Department of Medicine, Division of Hematology
Karolinska University Hospital Solna and
Karolinska Institutet, Stockholm, Sweden

CHRONIC MYELOID LEUKEMIA

CLINICAL, EXPERIMENTAL
AND HEALTH ECONOMIC STUDIES,
WITH SPECIAL REFERENCE TO IMATINIB TREATMENT

Lotta Ohm

Stockholm 2013
To my family and
in memory of my parents
ABSTRACT

CML is a malignant disease that originates in the bone marrow stem cell, carrying the Philadelphia chromosome with the BCR-ABL fusion gene. This gene translates into an active tyrosine kinase, Bcr-Abl, affecting hematopoiesis, particularly resulting in increased numbers of white blood cells in the peripheral blood. Left untreated, CML progresses from a silent chronic phase (CP) to a life-threatening blastic phase (BP). After the millennium shift imatinib was introduced for the treatment of CML. Specifically targeting the Bcr-Abl oncoprotein, it was the first tyrosine kinase inhibitor (TKI) employed in cancer. It induced spectacular responses among CML-CP patients, strikingly reducing the risk of disease progression, combined with excellent tolerability. In this thesis we have studied various aspects of imatinib treatment in CML.

In a cohort of 45 newly diagnosed CML-CP patients initiated on imatinib, we consecutively assessed treatment responses by FISH, PCR and chromosome banding analysis (CBA). In a landmark analysis, an early favourable response, defined as less than 10% BCR-ABL-positive cells by FISH after 3 months of treatment, was identified as a predictive marker of an improved long-term clinical outcome. Among evaluable patients 51% achieved this response. A large majority, 95% of such responders, reached complete cytogenetic response within 12 months and 100% an event-free survival at 48 months.

We assessed the effect of imatinib treatment on neutrophil leukotriene (LT) signaling to evaluate its possible role as a clinical biomarker predictive of treatment response. Increased LT signaling has previously been suggested as a driver of leukocytosis in CML. The activity and expression of LTC₄, catalyzing formation of LTC₄ from LTA₄, were determined in neutrophils from 11 CML-CP patients during their initial phase of imatinib treatment, and the results related to the parallel development of BCR-ABL-expression. CD16+ neutrophils were isolated, their LTC₄ activity measured and LTC₄ expression determined at the protein and mRNA levels. In parallel, BCR-ABL expression was assessed by bone marrow CBA and by FISH on peripheral blood cells, including a combined May Grünwald Giemsa staining and FISH technique (MGG-FISH) to score neutrophilic cells. An aberrant expression of LTC₄ in CML neutrophils was typically found, but it was rapidly normalized after initiation of imatinib treatment, later paralleled by a decreasing expression of BCR-ABL. The findings indicate that increased expression and activity of LTC₄ in CML is a down-stream step of BCR-ABL activity, i.e. the Bcr-Abl protein directly or indirectly causes an upregulation of LTC₄. It is possible that an early evaluation of LTC₄ expression during imatinib treatment could serve as a more rapid way of assessing treatment response than the current methods identifying BCR-ABL expression through CBA, FISH or qRT-PCR.

We also defined real life outcome of patients with CML in Sweden during four decades and related the relative survival (RS) patterns to imatinib treatment and other management strategies. We assessed trends in survival and short-and long-term excess mortality among all patients (n=3,173) regardless of clinical trial enrollment. Patients were categorized into five age groups (<50, 50-59, 60-69, 70-79 and >79 years) and five calendar periods (1973-1979, 1980-1986, 1987-1993, 1994-2000 and 2001-2008). We found that throughout all calendar periods, age was a strong predictor of survival, with superior survival for the youngest patients. In analyses including age and period of diagnosis, RS improved with calendar period in all age groups, but most markedly in patients younger than 79 years of age, particularly those 70-79 years of age. Survival among all age groups was greatest in the last calendar period, mainly as a result of an increasing use of imatinib. However, elderly patients still do poorly. The Swedish CML registry data show that patients diagnosed 2002-2008, at the age of 70-79 years received TKI in 66% and patients >80 years in only 18% of the cases.

Finally, we compared the costs during the last decades with earlier decades treatment regimens and related the costs to the expected improved survival. Using Swedish real world national data from CML patients diagnosed in the country from 1973 to 2008 (n=1,778), we evaluated the incremental cost-effectiveness ratio (ICER) between three periods associated with broad implementation of imatinib (III), interferon-α and allogeneic stem cell transplantation (II), and symptomatic treatment (I), respectively. We observed substantial health gains over time, paralleled by increased treatment costs. The mean survival was 2.9, 9.2 and 18.5 years during periods I-III, respectively. The resulting ICER was £45 700 per QALY gained comparing periods III and II using a societal perspective. In a separate analysis by groups of age at diagnosis showed lower ICERs for individuals <50 years at diagnosis: £38 500 for the societal perspective. Since the prevalence of CML patients is increasing and assuming that 75% of each incident cohort was to receive imatinib at current prices, the imatinib budget would need to double by 2050. A future potential discontinuation of imatinib for selected excellent responders would reduce the ICER per QALY gained. Reduced drug cost of imatinib linked to the patent expiry of the drug will probably have a greater impact on ICER per QALY. An estimated price reduction of 80% (global competition) or 30% (expected change for biological drugs) would be associated with an ICER of £20 000 and £36 000, respectively, per QALY gained.
LIST OF PUBLICATIONS

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


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LIST OF ABBREVIATIONS

5-LO | Five-lipoxygenase
ABL | Abelson 1 (gene)
ALL | Acute lymphoblastic leukemia
AML | Acute myeloid leukemia
AP | Accelerated phase
ASCT | Autologous stem cell transplantation
ATP | Adenosine-5'-triphosphate
BCR | Breakpoint cluster region (gene)
BCR-ABL | Breakpoint cluster region-Abelson fusion (gene)
Bcr-Abl | Breakpoint cluster region-Abelson fusion (protein)
BP | Blastic phase (or blastic crisis)
CBA | Chromosome banding analysis
CCA | Clonal chromosomal abnormalities
CCyR, CCgR | Complete cytogenetic response
CgR | Cytogenetic response
CHR | Complete hematologic response
CML-CP | Chronic myeloid leukemia chronic phase
CMR | Complete molecular response
CP | Chronic phase
CysLT | Cysteinyl Leukotriene
D-FISH | Dual fusion-FISH
DNA | Deoxyribonucleic acid
EFS | Event-free survival
ELN | European Leukemia Net
ES-FISH | Extra signal-FISH
FISH | Fluorescence in situ hybridization
HLA | Human leukocyte antigen
HR | High risk
HU | Hydroxyurea
IFN | Interferon-alpha
IR | Intermediate risk
LO | Lipoygenase
LR | Low risk
LT | Leukotriene
LTA₄ | Leukotriene A₄; 5(S)-trans-5,6-oxido-11,14-cis-eicosatetraenoic acid
LTB₄ | Leukotriene B₄; 5(S), 12(R)-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid
LTC₄ | Leukotriene C₄; 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans-11,14-cis- eicosatetraenoic acid
LTC₄S | Leukotriene C₄ synthase
LTD₄ | Leukotriene D₄; 5(S)-hydroxy-6(R)-S-cysteinylglycyl-7,9-trans-11,14-cis- eicosatetraenoic acid
LTE₄ | Leukotriene E₄; 5(S)-hydroxy-6(R)-S-cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>M-BCR</td>
<td>Major breakpoint cluster region</td>
</tr>
<tr>
<td>m-BCR</td>
<td>Minor breakpoint cluster region</td>
</tr>
<tr>
<td>MMR</td>
<td>Major molecular response</td>
</tr>
<tr>
<td>μ-BCR</td>
<td>Micro breakpoint cluster</td>
</tr>
<tr>
<td>MRD</td>
<td>Minimal residual disease</td>
</tr>
<tr>
<td>m-RNA</td>
<td>Messenger-RNA</td>
</tr>
<tr>
<td>OS</td>
<td>Overall survival</td>
</tr>
<tr>
<td>Ph</td>
<td>Philadelphia</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse-transcription polymerase chain reaction</td>
</tr>
<tr>
<td>q-RT-PCR</td>
<td>(quantitative) Real time reverse-transcription polymerase chain reaction</td>
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<tr>
<td>RS</td>
<td>Relative survival</td>
</tr>
<tr>
<td>RSR</td>
<td>Relative survival ratio</td>
</tr>
<tr>
<td>SCT</td>
<td>Stem cell transplantation</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
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<tr>
<td>WBC</td>
<td>White blood cell</td>
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<td>WHO</td>
<td>World Health Organization</td>
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</table>
1 INTRODUCTION TO CHRONIC MYELOID LEUKEMIA (CML)

1.1 BACKGROUND

The term leukemia was coined by Virchow in 1845 as he recognized several cases of splenomegaly, anemia and massive granulocytosis and understood the neoplastic nature in patients with “purulent” blood. \(^1,2\) The disease origin from the bone marrow was clarified by Neumann some years later. \(^3\) Nowell’s and Hungerford’s discovery of the Philadelphia chromosome in 1960 was a breakthrough in cancer biology and CML. For the first time it was demonstrated that a chromosome change was associated with a specific type of leukemia. \(^4\) In 1973 Rowley found that the Philadelphia chromosome (Ph) was a result of a reciprocal chromosomal translocation between the long arms of chromosomes 9 and 22. \(^5\) It took another 10 years before it was revealed that the proto-oncogene ABL on chromosome 9 and the previously unidentified BCR gene on chromosome 22 was involved and that the deregulated Abl tyrosine kinase was the pathogenic factor. \(^6,7,8\) In 1990 Daley et al reported the first evidence of ability of BCR-ABL to transform primary myeloid cells and induce a CML-like disease in mice, which finally confirmed that the BCR-ABL and the constitutively active Bcr-Abl tyrosine kinase was the underlying pathogenic factor in CML. \(^9\)

Prior to the 1950s, splenic irradiation was the mainstay of CML therapy. The treatment had no or minimal effect on survival. In the mid 1950s busulphan became the prevailing palliative
treatment of CML, reducing the leukocytosis and splenomegaly. Busulphan was some decades later replaced by hydroxyurea (HU) as the treatment of choice, due to less toxicity of the latter. Neither drugs affected cytogenetic response or progression to BP resulting in a median survival of 3.2 years in the 1970s. Until the 1980s, CML was regarded as incurable and inexorably fatal.

