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Towards the Cure of CML by the Molecular Approach Strategy

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1. Introduction

Chronic myeloid leukaemia (CML) is a hematopoietic stem cell (HSC) disorder accounting for about 15-20% of all leukemias of the adult (Goldman & Melo, 2003; Black et al., 1997). The main haematological features are represented by an increase in the number of circulating mature granulocytes and their precursors and, subsequently, by a secondary evolution in acute leukaemia.

In 1960, a major clue to the cause of CML was provided by Nowell and Hungerford who for the first time described an unusual small chromosome present in leukocytes from patients with this hematologic malignance (Nowell & Hungerford, 1960). This "minute chromosome" abnormality, designed as the Philadelphia (Ph) chromosome, after the city in which it was discovered, was found in all malignant cells of CML patients and is now considered the hallmark of this neoplasia (Nowell & Hungerford, 1960). Importantly this discovery was the first demonstration of a chromosomal rearrangement linked to a specific cancer, and had sparked searches for associations of additional chromosomal aberrations with specific forms of cancer. In 1973, Rowley demonstrated that the Ph chromosome resulted from a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9:22)(q34;q11) (Rowley, 1973). Later it was shown that this process fuses the c-ABL (human homologue of the Abelson Murine Leukaemia virus), a tyrosine kinase encoding oncogene on chromosome 9, and BCR (Breakpoint Cluster Region), on chromosome 22, the function of which is still not clear (Groffen et al., 1984). This balanced translocation leads to a fusion gene, the product of which is a chimeric BCR-ABL protein equipped with cellular transforming ability which is ascribed to the elevated tyrosine kinase (TK) activity of the molecule compared to the native c-ABL (Konopka et al., 1984; Daley et al., 1990).

The biochemical signal transduction pathways stimulated by BCR-ABL kinase activity are responsible for Ph+ CML oncogenesis (Ren, 2005; Calabretta & Perrotti, 2004; Krebs & Hilton, 2001; Neshat et al., 2000; Sattler et al., 2002; Sattler et al., 1999).

Further studies have established BCR-ABL as a leukaemogenic oncogene since both mouse models and in vitro assays have shown that BCR-ABL, is able to induce leukaemia (Daley & Baltimore, 1988).

2. Molecular mechanisms of BCR-ABL

Several BCR-ABL isoforms with different molecular weights have been reported (Melo & Deininger, 2004). Accordingly, while in all chimeric proteins the breakpoint within ABL gene is consistently located upstream of exon 2 (a2), the breakpoint in the BCR gene varies in its localization (Melo, 1996). A major breakpoint cluster region (M-bcr) and a minor breakpoint cluster region (m-bcr) have been defined (Kurzrock et al., 1988).
The M-bcr maps to a 5.8 Kilobase (Kb) area spanning exons 12 through 16. The resulting fusion transcripts with ABL generate a 210-kDa protein named p210 which is the most common BCR-ABL form, being observed in 99% of the CML patients and in one-third of Ph-positive B cell acute lymphoblastic leukaemia (Ph+ B-ALL) (Faderl et al., 1999). m-bcr localizes to a 54.4-kb area sited downstream of exon 1. It gives rise to a fusion transcript with ABL named p190. It is rarely observed in CML, but is the most frequent BCR-ABL isoform in Ph+ B-ALL. Finally, 3' breakpoints downstream of BCR exon 19 have also been described and they give rise to a 230-kDa fusion protein (p230 BCR-ABL), which is typically found in the rare chronic neutrophilic leukaemia (CNL) (Pane et al., 1996).
All three BCR-ABL fusion protein variants induce a similar CML-like syndrome in mice, but differ in their ability to induce lymphoid leukaemia (Li et al., 1999).

3. Cellular pathways involved in oncogenic BCR-ABL signalling

The oncogenic potential of BCR-ABL derives from its capacity to activate intracellular signalling cascades that lead to uncontrolled cell proliferation, altered cell adhesion, and apoptosis inhibition (Daley et al., 1990; Kelliher et al., 1990). To date several signalling pathways affected by the constitutively active BCR-ABL have been identified, as well as numerous binding partners and substrates that provide a link between this pathways and the defects that characterize CML. Increased susceptibility to proliferate derives from BCR-ABL's capacity to activate the RAS-mitogen activated protein (MAP) kinase signalling cascade and JAK/STAT signalling; the interaction with SRC is responsible for increased cell motility; resistance to apoptosis is thought to result from BCR-ABL-mediated activation of phosphatidylinositol- 3-phosphate kinase (PI3K) and thereby of AKT. In summary, the net effects of these molecular alterations include inhibition of apoptosis, increased cell proliferation, aberrant interaction with the bone marrow stroma and genetic instability. Importantly all these events drive disease progression (Deninger et al., 2000).
Consistent with these molecular sequelae, BCR-ABL was shown to transform hematopoietic progenitor cells in vitro and in vivo studies (Kantarjian et al., 2006; Hehlmann et al., 2007). Recent reports identified a role for other signalling cascades in CML biology, including Hedgehog, Wnt and Ikaros, suggesting that pharmacological inhibitors of these pathways may find application in the treatment of CML (Chen Zhao et al., 2009; Dierks et al., 2008; Mullighan & Dowing, 2008; Dierks et al., 2008). Finally, also micro RNA (miRNA) regulation appears to apply to CML biology since miR-203, which would normally suppress BCR-ABL expression, is either mutated or epigenetically silenced in CML. In the latter type of condition, demethylating drugs such as 5-azacytidine and 4-phenylbutyrate were shown to restore miR-203 and to thereby decrease BCR-ABL expression and proliferation rate of Ph+ human CML cell lines (Faber et al., 2008; Croce & Calin, 2005).

