

Prognostic factors, treatment and outcome in adult acute lymphoblastic leukemia

To my family

Örebro Studies in Medicine 134



PIOTR KOZLOWSKI

**Prognostic factors, treatment and outcome in adult
acute lymphoblastic leukemia**

Population-based studies in Sweden

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Abstract

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Acute lymphoblastic leukemia (ALL) has poor prognosis in older/elderly adults and in high-risk/relapsed disease. Recommended treatment of ALL was evaluated (study I-IV). Data was obtained from the Swedish Acute Leukemia registries and from patient records.

I. We assessed ALL relapse treatment and outcome in 76 patients aged 15-65 years (y). Complete remission (CR) was achieved in 50/71 patients (70%). Of them, 29 underwent allogeneic hematopoietic stem cell transplantation (hSCT). Five year overall survival (OS) was 15%, but close to 50% in 19 patients <35y after hSCT.

II. We studied outcome of treatment with the Hyper-CVAD protocol in 19 of 24 patients with T-ALL aged 18-72y. CR was reached in 89%, but 5y leukemia-free survival was only 29%, and 20% in 15 patients not transplanted in CR1. Six patients received hSCT in CR2. Finally, 5y OS in all 19 patients was 47%. The only negative prognostic factor found was age $\geq 35y$.

III. We evaluated minimal residual disease (MRD) monitoring in 35 patients with Philadelphia (Ph) negative B-ALL aged 46-79y and treated with the ABCDV protocol. The CR rate was 91%. MRD was measured by flow cytometry in 73% in CR1 (MRD1) and omitted in those >70y or with high-risk ALL. Five patients received hSCT (only one due to MRD). Five year OS in the whole cohort was 47%. Continuous CR but not OS was improved in patients with MRD1 <0.1 %.

IV. We studied 155 patients with ALL (Ph+ in 35%) aged 55-85y and treated with remission induction/palliation (124/31). Both, intensive, and palliative treatment resulted in the CR rates of 70/83/16% and 3y OS of 26/32/3%. OS was negatively influenced by age and platelet count $\leq 35 \times 10^9/L$ (but not Ph+). OS was not enhanced by introduction of an age-adapted protocol.

We concluded that intensive treatment with subsequent allogeneic hSCT is the most reasonable option in younger patients with ALL recurrence (I). Hyper-CVAD has low relapse-preventing efficacy (II). MRD guided intensification is probably feasible in only a minority of older patients (III). Prognosis in elderly ALL is poor, but no longer impaired by Ph+ (IV).

Keywords: Acute Lymphoblastic Leukemia, adult, chemotherapy, prognosis, population-based.

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List of papers

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

- I. **Kozlowski P**, Åström M, Ahlberg L, Bernell P, Hulegårdh E, Häggglund H, Karlsson K, Markuszewska-Kuczynska A, Tomaszewska-Toporska B, Smedmyr B, Hallböök H. High curability via intensive reinduction chemotherapy and stem cell transplantation in young adults with relapsed acute lymphoblastic leukemia in Sweden 2003-2007. *Haematologica*. 2012;97(9):1414-21.
- II. **Kozlowski P**, Åström M, Ahlberg L, Bernell P, Hulegårdh E, Häggglund H, Karlsson K, Markuszewska-Kuczynska A, Tomaszewska-Toporska B, Smedmyr B, Amini RM, Hallböök H. High relapse rate of T cell acute lymphoblastic leukemia in adults treated with Hyper-CVAD chemotherapy in Sweden. *Eur J Haematol*. 2014;92(5):377-81.
- III. Bergfelt E, **Kozlowski P**, Ahlberg L, Hulegårdh E, Häggglund H, Karlsson K, Markuszewska-Kuczynska A, Tomaszewska-Toporska B, Smedmyr B, Åström M, Amini RM, Hallböök H. Satisfactory outcome after intensive chemotherapy with pragmatic use of minimal residual disease (MRD) monitoring in older patients with Philadelphia-negative B cell precursor acute lymphoblastic leukaemia: a Swedish registry-based study. *Med Oncol*. 2015;32(4):135.
- IV. **Kozlowski P**, Bergfelt E, Ahlberg L, Hulegårdh E, Häggglund H, Karlsson K, Tomaszewska-Toporska B, Smedmyr B, Åström M, Hallböök H. Age but not Philadelphia positivity impairs outcome in older/elderly patients with Acute Lymphoblastic Leukemia in the Swedish population (in manuscript).

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Abbreviations

ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
AYA	adolescents and young adults
BM	bone marrow
CAR T-cells	chimeric antigen receptor modified T cells
CC	comorbidity component
CCR	continuous complete remission
CI	confidence interval
CNS	central nervous system
CR	complete remission
ED	early death
EFS	event free survival (EFS)
ETP	early thymic precursor
EWALL	the European Working Group on Adult ALL
FISH	fluorescence in situ hybridization
G-CSF	granulocyte colony-stimulating factor
GVHD	graft-versus-host disease
GVL	graft-versus-leukemia
HR	high-risk
hSCT	hematopoietic stem cell transplantation
ICD	International Classification of Diseases
LFS	leukemia-free survival
MAC	myeloablative conditioning
MRD	minimal residual disease
NOPHO	the Nordic Society of Paediatric Haematology and Oncology
OS	overall survival
PCR	polymerase chain reaction
PF	prognostic factors
Ph+	Philadelphia chromosome positive
PLT	platelets
PS	performance status
RD	related donor
RICT	reduced intensity conditioning transplant
RT-PCR	reverse-transcriptase polymerase chain reaction
SR	standard risk
SVALL	the Swedish Adult ALL group
TCR	T-cell-receptor

TKI	tyrosine kinase inhibitors
TRM	transplant-related mortality
URD	unrelated donor
WBC	white blood cell count
WHO	World Health Organization

Introduction

Acute Lymphoblastic Leukemia (ALL) is one of the most proliferative malignancies which can affect individuals at any age, but a minority of all patients are adults. Intense, prolonged, response/risk factor guided and complex regimes combining chemotherapy, steroids, biological and targeted therapy are used. Hematopoietic stem cell transplantation (hSCT) is applied in a proportion of patients with high risk ALL in order to prevent disease reoccurrence. The outcome in children/younger adults is improving (1-3) but elderly patients as well as those with relapsed /refractory disease still have poor prognosis (4, 5). New therapies such as bispecific antibodies and engineered T-cells (CAR T-cells) are emerging which even those groups can benefit of (4). Most of the protocols and prognostic factors were evaluated in retrospective studies. Prospective randomized trials are few due to rarity of the disease, and a population based perspective is almost lacking. Selection mechanisms are present in most trials with exclusion of patients not fit enough (elderly/with comorbidities or poor performance status) as well as those treated outside university hospitals. This leads to overestimation of the efficacy of the studied protocols (6). Establishment of nation-wide registries gave the opportunity to unbiased collection of data on patients' characteristics, treatment and outcome in this rare disease. Many countries/national ALL groups introduced guidelines in order to standardize ALL therapy. In Sweden, the guidelines were introduced 1986 and are continuously updated by the Swedish Adult ALL group (SVALL). All patients with the diagnosis are reported to nation-wide Acute Leukemia/ ALL registries. This gave us the opportunity to perform studies evaluating results of treatments with specific regimens (ABCDV, Hyper-CVAD, EWALL backbone) in well-defined ALL groups (T-ALL, relapsed ALL, older/elderly), as well as implementation and prognostic significance of minimal residual disease (MRD). The results reciprocally influenced/will influence the content of the Swedish guidelines.

History of ALL treatment

ALL was incurable until the 1960s. Development of effective ALL therapy was initiated by Sidney Farber ("father of the modern chemotherapy") who demonstrated for the first time that remission of the disease in children can be achieved with folic acid antagonists (7). Successive introduction of methotrexate, asparaginase, 6-mercaptopurine, vincristine, steroids

and finally combining systemic chemotherapy and central nervous system (CNS) prophylaxis led to cure in half of the patients in the 1970s. Allogeneic hSCT in refractory/relapsed disease was introduced in the 1980s, followed by intensification of post-induction therapies based on risk classification systems (1990s). Molecular targeted therapy with tyrosine kinase inhibitors (TKI) in Philadelphia chromosome positive (Ph+) ALL came into use in first decade of this century (8). The progress in therapy occurred mostly in children, with “adult ALL” clinicians utilizing experiences e.g. pediatric protocol use in younger adults in recent decades (9-11). Last years’ most exciting invention of bispecific antibodies and CAR T-cells will probably have substantial impact on ALL therapy in the future.

Epidemiology

ALL accounts for 30% of all acute leukemias. The incidence of ALL is estimated to 1/50 000/year and is increasing (12). It is highest in children of ages 1-4 years, and is rising again after age 60 years (12), as demon-

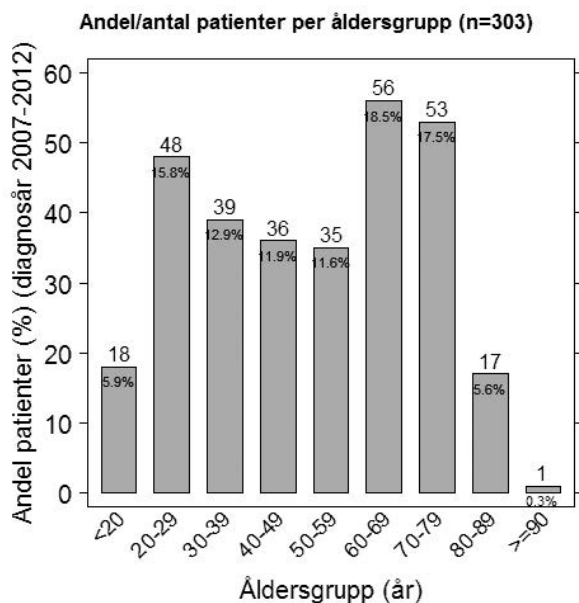


Figure 1. Number of adult patients (303 totally) diagnosed with ALL per age group according to the Swedish Acute Lymphoblastic Leukemia Registry (2007-12).

strated in Figure 1. Median age at diagnosis in adults in Sweden is 54 years (13). The incidence is higher in males than in females (12) but in the Swedish population this difference was more prominent from age 80 years and up (13). B-cell phenotype accounts for about 4/5, and T-cell for 1/5 of ALL in adults (1, 3). T-ALL is common in males and young adults but rare in children and the elderly. Ethnic differences are observed, with lowest ALL incidence in blacks and highest B-ALL in Hispanic whites (12).

Etiology

Most studies on etiology concern childhood ALL. The disease arises from hematopoietic precursors of the lymphoid lineage. Environmental exposures and genetics are involved, and the pathogenesis is probably a multi-step process. The strongest evidence for exposures as cause of ALL is demonstrated for ionic radiation according to studies of atomic bomb survivors (14). Prenatal X-ray in mothers increased the risk for childhood ALL (15). Interestingly, paternal but not maternal perinatal smoking was associated with ALL risk in the offspring (16). One of postulated exposures in adults was hair dye use (17). Epidemiological studies support a hypothesis about early life viral infection influence on leukemogenesis, based on number of siblings and birth order (18). Viral etiology is acknowledged in other lymphoid malignancies such as the human T-cell lymphotropic virus (HTLV) in adult T-cell leukemia (19), the Epstein-Barr virus (EBV) in endemic Burkitt leukemia/lymphoma (20), as well as the bovine leukemia virus (BLV) in animal leukemia (21). There is evidence of increased risk in first degree relatives of patients with ALL (22) which can be explained by the fact that some specific germline genomic variation predisposes for the disease (23). Down's syndrome is associated with excess of both Acute Myeloid Leukemia (AML) and ALL (24). Early genetic changes (in utero) are plausible causes of *MLL*-positive infant ALL as concordance in monozygotic twins is 100% (25).

Clinical presentation, diagnostics and classification

As ALL is a highly proliferative malignancy the symptoms usually have short duration and progress rapidly unless therapy is started. Bone marrow (BM) failure (with anemia, neutro- and thrombocytopenia), lymphadenopathy/splenomegaly and sometimes bone pain are main features. Very high white blood cell count (WBC) leading to leukostasis is uncommon. Fever due to infection or cytokine release by leukemic cells is present in

many patients. In the elderly though, proliferative potential seems to be less pronounced, with less common lymphadenopathy (26, 27), splenomegaly (27), and lower WBC (26). CNS disease affects 5-8% of the patients at diagnosis (28-30), with mainly meningeal and cranial nerve involvement. Other extramedullary manifestations are rare with exception of frequent mediastinal mass in T-ALL (31), with risk for esophageal/tracheal/vena cava superior obstruction. The T-cell phenotype is associated with higher WBC, more frequent hepatomegaly, and CNS involvement at diagnosis as compared with B-ALL (31). Diagnostics comprises pathological examination of BM (>20% of blasts required for diagnosis) with genetic evaluation and immunophenotyping as well as lumbar puncture and radiological imaging to investigate any other organ (liver, spleen, lymph nodes, CNS) involvement. Mature B-ALL is regarded as a separate entity (Burkitt leukemia) by the 2008 WHO classification (Table 1).

Table 1. The 2008 World Health Organization (WHO) classification of ALL (32).

<p>B lymphoblastic leukemia/lymphoma:</p> <ul style="list-style-type: none"> B lymphoblastic leukemia/lymphoma, NOS B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2), <i>BCR-ABL1</i> B lymphoblastic leukemia/lymphoma with t(v;11q23); <i>MLL</i> rearranged B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) <i>TEL-AML1</i> (ETV6-RUNX1) B lymphoblastic leukemia/lymphoma with hyperdiploidy B lymphoblastic leukemia/lymphoma with hypodiploidy B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) <i>IL3-IGH</i> B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3) <i>TCF3-PBX1</i> <p>T lymphoblastic leukemia/lymphoma</p>

Prognostic factors

The panorama of prognostic factors (PF) is changing as new protocols/specific therapies are emerging and new more reliable PF replace/complement those classical/historical. Some PF are used in clinical studies/ praxis to define risk groups in order to intensify consolidation

(hSCT included) in high-risk (HR) disease. Some common PF are listed in Table 2.

Age

High age is still the most important negative PF (1, 10, 13, 28, 33-37). Survival in adult ALL (Sweden) per age category is shown in Figure 2. Impact of age is however influenced by factors changing over lifetime as: disease biology, comorbidity, susceptibility to toxicity, and hSCT/intensive protocol eligibility. Some of those could be modified as for example with use of pediatric protocols in younger adults, or reduced intensity hSCT in older adults.

Table 2. Selected prognostic factors for survival in adult ALL.

Factor	Prognosis	Used as high risk criterion in clinical studies
Patient related:		
High age	poor	no
Male sex	(poor)	no
Performance status	poor	no
Disease related:		
High WBC	poor	yes
CNS involvement	(poor)	no/yes
<i>Immunophenotype</i>		
CD20+*	poor	no
CD10-*	poor	no
ETP/early T-ALL**	poor	no/yes
CD1a+**	good	no
<i>Genetics</i>		
Ph+/BCR-ABL*	poor	yes
t(4;11)/MLL	poor	yes
hyperdiploidy	good	no
hypodiploidy	poor	no
Complex karyotype	poor	no/yes
Ph like*	poor	no
Based on response:		
Late CR achievement	poor	yes
MRD	poor	yes

*in B-ALL, **in T-ALL

Performance status

Poor performance status (PS) according to WHO (32) is regarded as a negative PF by some authors (34, 37, 38) and is also often used as exclusion criterion in clinical studies.

Sex

Historically, boys had impaired survival as compared with girls (39). In adults, conflicting data were reported with no survival difference between sexes in most studies, and some reporting worse outcome in males (40, 41). According to the population-based study (40), disparity in overall survival (OS) between sexes disappeared during 2002-2006. Higher complete remission (CR) rate was found in males with T-ALL (42).

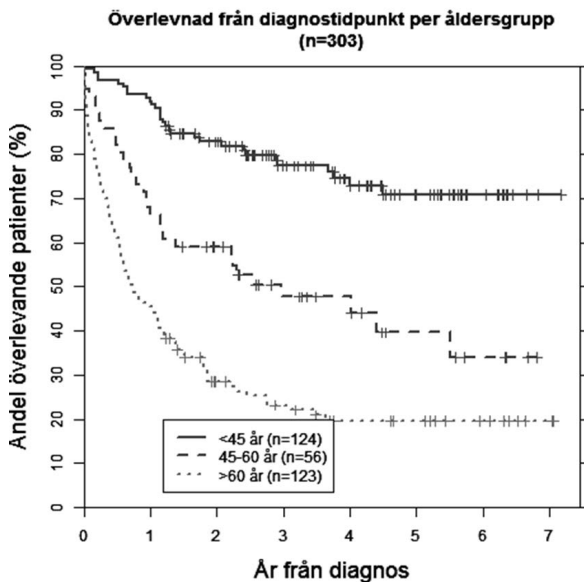


Figure 2. Overall survival in adult patients with ALL in three age groups according to the Swedish Acute Lymphoblastic Leukemia Registry (2007-12).

White Blood Cell count

High WBC, either as continuous variable or above a cutoff value, is still an acknowledged risk factor (10, 33, 35-37, 43). It is however often ex-

plained by other, more specific biological factors such as *MLL* rearrangement (44), T-cell phenotype (31) or Ph+ disease (45, 46).

CNS disease

CNS involvement was associated with high relapse rate and unfavorable prognosis historically (47). CNS directed prophylaxis abolished its poor prognostic value, and non-inferior outcome in patients with CNS leukemia at diagnosis as compared to those without it (48) with some exceptions (30, 49).

Phenotype

Data on the role of T-cell phenotype as PF is conflicting as according to some studies patients with T-ALL have favorable (33) or unfavorable survival (28, 50) compared with B-ALL. A population based study found that T-ALL was associated with enhanced survival in adults in contrast to children having impaired survival as compared with B-ALL (12). Most authors though failed to demonstrate a difference in outcome between the phenotypes. According to stage of maturation, T-ALL is classified as early thymic precursor (ETP) with frequent co-expression of myeloid markers, early non-ETP (pro-), cortical/thymic (CD1a positive), and mature/medullary (51, 52). ETP and non-ETP account for 50% of all T-ALL cases (53). Lower CR rate was achieved in immature T-ALL (51) and survival was impaired in ETP in adults (54). A lower proportion of patients with early and mature T-ALL reached molecular CR as compared with thymic (55). CD1a positivity was associated with favorable prognosis in T-ALL (31). CD10 negative pre-B ALL was associated with poor outcome and frequent *MLL* rearrangement (56). Impaired survival was observed in ALL with CD20 positivity (36).

Genetics

Cytogenetic aberrations are present in $\frac{3}{4}$ of adult ALL patients (57) and have major prognostic impact (58). Aside from classical cytogenetics the use of fluorescence in situ hybridization (FISH) and reverse-transcriptase polymerase chain reaction (RT-PCR) have become routine analyses in ALL diagnostics (59).

Philadelphia chromosome [t(9;22)(q34.1;q11.2)/*BCR-ABL1* rearrangement] is the most frequent single genetic aberration, present in about 11-29% of all adult ALL. There are two main variants of the *BCR/ABL* gene, depending on the length of the sequence of the *BCR* gene: p210 (in more

than 90% of chronic myeloid leukemia and one third of ALL) and p190 in 70-75% of ALL (60). Ph+ is regarded as a poor PF together with t(4;11)(11q23)/*MLL* rearrangement (7%), complex karyotype, and hypodiploidy (58, 59). In patients with hyperdiploidy and t(12;21)/*ETV6-RUNX1* (most common aberration in childhood ALL, uncommon in adults) favorable outcome is observed (59). With the development of genomic profiling, new genetic abnormalities get attention, such as *BCR-ABL* like ALL [with *IKZF1* (IKAROS) mutations] with poor prognosis; *CRLF2*; rearrangements of *ABL1*, *JAK2*, and *PDGFRB*; mutations of *JAK1* and *JAK2*; in T-ALL: *NOTCH1* (60% of patients with T-ALL) or *FBXW7* mutations (61). Frequency of favorable cytogenetic features (hyperdiploidy) decreases and unfavorable (hypodiploidy, complex karyotype, and the Ph+) increases with age (11, 57) with exception of *MLL* which is rare in adults >60 years (57).

Minimal Residual Disease (MRD)

MRD defines a presence of leukemic blasts in BM at a submicroscopic level in patients in CR, and reflects disease biology, pharmacokinetics and pharmacodynamics of the given chemotherapy. Measurement of MRD depends on ALL subtype and available method. Aberrant phenotype has to be established at diagnosis by flow cytometry or PCR [*BCR-ABL*, *MLL-AFF1*, *TCF3-PBX1*, *ETV6-RUNX1*, rearrangement the immunoglobulin or T-cell-receptor (TCR)]. Sensitivity of multicolor flow cytometry is reaching that of PCR. Next-generation sequencing (NGS) is applicable in MRD and as sensitive as real-time quantitative RT-PCR according to the recent study (62). MRD appears to be the single most important, based on response PF for relapse and death in all ALL subtypes/ages (35, 63-68). It is used as HR disease indicator in Ph- ALL defined as standard risk (SR) at diagnosis leading to treatment escalation (11, 55, 69). Deintensification of therapy based on MRD negativity in HR Ph- patients is less common in adults (70). Different cut-off levels (most common 10^{-3} to 10^{-4}) and time-points are used with postinduction MRD (29, 35, 66) or later measurement (55, 66, 67) both having strong impact on outcome. In Ph+ ALL late (3 months and later) but not early (at CR) MRD negativity was associated with lower relapse risk and with a prolonged overall survival (OS) (68). Prognostic value of pre-transplant (71) and post-transplant MRD (72) was demonstrated in the allogeneic hSCT setting.