In the 1980s it became clear that allogeneic stem cell transplantation (SCT), despite a relatively high transplantation-related mortality especially in the early years, could induce long-term Philadelphia chromosome negativity. It became the treatment of choice for eligible younger patients with access to a donor since it offered a chance of cure. Efforts to extend SCT to all patients with CML failed, due to lack of suitable donors and the increased incidence of lethal graft-versus-host disease (GVHD). The standard therapy for the majority of patients in CML chronic phase (CML-CP) in the mid 1980s was recombinant interferon-α (IFN). All studies that reported IFN as first line treatment, tested the drug in combination with other agents mostly hydroxyurea, low dose cytarabin or both. IFN prolonged life of patients in all ages, but lower doses were used in elderly patients. IFN showed promising results in different studies with 10-year overall survival (OS) of 25-53% and a median survival of 5-7.5 years. However, IFN therapy was associated with side effects, including fever, chills, muscle pain, asthenia, fatigue, that lead to considerably reduced quality of life and problems to maintain the required high doses of IFN.

In 1984 the Swedish CML Study Group was formed, and presented national recommendations for the management of patients with CML the same year. Between 1984 and 1989 the Swedish CML Study Group randomly allocated patients to treatment with either HU or busulphan. Approximately 35% of all patients with newly diagnosed CML (n=179) were included. No difference in overall or blast crisis-free survival was observed. Patients who underwent allogeneic SCT fared significantly better with a median survival of 4.7 years in comparison with 3.3 years in patients who did not. As a consequence of the study, younger patients were offered SCT. In patients younger than 55 years of age without a donor, the Swedish CML Study Group during the 1990s explored combined treatment with HU and IFN followed by one to three courses of intensive chemotherapy. Patients who achieved significant Ph reduction and negativity underwent high-dose chemotherapy and autologous SCT to further minimize the Ph-positive clone. During the same period (1989 to 1997), the Swedish CML Study Group used an intensive chemotherapy protocol in patients in accelerated phase (AP) and blastic phase (BP) in an attempt to restore CP, including allogeneic or double autologous (i.e., cells harvested in early chronic phase) SCT. The 1-year survival was 70% for allo-geneic SCT/Autologous-SCT(ASCT) patients (median survival 21 months), 50% in responding patients overall, but only 7% in non-responders.
1.2 CML – CLINICAL ASPECTS

1.2.1 Definition, diagnostic criteria, methods and predictors of prognosis.

CML is a myeloproliferative disease that originates in an abnormal pluripotent bone marrow stem cell carrying the Philadelphia (Ph) chromosome and/or the BCR-ABL fusion gene. It is found in all myeloid cell lineages, but also in some lymphoid cells, although the myelopoiesis is dominating. At diagnosis parallel to the malignant clone there is a suppressed normal hematopoiesis.

The diagnosis of CML should be suspected based on abnormal blood counts (leukocytosis, thrombocytosis), the presence of an enlarged spleen and/or general symptoms such as fatigue, weight loss, sweating and is formally diagnosed by

1) Typical morphological assessment of blood or bone marrow smears

AND

2) Detection of the BCR-ABL fusion gene in cells from blood or bone marrow.

Morphology

The peripheral blood shows increased white blood cell (WBC) counts, due mainly to segmented neutrophils and neutrophils in different stages of maturation such as myelocytes, metamyelocytes. Basophilia is invariably present and many patients may have eosinophilia as well. A low number of myeloblasts are often seen. The platelet count is normal or increased and may exceed 1000x10^9/l. Thrombocytopenia in chronic phase is rare. Most patients have a mild anemia.

The bone marrow shows hypercellularity due to increased numbers of neutrophils and their precursors. In chronic phase the blasts are usually fewer than 5%, while 10-19% indicates transformation to an accelerated and ≥ 20% to a blastic phase. The megakaryocytes are characteristically small and have hypolobated nuclei. Forty per cent of the patients display an increase of reticulin fibers that generally correlates with increased numbers of megakaryocytes, enlarged spleen and more severe degree of anemia.

Detection of BCR-ABL fusion gene

The presence of BCR-ABL can be detected by three different methods;

1) Chromosome metaphase analysis (chromosome level) shows an elongated chromosome 9 and a truncated chromosome 22, i.e. the Philadelphia chromosome. Karyotyping ("G-banding" or "conventional cytogenetics") is a screening analysis where all chromosomes are evaluated in metaphases. Normally 20-30 metaphases are analyzed. Since relatively few metaphases are studied the sensitivity is rather low. An advantage of this technique is that additional aberrations than Ph chromosome can be identified since the whole genome is analyzed. However, cryptic fusions between the BCR and ABL genes can not be detected by karyotyping.
Karyotyping is used to measure the response to therapy. The cytogenetic response is associated with prognostic significance, why this method is used until a complete cytogenetic response (CCyR) = 0% Ph chromosomes, has been achieved.

2) FISH (Fluorescence In Situ Hybridization) (DNA level) is a targeted analysis, showing the gene fusion BCR-ABL. In addition to the typical fusion gene, it is possible to detect cryptic fusions. FISH allows the assessment of a larger number of non-dividing (interphase) cells, both from peripheral blood and bone marrow and is thus more sensitive than chromosome banding analysis (CBA). The method can also be performed on cultured metaphase cells, hypermetaphase FISH.

Probes targeting the ABL and BCR genes are used and nucleated cells are then examined in an epifluorescence microscope. The fusion gene will appear yellow. In interphase FISH it is recommended that at least 200 cells should be analyzed. The sensitivity for FISH is higher than for karyotyping, less than 1 Ph-positive cell per 100 nucleated cells can be detected. The method has so far not been used to give prognostic information similar to the degree of cytogenetic responses using CBA (i.e. complete cytogenetic response, major cytogenetic response etc.)

3) PCR (Polymerase Chain Reaction) (mRNA level) can be performed on bone marrow and blood, usually on blood. Qualitative (RT-PCR) reveals the presence or absence of BCR-ABL mRNA. Quantitative (qRT-PCR), reveals the amount of mRNA. The method is highly sensitive and can detect 1 out of 10⁵ transcripts of mRNA. Q-RT-PCR is the only method that can measure minimal residual disease (MRD), after the patient reaches normal findings by karyotyping (CCgR) or FISH. The results of qRT-PCR are expressed as the ratio BCR-ABL/reference gene (ABL and/or GUS) as a percentage. A problem with the PCR technique is that the methods and results vary between laboratories. An international scale (IS) has been established to make results from different laboratories comparable, by giving each laboratory a conversion factor related to a reference laboratory. All over the world laboratories undergo a standardization work, in order to express their results according to the IS.

Clinical course

The natural course of the CML disease is generally divided into three stages/phases. The disease can present in any phase but most patients (90%) present in the chronic phase, during which mature granulocytes are still produced but with an increased numbers of myeloid progenitors in the peripheral blood. This early phase is often asymptomatic. In the absence of effective treatment, the disease progresses eventually into the more aggressive phases, AP and BP. In the pre-TKI era the average duration of the chronic phase was approximately 3-5 years. The following shorter AP is a poorly defined phase characterized by an increasing number of myeloblasts and basophils in peripheral blood and bone marrow, persistent thrombocytosis, and increasing spleen size unresponsive to therapy. In some cases there are evidence of clonal evolution. The most common cytogenetic events in the clonal evolution are the appearance of +8, +Ph, and i(17q) that denotes the activation of other oncogenes than BCR-ABL or a deletion of tumour suppressor genes. There are several definitions for the AP including the definitions by European Leukemia Net (ELN), World Health Organization...
and the American National Comprehensive Cancer Network (NCCN). In Sweden, the WHO classification from 2008 is the most widely used. The AP is a transition to the final blastic phase. Here the percentage of blast cell in the bone marrow or peripheral blood is increased to ≥20%, and the blasts can either be of lymphoid or myeloid origin. Myeloid BP is the most common BP 50%, lymphoid 25% and undifferentiated BP in 25%. Occasional patients have a combined myeloid/lymphoid CML-BP. In the CML-BP there is a clear maturation stop in the myelopoiesis and the clinical features are more like an acute leukemia. Isolated extramedullary proliferation of blasts that can be seen in the skin, lymph nodes, spleen, bone or in the central nervous system constitutes a CML-BP, independent of the picture in the peripheral blood and the bone marrow.

<table>
<thead>
<tr>
<th>CML-AP may be made when one or more of the following are present:</th>
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<tbody>
<tr>
<td>Blasts 10-19% of WBC in peripheral blood and/or of nucleated bone marrow cells</td>
</tr>
<tr>
<td>Peripheral blood basophils ≥20%</td>
</tr>
<tr>
<td>Persistent thrombocytopenia (&lt;100x10^9/l) unrelated to therapy or persistent</td>
</tr>
<tr>
<td>Thrombocytosis (&gt;1000x10^9/l) unresponsive to therapy</td>
</tr>
<tr>
<td>Increasing spleen size and increasing WBC count unresponsive to therapy</td>
</tr>
<tr>
<td>Cytogenetic evidence of clonal evolution</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CML-BP may be made if one or more is present:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts ≥ 20% of WBC in peripheral blood and/or of nucleated bone marrow cells</td>
</tr>
<tr>
<td>Extramedullary blast proliferation</td>
</tr>
<tr>
<td>Large foci or clusters of blasts in the bone marrow biopsy</td>
</tr>
</tbody>
</table>

Table 1. WHO definition of accelerated phase.

