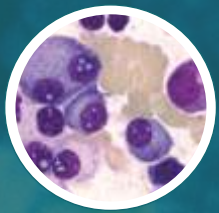
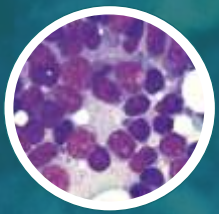
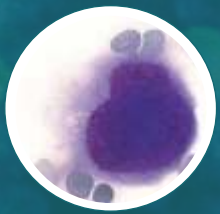
CHRONIC MYELOID LEUKEMIA

A Concise Clinical Guide







First Edition

Tariq I Mughal

Indian Cancer Network

CHRONIC MYELOID LEUKEMIA

A Concise Clinical Guide

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Tariq I Mughal

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For my parents

PREFACE

There can be little doubt that the study of chronic myeloid leukemia (CML), a clonal malignancy that results from an acquired genetic change in a single pluripotential hemopoietic stem cell has paved the efforts resulting in the successful incorporation of molecularly targeted therapies in cancer medicine. Moreover, it is truly remarkable to witness how rapidly the understanding of the cellular and molecular biology of this disease has been translated to improving the treatment of most, if not all patients diagnosed with CML. For CML patients, the introduction of the original tyrosine kinase inhibitor, imatinib mesylate, in 1998 was an important therapeutic milestone with most patients achieving a complete cytogenetic response and prolongation of survival compared with the previous therapies, other than stem cell transplantation. With the introduction of the second generation of tyrosine kinase inhibitors, dasatinib and nilotinib for front-line therapy of CML in chronic phase, and other candidate drugs, such as ponatinib and bosutinib, the pace at which the treatment algorithm for patients with CML is changing is unprecedented.

In this first edition of the concise guide to the management of CML, I aim to provide practical preclinical and clinical aspects of CML, for hematologists, oncologists and other health professionals interested in the disease. The opinions expressed are mine and I apologize for any errors or omissions.

Tariq Mughal, MD, FRCP, FACP
Boston, USA
September 26th, 2012

FOREWORD

The story of the unraveling of the molecular biology of chronic myeloid leukemia and the ensuing introduction of molecularly targeted therapy as treatment, must surely rank as one the real medical success stories of the latter part of the 20th century, despite the fact that the final chapter cannot yet be written. CML has been transformed from a uniformly fatal disease (except for the lucky patient who underwent a successful transplant) into one where the great majority of patients presenting today in chronic phase can expect a normal life span. On a wider front the demonstration of the remarkable efficacy of tyrosine kinase inhibitors in CML has re-directed research in other forms of leukemia and in solid tumors, and has resulted in important clinical benefits, if not yet in the same dramatic success as has been achieved in CML

Tariq Mughal has written a concise clinical guide summarizing succinctly the clinical features, criteria for diagnosis and approaches to management of CML for the second decade of the 21st century. The guide has at least three conspicuous merits. First, it is written by a single experienced individual and thus avoids the pitfalls of a multi-authored anthology with tedious repetition and often inconsistencies from chapter to chapter. Second, Professor Mughal is an accomplished clinician and has been able therefore to focus on clinical aspects of management relevant to the practising hematologist, and has avoided undue emphasis on molecular aspects that need not directly concern the clinician, though some of these are indeed essential background knowledge. Third, in an era when the production of a chapter in a textbook can take some years, while the internet sometimes carries a real plethora of indecipherable information, this guide is eminently up-to-date, clearly presented and can easily be read in a couple of hours, an important consideration for the busy hematologist.

The figures selected for inclusion in this guide are without exception clear and well explained. Another of guide's virtues is the inclusion of treatment algorithms, which can certainly help the hematologist confronted with a complicated CML case - when for example he/she needs to consider the indications for allografting in the current era.

In summary this guide will serve as a very valuable update for the established hematologist and a good introduction for any other person with an interest in malignant hematology.

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September 26th, 2012

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Iwould like to express heartfelt gratitude to my parents and my patients for having faith in me. I would like to thank John Goldman, Beppe Saglio, Rudiger Hehlmann, Rick Van Etten, and Jerry Radich for their invaluable advice & help with the book . My sincere thanks to Sabena and Alpa for their affection , Nababrata Ghosh for his professionalism and friendship & the ICN team for their help with compiling this e-book in a timely manner.

Chapter 1
Introduction and clinical aspects

Introduction

Chronic myeloid leukemia (CML) is a clonal BCR-ABL1 positive myeloid leukemia which arises from a pluripotent hematopoietic stem cell. In the majority of patients, this genetic change results in a balanced translocation between chromosomes 9 and 22 t(9;22)(q34;q11); the resulting 22q- is known as the Philadelphia (Ph) chromosome (Figure 1.1). This translocation results in a *BCR–ABL1* fusion gene, which is associated with an oncoprotein, P210. In the early 1990s, following the demonstration that introducing the *BCR–ABL1* gene into murine stem cells in experimental animals caused a disease simulating human CML, this fusion gene has generally been accepted as the principal pathogenetic event leading to the chronic phase (CP) of CML. Why this fusion gene occurs remains an enigma for the moment.

From a therapeutic perspective, the unprecedented clinical success of the original tyrosine kinase inhibitor (TKI), imatinib mesylate (now generally known as imatinib; Glivec or Gleveec, previously known as STI-571, Novartis, East Hanover, New Jersey, USA), means that for a significant majority of CML patients who achieve a complete cytogenetic response (CCyR) within 2 year of starting, the probability of achieving an overall survival is similar to that of the general population. Imatinib entered the clinics in 1998 and was approved by most global regulatory bodies by late 2001. It is of considerable interest to note how the drug revolutionized the conventional treatment of patients with CML in CP, with a significant shift from the use of allograft for the majority of patients under the age of 60 years, to the use of imatinib (Figure 1.2).

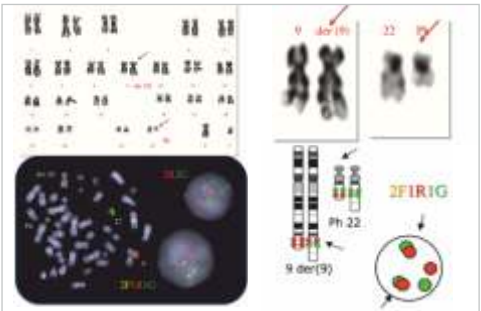


Figure 1.1: ***A photomicrograph and a florescent insitu hybridization (FISH) depiction of the***

Introduction and clinical aspects

Unfortunately, up to a third or more of patients in CP and almost all of those in blast crisis become resistant to the inhibitory effects of imatinib. Efforts have therefore focused on developing newer drugs, including the second and third generation TKIs. The first two of the second-generation TKIs, dasatinib (Spyrcel; previously known as BMS-354825; Bristol-Myers Squibb, New York, New York, USA) and nilotinib (Tasigna; previously known as AMN-107; Novartis, East Hanover, New Jersey, USA), entered randomized clinical studies assessing their potential first-line treatment role compared with standard dose imatinib (Figure 1.3). The studies demonstrated significantly higher rates of CCyR and of major molecular response (MMR) at the landmark 12 months of follow-up, resulting in the regulatory approval for first-line use in patients with CML in CP in the USA and many other countries thereafter.

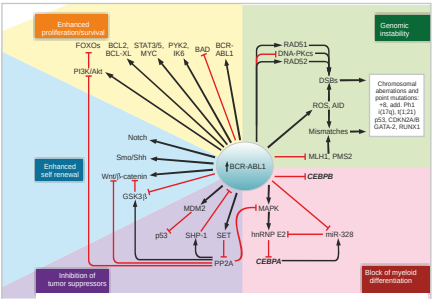


Figure 1.2: ***BCR-ABL1 dependent pathways to blast crisis (courtesy of Professor Tomasz Skorski)***

Further follow-up should help establish firmly the candidacy of the second-generation TKIs for front-line therapy of the newly diagnosed patient with CML in CP. Importantly, however, only a small minority of patients achieve long term complete molecular remissions (CMR) with any of the currently available TKIs. Moreover, in vitro studies suggest that none of the TKIs eradicates quiescent CML stem cells, which may account for relapse in most, but not all patients, once the drug is discontinued. It is therefore possible that none of the currently available TKI will ultimately translate to a cure, as defined by the absence of all malignant cells. It is, of course, likely that an 'operational' cure is achieved whereby most patients who achieve a CMP have very low levels of residual disease which might not shorten the overall survival.

In an attempt to achieve a conventional cure, many efforts are being directed

to develop other treatments, such as immunotherapy and innovative combinations of TKIs and other drugs. Other candidate drugs which remain in clinical trials include omacetaxine mepesuccinate (Omapro, previously known as homoharringtonine, Teva Pharmaceuticals, North Wales, Pennsylvania, USA) and rebastinib (previously known as DCC-2036; Deciphera Pharmaceuticals, Kansas City, USA).

The remarkable success obtained with TKIs when used as first-line treatment of patients with CML in chronic phase significantly changed the treatment algorithms that were in place a decade ago. The preferred treatment in the pre-imatinib era was an allogeneic stem cell transplant using an HLA-identical or a suitable matched unrelated donor, carried out as early as possible in the CP. Such a treatment was able to accord long-term success to the majority of patients who were eligible for the procedure; most ineligible patients received interferon alpha (IFN-α). There is now general agreement that most new patients, including children, should first receive treatment with a TKI.

Epidemiology

The incidence of CML worldwide, with the possible exception of India, appears to be fairly constant. It occurs in 1 to 2 per 100 000 of the adult population per annum. It represents, at least in the Western world, approximately 15% of all adult leukemias and less than 5% of all childhood leukemias. The precise incidence and, indeed, the prevalence in the developing world are currently unknown, but considered to represent a higher percentage of all leukemias in both adults and children. It is of considerable interest to note how the estimated prevalence, at least in the western countries, is anticipated to increase over the next few decades (Figure 1.4).

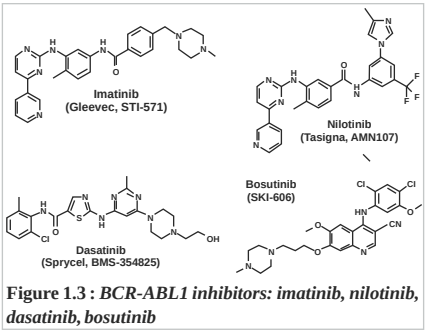


Figure 1.3 : ***BCR-ABL1 inhibitors: imatinib, nilotinib, dasatinib, bosutinib***

Introduction and clinical aspects

The median age of onset is around 55 years in the Western countries and probably around 35 years in the developing countries. The disease appears to afflict more males than females.

Risk factors

The only known risk of developing CML appears to be exposure to high doses of radiation, based on studies of survivors of the Nagasaki and Hiroshima atomic bombs in 1945. A very small number of families with a marginal higher incidence of the disease have also been reported, though no specific HLA genotypes have been identified.

Natural history

Historically, at least in the pre-TKI era, CML was a biphasic or triphasic disease that was usually diagnosed in the initial CP, which used to last typically 3 to 6 years. Following this, the disease evolved spontaneously into an advanced phase (AP), which could often be subdivided into an earlier accelerated phase and a later acute or blast phase (BP) (Table 1.1). This natural history of CML appears to have changed significantly in patients treated with TKIs, with the majority not progressing beyond the CP. There have been a few reports of patients who achieve a CCyR and subsequently relapse directly into AP.

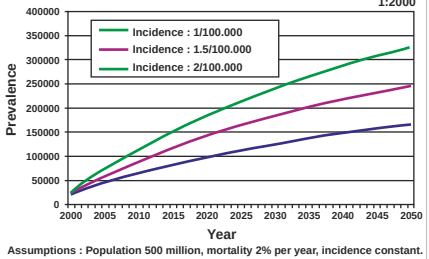


Figure 1.4: ***Estimated Prevalence of CML in Europe until 2050(courtesy of Professors R. Hehlmann, M. Lauseker, J. Hasford, M. Pfirmann and the German CML Study Group)***

Clinical features and diagnosis

About a third of the patients with CML in CP are asymptomatic at diagnosis. The remainder present with symptoms related to splenomegaly and leucocytosis (Figures 1.5 and 1.6). When symptoms are present, they may include lethargy, loss of energy, increased sweating, shortness of breath on exertion or weight

loss or hemorrhage from various sites. Spontaneous bruising or unexplained bleeding from gums, intestinal or urinary tract are relatively common. There may be pain or discomfort in the splenic area. Occasionally patients may present with an extramedullary event, such as a chloroma. Very rarely, male patients may present with features of priapism.

Examination of the bone marrow by aspiration or trephine biopsy is not necessary to confirm the diagnosis of CML, but is usually carried out to assess the degree of marrow fibrosis, to perform cytogenetic analysis on marrow cells and to exclude incipient transformation. The marrow aspirate is often hypercellular.