Treatment

ALL protocols consist commonly of induction (with aim of reaching CR), consolidation (intensification) and prolonged maintenance (up to 2-2.5 years) with reinduction courses. There are some exceptions such as the Hyper-CVAD regimen with two alternating courses (73). Induction is based on various combinations of prednisolone or dexamethasone, vincristine, anthracyclines, asparaginase; consolidation on cyclophosphamide, cytarabine (Ara-C), high dose methotrexate, vincristine, asparaginase, mercaptopurine. Maintenance is given in SR group, and HR group without hSCT prospect. CNS directed prophylaxis/therapy with intrathecal methotrexate with/without cytarabine and steroids is always recommended during induction/consolidation. Eligible patients with HR disease proceed to hSCT or receive intensified treatment. TKI are applied in Ph+ ALL from diagnosis. Combining CD20 antibodies and chemotherapy in B-ALL enhanced survival, mainly in younger patients but not to the same extent as in Burkitt leukemia (74). Standard therapy regimens used in adults rendered unsatisfactory long-term survival (30-40%) despite high CR rate of 74-90% (33, 37, 43, 73). Similarly estimated 3 year OS (29%) in patient treated with the ABCDV protocol (Table 3) in Sweden 1994-98 was observed (28).

Table 3. ABCDV protocol +/- imatinib.

	Drug	Dose	Days
	<i>Imatinib in Ph+ ALL</i>	<i>600 mg o.d.</i>	<i>continuously</i>
Pre-phase	Prednisolone	60 mg/m ² oral	-5-0
	Cyclophosphamide	200 mg/m ² i.v.	-5-0
Remission induction (ABCDV)	Methotrexate	10 mg/m ² i.t. (max 15 mg)	0
	Ara-C (cytarabine)	3 g/m ² b.i.d. i.v.	1-3
	Betamethasone	20 mg/m ² oral	1-5
	Cyclophosphamide	600 mg/m ² i.v.	1
	Daunorubicin	30 mg/m ² i.v.	1-3
	Vincristine	2 mg i.v.	1
Consolidation 1 (VABA)	Vincristine	2 mg i.v.	1
	Amsacrine	200 mg/m ² i.v.*	1-3
	Betamethasone	20 mg/m ² oral	1-5
	Ara-C	3 g/m ² i.v.*	1-4

Consolidation 2 (BCDE)	Betamethasone	20 mg/m ² oral	1-5
	Cyclophosphamide	1000 mg/m ² i.v.	1
	Daunorubicin	30 mg/m ² i.v.	1-2
	Etoposide	100 mg/m ² i.v.	1-5
Consolidation (mini-VABA)	Vincristine	2 mg i.v.	1
	Amsacrine	200 mg/m ² i.v.	1-2
	Betamethasone	20 mg/m ² oral	1-5
	Ara-C	3 g/m ² i.v.	1-3
Consolidation (MAP)	Methotrexate	1500 mg/m ² i.v.	1, 15
	PEG-Asparaginase	1000 E/m ² i.v.	2, 16
	6-Mercaptopurine	60 mg/m ² oral	1-21
Maintenance in 2 years from last consolidation	6-Mercaptopurine**	50-75 mg/m ² o.d.	continuously
	Methotrexate**	5-10 mg/m ² oral	continuously weekly
	Reinduction course- every 2:nd (1:st year) and every 3:rd (2:nd year) month:		
	Daunorubicin (1:st year)	40 mg/m ² i.v.	1
	Vincristine (1:st year)	2 mg i.v.	1
	Prednisolone	60 mg/m ² oral	1-7 (1:st year) 1-5 (2:nd year)
	Ara-C (2:nd year)	60 mg/m ² x1 sc	1-5
	Thioguanine (2:nd year)	80 mg/m ² oral	1-5

Optional pre-phase. VABA as second induction. MAP in high risk disease only.

*For patients >70 years of age, amsacrine is given d 1–2 and Ara-C d 1–3.

**Omitted in Ph+ ALL.

Younger adults

Outcome in patients 15-40 years was notably impaired as compared with children historically. Retrospective studies comparing outcome in adolescents treated either in “adult” or pediatric centers showed benefit of the latter (49, 75, 76). A number of studies (though not randomized) demonstrated substantial advantage of pediatric protocols over the traditional adult protocols in treatment of adolescents and young adults (AYA) (10, 77) and adults up to 55 years (9, 10). According to the latter study (10), toxic death was more common and survival impaired in adults >45 years as compared with <45. Advantage of a pediatric protocol in terms of event free survival (EFS) but not OS was demonstrated in patients 45-55 years

(9) as compared to AYA. Pediatric regimens consist of higher cumulative doses of asparaginase, cortisone, vincristine and methotrexate as compared with “adult” protocols. The Nordic Society of Paediatric Haematology and Oncology (NOPHO) protocol is currently used in younger adults in Sweden (11).

Older/elderly

Older/elderly patients (>55 years) are often treated according to standard adult protocols. However, the outcome is inferior as compared with younger adults included in the same trials in terms of CR achievement, disease free survival (DFS) and OS (27, 50, 78). CR rate in elderly is ranging from 34 to 84% (26, 27, 41, 50, 79-81) and long-term survival is poor reaching only 20-30% (27, 78, 79, 81). The main issue regarding induction treatment is high early death (ED) rate (11-34%) (26, 27, 41, 78-80). Comorbidities, poor performance status, toxicity, need for chemotherapeutics dose reductions and more frequent HR features (26, 27, 41, 78-80) are contributing to dismal outcome in this group. Results of treatment with an age-adapted protocol (Table 4) established by the European Working Group on Adult ALL (EWALL) were reported recently (82-84) showing high CR rate (85-90%) and substantial long-term survival.

Philadelphia positive ALL

Ph+ ALL constitutes a distinct entity in regard to biology, prognosis and therapy. Responses to standard chemotherapy were poor with low CR rate, high relapse rate as well as impaired survival as compared to Ph- (34, 37, 45, 58).

After introduction of imatinib (the first TKI), CR rates and OS improved markedly as compared with the “pre-imatinib era” (85, 86). Still, the vast majority of patients will subsequently relapse unless hSCT is performed, and the procedure is recommended in all eligible patients (87). TKI enabled more patients to be transplanted due to higher CR rate (86), and use of TKI post-transplant can prevent relapse (88). In elderly patients less intensive regimens combining chemotherapy/steroids and imatinib (82, 85, 89), nilotinib (83) or dasatinib (84) resulted in satisfactory short-term outcome. Relapse/imatinib resistance is associated with *BCR-ABL* kinase domain mutations in nearly all patients (mostly T315I) (90), who can be effectively treated with third generation TKI ponatinib which overcomes the resistance (91).

Table 4. EWALL backbone +/- imatinib.

	Drug	Dose	Days
	<i>Imatinib in Ph+ ALL</i>	<i>600 mg o.d.</i>	<i>continuously</i>
Remission induction I (d 1-16) and II (d 20-34)	Methotrexate	12 mg i.t.	1
	Dexamethasone	10 mg/m ² oral	1-7, 13-16
	Vincristine	1 mg i.v.	6, 13
	Idarubicin	10 mg i.v.	6, 7, 13, 14
	Ara-C (cytarabine)	60 mg/m ² i.v.	21-24, 28-31
	Cyclophosphamide	300 mg/m ² i.v.	20-22
	Methotrexate/Ara-C/prednisolone	12/40/12.5 mg i.t.	12, 20, 27, 34
	G-CSF	5 µg/kg s.c.	6-, 20-
Consolidation 1, 3, 5	Methotrexate*	1000 mg/m ² i.v.	1
	Asparaginase*	10 000 E/m ² i.v. or i.m.	2
Consolidation 2, 4, 6	Ara-C (cytarabine)	1000 mg/m ² i.v.	1, 3, 5
Maintenance in 2 years from start of induction	6-Mercaptopurine**	50-75 mg/m ² o.d.	continuously
	Methotrexate** weekly	5-10 mg/m ² oral	continuously
	Reinduction course- every 2:nd (1:st year) and every 3:rd (2:nd year) month during maintenance:		
	Vincristine	1 mg i.v.	1
	Dexamethasone	40 mg oral	1-2

*50% methotrexate and asparaginase dose reduction in patients aged >70 y. Asparaginase omitted in Ph+ALL. **omitted in Ph+ ALL.

T-ALL

As mentioned previously, disease with T-phenotype represents a distinctive subgroup of ALL in terms of epidemiology, presentation and genetics. The same protocols as in B-ALL are generally applied. However, at relapse of T-ALL, nelarabine (prodrug of guanine arabinoside) can be used with reported CR of 31% and one year OS of 28% (92). The drug can be applied even upfront in combination with standard regimens (93). Use of autologous hSCT as consolidation with subsequent prolonged mainte-

nance was reported to be exceptionally effective recently (94). Use of pediatric protocols in T-ALL (10) up to age of 69 showed significantly improved long-term survival as compared with classical ones (81 vs. 44%). As mentioned previously prognosis is worst in ETP. When allogeneic hSCT was performed in CR1 no difference in OS was found between all subtypes of T-ALL suggesting beneficial effect of the approach in ETP (53).

Allogeneic hSCT

Allogeneic hSCT is widely used as remission consolidation in HR ALL though its utilization is limited by patients' eligibility and donor availability. Prospective studies using donor/no donor "biological" randomization showed benefit of the procedure in SR disease (95, 96). The benefit was less pronounced (Ph+ included)(96) or absent in HR disease (Ph+ excluded)(95) though metaanalysis of seven studies (97, 98) could demonstrate OS improvement and cost effectiveness of the approach in the HR group. Results of transplant using stem cells from unrelated donor (URD) were similar to those with stem cell source from matched related donor (RD) (99). Utilization of hSCT in SR AYA is questioned by favorable results of pediatric inspired protocols (100). Autologous hSCT gave inferior outcome as compared with both allogeneic hSCT and standard chemotherapy (95). However, according to a population based study (101), the advantage of the allogeneic transplantation over autologous was observed in Ph+ disease only. In older adults not eligible for standard myeloablative conditioning (MAC) there is an option of reduced intensity conditioning transplant (RICT), though both relapse rate and transplant-related mortality (TRM) are still quite high in the latter group (40% and 28%, respectively) (102). Patients developing chronic graft-versus-host disease (GVHD) had improved OS (103), suggesting importance of a graft-versus-leukemia (GVL) effect in ALL. Implementation of RICT contributed to survival prolongation in recent years in patients 40-69 years according to a population-based study (1).

Relapse

Risk of ALL recurrence is increasing with age at diagnosis, and prognosis is dismal especially in those relapsing after hSCT (104). Most patients (90%) relapse in the BM, while CNS is the most common extramedullary relapse site (105). It was demonstrated that intensive therapy with the aim of reaching CR2 prolongs survival (106, 107), but long-term OS is seen

only in 7–12% of patients (105, 108, 109). There are a number of rescue protocols containing anthracyclines, vincristine, steroids, cyclophosphamide, cytarabine, and asparaginase, producing CR in 0-80% of patients (109). Remissions though are short-lived, and allogeneic hSCT is considered as the only curative option after CR2 achievement (105, 107-109). However, the procedure is available only for a minority of patients due to poor performance status, lack of a donor or second relapse. Transplantation without prior CR2 achievement is of questionable value (109, 110). Higher age and early relapse are regarded as unfavorable factors for achievement of CR2 and survival (105, 108). The new monoclonal antibodies blinatumomab (bispecific anti-CD19/CD3) (111) and inotuzumab ozogamicin (anti-CD22 bound to calicheamicin) (112) showed high activity in refractory/relapsed adult Ph- B-ALL, with low serious toxicity. Even advanced engineered T-cell therapies directed against the same target (CD19) have been developed, with CR rate of 90% in the relapsed/refractory setting (113). Long-term outcome of these therapies are to be awaited.

Population registries in Sweden

The Swedish Cancer Registry was founded in 1958 and covers the whole population. Subsequently the Swedish Acute Leukemia Registry in 1997 and Acute Lymphoblastic Leukemia Registry in 2007 were established. Coverage of the latter was estimated to 98% as compared with The Cancer Registry (13). Both leukemia records contain basic pathology/clinical data (as the date of diagnosis/relapse/death, ALL phenotype, hSCT), and more specific data (as Ph status, used protocol, and MRD) is reported to the recent one. The Swedish Cause of Death Registry was established in 1961. The causes of death are coded according to the International Classification of Diseases (ICD). Undernotification of acute leukemias in the Cancer Registry as compared with the Swedish Cause of Death Registry was reported previously (114). Obtaining data and linkage between various registries is possible through a unique personal identification code. This enables population-based epidemiological and clinical studies.

Summary of papers

Aims

Overall aim

Study of disease characteristics, implementation of guidelines, and results of therapy with focus on outcome, toxicity, and prognosis in specific ALL subsets in the Swedish population.

Specific aims

Paper I. Investigation of the characteristics of relapsed ALL and its treatment with specific protocols recommended by national guidelines, and efficacy of hSCT in second CR.

Paper II. Evaluation of an augmented protocol (Hyper-CVAD) for T-ALL treatment in terms of feasibility, outcome, and toxicity.

Paper III. Assessment of clinical use and prognostic implications of MRD in Ph negative B-ALL in older adults treated with the ABCDV protocol.

Paper IV. Evaluation of disease characteristics and different therapeutic approaches in older/elderly ALL

Materials and Methods

Data collection

The studies are population-based, semi prospective, and basic clinical/pathology/treatment data were obtained from the Swedish Acute Leukemia/ALL Registries. Additionally, search was performed using the Swedish Cause of Death Registry for study IV. Inclusion criteria are presented in Table 5.

Table 5. Inclusion criteria used in four studies.

Paper	I	II	III	IV
Diagnosis	Relapsed B-/T-ALL	T-ALL	Ph- B-ALL	B-/T-ALL
Treatment	guidelines	Hyper-CVAD	ABCDV	guidelines
Age	<66y	No age limit	>45y	55-85y
Period	2003-2007	10.2002-09.2006	2007-2011	2005-2012

Clinical and laboratory data as well as pathology and genetic reports were complemented directly from patient records. For study purposes, MRD levels were ascertained at each pathology department and later verified by one pathologist (Paper III). Performance status (PS) at diagnosis according to WHO (32), the comorbidity component (CC) of the Charlson Comorbidity Index (115), number of comorbidities and drugs were recorded (Paper IV), as well as cytostatics dose-reduction /omission and toxicities (grade III-IV) according to Common Terminology Criteria for Adverse Events (116). The studies were performed in accordance with the declaration of Helsinki, with approval from the Regional Ethical Review Board in Uppsala/Sweden (Paper I-III: Ups 03-520, Paper IV: Ups 2014/063).

Diagnostic procedures

The diagnosis of ALL was made using BM morphology, immunophenotype, and genetic analysis according to the WHO Classification (32) at each center. Genetic analyses included G-banding, FISH, and RT-PCR. CNS-leukemia was diagnosed in presence of blasts in cerebrospinal fluid or/and radiology, and bulky disease when lymph node conglomerate exceeded 10 cm or a mediastinal mass 1/3 of the thoracic diameter.

Response- and high risk criteria

CR criteria were: hematopoietic recovery, less than five percent blasts in the BM, and no extramedullary disease. MRD was assessed after induction (MRD1) and consolidations (MRD2-3) by flow cytometry (six-color since 2008) in Ph- B-ALL, RT-PCR in Ph+ ALL, and flow cytometry/PCR of TCR rearrangement in T-ALL. HR factors according to the guidelines included: WBC $>30 \times 10^9/L$ in B-ALL or $>100 \times 10^9/L$ in T-ALL, Ph+, t(4;11)/*MLL*, late CR achievement, and/or HR MRD ($>1\%$ after remission induction, not reaching $<0.1\%$ after consolidation therapy, or duplicate measurements $>0.1\%$ after the consolidation courses), and T-cell phenotype (from 2009).

Treatment recommendations

Treatment protocols recommended by the Swedish national guidelines during the study period are presented in Table 6.

Table 6. Treatment of ALL according to the national guidelines.

Period	2005-2009	2009-2012			
Age	All eligible adults	18-45y	Biological age 45-60y	Biological age 60-75y or younger with comorbidities	Biological age $>75y$ or younger with serious comorbidities
ALL					
Pre-B Ph-	ABCDV	NOPHO	ABCDV	EWALL	Reduced CHOP (75%), VAD, VCR+ steroids
Pre-B Ph+	ABCDV +imatinib*	ABCDV +imatinib	ABCDV +imatinib	EWALL +imatinib	Imatinib +steroids
T	Hyper-CVAD	NOPHO	ABCDV	EWALL	Reduced CHOP (75%), VAD, VCR+ steroids

*imatinib as standard treatment for Ph+ disease was introduced 2007.

The Hyper-CVAD protocol (73) was modified by replacing dexamethasone with equivalent doses of betamethasone (Paper II). Granulocyte colony-stimulating factor (G-CSF) (if not already part of the protocol) and anti-infectious prophylaxis were used according to local recommendations at each center. Allogeneic hSCT was recommended in eligible patients in the presence of HR factors. Local routines in terms of conditioning regi-

mens, donor type, and GVHD prophylaxis were used at the six transplantation centers. In patients with SR leukemia and HR, not eligible for hSCT/without donor, maintenance therapy was to be started after consolidation. ABCDV for late relapse (>2 years since initial diagnosis) and FLAG-Asp/MEA for early relapse were recommended (Table 7).

Table 7. Salvage regimens recommended in national guidelines 2003–2007.

	Drug	Dose	Days
FLAG-Asp	Fludarabine	30 mg/m ² i.v.	1, 2, 3, 4, 5
	Ara-C	2000 mg/m ² i.v.	1, 2, 3, 4, 5
	PEG-Asparaginase	500 E/m ² i.v.	2, 16
MEA	Mitoxantrone	12 mg/m ² i.v.	1, 2, 3, 4
	Etoposide	100 mg/m ² i.v.	1, 2, 3, 4
	Ara-C	1000 mg/m ² b.i.d. i.v.	1, 2, 3, 4

Allogeneic hSCT in CR2 was advised for younger, eligible patients if not transplanted in CR1.

Statistics

Distributions of survival (OS, LFS, EFS, CCR) were defined as shown in Table 8, and calculated by the Kaplan-Meier method, and 95% confidence intervals (95% CI) were obtained.

Table 8. Survival terms and definitions.

	From	To	Event	Paper
OS	Diagnosis*	Death, last follow up	Death	I, II, III, IV
Event free survival (EFS)	Diagnosis	Relapse, death, last follow up in CR	Relapse, death	IV
Continuous CR (CCR)	CR	Relapse, death, last follow up in CR	Relapse	III
Leukemia-free survival (LFS)	CR**	Relapse, death, last follow up	Relapse, death	II

*relapse in study I, **event at day 1 in patients with refractory ALL/no CR evaluation

Differences in survival according to risk factors were analysed by the log-rank test or Cox univariate regression analysis. Relevant covariates were included in Cox multivariate regression analysis (Paper I and IV). Logistic regression was used for evaluation of correlations between variables and CR achievement (Paper I, IV) as well as distribution of risk factors in two age groups (Paper I). Chi-square or two-tailed Fischer's exact test was applied for proportions and Mann-Whitney U test for continuous variable comparisons (Paper IV). Statistical tests were used with an alpha-significance level of 5%. Analyses were performed with SPSS (v.21-23, IBM) statistical package.

Results

Summary of results from all four papers is presented in Table 9.

Table 9. Patient characteristics, outcome, and risk factors per study.

paper	I	II	III	IV
Patients treated intensively (n)	71	19	35	124
Median age years (range)	39 (19-65)	32 (18-72)	61 (46-79)	65 (55-82)
Male:Female	42:29	15:4	12:23	57:67
Main protocol	ABCDV, MEA, FLAG-Asp	Hyper-CVAD	ABCDV	EWALL, ABCDV
CR%	70	89	91	83
Allogeneic hSCT (n)	29*	4	5	20
OS 1 year (95% CI)%	41 (29, 52)			59 (50, 67)
OS 3 year (95% CI)%	22 (13, 32)			32 (24, 40)
OS 5 year (95% CI)%	15 (7, 24)	47 (26, 69)	47 (30, 64)	
Negative prognostic factors	Age >35y, time to relapse <18 months	Age ≥35y, long time between course 1 and 3	Age >65y, PS ≥2, MRD1 >0.1%	Age ≥75y, PLT ≤35**, Male sex***

*in CR2, **platelet (PLT) count ($\times 10^9/L$), ***in patients 55-64 years

Paper I

76 patients with relapsed ALL were identified. Five patients received only palliation and were excluded. Median age at diagnosis in remaining 71 was 39 (range: 15-65) years. Pre-B phenotype was present in 58 (82%) and T in 13 (18%). 14/67 (21%) had Ph/*BCR-ABL* positivity and 2/67 *MLL* rearrangement. 14 patients received hSCT in CR1. In 39 of 71 patients (55%) disease recurred within 18 months (median 13, range: 2-82). Isolated BM relapse was observed in 72% and CNS involvement in 8% of individuals. Remission induction with MEA (n=9), FLAG-Asp (n=16), ABCDV (n=21), TKI (n=8) and other protocols (n=17) was attempted obtaining CR in respective: 67, 63, 43, 75, 35% of patients (not significant differences although ABCDV was recommended for late relapse). Totally 50/71 (70%) of the patients achieved CR2 [37/71 (50%) after first course]. Age >35 years at diagnosis and time to relapse <18 months influenced final CR2 achievement negatively ($P = 0.012$ and 0.001 , respectively). Both higher age and early relapse proved to be negative prognostic factors for CR2 achievement previously (108). Induction dead occurred in 3/71 (4%) patients. Allogeneic hSCT was performed in 29 of 57 (51%) eligible patients (not transplanted in CR1) with stem cells from RD (n=14), URD (n=14) or cord blood (n=1). The majority received MAC (n=25) and only four RICT. Two patients (+ one in active disease) underwent autologous SCT. Consolidation chemotherapy was given to remaining patients in CR2. None of patients transplanted in CR1 became long-term survivors, but those with URD transplant had superior OS as compared with RD ($P = 0.037$). OS in the whole cohort of 71 patients at one year was 41% (95%CI: 29, 52) and the projected 5 year OS rate was 15% (95%CI: 7, 24), which is in parity with previous results (105, 108, 109). Patients not receiving allogeneic SCT in CR1 (n=57) had CR2 rate of 70% and median OS of 9 months (range: 0.5–99).