### Prediction of prognosis

At diagnosis the most important clinical prognostic factor for survival is to define whether the patient is in the chronic, accelerated or blastic phase. In chronic phase the risk of progression to a more advanced phase can be calculated by the Sokal and Hasford scores, respectively. The two scores are calculated in a slightly different way, based on age of the patient, spleen size and blood counts. Depending on the results the patients will be designated into one of three risk groups; low, intermediate or high.

The Sokal score uses age at diagnosis, spleen size, platelet count and percentage of blasts in blood. It predicts survival of newly diagnosed CML patients treated with hydroxyurea. Data now indicate that Sokal score also predicts the chance of achieving CCgR and risk of progression to AP/BP in patients treated with imatinib. The Hasford (Euro) score, is a further development of the Sokal score, predicts survival of newly diagnosed CML patients with IFN treatment. It is based on data from 1303 patients treated within twelve different studies. It is possible, but not yet clearly demonstrated, that Hasford score can also be applied to patient groups treated with imatinib. Hasford score is calculated based on age, spleen size, platelet count and percentage of blasts, eosinophils and basophils in peripheral blood at diagnosis.
The EUTOS score is a novel, not yet widely spread score. It is calculated from the percentage of basophils and spleen size and predicts outcome of imatinib therapy.\textsuperscript{48} The score may be used to identify CML patients with significantly lower probabilities of responding to therapy and survival.

The risk scoring systems may have lost some of its impact, since the most important individual prognostic factor today is the degree and timing of the hematologic, cytogenetic and molecular responses.\textsuperscript{37,49}

1.2.2 Epidemiology

CML is a rare disease that has a reported world-wide incidence of 1.0-1.5 cases per 100 000 population per year.\textsuperscript{50,51} The incidence in Sweden during the last 8 year period was 0.8-1.0 per 100 000,\textsuperscript{52} which means that about 90 patients are diagnosed with CML in Sweden annually. CML is diagnosed in all ages, but the incidence increases with age. The median age at diagnosis in Sweden is approximately 60 years. Males are slightly more affected than females (1.3:1).\textsuperscript{51,53} Due to more effective treatment in the last decade, prevalence increases in all Western countries.\textsuperscript{54} No geographical or ethnical differences exist regarding the incidence, but the prevalence differs, likely due to differences in management strategies between countries, depending of availability of expensive drugs, modern diagnostic technologies and health-care system.\textsuperscript{55}

1.2.3 Etiology

Exposure to benzene and ionizing radiation constitute risk factors. Studies have shown that BCR-ABL transcripts can be induced in hematopoietic stem cells by ionizing radiation in vitro.\textsuperscript{56} A sharp increase of the incidence of CML was seen after the atomic bomb in Hiroshima 1945\textsuperscript{57-59}. No similar increase in CML incidence has been seen in connection with the Chernobyl accident in the Soviet Union in 1986, where the radiation doses were significantly lower compared to Hiroshima-Nagasaki.\textsuperscript{60}

An explanation for the spontaneous appearance of the fusion gene BCR-ABL may be the short physical distance between BCR and ABL genes in human lymphocytes\textsuperscript{61} and CD34 + cells that could predispose to translocation between the genes.\textsuperscript{62,63} The mere presence of the BCR-ABL translocation in a hematopoietic cell is not enough alone to cause CML. It is known that the BCR-ABL fusion transcripts of M-BCR and m-BCR type are detected in low frequency among 30\% of healthy individuals.\textsuperscript{64,65} It is unclear why CML occurs in only a minority of these individuals. In healthy subjects the translocation possibly occurs in the terminal portion of maturation and is eliminated by the normal immune system. Indirect evidence for the immune system effects is that certain HLA types (HLA-B8 HLA-A3) appear to protect against CML.\textsuperscript{66} It is also likely that the chromosomal change must occur in a sufficiently primitive hematopoietic progenitor cell, in order for the clone to expand. Another possibility is that BCR-ABL is not the only genetic lesion to induce CML-CP.\textsuperscript{67} However, in the vast majority of patients the etiology of the disease is still unknown and there does not appear to be a hereditary facto
1.2.4 Patophysiology

Blood cells develop from a multipotent hematopoietic stem cell located in the bone marrow. Normally the maturation from the stem cell into functional blood cells is very well regulated. In CML a reciprocal translocation of genes between the long arms of chromosome 9 and 22 has occurred in a stem cell. A part of the Abelson 1 gene (ABL1, hereafter called ABL) on chromosome 9 is translocated and forms a fusion gene with a part of the breakpoint cluster region gene (BCR), located on chromosome 22. The shorter derivate chromosome 22 carrying the BCR-ABL fusion gene, is now called the Ph chromosome t(9;22)(q34;q11).

The normal ABL proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response. The activity of the ABL gene is negatively regulated by its SH3 domain, and deletion of the SH3 domain turns ABL into an oncogene. After the t(9;22) translocation the negative regulation of ABL is lost. The new fusion gene, BCR-ABL encodes an unregulated, cytoplasm-targeted tyrosine kinase that allows the cells to proliferate without being regulated by cytokines. Although the ABL gene product and BCR-ABL fusion protein has been extensively studied, the function of the normal BCR gene product is not clear. Parts of the ABL gene remains on the derived chromosome 9 and parts of the BCR gene translocates to the 9th chromosome, resulting in a ABL-BCR gene. This fusion gene is encoding a protein without any known function.

Fig 2. After the translocation between chromosomes 9 and 22, the BCR-ABL fusion gene undergoes transcription. The resulting BCR-ABL messenger RNA (mRNA) is then translated into the Bcr-Abl tyrosine kinase protein, which enhances cell proliferation, adhesion and survival.
The site of the breakpoint in the BCR gene differs, but in CML it is almost always in the major breakpoint cluster region [M-BCR, BCR-ABL junctions e13a2 (b2a2) and e14a2 (b3a2)]. Rarely the breakpoint occurs in the minor breakpoint cluster region (m-BCR e1a2) as in Ph positive acute lymphoblastic leukemia (ALL), or in the micro-region (µ-BCR, e19a2) as in chronic granulocytic leukemia.\textsuperscript{11}

The BCR–ABL fusion gene is transcribed into a messenger-RNA (mRNA) which will be translated to a protein, a tyrosine kinase called Bcr-Abl, which activates mediators of the cell cycle regulation system, leading to CML. The Bcr-Abl protein has different weight depending of the different breakpoint in the BCR gene, 190kDa (m-region), 210 kDa (M-region) or 230kDa, (µ-region) but typically 210 kDa in CML.\textsuperscript{11}

The Bcr-Abl tyrosine kinase protein is constitutively active, catalyzing the transfer of phosphate from ATP to a tyrosine residue on a substrate protein downstream, resulting in the CML phenotype, i.e. inhibition of apoptosis, increased proliferation and decreased adhesion to stroma cells via effects on Bcr-Abl substrates like CRKL, CBL, RIN, GAP and paxillin and further phosphorylation activates intracellular signal pathways like RAS, MYC and STAT.\textsuperscript{11,74}

The mechanism behind disease progression and clonal evolution, when additional cytogenetic aberrations occur, is still largely unknown. Genetic instability as a consequence of the BCR-ABL translocation might be one explanation, alternatively, the mechanisms responsible for the translocation might trigger the changes leading to progression of the disease.\textsuperscript{55}

Five to 10% of CML patients have a variant translocation, which means that one or two additional chromosomes are involved in the 9:22 chromosomal translocation. All chromosomes have been described as participating in these variants, as an example t(3:9:22).\textsuperscript{75,76}

### 1.2.5 Clinical signs and symptoms

About 20-40% of the CML patients are asymptomatic at diagnosis.\textsuperscript{51} The disease is quite often detected at a routine medical examination. Others have symptoms like fatigue, sweats, weight loss and abdominal fullness. Sometimes symptoms caused by the large number of circulating leukocytes are seen eg. visual disturbances (due to vascular effects, including bleeding in the fundus of the eye), pain in the left flank (due to splenic infarction or enlarged spleen) and priapism.

In CML-CP, the peripheral blood shows an increasing amount of both mature and immature leukocytes together with mild anemia and sometimes increased number of platelets. Atypically the disease presents in CML-AP or CML-BP, without a previously detected CP. The more advanced phases of CML are generally accompanied by worsened performance status and by symptoms related to severe anemia, thrombocytopenia and/or marked splenic enlargement.
### Table 2. Typical full blood count in a newly diagnosed CML-CP patient.