The diagnosis is confirmed by demonstrating the presence of a Ph chromosome by conventional cytogenetics analysis on a bone marrow aspirate sample or a *BCR-ABL1* fusion gene by fluorescence in situ hybridization (FISH) on a peripheral blood sample (Figure 1.1). It is also useful to confirm the presence of the *BCR-ABL1* fusion gene and obtain the baseline transcript numbers, which are essential in the subsequent (optimal) monitoring of patients.

Figure 1.5: ***A patient with CML presenting with a massive splenomegaly (and a wicked sense of humor!)***

Risk stratification

In order to distinguish patients with more aggressive disease, it is conventional to classify them into various risk categories, based on criteria definable at diagnosis. Such criteria include both prognostic (disease-related) and predictive (treatment-related) factors, that may help to predict survival for individual patients. The most widely used methods are those established by

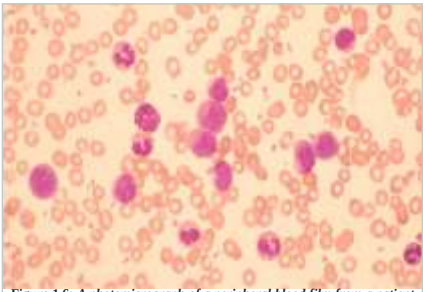


Figure 1.6: ***A photomicrograph of a peripheral blood film from a patient with CML in CP)***

Sokal's and Hasford's groups. Both were derived from clinical experience in the pre-imatinib era, but appear to work just as well today, at least in terms of predicting response to therapy. More recently the EUTOS score, a simplified version of the Hasford (also known as Euro) score, developed by the European Leukemia Net (ELN), based on the number of basophils and spleen size, has been introduced (Table 1.2). The initial attempts to validate this, at the Hammersmith Hospital, London and the MD Anderson Cancer Center have failed, but other attempts are on-going. Other efforts include developing risk scores based on functional and genetic studies but much further work remains prior to these being applied widely.

	Sokal 1984	Euro 1998	Eutos 2011
Parameters	Age	Age	
	Spleen	Spleen	Spleen
	Blasts	Blasts	
	Platelets	Platelets	
		Eosinophils	
		Basophils	Basophils
Treatment	Chemotherapy	IFN	Imatinib
Endpoint	Survival	Survival	CCyR

Table 1.2: ***Prediction of prognosis***

Chapter 2:
Cytogenetics, molecular anatomy and molecular biology

Cytogenetics

The Ph chromosome (9q-) is an acquired cytogenetic abnormality that characterizes all leukaemia cells in CML. It is formed as a result of a reciprocal translocation of chromosomal material between the long arms of chromosome 22 and chromosome 9, t(9;22)(q34;q11), shown schematically in Figure 2.1. This balanced translocation results in a BCR–ABL1 fusion gene on the Ph chromosome and also a 'reciprocal' fusion gene, designated ABL1-BCR, on the derivative 9q? chromosome. Such translocations involving just two chromosomes are described as 'simple', whereas about 10% of patients have either 'variant' or 'complex' translocations involving chromosomes 9, 22 and one or sometimes two other chromosomes.

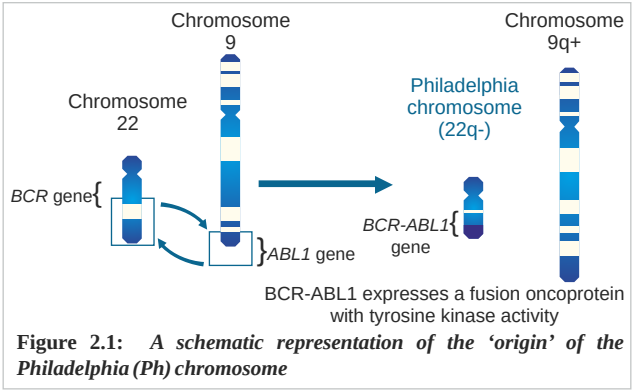


Figure 2.1: ***A schematic representation of the 'origin' of the Philadelphia (Ph) chromosome***

Molecular anatomy

It is likely that the acquisition of the BCR-ABL1 fusion gene by a hematopoietic stem cell and the ensuing expansion of the Ph-positive clone set the scene for acquisition and expansion of one or more Ph-positive subclones that are genetically more aggressive than the original Ph-positive population. The propensity of the Ph-positive clone to acquire such additional genetic changes is an example of 'genomic instability', but the molecular mechanisms underlying this instability are poorly defined. Such new events

may occur in the BCR-ABL1 fusion gene or indeed in other genes in the Ph-positive population of cells.

The Ph-positive cell is prone to acquire additional chromosomal changes, presumably as a result of acquired 'genetic instability', and this presumably underlies the progression to advanced phases of the disease. Recent work on the molecular pathogenesis of how CP

It is generally believed that the some CML stem cells, at a cytokinetic level, are in a quiescent or dormant (G0) phase. These quiescent CML cells appear to be able to exchange between a quiescent and a cycling status, allowing them to proliferate under certain circumstances. This perhaps provides some rationale for aficionados of autografting to pursue this clinical research approach for patients with CML, almost 37 years since investigators from Seattle reported their initial experience! There is also evidence that some Ph positive cells are quiescent and cannot be eradicated by cycle-dependent cytotoxic drugs, even at high doses, or indeed by any of the currently available TKIs (imatinib, dasatinib and nilotinib).

It was shown in the early 1980s that the ABL1 proto-oncogene, which encodes a non-receptor tyrosine kinase, was located normally on

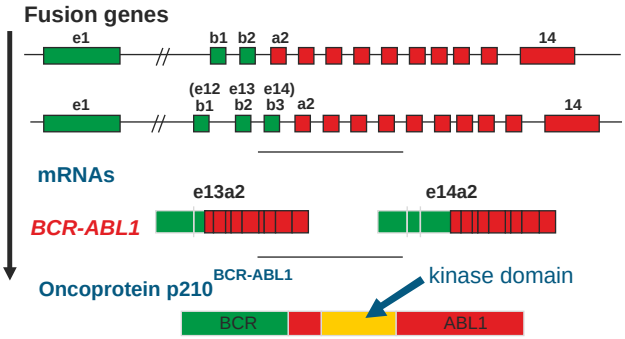


Figure 2.2: ***A schematic representation of the molecular anatomy of the Philadelphia (Ph) chromosome***

chromosome 9 but was translocated to chromosome 22 in CML patients. In 1984 the precise positions of the genomic breakpoint on chromosome 22 in different CML patients were found to be 'clustered' in a relatively small 5.8-kb region to which the name 'breakpoint cluster region' (BCR) was given. Later, it became clear that this region formed the central part of a relatively large gene now known as the BCR gene, whose normal function is not well defined, and the breakpoint region was renamed 'major breakpoint cluster region' (M-BCR). In contrast, the position of the genomic breakpoint in the *ABL1* gene (which is different from the *ABL*-related gene, *ARG* or *ABL2*) is very variable, but it always occurs upstream of the second (common) exon (a2). Thus, the Ph translocation results in juxtaposition of 5' sequences from the BCR gene with 3' ABL1 sequences derived from chromosome 9 (Figure 2.2). It produces a chimeric gene, designated *BCR–ABL*, or better *BCR-ABL1*, that is transcribed as an 8.5-kb mRNA and encodes a protein with a molecular weight of 210 kDa. This p210BCR–ABL1 oncoprotein has far greater tyrosine kinase activity than the normal ABL1 gene product.

In CML, there are two slightly different the *BCR–ABL1* transcripts, depending upon whether the break in M-BCR occurs in the intron between exons e13 and e14, or in the intron between exons e14 and e15. A break in the former intron yields an e13a2 mRNA junction and a break in the latter intron yields an e14a2 junction. (It should be noted that exon e13 was previously termed exon b2 and exon e14 was previously b3; thus the two RNA junctions were known originally as b2a2 and b3a2 respectively.) Most patients have transcripts with features of either e13a2 or e14a2, but occasional patients

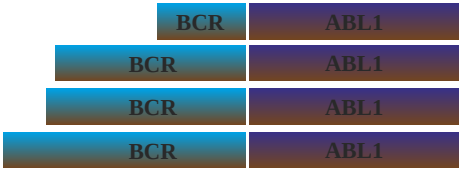


Figure 2.3 : ***The various Ph-positive associated oncoproteins in human leukemias: The different breakpoint positions within BCR lead to the formation of BCR-ABL1 transcripts and proteins with different BCR portions joined to the same ABL1 portion***

have both transcripts present in their leukaemia cells. The precise type of *BCR–ABL1* transcript probably has no prognostic significance for CML patients. Lucas and Clark have recently suggested that the larger transcript might actually be more ominous than the smaller one. Importantly, seminal work carried out by Daley, Van Etten and Baltimore, in Boston, and Heisterkamp and Groffen in Los Angeles in 1990, established that BCR–ABL1 gene played a pivotal role in the genesis of the CP of CML.

A minority of patients with Ph-positive acute lymphoblastic leukemia (ALL) also have *BCR–ABL1* fusion genes in their leukemia cells. In about one-third of Ph-positive ALL patients, the molecular features of the *BCR–ABL1* gene are indistinguishable from those of CML; in the remaining two-thirds the genomic breakpoint occurs in the first intron of the BCR gene (a zone designated 'minor breakpoint cluster region' or m-BCR) and the *BCR–ABL1* gene results from fusion of the first exon (designated e1) of the BCR gene with the second exon (a2) of the ABL1 gene. The mRNA is designated e1a2 and encodes a protein of 190 kDa (p190 BCR–ABL1) (sometimes reported in the literature as 'p185'). Very rare patients with CML have a p190 protein instead of the usual p210. Even rarer is the finding of a Ph chromosome in

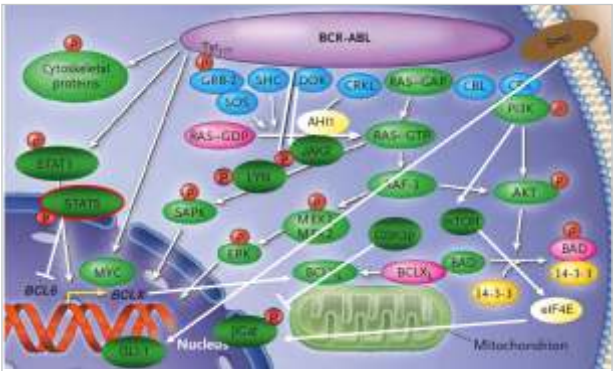


Figure 2.4: ***BCR-ABL1 activates a myriad of signaling pathways (Courtesy of*** **Professor John Goldman*)***

association with chronic neutrophilic leukemia. Such patients may have an mRNA formed from an e19a2 fusion gene associated with a p230 BCR–ABL1 oncoprotein (Figure 2.3).

Molecular biology

Molecular biology
The molecular basis of disease progression is still obscure, but it seems reasonable to infer that one or more probably a sequence of additional genetic events occurs in the Ph-positive clone. When the critical combination of additional events is achieved, clinically definable transformation ensues. At this stage, the leukemia cells usually harbor one or other of the additional cytogenetic changes referred to above. About 20% of patients with CML in myeloid transformation have point mutations or deletions in the coding sequence of the p53 tumor suppressor gene, a gene implicated in progression of a variety of solid tumors, notably colonic carcinoma. The retinoblastoma (RB) gene is deleted in rare cases of CML in megakaryoblastic transformation, and changes in the LYN, EVI-1 and MYC genes are described. About one-half of the patients with lymphoid blast transformations have homozygous deletions in the p16 gene, whose normal function is to inhibit cyclin-dependent kinase 4. Recent work by Mullighan and colleagues demonstrate that the majority of Ph positive B-ALL have loss-of-function mutations in genes regulating lymphoid development, including IKZF1, PAX5, and EBF; molecular changes underlying the non-random cytogenetic changes described above have not been identified.
Figure 2.4 depicts some of the pertinent molecular pathways which recognized to be involved in the CP of CML.

Conclusion

Though the observation that a small molecule such as imatinib could reverse the clinical and hematological features of CML constituted the final proof of the importance of the BCR-ABL1 oncoprotein to CML, there persisted some uncertainty about whether BCR-ABL1 was the initiating lesion or only a secondary event. Indirect evidence, collated by Fialkow and colleagues in 1981, had suggested that there may be a preceding predisposition to genomic instability in a Ph-negative population. There are also rare case reports of families where multiple individuals have different myeloproliferative neoplasms, including polycythemia vera, essential thrombocythemia and

CML. The *BCR–ABL1* gene has, of course, been cloned and inserted into a retroviral vector that has been used to transfect murine hematopoietic stem cells which can generate a disease resembling human CML in mice. Based on this it was generally accepted that the *BCR–ABL1* gene must play a principal role in the genesis of the CP of CML.