Best survival was noted in patients undergoing allogeneic SCT in CR2 as compared with patients who achieved CR2 but were treated without allogeneic SCT, and those without achievement of CR2 [5 year OS, (95%CI): 34%, (17, 52) vs. 9%, (0, 26) vs. 0%] as presented in Figure 3. Negative prognostic factors for OS in our cohort were: age >35 years, time from diagnosis to relapse <18 months, and additionally in non-transplanted (in CR1) patients isolated BM relapse (multivariate analysis). Influence of age and time to relapse on prognosis was demonstrated previously (105, 108, 109). When divided in four groups in terms of time to relapse </>18 months and age </>35 years, younger patients with longer

time to relapse had the longest, and older patients with short time to relapse had the shortest OS (Figure 4).

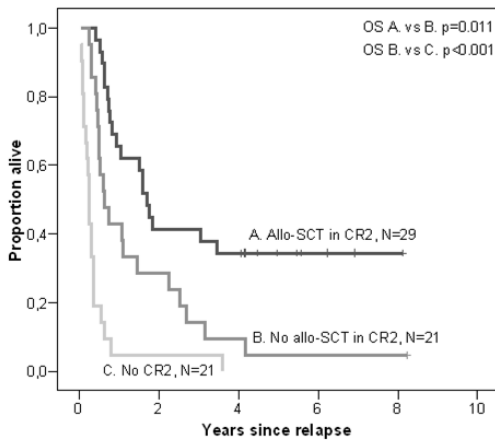


Figure 3. Effects of allogeneic SCT (allo-SCT) in CR2, and CR2 achievement, on overall survival.

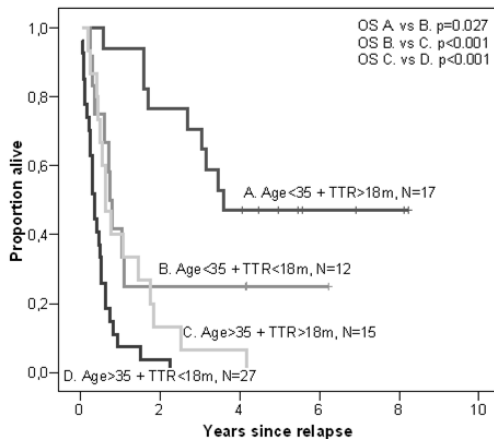


Figure 4. Overall survival according to age at diagnosis and time to relapse (TTR).

All patients older than 35 years at diagnosis (n=42) died regardless of postremission therapy.

As demonstrated previously intensive treatment of relapse can enhance survival (106) and a number of protocols are used with widely varying results (109) though both MEA and FLAG-Asp proved to be efficacious in CR2 induction. CR was achieved in a high proportion of patients with Ph+ disease (transplanted in CR1) using TKI only (6/8- 75%), but none of them have survived.

The only curative consolidation option in CR2 is allogeneic hSCT as confirmed in our cohort. High proportion of patients in our study (41%) underwent the procedure as compared to other reports (17–30%) (105, 108, 109). hSCT in active relapse is not a reasonable option (109, 110). The approach could be questioned in older populations as none of our patients older than 35 years survived regardless of remission consolidation used, and TRM was high. RICT could be a solution as indicated previously (100). Outcome in transplanted patients <35 years old was satisfactory suggesting that the procedure should be used rather in CR2 than CR1 in SR disease. Achieving CR2 even without subsequent SCT enhanced survival in our study. Even if allogeneic hSCT is to be preferred in relapsed disease, autologous hSCT could be a reasonable option, as prolonged survival was observed in two of our patients. Efficacy of autologous transplantation in CR2 was demonstrated by others (105, 108).

Beside age and time to relapse, other prognostic factors including Philadelphia chromosome status, WBC, blast count in BM, and lactate dehydrogenase in various scoring systems, were evaluated previously (110, 117). Patients experiencing disease recurrence at extramedullary site (mainly CNS) had improved survival as compared to isolated BM relapse in our study. However, the prognostic role of CNS involvement at relapse is not established (105, 108, 109).

Paper II

All patients diagnosed with T-ALL during the study period were identified (n=24). Five patients were excluded (four treated with palliative intention and one with a concomitant relapse of sarcoma). Remaining 19 patients were treated with the Hyper-CVAD protocol. The median age was 32 years (range: 18–72) with male dominance (15/19, 79%). Specific disease characteristics were: mediastinal mass (n=11), CNS involvement (n=1), WBC >100 × 10⁹/L (n=1). Of 14 evaluable patients five had immature (pro/pre), three cortical/thymic, and 6 had medullary T-cell phenotype.

Karyotype (16/19 evaluable) was complex (n=2) including one with the *MLL* fusion gene, showed miscellaneous aberrations (n=9), and was normal (n=5) respectively.

17/19 (89%) patients achieved CR: after one or two Hyper-CVAD cycles (n=15), after more than two cycles (n=2), after nelarabine therapy (n=1), and after allogeneic SCT (n=1). HR disease was present in 6 patients: hyperleukocytosis (n=1), MRD (n=1), no CR (n=2), and late CR achievement (n=2). The survival data in whole cohort was as follows: 2 and 5 year LFS - 29% (95%CI: 8, 51), the 2 year OS - 63% (95%CI: 42, 85), and 5-year OS 47% (95%CI: 26, 69). LFS was inferior as compared to a previous report of 5 year CR duration of 55% (34). Four patients (three with HR disease) underwent allogeneic hSCT upfront (MAC), with two long-term survivors (two deaths due to relapse). All but one non-transplanted patients (n=14, three with HR features) received all eight cycles of the Hyper-CVAD protocol (without significant dose reductions), with the median time between cycle one and eight of 5.7 (range: 4.9–8.5) months, which was similar to previously reported data (73). Mediastinal irradiation was applied in 3/11 patients with initial mediastinal involvement. Observed serious toxicities were: severe neuropathy (vincristine related, n=1), bacterial (n=5) and invasive fungal infections (n=1), and femoral head avascular necrosis (n=1). No toxic deaths due to chemotherapy only were observed. Five year LFS and OS in this group were 20% (95%CI: 0, 40) and 47% (95%CI: 21, 72) respectively. 12 of 15 (80%) patients relapsed after median 9 months (range: 2-23), and six patients were transplanted in CR2 (MAC), with three becoming long-term survivors.

Age ≥ 35 years at diagnosis influenced LFS [hazard ratio 2.7 (95%CI: 0.9, 8.3); Figure 5A] and OS [hazard ratio 5.1 (95%CI: 1.55, 16.7); Figure 5B] negatively, but not relapse rate [<35 years old - 7/9 (78%) vs. ≥ 35 years old - 5/6 (83%); $P = 0.79$] despite low transplantation rate in the older group [1/7 (14%) vs. 9/12 (75%); $P = 0.01$].

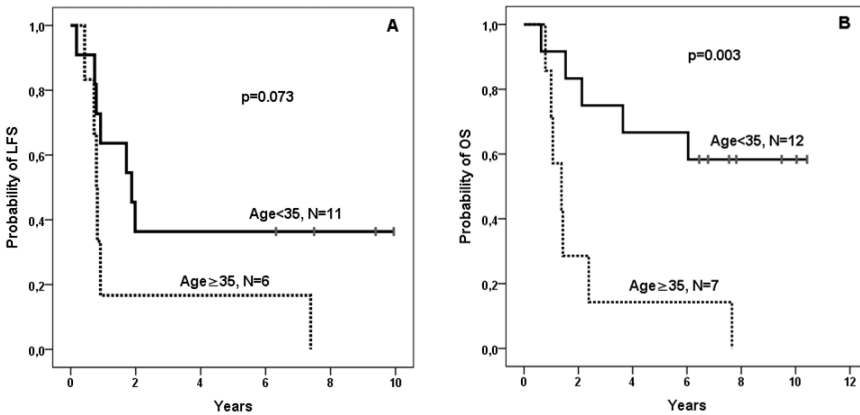


Figure 5. Leukemia-free (A) and overall survival (B) according to age (<35 years or ≥35 years) in patients treated with Hyper-CVAD.

Of all parameters, only time between cycle 1 and 3 (median 47 days) had a negative impact on OS [hazard ratio 1.05 (95%CI: 1.006, 1.09); $P = 0.03$; time as continuous variable], but not on LFS.

Although the T-ALL cohort was small due to rarity of the disease we could perform population-based assessment of efficacy of the Hyper-CVAD protocol. Adherence was acceptable. However, replacement of dexamethasone (used in original protocol) with betamethasone could have influenced the results. Outcome was similar to the previously reached by ABCDV in T-ALL (28). No toxic deaths and high relapse rate suggest possibility/need of protocol intensification, as relapsed disease has dismal prognosis (31). hSCT is the most effective treatment after ALL recurrence (107). The procedure is often unavailable for older patients, who had generally much worse outcome, as compared to younger despite equal relapse rate. One can speculate that clinicians failed to identify all patients with HR ALL, as MRD was not measured consistently. This group (HR T-ALL) can benefit from hSCT in CR1 (51, 118), but high TRM in older patients (95) limits the option and other approaches such as intensifying the Hyper-CVAD protocol (119), combining nelarabine and Hyper-CVAD, and using pediatric-like regimens (120) have been proposed.

Paper III

The ABCDV protocol was given to 35 patients with Ph- B-ALL and median age of 61 (range: 46–79) years. Relatively high proportion of the patients (91 %) achieved CR (30 after ABCDV, two after VABA). Two patients (6%) died during induction (within 30 days) which can be regarded as acceptable in this age group. Eight patients with CR (25 %) could not follow the protocol due to different reasons (mainly high age and infections), reflecting toxicity of the protocol and need of further studies in older patients (9). Allogeneic hSCT was performed in CR1 in five patients aged 47–64 years with HR factors [t(4;11) (n=2), WBC (n=2), or MRD (n=1)]. A total of 30 patients reached CR after induction with ABCDV. MRD1 measurement by flow cytometry was performed in 22 of 30 patients achieving CR after the first course, on day 19–26 (Table 10). MRD was not measured in eight patients over 70 years of age or with HR criteria reflecting pragmatic use of the tool by the clinicians, as treatment escalation was not a reasonable option in the former, and already indicated in the latter group. MRD1 was not evaluable in two cases (hypoplastic BM), not detectable in 8, and detectable at levels 0.01–0.099%, 0.1–0.9%, and >1% in 5, two, and 5 patients. After the second course of treatment, MRD2 (after VABA) was evaluable in 16 of 17 patients and detectable in three with analysis performed on day 48–78. Only one patient was assigned to the HR group due to persistent MRD. CCR at the last follow-up was still present in 17 patients (49%): 6 with HR and 11 with SR disease. Among patients with SR leukemia, 7 had MRD1 <0.1 %, one >1 %, and three no MRD measurement (two > 70 years of age, one not evaluable). Relapse occurred in 15 patients (7 with HR and 8 with SR features). One of 5 patients transplanted in CR2 became a long-term survivor. Surviving patients were followed up a median of 71 months. Five year OS and CCR was 47 % (95 % CI 30–64 %) and 51 % (95 % CI 33–70 %), respectively.

Negative prognostic factors for OS were age >65 years and PS ≥ 2 (Table 10), but neither high MRD1 nor other HR features. CCR was influenced positively (and reached almost 90% in patients in the SR group) by MRD1 <0.1 % (Table 10). However, it is difficult to assess the prognostic value of the procedure based on such a low number of patients.

Table 10. Factors affecting OS and CCR.

	n (%)	5 year OS (95%CI)%	P	n	5 year CCR (95%CI)%	P
Age <65	25 (71)	59 (39, 79)	0.01	24	58 (37, 79)	0.01
Age >65	10 (29)	20 (0, 45)		8	29 (0, 62)	
PS 0-1	27 (77)	55 (36, 74)	0.04	26	53 (33, 74)	NS
PS ≥2	8 (23)	25 (0, 55)		6	40 (0, 83)	
HR *	15 (43)	47 (21, 72)	NS	13	40 (11, 67)	NS
SR	20 (57)	49 (27, 71)		19	59 (19, 75)	
MRD1 >0.1%	7 (35)	54 (14, 93)	NS	7	43 (6, 80)	0.05
MRD1 <0.1%	13 (65)	69 (44, 94)		13	83 (62, 100)	
HR and/or MRD1 ≥0.1%	20 (71)	43 (20, 66)	NS	18	40 (16, 64)	0.04
SR including MRD1 <0.1%**	8 (29)	75 (45, 100)		8	87 (65, 100)	

* refractory: n= 2, late CR: n=2, WBC over $30 \times 10^9/L$: n=9, t(4;11): n=4, high risk MRD: n=1. Two patients had more than one HR factor. **omitting 7 patients regarded as SR according to the protocol but who did not have MRD1 measurement

Two main methods of MRD measurement are applied currently – PCR and flow cytometry (67), and analyses are used in different fashion depending on ALL phenotype (29, 35, 55, 66, 121). In our study MRD1 was measured by the latter method at median 25 days with discrimination level of 1%, though CCR was influenced at level of 0.1%. As only one patient was assigned to the HR group based on MRD positivity, and it was not measured in elderly, the serial MRD assessment could be questioned. On the other hand, MRD1 as a marker of HR disease could hypothetically indicate need of treatment de-escalation in elderly patients, as cure is not probable.

Paper IV

Patients from ALL registries (n=172) and the Swedish Cause of Death Registry (n=2) were identified. 19 patients with Burkitt leukemia (11%) were excluded. In the final study cohort of 155 patients with median age of 67 and male:female ratio of 72:83, 91% had B-, 9% T-, 1% B- UNS phenotype, 9% CNS involvement, only one patient bulky disease, and two mediastinal involvement. T-ALL was more common in males [10/72 (14%) vs. 2/83 (2%); $P = 0.01$]. Genetic analysis (G-band karyotyping, FISH or PCR for at least *BCR-ABL*) was performed in 140 of 155 (90%) patients. 35% had Ph+ ALL and 6% *MLL*. Characteristics of our cohort were similar to those from other studies (26, 27, 41, 78-80), with low T-ALL/bulky disease, and high Ph+ incidence. Intensive treatment was applied in 124 (80%) and palliative in 31 (20%) patients with different median age of 65 (55-82) and 79 (55-85) years, respectively. The proportions of patients with PS ≥ 2 and CC ≥ 1 were higher in the palliative cohort ($P < 0.001$ and 0.005). Only age (as continuous variable) and proportion of patients with PS ≥ 2 differed between the two cohorts ($P < 0.001$ and < 0.05 , respectively; multivariate analysis). CR, one and 3 year OS were: 67%, 50% (95%CI: 42, 58) and 26% (95%CI: 20, 33), respectively.

Patients treated intensively

Two main protocols, EWALL-backbone +/-TKI and ABCDV +/-TKI, were administered to 35 and 79 patients of which the former were older [median age of 69 (range 62-82) vs. 63 years (range: 55-79), respectively; $P < 0.001$]. Modification of treatment was performed in 34% of the patients for both protocols. Other regimens were: Hyper-CVAD (n=5), daunorubicin/cytarabine (n=4, due to misclassification as AML), pre-phase treatment only in one who died before continuation. TKI was started at induction in all 40 patients with Ph+ and one with Ph- ALL. When analyzing treatment in three age groups (55-64, 65-74, 75-82 years), ABCDV was most common in the youngest (given to 83% in this group).

In the total cohort, CR was achieved in 83% of the patients, compared to 34-84% in previous reports (not population-based) (26, 27, 41, 50, 79-81), with lowest proportion in oldest age group compared with youngest (59% vs. 90%; $P = 0.03$), and slightly higher proportion in Ph+ ALL than in Ph- (93% vs. 80%; $P = 0.07$). EWALL-backbone +/-TKI gave CR in 70% of patients compared to 85-97% in other studies assessing the protocol (82-84), and ABCDV +/-TKI gave CR in 89%. Median survival and median follow-up of survivors were 16 months (range 0-126) and 74 (33-

126) months, respectively. OS/EFS were 59% (95%CI: 50, 67)/ 47% (95%CI: 38, 56) after one and estimated to 32% (95%CI: 24, 40)/ 25% (95%CI: 17, 33) after 3 years. The latter was similar to results reported by others in older/elderly patients (78, 79). EWALL-backbone/ABCDV resulted in one year OS of 49% (95%CI: 32, 65)/ 63% (95%CI: 53, 74) and 3 year OS of 20% (95%CI: 7, 33)/ 39% (95%CI: 28, 50), respectively. According to the previously cited report (82), one year OS reached 60% in another EWALL-backbone treated cohort. Relapse was the main cause of death (56/124, 45%), as demonstrated also by others (27, 78-80). Early death (ED; within 60 days) occurred in 18/124 patients (15%), which is consistent with previous reports (11-34%) (26, 27, 41, 78-80). Higher proportion of patients with PS ≥ 2 experienced ED as compared to PS < 2 [7/26 (27%) vs. 11/97 (11%); $P = 0.046$]. ED for EWALL-backbone was 20% in contrast to other studies of the protocol with none (82, 83) or low early death (84). 13% of patients receiving ABCDV died within 60 days. Patients aged 65-74 years received EWALL-backbone and ABCDV in similar proportions (47% vs. 45%). The former was applied mainly after October 2009 (19/22, 86%) and the latter mainly in the preceding period (22/31, 71%; $P < 0.001$). CR after treatment with EWALL-backbone and ABCDV was achieved in 72% and 88% respectively ($P = 0.18$), and the ED rate was similar (20% vs 21%). OS was not different between EWALL and ABCDV cohorts, despite the same median age (69 years) in patients 65-74 years (Figure 6).

Toxicity

The toxicity profile among three age groups was not different (except more common kidney failure in the two oldest). Interestingly patients with diabetes as compared to those without were affected by invasive fungal infection more frequently [7/16 (44%) vs. 15/106 (14%); $P = 0.004$] which is a novel finding possibly indicating the need of prophylaxis in this group. Higher proportion of patients treated with ABCDV as compared to EWALL-backbone experienced serious infections [69/79 (87%) vs. 23/35 (66%); $P = 0.007$] and serious toxicity of TKI [(45%) vs. 1/12 (8%); $P = 0.03$].

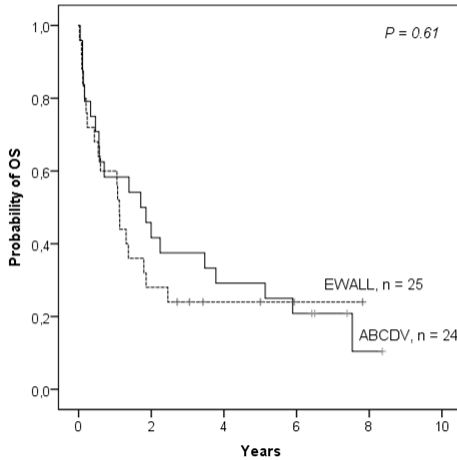


Figure 6. Overall survival in patients aged 65-74y according to protocol (EWALL-backbone vs. ABCDV).

Allogeneic hSCT in CR1

19% of patients reaching CR1, with median age of 60 years (range: 55-66), received allogeneic hSCT (RICT in 13 and MAC in 7) after ABCDV (n=17) and Hyper-CVAD (n=3) treatment. 14 of 20 patients had Ph+ ALL and 5 had other HR factors. HLA-identical RD and matched URD were in equal proportion. OS and EFS after 3 years were 40% (95%CI: 18, 62) and 25% (95%CI: 6, 44). Men (n=10) were of younger age [57.5 vs. 62 years in women ($P = 0.04$)] but had impaired OS ($P = 0.05$) and EFS ($P = 0.04$). TRM was 40% (8/20) and 6 patients relapsed. Transplantation in Ph+ ALL did not enhance survival (patients receiving palliative treatment included) (Figure 7).

Prognostic factors

Age (analyzed as three groups), $PLT \leq 35 \times 10^9/L$, sex, creatinine >90 , phenotype (B-/T-cell), $WBC >100 \times 10^9/L$ were included in multivariate analysis. Only high age ($P = 0.025$; Figure 8) and thrombocytopenia ($P = 0.008$) proved to be negative prognostic factors for OS and EFS (not shown) in multivariate analysis. Adverse effect of age in older/elderly (1, 3, 41, 80) and thrombocytopenia in adults (3, 34) were demonstrated previously. Neither presence of Ph+ nor $CC \geq 1/PS \geq 2$ influenced survival significantly. Thrombocytopenia was more common in females than in males in the

youngest age group [39/67 (58%) vs. 20/57 (35%); $P = 0.01$]; despite this fact, males in the age group had poorer OS than females (Figure 9) which was earlier demonstrated by only one study of elderly ALL (41).

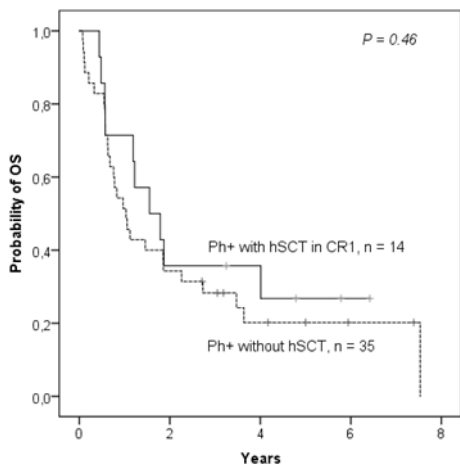


Figure 7. Overall survival in patients with Ph+ transplanted and not transplanted (intensive and palliative treated included, all receiving TKI)

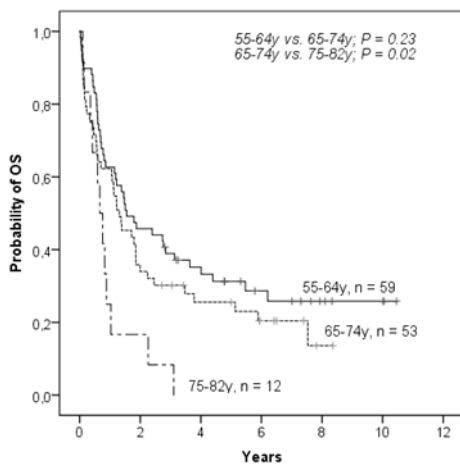


Figure 8. Overall survival in intensively treated patients with B- and T-ALL according to age group.