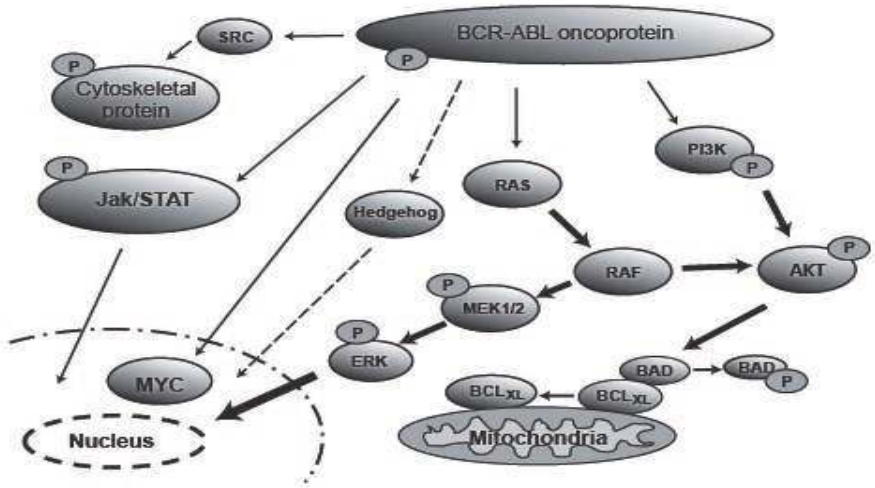


Fig. 2. Schematic view of the signal transduction pathways in cells transformed by BCR-ABL.

4. The CML leukaemia stem cell

Increasing evidence suggests that only a rare subset of immature cells within the tumor, named "leukaemia stem cells" (LSC), are able to propagate the CML (Reya et al, 2001). This cell has many common features with the hematopoietic stem cells - such as self-renewal and pluripotency pensions- unlike these, however, are refractory to conventional chemotherapy. Despite the remarkable improvements in the treatment of CML, the TKIs treatment is not curative, suppresses the disease but is not able to eradicate the CML Achilles hell, the leukaemia stem cell, causing recurrence of disease (Graham et al., 2002; Copland et al., 2006) The relapses in CML are thought to result from the outgrowth of quiescent LSC therapy-resistant, as the majority of leukemic cells in relapses represent (sub-) clones already present at diagnosis. To date the only long-term, sustainable remission derives from allogeneic bone marrow/peripheral blood stem cell transplantation which successfully restores normal hematopoiesis (Michor et al., 2005; Ljungman et al., 2009).

Recent data suggest that aberrant self-renewal is one of the central mechanisms underlying the pathogenesis of chronic myeloid leukaemia - acting either at the level of the BCR-ABL positive pluripotential stem cell in chronic phase or at the level of a more differentiated progenitor to cause blastic transformation, or most probably at both levels. Excessive self-renewal of LSCs may be mediated via several developmental pathways, including the Wnt/Frizzled/beta-catenin and Musashi-Numb pathway, or TWIST-1 oncogene and Polycomb-group protein BMI-1 (Hu et al., 2009; Ito et al., 2010; Cosset et al., 2011). An additional candidate is the Smoothened (SMO)/Sonic Hedgehog (sHH) signalling pathway, which is reasonably well characterised in solid tumours but is less well studied in leukaemia (Dierks et al., 2008; Chen Zhao et al., 2009;). Particularly it is essential during embryonic development, and might play a key role in human malignancies when aberrantly activated.

5. CML treatment options

The definition of the molecular structure of BCR-ABL tyrosine kinase domain has led to development of potent and specific tyrosine kinase inhibitor (TKIs) (Druker, 2008; Johnson et al., 2003). TKIs such as imatinib mesylate (Gleevec™, Novartis), nilotinib (Tasigna™, Novartis) and dasatinib (Sprycell™, Bristol-Myers Squibb) induce apoptosis in CML but not in healthy tissues, which is thought to result from addiction of CML cells to BCR-ABL signalling. The use of TKIs has led to remarkable improvements in disease outcome, in turn making TKIs the gold standard front line CML therapeutics. Importantly, although TKIs do induce disease remissions in most CML patients, they are not curative because of their incapacity to eradicate CML-LSC. Moreover, acquired resistance to TKIs is commonly observed and requires the prompt introduction of other TKIs that retain activity against BCR-ABL (Talpaz et al., 2002; Sawyers et al., 2002). Therefore, a timely and accurate follow-up is crucial for the management of CML and for effective therapeutic decisions (Druker et al., 2006; Kantarjian et al., 2008; O`Brien et al., 2003; Lahaye et al., 2005; Cervantes et al., 2003; Branford et al., 2003; Hughes & Branford, 2006). Additionally, such relapses are thought to result from the activation and proliferation of otherwise quiescent and therapy-resistant LSCs (Graham et al., 2002; Copland et al., 2006). Newer molecular therapies are being developed to eradicate the LSC pool by targeting critical signaling molecules that are essential for LSC maintenance.

6. CML monitoring

The remarkable progress in the treatment of CML over the past decade has been accompanied by steady improvements in our ability to accurately and sensitively monitor the status of the disease with the use of molecular markers, aimed at recognizing the depth of remission, and by use of readings to guide the choice of strategy for therapeutic interventions (Hughes et al., 2006).

However, the identification of patients that will experience a failure of TKI treatment, and appropriately altering the therapeutic strategy based on such monitoring, remains a challenge.

Routine CML diagnostics largely relies nowadays on traditional blood cell count, cytogenetic analysis (standard karyotype with or without fluorescence in situ hybridization-FISH), and real time quantitative polymerase chain reaction (RT-Q-PCR) for BCR-ABL messenger RNA (mRNA). These tests allow defining the haematological, cytogenetic, and molecular response to treatment, respectively (Kantarjian et al., 2008; Hughes et al., 2006). The haematological response to treatment is assessed by peripheral blood cell counts and by spleen size, and is classified as:

1. *Complete haematological response (CHR):* normalization of peripheral blood counts with no immature blood cells and with disappearance of any sign of disease
2. *Partial haematological response (PHR):* presence of immature blood cells and/or persistent splenomegaly. The next level of response is the cytogenetic one (CyR), defined as a decrease in the number of Ph+metaphases in a bone marrow aspirate (using ≥ 20 metaphases). This is categorized as:

1. Complete cytogenetic response (CCyR): 0% Ph+ metaphases
2. Partial cytogenetic response (PCyR): 1-35% Ph+ metaphases
3. *Minor cytogenetic response*: 36-65% Ph+ metaphases

4. Minimal cytogenetic response: 66-95% Ph+ metaphases

CCyR or PCyR configure a major cytogenetic response (MCyR). Finally, residual leukaemia cells (minimal residual disease, MRD) can be detected using RT-Q-PCR. Particularly, the molecular response is defined as a decrease of the BCR-ABL to control gene transcript ratio according to the International Scale (IS) (see below):

1. *Complete molecular response (CMR):* undetectable level of chimeric transcript
2. *Major molecular response (MMR):* reduction in transcript levels of at least 3-log from standard baseline level (which represent 100% on the International Scale) or ≤1%.