Chapter 3
Management of the newly diagnosed patient

General principles

Since the introduction of imatinib into the clinics in 1998, the drug has become the preferred treatment for the majority, if not all, newly diagnosed patients with CML in CP, including children. Imatinib reduces substantially the number of CML cells in a patient's body, resulting in a complete hematologic remission (CHR) in almost all such patients and a complete cytogenetic response (CCyR) in the vast majority. Table 3.1 re recently an improvement in survival has been confirmed. With imatinib, the estimated 7-year to 10-year survival is 80 to 85% increases to 90% to 93% if only CML-related deaths are considered (Figure 3.2). Current experience suggest that about 2% of all CP patients progress to advanced phase disease each year, which contrasts with estimated annual progression rates of more than 15 % for patients treated with hydroxycarbamide (previously known as hydroxyurea) and about 8–10 % for patients receiving interferon alpha (IFN-α), either with or without cytarabine.

Response by Type	Definitions
Hematologic	
Complete (CHR)	WBC < 10 x 10^9/L Basophils < 5% No myelocytes, promyelocytes, myeloblasts in the differential Platelet count < 450 x 10^9/L Spleen nonpalpable
Cytogenetic*	
Complete (CCgR)	No Ph+ metaphases
Partial (PCgR)	1% to 35% Ph+ metaphases
Minor (mCgR)	36% to 65% Ph+ metaphases
Minimal (minCgR)	66% to 95% Ph+ metaphases
None (noCgR)	> 95% Ph+ metaphases
Moleculart	
Complete (CMolR)	Undetectable BCR-ABL mRNA transcripts by real time quantitative and/or nested PCR in two consecutive blood samples of adequate quality (sensitivity > 10^4)
Major (MMolR)	Ratio of BCR-ABL to ABL (or other housekeeping genes) ≤ 0.1% on the international scale

Table 3.1 : ***Definitions of Response in CML***

Complete molecular responses (CMR) are, however, infrequent and then only after some years of treatment and probably in less than 50% of patients. It is therefore highly probable that imatinib will not eradicate residual CML in the vast majority of patients. A current central issue is therefore whether total eradication of all residual leukemia stem cells is actually necessary, since the survival of small numbers of residual leukaemia stem cells might be compatible with long-term survival in an individual patient. This would be tantamount to cure at an operational

level, as may well be the case after allogeneic stem cell transplantation (SCT). Allogeneic SCT was the preferred first-line therapy for patients with CML in chronic phase in the pre-TKI era, but it is now reserved for those who do not achieve an optimal response on TKI, develop progressive disease on TKI, children and in some parts of the world for economic

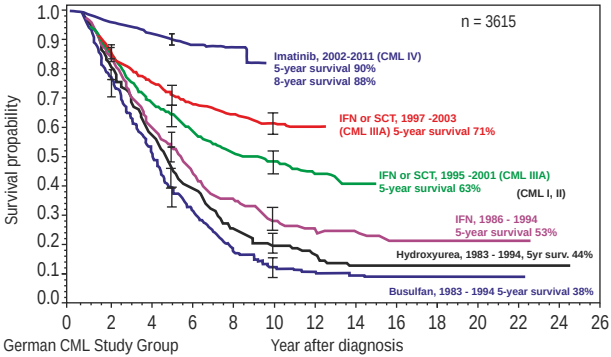


Figure 3.1: ***(Courtesy of Professor Rudiger Hehlmann and German CML Study Group)***

reasons.

Imatinib for the treatment of newly diagnosed patients with CML:

Most specialists today will commence a newly diagnosed adult patient on imatinib at 400mg orally, once daily. Imatinib was thought originally to act by occupying the ATP-binding pocket of the Abl kinase component of the BCR–ABL oncoprotein, and thereby blocking the capacity of the enzyme to phosphorylate downstream effector molecules; it is now thought to act also by binding to an adjacent domain in a manner that holds the Abl component of the BCR–ABL1 oncoprotein molecule in an inactive configuration (Figure 3.3). The drug rapidly reverses the clinical and haematological abnormalities

and induces major and complete cytogenetic responses in over 80% of previously untreated CP patients.

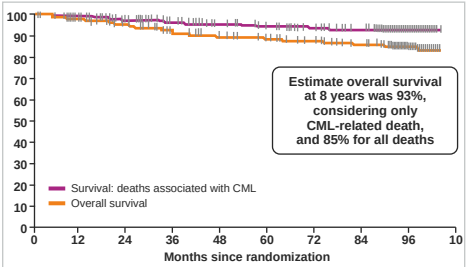


Figure 3.2: ***Leukemia-free survival in patients with CML based on the IRIS trial (an intention to treat analysis) (courtesy of Professor Michael Deininger, presented at ASH 2009)***

Side-effects include nausea, headache, rashes, infraorbital oedema, bone pains and, sometimes, more generalized fluid retention. The rashes can from time to time be treated by temporarily interrupting IM and then re-instituting it under short-term corticosteroid cover. Hepatotoxicity characterized by raised serum transaminases is occasionally seen and may necessitate stopping the drug. Some caution must be also exercised in the light of very rare reports of potentially fatal cerebral edema. Patients with black skin may sustain areas of depigmentation. An interesting non-sinister effect, repigmentation of grey hair, has been reported in a small group of responders. The toxicity in general seems to be appreciably less than that associated with interferon alfa (IFN-α), but long term vigilance is important.

The issue of how long to continue imatinib remains unresolved. For the patient who has achieved a CMR (Complete molecular remission; equivalent to a 4.5 log reduction in BCR-ABL1 transcript numbers), stopping the drug usually leads to recurrence of Ph positivity and eventually leucocytosis in about 60% of the cases, based on the work done by Mahon and colleagues, from Bordeaux, France (Figure 3.4). Many efforts are Figure 3.4 addressing the notion of identifying risk factors which could help predict relapse. In the meantime the best advice for the responding patient is to continue the drug indefinitely, unless of course, they choose to participate in a clinical trial assessing this issue.

A prospective randomized phase III trial (the 'IRIS' trial) designed to

compare imatinib as a single agent with the combination of IFN-α with cytarabine in previously untreated patients started in June 2000. Analysis after 8 years follow-up showed that 55% of the patients who remained on imatinib therapy, had achieved a CCyR. The cumulative best CCyR rate was 82% of all patients randomized to receive imatinib (Figure 3.5). The Figure 3.5event free survival was 83% and the overall survival 88%. A substantial proportion of the patients in CCyR also achieved a major molecular response (MMR; a 3-log reduction of the BCR-ABL1 transcript numbers) and this proportion seems to have continued to increase steadily with time of imatinib. The overall survival of patients treated with imatinib with that of historical control patients showed highly significant superiority for those who received imatinib Figure 3.6

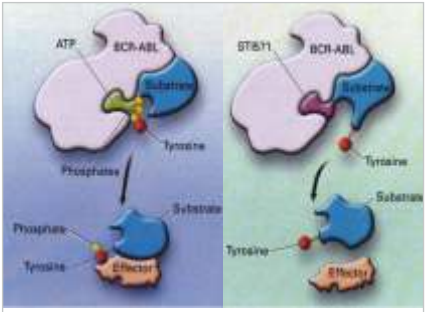


Figure 3.3: ***The presumed initial mechanism of action of imatinib (previously known as imatinib);(published with permission, from Goldman & Mughal, Postgraduate Haematology, Wiley, 2005)***

Resistance to imatinib may be primary or secondary and occurs in about 30% of patients in the chronic phase; the prevalence is substantially higher for patients in the advanced phases of the disease. Primary resistance is very rare and it is likely to reflect underlying heterogeneity of CML at diagnosis. Secondary resistance is associated with a variety of diverse mechanisms, including overexpression of the BCR-ABL1 oncoprotein and overexpression of P-glycoprotein, which expedites efflux of the drug from individual cells, and the 'acquisition' of point mutations in the ABL1 kinase domain. Thus far Ph-positive sub-clones with over 80 different point mutations have been identified in leukaemia cells obtained from patients with variable degrees of resistance to imatinib, and some of these, but by no means all, are clearly the cause of the resistance (Figure 3.7)
Each mutation encodes a different amino acid substitution in the Abl kinase component of the BCR-ABL1 oncoprotein. Cells with one such substitution, the replacement of a threonine by an isoleucine at position

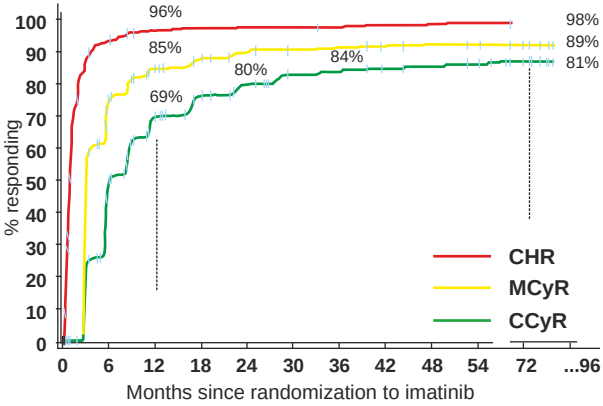


Figure 3.4: ***IRIS trial Kaplan-Meir estimates following 8 years of follow-up (courtesy of Professor Michael Deininger, presented at ASH 2009)***

315 (referred to as the T315I mutation; also known as the gatekeeper mutation), seem to be especially resistant to the inhibitory action of imatinib and all currently available TKIs. Cells with other substitutions are relatively less resistant. It is probable that some of these subclones pre-exist the administration of IM, or indeed any other TKI, but are allowed to expand when the unmutated oncoprotein molecule is inhibited by TKI; in other cases the mutation may develop de novo after initiation of TKI.

Second generation TKIs as potential first-line therapy for patients with CML

The remarkable success of imatinib in CP-CML led rapidly to development of the second generation TKIs, notably dasatinib and nilotinib, both of which are clearly more potent than the original TKI. Recent studies have confirmed the candidacy of both drugs as front-line therapy. Its truly remarkable that the pace of this clinical progress is such that we are already witnessing the entry of the third-generation TKIs as potential candidates for front-line therapy!

(a) Dasatinib

Still On First-Line Imatinib	304 (55%)
Discontinued Imatinib	249 (45%)
Adverse events/Abnormal Labs	30 (5.4%)
Suboptimal Response	77 (13.9%)
Death	16 (2.9%)
SCT	16 (2.9%)
Withdrawal Consent	44 (8%)
No Reconsent to Amendment	19 (3.4%)
Crossed Over to IFN+Ara-C*	14 (2.5%)
Other Reasons**	3 (6%)

*Due to intolerance (0.7%), lack of MCyR at 12 months or progression (1.8%)
**Includes administrative problems, protocol violation, lost to follow-up

Table 3.2: ***8 year follow-up results of the IRIS trial***

Dasatinib was first used to treat CML in 2003. It is an orally available potent dual kinase inhibitor, inhibiting the BCR-ABL1 and SRC kinases. Following the success in the treatment of patients with CML-CP resistant/refractory or intolerant to imatinib, the drug was approved for the treatment of all phases of CML with intolerance or resistance/refractoriness to imatinib and all patients with Ph-positive ALL. Dasatinib was noted to overcome most mechanisms of resistance to imatinib, with the exception of the T315I mutation.

The drug thereafter entered an international randomized phase 3 trial comparing it with imatinib for front-line therapy of newly diagnosed patients with CML-CP. A total of 519 such patients were recruited into the Dasatinib versus Imatinib Study in Treatment-Naïve CML Patients (DASISION) trial and the initial results which led to the regulatory approval by the US Food and Drug Administration (FDA) for the drug's first-line use for newly diagnosed patients with CML in CP, were published in June

Adverse events Grades 1/2	% patients	Adverse events Grades 3/4	% patients
Oedema	60	Neutropenia	17
Muscle cramps	49	Thrombocytopenia	9
Diarrhoea	45	Anemia	4
Nausea	50	Hepatotoxicity	5
Musculoskeletal pain	47	Other	17
Rash/skin	40		
Abdominal pain	37		
Fatigue	39		
Joint pain	31		
Headache	37		

Table 3.3 : ***Principal short term side-effects of imatinib***

2010 (Table 3.6).

