Acute Lymphoblastic Leukaemia in Adult Patients

Studies of Prognostic Factors, Treatment Results and in vitro Cellular Drug Resistance

HELENE HALLBÖÖK

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 30

ISSN 1651-6206
ISBN 91-554-6229-4
urn:nbn:se:uu:diva-5768
Dissertation presented at Uppsala University to be publicly examined in Enghoffsalen, Akademiska sjukhuset, Ingång 50, Uppsala, Thursday, May 19, 2005 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

**Abstract**


Treatment results and clinical characteristics in adult acute lymphoblastic leukaemia (ALL) were evaluated regarding three issues: a new treatment with cytarabine up-front, stem cell transplantation and a comparison between adult and paediatric treatment protocols. All studies were conducted on a national basis. Furthermore, activity of imatinib was investigated by in vitro cytotoxicity assay.

The national protocol was evaluated in 153 adult ALL patients. A high complete remission rate, 86%, was achieved with 29% overall survival at 3-years. Favourable outcome was identified in patients < 40 years with precursor B phenotype and continuous complete remission was higher for precursor B compared to T-ALL.

Stem cell transplantation was evaluated in 187 patients. No differences in outcome between allogeneic and autologous transplantation were found, with the exception of Philadelphia-positive ALL, in which allogeneic transplantation was preferable. Limited chronic graft-versus-host disease (compared to none) resulted in superior disease free survival.

The paediatric NOPHO-92 and the Adult protocols were evaluated for 243 ALL-patients. Superior remission rate and survival were achieved for 10-18 year-olds treated according to the Paediatric protocol compared to both 15-25 and 25-40 year-olds treated according to the Adult protocol. Treatment protocol was a significant prognostic factor for patients aged 15-20 years.

Fluorometric Microculture Cytotoxicity Assay was used to analyze 15 tumour cell samples from ALL patients. High concordance was determined between in vitro sensitivity to imatinib and presence of BCR-ABL. Daunorubicin, prednisolone and cytarabine had the greatest benefit from a combination with imatinib.

The national adult treatment protocol’s results were consistent with international trials regarding precursor B ALL but may be under performing for T-ALL. Adolescents may benefit from treatment according to the Paediatric protocol. No difference in outcome between allogeneic and autologous stem cell transplantation was determined except for Philadelphia-positive patients, despite the indication of a graft-versus-leukaemia effect.

**Keywords:** acute lymphoblastic leukaemia, adult, adolescent, chemotherapy, stem cell transplantation, in vitro assay, imatinib

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ISSN 1651-6206
ISBN 91-554-6229-4
urn:nbn:se:uu:diva-5768 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-5768)
To my family
List of Papers

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


II H Hallböök, H Hägglund, D Stockelberg, P-G Nilsson, K Karlsson, M Björkholm, M Linderholm, A Wahlin, O Linder, B Smedmyr for the Swedish Adult ALL Group. Autologous and allogeneic stem cell transplantation in adult ALL. The Swedish Adult ALL Group experience. Accepted for publication in Bone Marrow Transplantation.

III H Hallböök, G Gustafsson, B Smedmyr, S Söderhäll and M Heyman. For the Swedish Adult ALL group and the Swedish Childhood Leukemia group. Treatment outcome of children over 10 years and young adults in Sweden: A comparison between paediatric and adult protocols. Manuscript.

IV H Hallböök, G Barbany, A Åleskog, A Björnberg, R Larsson, C Sundström, E Lindhagen. In vitro activity of imatinib in cells from patients with adult acute lymphoblastic leukemia. Accepted for publication in Anti-Cancer Drugs.

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<td>ABL</td>
<td>non receptor tyrosine kinase Abelson</td>
</tr>
<tr>
<td>AML</td>
<td>acute myeloid leukaemia</td>
</tr>
<tr>
<td>ALL</td>
<td>acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td>Ara-C</td>
<td>cytarabine</td>
</tr>
<tr>
<td>ARDS</td>
<td>adult respiratory distress syndrome</td>
</tr>
<tr>
<td>AUL</td>
<td>acute undifferentiated leukaemia</td>
</tr>
<tr>
<td>BCR</td>
<td>breakpoint cluster region</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>fusion gene BCR-ABL</td>
</tr>
<tr>
<td>BFM</td>
<td>Berliner-Frankfurt-Munster</td>
</tr>
<tr>
<td>BGMT</td>
<td>Bordeaux-Grenoble-Marseille-Toulouse cooperative group</td>
</tr>
<tr>
<td>CI</td>
<td>confidence intervals</td>
</tr>
<tr>
<td>CML</td>
<td>chronic myeloid leukaemia</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CR</td>
<td>complete remission</td>
</tr>
<tr>
<td>CCR</td>
<td>continuous complete remission</td>
</tr>
<tr>
<td>DFS</td>
<td>disease free survival</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimetyl sulphoxide</td>
</tr>
<tr>
<td>EBMT</td>
<td>European Cooperative Group for Blood and Marrow Transplantation</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>EFS</td>
<td>event free survival</td>
</tr>
<tr>
<td>FDA</td>
<td>fluorescein diacetate</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescent in situ hybridisation</td>
</tr>
<tr>
<td>FMCA</td>
<td>fluormetric microculture cytotoxic assay</td>
</tr>
<tr>
<td>FRALLE</td>
<td>French Acute Lymphoblastic Leukemia Study Group</td>
</tr>
<tr>
<td>GAPDH</td>
<td>glyceraldehyde-3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>GMALL</td>
<td>German Multicenter Study for Adult ALL</td>
</tr>
<tr>
<td>GVHD</td>
<td>graft-versus-host disease</td>
</tr>
<tr>
<td>GVL</td>
<td>graft versus leukaemia</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>i.t.</td>
<td>intrathecal</td>
</tr>
<tr>
<td>LALA</td>
<td>Leucémie Aigue Lymphoblastique de l’Adulte</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>MRD</td>
<td>minimal residual disease</td>
</tr>
<tr>
<td>mRNA</td>
<td>messengerRNA (RiboNucleicAcid)</td>
</tr>
</tbody>
</table>
NHL  non-Hodgkin’s lymphoma
NOPHO  The Nordic Society of Paediatric Haematology and Oncology
OS  overall survival
PBS  phosphate buffered saline
PCR  polymerase chain reaction
PETHEMA  Program for the Study and Treatment of Malignant Hemopathies, Spanish Society of Hematology
Ph  Philadelphia chromosome
RD  related donor
SCT  stem cell transplantation
URD  unrelated donor
WBC  white blood cell
QR-PCR  quantitative real-time reverse transcriptase PCR
Introduction

Acute lymphoblastic leukaemia (ALL) is a disease originating in the bone marrow. The proliferating malignant clone expands and forces the normal haematopoietic cells aside. Without treatment, the disease is fatal.

Therapy for childhood ALL has developed rapidly; fifty years ago almost all patients with ALL died of the disease, whereas today approximately 75% of the children are cured (1-3). Unfortunately, adult ALL has not developed at the same rate and despite high remissions rates approximately 30% of the adults are cured (4-10). Relapse remains as the main cause of death. The characteristics of the disease in adults have been surveyed and a differentiation of the treatment has been proposed, regarding chemotherapy, stem cell transplantation and recently also regarding signal transduction modulators.

Adult ALL is a rare disease and approximately 50 patients are diagnosed each year in Sweden. In order to give equal treatment over the whole country and evaluate outcome and adverse events there has been national treatment protocols for adult ALL since 1986. A national group is responsible for organizing treatment protocols and performing evaluations. Three of the papers from this thesis are written on behalf of this national group, the "The Swedish Adult ALL group", and would otherwise not have been possible to realize.

Acute lymphoblastic leukaemia (ALL)

History

The word leukaemia, derived from the Greek words leukos – white and haima – blood, means white blood. In 1845 and only a few weeks apart, both John Hughes Bennet and Rudolph Virchow published papers describing patients with leukaemia. However, a French physician, Alfred Donné had already in 1844 written a book in which he described that “several cases exist with a great excess of white blood cells.” and poised a hypothesis that “the overabundance of white blood cells should be the result of an arrest of development of intermediate cells” (11). Ernst Neuman (12) described acute leukaemia in 1868, and in 1872 he stated that leukaemia was a disease of the bone marrow. The development of staining slides to differentiate between different types of blood cells (Erlich 1877 and 1880) (12), enabled the classi-
fication of leukaemia into two groups; a myeloid and lymphoid. Modern chemotherapy was first introduced for patients with ALL by Franklin in 1947 (folic acid antagonists) followed by introduction of corticosteroids (Paerson 1949 and Farber 1950) and 6-mercaptopurin by Elion 1951 (12). These chemotherapy agents are still important in modern ALL treatment.

Clinical presentation
The expansion of the leukaemic clone results in a depressed production of the normal haematopoesis. Symptoms such as fatigue and dyspnea due to anaemia, petechiae and ecchymoses due to thrombocytopenia, and infections due to granulocytopenia are common. Hepatosplenomegaly may occur but is rarely the cause of symptoms. Central nervous system (CNS) or extradural nerve involvement occurs in 5-10% of patients at diagnosis (13). Other sites can also infrequently be involved such as testicles, ovary, pericardium, pleura and skin. Extremity and joint pain are present in approximately every second patient with adult ALL (unpublished data from the Swedish Adult ALL Group). Lymphadenopathy is common and mediastinal lymph nodes can, particularly in T-lineage ALL, give rise to a mediastinal mass that infrequently causes a vena cava superior syndrome (14). Mature B-ALL (Burkitt’s lymphoma/leukaemia) is characterised by more frequent CNS-involvement and large tumour mass with lymph node and organ involvement. Thereby, at the start of treatment, these patients have a higher risk of tumour lysis syndrome (15). High white blood cell count (WBC) at diagnosis (>100 x 10^9/l) rarely leads to leukostasis syndrome, associated with respiratory failure and intracranial bleeding (16).

