \(\geq 10p\) compared to 6\% (12 of 193) if \(<10p\) \((P < 0.01)\). These results suggest that depression might be underdiagnosed and/or untreated in this group of patients.

As it is known that pre-treatment counselling about the impact of AL treatment on fertility can affect QoL, patients were asked about this \(^{252}\). The majority of patients received such information, but young female patients reported a great concern for the risk of impaired fertility and scored high on PHQ-8.

Most patients were very pleased with their leukemia care, but a few (10\%) had not been informed about their right to a new medical assessment (“second opinion”) and only some (27\%) were informed about whom to contact in case of complaints. Patients were pleased with supportive care, but they requested more practical and psychological support. This illustrates that PRO in a registry can be informative, although it is the local staff that have the possibilities to change the situation for the individual patient.

Limitations

The strengths of these studies are the unselected population-based cohorts, given the excellent coverage compared to the Swedish Cancer Registry. Also, the equal access to advanced medical care in Sweden regardless of economic situation reduces socioeconomic and referral biases. The social security identification number makes OS calculations undisputable.

While the advantages include patient coverage thereby minimizing selection bias, the drawback of registry-based data from a nationwide healthcare system is the quality of the data, which is hampered by the lack of systemic monitoring and central revision of results as performed in clinical trials. In addition, no systematic validation of the entire registry was done.

In Paper I, we verified MRD measurements, diagnosis, and treatment. The pathology departments were already accredited within other diagnostic projects, but a central review of the MRD plots and diagnostic phenotypes was not realistic. Furthermore, a challenge with national guidelines is their ability to motivate clinicians to perform more extensive diagnostics and follow-up in routine care. MRD was not measured in elderly patients. This makes retrospective analysis more difficult due to missing data.
In *Paper II* and *III*, we retrospectively scrutinized patient records to verify registry data and collected information about comorbidities and toxicity. This involves the risk of missing events not properly recorded, the risk of misinterpreting information, and the risk of observation bias (as the observer was aware of patient outcome). To minimize misinterpretation, only two physicians scrutinized patient records and discussed borderline cases for proper classification.

In *Paper IV*, we analyzed data from the whole ALL registry. We knew from *Paper II* that about 5% of patients >55y might not have a proper ALL diagnosis, but they were treated as such and reported to the registry, reflecting standard of care. The collection of genetic data was essential for interpretation of results; although no central review was performed, results agreed with international data.

In *Paper V*, the collection of data in an out-of-hospital setting was problematic in terms of timing, so the results should be interpreted with caution. Patients wanting to please their care giver can also be a problem but might be mitigated if no direct coupling of answers is done as in the present study. As a clinician, an important limitation with this type of data collection is the lack of possibility to give feedback to patients, especially in case of symptoms of depression. Validated forms were used to assess HRQoL, depression, and experiences of cancer care. However, some questions were not validated leading to impaired data quality.
Conclusions

Based on the studies in this thesis, the following conclusions were formed

- Minimal residual disease assessed by flow cytometry in Ph-neg B-ALL is feasible but selectively used as a treatment escalation tool in routine care (Paper I).

- The introduction of an age-adopted protocol in the Swedish National Guidelines for older patients did not translate into better outcome with regard to toxicity or efficacy (Paper II).

- The outcome after relapse of ALL is dismal in older patients, especially if time to relapse is less than one year (Paper III).

- Overall survival improved in adult ALL patients between the periods 1997-2006 and 2007-2015. However, older patients and middle age men with Ph-neg B-ALL have a poor outcome (Paper II and IV).

- The frequency of Ph-pos ALL was 34% of tested B-ALL. After the introduction of TKI, Ph-pos disease was no longer associated with poor survival in both younger and older patients (Paper II and IV).

- Allogeneic HSCT was reported in first remission in 24% of patients in the ALL registry and OS did not differ between Ph-pos and Ph-neg ALL (Paper IV).

- Collection of PRO in the AL registries is feasible and adds information otherwise not retrieved. However, proper timing and the adequate selection of questionnaires are challenging (Paper V).

- Depressive symptoms are frequent in patients six months after acute leukemia diagnosis, especially in young females worrying about fertility (Paper V).
In view of the advances in ALL treatment over the last century, it is easy to admire the contributions of patients and physicians taking the first steps towards cure of this disease without modern supportive care, computers, internet, statistics, and other technology. The treatment success in childhood ALL has been nothing less than a giant leap in medicine made possible only by millions of small steps across the world.

In contrast to the focus on numbers and statistics in studies, the outcome for the individual patient is binary in terms of life and death. However, it is also true that refined measures such as PRO add dimensions to the survival aspect. Hopefully, more individualized medicine will enable us to avoid many of those side effects that hamper quality of life and potentially increase the risk of death for ALL survivors. In an optimistic scenario, disease-specific drugs can be combined with the ultimate immunotherapy – allogeneic stem cell transplantation – to cure leukemia in terms of efficacious graft versus leukemia effect and negligible graft versus host disease.