<table>
<thead>
<tr>
<th>Blood count</th>
<th>Reference values</th>
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<tbody>
<tr>
<td><strong>B-Hb (g/l)</strong></td>
<td>105 134-170</td>
</tr>
<tr>
<td><strong>B-Platelets (10^9/l)</strong></td>
<td>694 145-348</td>
</tr>
<tr>
<td><strong>B-Leukocytes (10^9/l)</strong></td>
<td>124 3.5-8.8</td>
</tr>
<tr>
<td>B-Neutrophil granulocytes</td>
<td>47 1.8-7.5</td>
</tr>
<tr>
<td>B-Eosinophil granulocytes</td>
<td>2.5 0.04-0.4</td>
</tr>
<tr>
<td>B-Basophil granulocytes</td>
<td>3.7 0.0-0.1</td>
</tr>
<tr>
<td>B-Lymphocytes</td>
<td>9.9 2.9-4.5</td>
</tr>
<tr>
<td>B-Monocytes</td>
<td>9.9 0.1-1.0</td>
</tr>
<tr>
<td><strong>Immature myeloid cells</strong></td>
<td></td>
</tr>
<tr>
<td>B-Band-formed granulocytes</td>
<td>17.4 0</td>
</tr>
<tr>
<td>B-Metamyelocytes</td>
<td>8.7 0</td>
</tr>
<tr>
<td>B-Myelocytes</td>
<td>14.9 0</td>
</tr>
<tr>
<td>B-Promyelocytes</td>
<td>5.0 0</td>
</tr>
<tr>
<td>B-Blasts</td>
<td>3.7 0</td>
</tr>
<tr>
<td>B-Erythroblasts</td>
<td>1.2 0</td>
</tr>
</tbody>
</table>

**1.2.6 Treatment**

### Tyrosine Kinase Inhibitors

With improved understanding of the molecular mechanism involved in CML, the development of targeted therapies, such as tyrosine kinase inhibitors (TKI) has gradually improved. TKI inhibit the Bcr-Abl protein activity, thus stopping the leukemic phenotype. The first TKI for CML, imatinib mesylate was a major breakthrough in CML treatment. The large phase 3 clinical trial International Randomized IFN versus STI571=imatinib mesylate (IRIS) for newly diagnosed CML-CP patients started in 2000. The superiority of imatinib over IFN soon became obvious. After 12 months of treatment 69% of the imatinib treated patients had achieved CCyR compared to 7% in the IFN group. After 6 years of treatment the OS was 88% (95% when only CML related deaths were considered) in the imatinib arm. Since a majority of the patients (65%) randomized to the IFN arm, crossed over to the imatinib arm mainly due to intolerance it is difficult to correctly compare data between the two arms. However, in the British CML III study the 6-year OS was only 5% for patients treated with the IFN. At a median of 8 years of follow up of the IRIS trial, the estimated OS of all patients randomized to receive imatinib was 85%. When only CML-related deaths was considered the figure was 93%.

Imatinib and later generations of TKI have improved the prognosis for CML patients in all disease phases, but most particularly for patients in CML-CP. TKI induce a much faster reduction of BCR-ABL transcript levels than previously available drug therapies. Today TKI is formally approved as first line treatment for CML-CP in all ages. Currently there are three TKI approved by European Medicines Agency (EMA) and Food and Drug Administration (FDA) as first-line treatment in CML; imatinib, nilotinib and dasatinib. Additional TKIs (bosutinib and ponatinib) are currently being evaluated in clinical trials.
A recent French study has shown that approximately 40% of 100 imatinib treated patients with a deep molecular response could discontinue treatment, without any signs of recurrent disease within 2-3 years.\cite{81,82} Since 2nd generation TKIs as first line treatment seem to generate a larger number of patients with deep molecular response, it is possible that more CML-CP patients can discontinue the treatment in the future. Although imatinib has dramatically increased survival for CML patients in CP the outcome of patients in more advanced phases has not been as successful. A treatment effect can be seen, but is usually transient. TKIs may play a role as a bridge to allogeneic SCT for patients who progress to BP. For patients progressing to AP during imatinib treatment a switch to a 2nd generation TKI may revert the disease into the CP.

**Imatinib** (Gleevec® or Glenvec®) is a small molecule, created by using the structure of the ATP binding site of the ABL protein kinase as a template. It binds to the adenosine triphosphate-binding (ATP) site of Abl, thereby keeping the protein in an inactive form, thus inhibiting the phosphorylation of substrate and subsequent blocking the activity of the Bcr-Abl tyrosine kinase. The transmission of the oncogenic signal to the nucleus is by this interrupted and thereby the malignant transformation, since unphosphorylated Bcr-Abl tyrosine kinase is inactive.\cite{83} Imatinib is relatively specific for Bcr-Abl tyrosine kinase but also active against platelet-derived growth factor (PDGF) receptor\cite{84,85} and c-KIT receptor kinase.\cite{86} In Sweden imatinib was approved for clinical use in 2001 and was the only TKI approved for newly diagnosed CML patients until 2010. The most frequent side-effects are oedema, muscle cramps, rash, nausea, diarrhoea, and hepatotoxicity.\cite{87,88,89}

Imatinib is also used in the treatment of gastrointestinal stroma tumor (GIST)\cite{90,91} in hypereosinophilic syndrome (HES)\cite{92} and chronic eosinophilic leukemia (CEL)\cite{93} with FIP1L1-PDGFRA rearrangement,\cite{92,94,95} (PDGFR) gene, in unresectable dermatofibrosarcoma protuberans (DFSP)\cite{96} and in Ph positive acute leukemias, mainly in ALL.\cite{97,98,99,100} However, except for newly diagnosed CML-CP patients, there are no controlled trials demonstrating a clinical benefit or increased survival for these diseases.

**Dasatinib** (Sprycel®) a second generation TKI, is a dual Abl/Src kinase inhibitor that binds to Abl kinase domain irrespective of the configuration of the activation loop. Dasatinib inhibits a lot more tyrosine kinases than imatinib and nilotinib. Dasatinib was approved in 2006 for CML (CP, AP and BC) with resistance or intolerance to imatinib and to Ph positive ALL in resistance or intolerance to prior therapy. It was approved for treatment of newly diagnosed CML patients in 2010 but the Swedish The Dental and Pharmaceutical Benefits Agency (TLV) does not reimburse the drug as first line treatment. Consequently it is not used beyond clinical trials as first line drug.

The DASISION (Dasatinib vs Imatinib Study in Treatment–Naïve CML patients) study in newly diagnosed patients, has shown a significantly superior response for the dasatinib vs. imatinib with a major molecular response (MMR) rates at 3 years of 68% vs. 55% treated.\cite{101} The rate of transformation to AP or BP was numerically (but not statistically significantly) lower for dasatinib: 4.7% vs. 6.7%.\cite{101} Treatment with dasatinib cause pleural effusion in 14-
26% of the patients, which seems more frequent in patients with previous cardiovascular/pulmonary disease, and autoimmune diseases.

**Nilotinib** (Tasigna®) a second generation TKI derived from imatinib, is a selective Abl inhibitor that binds to the inactive/closed conformation of the Abl kinase that also inhibits c-KIT, ARG, PDGFα and PDGFβ. Nilotinib was approved in 2007 for treatment of CML patients with resistance or intolerance to imatinib and was in 2010 approved for newly diagnosed patients since the large randomized ENESTnd (Nilotinib Efficacy and Safety in Clinical trial-Newly Diagnosed Patients) study showed that nilotinib compared to imatinib resulted in deeper and faster short term treatment responses. The cumulative rates of MMR by 3 years was 70% to 73% with nilotinib (two different doses) and 53% with imatinib, combined with a significantly lower rate of transformation to AP or BP was observed, 2.1-3.2% vs 6.7%, respectively. Nilotinib may cause pancreatitis (about 1%), liver (5-10%), lipase increase (about 10%) and cause hyperglycaemia.

### Table 3. Tyrosine kinase “targets” of imatinib, nilotinib and dasatinib

<table>
<thead>
<tr>
<th>Imatinib</th>
<th>ABL, ARG, BCR-ABL, KIT, PDGFR, DDR1/2, NQO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nilotinib</td>
<td>ABL, ARG, BCR-ABL, KIT, PDGFR, DDR1/2, NQO2</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>ABL, ARG, BCR-ABL, KIT, PDGFR, DDR1/2, SRC, YES, FYN, LYN, HCK, LCK, FRG, BLK, FRK, CSK, BTK, TEC, BMX, TXK, ACK, ACTR2B, ACVR2, BRAF, EGFR/ERBB, EPHA2, EPHA3, EPHA4, EPHA5, FAK, GAK, GCK, HH498/TNN13K ILK LIMK1, LIMK2, MYT1, NLK, PTK6/Brk, QIK, QSK, RAF1, RET, RIPK2, SLK, STK36/ULK, SYK, TAO3, TESK2, TYK2, ZAK</td>
</tr>
</tbody>
</table>

**Treatment objectives**

Today the key objectives of front-line therapy in CML chronic phase are to

1) Prevent progression to the advanced phases (AP and BP)
2) Maximize achievement of CCyR and MMR which gives the patient a platform to achieve CMR and thereby a possibility of drug cessation.

There are three clinical milestones (interim targets) in the treatment response of CML-CP (Table 4)

| 1) Complete hematologic response (CHR) |
| 2) Complete cytogenetic response (CCyR) |
| 3) Deep molecular response, i.e. achieving major molecular response (MMR) and “complete molecular response” (CMR, signifying non-detectable transcripts, normally <MR4.0 – MR5.0). |

The response to TKI treatment is generally fast, with normalization of leukocytosis/thrombocytosis within weeks, followed by a gradual reduction of Ph positive cells in blood and bone marrow until achieving CCyR and MMR/CMR. To guide the physicians European Leukemia Net (ELN) and other organizations give valuable recommendations, which among other things specify treatment goals at certain time points.
In trials, second generation TKIs as first line treatment seem to give a more rapid treatment response and with fewer patients progressing to AP and BP, compared to imatinib, but so far, no overall survival benefits have yet been reported.