Epidemiology and aetiology
Acute leukaemia is a rare disease with an incidence in Sweden of 5.5 per 100 000 inhabitants per year in adults. ALL is more common in childhood but occurs in all ages and constitutes 13% of acute leukaemia in adults, with a male predominance (17).

Chemicals and ionising radiation
For the majority of patients, the aetiology of ALL is unknown. For both childhood and adult ALL, exposure to cigarette smoke and petrochemicals, such as benzene, has been suggested as risk factors, but recent studies (18, 19) show a significant connection with AML rather than with ALL. Ionising radiation, especially in high doses and with acute exposure has been implicated as a risk factor in leukaemogenesis (20). In The Ukraine after the Chernobyl accident, the risk ratio was increased three-fold for ALL in children exposed to radiation in utero (21). Similar studies have not disclosed any increased risk for childhood ALL in Sweden after the reactor accident (22).
Infections

There is no profound evidence that infections may be the cause of adult ALL; however, infections may be co-factors for diseases related with ALL. Human T cell lymphotropic virus type-1 (HTLV1) is endemic in Japan, Taiwan and the Caribbean Islands and is strongly associated with an increased incidence of Adult T cell leukaemia/lymphoma (23, 24). The combination of Epstein-Barr virus (EBV) and malaria are probably cofactors for endemic Burkitt lymphoma, and a third cofactor, such as arboviruses, has been discussed as responsible for the clusters of cases in the "lymphoma belt" of Africa (25).

Genetic factors and other special considerations in childhood ALL

There is evidence to suggest a genetic factor in a few cases, as individuals with, for example, Down syndrome have an increased incidence of ALL (26). Recent studies (27, 28) have suggested that a part of childhood ALL arises in utero, as leukaemic clones have been detected retrospectively in the patients’ new-born screening ('Guthrie') cards; however, whether the clones detected at birth represent the malign phenotype or are a preleukaemic phenotype that needs a second genetic event to develop the disease is unknown. Reported concordance rate for childhood ALL in twins (approximately 10% excluding infant ALL) may be due to an initiation of leukaemia in one twin foetus and a clonal progeny spread to the co-twin via vascular anastomoses within the (single) placenta (27).

An infectious aetiology has also been proposed, epidemiological studies have implied that maternal infections during pregnancy are associated with increased risk of ALL (29, 30). Different childhood infections has been suggested both protective and giving an increased risk of ALL in different studies (31) and the evidence is at this point not conclusive. There is no current evidence that adult ALL has a similar aetiology as proposed for some childhood ALL.

Diagnostic criteria and classification

The diagnosis of ALL is based on morphology, cytochemistry and immunophenotyping as proposed by the World Heath Organisation 2001 (32). The French-American–British group classifies ALL, based on morphology, into three subgroups: L1, L2 and L3. This classification is of limited value for recognising prognostically important subsets and instead a classification based on immunophenotyping is used (32-34). Precursor B ALL can be divided into at least three subgroups, depending on there degree of differentiation: early precursor (pro) B-ALL, common B-ALL and preB-ALL. Precursor T-ALL (T-ALL) can also be divided into subgroups but this is neither described by the WHO-classification nor generally used in Sweden. Mature B-ALL is characterised by morphology (L3 according to the French-
American–British classification) and immunophenotype, including membrane light chain restricted immunoglobulin, and is now classified as Burkitt lymphoma (32).

A few cases of ALL can express mixed myeloid-lymphoid lineage markers in the same population, and is called biphenotypic leukaemia. The leukaemia that does not show differentiation in cytochemistry or immunophenotyping is called acute undifferentiated leukaemia (AUL). This is a rare disease that have until now been treated as ALL in Sweden.

Cytogenetics

Clonal chromosomal abnormalities are found in approximately 85% of all adult ALL cases (35). The most common structural change is the Philadelphia chromosome (Ph), t(9,22)(q34;q11) which is present in 20-30% of cases (35, 36). The translocation results in either a p190 or a p210 Bcr-Abl fusion protein, which can be detected by fluorescent in situ hybridisation (FISH) or polymerase chain reaction (PCR) technique. Present in only 4% of the cases, t(4,11) is a rare but important structural change and together with BCR-ABL are two of the most important adverse prognostic factors for survival in adult ALL (35).

There are differences in the presence of chromosomal abnormalities between childhood and adult ALL (37). The incidence of Ph-positive ALL is only 3% in childhood ALL; hyperdiploidy (>50 chromosomes) is common in childhood ALL (25% versus 7% in adult ALL) as well as t(12,21) (22% in childhood versus 2% in adult ALL).

Prognostic factors

Prognostic systems have been developed, including diagnostic and clinical features at diagnosis and after induction therapy, and are used for predicting durability of remissions and as support for differentiation of the treatment. The German Study Group for Adult ALL (GMAILL) trials (38, 39) has been important in the development of the models. Several models with slight variations exist (13). Late achievement of remission (defined as 3-4 weeks from the start of treatment to remission or more than one course to achieve remission); an elevated white blood cell (WBC) count over 30x 10^9/l; and high age (above 35 years) (39) are together with BCR-ABL and t(4,11) (35, 38) recognized as adverse prognostic factors on diagnosis. The presence of CNS leukaemia has been debated as a high risk factor for relapse, but is not included in many of the current prognostic systems (13).

Age is recognised as an important risk factor, not only in adult but also in childhood ALL. The Medical Research Council (MRC) trials UKALL X (for children) and Xa (for adults) (40) consisted of a similar treatment for both childhood and adult ALL. The survival strongly correlated with age, with the
highest survival rates for children aged 1-9 years and the lowest for adults aged over 40 years.

The impact of immunophenotype regarding outcome has shifted over time. T-ALL and mature B-cell were previously adverse prognostic factors but have with modern treatment become favourable immunophenotypes regarding risk for relapse in adult ALL (13, 41). However, for T-ALL, the GMALL-group has proposed that patients with WBC over 100x 10^9/l, late remission or early/mature T immunophenotype should still be considered as a high risk group (42).

Minimal residual disease (MRD)

Late achievement of remission is recognised as a high risk factor (39). A normocellular bone marrow with less than 5% blast is commonly used as one criterion for complete remission (CR) but has not been able to account for low amounts of remaining leukaemic cells. Modern techniques have opened new possibilities both regarding evaluation of the quality of remission and measurement of the leukaemic tumour burden during the following treatment. The detection and quantification of the residual tumour burden is called minimal residual disease (MRD). There are two predominant MRD techniques: flow cytometric immunophenotyping, based on occurrence of leukaemia-associated immunophenotypes; and quantitative real time (QR) PCR based on tumour specific sequences in junctional regions of rearranged immunoglobulins or T-cell receptor genes. The sensitivity level is 10^{-3} - 10^{-6} for both methods (43). One drawback is the difficulty in identifying markers for QR-PCR and immunphenotyping and that ongoing and secondary rearrangements within the leukaemic clone may cause false-negative results (43).

There are experience of both flow cytometric immunophenotyping and QR-PCR MRD in childhood ALL and early tests appear important for predicting durability of remissions (44). In addition, MRD was found to be an independent risk factor in multivariate analysis (44, 45). In adult ALL, the experience is not as extensive. Later time points (after 3-9 months) are important for predicting durability of remissions or relapse (46, 47). In the GMALL study 06/99 (38), MRD at the level 10^{-4} (measured by QR-PCR) was used as a risk factor for a second stratification after one year of therapy. For MRD-low risk patients, therapy ceased after one year and the relapse risk in this cohort was approximately 20%. Due to early relapses during the first year's therapy, only 10% of the MRD-high risk patients were identified after one year and consequently the planned therapy-changes, stem cell transplantation (SCT), could not be carried out to full extent. Therefore, the time point for stratification due to MRD is performed earlier (month 4) in the current GMALL study. It remains to be proven if intervention with, for example, SCT can change the prognosis when MRD is positive.
Treatment of adult ALL

Chemotherapy

The treatment of adults with ALL is based on chemotherapy and is usually divided into an induction, consolidation and maintenance phase. Intensive cyclic systemic chemotherapy and CNS prophylactics are used in induction and consolidation therapy. The CNS prophylactics usually consist of intrathecal chemotherapy (mainly methotrexate), combined with CNS irradiation in some protocols.

The chemotherapy drugs frequently used in therapy are a combination of prednisolone and vincristine together with an anthracycline. Most current protocols constitute additional chemotherapy agents in both the induction course and the following courses, for example asparaginase, cytarabine, cyclophosphamide, etoposide, and methotrexate. As maintenance therapy, a two-year chemotherapy treatment usually with 6-mercaptopurin and methotrexate, together with reinduction courses are given and trials omitting the maintenance phase have shown inferior results for precursor B and T-ALL (48, 49). There is no current consensus regarding chemotherapy concepts for adult patients with ALL. A broad variety of protocols exists both concerning intensity and duration of therapy (50). For example, the Swedish adult ALL protocols are shown in Table 1. The 1986-1993 protocol exemplifies a 'standard' protocol including asparaginase (Table 1a) and the 1994-2000 is a more intensive protocol with high-dose Ara-C (Table 1b). Both protocols were followed by a 2-year maintenance therapy (Table 1c). Based on the START methodology for evidence based medicine, Bassan et al (13) have reviewed the current knowledge and evidence regarding chemotherapy.

Table 1. The Swedish Adult ALL Group’s treatment protocols.

