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Modern Therapy of Chronic Myeloid Leukemia

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1. Introduction

Chronic myeloid leukemia is one of the most thoroughly studied and, undoubtedly, best understood neoplasms. There are about 4000 and 5000 new cases of CML every year in the USA. CML is a hematologic stem cell malignancy that typically evolves in 3 distinct clinical stages: chronic and accelerated phases and blast crisis. The chronic phase lasts several years and is characterized by accumulation of myeloid precursors and mature cells in bone marrow, peripheral blood, and extramedullary sites. The accelerated phase lasts 4 to 6 months and is characterized by an increase in disease burden and in the frequency of progenitor/precursor cells. The blast crisis lasts a few months and is the terminal phase of chronic myelogenous leukemia, characterized by the rapid expansion of a population of myeloid or lymphoid differentiation-arrested blast cells (Calabretta and Perrotti 2004; Radich 2007).

The cytogenetic hallmark of CML is the Philadelphia chromosome (Ph). It is a product of a reciprocal translocation between chromosomes 9 and 22 (t[9;22][q34;q11]). The *Bcr-abl* oncogene, responsible for the deregulated tyrosine kinase, arises out of the conjugation between the breakpoint cluster region gene (*Bcr*) on chromosome 22 and the Abelson kinase (*Abl*) gene on chromosome 9. This oncogene activates multiple signal transduction pathways such as Ras/Raf/mitogen-activated protein kinase [MAPK], phosphatidylinositol 3 kinase, STAT5/Janus kinase, and Myc (Fig. 1). The Bcr-Abl tyrosine kinase activity leads to uncontrolled cell proliferation and significantly reduced apoptosis and so starts the malignant expansion of pluripotent stem cells in the bone marrow. The Wnt/ β -catenin pathway was also found to be critical for the evolution to blast crisis as far as its activation was observed in primary cell samples from patients with CML. Most of the pathways activated by Bcr-Abl are known, but the pathways downstream Bcr-Abl that are critical for oncogenesis and transformation are yet not well understood. For example, several transcription factors like Jun B, MZF1

and δ EF1 appear to be involved in CML progression. Jun B has been shown to be down-regulated in CML progression. The transcription factors MZF1 and δ EF1 that play key role in hematopoietic stem cell differentiation, including modulation of CD34 and *cMyb* expression, are also deregulated during CML progression (Calabretta and Perrotti 2004; Steelman, Pohnert et al. 2004; Jabbour, Cortes et al. 2007; Radich 2007; Druker 2008; Roychowdhury and Talpaz 2011). All these findings reflect the recent knowledge about the molecular disease pathogenesis and are platform for translating bench research into clinical application of target-driven therapeutic strategies.

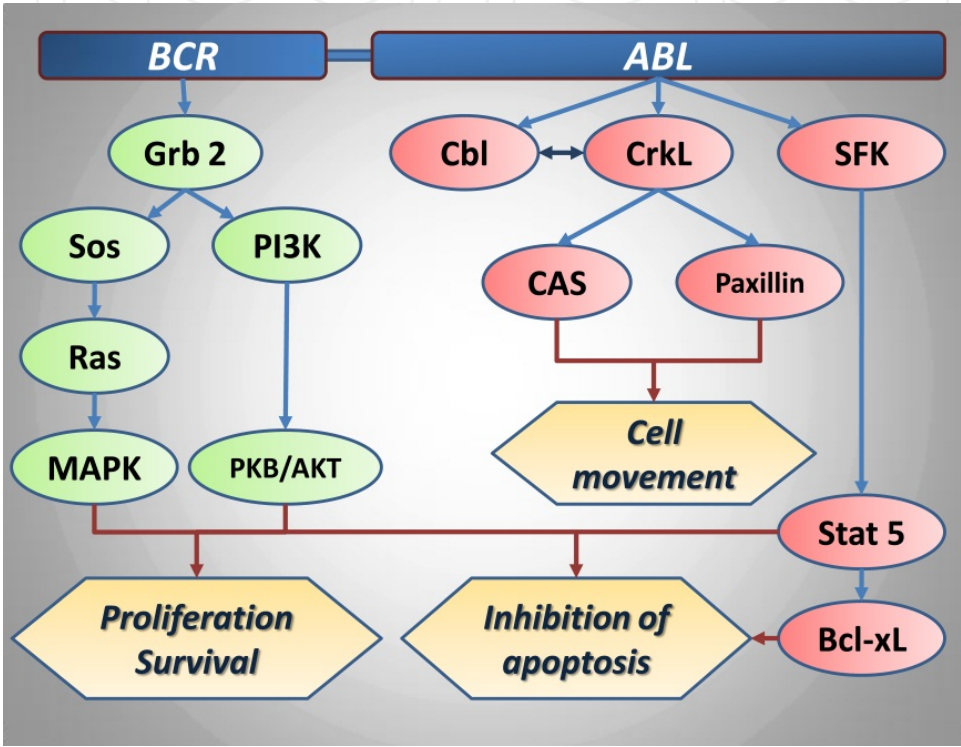


Figure 1. Pathways activated by the fusion oncoprotein Bcr-Abl. The RAS pathway becomes constitutively activated by mechanisms involving the interaction of Bcr-Abl with the growth factor receptor-binding protein (Grb-2)/Gab2 complex. This leads to enhanced activity of the guanosine diphosphate/guanosine triphosphate (GDP/GTP) exchange factor Sos, which promotes the accumulation of the active GTP-bound form of Ras. Bcr-Abl interacts also indirectly with the p85 regulatory subunit of PI3K via various docking proteins including GRB-2/Gab2 and c-cbl. Activation of the PI3K pathway triggers an Akt-dependent cascade that has a critical role in BCR/ABL transformation by regulating the subcellular localization or activity of several targets such as BAD, MDM2, I κ B-kinase α , and members of the Forkhead family of transcription factors. Another signaling pathway activated by BCR/ABL is that dependent on signal transducer and activator of transcription 5 (STAT5). The consequence of this activation is inhibition of apoptosis and enhanced survival.