6.1 Cytogenetic and FISH

The Ph chromosome can be detected by standard cytogenetic techniques in the vast majority of patients (Osarogiagbon, 1999). In patients who are cytogenetically Ph chromosome negative (Ph–), molecular techniques such as FISH and RT-Q-PCR may be useful in detecting BCR-ABL. Cytogenetic analysis is typically performed by chromosome banding of at least 20 bone marrow cells in metaphase allowing to identify the t(9:22) translocation (Haferlach et al., 2007). In addition, cytogenetic also allows to define any additional chromosomal abnormality (i.e. additional Ph chromosome, isochromosome 17q, trisomy 8, or trisomy 19), thereby providing additional prognostic information. Baccarani et al. recommend that, at diagnosis, two cytogenetic analyses are performed in order to increase the sensitivity of the method. Furthermore, if less than 20 metaphases are visualized, the cytogenetic analysis should be validated by FISH or by RT-Q-PCR (see below) (Baccarani et al., 2008). Importantly, in 5% of CML cases no cytogenetically-detectable Ph chromosome can be demonstrated, since the BCR-ABL fusion oncogene derives from a submicroscopic genetic fusion. In these cases, FISH or RT-Q-PCR will demonstrate the presence of the specific genetic abnormality. Traditional FISH uses 5′ BCR and 3′ ABL fluorescent probes of different colours while more recent FISH reagents use 3-4 probes (D-FISH). Such probes can detect the variant translocations leading to Ph chromosome formation and are also associated with low false positive rates (Dewald et al., 1998; Wang et al., 2001; Landstrom & Tefferi, 2006; Sinclair et al., 1997; Seong et al., 1995). Interphase or hypermetaphase FISH can be performed on peripheral blood specimen or bone marrow aspirates, respectively. Interphase FISH is applicable to a larger population of cells since does not require cycling cells. On the other hand, this technique is associated with a background signal greater than 1-5% (depending on the specific probe used in the assay) (Cuneo et al., 1998; Le Gouill et al., 2000; Lesser et al., 2002; Raanani et al., 2004). Hypermetaphase FISH is applicable only to dividing bone marrow cells (Schoch et al., 2002). This approach is more sensitive and can analyze up to 500 metaphases at a time. Usually, FISH results correlate with traditional cytogenetic analysis and with RT-Q-PCR results, thus remaining a convenient and sensitive diagnostic tool (see below).

6.2 PCR-based approaches to CML monitoring

Nested reverse transcriptase PCR can detect one CML cell in a background of ≥ 100.000 normal cells (Martinelli et al., 2006). However, it remains a purely qualitative assay which is only capable of demonstrating the presence or absence of CML cells. Nested-PCR is normally only used to confirm the achievement of CMR. RT-Q-PCR methods are less sensitive than qualitative PCR (by 0.5-1 order of magnitude) but they have the advantage of determining the actual percentage of BCR-ABL transcripts and can therefore be used to

track changes in the number of leukemic cells over time (Lowemberg, 2003; Hughes et al., 2003; Merante et al., 2005; Mauro et al., 2004; Cortes et al., 2004). Currently, RT-QPCR for BCR-ABL is the recommended approach for routine follow-up of CML patients and is considered the gold standard test for routine therapeutics decision. The BCR-ABL transcript levels are expressed as a percentage ratio of BCR-ABL compared to ABL transcripts. ABL acts as control gene to compensate for variations in the quality of the RNA and for differences in the efficiency of the reverse transcription reaction. The last years have seen numerous efforts to standardize the molecular approaches to CML monitoring as well as their interpretation criteria. In order to harmonize the results across laboratories worldwide, a standard pre-treatment baseline value for each laboratory was established. Thus, a molecular response is defined by reductions from an absolute baseline (common to all) rather than a relative baseline (individualized). This ensures that patients with the same level of response have the same degree of residual disease. Additionally, under- or over-estimation of the extent of response due to individual variations is avoided by using a common standard baseline. According to the international reporting scale (IS) the absolute BCR-ABL value to define major molecular response is standardized at 0.1% (or 3 log) reduction from the laboratory-specific pretreatment standard baseline (Hochhaus & Dreyling, 2008; Hochhaus et al., 1996). A value of 1.0% is approximately equivalent to the achievement of a CCyR and a CMR is achieved when transcripts are undetectable (Branford et al., 2006; Muller et al., 2007, 2008). Because of its high sensitivity, CML monitoring by RT-Q-PCR enables to define an early loss of response once CCyR has been achieved (Wang, 2000, Press et al., 2006). Additionally, early molecular monitoring after initiation of treatment helps to identify patients at higher risk of relapse after pharmacological treatment onset as well as after allogeneic bone marrow transplantation (Olavarria et al. 2002; Lange et al., 2004; Asnafi et al., 2006). Finally, another advantage of CML monitoring by RT-Q-PCR is the feasibility of this method on peripheral blood samples. In a large cohort of patients monitored to BCR-ABL mRNA levels after allogeneic bone marrow transplantation, we found that peripheral blood and bone marrow samples perform equally well in terms of sensitivity in relapse detection and show a very good correlation of results. Thus, molecular monitoring of CML with RT-Q-PCR can be performed using peripheral blood samples instead of bone marrow (Ballestrero et al., 2009). The drawbacks of this method include a substantial incidence of false negative tests, which on the other hand, is strongly reduced when serial evaluations are performed. Nowadays, RT-Q-PCR monitoring is included as integral part of the management of CML patient treated with TKIs and must be performed every 3 months even in patients in MMR. An increase in BCR-ABL levels of 2 to 5 fold is an early sign of relapse, and suggests the need to switch to another type of treatment as soon as possible.