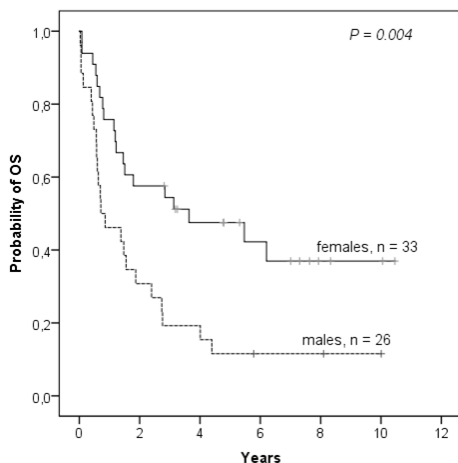


Figure 9. Overall survival in youngest age group (55-64y) according to sex.

MRD1 was not analyzed consistently (59% of all patients in CR1) and positivity (>0.1%) did not influence survival (not shown).

Outcome before and after the introduction of new guidelines

Of 155 patients, 92 (59%) were diagnosed before and 63 (41%) after October 2009, receiving intensive treatment in 79% and 80% respectively. Median age [64 and 67 years ($P = 0.1$)] and distribution of prognostic factors were not significantly different between the two periods. Contemporary guidelines were followed for remission induction in 93% of the patients. EWALL-backbone was used mainly after October 2009 [29/50 (58%) vs. 6/74 (8%) in the previous period; $P < 0.001$] which neither influenced survival in the whole cohort nor in the three age groups.

Palliation

Of 31 patients receiving palliation, 17 (55%) were treated more intensively with intravenous cytostatic combinations and/or TKI (with 29% achieving CR), and 14 with oral cytostatics and/or cortisone, or supportive care only. OS was 13% (95%CI: 1, 25) after one and 3% (95%CI: 0, 9) after 3 years. When comparing with the oldest age group (75-85y) given palliation (n=23), OS was not significantly different in 12 patients (75-82y) receiving remission induction therapy ($P = 0.12$).

Discussion

Our study is quite unique, as it specifies patients', disease, and therapy characteristics in a population based cohort of older/elderly ALL treated according to national guidelines, which were followed to a large extent. High proportion of patients <75 years received remission induction (90%) in contrast to those >75 years. The latter group had dismal outcome, comparable with those receiving palliation, suggesting use of "intensive palliation" (+ TKI in Ph+ disease) rather than standard ALL protocols in patients >75 years. Age-adapted EWALL-backbone did not enhance survival in Sweden, and at age 65-74 years gave similar outcome as ABCDV. Our finding of impaired survival in males <65 years may be protocol specific, as differences in survival between sexes have disappeared during population-based study period (40) and when applying modern pediatric protocol (122).

There were no survivors among patients with T-cell phenotype, though their number was probably too low to draw any conclusions. The phenotype carried poor prognosis in elderly according to one study only (50).

OS in Ph- and Ph+ ALL did not differ in our cohort although Ph+ is considered a HR factor by most authors (123). Even possibly improved outcome in elderly with Ph+ was reported previously (79), and TKI had substantial impact on enhancement of the survival in this ALL subgroup (124).

High proportion of patients <65 years underwent hSCT mostly due to Ph positivity. hSCT procedure in CR1 is encouraged by some authors (78, 124), particularly in Ph+ ALL (123). We found no advantage of hSCT in Ph+ disease in terms of OS. It is possible that the procedure is too toxic in older individuals, as TRM was high.

Conclusions

Paper I

Patients with ALL recurrence are rarely cured. In our study, a proportion of younger patients <35y could achieve long-term survival by means of intensive chemotherapy (MEA and FLAG-Asp appearing effective) with subsequent hSCT. However, older patients and those relapsing after hSCT were beyond rescue. This implicates the strong need of preventing relapse in older patients and novel rescue therapies in the high risk group. A combination of time to relapse and age predicts outcome in relapsed ALL.

Paper II

With Hyper-CVAD, CR was achieved in a high proportion of patients with T-ALL, without serious toxicity. However, survival (especially LFS) was unsatisfactory, and a high relapse rate was observed both in older and younger patients. Probably due to high hSCT rate in CR2, the latter had favorable OS. The protocol was abandoned in upcoming guidelines and replaced by the Nordic NOPHO 2008 study protocol in patients <45 years, and hSCT in CR1 following the ABCDV protocol in eligible patients older than 45 years.

Paper III

By applying the ABCDV protocol, around half of treated ALL patients >45 years can be cured, experiencing acceptable toxicity. MRD measurement was performed only in patients potentially benefitting of treatment escalation. Continuous remission in patients reaching MRD negativity after induction treatment (<0.1%) was excellent. MRD1 cut-off level will be reduced from 1% to 0.1% in the upcoming guidelines.

Paper IV

The outcome in older/elderly ALL patients was poor, with high early mortality and relapse rate, despite the use of an age-adapted protocol mainly in those 65-74 years old. Allogeneic hSCT did not enhance survival in Ph+ ALL, and the aberration had no impact on outcome in our cohort, probably due to consistent use of TKI. Poor prognostic factors (high age and thrombocytopenia) can be identified at diagnosis. Impact of male sex on survival in older patients was demonstrated but could not be explained. Intensive treatment should primarily be withheld in patients older than 75 years.

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High curability via intensive reinduction chemotherapy and stem cell transplantation in young adults with relapsed acute lymphoblastic leukemia in Sweden 2003–2007

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ABSTRACT

Background

A minority of patients with adult acute lymphoblastic leukemia who relapse are rescued. The aim of this population-based study was to assess the results of reinduction treatment and allogeneic stem cell transplantation in patients in second complete remission.

Design and Methods

Between 2003–2007, 76 adults (<66 years) with relapsed acute lymphoblastic leukemia (Burkitt's leukemia excluded) were prospectively reported to The Swedish Adult Acute Leukemia Registry and later evaluated.

Results

Reinduction with: (i) mitoxantrone, etoposide, and cytarabine (MEA); (ii) fludarabine, cytarabine, pegylated-asparaginase plus granulocyte colony-stimulating factor (FLAG-Asp); and (iii) cytarabine, betamethasone, cyclophosphamide, daunorubicin, and vincristine (ABCDV) resulted in complete remission in 6/9 (67%), 10/16 (63%) and 9/21 (43%) of the patients, respectively. Allogeneic stem cell transplantation was performed during second complete remission in 29 patients. Multivariate analysis regarding overall survival after relapse revealed that age over 35 years at diagnosis and relapse within 18 months were negative prognostic factors. Overall survival rates at 3 and 5 years were 22% (95% CI: 13–32) and 15% (95% CI: 7–24). Of 19 patients less than 35 years at diagnosis who underwent allogeneic stem cell transplantation in second remission, ten (53%) are still alive at a median of 5.5 years (range, 4.2–8.3) after relapse, whereas all patients over 35 years old at diagnosis have died.

Conclusions

Allogeneic stem cell transplantation remains the treatment of choice for young adults with relapsed acute lymphoblastic leukemia. Both (i) mitoxantrone, etoposide, and cytarabine and (ii) fludarabine, cytarabine, pegylated-asparaginase plus granulocyte colony-stimulating factor seem effective as reinduction treatments and should be further evaluated. New salvage strategies are needed, especially for patients over 35 years old at diagnosis.

Key words: adult acute lymphoblastic leukemia, relapse, salvage therapy, allogeneic stem cell transplantation, prognostic factors

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Introduction

Acute lymphoblastic leukemia (ALL) in adults has an unsatisfactory prognosis despite efforts to improve long-term outcome with strategies such as adaptation of pediatric protocols,^{1,2} use of tyrosine kinase inhibitors in Philadelphia chromosome-positive disease³ and allogeneic stem cell transplantation (SCT) in first complete remission (CR1).^{4,6} Primary chemotherapy-resistant disease is unusual and CR1 can be reached in 90% of Philadelphia chromosome-negative patients with novel protocols,^{1,7} but relapse is frequent, especially in patients over 35 years of age at diagnosis. This age group is considered as high-risk both at diagnosis^{7,8} and relapse.⁹ Prognosis remains very poor for relapsing patients, with approximately 40% achieving second complete remission (CR2) through salvage regimens and only 7–12% becoming long-term survivors.^{10,12} Allogeneic SCT seems to be the best consolidation option for adult patients with relapsed ALL,^{10–12} although only a minority of such patients are eligible for the procedure as a result of poor performance status, lack of a donor or short duration of CR2. Achieving CR2 after relapse is an important aim for patients who are considered for allogeneic SCT.¹¹ If relapse occurs after SCT the long-term outcome is dismal, although donor lymphocyte infusions with or without chemotherapy or a second transplant are used in attempts to improve survival.¹¹

We present here the results of a national, multicenter, prospective, population-based study of outcome among adult patients aged 19 to 65 years with ALL relapse diagnosed during 2003–2007 in Sweden. Most of the patients had primary treatment according to national guidelines for ALL, as recommended by the Swedish Adult ALL Group (SVALL). The guidelines suggest two different treatment protocols for early relapses, and retreatment according to the initial therapy for late relapses, with the intention to proceed to allogeneic SCT in CR2 for eligible patients. The aim of the current study was to assess outcome among adult patients after first ALL relapse, and the utilization and efficacy of recommended protocols and of allogeneic SCT in this setting.

Design and Methods

Patients

Adult patients with first relapse of ALL were prospectively reported to The Swedish Acute Leukemia Registry in 2003–2007. The Swedish Acute Leukemia Registry is a truly population-based registry containing data on patients diagnosed with acute leukemia since 1997, with 98% coverage.¹⁵ Missing data were added retrospectively. Patients older than 66 years at relapse were excluded from this analysis as not being eligible for allogeneic SCT, as were patients with Burkitt's leukemia. Informed consent was obtained from all patients. The date of last follow-up of the survivors was 3rd June 2011. The study was approved by the regional ethical review board in Uppsala.

Initial diagnostics and treatment

Diagnostics and treatment at primary diagnosis of ALL were performed at each center according to the national guidelines. Induction therapy consisted of cytarabine, betamethasone, cyclophosphamide, daunorubicin, and vincristine (ABCDV)/vincristine, cytarabine, betamethasone, and amsacrine (VABA) for precursor ALL, as previously described,¹⁴ and hyper-CVAD for T-

ALL.¹⁵ Asparaginase was not incorporated in either the induction or consolidation protocol. Complementary use of tyrosine kinase inhibitors in Philadelphia chromosome-positive ALL was not mandatory in this period except for refractory or relapsed disease.

High-risk ALL was defined by the presence of at least one of the following criteria: white blood cell (WBC) count $>30 \times 10^9/L$ ($>100 \times 10^9/L$ for T-ALL), central nervous system disease, more than one course required to achieve CR1, Philadelphia-positive chromosome-positive or t(4;11), and for patients first diagnosed in 2003–2007, high levels of minimal residual disease ($>1\%$ after induction or $>0.1\%$ after consolidation). Myeloablative allogeneic SCT in CR1 was recommended for these patients but not for patients with standard-risk ALL. The CR1 rate and 3-year overall survival after diagnosis for all patients treated with the ABCDV/VABA protocol were 86% and 29%, respectively, as reported previously.¹⁴

Relapse treatment

Between 2003 and 2007, the national guidelines recommended retreatment with ABCDV for late relapses (>2 years since initial diagnosis) and two treatment alternatives for early relapses: fludarabine, cytarabine, pegylated-asparaginase plus granulocyte colony-stimulating factor (FLAG-Asp) and mitoxantrone, etoposide, and cytarabine (MEA) (Table 1). For patients not undergoing transplantation in CR1 the aim was to perform myeloablative allogeneic SCT in CR2. The final decision on the choice of relapse treatment was left to the treating physicians.

Statistical methods

Overall survival was calculated from the time of first relapse to death or time of last follow-up. Distributions of overall survival were estimated by the Kaplan-Meier method and differences in overall survival according to risk factors were analyzed by the log-rank test. In addition, univariate and multivariate Cox regression analyses were performed to evaluate the effects of relevant covariates on overall survival. Ninety-five percent confidence intervals (95% CI) for hazard ratios (HR) were obtained. Correlations between variables and achievement of CR2 were evaluated by logistic regression. This method was also used to estimate differences in the distribution of risk factors in two age groups (<35 and >35 years at diagnosis). Statistical analyses were performed with SPSS or StatView statistical packages.

Results

Patients' characteristics

According to The Swedish Acute Leukemia Registry there were 76 adult patients aged <66 years with ALL relapse in the years between 2003 and 2007 in Sweden. A flow chart illustrating treatment in the whole cohort is presented in Figure 1. Five patients (7%) received palliative treatment: with cyclophosphamide ($n=1$), thioguanine/mercaptopurine ($n=1$), corticosteroids ($n=1$) or supportive care only ($n=2$). The median age in this group was 60 years (range, 30–63) and the median overall survival was 1 month (range, 0.5–14). These five patients were excluded from further analysis.

The characteristics of the 71 remaining patients treated with intensive chemotherapy and/or tyrosine kinase inhibitors are shown in Table 2. There was a slight male predominance (60%). The median age at first ALL diagnosis was 39 years (range, 15–65) for all patients and 36 years (range, 15–65) for the 57 patients not undergoing transplantation in CR1. The median age at relapse for all

patients was 39 years (range, 19-65). B-precursor ALL was more common (82%) than T-ALL (18%). Data on cytogenetics were available for 67 patients (94%). Fourteen cases (20%) had Philadelphia chromosome-positive ALL, detected by the presence of t(9;22) and/or bcr-abl, and two cases (3%) had t(4;11). Twenty-seven patients had normal karyotypes (38%) and 24 had miscellaneous abnormalities (34%). A high WBC count (as defined previously) was present in 13 cases, all with B-precursor ALL.

The median time from diagnosis to relapse was 13 months (range, 2-82), with 55% relapsing within 18 months.

Isolated bone marrow relapse was most common (51/71, 72%). Extramedullary relapse at a single site was

seen in five patients: central nervous system (n=3), testes (n=1) and extremities (n=1). Disease recurrence in both bone marrow and an extramedullary site occurred in nine patients: central nervous system (n=3), testes (n=1), mediastinum (n=1), uterus (n=1), lymph nodes (n=2) and base of the skull (n=1).

Overall, allogeneic SCT was performed in 14 patients and autologous SCT in three patients in CR1. This cohort of 17 patients included 13 patients with the pretreatment high-risk factors of high WBC count and/or adverse cytogenetics [t(4;11) or Philadelphia chromosome], two patients with late CR1, one with a complex karyotype and one patient unable to tolerate maintenance therapy.

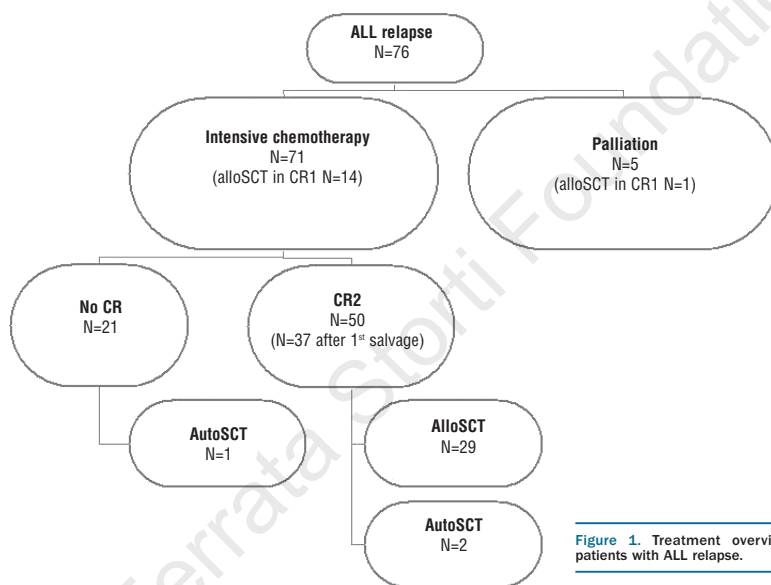


Figure 1. Treatment overview regarding 76 patients with ALL relapse.

Table 1. The most commonly used salvage regimens, recommended in national guidelines 2003-2007.

ABCDV		
Cytarabine	3000 mg/m ² b.i.d. every 12 h i.v.	d 1,2,3
Betamethasone	20 mg/m ² oral	d 1,2,3,4,5
Cyclophosphamide	600 mg/m ² i.v.	d 1
Daurorubicin	30 mg/m ² i.v.	d 1,2,3
Vincristine	2 mg i.v.	d 1
FLAG asparaginase		
Fludarabine	30 mg/m ² i.v.	d 1, 2, 3, 4, 5
Cytarabine	2000 mg/m ² i.v.	d 1, 2, 3, 4, 5
PEG asparaginase	500 E/m ² i.v.	d 2, 16
MEA		
Mitoxantrone	12 mg/m ² i.v.	d 1, 2, 3, 4
Etoposide	100 mg/m ² i.v.	d 1, 2, 3, 4
Cytarabine	1000 mg/m ² b.i.d. every 12 h i.v.	d 1, 2, 3, 4

Second complete remission rate after salvage therapy

The CR2 rate after the first salvage treatment was 52% (37/71). With additional treatment CR2 was achieved in 70% (50/71) of patients: 11 and two patients received two and three reinduction courses, respectively. Three patients died as a result of toxicity/infection after reinduction without reaching CR2. Gender, immunophenotype, cytogenetics, WBC count at diagnosis, site of relapse and allogeneic SCT performed during CR1 did not significantly influence either the CR2 rate after first salvage or the final CR2 rate. Final achievement of CR2 was negatively influenced by age >35 years at diagnosis (60% versus 86%) ($P=0.012$), and also by time to relapse <18 months (54% versus 91%) ($P=0.001$).

The outcomes of the applied salvage regimens and characteristics of the patients treated are presented in Table 3. Both recommended options for early relapsing patients, i.e. MEA and FLAG-Asp, resulted in CR2 in high proportions of patients (67% and 63%, respectively). Reinduction with ABCDV, which was recommended for late-relapsing patients, induced CR2 in 43% of cases after one course, suggesting poorer efficacy than for MEA and FLAG-Asp, although statistically not proven. Other therapy options used as first salvage treatment were: hyper-CVAD (n=2), hyper-CVAD in combination with a tyrosine kinase inhibitor (n=2), a tyrosine kinase inhibitor (n=8),

FLAG-idarubicin (n=2), FLAG (n=2), MEA with nelarabine (n=1) or rituximab (n=1), high-dose cytarabine (n=1), combinations of cytarabine and anthracyclines (n=1) or cytarabine and methotrexate (n=1), a combination of radio- and chemotherapy (n=1), and a modified NHL-BFM 90 protocol¹⁶ (n=3). Subsequent treatments in refractory disease were heterogeneous and not reported for all patients. Those used as second-line therapy achieving CR2 were VABA¹⁴ (n=4), a tyrosine kinase inhibitor (n=1), FLAG-Asp (n=4), hyper-CVAD (n=1), and the NHL-BFM 90 protocol (n=1). Effective third-line salvage therapies were hyper-CVAD with gemtuzumab ozogamicin (n=1) and induction according to a pediatric protocol (NOPHO-92)¹⁷ (n=1).

Overall survival after relapse

The median overall survival of the 71 patients receiving intensive chemotherapy was 9 months (range, 0.5-100). The overall survival rate at 1 year was 41% (95% CI: 29-52%), after 3 years it was 22% (95% CI: 13-32%), and the projected 5-year overall survival rate was 15% (95% CI: 7-24%). Eleven patients (15%), all below 35 years of age at diagnosis, are still alive at a median of 5.5 years (range, 4-8.2) after relapse. A majority of the 60 dead patients (n=43, 72%) died of leukemia. Other common causes of death were infection (n=8, 13%) and graft-versus-host disease (n=3, 5%).

Table 2. Patients' characteristics and treatment factors analyzed by way of univariate and multivariate Cox regression for effects on overall survival.

	All patients N=71			Patients not transplanted in CR1 N=57		
	N.	Univariate analysis HR-OS (95%CI)	P	Multivariate analysis HR-OS (95%CI)	P	Multivariate analysis HR-OS (95%CI)
Gender						
male	42					34
female	29	1.34(0.80-2.24)	$P=0.264$			23
Age at diagnosis						
15-35	29					26
35-65	42	4.82(2.65-8.78)	$P<0.001$	3.65(1.83-7.25)	$P<0.001$	31
						3.81(1.70-8.55)
						$P=0.001$
Immunophenotype						
T-cell	13					12
pre-B	58	1.51(0.74-3.08)	$P=0.257$			45
Cytogenetics						
others	54					49
adverse *	16	2.17(1.20-3.89)	$P=0.01$			5
WBC count at diagnosis						
low	51					43
high **	13	2.19(1.16-4.14)	$P=0.016$			8
Time from diagnosis to relapse						
>18 months	32					26
<18 months	39	2.89(1.69-4.94)	$P<0.001$	2.19(1.19-4.02)	$P=0.012$	31
						3.43(1.56-7.58)
						$P=0.002$
Site of relapse ***						
extramedullary +/- BM	14					10
isolated BM	52	2.15(1.05-4.41)	$P=0.036$	1.67(0.80-3.50)	$P=0.174$	44
						3.54(1.26-9.99)
						$P=0.017$
AlloSCT in CR1						
no	57					
yes	14	1.54(0.84-2.81)	$P=0.16$			
Treatment after relapse						
alloSCT in CR2	29					29
no alloSCT in CR2	21	2.28(1.21-4.29)	$P=0.011$	1.48(0.74-2.95)	$P=0.265$	11
CR2 not achieved	21	7.59(3.94-14.62)	$P<0.001$	5.60(2.72-11.52)	$P<0.001$	17
						3.06(1.24-7.54)
						4.90(2.22-10.79)
						$P<0.001$
						$P=0.015$
						$P<0.001$

*t(9;22), t(4;11); one missing value (three missing values in FAL1 were regarded as "other"); ** >30x10⁹/L for B-ALL and >100x10⁹/L for T-ALL; seven missing values; ***five missing values. AlloSCT: allogeneic stem cell transplantation.

