Prognostic factors in Chronic Myeloid Leukaemia therapy

MD (Res) thesis

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To my

Grandparents

With this work, their grandson

made one of their dreams

come true...

Acknowledgements

First of all, I am greatly indebted to God, for facilitating the means and giving me the strength to finish this work. It is with great pleasure that I thank all those who made this work possible. I would like to express my deepest and honest thanks to my supervisor, Dr David Marin, who through his continuous encouragement and motivation made this work see the light. His sometimes untraditional teaching methods and learning techniques proved to be efficient not only to my haematology experience, but for my benefit later in life. I would also like to thank my co-supervisor, Prof Letizia Foroni for her guidance and support.

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Declaration of originality

I, Amr Reda Ahmed El Bahy Ibrahim, declare that the work presented in this thesis is my own and that it has not been submitted elsewhere. I have referenced all other material, and clearly indicated the work of others whenever possible.

Contributions from others

The work included in this thesis was carried out at Department of Haematology, Hammersmith Hospital, Imperial College London during the period from February 2009 until June 2011 under the supervision of Dr David Marin and Professor Letizia Foroni. Patients were followed up by the consultants and physicians at the Hammersmith Hospital. Data collection was done by me and others that contributed as the database is almost 10 years old. The cytogenetics data was provided by the Cytogenetics unit headed by Dr Alistair Reid, while the PCR data was provided by the MRD research unit at the Hammersmith Hospital headed by Prof Letizia Foroni.

Abstract

Chronic myeloid leukaemia (CML) is a chronic myeloproliferative disease that is characterized by the presence of the *BCR-ABL1* protein, with subsequent increased tyrosine kinase activity. The Introduction of imatinib and second generation tyrosine kinase inhibitors revolutionized CML therapy; however, prognostic factors that can predict disease outcome are still measured using scores that have been developed during the chemotherapy and IFN-α era. This raises the need for modern scores that match the current era. The EUTOS score that has been recently introduced by the ELN promised significant accuracy in determining patients' outcomes. One drawback however is that the EUTOS score hasn't been independently validated; hence it failed to predict outcomes in our patient sample. Knowing that the achievement of an early response is an important factor in determining outcomes, we investigated the use of molecular markers as a prognostic factor and we managed to prove that the measurement of transcript levels at 3 months is the single most important factor to determine patients' outcomes. Current therapy has managed to convert CML into merely a chronic illness in most of the cases, therefore patients and clinicians are faced by problems that are characteristic of long term therapy, including issues of non-adherence and fertility. We managed to show that non-adherence to imatinib can lead to significant loss of responses and treatment failure. We also managed to show that an adequate response is required prior to imatinib interruption for those women who wish to conceive. Unfortunately, not all patients manage to achieve responses on imatinib, and therefore need to change to second line therapy. Funding agencies argue about the survival benefit of second generation TKIs, so here we don't only show that second generation TKIs offer a survival benefit, but also that the responses achieved on 2G – TKIs are also durable and long lasting. Finally, we explore of the use of TKIs as third line therapy in CML. With future research directed towards the introduction of even better TKIs, especially those targeted against the T315I mutations and vaccination therapy, perhaps in the near future a cure for CML might be achieved.

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List of abbreviations

2G-TKI	Second generation tyrosine kinase inhibitor
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1
ABL1	Human homologue of the Abelson leukaemia virus oncogene
AE	Adverse events
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
AP	Accelerated phase
Ara-C	Cytarabine
aRR	Adjusted relative risk
ATP	Adenosine triphosphate
BC	Blast crisis
BCR	Breakpoint cluster region
BP	Blastic phase
CA	Chromosomal abnormalities
CBA	Chromosome banding analysis
CCyR	Complete cytogenetic response
CE	Clonal evolution / additional cytogenetic abnormalities
CHR	Complete haematological response
CI	Cumulative incidence
CML	Chronic myeloid leukaemia
CMR	Complete molecular response
CMV	Cytomegalovirus
CNS	Central nervous system
CP	Chronic phase
CYP3A4	Cytochrome P450 family
DLI	Donor lymphocyte infusion
DNA	Deoxyribonucleic acid
EBMT	European group for blood and marrow transplantation
EFS	Event free survival
ELN	European Leukaemia-Net
EUTOS	European Treatment and Outcome Study
EVI-1	Ecotropic virus integration site-1 protein homolog
FBC	Full blood count
FDA	Food and drug administration
FISH	Fluorescence in situ hybridisation
GIST	Gastrointestinal stromal tumour
GRB	Growth factor receptor-bound protein
GUSB	β - glucuronidase
GVHD	Graft versus host disease
GVL	Graft versus leukaemia

HLA	Human leukocytic antigen
hOCT1	Human organic cation transporter 1
IFN-α	Interferon alpha
IRIS	International randomized study of interferon and STI571
KD	Kinase domain
KIR	Killer cell immunoglobulin-like receptor
MAP	Mitogen activated protein
MCyR	Major cytogenetic response
MDR-1	Multi-drug resistance protein-1
MEMS	Microelectronic monitoring system
MiCyR	Minimal cytogenetic response
MMR	Major molecular response
MRD	Minimal residual disease
NICE	National institute for health and clinical excellence
OS	Overall survival
PCR	Polymerase chain reaction
PCyR	Partial cytogenetic response
PDGFR	Platelet derived growth factor receptor
PEG	Polyethylene glycol
PFS	Progression free survival
Ph	Philadelphia chromosome
PI3-K	Phosphatidyl inositol 3 - kinase
P-loop	Phosphate binding loop
RIC	Reduced intensity conditioning
RNA	Ribonucleic acid
ROC	Receiver operating characteristic curve
RQ-PCR	Real-time quantitative reverse transcriptase
RR	Relative risk
RUNX1	Runt-related transcription factor gene 1
SCT	Stem cell transplant
SH (1, 2)	Src homology domain
SRC	Sarcoma viral oncogene homolog
STAT	Signal transducers and activators of transcription
TKI	Tyrosine kinase inhibitors
TRM	Transplant related mortality
WBC	White blood cell
WHO	World health organization
WT1	Wilms tumour protein

Chapter I

Introduction

Chronic myeloid leukaemia, *BCR-ABL1* +ve (CML) is a myeloproliferative neoplasm that originates in a pluripotent stem cell in the bone marrow, and is consistently associated with the *BCR-ABL1* fusion gene (1). No other myeloid neoplasm gave science this chance of studying genetic defects behind a disease that eventually led to the development of treatment as CML. In other words, the research into the disease offered scientists a blueprint of how a cure for cancer might be generally achieved.

Incidence, Epidemiology & Aetiology

CML is a relatively rare disease with an incidence rate that ranges from 0.6 to 2.0 per 100,000 persons worldwide. This incidence increases with age, with greater frequency in men than in women with a ratio that ranges from 1.3 to 1.8 (2). Although more population-based data are necessary to determine geographic or ethnic variations of the disease, an older review reported higher rates from Switzerland, the USA, Italy, Australia, Germany and the United Kingdom, whereas lower rates were reported from the Netherlands, Sweden and China; with the disease more common in Whites and Blacks than in other races (3). In the USA, 4870 new cases are estimated to be diagnosed annually (4) and around 700 new cases in the UK. Overall, CML accounts for 7 - 20 % of all leukaemias.

The main aetiological factor behind the disease remains unknown, as there are no known hereditary, familial, geographic, ethnic, or economic associations with CML; which makes the disease neither preventable nor inherited (5). However, the disease has been observed with increased frequency in Japan after the 1945 atomic bombing; in radiologists and in ankylosing spondylitis patients who have been treated with radiation. Individuals expressing HLA-B8, HLA-A3 and HLA-DR4 have been reported to have a reduced incidence of developing CML (6, 7) indicating that certain HLA types offer protection against disease occurrence.

History

Chronic myeloid leukaemia (CML) was the first form of leukaemia to be recognized as a distinct entity (8). It was in 1845 that David Craigie in Glasgow saw a patient with fever, splenomegaly and leukocytosis; so when he saw a similar patient three years later, this led his colleague John Hughes Bennett, a physician and pathologist, to perform an autopsy and to describe his findings in the *Edinburgh Medical and Surgical Journal* (9). Simultaneously Rudolph Virchow saw a similar patient at the Berlin Charité Hospital. The patient died 4 months later and Virchow noted on autopsy enlargement of the spleen. His report was published in German 5 weeks after Bennett's paper (10).

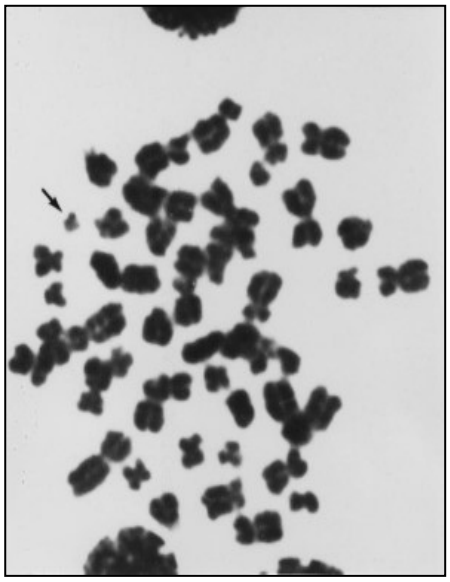
Bennett concluded that his patient had died from "the presence of purulent matter in the blood," implying some occult infection as the cause. Virchow came up with the term "Weisses Blut" or "white blood"; this was based on the Greek (λευκος and αίμα) and this became in German "Leukaemia." Virchow took the view that the cause of the disease was a primary alteration in haematopoiesis (11). The sequence of events that followed is reviewed in detail by Geary (12) and later by Piller (13).

A vital discovery came almost a decade later when Ernst Neumann reported changes in the bone marrow, and established links between the source of blood and the bone marrow. He stated in 1872 that leukaemia was a disease of the bone marrow (14). Six years later, he added the classification "myelogenous leukaemia" to the splenic and lymphatic forms of the disease (15).

In the first documented attempt of therapy, Heinrich Lissauer reported in 1865 that arsenous oxide was effective in the treatment of CML (16). The discovery of X-rays by Wilhelm Roentgen in 1895 brought a new treatment for leukaemia (17), with initial temporary results similar to those produced by arsenic.

The first important clue to the pathogenesis came 100 years later, in 1960, the newly developed techniques for studying cells in mitosis allowed Nowell and Hungerford to detect a consistent chromosomal abnormality, later termed the Philadelphia (Ph^1, or just Ph) chromosome and identified as 22q-, in persons with this disease (18) (figure 1).

Figure 1: Metaphase picture showing the small abnormal Ph chromosome



With the introduction of improved banding techniques the Ph chromosome was further characterised by Rowley (19), who was able to show in 1973 that it was a truncated version of chromosome 22 (designated 22q-) and was the result of reciprocal translocation of genetic material between chromosomes 9 and 22 [designated t(9;22)].

The early 1980s saw the identification of the two genes that flank the translocation breakpoint. The *ABL* gene (now renamed *ABL1*) from chromosome 9 had been known as the human homolog of a murine leukaemia virus (20, 21); while the translocation partner from chromosome 22 was termed *BCR* for breakpoint cluster region, since DNA breaks occurred in a relatively small genomic region (22). It was later confirmed the relation between the *BCR-ABL1* fusion gene and the Ph chromosome in CML patients (23).

Of great importance was the discovery that the protein derived from the *BCR-ABL1* gene had protein-tyrosine kinase (TK) activity that was deregulated compared with normal *ABL1* and correlated with its ability to transform cells to a malignant phenotype (24). Several groups later reported that a CML-like disease could be induced in mice transplanted with bone marrow infected with a *BCR-ABL1* retrovirus (25, 26). This proved that *BCR-ABL1* is the causative agent of the disease and not just a marker (27).

With the introduction of radiotherapy in the early half of the 20th century, treatment options improved and it became the first reliable option for the disease. It remained the standard therapy until later replaced by busulfan in the 1960's (28). Hydroxyurea followed later but was slow to gain popularity although proved superior to busulfan.

It was during the 1980's, that interferon alpha (IFN-α) was introduced for the management of CML in the chronic phase with the ability to induce Philadelphia negativity in a minority of patients (29).

Research conducted during the 1980's in London showed that allogeneic stem cell transplantation, although hazardous and even sometimes fatal, could if induce long term remissions and probably cure some patients (30). Therefore, from the 1990's onwards, the treatment of choice for all relatively young patients (i.e., under the age of 50) who presented in chronic phase was an allogeneic stem cell transplant (SCT). Later on, evidence from France suggested that the best treatment for CML patients who aren't eligible for allografting was a combination of IFN-α plus cytarabine (Ara - C) (31), though later data came to cast some doubt on this conclusion (32).

It was in 1996, when Druker and colleagues described CGP57148, a highly specific pharmacologic inhibitor of the *ABL1*-tyrosine kinase that selectively suppressed the growth of *BCR-ABL1*-positive cells (33) that CML therapy was changed. This compound, first renamed STI571 and then imatinib (34), revolutionized the treatment of CML and set an example for the development of other targeted therapies. The use of Imatinib and the new generations of tyrosine kinase inhibitors (TKIs) and the modern challenges that arise in the management are the subject of this thesis.

Pathogenesis

Cytogenetics

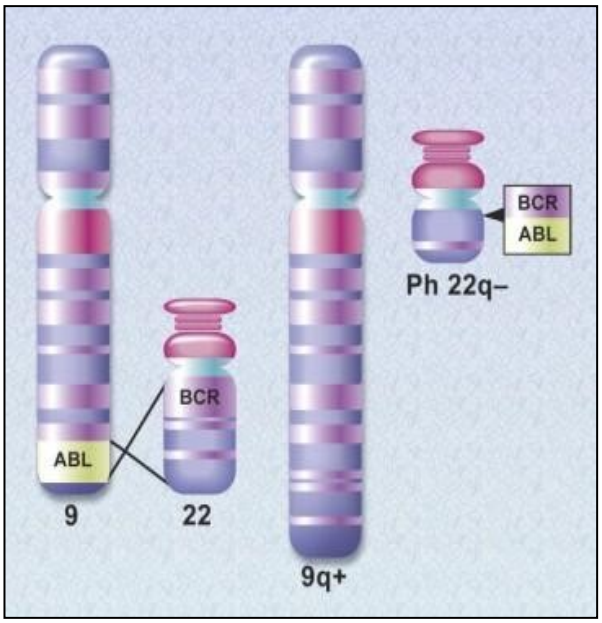
It has been established that CML develops when a single, pluripotential, haematopoietic stem cell acquires the Philadelphia (Ph) chromosome carrying the *BCR-ABL1* fusion gene, which provides its descendants with a proliferative advantage over normal haematopoietic elements and thus allows the Ph-positive clone gradually to displace residual normal haematopoiesis (8).

The Ph chromosome is a shortened chromosome 22 (22q-) (18) that results from a balanced, reciprocal translocation between the long arms of chromosome 9 and 22, designated t(9;22)(q34;q11) (19) (figure 2).

As a result of this translocation the bulk of the *ABL1* (Abelson) proto-oncogene normally found in chromosome 9 is translocated into a relatively small, 5.8Kb genomic region on chromosome 22, that was named the breakpoint cluster region or *BCR* (22).

Molecular biology of *BCR-ABL1* +ve CML

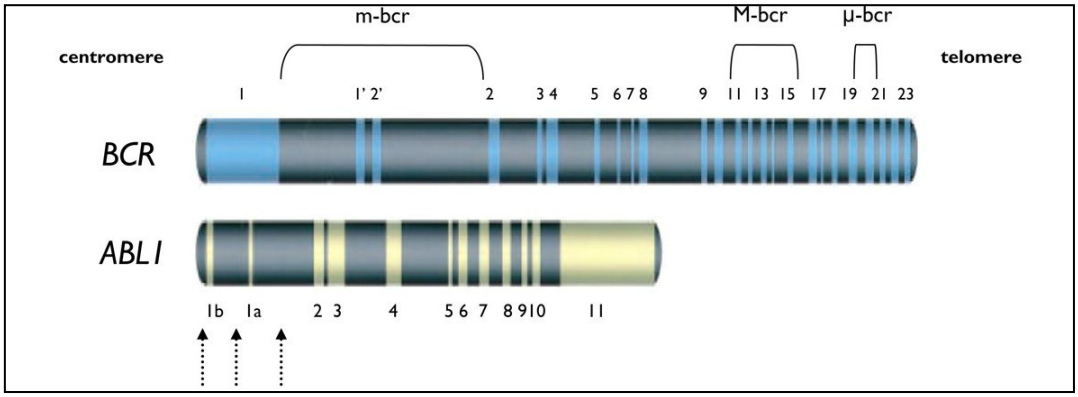
Figure 2: Schematic diagram of the translocation that creates the Ph chromosome



Over the years, several experimental models have managed to establish a relationship between *BCR-ABL1* and CML. This was later confirmed by studies on murine models in which a CML-like disease has been produced by tranfecting stem cells with a *BCR-ABL1* gene (25, 26, 35, 36).

The classic *BCR-ABL1* gene of CML results from the fusion of parts of two normal genes: the *ABL* gene on chromosome 9 (now renamed *ABL1*) and the *BCR* gene on chromosome 22 (8). The breakpoints within the *ABL1* occur either upstream of exon Ib, downstream of exon Ia, or more frequently, between exons Ib and Ia (37) (figure 3).

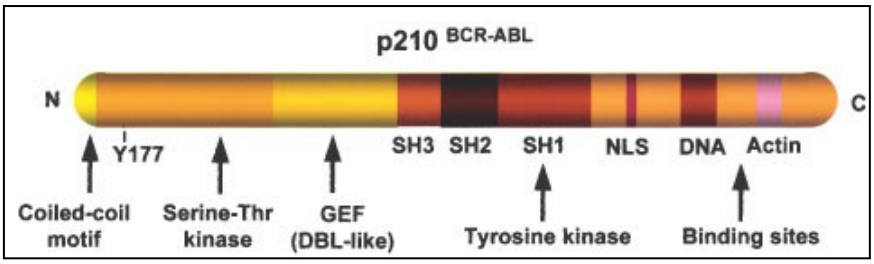
Figure 3: Schematic representation of the *ABL1* and *BCR* genes



In most patients with CML and in one-third of those with Ph-positive B-cell acute lymphoblastic leukaemia (Ph+ B-ALL) the breakpoints within *BCR* occur at a 5.8-kilobase (kb) area spanning exons e12-e16 (formerly called b1-b5), referred to as the major breakpoint cluster region (M-*bcr*). Alternative splicing gives rise to fusion transcripts with either b2a2 or b3a2 junctions that generate a 210-kDa protein ($p210^{BCR-ABL1}$) (38).

The resulting *BCR-ABL1* kinase contains a series of functionally distinct domains (figure 4). The tyrosine kinase encoded by the SRC-homology 1 (SH1) domain of the *ABL1* component is undoubtedly the most crucial for oncogenic transformation. Other important motifs in the *ABL1* portion are the protein interaction SRC-homology 2 (SH2) and the C-terminal actin-binding and DNA binding domains. On the *BCR* moiety, the coiled-coil motif encoded by the first *BCR* exon is responsible for the dimerization of the oncoprotein; a tyrosine at position 177 is crucial for the binding of adaptor proteins such as growth factor receptor bound protein 2; the N-terminal phosphoserine and phosphothreonine residues are required for interaction with SH2 containing proteins, including *ABL1* itself (figure 4).

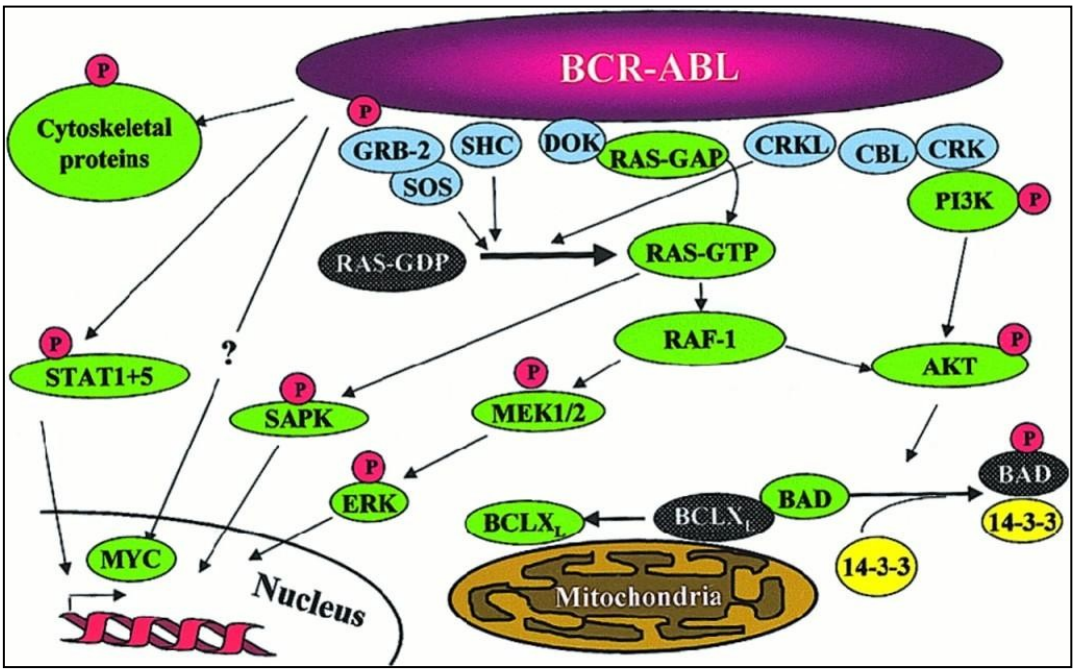
Figure 4: Functional domains of $p210^{BCR-ABL1}$



The leukaemogenic potential of $p210^{BCR-ABL1}$ resides in the fact that the normally regulated tyrosine kinase activity of the *ABL1* protein is constitutively activated by the juxtaposition of 'alien' *BCR* sequences. *BCR* acts by promoting dimerization of the oncoprotein, such that the two adjacent BCR-ABL molecules phosphorylate each other on tyrosine residues.

The uncontrolled kinase activity of *BCR-ABL1* then supersedes the physiologic functions of the normal *ABL1* enzyme by interacting with a variety of effector proteins. The key pathways implicated (figure 5) are those involving RAS, mitogen-activated protein (MAP) kinases, signal transducers and activators of transcription (STAT), phosphatidylinositol 3-kinase (PI3K) and MYC. Most of the interactions are mediated by tyrosine phosphorylation and require the binding of *BCR-ABL1* to adapter proteins such as growth factor receptor-bound protein 2 (GRB2), DOK, CRK, CRK-like proteins, SHC and casitas B lineage lymphoma protein (8). The net result is deregulated cellular proliferation, decreased adherence of leukaemia cells to the bone marrow stroma and a reduced apoptotic response to mutagenic stimuli.

Figure 5: Signaling pathways impacted by *BCR-ABL1* expression



CML blastic crisis

Prior to the introduction of *BCR-ABL1* tyrosine kinase inhibitors (TKIs), patients with CML used to progress to an advanced phase within a median time of 5 years. This phase has been divided into: an initial accelerated phase (AP), and a subsequent more aggressive blastic phase (BP). The characteristics and definitions of the phases are still under debate.

At the present time, the molecular mechanisms that lead to disease progression are still uncertain (39), but it is most likely to involve the activation of oncogenic factors and/or inactivation of tumour suppressors genes (40). An acceptable assumption is that BP is a multi-step process that is activated by both *BCR-ABL1* - dependant and independent DNA damage that is associated with inefficient repair. This, in addition to an increased activity of *BCR-ABL1* leads to the evolution of one or more CML-BP clones.

The occurring lesions may include chromosomal aberrations, gene deletions or insertions, and/or point mutations (including *BCR-ABL1* mutations). At the molecular level, the most common detectable lesions (other than those in the *BCR-ABL1* kinase domain) occur at the loci of the tumour suppressor genes *P53*, the runt-related transcription factor gene (*RUNX1*), the loci of cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/B*) and the Ikaros transcription factor (*IKZF1*).

Expression studies have also revealed that the constitutive *BCR-ABL1* activity can influence the CML transcriptome, resulting in altered expression of some genes (e.g., PRAME, MZF1, EVI-1, WT1 and JUN-B), the level of which start to increase in AP and might play a role in BP.

A number of chromosomal aberrations are also believed to be responsible for the transition of the relatively benign CP to the aggressive BP (41). The frequency of these of these chromosomal abnormalities is approximately 7% in CP and increase to 40 - 70% in advanced phase disease. Examples of such include: acquisition of additional chromosomes (e.g., +Ph, +8, +19); acquisition of iso-chromosome i(17q); acquisition of t(1;17), which is associated with loss of p53; acquisition of t(1;21), which affects RUNX1; acquisition of t(3;21), which generates the AML-1/EVI-1 fusion protein; acquisition of t(7;11), which produces the NUP98-HOXA9 fusion protein that causes aberrant self renewal; and acquisition of translocations and inversions associated with AML/myelodysplasia (e.g., inv[3] and t[15;17])(39).

Clinical manifestations

From the clinical point of view, CML is characterized by a biphasic or triphasic course that includes a chronic phase (CP), an intermediate or accelerated phase (AP) and an acute or blastic phase (BP) (42). At the time of diagnosis, more than 80% of patients are in CP, which is asymptomatic in approximately 40% of patients, being discovered during routine laboratory workup.

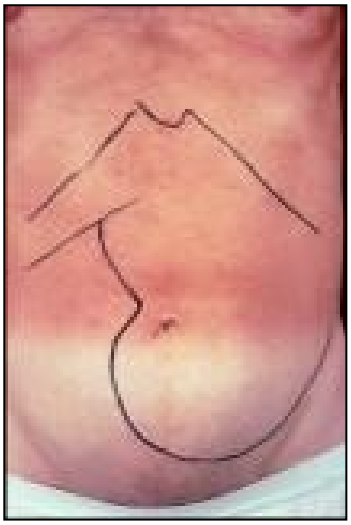
From historical data, patients diagnosed with CP had a median survival of approximately 5 years, and unless the disease was controlled or eliminated, they eventually transformed to a terminal or BP after a median of 3 to 5 years.

The prognosis for patients with BP is poor, with a median survival of 3 – 6 months. Approximately two thirds of the patients will go through an intermediate phase known as AP before progressing to BP with a median survival of 1 – 2 years (43). These numbers have changed since the introduction of TKIs.

Chronic Phase

As a result of routine examination, the incidence of asymptomatic presentations in CP-CML has increased dramatically from 15% to 40% – 50%. There has been also a decrease in the presenting features of advanced phase disease (44).

Figure 6: Patient with massive splenomegaly in chronic phase CML



The commonest physical finding present in more than three-quarters of CML patients is splenomegaly (figure 6) and symptoms at the time of presentation are often attributed either to it or to anaemia. These include fatigue and left upper abdominal pain or mass (45). Patients with CML are often diagnosed with leukocytosis, with white blood cell counts > 50 x 10^9/L. In rare situations, the leukocytosis can lead to retinal haemorrhage and signs of hyper viscosity such as priapism, cerebrovascular accidents, tinnitus, confusion, and stupor.

Advanced phase (accelerated and blastic phases)

In contrast to patients in chronic phase, patients in the advanced phase are more likely to experience symptoms, including weight loss, fever, night sweats and bone pains. Anaemia, infectious complications and bleeding are also common among these patients. Subcutaneous nodules or haemorrhagic tender skin lesions, lymphadenopathy and central nervous system (CNS) leukaemia may also occur (46). Other features of progressive disease include increased blasts and basophils, resistance to therapy, increasing splenomegaly, cytogenetic clonal evolution, thrombocytosis or thrombocytopenia (45, 47). In the majority of patients, the progression in between phases is relatively insidious but distinct.

Diagnosis

In the majority of cases, the diagnosis is based on blood counts (FBC) and differential. Leukocytosis is present (median WBC count, ~100,000/µL [100 × 10^9/L]; range, 12,000-1,000,000/µL [12-1,000 × 10^9/L]), with immature granulocytes (from metamyelocyte to myeloblast) and basophilia.

Thrombocytosis is also frequent with ranges from normal to more than 1,000 × 10^3/μL (1,000 × 10^9/L). Splenomegaly is present in >50% of cases and ~ 50% are asymptomatic on presentation. Bone marrow shows increased cellularity with blasts accounting for fewer than 5% of the nucleated bone marrow cells (figure 7) (48). Megakaryocytes vary in number and shape from slightly decreased to markedly increased and might show characteristic features (smaller and have hypolobated nuclei – dwarf megakaryocytes) (figure 8). Diagnosis is further confirmed by the demonstration of the Ph chromosome and the *BCR-ABL1* rearrangement in the PB or BM cells.

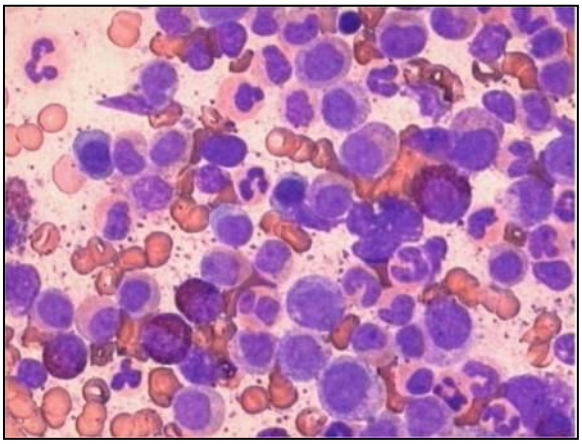


Figure 7: CML - Bone marrow aspirate

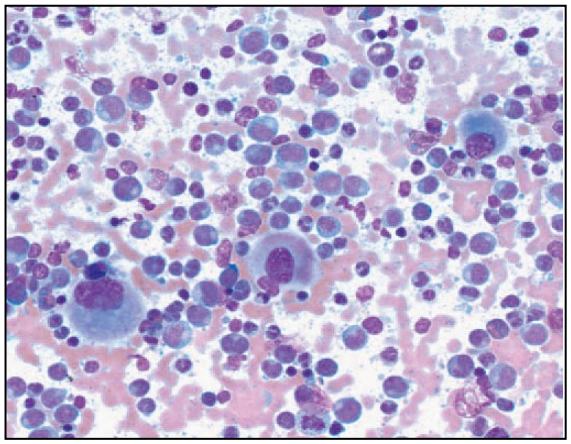


Figure 8: CML - Characteristic granulocytic proliferation and “dwarf megakaryocytes”

Definition of CML phases

Over the years several definitions were proposed in order to differentiate between the 3 phases, the subject of which is still under debate. A definition that was proposed in 1988 (47) was used frequently in studies with interferon - α (IFN-α) (31, 49, 50) and, more recently, in most studies with imatinib (51-54)(table 1).

In 2001, the WHO proposed a classification system with the intention to refine the criteria for AP and BP (55). For this purpose, they relied on the published literature and the experience of a clinical advisory committee. The classification has been revised in 2008 to include newly refined diagnostic criteria and to introduce new disease entities (56) (table 1).

Table 1: Criteria for AP/BP in CML

Kantarjian Criteria (most widely used) (47)	**Revised 2008 WHO Criteria** (56)
Accelerated Phase (one of the following) • 15 - 29 % blasts in peripheral blood or bone marrow • > 30 % blasts plus promyelocytes in peripheral blood or bone marrow • > 20 % basophils in peripheral blood or bone marrow • Platelets < 100 x 10^9/L unrelated to therapy	Accelerated Phase: • 10 % - 19 % myeloblasts in the blood or bone marrow • $\geq$ 20 % basophils in the peripheral blood • persistent or increasing WBC count > 10,000/μL (10 × 10^9/L) and/or persistent or increasing splenomegaly unresponsive to therapy • persistent thrombocytosis, with a platelet count > 1,000 × 10^3/μL (1,000 × 10^9/L) uncontrolled by therapy, or • persistent thrombocytopenia, with a platelet < 100 × 10^3/μL (100 × 10^9/L) unrelated to therapy • evidence of cytogenetic evolution
Blastic Phase (one of the following) • Blasts in peripheral blood or bone marrow ≥ 30 % • Presence of extramedullary blastic disease	Blastic Phase: • Blasts in peripheral blood or bone marrow ≥ 20% • Extramedullary proliferation of blasts

Management of chronic myeloid leukaemia prior to TKIs

Prior to the discovery and introduction of imatinib and TKIs, the accepted standard curative treatment for CML was allogeneic stem cell transplantation (allo-SCT). Other therapies have been previously tried without much success. Total body or splenic irradiation was the mainstay of therapy until the 1950's (17). It's rarely used nowadays, if at all.