6.3 Mechanisms of resistance

A growing problem in the treatment of CML is resistance to treatment since most patients in chronic phase initially respond to TKIs but subsequently relapse and/or progress to accelerated phase or blast crisis (Talpaz et al., 2002; Sawyers et al., 2002). Primary resistance or, perhaps more appropriately, primary refractoriness (typically BCR-ABL independent), is defined as the failure to achieve initial response to therapy and is only seen in approximately 5% of newly diagnosed patients in chronic phase of CML. (Apperley, 2007) Acquired resistance, defined as the loss of previous response, is more common. About 10-

15% of patients in TKIs treatment develop treatment failure at a rate of approximately of 1-4%/year). Resistance to TKIs may be primary or secondary and is usually classified in BCR-ABL-dependent or -independent. The BCR-ABL-dependent mechanisms include reactivation of BCR-ABL signaling through mutations in the ABL kinase domain (KD), and increased production of BCR-ABL at the genomic (gene amplification) or transcript (overexpression) levels (Campbel et al., 2002, Morel et al., 2003; Hochhaus et al., 2002). Conversely, BCR-ABL independent resistance mechanisms involve: i) a drop in the intracellular drug concentration through expression of drug efflux (such as multidrug-resistant P-glycoprotein MDR-1) (Mahon et al., 2000; le Coutre et al., 2000) or drug influx (such as hOCT1 that affects intracellular drug availability) (Thomas et al., 2004) genes; ii) activation of Src family of kinases (SFKs); and iii) acquisition of additional chromosomal abnormalities with Ph-chromosome (O'Dwyer et al., 2002, 2004; Schoch et al., 2003). Although gene amplification occurs more frequently than point mutations (10^{-4} per cell division vs. 10^{-9}(Hochhaus A et al., 2002) clinical resistance is much more likely to be due to a point mutation in the BCR-ABL TK domain than to BCR-ABL amplification (Willis et al., 2005). To date more than 50 mutations have been identified, each of which arises at variable frequencies and with different consequences (Jabbour et al., 2006; Shah et al., 2002; Branford et al., 2002; Hofmann et al., 2002; Roche-Lestienne et al., 2002; Deninger et al., 2000; Soverini et al., 2004, 2005; Chu et al., 2005; Nicolini et al., 2006; Barthe et al., 2002; Irving et al., 2004; Wei et al., 2006; Wang et al., 2006). Mutations may occur in various ATP-binding sites, such as the phosphate-binding loop (P-loop), activation site, catalytic site, or other areas in the BCR-ABL structure. Depending on the mutation site, resistance to imatinib will either be absolute or relative, or it will be clinically irrelevant. Earlier studies have associated P-loop mutations and the T315I mutation with the worst outcomes (Cortes et al., 2007). Mutations within the P-loop site are found in 30-40% of the resistant cases and reduce susceptibility to imatinib by 70 to 100 folds. The T315I mutation in BCR-ABL occurs in 0.16-0.32% of newly diagnosed patients in chronic phase, leading to substitution of threonine 315 with isoleucine. This "gatekeeper" mutation also affects the response to the currently existing second-generation TKIs. Therefore, upon its identification, patients should be considered for alternative pharmacological treatments or for allogeneic bone marrow transplantation.

6.4 Mutational analysis

A careful mutational screening allows the timely identification of potential mutant clones and suggests the most suitable second-line treatment based on the in vitro sensitivity of the specific mutation. The technologies used to identify and quantify the ABL KD mutations include: direct sequencing (Branford et al., 2003), subcloning and sequencing, denaturing-high performance liquid chromatography analysis (DHPLC), pyrosequencing and allele specific oligonucleotide PCR. Direct sequencing represents the most widespread method used for routine monitoring. Its main drawback is the low detection limit (20%) which is responsible for false negative results. Fluorescent-based allele-specific oligonucleotide PCR (ASO-PCR) assays have higher detection limit (0.1%), although their main drawback is that the search for specific mutations does not include screening of the entire KD region of the BCR-ABL gene. Nowadays, numerous groups perform DHPLC to monitor CML patients, followed by a sequence analysis to confirm the data. DHPLC has a detection limit of 1-5% (Deininger et al., 2004). Mutation studies might be performed on peripheral blood or bone

marrow although a direct comparison of these two types of samples has not been done yet. The search for BCR-ABL mutations should be performed, according to NCCN CML guidelines (NCCN Clinical Practice Guidelines in Oncology, 2010), in the following conditions:

1. Progression to accelerated or blast phase
2. Treatment failure
3. Suboptimal therapeutic responses
4. Increasing BCR-ABL levels (5 to 10 fold in mRNA)

6.5 Scheduling CML diagnostics and monitoring

An effective CML monitoring entails an appropriate follow up-schedule (Baccarani et al., 2006). Evidence obtained in clinical trials has prompted experts to formulate consensus recommendations to assess the response to treatment in patients with Ph+ CML (Quintas-Cardama & Cortes, 2005). In the diagnostic setting, bone marrow cytogenetics is recommended before initiation of treatment. Additionally, a nested PCR confirms the diagnosis of CML and establishes the type of BCR-ABL fusion transcript present. Bone marrow cytogenetics is able to detect chromosomal abnormalities that FISH is not able to detect. However, if bone marrow collection is not feasible, FISH on peripheral blood specimen with dual probe (BCR and ABL genes) is a suitable tool to confirm the diagnosis. Subsequently, the cytogenetic evaluation is recommended at 6 and 12 months from the beginning of treatment. If a CCyR is achieved at 6 months, it is not necessary to repeat the cytogenetic evaluation at 12 months. If patients is not in a CCyR at 12 months, a cytogenetic evaluation should be repeated at 18 months. Once cytogenetic remission is achieved, residual disease should be monitored using BCR-ABL transcript levels by RT-Q-PCR, which is the most sensitive technique to monitor BCR-ABL. The hybrid transcript levels should be measured every 3 months at the beginning of treatment and then every 3-6 months since a CCyR is achieved. A steady decline in BCR-ABL transcripts indicates an ideal response to therapy. Rising level of BCR-ABL transcript (1 log increase) following the achievement of a MMR, mandates to repeat the molecular analysis after 1 month (Baccarani et al., 2006). If the result is confirmed, bone marrow cytogenetics should be performed, BCR-ABL quantifications by RT-Q-PCR should be scheduled every month, and a kinase domain mutational analysis should also be done (Wang et al., 2003). The evaluation of the hematologic response foresees that, starting from treatment onset, blood cell counts are performed every 2 weeks until a stable CHR is achieved, then every 3 months (Deininger, 2005). If the patient fails to achieve CHR by 3 months, the treatment is generally regarded as a failure, indicating the need to consider alternative therapeutic strategies.