<table>
<thead>
<tr>
<th><strong>Definitions of treatment response</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic response</strong></td>
</tr>
<tr>
<td>Complete (CHR):</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Cytogenetic response</strong></td>
</tr>
<tr>
<td>Complete (CCyR)</td>
</tr>
<tr>
<td>Partial</td>
</tr>
<tr>
<td>Minor</td>
</tr>
<tr>
<td>Minimal</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td><strong>Molecular response</strong></td>
</tr>
<tr>
<td>Major (MMR, 3.0 log reduction)</td>
</tr>
<tr>
<td>MR⁴.0 (4.0 log reduction)</td>
</tr>
<tr>
<td>MR⁴.5 (4.5 log reduction)</td>
</tr>
<tr>
<td>MR⁵.0 (5.0 log reduction)</td>
</tr>
</tbody>
</table>

*Table 4. Definitions of treatment response.*

<table>
<thead>
<tr>
<th>Time</th>
<th>Optimal</th>
<th>Suboptimal</th>
<th>Failure</th>
<th>Warnings</th>
</tr>
</thead>
<tbody>
<tr>
<td>At diagnosis</td>
<td>CHR and at least minor CR</td>
<td>No CgR</td>
<td>Less than CHR</td>
<td>High risk. CCA/Ph+</td>
</tr>
<tr>
<td>3 month</td>
<td>At least PCgR</td>
<td>Less than PCgR</td>
<td>No CgR</td>
<td></td>
</tr>
<tr>
<td>6 month</td>
<td>CCgR</td>
<td>PCgR</td>
<td>Less than PCgR</td>
<td>Less than MMR</td>
</tr>
<tr>
<td>12 month</td>
<td>MMR</td>
<td>Less than MMR</td>
<td>Less than CCgR</td>
<td></td>
</tr>
<tr>
<td>Any time</td>
<td>Stable or improving MMR</td>
<td>Loss of MMR Mutations A</td>
<td>Loss of CHR or CCgR Mutations B CCA/ Ph+</td>
<td></td>
</tr>
</tbody>
</table>

*Table 5. Definitions according to ELN, of optimal and suboptimal response, failure and warnings for previously untreated patients with early chronic phase CML, treated with imatinib at certain time points. Mutation A= Still sensitive to imatinib. Mutation B= Poorly sensitive to imatinib. CCA= Clonal chromosome abnormalities.*

**Treatment in AP and BP**

Patients diagnosed in AP may initiate treatment with a 2nd generation TKI. They should be carefully monitored, and if the patients do not reach the criteria for CP, allogeneic SCT should
be considered. For imatinib treated patients progressing to AP a switch to a 2nd generation TKI or SCT is needed.

For patients diagnosed in or that have progressed to BP, the prognosis is very poor. If CML is diagnosed in BP the patients should start TKI treatment in combination with ALL- or AML-like chemotherapy (depending on lymphoid or myeloid phenotype) and be considered for allogeneic SCT as soon as possible. If progressing to BP during imatinib treatment the patients should be prepared for allogeneic SCT if possible, and a switch to high dose dasatinib is then preferred (unless mutations resistant to dasatinib) to optimize the conditions for SCT. AML - or ALL–like chemotherapy could be given before transplantation.

The response to TKI in BP is expected to be transient. Allogeneic SCT has a curative potential for CML and the best results are achieved for patients transplanted in CP.\textsuperscript{115-118} SCT in overt BP is not recommended.\textsuperscript{116}

Treatment failure

If imatinib treatment of a CML-CP patient does not meet the ELN criteria for “optimal” response at a certain time point (see table 1), the response may be regarded as “suboptimal” or as a “failure”. There are several explanations for not achieving optimal response. Both BCR-ABL dependent and independent mechanisms have been suggested; poor compliance, reduced bioavailability, mutations in the Bcr-Abl tyrosine protein pocket, clonal evolution or resistance to the drug etc. When treatment failure is a fact, a change of treatment is needed. Both dasatinib and nilotinib have been shown to have clinical activity in CML patients with intolerance or resistance to imatinib.\textsuperscript{119-125} Another options is allogeneic SCT. In the case of BCR-ABL independent factors, a dose escalation of imatinib may be successful. There are currently about 100 known mutations, rendering imatinib therapy suboptimal or failing. In most cases, 2nd generation TKI can overcome this.\textsuperscript{124,125} Nilotinib is sometimes a better choice than dasatinib, or vice versa, related to specific mutations. Dasatinib thus seems to be more effective than nilotinib in the presence of BCR-ABL mutations such as Y253F/H, E255K/V, F359C/V while in the presence of Q252H, V299L and F317L, nilotinib is preferable to dasatinib.\textsuperscript{126} Only one mutation, T315I (threonine 315 isoleucine) is completely resistant to imatinib, dasatinib and nilotinib.\textsuperscript{127} However, ponatinib, a multi-targeted tyrosine kinase inhibitor, has in an on-going clinical trial shown efficacy against this mutation.\textsuperscript{128} The AP may after a switch of TKI be induced to revert into the CP. In BP the effect of TKIs is very limited.

If any effect, it is not durable, but can act as a bridge to allogeneic SCT, which is the only treatment offering long-term survival for patients in BP.\textsuperscript{129}

Surrogate markers for long-term outcome

CCyR, MMR and CMR are the therapeutic goal in treatment of CML-CP in order to minimize the risk of transformation into advances phases. For many years, the main goal of treatment was to achieve CCyR, since studies had shown that CCyR was associated with survival benefits.\textsuperscript{130-132} With the introduction of imatinib treatment, a majority of the patients achieved CCyR. Many had an even better response, major molecular response (MMR), which gave further improved OS compared to patients with CCyR but without MMR.\textsuperscript{133} Furthermore, 2nd generation TKI (dasatinib and nilotinib) as first line treatment seem to give an even deeper and
faster response, and the proportion of patients achieving MMR is higher at 12 months than with imatinib.\textsuperscript{109,136,137} Today the IRIS study has given the clinicians more than 10 years of experience regarding imatinib treatment. At 5-year the cumulative incidence of CCyR in the imatinib arm was 87%.\textsuperscript{130} At that time 93% of the patients had not progressed to the accelerated or blastic phases. The estimated 5 year OS was 89%.\textsuperscript{130} However at 5 years 31% of the patients in the IRIS study had discontinued imatinib treatment and were censored.\textsuperscript{131} Clinical trials using “intention to treat” criteria may be difficult to interpret as for instance in the IRIS study. The achieved OS is not only reflecting imatinib treatment, but eventually also second line TKI and/or allogeneic SCT, thus the OS in the IRIS study is likely overestimated, reflecting only imatinib treatment.

However, achieving CCyR and MMR seems to give the patients long-term event free and overall survival, but there is also a time aspect when the goals are achieved. Reducing the tumour load is important to reduce the risk of progression to more advanced phases. Having achieved CCyR and MMR the annual risk of progressing is low. For example, in the IRIS study no patient that had achieved MMR at 12 months subsequently progressed into AP or BP in the 5-year follow-up\textsuperscript{130}. Detailed recommendations are available for monitoring and timing of treatment goals, provided by groups and individual centers. In Sweden the national recommendations\textsuperscript{138} are used which are similar to the guidelines of ELN. To further improve the outcome, we probably need to act earlier in the disease process, at an earlier time than guidelines advise us today.

1.3 SWEDISH POPULATION REGISTRIES

Since 1947 all Swedish citizens are given a unique identification code at birth. For each individual, date of death is centrally registered in the nation wide “Cause of Death Registry”\textsuperscript{139}. Furthermore, every physician, pathologist and cytologist are obliged by law to report occurrence of cancer to the population-based national wide “Swedish Cancer Registry”\textsuperscript{140} established in 1958. Through these high quality registers it is possible to follow patients from the date of cancer diagnosis to death. The Swedish CML Registry, established in 2002, collects information once a year from physicians treating CML patients throughout the course of the disease. The registry is unique, with nearly all CML cases in Sweden included.
2 AIMS

Overall aims
To improve management of patients with CML by
i) identifying early biological markers linked to long-term clinical outcome of imatinib treatment and
ii) establishing population-based survival data including health economic aspects with special focus on imatinib treatment

Specific aims
I. To evaluate the impact of early reduction of disease burden on long-term outcome (i.e. landmark analysis) in imatinib treated patients.

II. To evaluate the effect of imatinib treatment on neutrophil leukotriene signaling and assess its possible role as a clinical marker of CML treatment response.

III. To define real life outcome of patients with CML in Sweden during four decades and to relate the survival patterns to imatinib treatment and other management strategies.

IV. To evaluate health economic aspects of real life treatment of CML in Sweden, comparing imatinib with previous and alternative therapeutic strategies.
3 STUDY OF PROGNOSTIC FACTORS-LANDMARK ANALYSIS (I)

3.1 METHODOLOGICAL ASPECTS

Imatinib has dramatically improved the outcome in CML-CP, but still some patients continue to respond sub optimally or become resistant to imatinib. These patients need alternative treatment with an early switch to 2nd generation TKI or allogeneic SCT. Standard dose imatinib (400mg q.d.) has been reported to induce CHR in the majority of patients and CCyR in 69% within 12 months of treatment, but a minority of the patients still does poorly. In the IRIS study, 34% of the patients had discontinued their initial imatinib therapy at 5 years because of different reasons, mostly intolerance or resistance. Seven percent of the IRIS study patients had progressed to AP or BP within 2 years. The risk of progression seems to peak during the first year of treatment, before the tumour burden radically has been decreased. Apparently, we need to act early of signs of non-optimal response, to improve outcome in imatinib treated patients.

CBA has for many years been the gold standard for evaluating treatment effects and to reach a status of non-detectable Ph-chromosomes (CCyR) by 12 months, has been regarded as one of the treatment goals. Since 30% of the patients do not reach that goal it is of interest to early identify patients having an increased risk of progression of the disease.

In an attempt to identify markers of early non-responders, we followed 45 newly diagnosed CML-CP patients initiated on imatinib treatment at standard dose at Karolinska University Hospital. We evaluated the treatment responses by repeated FISH, PCR and cytogenetics assessments initially at 3 months intervals.