In order to analyze the influence of CR2 and subsequent allogeneic SCT on overall survival the study population was divided into three groups. Kaplan-Meier survival curves for these groups are shown in Figure 2, with advantageous outcome for patients receiving allogeneic SCT in CR2 (Figure 2, curve A) compared to patients achieving CR2 but with no subsequent allogeneic SCT (Figure 2, curve B), and the worst outcome for patients not achieving CR2 (Figure 2, curve C). Three patients from group B died within 119 days of relapse (the median time until allogeneic SCT in CR2). Their causes of death were second relapse of ALL, aspergillosis and complications after a liver biopsy. The difference in overall survival between patients in groups A and B remained statistically significant even when these patients were excluded (*data not shown*).

Along with a failure to achieve CR2 and no allogeneic SCT in CR2, other negative prognostic factors with regards to overall survival in univariate analyses were age >35 years at diagnosis ($P<0.001$), time to relapse <18 months ($P<0.001$), adverse cytogenetics ($P=0.007$), high WBC count ($P=0.016$) and isolated bone marrow relapse ($P=0.036$) (Table 2).

In the multivariate model for overall survival the variables cytogenetics and WBC count at diagnosis had to be omitted, as no patients with high-risk features (high WBC count and/or adverse cytogenetics) underwent transplantation in CR2. Five of the 71 patients had missing information on relapse site and these patients were excluded from the multivariate analysis. Age >35 years at diagnosis ($P<0.001$), time to relapse <18 months ($P=0.011$) and lack of achievement of CR2 ($P<0.001$) had significant negative impacts on overall survival after relapse (Table 2).

The patients were divided into four subgroups with differing overall survival rates depending on age and time to relapse: <35 years with relapse after >18 months, <35 years with relapse in <18 months, >35 years with relapse after >18 months, and >35 years with relapse in <18 months (Figure 3). No patients older than 35 years were rescued compared with projected 5-year overall survival rates of 25% (95% CI: 0-50%) and 47% (95% CI: 23-71%) among young adults with relapse in <18 months and after >18 months, respectively.

Patients >35 years old at diagnosis had more high-risk features compared with those <35 years old: adverse cytogenetics (36% versus 7%, $P=0.004$) and high WBC counts

(32% versus 8%, $P=0.017$). Isolated bone marrow relapse (86.5% versus 69%, $P=0.084$) and time to relapse <18 months (64% versus 41%, $P=0.056$) were also slightly more common in older patients. Fewer patients >35 years old versus <35 years old proceeded to allogeneic SCT in CR2 (24% versus 65.5%, $P<0.001$).

Outcome in patients who relapsed after allogeneic stem cell transplantation in first complete remission

All patients who relapsed after allogeneic SCT in CR1 ($n=14$) died at a median of 7 months (range, 1-51) after relapse. As shown in Table 2, their overall survival was not significantly different from that of patients who relapsed and who had not undergone transplantation in CR1 ($P=0.16$) when analyzed overall. Ten patients carried the Philadelphia chromosome and one had $t(4;11)$. The median time between SCT and relapse was 10 months (range, 2-45). Having a transplant from an unrelated donor (7/14) was associated with a longer time to first relapse ($P=0.042$, log-rank test) and longer overall survival after first relapse ($P=0.037$, log-rank test) compared with having a transplant from a related donor (7/14). Donor lymphocyte infusions were given to 9/14 patients, in combination with tyrosine kinase inhibitors, chemotherapy or both. Donor lymphocyte infusions had no significant influence on overall survival (log-rank test). One relapsed patient underwent a second allogeneic transplant but died from an early second relapse.

Outcome in patients who did not receive allogeneic stem cell transplantation in first complete remission

The median overall survival of the 57 patients who did not undergo allogeneic SCT in CR1 was 9 months (range, 0.5-99), with 11 patients still alive belonging to this group. Forty (70%) achieved CR2 after the first ($n=28$), second ($n=10$) or third ($n=2$) salvage treatment and 29 (51%) proceeded subsequently to allogeneic SCT from an HLA identical related donor ($n=13$), a mismatched related donor ($n=1$), a 10/10 antigen matched unrelated donor ($n=7$), a mismatched unrelated donor ($n=7$) and unrelated cord blood ($n=1$). Myeloablative conditioning (mainly cyclophosphamide/total body irradiation) was used in 25 cases and reduced-intensity conditioning in four cases. The source of stem cells was peripheral blood ($n=25$), bone marrow ($n=2$), peripheral blood and bone marrow

Table 3. Complete remission rates after the first reinduction course and patients' characteristics according to the applied salvage regimens.

Salvage regimen	Complete remission rate	5-year overall survival	Median age (range) years	Proportion of patients older than 35 years	Proportion of patients with CR1 lasting <18 months	AlloSCT in CR1	AlloSCT in CR2
MEA	6/9 67%	11%	45 (22-62)	8/9 89%	4/9 44%	0/9 0%	5/9 56%
FLAG-Asp	10/16 63%	19%	37 (18-65)	7/16 44%	14/16 88%	1/16 6%	8/16 50%
ABCDV	9/21 43%	29%	36 (15-61)	11/21 52%	6/21 29%	1/21 5%	13/21 62%
TKI alone	6/8 75%	0%	43 (21-59)	6/8 75%	6/8 75%	8/8 100%	0/8 0%
Other	6/17 35%	6%	40 (19-62)	10/17 59%	10/17 59%	4/17 24%	3/17 18%

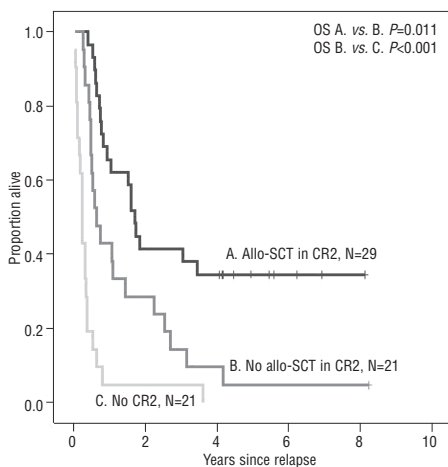


Figure 2. Effects of allogeneic SCT (allo-SCT) in CR2, and CR2 achievement on overall survival (OS).

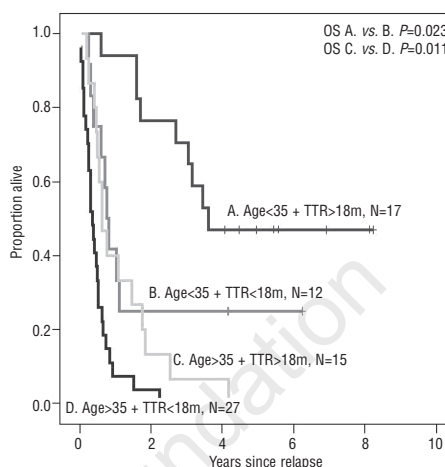


Figure 3. Overall survival (OS) according to age at diagnosis and time to relapse (TTR).

(n=1) and cord blood (n=1). The median time from relapse to transplantation was 119 days (range, 51-226). The remaining patients (n=23) received chemotherapy only (n=21) or autologous SCT (n=2). Autologous SCT was performed during active disease in one patient who died 31 months later (CR2 was reached after the SCT) and in one patient with isolated testis relapse who is alive after 8 years of follow-up. None of the patients who achieved CR2 but did not proceed to SCT became long-time survivors.

For the eight patients with pretreatment high-risk factors whose treatment did not include SCT in CR1, the outcome was dismal. Their median age was 52 years (range, 22-61) and the median time from diagnosis to relapse was 6 months (range, 2-12). In this cohort of patients all but one had a WBC count of $>30 \times 10^9/L$, three had Philadelphia chromosome-positive leukemia, one had t(4;11), one had a complex karyotype and two had tetraploid leukemia; only one patient reached CR2 and their median overall survival was 3.9 months (range, 0.5-7.7).

A projected 5-year overall survival rate of 34% (95% CI: 17-52%) for patients who underwent allogeneic SCT in CR2 compares with 9% (95% CI: 0-26%) for patients who achieved CR2 but were treated without allogeneic SCT, and 0% for those without achievement of CR2. Cox multivariate regression concerning overall survival was performed. Other variables tested in this model were time to relapse, age, and site of relapse (isolated bone marrow versus extramedullary ± bone marrow), with the results shown in Table 2. A survival advantage for the allogeneic SCT group was found compared with both the group of patients not reaching CR2 (HR 4.90, $P<0.001$) and those reaching CR2 but not treated with allogeneic SCT (HR

3.06, $P=0.015$). Along with age >35 years at diagnosis and time to relapse <18 months, isolated bone marrow relapse also had a negative impact on overall survival compared with extramedullary ± bone marrow relapse (HR 3.54, $P=0.017$).

Within the cohort of 29 patients who underwent allogeneic SCT in CR2, all ten patients >35 years old at diagnosis were identified as a high-risk group, with all patients dying because of leukemia (5/10), infection (3/10) or graft-versus-host disease (2/10) within a median period of 5.5 months (range, 1-18) after transplantation. In comparison, among 19 patients treated with allogeneic SCT who were <35 years old at diagnosis, ten are still alive after a median of 5.5 years (range, 4.2-8.3). Among the patients <35 years at diagnosis, the projected 5-year overall survival rate in allogeneic SCT-treated patients is 53%. The only young patient surviving in the non-allogeneic SCT-treated group underwent autologous transplantation, as mentioned above.

Overall survival was not significantly influenced by type of donor, with projected 5-year overall survival rates of 43% (95% CI: 17-69%) versus 29% (95% CI: 5-52%) for unrelated and related donors, respectively.

Discussion

This study is unique as a population-based survey of adult patients (age <66 years) with relapsed ALL potentially eligible for allogeneic SCT, covering all relapsing patients reported to the Swedish Acute Leukemia Registry during the period from 2003 to 2007. Reinduction protocols used in cases of relapsed ALL lead to CR2 in 0-80%

of patients.¹¹ It is, however, extremely difficult to compare their efficacy, as no randomized or population-based studies are available and selection is often present in the populations of patients. Intensified chemotherapy at relapse can increase overall survival.¹⁸ Many protocols involving various combinations of anthracyclines, vincristine, steroids, cyclophosphamide, cytarabine and other cytostatics are available, illustrating the lack of uniform treatment guidelines for relapsed ALL.

Both recommended reinduction protocols for early relapsing patients, MEA and FLAG-Asp, were effective in achieving CR2 in our study, whereas ABCDV, which was used for late relapses, resulted in a somewhat lower CR2 rate. However, no significant differences in CR2 achievement among the applied protocols were observed. Final achievement of CR2 was more likely in younger patients and in cases of late relapse, as reported previously.¹⁰ One could speculate that the MEA and FLAG-Asp regimens might produce at least as good CR2 results if applied in cases of late relapse and thereby be more suitable choices for these patients also. If CR2 is not reached by means of front-line salvage it can be reached via second-line regimens, as we and other groups have shown.

Achieving CR2 is essential with regards to the outcome of allogeneic SCT, as patients undergoing transplantation during active relapse have little chance of long-term survival.^{11,19} Even though no patients became long-time survivors without SCT, achieving CR2 as such can improve survival time, as illustrated in our study. Patients who underwent transplantation in CR1 and who subsequently suffered relapse were beyond rescue, although it is noteworthy that CR2 was reached in a high proportion of Philadelphia chromosome-positive cases after administration of tyrosine kinase inhibitors (Table 3).

Age is an important prognostic factor in adult ALL and a high-risk disease pattern in ALL (adverse cytogenetics, high WBC count) in older patients is well known.⁸ Other factors often contributing to worse outcome in this population are poor performance status and chemotherapy-related complications. Probably a combination of the above-mentioned factors meant that none of the patients aged over 35 years old at diagnosis became long-term survivors in our study.

There are conflicting data regarding whether or not central nervous system disease, which is the most common extramedullary ALL manifestation, has an adverse effect on survival at diagnosis⁹ and/or relapse.¹⁰⁻¹² In our limited number of patients we found that isolated bone marrow relapse was correlated with shorter overall survival time (at least in the SCT setting) compared with extramedullary disease alone or in combination with bone marrow relapse. This finding needs to be confirmed in other studies but it indicates that allogeneic SCT can be an effective treatment option in CR2 for patients with extramedullary relapse.

Scoring systems and risk-group stratification regarding outcome in cases of ALL relapse based on parameters such as age, duration of CR1, Philadelphia chromosome status, WBC count, blast count and lactate dehydrogenase level have been proposed.^{19,20} Our results confirm the previously reported, strong prognostic value of age and time to relapse.¹⁰⁻¹² These factors, together with the effect of allogeneic SCT in CR2, seem to influence the possibility of

long-term survival strongly. Since patients with high-risk factors had undergone SCT in CR1 or had been found not eligible or suffered from early relapses prohibiting SCT, a scoring system including pretreatment high-risk factors was not really possible.

Intensive chemotherapy followed by allogeneic SCT is regarded as the optimal treatment strategy for relapsed adult ALL, but usually only a minority of patients (17-30%) are considered eligible for such an approach.^{10,12} In comparison, the proportion of patients among the intensively treated subjects who underwent allogeneic SCT in CR2 in our study was as high as 41% (51% among those who did not undergo transplantation in CR1), with a high overall survival rate of 38%. Whereas 53% of post-relapse allogeneic SCT patients in the younger age group showed sustained survival, no positive long-term effect was seen among patients >35 years of age at the time of diagnosis. We interpret this apparent lack of success in "older" adults as being partly a result of our low numbers of patients, and also a result of the well-known efficacy and toxicity problems of allogeneic SCT in this age group, in which five of our ten transplanted patients died of infection/graft-versus-host disease. Likewise, very few older survivors have been reported in other relapse studies,^{11,12} but non-myceloablative SCT may be a possible approach in this age group.²¹ Conversely, for young, standard-risk patients, the results of allogeneic SCT after relapse seem good enough to indicate that withholding transplantation in CR1 may be advisable, especially in the era of more effective pediatric-based protocols. When relapses occur in such young patients, an urgent search for a donor should be performed, regardless of the availability of related donors.

It is noteworthy that autologous SCT after relapse can also be effective in selected cases, as exemplified by two of our patients, of whom one is showing sustained survival and the other (SCT without prior CR2 achievement) survived for 3.5 years. In a recently published study, six of 14 relapsed patients who received autologous SCT showed sustained survival.¹⁰ Long-term survival proportions after autologous SCT (15%) and unrelated donor allogeneic SCT (16%) performed as treatment after relapse were comparable in another large study, in which sibling allogeneic SCT was associated with the highest survival rate (23%) and chemotherapy alone with the lowest (4%).¹²

In summary, our results show encouraging long-term survival rates after intensive salvage chemotherapy followed by allogeneic SCT in CR2 for relapsed patients <35 years of age at diagnosis. The overall prognosis after ALL recurrence is, however, still unsatisfactory, with only a minority of adult patients being rescued. Simple risk-group stratification based on age and time to relapse aid in predicting outcome. MEA and FLAG-Asp seem effective as reinduction therapies, although our small numbers treated preclude solid conclusions. Efforts should continue to identify effective reinduction protocols to increase the number of patients eligible for allogeneic SCT in CR2. However, as regards relapses after allogeneic SCT performed in CR1, and patients >35 years of age at diagnosis, allogeneic SCT in CR2 is often either not achievable or is ineffective at improving survival. Prevention of relapse is essential to improve prognosis, especially in older patients, and new salvage treatments are urgently needed.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with

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ORIGINAL ARTICLE

High relapse rate of T cell acute lymphoblastic leukemia in adults treated with Hyper-CVAD chemotherapy in Sweden

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Abstract

Background: Hyper-CVAD is widely used to treat acute lymphoblastic leukemia (ALL) and aggressive lymphomas. This multicenter, population-based study assessed the efficacy of Hyper-CVAD as first-line therapy in patients with T-cell ALL (T-ALL). **Patients and methods:** Between October 2002 and September 2006, 24 patients were diagnosed with T-ALL in Sweden; 19 were eligible for treatment with the protocol. **Results:** The median age was 32 yr (range 18–72 yr). Complete remission (CR) was obtained in 17 of 19 (89%) patients, and the treatment was relatively well tolerated. Allogeneic stem cell transplantation (SCT) was recommended in high-risk disease and was performed in four patients upfront. Two- and 5-yr leukemia-free survivals (LFS) in 17 patients with CR achievement were identical, at 29% (95% confidence interval [CI]: 8–51). Two- and 5-yr overall survival (OS) in whole cohort was 63% (95% CI: 42–85) and 47% (95% CI: 26–69), respectively. The 5-yr LFS for 15 patients who did not receive allogeneic SCT upfront were 20% (95% CI: 0–40), although 14 of 15 completed the protocol (eight cycles). Relapse occurred in 2 of 4 upfront-transplanted patients and in 12 of 15 patients treated with chemotherapy alone, six of whom received allogeneic SCT in CR2. Age ≥ 35 yr influenced OS negatively in univariate analysis (HR 5.1, 95% CI: 1.55–16.7). **Conclusions:** Hyper-CVAD treatment resulted in a high CR rate and appeared safe, but it showed poor efficacy at preventing relapse. Therefore, this treatment is no longer recommended for adults with T-ALL in Sweden.

Key words precursor T-cell lymphoblastic leukemia–lymphoma; antineoplastic combined chemotherapy protocols; stem cell transplantation; treatment outcome

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T-cell acute lymphoblastic leukemia (T-ALL), which constitutes about one-fifth of adult ALL cases, differs in some features from B-ALL; it is characterized by male predominance, tumor growth in the mediastinum, more frequent hyperleukocytosis, central nervous system (CNS) involvement, and lymph node and organ enlargement. The outcome in T-ALL is influenced negatively by hyperleukocytosis, non-thymic phenotype (CD1a⁻), complex karyotype, female gender, and age >35 yr (1). As in B-ALL, minimal residual disease (MRD) has been shown to be an independent prognostic factor for T-ALL (2).

Treatment strategies for T- and B-ALL were very similar in Sweden until 2002 (ABCDV/VABA); this resulted in an inferior 3-yr leukemia-free survival (LFS) for T-ALL compared with B-ALL (25% vs. 38%, respectively) (3). An attempt to optimize T-ALL therapy was made, and Hyper-CVAD (4) was introduced as standard treatment for T-ALL, leaving the recommendation for B-ALL unmodified (ABCDV/VABA). The Hyper-CVAD protocol was recommended for T-ALL from 2002 to 2006 in the national guidelines of the Swedish Adult ALL Group. The decision to recommend this protocol was based on an earlier

single-center study (2000) that described favorable outcome after Hyper-CVAD treatment in 24 adult T-ALL patients, with a complete remission (CR) rate of 100%, estimated 5-yr overall survival (OS) of 43%, and estimated 5-yr CR duration of 53% (4). In this study, we prospectively evaluated the efficacy of Hyper-CVAD in a population-based national multicenter T-ALL cohort.

Methods

Patients and treatment

The study cohort consisted of patients prospectively reported to the Swedish Adult Acute Leukemia Registry who were diagnosed with T-ALL between October 2002 and September 2006. The Registry is truly population based; it contains data on patients diagnosed with acute leukemia since 1997, with 98% coverage compared with the Swedish Cancer Registry (5). Missing data were complemented retrospectively. The study was approved by the regional ethical review board in Uppsala (3–520 and 10–258), and the patients gave informed consent in accordance with the ethical approval.

Hyper-CVAD treatment with a total of eight courses (four of each of fractionated cyclophosphamide, vincristine, doxorubicin, and betamethasone alternating with high-dose methotrexate and cytarabine, prophylactic intrathecal methotrexate included) was recommended to all adult (≥ 18 yr) T-ALL patients without severe comorbidity. Dexamethasone, which was recommended in the original publication (4), was replaced by equivalent doses of betamethasone. G-CSF was recommended between courses to shorten the duration of neutropenia. No upper age limit was stipulated. Allogeneic stem cell transplantation (SCT) was recommended for patients with high-risk T-ALL, defined as disease with hyperleukocytosis [white blood cell (WBC) count $> 100 \times 10^9/L$], CR achievement after >2 courses, or high MRD level measured by flow cytometry ($\geq 1\%$ after the second course or $\geq 0.1\%$ after the third course), as well as relapsed disease (after CR2 achievement). In patients without high-risk factors, maintenance consisted of oral mercaptopurine and methotrexate for 2 yr, as well as the following reinduction courses: daunorubicine, vincristine, and prednisolone every second month (for the first year) and cytarabine, thioguanine, and prednisolone every third month (for the second year). Mediastinal radiotherapy was recommended if residual mediastinal disease was present (if the patient was not a candidate for allogeneic SCT with total body irradiation).

Statistics

OS was calculated from the time of diagnosis to death or last follow-up and LFS from achievement of first remission (CR1) to the time of first relapse, death in CR1, or last follow-up. The chi-squared test was used to compare relapse

rates and frequencies of transplantation between age groups. OS and LFS with 95% confidence intervals (95% CI) were estimated by the Kaplan–Meier method complemented with the log-rank test. Univariate Cox regression analyses were also performed to evaluate the effects of relevant covariates on overall survival; hazard ratios (HR) with 95% CI were obtained. Statistical analyses were performed with the IBM SPSS software package, version 21.0 (Armonk, NY, USA).

Results

Patient characteristics

In total, 24 T-ALL patients were diagnosed during the study period, with last follow-up on 25 February 2013. Median follow-up was 93 months (range 77–125 months) for patients who were alive at the end of the study period. Therapy and outcomes are presented in Fig. 1. Four patients were treated with palliative intentions and were excluded from further analysis, as was one patient with a relapse of sarcoma and concomitant secondary T-ALL.

The remaining 19 patients were treated in ten hospitals according to the Hyper-CVAD protocol. Their median age at diagnosis was 32 yr (range 18–72 yr) with 17 of 19 patients (89%) younger than 60 yr. Four were women (21%), and 15 were men (79%). A mediastinal mass was present in 11 patients, CNS involvement in one, and WBC count $> 100 \times 10^9/L$ in one patient. The median blast count in the bone marrow was 85% (range 25–99%). The differentiation stages were defined by flow cytometry in 14 patients as follows: five immature (pro/pre), three cortical/thymic, and six medullary T-ALL. Cytogenetic analysis (16/19 evaluable) revealed two cases with complex karyotype (including one with the MLL fusion gene), nine with other chromosomal

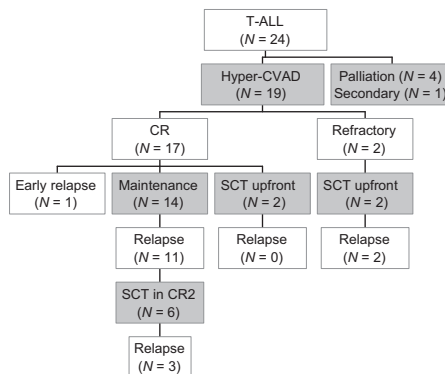


Figure 1 Treatment overview for the 24 patients with T-ALL.

abnormalities, and five with normal karyotypes. There were no cases of hyperdiploidy or $t(10;14)$.