Treatment of this disease improved dramatically with the development of tyrosine kinase inhibitors (TKIs). Recently, the modern therapy of CML includes the use of TKIs (first and second generation), stem cell transplantation and clinical trials with novel agents such as novel multiple kinase inhibitors, Aurora kinase inhibitors, arsenic trioxide, hystone deacetylase inhibitors, proteasome inhibitors, other semi-synthetic drugs (Fig. 2). Imatinib is the golden standard in CML therapy and serves as first-line drug of choice in the chronic phase of CML

(CP-CML) whereas second generation TKIs are taken in consideration after appearance of resistance to imatinib due to Bcr-Abl mutations. According to de Lavallade et al. only 62,7% of the patients treated with imatinib achieved complete cytogenetic response (CCyR) and up to 5% of the patients present with advanced disease are poorly responsive to TKIs in general (de Lavallade, Apperley et al. 2008; Roychowdhury and Talpaz 2011). In other words, about one third of CML patients develop intolerance or resistance to TKIs and therefore they need alternative therapies.

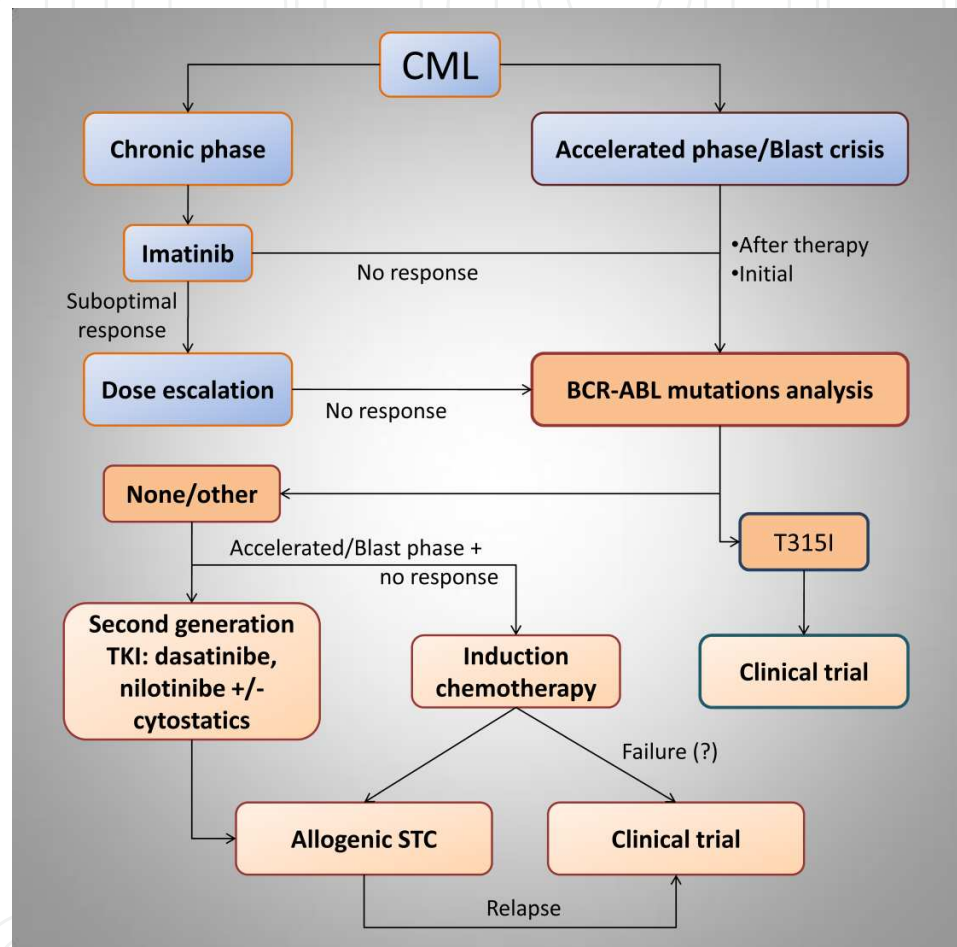


Figure 2. Clinical algorithm for CML therapy: chronic, accelerated and blast phase (adapted from Roychowdhury et al., 2011, 25(6): 279-90).

2. Tyrosine-kinase inhibitors

Imatinib, dasatinib and nilotinib are today the three clinically applied tyrosine kinase inhibitors of the fusion oncoprotein Bcr-BL for the treatment of CML and Ph (+) ALL (Fig. 3, Table 1). The constitutively active Bcr-Abl tyrosine kinase functions by transferring a phosphate group from ATP to tyrosine residues on various substrates (signaling molecules) to cause

excess proliferation of myeloid cells typical for CML. Imatinib and the other two tyrosine kinase-inhibitors block the binding of ATP to Bcr-Abl, thus inhibiting its kinase activity (Fig. 4). They also act on other components of the cellular metabolism and signalling, e.g. Abl and ARG (Abl-related gene), C-kit receptor (KIT), receptors for platelet growth factor alpha (PDGFR- α) and beta (PDGFR- β), receptor for colony stimulating factor 1 (c-FMS) etc. (Kantarjian, Cortes et al. 2010). All tyrosine kinase inhibitors have good gastrointestinal absorption, which makes them suitable for oral application.