Table 1a. 1986-1993

<table>
<thead>
<tr>
<th>Treatment phase/drug</th>
<th>Days given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission induction</td>
<td></td>
</tr>
<tr>
<td>Methotrexate 10 mg/m² i.t. (max 15 mg)</td>
<td>1</td>
</tr>
<tr>
<td>Vincristine 2 mg i.v.</td>
<td>1, 8, 15, 22</td>
</tr>
<tr>
<td>Daunorubicin 30 mg/m² i.v.</td>
<td>1, 2, 15, 16</td>
</tr>
<tr>
<td>Cyclophosphamide 600 mg/m² i.v.</td>
<td>1</td>
</tr>
<tr>
<td>L-Asparaginase 15 000 E/m² i.v.</td>
<td>15-28</td>
</tr>
<tr>
<td>Prednisone 60mg/ m² oral</td>
<td>1-28</td>
</tr>
</tbody>
</table>

Consolidation 1 and 2*

<table>
<thead>
<tr>
<th>Treatment phase/drug</th>
<th>Days given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine 2 mg i.v.</td>
<td>1</td>
</tr>
<tr>
<td>Daunorubicin 30 mg/m² i.v.</td>
<td>1</td>
</tr>
<tr>
<td>Etoposide 100 mg/m² i.v.</td>
<td>1-5</td>
</tr>
<tr>
<td>Ara-C 100 mg/m² i.v. twice daily</td>
<td>1-5</td>
</tr>
<tr>
<td>Prednisone 60mg/ m² oral</td>
<td>1-5</td>
</tr>
</tbody>
</table>

*The 2nd consolidation was only given to high risk patients.
### Table 1b. 1994-2000

<table>
<thead>
<tr>
<th>Treatment phase/drug</th>
<th>Days given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remission induction</td>
<td></td>
</tr>
<tr>
<td>Methotrexate 10 mg/m² i.t. (max 15 mg)</td>
<td>1</td>
</tr>
<tr>
<td>Cyclophosphamide 600 mg/m² i.v.</td>
<td>1</td>
</tr>
<tr>
<td>Vincristine 2 mg i.v.</td>
<td>1</td>
</tr>
<tr>
<td>Daunorubicin 30 mg/m² i.v.</td>
<td>1-3</td>
</tr>
<tr>
<td>Ara-C 3 g/m² i.v. twice daily</td>
<td>1-3</td>
</tr>
<tr>
<td>Betamethasone 20 mg/m² oral</td>
<td>1-5</td>
</tr>
</tbody>
</table>

| Consolidation 1 or 2<sup>nd</sup> induction   |            |
| Vincristine 2 mg i.v.                         | 1          |
| Amsacrine 200 mg/m² i.v.                     | 1-3        |
| Ara-C 3 g/m² i.v.                            | 1-4        |
| Betamethasone 20 mg/m² oral                   | 1-5        |

| Consolidation 2                               |            |
| Cyclophosphamide 1000 mg/m² i.v.              | 1          |
| Daunorubicin 30 mg/m² i.v.                    | 1-2        |
| Etoposide 100 mg/m² i.v.                      | 1-5        |
| Betamethasone 20 mg/m² oral                   | 1-5        |

| Consolidation*                                |            |
| Vincristine 2 mg i.v.                         | 1          |
| Amsacrine 200 mg/m² i.v.                     | 1-2        |
| Ara-C 3 g/m² i.v.                            | 1-3        |
| Betamethasone 20 mg/m² oral                   | 1-5        |

*The course was only given if 2<sup>nd</sup> induction was required.

### Table 1c. Maintenance therapy 1986-1993 and 1994-2000

<table>
<thead>
<tr>
<th>Treatment phase/drug</th>
<th>Days given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance therapy</td>
<td>2 years</td>
</tr>
<tr>
<td>6-Mercaptopurine 50-75 mg/m² oral</td>
<td>daily</td>
</tr>
<tr>
<td>Methotrexate 5-10 mg/m² oral</td>
<td>once weekly</td>
</tr>
</tbody>
</table>

| First year                                    |            |
| Reinduction: every second month               |            |
| Daunorubicin 40 mg/m² i.v.                    | 1          |
| Vincristine 2 mg i.v.                         | 1          |
| Prednisolone 60 mg/m² oral                    | 1-7        |

| Second year                                   |            |
| Reinduction: every third month                |            |
| Ara-C 60 mg/m² s.c                            | 1-5        |
| Thioguanine 80 mg/m² oral                     | 1-5        |
| Prednisone 60 mg/m² oral                      | 1-5        |
Stem cell transplantation

SCT is an important alternative consolidation therapy. Even though several studies have discussed transplantation versus chemotherapy, the optimal strategy when to recommend an allogeneic or autologous SCT is still unclear regarding age, remission status and risk factors. The strength of graft versus leukaemia (GVL) has been an issue in ALL. In the LALA-87 and LALA-94 trials, allogeneic SCT, autologous SCT and chemotherapy in first remission are compared. A favourable outcome is found for allogeneic SCT in high-risk patients, but no significant difference between autologous SCT and chemotherapy is revealed (4, 51, 52). The International Bone Marrow Transplant Registry do not find any differences between related donor SCT and chemotherapy in first remission (53, 54) nor do the PETHEMA, group find any differences in outcome between chemotherapy, allogeneic SCT or autologous SCT for high-risk patients (55). In a report from the BGMT group, a higher DFS were determined in SCT with HLA-identical sibling versus autologous SCT (56). In the ongoing international trial (MRC UKALL XII/ ECOG) Ph-negative patients with a histocompatible donor received an allogeneic SCT whereas the remaining patients were randomised to autologous SCT or maintenance therapy. Preliminary results suggest that an allogeneic SCT is beneficial for all risk groups in first CR (57). Further results from this large study will also provide information of the outcome for autologous SCT compared to chemotherapy. For the Ph-positive patients, superior DFS has been determined for allogeneic SCT compared to autologous SCT or chemotherapy (58, 59) and is recommended for all suitable patients in first remission.

Treatment of adolescents

Adolescents, aged 15-20 years, can be treated either in paediatric unit according to paediatric protocols or in adult haematological unit according to adult protocols. In Sweden, as in many other counties, the initial referral decides where the patients will be treated. Three principal studies have compared the outcome for adolescents treated according to either paediatric or adult protocol: one French study compare the paediatric FRALLE-93 versus the adult LALA -94 trials (60); one from The Netherlands compare the Dutch Childhood Oncology Group, DCOG and Dutch-Belgian Hemato-Oncology Group, HOVON protocols (61); and one from the US compare Children’s Cancer Group, CCG and Cancer and Leukemia Group B, CALGB regimens (62). All three comparisons determined a superior EFS for patients treated according to paediatric versus adult protocols. The treatment results are similar with 5 (or 6) year EFS for the paediatric protocols 64-69% versus 34 -41% for the adult protocols. These studies initiated a discussion as to whether adolescents and young adults should be treated according to paediatric or paediatric inspired protocols.
Treatment of elderly patients

Patients over 60 years have a higher incidence of coexistent diseases that may interfere with intensive treatment by chemotherapy. The frequency of non-leukaemic deaths during induction is higher than for younger patients (63). Although a lower frequency of T-ALL has been reported (64, 65), it is unclear if the characteristics of adult ALL change with increasing age.

Many treatment studies exclude patients over the age of 60-65 years (4-6, 10, 66), even though patients over 60 years of age constitute approximately 30% of all adult patients with ALL (65). The treatment studies for this age group, have been have been examined in a review by Pagano et al (67). The number of patients in each study has not exceeded 80 patients and an overall response rate of 36-85% has been reported. Median overall survival ranged from 3-14 months (or expressed as 2 or 3 year OS 10-19%) (67). In the population based study by Taylor et al (65), 49 patients were diagnosed with ALL and 19 received a curative intending treatment. The OS at 5 years was 4% for the whole group. Even though the attitude towards intensive chemotherapy for elderly patients may change over time, Taylor et al’s study implies that the selection of patients regarding age, performance status and concomitant diseases ought to be of importance when comparing different studies for this age group. The new therapeutic options (as described below) that are becoming available for Ph-positive ALL may have an impact on prognosis for some elderly patients.

New therapeutic options

Signal transduction modulators

Imatinib (Imatinib mesylate, STI 571, Glivec) is a competitive inhibitor of the Bcr-Abl protein tyrosine kinase that is currently used for the treatment of chronic myeloid leukaemia (CML) in chronic phase, but has also shown initial response in the treatment of Ph-positive ALL. For Ph-positive ALL the remissions achieved with imatinib alone have been short (68) due to development of resistance. Therefore combinations of imatinib with chemotherapy, imatinib as maintenance therapy, or imatinib together with SCT is currently discussed in Ph-positive ALL and is the subject of trials.
A “second generation” of tyrosine kinase inhibitors are under development and these compounds may be able to overcome some of the poor responses in ALL. The BMS-354825 is a dual Src and Abl kinase inhibitor (69) that has reached clinical trials. The compound is more potent than imatinib in inhibiting non-mutated BCR-ABL kinase activity in vitro, and can also inhibit 14 out of 15 clinically important, imatinib-resistant mutated-BCR-ABL variants (69). There are several other signal transduction modulators, but no compound has yet reached clinical practice in ALL. This group of agents could in the future provide an important development in treatment, possible not only with respect to BCR-ABL-positive ALL.