After a median follow-up of 36 months, 183 (71%) dasatinib-treated patients and 179 (70%) imatinib-treated patients remained on study; a similar proportion of patients discontinued therapy: dasatinib 75 (30%) and imatinib 79 (31%). The rates of cumulative CCyR were superior in those patients receiving dasatinib therapy: at 12 (79% versus 68%), at 24 months (80% versus 74%) and at 36 months (83% versus 77%); the cumulative CCyR rate was higher for dasatinib versus imatinib across the

- Cardiac toxicities
- Secondary malignancies
- Myositis
- Renal failure
- Dermatitis
- Pancreatitis
- Hypophosphatemia
- Gynecomastia
- Hypogammaglobinemia opportunistic infections
- Endocrinopathies
- Weight gain

Table 3.4 : ***Principal long term side-effects of imatinib***

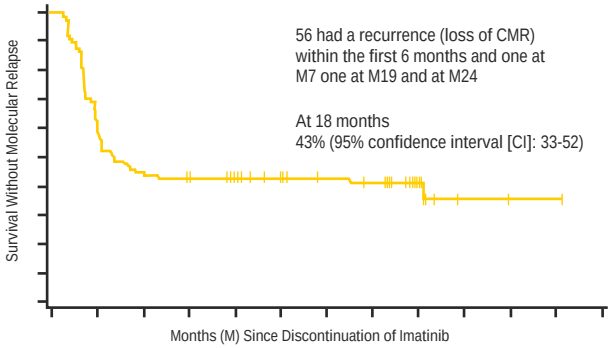


Figure 3.5: ***Preliminary Kaplan-Meier estimates of sustained CMR after discontinuation of imatinib from the French STIM (Stop Imatinib) study (courtesy of Professor Francoise Mahon; adapted from Lancet Oncol. 2010;11:1029-1035)***

period analyzed (p=0.0002). At 3 months, CCyR rates were 54% with dasatinib versus 31% with imatinib, increasing to 73% versus 59%, respectively, at 6 months.; the median time to CCyR was 3.2 months for the dasatinib-treated patients, compared to 6.0 months for the imatinib-treated cohort. MMR rates by 12 and 36 months were significantly higher with dasatinib compared with imatinib (46% and 67% versus 28% and 55%, respectively; p<0.0001). Among the subgroup of patients who achieved MMR, median time to MMR was 15 months for dasatinib and 36 months for imatinib. CMR (defined in this study as a 4.5 log reduction in the *BCR-ABL1* transcripts, compared to baseline) was achieved in 22% of dasatinib and 12% of the imatinib-treated patients (p = 0.002) at 36 months (Figure 3.9).

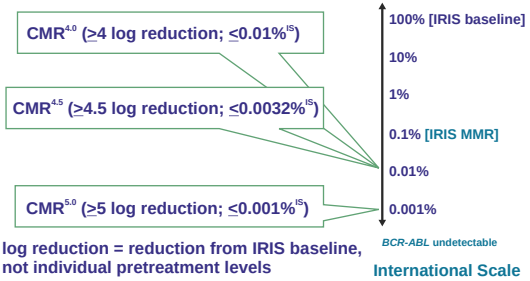


Table 3.5: ***Definitions of complete molecular response (CMR)***

Following a minimum follow-up of 24 months, transformation to the advanced phases of the disease was noted in 2.3% of the dasatinib and 5.0% of the imatinib-treated cohorts. The toxicity profile revealed 14% of patients treated with dasatinib, compared to none treated with imatinib, developed pleural effusion, but only 5 (1.9%) discontinued therapy for such toxicity. The rates of fluid retention, superficial edema, myalgia, vomiting, and rash were more common with imatinib, whilst the rates of diarrhea, fatigue, and headache were similar for both treatments. Drug-related pulmonary hypertension was noted in three (1.2%) dasatinib-treated patients, although in one patient, no evidence of pulmonary arterial hypertension was found on right heart catheterization; none of these three patients discontinued dasatinib.

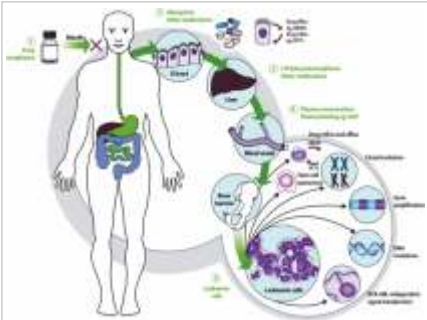


Figure 3.6: ***Mechanisms of imatinib resistance in patients with CML in chronic phase (adapted, with permission, from Apperley JF, Lancet Oncology, 2007;8(11):1018-1029)***

Seventeen dasatinib-treated patients (6.6%) and fourteen (5.4%) imatinib-treated patients were reported to have a drug-related cardiac event.

The principal biochemistry abnormality was hypophosphatemia, which was of grade 3/4 hypophosphatemia in 7% of dasatinib and 25% of imatinib-treated patients. Rates of grade 3-4 anemia (11% versus 8%) and neutropenia (24% versus 21%) were similar but more patients treated with dasatinib developed grade 3-4 thrombocytopenia compared with those treated with imatinib (19% versus 11%). Figure 3.10 depicts a forest plot of the toxicity as seen in the

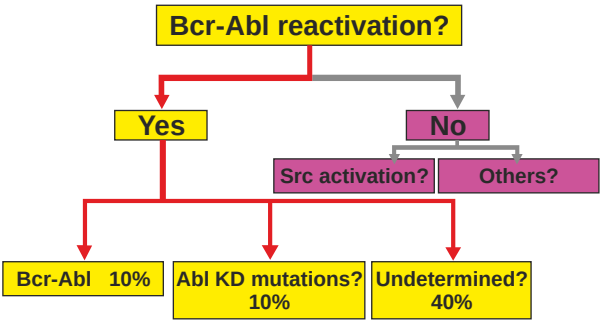


Figure 3.7 : ***Mechanisms of acquired or secondary resistance in patients with CML in chronic phase, being treated with TKI therapy***

Management of the newly diagnosed patient

DASISION trial by 24 months of follow-up. The adverse events at 36 months remained similar.

Overall, the results reported by the DASISION studies suggest that frontline therapy with dasatinib renders higher response rates with a comparable toxicity profile compared to imatinib by 24 months of minimum follow-up. It remains unknown whether these higher rates of early response will translate into improved EFS and/or OS rates.

(b) Nilotinib

Nilotinib is an oral ABL1 TKI structurally and biologically similar to imatinib, but in vitro approximately 30 times more potent. It entered the clinic in 2004 and following confirmation of its safety and efficacy profile in patients who were either resistant or intolerant to imatinib, the drug was evaluated in the front-line use in patients with CML in CP. The Evaluating Nilotinib Efficacy and Safety in Clinical Trials-Newly Diagnosed Patients (ENESTnd) trial is a phase 3, randomized, open-label, multicenter study comparing the efficacy and safety of nilotinib with imatinib. 846 patients with CML in CP were randomly assigned 1:1:1 to nilotinib 300 mg twice daily (n = 282), nilotinib 400 mg twice daily (n = 281), or imatinib 400 mg/day (n =

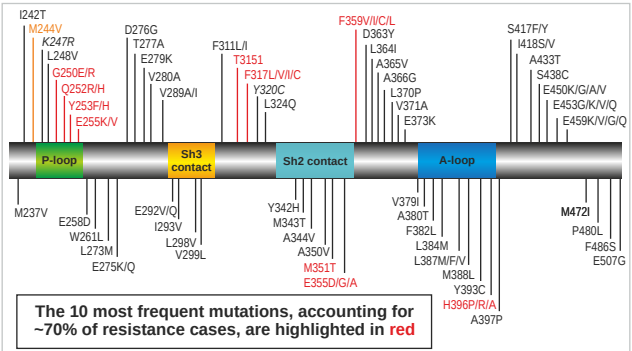


Figure 3.8: ***A schematic depiction of some of the currently established ABL1 kinase domain mutations (courtesy of Dr Simona Soverini)***

283).The primary endpoint was MMR at 12 months, and patients were stratified by Sokal risk score, which resulted in equal distributions of low, intermediate, and high Sokal risk scores in each arm of the trial.

On an intention-to-treat basis, the MMR rate at 12 months was significantly higher for nilotinib 300 mg twice daily (44%, P < .0001) and nilotinib 400 mg twice daily (43%, P < .0001) than for imatinib 400mg once daily (22%); for evaluable patients only, the MMR rates were again higher for nilotinib 300 mg twice daily (51%, P < .0001) and nilotinib 400 mg twice daily (50%, P < .0001) than for imatinib (27%). The cumulative rates of CCyR by 12 months were significantly higher for nilotinib 300 mg twice daily (80%, P < .0001) and 400 mg twice daily (78%, P < .0005) than for imatinib (65%); for evaluable patients, the CCyR at 12 months was higher for nilotinib 300 mg twice daily (93%) and nilotinib 400 mg twice daily (93%) than for imatinib (76%) (Table 3.1). Reponses were rapidly achieved with nilotinib, with 6-month MMR rates of 33%, 30%, and 12% and 9-month MMR rates of 43%, 38%, and 18% for nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively. These results led to the drug's approval for the treatment of newly diagnosed patients with CML in CP by the FDA.

		Response Rates (Intention to treat)								
		12 mos			24 mos			36 mos		
Trial	N	CCyR	MMR	CMR	CCyR	MMR	CMR	CCyR	MMR	CMR
DASISION*										
Dasatinib	258	76%	46%	NA	80%	64%	17%	83%	67%	22%
Imatinib	258	68%	28%	NA	74%	46%	8%	77%	55%	12%
ENESTnd**										
Nilotinib (300)	282	65%	55%	11%	87%	71%	26%	NA	73%	32%
Nilotinib (400)	281	55%	51%	7%	85%	67%	21%	NA	70%	28%
Imatinib	283	22%	27%	1%	77%	44%	10%	NA	53%	15%

Abbreviations: N=Number of patients; NA=Not applicable; CCyR = Complete Cytogenetic Remission; MMR=Major Molecular Response; CMR=Complete Molecular Response (4.5 log);
*DASISION trial: Kantarjian H, et al, Blood, 2012
**ENESTnd trial: Saglio G, et al, ASH, 2011; EHA-iCMLf-Sep 2011

Table 3.6: ***Current results of clinical trials of dasatinib and nilotinib as initial therapy in CML in CP***

The updated results following a minimum follow-up of 36 months were presented recently (Table 3.6, above). New progressions to AP/BP were not observed in the third year of treatment and the differences between the number of progressions observed in both nilotinib arms were significantly lower with respect to those observed in the imatinib arm and remain significant not only for patients still in the core study (p = 0.0059, nilotinib

300 mg BID versus imatinib; p = 0.0185 nilotinib 400 mg BID versus imatinib), but also including those patients who discontinued from the study, in an intention to treat analysis (p = 0.0496, nilotinib 300 mg BID versus imatinib; p = 0.0086 nilotinib 400 mg BID versus imatinib).

Although a statistically significant OS advantage has not been so far observed for nilotinib versus imatinib treated patients, deaths due to CML progressions are significantly lower in both nilotinib arms. The cumulative rates of MMR were significantly higher for nilotinib 300 mg twice daily (73%, p < .0001)

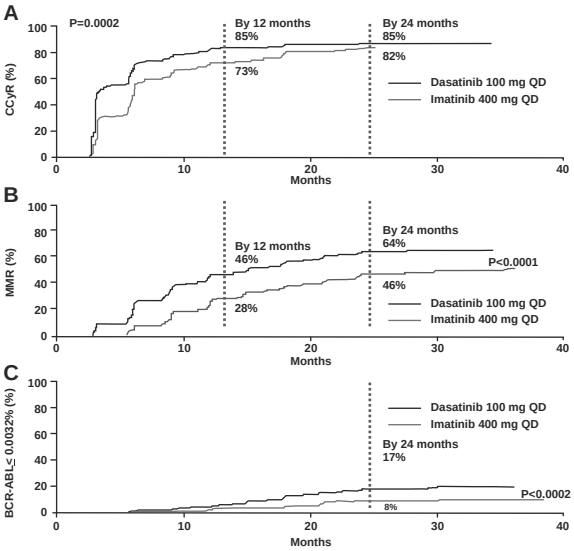


Figure 3.9: ***Cumulative incidences of response in dasatinib and imatinib arms (courtesy of Professor Hagop Kantrajian; adapted, with permission, from Kantarjian et al, Blood, 2012, 119: 1123-1129). (A) Complete cytogenetic response. (B) Major molecular response. (C) BCR-ABL transcript level reduction to 0.0032%. CCyR indicates complete cytogenetic response; and MMR, major molecular response.***

and nilotinib 400 mg twice daily (70%, p < .0001) than for imatinib (53%). Furthermore, a greater proportion of patients achieved a CMR, compared to imatinib.

In general therapy was well tolerated in all the study cohorts and treatment discontinuation due to adverse events was observed in 8%, 12%, and 10% of patients on nilotinib 300 mg twice daily, nilotinib 400 mg twice daily, and imatinib, respectively. Grade 3-4 thrombocytopenia was more common with nilotinib, compared to imatinib. Grade 3-4 biochemical abnormalities with nilotinib such as elevated levels of lipase, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and glucose were seen less often than previously reported. Other grade 3-4 toxicities were rare and included rash, headache. Figure 3.11 depicts a Forest plot comparing the differences in rates of drug-related non-hematological and grade 3/4 hematogical adverse events for patients treated with nilotinib or imatinib. Overall, nilotinib, at either dose, accords better efficacy than imatinib for the treatment of patients with newly diagnosed CML in chronic phase.