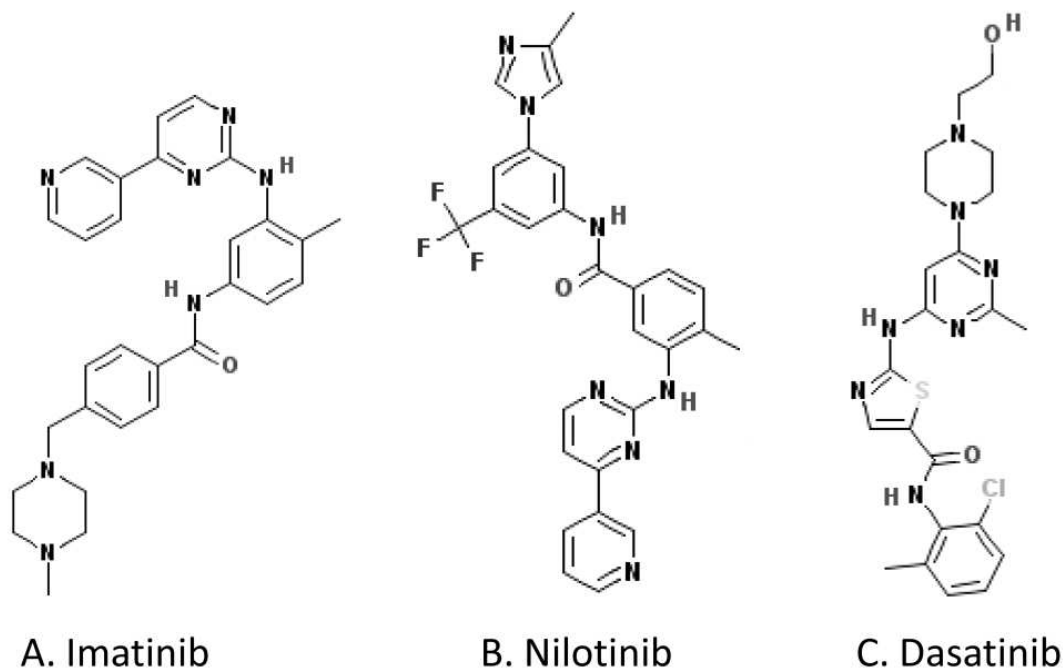


Figure 3. Chemical structures of first and second line tyrosine kinase-inhibitors.

2.1. First generation tyrosine-kinase inhibitors — Imatinib

Imatinib was introduced for clinical use in 1998 for the treatment of CML. It showed high efficacy and revolutionized the disease management. Nowadays, Imatinib is the first-line drug of choice for the treatment of patients with CML in chronic phase and Ph (+) ALL as it induces long lasting remissions and is well tolerated as compared to conventional cytostatics (Fig. 2). Imatinib stops the progression of CML at early stages (Baccarani and Dreyling 2009).

Imatinib reaches C_{\max} within 2 to 4 h. Its total bioavailability is 98%. The half-lives of Imatinib and its main metabolite, N-demethyl derivative (CGP74588), are 18 h and 40 h, respectively. Plasma protein binding (mainly albumin and α 1-acid glycoprotein) for imatinib reaches 95%. The drug is primarily metabolized by CYP3A4 and to a lesser extent by CYP1A2, CYP2D6, CYP2C9 and CYP2C19. The main active metabolite of Imatinib is one N-demethylated piperazine derivative, formed by CYP3A4 and has *in vitro* activity similar to that of Imatinib. Imatinib is a potent inhibitor of CYP2C9, CYP2D6 and CYP3A4/5, which is described in *in*

vitro studies conducted with human liver microsomes. It is eliminated with the faeces, mainly in the form of respective metabolites. Imatinib clearance in one 50 year-old-patient, weighing 50 kg is 8 L/h, while in patient weighing 100 kg, it increases to 14 L/h (Roychowdhury and Talpaz 2011).

The superiority of Imatinib against IFN α was confirmed in the third phase of the International Randomized Study of Interferon and STI-571 (IRIS). A substantial cytogenetic response was achieved in 87% of the patients receiving Imatinib after 18 months, compared to 35% in those who received IFN. Complete cytogenetic remission was observed in 76.2% of the patients treated with Imatinib, compared to 14.5% of the treated with IFN. Molecular remission with reduction of the Bcr-Abl transcripts was found in 39% of the patients treated with Imatinib, against 2% for IFN. 325 (71%) of 456 patients who had achieved complete cytogenetic remission with Imatinib sustained their remission on the sixth year of treatment. Tracking patients over the 6-year exploration period indicated that Imatinib had a favourable and long-lasting safety profile, as there were no new adverse events during the 5th and 6th year of study. The use of Imatinib as first-line drug for CML did not affect subsequent treatment of patients with allogeneic hematopoietic stem cells transplantation (Kantarjian, O'Brien et al. 2003; Deininger 2008; Baccarani and Dreyling 2009).