Busulfan:

Busulfan, introduced in the 1950s (28), controlled haematologic variables, was given orally, and was inexpensive. Side effects included severe, prolonged myelosuppression in 10 % of patients; idiosyncratic pulmonary reactions ("busulfan lung"); marrow fibrosis; endocardial fibrosis; and, occasionally, an Addison disease–like picture (57).

Busulfan was given at 0.1 mg/kg daily until the leukocyte count decreases by 50 %; at that point, the dose is reduced by 50 %. Courses were repeated at 2 to 8 mg daily for 5 to 10 days (58). Its use nowadays is limited mainly as part of some conditioning regimes for SCT. It acts on the primitive stem cell and its effects persist for several weeks after stoppage, which proves an advantage for patients who are known to be non compliant as it can be given in single oral doses at 50 – 150 mg at 4 weekly intervals depending on the leukocytic count.

Hydroxyurea (hydroxycarbamide):

In 1972, hydroxyurea, a cell cycle–specific inhibitor of DNA synthesis, became available for the treatment of chronic myelogenous leukaemia. It allowed rapid but transient haematologic control, was well tolerated, and had few side effects (nausea, vomiting, diarrhoea, mucosal ulcers, and skin manifestations). Hydroxyurea is given orally at 40 mg/kg daily, and doses are adjusted to maintain a leukocyte count of 2 to 10 cells $x10^9$/L (58). Although hydroxyurea is effective in controlling the white blood cell count and reduces the splenomegaly of CML, it does not eradicate the Ph clone. Its superiority over busulfan, quite apart from the lower toxic profile, was shown by a multicenter randomized trial in Germany (59). Patients who initially were treated with busulfan and then given hydroxycarbamide survived longer than those treated with busulfan alone.

Interferon-α

The use of interferon-α (IFN-α) for the treatment of CML was pioneered by the MD Anderson group in Texas during the 1980's (60, 61). Its role in CML treatment was reviewed by an international panel convened by the American Society of Haematology (62). Subsequent studies agreed that it was more effective than busulfan or hydroxyurea (63), and that hydroxyurea was more effective than busulfan (64).

All studies reporting IFN-α as the first choice treatment tested the drug in combination with other agents, mostly with hydroxyurea, low-dose cytarabine, or both. Results of a randomised comparison of IFN-α versus IFN-α plus low-dose cytarabine (32) came in line with those of an earlier study (31), recording the best cytogenetic response with the combination therapy, but with no improved survival. IFN-α prolonged life in patients of all ages, but lower doses were needed for elderly patients (65).

In a comparison of low-dose (15 MIU per week) and high-dose IFN-α (5 MIU/m² per day), the low dose was as effective as the high dose, but better tolerated (66). In the follow-up of 2 large studies of IFN-α for CML, 10 years overall survival was 20–53% (63, 67). In a meta-analysis of 317 patients who had achieved complete cytogenetic remission (CCyR), after 10 years 50% were still in complete remission and 70% were alive (68).

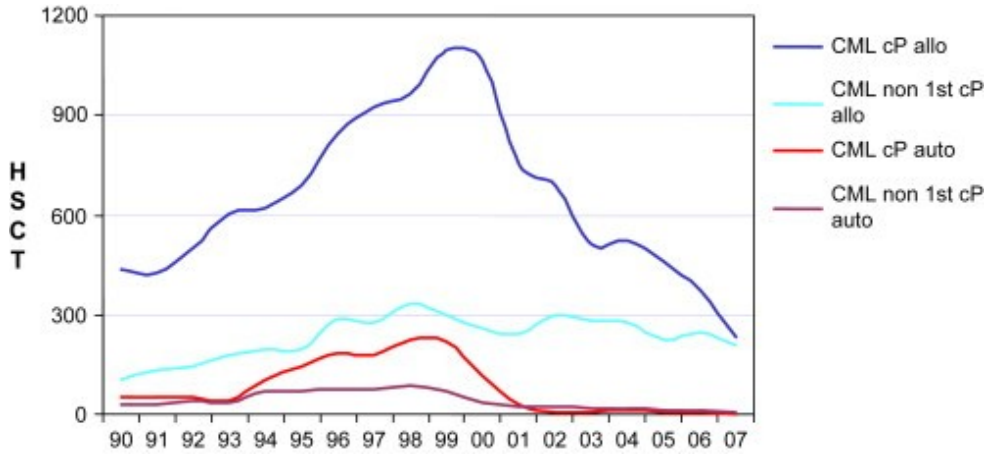
Two pegylated formulations of IFN-α with improved pharmacokinetic and pharmacodynamic properties were tested by randomised comparison with non-pegylated IFN-α. Peg IFN-α -2b did not give better results than IFN-α (69). In a trial on 146 patients (70), peg IFN-α -2a gave better results than IFN-α. Randomised comparison of IFN-α with Imatinib showed best response rates (haematological, cytogenetic, and molecular) and least adverse effects with imatinib (54). The main drawback of IFN-α therapy is its adverse events; the commonest are 'flu-like' symptoms including fever, shivers, myalgia and tiredness which reduce the quality of life in many patients (71).

Stem cell transplantation (SCT)

The disappearance of the Philadelphia chromosome in patients given intensive chemo/radiotherapy and bone marrow from their twin donors more than 30 years ago marked the beginning of a new era in the treatment of CML (72). It was the first therapy that was able to eradicate the Philadelphia +ve clone. These few patients demonstrated that it could become possible to cure patients from their disease. The concept was rapidly adopted and followed by transplantations of bone marrow from human leukocyte antigen (HLA) identical sibling donors (30, 73-75) and later of unrelated donors (76, 77). The efficacy of allogeneic SCT was proven in several large series in Europe and the USA.

Allogeneic SCT became the treatment of choice for young patients with CML and a compatible donor. At the end of the last decade, CML was the most frequent indication for an allogeneic transplant worldwide, and more than 1000 SCTs were reported to the European Group for Blood and Marrow Transplantation (EBMT) activity survey in 1999 (78). With the introduction of the TKI imatinib (79) the trend of transplantation in CML changed completely and the numbers of SCT for CML began to decline. In 2007 there were a total of 434 allogeneic HSCTs for CML, 228 in first chronic phase and 206 in advanced stages of the disease (figure 9).

Figure 9: Numbers of allogeneic and autologous SCT for CML reported to the EBMT activity survey from 1990 to 2007



More than 2600 patients were transplanted in Europe between 1980 and 1990. About 40%, 20% and 10% of the patients transplanted in the first chronic phase, in the accelerated phase and in the blast crisis, respectively, are alive without the disease beyond 20 years after transplantation (80). No other form of treatment was available in this period of time to achieve comparable results.

Transplantation risk assessment:

Although allo-SCT offers a clear benefit of providing a cure to a rather lethal condition, one clear disadvantage is that it is associated with a high rate of morbidity and mortality. Outcome has been improved over the years by selecting those patients who are most likely to benefit from the procedure. The European Group for Blood and Marrow Transplantation (EBMT) developed a risk score system for patients with CML undergoing transplant.

The EBMT score is based on 5 variables: donor type, disease phase, recipient age, donor/recipient sex combination, and interval from diagnosis to transplantation (81). These five factors provide a score from 0 as lowest up to 7 as highest risk (table 2).

Risk assessment can be further adjusted by integrating additional elements known to influence transplant related mortality (TRM), such as cytomegalovirus (CMV) sero-status of the recipient, cytokine polymorphisms or the co-morbidity score (82-84). Expression of the polycomb group gene BMI-1 was shown to reduce TRM in patients with CML (85).

A defined natural killer receptor, KIR2DS5, was shown to be associated with a higher risk for relapse despite allogeneic SCT (86) and recently, C-reactive protein level less than 10mg/L (regardless of infective status) has been also shown to be an independent predictor for survival (87).

Graft-versus-host-disease and T-cell depletion:

The major causes of morbidity and mortality during SCT are infections and graft-versus-host disease (GVHD). Now GVHD could be prevented using a combination of methotrexate and cyclosporine post-transplant. This combination offers good protection without a significant increase in relapse (88). The 1980s saw the introduction of T-cell depletion, which was effective in decreasing the severity and frequency of GVHD, but was associated with a higher frequency of graft failure (89).

Table 2: EBMT risk score

Risk Factor	Score
Age of patient: < 20 years 20 – 40 years > 40 years	 0 1 2
Disease stage: Chronic phase Accelerated phase Blastic phase	 0 1 2
Time interval from diagnosis: < 12 months > 12 months	 0 1
Donor type: HLA identical sibling donor Unrelated donor	 0 1
Donor/recipient sex match: All other Donor female – male recipient	 0 1

This was later overcome by increasing the intensity of conditioning or the immune suppressive efficacy by the in vivo use of the same anti T-cell antibodies that were used for T-cell depletion of the graft pre-transplant (90). Unfortunately this soon revealed an increased relapse rate, which first came apparent in patients who underwent transplantation for CML (91). Although use of T-cell depletion in unrelated allo-SCT showed shorter hospital stay and increased cost effectiveness compared with non–T-cell–depleted transplantations (92), no clear survival advantage of this approach has been demonstrated.

Reduced intensity conditioning:

Recognizing that much of the curative effect of allo-SCT was a result of the allo-immune effect led to the development of reduced intensity conditioning (RIC) regimens (93, 94). This enabled transplantation of older patients and those with co-morbidities.

However, the introduction of TKIs prevented randomized trials of RIC versus myeloablative conditioning, but TKIs on the other hand offered new possibilities for combinations with RIC regimens, as TKIs and cellular immunotherapy act synergically (95).

Relapse & Donor Lymphocyte infusions (DLI):

Risk of relapse is primarily influenced by the EBMT risk score. The risk of relapse at 5 years is about 15% for patients transplanted in the first chronic phase, but increases to 30% when the transplantation is performed in AP and to > 60% for patients transplanted in BP. The risk of relapse is further enhanced by the GVHD prevention method as there are more relapses after intensive T-cell depletion. The risk of relapse is also influenced by the conditioning regimen with the risk is higher for patients given RIC-SCT (80).

In order to restore remission to transplanted patients who have relapsed, additional donor lymphocytes were given (96). Their therapeutic principle relies on the evidence that donor T lymphocytes can exert a graft-versus-leukaemia effect (GVL), potentially able to eradicate the residual host disease (97).

Administration of DLIs can re-induce remission in 60% to 90% of patients with CML who underwent transplantation, and relapsed in chronic phase. The use of escalating doses in case of persistent disease limits the risks for GVHD (98, 99). A European study showed a 69% 5-year survival in 328 of patients who received DLI for relapsed CML (100).

DLI-related mortality was 11% and disease related mortality was 20%. Some form of GVHD was observed in 38% of patients. Risk factors for developing GVHD after DLI were T-cell dose at first DLI, time interval from transplantation to DLI, and donor type. In a time-dependent multivariate analysis, GVHD after DLI was associated with a risk of death of 2.3-fold compared with patients without GVHD (101).

Recent results of allo-SCT:

Lee et al (102) analyzed the outcome of transplantation in 1309 patients treated between 1999 and 2004. The 3-year survival rate of patients who underwent transplantation with or without prior imatinib was 72% and 65% in CP and 34% and 36% in advanced phase. This suggests that prior treatment with imatinib does not adversely affect transplantation outcome.

Similarly was a report from a German multicenter study, describing 84 patients who underwent transplantation after pre-treatment with imatinib. Nineteen patients with EBMT score 0 or 1 had early transplantation, 37 underwent transplantation after imatinib failure, and 28 for advanced phase disease. The estimated 3-year survival rate of patients in chronic and advanced phase was 91% and 59%, respectively (103).

These results show that the results of SCT have improved over time. This is due to better supportive care that reduced TRM for most patients. Better HLA typing methods have also reduced the risk of unrelated transplants. And thanks to the increasing pool of unrelated volunteer donors, which gives access to more than 12 million donors worldwide, patients without a family donor can now have hope of having a SCT when needed.

Current role of transplantation in CML:

The current European LeukaemiaNet (ELN) recommendations update (104) states that transplantation remains the only potentially curative option for patients in accelerated or blast phase, patients with T315I mutation and after failure or intolerance of second-generation TKI (table 3). The decision for transplantation however should involve careful balancing of the risks for allo-SCT against the risk for disease progression in each individual patient.

Table 3: Summary of current ELN recommendations for SCT

Disease phase	Indication
First chronic	- Failure of second-generation TKI (donor search should be undertaken after failure of imatinib) - Imatinib failure and T315I *BCR-ABL1* mutation
Accelerated	- Less advanced accelerated phase at diagnosis: treat as first chronic phase - Near blast phase or evolution into accelerated phase on TKI: treat as blast phase
Blast	As soon as possible after re-establishing chronic phase with TKI or chemotherapy (consider use post-engraftment therapy with second generation TKI)

Autologous SCT:

Autologous SCT was introduced as a treatment attempt to try to restore the chronic phase in patients with advanced disease (8). The idea behind this concept was to reduce the tumour burden, therefore reducing the number of leukemic cells at risk for transformation and to eradicate the already mutated leukemic cells. Later, autologous SCT was tried in the early chronic phase in an attempt to eradicate the leukemic stem cells in those patients responding to interferon. Autologous SCT was integrated into the treatment protocol in many European CML studies but they coincided with the introduction of imatinib and TKIs. These studies never concluded and the number of auto-transplants fell rapidly.

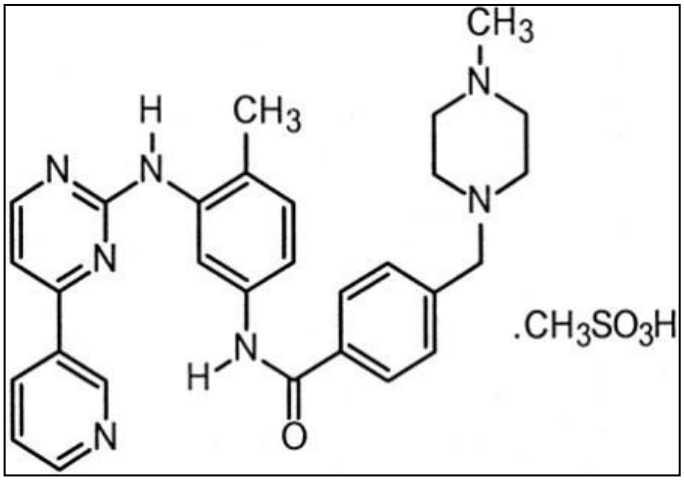
The European LeukaemiaNet (ELN) and EBMT guidelines currently do not consider any indication for autologous SCT for CML outside the context of an experimental study protocol (78). Only theoretical considerations currently remain for future use and in combinations with TKIs. These could include: to reverse blastic transformation to chronic phase in order to save time; to reverse TKI resistance; to eliminate leukemic cells which harbour mutations such as T315I and to reduce residual leukemic burden in patients with complete cytogenetic remission and a *BCR-ABL1* - negative stem cell product. Cases of achieving complete molecular remissions after such attempts in combination with TKIs have been published (105-107). The exact role remains questionable.

Imatinib & TKIs

The introduction of imatinib came in the early 1990s when Druker approached scientists at what was then Ciba – Geigy with a proposal to find a small molecule that would inhibit the *BCR-ABL1* tyrosine kinase activity. The collaboration yielded a phenyl-aminopyrimidine molecule, then called CGP57148B, that occupied the kinase pocket of the *BCR-ABL1* protein and blocked access to ATP, thereby preventing phosphorylation of any substrate (figure 10) (34).

Preclinical studies showed that the molecule was highly effective in blocking the tyrosine kinase activity of *ABL1*; the stem-cell factor receptor, c-kit; and the platelet-derived growth factor receptor (PDGFR) but had little effect on other tyrosine kinases.

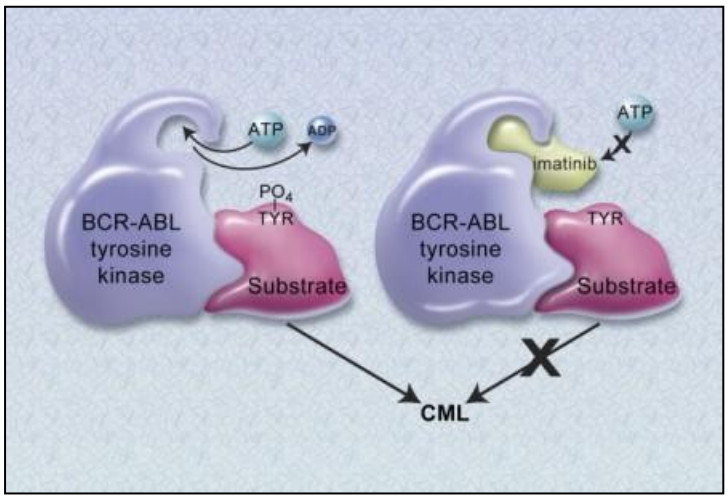
Figure 10: Structure of imatinib



CGP57148B inhibited proliferation of CML cell lines from patients with CML but did not affect equivalent control cells (108). The compound had low potency and poor specificity, inhibiting both serine/threonine and tyrosine kinases, but from this starting point, a series of derivatives were synthesized.

The activity of the compound against tyrosine kinases was enhanced by introduction of a benzamide group at the phenyl ring. The first series of compounds had poor oral bioavailability, with low water solubility. The attachment of a highly polar side chain markedly improved solubility and oral bioavailability. STI571 (formerly CGP57148B, now Imatinib mesylate; Gleevec or Glivec, Novartis, Basel, Switzerland) emerged as the most promising compound for clinical development, since it had the highest selectivity for growth inhibition of *BCR-ABL1* expressing cells (109).

Figure 11: Mechanism of action of imatinib



Mechanism of Action

Imatinib mesylate binds to the amino acids of the *BCR-ABL1* tyrosine kinase ATP binding site and stabilizes the inactive, non-ATP-binding form of *BCR-ABL1*, thereby preventing tyrosine auto- phosphorylation and, in turn, phosphorylation of its substrates. This process ultimately results in "switching off" the downstream signalling pathways that promote leukemogenesis (figure 11). Preclinical *in vitro* and *in vivo* data indicated a selective activity of Imatinib mesylate on cells expressing *BCR-ABL1*, and supported a rapid transition of the compound from the bench to the clinic.

The striking in vitro results led to experiments to determine the effectiveness of Imatinib in vivo. Since apoptosis could be averted in cells expressing *BCR-ABL1* if exposure to Imatinib was limited to 16 hours or less, Druker and colleagues reasoned that continual suppression of *BCR-ABL1* would require long-term treatment with a well-tolerated oral formulation (110).

Phase 1 clinical trials

A standard dose-escalation phase 1 study of imatinib began in June 1998. The study population consisted of patients with CML in chronic phase, refractory or resistant to IFN-α - based therapy, or intolerant of the drug (111). At later stages of the study, patients with CML in blast crisis and patients with Ph chromosome–positive acute lymphoblastic leukaemia were also enrolled (112).

Imatinib was well tolerated with the most common side effects including occasional nausea, periorbital oedema, and muscle cramps. Despite dose escalation from 25 mg to 1000 mg in 14 cohorts of patients, a maximally tolerated dose could not be defined (113). Imatinib was administered once daily and pharmacokinetics showed a half-life of 13 to 16 hours.

At doses of 300 mg and above, significant therapeutic benefits were observed. In chronic phase patients who had failed therapy with IFN-α, 53 of 54 (98%) patients treated at 300 mg and above achieved a complete haematologic response, and with one year of follow-up, only one of these patients relapsed (111). In myeloid blast crisis patients, 21 of 38 (55%) patients treated at doses of 300 mg or more per day responded, with 18% having responses lasting beyond one year (112).

Phase 2 studies

This remarkable phase 1 data led to rapidly accruing phase 2 clinical trials. These clinical trials confirmed the results seen in the phase 1 studies and led to FDA approval of imatinib in May 2001, less than 3 years after the start of the phase 1 study. In chronic phase patients who had failed IFN-α therapy, 95% of patients achieved a complete haematologic response (CHR) and 60%, a major cytogenetic response (MCyR), defined as a reduction in the percentage of Ph chromosome–positive metaphases to less than 35% (53). The estimated rates of freedom from progression to AP and BP and overall survival at 6 years were 61% and 76%, respectively (114).

In AP and BP patients, the response rates were also quite high, but relapses have been much more common, with the majority of BP patients relapsing during the first year of therapy (51, 52).

The IRIS trial

The IRIS trial [International Randomized Study of Interferon and STI571] was initiated in June 2000 for newly diagnosed patients with CML in the chronic phase. It reached its goal of more than 1000 patients in less than 7 months (54). 553 patients were randomized to each of the two treatments, imatinib at 400 mg per day or IFN-α plus cytarabine (Ara – C) (113).

With a median follow-up of 19 months, patients randomized to imatinib had significantly better results than patients treated with IFN-α plus Ara - C in all parameters measured, including rates of CHR (97% vs. 56%, $P < 0.001$), MCyR and complete cytogenetic responses (CCyR) (85% and 74% vs. 22% and 8%, respectively, $P < 0.001$), discontinuation of assigned therapy due to intolerance (3% vs. 31%), and progression to AP or BP (3% vs. 8%, $P < 0.001$)(54).

The substantial superiority of imatinib resulted in study results being disclosed early and most patients being crossed over to the imatinib arm. Accordingly, this study is now a long-term follow-up study of patients who received imatinib as initial therapy (113).

In the most recent update of the IRIS study, estimated event free survival (EFS) at 8 yr was 81% and freedom from progression to AP/BP was 92%. Estimated OS was 85% at 8 yr and 93% when only CML-related deaths and those prior to SCT were considered. At the 8-yr data cut-off, 304 (55%) pts remained on imatinib study treatment, and 45% had discontinued treatment due to adverse events/safety (6%), unsatisfactory therapeutic outcome (16%), SCT (3%), death (3%) or other reasons (17% for withdrawal or lack of renewal of consent and miscellaneous) (115).

IRIS limitations:

The IRIS trial had its limitations. As 20% of patients in the study were censored for diverse reasons including unsatisfactory response (11%), consent withdrawal (5%), and adverse events (4%) (116) and there was no evidence that discontinuation was random; patients with a worse prognosis may have been more likely to have been censored for these reasons. Consequently, the real impact of imatinib therapy can be assessed only by an intention-to- treat analysis (117).

Pharmacokinetics:

Absorption:

After oral intake, Imatinib is rapidly absorbed from the gut, because of its rapid dissolution at acidic pH. Imatinib should be taken with a meal and water to reduce gastrointestinal side effects. However, a high fat meal can prolong the uptake and therefore the time to reach a maximum plasma concentration (tmax) (118).

Protein Binding:

Imatinib is highly protein bound. It binds mostly to albumin. Protein binding is approximately 95%. Imatinib also binds to α1-acid-glycoprotein. α1- acid-glycoprotein levels are elevated in patients with CML and GIST. However, it is thought that at clinically relevant doses, α1-acid glycoprotein binding does not influence the activity of Imatinib.

Elimination:

On average, 75% of the dose of Imatinib undergoes biotransformation. Imatinib has a terminal half-life of 19 hours while its main metabolite has a terminal half-life of 40 hours. Excretion takes place mainly via the bile, renal excretion is low.

Drug Interactions:

The metabolism of Imatinib is largely dependent on CYP3A4. Therefore, caution should be undertaken when patients are treated with inducers and/or inhibitors of CYP3A4 during Imatinib therapy. Inhibitors, such as ketoconazole and erythromycin, led to higher plasma concentrations of Imatinib in clinical trials. Inducers, such as rifampicin and St. John's Wort, can lower the terminal half-life of Imatinib two to four fold. As Imatinib itself is also an inhibitor of CYP3A4, competitive inhibition of certain drugs, such as simvastatin and cyclosporine, can occur. This can increase drug exposure to both compounds (119)

Side Effects:

Imatinib is associated with a variety of side effects, most of which are mild to moderate intensity and settle after the first few months of treatment. Most patients don't require dose reduction or interruption of therapy. Transient neutropenia or thrombocytopenia, which can occur during the first 1 to 2 months of treatment, is due to variability in the rate of regeneration of normal haematopoiesis after Imatinib suppression of Ph derived haematopoiesis (120).

The most commonly reported side effects are oedema (including peripheral and periorbital) (60%), muscle cramps (49%), diarrhoea (45%), nausea (50%), musculoskeletal pain (47%), rash and other skin problems (40%), abdominal pain (37%), fatigue (39%), joint pain (31%), and headache (37%). Grade 3 or 4 side effects consist of neutropenia (17%), thrombocytopenia (9%), anaemia (4%), elevated liver enzymes (5%), and other drug-related adverse events (17%). Congestive heart failure was reported as being drug-related in one patient (<1%) (79).

ELN current recommendations

An international expert panel was convened to prepare treatment recommendations, on behalf of the European LeukaemiaNet (121). The panel consisted of 19 members with recognised clinical and research expertise in CML, of whom 11 came from Europe, seven from the USA, and one from Australia. The panel's recommendations corroborated the value of imatinib and allogeneic stem cell transplantation. These recommendations have been recently revised and updated (104).

The first-line treatment of a CP patient who is TKIs-naive is imatinib; 400 mg daily (table 4). In case of imatinib intolerance, and or resistance, it is recommended to switch to newer generation TKIs like nilotinib or dasatinib, at the registered dose of 400mg twice daily and 100 mg once daily respectively. The choice between nilotinib and dasatinib may be influenced by the presence of *BCR-ABL1* kinase domain (KD) mutations and by a difference in the expected side effects (122).

Table 4: Current treatment recommendations

Chronic phase, frontline	Imatinib 400mg daily
Chronic phase, 2nd line: - Imatinib intolerance - Imatinib failure	- Nilotinib 400mg twice daily or dasatinib 100mg once daily - Nilotinib or dasatinib, as above - Allo-SCT in high disease risk and low EBMT score
Chronic phase, 3rd line: - Nilotinib or dasatinib failure	- Allo-SCT
Accelerated and blastic phase: - Frontline (TKI naive) - Second line (imatinib resistant)	- Imatinib followed by allo-SCT, as soon as possible - Nilotinib or dasatinib, followed by allo-SCT as soon as possible

In case of suboptimal response to imatinib, it is possible to continue the same dose, to increase imatinib dose, or to test nilotinib or dasatinib. In case of suboptimal response to nilotinib or dasatinib, it is possible to continue with TKIs, or to move to allo-SCT.

Monitoring the response:

Blood counts and differential should be performed at least every 2 weeks until a CHR has been achieved and confirmed, then at least every 3 months. Cytogenetic examination of marrow cell metaphases by chromosome banding analysis (CBA) is required at 3 and 6 months, then every 6 months until a CCyR has been achieved and confirmed, then every 12 months. Interphase-fluorescence- in situ hybridisation (I-FISH) of marrow or blood cells may substitute for CBA only to monitor the CCyR. CBA of marrow cell metaphases is required to detect clonal chromosome abnormalities in Ph+ as well as in Ph– cells. *BCR-ABL1* transcript level with RT-Q-PCR of blood cells is required every 3 months until a major molecular response (MMR) has been achieved and confirmed, then every 6 months.

Mutational analysis by direct sequencing or denaturing high performance liquid chromatography (D-HPLC) is recommended in case of suboptimal response and failure, and always before switching from one TKI to another (122).

Definition of response to imatinib:

The response is measured based on haematologic, cytogenetic and molecular tests (table 4). Based on the degree of each respective response, it has been proposed to classify the overall response to treatment as optimal, suboptimal and failure (tables 5, 6).

Table 5: Definition of haematologic, cytogenetic and molecular response

Complete haematologic response (CHR)	WBC < 10 x 10^9/L, no immature granulocytes, basophils < 5%, platelets < 450 x 10^9/L, non palpable spleen
Complete cytogenetic response (CCyR)	No Ph +ve metaphases
Partial cytogenetic response (PCyR)	1 – 35 % Ph +ve metaphases
Minor cytogenetic response (MiCyR)	36 – 65 % Ph +ve metaphases
Minimal cytogenetic response	66 – 94 % Ph +ve metaphases
No cytogenetic response	$\geq$ 95% Ph +ve metaphases
Major molecular response (MMR)	*BCR-ABL1: ABL1* $\leq$ 0.1% on international scale
Complete molecular response (CMR)	*BCR-ABL1* transcripts undetectable by RQ-PCR

'Warning' means that the patient may have a lower probability of achieving an optimal response, and should be monitored more carefully than the standard patient. A CML patient who fails imatinib therapy may do well for years, but will never become an optimal responder, and for that reason should be moved to another therapy, whenever whenever available and applicable.

'Suboptimal response' is a grey area between failure and optimal response. The condition of a suboptimal responder is transitory by nature (122), but there is now massive evidence that they do as bad as failures (123-126). 'Optimal response' means that based on current knowledge, the patient is expected to have a normal survival. Although this definition may be overconfident, it emphasizes that the goal of therapy is to achieve a normal survival.

Table 6: Definition of the response to treatment with IM, early chronic phase, frontline

	Warnings	Failure	Suboptimal response	Optimal response
Baseline	- High risk score [a] - CA/Ph +ve [b]	-	-	-
3 months	-	No CHR	No CyR	At least MiCyR
6 months	-	No CyR	Less than PCyR	At least PCyR
12 months	Less than MMR	Less than PCyR	PCyR	CCyR
18 months	-	Less than CCyR	Less than MMR	MMR
Any time	- rise in transcript levels - CA/Ph -ve [c]	- Loss of CHR - Loss of CCyR - KD mutations - CA/Ph +ve [b]	- Loss of MMR - KD mutations	Stable or improving MMR

a: High risk either by Sokal or Hasford
b: Chromosomal abnormalities (CA) in Ph +ve cells
c: Chromosomal abnormalities (CA) in Ph -ve cells

Recommendations: *Accelerated and blastic phase (AP, BP)*

All BP patients, and the majority of AP patients who are eligible to allo-SCT, are candidates for allo-SCT, from any donor, even if the response is optimal, because most optimal responses are short. If a patient is TKI-naive, and does not carry an imatinib poorly sensitive *BCR-ABL1* mutation, the recommended treatment before allo-SCT is imatinib 600 or 800 mg daily. If a patient progresses on imatinib, the recommended treatment before allo-SCT is Nilotinib or Dasatinib (122).

Prediction of patient response

Sokal and Hasford scores:

The Sokal prognostic score (127) arose from a study of 813 patients with non-blastic Ph+ CML, which was designed to distinguish between patients with good and poor prognosis. This study was conducted at a time when busulfan was the most common treatment modality. Clinical parameters (spleen size; blast-cell, platelet, and white cell count; and haemoglobin) were assessed as possible prognostic indicators. Importantly, all of the variables were assessed prior to the commencement of any therapy. Using a hazard ratio analysis, the most discriminatory factors were found to be spleen size, blast-cell percentage, patient age, and platelet count. The hazard ratio was then calculated for each patient, and patients were grouped into low-risk (Sokal score < 0.8), intermediate-risk (Sokal score 0.8–1.2), and high-risk groups (Sokal score > 1.2). In the busulfan era, the Sokal score was found to be predictive of long-term survival and became a universal prognostic indicator for CML; it remains in use today.