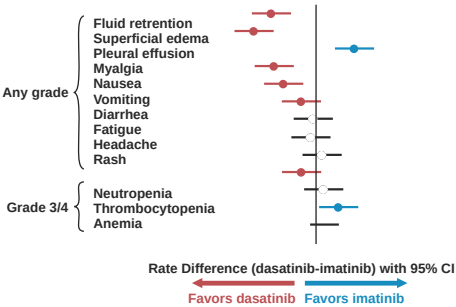


Figure 3.10 : ***Forest plot comparing differences in adverse events rates for dasatinib and imatinib (courtesy of Professor Hagop Kantarjian; adapted from the 24 months follow-up data on DASISION)***

Conclusions

The successful introduction of imatinib, followed by dasatinib and nilotinib, as targeted therapy for CML has made the approach to management of the newly diagnosed patient fairly complex. Imatinib unequivocally established

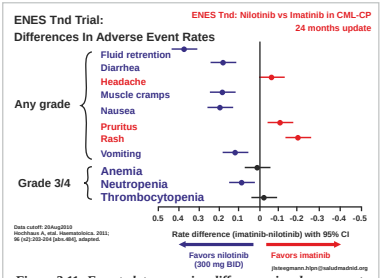


Figure 3.11: ***Forest plot comparing differences in adverse events rates for nilotinib and imatinib (Courtesy of Professor Giuseppe Saglio)***

the principle that molecularly targeted treatment can work and the second generation of TKIs, dasatinib and nilotinib, appear to be more effective in terms of achieving a faster CCyR and MMR, but the follow-up period is still relatively short. There is however, little doubt that both drugs appear to be more efficacious than imatinib in the first line use and the current safety analysis appears to suggest the notion that these drugs appear to be at least as safe for use as first-line therapy. Furthermore, both drugs appear to induce CMR, an emerging end-point for discontinuing TKI therapy safely, to a greater proportion of patients. The treatment algorithm for a newly diagnosed patient with CML-CP can therefore be anticipated to evolve substantially with a longer follow-up period for the second generation TKIs. I discuss the potential role of allogeneic SCT in the management of a newly diagnosed patient with CML in CP in chapter 4.

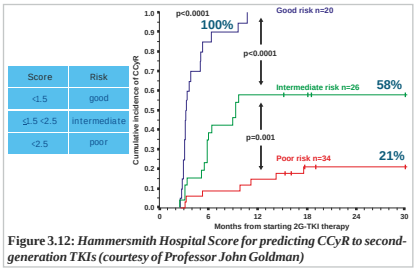


Figure 3.12: ***Hammersmith Hospital Score for predicting CCyR to second-generation TKIs (courtesy of Professor John Goldman)***

Chapter 4
Secondary treatment and stem cell transplantation

Current experience with the use of imatinib as primary therapy suggests that up to a third of all patients with CML in chronic phase, and significantly more in the advanced phase will require an alternative therapy within the first two years of treatment. The long term data following the use of a second generation TKIs, dasatinib or nilotinib for first line therapy is not known at the present time, but the failure, though not necessarily the tolerance is generally anticipated to be lower than that experienced with imatinib, but we cannot be sure at this time. Current results from randomized trials suggest better outcomes with both dasatinib and nilotinb, compared to standard dose imatinib, in particular the rate of MMR and the EFS. Thus far, no differences in OS have been observed with either dasatinib, nor nilotinib. Allogeneic SCT was the preferred first-line therapy for patients with CML in chronic phase in the pre-TKI era, but it is now reserved for those who do not achieve an optimal response on TKI, develop progressive disease on TKI, children and in some parts of the world for economic reasons.

	CML CP (2n[1] Line)			CML AP (2[nd] Line)	
	Dasatinib 100 mg qd	Nilotinib 40 mg bid	Bosutinib 500 mg qd	Dasatinib 140 mg qd	Nilotinib 400 mg bid
No.	167[1]	321[1]	169[3]	157[4]	
Time	24M	19M	7.3M	14M	9M
CHR	92%	94%	81%	47%	31%
MCyR	63%	59%	45%	39%	32%
CCyR	50%	44%	32%	32%	20%
MMR	37%		42%		
CMR			22%		
PFS	2Y80%	2Y64%		2Y51%	
OS	2Y90%	2Y90%		2Y63%	2Y63%

Adapted, with permission, from1) 100 mg qd arm BMS CA 180-034 study, Shah NP, et al. Abst #3225, ASH 2008. 2) Kantarjian H, et al. Abst #3238, ASH 2008. 3) Cortez J, et al. Abst #1098, ASH 2008. 4) Kantarjian H, et al. Abst #3224, ASH 2008. 5) Le Courtre P, et

Figure 4.1: ***Efficacy of second generation TKIs after imatinib failure***

Treatment algorithm for a patient with CML who is resistant or intolerant to imatinib

Intolerance to imatinib occurs in 8% to 10%, but resistance, both primary and secondary, is being increasingly recognized in a significant minority of patients in chronic phase. About 30% of patients with CML in chronic phase eventually become resistant to imatinib. Resistance is more common in patients who start imatinib in late CP and AP. It occurs in about 70% of patients treated in myeloid blast crisis and in almost all of the patients in lymphoid blast crisis.

The majority of patients who are resistant or intolerant to imatinib should receive either dasatinib or nilotinib, both of which are approved for this indication in many parts of the world. Current experience with dasatinib in patients with CML in chronic phase resistant and/or refractory to imatinib suggests that about 90% of patients have a complete hematological response and 52% of patients have a CCyR (Figure 4.1). About 25% of Figure

Drug	3 months response level	Outcomes	Abstract*
Imatinib	CCyR	EFS 83% vs 35%	3783
Imatinib	BCR-ABL1 transcripts ≥10% IS	cCCyR 91% vs 47% OS 93% vs 57%	1680
Imatinib +/- Interferon	BCR-ABL1 transcripts ≥10% IS	FFS 94% vs 86% EFS 86% vs 65%	1684
Nilotinib or Dasatinib	BCR-ABL1 transcripts ≥10% IS	OS 97% vs 87%	783
Dasatinib	BCR-ABL1 transcripts ≥10% IS	CCyR 93% vs 76% MMR 83% vs 54% CMR 20% vs 0%	785

Abbreviations: CCyR=Complete Cytogeentic Remission; cCCyR=continuous complete cytogenetic remission; MMR=Major molecular response; CMR=Completemolecular response; EFS=Event-free survival; OS=Overall survival; FFS=failure-free survival; vs=versus; TKI=Tyrosine kinase inhibitors; IS=International scale;
*Abstracts presented at the 54th American Society of Hematology Annual meeting (December 2011), #3783-Latagliata et al, #1680-Milojkovic et al, #1684-Nicolini et al, #783-Hanfstein et al, #785-Marin et al.

Table 4.1: ***Three-month responses and outcomes on TKI therapy***

4.1patients with the AP of CML and Ph-positive ALL also have reasonable responses. Responses are seen in patients with most of the currently known ABL kinase domain (KD) mutations, except the T315I mutation (also known as the 'gatekeeper' mutation). Hematological toxicity is common, particularly in those with the advanced phases of CML and Ph-positive ALL. These include neutropenia (49%), thrombocytopenia (48%), and anemia (20%). Non-hematological toxicity includes diarrhea, headaches, superficial edema,

pleural effusions, and occasional pericardial effusions. Grade 3/4 side-effects are rare and grade 3/4 pleural effusions occurred in 6% of patients. The prospective randomized dasatinib dose optimization study confirmed the notion that a lower dose of dasatinib (100 mg daily) was as effective as the previously approved higher dose (70 mg twice daily) in terms of the hematological, and major and complete cytogenetic responses, including the time to achieve these responses, but the toxicity profile confirmed a much lower incidence of pleural and pericardial effusions. Following this, the approved dose of dasatinib for patients with CML in chronic phase was adjusted to 100mg daily.

Current experience with nilotinib in patients with CML in chronic phase resistant or intolerant to imatinib suggests a CHR of about 70% and a CCyR of about 40% (Figure 4.2). Patients in the advanced phases of CML also respond, but to a lesser degree. The Figure 4.2 most common treatment-related toxicity is myelosuppression, followed by headaches, pruritus, and rashes. Overall, 22% of the patients experienced thrombocytopenia, with 19% having either grade 3 or 4 severity; 16% had neutropenia and a further 16% had anemia. Most of the non-hematological side-effects were of a grade 1/2 severity. All, including the hematological effects were fully reversible. About 19% of all patients experience arthralgias and about 14% experience fluid retention, particularly pleural and pericardial effusions. Importantly, patients with the imatinib-acquired T315I mutation appear to be refractory to nilotinib.

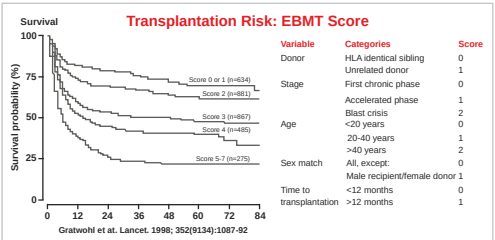


Figure 4.2: ***(adapted, with permission, from Gratwohl A, et al, Lancet, 1998)***

Until recently, it was less clear whether the responses accorded by these second generation TKIs in imatinib resistant or intolerant patients were durable. In December 2011, the Hammersmith group published a report confirming the durability of these responses, based on an intention to

treat analysis of 119 consecutive patients (including 3 who received bosutinib). The 4-year probabilities of OS and EFS were 81.9% and 35.3%, respectively. To assess the durability of cytogenetic responses further, irrespective of the need for a third line treatment, the group adopted the concept of 'current CCyR survival' (c-CCyRS), defined as the probability of being alive and in CCyR at a given time point. This essentially is the analog of 'current leukemia-free survival', which was developed to describe how patients may relapse but regain remission with an alternative therapy. The c-CCyRS at 4-years was 54.4%. Furthermore, they demonstrated that by assessing BCR-ABL1 transcript results at 3-months, one could potentially identify patients destined to fare poorly (those with >10% BCR-ABL1 transcripts on the IS relative to baseline) (see Table 4.1). It is of note that this landmark analysis is now part of the NCCN 2013 CML guidelines, despite the lack of an independent validation at present.able 4.1

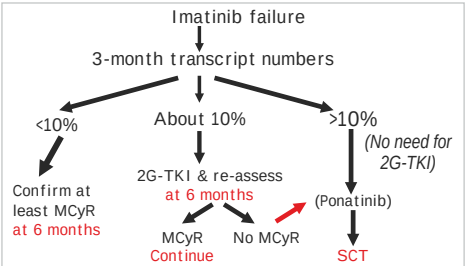


Figure 4.3: ***Potential treatment options for patients who are imatinib failure (Courtesy of Professor John Goldman); 2G-TKI = Second generation TKI.***

As discussed earlier, based on current EBMT experience, it is reasonable to consider an early allogeneic SCT for those patients who are resistant to imatinib and have high-risk disease, by Sokal and/or Hasford risk stratification, and a low-transplant risk, by EBMT criteria (also known as the Gratwohl score), and wish to be transplanted, rather than receiving a second generation TKI (see Table 4.2).

An alternative approach would be to prescribe a second TKI for a defined period and then proceed with an allogeneic SCT if the response is suboptimal. In practice, however, many patients will opt to receive a trial of dasatinib or nilotinib. Efforts to develop predictive and prognostic scores based on factors known prior to commencing either dasatinib or nilotinib, are being developed

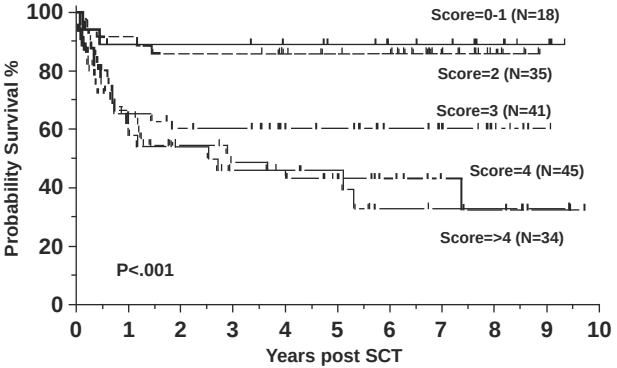


Figure 4.4: ***Survival of patients allografted for CML at the Hammersmith Hospital, London, from January 2000 to December 2010 stratified by EBMT risk score (courtesy of Professor John Goldman; adapted, with permission, from Pavlu° J et al, Blood. 2011;117:755-763)***

on both sides of the Atlantic, which might make the decision making process easier, in particular with regards to balancing the risks associated with an allograft against the risk for disease progression. Clearly if the notion of the 3-months BCR-ABL1 transcripts are confirmed in larger studies, one could use these results to identify patients who should be considered for an alternative therapy. Figure 4.3 depicts the potential treatment options for patients who are imatinib-failures.Figure 4.3

Treatment algorithm for a patient with CML who is resistant or intolerant to all currently available TKIs

For patients who are resistant/refractory to all of the currently available TKIs, and are under the age of 50 years, it is probably best to consider an allogeneic SCT, provided that a suitable donor is identified, the patient remains in chronic phase and, of course, wishes to be considered for an allogeneic SCT. It is of note that several candidate drugs, including ponatinib and omacetaxine mepesuccinate, are now in clinical trials for patients who are either resistant or intolerant of the second generation TKIs. I discuss these drugs further in

For patients who proceed to an allogeneic SCT after prior treatment with TKIs, there is some concern that there might be a higher relapse incidence than those who have not previously received TKI. This most likely represents a selection bias for relatively resistant disease. Preliminary data based on small patient series who had previously received imatinib, but not dasatinib or nilotinib, do not, however, suggest that prior treatment with a TKI increases the probability of transplant-related mortality (Figure 4.4).