In summary, the international guidelines recommend the following testing schedule when monitoring treatment of CML patients:

1. Hematologic responses should be assessed at diagnosis, then every 2 weeks until a CHR has been achieved and confirmed, then every 3 months or as required.
2. Cytogenetic responses should be assessed at diagnosis, and every 6 months until a CCyR is achieved and confirmed, then every 12 to 36 months as long as MMR is stable
3. Molecular responses should be assessed every 3 months, or monthly if an increasing BCR-ABL transcript level is detected.
4. Mutational analysis in occurrences of suboptimal response or failure; recommended before changing to other TKIs or other therapies

FISH may be preferred over conventional cytogenetics as it can evaluate more cells and peripheral blood can be used instead of bone marrow. However it is only recommended prior to treatment to identify cases of Ph-, BCR-ABL CML and those with variant translocations, Ph amplification, or del9q+.

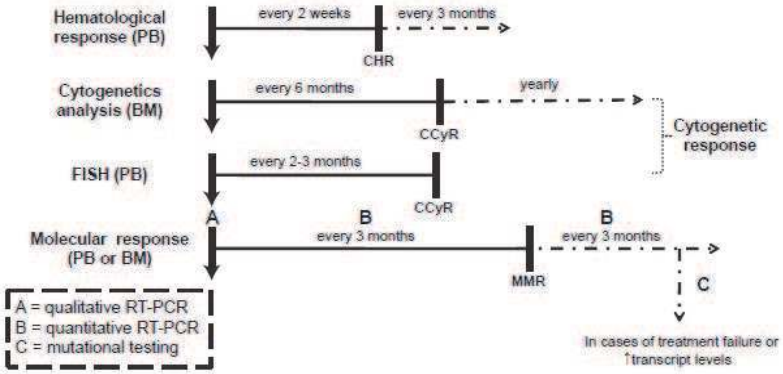


Fig. 1. Proposed algorithm for CML monitoring according to the National Comprehensive Cancer Network guidelines.

7. Conclusions

Chronic myeloid leukaemia is a biological model of how the molecular understanding of a disease is able to provide the substrate for therapy and diagnostics. The recent molecular analysis of the leukaemia cell has generated an extraordinary range of discoveries about the anomalies developed during the cell growth, promoting the development of innovative therapeutic approaches for this type of hematopoietic neoplasia. In particular with the introduction of TKIs we have embarked on a journey aiming to reduce disease burden and prolonging survival.

Additionally the molecular tools to monitor disease and characterize resistance are remarkably effective not only in the diagnostic evaluation but even in the management of CML patients. While traditional cytogenetics with or without FISH and qualitative nested-PCR are essential for the diagnosis of CML, serial RT-Q-PCRs are the mainstay of therapeutic monitoring and MDR assessment (Kantarjian et al., 2008). In cases of treatment failure, highlighted by increasing BCR-ABL levels and/or by loss of hematologic and cytogenetic responses, mutational analysis to identify KD mutations should be considered in order to meet the better treatment decisions (i.e. use alternative TKIs or stem cell transplantation) (Hughes et al., 2006). Additionally, an early identification of treatment failure increases the chance that alternative treatments will be effective (Jabbour et al., 2009).

However the major current impediment to cure for CML patients resides in the cancer stem cell population that is neither oncogene addicted nor sensitive to TKIs. Thus, one of the major challenges is to recognize as early as possible the patient destined to fail TKIs to revise the therapeutic strategy. Additionally, an early identification of treatment failure increases the chance that alternative treatments will be effective.

Hence the need for increasingly sophisticated technologies for an early detection of molecular relapse. In this field the comprehensive analysis of the CML genome, by the

single nucleotide polymorphism arrays, will provide the basis for a molecular approach to guide therapeutic decisions. (Boultwood et al., 2010)In summary the CML represents one of the best examples of tumour malignancies and despite the numerous advantages of modern technologies, it is important to continue interpreting laboratory data within the clinical context of the patient in order to effectively and inexpensively utilize current and nascent laboratory tools.

8. Acknowledgment

This work was supported by an American-Italian Cancer Foundation Post-Doctoral Research Fellowship and by Associazione Cristina Bassi Onlus

9. References

Apperley JF. (2007). Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia. Lancet Oncol 8: 1018-1029.

Asnafi V, Rubio MT, Delabesse E et al. (2006). Prediction of relapse by day 100 BCR-ABL quantification after allogeneic stem cell transplantation for chronic myeloid leukemia. Leukemia 20: 793-799.

Baccarani M, Pane F & Saglio G. (2008). Monitoring treatment of chronic myeloid leukemia. Haematologica 93: 161-169.

Baccarani M, Saglio G, Goldman J et al. (2006). Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood 108: 1809-1820.

Ballestrero A, Cirmena G, Dominietto A et al. (2010). Peripheral blood vs. bone marrow for molecular monitoring of BCRABL levels in chronic myelogenous leukemia; a retrospective analysis in allogeneic bone marrow recipients. Int J Clin Lab 32(4):387-91

Barthe C, Gharbi MJ, Lagarde V et al. (2002). Mutation in the ATPbinding site of BCR-ABL in a patient with chronic myeloid leukaemia with increasing resistance to STI571. Br J Haematol 119: 109-111.

Black RJ, Bray F, Ferlay J & Parkin DM. (1997). Cancer incidence and mortality in the European Union: cancer registry data and estimates of national incidence for 1990. Eur J Cancer 33: 1075-1107.

Boultwood J, Perry J, Zaman R et al. (2010). High-density single nucleotide polymorphism array analysis and ASXL1 gene mutation screening in chronic myeloid leukemia during disease progression. Leukemia 24: 1139-1145.

Branford S, Cross NC, Hochhaus A et al. (2006). Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukaemia. Leukemia 20: 1925-1930.

Branford S, Rudzki Z, Harper A et al. (2003). Imatinib produces significantly superior molecular responses compared to interferon alfa plus cytarabine in patients with newly diagnosed chronic myeloid leukemia in chronic phase. Leukemia 17: 2401-2409.

Branford S, Rudzki Z, Walsh S et al. (2002). High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 99: 3472-3475.

Calabretta B & Perrotti D. (2004). The biology of CML blast crisis. Blood 103: 4010-4022.

Campbell LJ, Patsouris C, Rayeroux KC, Somana K, Januszewicz EH & Szer J. (2002). BCR/ABL amplification in chronic myelocytic leukemia blast crisis following imatinib mesylate administration. Cancer Genet Cytogenet 139: 30-33.