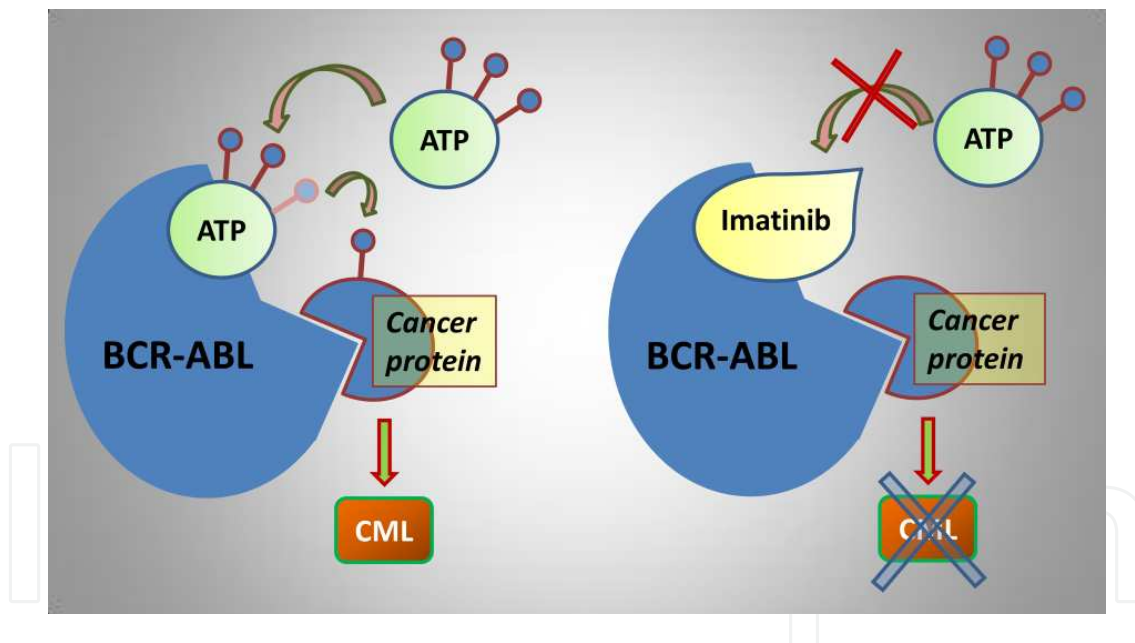


Figure 4. Mode of action of Imatinib. Imatinib blocks the ATP binding center of Bcr-Abl thus inhibiting its phosphorylation activity.

2.2. Second generation tyrosine-kinase inhibitors

2.2.1. Nilotinib

Nilotinib is a highly selective ABL inhibitor and derivative of Imatinib and can overcome some mutations that can cause imatinib resistance with the exception of T315I. During first phase

clinical trial, Nilotinib was tested at doses of 50 mg once daily to 600 mg twice daily in 119 Imatinib-resistant CML patients at various stages of the disease. 92% of the patients in chronic phase achieved complete hematologic remission. 72% of the patients in the acute phase of CML presented hematologic remission and 48% of them had a cytogenetic response. 39% of patients with CML in blast crisis demonstrated a hematologic response and 9 of them (27%) had a cytogenetic response. The results of the study defined a recommended dose of 400 mg twice daily (Table 1). Nilotinib adverse reactions include small pleural or pericardial effusions. The most common adverse effects during the study were thrombocytopenia (20% -33%), neutropenia (13% -31%), elevated bilirubin (7%), and increased serum lipase (5% -15%). Grapefruit and other food that inhibits hepatic metabolism (mainly CYP3A4 inhibitors) should be avoided. Nilotinib is not recommended for patients with prolonged QT-segment, and drugs that prolong the QT-segment should not be given during treatment with Nilotinib (Kantarjian, Giles et al. 2007; Olivieri and Manzione 2007; Weisberg, Catley et al. 2007; Melo and Chuah 2008; Giles, Rosti et al. 2010; Kantarjian, Cortes et al. 2010; Kantarjian, Giles et al. 2010; Quintas-Cardama, Kim et al. 2010).

2.2.2. Dasatinib

Dasatinib is another ABL kinase inhibitor that binds to the active conformation of the ABL-kinase domain and to the structurally related kinases of the Src-family. *In-vitro* dasatinib is 300 times more potent than imatinib against Bcr-Abl mutated blasts. Dasatinib inhibited almost all Imatinib-resistant Bcr-Abl mutants with the exception of T315I and was shown to block PDGFRs and KIT. After successful phase II clinical trials dasatinib was approved in North America and Europe and in many other countries. It should be given to patients with imatinib resistance. During the trials some severe hematologic adverse effects were observed in imatinib resistant or imatinib intolerant patients with CML, e.g. thrombocytopenia (48%), anemia (22%), neutropenia (49%), and leukopenia (27%). There were some non-hematological adverse reactions as well, e.g. pleural effusions (6%). In the phase III trials, in order to reduce Dasatinib-related toxicity while maintaining efficacy of treatment, 670 randomly selected imatinib resistant or imatinib intolerant patients with CML in the chronic phase received dasatinib in 1 of 4 dose regimens: 100 mg once daily, 50 mg twice daily, 140 mg once daily, or 70 mg twice daily. It was found that non-hematological and hematological adverse reactions are rarer at a dose of 100 mg once daily (Hochhaus, Kantarjian et al. 2007). Currently, this is the recommended dosage for patients with chronic phase CML, while the 70 mg twice daily or 140 mg once daily regimens are options for patients in the acute phase or blast crisis CML (Table 1).

Nowadays, the choice between treatment with dasatinib and nilotinib in imatinib resistant patients is made on the basis of the history of comorbidities, since no such very important comparative study of both drugs has been made so far. Therefore, patients with a history of lung disease may be more suitable for treatment with nilotinib, while patients with pancreatic disease could benefit from treatment with dasatinib. Patients bearing the T315I mutation are resistant to treatment with both dasatinib and nilotinib. Such patients should be offered the option for inclusion in clinical trials or allogeneic bone marrow transplantation (Kantarjian, Cortes et al. 2010).