**Monoclonal antibodies**

CD 20-antigen expression (defined as expression in more than 20% of the blast cells) are present in approximately 80-90% of the Mature B-ALL (Burkitt Leukemia/Lymphoma) and 40% of the precursor B-ALL (70). A trial (71) incorporating the monoclonal CD 20-antibody Rituximab with hyper CVAD-regimen for 20 patients with Burkitt (or Burkitt-like) leukae-mia/lymphoma indicate acceptable toxicity: the survival rates appear promising but the observation time was, at time of publication, short. Further trials are warranted both for Burkitt lymphoma and for precursor B--ALL.

CD 19-antigen is highly expressed in B-lineage ALL and monoclonal CD-19 antibodies, some conjugated with immunotoxins, have been developed. They are not clinically available and their effect and role is undetermined (70). CD 52-antigen is expressed in approximately 70% of B and T-lineage ALL, with predominance for T-ALL. There is to date no data that
single therapy with monoclonal CD-52 antibodies has a substantial effect in ALL (72) and further trials, including combination therapy, are needed.

Monoclonal antibodies targeting CD 22 (expressed in less than 20% of precursor B-ALL), CD 25 (interleukin-2 receptor) and CD 33 (expressed in some of the early subtypes where myeloid antigens are present) are available and may be a part of future studies in ALL.

In vitro cytotoxicity assays

As part of the cytotoxic drug development, in vitro cytotoxicity assays can indicate sensitivity to the drugs in tumour cells. The effect of survival or proliferation is examined. There are two main groups of in vitro assays: total cell kill assays; and cell proliferation assays.

In vitro cytotoxic assays can be used in different ways. The assays can be used in preclinical development of new cytotoxic agents to evaluate drug sensitivity pattern for groups of patients with different diagnoses (73) or subgroups of diagnoses (for example genetic aberrations (74)). Combinations of cytotoxic agents can be evaluated regarding antagonistic or synergistic effects and can be the basis of future phase I and phase II trials. For the individual patient, the assay can provide prognostic information (75) or evaluation of drug sensitivity, and thereby serve as a guide in the choice of treatment, for example in salvage treatment. Either fresh or cryopreserved human tumour cell samples or tumour cell lines can be used. The human tumour cell samples may predict clinically relevant drug activity patterns better than tumour cell lines (76), whereas the latter provides practically unlimited samples for facilitating multiple tests.
Aims for the doctoral project

- To evaluate the results of the national treatment protocol for adult ALL used in Sweden between 1994-1998 and in particular regarding adverse events, survival and risk factors including age.

- To evaluate treatment results and complications for SCT in adult ALL, with special focus on differences between allogeneic and autologous transplantation and the effect of GVL.

- To compare clinical characteristics and treatment for the age groups 10-14 years, 15-20 years and older young adults (20-40 years). To investigate the outcome for these age groups regarding treatment protocol and treatment unit (paediatric protocol and units and adult haematology protocol and units, respectively).

- To investigate the in vitro activity of imatinib on BCR-ABL-positive and -negative tumour cells from adult ALL patients and to determine if the combination of imatinib with conventional agents used in ALL treatment could enhance or reduce activity of those agents in vitro.
Material and Methods

Papers I-III

Classification and diagnostic criteria of ALL

In the papers included in this thesis, the patients were diagnosed with ALL before the introduction of the WHO-classification in 2001. Diagnosis was based on morphology (with more than 30% blasts in the bone marrow), cytochemistry and immunophenotyping. No sub classification of Precursor B- and T-ALL were used. Precursor B-ALL was called “preB-ALL” in Papers I, II and IV and “B precursor ALL” in Paper III. Diagnostic and cytogenetic analyses were performed at each centre.

Adult national protocols and definition of high risk for relapse

In Sweden, patients with ALL and AUL were treated according to uniform national protocols between 1986-1993 and 1994-2000 and were reported to a national register during the later period. The 1986 –1993 protocol was based on asparaginase, corticosteroids and vincristine (Table 1a) and was combined with CNS-irradiation (if a SCT was not intended). The 1994-2000 protocol (for precursor B, T-ALL and AUL) aimed at providing a more intensive early phase of treatment with high-dose Ara-C upfront and without asparaginase or CNS irradiation (Table 1b). In both periods, a two-year maintenance therapy phase was recommended for standard risk patients. For the patients with high-risk leukaemia, allogeneic SCT was recommended (in the first period mainly for patients younger than 45 years) and autologous SCT was recommended for biologically older patients or if a suitable donor could not be identified. The patients were classified as having high risk of relapse if one or more of the following characteristics were present: WBC > 30 x 10^9/l; CNS leukaemia; Ph chromosome; late remission (more than one course to remission); or AUL. In the 1986-1993 protocol, mature B-ALL phenotype was included in the high risk criteria and in the 1994-1998 protocol t(4,11) was considered as a high risk criterion.

Patients with mature B-ALL were from 1994 treated according to a modified Berlin-Frankfurt-Munster protocol (‘NHL-BFM 90’) (80, 81) originally introduced for B-cell lymphoma.
Paediatric national protocol and definition of high risk for relapse

Children diagnosed with Precursor B or T-cell ALL between January 1992 and December 2000 were treated according to the NOPHO-92 protocol (Paper III, Table 1) (3, 77). The CNS-directed prophylactic treatment consisted of mainly methotrexate (high-dose intravenous and intrathecal) and less than 10% of patients received cranial irradiation.

High risk leukaemia was defined as the presence of: Philadelphia-chromosome; t(4,11); WBC >50x10⁹ /l; mediastinal mass; testicular involvement; or T-cell leukaemia. Very high risk was defined as slow response, CNS involvement, lymphomatous features or T-cell leukaemia, together with one more high-risk factor. Leukaemia with either of these features was (in Paper III) referred to as "higher-risk" leukaemia. Patients with mature B-ALL (Burkitt leukaemia) were treated according to the NHL-BFM 90 protocol.

SCT procedures

Conditioning regimen, stem cell source and GVHD prophylactics were decided by institutional guidelines. Donor and recipient were considered matched when identical at HLA-A, -B and -DRB1 loci.

Grading of graft versus host disease (GVHD)

Estimation of the patients GVHD was acquired for the patients who underwent an allogeneic SCT. Chronic GVHD was assessed in patients who survived more than 100 days after SCT. Acute and chronic GVHD were graded according to the previously published criteria (78, 79).

Study subjects and data collection

In the first study (Paper I), the national adult ALL register was retrospectively surveyed for adult patients (>16 years) diagnosed with ALL during the period January 1994 to October 1998. In addition, the National Cancer Register was searched to estimate the number of adult patients diagnosed with ALL during this period and the extent they were treated according to the protocol was investigated through the patients case sheets. All adult patients with precursor B-, T-ALL and AUL treated according to the protocol were eligible for the study. Patients with mature B-ALL were excluded as during this period they had been treated according to a modified NHL-BFM 90 protocol (80, 81). Reporting and initial analysis of all patient data were undertaken at the Oncology Centre in the Uppsala/Örebro region.
In the second study (Paper II), all patients diagnosed between 1986-2000 who underwent SCT were eligible. The patients were identified through: the national 1994-1998 study; a previous national study; the participating centres’ EBMT register; and the transplantation records of each of the eight transplantation centres in the country. Data concerning the classification of leukaemia, risk factors and transplantation together with outcome were collected from these sources and the patients case sheets.

The third study (Paper III) was collaboration between the Swedish Adult ALL Group and the Swedish Childhood Leukemia group. Patients eligible for the study were aged 10-40 years, were diagnosed with ALL between 1992-2000 and treated according to the Nordic Society of Paediatric Haematology and Oncology, NOPHO-92 protocol, or diagnosed between 1994-2000 and treated according to the Adult protocol. Data concerning the children (aged 10-18 years) were collected from the Nordic childhood leukaemia registry (Childhood Cancer Research Unit, Karolinska Institute) and only included Swedish children. The adult patients were identified through previous studies and a new search in the National Cancer Register. Additional data and clinical follow-up were obtained from patients’ case sheets.

The studies was scrutinised and approved by the Swedish ethical committees in all regions.

Statistical analysis

Overall survival (OS) was defined as the time from diagnosis to death, censoring patients alive at last follow up. Event free survival (EFS) was defined as the time from diagnosis to relapse or death in remission, censoring patients alive in first remission and presuming that patients not reaching remission had an event at day one. Continuous complete remission (CCR) was defined as the time from remission to relapse, censoring the patients (alive or dead) in first remission. Disease free survival (DFS) was defined as the time to relapse or death, censoring patients alive in remission. Both CCR and DFS only included patients reaching remission according to protocol. The probability of OS, EFS, CCR and DFS were estimated by the Kaplan-Meier life table analysis. Confidence intervals (CI) of 95% were obtained. Differences between patient subgroups were tested using a log-rank test (Mantel-Cox). The prognostic power of high risk factors was also tested using univariate and multiple-variate analyses (Cox regression). Differences between subgroups of patients were tested using Students T-test (Paper I), chi-square test and Fisher’s exact test (Paper II and III). The level of statistical significance was set at $p = 0.05$.

In Paper II, the patient who received a syngeneic SCT in CR1 was statistically analysed together with the autologous SCT.
Paper IV

Tumour cell samples and cell preparation

Cryopreserved tumour cells from 25 adult ALL patients were used in the study. The samples, obtained at diagnosis or at relapse, originated from either bone marrow or peripheral blood, and none of the patients had received imatinib. Normal peripheral blood mononuclear cells from two healthy donors were also obtained.

Before cryopreservation, mononuclear cells were isolated by density gradient centrifugation with 1.077 g/ml Ficoll-Isopaque (82). Before each experiment, the cells were thawed and washed twice with a culture medium containing RPMI 1640 medium with 10% heat inactivated foetal calf serum, 2 mM L-glutamine, 50 μg/ml streptomycin and 60 μg/ml penicillin.