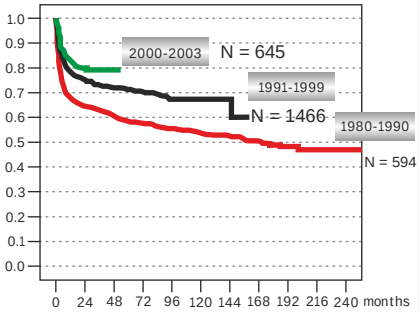


Figure 4.5: ***Improvements in survival rates by decades of transplantation for patients with CML in chronic phase (the EBMT registry data; courtesy of Professor Alois Gratwohl)***

Allogeneic stem cell transplantation

Younger patients with suitable stem cell donors who fail treatment with TKI may be offered the option of treatment by allogeneic SCT. The major factors influencing survival are patient age, disease phase at time of SCT, disease duration, degree of histocompatibility between donor and recipient, and gender of donor. In general, patients are 'conditioned' for a myeloablative (conventional) transplant with cyclophosphamide at high dosage followed by total body irradiation, or with the combination of busulphan and cyclophosphamide at high dosage. Reasonable marrow function is typically achieved in 3–4 weeks after the infusion of donor hematopoietic stem cells.

The possible major complications include graft-versus-host disease, reactivation of infection with cytomegalovirus or other viruses, idiopathic pneumonitis and sinusoidal obstruction syndrome (previously known as

veno-occlusive disease of the liver). For patients with CML treated by SCT with marrow from HLA-identical siblings, the overall leukemia-free survival at 5 years is now 60–80%; patients with the lowest Gratwohl score fare best (Gratwohl et al, 1998) (Figures 4.5 and 4.6). There is a roughly 20% chance of Figure 4.5 Figure 4.6 transplant-related mortality and a 15% chance of relapse. Patients surviving without hematological evidence of disease can be monitored by serial cytogenetic studies and by use of the much more sensitive RT-PCR, which can detect very low numbers of BCR–ABL1 transcripts in the blood or marrow. These studies suggest that in the majority of long-term survivors the CML may truly have been eradicated.

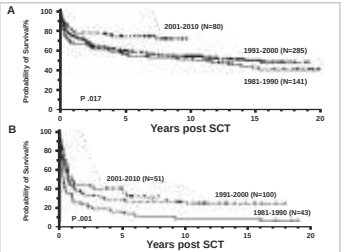


Figure 4.5: *Survival rates by decade of transplantation. Probability of overall survival for patients with CML in first chronic (A) and advanced (B) phase after allogeneic transplantation at the Hammersmith Hospital, London, stratified by decade of transplantation (courtesy of Professor John Goldman; adapted, with permission, from Pavlu' J et al, Blood. 2011;117:755-763)*

The recognition that the graft-versus-leukemia (GvL) effect plays a major role in eradicating CML after allografting led to the concept that the toxicity of the transplant procedure could be substantially reduced by decreasing the intensity of the pretransplant conditioning. The resulting strategy is thus to focus predominantly on the use of immunosuppressive rather than myeloablative agents, to maximize the numbers of hematopoietic stem cells transfused and to exploit the GvL effect mediated by donor alloreactive immunocompetent cells to eliminate the leukaemia cells. Such procedures non-myeloablative SCTs have been termed variously reduced-intensity conditioning (RIC) SCTs or mini-SCTs, and reflect advances in our understanding of how SCT actually works. It is still too early to say whether such RIC SCTs will prove superior to conventional transplants in the longer term for the younger patient, but the technique could make SCT more widely available to higher risk and perhaps also to older patients.

The qualified success of conventional SCTs using matched siblings led in the late 1980s to increasing use of 'matched' unrelated donors for SCT for patients with CML. At present, serologically matched unrelated donors can be

identified for about 50% of white patients and for lower percentages of patients of other ethnic origins. However, molecular methods for typing HLA class I and II have now largely superseded serological techniques, and complete matches for a given patient for five gene pairs, HLA-A, -B, -C, -DR and -DQ, are relatively rare. Thus, in the absence of a 'perfect match' the clinician has to decide what degree of mismatch may be acceptable for a given transplant. In general, the results of transplants using such unrelated donors are less good at present than results of using HLA-identical siblings, but some patients will probably prove to be cured.

About 10–30% of patients submitted to allogeneic SCT relapse within the first 3 years post transplant. The relapse in usually insidious and characterized first by rising levels of BCR–ABL1 transcripts, then by increasing number of Ph-positive marrow metaphases and, finally (if untreated), by hematological features of chronic-phase disease. This provides some rationale for the recommendation that patients should be monitored post-transplant by regular RT-PCR and cytogenetic studies. Rare patients in cytogenetic remission relapse directly to advanced-phase disease without any identified intervening period of chronic-phase disease.

Conclusions

It is of considerable interest to witness how rapidly the potential therapeutic algorithms for patients with CML who do not fare well on first line therapy, have evolved. The clinical availability of the second generation TKIs and more recently other novel drugs, such as ponatinib, the next line therapy have improved much, both in terms of efficacy and safety. The improvements in allogeneic SCT technology over the past decade have accorded this modality to even more prospective candidates and significant gains appear to have been made in the reduction of transplant-associated mortality and morbidity. Importantly, transplantation currently remains the only potential 'curative' treatment option for all

patients with CML, but particularly so for those in the advanced phase, or harbour a T315I mutation. Table 6.1 depicts the potential indications for an allogeneic SCT today.

Finally, the lessons from transplantation have been instructive in a renewed interest in immunotherapy and the use of TKIs in conjunction with various immunotherapeutic strategies is now being studied. Parenthetically it should be noted that globally, so far, our efforts to optimize the clinical management of the newly diagnosed patient have failed. Current estimates suggest that although about 80% of all patients receive imatinib therapy at some time, most patients are not monitored satisfactorily and therefore have suboptimal outcomes. For some of these patients it remains reasonable to offer an allogeneic SCT sooner rather than later.

- **First Chronic Phase**:
  Failure of second generation TKI
  Imatinib failure and T315I mutation
- **Accelerated phase**:
  Treat like blast crisis if near blast crisis or if enters accelerated phase whilst on TKI, otherwise as chronic phase
- **Blast crisis**:
  Urgently once chronic phase is re-established with TKI or chemotherapy; consider second generation TKI post allograft (maintenance)

Table 4.2: ***Potential indications for an allogeneic SCT in CML in 2012 - 2013***

Chapter 5
Emerging and investigational approaches

Just over a decade's use of TKI first-line therapy for patients with CML in chronic phase has confirmed the notion that imatinib accords long term resmission to about 60-65% of patients. About half of the patients who do not fare well on imatinib are able to achieve durable remissions with currently available second-generation TKIs, dasatinib and nilotinib. For the remaining patients a clinical trail is often the best option. In this chapter I address some of the emerging and investigational approaches available.

(1) Ponatinib

Ponatinib (previously known as AP24534; Ariad Pharmaceuticals) is a rationally designed inhibitor of BCR-ABL1 that binds both active and inactive conformations of the enzyme and is active against a broad array of BCR-ABL1 mutants (including T315I) as well as other kinases such as VEGF, FGF, c-KIT, and SRC. Much of what we have learne about this drug comes from the results of a phase II trial, PACE (Ponatinib Ph+ ALL and CML Evaluation), in which 449 patients who were either resistant or intolerant to dasatinib or nilotinib, or had a T315I mutation were enrolled, demonstrated that. 47% of all patients in CP were able to achieve a major cytogenetic responses (MCyR). 39% of these patients achieved a CCyR

Response	n Response / N Evaluable (%)		
	Overall	R/I Cohort	T315I Cohort
CHR	248/271 (92)	193/207 (93)	55/64 (86)
MCyR*	116/248 (47)	79/191 (41)	37/57 (65)
CCyR	96/248 (39)	63/191 (33)	33/57 (58)
MMR	51/265 (19)	31/205 (15)	20/60 (33)

*MCyR is the primary endpoint

Figure 5.1: ***PACE initial results in patients with CML in CP (Courtesy of Professor Jorge Cortes, Dec 2011)***

(Figure 5.1). The toxicity data from this PACE trial Figure 5.1confirmed grade 3 (or more) pancreatitis was noted in 6%. Clearly longer follow-up is required to establish the precise place of ponatinib in the management of patients with CML who are intolerant or resistant to dasatinib or nilotinib. The data thus far is indeed impressive and confirms the substantial activity of ponatinib in heavily pretreated patients in the various phases of CML and also Ph positive ALL. Furthermore, it is of note that response rates continue to improve with longer follow-up.

The drug's place in the management of those with a T315I mutation is accepted and regulatory approval in the US in late 2012 anticipated. It is likely that the drug's label may include its potential use as salvage therapy for second-generation TKI failures. An international phase III trial comparing ponatinib to standard dose imatinib in newly diagnosed patients with CML in CP has commenced recently.

(2) Bosutinib

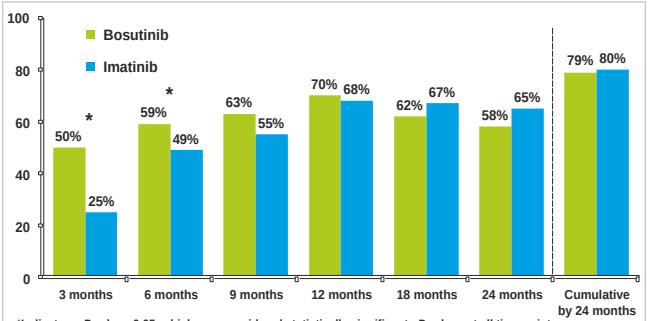
Bosutinib (previously known as SKI606; Pfizer) is chemically different from both dasatinib and nilotinib but not yet licensed. It is an orally administered second generation TKI that targets a relatively wide spectrum of tyrosine kinases, including ABL1 and SRC. It appears to be about 200 times more potent than imatinib, and unlike IM and dasatinib, does not inhibit other targets such as KIT or platelet-derived growth factor receptor, making it less likely to be associated with serious hematological toxicity. Following initial studies in 2006, assessing bosutinib's role in the treatment of patients with CML in CP intolerant or resistant/refractory to IM, the drug entered an international randomized, phase III, open-label study of bosutinib versus standard dose IM in newly diagnosed patients with CML in chronic phase, called BELA (Bosutinib efficacy and safety in chronic myeloid leukemia study).

The primary endpoint of the trial was confirmed CCyR at 12 months, and the drug failed (70% vs 68% for IM). Following a median follow-up of 24 months, the cumulative rates of CCyR and MMR were 87% with bosutinib versus 81% with IM, and 67% with bosutinib versus 52% with IM, respectively ($p = .002$) (Figure 5.2). Treatment Figure 5.2 discontinuation was reported in 37% of patients treated with bosutinib and 29% of those

receiving IM. The primary reason for discontinuation of bosutinib was adverse events. Bosutinib was associated with more diarrhea, nausea, vomiting, and rash compared with IM; the most frequent grade 3 and 4 adverse events were diarrhea (12%) and rash (2%). More muscle cramps, bone pain, and periorbital edema were associated with imatinib therapy.

(3) Omacetaxine mepesuccinate

Omacetaxine mepesuccinate is a first in class cetaxine which has been in clinical trials for almost two decades, in patients with a variety of haematological malignancies, including CML in various phases. The drug is a



*Indicates a *P* value <0.05, which was considered statistically significant. *P* values at all time points with the exception of at Month 12 were considered exploratory.