Outcome

CR was obtained in 17 of 19 (89%) patients: after one or two Hyper-CVAD cycles for 15 patients (79%) and after more than two cycles for two patients. In the remaining two patients, CR was achieved after nelarabine therapy and allogeneic SCT, respectively. The disease was assessed as high-risk T-ALL in six patients due to hyperleukocytosis in one patient, a high level of MRD in one patient, no CR in two patients, and late CR achievement in two patients. The median OS (for all 19 patients) was 43 (7–125) months, and LFS (for 17 patients with CR achievement after Hyper-CVAD) was 11 (2–119) months. Two-year and 5-yr LFS were identical: 29% (95% CI: 8–51). The 2 and 5-yr OS was 63% (95% CI: 42–85) and 47% (95% CI: 26–69), respectively.

Allogeneic SCT (sibling donor for three patients and unrelated donor for one patient; myeloablative, total body irradiation containing conditioning) was performed upfront in four patients after 3–5 cycles of chemotherapy (three high-risk, including one with active disease at transplantation, one with CR after nelarabine salvage therapy, and one with standard risk disease with CNS involvement). Two of these patients were long-term survivors at the time of publication, whereas the other two relapsed and died.

The Hyper-CVAD protocol (eight cycles in total) was completed (without significant dose reductions) in 14 of 15 (93%) of the patients who were not transplanted upfront. Three patients had high-risk factors (late CR achievement or high MRD) but did not receive an allogeneic SCT upfront: one patient due to high age (72 yr), two of unknown cause (aged 18 and 25 yr). The median time lapse between initiation of cycles one and eight was 5.7 (range: 4.9–8.5) months, yielding an average of 24 d between cycles. Mediastinal irradiation was given after completion of the Hyper-CVAD protocol to 3 of 11 patients with initial mediastinal involvement. Vincristine caused severe neuropathy in one patient and was withdrawn after the fifth cycle. Other toxicities in single cases included transient liver and kidney function impairment, infections (septicemia, pneumocystis pneumonia, pyomyositis, colitis, and appendicitis), and femoral head avascular necrosis. One patient received only two cycles due to agranulocytosis and invasive candidiasis and experienced a subsequent early relapse. No mortality exclusively related to treatment with Hyper-CVAD was observed.

Five-year LFS and OS for the 15 patients who did not receive allogeneic SCT upfront were 20% (95% CI: 0–40) and 47% (95% CI: 21–72), respectively. Twelve of the 15 patients not transplanted upfront (80%) relapsed after a median of 9 (range 2–23) months from CR1 achievement. Six of the relapsed patients received allogeneic SCT in CR2

(sibling donor, 1; unrelated donor, 4; cord blood, 1; myeloablative conditioning), with three still alive and in persistent CR2 after >6 yr. The other three died of relapse ($n = 2$) or transplantation-related toxicity ($n = 1$). Five-year OS in all relapsed patients (14/19) was 29% (95% CI: 5–52).

Prognostic factors

There was a trend toward shorter LFS in patients ≥ 35 yr at diagnosis ($n = 6$) compared with the younger group (HR 2.7, 95% CI: 0.9–8.3; Fig. 2A). A negative impact of age

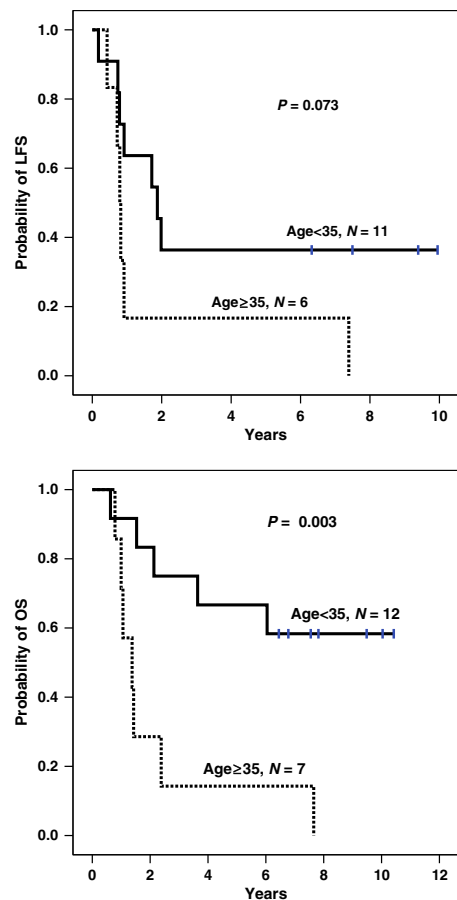


Figure 2 Leukemia-free survival (LFS) (A) and overall survival (OS) (B) according to age (<35 or ≥ 35 yr) in patients treated with Hyper-CVAD.

≥ 35 yr was, however, more evident with regard to OS (HR 5.1, 95% CI: 1.55–16.7; Fig. 2B). There were no long-term survivors in this older group ($n = 7$), except for a 72-yr-old man who died after 91 months in CR1, compared with 67% (95% CI: 40–93) OS at 5 yr in 12 younger patients. There was no statistical difference in the relapse rate according to age group [<35 yr old, 7/9 (78%); ≥ 35 yr old, 5/6 (83%); $P = 0.79$ by χ^2 test] in the 15 patients not transplanted upfront. Only 1 of 7 (14%) patients aged ≥ 35 yr was treated with allogeneic SCT (upfront) compared with 9 of 12 (75%) patients <35 yr, of whom three were transplanted upfront and six in CR2 ($P = 0.01$, χ^2 test). A long time lapse between the initiation of cycles 1 and 3 (median 47 d) had a negative impact on OS (HR 1.05, 95% CI: 1.006–1.09, $P = 0.03$; time as continuous variable), but not on LFS. No other clinical or laboratory parameters had a significant impact on LFS and OS. Samples for MRD were not consistently collected.

Discussion

To our knowledge, this study is the first population-based report of Hyper-CVAD efficacy in adult T-ALL, although the cohort size was small. Chemotherapy was well tolerated and yielded a high CR rate. Disappointingly, treatment without subsequent SCT did not prevent relapse in the majority of patients, resulting in a 5-yr LFS of 20%. This finding is very similar to that of our previous study in Sweden of AB-CDV/VABA chemotherapy (3), although it is inferior to the previously published single-center long-term results with the Hyper-CVAD regimen in totally 36 T-ALL patients, which showed a 5-yr CR duration rate of 55% (6). However, the 5-yr OS is equivalent, with 47% in our study compared with 48% in that report (6). This indicates that our high relapse rate does not reflect an extremely high-risk cohort, as a significant proportion of relapsed patients still had a chemotherapy-sensitive disease, which allowed them to be allocated to allogeneic transplantation in CR2. It is also possible that our high-risk factors were suboptimal and that, for instance, a more stringent use of MRD could have allocated a higher proportion of patients to allogeneic SCT in first remission, which might have been advantageous for the outcome.

T-ALL is a rare disease, and Sweden has relatively few inhabitants, resulting in a small treatment cohort (5). One could speculate as to whether the decentralized organization for ALL treatment might have had a negative effect on treatment results. OS was influenced negatively by long lapses in time between the first and third courses in our investigation, but total time between initiation of the first and eighth courses was similar to that of the original publication describing the Hyper-CVAD protocol (4), indicating an acceptable adherence to the protocol. However, dexamethasone previously showed superior efficacy compared with prednisolone, particularly with regard to reduced risk of

CNS relapse (7). Betamethasone has historically been recommended for ALL treatment in Sweden, and the use of betamethasone instead of dexamethasone in the Hyper-CVAD regimen might have influenced the outcome. No mortality due to toxicities from Hyper-CVAD alone is a favorable finding that indicates good tolerability and appropriate supportive care, but it could also indicate that the treatment schedule might be intensified.

Relapse of T-ALL is known to result in extremely poor outcome with only a few survivors (1). In our study, the 5-yr OS after relapse was 29%, due to the high frequency of SCT in CR2. This procedure is equally effective for relapsed B- and T-ALL (8,9), but unfortunately none of the relapsed patients ≥ 35 yr old in our study reached SCT in CR2. Together with factors related to disease biology and toxicity, this low rate of SCT probably contributed to the impaired OS for older compared with younger patients, as the relapse rate was high for all ages.

The efficacy of upfront allogeneic SCT could not be assessed in our study, as only four patients received this treatment; however, in a previous study (1), having a sibling donor influenced survival positively, which indicates a beneficial role for SCT in CR1. This effect could be even greater for high-risk T-ALL (10,11), but could be weaker for older patients, mainly due to increased transplant-related mortality in patients >40 yr old (12). Intensifying the Hyper-CVAD protocol (13), incorporating nelarabine, and adapting pediatric-like regimens (14) might improve prognosis for patients with adult T-ALL, although in well-defined high-risk cases, upfront allogeneic SCT remains a reasonable option.

To conclude, Hyper-CVAD gave a high CR rate and appeared safe but showed a poor relapse-preventing efficacy and unsatisfactory survival rate in a population-based setting. As a consequence of the results of this study, the Hyper-CVAD treatment is no longer recommended for adult patients with T-ALL in Sweden. In the new guidelines, allogeneic SCT is recommended in all eligible high-risk patients >45 yr old after CR1 achievement (ABCDV/VABA induction). Patients <45 yr old are treated (as are Philadelphia negative B-ALL patients of the same age group) according to the common pediatric and adult Nordic NOPHO 2008 study protocol (15).

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Satisfactory outcome after intensive chemotherapy with pragmatic use of minimal residual disease (MRD) monitoring in older patients with Philadelphia-negative B cell precursor acute lymphoblastic leukaemia: a Swedish registry-based study

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Abstract The introduction of minimal residual disease (MRD) monitoring, in the Swedish national guidelines for acute lymphoblastic leukaemia, was evaluated in 35 patients aged 46–79 years (median 61), who were diagnosed from 2007 to 2011 and treated with high-intensity, block-based chemotherapy (ABCDV/VABA induction). Both a high complete remission rate (91 %) and acceptable overall survival (OS) rate (47 %) at 5 years were achieved. MRD by flow cytometry was measured in 73 % of the patients reaching complete remission after the first course, but was omitted by the clinicians for eight patients who were either over 70 years of age or already met conventional high-risk criteria. Factors negatively influencing

OS were age over 65 years and WHO status ≥ 2 . MRD < 0.1 % after induction had positive impact on continuous complete remission but not on OS. Only five patients were allocated to allogeneic haematopoietic stem cell transplantation in first remission, mainly due to conventional high risk factors. Thus, use of intensive remission induction therapy is effective in a selection of older patients. In a population for whom the possibilities of treatment escalation are limited, the optimal role of MRD monitoring remains to be determined.

Keywords Acute lymphoblastic leukaemia · Adults · Minimal residual disease · Flow cytometry

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Introduction

While substantial advancements have been made in the treatment of paediatric acute lymphoblastic leukaemia (ALL) over the last decades, the prognosis for adult ALL patients is still dismal. Younger adults treated according to paediatric protocols have superior outcome compared with patients over 45 years of age, a majority of whom die of the disease [1]. Minimal residual disease (MRD) observed after remission induction, consolidation, or recurrence after haematopoietic stem cell transplantation (hSCT) are recognized as risk factors for relapse and death in both paediatric and adult ALL patients, regardless of type of leukaemia or detection method. However, repeated MRD analyses are expensive and little information is available about the use of MRD outside controlled clinical trials.

In the 2003 national guidelines of the Swedish ALL group, MRD measurement by flow cytometry was introduced for Philadelphia-negative B cell precursor (Ph-BCP) ALL and by PCR for T-ALL. The aim of this study was to evaluate MRD monitoring for Ph-BCP ALL outside of randomized

controlled trials in a middle-aged/older population treated in a decentralized national setting, with respect to feasibility, cut-off adequacy, and correlation with treatment outcome.

Materials and methods

Patients over 45 years old who were diagnosed with Ph-BCP ALL between 2007 and 2011 were identified using the Swedish ALL registry. Patients treated according to the high-intensity ABCDV/VABA protocol [2] were included in this study. Baseline characteristics and treatment data were obtained from the registry and supplemented from data collected from patient records. The national guidelines recommended allogeneic hSCT in the presence of conventional high risk (HR) factors [white blood cell count (WBC) $> 30 \times 10^9/l$, $t(4;11)$, late complete morphologic remission (CR) achievement, refractory disease] and/or HR MRD. HR MRD was, at the time, defined as MRD $> 1\%$ after remission induction (MRD1), not reaching $< 0.1\%$ after consolidation therapy or duplicate MRD measurements $> 0.1\%$ after the consolidation courses. Maintenance therapy was recommended for patients with standard risk (SR) leukaemia and patients not eligible for hSCT.

MRD levels were prospectively reported to the ALL registry and, at the time of the study, were confirmed directly with the five laboratories that had performed the analyses. Since 2008, MRD was measured by six-colour flow cytometry, which was then used throughout the study period.

Vital status was obtained through 30 August 2014. The study was approved by the regional ethical committee in Uppsala, Sweden, Dnr 2012/415, in accordance with the Declaration of Helsinki. Informed consent was obtained.

Statistical analysis

Overall survival (OS) was defined as the time from diagnosis until last follow-up or death. Continuous complete remission (CCR) was defined as the time from morphologic complete remission to relapse, death in remission, or date of last follow-up. The probability of OS and CCR was estimated by the Kaplan–Meier method. The log-rank method was used for comparisons between groups. Confidence intervals (CI) of 95 % were calculated. Analyses were performed using the SPSS package (v.22, IBM).

Results

A total of 35 patients received ABCDV induction treatment and were included in the study. Patient characteristics are shown in Table 1. Remission induction treatment

comprised ABCDV followed by VABA. Cytarabine and daunorubicin doses were reduced for the three patients over 70 years of age. CR was attained for 32 (91 %) of the patients (30 after ABCDV, two after VABA). Induction death was 6 % within 30 days (2/35). One additional patient received two courses and died without reaching remission, 49 days after start of induction therapy. Eight patients (25 %) reached CR but deviated from the protocol because of comorbidity/age ($n = 2$) or severe infections, such as aspergillosis ($n = 4$); two died in CR due to complications after 32 and 80 days, respectively, and one more patient died in CR during maintenance. Allogeneic hSCT was performed in five patients in CR1, aged 47–64 years, due to HR factors of $t(4;11)$ ($n = 2$), WBC ($n = 2$), or MRD ($n = 1$).

A total of 30 patients reached CR after induction with ABCDV. MRD1 was measured in 22 of these patients on day 19–26 (Table 1). Due to hypoplastic bone marrow, MRD was not evaluable in two cases. The remaining eight patients without MRD measurements were either over 70 years of age or already met HR criteria.

After the second course of treatment, MRD2 measurements were taken in 17 patients, on day 48–78 from start of the first course, and proved evaluable in 16 (Table 1). One patient met the criteria for the HR group because of persistent MRD.

Seventeen patients (49 %) were in CCR at the time of last follow-up. Six had HR leukaemia, of which three underwent hSCT in CR1. The remaining 11 patients had SR leukaemia, seven with MRD1 $< 0.1\%$, one with MRD1 $> 1\%$, and three without MRD measurement (two over 70 years of age, one not evaluable). Fifteen patients relapsed (seven with HR features, including two who had undergone hSCT and four with MRD1 $> 0.1\%$; eight with SR leukaemia). Five patients received allografts in CR2, but no one became a long-term survivor.

Median follow-up among surviving patients was 71 months. OS and CCR at 5 years were 47 % (95 % CI 30–64 %) and 51 % (95 % CI 33–70 %), respectively. Factors negatively influencing OS were age over 65 years and WHO status ≥ 2 (Table 2). OS did not differ significantly between patients with MRD1 $> 0.1\%$ and $< 0.1\%$ nor between the SR and HR group, according to our protocol. MRD1 $< 0.1\%$ showed positive impact on CCR, especially in the absence of conventional HR factors (Table 2).

Discussion

In the present study, we document a 47 % overall survival rate 5 years after ALL diagnosis in a nationwide older cohort. The selection of patients eligible for intensive

Table 1 Clinical and treatment characteristics

<i>Patient characteristics</i>	
Patients included, <i>n</i>	35
Male/female, <i>n</i>	12/23 (34 %/66 %)
Median age, years	61 (range 46–79)
Previous chemotherapy*	2 (6 %)
Previous radiation therapy*	3 (9 %)
Previous haematologic diagnosis	2 (6 %)
PCR or FISH for <i>t</i> (9;22) performed and negative	34 (97 %)
<i>t</i> (4;11), <i>n</i>	4 (11 %)
WBC > 30 x 10 ⁹ /l, <i>n</i>	9 (26 %)
CNS leukaemia, <i>n</i>	1 (3 %)
<i>Allogeneic stem cell transplantation</i>	
in CR1/CR2, <i>n</i>	5/5 (14 %/14 %)
<i>Response to treatment, n = 35</i>	
CR after first course/second, <i>n</i>	30/2 (86 %/6 %)
<i>MRD1-evaluation, n = 30**</i>	
MRD1 measured, <i>n</i>	22 (73 %)
Evaluable, <i>n</i>	20/22 (91 %)
<i>MRD1, evaluable, n = 20**</i>	
MRD1 not detectable (<0.1 or <0.01 %), <i>n</i>	8 (40 %)
MRD1 detectable 0.01–0.099 %, <i>n</i>	5 (25 %)
MRD1 detectable 0.1–0.09 %, <i>n</i>	2 (10 %)
MRD1 detectable >1 %, <i>n</i>	5 (25 %)
<i>MRD2, evaluable, n = 16</i>	
MRD2 not detectable (<0.1 or <0.01 %), <i>n</i>	13 (81 %)
MRD2 detectable 0.01–0.099 %	2 (13 %)
MRD2 detectable 0.1–0.09 %	1 (6 %)

* Therapy given for previous cancer

** Only including patients with morphological CR after first induction

Table 2 Factors affecting OS and CCR

	OS			CCR		
	<i>n</i> (%)	5 years OS (95 % CI)	<i>P</i>	<i>n</i>	5 years CCR (95 % CI)	<i>P</i>
Age < 65	25 (71)	59 (39–79) %	0.01	24	58 (37–79) %	0.01
Age > 65	10 (29)	20 (0–45) %		8	29 (0–62) %	
WHO 0–1	27 (77)	55 (36–74) %	0.04	26	53 (33–74) %	NS
WHO ≥ 2	8 (23)	25 (0–55) %		6	40 (0–83) %	
HR according to protocol*	15 (43)	47 (21–72) %	NS	13	40 (11–67) %	NS
SR according to protocol	20 (57)	49 (27–71) %		19	59 (19–75) %	
MRD1 > 0.1 % after ABCDV	7 (35)	54 (14–93) %	NS	7	43 (6–80) %	0.05
MRD1 < 0.1 % after ABCDV	13 (65)	69 (44–94) %		13	83 (62–100) %	
HR according to protocol and/or MRD1 ≥ 0.1	20 (71)	43 (20–66) %	NS	18	40 (16–64) %	0.04
SR including MRD1 < 0.1**	8 (29)	75 (45–100) %		8	87 (65–100) %	

* Refractory: *n* = 2, late CR: *n* = 2, WBC count over 30 x 10⁹/l: *n* = 9, *t*(4;11): *n* = 4, high-risk MRD: *n* = 1. Two patients had more than one HR factor

** Omitting seven patients regarded as SR according to the protocol but who did not have MRD1 measurement

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chemotherapy was made by clinicians in daily practise and likely has influenced these results. Nevertheless, the high remission frequency (91 %) and long-term survival of almost half of the patients are encouraging, considering the high median age of this cohort. Rates of induction death and death in CR were acceptable; however, only 75 % of the patients who reached CR could complete consolidation therapy as the protocol recommends, due to comorbidity and toxicity. This observation illustrates the necessity of treatment adjustment in older patients, and the need for further studies in this population [3].

The prognostic value of MRD in this cohort cannot be thoroughly evaluated because of the limited number of patients. However, the excellent CCR observed in patients with low MRD is promising, and it supports the utility of MRD measurements in making treatment decisions in an older population. MRD < 0.1 %, as measured by flow cytometry approximately 25 days after the start of induction, seems to be an adequate level in this setting. This limit is lower than in the current Swedish guidelines, which will be revised accordingly. From a health-economic perspective, we noted that assessment of MRD was performed judiciously, that is, only when the results would influence further treatment.

Use of MRD as a treatment-escalating tool is established, but the measurement timepoint and discrimination level vary among children, adults, and B cell- and T cell-derived leukaemias [4–8]. Furthermore, the two alternative methods of MRD detection, PCR and flow cytometry, have various advantages and disadvantages [9]. Additional studies and new diagnostic methods such as next-generation sequencing may improve the prognostic stratification, including MRD, in older patients as well as younger patients [10].

The cost-effectiveness of using MRD as a tool for treatment escalation could be questioned in a population that is not eligible for hSCT. However, one could hypothesize that a high MRD after remission induction, along with conventional HR factors, could serve as a signal for transition to less toxic regimens and palliative care.

We conclude that the ABCDV-based regimen is both feasible and effective in a selected fraction of older ALL patients. We also find that MRD is used in a pragmatic way in a decentralized setting in Sweden. However, we still lack knowledge about how to most optimally treat older patients with regard to overall survival, time spent in hospital, quality of life, and economic costs. Additional population-based and randomized interventional studies will be necessary to determine the value of intensive treatment in the older ALL patient.

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Conflict of interest None.