Cervantes F, Hernandez-Boluda JC, Steegmann JL et al. (2003). Imatinib mesylate therapy of chronic phase chronic myeloid leukemia resistant or intolerant to interferon: results and prognostic factors for response and progression-free survival in 150 patients. Haematologica 88: 1117-1122.

Chen Zhao AC, Jamieson CH, Fereshteh M et al. (2009). Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature 458: 776-780.

Chu S, Xu H, Shah NP et al. (2005). Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment. Blood 105: 2093-2098.

Copland M, Hamilton A, Elrick LJ et al. (2006). Dasatinib (BMS-354825) targets an erlier progenitor population than imatinib in primary CML, but does not eliminate the quiescent fraction. Blood 107(11): 4532-4539.

Cortes J, O'Brien S & Kantarjian H. (2004). Discontinuation of imatinib therapy after achieving a molecular response. Blood 104: 2204-2205.

Cortes J, Rousselot P, Kim DW et al. (2007). Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood 109: 3207-3213.

Cosset E, Hamdan G, Jeanpierre S, Voeltzel T, Sagorny K, Hayette S, Mahon FX, Dumontet C, Puisieux A, Nicolini FE & Maguer-Satta V.(2011) Deregulation of TWIST-1 in the CD34+ compartment represents a novel prognostic factor in chronic myeloid leukemia. Blood.117(5):1673-6.

Croce CM & Calin GA. (2005). miRNAs, cancer, and stem cell division. Cell 122: 6-7.

Cuneo A, Bigoni R, Emmanuel B et al. (1998). Fluorescence in situ hybridization for the detection and monitoring of the Ph-positive clone in chronic myelogenous leukemia: comparison with 572 metaphase banding analysis. Leukemia 12: 1718-1723.

Daley GQ & Baltimore D. (1988) Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. Proc Natl Acad Sci U S A. 85(23):9312-9316..

Daley GQ, Van Etten RA & Baltimore D. (1990). Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 247: 824-830.

Deininger MW, McGreevey L, Willis S, Bainbridge TM, Druker BJ & Heinrich MC. (2004). Detection of ABL kinase domain mutations with denaturing high-performance liquid chromatography. Leukemia 18: 864-871.

Deininger MW. (2005). Management of early stage disease. Hematology Am Soc Hematol Educ Program 174-182.

Deninger MW, Goldman JM & Melo JV. (2000).The molecular biology of chronic myeloid leukemia. Blood 96: 3343-3356.

Dewald G, Stallard R, Alsaadi A et al. (2000). A multicenter investigation with D-FISH BCR/ABL1 probes. Cancer Genet Cytogenet 116: 97-104.

Dewald GW, Wyatt WA, Juneau AL et al. (1998). Highly sensitive fluorescence in situ hybridization method to detect double BCR/ABL fusion and monitor response to therapy in chronic myeloid leukemia. Blood 91: 3357-3365.

Dierks C, Beigi R, Guo GR et al. (2008). Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell 14: 238-249.

Druker BJ, Guilhot F, O'Brien SG et al. (2006). Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 355: 2408-2417.

Druker BJ. (2008). Translation of the Philadelphia chromosome into therapy for CML. Blood 112: 4808-4817.

Faber J, Gregory RI & Armstrong SA. (2008). Linking miRNA regulation to BCR-ABL expression: the next dimension. Cancer Cell 13: 467-469.

Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R & Kantarjian HM. (1999). The biology of chronic myeloid leukemia. N Engl J 341: 164-172.

Goldman JM & Melo JV. (2004). Chronic myeloid leukemia-advances in biology and new approaches to treatment. N Engl J Med 349: 1451-1464.

Graham SM, Jorgensen HG, Allan E et al. (2002). Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 99; (1): 319-325.

Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR & Grosveld G. (1984) Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell. 36(1):93-99.

Haferlach C, Rieder H, Lillington DM et al. (2007). Proposals for standardized protocols for cytogenetic analyses of acute leukemias, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myeloproliferative disorders, and myelodysplastic syndromes. Genes Chromosomes Cancer 46: 494-499.

Hehlmann R, Berger U, Pfirrmann M et al. (2007). Drug treatment is superior to allografting as first-line therapy in chronic myeloid leukemia. Blood 109: 4686-4692.

Hochhaus A & Dreyling M. (2008). Chronic myelogenous leukemia: ESMO clinical recommendations for the diagnosis, treatment and follow-up. Ann Oncol 19 (Suppl 2): ii63-64.

Hochhaus A, Kreil S, Corbin AS et al. (2002). Molecular and chromosomal mechanisms of resistance to imatinib (STI571) therapy. Leukemia 16: 2190-2196.

Hochhaus A, Lin F, Reiter A et al. (1996). Quantification of residual disease in chronic myelogenous leukemia patients on interferon- alpha therapy by competitive polymerase chain reaction. Blood 87: 1549-1555.

Hofmann WK, Jones LC, Lemp NA et al. (2002). Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique BCR-ABL gene mutation. Blood 99: 1860-1862.

Hu Y, Chen Y, Douglas L & Li S. (2009) beta-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABL-induced chronic myeloid leukemia. Leukemia. 23(1):109-16.

Hughes T, Branford S. (2006). Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. Blood Rev 20: 29-41.

Hughes T, Deininger M, Hochhaus A et al. (2006). Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood 108: 28-37.

Hughes TP, Kaeda J, Branford S et al. (2003). Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med 349: 1423-1432.

Irving JA, O'Brien S, Lennard AL, Minto L, Lin F & Hall AG. (2004). Use of denaturing HPLC for detection of mutations in the BCR-ABL kinase domain in patients resistant to Imatinib. Clin Chem 50: 1233-1237.

Ito T, Kwon HY, Zimdahl B, Congdon KL, Blum J, Lento WE, Zhao C, Lagoo A, Gerrard G, Foroni L, Goldman J, Goh H, Kim SH, Kim DW, Chuah C, Oehler VG, Radich JP, Jordan CT & Reya T (2010). Regulation of myeloid leukaemia by the cell-fate determinant Musashi. Nature. Aug 5;466(7307):765-8.

Jabbour E, Cortes JE, Kantarjian HM. (2009). Suboptimal response to or failure of imatinib treatment for chronic myeloid leukemia: what is the optimal strategy? Mayo Clin Proc 84: 161-169

Jabbour E, Kantarjian H, Jones D et al. (2006). Frequency and clinical significance of BCR-ABL mutations in patients with chronic myeloid leukemia treated with imatinib mesylate. Leukemia 20: 1767-1773.