The Hasford prognostic score was developed in the interferon era (128). It was based on the analysis of 908 patients and generated an algorithm based on several covariates: age, spleen size, blast count, platelet count, eosinophil count, and basophil count. Both the Sokal and Hasford scores were found to be predictive of survival in interferon-treated patients (59, 129).

A degree of uncertainty remains about the value of these prognostic indicators in the imatinib era. For this purpose, a European registry for CML patients was created by the European LeukaemiaNet (ELN) containing data from 2060 patients gathered from 5 national studies. The European Treatment and Outcome Study for CML (EUTOS) project was setup (130), in order to provide a score that predicts the probability of achieving a CCyR within 18 months, being the most accurate marker of survival (79, 104, 115).

Pre-therapy predictors:

The IC_{50}imatinib:

In 2005, researchers launched an in vitro study to determine the specific 50% inhibitory concentration (IC_{50}) of imatinib (IC_{50}imatinib) for individuals in a series of 62 previously untreated patients with CP CML who were enrolled in the Australian Trial of Imatinib with Dose Escalation in chronic myeloid Leukaemia (TIDEL) study, which used 600mg of imatinib as front line therapy (131).

The phosphorylation status of the adaptor protein Crkl (p-Crkl) was used as a surrogate marker for *BCR-ABL1* protein (132). The study demonstrated that the degree of kinase inhibition achieved was a critical determinant of the subsequent molecular response to imatinib therapy and those intrinsic factors in the patient (yet to be defined) determine this sensitivity (131). The study also demonstrated that there was no significant association between the IC_{50}imatinib and classic indicators such as the Sokal score or deletions of the long arm of der(9). The IC_{50}imatinib is predictive, but the underlying basis of the intrinsic sensitivity in some patients remains to be determined.

Wilms tumour gene

Wilms tumour (*WT1*) is a tumour suppressor gene encoding a zinc finger transcription factor (133). In normal haemopoietic cells, *WT1* is expressed at very low levels, but it is elevated in several acute and chronic leukaemias (134). Cilloni et al investigated *WT1* as a potential prognostic indicator in 36 patients and found that a short incubation with imatinib reduced the elevated level of *WT1* in patients who were later defined as good imatinib responders but not in patients who demonstrated a suboptimal response to imatinib.

hOCT1

The molecular transporter *hOCT1* is responsible for the active intracellular intake of imatinib. Low levels of *hOCT1* (or low activity), which result in low intracellular concentration of imatinib, have been associated with poor cytogenetic and molecular responses to imatinib (135-137). Furthermore, patients with low *hOCT1* activity have been shown to have worse OS and EFS (137). It should be noted that dasatinib and nilotinib are not substrates for this transporter, so *hOCT1* has only prognostic value in patients receiving imatinib. Despite the fact that several groups have confirmed the prognostic utility of *hOCT1*, the level or the activity of this molecular transporter is not routinely measured in patients undergoing therapy, nor is there any agreement about the best technique to measure it.

MDR-1

Proteins belonging to the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family, in particular ABCB1, also known as P-glycoprotein or MDR-1, are involved in the active extrusion of imatinib from the cells. High expression of these proteins has been shown in patients with imatinib resistance and patients who have progressed to advanced phase (138).

A number of single-nucleotide polymorphisms in genes encoding for these proteins have been found to be predictive for response in CML patients treated with imatinib; in particular polymorphisms in the *MDR-1* gene have been associated with the achievement of MMR (139). Most of these associations have not been independently confirmed and patients are not routinely tested for these polymorphisms.

Gene Expression Profiling

Several groups have reported gene expression signatures that allow one to separate patients according to the phase of the disease (early chronic phase, late chronic phase, and advanced phase) (140, 141) and to differentiate patients with primary or secondary (or acquired) resistance (142). Ideally one would like to identify at diagnosis patients who are less likely to fare well and to adjust therapy accordingly. Some groups have reported that it is possible to separate patients likely to fail to achieve cytogenetic responses (143, 144) or more likely to progress (145) according to their gene expression signature using diagnostic samples. However, up to now gene expression profiling has not achieved the discriminative power that one would like and it is not used in the routine management of patients.

On-therapy indicators

Imatinib plasma levels

In a publication by Picard et al (146), the association between trough plasma levels of imatinib and the likelihood of achieving CCyR and MMR was assessed. The authors concluded that sensitivity and specificity for cytogenetic and molecular responses are associated with a trough level of 1002ng/mL, and that plasma trough levels should form part of routine on-therapy disease response monitoring. These data were preliminary, and provided no indication of the best time to monitor plasma levels (147).

Several studies later reported better rates of cytogenetic and molecular responses have in groups of patients with higher imatinib exposure (135, 137, 146, 148), but it is not clear how to interpret the results of individual patients.

A recent study has shown that the predictive value of imatinib levels disappeared when adherence to imatinib therapy was taken into account (135). Imatinib plasma levels are not routinely used as a prognostic marker.

Early molecular response

BCR-ABL1 measurements performed early in imatinib therapy were found to predict the subsequent imatinib response (149, 150). In these analyses, RQ-PCR for *BCR-ABL1* was performed at 3 months after the start of imatinib therapy. Patients were divided into those who failed to achieve a 1-log reduction, those achieving a 1 log to 2 log reductions, and those achieving a reduction greater than 2 logs. Kaplan-Meier analysis on these three groups revealed a significant outcome advantage for those patients who achieved a good molecular response by 3 months. Assessing the IRIS trial patients, 100% of patients who achieved greater than 2-log reduction by 3 months achieved an MMR by 24 months, and none demonstrated primary or secondary resistance out to 30 months of therapy.

Imatinib resistance

Patients with CML can vary in their response to imatinib. Rarely, some patients are entirely unresponsive to imatinib and thus have primary resistance [failure to achieve CHR by 3 months, CyR by 6 months, partial cytogenetic response (PCyR) by 12 months, or CCyR by 18 months]. Additionally, some patients develop an acquired secondary resistance to imatinib, whereby they can lose the initial response they had achieved.

Patients develop imatinib resistance through multiple mechanisms, with some being *BCR-ABL1* dependent and others *BCR-ABL1* independent (table 7) (151). The acquisition of point mutations in the kinase domain of the *BCR-ABL1* gene can lead to conformational changes that abrogate the ability of imatinib to bind to and/or inhibit the kinase activity of *BCR-ABL1* (152). Molecular sequencing has uncovered over 100 different mutations in clinical specimens of patients who have failed imatinib (figure 12). The development of a T315I mutation is particularly concerning as it confers resistance to all available TKIs. While P-loop mutations also can lead to resistance to imatinib, most can be overcome with second generation TKIs (153).

BCR-ABL1 independent mechanisms of resistance can include over expression of the Src family of kinases (SFKs), variable expression of *hOCT1* and MDR-1 (154). The persistence of quiescent stem cells can prevent the eradication of disease (155). This latter finding has led many investigators to develop strategies to eliminate residual disease (156).

Table 7: Mechanisms of resistance to TKI

BCR-ABL1 dependant
▪ *BCR-ABL1* amplification and/or over expression ▪ Mutations within the ABL1 kinase domain
BCR-ABL1-independent
▪ Treatment compliance ▪ Plasma levels ▪ CYP3A4/A5 single nucleotide polymorphisms ▪ Increased α-1 acid glycoprotein ▪ Decreased *hOCT1* expression ▪ Increased ABCB1 (MDR-1) expression ▪ Over expression of SRC-related kinases ▪ Clonal evolution ▪ CML stem cell quiescence (persistence)

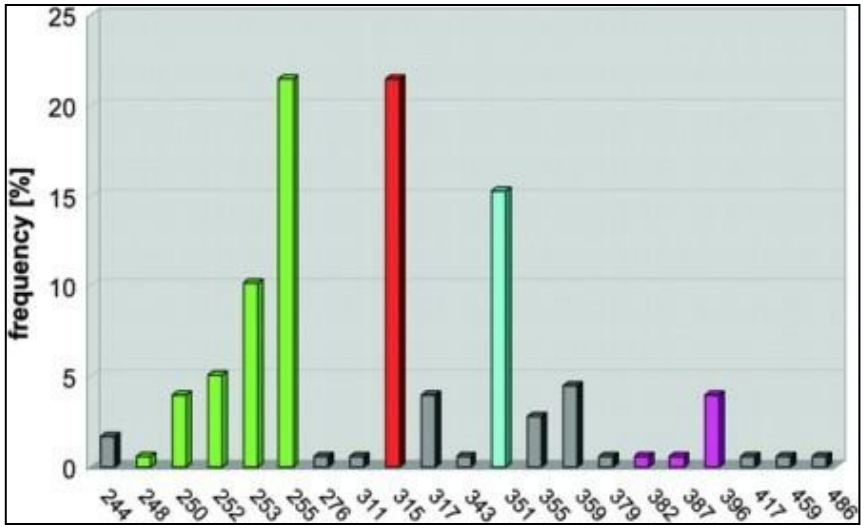
Possible solutions to the problem:

Primary and secondary resistance to imatinib remain a challenging problem. Guidelines indicate that patients failing imatinib should be checked for compliance issues, potential drug–drug interactions and *BCR-ABL1* KD mutations. Patients may then be considered for imatinib dose escalation, changing to a second generation TKI, stem cell transplantation (SCT) or clinical trial participation (78, 104).

Imatinib dose escalation

Suboptimal response due to low imatinib plasma concentrations or as a consequence of low *hOCT1* activity and failure due to mutations causing low-level resistance can be overcome by imatinib dose escalation (157-159). Conflicting results have been reported on durability of responses. In some reports the beneficial effect of dose escalation is short-lived (160). In more recent reports, durable responses have been described.

Figure 12: Schematic diagram of point mutations in the *BCR-ABL1* kinase domain



Jabbour et al (161) assessed 84 CML patients with failure to standard dose imatinib and reported durable responses after dose escalation to 600 or 800 mg imatinib. CCyR was achieved in 40, and 69% of patients remained alive after a median follow-up of 61 months from dose escalation. Eighty-eight per cent of patients with MCyR sustained their response beyond 2 years. Rea et al (124) analyzed imatinib dose escalation in 23 newly diagnosed CP-CML patients with suboptimal cytogenetic and molecular responses and showed that in the vast majority the incidence of MMR increased significantly, which was durable in most cases 21 months after dose escalation.

In a randomized phase III trial, Kantarjian and colleagues (162) evaluated switching to dasatinib (70mg twice daily) versus dose escalation of imatinib (400mg twice daily) in 150 patients with secondary resistance to imatinib. After a median follow-up of 26 months, there was no statistical difference in the primary endpoint of a MCyR at 12 weeks in the dasatinib-treated patients (36 %) compared with those receiving imatinib (29 %) (P = 0.40). However, the rates of CHR, MCyR, CCyR, MMR, PFS and treatment failure were all superior in the dasatinib-treated patients at later time points (163). The efficacy of imatinib dose escalation has been confirmed by three recent trials (125, 164, 165). The choice between imatinib dose escalation and crossover to second generation TKI frequently depends on clinical judgment. In view of the causes of suboptimal response (low *hOCT1* activity, lack of adherence, low level resistance mutations), dose escalation is a reasonable first choice (166).

Second-generation TKIs

At present, two second-generation TKIs are available for the treatment of imatinib-resistant or -intolerant CML: dasatinib and nilotinib. A third second-generation TKI, bosutinib, is still under trial. Various phase II studies in CP, AP and BC CML have established dasatinib and nilotinib potency after imatinib failure.

<u>Dasatinib:</u>

Dasatinib is a potent orally available ABL kinase inhibitor with 325-fold greater in vitro selectivity for un-mutated *BCR-ABL1* than imatinib (152, 167). In addition to blocking *BCR-ABL1* kinase activity, it also inhibits a wide spectrum of other kinases, including SFKs, c-Kit, platelet-derived growth factor-receptor (PDGFR), and ephrin-A receptor (168).

Dasatinib, unlike imatinib, binds to both the active and inactive conformations of *BCR-ABL1* and is active in vitro against all imatinib resistant mutations, with the exception of the threnoine to isoleucine mutation at codon 315 (T315I) ‘‘gatekeeper’’ mutation (169).

Several phase (II) studies (the START-series) were carried out leading to approval of dasatinib in 2006. In the first phase (II) trial (START C), 387 imatinib resistant or intolerant CP-CML patients were studied. CHR was attained in 91% of patients and MCyR in 59%, which was complete (CCyR) in 49% of patients (40% of imatinib resistant and 75% of imatinib-intolerant patients) (170). Fifteen months overall survival (OS) was 96%. Grade 3/4 thrombocytopenia and neutropenia were reported in 48 and 49% of patients, respectively. Non-haematologic toxicity consisted primarily of diarrhoea (37%, mostly self limiting), headache (32%), fatigue (31%), dyspnoea (30%) and pleural effusion (27%, none of grade 4). Although efficacy in patients with imatinib-resistant CML was considered a major breakthrough in CML-therapy, the limitation of dasatinib was the disappointingly low CCyR rate of <50%.

In addition, the high frequency of pleural effusions was disturbing. To determine whether a lower dose might improve tolerability to the drug but the same efficacy, various dasatinib regimes were analyzed. In a Phase (III) study on 670 CP-CML patients, dasatinib 100 mg once daily was compared with 50 mg twice daily, 140 mg once daily and 70 mg twice daily. Dasatinib 100 mg once daily was found to preserve efficacy at improved tolerability and resulted in significantly lower rates of pleural effusion and grade 3/4 thrombocytopenia, while maintaining comparable haematologic and cytogenetic remission rates and survival at 2 years (171).

In contrast to other TKIs, dasatinib crosses the blood--brain barrier and can be used for central nervous system involvement by Ph-positive leukaemia (e.g., Ph-positive ALL or lymphoid BC) (172). Phase (II) trials were carried out in AP, BC and Ph-positive ALL.

In a phase (II) trial with dasatinib 70 mg twice daily in 161 imatinib-resistant or -intolerant patients with CML in AP (START A), CHR was attained in 45% and CCR in 32% of patients. Twelve-month OS was 82%. Diarrhoea occurred in 52% and pleural effusion in 27% of patients (173). In a phase (II) trial with dasatinib 70 mg (up to 100 mg) twice daily in 116 patients with imatinib-resistant or -intolerant BC (74 myeloid, 42 lymphoid; START B), MCyR was achieved in 31 and 50% of patients, respectively (174).

In another phase (II) trial with dasatinib of 140 mg/day in 36 patients with Ph-positive ALL (START L), major haematologic responses were achieved in 42% and CCR in 58% (21/36) of patients. Tolerability was acceptable and adverse events were mostly grade 1-2 (175).

The Dasatinib versus Imatinib study in Treatment-Naive CML patients (DASISION) was a phase (III) multinational trial in which 519 patients with newly diagnosed chronic-phase CML were randomized to receive either dasatinib 100mg once daily or imatinib 400mg once daily (176). After a minimum follow-up of 12 months, the rate of confirmed complete cytogenetic response was higher with dasatinib than with imatinib (77 % vs. 66 %, P = 0.007), as was the rate of complete cytogenetic response observed on at least one assessment (83 % vs. 72 %, P = 0.001). The rate of major molecular response was higher with dasatinib than with imatinib (46 % vs. 28 %, P<0.0001), and responses were achieved in a shorter time with dasatinib (P<0.0001).

Progression to the accelerated or blastic phase of CML occurred in 5 patients who were receiving dasatinib (1.9 %) and in 9 patients who were receiving imatinib (3.5 %). Dasatinib was relatively well tolerated, with grade 3 – 4 cytopenias (10 - 21%) and effusions (10%) the most common side effects.

Nilotinib:

Another orally available TKI that can be used after failure of first-line imatinib is nilotinib. It was approved by the FDA in 2007 for the treatment of Ph-positive CML in CP and AP (but not in BP or Ph-positive ALL) who are resistant or intolerant to imatinib. Nilotinib is an imatinib analogue with 30 times more potency at in-vitro *BCR-ABL1* inhibition (152, 177). Nilotinib is active against nearly all imatinib-resistant *BCR-ABL1* mutations with the exception of T315I (178). Like imatinib, nilotinib binds an inactive conformation of *ABL1* but may overcome most mutations through its greater potency (179).

Nilotinib activity against imatinib resistant or imatinib intolerant CML was demonstrated in 2 open-label, phase (II) studies; one in patients with CP-CML (180) and the other with CML in AP (181). The CP-CML trial involved 321 patients who were imatinib resistant (30%) or imatinib intolerant (70%) and had a minimum follow-up of 19 months. A MCyR was achieved in 59% of patients at a median of 2.8 months, and a CCyR was achieved by 44% of patients at a median of 3.3 months.

Responses were durable, with 78% and 83% of patients maintaining MCyRs and CCyRs, respectively at 24 months. The OS rate was 88% at 24 months. At 2-year follow-up, treatment had been discontinued in 59% of participants because of disease progression (27%), drug-related adverse events (15%), or other reasons (17%) (180).

The AP CML trial involved 137 patients who were imatinib resistant (80%) or imatinib intolerant (20%) and had a minimum follow-up of 11 months. A CHR was achieved in 31% of patients at a median of 1 month after the initiation of therapy, and a MCyR was achieved in 32% of patients at a median of 2.8 months. A CCyR was achieved in 20% of patients, and >70% of those patients remained in CCyR at 24 months. The OS rate was 67% at 24 months (181).

The recommended dose for nilotinib is 400mg administered twice daily approximately 12 hours apart. As food increases the bioavailability of nilotinib, it is recommended to take the dose on an empty stomach, i.e.: either 2 hours after or 1 hour before the last meal. Nilotinib is well tolerated with the most common grade 3 or 4 laboratory abnormalities were; elevated lipase (18%), hypophosphatemia (17%), hyperglycemia (12%), and elevated total bilirubin (7%).

Grade 3 or 4 haematologic adverse events were infrequent, the most common of them were; neutropenia (31%), thrombocytopenia (30%), and anaemia (11%). Pleural or pericardial effusions (all grades) occurred in 2% of patients, and grade 3 or 4 pleural or pericardial effusions were rare (<1%).

To determine its efficacy as first line therapy, a phase III randomized, multicentre trial, called the Evaluating Nilotinib Efficacy and Safety in Clinical Trials – Newly Diagnosed Patients (ENESTnd) study was designed. The study involved 846 patients with chronic-phase Philadelphia chromosome positive CML who were given in a 1:1:1 ratio either nilotinib (at a dose of 300 mg or 400 mg twice daily) or imatinib (at a dose of 400 mg once daily). The 12 months follow-up showed that the rates of MMR for Nilotinib (44% for the 300mg dose and 43% for the 400mg dose) were nearly twice that for Imatinib (22%) with (P<0.001) for both comparisons.

The rates of CCyR were significantly higher for Nilotinib (80% for the 300mg dose and 78% for the 400mg dose) than for Imatinib (65%) with (P<0.001) for both comparisons, indicating Nilotinib superiority (182).

Bosutinib:

Bosutinib (formerly known as SKI-606; Wyeth/Pfizer, New York, New York, USA) is a dual *ABL1/SRC* TKI which, like nilotinib and dasatinib, is more potent than imatinib and offers activity against several imatinib resistant ABL1 mutations.

Bosutinib has shown activity in CML patients refractory to imatinib and other TKIs in phase II trials (183-185) and has recently completed the phase III randomized BELA trial (Bosutinib Efficacy and Safety in Chronic Myeloid Leukaemia) in which its results were presented at the ASH meeting in 2010 (186) and the results have been recently published (187).

The study involved 502 newly diagnosed CML-CP patients, randomised either to bosutinib 500mg or imatinib 400mg daily with CCyR at 12 months as the primary end point. Unfortunately, there was no significant difference in CCyR achievement rates between the bosutinib and imatinib arms (70% vs. 68% respectively, P = 0.601), whereas the MMR rate at 12 months showed significance between both arms (39% vs. 26% respectively, P = 0.002). Drug related adverse events (AE) leading to discontinuation occurred in 19% of patients on bosutinib compared to 5% on imatinib, especially diarrhoea (68% on bosutinib, compared with 21% on imatinib) that is transient and occurs early in the course of treatment.

Newer TKIs including anti-T315I agents

Ponatinib

Ponatinib (AP24534; Ariad Pharmaceuticals, Cambridge, MA) is a novel TKI of *BCR-ABL1* with activity against all known *BCR-ABL1* mutants, including T315I (188). Phase I trials data, showed that ponatinib had encouraging activity, including haematologic and cytogenetic responses achieved in patients harbouring T315I (189-191). Pancreatitis developed at 60mg daily, and 45mg daily was selected as the recommended dose for further testing.

There was clear evidence of anti-leukaemia activity, with major cytogenetic responses in 46% of chronic-phase patients resistant to second-line tyrosine kinase inhibitors, including 67% of those with the T315I mutation, as well as MMRs. A pivotal phase II trial of ponatinib is now under way. A number of similarly targeted third-generation TKIs have recently initiated clinical trials evaluation and are summarized in table 8.

Table 8: Investigational agents in chronic myeloid leukaemia

Drug	Company	Targets	T315I activity	Clinical results
INNO-406 (192, 193)	CytRx, Los Angeles, CA, USA	*ABL1*, KIT, LYN, PDGFR	No	n = 40 MCyR – 15% CCyR – 8%
XL228 (194)	Exelixis, South San Francisco, CA, USA	*ABL1*, Aurora A, FGFR1-3, IGF1R, SRC	Yes	n = 27 MCyR – 7% CCyR – 4%
Danusertib (195-197)	Nerivano Medical Sciences, Milan, Italy	*ABL1*, Aurora A&B, FGFR1, RET, TRK	Yes	n = 23 CHR – 22% MCyR – 9% CCyR – 4%
AT9283 (198-200)	Astex, Cambridge, England	*ABL1*, Aurora A&B, FLT3, JAK2, JAK3	Yes	N/A
KW-2449 (201-203)	Kyowa Hakko Kirin Pharma, Tokyo, Japan	*ABL1*, Aurora A, FGFR1, FLT3	Yes	N/A
DCC-2036 (204, 205)	Deciphera, Lawrence, KS, USA	*ABL1*, FLT3, KDR, SFK, TIE2	Yes	Phase 1 study results published
Omacetaxine (206, 207)	ChemGenex, VIC, Australia	cytochrome c, MCL-1	Yes	Phase 2/3 study results published

Aims of this thesis

The prognosis of CML has changed dramatically with the introduction and use of imatinib and second generation tyrosine kinase inhibitors into clinical practise, transforming what used to be a lethal disease to a relatively chronic condition. Recent data suggest that chronic phase patients treated with TKIs could have almost a normal life span, with a good quality of life.

Disease management is currently being shifted from managing resistant conditions and blastic transformation into dealing with the new challenges that arise with long-term therapy such as: early identification of patients who won't do well and improving patients' outcome.

Despite the use of imatinib and TKIs for almost a decade, proper prognostic tools that can predict patients' responses are still lacking. And also, as these drugs need to be used life-long, management should also be directed towards tackling problems that face patients on long-term therapy including those of compliance and pregnancy to try to offer patients the best quality of life possible.

Therefore, the current aims of this thesis are:

- Examining current prognostic scores used and attempting their validation
- Establishing the use of molecular markers as a prognostic tool
- Assessment of extent of non-adherence to therapy
- Demonstrating the effect of non-adherence on achievement or loss of responses
- Assessment of the effect of treatment interruption in pregnancy
- Determining the survival benefit of second generation TKIs
- Assessment of durability of responses to second generation TKIs
- Assessment of efficacy of TKIs as third line therapy

Chapter II

Patients and Methods

Patients & database

For our analyses, we included a total of 289 patients with Ph +ve, *BCR-ABL1* +ve CML patients in chronic phase who were treated with first line imatinib at the Hammersmith Hospital. These patients included: patients enrolled in current clinical trials; patients who are on follow up from older trials; and newly diagnosed patients who started imatinib therapy within 6 months of diagnosis as imatinib became available within the NHS (non trial patients).

Data for the patients were entered into an Access database comprising three tables. Table (I) contains the basic demographic data, details of diagnosis and of treatment and response before starting treatment with Tyrosine Kinase Inhibitors (TKIs). Table (II) contains descriptive data applicable at the actual time of starting TKIs. Tables (I) and (II) both contain a register for each patient. Table (III) contains the TKI data, where each patient is represented by an unlimited number of registers each showing the situation of the patient at a given time point (visit).

Although the database was originally created to monitor patients enrolled in imatinib trials it has been updated to include consecutive changes in therapy, including the introduction of the new generation TKIs such as dasatinib, nilotinib and bosutinib. All three tables are linked by the patient identifier number (N). The variables and codes included in the database are shown in Appendix (I).

TKI administration

Imatinib:

Imatinib was prescribed to patients at a dose of 400 mg orally daily. The dose was adjusted according to tolerance and response (54, 79): it was reduced in the presence of grades 3-4 toxicity (208) and hematopoietic growth factors were administered with the aim of maintaining imatinib higher than 300 mg/d.

Dose escalation for patients on imatinib who failed to achieve a complete haematologic response at 3 months or at least a minor cytogenetic response (defined as between 36 to 65% Ph-positive metaphases) at 12 months were applied as in the phase II trial or the IRIS study (53, 54), but as more evidence accumulated, the criteria evolved and resembled the subsequent recommendations from the European Leukaemia-Net (104, 121).

Dasatinib & Nilotinib:

Dasatinib was administered at a dose of either 70mg every 12 hours (twice daily) or 100mg once daily while nilotinib was given at a dose of 400mg twice daily. Doses were adjusted according to tolerance (209, 210).

Bosutinib:

Bosutinib was prescribed to some of our patients who are participating in phase III clinical trials. Bosutinib was given in a dose of 500mg/day, which was also adjusted according to tolerance (187).

Definitions and monitoring of responses

Chronic phase (CP) and complete hematologic response (CHR) are defined by standard criteria (47, 54, 104, 121). Bone marrow morphology and cytogenetics are assessed at diagnosis and then every 3 months, until patients achieve CCyR using Giemsa banding. Thereafter, patients are monitored by real-time quantitative polymerase chain reaction (RQ-PCR) and annual bone marrow examinations.

A complete cytogenetic response (CCyR) is defined by the failure to detect any Philadelphia chromosome (Ph)-positive metaphases in two consecutive bone marrow examinations with a minimum of 20 metaphases examined. A partial cytogenetic response (PCyR) is defined as a decrease in the proportion of Ph-positive metaphases to between 1 and 35%, while a major cytogenetic response (MCyR) is defined by combining the number of complete and partial cytogenetic responses ($\leq$35% Ph +ve metaphases), and a minor cytogenetic response (MiCyR) is defined as a decrease in the proportion of Ph +ve metaphases to between 35 and 95%.

Measurement of *BCR-ABL1* transcripts

BCR-ABL1 transcripts were quantitated by RQ-PCR using the TaqMan Real-time PCR apparatus (Perkin-Elmer, Wellesley MA, USA) in the Minimal Residual Disease (MRD) Laboratory of the Hammersmith Hospital. *BCR-ABL1* transcripts and *ABL1* transcripts selected as internal control were quantified separately. Results were expressed as percent ratios relative to an *ABL1* internal control with original laboratory values converted to the international scale (211). Peripheral blood cellular RNA was reverse transcribed to cDNA using standard molecular biology techniques (212, 213). The cDNA was then subjected to 50 cycles of analysis using the ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and TaqMan Universal Master Mix in accordance with the manufacturer's instructions in a final reaction volume of 25 µl. Probes and primers were designed using the Primer Express software (Applied Biosystems) to detect e13a2 and e14a2 junctions in a single reaction by RQ-PCR.

Probes were designed for *BCR-ABL1* (-cccttcagcggccagtagcatctga-TAMRA) and *ABL1* (tgcttctgatggcaagctctacgtctcct-TAMRA) and dual labelled with 6-carboxyfluorescein (FAM) and 6-carboxy-tetramethyl-rhodamine (TAMRA). The primers used in Q-PCR were: *BCR-ABL1* forward primer: 5'-tccgctgaccatcaayaagga-3'; *BCR-ABL1* reverse primer: 5'-cactcagaccctgaggctcaa-3'; *ABL1* forward primer: 5'-gatacgaagggagggtgtacca-3': *ABL1* reverse primer: 5'ctcggccagggtgttgaa-3'. The probe and primer concentrations for *ABL1* mRNA quantification were 200 nM and 300 nM respectively and 3 µl of cDNA. The *BCR-ABL1* mRNA levels were measured using 100 nM of probe and 300 nM of each primer with 5 µl of cDNA.

BCR-ABL1 and *ABL1* copy numbers were calculated by comparison with the standard curve generated using serial dilutions of linearized pNC210/G plasmid, containing the *BCR-ABL1* insert described previously. The sensitivity in our laboratory of quantitative RQ-PCR is equal to that of conventional nested PCR, i.e. $1x10^{-5}$, so the failure to detect any *BCR-ABL1* transcripts using RQ-PCR represents a reduction of at least 5 logs.

Detection of tyrosine kinase mutations

Direct Sequencing

The *BCR-ABL1* amplicon from each subject in the study was subjected to nested primer PCR using primers NTPB+ and NTPE- to generate an 863-bp fragment containing the entire *BCR-ABL1* kinase domain.

An aliquot of the PCR products was electrophoresed through 2.0 % agarose gel; if a single amplicon was observed the remaining PCR products were purified using Magna-PCR clean up kit in accordance with the manufacturer's instructions (DRI, UK). Otherwise the PCR fragment containing the Abl kinase domain was isolated using commercially available gel purification kit (Qiagen, UK) as recommended by the manufacturer. The purified amplicons were then subjected to Sanger's dideoxy chain termination reaction using a Big-Dye ABI 310 sequencer (Applied Biosystems, Foster City, USA). In each case the sequence obtained was compared with the published *ABL1* type 1a sequence, GenBank M14752, using BLAST 2 software. All observed base substitutions were confirmed by sequencing the complementary strand.

Statistical analysis

The data was analysed using the statistical package SPSS statistics 17.0 (August 2008, SPSS Inc., Chicago, Illinois). Data was captured from the Access database mentioned above and described in Appendix (I). Probabilities of OS, PFS and event-free survival (EFS) were calculated using the Kaplan-Meier method. Events were defined as loss of a CCyR or CHR, progression to advanced phase, death or imatinib discontinuation. Univariate analyses to calculate the probabilities of cytogenetic and molecular responses were done using the cumulative incidence (CI) procedure. The probabilities were compared using the log rank test or a Cox regression model. Variables found to be significant at $p < 0.1$ level was entered in the multivariate analysis unless otherwise mentioned in relevant sections.

In certain chapters we used the concept of "Current CCyR survival" (c-CCyRS), which is the analogue of 'current leukaemia free-survival'. This concept was originally developed to describe the behaviour of patients after allogeneic stem cell transplants; as it recognizes the fact that patients may relapse but regain remission with alternative therapy (214). In other words, c-CCyRS is defined as the probability of being alive and in CCyR at a given time point. Probabilities of c-CCyRS were examined using Fine-Gray regression or a modified Cox regression model for recurrent events respectively (215-218). All the analyses mentioned were performed on an "intention-to-treat" basis whenever possible.

Chapter III

New prognostic factors for CML

Introduction

As shown from the literature, the introduction of imatinib and other tyrosine kinase inhibitors (TKIs) have proved to be a major advance in the management of patients with chronic myeloid leukaemia (CML) in chronic phase (CP). However patients' prognosis is still evaluated using prognostic scores that have been developed during the chemotherapy and interferon era, i.e.: Sokal (127) & Hasford (128) scores. Although both scores are still in use, researchers argue that a new score is needed to fit with the current era of imatinib and TKIs (219).