Ethical standard All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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Age but not Philadelphia positivity impairs outcome in older/elderly patients with Acute Lymphoblastic Leukemia in the Swedish population

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ABSTRACT

Few older/elderly patients with Acute Lymphoblastic Leukemia (ALL) are included in clinical trials. Based on data from the Swedish leukemia registries and the medical records for patients aged 55- 85 years (y) diagnosed 2005-2012, we investigated the disease/patient characteristics, choice of treatment, and outcome in this population based cohort, and examined if survival had improved with the introduction of an age-adapted protocol from 2009. Among 174 patients, 81% had B-ALL, 11% Burkitt leukemia (excluded from further analysis), and 7% T-ALL. The frequency of Philadelphia chromosome positivity (Ph+) among 155 B- and T-ALL patients was 35% (found only in B-ALL). The majority, 124/155 (80%) patients (median age 65y, range: 55-82), were treated with intensive protocols (+ tyrosine kinase inhibitor in all 42 with Ph+) and 31 (median age 79y, range: 55-85) with palliative intention. Higher age and performance status ≥ 2 were factors for the choice of palliation. Intensive, palliative and both treatments resulted in the complete remission rate of 83/16/70% and 3y overall survival (OS) of 32/3/26%. The introduction of an age-adapted protocol did not improve OS. In the intensively treated cohort, platelet count $\leq 35 \times 10^9/L$ and age $\geq 75y$ were negative prognostic factors for OS in a multivariate analysis, but not Ph+. Males had impaired OS in the 55-64y group. In conclusion, we confirm a high frequency of Ph+ ALL in older/elderly patients, with a non-inferior outcome compared to Ph- ALL. However, overall prognosis of ALL in the elderly remains mostly dismal despite use of intensive, including age-adapted, treatments.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a highly proliferative blood malignancy, treated with complex and intense chemotherapy protocols with improving results in children and young adults over the last decades. Survival has not improved in patients >70 years (y) and improved only modestly in those aged 60-70 y.¹ In studies with intensive chemotherapy, elderly patients had inferior complete remission (CR) rate, overall survival (OS), and disease free survival (DFS) compared with younger adults.²⁻⁴ This is partly explained by comorbidities, poor performance status at diagnosis, toxicity, need for dose reductions, and a higher proportion of adverse risk cytogenetics including Philadelphia chromosome positivity (Ph+).^{2,4-8} The latter has historically been recognized as a high-risk factor, but its impact on outcome in elderly patients has declined since the introduction of tyrosine kinase inhibitors (TKI).⁵ Due to the poor outcome for patients >55y according to the Swedish ALL registry⁹, and based on the promising results of the European Working Group on Adult ALL (EWALL) backbone¹⁰, this age-adapted protocol +/- TKI was introduced in Sweden for elderly (and older unfit) patients as of October 2009.

By using the Swedish Acute Leukemia Registry/ALL Registry, we performed a population based study in order to assess different therapy strategies in patients 55-85y treated for ALL 2005-2012 according to the Swedish national guidelines. We hypothesized that the outcome had improved after October 2009. The aim was also, in this unselected population, to investigate the disease and patient characteristics in relation to age, clinician treatment choice, and outcome.

PATIENTS AND METHODS

Every Swedish citizen has a unique social security number which enables disease surveillance in population-based registries. Patients are reported to the Swedish ALL Registry by their treating physician. As previously described, the coverage of the leukemia registries compared to the compulsory Swedish Cancer Registry has been 98%.⁹ In this study, patients were identified through the ALL registry and additionally through the Swedish Cause of Death Registry. Vital status was obtained through 30th of June 2015. The study was approved by the Regional Ethical Review Board in Uppsala/Sweden (2014/063) in accordance with the declaration of Helsinki, including informed consent from the patients.

Data collection

Clinical and laboratory data along with pathology and genetic reports were verified from medical records by P.K and E.B. Registry data were supplemented regarding co-morbidities, treatment description, and toxicity. The diagnoses were verified by morphology reports, immunophenotype and genetics according to the World Health Organization (WHO) Classification of 2005.¹¹ In the case of

Burkitt leukemia, t(8;14), *C-MYC* rearrangement [by fluorescence in situ hybridization (FISH) or immunohistochemistry] or typical immunophenotype was verified. Cytogenetic classification was based on G-banding analysis and considered normal when ≥ 20 normal metaphases were analyzed. Ph+ ALL diagnosis was based on t(9;22), *BCR-ABL* detection by G-banding and /or FISH and/or reverse transcription polymerase chain reaction (RT-PCR), and *MLL* rearrangement by the presence of t(4;11) or FISH for 11q23 (regardless of fusion-partner). Bulky disease was defined as a lymph node conglomerate > 10 cm or a mediastinal mass larger than 1/3 of the thoracic diameter. CNS-leukemia was diagnosed if blasts in cerebrospinal fluid and/or radiological findings of CNS-involvement were present.

Performance status (PS) at diagnosis was reported according to the WHO classification.¹² The comorbidity component (CC) from the adjusted Charlson Comorbidity Index was estimated retrospectively.¹³ The ALL diagnosis was not included in the score. The number of drugs (including inhaled therapy) was counted from medical records at the first admission. Treatment adjustments were considered in specific regimens if a dose was reduced by $> 25\%$ or a drug was omitted. The only hematologic toxicity registered was time from start of first treatment (pre-phase treatment included) to the neutrophilic recovery ($> 0.5 \times 10^9/L$ for three consecutive days). Proven or probable invasive fungal and pneumocystis jiroveci infections were noted. Septicemia was reported when a clinical infection was documented and bacteria detected in one blood culture (two cultures in the case of coagulase negative staphylococci).

Renal- and hepatic failure of grade III-IV (Common Terminology Criteria of Adverse Events)¹⁴, heart failure, enterocolitis, mucositis, and neuropathy of grade III-IV influencing protocol adherence were reported. Pancreatitis and thrombosis were recorded if observed. Toxicity was assessed from initiation until start of maintenance therapy or allogeneic hematopoietic stem cell transplantation (hSCT) with the exception of osteonecrosis and neuropathy recorded throughout the whole follow up period. CR was defined as morphologic complete remission, with less than five percent blasts in the bone marrow (BM) and absence of extramedullary disease. Minimal Residual Disease (MRD) was measured by flow cytometry in B-ALL, RT-PCR in Ph+ ALL or PCR of T-cell receptor rearrangement in T-ALL.

Treatment and risk classification

National guidelines for treatment of patients older than 55y with the two main protocols (ABCDV¹⁵ and EWALL-backbone) are presented in supplemental Table S1 and S2, respectively. Allogeneic hSCT in CR1 was recommended in fit patients mainly up to 65y fulfilling at least one of the following high-risk criteria: white blood cells (WBC) $> 30 \times 10^9/L$ in B-ALL or $> 100 \times 10^9/L$ in T-ALL, Ph+ disease, *MLL* rearrangement, CR achievement after more than one course (two courses if T-ALL treated with hyper-CVAD), MRD $> 1\%$ after remission induction, increasing MRD levels or not reaching $< 0.1\%$ after consolidation therapy. From 2009, T-cell phenotype was regarded as high-risk feature. Decisions on

conditioning regimens, donor type and graft-versus-host prophylaxis were made by transplantation centers at their own discretion. The extent of human leukocyte antigen (HLA)-match (full/mismatch) was not scrutinized. Viral-, bacterial-, and fungal prophylaxis were given according to local routine.

Statistical methods

For categorical data differences in proportions were compared with the Chi-square or two-tailed Fischer's exact test when appropriate. Continuous variables were compared with Mann-Whitney U test. OS was calculated from diagnosis to death or date of last follow up. Event-free survival (EFS) was estimated from diagnosis to relapse, death or last follow up in CR. Event was considered on day one for patients who died without CR evaluation or because of refractory disease. Distributions of OS and EFS were estimated by the Kaplan-Meier method and differences analyzed using the log-rank test. In addition, univariate and multivariate Cox regression analyses were performed to evaluate the effects [calculating hazard ratio (HR)] of relevant covariates on OS and EFS. Association of CR achievement with different variables was evaluated by logistic regression estimating odds ratio (OR). Cut-off points providing the most significant discrimination for OS were used for continuous variables when converted to dichotomous. Statistical tests were used with an alpha-significance level of 5% and 95% confidence intervals. No adjustment of multiplicity was performed and *P* values should be interpreted as explorative. The IBM SPSS software package, version 23.0 (Armonk, NY, USA) was used.

RESULTS

Patients and treatments in the population based ALL cohort

A total of 183 patients were identified through the ALL-registry. Eleven patients were excluded [ten because of diagnosis misclassification (5%) and one because of withdrawn consent]. Additionally, two patients with ALL were identified through the Swedish Cause of Death Registry giving a coverage reaching 99% for B- and T-ALL. Among 174 patients, B-ALL was the most common phenotype (81%), followed by Burkitt leukemia (11%) and T-ALL (7%). The median age for the respective ALL subtype was: 67, 73 and 70.5y. Patients with Burkitt leukemia were excluded from further analysis as being a separate entity according to the WHO 2005 classification. Characteristics of the final study cohort of 155 patients are presented in Table 1. The male/female distribution was equal except that T-ALL was more common in males [10/72 (14%)] compared to females [2/83 (2%); *P* = 0.01]. Bulky disease was documented in one and mediastinal mass in two patients. Cytogenetic abnormalities are summarized in Table 1. G-band karyotyping, and FISH or PCR for at least *BCR-ABL*, were performed and evaluable for 140/155 (90%) of the cohort. Ph+ ALL was diagnosed in 49/132 (37%) of the B-ALL cohort. The vast majority of patients were treated with intensive (remission inducing) protocols (Table 1). Median age in the cohort was lower compared with those treated with a palliative approach (*P* < 0.001). The most common comorbidities were diabetes 16/123 (13%) and history of myocardial infarction 12/123

(10%). The proportions of patients with PS ≥ 2 and CC ≥ 1 were significantly higher in those receiving palliation ($P < 0.001$ and 0.005), as also for comorbidities ≥ 2 and median number of drugs (Table 1). CC was used in further analyses for comorbidity assessment. In multivariate analysis (PS, CC, age; logistic regression), only age (as continuous variable) and proportion of patients with PS ≥ 2 remained significantly different between the intensive and palliative cohort ($P < 0.001$ and < 0.05 , respectively). CR was reached in 108/155 (70%) in the entire B- and T-ALL cohort, and one and 3 year OS were 50% (95%CI: 42, 58) and 26% (95%CI: 20, 33) respectively.

Patients with B- and T-ALL treated with remission intention

Treatment characteristics

Remission inducing therapy was given to 124 patients aged 55-82y. Characteristics of patients and treatments given in three age groups are presented in Table 2. Patients receiving EWALL-backbone +/- TKI (n = 35) were older than those treated with ABCDV +/- TKI (n = 79) [median age of 69y (range 62-82) and 63y (range: 55-79) respectively; $P < 0.001$]. Treatment was modified in equal proportions of patients for both protocols, 12/35 (34%) and 27/79 (34%) respectively. Totally 12/35 (34%) of patients completed EWALL-backbone and 48/79 (61%) the ABCDV protocol. Of the 10 remaining patients, five received hyper-CVAD¹⁶ and four remission induction with daunorubicin/cytarabine (DA) due to initial misclassification as Acute Myeloid Leukemia (AML). One patient received pre-phase treatment only and died before induction was started. All 42 patients with Ph+ disease (and one with Ph-) started with TKI at induction (41 imatinib and two dasatinib) according to EWALL (n = 12), ABCDV (n = 29), hyper-CVAD (n = 1) and DA (n = 1).

Remission rate and survival for all patients

The proportion of patients achieving CR (83%) was not influenced by age analyzed as continuous variable (OR 0.95, 95%CI: 0.89, 1.03; $P = 0.20$), but was significantly lower in the oldest as compared to youngest age group ($P = 0.03$, Table 2). CR frequency was slightly higher in Ph+ ALL (93% vs. 80%; $P = 0.07$). No other factors influenced probability of CR achievement (data not shown). Median survival was 16 months (range 0-126). OS was 59% (95%CI: 50, 67) after one and estimated to 32% (95%CI: 24, 40) after 3 years. EFS was 47% (95%CI: 38, 56) and 25% (95%CI: 17, 33), respectively. Median follow-up of survivors was 74 (33-126) months. Of the patients treated with remission intention, 96/124 (77%) died during follow up. Causes of death were: early death (ED; within 60 days) in 18/124 (15%) patients (four in CR, two with refractory disease), relapse in 56 (45%), transplant-related mortality (TRM) in 9 (7%; eight after hSCT in CR1 and one in CR2), refractory disease in five (4%), secondary AML in two, late induction treatment complication in one. Five (4%) patients died later on in remission. The only factor associated with ED was PS ≥ 2 at diagnosis [7 of 26 (27%) vs. 11 of 97 patients (11%) with PS < 2 ; $P = 0.046$].

Outcome according to treatment protocol (EWALL/ABCDV+/-TKI):***All patients***

Induction according to EWALL-backbone resulted in CR in 25 of 35 (71%) patients, and ABCDV in 79 of 79 (89%; in seven after ≥ 2 courses). ED occurred in 20% and 13% of the patients respectively. One and 3 year OS in the cohort treated with EWALL-backbone was: 49% (95%CI: 32, 65) and 20% (95%CI: 7, 33) respectively, and with the ABCDV protocol: 63% (95%CI: 53, 74) and 39% (95%CI: 28, 50).

Patients aged 65-74y

A shift from ABCDV before October 2009 (22/31, 71%) to the EWALL-backbone in the later period (19/22, 86%) occurred in the 65-74 age group ($P < 0.001$) as a consequence of the new guidelines, and approximately the same proportion of patients had received treatment by the respective protocol at the end of the study period (Table 2). Median age was 69y for each protocol. Proportion of patients with PS ≥ 2 (16 vs 25%) and CC ≥ 1 (44 vs 50%) did not differ significantly between EWALL-backbone and ABCDV. CR was achieved in 18/25 (72%) after EWALL induction and 21/24 after ABCDV (88%; $P = 0.18$). Neither ED-rate (20% vs 21%) nor OS differed between protocols in this age group (Figure 1).

Toxicity:***All patients***

The proportion of patients affected by toxicity (displayed in Table 2) did not differ significantly among three age groups with exception of kidney failure which was more common in patients aged $\geq 65y$. The frequency of probable or proven invasive fungal infection was higher in patients with diabetes as compared to those without [7/16 (44%) vs. 15/106 (14%); $P = 0.004$]. Invasive candidiasis (with positive blood cultures) was diagnosed in 4 patients, aspergillosis in 4 and the agent was unknown in 14. No case of pancreatitis was recorded. Median time to neutrophil recovery was 23 days from start of chemotherapy in patients reaching CR.

According to protocol (EWALL/ABCDV+/-TKI)

Serious infections (mainly septicemia) during induction/consolidation were more common in ABCDV as compared to EWALL-backbone treated patients [69/79 (87%) vs. 23/35 (66%); $P = 0.007$], and pneumocystis jiroveci pneumonia occurred only in the former cohort. Median time to neutrophil recovery was equal for both protocols (ABCDV-23 vs. EWALL-24 days; $P = 0.3$) despite more common use of G-CSF in the latter [34/73 (47%) vs. 31/32 (97%); $P < 0.001$]. Serious toxicity of TKI (leading to transition to another TKI or long-lasting intermissions) was less frequent in EWALL-backbone compared to ABCDV [1/12 (8%) vs. 13/29 (45%), $P = 0.03$] but did not differ among the three age groups ($P = 0.64$).

Allogeneic hSCT in CRI

Allogeneic hSCT was performed in 20 of 103 (19%) patients in first remission (10 males and 10 females) after ABCDV (n = 17) and hyper-CVAD (n = 3) treatment. Median age was 60y (range 55-66). High-risk disease according to guidelines was considered in 19 patients due to: Ph+ (n = 14), T-ALL (n = 1), high WBC at diagnosis (n = 3, as only high-risk criterion), late remission (n = 1), and high MRD (n = 1). Donors were HLA-identical siblings (n = 10) or matched unrelated (n = 10). Peripheral blood as stem cell source was used in all but one patient. Reduced-intensity conditioning [fludarabine-based including total body irradiation (TBI) in five patients] was given to 13 patients and myeloablative to the remaining seven (mainly cyclophosphamide and TBI). Three year OS and EFS were 40% (95%CI: 18, 62) and 25% (95%CI: 6, 44). OS in men was impaired ($P = 0.05$), as well as EFS ($P = 0.04$), despite lower median age 57.5 vs. 62y in women ($P = 0.04$). Transplanted patients died mainly because of TRM (8/20) and relapse (6/20). OS in 14 transplanted Ph+ patients was not different from all non-transplanted Ph+ (receiving palliative treatment included) (Figure 2).

Prognostic factors

Univariate analysis of prognostic factors for OS is demonstrated in Table 3. Significant/borderline significant factors from univariate analysis (Table 3) were included in a multivariate model which revealed age ≥ 75 (Fig 3A) and $PLT \leq 35 \times 10^9/l$ as negative prognostic factors for OS (Table 3) and EFS (data not shown). No impact on survival was observed for: Ph+, PS, $CC \geq 2$. When analyzing three age groups versus Ph status, age negatively influenced OS in Ph- but not as clearly in Ph+ ALL, as presented in Figure 3B-C.

EFS (not shown) and OS were significantly impaired in males in the youngest age group (Figure 4), even though thrombocytopenia ($PLT < 35 \times 10^9/l$) was more common in females in this age group [39/67 (58%) vs. 20/57 (35%); $P = 0.01$].

MRD analysis after remission induction was performed in only 55 of 94 patients achieving CR (13 after EWALL induction, 39 after ABCDV, and three after other protocols). Detectable MRD1 ($>0.1\%$) neither had impact on OS nor EFS.

Outcome before and after the introduction of new guidelines

In total, 92 of 155 (59%) patients with B- and T-ALL were diagnosed before and 63 of 155 (41%) after October 2009. The proportion of patients treated with remission intention was equal in both periods [74/92 (80%) vs. 50/63 (79%)]. Median age was 64y and 67y ($P = 0.1$), respectively. There was no significant difference in previously defined prognostic factors (data not shown) between the two periods. Of 124 intensively treated patients, 115 (93%) started treatment according to the contemporary guidelines. EWALL-backbone was applied to 6/74 (8%) patients before October 2009 and 29/50 (58%) after ($P < 0.001$). Neither OS nor EFS differed between periods in the whole cohort or in the three age groups (not shown).

Palliation

Characteristics of 31 patients are shown in Table 1. Of them 17 (55%) received more intense palliative treatment: modified (heavily reduced) induction according to ABCDV (n = 2) or EWALL (n = 1), COP/CHOP [n = 9: with rituximab (n = 1) or with TKI (n = 2)], VAD (n = 2; with TKI n = 1), vincristine/thioguanine/cortisone combination (n = 1) or cortisone/TKI combination (n = 2). Five of 17 (29%) achieved CR. Less intensive palliation (14/31) consisted of: oral cyclophosphamide/cortisone (n = 1), thioguanine/cortisone (n = 1), cortisone alone (n = 2), hydroxyurea (n = 1) or no specific antileukemic therapy (n = 9). OS after one and three years was 13% (95%CI%: 1, 25) and 3% (95%CI: 0, 9). OS was not significantly impaired in the oldest age group (75-85y) given palliation (n = 23) as compared to the 12 patients (75-82y) receiving remission induction therapy ($P = 0.12$).

DISCUSSION

We present a truly population based study of Swedish older/elderly ALL patients. Disease characteristics, with low T-ALL-, bulky disease- and high Ph+ incidence compared to younger adults, were similar to other studies.^{2,4-8} As regards therapy, the national guidelines were followed to a large extent. The decision to refrain from intensive treatment appeared to be based mainly on age and PS, and a minority of patients ≥ 75 y received remission induction compared to over 90% of those < 75 y old.

Complete remission rate (83%) was at least as high as compared to other protocols (34-84%)^{2,3,5-8,17}, having in mind the population-based character of our study. The drawback was the high early mortality (15%), even if the frequency reported by others is comparable (11-34%).^{2,4-8} The 3y OS was similar to other ALL studies including older/elderly patients.^{4,5} Toxicity, especially infectious, was high in previous reports.^{2,4,17} A new finding was the increased frequency of invasive fungal infections in diabetic patients. This could indicate the need for broad spectrum antimycotic prophylaxis in this group. ALL recurrence was the main cause of death, as found also by others^{2,4,5,7}, suggesting need of intensification during consolidation to reduce the relapse risk.

The introduction of the age-adapted EWALL-backbone, which was used mainly for the ≥ 65 y old patients, did not improve overall outcome. Even if CR achievement was satisfactory (71%) it was not translated into prolonged survival, and a high early death-rate (20%) was observed. In contrast to our results of population-based use of the protocol, others reported none^{10,18} or low early death.¹⁹ The CR-rates previously reported using EWALL-backbone¹⁰, EWALL backbone + nilotinib¹⁸, or EWALL-backbone +dasatinib¹⁹ were 85, 97, and 90% respectively. One year OS in our study was 49% (46% in Ph-) as compared to 61% in the previously cited.¹⁰ A straightforward comparison of ABCDV and EWALL in terms of efficacy and toxicity is not fair, as they were used in different (but overlapping) age cohorts. Though when looking at ages 65-74y, where comparable numbers of patients received each

treatment option and baseline patient characteristics were similar, we could not see any significant difference in outcome/toxicity.

Age as adverse prognostic factor in older/elderly patients was demonstrated by us and other authors^{6,7,17}, but not all.^{5,8} In epidemiological studies outcome according to age is striking.^{1,20} Intensive chemotherapy beyond the 75y age-limit rendered few survivors and an OS similar to palliative treatment. A randomized trial is needed to address the question how to best treat the >75y patients. To speculate, less intensive chemotherapy (“intensive palliation”) in the age group inclusive the use of TKI in Ph+ disease can be an option, especially as age had limited impact on outcome in Ph+ ALL.

Thrombocytopenia impaired OS and EFS. It was not reported previously in elderly ALL, but in a population based study from Denmark²⁰ and in patients treated with Hyper-CVAD.¹⁶ In our study, the impaired outcome was not an effect of major hemorrhage as only a few patients were affected by the latter. Instead, we interpreted the thrombocytopenia as a pseudo-marker for more aggressive disease.