Johnson JR, Bross P, Cohen M et al. (2003). Approval summary: imatinib mesylate capsules for treatment of adult patients with newly diagnosed Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase. Clin Cancer Res 9: 1972-1979.

Kantarjian H, O'Brien S, Shan J et al. (2008). Cytogenetic and molecular responses and outcome in chronic myelogenous leukemia: need for new response definitions? Cancer 112: 837-845.

Kantarjian H, Schiffer C, Jones D & Cortes J. (2008). Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods. Blood 111: 1774-1780.

Kantarjian HM, Talpaz M, Giles F, O'Brien S & Cortes J. (2006). New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. Ann Intern Med 145: 913-923.

Kelliher MA, McLaughlin J, Witte ON & Rosenberg N. (1990). Induction of a chronic myelogenous leukemia-like syndrome in mice with v-abl and BCR/ABL. Proc Natl Acad Sci USA 87: 6649-6653.

Konopka JB, Watanabe SM,& Witte ON. (1984) An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell. 37(3):1035-1042.

Krebs DL & Hilton DJ. (2001). SOCS proteins: negative regulators of cytokine signaling. Stem Cells 19: 378-387.

Kurzrock RG, Gutterman JU & Talpaz M. (1988). The molecular genetics of Philadelphia chromosome-positive leukemias. N Engl J Med 319: 990-998.

Lahaye T, Riehm B, Berger U et al. (2005). Response and resistance in 300 patients with BCR-ABL-positive leukemias treated with imatinib in a single center: a 4.5-year follow-up. Cancer 103: 1659-1669.

Landstrom AP & Tefferi A. (2006). Fluorescent in situ hybridization in the diagnosis, prognosis, and treatment monitoring of chronic myeloid leukemia. Leuk Lymphoma 47: 397-402.

Lange T, Deininger M, Brand R et al. (2004). BCR-ABL transcripts are early predictors for hematological relapse in chronic myeloid leukemia after hematopoietic cell transplantation with reduced intensity conditioning. Leukemia 18: 1468-1475.

le Coutre P, Tassi E, Varella-Garcia M et al. (2000). Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood 95: 1758-1766.

Le Gouill S, Milpied N, Daviet A et al. (2000). Fluorescence in situ hybridization on peripheral-blood specimens is a reliable method to evaluate cytogenetic response in chronic myeloid leukemia. J Clin Oncol 18: 1533-1538.

Lesser ML, Dewald GW, Sison CP & Silver RT.(2002). Correlation of three methods of measuring cytogenetic response in chronic myelocytic leukemia. Cancer Genet Cytogenet 137: 79-84.

Li S, Ilaria RL, Jr., Million RP, Daley GQ & Van Etten RA. (1999) The P190, P210, and P230 forms of the BCR/ABL oncogene induce a similar chronic myeloid leukemia-like syndrome in mice but have different lymphoid leukemogenic activity. JExp Med. 189(9):1399-1412.

Ljungman P, Bregni M, Brune M et al. (2010). Allogeneic and autologous transplantation for haematological diseases, solid tumours and immune disorders: current practice in Europe 2009. Bone Marrow Transplant 45(2):219-34.

Lowenberg B. (2003). Minimal residual disease in chronic myeloid leukemia. N Engl J Med 349: 1399-1401.

Mahon FX, Deininger MW, Schultheis B et al. (2000). Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood 96: 1070-1079.

Martinelli G, Soverini S, Cilloni D et al. (2006). Monitoring minimal residual disease and controlling drug resistance in chronic myeloid leukaemia patients in treatment with imatinib as a guide to clinical management. Hematol Oncol 24: 196-204.

Mauro MJ, Druker BJ & Maziarz RT. (2004). Divergent clinical outcome in two CML patients who discontinued imatinib therapy after achieving a molecular remission. Leuk Res 28 (Suppl 1): S71-73.

Melo JD & Deininger MW. (2004). Biology of chronic myelogenous leukemia-signaling pathways of initiation and transformation. Hematol Oncol Clin North Am 18: 545-568.

Melo JD. (1996). The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 88: 2375-

Merante S, Orlandi E, Bernasconi P et al. (2005). Outcome of four patients with chronic myeloid leukemia after imatinib mesylate discontinuation. Haematologica 979-981.

Michor F, Hughes TP, Iwasa Y et al. (2005). Dynamics of chronic myeloid leukaemia. Nature 435: 1267-1270.

Morel F, Bris MJ, Herry A et al. (2003). Double minutes containing amplified bcr-abl fusion gene in a case of chronic myeloid leukemia treated by imatinib. Eur J Haematol 70: 235-239.

Muller MC, Erben P, Saglio G et al. (2008). Harmonization of BCRABL mRNA quantification using a uniform multifunctional control plasmid in 37 international laboratories. Leukemia 22: 96-102.

Muller MC, Saglio G, Lin F et al. (2007). An international study to standardize the detection and quantitation of BCR-ABL transcripts from stabilized peripheral blood preparations by quantitative RT-PCR. Haematologica 92: 970-973.

Mullighan C & Downing J. (2008). Ikaros and acute leukemia. Leuk Lymphoma 49: 847-849.

NCCN Clinical Practice Guidelines in Oncology. Chronic Myelogenous Leukemia. V.I. 2010. www.nccn.org

Neshat MS, Raitano AB, Wang HG, Reed JC & Sawyers CL. (2000). The survival function of the Bcr-Abl oncogene is mediated by Bad-dependent and -independent pathways: roles for phosphatidylinositol 3-kinase and Raf. Mol Cell Biol 20: 1179-1186.

Nicolini FE, Corm S, Le QH et al. (2006). Mutation status and clinical outcome of 89 imatinib mesylate-resistant chronic myelogenous leukemia patients: a retrospective analysis from the French intergroup of CML (Fi(phi)-LMC GROUP). Leukemia 20: 1061-1066.

Nowell PC & Hungerford DA. (1960) A minute chromosome in human chronic granulocytic leukemia. Science 142: 1497.

Nowell PC & Hungerford DA. (1960) Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 25:85-109.

O'Brien SG, Guilhot F, Larson RA et al. (2003). Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 348: 994-1004.

O'Dwyer ME, Mauro MJ, Blasdel C et al. (2004). Clonal evolution and lack of cytogenetic response are adverse prognostic factors for hematologic relapse of chronic phase CML patients treated with imatinib mesylate. Blood 103: 451-455.