Research has been ongoing to try to find new prognostic factors to match the current era. A recent publication by the Hammersmith group examined the prognostic significance of some of the patients' factors at diagnosis, including: patient's age; male to female ratio; Sokal risk score; time till start of therapy; splenomegaly; leukocyte count; platelet count; haemoglobin; blast count; basophil count and *BCR-ABL1* transcript type at diagnosis. The Sokal risk score at diagnosis showed to be the only predictive variable for achievement of CCyR (220), being the most accurate marker of survival (79, 104, 115)

Recently, a European registry for CML patients was created by the European Leukaemia-Net (ELN) containing data from 2060 patients gathered from 5 national studies to make the "European Treatment and Outcome Study" (EUTOS) for CML (130), in order to provide a score that predicts the probability of achieving a CCyR within 18 months. The simple formula for calculating the new (EUTOS) score can be shortened into:

EUTOS score = (7 X basophils) + (4 X spleen size)

The spleen was measured in centimetres below the costal margin and basophils as a percentage at baseline. A EUTOS score of > 87 indicates high risk and < 87 low risk.

As it only requires the basophil percentage in the blood and the spleen size, the authors suggest that it can be applied in any routine healthcare practice worldwide because of its simplicity (130). However, one drawback of the new EUTOS score is that it hasn't been validated independently. Therefore, in the first part of this chapter we applied the new score to an independent cohort of patients at our institution in order to check its prognostic value compared with the widely used Sokal.

Part I: EUTOS score validation

Patients and methods

We included 282 consecutive patients with CML in chronic phase received imatinib as first line therapy in treated at the Hammersmith hospital. The median age was 43 years (range 13-86 years); 157 (55.7%) were male. After starting imatinib 95 patients became intolerant of or resistant to imatinib and continued treatment with a second generation TKI therapy. The median follow up was 5.7 years (range 1.3 - 10.8 years). Table 9 shows the 8-year probabilities of overall survival (OS), progression-free survival (PFS) and the cumulate incidences of complete cytogenetic (CCyR) response and major molecular response (MMR).

Table 9: 8 year probabilities of OS, PFS, CCyR and MMR according to EUTOS and Sokal

	n*	OS	PFS	CCyR	MMR
EUTOS:		*p*=0.10	*p*=0.24	*p*=0.35	*p*=0.15
Low risk	241	89.8%	88.6%	87.5%	68.1%
High risk	31	79.1%	81.1%	86.8%	66.1%
Sokal:		*p*=0.001	*p*=0.002	*p*<0.0001	*p*=0.05
Low risk	88	94.6%	92.3%	93.6%	71.2%
Intermediate risk	111	86.5%	87.5%	86.7%	66.5%
High Risk	78	70.2%	74.3%	77.3%	58.1%

*We could not calculate the EUTOS and the Sokal score in 3 and 5 patients respectively due to missing data.

Results & discussion

We had data to calculate the EUTOS score in 277 (98.2%) of the patients. 246 (88.8%) were classified as low risk and 31 (11.2%) as high risk. Using the EUTOS scoring system the proportion of high risk patients was very similar to that of patients reported by Hasford et al (130)(11.2% vs. 10.1%, p= 0.58). As shown in table 3.1 the new EUTOS score calculated for our patients failed to significantly predict for any of the outcomes, namely OS, p=0.1; PFS, p=0.24; CCyR, p=0.35 and MMR p=0.15. Conversely, the Sokal score significantly predicted for all these outcomes (OS, p=0.001; PFS, p=0.002; CCyR, p<0.0001 and MMR, p=0.05). This should warn clinicians not to rush in before adopting the new system.

It has been shown that probably progress in assessing molecular responses would provide a better prognostic classification (211, 221-224), as in patients who manage to achieve CCyR, a rise in the *BCR-ABL1* transcript levels (which is detectable by RQ-PCR) can identify those that are prone to relapse (225). Therefore in the second part of this chapter, we explored the use of molecular responses as a prognostic factor in achieving responses.

Part II: *BCR-ABL1* as a prognostic factor in CML

Introduction

Within the last years, efforts have been made in order to define molecular milestones that can predict patients' responses (79, 226, 227), but unfortunately such findings haven't been entirely reliable. This has been partially attributed to the lack of standardization of the RQ-PCR technology used, an issue which is currently being addressed (211, 228); and also due to the fact that the achievement of MMR - commonest molecular response, which is equivalent to 3-log reduction in *BCR-ABL1* levels - doesn't always predict the most relevant outcomes such as OS or PFS (123).

Therefore we tried to identify molecular milestones that would strongly predict for OS and other outcomes. Using a conversion factor, these milestones can be applied in other centres to allow laboratories to express transcript levels on a widely accepted international scale (211) which can be later used to guide therapeutic decisions.

Patients

Between June 2000 and December 2010, 282 consecutive adult patients with CML in CP received imatinib 400 mg daily as first line therapy were recruited at our institution. Median follow-up was 69 months (range 17-131). Patient characteristics are shown in table 10.

During follow-up 118 patients discontinued imatinib and received either nilotinib (n=37), dasatinib (n=72) or an allogeneic stem cell transplant (n=9). Additionally, 22 patients underwent transplant after having failed second line therapy. Dasatinib and nilotinib were administered as described previously. CP, CHR, CCyR, and MCyR were defined by conventional criteria.

Detection of *BCR-ABL1* transcripts

BCR-ABL1 transcripts were measured in the blood at 6 to 12 week intervals using RQ-PCR as previously described. Results were expressed as percent ratios relative to an *ABL1* internal control with original laboratory values converted to the international scale (211). CMR was defined by the finding of two consecutive samples with no detectable transcripts with an *ABL1* control >40,000 copies. The median *ABL1* control in the CMR samples was 84,000 copies.

Statistical methods

Probabilities of OS, PFS and EFS were calculated using the Kaplan-Meier method and analyzed as mentioned previously. Here we put in the use the Current CCyR survival (c-CCyRS) which is the analogue of “current leukaemia free-survival” as it recognizes the fact that patients may relapse and regain remission with alternative therapy. All the analyses were performed on an ‘intention-to-treat’ basis.

Table 10: Patients' characteristics

Characteristics	
Age:	
Median-yr	46.3
Range-yr	13-86.4
Sex- no. (%):	
Male	157 (55.7)
Female	125 (44.3)
Sokal risk group - no. (%):*	
Low	88 (31.8)
Intermediate	111 (40.1)
High	78 (28.1)
Interval since diagnosis – (months)	
Median	1.5
Range	0-6
Chromosomal abnormalities in addition to the Ph chromosome no. (%)**	16 (6.0)
Splenomegaly***	
n (%)	186 (66.4)
Spleen size ≥10cm belo the costal margin, n (%)	75 (26.8)
White cell count- ($x10^9$/l)	
Median	140.5
Range	5.8-645
Platelet count - ($x10^9$/l)	
Median	396
Range	108-2267
Haemoglobin – (g/l)	
Median	116.0
Range	33.0-172.0
Peripheral blood blasts (%)	
Median	1
Range	0-14
Peripheral blood basophils (%)	
Median	2.6
Range	0-18

* We could not calculate the Sokal score in five patients due to missing data

** The commonest additional abnormalities were a second Ph chromosome and chromosome 8 trisomy. Data was missing in 17 patients.

*** Two patients had missing data; splenomegaly was defined as a palpable spleen in the physical examination and measured in cm below the costal margin.

Results

Outcome and responses

The 8-year CI of CCyR, MMR and CMR on imatinib therapy were 76.8%, 51.8% and 13.5% respectively. The 8-year probabilities of OS and PFS were 84.3% and 83.7% respectively. During follow-up, 118 (41.8%) patients discontinued imatinib on account of lack of efficacy (85 cases) or intolerance (33 cases) resulting in an 8-year probability of imatinib failure-free survival of 50.7%. The prognostic influence of patient characteristics on different outcomes is shown in tables 11 and 12.

Table 11: 8 years relative risk (RR) for OS, PFS, EFS according to patients' characteristics

	Relative risk (RR)		
Variable	**OS**	**PFS**	**EFS**
Age (years)	*p*=0.14 1.021	*p*=0.14 1.017	*p*=0.36 1.006
Sex- (female)	*p*=0.96 0.983	*p*=0.91 0.963	*p*=0.96 1.000
Sokal risk group Low Intermediate High	*p*=0.002 1 2.279 5.532	*p*=0.003 1 1.646 3.944	*p*=0.27 1 1.260 1.388
CE*	*p*=0.5 1.655	*p*=0.21 2.180	*p*=0.52 0.761
Splenomegaly (cm)	*p*=0.03 1.056	*p*=0.06 1.045	*p*=0.007 1.029
White cell count ($x10^9$/l)	*p*=0.01 1.003	*p*=0.05 1.002	*p*<0.001 1.002
Platelet count ($x10^9$/l)	*p*=0.91 1.000	*p*=0.572 1.000	*p*=0.006 1.001
Haemoglobin (g/l)	*p*=0.001 0.733	*p*=0.005 0.790	*p*=0.01 0.878
Peripheral blood blasts (%)	*p*=0.07 1.082	*p*=0.06 1.094	*p*=0.001 1.121
Peripheral blood basophils (%)	*p*=0.25 1.058	*p*=0.51 1.033	*p*=0.31 0.974

Table 12: 8 year cumulative incidences (CI) of CCyR, MMR, CMR, c-CCyR according to patients' characteristics

	Cumulative incidence (CI)			
Variable	**CCyR**	**MMR**	**CMR**	**c-CCyRS**
Age (years)	*p*=0.74 0.990	*p*=0.75 1.002	*p*=0.23 1.013	*p*=0.64 0.998
Sex- (female)	*p*=0.31 1.142	*p*=0.04 1.719	*p*<0.001 6.491	*p*=0.953 1.007
Sokal risk group Low Intermediate High	*p*<0.001 1 0.800 0.506	*p*=0.02 1 0.814 0.622	*p*=0.09 1 0.570 0.492	*p*<0.001 1 0.786 0.498
CE*	*p*=0.51 1.171	*p*=0.29 1.411	*p*=0.47 1.101	*p*=0.75 1.096
Splenomegaly (cm)	*p*<0.001 0.958	*p*<0.001 0.938	*p*<0.001 0.847	*p*<0.001 0.935
White cell count ($x10^9$/l)	*p*<0.001 0.997	*p*<0.001 0.996	*p*<0.001 0.989	*p*=0.01 0.998
Platelet count ($x10^9$/l)	*p*=0.005 1.001	*p*=0.03 1.001	*p*=0.001 1.001	*p*=0.64 1.000
Haemoglobin (gm/l)	*p*<0.001 1.207	*p*<0.001 1.221	*p*=0.02 1.258	*p*=0.001 1.116
Peripheral blood blasts (%)	*p*=0.005 0.928	*p*=0.12 0.953	*p*=0.01 0.648	*p*=0.108 0.964
Peripheral blood basophils (%)	*p*=0.18 1.023	*p*=0.13 1.030	*p*=0.19 1.027	*p*=0.34 1.017

The *BCR-ABL1* transcript levels at 3, 6 and 12 months strongly predicted for the most relevant clinical outcomes

The *BCR-ABL1* transcript levels at 3, 6 and 12 months significantly predicted for OS, PFS, and EFS, and also for the probabilities of achieving CCyR, MMR and CMR (tables 13, 14, 15). We employed a receiver operating characteristic (ROC) curve to identify the optimal cut-off in transcript level that would allow us to classify the patients as good or poor risk with maximal sensitivity and specificity for each individual outcome and time point. For example, at 3 months patients with transcript levels below 9.84% had significantly better 8-year OS (93.3% vs. 56.9%, *p*<0.0001, Figure 13), while the cut-off with maximal sensitivity and specificity for CMR was 0.61% (8-year CI of CMR 84.7% vs. 1.5%, *p*<0.0001, figure 13).

Interestingly, we found that at each time point the optimal cut-offs identified for OS, PFS, EFS and CI of CCyR were very similar; for example at 3 months the cut-offs identified for OS, PFS, EFS and CI of CCyR were 9.84%, 9.54%, 9.84% and 8.58% respectively, while the optimally predictive cut-offs for MMR and in particular for CMR were significantly lower (i.e. 2.81% and 0.61% respectively), indicating that a sub-set of patients destined to achieve CMR can be identified at early time points based on the speed of their response. The cut-offs identified for OS, namely 9.84% at 3 months, 1.67% at 6 months, and 0.53% at 12 months also strongly predicted for all the other outcomes (tables 13, 14, 15). This together with the fact that OS is the most relevant outcome made us choose the cut-offs identified for OS to define the transcript-based prognostic system.

Table 13: RR & CI at 8 years according to transcript level at 3 months

***BCR-ABL1* transcript level at 3 months**	**Outcome**	**RR, transcript level (log)**	**cut-off (%)**	**Number of patients at risk**	**Eight years probability of the outcome**	**Eight year probability of the outcome according to the risk group (n)**	
	OS	0.161 *p*<0.0001	≤9.84 >9.84	211 68	*p*<0.0001 93.3 56.9	N / A	
	PFS	0.162 *p*<0.0001	≤9.54 >9.54	208 71	*p*<0.0001 92.8 57.0	Low (211) High (68)	*p*<0.0001 92.8 55.5
	EFS	0.102 *p*<0.0001	≤9.84 >9.84	211 66	*p* <0.0001 65.1 6.9	Low (211) High (66)	*p* <0.0001 65.1 6.9
	CCyR	5.17 *p*<0.0001	≤8.58 >8.58	169 79	*p*<0.0001 99.4 21.7	Low (180) High (68)	*p* <0.0001 97.9 14.8
	MMR	12.98 *p*<0.0001	≤2.81 >2.81	141 137	*p*<0.0001 82.5 21.1	Low (210) High (68)	*p*<0.0001 70.1 0
	CMR	10.95 *p*<0.0001	≤0.61 >0.61	57 222	*p*<0.0001 84.7 1.5	Low (211) High (68)	*p*<0.0001 19.3 0

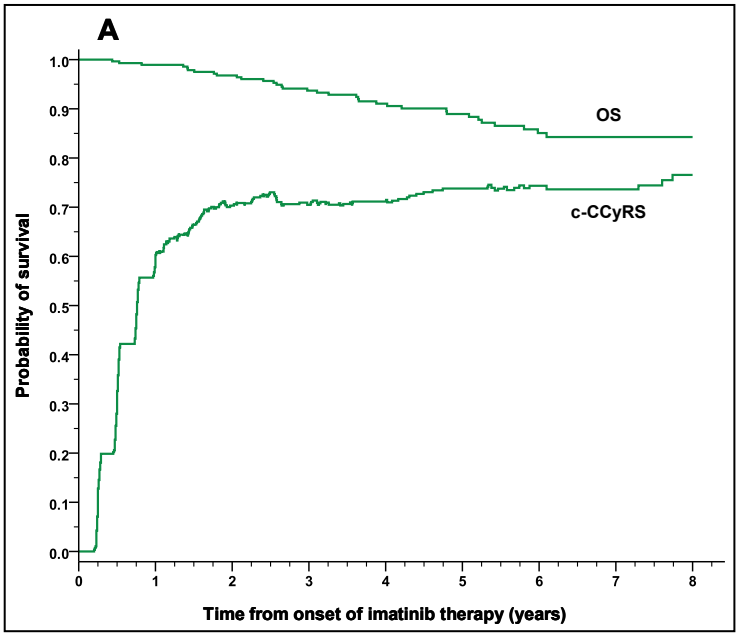
Table 14: RR & CI at 8 years according to transcript level at 6 months

BCR-ABL1 transcript level at 6 months	Outcome	RR, transcript level (log)	cut-off (%)	Number of patients at risk	Eight years probability of the outcome	Eight year probability of the outcome according to the risk group (n)	
	OS	0.342 *p*<0.0001	≤1.67 >1.67	187 87	*p*<0.0001 93.7 74.7	N / A	
	PFS	0.328 *p*<0.0001	≤1.73 >1.73	188 86	*p*<0.0001 92.8 68.9	Low (187) High (87)	0.328 *p*<0.0001
	EFS	0.195 *p*<0.0001	≤1.67 >1.67	186 87	*p*<0.0001 70.7 18.3	Low (186) High (87)	0.195 *p*<0.0001
	CCyR	5.033 *p*<0.0001	≤2.70 >2.70	98 66	*p*<0.0001 92.0 24.7	Low (82) High (82)	5.033 *p*<0.0001
	MMR	6.854 *p*<0.0001	≤0.73 >0.73	136 123	*p*<0.0001 81.6 20.4	Low (176) High (83)	6.854 *p*<0.0001
	CMR	4.405 *p*<0.0001	≤0.21 >0.21	73 197	*p*<0.0001 42.7 6.1	Low (187) High (83)	4.405 *p*<0.0001

Table 15: RR & CI at 8 years according to transcript level at 12 months

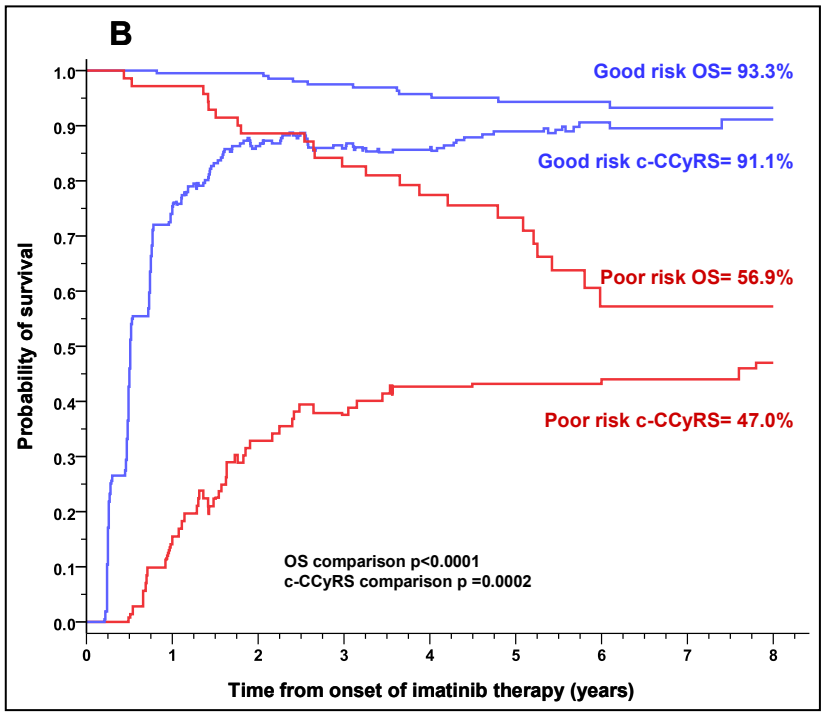
BCR-ABL1 transcript level at 12 months	Outcome	RR, transcript level (log)	cut-off (%)	Number of patients at risk	Eight years probability of the outcome	Eight year probability of the outcome according to the risk group (n)	
	OS	0.398 *p*<0.0001	≤0.53 >0.53	164 93	*p*<0.0001 95.4 74.7	N / A	
	PFS	0.333 *p*<0.0001	≤0.53 >0.53	164 92	*p*<0.0001 94.9 73.1	Low (164) High (92)	*p*<0.0001 94.9 73.1
	EFS	0.280 *p*<0.0001	≤ 0.57 >0.57	168 78	*p*<0.0001 82.1 41.4	Low (164) High (82)	*p*<0.0001 75.0 27.6
	MMR	5.639 *p*<0.0001	≤0.22 >0.22	90 114	*p*<0.0001 81.6 20.4	Low (121) High (83)	*p*<0.0001 78.4 12.3
	CMR	4.139 *p*<0.0001	≤0.036 >0.036	59 182	*p*<0.0001 52.1 4.1	Low (158) High (83)	*p*=0.005 18.8 4.1

Figure 13: 8-year probability of OS and Current CCyR survival (c-CCyRS)



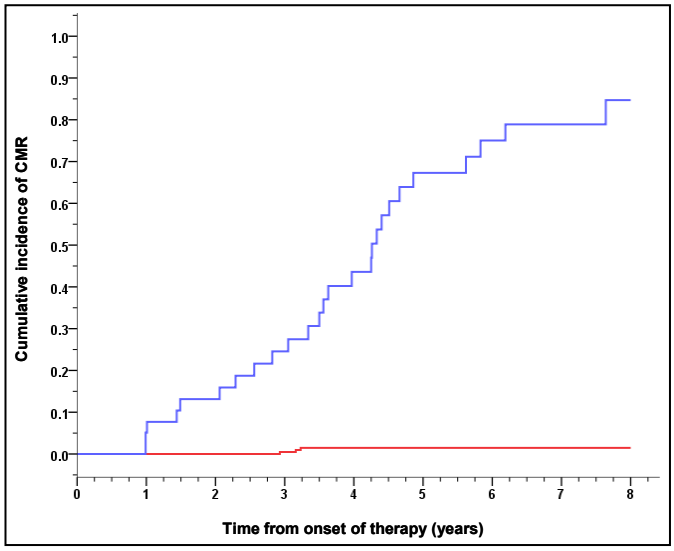
Panel A: The 8-year probability of OS in the whole population was 84.3%. During follow-up 34 patients died, 12 of them of causes unrelated to the leukaemia. When only leukaemia related deaths were taken into account, the 8-year probability of OS was 89.2%.

The 8-year probability of c-CCyRS was 76.6% which represents the probability of being alive and in CCyR at a given time points, thus the curve fluctuates as patients gain or lose CCyR (or die). It is interesting to observe that after the third year the c-CCyRS curve remain practically unchanged.



Panel B: shows the 8-year probability of OS and c-CCyRS for the patients according to the risk group defined by transcript levels at 3 months. The high risk group, represented by red lines, had a significantly lower OS (56.9 vs. 93.3%, p<0.0001) and c-CCyRS (47.0% vs. 91.1%, p=0.0002) than the low risk groups patients, shown in blue.

Figure 14: 8-year CI of CMR on imatinib according to *BCR-ABL1* transcript level at 3 months



The transcript level at 3 months identifies those patients with higher probability of achieving CMR on imatinib therapy. The 57 patients who had a 3-month transcript ratio ≤0.61% (blue line) had an 8-year CI of CMR of 84.7%, while the 222 patients with a ratio >0.61% (red line) had a CI of CMR of only 1.5% (p<0.0001).

Similar thresholds with high predictive power could be identified for 6 and 12 months (see tables 3.6 & 3.7). Patients in the good risk group defined at 3, 6 and 12 months had also significantly higher CI of CMR.

The transcript-based prognostic categories can be applied to other patient populations when the transcript ratio is expressed on the international scale

We classified 95 patients treated with imatinib first line at the Royal Liverpool University Hospital based on their transcript levels at 3, 6 and 12 months as explained above after converting their local their RQ-PCR results to the international scale. At 3, 6 and 12 months, 32 of 95, 40 of 94 and 37 of 83 patients still on imatinib therapy belonged to the high risk group as defined by RQ-PCR. At each time point the high risk patients had significantly poorer OS, namely 69.1% vs. 98.3% (p=0.003); 71.2% vs. 98.1% (p=0.009) and 74.4% vs. 98.0% (p= 0.01) than the good risk patients. Similarly patients in the high risk group had significantly lower PFS, EFS, c-CCyR, CI of CCyR and CI CMR (data not shown).

The use of second-line or subsequent therapies had very little impact on the prognostic value of early measurement of *BCR-ABL1* transcript levels

During the follow-up 118 patients failed imatinib and required alternative therapies. In order to further explore the impact of early measurement of transcript levels on outcome irrespective of the use of the rescue treatment, we calculated the 'current CCyR survival' (c-CCyRS, i.e. the probability of being alive and in CCyR at a given time point as defined previously).

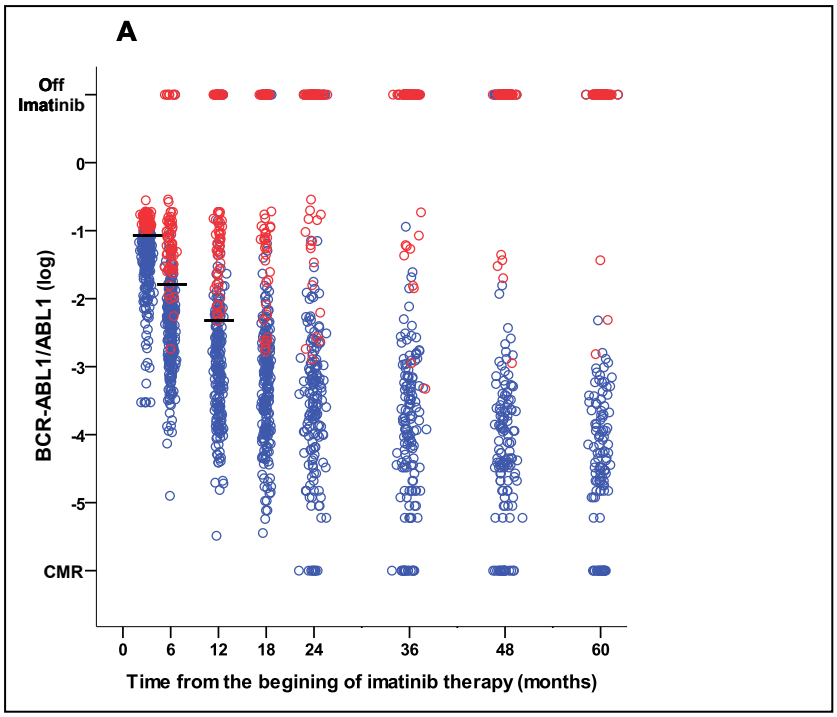
The 8-year c-CCyRS for the whole population was 76.6% (figure 13 - Panel A). High risk patients had a significantly worse 8-year probability of c-CCyRS than good risk patients, namely 47.0% vs. 91.1% at 3 months (p=0.0002) and 53.1% vs. 91.3% (p=0.01) at 6 months (figure 13 - Panel B). These findings indicate that the prognostic value of early measurement of the residual leukaemia burden retains its value even for patients requiring treatment with second generation TKI.

Patients' outcome can be best predicted at 3 months

We classified the patients as good or poor risk according to their transcript levels at 3, 6, and 12 months as described above, and entered the resulting variables in a Cox regression model together with the appropriate pre-therapy variables shown in table 10 (see methods section). The 3-month transcript level (higher or lower than 9.84%) was the only independent predictor for OS (RR= 7.33, p<0.0001), PFS (RR= 7.16, p<0.0001), EFS (RR=9.71, p<0.0001) and c-CCyRS (RR=0.431, p=0.0002), which indicates that the measurement at 3 months is the most informative.

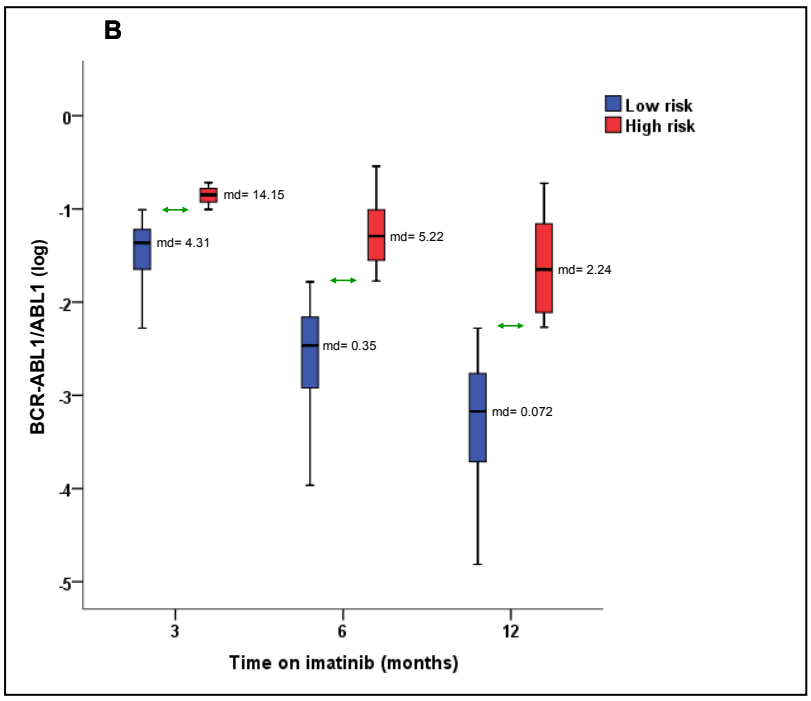
In fact, 18 of the 23 patients who eventually died of CML-related causes and 28 of the 37 who are still alive but not in CCyR at their latest follow up belonged to the high risk group defined at 3 months, and the use of transcript cut-offs at 6 and 12 months did not identify any additional deaths or cytogenetic failures. On the other hand, the high risk group defined at 3 months only contained 68 patients in total while the 6 and 12 month groups contained many more patients, namely 87 and 93 patients respectively, thus the 3-month time point is the one with the best sensitivity and specificity. Figure 15 shows the evolution of the transcript level according to the prognostic risk group defined at 3 months.

Figure 15: Evolution of the transcript level according to the 3, 6 and 12 month risk group



Panel A: represents the evolution of the transcript level over time. Patients are classified as high risk (red circles) and low risk (blue circles) according to their transcript level at 3 months (higher or lower than 9.84%). The horizontal black lines represent the transcript level that defines the 3-month (9.84%), 6-month (1.67%) and 12-month (0.53%) risk groups.

The majority of patients (all but 6) who were classified as high risk at 3 months were also classified as high risk at 6, and all are classified as high risk at 12 months. The transcript level declines over time in the good risk patients, while it remains comparatively high in the poor risk population. Also many of the high risk patients eventually abandon imatinib on account of unsatisfactory response, loss of response or progression.



Panel B: shows the comparison of transcript levels in the two risk groups defined at 3, 6 and 12 months. Although the optimal cut-offs to define the groups at 3, 6 and 12 months are 9.84%, 1.67% and 0.53%, the majority of the patients had a transcript level much higher or much lower than the cut-off selected for a given time. The median value (md) of the transcript level is also specified in the figure as a *BCR-ABL1/ABL1* ratio (%).The green double arrowed lines represent the value of the cut-offs used to define the groups on a logarithmic scale (9.84%, 1.67% and 0.53%).

The prognostic value of the 3-month transcript level is independent of the imatinib dose received by the patient

Ninety-three patients (33.0%) had imatinib temporarily discontinued or the dose reduced on account of side-effects. These patients had a significantly higher 3-month transcript levels than the remaining 186 patients (8.49% vs. 5.83%, p=0.001). This raised the question of whether the transcript level in these patients can be interpreted in the same way as in the rest of the population.

In order to investigate this we sub-classified these patients into two groups: (a) patients with non-haematological toxicity; and (b) patients with haematological toxicity. The first group (23 patients) had transcript levels similar to those of the 186 tolerant patients (7.15% vs. 5.83%, p=0.82), while the 70 patients who developed haematological toxicity had a significantly higher level (8.91% vs. 5.83%, p<0.0001). Patients with non-haematological toxicity had prognosis identical to those of the tolerant patients (data not shown) but the 70 patients with haematological intolerance to imatinib had a worse 8-year CI of CCyR (RR=0.44, p<0.0001), c-CCyRS (RR=0.531, p<0.0001) and OS (RR=2.33, p=0.02).

Furthermore, imatinib dose intensity lost its prognostic significance when the transcript level was introduced into the multivariate model (data not shown), reinforcing the idea that the transcript level at early time points affects prognosis independently of the imatinib dose intensity that the patient has received.

Transcript levels at 6 and 12 months identify those patients in CCyR who are destined to fare poorly

Of the 166 patients who had achieved CCyR at 12 months, 41 had also achieved MMR. Patients who had achieved MMR had an OS (96.3% vs. 92.4%, p=0.5), and an EFS (93.7% vs. 80.4%, p=0.08) similar to those of the patients who had failed to achieve MMR. The achievement of MMR at 18 months also lacked prognostic value (data not shown). We tried to identify transcript levels at 6 and 12 months above which patients in CCyR were destined to fare worse than those with lower values.