1. Chronic phase of CML:			
<i>a Initial therapy:</i>			
Imatinib	400 mg	p.o.	Daily
<i>b Salvage therapy:</i>			
Dasatinib	70 mg	b.i.d.p.o.	Daily
	100 mg	p.o.	
Nilotinib	400 mg	b.i.d.p.o.	Daily
Imatinib (high-dose)	400 mg	b.i.d.p.o.	Daily
2. Accelerated phase and blast crisis of CML:			
Imatinib	400-600 mg	p.o.	Daily
Dasatinib	70 mg	b.i.d.p.o.	Daily
Nilotinib	400 mg	b.i.d.p.o.	Daily
3. Pediatric patients with CML:			
Imatinib	260 mg/m ² /d	p.o	daily
	340 mg/m ² /d	p.o..	daily

Table 1. Treatment schedule with tyrosine kinase-inhibitors.

2.3. Resistance towards TKI — Mechanisms and management

The most important problem in the target therapy of CML today is the development of resistance towards TKIs. Lucas et al. reported after a population-based study in Northern England that about 50% of the patients treated with imatinib developed intolerance or failure up to two years after initiation (Lucas, Wang et al. 2008). Responses obtained in patients with advanced disease were also not durable. CCyR at 6 years after initiating imatinib therapy is determined by 57% patients and 55% remain on the treatment after 8 years (Burke, Swords et al. 2011). Even nilotinib at best achieved CCyR in 80% of the treated patients (Rosti, Palandri et al. 2009). In summary, there is a significant minority of patients developing resistance towards imatinib and second generation TKIs. Investigators distinguish between primary and secondary resistance. Secondary treatment failure or disease relapse is characterized by the loss of already achieved complete cytogenetic or hematological response and is dependent on different factors such as age, stage of disease, duration of INF therapy and duration of response to initial therapy. In contrast, primary resistance is the failure to achieve CCyR or hematologic response and is not very well investigated and understood. Concerning leukemic cell proliferation and survival two other models of imatinib resistance could be outlined: on one hand leukemic cells became independent from the fusion oncoprotein for their survival and proliferation and, on the other hand the tyrosine kinase Bcr-Abl circumvented the inhibition by imatinib or other TKIs, e.g. appearance of the mutation T315I (Burke, Swords et al. 2011; Roychowdhury and Talpaz 2011).

The mechanisms of imatinib resistance could be classified as either Bcr-Abl dependent or Bcr-Abl independent. Bcr-Abl dependent mechanisms include Bcr-Abl amplification, kinase domain mutations and Bcr-Abl induced genomic instability. Bcr-Abl independent mechanisms are usually related to drug compliance and metabolism, drug transport, clonal evolution,

escape of the primitive progenitors from therapy and activation of alternative signal transduction pathways.

Kinase domain mutations belong to the most important mechanisms of resistance towards TKIs. They were identified in clinical studies with patients with imatinib resistance (initial or developed during the therapy) where 30% of the patients had primary resistance and 57% - secondary resistance (Shah, Nicoll et al. 2002; Soverini, Martinelli et al. 2005). Today over a 90 mutations in the Bcr-Abl fusion gene are described and they affect 57% of the amino-acid residues. Some of these mutations cause a direct steric interference to drug-enzyme binding or alter allosterically the kinase domain activity and are reported to be the main reason for treatment failure in many clinical trials. Mutations in the P-loop are assigned as poor prognostic factor. In particular, the T315I mutation accounts for the majority of secondary resistance (Burke, Swords et al. 2011). The T315I mutation is a single amino-acid substitution of isoleucine with threonine at position 315 on c-Abl. It alters the ATP-binding site of the tyrosine kinase, confers cross-resistance to nearly all clinically applied TKIs and correlates with decreased cytogenetic response and progression free survival rates. In summary, in clinical studies about 50% incidence of this mutation had been reported for patients with secondary resistance. Therefore, introduction of new drugs is substantially needed (Bradeen, Eide et al. 2006). However, there are studies reporting that kinase domain mutations were found also in patients with CCyR and in remission, but only few of them eventually developed disease relapse. It is evident that not all kinds of resistance are due to Abl-mutations and not all mutations could lead to resistance (Branford, Melo et al. 2009; Roychowdhury and Talpaz 2011). Correlations based on *in vitro* sensitivity have not been consistent in all studies. Thus Bcr-Abl induced genomic instability or unknown Bcr-Abl independent mechanisms are suspected to be also responsible for disease resistance development.

In vitro studies showed that Bcr-Abl could promote genomic instability and resistance. There are additional facts that support the hypothesis that Bcr-Abl can affect the mismatch repair activity. The fusion protein caused down-regulation of nucleotide excision repair in leukemic cell lines and promoted double-stranded DNA breaks through single strand annealing (Stoklosa, Poplawski et al. 2008; Fernandes, Reddy et al. 2009).

Pharmacokinetic characteristics of the TKIs were also demonstrated to be important for better clinical outcomes. Imatinib is substrate for the liver enzyme CYP3A4 which converts it into active metabolite. A higher enzyme activity correlated with higher rate of complete molecular responses (Green, Skoglund et al. 2010). The α -1-acid glycoprotein directly binds imatinib, thus increasing its clearance. Therefore, patients with high levels of this protein demonstrated lower plasma concentrations of imatinib which worsened the therapeutic response (Delbaldo, Chatelut et al. 2006). Through *in vitro* experiments with radio-labeled imatinib it was demonstrated that cellular mechanisms for drug transport (influx and efflux) are also important determinants of the drug sensitivity. The imatinib influx is an active process which involves the human organic cation transporter 1 (hOCT1). In a study with 56 patients it was reported that high activity of hOCT1 is associated with significantly improved overall survival and response rates (Giles, Kantarjian et al. 1999; Roychowdhury and Talpaz 2011). The P-glycoprotein (MDR1 or ABCB1) which is responsible for the drug efflux out of the leukemic cells

also mediated resistance but did not appear to be essential for the development of imatinib resistance even in primitive CD34⁺ CML progenitors *in vitro*.