O'Dwyer ME, Mauro MJ, Kurilik G et al. (2002). The impact of clonal evolution on response to imatinib mesylate (STI571) in accelerated phase CML. Blood 100: 1628-1633.

Olavarria E, Craddock C, Dazzi F et al. (2002). Imatinib mesylate (STI571) in the treatment of relapse of chronic myeloid leukemia after allogeneic stem cell transplantation. Blood 99: 3861-3862.

Osarogiagbon UR MP. (1999). Chronic myelogenous leukemia. Curr Opin Hematol 6: 241-246.

Pane F, Frigeri F, Sindona M et al. (1996). Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker (BCR/ABL with C3/A2 junction). Blood 88: 2410-2414.

Press RD, Love Z, Tronnes AA et al. (2006). BCR-ABL mRNA levels at and after the time of a complete cytogenetic response (CCR) predict the duration of CCR in imatinib mesylatetreated patients with CML. Blood 107: 4250-4256.

Quintas-Cardama A & Cortes JE. (2006). Chronic myeloid leukemia: diagnosis and treatment. Mayo Clin Proc 81: 973-988.

Raanani P, Ben-Bassat I, Gan S et al. (2004). Assessment of the response to imatinib in chronic myeloid leukemia patients-comparison between the FISH, multiplex and RT-PCR methods. Eur J Haematol 73: 243-250.

Ren R. (2005). Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. Nat Rev Cancer 5: 172-183.

Reya T, Morrison SJ, Clarke MF, & Weissman IL (2001). Stem cells, cancer, and cancer stem cells. Nature. 1;414(6859):105-11. Review.

Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N et al. (2002). Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood 100: 1014 -1018.

Rowley JD. (1973) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 243(5405):290-293.

Sattler M, Mohi MG, Pride YB et al. (2002). Critical role for Gab2 in transformation by BCR/ABL. Cancer Cell 1: 479-492.

Sattler M, Verma S, Byrne CH et al. (1999). BCR/ABL directly inhibits expression of SHIP, an SH2-containing polyinositol- 5-phosphatase involved in the regulation of hematopoiesis. Mol Cell Biol 19: 7473-7480.

Sawyers CL, Hochhaus A, Feldman E et al. (2002). Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood 99: 3530-3539.

Schoch C, Haferlach T, Kern W et al. (2003). Occurrence of additional chromosome aberrations in chronic myeloid leukemia patients treated with imatinib mesylate. Leukemia 17: 461-463.

Schoch C, Schnittger S, Bursch S et al. (2002). Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia 16: 53-59.

Seong DC, Kantarjian HM, Ro JY et al. (1995). Hypermetaphase fluorescence in situ hybridization for quantitative monitoring of Philadelphia chromosome-positive cells in patients with chronic myelogenous leukemia during treatment. Blood 86: 2343-2349.

Shah NP, Nicoll JM, Nagar B et al. (2002). Multiple BCR-ABL kinase 573 domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2: 117-125.

Sinclair PB, Green AR, Grace C & Nacheva EP. (1997). Improved sensitivity of BCR-ABL detection: a triple-probe three-color fluorescence in situ hybridization system. Blood 90: 1395-1402.

Soverini S, Martinelli G, Amabile M et al. (2004). Denaturing-HPLC -based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. Clin Chem 50: 1205-1213.

Soverini S, Martinelli G, Rosti G et al. (2005). ABL mutations in late chronic phase chronic myeloid leukemia patients with upfront cytogenetic resistance to imatinib are associated with a greater likelihood of progression to blast crisis and shorter survival: a study by the GIMEMA Working Party on Chronic Myeloid Leukemia. J Clin Oncol 23: 4100-4109.

Talpaz M, Silver RT, Druker BJ et al. (2002). Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a phase 2 study. Blood 99: 1928-1937.

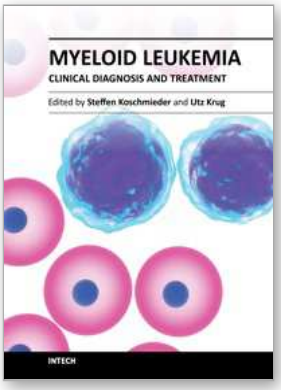
Wang JY. (2000). Regulation of cell death by the Abl tyrosine kinase. Oncogene 19: 5643-5650.

Wang L, Knight K, Lucas C & Clark RE. (2006). The role of serial BCRABL transcript monitoring in predicting the emergence of BCRABL kinase mutations in imatinib-treated patients with chronic myeloid leukemia. Haematologica 91: 235-239.

Wang YL, Bagg A, Pear W, Nowell PC & Hess JL. (2001).Chronic myelogenous leukemia: laboratory diagnosis and monitoring. Genes Chromosomes Cancer 32: 97-111.

Wei Y, Hardling M, Olsson B et al. (2006). Not all imatinib resistance in CML are BCR-ABL kinase domain mutations. Ann Hematol 85: 841-847.

Willis SG, Lange T, Demehri S et al. (2005). High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naïve patients: correlation with clonal cytogenetic evolution but not response to therapy. Blood 106: 2128-2137.



Myeloid Leukemia - Clinical Diagnosis and Treatment

Edited by Dr Steffen Koschmieder

ISBN 978-953-307-886-1
Hard cover, 296 pages
Publisher InTech
Published online 05, January, 2012
Published in print edition January, 2012

This book comprises a series of chapters from experts in the field of diagnosis and treatment of myeloid leukemias from all over the world, including America, Europe, Africa and Asia. It contains both reviews on clinical aspects of acute (AML) and chronic myeloid leukemias (CML) and original publications covering specific clinical aspects of these important diseases. Covering the specifics of myeloid leukemia epidemiology, diagnosis, risk stratification and management by authors from different parts of the world, this book will be of interest to experienced hematologists as well as physicians in training and students from all around the globe.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Michele Cea, Antonia Cagnetta, Marco Gobbi and Franco Patrone (2012). Towards the Cure of CML by the Molecular Approach Strategy, Myeloid Leukemia - Clinical Diagnosis and Treatment, Dr Steffen Koschmieder (Ed.), ISBN: 978-953-307-886-1, InTech, Available from: http://www.intechopen.com/books/myeloid-leukemia-clinical-diagnosis-and-treatment/towards-the-cure-of-cml-by-the-molecular-approach-strategy

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