While I envision this future scenario, the next large clinical study in northern Europe is about to start. The ALLTogether is a consortium where study groups including SVALL have co-operated and designed a uniform backbone protocol for children and adults with Ph-neg ALL up to the age of 45y. By recruiting patients with a rare disease internationally, study endpoints can be evaluated more rapidly and the power to detect changes in relevant outcome reached faster. Add on studies with randomizations are planned in different phases and patient subgroups. International collaboration has been and still is one way of improving leukemia treatment.

National quality registries also play an important role in evaluating leukemia care. Complementing randomized controlled trials, quality registries can be used to identify patients not eligible for studies but with equal medical need. The next step must be to reach results for adult patients of all ages comparable to childhood ALL. With new drugs such as the T-cell engaging bispecific antibody blinatumomab, toxin-coupled antibodies such as inotuzumab ozogamicin, signal transduction inhibitors, and CAR-T cell therapy in frontline treatment, long-term cure without long-term toxicity might be reachable. The challenge will not only be to choose wisely from many possibilities but also to deal with cost-benefit questions and health economics. I hope to have the possibility to focus on the former task in future research and patient care.
Akut lymfatisk leukemi (ALL) är en elakartad blodsjukdom och den vanligaste cancerformen hos barn. Bland vuxna är det en ovanlig sjukdom med 50 nya fall per år i Sverige. Överlevnaden för barn har successivt förbättrats - mer än 90% av barnen lever numera fem år efter diagnos. Tyvärr har utvecklingen för vuxna inte varit lika gynnsam. Chansen att överleva minskar drastiskt med stigande ålder.


Den här avhandlingen består av fem delarbeten som återfinns i slutet av boken. Det övergripande syftet med avhandlingen var att, med hjälp av det svenska ALL registret, studera vuxna patienter med ALL i Sverige sedan 1997 med avseende på behandling och överlevnad.

ALL kan delas upp i olika undergrupper där B-ALL och T-ALL är två huvudgrupper liksom om leukemicellerna har en genetisk (ej medfödd) avvikelse som kallas Philadelphiakromosom (Ph-pos) eller ej (Ph-neg).


I delarbete I studerades införandet av en ny mätmetod av kvarvarande leukemisjukdom (minimal residual disease, MRD). I riktlinjerna föreslogs att man skulle kontrollera MRD efter bland annat första cellgiftskur. Resultatet kunde sedan ge vägledning om fortsatt behandling. MRD eftersökes därför för


I delarbete III studerades överlevanden efter återfall i ALL hos äldre. Av de patienter som i delarbete II nått en första komplett remission var det slutligen 63 som drabbades av återfall. Medianöverlevnaden efter återfall var 3,6 månader. Efter 1 år var 19% vid liv och efter 2 år endast 14%. Återigen gjorde det ringa antalet patienter att man inte kan dra några säkra slutsatser. Om man däremot detaljstudierade de patienter som behandlats mer intensivt vid återfallet så fanns ändå en chans jämförbar med yngre vuxna att få sjukdomskontroll. Behandlingsalternativen i händelse av återfall i leukemi är få men nya läkemedel är på väg in i klinisk rutin.


I delarbete V studerades slutligen hur patientrapporterade utfallsmått kan samlas in och användas i ALL och AML-registret. Sex månader efter diagnos skickades ett brev ut till patienter vid liv med inbjudan att deltaga i studien. Av 398 tillfrågade så svarade 64% (255 st); 60% svarade digitalt och 40% besvarade ett pappersformulär vilket i sig var förvånande många. Det visade sig att symptom på depression var vanligt (18%) hos patienterna och särskilt hos unga kvinnor med stor oro för påverkan av behandling på framtida fertilitet. Man var generellt nöjd med vården men en del önskade mer hjälp med praktiska saker och mer psykologiskt stöd. Vi upptäckte också att endast 10% av patienterna uppgav att de fått information om förnyad medicinsk bedömning (second opinion). Slutsatsen blev också att man måste man fundera noggrant på hur och när man ska samla in patientrapporterade symptom och upplevelser och på vilket sätt man ska analysera och förbättra vården med vägledning av resultaten.

Avslutningsvis så kan man sammanfatta att även om överlevnaden vid ALL hos vuxna har förbättrats så är det alltjämt är en mycket allvarlig sjukdom där det återstår stora utmaningar. Nya läkemedel med helt andra verkningsmekanismer än cellgifter studeras just nu i kliniska studier. Om de visar sig effektiva vid initial behandling av ALL blir de förhoppningsvis snart därefter tillgängliga för våra patienter. Jag hoppas få vara en del av den framtiden.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)