Figure 5.2: ***BELA CCyR Rates at Specified Times on Therapy: ITT Population***

natural plant alkaloid from the Chinese plum yew tree, cephalotoxus fortuneii, which inhibits synthesis of the anti-apoptotic Bcl-2 proteins and is a potent myelosuppressive agent. It appears to be a reversible, transient inhibitor of protein elongation that facilitates tumor cell death without depending on BCR-ABL1 signaling. Studies in the 1990s confirmed a modest activity in patients with CML but there were concerns with regards to the route of administration and schedule of delivery largely due to the occurrence of cardiovascular side-effects, such as hypotension and arrhythmias. More recently it has been tested, in a subcutaneously administered formulation, in CML patients resistant to all current TKIs and

those who harbor the T315I mutation. Recent phase II studies have confirmed the drug's clinical activity in 'conventional-treatment' resistant patients with different phases of CML. The results from one of these studies in which 122 such patients resistant/intolerant to ≥ 2 approved TKIs, were presented in June 2012. Sixty-two of these patients had received 2 TKIs (100% imatinib; 76% dasatinib; 24% nilotinib) and 60 had received all 3 of these TKIs. In the 45 patients who had received at least two prior TKIs but remained in chronic phase, there were 12 (27%) major cytogenetic responses (median duration of 17.7 months); in the 36 chronic phase patients subjected to at least 3 TKIs, there were 4 (11%) had MCyR (median duration not reached). Of the 17 patients in the advanced phases, there were 35% major hematological responses in the two prior TKIs cohort and in the 24 such patients who had received 3 TKIs, 21% had major hematological responses. Median survival in the 2 and 3 TKI groups were 30.1 months and not reached for the chronic phase cohort and 12.0 months and 24.6 months for the advanced phase cohort. Treatment-related grade 3/4 adverse events were noted in 52 (84%) patients in the 2 TKI group and 42 (70%) in the 3 TKI group (most common: thrombocytopenia [71%, 48%]) (Figure 5.3). Figure 5.3 Based on these encouraging results in heavily pretreated patients with CML, further studies are on-going. Should longer follow-up confirm the durability of the responses noted so far, the drug should be a candidate as a salvage agent.

(4) Rebastinib

Rebastinib (formerly called DCC-2036, Deciphera Pharmaceuticals), is a novel and potent TKI which binds to a novel region called the switch pocket, thereby preventing BCR-ABL1 from adopting a conformationally active state. Efficacy against multiple imatinib-resistant BCR-ABL1 mutants has been demonstrated both in vitro and in vivo. Importantly, DCC-2036 retains full potency against the T315I mutant in preclinical efficacy studies. The drug is currently in a phase I study designed to find the maximal tolerated dose (MTD) when administered daily as a single-agent on a 28-day cycle. Two reversible dose-limiting toxicities (Grade 3 peripheral neuropathy and Grade 4 lower extremity weakness) occurred during the initial treatment cycle at the 200 mg tablets twice daily dose level. Evaluation of 6 patients at the 150 mg tablets twice daily dose level determined that dose to be the

maximum tolerated dose (MTD).The preliminary results presented in December 2011, from 30 patients with CML in various phases, including 11 patients with the T315I mutation: 19 in CP, 8 in accelerated phase and 3 in blast phase demonstrate responses in CP patients: one MMR in a patient with T315I mutation, one CCyR, and one partial cytogenetic response. Hematologic responses were also seen in two patients in AP. These preliminary results suggest that rebastinib is well tolerated and has anti-leukemia activity in subjects with refractory CML and T315I positive disease. Pharmacokinetics results are consistent with inhibition of BCR-ABL1 signaling in this first-in-man study of a switch pocket TKI.(5)

(5) Immunotherapy

Following the realization that a CMR and 'cure' might not be possible with TKI therapy alone, efforts were directed to exploring the potential of developing an active specific immunotherapy strategy for patients with CML by inducing an immune response to a tumor specific antigen. Furthermore, the demonstration of a powerful graft-versus-leukemia (GvL) effect in CML has renewed interest in the possibility that some form of immunotherapeutic manipulation could be effective in CML. Some evidence suggests that patients vaccinated with oligopeptides corresponding to the junctional region of the BCR–ABL1 protein generate immune responses that may be of clinical benefit.

The principle of immunotherapy in CML involves generating an immune response to the unique amino-acid sequence of p210 at the fusion point. Clinical responses to the BCR-ABL1 peptide vaccination, including CCyRs, have been reported in a small series. In contrast to previous earlier unsuccessful attempts, the current series included administration of GM-CSF as an immune adjuvant and patients were only enrolled if they had measurable residual disease and HLA alleles to which the selected fusion peptides were predicted to bind avidly. If these results can be confirmed, vaccine development against BCR-ABL1 and other CML-specific antigens could become an attractive treatment for patients who have achieved a minimal residual disease status with imatinib. Other targets for vaccine therapy now being studied include peptides derived from the Wilms tumour-1 (WT1) protein, proteinase-3 (PR1), PRAME, and elastase, all of which are

overexpressed in CML cells. Another vaccine strategy that may prove useful for patients who do not achieve a CCyR to IM is use of the K562 CML cell line engineered to produce GM-CSF.

Conclusion

Ponatinib efficacy and safety data from the current phase II study supports the drug's candidacy as a potential salvage agent for second-generation TKI failures and the drug is now being compared to imatinib in a phase III trial for newly diagnosed patients. Ponatinib has a firm role in the treatment of T315I Ph positive leukemias. The updated results of bosutinib, a second generation TKI, lend some support towards the drug's candidacy for first-line use, but it is unclear how the drug would fare against dasatinib or nilotinib. Immunotherapy is also garnering support, in particular with the BCR-ABL1 and other CML-specific antigens' targeted vaccines for patients following TKI-induced minimal residual disease status.

Chapter 6
Definitions of response and monitoring response

Introduction

A decade following the introduction of imatinib into the clinics for the treatment of patients with CML in chronic phase, it is abundantly clear that the overall safety and efficacy of the drug are impressive, but not optimal. It induces complete cytogenetic response (CCyR) rates of 65% to 85%, major molecular response (MMR, defined as at least a 3-log reduction in the BCR-ABL1 transcript levels compared to the baseline) rates of 40% to 70%, and a

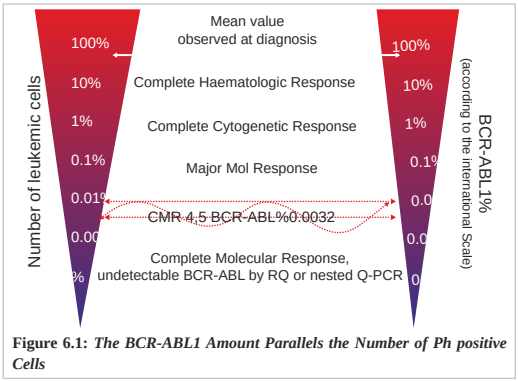


Figure 6.1: ***The BCR-ABL1 Amount Parallels the Number of Ph positive Cells***

complete molecular response (CMR; defined as the absence of any detectable BCR-ABL1 transcripts) rates of 10% to 40%. These results appear to have improved further with the second generation TKIs, dasatinib and nilotinib.

Monitoring strategies

The principal objective of monitoring patients with CML is to accurately determine response to treatment and be able to detect relapse at an early stage, particularly if a change of treatment might be indicated. Remarkably similar monitoring approaches have been proposed by the European LeukemiaNet (ELN) and many CML-interested consortia

Definitions of response and monitoring response

Despite these efforts, there appears to be a monitoring paradigm shift, initially in the USA and now global, of using molecular monitoring in preference to cytogenetics (see below). Molecular monitoring is indeed an important aspect of the management of patients with CML, but its principal role, outside of clinical trials, appears to be in the patient who has achieved a firm CCyR. Table 6.1 depicts the revised ELN criteria for responses in patients with CML in chronic phase initially treated with TKIs. Table 6.1

The frequency of performing a specific test has been based largely on the results from the IRIS study and other global single institutions and consortia trials. For example, in patients with CCyR, molecular monitoring with FISH and RQ-PCR is recommended every 6 months, rather than every 3 months, based on the IRIS study demonstrating the low risk of transformation to the advanced phases beyond the second year. Most experts appear to prefer peripheral blood analyses for monitoring, rather than bone marrow studies, except at diagnosis. The ELN guidelines require bone marrow conventional cytogenetics at diagnosis, at 3 months and at 6 months, and then every 6 months until CCyR has been confirmed (Table 6.2). Once a stable CCyR has been achieved, it is Table 6.2reasonable to monitor responses every 6 months,

Milestone	Response Definition and Criteria		
	Optimal	***Suboptimal***	**Failure**
3 months	CHR + minor CyR	*No CyR*	<CHR
6 months	PCyR	*<PCyR*	No CyR
12 months	CCyR	*PCyR*	*<PCyR*
18 months	MMR	*<MMR*	<PCyR
Any time	Stable or improving MMR	*Loss of MMR imatinib sensitive mutations*	Loss of CyR or CHR, imatinib insensitive mutations

Table 6.1: ***Revised European LeukemiaNet (ELN) criteria for responses in patients with chronic myeloid leukemia in chronic phase initially treated with TKIs (courtesy of Professor Michele Baccarani, June 2009)***

since abrupt transformation to advanced phases are quite rare. Finally it is important to monitor compliance throughout the treatment period. Several studies have demonstrated the critical importance of adherence in terms of achieving optimal outcomes.

Baseline investigation

All CML patients should be assessed thoroughly as any patient, with a detailed history and clinical examination. All patients should then have a complete blood count, blood chemistry (renal, hepatic profile) bone marrow aspirate/biopsy for morphology and conventional cytogenetic analysis, and RQ-PCR on peripheral blood sample. The conventional cytogenetic will confirm the diagnosis, provide information for Sokal index, and also detect clonal evolution (if any). A FISH can detect Ph-negative but BCR-ABL1 positive disease. Patients who are commenced on TKI therapy, should be followed regularly for hematological, cytogenetic and molecular response (Table 6.3).

Hematological response

Complete hematological response (CHR) is defined as a white blood count (WBC) <10 x 109/l with the differential count showing no immature granulocyte, basophils <5%, platelet count <450 x 109/l, and no palpable

Hematologic: At diagnosis, then every 2 weeks until complete hematologic response (CHR), then every 3 months for 2 years, then 3-6 monthly
Cytogenetic (Bone marrow): At diagnosis, at 3 months, and at 6 months; thereafter every 6 monthly until CCyR confirmed. Once CCyR confirmed, monitor with FISH or Q-PCR. Repaeat bone marrow if clinically indicated
Molecular by RT-qPCR (Peripheral blood): RT-qPCR every 3 months until MMR confirmed, then every 6 months
FISH (Peripheral blood): If unable to perform conventional cytogeentics on bone marrow; or once CCyR confirmed, can be used to supplement Q-PCR results
Mutational analysis (Peripheral blood): Only if failure (required before decision to change treatment)

Table 6.2: ***Monitoring patients with CML in CP who are on TKI therapy***

spleen. In the IRIS study, 96% of all patients achieved CHR by 12 months and 98% at 60 months. A failure to achieve CHR by 3 months is considered as imatinib failure. In the IRIS study some patients develop grade 3-4

cytopenias, in particular neutropenia (17%), thrombocytopenia (9%) and anemia (4%) and might require discontinuing the drug or reducing the dose (preferable). In most patients the cytopenias are short-lived, but some patients with severe neutropenia might require G-CSF support. It is important to maintain the dose intensity of the TKI as best as possible. The ELN guidelines suggest that peripheral blood count should be monitored 2 weekly until CHR is achieved and then 3 monthly unless otherwise required.

Cytogenetic response

Most experts concur that a baseline bone marrow examination is desirable and conventional cytogenetics carried out. The bone marrow examination with conventional cytogenetics should be repeated 3-monthly until CCyR and then cytogenetics can be monitored solely by FISH analysis, carried out 3-monthly. Some clinicians prefer not do bone marrow examinations at all and rather obtain FISH analysis on peripheral blood sample. This is not preferred for the reasons discussed above, but if it is carried out, FISH should be repeated every 3 months until the FISH levels are less than 5% to 10%, when a bone marrow evaluation with conventional cytogenetics be done to confirm a CCyR. Thereafter, it is reasonable to monitor the patient with regular FISH studies, provided they are reported as negative; persistent low levels of FISH positivity should trigger a conventional cytogenetic analysis.

The IRIS study established that cytogenetic response at 3 and 6 months predicts CCyR and progression-free survival at 24 months. Subsequent follow-up of the trial suggested that a cytogenetic response at 6 months is a better predictor than a cytogenetic response at 6 months. It is therefore reasonable to perform a bone marrow conventional cytogenetic analysis at baseline, at 6 months, and then 6 monthly until the patient achieves a CCyR. Patients who experience a significant rise in the BCR-ABL1 transcripts levels and loss of their MMR by RQ-PCR, should be considered for a repeat bone marrow conventional cytogenetic examination. If there is evidence of an additional clonal event, then the clinician might contemplate a change of therapy.

Molecular monitoring

It is desirable, but not mandatory, for all patients to have a baseline RT-qPCR

for BCR-ABL1 on peripheral blood and thereafter 3-monthly after the confirmation of CCyR. The IRIS trial is considered to have provided evidence that a reduction of the BCR-ABL1 transcripts was predictive of PFS. In the landmark analysis of the trial, achievement of MMR versus no MMR by 12 months was associated with improved EFS, but not with improved OS. A subsequent re-analysis showed the 18 months MMR did correlate with sustained CCyR and OS. Thereafter many studies have addressed the precise significance of achieving MMR at specific milestones.