Viability was determined by the trypan blue exclusion test. On day zero and day three, the proportion of leukaemic cells was estimated by light microscopy on May-Grunwald-Giemsa stained cytocentrifugate preparations.

Data concerning immunophenotype and Ph-status were obtained from the patients’ medical charts and the experiments were thereafter performed with coded samples. The local ethical committee approved the study.

Reagents and drugs

Cytotoxic agents were primarily selected on clinical usefulness in ALL and regarding diversity in mechanism of action. The choice of concentrations of the cytotoxic agents was based on data from previous studies in primary ALL cells (cytarabine 10 μM, prednisolone 30 μM, vincristine 0.5 μM, daunorubicin 0.2 μM, asparaginase 10 U/ml and mercaptopurine 300 μM) (75). The intention was to produce an intermediate drug effect.

Imatinib was supplied from Novartis Pharma and dissolved in dimethyl sulphoxide (DMSO) to a concentration of 10 mM. To examine the concentration-response relation, the drug was tested at five solutions of ten-fold ranging from 100 μM to 0.01 μM. For the Ph-positive samples, the concentration 1.0 μM of imatinib was chosen for the combination study as it resulted in approximately 50% cell survival. Experimental microtitre plates were prepared with 20 μl/well of solution and with the cytotoxic agents at 10x the desired final concentration. All agents and concentrations were tested in triplicate.

Fluorometric Microculture Cytotoxicity Assay (FMCA)

FMCA is a total cell kill assay based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescent fluorescein in cells with intact cell membranes, as previously described (75).
Microtitre plates with 96-wells were used in the experiment. To each well, pre-prepared with the cytotoxic agents as described above, 100 000 viable cells were seeded in 180 µl culture medium. After exposure to the cytotoxic agents for 72-hours at 37°C, the plates were centrifuged and the medium was removed: the plates were then washed with phosphate buffered saline (PBS). Hepes buffered saline containing FDA (10µg/ml) 100 µl/well was added and incubated for 40 minutes: the fluorescence was then read. As a blank, the fluorescence was read in wells containing culture medium without cells, and as control served six wells with culture medium and cells. Cell survival was calculated as the ratio between the fluorescence in drug-treated wells and the fluorescence in control wells with blank values subtracted and was expressed as percentage. Low numerical values indicated a high cytotoxic effect.

Measurement of the BCR-ABL fusion transcript

The presence of BCR/ABL in the samples was detected in quantitative real-time reverse transcriptase-PCR (QR-PCR) using the ABI 7700 from Aplied Biosystems. The analysis was performed as previously described (83, 84) using the known sequences for BCR-ABL minor and major and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), where GAPDH was used as a control gene.

Statistical analysis

The Mann-Whitney test for unpaired comparisons compared mean cell survival between BCR-ABL-positive and -negative samples. Spearman Rank Correlation determined for relationships between continuous values. The level of significance was set at p=0.05.
Results and Discussion

High dose cytarabine in upfront therapy (Paper I)

This was an evaluation of a new national treatment protocol for adult patients with precursor B, T-ALL and AUL. Of the 269 patients diagnosed with ALL, according to the National Cancer Register, between 1994-1998, 220 (82%) were reported into the study. Precursor B, T-phenotype and AUL were determined in 185 of these 220 patients. This report was based on the treatment results of the 153/185 patients who were treated according to the protocol (this included 90% of the patients <60 years and 63% of the patients >60 years). The median age was 42 (16-82) years with a male predominance (male n=84 versus female n=69).

Precursor B-ALL was the most common phenotype (131/153) and this group was characterized by an equal distribution between the sexes and a median age of 42 (16-80) years. T-cell ALL was diagnosed in 18 patients and this group had a lower median age 37 (18-60) years and a clear male dominance (14 males versus 4 females). Only four patients were diagnosed with AUL.

Successful cytogenetic analyses were reported for 112/153 (73%) patients and of these 7/112 (6%) were diagnosed with t(4,11). Results of molecular analyses for BCR-ABL with PCR or FISH were available for 41 patients. In total 25% (32/129) of all patients possible to evaluate for BCR/ABL were BCR-ABL-positive and all had a Precursor B-phenotype.

The median age was relatively high; probably due to the lack of upper age limit and lack of pronounced selection of patients as the study evaluated a national protocol. The penetrance of use of the national protocol differed when comparing patients <60 and >60 years. As this was a retrospective study, no detailed data were available as to why some patients were not treated according to the protocol. For younger patients, there was evidence that some patients were initially diagnosed and treated as AML or mature B ALL and that others were diagnosed abroad. For older patients, some had received treatment with reduced intensity or only supportive care. The distribution between the immunophenotypes, the proportion between males and females and prevalence of Ph-positive leukaemia were as expected (41).

The CR rate was significantly higher (90%) for younger patients (<60 years) compared to 70% for patients over 60 years (p=0.004, t-test). Patients
with a WBC-count >30x 10^9/l had, as the only other subgroup, a significantly lower CR-rate (74% versus 91%, p=0.01, t-test).

Fever and infections were the main toxicity. Culture verified sepsis was reported in 49% of the patients after the first course and 36% after the second course. Group A streptococci sepsis was reported in 25 cases and of these eight patients had simultaneously reversible pulmonary symptoms and/or pathological chest X-ray changes. Three of these eight patients were treated with steroids due to adult respiratory distress syndrome (ARDS). A staphylococcus aureus sepsis was reported in 12 cases after the first course, whereas no case was reported after the second course. The mortality within 30 days was 4% and within three months 8%, this group of patients were characterised by a high median age 61(26-82) years.

The protocol resulted in a high remission rate, both for patients <60 years and >60 years and can be compared to other studies (5-10, 49, 56, 66, 85-92). The rate of culture verified sepsis was high but the frequency of early death did not exceed that in other trials (7, 8). High dose Ara-C, alone or in conjunction with Group A streptococci sepsis, has previously been reported to predispose to ARDS (93-95). That the combination of high-dose Ara-C and high frequency of sepsis, after treatment according to this protocol, implicate a high risk for ARDS is important and provides an opportunity for early treatment for these patients. Cerebellar toxicity has been well described after treatment with Ara-C (7, 95) but was only reported in one patient in this study, despite the repeated Ara-C doses. There was a difference in distribution of cases of *Staphylococcus aureus* sepsis between the first and second courses; although there was no conclusive explanation for this, it might be related to the insertion of a central venous line before start of treatment, giving a possible entrance for infections.

An SCT was carried through for 61 of the patients reaching CR according to the protocol (19 autologous, 23 related donor (RD) and 19 unrelated donor (URD) SCT). In first remission, 44 patients underwent SCT, of which 37 were classified as high-risk patients. An additional 17 patients with high-risk factors and <60 years did not receive a SCT in first remission: of these patients, seven had suffered from an early relapse and four had contraindications for SCT. The protocol’s intentions of SCT for high-risk patients were adhered to in 69% of the patients <60 years. The reasons that seven patients without any known high-risk factors underwent SCT and that the six patients with high-risk leukaemia (but without contraindications) did not receive a SCT were unknown and illustrated one of the weaknesses with a retrospective study.

The estimated three-year OS was 29% (CI 21-36%) and the CCR 36% (CI 7-45%). The probability of three-year CCR was significantly higher for precursor B ALL (38%; CI 28-48%) versus T-cell ALL (25%; CI 4-46%) and AUL (not reached) (p=0.003 log-rank test). No significant difference for CCR between the high-risk and standard-risk groups, or for any specific
high-risk factor, was determined; however, in the group of patients with precursor B immunophenotype and ≤40 years, a significant difference in three-year CCR between the high-risk (44%; CI 25-62%) and standard-risk group (62%; CI 41-82%, p=0.04%) was determined. Consequently, in the cohort of adult ALL patients a ‘good-risk’ group consisting of patients ≤40 years with standard-risk precursor B-ALL was identified.

There was a significant difference between the projected DFS after SCT in first remission (39%; CI 24-54%) compared to beyond first remission (not reached, p<0.0001). A comparison of autologous, RD and URD transplantation in first remission revealed no difference for DFS either for all patients or the precursor B-patients (Fig. 4, Paper I). The dominating cause of death was leukaemia both for patients treated only with chemotherapy and for patients receiving SCT.

Age has been recognised as an important risk factor for relapse (39), and a comparison of these treatment results to other studies was difficult as this national study had relatively high median age. The study has an asset in including a majority of cases in a large geographical region, which indicated a low degree of selection, which was unusual in other ALL studies. However, CCR and OS for precursor B ALL were comparable with other trials (5-10, 49, 56, 66, 85-90). The treatment protocol may be under performing for T-cell ALL (6, 8, 90) but the comparison was precarious due to the limited number of patients.

The majority of autologous SCT was performed in the first part of the period with URD SCT (including mismatch grafts) becoming successively more common. The reason for this shift in transplantation type is unknown and might have involved increased knowledge and experience of URD SCT or a notion that URD was superior to autologous SCT. DFS for autologous, RD and URD transplantation was similar, and this warranted further study in a larger patient group and constituted one of the rationales for Paper II.

Autologous and allogeneic SCT (Paper II)

Altogether 187 patients were included in this study: 65 patients treated according to the 1986-1993 protocol; 103 according to the 1994-2000 protocol; and 19 according to other protocols. The majority of patients transplanted in first remission had high risk factors for relapse (102/124). However, 22 patients were classified as having standard risk leukaemia. The clinical characteristics are summarised in Table 2; Paper II. The median age were 34 (range 17-66) years and with a male dominance (male 111/ female 76). The distribution of pre-treatment high risk factors did not differ significantly between autologous, RD and URD SCT group. Ph-positive ALL were present in 22% of the patients: 15% in autologous SCT; 26% in RD SCT; and 29% in URD SCT. A majority of the patients, who received an allogeneic graft versus an
autologous graft, were beyond first remission (p=0.04 RD versus autologous and p=0.006 URD versus autologous SCT).