At 6 months and 12 months, 23 of the 109 and 20 of the 166 patients who were in CCyR had a transcript level higher than 0.53% (the value that we had previously identified as defining the 12 months high risk group); these patients had significantly worse OS (65.8% vs. 95.9%, p=0.0002 and 81.5% vs. 94.9%, p=0.01) and EFS (37.3% vs. 72.0%, p<0.0001 and 29.5% vs. 74.3%, p<0.0001) than the 86 and 146 patients with lower transcript levels.

Measurement of transcript levels at 6 and 12 months is more informative than bone marrow cytogenetic data for identifying patients with worse prognosis

The assessment of bone marrow cytogenetics at 6 and 12 months is probably the commonest method of assessing the response of patients. Table 16 shows the RR for OS and c-CCyR according to the cytogenetic and molecular responses.

Table 16: RR for 8-year OS and c-CCyRS according to the cytogenetic and molecular responses achieved at 6 or 12 months

	n	OS	c-CCyRS
6 month CCyR *		p=0.29	p=0.12
Yes	106	1	1
No	157	3.44	0.815
6 month MCyR *		p=0.033	p=0.01
Yes	170	1	1
No	93	2.30	0.715
6 month MiCyR *		p=0.29	p=0.23
Yes	223	1	1
No	40	2.15	0.727
6 months *BCR-ABL1/ABL1*		p<0.0001	p<0.0001
good response (≤1.67%)	87	1	1
poor response (≥1.67%)	187	4.83	0.486
12 months CCyR **		p=0.016	p=0.003
Yes	164	1	1
No	89	2.88	0.674
12 months MCyR **		p=0.08	p=0.007
Yes	199	1	1
No	54	2.40	0.714
12 months *BCR-ABL1/ABL1*		p<0.0001	p<0.0001
good response (≤0.53%)	164	1	1
poor response (≥0.53%)	93	6.33	0.545

* Data missing in 11 patients

** Data missing in 4 patients

Patients who achieved a MCyR at 6 months and patients who achieved MCyR or CCyR at 12 months had better outcomes. Using OS and c-CCyRS as endpoints we compared the prognostic values of cytogenetic assessment with the RQ-PCR results by performing multivariate analysis for each time point, including the appropriate variables shown in tables (11, 12) and (16). The transcript levels at 6 months (higher or lower than 1.67%) and at 12 months (higher or lower than 0.53%) were the only independent predictors for OS (RR= 4.83, p<0.0001 and RR=6.33, p<0.0001) and c-CCyRS (0.486, p<0.0001 and 0.545, p<0.0001).

Discussion

The observation that *BCR-ABL1* transcript levels measured early in the course of treatment with imatinib for CML in CP may have prognostic value is not new; as it has been shown that patients with relatively low transcript levels at early time-points had superior outcomes than those with higher values (117, 229-231).

Here we showed that *BCR-ABL1* transcript measurements performed at 3, 6 or 12 months can identify those patients with relatively poor OS, poor PFS and poor c-CCyRS. The data also suggests that the 3-month assessment is the single most important one and may abolish the need for later measurements. Therefore we have established transcript cut-off values at 3 time points based on outcomes observed for the patients' population. These may be preferable to use more than depending on the somewhat biased levels of log reduction from a starting baseline value.

This discovery led to a new set of definitions for molecular responses, namely transcript levels of ≤9.84% at 3 months, of ≤1.67% at 6 months and of ≤0.53% at 12 months. It is also possible to refine the proposed definitions of molecular response to better identify those patients who are more likely to achieve CMR on imatinib (figure 14). We have also validated our results by applying these new definitions to an independent cohort of patients who were treated and had their transcripts levels analyzed in a different specialist centre.

We found that the 3-month milestone is the most informative and that the 6- and 12- month assessments contribute very little (if anything) more than identifying patients with a high risk of progression.

This would suggest that current therapeutic strategies that depend on patients achieving successive milestones at specific time points - for example MCyR at 6 months and then CCyR at 12 months and so on - could be abandoned in favour of a single assessment at an early time point that would therefore direct those patients with high risk to alternative therapy.

The prognostic value of the 3-month assessment was independent of whether or not patients have had their imatinib dose temporarily reduced or discontinued on account of side-effects. Thus the finding of high transcript levels at 3 months in a given patient has to be a cause of major concern for clinicians, as this also applies in cases that can be attributed to haematological toxicity. This cohort of patients has lower OS, PFS and CI of CCyR than the rest of the population.

The 8-year c-CCyRS, which reflects the probability of being alive and in CCyR, was 76.6%. Interestingly the c-CCyRS remained relatively constant after the 3rd year reflecting the low probability of later events in patients with CML treated with TKIs. Patients with low transcript levels at 3, 6 or 12 months had an excellent c-CCyRS (figure 13). For example the probability of being alive and in CCyR at 8 years for patients with a good 3-month molecular response was 91.2% (95.4% if the non leukaemia related deaths were excluded), whereas poor risk patients had only a 47.0% probability of being alive and in CCyR at 8 years.

This is consistent with the conclusion that second line TKIs have only partial success in 'rescuing' patients who fail imatinib. Thus the poor prognosis reflected by the high transcript level at early time points was maintained despite the fact that an individual patient may fail imatinib and subsequently receive a variety of more effective agents.

As previously shown (123, 232), we found that the patients who were in MMR at 12 or 18 months had better EFS both when we analyzed the whole patient population or when we limited the analysis to patients who were in CCyR, but the achievement of MMR at 12 or 18 months didn't have any predictive value for OS (p=0.21 and p=0.42), PFS (p=0.26 and p=0.35) or for c-CCyRS (p=0.32 and p=0.44) in the whole population or indeed when the analysis was limited to patients who were in CCyR.

This reflects the limitations of basing the definition of therapeutic milestones on approximate predefined points (i.e. 3 log reduction in transcripts or 0.1% on the international scale) rather than trying to find the appropriate cut-offs that discriminate between patients with different clinical outcomes.

Finally we compared our risk groups based on molecular response at 6 and 12 months with the cytogenetic milestones normally used in clinic. Patients who achieved MCyR at 6 months and patients who achieved MCyR or CCyR at 12 months had significantly better OS and CR (table 16), but cytogenetic responses were not independent predictors for these outcomes when the molecular responses were taken in account. The superior value of early molecular assessment over serial cytogenetic studies is not easily explained. It could reflect the statistical unreliability of concluding cytogenetic results from a small number of analyzable metaphases. It could also reflect the fact that the population of dividing Ph-positive cells that is identified in the bone marrow is a poor representative of a specialized more primitive population of leukaemia cells less susceptible to eradication by TKI *ab initio* or perhaps able to acquire resistance in the first few years of therapy. This hypothetical population might be better characterized by their capacity to express more *BCR-ABL1* transcripts.

Conclusion

Determining patients' responses to therapy is a vital challenge that faces clinicians in this era of TKIs as not all of them will benefit from the use of imatinib and will be required to change to alternatives. Over the years, several groups have looked at several factors that could predict responses at different times of therapy, among them only Sokal stands the test of time and proves to be rather accurate in predicting responses.

A new score that was set up by the EUTOS group seemed promising, but didn't manage to predict accurately the outcome of our patients. Knowing that the achievement of an early molecular response is an important factor in determining outcomes, we investigated the use of molecular responses as a method of predicting outcome. We managed to establish cut off points at 3, 6 and 12 months that would determine a patient response. Not only that, but also we managed to show that a single measurement of *BCR-ABL1* transcripts performed at 3 months is the most accurate way to identify those patients with CML in CP treated with imatinib who are destined to fare poorly, thus allowing early clinical intervention.

Chapter IV
Emerging problems on long term therapy

Introduction

Current therapy for CML has been dramatically changed since the introduction of imatinib and new generation TKIs, transforming the disease from what used to be a lethal condition to a mere chronic illness. The efficacy of the drug coupled with its ease of administration (in comparison with intravenous therapies) and a low level of toxicity has resulted in many patients leading relatively normal lives. It has been estimated that imatinib could prolong the chronic phase of CML to an average of 12 to 15 years (233). This in turn has changed the therapeutic targets in CML patients to other issues such as improving the quality of life. Clinicians are now faced by problems that are of those related of management of chronic diseases, such as better control of side effects, poor adherence to therapy, and issues of fertility.

In the first part of this chapter, we investigate the effect of poor adherence on responses gained while on imatinib therapy by following up a group of patients who were enrolled in the Adherence trial at the Hammersmith Hospital (135). While in the second part we investigate the effect of imatinib discontinuation on a group of female patients who interrupted therapy at different points of response in order to conceive to determine the best timing.

Part I: Non - adherence to medication

Introduction

Non –adherence to medication is a well recognised problem among patients taking long term therapy (234, 235). This led the WHO to issue a report defining adherence as the extent to which a person's behaviour corresponds with agreed recommendations of a healthcare provider (236). Disappointingly, adherence rates for many long-term drug therapies have been shown to be strikingly low, often no more than 40%–50 (234, 237).

Although non-adherence to a potentially lifesaving medication such as oral anti-cancer therapies might seem unreasonable, yet the adherence rates show great disparity; depending on the method used to measure adherence. It has been shown that average non-adherence rate to oral anticancer treatments has been estimated as low as 21% (238).

Patients usually have different reasons on why they aren't adherent to medication, but typical reasons include forgetfulness (30%), other priorities (16%), own decision to omit doses (11%), lack of information (9%), and emotional factors (7%); an astonishing 27% however did not provide a reason for poor adherence to medication at all (239).

Physicians often fail to recognize non-adherence (as they usually assume that their patients are taking the drugs as prescribed), they can incorrectly attribute the patient's worsening condition to an absence of drug activity. This incorrect conclusion may lead to unnecessary diagnostic testing, hospitalizations, and changes in dose or regimen (240).

Measuring adherence

Several methods are used to measure adherence, each of them have their own benefits and discrepancies, among the most commonly used: self-reporting has been used to measure patient adherence to oral therapies, but it's considered inaccurate as patients may be reluctant to admit bad behaviour, especially if asked by people involved in their daily care (240); pill counts, requiring patients to return unused pills at each visit so that the number of missed doses can be calculated are also subject to many problems as patients can switch medicines between bottles and may discard pills before visits in order to appear to be following the regimen (239); and drug levels which are expensive and also susceptible to distortion by the patients, as they may take additional doses just before a visit, therefore making their drug levels falsely suggesting good adherence (239, 240) (table 17).

A more sophisticated method for the measurement of adherence is by using the microelectronic monitoring system (MEMS). The MEMS consists of an "intelligent" cap on a pill bottle that electronically records every time the cap of the pill bottle is removed (figure 16). MEMS data thus provide a computerized record of each date and time the bottle is opened.

Table 17: Methods of measuring adherence

Test	Advantages	Disadvantages
Self reporting	Simple; inexpensive; most useful method in the clinical setting	Susceptible to error with increases in time between visits; results are easily distorted by the patient
Pill counts	Objective, quantifiable, and easy to perform	Data easily altered by the patient (e.g., pill dumping)
Drug levels	Objective	Variations in metabolism and "whitecoat adherence" can give a false impression of adherence; expensive
MEMS	Precise; results are easily quantified; tracks patterns of taking medication	Expensive; requires return visits and downloading data from medication vials

MEMS is often referred to as the "gold standard" of adherence measurements (235, 239-242), however it still has its limitations. Patients may open the bottle without ingesting the medication, or may take out extra medication to store and subsequently ingest from another container (243-245). Furthermore, patients who use monitored dosing boxes (e.g. Dosette) cannot use these adherence aids at the same time while using MEMS.

Several recent studies have compared the use of MEMS against other methods for measuring adherence; all have demonstrated MEMS superiority over other methods such as self reporting (246, 247), pill counts (248) and with the unreliability of drug levels (238, 240), MEMS remains the most valid and reliable method available to measure adherence.

Effects of non-adherence to imatinib:

Studies have shown that non-adherence to imatinib is frequent and may have a significant impact on a patient's outcome. In a Belgian study, one third of 169 patients were non-adherent, and only 14% took all of their prescribed imatinib; which was associated with reduced clinical response (249).

A previous study from our team reported that 26% of the patients on long term imatinib therapy had an adherence rate lower than 90% and 14% had a rate lower than 80%. In that study, multivariate analysis identified adherence and the level of expression of the molecular transporter *hOCT1* as the two independent predictors for achieving MMR (135).

We now present the follow up data, where we investigated the relation between adherence to imatinib and the probabilities of losing CCyR and imatinib failure in patients receiving long term imatinib therapy.

Patients & methods

Patients:

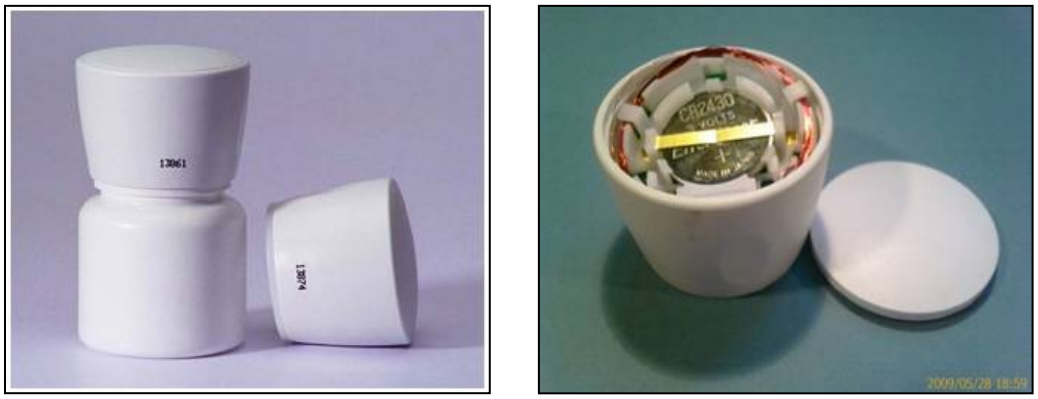
Between April 2008 and February 2009, adherence to imatinib was measured in 87 chronic phase CML patients who had received imatinib 400 mg/day as first line therapy for a median of 59.7 months before enrolment in the previous study (135)(ClinicalTrials.gov identifier: NCT00632255).

All patients were in CCyR at the time of enrolment. Patient characteristics are shown in table 18. Imatinib was administered as described previously. Adherence levels were monitored during a 3-month period and after the monitoring period the patients were followed subsequently for a median of 19 months (range 14-24).

Adherence monitoring:

Adherence levels were monitored using microelectronic monitoring systems (MEMS) (Aardex, Zug, Switzerland) (figure 16). Because the half-life of imatinib is long, the adherence rate for each patient was defined as the dose that was taken according to the MEMS reading expressed as a percentage of the dose prescribed during the total duration of the study (135).

Figure 16: Microelectronic monitoring system (MEMS)



Laboratory assessments:

BCR-ABL1 transcript levels were measured in the blood at 6- to 12 week intervals as previously described. Trough imatinib plasma levels were measured as reported by others (146). Patients who took imatinib in the evenings were instructed to take it in the mornings for the 3 days before the clinic visit. Results were later verified using the MEMS. Levels of *hOCT1* transcripts were measured by RQ-PCR in a peripheral-blood sample obtained at diagnosis and levels were expressed as the ratio of *OCT1* to β - glucuronidase (GUSB) as control from the same sample (250). The polymorphism 1236C/T in ABCB1 was genotyped using pyrosequencing (135, 251, 252).

Statistical analysis:

The probabilities of EFS (the reverse of imatinib failure) as well as of molecular response were calculated as previously mentioned. Variables significant at $p<0.2$ were entered into a proportional hazards regression analysis; a forward-stepping procedure was employed to find the best model. The proportional hazards assumption was confirmed by adding a time-dependent covariate for each covariate. Tests for interactions were carried out, but none was found to have statistical significance.

The relation between the different prognostic factors and the response at 18 months was explored by using a logistic regression model. Groups were compared by using Fisher's exact test for categorical data and the Mann-Whitney Test for quantitative data. *P* values were two sided.

Table 18: Patients characteristics prior to enrolment

Variables at diagnosis	n
Gender	
male	49
female	38
Age (years)	
≤45	42
>45	45
Sokal risk group	
Low	33
Intermediate	32
high	22
Haemoglobin	
≤115 gm/l	40
>115 gm/l	47
Leukocytes	
≤140 x 10^9/l	44
>140 x 10^9/l	43
BCR-ABL1 transcript type	
e14a2	40
e13a2	33
e13a2 and e14a2	14
hOCT1 transcript level [(a)]	
≤0.16	30
>0.1	30
MDR-1 polymorphism	
C/C	75
T/C	12
Early molecular response[(b)]	
Yes	55
No	32
Baseline response	
CCyR and no MMR	34
MMR	53

(a) Samples weren't available in 27 patients.

(b) Early molecular response is defined as having achieved a 1-log reduction (*BCR-ABL1/ABL1* ratio ≤10%) by 3 months

Results

Cytogenetic responses:

The median adherence rate was 97.6% (range 24-103.8%). In 23 (26.4%) patients, adherence was ≤90% (median 76%) and in 18 (20.7%) ≤85% (median 73.8%). During the follow up, seven (8%) patients lost their CCyR (six of whom became >35% Ph-positive before alternative therapy could be commenced) and 12 (13.8%) discontinued the imatinib therapy (seven because of loss of CCyR and five because of side effects).

In order to identify factors associated with loss of CCyR and EFS, we performed univariate analysis (see tables 19 and 20). The 18 patients with an adherence rate ≤85% had a higher probability of losing their CCyR than the 69 patients with an adherence rate >85%, namely 36.3 vs. 1.4% (p<0.0001). None of the 57 patients with an adherence rate >95% lost their CCyR (p=0.0001). Patients who were in MMR at the beginning of the study had a significantly lower probability of losing their CCyR by the end of follow up (see table 20 and figure 17).

EFS and Imatinib failure

The median adherence rate in the 12 patients who eventually failed imatinib therapy was significantly lower than that of the patients who continued on therapy, namely 78.1% vs. 97.8% (p=0.02). Patients with an adherence rate ≤85% had a significantly lower EFS than the adherent patients (54.4% vs. 91.4%, p=0.0002). Again we performed univariate and multivariate analysis in order to identify prognostic factors for imatinib failure (table 20). An adherence rate ≤85% (RR=0.22, p=0.01) and having failed to achieve a MMR (RR=0.14, p=0.15) were the only independent predictors for EFS.

Table 19: 24 months probabilities of cytogenetic relapse and EFS for variables at diagnosis

Variables at diagnosis	n	24 month probability of losing CCyR (%)	24 month probability of EFS (%)
Gender		*p*=0.41	*p*=0.12
male	49	10.3	79.0
female	38	5.8	90.9
Age (years)		*p*=0.55	*p*=0.20
≤45	42	9.6	76.6
>45	45	6.9	89.7
Sokal risk group		*p*=0.13	*p*=0.79
Low	33	6.6	85.8
Intermediate	32	3.4	85.3
high	22	17.9	79.7
Haemoglobin		*p*=0.82	*p*=0.77
≤115 gm/l	40	8.1	81.9
>115 gm/l	47	7.3	83.7
Leukocytes		*p*=0.95	*p*=0.99
≤140 x 10^9/l	44	7.2	85.1
>140 x 10^9/l	?3	7.1	82.3
BCR-ABL1 transcript type		*p*=0.72	*p*=0.43
e14a2	40	10.9	89.0
e13a2	33	9.4	74.6
?13a2 and e14a2	14	4.6	75.0
hOCT1 transcript level [(a)]		*p*=0.30	*p*=0.51
≤0.16	30	11.2	81.1
>0.16	30	3.4	86.7
MDR-1 polymorphism		*p*=0.82	*p*=0.79
C/C	75	10.1	82.3
T/C	12	7.9	80.2
Early molecular response[(b)]		*p*=0.08	*p*=0.13
Yes	55	4.1	92.9
No	?2	15.0	78.0
Baseline response		*p*=0.0006	*p*=0.0007
CCyR and no MMR	34	21.1	67.5
MMR	53	0	94.4

(a) Samples weren't available in 27 patients.
(b) Early molecular response is defined as having achieved a 1-log reduction (*BCR-ABL1/ABL1* ratio ≤10%) by 3 months

The multivariate analysis identified adherence rate and the degree of molecular response as the only independent predictors for loss of CCyR and imatinib failure. Figure (17) shows the 2 year probabilities of loss of CCyR and EFS according to the combination of both variables. The 53 patients who started the study in MMR fared well, with a 2 year probability of losing the CCyR of 0% and EFS of 94.4%.

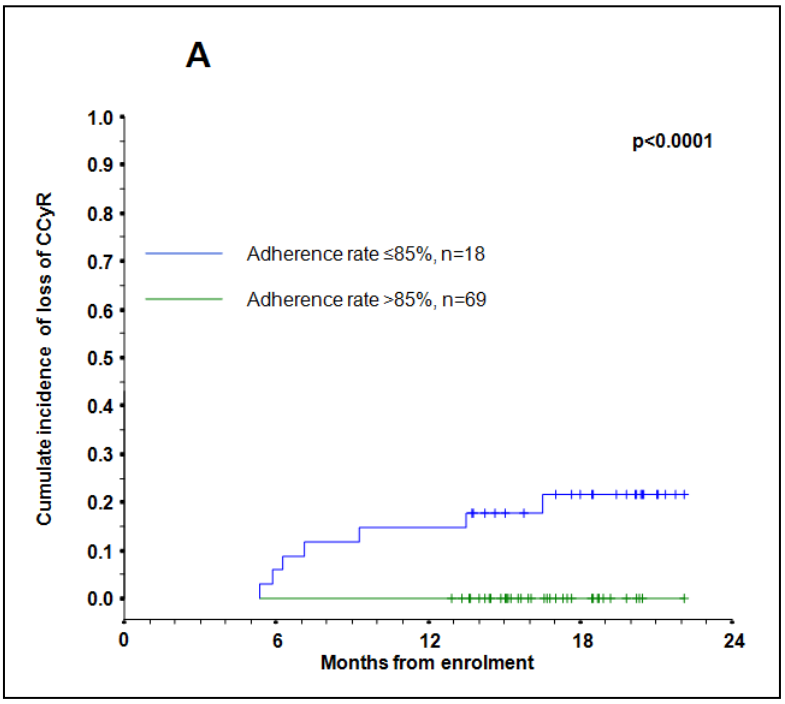
Table 20: 24 months probabilities of cytogenetic relapse and EFS for on study variables

On study variables	n	24 month probability of losing the CCyR (%)	24 month probability of EFS (%)
Age (years)		*p*=0.18	*p*=0.03
≤50	42	12.5	74.7
>50	45	4.6	91.7
Weight (kg)		*p*=0.57	*p*=0.14
≤74	47	6.5	90.9
>74	40	10.4	75.5
Imatinib plasma level [(c)]		*p*=0.98	*p*=0.80
≤1 μg/ml	43	6.8	86.7
>1 μg/ml	41	7.0	83.9
Adherence rate [(d)]		*p*<0.0001	*p*=0.0002
>85%	69	1.4	91.4
≤85%	18	36.3	54.2
Dose of imatinib		*p*=0.73	*p*=0.79
400mg day	55	7.7	81.6
>400mg day	32	9.2	87.1

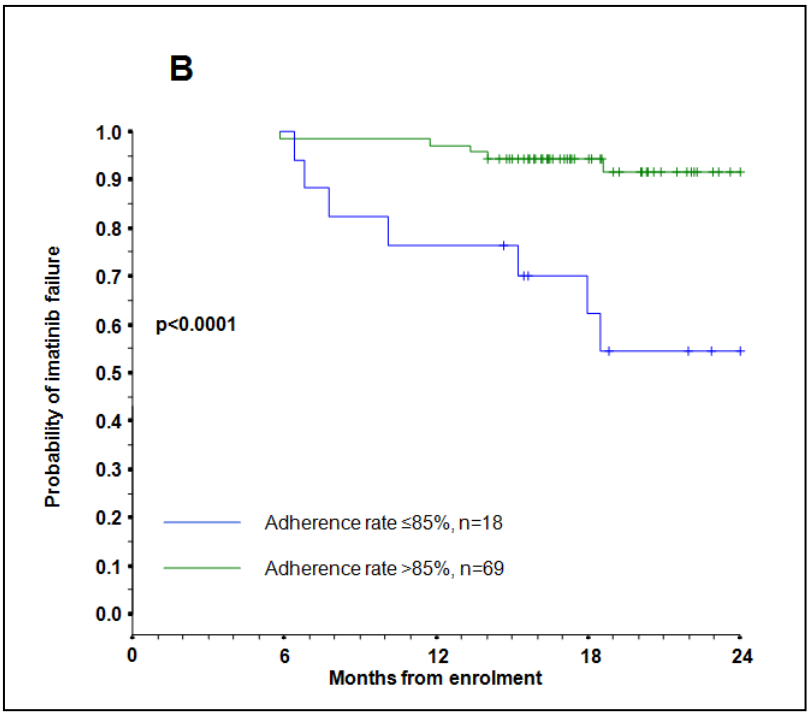
(c) In three patients the trough plasma level was not available.

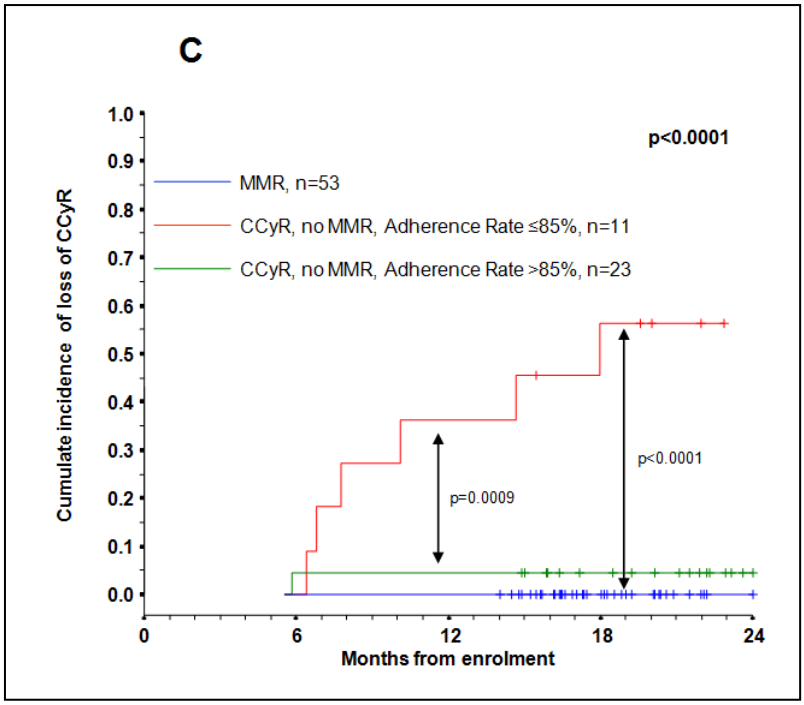
(d) We explored different cut-offs for the adherence rate. In all cases the patients in the group with the lower adherence rate were more likely to lose their CCyR during the follow up, namely ≤80 vs. >80 (54.5 vs. 2, $p<0.0001$), ≤85 vs. >85 (36.3 vs. 1.4, $p<0.0001$), ≤90 vs. >90 (26.9 vs. 1.6, $p=0.0002$) and ≤95 vs. >95 (24.5 vs. 0, $p=0.0001$). We used a multivariate Cox model to choose the better cut-off which was 85. The adherence rate also predicted for loss of CCyR when considered as a continuous variable (RR=1.05, $p=0.0001$).

Figure 17: Two year probability of losing the CCyR and imatinib failure (EFS)

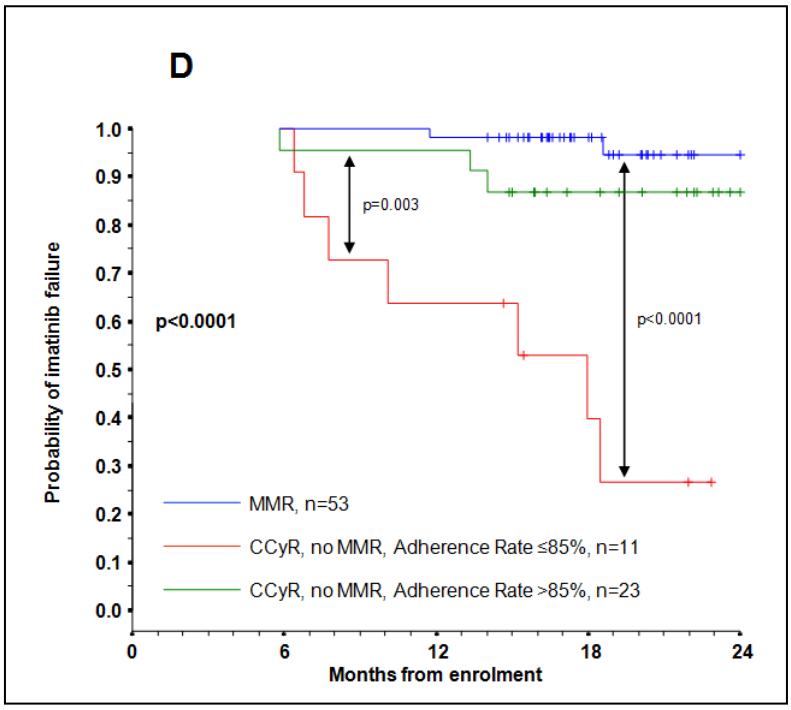


The 18 patients with an adherence rate ≤85% had higher two year probability of both losing their CCyR (panel A; 36.3% vs. 1.4%, p<0.0001) and of failing imatinib therapy (panel B; 54.4% vs. 91.4%, p=0.0002) than the 69 patients with an adherence rate >85%





The 53 patients who had achieved MMR at the moment of enrolment had a similar 2-year probability of losing their CCyR during follow up (panel C) and a 2-year EFS (Panel D) similar to that of the 23 patients who were not in MMR at enrolment but who had a adherence rate >85%, namely 0% vs. 4.3% (p=0.31) and 94.4% vs. 86.1% (p=0.14). However the 11 patients who were not in MMR at the moment of enrolment and who had a adherence rate ≤85% had a significantly higher probability of losing their CCyR (54.5%) and lower EFS (28.3%), panels C and D.



Discussion

It has been previously shown that patients who achieve MMR are less likely to lose their CCyR (123) and that the achievement of MMR depends on the adherence to imatinib therapy, and to a lesser extent on ill-defined intrinsic biological factors, such as higher expression of *hOCT1* (135). It is impossible, however, to establish the relative contributions of these two factors to the sustained responses.

The outcome for the 34 patients who were not in MMR at the beginning of the study was rather disappointing (probability of losing their CCyR 67.5%, p=0.0007). Within this second cohort the impact of adherence on their outcome was dramatic. The 23 of those patients who had an adherence rate >85% had a prognosis similar to that of the MMR patients (probability of losing their CCyR 4.3%) while the 11 patients with a low adherence rate had 54.5% cumulative incidence of loss of CCyR (p<0.0001) (figure 17).

Good adherence to therapy seemed to be able to overcome any possible biological difference as patients who had failed to achieve MMR but had a good adherence to therapy had a prognosis similar to that of MMR patients. This must mean that for patients who have achieved CCyR, good adherence to therapy should be the main clinical focus and not the further achievement of MMR. Therefore, non-adherence should be examined as a possible reason for patient non- or reduced response to imatinib before considering these patients to be imatinib-resistant and switching them to second-generation tyrosine kinase therapy. Non-adherence to imatinib must be ruled out as a possible cause of lack of optimal response.

Part II: Drug interruption due to pregnancy

Introduction

Although responses to imatinib can be regained after a prolonged period of interruption on account of side effects or pregnancy (253) the ideal degree of achieved responses needed for female patients wishing to conceive before therapy discontinuation isn't clear. Neither is whether a lengthy discontinuation of the treatment is safe in all the cases. Therefore we decided to report the effect of discontinuing imatinib on the clinical response in 7 female patients observed at our institution.