3.1.1 Cytogenetic Banding Analysis (CBA)

Conventional cytogenetic analyses of bone marrow samples were performed at the Department of Clinical Genetics, Karolinska University Hospital. All investigations were done using standard G-banding technique and followed the rules of International System for Human Cytogenetic Nomenclature, ISCN. Cytogenetic response evaluations were regularly based on full analysis of at least 20 metaphases. Of a total of 257 samples in this study, 246 contained 20 or more metaphases, with a median of 29 metaphases analysed per sample.

3.1.2 Fluorescence In Situ Hybridization (FISH)

DNA-probes directed against the BCR and ABL genes are added to a smear of blood or bone marrow cells and are analyzed in an epifluorescence microscope. The reliability of FISH is largely dependent on the quality of the DNA probe used. Different probes show different signal patterns. The Extra Signal Dual Color Translocation Probe-FISH (ES-FISH) reduces the number of falsely BCR-ABL positive cells, compared to the earlier used Dual Color Single Fusion Translocation Probe-FISH (S-FISH). Our control studies showed that the ES probe, in our hands, had high sensitivity and specificity, similar to the Dual Color Dual
Fusion translocation Probe (D-FISH). One disadvantage with ES-FISH compared to D-FISH is that Ph-clones with minor deletions of the long arm of chromosome 9 can be missed because these aberrations will give the typical signal pattern 1G1O1F.

In our study we performed interphase FISH on unseparated nucleated cells on bone marrow smears, using an extra signal dual-color DNA probe (LSI BCR-ABL ES Dual Color Translocation Probe, Vysis, IL). The probe is a mixture of the LSI ABL probe labeled with spectrum orange and the LSI BCR probe labeled with spectrum green. The spanning ABL probe is approximately 650kb extending from an area centromeric of ASS gene well telomeric of the last ABL exon. The spectrum green BCR probe is approximately 300 kb beginning between BCR exons 13 and 14 (M-BCR region) A cell lacking the BCR-ABL fusion gene will exhibit a two orange and two green signal pattern (2O2G). In a cell carrying the fusion gene one green (native BCR) one large orange (native ABL), one smaller orange (rest signal of ABL on the derivate chromosome 9) and one fused green/orange (BCR-ABL) (2O1G1F).

On each patient smear a median of 400 nucleated cells were analysed.

Figure 3. A BCR-ABL positive cell with the typical ES probe signal pattern 2O1G1F. (Photo Ingrid Arvidsson)

Control studies for FISH

Bone marrow smears from nine Ph-negative controls were analyzed using the ES probe. In each case 500 cells were scored. The mean percentage of “not true” BCR-ABL positive cells among these controls was 0.031% (SD 0.075%) For the purpose of our study (I) we decided to define the cut-off level for a “true” positive sample to ≥0.25% (mean +2.576xSD).
Further cytospin preparations from the CML cell line K562 were used as positive controls. All (100%) of 2500 K562 cells examined with the ES-probe depicted the typical fusion pattern (2O1G1F).

To compare DS-FISH with ES-FISH, we analyzed nine patient samples with both probes. We found a strong correlation (r= 0.87; p= 0.0003).

### 3.1.3 Quantitative reverse-transcriptase Polymerase Chain Reaction (qRT-PCR)

The Department of Clinical Genetics, Karolinska University Hospital, Stockholm, performed the PCR analysis. For samples prior to October 2008 peripheral blood mononuclear cells were separated by the Ficoll-Paque™PLUS (GE Healthcare, UK) and total RNA was isolated using Trizol® reagent (Invitrogen, Carlsbad). From October 2008 and onwards, total blood leukocytes were used for RNA isolation. RNA (1µg) was reverse-transcribed into cDNA as described elsewhere using pd(N)6 random hexamer (GE Healthcare, UK) and M-MLV enzyme (Invitrogen, Carlsbad). ABL and GUS were used as control genes to correct for variations in RNA quality and quantity. The BCR-ABLp210 and BCR-ABLp190 mRNA transcripts were assessed by quantitative real-time RT-PCR, as described previously. ABL and GUS were used as reference genes. In this studies, we consequently expressed the amount of BCR-ABL transcript as a ratio of BCR-ABL copy number relative to 100 ABL copies, since the IS not yet was established in our laboratory at that time.

### 3.1.4 Statistical methods

The probabilities of OS and event free survival (EFS), where events were defined as response loss, progression or death, in line with ELN recommendations. OS and EFS were calculated using Kaplan-Meier method according to the intention-to-treat principle. The association between early determinations of BCR-ABL or Ph-expression, as assessed by the three different techniques and the long-term clinical outcome was examined using contingency tables and Fisher’s exact test.

### 3.2 RESULTS AND DISCUSSION

The median follow up was 58 (range 15-115) months. Within 12 months of treatment 80% achieved CCyR and 42% MMR. Corresponding figures at 24 months were 97 and 84%. After 3 months of treatment the median BCR-ABL expression was 9.8 (range 0-95)% according to FISH and 16.0 (range 0-100)% according to PCR. Corresponding figures for 6 months were 0.25 (range 0-65)% for FISH, 1.2 (range 0-85)% for PCR and 0 (range 0-100)% for CBA. Four patients (8.8%) progressed to AP within 16 months. One patient later progressed into BP after initially having achieved CCyR.

In a landmark analysis, an early favourable response, defined as < 10% BCR-ABL positivity by FISH after 3 month of treatment, was identified as a predictive marker of an improved long-term clinical outcome. Thus of evaluable patients, 51% achieved this response. Ninety-five per cent of such responders reached CCyR within 12 months and 100% had an event-free survival
at 48 months as compared to 67 and 65%, respectively, of patients with higher (≥ 10%) BCR-ABL positivity at 3 months.

<table>
<thead>
<tr>
<th>3-month landmark</th>
<th>BCR-ABL positive cells by FISH</th>
<th>BCR-ABL mRNA by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10%</td>
<td>≥10%</td>
</tr>
<tr>
<td>CCyR at 12 months</td>
<td>18/19</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>EFS at 36 months</td>
<td>19/19</td>
<td>12/18 (67%)</td>
</tr>
<tr>
<td>EFS at 48 months</td>
<td>19/19</td>
<td>11/17 (65%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>6-months landmark</th>
<th>BCR-ABL positive cells by FISH</th>
<th>BCR-ABL mRNA by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10%</td>
<td>≥10%</td>
</tr>
<tr>
<td>CCyR at 12 months</td>
<td>21/24</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>EFS at 36 months</td>
<td>21/24</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>EFS at 48 months</td>
<td>21/24</td>
<td>2/5 (40%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>12-months landmark</th>
<th>BCR-ABL positive cells by FISH</th>
<th>BCR-ABL mRNA by PCR</th>
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<tbody>
<tr>
<td></td>
<td>&lt;10%</td>
<td>≥10%</td>
</tr>
<tr>
<td>EFS at 36 months</td>
<td>17/18</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>EFS at 48 months</td>
<td>17/18</td>
<td>0/3 (0%)</td>
</tr>
</tbody>
</table>

Table 6. Number of patients with CCyR at 12 months, EFS at 36 months and EFS at 48 months, related to responses at 3 and 6 months assessed by FISH and PCR.

We showed that significantly more patients with a reduction of the malignant clone to <10% at 3 months of imatinib treatment achieved CCyR within 12 months compared to those that failed to reach this level of reduction. They had also a significantly better EFS both at 36 and 48 months compared to patients with BCR-ABL ≥10% by FISH at 3 months. This finding is consistent with other research groups,¹⁵¹,¹⁵² using the qRT-PCR method, observing that patients with PCR levels ≥10% at 3 month are likely to respond poorly on imatinib therapy. Marin et al found that patients with transcript levels of more than 9.84% (n = 68) at 3 months had significantly lower 8-year probabilities of OS (56.9% v 93.3%; p <0.001), progression-free survival, cumulative incidence of CCyR, and CMR than those with lower transcript levels.¹⁵¹ Hanfstein et al found persistence of BCR-ABL transcript levels >10%, according to the IS at 3 months, to separate a high-risk group (28% of patients; 5-year OS: 87%) from a group with 1-10% BCR-ABL.¹⁵² In contrast, when we performed landmark analysis on our patient cohort with PCR, we did not observe the same predictive value as with FISH. The fact that we did not use IS in our study may explain why we did not find the same predictive value with PCR<10% as other groups have. Although PCR has become the dominating method for monitoring TKI treatment, particularly to detect minimal residual disease (MRD), the technique can be inconsistent with considerable intralaboratory and interlaboratory variations. Thus, this will be a minor problem when laboratories have harmonized the PCR standard to the IS. Despite this, many physicians do not have access to laboratory service with the semi-quantitative PCR method. The well-established FISH method could then be of great value.
In our study four patients (8.8%) progressed to AP within 16 months using the WHO classification, which is slightly more than is reported in the IRIS trial where 7.9% progressed within 18 months.\textsuperscript{22} However, in the IRIS study, a different definition of AP and BP was used. In the IRIS study definition of AP was $\geq 15\%$ blasts in peripheral blood or bone marrow and for BP $\geq 30\%$ blasts, compared to 10-19\% and $\geq 20\%$ respectively, in the WHO classification. If WHO classification would have been used in the IRIS study, probably more patients would have been considered to have progressed to AP or BP.