The overall 5-year OS was 30% (CI 23-36%) and 5-year DFS 26% (CI 20-32%). The two patients who received a reduced intensity conditioning were excluded from the rest of the analysis. The rationale was to preserve as uniform treatment as possible in the allogeneic SCT groups, as the place for reduced intensity conditioning in ALL is so far undetermined (13). There was a significant difference in the 5-year DFS between patients who underwent an SCT in CR1 (32%; CI 24-40%) versus beyond CR1 (14%; CI 5-23%, p<0.0001). The observation was confirmed in univariate analysis (Table 4, Paper II) and in multiple model of Cox Regression both for autologous (hazard risk 2.62; CI 1.34-5.46) and allogeneic SCT (Table 5 in Paper II). A difference was determined, although, the majority of patients who received an SCT in first remission had high-risk leukaemia. Similar findings are reported in other studies (96, 97), however, not all have detected this difference (98).

For SCT beyond CR1, there was a significant difference (p=0.04) in 5-year DFS between the patients suffering an early relapse (7% (CI 0-15%): <two years from diagnosis to SCT) compared to a late relapse (28% (CI 8-47%): >two years from diagnose to SCT). No significant differences in DFS between the SCT types were determined. The outcome for patients with an early relapse cannot be considered as satisfactory. For these patients, trials including experimental therapy could be considered. Furthermore, it might be important to discern time from diagnosis to relapse and SCT when comparing studies including patients receiving SCT beyond first remission.

Age, sex, immune phenotype and total body irradiation, TBI, in the conditioning or stem cell source did not predict DFS in univariate regression analysis, neither for autologous nor for allogeneic SCT.

There was no significant difference (p=0.06) in 5-year DFS between autologous (22%: CI 12-32%), RD (32%: CI 22-42%) and URD SCT (22%: CI 10-35%), even if there was a trend towards higher DFS for RD SCT versus autologous and URD SCT. Furthermore, the hazard ratio for DFS regarding URD versus RD SCT was significant in both univariate analysis and multiple-model of Cox regression. As the difference remained in the multiple-model, it should be interpreted as an independent factor and that more patients receiving an URD graft were beyond first remission could not fully explain the result. The determination from the multiple-model should however, due to the number of analyses conducted, be interpreted with caution.

In the allogeneic SCT group, both matched and mismatched RD and URD grafts were included. DFS between autologous SCT, matched RD SCT and matched URD SCT were also compared using the log-rank test, and no significant differences were determined (Figure 2). The lack of difference between DFS for the autologous and URD SCT was an interesting result, and Wiesdorf et al (99) present a similar outcome.
The dominating cause of death for autologous, RD and URD SCT patients both in first and beyond first remission was leukaemia, i.e. death after relapse (Table 6, Paper II). As in other studies (53, 54, 99), transplantation-related mortality was higher and relapse rate lower for RD and URD SCT compared with autologous SCT (Fisher's Exact test p=0.04). These conditions compensated for each other regarding DFS and might in part explain the lack of difference between the SCT types.

For patients who received an allogeneic SCT, a superior DFS was determined for limited versus no chronic GVHD (Figure 3), and limited chronic GVHD was also established as an independent factor in multiple model of Cox regression (Table 5, Paper II). No significant difference in DFS was determined between grades I-II and no acute GVHD and none of the 10 patients with grades III-IV acute GVHD survived. Superior DFS for limited chronic GVHD was supported by some previous studies (100, 101, 102) but not all (96), and strongly supported the presence of a clinically important GVL effect in ALL.
Higher age as a continuous variable (p=0.03) and the combination male recipient/female donor (p=0.06) might be associated with impaired DFS for allogeneic SCT according to the multiple model of Cox regression (Table 5, Paper II). The male recipient/female donor combination and age are described by Gorin (103) (together with more than one course to remission) as the three most important factors for the outcome of allogeneic SCT in adult ALL. The results from this study indicated the importance of these factors, but a larger study cohort would be needed to confirm the results.

Ph-positive ALL were reported in 35/124 patients who underwent SCT in first remission and their 5-year DFS was 22% (CI 8-36%), which not were significantly different from the outcome of Ph-negative ALL (36%; CI 26-46%, p=0.12). In the univariate analysis, Ph-positive leukaemia did not correlate with impaired DFS in allogeneic SCT but in autologous SCT. None of the patients who received an autologous SCT or any SCT beyond first remission survived. The development of treatment with signal transduction modulators such as imatinib (as discussed in Paper IV) might present new options. The present results indicated that an allogeneic SCT in first remission should be recommended for all eligible Ph-positive patients: this is uncontroversial and has been confirmed by other studies (58, 59).

In Ph-negative ALL, there are conflicting data regarding when to recommend allogeneic SCT, autologous SCT and chemotherapy as maintenance therapy. Several large studies have compared allogeneic SCT (mainly with a matched related donor) with autologous SCT and/or chemotherapy. Superior survival has been found for allogeneic SCT for high-risk patients (4, 52) and for both standard and high-risk patients (56, 57); however, other studies not
have found any differences between allogeneic SCT, autologous SCT and chemotherapy (53, 54). This study indicated that DFS after RD SCT might be favourable in high risk ALL compared to autologous and URD SCT, although the results were not significant. The superior DFS for patients with limited chronic GVHD (supporting a clinical important GVL-effect in ALL) could be a contributing cause, whereas the higher treatment related mortality in allogeneic versus autologous SCT results in a counterbalancing effect. The lack of difference between autologous and URD SCT should be noticed, as well as that higher age (as a continuous variable) was associated with impaired DFS in allogeneic SCT. These findings might be taken into consideration in discussing treatment options for middle-aged patients with high-risk (Ph-negative) ALL who are lacking a family donor.

Treatment of children over 10 years and young adults (Paper III)

Of the 266 patients (aged 10-40 years) diagnosed with ALL within the stipulated period, 253 (95%) were treated according to either of the national protocols in paediatric units (148/148: 100%) or in adult units (105/118: 89%) (Chi-square test p<0.01).

Of the 253 protocol patients, 144 were treated according to the paediatric NOPHO-92 protocol for precursor B and T-cell ALL in paediatric units and 99 were treated according to the Adult protocol for precursor B and T-cell ALL in adults unit. These 243 patients were included in the protocol-evaluation. The nine patients with mature B-cell ALL treated according to the NHL-BFM 90 protocol and one patient with NK-cell ALL were excluded from further statistical analyses.

The proportion of adult patients was lower than the proportion of paediatric patients who entered into the national studies: this pattern was consistent with previous reports (104, 105). However, in both groups, participation was high and the results should thereby not have been affected by selection mechanisms to any higher degree.

Clinical characteristics and risk factors regardless of treatment protocol

Considering the whole patient population (regardless of treatment), a trend towards higher WBC-counts and an increased frequency of CNS involvement and Ph-chromosome was determined in patients over 20 years (Paper III, Table 3). T-cell ALL was less frequent among patients aged 15-25 than both younger and older patients. Hyperdiploidy (51-61 chromosomes) was more common in patients aged 10-25 years but rarely seen in older patients.
The distribution of these characteristics confirmed previous findings (40) except for T-cell phenotype distribution, which was unexpectedly low in the 15-25 year cohort.

For precursor B and T-cell ALL, univariate and multiple model Cox regression analyses were used to evaluate the impact of prognostic factors regarding EFS (Paper III, Tables 6 and 7b). To analyse the effect of age and clinical characteristics, disregarding impact of treatment protocol, the cohorts treated at paediatric and adult units were analysed separately. In the paediatric cohort (10-18 yrs), WBC was the only significant prognostic factor in both the univariate and multiple model analyses. In the adult cohort (15-40 yrs), age, CSN-leukaemia and Ph-chromosome (but not WBC) were identified as independent prognostic factors for EFS. The correlation for CNS-leukaemia and age should be interpreted with caution considering the high number of analyses done.

Both the Paediatric and Adult protocols had adjusted the treatment protocols for patients with known high-risk criteria. Yet, even though intensified treatment, the above stated factors were associated with impaired prognosis. In both childhood and adult ALL, WBC is recognised as an important prognostic factor(106); however, WBC was only a prognostic factor in the paediatric cohort. The result remains when looking separately at the precursor B cohort for adults (data not shown). The reason was unclear, but could be a reflection of different biology of the disease in different ages, whereas other factors might have a greater influence in adult ALL. As expected, Ph-chromosome was a strong independent factor for impaired prognosis(13, 35). CNS-leukaemia surprisingly also remained a possible high risk factor in the adults.

With modern treatment according to a paediatric protocol, age no longer appeared a prognostic factor in the 10-18 yr cohort. According to an evaluation by NOPHO, the difference in prognosis between patients >10 years and 1-9 years old is less pronounced in the NOPHO-92 protocol compared with previous protocols (3). The remaining (border-) significance for age in the multiple model for the adult cohort can not be explained by difficulties in tolerating chemotherapy (107), and other known risk-factors are compensated for in the analysis. A potential explanation is a different biology in adult ALL, which varies in correlation with age.