Patients

We included in our report, 7 female CML patients who conceived while on imatinib at the Hammersmith Hospital. The median age was 32 years (range, 25-34 years). All patients were being treated with imatinib 400 mg daily as initial therapy for CML in chronic phase. In 4 patients, imatinib was discontinued as soon as the pregnancy was confirmed, and 3 patients stopped imatinib in order to conceive. Before discontinuation, imatinib was received for a median time of 19 months (range, 7-42 months, table 21), and all patients were receiving 400 mg.

Results

The median period of drug interruption was 9 months (range, 6-23 months). During the time of interruption, all patients lost their cytogenetic response, and 4 lost their CHR before imatinib was reintroduced. Only patient no. 3 required therapeutic intervention (leukapheresis) during imatinib discontinuation. Imatinib therapy was reintroduced directly after delivery.

Before Imatinib discontinuation, patients 2, 3, 4, and 6 were suboptimal responders (table 21). None of these 4 patients obtained an appropriate response when imatinib was reintroduced after the delivery. Patients 2, 4, and 6 (table 21) failed to achieve CCyR on subsequently restarting imatinib therapy, with patients (nos. 2 & 6) requiring imatinib dose increase and eventually change to second line dasatinib before regaining CCyR. Patient 3 achieved CCyR but failed to achieve MMR after 26 months of therapy. The 3 patients (nos. 1, 5, and 7) who had obtained an optimal response at the time of stopping Imatinib regained at least MMR after restarting therapy (table 21). None of the 7 patients had developed kinase domain mutations.

Table 21: Summary of patients' data and outcomes

N	Sokal risk group	Months on Imatinib prior to discontinuation	Clinical response at time of discontinuation	Clinical response at time of discontinuation according to ELN	Months without Imatinib	WBC and cytogenetic response before reintroduction	Months of Imatinib therapy after reintroduction	Clinical response after restarting Imatinib (time to response, months)	Outcome
1	Low	9	MMR	optimal	9	7.3, no MCyR	30	CMR (12)	Maintained CMR on Imatinib therapy
2*	Low	42	CCyR	suboptimal	9	48.2, no MCyR	18	Failure to achieve MCyR, subsequent lost of CHR	Imatinib increased to 600 mg after 8 months, and then changed to Dasatinib. The patient achieved CCyR at 12 months
3	Low	21	CCyR	suboptimal	13	88.8**, no MCyR	26	CCyR (24)	On Imatinib; no MMR after 26 months
4	Low	19	CCyR	suboptimal	23	60.3, no MCyR	29	no MCyR	Failure to achieve any degree of cytogenetic response. Patient lost to follow up after 29 months

* Patient 2 had two pregnancies; the data shown in the table correspond to the second pregnancy. In the first pregnancy the Imatinib was discontinued after 3 months of therapy for 11 months.

** Patient required leukapheresis. The leukapheresis was started when the WBC rise over 100 x 10^9 cell/l.

Table 21 (continued): Summary of patients' data and outcomes

N	Sokal risk group	Months on Imatinib prior to discontinuation	Clinical response at time of discontinuation	Clinical response at time of discontinuation according to ELN	Months without Imatinib	WBC and cytogenetic response before reintroduction	Months of Imatinib therapy after reintroduction	Clinical response after restarting Imatinib (time to response, months)	Outcome
5	Low	14	MMR	optimal	6	5.3, no MCyR	90	MMR (9)	Patient remains in MMR
6	High	7	MCyR	suboptimal	8	79.4, no MCyR	50	MCyR (4)	Patient failed to regain MCyR; the dose of Imatinib was increased to 600mg after 11 months and then changed to Dasatinib. Subsequently the patient achieved CCyR after 6 months of Dasatinib therapy
7	Low	50	MMR	optimal	13	6.5, MCyR	14	MMR (3)	Patient remains in MMR with a continuing decline in transcript levels

Discussion

The results show that an adequate response on restarting imatinib after its discontinuation for pregnancy is seen only in patients who had an optimal response (MMR) before stopping the drug. Suboptimal responders to initial imatinib treatment either demonstrated the same response after discontinuation or, more of concern, deterioration of the response eventually meeting criteria for imatinib failure. It wasn't clear whether the poor results after reintroduction occurred because the patients hadn't yet achieved an appropriate response before conception or because they were poor-risk patients.

It's advisable to use second generation TKIs nilotinib or dasatinib in these patients (suboptimal responders) in order to obtain MMR before therapy interruption as this might reduce the risk of treatment failure after re-introduction of imatinib therapy. Our findings emphasize the need for further investigation into the effects of imatinib discontinuation in for conception and pregnancy issues.

Conclusion

We have shown that adherence to medication is an important determinant of achievement of responses. Our results show that poor adherence to imatinib is the most important factor contributing to cytogenetic relapse and imatinib failure. Therefore, non-adherence should be examined as a possible reason for patient non- or reduced response to imatinib before considering these patients to be imatinib-resistant and switching them to second-generation tyrosine kinase therapy. It is also important that clinicians recognize this problem and work towards improving adherence in CML patients. As for pregnancy issues, our results show that pregnant women should have achieved an adequate response to therapy, preferably a MMR, before imatinib interruption in order to conceive.

It's worth noting how a prolonged period of interruption doesn't seem to affect the responses as much as missing doses for short periods. That can be explained by the fact that achieving an adequate response necessitates taking the drug regularly and therefore manages to acquire durable responses, while interrupting therapy without achievement of responses allows the proliferation of a subset of cells that might be resistant to therapy.

Chapter V

Second generation TKIs improve survival and provide durable responses

Introduction

Imatinib is an extremely effective therapy for CML, yet unfortunately not all patients will benefit from it, either because of inadequate responses or intolerance. At 5 years approximately 40% of the patients will have discontinued imatinib on account of an unsatisfactory response or toxicity (117).

With the availability of second generation tyrosine kinase inhibitors (2G-TKIs) such as dasatinib or nilotinib, patients who fail imatinib are changed to either one of them while still in CP. These drugs have been shown to be able to induce complete cytogenetic responses (CCyR) in approximately 50% of these patients (171, 177, 180, 254, 255).

However, two issues have been raised regarding the use of 2G-TKIs after imatinib failure; the first is whether these drugs actually improve patients' survival; and the second is whether the responses obtained on 2G-therapy can be maintained on the long term.

The lack of data regarding the survival benefit of 2G-TKIs has allowed funding agencies in the UK to question their use (256). Also most of our data relating to these drugs' efficacy is derived from phase (II) sponsored studies in which patients were censored when they discontinued the drugs (171, 180).

This practice although common in clinical studies, yet it offers a limitation when trying to define long term outcomes as some patients who discontinue the study drug might still obtain excellent responses on alternative therapies. On the other hand, the unresponsive patients are removed from the study (and censored) before they progress to advanced phase or death takes place. In order to address these issues, we have conducted two separate studies in order to prove the survival benefit of 2G-TKIs and to determine the durability of responses achieved.

Part I:

2G - TKI improve survival

Patients & methods

In order to address the survival issue, we used data from 246 patients who had failed IFN-α therapy in the UK Medical Research Council's (MRC) CML-III trial (257) as control. We compared it to the data of 283 patients who received imatinib as first line therapy (followed by second generation TKI in case of imatinib failure) at our institution.

Hammersmith patients

Between June 2000 and September 2009, 283 consecutive adult patients with *BCR-ABL1* positive CML in CP received imatinib 400 mg daily as first line therapy. The median follow up was 67.9 months (range 14-122). Patients' characteristics are shown in table 22.

Of the 283 patients, 104 patients required second line therapy with dasatinib (n=67) or nilotinib (n=37) at some point after having failed imatinib therapy. Dasatinib and nilotinib were given as previously mentioned. Twenty-one patients who failed dasatinib or nilotinib second line were treated with the alternative TKI as described. Standard criteria were used to define CCyR, PCyR and MCyR.

UK-MRC CML-III trial control patients

Between September 1986 and April 1994, 587 patients with CML in CP were randomly allocated to receive either IFN-α or chemotherapy (busulfan or hydroxyurea) as maintenance therapy after initial chemotherapy induction treatment as part of the UK Medical Research Council's CML-III trial (257). 293 patients were allocated to the IFN-α arm, of which 246 failed to respond to IFN-α at some stage as described elsewhere (258). Thus data on these 246 patients were eventually used for this study. Patients' characteristics are shown in table 22.

After satisfying the criteria for IFN-α failure, 122 (49.6%) patients remained on IFN-α-containing regimens until disease progression, whereas 124 (50.4%) abandoned IFN-α therapy at some stage after failure; of these 117 (94.3%) were treated with hydroxyurea and 7 (5.7%) with busulfan. The median follow up was 50.4 months (range 2-202).

Table 22: Patients characteristics

	Hammersmith pts (HH)	UK MRC-CML III pts (Control sample)	Total pts
	(n = 283)	(n = 246)	(n = 529)
Sokal:			
Low risk:	88	50	138
Intermediate risk:	112	90	202
High risk:	83	106	189
Sex:			
Male:	158	134	292
Female:	125	112	237
CE at diagnosis:*			
Yes:	15	20	35
No:	193	226	419
Age at diagnosis:			
≤50 years:	169	101	270
>50 years:	114	143	259
Haemoglobin at diagnosis:			
≤11 g/dl	105	146	251
>11 g/dl	158	100	258

*CE stands for additional cytogenetic abnormalities other than the Ph chromosome. Data was missing in 75 patients.

Statistical methods

Probability of OS (from the time point of diagnosis) was calculated using the Kaplan-Meier method; univariate analysis was carried out using the log-rank test and a Cox regression model of time to death was used to compare the outcomes as previously mentioned. Patients were censored at the time of allogeneic stem cell transplant (28 and 63 patients for the TKI and the control group respectively).

In addition to the treatment group the model was adjusted for the independently significant variables shown in table 23 (age at diagnosis and Sokal risk group). The influence of cytogenetic response on OS was studied in a time-dependent Cox model (also adjusted as described above).

Results

Improved survival on TKI therapy is limited only to those patients who achieved CCyR on first or subsequent line of therapy

Table 23 shows the seven year probabilities of OS according to the characteristics of the patients. Unsurprisingly the 283 patients who received imatinib as first line therapy (HH patients) had an OS dramatically superior to that of the 246 IFN-α treated controls, (adjusted Relative Risk (aRR) =0.11, (p<0.0001). Equally, the 179 (63.2%) patients who achieved and sustained CCyR on imatinib first line had an even greater advantage in OS over the IFN-α controls (aRR=0.02, 95CI= 0.002-0.126, p<0.0001).

The remaining 104 (36.8%) patients failed imatinib at some point (73 had primary cytogenetic resistance, 8 lost their CCyR and 23 were imatinib intolerant). These patients had an OS longer than the IFN-α failure control patients (aRR=0.28, 95CI=0.145-0.531, p=0.0001; figure 18). Sixty-seven (64.4%) of the 104 patients who had failed imatinib achieved CCyR on second (n=49) or third (n=14) line therapy with another TKI. These patients also had an OS longer than the IFN-α controls (aRR=0.05, 95CI=0.007-0.36, p=0.003) and similar to the OS of imatinib responders; in contrast the 37 patients who failed to achieve CCyR on second or third line TKI therapy had an OS similar to that of the control patients (aRR=0.76, p=0.65).

Thus the survival benefit conferred by TKI therapy is limited to the majority of patients who achieve a sustained CCyR either on imatinib or on subsequent TKI therapy, while the patients who failed imatinib and then failed to achieve a CCyR with subsequent TKI(s) had a prognosis similar to the IFN-α failure controls.

Table 23: Seven year probability of OS according to the patients' characteristics

	Seven Year Probability of OS					
	HH pts		Control pts		Total pts	
Sokal:	N	*p*<0.0001	N	*p*<0.0001	N	*p*<0.0001
Low risk:	88	95.4	50	55.3	138	77.8
Intermediate risk:	112	94.4	90	43.2	202	65.6
High risk:	83	72.5	106	21.7	189	38.1
Sex:	N	*p*=0.27	N	*p*=0.52	N	*p*=0.31
Male:	158	85.6	134	31.3	292	54.0
Female:	125	91.5	112	38.1	237	61.3
CE at diagnosis:*	N	*p*=0.17	N	*p*=0.06	N	*p*=0.035
Yes:	15	74.7	20	17.91	35	36.4
No:	193	93.2	226	35.9	419	56.2
Age at diagnosis:	N	*p*=0.05	N	*p*=0.0005	N	*p*<0.0001
≤50 years:	169	92.5	101	48.7	270	74.6
>50 years:	114	82.0	143	27.9	259	44.5
Haemoglobin at diagnosis:	N	*p*=0.01	N	*p*=0.14	N	*p*<0.0001
≤11 g/dl	105	78.6	146	32.0	251	46.2
>11 g/dl	158	93.7	100	38.1	258	66.8

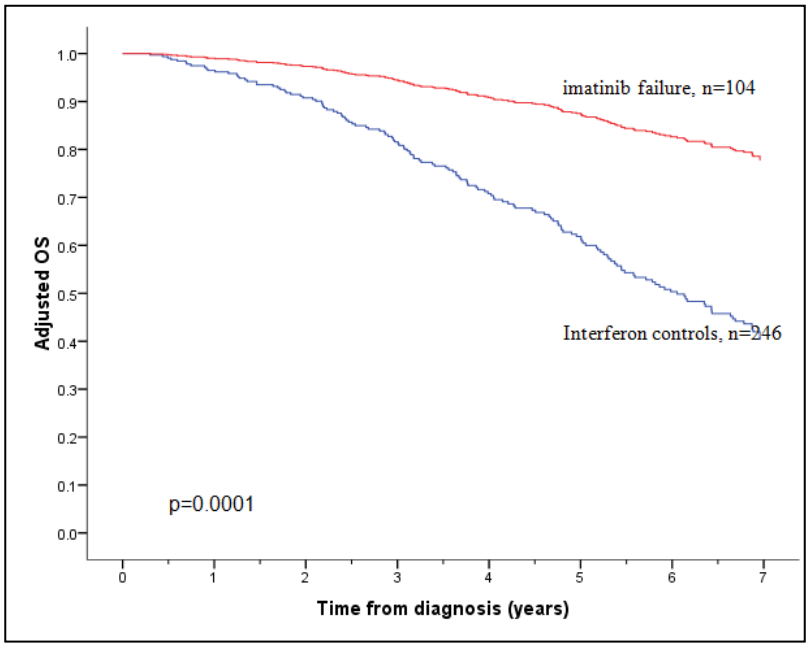
* CE stands for additional cytogenetic abnormalities other than the Ph chromosome. Data was missing in 75 patients.

** Sokal risk group and the age at diagnosis were the only independent predictors for OS in the global population as well as in the separate cohorts of patients.

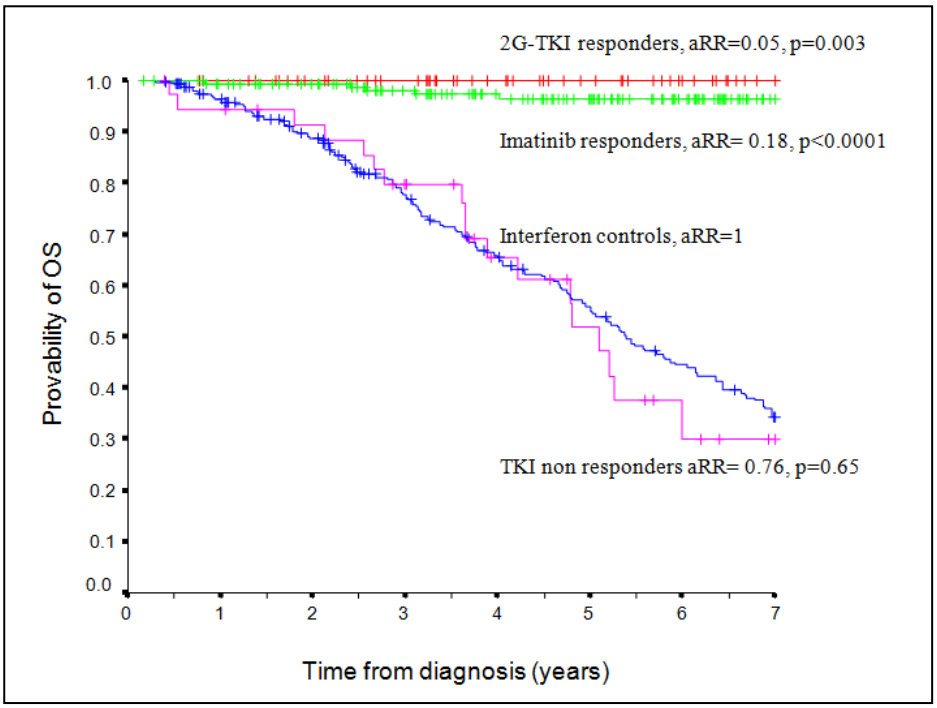
Achievement of PCyR does not confer survival advantage

Fourteen (5.5%) of the 256 patients who achieved PCyR (≤35% Ph-positivity) on TKI therapy (either on first second or third line) failed to achieve CCyR (i.e. the response did not improve to CCyR). These 14 patients had significantly worse OS than the 242 patients who eventually achieved CCyR (aRR=6.64, 95CI=1.74-25.4, *p*=0.006), and an OS similar to that of the IFN-α failure controls (aRR=0.6, *p*=0.4), indicating that PCyR *per se* may not be an adequate therapeutic target.

Figure 18: Adjusted and unadjusted probabilities of survival for patients in different groups



Panel A: shows the adjusted probabilities of OS for the 104 patients who failed imatinib and the 246 IFN-α failure controls (adjusted RR (aRR) = 0.28, 95CI=0.145-0.531, p=0.0001). The unadjusted probabilities of 7-year survival for both groups were 73.4% and 34.4% respectively.



Panel B: shows the unadjusted 7-year probability of OS for the 246 IFN-α controls (34.4%), the 179 patients who achieved and sustained CCyR on imatinib (96.6%), the 67 patients who achieved CCyR on second line TKI therapy (100%) and the 37 patients who failed to achieve a satisfactory response on TKI therapy (30.2%). We also show the adjusted RR for OS with respect the IFN-α controls.

Part II:

Responses on 2G-TKI are durable

Patients & methods

Patients in the 2G-TKI responses study

Between March 2005 and March 2011, 119 consecutive patients with CML in CP resistant to imatinib were treated with dasatinib (n=91), nilotinib (n=25) or bosutinib (n=3) at our institution. The characteristics of the patients were typical of those with imatinib-treated 'late' CP (Table 24). The median follow-up for the surviving patients after starting 2G-TKI was 36.3 months (range 6-73).

Dasatinib, nilotinib and bosutinib were administered as previously described and doses were adjusted according to tolerance. Eleven patients received allogeneic stem cell transplantation after having failed second or third line TKI.

Detection of *BCR-ABL1* transcripts and kinase domain (KD) mutation analysis

BCR-ABL1 transcripts were measured in the blood at 6 to 12 week intervals using RQ-PCR as mentioned previously. Samples obtained for RQ-PCR were also analyzed for KD mutations before starting therapy with 2G-TKI using direct sequencing (251) and again when resistance to therapy was suspected (252).

Statistical analysis

Probabilities of OS, PFS and EFS were calculated using the Kaplan-Meier method; univariate and multivariate analyses were performed with standard methodology as previously mentioned. Probabilities of cytogenetic and molecular responses were calculated using CI procedure, whereby cytogenetic or molecular responses were the events of interest, and death or first choice 2G-TKI therapy discontinuation were the competitors.

Here we used the "current CCyR survival" (c-CCyRS) which is the analogue of 'current leukaemia free-survival' which denotes the probability of being alive and in CCyR at a given time point (214) (see statistical methodology section before).

Table 24: Patient characteristics at the time of starting 2G-TKI and 4-year probabilities of OS and EFS

Variable	n	Survival (%)	EFS (%)
Age:*		*p*=0.04	*p*=0.08
>50 years:	50	68.5	27.8
≤50 years:	69	91.6	41.1
Sex:		*p*=0.8	*p*=0.6
Male:	65	83.9	31.6
Female:	54	78.2	39.9
Sokal risk group:		*p*=0.03	*p*=0.7
Low:	30	100	45.5
Intermediate:	48	83.9	28.6
High:	41	66.5	34.7
Status at the onset of imatinib therapy:		*p*=0.4	*p*=0.3
Newly diagnosed CP patients:	60	77.8	30.7
Late CP:	59	84.6	39.1
Additional cytogenetic abnormalities:		*p*=0.2	*p*=0.07
No:	84	85.2	41.8
Yes:	35	75	21.3
Time from diagnosis to 2G-TKI: **		*p*=0.7	*p*=0.3
≤6 years:	59	81.1	31.8
>6 years:	60	82.2	38.3
Hematologic resistance to imatinib:		*p*=0.4	*p*=0.01
Yes:	31	76.7	22.5
No:	88	84.5	40.5

* The median age was 48.3 years (range 25-78.5)

** The median time was 5.9 years (range 0.4-18)

Results

Responses and outcomes

The 4-year probabilities of OS, PFS and EFS were 81.9%, 80.6% and 35.3% respectively. The 4-year CI of CCyR in patients who were not in CCyR at the time of starting 2G-TKI was 50.0% (in accordance with previous studies) and 62.6% if all patients were included. The 4-year CI of MMR and CMR were 32.0% and 6.8% respectively. Table 5.3 shows the probabilities of OS and EFS according to baseline patient characteristics. High Sokal risk group (RR=3.0, *p*=0.04) and up-front cytogenetic resistance to imatinib (RR=3.1, p=0.03) were the only pre-second line therapy independent predictors for OS.

Table 25: Patient characteristics at the time of starting 2G-TKI and 4-year probabilities of OS and EFS (continued)

Variable	n	Survival (%)	EFS (%)
Primary cytogenetic resistance to imatinib:		*p*=0.03	*p*=0.001
Yes:	37	70.5	13.5
No:	82	87.9	46.7
Acquired cytogenetic resistance to imatinib:		*p*=0.6	*p*=0.08
Yes:	34	83.5	26.8
No:	85	81.4	50.1
Imatinib intolerant:		*p*=0.06	*p*=0.08
Yes:	43	97.1	50.4
No:	76	77.3	30.2
KD mutation:		*p*=0.2	*p*=0.9
Yes:	26	89.3	39.0
No:	93	80.2	32.9

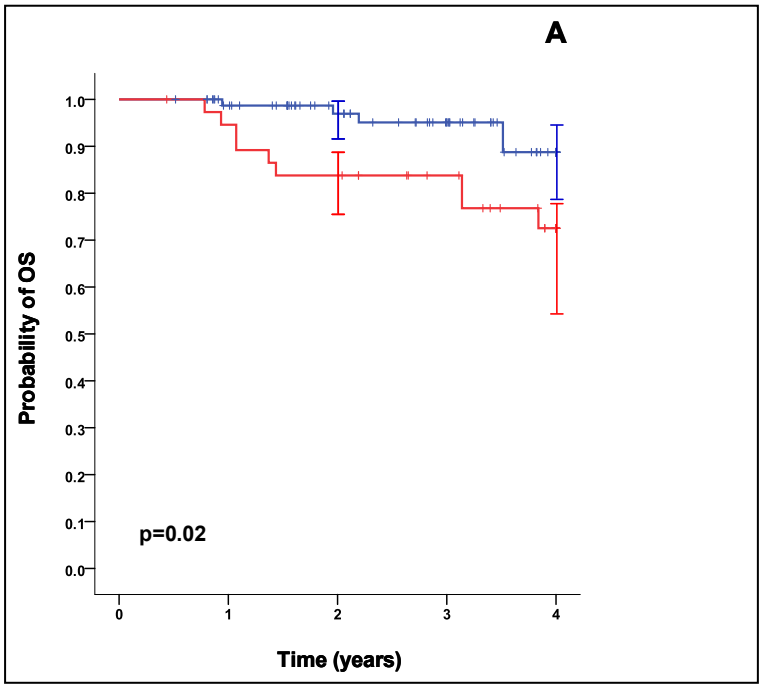
The *BCR-ABL1* transcript levels after 3 months on first choice 2G-TKI therapy strongly predicted for the most relevant clinical outcomes

The 77 patients who after 3 months of second line therapy had a *BCR-ABL1/ABL1* ratio of ≤ 10% had a significantly superior OS (91.3% vs. 72.1%, *p*=0.02), PFS (91.0% vs. 67.9%, *p*=0.007), EFS (49.3% vs. 13.0%, *p*<0.001), CI of CCyR (75.6% vs. 4.2%, *p*<0.001), CI of MMR (45.3% vs. 0%, *p*<0.001) and CI of CMR (8.8% vs. 0%, *p*=0.001) compared to the 30 patients with values greater than 10% (10 patients had missing data and 2 were already receiving third line TKI therapy) (figure 19).

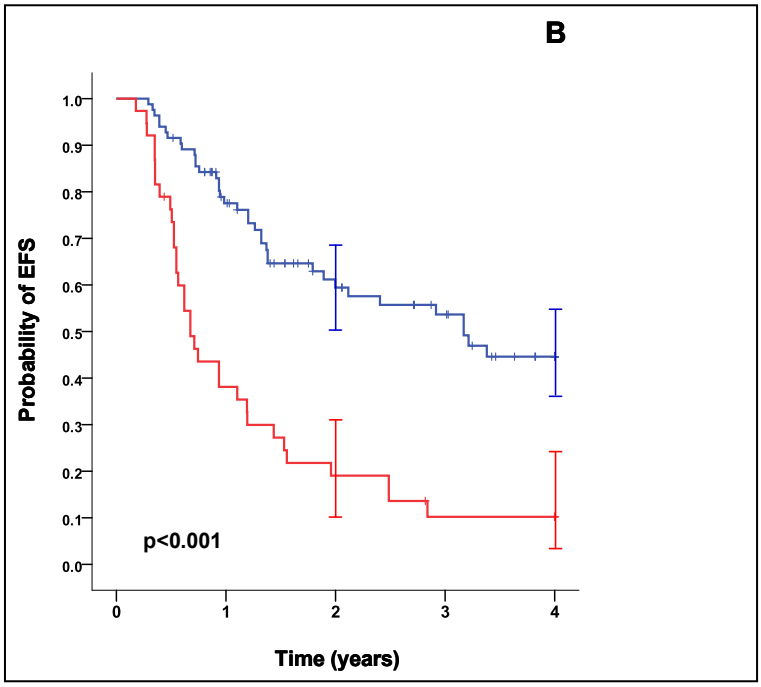
The 64 patients who were in MCyR at 3 months also had significantly superior OS (92.2% vs. 72.4%, *p*=0.04); also there was a trend towards superior OS for the 47 patients who were in CCyR at 3 months (93.1 vs. 72.8, *p*=0.13). When we performed multivariate analysis for OS (we included the molecular and cytogenetic responses at 3 months and the baseline variables found to be significant in the univariate analysis), the transcript level >10% (RR=5.6, *p*=0.01) was the only independent predictor for OS.

We also examined the predictive value of the transcript level at 6 months. The 59 patients with a *BCR-ABL1/ABL1* ratio of ≤1% had a significantly superior EFS (58.5% vs. 18.3%, p<0.001), but not OS (90.8% vs. 77.0%, p=0.1) or PFS (91.5% vs. 74.2%, p=0.06). We then employed a receiver operating characteristic (ROC) curve to identify the optimal transcript level cut-off at 6 months that would be predictive for OS with the maximal sensitivity and specificity, the result was 1.1%. The use of this cut-off did not improve the predictive power of the transcript level on OS or PFS.

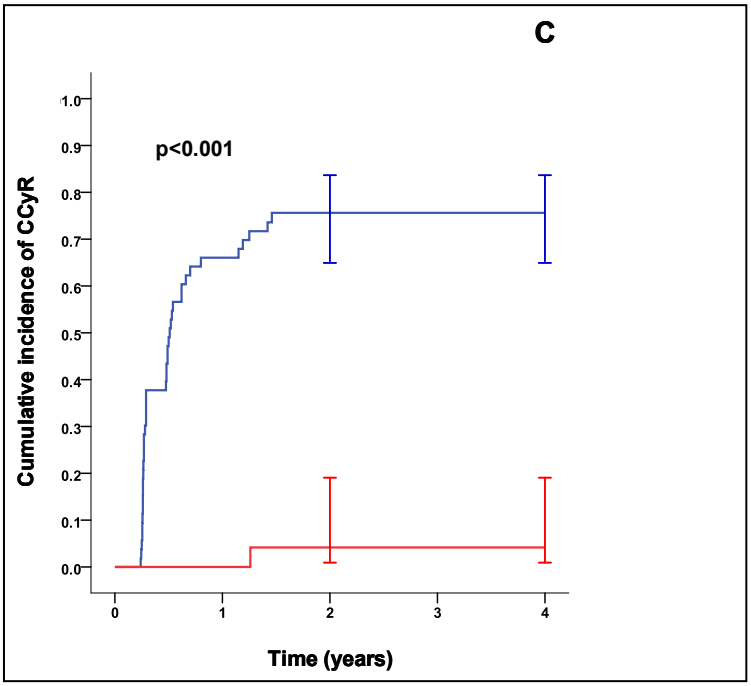
Figure 19: OS, EFS, CI of CCyR and current CCyR-Survival according to the molecular response to 2G-TKI at 3 months



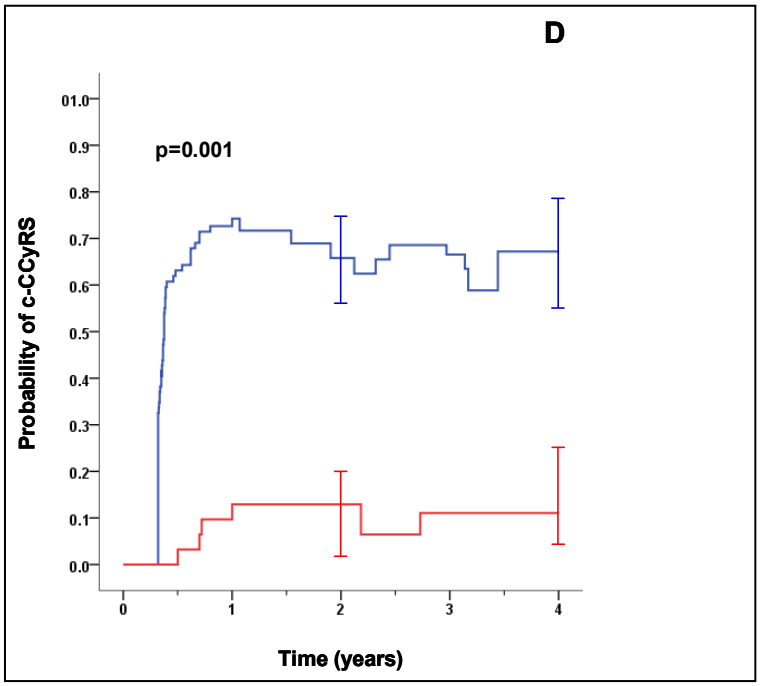
Panel A shows the 3 months OS of 77 patients who had *BCR-ABL1/ABL1* ratios of ≤10% (blue line) compared with the 30 patients with transcript ratios >10% (red line). Whiskers represent 95% confidence intervals. Ten patients had missing Q-PCR data and 2 were already receiving third line TKI therapy and therefore not included in the analysis. The panel shows significantly superior OS between the 2 groups (91.3% vs. 72.1%, p=0.02).



Panel B shows significantly superior EFS between the same comparison groups (49.3% vs. 13.0%, p<0.001).



Panel C shows higher CI of achieving CCyR between the 2 groups (76% vs. 4%, p<0.001).



The patients with a transcript ratio ≤10% had also a significantly higher probability of c-CCyRS (Panel D) (defined as the probability of being alive and in CCyR at a given time point) namely 67.2% for patients with a transcript level ≤10% (blue line) and 11.2% (p=0.001) for patients with a higher transcript level (red line).