All of our four patients had clonal evolution in their Ph+ cell clones. Thus, undoubtedly, they had progressed to AP. They were all scored as Sokal intermediate risk at diagnosis. Two of these four patients regained chronic phase after treatment with second generation TKI, while the remaining two died. All patients with Sokal high-risk score achieved CCyR within 12 months, although high-risk patients in general have an increased risk of progression.\textsuperscript{130,153 154}

Our data, even though based on a limited patient cohort, indicate that FISH can effectively be used in the early assessment of remaining Ph-positive cells to identify patients at risk for a long-term non-optimal response to imatinib. In clinical practice; sufficient data presently indicate that a change of treatment to 2nd generation TKI should be considered if BCR-ABL levels persist above 10\% after 3 months of treatment with imatinib, as measured with PCR or FISH or PCR.
4 STUDY OF PROGNOSTIC FACTORS - LEUKOTRIENE SIGNALING (II)

4.1 BACKGROUND

Leukotrienes (LTs) are a family of lipid mediators derived from arachnoid acid with important signaling molecules in inflammatory and allergic conditions.\(^{155-158,156-159}\) Leukocytes can form various types of leukotrienes (hence their name) via an initial conversion of arachnoid acid to LTA\(_4\), a reaction mediated by the 5-lipoxygenase (5-LO) enzyme. Members of our group have previously reported data indicating a role for certain leukotrienes also in CML. Thus, we have found that the bioactive cysteinyl-containing leukotriene C\(_4\) (LTC\(_4\)) can stimulate normal human myelopoiesis\(^{160}\) and that myeloid cells from CML patients have a markedly increased capacity to synthesize LTC\(_4\).\(^{160,161}\) Moreover, neutrophils from CML patients were noted to possess significant leukotriene C\(_4\) synthase (LTC\(_4\)S) expression and activity, while this is not the case for normal neutrophils.\(^{162,163}\) Recently, the 5-LO was suggested as a crucial growth regulator for leukemia stem cells (LSC) in CML, since gene expression profiles using DNA micro array technique identified Alox5, the gene encoding 5-LO, as highly and selectively expressed in LSC. Blocking the Alox5 activity impaired LSC function and prolonged survival in mice with CML.\(^{164}\) These data indicate a role for Alox5 and LTC\(_4\) in CML leukemogenesis.

In this study we examined and followed the response of imatinib treated CML-patients over time, to analyse whether imatinib could regulate neutrophil LTC\(_4\)S expression and activity in vivo and if so, whether such changes were paralleled by changes in the expression of BCR-ABL. If so, a normalization of neutrophil LTC\(_4\)S and/or activity might be used as a novel indirect biomarker of early treatment efficacy. Furthermore, we also aimed at estimating the LTC\(_4\) production in vivo, by assessing urinary excretion of the LTC\(_4\) metabolite LTE\(_4\) in individual patients with CML and healthy controls.
Fig 4. Enzymatic biosynthesis of leukotrienes from arachidonic acid. 5-LO= 5-lipoxygenase. LTC₄S=Leukotriene C₄ synthase.

4.2 PATIENTS AND METHODS

We sequentially studied the activity and expression of LTC₄S in neutrophils from CML patients subjected to imatinib treatment, and related these data to the parallel development of BCR-ABL-expression. A total of eleven CML-CP patients, three newly diagnosed and eight with previous IFN therapy, were assessed. Blood samples were drawn before start of imatinib therapy and then after 2 weeks, 1, 4, 6 and 9 months of treatment. Peripheral blood CD16⁺ neutrophils were isolated using magnetic cell sorting (MACS). Their LTC₄S activity assessed (i.e. Formation of LTC₄ from LTA₄) as measured by high performance liquid chromatography, (HPLC) and LTC₄S expression determined at the protein (immunoblot) and mRNA (RT-PCR) levels. In parallel, Ph-chromosome expression was assessed by bone marrow cytogenetics and by performing FISH on smears of peripheral blood identifying and specifically scoring neutrophilic granulocytes by a combined May-Grünwald-Giemsa staining and regular FISH technique cells, which makes it possible to allocate specific chromosomal aberrations (in this study BCR-ABL) in morphologically defined cells. ¹⁶⁵,¹⁶⁶
4.2.1 Statistical methods

The two-sided Student’s test for unpaired samples was employed to compare mean values of LT production in the different cell fractions.

4.3 RESULTS AND DISCUSSION

We demonstrated that in vivo-treatment of CML patients with imatinib led to a normalization of the aberrant LTC₄S expression and activity. In general, this normalization occurred in parallel to a reduced DNA-expression of BCR-ABL, but the time courses of these changes were different. Already after 14 days of imatinib treatment we found a significant decrease of LTC₄S activity in the neutrophils while the BCR-ABL expression remained unaltered in these cells at the same time point. We propose that a block of p210/Bcr-Abl protein activity in neutrophils by imatinib blocks down-stream 5-LO/Alox5 activity and thereby LTC₄S and LTC₄ formation. This can occur in CML cells that still express the Ph chromosome. Later, when imatinib treatment has resulted in replacement of Ph-positive CML cells with normal Ph-negative cells, LTC₄S activity remains low or non-detectable while FISH and cytogenetics show no or low BCR-ABL expression.

Thus, it is possible that analyses of neutrophil LTC₄S and/or LTC₄ expression may serve as early biomarkers of initial phase clinical imatinib response in CML-CP. Although TKIs are the standard treatment of CML today, resistance to these drugs remains a problem for certain patients. Targeting complementary, aberrant signaling pathways down-streams of or parallel to Bcr-Abl tyrosine kinase may provide a constructive option.

Furthermore, urine samples from 23 CML-CP patients were analyzed and compared to urine samples from 18 healthy controls to look for differences in LTE₄ excretion, which is considered to represent the combined in vivo formation of the LTC₄, LTD₄ and LTE₄, the cysteinyl LTs (cysLTs) in humans. Notably, a significantly higher level of LTE₄ was observed in the CML group. This indicates indeed a higher in vivo production of LTC₄ in the leukemia patients and further supports a pathophysiological role for the cysLTs in CML.

The recent results by Chen et al demonstrating that blocking the 5-LO with the asthma drug zileuton inhibited the leukomogenesis in CML induced mice are consistent with our findings of a relationship between 5-LO, LTC₄ and BCR-ABL in CML. An ongoing clinical study in USA is examining the effect of combining the 5-LO inhibitor zileuton with imatinib in newly diagnosed CML-CP patients. This study is planed to be completed in 2014.
Figure 5. LTC⁴S activity in CD16 (+) neutrophils (left panel) and the parallel occurrence of BCR-ABL, investigated by FISH (right panel) from CML patients before, after 7 and 14 days of treatment with imatinib. The lines represent individual patients and the bars show mean± SEM (n=4) of experiments performed in duplicate.
5 POPULATION-BASED STUDIES OF SURVIVAL IN CML (III)

5.1 PATIENTS AND METHODS

Our aim was to assess trends in survival, among all patients diagnosed with CML in Sweden, regardless of clinical trial enrollment, during a 36-year period. In the 1970s, at the start of the study period, busulphan was the dominating therapeutic agent. It was followed by a more widespread use of hydroxyurea and the introduction of IFN and allogeneic SCT. Most importantly, imatinib was available in study protocols from 2000 and it was approved by the Swedish Medical Products Agency (MPA) in November 2001 for CML patients in advanced phase CML and in “interferon failing” CP.

We included information on all newly diagnosed CML patients (n=3173; 1796 males and 1377 females; median age 62 years), reported to the Swedish Cancer Registry140 from 1973 to 2008 using the International Classification of Diseases Version 7 (ICD 7). The diagnosis was coded as 2051. Patients in all phases of CML were included. The exact proportion of patients diagnosed in the advanced phases is known only for period the 2002-2008: 4% in AP and 3% in BP. The Swedish CML Registry, to which more detailed information about disease characteristics is reported, was established in 2002,53. Information from the Swedish Cancer Registry140 included date of birth, gender, date of diagnosis, region, and hospital where the diagnosis was made. All patients were observed from the date of diagnosis until death, emigration, or end of follow-up (December 31, 2009). We excluded individuals who were diagnosed incidentally at autopsy. The choice to include patients from 1973 was based on the fact that by then, the Swedish Cancer Registry, established 1958, had reached a high rate of coverage.168,169 Patients with a preceding cancer diagnosis, including those with a hematological malignancy, were included. Date of death was obtained from Cause of Death Registry.139 Information of the use of upfront imatinib was collected from the Swedish CML registry. Information on the number of SCTs performed in CML patients during this time period was obtained from the registry of the European Group for Blood and Marrow Transplantation (EBMT), established in 1974.

5.1.1 Statistical methods

Relative survival ratios (RSR) were computed as measures of CML survival.170,171 The important advantage of using RS is that it does not rely on the accurate classification of cause of death. Instead, in this project it provides a measure of total excess mortality associated with the diagnosis of CML, irrespective of whether the excess mortality was a direct or indirect result of the cancer (here CML). As such, the RSR allows capturing the excess mortality resulting from, e.g. infection or second primary malignancies, which is not possible when using cause-specific survival. The RSR is defined as observed survival in the patient group (where all deaths are considered as events) divided by the expected survival of a comparable group from the general population, which is assumed to be free from the cancer in question. One, 5 and 10 year RSRs can be interpreted as the proportion of patients with CML who survived the malignancy at 1, 5 and 10 years, respectively. Expected survival was estimated using the Ederer II method172 from
Swedish population life tables stratified by age, sex, and calendar period. One, 5, and 10 year RSRs were calculated for patients diagnosed during five calendar periods and five age categories. Poisson regression was used to model excess mortality\textsuperscript{173} to estimate the effects of the factor described, while controlling for potential confounding factors. Parameter estimates from this model are interpreted as excess mortality rate ratios (EMRRs). An EMRR of e.g. 1.5 for men/women indicates that men experience a 50% higher excess mortality rate (difference between observed and expected mortality) than women. All calculations were performed using Stata 11 (StataCorp, College Station, TX).