Another reason for developing resistance towards targeted CML therapy is provided by the stem cell hypothesis. According to some investigators there is a reservoir of primitive progenitors that are capable of self-renewal or progression *in vitro* or *in vivo* in immunodeficient mice. Another proof for this hypothesis is the fact that patients with deep remissions in the chronic phase of CML can relapse after discontinuation of imatinib therapy. There are different biological mechanisms responsible for the behavior of the stem cell compartment. The Wnt/ β -catenin signal pathway and the Foxo transcription factors were found to be involved in the renewal of malignant and normal haematopoietic stem cells. Additionally, the Hedgehog signaling pathway seems to be important for CML stem renewal. Thus far, it is clear that CML biology involves a stem cell component, but its exact role and biological mechanisms have not yet been determined.

The strategies to overcome resistance include first optimization of the front line therapy with imatinib such as escalation of the daily dose up to 800 mg and second the introduction of second generation TKIs such as dasatinib, nilotinib (Table 1) and other new compounds that are still in clinical trials (Jabbour, Kantarjian et al. 2009; Roychowdhury and Talpaz 2011). Dasatinib and nilotinib were successfully used in patients in accelerated phase or blast crisis after Bcr-Abl mutation analysis that demonstrated other mutations than T315I. Ponatinib, a new multiple kinase inhibitor, showed high activity against T315I mutants *in vitro* and low toxicity *in vivo* during a Phase I clinical trial and is a very promising new TKI for the treatment of patients bearing the T315I mutation. There have been no randomized studies to evaluate the predictive value of kinase domain mutations for response to specific TKIs until now. However, a summary of the data can be taken from retrospective studies. Dasatinib is recommended for patients with the following mutations: Y253H, E255K/V and F359V/C. Nilotinib is beneficial for patients with F317L and V299L mutations. The side effect profile and patient's characteristics are also important for the choice of an appropriate TKI (Hughes, Saglio et al. 2009; Jabbour, Jones et al. 2009; Roychowdhury and Talpaz 2011).

After failure of imatinib and a second generation TKI, most patients could be included in ongoing clinical trials. Next, some of the novel agents being evaluated in Ph(+) leukemias are listed.

3. Experimental drugs for CML therapy

3.1. Tyrosine kinase inhibitors

3.1.1. Bosutinib

Bosutinib was developed to overcome the resistance to first and second generation TKIs and is still in clinical trials. It was shown to possess a high antiproliferative activity *in vitro* and in xenografts against most of the Bcr-Abl mutants except T315I. It is a dual Abl and Src kinase

inhibitor and binds to both active and intermediate conformations of the fusion oncoprotein Bcr-Abl. Bosutinib was found to inhibit the proliferation of CML progenitors about 200 times better than imatinib. However, it was not able to eliminate all mutant populations and was moderately effective in inducing apoptosis. The Phase II studies with bosutinib showed that about 36% of all patients in chronic phase, 22% in accelerated phase and 9% in blast crisis achieved complete cytogenetic responses. Preliminary data indicate that bosutinib was well tolerated and the most common adverse events were gastrointestinal discomfort and grade 3-4 myelosuppression which occurred in the advanced disease phases (Melo and Chuah 2008; Roychowdhury and Talpaz 2011).

3.1.2. Other experimental tyrosine kinase inhibitors

There are also new types of compounds inhibiting pathobiochemical pathways, resulting from the *bcr-abl* oncogene. These allosteric inhibitors use a newly described allosteric, non-ATP competitive mechanism, potentially involving binding to the myristate pocket in the C-lobe of the Bcr-Abl kinase domain. The most promising of these compounds is the GNF-2. It has practically no activity against most kinases, including Kit, PDGFR and SFK. GNF-2 inhibited the growth of cells with the Y253F and E255V mutations, but not the other P-loop mutants, the T315I or F317L mutants. Another promising new agent is INNO-406 (Bafetinib) which is Abl/Lyn kinase inhibitor and is about 55 times more potent than imatinib *in vitro*. It has been shown to inhibit numerous Bcr-Abl mutants except T315I. The drug underwent a Phase I study in patients with resistance to imatinib. It was well tolerated and 2 of 7 patients achieved complete cytogenetic response. The novel multiple kinase inhibitor, the purine derivative AP24534 (ponatinib) inhibits FLT3, Src kinases, Bcr-Abl and multiple Bcr-Abl mutants, including the T315I mutation. During the preclinical studies it was evidenced that ponatinib is highly effective mutant Bcr-Abl inhibitor *in vitro* and *in vivo* in mouse models. Interestingly, no dose-limiting toxicity was found in a dose-escalating Phase I clinical trial. Ponatinib was effective in patients who had failed to respond to all other approved therapies, including patients with the T315I mutation of the fusion oncoprotein Bcr-Abl. Therefore ponatinib is fastly moving to phase II studies in patients with Ph+ leukemias.

Other new TKIs are the SFK/Abl kinase inhibitor, the anilino-quinazoline AZD0530; the purine derivatives AP23464 and its analogue AP23848; the pyrido-pyrimidines, PDI66326, PDI73955 and PDI180970; the pyrazolo-pyrimidines, PP1 and PP2; the acetylans AC22 and K1P. These compounds have not been developed for clinical use yet (Melo and Chuah 2008; Bixby and Talpaz 2009; Burke, Swords et al. 2011).

3.2. Aurora kinase inhibitors

The Aurora kinase family consists of serine-threonine kinases that are crucial for different stages of the mitosis. There are two family members Aurora A and B and are overexpressed in some neoplasias. Following Aurora kinase inhibitors are in pre-clinical and clinical evaluation: PHA-739358 (danusertib), AT9283, MLN8237XL-228, KW-2449 and MK-0457. Danusertib showed safety profile and efficacy in patients bearing the T315I mutation in phase I clinical study. AT9238 is a multi-kinase inhibitor which also inhibited cells with the T315I mutation.