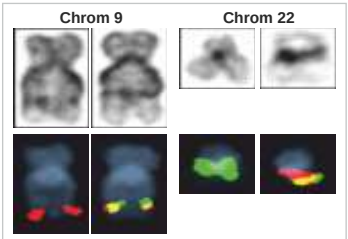


Figure 6.2: ***A photomicrograph of dual FISH analysis for the BCR-ABL1 fusion gene***

In general the importance of achieving MMR has been recognized, but the notion of defining the patients who do not achieve MMR is challenging. These patients represent a rather motley group, including those who are in CCyR but not MMR and some in CHR but neither CCyR nor MMR.

An important predictor of long-term response to TKI therapy is the depth of response at early time points. The Adelaide group have demonstrated that BCR-ABL1 mRNA levels assessed by PCR after only 3 months of therapy is strongly associated with achievement of CCyR, MMR and PFS. Conversely, patients who did not have a 1-log (10-fold) reduction of their BCR-ABL1 transcripts by 3 months had a very low probability of achieving MMR (13% at 30 months). Those who achieved a >2-log (100-fold) reduction at 3 months (this is 'equivalent to achieving a CCyR, by conventional cytogenetics) had a 100% probability of achieving MMR. More recently studies have addressed the usefulness of cytogenetic and BCR-ABL1 transcripts results following 3 months of first-line TKI therapy (Table 6.4).

The efforts reporting the usefulness of the BCR-ABL1 transcripts at 3 months as a predictive parameter for patients receiving TKI therapy suggest the critical cut-off point to be at the 10% international scale (IS) level, where patients with a BCR-ABL1 of >10% IS fared poorly compared to those whose

disease burden was <10%. These potentially useful parameters need further validation prior to being used in the clinics to identify patients who should be considered for an alternative therapy.

The measurement of BCR-ABL1 transcripts by RT-PCR is most relevant in patients that have achieved a CCyR. After 7 years of follow-up in the IRIS study, no patients achieving CCyR and MMR at 18 months had progressed to advanced phase. The rate of progression for those that had a CCyR but less than 3 log reduction in BCR-ABL1 was only 3%. Subsequent studies have confirmed the IRIS PCR data and demonstrate that patients with a deeper molecular response at the time of initial CCyR, or a >3-log reduction of BCR-ABL1 during CCyR, have very low odds of progression and a superior PFS compared to patients with an inferior response.

Despite being the qualified method of choice to monitor patients who have achieved a CCyR, there are several challenges. There appears much diversity in not only how the test is carried out, but also how the results are reported in different laboratories. Many of the methods appear not to have been standardized and there appear to be some variability in the guidelines for acceptable levels of reproducibility and sensitivity of the procedure. In the context of the IRIS trial, the standardized baseline was defined as the average ratio of BCR-ABL transcipts to a control gene, in this case BCR, from 30 untreated patients, was 36%. A MMR was therefore 'defined' as achieving level a 3-log reduction from the starting level, namely 0.036% or less. The use of different control genes and the considerable range in the values amongst the study cohort introduces some uncertainty to the results. It was therefore decided to introduce and International Scale on which the starting value for untreated patients would be designated 100%. On this scale an MMR would be 0.1%. However, this standardized baseline needs to be stringently applied in individual laboratories, a feat not easily accepted by many commercial laboratories, resulting in significant inter-laboratory variations; some laboratories do not even include this baseline in the final report.

A major effort led by John Goldman (London) is to establish a harmonization of results from diverse laboratories in diverse countries began in Bethesda in October 2006 and is currently on-going. A significant step has been to develop accredited reference reagents that are directly linked to the BCR-

Definitions of response and monitoring response

ABL1 international scale, under the aegis of the WHO (as the WHO International Genetic Reference Panel. Once this has been accomplished, a conversion factor should follow and the individual laboratories can adjust their values uniformly to define MMR as a value of 0.1% or less on the adjusted scale. It is of interest that even in the 'best' laboratories there can be a log-0.5 (5-fold) variation in the reported results. Efforts on the use of a DNA-based RT-qPCR, which would be 'patient specific' rather than the RNA-based 'disease specific', are also ongoing.

Mutational analysis

Studies designed to detect acquired mutations in the kinase domain of the BCR-ABL1 gene are generally not indicated when treatment with TKI therapy is commenced. They are also of very limited value in patients who are responding appropriately on therapy. The studies themselves are costly and not readily available, so it may not always be essential to perform them, unless the patients are in a clinical trial that stipulates the need. Figure 6.1 depicts some of the currently known mutations in patients with CML in CP.

Figure 6.1The 2011 ELN-led BCR-ABL1 kinase domain mutation analysis guidelines recommend mutational studies to be performed only with evidence of failure or suboptimal response or if there is a therapy change. The latter is particularly important since the choice of the next therapy might well be dictated in part by the demonstration of specific mutations, for example if the T315I mutation, a preferred treatment might be an allogeneic SCT, or perhaps ponatinib, if the mature analysis confirm its efficacy and safety.

Blood levels of imatinib

There has been some interest in monitoring imatinib blood levels to optimize the imatinib dose-intensity. This was based on pharmacokinetic studies of the 4-week trough blood level of imatinib and its correlation with cytogenetic and molecular response and suggestions that high blood levels might correlate with some imatinib-related toxicities. Patients who maintained an imatinib trough level > 1000 ng/mL were noted to have a greater probability of achieving a CCyR. A Hammersmith hospital (London) study assessing potential independent prognostic factors for optimal long-term outcome confirmed that imatinib blood level per se was not an independent prognostic

factor. At present there are no data from randomized studies necessitating a change in imatinib dose based on blood levels and most experts would agree that imatinib blood levels, outside of a clinical trial, are not required.

Conclusions

Optimal response definitions, homogeneous definitions of different end-points and events in clinical CML trials and how best to monitor patients on TKI therapy are some of the important challenges facing the clinician managing patients with CML today. Imatinib therapy has accorded for patients with CML in chronic phase a survival (OS) of at least 85% at 10 years. Significant survival benefit for the second generation TKIs remains to be established but the importance of molecular monitoring has been established. It is likely, though not definite, that the 3 months BCR-ABL1 transcripts will become a principal tool to assess this in the future.

Chapter 7
Concluding thoughts

For patients with CML, the introduction of imatinib into the clinics in 1998, resulted in being both a classic and a landmark achievement. It was classic since it established the notion of the BCR-ABL1 being of a principal pathogenetic importance, and a landmark, since it established the usefulness of TKIs to accord a survival benefit to the majority of patients with CML in chronic phase. It is interesting to recall that in the early 1990s much scepticism was expressed, from both academic and industry experts, about any possible clinical value of TKIs!

After 12 months of therapy with imatinib, 69% of patients with CML in chronic phase achieve a CCyR and after 8 years of follow-up, such response rates increases to 83%. This remarkable activity translates into an estimated overall survival of 93% (when only CML-related deaths are accounted for), which is substantially higher than that achieved by any previous medical treatment, including allogeneic SCT. The success in the treatment of patients with CML in advanced phases and the Ph-positive ALL has also been improved with the addition of imatinib to cytotoxic drugs, though less remarkably.

The adverse events attributable to imatinib, and indeed dasatinib and nilotinib (so far), appear to be relatively mild, but not innocuous, and generally easily manageable. In contrast, intolerance and resistance, in particular secondary, has been more challenging, with about a third of all patients with CML in chronic phase and substantially higher proportions with CML in advanced phases and Ph-positive ALL, not being able to tolerate imatinib or have a leukemia that becomes resistant or refractory to imatinib; precise data on the use of second generation TKI is currently not known, but probably better compared with imatinib, albeit with a relatively short period of follow-up.

Current observations suggest that about 20% of imatinib treated patients never achieve a CCyR, and 10% who do will lose such response over time. Furthermore about 26% of patients are intolerant of imatinib. Novel risk stratification methods and optimal molecular monitoring can be used to judge response and predict future risk of progression for patients with CML in chronic phase. These are complemented by recent insights into the mechanisms of resistance to TKIs as well as by knowledge gained regarding aspects of the cellular and molecular biology of BCR-ABL1 positive cells, such as their underlying genomic instability. Given the limited activity of TKI therapy in advanced phases of the disease, the most immediate goal of CML therapy is the prevention of progression, which has been associated with the achievement of deep responses at early time points during the course of TKI

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therapy. In this regard, the use of second generation TKIs as frontline therapy has led to an increase in the number of patients capable of study demonstrate that over half of CML patients in CMR on imatinib relapse quickly when TKI therapy is stopped. It is postulated, but not proven, that these relapses are a consequence of quiescent CML stem cells that are resistant to killing by conventional TKIs. Indeed, these malignant progenitors can be detected in bone marrow from CML patients in CCyR on imatinib.

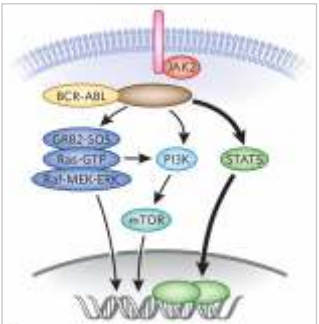

Figure 7.1: ***Schematic representation of STAT5 activation in Ph positive CML(Courtesy of Dr Doriano Fabbro)***

Studies have demonstrated the presence of BCR-ABL1 positive clonogenic progenitors, including LTCIC in CML patients in CMR, whose disease is undetectable by conventional PCR technology. Hence, there is much interest in identifying targets and strategies for eliminating leukemic stem cells (LSC) in CML.

The phenotype of leukemia-initiating cells in a conditional transgenic mouse model of CML have been recently defined and demonstrated that treatment of mice with the Hh inhibitor LDE225 together with nilotinib decreased phenotypic CML LSCs in spleen, but not bone marrow, and further decreased engraftment of NSG mice with human CD34+ CML progenitors. Given the recent launch of clinical trials of Hh pathway antagonists in refractory Ph-positive leukemia, further preclinical studies of these agents are warranted to aid in their clinical development. The possible role of JAK2 in the maintenance of quiescent, TKI-resistant BCR-ABL1-expressing stem cells in CML was also explored by several groups, where JAK2 may be activated by an extrinsic pathway through stroma-mediated cytokines, or through an intrinsic pathway via inhibition of a protein phosphatase, PP2A (Figure 7.1). These results open the possibility of targeting JAK2 in Figure 7.1 CML either through a specific JAK2 TKI, or the PP2A activator FTY720. Together, these exciting basic and preclinical studies continue to define CML as perhaps the best understood human cancer, and offer the hope that one day we will be able to eradicate the leukemia and 'cure' patients without the need for lifelong drug

Concluding thoughts

therapy.

The natural history of all BCR-ABL1 positive leukemias has been modified positively by the introduction of TKI therapy, which renders high rates of CCyR that translate into an 8-year EFS and OS rates of approximately 80% and 85%, respectively. Second generation TKIs such as dasatinib and nilotinib produce CCyR and MMR at higher rates and at a much faster pace than imatinib and current results suggest a superior rates of freedom from progression.

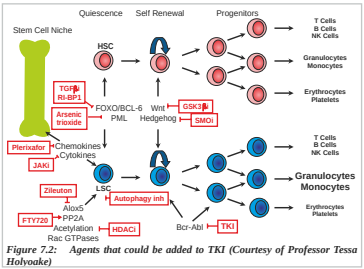

Figure 7.2: Agents that could be added to TKI (Courtesy of Professor Tessa Holyoake)

Efforts are also addressing potential strategies to eradicate the quiescent CML stem cells, which appear to be resistant to all currently available TKIs. These include combining TKIs with other agents, old and new, for patients with CML in chronic phase, in addition to consider various ways in which TKIs could be combined or used in sequence. It is of some interest that in addition to assessing combinations with novel agents such as histone deacetylase inhibitors, antagonists of the hedgehog signaling pathway, inhibitors of autophagy, JAK2 inhibitors, such as ruxolitinib, and IFNα, both as part of initial therapy and also once a CMR has been achieved.

It is of course important to develop treatment strategies where patients do not have to continue lifelong therapy with TKI at considerable expense, both financial and perhaps personal. Efforts in improving the technology of allo-SCT, such as the ability to prevent graft-vs-host disease without abrogation of graft-vs-leukemia, are also in progress.

Finally as our efforts in improving on the primary and secondary therapies continue, we can anticipate an improvement in the way progression and resistance to TKI risk is classified, based on the emerging tools. These tools may include of set of different levels of genetics-mutated genes that become evident in studies utilizing whole genome sequencing; microRNAs; and gene expression. In addition, the advent of DNA sequencing may uncover new cryptic translocations, or splicing variants, which define disease biology.

APPENDIX

Appendix

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- The book has been reviewed and highly recommended for health care professionals interested in CML by notable key CML experts from both sides of the Atlantic [Professors Giuseppe Saglio (Turin, Italy), Rudiger Hehlmann (Mannheim, Germany), Rick Van Etten (Boston, USA) and Jerald Radich (Seattle, USA)]

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