5.2 RESULTS AND DISCUSSION


Relative survival improved with each calendar period, with the greatest improvement between 1994-2000 and 2001-2008. Five-year cumulative relative survival ratios (95% Cls) were 0.21 (0.17-0.24) for patients diagnosed 1973-1979, 0.23 (0.20-0.27), 0.37 (0.33-0.41) 0.54 (0.50-0.58) 1994-2000, and 0.80 (0.75-0.83) for 1980-1986, 1987-1993, 1994-2000 and 2001-2008, respectively. This improvement was confined to patients younger than 79 years of age. Five-year RSRs for patients diagnosed from 2001 to 2008 were 0.91 (95% CI, 0.85 to 0.94) and 0.25 (95% CI, 0.10-0.47) for patients younger than 50 and older than 79 years, respectively. Men had inferior outcome. Upfront overall use of imatinib increased from 40% (2002) to 84% (2006). Only 18% of patients older than 80 years of age received imatinib as first-line therapy.

Throughout all calendar periods age was a strong predictor of survival, with superior survival in the youngest patients. In analyses including age and period of diagnosis, RS clearly improved with calendar period in all age groups, but was most pronounced in patients younger than 79 years of age, particularly those 70 to 79 years of age. The use of imatinib as first line treatment was confirmed by data from the national CML registry. Imatinib was used in 40% of the newly diagnosed CML patients in 2002, in 78% 2004, and in 90% in 2007. This large population-based study reveals a major improvement in outcome of patients with CML up to 79 years of age diagnosed from 2001 to 2008, mainly caused by an increasing use of imatinib. The elderly patients still had a poor outcome, partly because of a limited use of imatinib. However, according to a later report from the Swedish CML registry\textsuperscript{53}, the primary use of imatinib (or other TKI) in patients >80 years of age, diagnosed 2007 - 2010, has increased to 72%, showing an attitude change in the approach to the treatment of CML in the elderly, in favor of more active treatment.
Figure 6. Cumulative relative survival ratios by calendar period of diagnosis.

Figure 7. Cumulative relative survival by age (years) and period of diagnosis.
6 HEALTH ECONOMIC ASPECTS OF CML TREATMENT (IV)

6.1 PATIENTS AND METHODS

Medical advances have improved the outcome for patients with CML, especially during the most recent decade. The tyrosine kinase inhibitor imatinib was introduced in the treatment of CML 2000-2001 and has been a breakthrough in treatment of CML. Imatinib allows a faster, deeper and more pronounced reduction of BCR-ABL transcript levels than previously available drug therapy. This is associated with a lower rate of progression and an improvement of survival leading to an increased prevalence. One disadvantage with imatinib and other TKIs is that they are costly to the society. We wanted to compare the costs of different decades and to relate the costs to the expected improved survival. Using the unique real world national data from CML patients diagnosed in Sweden from 1973 to 2008, (n=1778) we evaluated the incremental cost-effectiveness ratio (ICER) between periods associated with broad implementation of imatinib, (Glivec®), IFN and allogeneic SCT, and symptomatic treatment, respectively.

6.1.1 Statistical methods

A lifetime cohort cost-effectiveness model with three age cohorts (<50; 50-69; and 70+ years) diagnosed during three calendar periods (I: 1973–1979; II: 1991–1997; III: 2002–2008) was developed. The number of patients diagnosed in each calendar period was I: 609, II: 576, III: 593. The three periods were selected in order to capture broad implementation of three different treatment therapies prevailing at the respective time period, namely; I. Busulphan II. Allogeneic SCT or a combination of IFN and hydroxyurea and III. Imatinib. The stratification of data into age cohorts reflected the fact that treatment decisions were made partly with respect to age, where patients eligible to allogeneic SCT were typically younger than 50 years. The model allowed separate distributions of therapies (type, resource use) and outcome (survival, quality of life) for each age cohort and period (I-III)

We compared the quality adjusted life years (QALYs) and lifetime costs for an average person if treated for CML with therapies as provided in each of the three periods. The value of continued medical advances was compared by calculating the incremental cost-effectiveness ratio (ICER) per QALY gained for each period compared to the previous period. For example, the ICER of period III versus period II was:

\[
ICER = \frac{\text{Lifetime costs (period III)} - \text{Lifetime costs (period II)}}{\text{QALYs (period III)} - \text{QALYs (period II)}}
\]

We applied a health-sector perspective and a societal perspective. All prices reflected Swedish prices in year 2011 and were expressed in pound sterling (1GBP=10.4 SEK). Costs and health gains were discounted by 3% following Swedish health economic guidelines. Survival data from all patients included was obtained from the national wide Swedish Cancer Registry and Cause of Death Registry. All patients were followed from date of diagnosis until death, emigration or end of follow up, December 31st 2009. Health-care costs for each therapy were
estimated from Swedish clinical practice, following the Swedish guidelines. We included resources as blood tests, bone marrow tests, chromosomal analyses, qRT-PCR, allogeneic SCT, special visits, inpatient days, and costs for the drugs (busulphan, IFN, hydroxyurea and imatinib) and finally care related to BP. According to Swedish health economic guidelines, economic evaluations should also consider the effect of net consumption (total consumption minus total production) from increased survival. The health-sector perspective only includes health care costs while the societal perspective includes both health care costs and net consumption.

A recently published study by Mahon et al has suggested that imatinib may be successfully discontinued in a subpopulation of patients with excellent treatment response showing no PCR-detectable minimal residual disease. Furthermore, since the imatinib (Glivec®) patent in Sweden will expire in 2016, opening the market for biosimilars, it is estimated that the overall drug costs for CML may then be reduced by approximately 30-80%. To compute these future perspective we also analysed two different, possible upcoming scenarios A) Discontinuation of imatinib and B) Price reduction of imatinib.

6.2 RESULTS AND DISCUSSION

We observed substantial health gains over time, paralleled by increased treatment costs. The mean survival was 2.9, 9.2 and 18.5 years during periods I-III, respectively. The resulting ICER was £45 700 per QALY gained comparing periods III and II using a societal perspective and a similar result adopting the limited health-sector perspective. In a separate analysis by age groups showed lower ICERs for individuals <50 years at diagnosis: £38 500 for the societal perspective and £47 700 per QALY gained for the health-sector perspective. Since the prevalence of CML patients is increasing and assuming that 75% of each incident cohort was to receive imatinib at current prices, the imatinib budget would need to double by 2050. A future potential discontinuation of imatinib for selected excellent responders would reduce the ICER per QALY gained. Reduced drug cost of imatinib linked to the patent expiry of Glivec will probably have a greater impact of ICER per QALY. An estimated price reduction of 80% (global competition) or 30% (expected change for biological drugs) would be associated with an ICER of £20 000 and £36 000, respectively, per QALY gained.

Our study focused on imatinib treated patients. However, imatinib has for the last 5 years gradually been replaced by 2nd generation TKIs for many patients, partly as second line treatment because of intolerance or resistance to imatinib and partly as first line treatment, as trials have shown that these drugs provide a faster and deeper molecular response. This change in prescribing pattern is not taken into account in this analysis.

Nevertheless these health economic analyses reveal a dramatically prolonged survival for CML patients during four decades of development, paralleled by incremental cost-effectiveness ratios at levels generally accepted by some, but not all Western national health authorities.
Figure 8. Prevalence of CML per 100 000 inhabitants year 1980-2050 and the predicted drug costs for imatinib years 2008-2050 using three levels after patent expire in year 2016.
7 SUMMARY AND CONCLUSIONS

I
Our landmark data indicate that ES-FISH may be used already after 3 months of imatinib treatment, to identify a patient cohort with inferior long-term EFS. From our data FISH appears to be more sensitive than PCR. The observation that PCR did not give the predictive values as other groups have found, may be explained by the fact that we did not use the IS. Of patients with FISH levels < 10% after 3 months of imatinib treatment 95% achieved CCyR within 12 months and 100% had an event free survival at 48 months. FISH can effectively be used in the early assessment of remaining Ph-positive cells to identify patients at risk for a long-term non-optimal response to imatinib.

II
Neutrophils from CML patients showed an increased expression and activity of LTC₄S. In vivo treatment with imatinib normalized this pattern. This normalization occurred faster than the cytogenetic response. The observation indicates that expression and activity of LTC₄S may be a down-stream step in the signal transduction events initiated by the Bcr-Abl tyrosine kinase. It is possible that an early evaluation of LTC₄S expression during imatinib treatment could be a more rapid way of assessing treatment response than by conventional methods identifying BCR-ABL expression (through CBA, FISH or qRT-PCR).

III
Survival in CML has improved significantly since the 1970s. In all calendar periods, age was a strong predictor of survival, with superior survival for the youngest patients. In analyses including age and period of diagnosis, RS improved with calendar period in all age groups, but most pronounced in patients younger than 79 years of age, particular those 70-79 years of age. We observed a moderate but statistically significant better survival for women, when adjusted for age and calendar period.
The improvement of survival started 1987-1993. Major contributing factor to the improvement observed were an increasing number of allogeneic SCT, the introduction of IFN, better supportive care and more aggressive treatment in both chronic and accelerated phases.
Survival among all age groups was greatest in the last calendar period, but patients older than 79 still do poorly. The Swedish CML registry data shows that patients diagnosed 2002-2008, 70-79 years old received TKIs in 66% and patients >80 years in only 18%. This observation has resulted in an increased use of the TKIs in elderly patients. A recent report from the CML registry, reveals that the primary use of imatinib in patients >80 years diagnosed 2007-2010 has increased to 72%. This shows an attitude change in the approach to treatment of CML in elderly individuals.

IV
Substantial health gains were noted over the three calendar periods (I: 1973-1979, II: 1991-1997, and III: 2002-2008), paralleled by increased treatment costs. The mean survival was 2.9,
9.2 and 18.5 years during periods I-III. The health economic analyses reveal dramatically prolonged survival for CML patients during the four decades, with incremental cost-effectiveness ratios at levels generally accepted by Swedish and some but not all Western national health authorities.
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