It was well-tolerated during phase I clinical trial and showed promising anti-leukemic activity (Cortes-Franco, Dombret et al. 2009; Howard, Berdini et al. 2009; Moore, Blagg et al. 2010; Tanaka, Squires et al. 2010; Burke, Swords et al. 2011).

3.3. Heat shock protein 90 inhibitor

As a molecular chaperone that interacts with various proteins (Raf, Akt, FLT-3 and Bcr-Abl) Hsp90 maintains those proteins in a stable and functional conformation. Geldanamycin and its derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG) bind to the ATP-binding pocket of Hsp90 and inhibit its chaperone activity. This leads to downregulation of Bcr-Abl and also induction of apoptosis in CML cell lines. Geldanamycin and 17-AAG inhibited the cell growth of some mutant lines (E255K and T315I). Hsp90 has its limits and there are some cross-resistant types. Combination therapy with imatinib and 17-AAG led to synergistic inhibition of growth and induction of apoptosis in cross-resistant cell lines but not in the imatinib-sensitive counterparts. 17-AAG may also block the imatinib efflux (Melo and Chuah 2008; Burke, Swords et al. 2011).

3.4. Arsenic trioxide

Another compound that induces apoptosis in Bcr-Abl-positive cell lines and reduces proliferation of CML blasts without affecting the CD34⁺ progenitors, is the arsenic trioxide (As₂O₃). The combination of As₂O₃ with imatinib exerted additive to synergistic effect. This combination induced cell death in imatinib-resistant cell lines with overexpressed Bcr-Abl or bearing M351T or Y253F mutations, but it does not affect the T315I mutants (Melo and Chuah 2008; Roychowdhury and Talpaz 2011).

3.5. Homoharringtonine

Homoharringtonine (HHT), a by-product of a plant alkaloid, inhibits protein synthesis and induces apoptosis. The combination of HHT with imatinib is synergistic or additive on CML derived cell lines. Omacetaxine and chemgenex are semisynthetic HHT derivatives that combined with imatinib showed promising activities. Omacetaxine is now in phase II trials with TKIs-resistant patients with or without the T315I mutation (Melo and Chuah 2008; Burke, Swords et al. 2011).

3.6. Histone deacetylase inhibitors

Histone deacetylases (HDAC) are the catalysts in deacetylation of lysine residues at the amino termini of core nucleosomal histones. Histone deacetylase inhibitors (HDI) such as suberoylanilide hydroxamic acid (SAHA, vorinostat), generate hyperacetylated histones, causing transcriptional upregulation of the cyclin-dependent kinase inhibitor, p21, cell-cycle arrest and apoptosis in tumor cells. SAHA also induces expression of a key cell-cycle regulator p27, and its application is associated with downregulation of the p210 Bcr-Abl protein. There is a synergistic interaction between SAHA and imatinib on CML cell lines. The mentioned combination induces apoptosis in imatinib-resistant CML cell lines as well. The co-treatment with

nilotinib and the HDI LBH589 (panobinostat) was very effective in inducing apoptosis in K-562 and LAMA-84 CML cell lines. LBH589 showed efficacy in imatinib-resistant cell lines bearing the T315I and E255K mutations and this was associated with depletion of Bcr-Abl. A published study showed that when combined with imatinib, the HDI valproate can increase the antileukemic efficacy and sensitize imatinib-resistant CML cells (Kantarjian, O'Brien et al. 2003; Melo and Chuah 2008; Burke, Swords et al. 2011).

3.7. Proteasome inhibitors

Proteasomes are responsible for the degradation of different cellular proteins. The proteasome inhibitor bortezomib was shown to inhibit proliferation, to stop the cell cycle in the G₂/M phase and to promote apoptosis in imatinib-sensitive and imatinib-resistant CML cell lines. Co-treatment with bortezomib and imatinib isn't recommended because there are some antagonistic interactions. However, if a low dose bortezomib exposure of CML cell lines is followed by imatinib, there are some additive effects. Synergistic interactions between bortezomib and the HDI SAHA are reported, and between bortezomib and flavopiridol in *in vitro* as well (Melo and Chuah 2008).

3.8. Semi-synthetic drugs

Semi-synthetic drugs flavone and flavopiridol are going through clinical trials. They target multiple cyclin-dependent kinases. Flavopiridol showed a very promising activity in combination with imatinib for inducing apoptosis in Bcr-Abl-positive CML cell lines (Melo and Chuah 2008).

3.9. Farnesyl Transferase Inhibitors (FTIs)

Current FTIs under investigation and with a potential as antileukemic agents are tipifarnib and lonafarnib. They are inhibitors of the Ras-MAPK signal pathway which was shown to couple to Bcr-Abl through protein-protein interactions and to play a central role in leukemogenic transformation. Tipifarnib is in Phase II trial involving 22 patients with CML. Complete or partial hematological response was achieved in 32% of the patients. The combination imatinib and tipifarnib was well tolerated and active in patients with imatinib-resistant CML – a partial cytogenetic response was achieved in patients harboring the T315I mutant. Lonafarnib is a selective inhibitor of primary progenitor cells derived from CML patients. It reduced colony formation of progenitor cells and showed activity in imatinib-resistant CML cell lines. However, the reports from a pilot study demonstrated that only 2 of 13 patients achieved a clinical response.