Survival was noticeably impaired in males in the youngest age group. Previously reported data on prognostic significance of sex are conflicting. Historically, male sex was regarded as a negative prognostic factor mainly in children. In Poland, OS and probability of CR achievement was superior in elderly females.⁶ To speculate, the differences in outcome may be protocol specific, and possibly related to pharmacokinetics, as survival impairment in males has disappeared during population-based study periods²¹ as in modern pediatric protocols.²²

No patient with T-ALL survived beyond two years. Inferior survival in T-ALL treated with ABCDV was demonstrated previously in Sweden¹⁵ and was not improved by the introduction of hyper-CVAD.²³ T-phenotype was found to be a negative prognostic factor for survival³ and CR achievement¹⁷ in elderly patients. The findings were not confirmed by others.^{4,8} Numbers of patients with T-phenotype were low in all studies making thorough analysis difficult.

Ph+ disease is classically regarded as a high-risk factor in ALL,²⁴ including in our national guidelines. Introduction of TKI has challenged the paradigm²⁵ and even a trend towards favorable outcome compared to Ph-disease was reported in a small Ph+ cohort of elderly patients.⁵ We found similar OS in Ph- and Ph+ ALL, given the fact that every patient with Ph+ disease received TKI.

The hSCT frequency in patients <65 years was high. According to some authors^{4,26}, hSCT in CR1 in eligible older patients with high-risk features (particularly Ph+)²⁴ should be considered. In our presented study, the overall survival was similar in Ph+ patients treated with chemotherapy + TKI (palliative included) and patients receiving hSCT. Even if the number of patients was low, one can speculate if more

intensive protocols (such as ABCDV) plus TKI with subsequent hSCT are too toxic in this age group as reflected by high TRM. Low intensity protocols +TKI rendered acceptable results in elderly.^{27,28}

Comorbidities had no clear impact on survival in our intensively treated cohort, probably partly because of “wise” selection of patients ineligible for remission induction, demonstrated by a larger fraction of patients with higher age and poor PS in the palliatively treated cohort. Lack of prognostic value of different comorbidity scoring systems was found also previously.⁷

Conclusions

The prognosis remains mostly dismal for older/elderly ALL patients despite intensive treatment. The use of an age-adapted protocol did not improve outcome in Sweden. The challenge remains – to decrease early mortality and the frequency of relapse. Risk factors based on disease and patient characteristics including age can probably predict response to intensive treatment, but Ph+ ALL is, in the TKI-era, no longer a negative prognostic factor for the age group. Male sex, as an adverse prognostic factor in patients aged 55-65y, warrants further investigation. We conclude that intensive treatment should primarily be reserved for patients aged <75y, and new treatment modalities with less toxicity are needed for elderly patients with ALL.

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TABLES AND FIGURES

Table 1. Characteristics and treatment type for all patients with B- or T-ALL.

	n (%)	Treatment	
		Intensive (%)	Palliation (%)
Number of patients	155	124 (80)	31 (20)
Median age (range)	67 (55-85)	65 (55-82)	79 (55-85)
Age group:			
55-64y	60 (39)	59 (47)	1 (3)
65-74y	60 (39)	53 (43)	7 (23)
75-85y	35 (22)	12 (10)	23 (74)
Male: Female	72:83	57:67	15:16
Phenotype:			
B	141 (91)	116 (93)	25 (81)
T	12 (8)	8 (7)	4(13)
B-ALL UNS	2 (1)	0	2 (6)
Cytogenetics:			
<i>BCR-ABL</i> /Ph	49 (35)	42 (35)	7 (35)
<i>MLL</i> /t(4;11)	8 (6)	7 (6)	1(5)
complex	8 (6)	6 (5)	2 (10)
normal	10 (7)	10 (8)	0
other	41 (31)	36 (30)	7 (35)
unknown, not Ph	22 (15)	19 (16)	3 (15)
<i>not performed/ not evaluable</i>	15	4	11
CNS leukemia:			
Yes	9 (9)	6 (6)	3 (43)
No	104 (91)	100 (94)	4 (57)
<i>not performed/ not evaluable</i>	41	18	23
WBC median (range) x10 ⁹ /l	11.9 (0.6-420)	13.0 (0.6-420)	8.8 (1.7-105)
Plt median (range)x10 ⁹ /l	41 (6-654)	37 (6-654)	53 (9-200)
LDH ratio* median (range)	2.7 (0,5-42.7)	2.7 (0.5-33.3)	2.8 (0.5-42.7)
Hb median (range) g/l	10.1 (4.0-15.0)	9.9 (4.0-15.0)	11.1 (6.0-13.9)
Creatinine median (range)	8.5 (3.3-34.3)	8.5 (3.3-26.1)	8.6 (3.9-34.3)
Performance status (WHO):			
0	40 (26)	35 (28)	5 (17)
1	70 (46)	62 (50)	8 (28)
2	25 (16)	18 (15)	7 (24)
3	10 (7)	6 (5)	4 (14)
4	7 (5)	2 (2)	5 (17)
Number of comorbidities:			
0	52 (34)	49 (40)	3 (10)
1	48 (31)	40 (32)	8 (26)
≥2	54 (35)	34 (28)	20 (64)
Comorbidity component:			
0	85 (55)	75 (61)	10 (32)
1	31 (20)	24 (19)	7 (23)
2	19 (12)	12 (10)	7 (23)
3	12 (8)	10 (8)	2 (6)
≥4	7 (5)	2 (2)	5 (16)
Median number of drugs	2 (0-14)	1 (0-13)	6 (0-14)

*a ratio of serum lactate dehydrogenase and upper limit of normal value

Table 2. Patient and treatment characteristics according to age group for intensively treated B- or T-ALL.

	n (%)	Age group		
		55-64y (%)	65-74y (%)	75-82y (%)
Number of patients	124	59 (47)	53 (43)	12 (10)
Median age years	65	60	69	77
Male: Female	57:67	26:33	23:30	8:4
T phenotype	8 (7)	3 (5)	4 (8)	1 (8)
Ph+*	42 (35)	21 (36)	16 (32)	5 (46)
Performance status ≥ 2	26 (21)	13 (22)	11 (21)	2 (17)
Comorbidity component ≥ 1	49 (40)	17 (29)	25 (47) ^a	7 (58)
Protocol used:				
ABCDV	79 (64)	49 (83)	24 (45)	6 (50)
EWALL	35 (28)	5 (8.5)	25 (47) ^a	5 (42) ^a
Other	10 (8)	5 (8.5)	4 (8)	1 (8)
Protocol adherence	86 (70)	44 (76)	37 (70)	5 (42) ^a
CR	103 (83)	53 (90)	43 (81)	7 (59) ^a
Early death	18 (15)	6 (10)	10 (19)	2 (17)
Infectious toxicity	97 (79)	46 (79)	40 (76)	11 (92)
Septicemia	80 (65)	39 (67)	34 (64)	7 (58)
Pneumonia	28 (23)	12 (21)	12 (23)	4 (33)
Pneumocystis pneumonia	4 (3)	2 (4)	2 (4)	0
Invasive fungal infection	22 (18)	15 (26)	6 (11)	1 (8)
Other toxicity	52 (42)	19 (33)	27 (51)	6 (50)
Enterocolitis	14 (11)	7 (12)	6 (11)	1 (8)
Mucositis	4 (3)	0	3 (6)	1 (8)
Heart failure	13 (11)	3 (5)	8 (15)	2 (17)
Kidney failure	9 (7)	0	7 (13) ^a	2 (17) ^a
Liver failure	6 (5)	1 (2)	4 (8)	1 (8)
Thrombosis	6 (5)	3 (5)	3 (6)	0
Neuropathy	8 (7)	4 (7)	4 (8)	0
Diabetes at discharge	3 (2)	1 (2)	2 (4)	0
Bleeding	3 (2)	1 (2)	2 (4)	0
ICU** admission	21 (17)	11 (19)	10 (19)	0
hSCT in CR1	20 (16)	18 (31)	2 (4) ^a	0 ^a

*4 missing values, **intensive care unit

^a significant difference as compared to 55-64y

Table 3. Univariate and multivariate analyses of pretreatment prognostic factors for overall survival (OS) in intensively treated patients with B- or T-ALL.

	n	3y OS% (95%CI)	Univariate analysis:	Multivariate analysis*:
			HR (95%CI); <i>P</i> value	HR (95%CI); <i>P</i> value
Age group: 55-64y	59	39 (26, 51)	; 0.01	; 0.025
65-74y	53	30 (18, 43)	1.30 (0.85, 1.99); 0.23 ^a	1.20 (0.77, 1.85); 0.42 ^a
75-82y	12	8 (0, 24)	2.71 (1.40, 5.22); 0.003 ^a 2.08 (1.08, 4.00); 0.028 ^b	2.60 (1.30, 5.19); 0.007 ^a 2.17 (1.10, 4.31); 0.026 ^b
Female	67	40 (28, 52)		
Male	57	23 (12, 34)	1.52 (1.02, 2.27); 0.04	1.37 (0.82, 2.29); 0.22
B-cell phenotype	116	34 (26, 43)		
T-cell phenotype	8	0	2.04 (0.98, 4.27); 0.06	1.49 (0.68, 3.28); 0.32
Ph-	78	31 (21, 41)		
Ph+	42	33 (19, 48)	0.96 (0.62, 1.47); 0.84	
No <i>MLL</i> rearrangement	100	31 (22,40)		
<i>MLL</i> rearrangement	7	13 (0, 40)	1.06 (0.92, 1.21); 0.45	
No CNS leukemia	100	37 (27,46)		
CNS leukemia	6	17 (0, 46)	1.36 (0.55, 3.38); 0.50	
WBC ≤100 x10 ⁹ /l	108	35 (26, 44)		
WBC >100 x10 ⁹ /l	16	13 (0, 29)	1.64 (0.94, 2.87); 0.08	1.63 (0.92, 2.87); 0.09
Plt >35 x10 ⁹ /l	65	39 (27, 50)		
Plt ≤35 x10 ⁹ /l	59	25 (15, 36)	1.53 (1.02, 2.29); 0.04	1.81 (1.16, 2.79); 0.008
LDH ratio <3**	61	41 (29, 53)		
LDH ratio ≥3	49	25 (15, 36)	1.41 (0.92, 2.17); 0.11	
Creatinine ≤90 mg/dL	74	40 (29, 52)		
Creatinine >90 mg/dL	48	19 (8, 30)	1.73 (1.15, 2.61); 0.009	1.26 (0.76, 2.09); 0.37
Performance status <2	97	34 (24,43)		
Performance status ≥2	26	27 (10, 44)	1.34 (0.82, 2.18); 0.24	
Comorbidity component <1	75	32 (21, 42)		
Comorbidity component ≥1	49	33 (20, 46)	1.01 (0.67, 1.53); 0.95	

*Two patients excluded due to missing values.

^a as compared with 55-64y group, ^b as compared with 65-74y group

**a ratio of serum lactate dehydrogenase and upper limit of normal value

Figure 1. Overall survival in patients aged 65-74y according to protocol (EWALL-backbone vs. ABCDV).

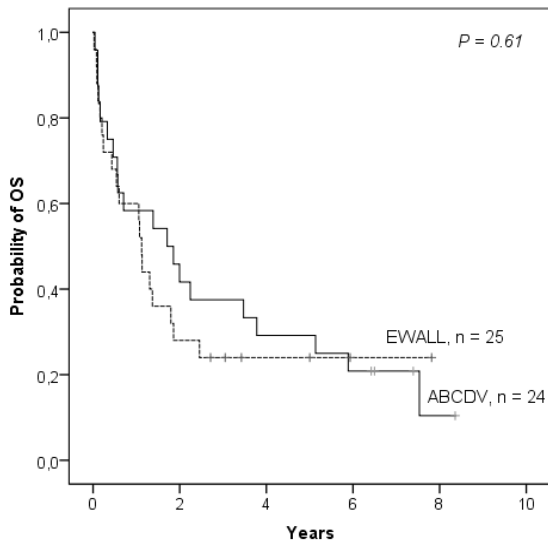


Figure 2. Overall survival in patients with Ph+ transplanted and not transplanted (intensively and palliatively treated included, all receiving TKI).

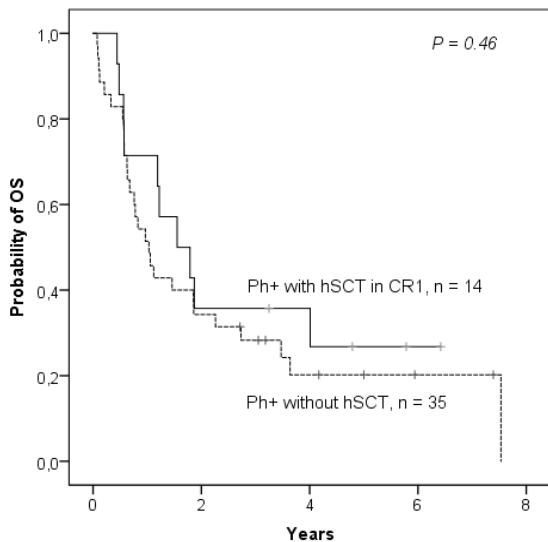
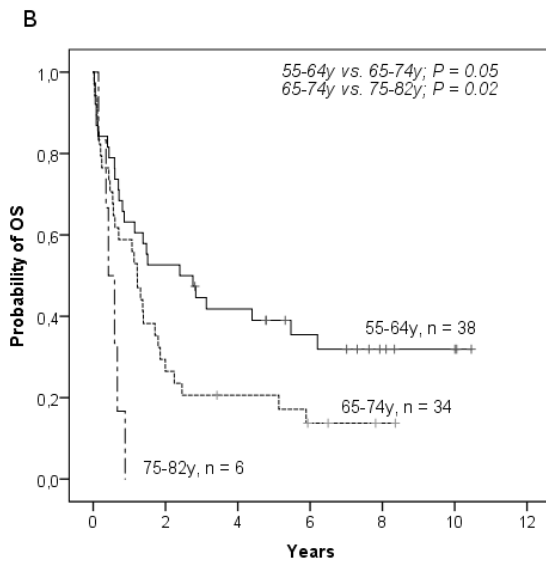
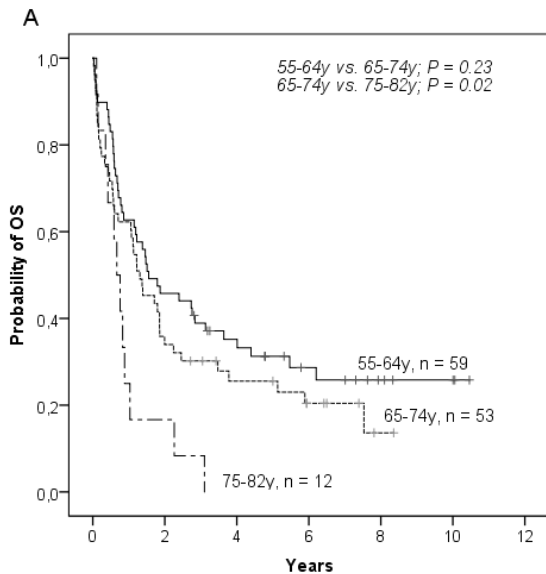


Figure 3. Overall survival in intensively treated patients with B- or T-ALL according to age group in whole cohort (A), Ph- (B), and Ph+ (C) disease.



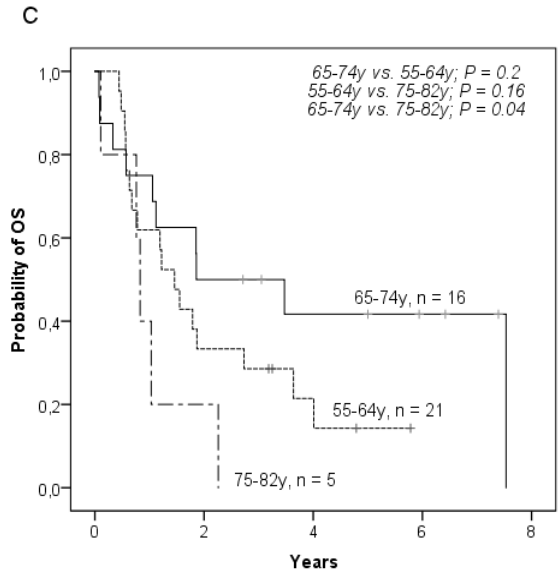


Figure 4. Overall survival in youngest age group (55-64y) according to sex.

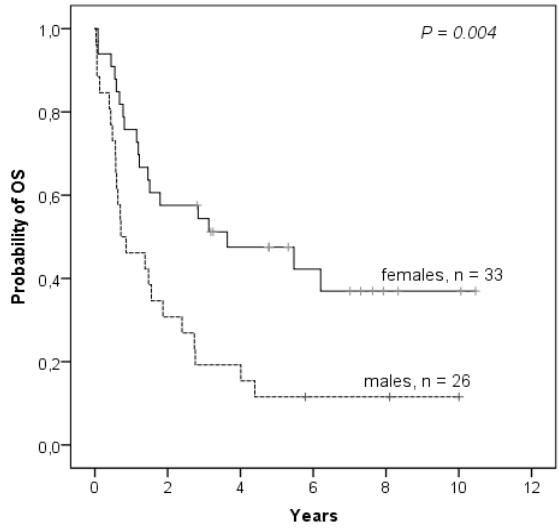


Table S1 (supplemental). National guidelines for treatment of older/elderly ALL patients in Sweden.

Phenotype	Until year 2009	After year 2009		
	All eligible adults	Biological age 45-60y	Biological age 60-75y or younger with comorbidities	Biological age >75y or younger with serious comorbidities
B-ALL Ph-	ABCDV	ABCDV	EWALL backbone	Reduced CHOP (75%), VAD, Vincristine+ steroids
B-ALL Ph+	ABCDV (+imatinib*)	ABCDV +imatinib	EWALL backbone+ imatinib	Imatinib + steroids
T-ALL	Hyper-CVAD	ABCDV	EWALL	Reduced CHOP (75%), VAD, Vincristine+ steroids

*imatinib as standard treatment for Ph+ disease was introduced 2007

Table S2 (supplemental). Protocol specifications.

ABCDV +/- Imatinib			EWALL backbone +/- Imatinib		
Drug	Dose	Days	Drug	Dose	Days
<i>Imatinib in Ph+ALL</i>	<i>600 mg o.d.</i>	<i>continuously</i>	<i>Imatinib in Ph+ ALL</i>	<i>600 mg o.d.</i>	<i>continuously</i>
Pre-phase (optional)					
Prednisolone	60 mg/m ² oral	-5-0			
Cyclophosphamide	200 mg/m ² i.v.	-5-0			
Remission induction (ABCDV)			Remission induction I (d 1-16) and II (d 20-34)		
Methotrexate	10 mg/m ² i.t. (max 15 mg)	0	Methotrexate	12 mg i.t.	1
Ara-C (cytarabine)	3 g/m ² b.i.d. i.v.	1-3	Dexamethasone	10 mg/m ² oral	1-7, 13-16
Betamethasone	20 mg/m ² oral	1-5	Vincristine	1 mg i.v.	6, 13
Cyclophosphamide	600 mg/m ² i.v.	1	Idarubicin	10 mg i.v.	6, 7, 13, 14
Daunorubicin	30 mg/m ² i.v.	1-3	Ara-C (cytarabine)	60 mg/m ² i.v.	21-24, 28-31
Vincristine	2 mg i.v.	1	Cyclophosphamide	300 mg/m ² i.v.	20-22
			Methotrexate/Ara-C/prednisolone	12/40/12.5 mg i.t.	12, 20, 27, 34
			G-CSF	5 µg/kg s.c.	6-, 20-
Consolidation 1 or 2:nd induction (VABA)			Consolidation 1, 3, 5		
Vincristine	2 mg i.v.	1	Methotrexate**	1000 mg/m ² i.v.	1
Amsacrine	200 mg/m ² i.v.*	1-3	Asparaginase**	10 000 E/m ² i.v. or i.m.	2
Betamethasone	20 mg/m ² oral	1-5			
Ara-C (cytarabine)	3 g/m ² i.v.*	1-4			
Consolidation 2 (BCDE)			Consolidation 2, 4, 6		
Betamethasone	20 mg/m ² oral	1-5	Ara-C (cytarabine)	1000 mg/m ² i.v.	1, 3, 5
Cyclophosphamide	1000 mg/m ² i.v.	1			
Daunorubicin	30 mg/m ² i.v.	1-2			
Etoposide	100 mg/m ² i.v.	1-5			
Consolidation (mini-VABA)					
Vincristine	2 mg i.v.	1			
Amsacrine	200 mg/m ² i.v.	1-2			
Betamethasone	20 mg/m ² oral	1-5			
Ara-C (cytarabine)	3 g/m ² i.v.	1-3			
Consolidation (MAP)***					
Methotrexate	1500 mg/m ² i.v.	1, 15			
PEG-Asparaginase	1000 E/m ² i.v.	2, 16			
Mercaptopurine	60 mg/m ² oral	1-21			

Maintenance in 2 y from last consolidation			Maintenance in 2 y from start of induction		
Mercaptopurine****	50-75 mg/m ² o.d.	continuously	Mercaptopurine****	50-75 mg/m ² o.d.	continuously
Methotrexate****	5-10 mg/m ² oral, once weekly	continuously	Methotrexate****	5-10 mg/m ² oral once weekly	continuously
Reinduction course- every 2:nd (1:st y) and every 3:rd (2:nd y) month during maintenance:			Reinduction course- every 2:nd (1:st y) and every 3:rd (2:nd y) month during maintenance:		
Daunorubicin (1:st y)	40 mg/m ² i.v.	1	Vincristine	1 mg i.v.	1
Vincristine (1:st y)	2 mg i.v.	1	Dexamethasone	40 mg oral	1-2
Prednisolone	60 mg/m ² oral	1-7 (1:st y) 1-5 (2:nd y)			
Ara-C (2:nd y)	60 mg/m ² s.c.	1-5			
Thioguanine (2:nd y)	80 mg/m ² oral	1-5			

*For patients >70 years of age, amsacrine is given d 1-2 and Ara-C d 1-3

** 50% methotrexate and asparaginase dose reduction in patients aged >70 y. Asparaginase omitted in Ph+ALL.

***Only for high-risk patients

****omitted in Ph+ ALL

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