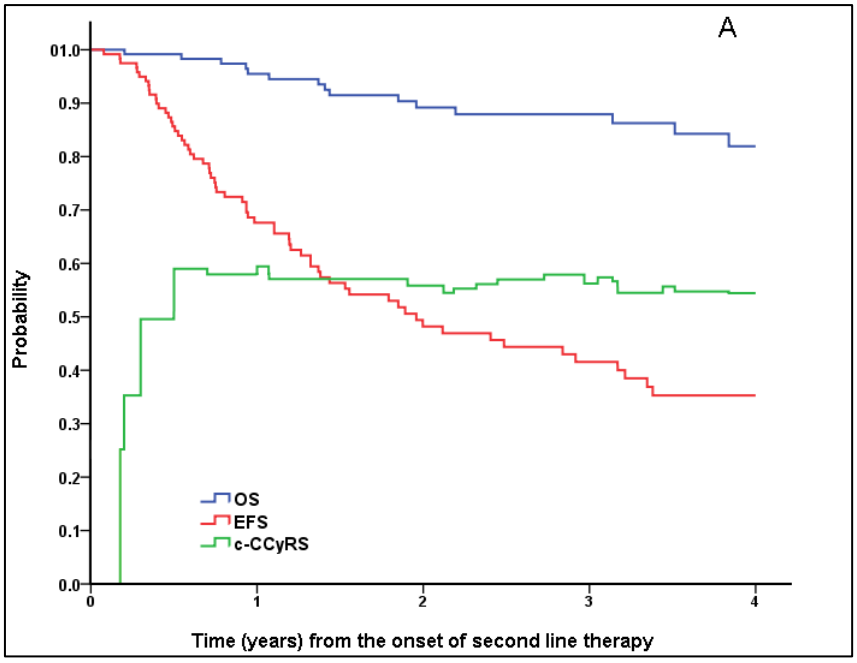
Responses obtained on second line therapy are durable

Sixty-two patients (52%) discontinued the first choice 2G-TKI on account of primary resistance (n=23), acquired resistance (n=8) and intolerance (n=31), whereas 57 patients were still on first choice 2G-TKI therapy at last follow-up. *ABL1* KD mutations were identified in 26 patients prior to 2G-TKI therapy and 16 of these (62%) developed further mutations on subsequent therapy in contrast to only 10 (11%) of the 93 patients without mutations at start of 2G-TKI (p<0.001). 74 patients achieved/maintained CCyR on first choice 2G-TKI therapy. Of these 8 subsequently lost their CCyR (3 patients regained CCyR on alternative therapy). The 4-year CI of loss of CCyR was 14.8%. Of the remaining 66 patients, 14 changed 2G-TKI as a result of intolerance, and 52 continued on first choice 2G-TKI. 45 patients did not achieve CCyR on first choice 2G-TKI, of who 32 then received third line therapy (alternative TKI, allo-SCT and others).

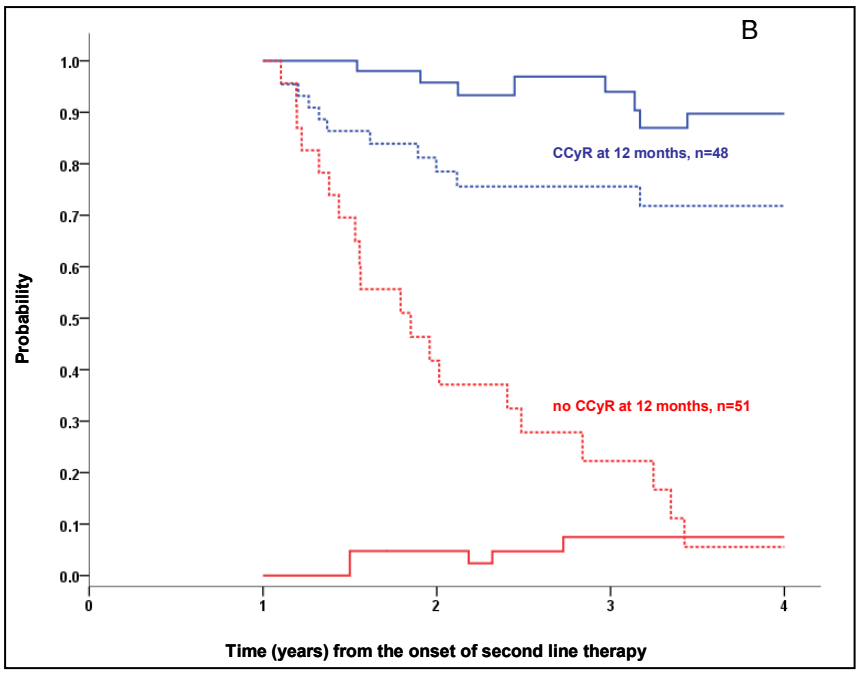
As we have shown above, the discontinuation rate of the first choice 2G-TKI is relatively high and to explore the durability of CCyR, irrespective of the need of an alternative TKI, we have used the concept of current CCyR survival (c-CCyRS) (see statistics methods section). The c-CCyRS at 4 years was 54% (figure 20 – Panel A). The c-CCyRS reaches a plateau after the first year.

Furthermore, in a landmark analysis of patients in CCyR at 12 months (figure 20 – Panel B) the c-CCyRS at 4 years was 90%. However, three of these patients died of causes not related to CML, and when these patients were excluded the 4-year probability of c-CCyRS was 100%. This shows a remarkable stability of the responses.

Figure 20: OS, EFS and current CCyR-Survival (c-CCyRS) in the whole population (Panel A) and according to the cytogenetic response at 12 month (Panel B)



Panel A shows the probabilities of OS (blue line), EFS (red line) and c-CCyRS (green line) in the whole population. The 4-year probability of OS, EFS and c-CCyRS were 81.9%, 35.3% and 54.4% respectively. The figure shows how the EFS continues to decline over time while the c-CCyRS plateaus around 55% after the first year of therapy, reflecting on the one hand that the responses are stable once they have been achieved and on the other hand that an alternative TKI is very effective in maintaining a cytogenetic response that already been achieved in patients who require a change of therapy on account of toxicity.



Panel B shows the EFS (broken line) and c-CCyRS (solid line) according to the cytogenetic response achieved at 12 months. The 48 patients who were in CCyR at 12 months, represented in blue, had a 4-year probability of EFS of 71.8%; on the other hand they had an excellent 4-year probability of c-CCyRS, namely 90%. In contrast the 51 patients who were not in CCyR at 12 months, represented in red, had an EFS of 5.6% (p<0.001) and a c-CCyRS of 7.5% (p=0.008).

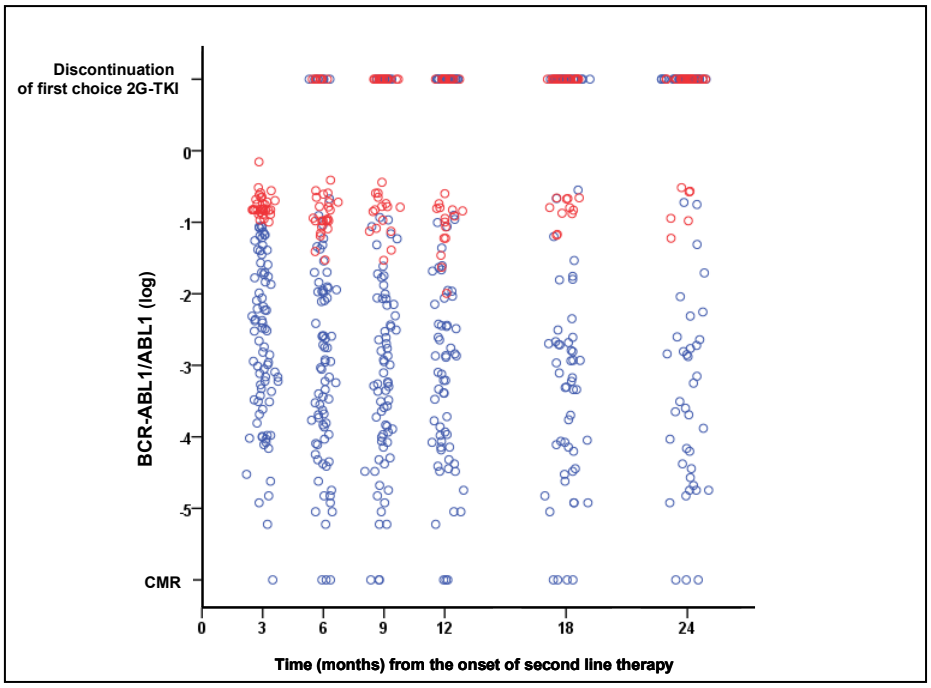
The transcript level >10% at three months of first choice 2G-TKI remains high during the follow up in spite of changing therapy

Figure 21 shows the evolution of the transcript levels over time. The 30 patients who had a *BCR-ABL1/ABL1* ratio >10% at 3 months only gained a very modest reduction in their transcript levels during follow-up. The 4-year CI of CCyR for these patients was only of 4.2% (figure 19 – Panel C). These patients obtained a limited benefit from changing therapy as shown by the fact that 4-year c-CCyRS for this cohort was only 11.2% (vs. 67.2 % for the rest of the population, p=0.0001, figure 19 – Panel D).

The *BCR-ABL1* transcript kinetics is influenced by the early dose intensity of the 2G-TKI

Sixty two patients required dose reduction or dose interruption during the first three months of therapy. These patients had significantly lower 4-year CI of CCyR and EFS, namely 29.3% vs. 76.0% (p<0.0001) and 29.5% vs. 44.4% (p=0.03) respectively compared with the 54 patients who received the full dose (2 patients had already permanently discontinued the first choice 2G-TKI). Again these poor risk patients only obtained a limited benefit from changing therapy with a 4-year c-CCyRS of 32.4% (versus 64.5% for the rest of the patients, p=0.005). Furthermore 25 of 30 patients with a *BCR-ABL1/ABL1* ratio >10% at 3 months received a dose intensity lower than 100 mg daily of dasatinib, 400 mg twice daily of nilotinib or 500 mg daily of bosutinib.

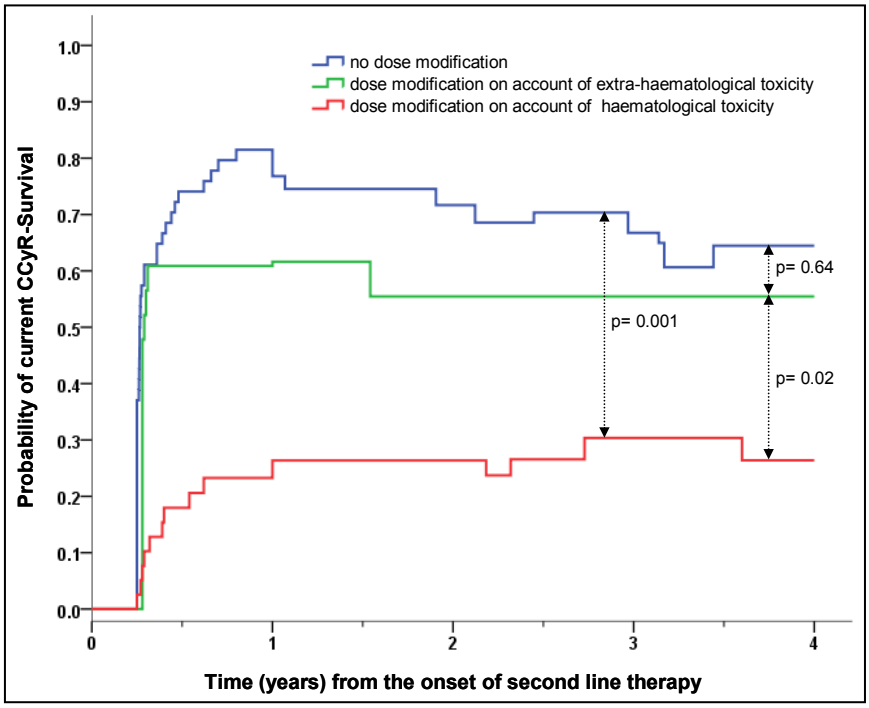
Figure 21 : Evolution in the *BCR-ABL1* transcript level on first choice second line 2G-TKI according to the molecular response achieved after 3 months on second line therapy.



Patients who had a *BCR-ABL1/ABL1* ratio >10% after three months of therapy (represented by red circles) had a minimal reduction in the transcript level during subsequent follow-up, remaining always above the 1% mark (equivalent to a 2 log reduction commonly identified with CCyR). Patients with a transcript level at 3 months ≤10% are represented by blue circles. The top row represents patients who discontinued their first choice 2G-TKI (not the transcript level) at the various time points according to their transcript levels at 3 months.

To further investigate the effect on outcome of low dose intensity during the first three months of therapy we sub-classified the poor tolerance as haematological (n=39) or non-haematological (n=23). We found that patients with haematological intolerance fared worst while the patients with non haematological toxicity had a 4-year c-CCyRS comparable to that of patients with good tolerance to therapy (figure 22); thus the c-CCyRS for the patients with haematological intolerance was 26.4% (p=0.001 with respect the patient with good tolerance) while the c-CCyRS for the patients with non-haematological intolerance was 55.5% (p=0.64 with respect the patient with good tolerance).

Figure 22: c-CCyRS according to the dose intensity received by the patient during the first three months of therapy and reason for dose adjustment



The 39 patients (red line) who had their therapy with the first choice 2G-TKI temporarily discontinued or dose reduced on account of haematological toxicity during the first three months of treatment had a significantly worse 4-year probability of c-CCyRS than the 54 patients who did not require dose modification, represented by a blue line, (26.4% vs. 64.5%, p=0.001). On the other hand the 23 patients who had their dose of 2G-TKI modified on account of extra-haematological toxicity (green line) had a 4- year c-CCyRS comparable to that of patients with good tolerance to therapy (55.5% vs. 64.5%, p=0.64) which is significantly higher than that of patients with haematological intolerance (p=0.02).

Discussion

The results from the first part of our study, like others that involve historical comparisons, have been regarded by some researchers as less reliable than normal randomized studies. However, evidence suggests that results from well designed observational studies don't differ much from those of normal trials (259, 260). No to mention that in some conditions, prospective randomized trials would be either impossible to design or un-ethical (258).

And although efforts are always done in order to minimise bias, yet sometimes it becomes inevitable, especially if the used prognostic factors strongly predict outcome (261, 262), and the use of other therapies is prevented even for the patients with poor prognosis (259).

Our study fits within these criteria; as it's very unlikely that a prospective randomised trial which involves the patients that we have studied will be conducted; secondly, our model was designed for strong predictive factors; and finally the clinicians had no opportunity to influence treatment as patients in the MRC CML-III study could only continue IFN-α or switch to palliative treatment measures such as (hydroxyurea or busulphan), since TKIs weren't available at that time.

When using the adjusted Cox model to compare outcomes between the 2 groups, it wasn't surprising that the imatinib responders had a dramatically better outcome than the CML patients treated with the palliative therapy (control group). Patients who failed imatinib and went on to receive sequential therapy with 2G-TKIs also had an enormous survival advantage over the controls (aRR=0.28, *p*=0.0001, figure 18). However, we found that this survival advantage was only limited to those patients who had achieved CCyR after imatinib failure, while the others had prognosis similar to the control group.

In the second part of our study, we have shown that 50% of patients who aren't in CCyR by the start of the second line therapy will achieve it at some point during the follow up. This has been also shown by others (171, 177, 180, 254, 255). However, there has been no data published referring to the durability of these achieved responses. This is an important issue as patients who fail imatinib are likely to have developed resistant or mutated clones that are responsible for early replace. Furthermore, a number of patients who eventually respond fail the first choice of 2G-TKI on account of toxicity and require alternative therapies.

To study these issues, we have exploited the concept of "current leukaemia-free survival". The 4 year c-CCyRS, was 54.4% (figure 20 – Panel A) for the whole population and 90% (figure 20 – Panel B) for those patients who were in CCyR after 12 months of second line therapy (100% if non-leukaemia causes of death were excluded). This shows that the responses are remarkably stable over time.

Both curves reach a plateau after the first 12 months, although the patients continue to have "events". This indicates that patients who discontinue their second line therapy because of side-effects fared well on third line therapy (263).

Primary cytogenetic resistance to imatinib and the high Sokal risk were the only pre-therapy independent predictors for OS as previously reported by a previous study from our group (254) and others (264, 265).

The *BCR-ABL1* transcript levels at 3 months on second line therapy accurately predicted the outcome of patients (figure 19) as the 30% of patients who had transcript levels >10% had a very low probability of achieving CCyR (4.2%) and a significantly lower OS (72.1% vs. 91.3%, p=0.02). Transcript levels of patients who had a poor 3-month molecular response remained comparatively high during the follow-up (figure 21) and further assessments didn't come up with better definitions of poor risk patients.

In a previous report, we have mentioned that poor responders to second line 2G-TKI obtained limited benefit from 3rd line TKI (263), this now shows as patients who had a *BCR-ABL1* ratio >10% after 3 months from starting 2G-TKI had a very low 4 year c-CCyR, namely 11.2% (compared with 67.2% for the rest, p=0.0001, figure 19 – Panel D). This clearly shows that a high 3-month transcript level identifies patients with intrinsic resistance to TKIs and won't benefit from changing to an alternative TKI.

Patients who had difficulties tolerating the first 2G-TKI during the first 3 or 6 months on account of side-effects fared worst (data not shown), but the harmful effects of the low dose intensity during the initial period of second line therapy was limited to patients with haematological toxicities.

Patients with non-haematological side-effects responded well to alternative TKI therapy and had a 4 year c-CCyR comparable to those patients who received full dose intensity during the first months of therapy; while patients who suffered haematological toxicities had a low 4-year c-CCyRS (figure 22). Again changing to an alternative therapy made little impression on the long term outcome. A possible explanation to that is that haematological toxicity in some cases is linked to an intrinsic characteristic of the leukaemia, such as the lack of an expandable Ph-negative stem cell population that can restore effective normal haematopoiesis (117, 266). This prevents a given patient from responding satisfactorily to TKI therapy.

Conclusion

The use of second generation tyrosine kinase inhibitors after imatinib failure offers a survival benefit over palliative regimens as compared with historical data. However this benefit is only noticed in patients who achieved CCyR on imatinib. Patients who fail to achieve CCyR didn't do better than if they were given palliative therapy, and therefore it is important to make sure those patients who fail imatinib are treated with at least one other TKI and if necessary with two TKIs.

We also showed that the responses achieved on second line are durable, although patients with side-effects may need to be treated with more than one 2G-TKI after imatinib failure in order to achieve optimal outcome. Those patients can be identified as early as 3 months using *BCR-ABL1* transcript levels. Those patients who fare poorly can be offered alternative therapy.

Chapter VI

Use of TKIs as third line therapy

Introduction

Dasatinib and nilotinib have been shown to be effective in the treatment of CP – CML in patients who are imatinib resistant or intolerant (170, 177, 209, 210, 255, 267). Patients who currently fail second line treatment either proceed to allogeneic stem cell transplantation, or offered to receive the alternate TKI as alternative third line therapy.

A previous study has shown that the efficacy of dasatinib or nilotinib after failure of 2 prior TKIs is limited (268), so it was important to identify which patients are most likely to benefit from this approach. We also tried to identify prognostic factors that can used to help guide clinical decisions in this group of patients.

Patients & methods

Patients

Data from 26 patients with CP – CML who were treated either with dasatinib (n=20) or nilotinib (n=6) after imatinib failure at the Hammersmith Hospital between March 2005 and January 2008 were evaluated. Patients' criteria are mentioned in table 25. CP, CHR, MiCyR, MCyR, CCyR and MMR were defined by standard criteria as previously mentioned. The median follow up after starting third line therapy for the surviving patients was 21.5 months (range, 6–46.5 months).

Failure to second line therapy was defined based on ELN criteria (121). Patients with a T315I kinase domain (KD) mutation were not included. Nilotinib and dasatinib were administered as previously mentioned and doses were adjusted according to tolerance.

Patients were classified not only according to the degree of cytogenetic response to imatinib or the other TKI, but also according to their tolerance to previous therapies.

Table 25: Patients' baseline criteria

Variable:	N
Age:	
≤ 64 years:	13
> 64 years:	13
Sex:	
Female:	12
Male:	14
Status at the onset of imatinib therapy:	
Early CP:	19
Late CP:	7
Sokal risk group:	
Low + intermediate[III]	14
High	11
Best cytogenetic response on imatinib:	
No response:	16
At least MiCyR:	10
Best cytogenetic response on second line therapy:	
No response:	14
At least MiCyR:	12
Prior history of clonal evolution:	
No:	18
Yes:	8
Prior history of KD mutation:	
No:	14
Yes:	12
Prior history of haematologic resistance to TKI[IV]:	
No:	19
Yes:	7
Prior history of intolerance to TKI therapy:	
No:	9
Yes:	17
Percentage of Ph +ve at start of third line therapy:	
≥95%:	22
<95%:	4
Time from diagnosis to 3rd line therapy[V]:	
≤ 63 months:	11
> 63 months:	12

We defined intolerant patients as those who required dose reduction during prior TKI therapy on account of recurrent toxicity or who permanently discontinued the imatinib or second line therapy because of grade 3-4 non-haematologic side effects (54).

Statistical methods

Statistical analyses for the probabilities of OS, EFS were calculated using the Kaplan-Meier method. Data from the six patients who underwent allogeneic stem cell transplantation were censored at the moment of transplantation for the analysis of cytogenetic responses but not for EFS, PFS and OS. Univariate analysis was carried out using the log rank test, and variables found to be significant at $p < 0.25$ were entered into a hazard regression analysis and a forward stepping procedure was employed to find the best model.

[I] 64 years was the median age in population at the onset of the third line therapy

[II] Patients were considered to be in late chronic phase at the moment of starting imatinib if they had commenced the imatinib more than 6 months after diagnosis or had received prior IFN-α therapy.

[III] One patient was classified as low risk and 13 as intermediate risk. The Sokal score could not be calculated in one patient.

[IV] Haematological resistance was defined as either failure to achieve a CHR or as the loss of a previously achieved CHR.

[V] 63 months was the median time from diagnosis of CML to the start of third line therapy.

Results

Responses to third line

During the follow up 13 (50.0%), 9 (34.6%) and 5 (19.2%) patients achieved a MCyR, CCyR and MMR respectively. The 2.5 years (30 months) cumulative incidences of MCyR, CCyR and MMR were 48.2%, 32.4%, 21.1% respectively. We performed univariate and multivariate analyses to identify factors that can predict responses. Table 26 shows responses based on patients' baseline criteria (MMR data not shown).

We found that patients who had achieved a cytogenetic response either on imatinib or on second line therapy had a higher probability of also achieving cytogenetic response on third line, while patients who had developed haematologic resistance during prior TKI therapy were less likely to do so. The presence of a KD mutation at the start of the third line therapy didn't affect the response.

When performing multivariate analysis, the achievement of a cytogenetic response on imatinib (RR=5.6, p=0.03) or on second line therapy (RR=11.8, p=0.006) were the only independent predictors for the achievement of CCyR on third line. The achievement of cytogenetic response on second line was the only independent predictor for MCyR (RR=15.4, p<0.001).

When combining both factors, we found that the patients who had achieved cytogenetic responses on one of the two prior therapies had a higher probability of achieving cytogenetic response on third line therapy. The 30 month probability of MCyR and CCyR were 100% vs. 12.5% (p<0.0001) and of 71.4% vs. 0% (p=0.0005) respectively (figure 23).

Table 26: Responses to 3rd line according to patients' criteria

		Response on 3rd line	
Variable:	**N**	**MCyR (%)**	**CCyR (%)**
Age:		*p*=0.3	*p*=0.5
≤ 64 years:	13	52.0	41.0
> 64 years:	13	42.3	53.8
Sex:		*p*=0.9	*p*=0.9
Female:	12	44.4	45.3
Male:	14	53.1	55.1
Status at the onset of imatinib therapy:		*p*=0.3	*p*=0.3
Early CP:	19	57.1	64.3
Late CP:	7	47.8	52.6
Sokal risk group:		*p*=0.9	*p*=0.6
Low + intermediate[III]	14	44.9	38.6
High	11	60.7	49.3
Best cytogenetic response on imatinib:		*p*=0.004	*p*=0.01
No response:	16	28.6	13.5
At least MiCyR:	10	85.0	62.5
Best cytogenetic response on second line therapy:		*p*<0.001	*p*<0.001
No response:	14	16.7	0
At least MiCyR:	12	83.3	70.0
Prior history of clonal evolution:		*p*=0.1	*p*=0.3
No:	18	49.5	27.8
Yes:	8	0	0
Prior history of KD mutation:		*p*=0.9	*p*=0.4
No:	14	45.5	53.2
Yes:	12	53.1	50.6
Prior history of haematologic resistance to TKI[IV]:		*p*=0.007	*p*=0.04
No:	19	67.8	63.9
Yes:	7	0	0
Prior history of intolerance to TKI therapy:		*p*=0.5	*p*=0.5
No:	9	52.6	46.6
Yes:	17	59.7	52.9
Percentage of Ph +ve at start of third line therapy:		*p*=0.04	*p*=0.03
≥95%:	22	48.5	39.1
<95%:	4	100	100
Time from diagnosis to 3rd line therapy[V]:		*p*=0.9	*p*=0.7
≤ 63 months:	11	46.7	66.7
> 63 months:	12	50.7	53.5

EFS and OS

During follow up 11 (42.3%) patients failed third line therapy and 9 (34.6%) died. One patient died of non leukaemia causes (myocardial infarction) while the remaining 8 died after progression to blastic phase (BP). The 30-month probabilities of EFS and OS were 45.7% and 46.7% respectively (table 27). Patients with primary cytogenetic resistance to second line therapy had a worse EFS and OS. Multivariate analysis showed that the development of haematologic resistance was the only independent predictor for EFS (RR= 0.4.3, *p*=0.02). The achievement of a cytogenetic response on second line and age younger than 64 were the only independent predictors for OS (RR=6.5, *p*=0.02 and RR=0.13, *p*= 0.02).

Influence of responses on outcome

At 3 months, all of our 26 patients remained on follow up, of whom 9 had achieved at least MiCyR (<95% Ph-positive metaphases). These 9 patients had better 30 month probabilities of CCyR, EFS and OS than the patients who had failed to achieve MiCyR, namely 88.9% vs. 13.3% (*p*<0.0001), 87.5% vs. 28.0% (*p*=0.007) and 87.5% vs. 35.0% (*p*=0.1). When we excluded the only patient who died of non leukaemia cause while in CCyR, the probabilities of EFS and OS were 100% vs. 28% (*p*=0.007) and 100% vs. 35.0% (*p*=0.04) respectively (figure 23).

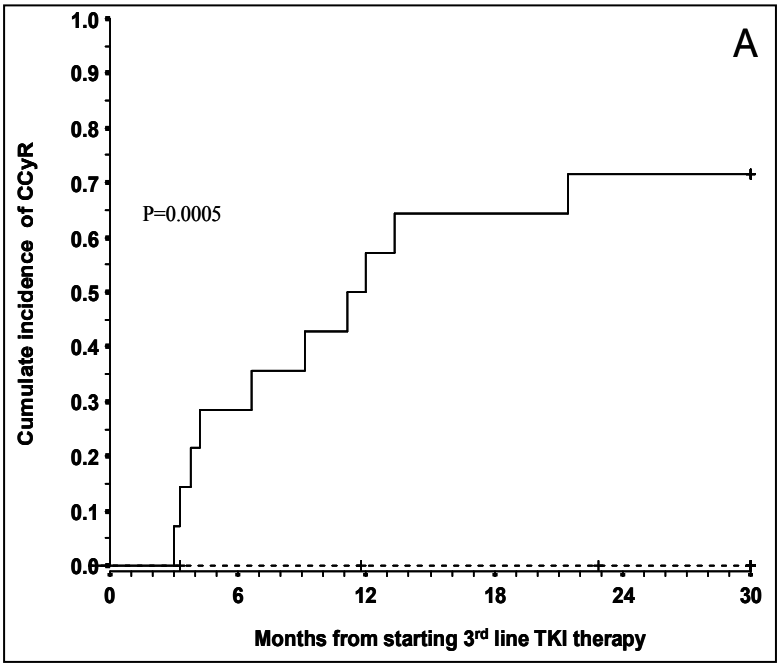
Intolerant patients

Seventeen patients (65%) met our criteria for intolerance (table 25). Intolerant patients had probabilities of response similar to those of resistant patients. When we subdivided this cohort according to the type of intolerance we found that the 11 patients who had haematologic toxicity with either the imatinib or the second line therapy, these patients had a probability of 30-month CCyR lower than that of the remaining 15 patients (11.1% vs. 47.5 %, *p*=0.03), while the 8 patients with non-haematologic intolerance to the imatinib or to the second line had a probability of 30-month CCyR greater than that of the remaining 18 patients (87.5% vs. 5.6%, *p*<0.001).

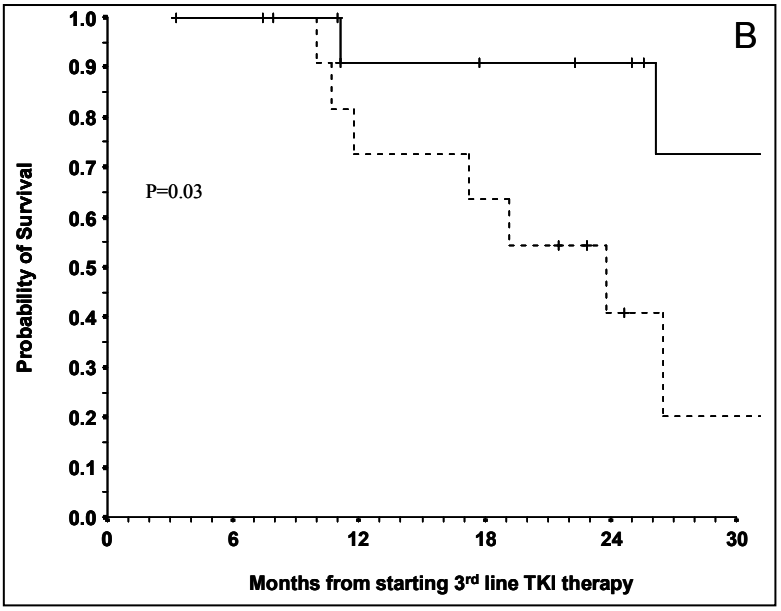
Table 27: OS and EFS on 3rd line according to patients' criteria

		Response on 3rd line	
Variable:	**N**	**OS (%)**	**EFS (%)**
Age:		*p*=0.03	*p*=0.7
≤ 64 years:	13	60.6	49.9
> 64 years:	13	24.9	39.8
Sex:		*p*=0.9	*p*=0.8
Female:	12	45.0	51.3
Male:	14	47.6	41.4
Status at the onset of imatinib therapy:		*p*=0.4	*p*=0.8
Early CP:	19	80.0	53.6
Late CP:	7	40.4	43.5
Sokal risk group:		*p*=0.9	*p*=0.8
Low + intermediate[III]	14	39.0	33.3
High	11	50.0	56.6
Best cytogenetic response on imatinib:		*p*=0.4	*p*=0.1
No response:	16	42.4	32.7
At least MiCyR:	10	57.1	72.0
Best cytogenetic response on second line therapy:		*p*=0.03	*p*=0.04
No response:	14	25.6	23.4
At least MiCyR:	12	88.9	83.3
Prior history of clonal evolution:		*p*=0.9	*p*=0.9
No:	18	57.0	46.1
Yes:	8	50.0	50.0
Prior history of KD mutation:		*p*=0.2	*p*=0.3
No:	14	50.0	36.9
Yes:	12	53.9	54.0
Prior history of haematologic resistance to TKI[IV]:		*p*=0.4	*p*=0.04
No:	19	61.9	64.7
Yes:	7	44.4	28.6
Prior history of intolerance to TKI therapy:		*p*=0.6	*p*=0.3
No:	9	50.0	40.0
Yes:	17	46.5	45.4
Percentage of Ph +ve at start of third line therapy:		*p*=0.1	*p*=0.2
≥95%:	22	50.9	55.9
<95%:	4	100	100
Time from diagnosis to 3rd line therapy[V]:		*p*=0.9	*p*=0.9
≤ 63 months:	11	45.0	47.0
> 63 months:	12	51.6	49.0

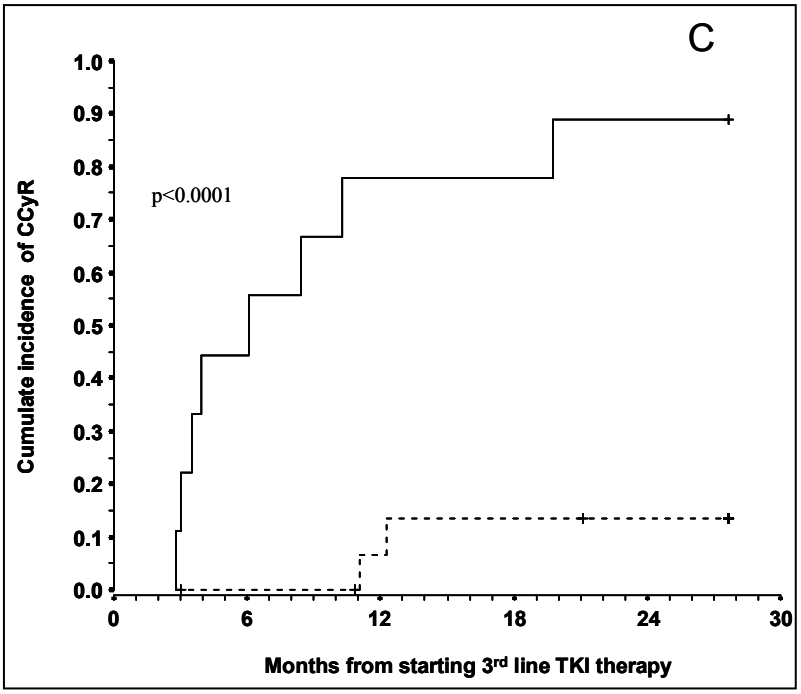
Figure 23: Outcome of patients treated with third line TKI according to the cytogenetic response obtained with the prior TKI therapy and to the cytogenetic response obtained after 3 months on third line therapy.