3.10. Raf-1 inhibitors

Sorafenib (BAY 43-9006) is a multi kinase inhibitor of the RAS/Raf pathway which is involved in leukemogenesis downstream from Bcr-Abl. Drug concentrations of 5-10 μ M were found to induce apoptosis via the mitochondrial pathway in imatinib-resistant cell lines. The combination between sorafenib and vorinostat (HDAC inhibitor) triggers cell dysfunction through

Mcl-1 downregulation and p21 inhibition. Sorafenib is approved for the treatment of hepatocellular and renal cancers and Phase I and Phase II trials are carried out in CML patients (Burke, Swords et al. 2011).

3.11. MEK inhibitors

CI-1040 was the first MEK inhibitor which entered a clinical trial in CML patients. It has been studied in combinations with imatinib, dasatinib, HDAC inhibitors, arsenic trioxide and HSP 90 inhibitors. The last two combinations were tested with a positive effect in patients with T315I mutation. Because of the challenging pharmacokinetic properties, clinical advancement is unlikely, but a derivative (PD0325901) is under development (Burke, Swords et al. 2011).

3.12. mTOR inhibitors

The protein kinase, mTOR (mammalian target of rapamycin), is a downstream mediator in the PI3K/Akt pathway which controls cell growth and survival. Rapamycin (sirolimus) is the prototype compound of this group, but it has poor aqueous solubility and chemical stability, limiting its clinical usefulness. However, in a small clinical trial four out of six patients with imatinib-resistant disease responded to oral rapamycin. Rapamycin also significantly inhibited the cell growth in Ph⁺ cell lines with or without the T315I mutation. The combination between imatinib and another mTOR inhibitor, everolimus, was associated with an increased expression of c-Abl and inhibition of Bcr-Abl. In the presence of inhibited Bcr-Abl, c-Abl enters the nucleus and modulates apoptosis. A Phase I trial with imatinib and everolimus has been completed while a study with temsirolimus is currently in accrual (Burke, Swords et al. 2011).

4. Interferons

Before the era of TKIs and since the 1980s interferon- α had constituted the first-line therapy for CML patients in the chronic phase of the disease. Interferon induced long-lasting remissions in up to 80% of the patients with complete cytogenetic response. After interferon therapy they had disease-free survival beyond 10 years. There are some clinical trials that showed successful combinations between TKIs and interferon which provided an adjunct immunologic response during induction or maintenance therapies. Treatment with interferon after imatinib was shown to be followed by a possible remission status (Burchert, Muller et al. 2010; Roychowdhury and Talpaz 2011). However, additional data are required in order to clarify the role of interferon in conjunction to the TKI therapy.

5. Stem cell transplantation

Allogeneic stem cell transplantation (ASCT) was first line therapy for CML patients for many years. In the imatinib era, however, ASCT is becoming second or even third option for these patients if hematological, cytogenetic or molecular remission with imatinib is not achieved

after 3, 12 and 18 months, respectively (Baccarani and Dreyling 2009). ASCT is still the only treatment that offers a definitive cure. The risks of ASCT are some mortality rate, graft-versus-host disease (GVHD), potentially lethal acute or chronic infections death and risk of second malignancy. There are no data about negative influence on ASCT of pre-treatment with tyrosine-kinase inhibitors. In a single institution in the USA between 1995 and 2000 131 CP CML patients underwent allogeneic SCT with bone marrow or peripheral blood from related donors. In the 3 year long period, the probability of disease-free survival was 78%. The survival and disease recurrence rates were estimated at 86% and 8% respectively. The Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT) published own data. In the period between 2000 and 2003 3018 patients were treated with ASCT for CML. The 2-year survival rate was 61%, the transplant-related mortality rate was 30%, and the rate of disease recurrence was 22%. Better results were observed in patients who underwent ASCT at the time of first CP using as a donor an HLA-identical sibling. The 2-year survival rate in this case was 74%, transplant-related mortality rate 22% and disease recurrence rate 18%. This confirms the fact that the outcome of ASCT is highly dependent on risk factors. EBMT study showed that favorable factors are sibling donor, treatment at early stage of the disease, under 12 months after diagnosis, and younger age of the patient (age under 20 years is better than 20-40 years, and above 40 the risks are higher). If successful, ASCT can lead to long-lasting results. In a 10-year study of patients transplanted with an allogeneic bone marrow from siblings, the mean time of hematologic or cytogenetic disease recurrence was 7.7 years and 46% of the long-term survivors never developed disease recurrence.

One of the main problems standing in front of ASCT is that most of the patients don't have a suitable HLA-matched sibling. National Marrow Donor Program institutions in the U.S. conducted a study in the period between 1988 and 1999 that compared results from 2464 unrelated donor bone marrow transplantations with 450 HLA-identical sibling donor transplantations. The results from this study confirm that patients transplanted with bone marrow from a non-relative donor have greater risk of complications. However it is important to mention that data from this study didn't show a significant difference in the 5-year survival rate between the two types of donors if the transplantation was made within 1 year after diagnosis (Jabbour, Cortes et al. 2007).

6. Conclusion

Imatinib is now the most common first line drug for the treatment of CML and is a hallmark of target drug therapies for malignant diseases. However resistance to this drug is a major problem that can't be overcome by increasing the dosage (Jabbour, Kantarjian et al. 2007; O'Hare, Eide et al. 2007). Single agent therapy with imatinib may not be the best long-term option in many of the CML patients and other approaches should be considered. There are many novel compounds that are in development and in preclinical and clinical trials, some of them showed very promising results. Dasatinib, nilotinib and bosutinib are representatives of the newer generation TKIs which are effective and safe to use in imatinib-resistant and/or -intolerant CML patients. It is very likely that new Bcr-Abl mutants will become resistant to

these small-molecule inhibitors. Therefore, other therapeutic approaches are required. The combination of TKIs with other inhibitors of non-Bcr-Abl targets is needed to overcome the resistance.

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