Outcome according to treatment protocol

The CR rate for patients treated according to the Paediatric protocol was 99% (CR after the 29 day induction phase) and for patients treated according to the Adult protocol 90% (CR after one or two courses) (p<0.01). There was no difference between the two adult age groups (15-25 yr and 26-40 yr). The frequency of induction deaths was 1% for the Paediatric protocol versus 2% for the Adult protocol (ns).
The OS, DFS and EFS are presented in Table 2. Significant differences were determined between all the groups: the Paediatric protocol 10-18 yrs; Adult 15-25 yrs; and Adult 26-40 yrs. There were no significant differences in 5-year EFS between patients with precursor B and T-cell ALL within either group, but when the two Adult groups were analysed together, a difference (p=0.05) between the EFS-values for the precursor B (EFS 34%; CI 24-45%) and T-cell groups (EFS 13%; CI 0-30%) was found. The Paediatric and Adult protocol had stated different high-risk criteria. To enable a comparison between patients with similar pre-treatment characteristics, uniform pre-treatment risk criteria were applied: WBC >30 x 10^9/l; Philadelphia-chromosome; t(4,11); or CNS-involvement.

Table 2. Outcome of therapy according to protocol and age.

<table>
<thead>
<tr>
<th>Protocol / age</th>
<th>5-year OS (CI 95%)</th>
<th>5-year EFS (CI 95%)</th>
<th>5-year DFS (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paediatric / 10-18 y</td>
<td>79% (CI 72-86%)</td>
<td>66% (CI 58-74%)</td>
<td>68% (CI 60-76%)</td>
</tr>
<tr>
<td>p-value^1</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Adult / 15-25 y</td>
<td>52% (CI 37-66%)</td>
<td>42% (CI 28-56%)</td>
<td>46% (CI 31-61%)</td>
</tr>
<tr>
<td>p-value^2</td>
<td>P&lt;0.01</td>
<td>p=0.01</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Adult /26-40 y</td>
<td>26% (CI 13-39%)</td>
<td>19% (CI 7-31%)</td>
<td>21% (CI 8-34%)</td>
</tr>
<tr>
<td>p-value^3</td>
<td>P&lt;0.01</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

p-value^1= Paediatric 10-18 y versus Adult 15-25 y, p-value^2= Adult 15-25 y versus 26-40 y, p-value^3= Paediatric 10-18 y versus Adult 25-40 y

For the lower-risk group, the 5-year EFS for the Paediatric 10-18 yr group was 71% (CI 62-80%), the Adult 15-25 yr group was 47% (CI 29-66%) and the Adult 26-40 yr group was 22% (CI 4-40%) (p<0.01) (Figure 4a). For the higher-risk group, the 5-year EFS-values was 53% (CI 37-68%) for the Paediatric 10-18 yr group, 33% (CI 13-54%) for the Adult 15-25 yr group and 17% (CI 2-32%) for the 26-40 yr group (p<0.01) (Figure 4b). There was no significant difference in outcome between the two adult protocol groups in the higher-risk population.

SCT in first remission was part of the treatment for 14/144 (10%) of the Paediatric protocol patients and 41/99 (41%) of the Adult protocol patients. The 5-year OS and EFS for the patients who received a SCT were: for the Paediatric group 42% (CI 15-68%) for OS and 42% (CI 15-68%) for EFS, and in the Adult group, 31% (CI 16-45%) for OS and 29% (CI 15-43%) for EFS (ns).
Univariate regression analyses and multiple model analyses (Tables 6 and 7a, Paper III) were used again to evaluate the impact of prognostic factors regarding EFS for the entire cohort. WBC-count, CNS-leukaemia, Ph-chromosome and treatment protocol were determined as independent factors for EFS. Age (as a continuous variable) was only significant in the univariate analysis. When the 15-20 yr cohort (59 patients) were analysed separately in a multiple-model, treatment protocol became the only significant factor.

The cause of death was mainly associated with relapse and the frequency of deaths in complete remission including treatment related mortality after transplantation in first remission, was low for all groups (3-8%).
A comparison of the total doses of chemotherapeutic drugs for lower risk patients in the Paediatric and Adult protocols is presented in Table 8, Paper III. The main differences between the protocols included: the use of asparaginase; dexamethasone; and an approximately twice as high cumulative dose of vincristine and cyclophosphamide together with a more extensive use of methotrexate (both as i.v. high-dose infusions and i.t. injections) in the Paediatric protocol. The Adult protocol included high-dose Ara-C for all patients.

Asparaginase and high dose methotrexate are well recognised as efficacious drugs in ALL—treatment (108). A better outcome has been observed when dexamethasone is used instead of Prednisone in clinical trials for children with ALL (109). Dexamethasone has been shown to have a better CNS penetration than prednisone (110) and thus a better preventive effect against CNS relapses (109). The high cumulative dose of vincristine could also be of importance, as vincristine is one of the backbones of ALL treatments.

One other important difference between the protocols may be the disposition of the treatment. The Paediatric protocol had a prolonged induction and consolidation phase with a late start of the maintenance phase compared with the Adult protocol. In contrast, the Adult protocol recommended SCT for all patients with high-risk leukaemia.

Previous studies from France (60), the Netherlands (61) and the US (62) comparing the outcome for adolescents treated on paediatric versus adult protocols all report similar results to this study. There are difficulties in comparing all protocols, but some common features can be recognised. In both the French and the Dutch adult protocols, the total doses of vincristine and asparaginase were lower than in the corresponding paediatric protocol (or the drug was not included in the treatment). These differences were also present in the Swedish comparison.

The results from the present study indicated that adolescents with ALL would benefit from treatment according to the Paediatric protocol. Could young adults benefit from a similar protocol; would the treatment be feasible; and is the biology of the leukaemia in young adults sufficiently similar to the leukaemia of children and adolescents?

Asparaginase is and has been used in several adult protocols. The side effects are well known, including disturbance of the coagulation system and thromboembolism (111). High-dose methotrexate is used (and feasible) in several adult protocols even though the dose rarely exceeds 3 g/m². For patients with high-risk criteria, the NOPHO-92 protocol includes methotrexate 8 g/m², which is not common in adult protocols and the feasibility is uncertain. Vincristine is well known for its neurotoxicity (mainly peripheral, mixed sensory-motor, and autonomic polyneuropathy) (112). In the present Adult protocol, some patients have difficulties in proceeding with treatment with vincristine during the maintenance therapy due to, for example, polyneuropathy. Side effects from vincristine are present also in the treatment of
children according to paediatric protocols but can in the currently used NO-PHO protocol usually be managed with minor dose-reductions or temporary suspension of vincristine-treatment (Mats Heyman, Astrid Lindgren’s Childrens Hospital, personal communication). A crucial difference between children and adults may be a plasticity of the nervous system, and the effects on the nervous system is likely to be reversible to a higher degree in children compared with adults. However, long-term sequelae have been described both in both children and adults (112).

The proportion of patients with high-risk criteria increased with age in the adult cohort and, together with age itself, remained as a high-risk criterion: this indicates a different biology in young and middle-aged patients with ALL.

Further trials are needed to determine if a “paediatric treatment” would be beneficial and feasible for young adults.

In vitro activity of imatinib (Paper IV)

The FMCA was used to investigate the in vitro activity of imatinib in tumour cell samples from adult ALL patients. A successful drug sensitivity analysis and QR-PCR was performed on 15 of the 25 tumour samples according to the quality criteria of the tests (75). In four samples, \( p^{190} \text{BCR-ABL} \) fusion transcripts were detected, \( p^{210} \text{BCR-ABL} \) were detected in two samples and \( \text{BCR-ABL} \) was absent in nine samples.

The \( \text{BCR-ABL} \)-negative samples responded in a similar way as the normal peripheral blood mononuclear cells to imatinib and displayed a sensitivity at 100 \( \mu \text{M} \), and to a minor extent at 10 \( \mu \text{M} \). Comparable responses to the high concentrations of imatinib were observed in tumour cells from AML and chronic lymphocytic leukaemia, which were analysed as reference material (unpublished results).

The \( \text{BCR-ABL} \)-positive samples were significantly \((p<0.05, \text{Mann-Whitney test})\) more sensitive to imatinib than the \( \text{BCR-ABL} \)-negative at concentrations of: 0.1 \( \mu \text{M} \); 1 \( \mu \text{M} \); and 10 \( \mu \text{M} \). The six \( \text{BCR-ABL} \)-positive samples had high levels of \( \text{BCR-ABL} \) with mRNA ranging from 107 to 404 \( \text{BCR-ABL} \) molecules relative to 10e4 GAPDH. No correlation was determined between the level of expression of \( \text{BCR-ABL} \) mRNA and response to imatinib. However, particularly at 1\( \mu \text{M} \), samples expressing the \( p^{190} \text{BCR-ABL} \) fusion transcript tended to be more sensitive than samples expressing the \( p^{210} \text{fusion} \).

Imatinib is a relatively specific inhibitor of Bcr-Abl, Pdgfr and c-Kit. The results for primary ALL-cells confirmed the selective effect for \( \text{BCR-ABL} \)-positive acute leukaemia as described in previous in vitro tests and in clinical trials (68, 113, 114). The activity of high concentrations of imatinib in \( \text{BCR-ABL} \)-negative samples was less specific, and may be independent of Bcr-Abl
tyrosine kinase. It is unknown if any clinical effect could be achieved for BCR-ABL-negative ALL.

Are the selected concentrations of imatinib in this study of clinical interest? A direct comparison between the concentration of drugs in in vitro tests and in clinical use is difficult and should be undertaken with caution. However, patients receiving 350 mg imatinib a day have steady-state plasma-concentration levels at ≥1 μM (115). This suggests that the selected concentrations in this study were in a range of clinical interest. Compared to the p210 version, p190 Bcr-Abl has been shown to have a higher tyrosine kinase activity (116), which gives a potential for a different sensitivity to imatinib in p190 versus p210 BCR-ABL-positive leukaemia. Results indicated that p190 BCR-ABL-positive cells might have a higher sensitivity to imatinib than p210, but the number of samples was too few and additional experiments are warranted.