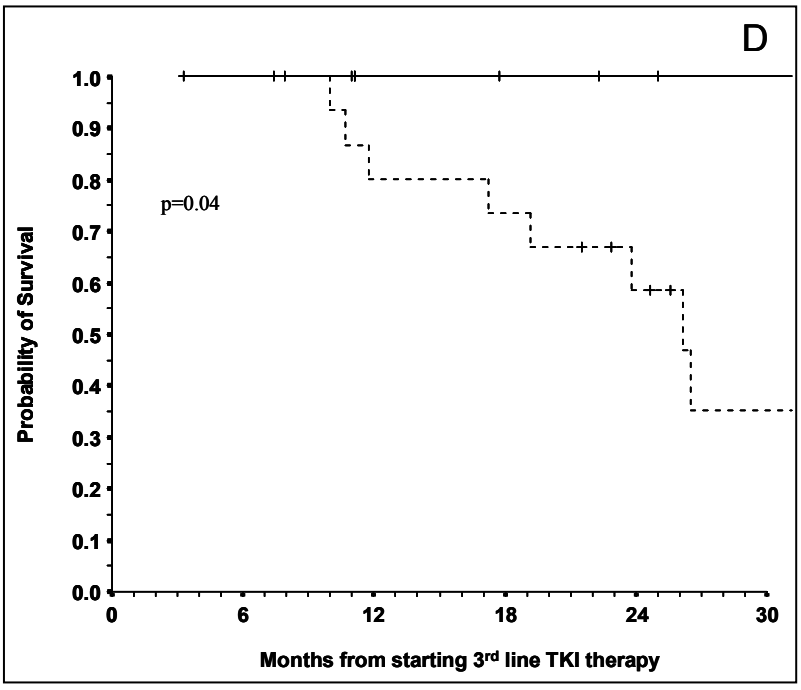
The 14 patients who achieved at least MiCyR on imatinib or on second line therapy (solid line) had a significantly better probability of achieving CCyR (panel A) and a better overall survival (panel B) on third line nilotinib or dasatinib than the 12 patients who had primary cytogenetic resistance to the prior two lines of TKI therapy (broken line).



The 30-month cumulative incidence of CCyR for the two groups was of 71.4% vs. 0% (p=0.0005), and the 30-month OS was 72.7% vs. 20.4% (p=0.03). When we excluded the only patient who died of non-leukaemia related reasons and in CCyR the OS were 90.1% vs. 20.4% (p=0.01). The 14 patients who achieved at least MiCyR on one of the prior TKI therapies had also a better EFS that the 14 patients with primary cytogenetic resistance, namely 70.5% vs. 16.2% (p=0.02).



Panels (C) and (D) show the results of the landmark analyses for the achievement of CCyR and OS (excluding the non-leukaemia related death) according to the cytogenetic response at 3 months on nilotinib or dasatinib as third line therapy. At 3 months 9 patients had achieved at least MiCyR (solid line). These patients had higher probabilities of achieving CCyR (panel C) and OS (panel D) than the 17 patients who had failed to do so (broken line), namely 88.9% vs. 13.3% (p<0.0001), and 100% vs. 35.0% (p=0.04).



Discussion

Although disappointing, yet the results from 2 trials conducted to explore the efficacy of dasatinib (269) or nilotinib (270) after failure of the other TKI in those patients after previously failing imatinib encouraged clinicians to use sequential TKIs to try to control the disease progression or if possible, to revert the patient back to CP. As far as we know, our study is the largest series of CML patients in CP treated with a third line TKI after failing both imatinib and another TKI.

The cytogenetic response obtained on previous therapies was highly informative. Patients who have achieved some degree of cytogenetic response during the first or second line therapy had a higher probability of achieving MCyR and CCyR and better EFS and OS than the patients who had primary cytogenetic resistance to both first and second line therapy (figure 23). On the contrary, none of the patients with primary cytogenetic resistance to both initial lines achieved CCyR on the third line, clearly indicating that third line TKI is not useful for these patients.

We also show that patients who their main reason of change of therapy was non-haematologic intolerance fared well, while those with a history of haematologic toxicity to one of the prior TKIs fared worse. Our research group has previously reported the association between haematologic toxicity and lack of cytogenetic response in CP patients treated with imatinib as first line therapy (117), in patients treated with imatinib after IFN-α failure (266), and in patients treated with nilotinib or dasatinib after imatinib failure (254). In these cases the poor prognosis associated with haematologic toxicity may in part explained by the lack of an expandable population of Ph-negative cells (266).

Conclusion

Our study showed that TKIs used as third line therapy is limited only to those patients who have achieved a degree of cytogenetic response (at least MiCyR) on one of the previous therapies. Furthermore, TKIs also offer benefit to patients who have changed therapy because of non-haematological toxicities. Our disappointing results stress the need to select more carefully the patients who may benefit from a third line TKI, as for many patients allogeneic stem cell transplant or an alternative experimental therapy may be a more appropriate.

Chapter VII

Conclusions

It has been a very exciting decade in the management of CML. Current therapy with imatinib and second generation tyrosine kinase inhibitors have transformed what used to be a previously lethal condition to a relatively chronic disease that can be easily managed in the majority of cases, yet remains the challenge of finding true prognostic factors which can determine patients' responses to therapy.

Over the years, several groups have tried to predict the achievement of responses using factors prior to commencing therapy; some of them even date to the pre-imatinib era, among them are the Hasford (128) and the Sokal scores (127), the latter of which is the most widely used. Other factors have been recently developed such as *hOCT-1* and the molecular marker MDR-1 but as shown from our research that they aren't always reliable.

Recently, the European LeukaemiaNet has developed a new score (EUTOS) that classifies patients either as high risk or low risk depending on the pre-therapy spleen size and basophil count. Although the developers suggested that it has superior prognostic power over the Sokal (130), but when we tried applying the new score on our patients, it failed to reproduce similar results denoting that it still requires further validation.

This casts a shadow over whether one should entirely depend on pre-therapy criteria as prognostic factors, whereas it has been shown that the achievement of an early response on therapy is a major treatment endpoint regarding outcome of patients as it is associated with improved OS (123, 223), thus clearly establishing it as an important prognostic factor in CML. Our results confirm that the achievement of responses as early as 3 months can offer a survival benefit to patients either on imatinib, or on 2G-TKIs when used as 2nd or 3rd line therapies. Given that early molecular response can also predict long-term responses we managed to show that *BCR-ABL1* transcript measurements performed at 3, 6 or 12 months can successfully identify those patients with relatively poor OS, PFS and c-CCyR.

To facilitate this, we also established cut-off values determined from the patient population namely *BCR-ABL1* transcript levels of ≤9.84% at 3 months, of ≤1.67% at 6 months and of ≤0.53% at 12 months. Our data suggests that a single measurement at 3 months is the most important measurement needed in order to predict outcomes.

This is majorly dependant however on the regular and accurate intake of imatinib, as our group has previously shown that adherence to imatinib is an important prognostic factor regarding the achievement of responses (135). Astonishingly however it was also reported that adherence to therapy can be as low as 14%. The results of our follow up study show that non-adherent patients with an adherence rate lower than 85% had higher 2-year probability of both losing their responses and of failing imatinib therapy.

Why do patients don't adhere to a life-saving and relatively nontoxic therapy? In another study that was conducted in parallel with our research, interviews were conducted with patients who participated in order to explore their reasons of non-adherence (271). The results show that patients reasons can be divided to either "intentional" or "un-intentional", with a degree of overlap in-between, denoting the need for further strategies to help patients improve their adherence.

Despite the success of imatinib, not all patients respond to therapy either due to resistance or intolerance and require change to second generation therapy. With the availability of dasatinib and nilotinib that current phase II studies have shown that they are able to produce even quicker and deeper responses (272, 273), perhaps soon they might replace imatinib as first line therapies in the near future.

We have demonstrated the survival benefit of 2G-TKIs in patients who failed imatinib and have also shown that the responses achieved either on 2nd line TKIs are durable on the long term. These responses are stable once they have been achieved and aren't affected by change of therapy. Responses on third line on the other hand are only limited to those who have acquired a previous response. This group of patients require further and novel therapies as they tend to progress quickly.

Future challenges:

The biggest challenge that will face researchers in the future is to discover drugs that one day can target the leukaemia cells. Perhaps imatinib was the first step in the path to cure leukaemia, but can it be discontinued without patients requiring further therapy? Results have shown that imatinib can be safely discontinued in a group of 100 patients who had achieved CMR for at least 2 years with a relapse-free survival of 41% (274). This data is very encouraging as with the availability of even better TKIs that offer deeper and more durable responses, CML might be one day cured in some patients.

Another challenge that is still present is the treatment of advanced phase disease. Despite the fact that imatinib and 2G-TKIs have reduced the incidences of progression, blastic crisis remains the ticking time bomb that faces this group of patients who progress. Several approaches that focus on combination chemotherapy with imatinib has been introduced (275-277), but all have shown minimal survival improvement.

A recent study from our group tested the efficacy of combining dasatinib and high dose chemotherapy (FLAG-IDA) to induce remission in BC patients (278). Results showed good responses after 2 cycles of therapy followed by allo-SCT. This approach still needs to be tested in a larger study cohort as it seems to induce deep, durable remissions.

Using the fact that the leukaemic cells are responsive to T-cells immune modulation, another future direction would be to eliminate the residual leukaemia using vaccines; the use of which improves the molecular response in patients with stable cytogenetic responses on imatinib but still carry detectable levels of *BCR-ABL1* transcripts. These vaccines might lead to eradication of minimal residual disease in this subset of patients.

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Appendix I

Database tables

Table I (prior to onset of TKI)

Field name	Description	Codification
N	Patient identifier	Numeric
HN	Hospital number	Numeric
TRIAL NO	Patient trial number	Numeric
TRIAL	Trial code	Numeric: 102;109;110 999: no trial
DB	Patient date of birth	Date (day, month, year)
SEX	Patient sex	Binary 0: male 1: female
RACE	Patient race	Categorical 1: White 2: Asian 3: Black 4: African 5: Middle Eastern 6: Other For subdivisions, see end of history
DIAGCML	Date of diagnosis	Date (day, month, year)
DIAGACEL	Date of progression to AP	Date (day, month, year)
DIAGACELCRI	Criteria of AP	Categorical 999: no AP 1: cytogenetic minor abnormality 2: cytogenetic major abnormality 3: Bl + pm > 30 4: Bl + pro > 20 5: Bl > 20 6: Bl > 10 (was >15) 7: plts > 1000 8: loss of haematological control (ex spleen) 9: increase in early form in trephine but not bm done 10: plt < 100
DIAGBLAS	Date of progression to BP	Date (day, month, year)
DIAGBLASCRI	Criteria of BP	Categorical 999: no blastic phase 1: blasts > 30%
DBLATYPE	blastic phase phenotype	Binary 0: Myeloid 1: Lymphoid 3: Biphenotypic

Field name	Description	Codification
DIECOG	ECOG score at diagnosis	0:normal to 4: almost death
DISPL	Spleen size at diagnosis (cm below costal margin)	Numeric 999: Not measured splenomegaly.
DILIV	Liver size at diagnosis (cm below costal margin)	Numeric 999: Not measured hepatomegaly.
DIABL	at diagnosis % of blast in BM	Numeric
DIAPR	at diagnosis % of promyelocytes in BM	Numeric
DIABS	at diagnosis % of basophiles in BM	Numeric
DITR	at diagnosis cellularity % of BM	Numeric
DITRC	diagnosis trephine code in BM	1: CR 2: CP 3: excess of blast or immature cells 4: increase in megakaryocytes or occurring in clusters 5: aplastic marrow new: other cell : lymphoid infiltrate 0= no, 1=yes
DITRCD34	at diagnosis %CD34 expressing cells in the trephine	Numeric
DITRCKIT	at diagnosis % of c-kit expressing cells in the trephine	Numeric
DITRRET	at diagnosis presence of reticulin in the trephine	0: normal 1: grade I 2: grade II 3: grade III 4: grade IV
DIMORPHO	overall morphologic diagnosis	0: not evaluable 1: CHR 2: CP 3: AP/PR 4: BP 5: low counts and excess of blast
DIPHI	at diagnosis % of Ph+	Numeric
DIFISH	at diagnosis FISH	Numeric
DICYTEXT	at diagnosis cytogenetics	Text
DIPCR	at diagnosis PCR *BCR-ABL1* ratio %	Numeric
DIPCRABL	at diagnosis PCR *ABL1* control	Numeric
TT	at diagnosis transcript type	0: Not Available/Unknown 1: e14a2 (or b3a2) 2: e13a2 (or b2a2) 3: e8a2 4: e6a2 5: e1a2

Field name	Description	Codification
DSHB	at diagnosis Hb	Numeric
DIWBC	at diagnosis WBC	Numeric
DINEU	at diagnosis neutrophil % in PB	Numeric
DIBS	at diagnosis basophil % in PB	Numeric
DIEO	at diagnosis eosinophil % in PB	Numeric
DIPRO	at diagnosis promyelocytes % in PB	Numeric
DIBL	at diagnosis blasts % in PB	Numeric
DIPLA	at diagnosis Platelet count	Numeric
DISOKAL	Sokal risk group (reported)	0:Low 1: Intermediate 2: High
DIEURO	Hasford risk group (reported)	0: Low 1: Intermediate 2: High
INFDATE	Date of starting interferon	Date (day, month, year)
INFEND	Date of interferon discontinuation	Date (day, month, year)
INFDOSE	Maximal dose of interferon tolerated (MU/week)	Numeric
INFBR	Best response achieved on interferon	999: Haematological refractoriness 888: Intolerance 0 – 100: Best cytogenetic response obtained; 0 being CCyR; 100 lack of cytogenetic response but CHR
INFBRDATE	Date of best response on interferon	Date (day, month, year)
INFBRLOST	Date of loss of best response on interferon	Date (day, month, year)
AUTO	Autologous stem cell transplant	0: no 1: yes
AUTODATE	Date of auto-BMT	Date (day, month, year)
AUTOc	Conditioning regiment for the autograft	1: Bu 16 2: Cy-TBI 3: Cy-BU 4 : Bu 8 5: unknown 6: autologous rescue for failed allo
AUTOBR	Best response after autograft	999: Haematological refractoriness 888: Intolerance 0-100: Best cytogenetic response obtained: 0 being CCyR; 100 lack of cytogenetic response but CHR
AUTOBRLOST	Date of loss of best response	Date (day, month, year)

Field name	Description	Codification
ALLO	Allogeneic stem cell transplant	0: No 1: Yes
ALLOC	Type of allogeneic transplant	1: Sib 2: MUD 3: Mini-sib 4: Mini-MUD
ALLOBR	Best response after allograft	1: Molecular remission 2: Cytogenetic remission 3: Haematological remission
ALLOBRLOST	Date of loss of best response obtained after allograft	Date (day, month, year)
DLI	Donor lymphocyte infusion (DLI)	0: No 1: Yes
DLIDATE	Date of the first DLI	Date (day, month, year)
DLIN	Number of DLI	Numeric
DLIDOSE	Dose of last DLI	Numeric
DLIBR	Best response DLI	1: Molecular remission 2: Cytogenetic remission 3: Haematological remission 4: No response
DLIBRDT	Date of DLI best response	Date (day, month, year)
DLILOST	Date of DLI loss of best response	Date (day, month, year)
TP1, TP2, TP#	Other treatments received	0: None 1: Hydroxyurea (HU) 2: Interferon 3: Cytarabine (low dose) 4: Thioguanine 5: FLAG-IDA 6: auto-BMT 7: allo-BMT 8: DLI 9: Anagrelide 10: Ara-C 11: Busulphan 12: ICE or mini-ICE 13: High dose HU 14: Splenectomy 15: Mercaptopurine 16: AML chemo 17: UKALL XII 18: Leucopharesis 19: Imatinib
DTP1, DPT2	dates of TP#: no dates if HU	Date (day, month, year)
DETP1	Date stop TP1: only if imatinib	Date (day, month, year)
TN	Total number of treatments (including IFN)	Numeric

Field name	Description	Codification
TCA	Treatment given for control AP	0: none 1: HU 2: HU+INF 3: Thioguanine or chemotherapeutical agent in monotherapy 5: FLAG-IDA or polychemotherapy 6: auto-BMT 7: allo-BMT
TCB	Treatment given for control BP	0: none 1: HU 2: HU+INF 3: Thioguanine or chemotherapeutical agent in monotherapy 5: FLAG-IDA or polychemotherapy 6: auto-BMT 7: allo-BMT 8: UKALL 12
TR(comments)	comments (treatment)	Text

Table II (status at the onset of imatinib)

Field name	Description	Codification
N	Patient identifier	Numeric
TRIAL	Trial code	Numeric
DSDATE	Date of onset of imatinib therapy	Date (day, month, year)
DSECOG	ECOG on day of screening	ECOGH 0 ECOGH 1 ECOGH 2 ECOGH 3 ECOGH 4
DSSPL	Spleen size (cm below costal margin)	Numeric
DSSLIV	Liver size (cm below costal margin)	Numeric Not measured hepatomegaly.
DSABL	day of screening % of blast in BM	Numeric
DSAPR	day of screening % of promyelocytes in BM	Numeric
DSABS	day of screening % of basophiles in BM	Numeric
DSLIN	day of screening % of lymphocytes in BM	Numeric
DSTR	day of screening % cellularity in BM	Numeric
DSTRC	day of screening trephine code	1: CR 2: CP 3: excess of blast or immature cells 4: increase in megakaryocytes or megakaryocytes occurs in clusters
DSTRCD34	%CD34 expressing cells in the trephine on the day of screening	Numeric
DSTRCKIT	% of c-kit expressing cells on the trephine on the day of screening	Numeric
DSTRRET	presence of reticulin on the trephine on the day of screening	0: normal 1: grade I 2: grade II 3 grade III 4 grade IV
DSTLIN	Day of screening lymphocytes on the trephine	0: no lymphoid infiltrate 1: lymphoid infiltrate
DSMORPHO	overall morphologic diagnosis	0: not evaluable 1: CHR 2: CP 3: AP 4: BP 5: low counts with excess blasts

Field name	Description	Codification
DSPHI	day of screening % of Ph +ve cells	Numeric
DSCY	day of screening cytogenetic abnormalities	Numeric
DSFISH	day of screening FISH	Numeric
DSCYTEXT	day of screening cytogenetics	Text
DSPCR	Closest to day of screening *BCR-ABL1* ratio %	Numeric
DSPCRABL	Closest to day of screening PCR *ABL1* control	Numeric
DSPCRD	PCR date closest to day of screening	Date (day, month, year)
EVI-1	expression of EVI-1	0: negative 1: positive
DSHB	day of screening Hb	Numeric
DSWBC	day of screening WBC	Numeric
DSNEU	day of screening neutrophil count (%)	Numeric
DSBS	day of screening basophile count (%)	Numeric
DSEO	day of screening promyelocytes + myelocytes (%)	Numeric
DSBL	day of screening blast count (%)	Numeric
DSPLA	day of screening platelet count	Numeric
CRITERIA	criteria of inclusion	1: Hematologic resistance (including also previous 2: Hematologic refractoriness) 3: Cytogenetic resistance (including also previous 4: Cytogenetic refractoriness 5: INF intolerant 6: new patient who's never had INF and is <6 months post diagnosis 7: latter CP patient but never treated with INF 8: AP 9: BP 10: 2nd CP 11: INF first due to pregnancy 12: TKI post allo-BMT 13: other
DSSTIDOSE	STI dose, or dose of TKI	Numeric
DSANTH	Concomitant therapy	0: none 1: HU 2: anagrelide 4: ARA-C

Field name	Description	Codification
STIMOD		0: STI alone 10: STI + INF 11: STI+PEG IFN (dose in µg/kg/week) 20: STI + Zoledronic Acid 31: STI+HHT ref 40 32: STI+HHT ccr 41 33: HHT compassionate 51: HSP70+STI 60: auto 16 61: auto 8 62: allo-Sib; (621 mini) 63: allo-MUD; (631 mini) 64: DLI+STI 65: stem cell support 70: Flag-IDA 71: ADE 72: MAE 73: UKALL XII 800: Nilotinib first line therapy 810: Dasatinib first line therapy 9 followed by any other STImod number means that the patient can't be used for analysis (e.g.: pharmacological interactions)
DSCONDOSE	dose of the combination drug or frequency of the dose	Numeric
DSGCSFdose	G-CSF dose	Numeric

Table III (on treatment)

A new entry is done in the database when there is:

1. Change in treatment/dose of drug
2. Change in response
3. BM/trephine examination
4. PCR result

Field name	Description	Codification
N	Patient identifier	Numeric
DATE	Date	Date (day, month, year)
COMMENT	Comments	Text
SEC	Automatic sequence number	Numeric
NAME	Patient name	Text
HN	Hospital number	Numeric
STATUS	Status	0: dead; 1: alive; 2: lost
ECOG	ECOGH	ECOGH 0 - 4
SPL	spleen in cm	Numeric
LIV	liver in cm	Numeric
HARV	Harvest	0: no; 1: yes; 2: BM harvest
STIDOSE	Dose of imatinib (mg/day)	Numeric
STOP	reason to stop TKI	1: low neutro 2: low plt 3: pancytopenia 4: liver/hepatic toxicity 5: cramps 6: asthenia 7: skin rash 8: mistake 9: unknown 10: off trial/lack of efficacy 11: stop for harvest 12: oedema/fluid retention 13: diarrhoea 14: infection 15: other extra-haematological 16: for pregnancy (elective) 17: nausea 18: poor compliance 19: flu like symptoms 20: stop for transplant 21: pleural effusion 22: elevated cardiac enzymes 23: MDS/AML 24: optimal responses 25: gastrointestinal other 26: other reason

Field name	Description	Codification
ANTH	Concomitant therapy	0: none 1: HU 2: anagrelide 3: splenectomy 4: Ara-C 5: Mitoxantrone 6: 6TG 7: Bu 8: HHT 9: Mercaptopurine
BASELINE	reason for changing to another therapy	a: Primary haematological resistance to STI b: Secondary haematological resistance /loss of haematological response c: Primary cytogenetic resistance to STI d: Secondary cytogenetic resistance to STI e: Primary cytogenetic resistance to STI with cytopenia (plt<100 or Neutrophils <1 or need for GCSF under STI 400mg or less) f: suboptimal cytogenetic response g: suboptimal molecular response h: extra-hematologic toxicities u: haematological toxicity i: AP : CE j: AP : > 10% blasts k: AP : > 15% blasts l: AP: > 30% (blasts + PM) m: AP: > 20% basophils n: AP : <100plts o: BP Myeloid p: BP Lymphoid q: Major molecular response but plateau in PCR r: pharmacological interaction
LINE	Line of therapy	1: first line 2: second line 3: third line

Field name	Description	Codification
STIM	Alternative therapy	0: STI alone 1: 2nd attempt with STI 10: STI+INF 11: STI+PEG IFN (dose in µg/kg/week) 12: INF alone – no STI 13: hydroxyurea 14: MTX + 6MP 20: STI + Zoledronic Acid 31: STI+HHT ref 40 32: STI+HHT ccr 41 33: HHT compassionate 51: STI+HSP70 60: auto 16 (bulsufan) 61: auto 8 (bulsufan) 611: FLAG-IDA + cells 62: allo-Sib; (620 mini); (622 –Eduardo protocol – followed with STI) 63: allo-MUD; (631 mini); (632 –Eduardo protocol – followed with STI) 64: STI+DLI 65: Stem cell support 70: Flag-IDA 7081: Flag-IDA + Dasatinib 71: ADE 72: MAE 73: UKALL XII 74: MIDAC 80: Nilotinib second line 800: Nilotinib first line 81: Dasatinib second line 810: Dasatinib first line 82: Bosutinib 90: No treatment 91: not applicable 92: TKI discontinuation in CMR
CONDOSE	Dose of alternative drug	Numeric
CONDOSE2	Number of times/day alternative drug is given	Numeric
COURSE	Course number	Numeric
EPO	if taking erythropoietin	0: no; 1: yes
GCSF	If taking GCSF	0: no; 1: yes
ABL	% of blasts in BM	Numeric
APR	% of promyelocytes in BM	Numeric
ABS	% of basophiles in BM	Numeric
LIN	% lymphocytes	Numeric
TR	BM cellularity %	Numeric

Field name	Description	Codification
RET	presence of reticulin on the trephine	0: normal (if patient in remission) 1: grade I 2: grade II 3: grade III 4: grade IV
TR34	% CD34 expressing cells in BM	Numeric
TCKIT	% c-kit expressing cells in BM	Numeric
MORPH	overall morphologic diagnosis	0: not evaluable 1:CHR 11: CP under treatment 2:CP 3:AP 4: BP
BLPHEN	blast phenotype	1: myeloid 2: lymphoid 3: biphenotypic 4: unknown 5: megakaryocytic
PHI	% of Ph +ve cells	Numeric
CY	Cytogenetic abnormalities	Numeric
FISH	FISH	Numeric
CYCRTEXT	Cytogenetics	Text
PCR	*BCR-ABL1* ratio %	Numeric
PCRBL	*ABL1* control	Numeric
CYRES	Cytogenetic response	1: major cytogenetic response (PCR <12%) 2: complete cytogenetic response (PCR ≤ 2%) 3: major molecular response (PCR < 0.07%) 4: four log reduction (PCR < 0.007%) 5: complete molecular response (PCR = 0.0007%, actually appears as 0)
MUT	number of KD mutations	Numeric
AA	amino acid mutated	Alpha-numeric
%MUT	mutation %	Numeric
HB	HB	Numeric
WBC	WBC	Numeric
NEU	% neutrophils in PB	Numeric
BS	% basophils in PB	Numeric
EO	% eosinophils in PB	Numeric
PRO	% promyelocytes in PB	Numeric
MYE	% myelocytes in PB	Numeric
META	% metamyelocytes in PB	Numeric
BL	% blasts in PB	Numeric
PLA	Platelet count	Numeric

Field name	Description	Codification
RES	Response status	1: CHR 2: criteria 1 with low count (n<1.0, pla<100) 3: Chronic phase 4: no response (i.e. BP after 2 weeks of therapy in a BP patient) 5: AP 6: BP 7: low peripheral counts and excess of blast in BM 8: CP but loss of haematological control in other words, haematological resistance 9: N/A Single digit; Double digit (double digit means there is a loss of response)

Appendix II

Publications arising from work included in this thesis

1. Milojkovic D, Apperley JF, Gerrard G, ***Ibrahim AR***, Szydlo R, Bua M, et al. Responses to second line tyrosine kinase inhibitors are durable: an intention to treat analysis in chronic myeloid leukemia patients. Blood. 2011.

2. Milojkovic D, ***Ibrahim A***, Reid A, Foroni L, Apperley J, Marin D. Efficacy of combining dasatinib and intensive chemotherapy for patients with chronic myeloid leukemia in blastic transformation. Haematologica 2011 December 1, 2011.

3. Marin D, ***Ibrahim AR***, Lucas C, Gerrard G, Wang L, Szydlo RM, Clark RE, Apperley JF, Milojkovic D, Bua M, Pavlu J, Paliompeis C, Reid A, Rezvani K, Goldman JM, Foroni L. Assessment of *BCR-ABL1* Transcript Levels at 3 Months Is the Only Requirement for Predicting Outcome for Patients With Chronic Myeloid Leukemia Treated With Tyrosine Kinase Inhibitors. J Clin Oncol. 2011 Nov 7.

4. Marin D, ***Ibrahim AR***, Goldman JM. European Treatment and Outcome Study (EUTOS) Score for Chronic Myeloid Leukemia Still Requires More Confirmation. J Clin Oncol. 2011 Sep 6.

5. ***Ibrahim AR***, Clark RE, Holyoake TL, Byrne J, Shepherd P, Apperley JF, Milojkovic D, Szydlo RM, Goldman JM, Marin D. Second generation tyrosine kinase inhibitors improve the survival of patients with chronic myeloid leukemia who have failed imatinib therapy. Haematologica. 2011 August 22, 2011:haematol.2011.049759.

6. ***Ibrahim AR***, Eliasson L, Apperley JF, Milojkovic D, Bua M, Szydlo R, Mahon FX, Kozlowski K, Paliompeis C, Foroni L, Khorashad JS, Bazeos A, Molimard M, Reid A, Rezvani K, Gerrard G, Goldman J, Marin D. Poor adherence is the main reason for loss of CCyR and imatinib failure

for chronic myeloid leukemia patients on long-term therapy. Blood. 2011 Apr 7;117(14):3733-6.

7. ***Ibrahim AR***, Paliompeis C, Bua M, Milojkovic D, Szydlo R, Khorashad JS, Foroni L, Reid A, de Lavallade H, Rezvani K, Dazzi F, Apperley JF, Goldman JM, Marin D. Efficacy of tyrosine kinase inhibitors (TKIs) as third-line therapy in patients with chronic myeloid leukemia in chronic phase who have failed 2 prior lines of TKI therapy. Blood. 2010 Dec 16;116(25):5497-500.

8. Kuwabara A, Babb A, ***Ibrahim A***, Milojkovic D, Apperley J, Bua M, Reid A, Foroni L, Rezvani K, Goldman J, Marin D. Poor outcome after reintroduction of imatinib in patients with chronic myeloid leukemia who interrupt therapy on account of pregnancy without having achieved an optimal response. Blood. 2010 Aug 12;116(6):1014-6.