Figure 5. Mean cell survival of primary ALL cells when exposed to imatinib, single agent ± SEM

The second aim of the study was to determine if the combination of imatinib with conventional agents could enhance or reduce activity of the single agent in vitro. The highest mean difference in cell survival (%) of the combination of imatinib and conventional cytotoxic agent was compared to the most active single agent. The drugs indicating the largest benefit from a combination with imatinib for BCR-ABL-positive samples were: daunorubicin (51% higher effect of combination); prednisolone (41%); and cytarabine (26%). A less prominent effect was determined for vincristine (17% better effect of combination) and asparaginase (12%). In one sample, a possible
antagonistic interaction was observed for imatinib combined with mercaptopurine and a mean difference of only 8% was found for this combination. No enhanced activity was determined for the combination of imatinib with conventional agents for BCR-ABL-negative samples. The mean cell survival for BCR-ABL-positive samples to imatinib, single agent and imatinib in combination with the single agent are shown in Figure 5.

Imatinib has only achieved short remissions in Ph-positive ALL, probably due to development of drug resistance by amplification or mutations in the Bcr-Abl tyrosine kinase. To achieve an enhanced drug effect and, if possible, prevent development of resistance, combinations of imatinib and cytotoxic agents (117, 118) are of importance. Imatinib in conjunction with interferon alfa as maintenance treatment (119) and imatinib as addition to induction and consolidation therapy have shown promising results (120). It may be crucial for BCR-ABL-positive ALL patients to be able to optimise combination therapy. Previous in vitro studies, mainly using cell lines or samples from CML-patients, have determined synergistic or additive interactions between imatinib and for example cytarabine, daunorubicin or vincristine (121-124). Our observations in primary tumour cells from patients with BCR-ABL-positive ALL were in accordance with others results, and combinations of imatinib with cytotoxic drugs, such as cytarabine, daunorubicin and predisolone, are potentials for further evaluation in treatment of Ph-positive ALL. Mercaptopurine is commonly used in maintenance therapy (Table 1c); however, these results did not suggest that a combination with imatinib would create synergistic effects advantageous for maintenance therapy.

Supplementary discussion and some unpublished results

Papers I – III included, in part, the same cohort of patients. When comparing treatment results for the adult cohort in Paper III with the results for the patients in Paper I, additional questions emerged. In the first study, patients <40 years with precursor B ALL had been identified as a “good-risk” group. In Paper III, age itself was determined as a possible independent risk factor (for patients aged 15-40 years) and survival for the 26-40 year-old patients was discouraging. This was the rationale for a long-time follow-up regarding survival of the patients in the first study and the data for the additional cohort of adult patients in study III was added to the data-set (as they were treated according to the same protocol). The OS for these patients, all treated according to the adult national protocol, is presented in Figures 6a and 6b. The two graphs both show OS for exactly the same population and only differ in the division of age groups.
Figure 6. OS for patients treated according to the national adult protocol.
a. Patients aged 15-40 yrs 39 (CI 29-49)%, 41-60 yrs 23 (CI 10-35)%, and >60 yrs 9 (CI 0-18)%.  
b. Patients aged 15-25 yrs 52% (CI 38-66%), 26-40 yrs 26% (CI 13-38%), 41-60 yrs and >60 yrs as Figure 6a.

Age is, as mentioned previously, well recognised as a risk factor and has been described as a (probably) continuous variable (88). Age as a risk factor is a difficult issue to address, perhaps because it might be a “pseudo marker” for other biological features of the leukaemia. This issue is of interest as the results from Paper III raised the question of which patients might benefit from “paediatric inspired” protocols.
Conclusions

Paper I
This national protocol with high-dose cytarabine up-front generated higher remission rates in patients <60 years (90%) compared to >60 years (70%). The side effects were acceptable and main adverse events consisted of fever, infections and transient elevation of liver-enzymes. The 3-year OS was 29% and CCR was 36%. A favourable pre-treatment characteristic was precursor B phenotype, especially for patients <40 years without high risk factors, and the survival for the precursor B patients was consistent with other international studies. The survival was significantly lower for T-cell ALL and the protocol may be under-performing for this phenotype, even if the small number of patients made the comparison uncertain. Consequently, the Swedish Adult ALL group has maintained the protocol for precursor B ALL in the 2004 revised National treatment protocol, but exchanged the treatment for T-cell ALL.

Paper II
Improved DFS in favour of SCT in first remission versus beyond first remission was determined, despite the majority of patients in first remission having high-risk leukaemia. No significant differences between RD, URD and autologous SCT were determined, even if the results indicated a favourable outcome for RD SCT. The exception was patients with Ph-positive ALL, for whom an allogeneic SCT in first remission was preferable. The presence of a limited chronic GVHD (compared to no chronic GVHD) resulted in higher DFS, strongly indicating a clinically important GVL-effect in adult ALL.

Paper III
Superior remission rates and survival was determined for 10-18 year-old ALL patients treated according to the Paediatric protocol compared to both 15-25 years and 25-40 years treated according to the Adult protocol. Treatment protocol remained as a significant factor for EFS in patients aged 15-20 years. Ph-chromosome, age and CNS-leukaemia were determined as signifi-
cant factors for EFS in adult patients whereas only WBC was of significance for paediatric patients. The results indicate that adolescents with ALL would benefit from treatment according to the Paediatric protocol. Further trials are needed to determine if similar treatment would be beneficial and feasible for young adults. The results have lead to reconsideration of treatment protocols for adolescents and young adults with ALL in Sweden.

Paper IV

There was concordance between in vitro sensitivity to imatinib and the presence of BCR-ABL in primary tumour cells from patients with adult ALL. The sensitivity of BCR-ABL-negative samples was similar to the sensitivity for normal peripheral blood mononuclear cells from healthy donors. In the BCR-ABL-positive samples, the combination of imatinib with conventional cytotoxic agents in vitro resulted in an enhanced activity compared to the single best agent. Daunorubicin, prednisolone and cytarabine were determined as having the greatest benefit from a combination with imatinib and are potentials for further clinical studies.
Acknowledgments

I would like to express my sincere gratitude to all the people who made this thesis possible. My special thanks to:

**Bengt Smedmyr**, my principal tutor, for introducing me to and giving me the opportunity to work in this field, for continuous support, having confidence in my work and sharing his expert knowledge in ALL.

**Elin Lindhagen**, my second tutor for introducing and guiding me into the new world of the “FMCA” and for being straight-forward and pleasant to work with.

All the Colleagues in the Swedish Adult ALL group during these years, for giving me the opportunity to work with ALL in a national perspective, for valuable discussions and for scrutinizing both content and language in presentations and manuscripts.

**Mats Heyman** and **Göran Gustafsson**, at the Childhood Cancer Research Unit, Astrid Lindgren’s Children’s Hospital, for valuable collaboration. Our discussions have been productive, revealing both differences and similarity in the worlds of childhood and adult ALL.

**Gisela Barbany** for good collaboration and expert knowledge in the complicated field of QR-PCR.

**Rolf Larsson** and the staff at the Clinical Pharmacology, for giving me the opportunity to come to your lab, for collaboration and for providing excellent laboratory facilities.

**Annelie Björnberg**, for doing such good work in the degree project.

**Christer Sundström**, for good collaboration.

**Karin Hellström** at the ROC for help with the data collection and register.

**Inger Persson, Niclas Eriksson** and **Lars Berglund** for well needed statistical advice.
Sue Avalon, for skilful linguistic revision.

Gunilla Eriksson, for secretarial help.

Anna, Elisabeth and Ulla, my roommates, for good companionship and all info regarding the pit-falls, disasters and timetables for writing a thesis. Special thanks to Anna for all your useful help and discussions regarding the “FMCA” project.

My other colleagues at the Haematology department, Bengt Si, Gunnar B, Gunnar Ö, Inaam, Kristina, Martin, Max and Simon for all good clinical discussions, friendship and for letting me off clinical work! Special thanks to Bengt Simonsson and Ulla Lindquist for providing good working facilities.

All the staff at “Blodavdelningen” and “Blodmottagningen” for creating a good and stimulating atmosphere at work.

Rolf Billström and Annelie Cervin for introducing me into the world of Haematology from the very beginning.

My family: My mother Elvi for your support and for helping out with the kids, especially at the school-holidays. Eva, Nisse and “Kussarna” – everybody needs an older sister! Siv, Torgil and the other “Hallbööks” for your support.

And finally, not only for support and distraction in the process of the thesis, but for being more important than the thesis – Finn, Filip and Ylva!

Financial support from the Children’s Cancer Foundation is gratefully acknowledged.
References

30. Hematologique GFdC. Cytogenetic abnormalities in adult acute lymphoblastic leukaemia:correlation with hematologic findings and outcome. A Collaborative


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84. Barbany G, Hagberg A, Olsson-Stromberg U et al. Manifold-assisted reverse transcription-PCR with real-time detection for measurement of the BCR-ABL
98. Jamieson CH, Amylon MD, Wong RM, Blume KG. Allogeneic hematopoietic cell transplantation for patients with high-risk acute lymphoblastic leukemia in


113. Druker BJ, Sawyers CL, Kantarjian H *et al.* Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia


122. Avramis IA, Laug WE, Sausville EA, Avramis VI. Determination of drug synergism between the tyrosine kinase inhibitors NSC 680410 (adaphostin) and/or STI571 (imatinib mesylate, Gleevec) with cytotoxic drugs against human leukemia cell lines. Cancer Chemother Pharmacol 2003;52(4): 307-318.


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