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New drugs to overcome meccanisms of resistance in Ph+ leukemia: bosutinib

A B S T R A C T

The outcome for adult with Ph+ leukemias (ALL and CML) has improved dramatically with current therapy including use of Tyrosine Kinase Inhibitors (TKIs), such as imatinib, nilotinib or dasatinib. The complete hematological remission is obtained in about 98% of early chronic phase CML patients treated with TKIs. But the emergence of resistance to imatinib has become a significant problem: the most common cause of imatinib resistance is the selection of leukemic clones with point mutations in the Abl kinase domain. These mutations lead to amino acid substitutions and prevent the appropriate binding of imatinib. These is an unexpected event in about 4% of CML patients, during first year of TKI therapy. Current approaches to risk classification based not only on well-established clinical parameters such as Sokal's Score for CML, but including genetic lesions of acute Ph+ leukemia cell at diagnosis, as well as early response parameters are proposed. Several novel agents have been developed showing efficacy in overcoming imatinib resistance: new therapeutic approaches that interfere specifically with mutated forms of Ph+ leukemias and activate specifically the apoptotic pathway on leukemic blast cells are now available, such as Dasatinib, Nilotinib, Bosutinib and we highlights the latter as that may be applicable to the treatment of adult Ph+ leukemias.

Overview of emerging mechanisms of therapy resistance at genomics level in Ph+ leukemias

The Philadelphia chromosome,[1] a translocation arising from chromosomes 9 and 22,[2] was the first defined cytogenetic abnormality recognized as linked to both chronic myeloid leukemia (CML) and Ph+ acute lymphoblastic leukemia (ALL). This translocation fuses the Abelson (*ABL1*) oncogene on chromosome 9 to a breakpoint cluster region (*BCR*) from chromosome 22. It generates the constitutively activated BCR-ABL tyrosine kinase, which is responsible for both acute and chronic diseases.[3-5] In CML, a p210BCR-ABL isoform is initially expressed in hematopoietic stem cells (HSCs) capable of giving rise to both differentiated myeloid and lymphoid progeny, whereas in de novo Ph<ALL, the expression of either of two alternative p185 and p210 isoforms is restricted to the B-cell lineage.[6] CML typically presents as an indolent myeloproliferative disease (so called chronic phase or CML-CP) which, if untreated, invariably evolves to blast crisis (CML-BC), in which poorly differentiated malignant

myeloid or lymphoid blast cell became resistant to any therapy approach. BCR-ABL expression increases during disease progression, and promotes the acquisition of additional genetic changes (genomic instability) that are essential for the expansion of clones with greater malignant potential.[7]

From a clinical perspective, *de novo* Ph+ ALL resembles CML lymphoid blast crisis, but without a preceding chronic phase. Although they are triggered by BCR-ABL tyrosine kinase, CML-CP and Ph+ ALL clearly differ in their aggressiveness and response to therapy. Ph+ ALL is associated with rapid response to treatments but with frequent relapse and with poorer outcome, regardless of the therapeutic modalities used in treating these patients. Rare in children (5%) but common in adults (35%), these forms of leukemia are associated with poor prognosis in both age groups.[8-9] Drugs that target and inhibit the BCR-ABL kinase have revolutionized the treatment of CML. Imatinib (Gleevec) was the first such FDA-approved drug and has been considered as a prototype example for rational targeted therapy in cancer, since long-term remissions are achieved in virtually all CML-CP patients who are continuously treated.[6,10,11] However, CML Ph+ patients still harbor leukemic stem cells, since those who terminate therapy almost invariably restart disease. A small percentage of treated patients relapse (about 5% in the first two year and fewer thereafter)[12-13] and, in general, most harbor leukemic clones that express mutant forms of BCR-ABL to which imatinib no longer binds.[14] The advent of broader spectrum and more potent kinase inhibitors such as dasatinib (Sprycel) or nilotinib (Tasigna), or bosutinib (SKI606) "covers" most mutant forms of BCR-ABL and can reinduce remissions in many CML patients who fail imatinib therapy.[15-18] Treatment of Ph+ ALL patients with these same drugs typically induces high rate of hematological and cytogenetic responses.19 Significant advances and clinical responses have been made since the discovery of the selective ABL tyrosine kinase inhibitors (TKIs): whereas the outcome with standard chemotherapy was previously dismal, incorporation of imatinib mesylate into front-line therapy has improved relapse-free and overall survival. Unfortunately, in most cases these responses are rapidly lost despite persistent treatment.

The adult Ph+ leukemias cells: cytogenetic and molecular characteristics

Ph+ Acute Lymphoblastic Leukemia cells (blasts) contain genetic abnormalities, acquired somatically. These provide insight into pathogenesis and strongly influence prognosis. Additional cytogenetic abnormality to Ph1 chromosome or complex Ph+ karyotyping is presents in approximately one third of cases of adult leukemias. Overexpression of the *BCR-ABL* fusion gene, due to double Ph+ chromosome, activates a number of downstream signaling pathways involving Ras/Raf/mitogen activated protein kinase and Jak-STAT (Janus kinase signal transducer and transcription activator of transcription). Development of growth factor–independent malignant clones ensues, contributing further to the progression of the disease.

Clinical relapse is frequently associated with a bcr-abl kinase domain point mutation

In Ph+ ALL about 50-80% of the patients who achieved a CR with imatinib relapsed within one year, relapse being frequently associated with a BCR-ABL kinase domain point mutation. Soverini et Al reported a high rate of BCR-ABL mutations which have been recognized in resistant Ph+ patients13 In all these

patients additional or different mechanisms of resistance to TKI therapy have been suggested. These mechanisms of acquired resistance are predominantly unknown but additional mutations or genomic deletions located "downstream" from the BCR-ABL kinase could contribute to more aggressive disease and to the reduced therapeutic response. What might these additional mutations be, and how might they contribute to disease have recently been investigated (aggiungere reference). Treatment outcome is dependent not only on the therapy applied, but importantly, also on the underlying biology of the tumor and the host: each of these variables must be factored into initial treatment decisions, as well as later refinements based on initial response, and several biological features.

Second generation ATP-competitive bcr-abl inhibitors

Since point mutation are the major mechanism of resistance to first line imatinib therapy in Ph^+ leukemia a different group of drug active on mutant bcr-abl variant have been developed and tested at clinical level.[20-28]

Nilotinib. The substitution of N-methylpiperazine moiety with alternative binding groups, pilot to the discovery of a more potent compound, nilotinib (AMN107, Tasigna; Novartis).[20] Nilotinib does not inhibit the activity of Src-family kinases (SFK) but maintains the inhibitory activity on Arg, Kit, and platelet-derived growth factor receptor (PDGFR). Nilotinib is 10-50 times more potent than imatinib in inhibiting the autophosphorylation of wild-type Bcr-Abl cell lines and most of the Bcr-Abl mutants, except the T315I mutant. It is superior to imatinib in prolonging the survival of mice transplanted with wild-type Bcr-Abl, the M351T and E255V mutants. Results from phase II clinical trials with nilotinib are summarized in Figure 1. Nilotinib is well tolerated and common adverse events included grade 3-4 myelosuppression, elevated bilirubin and lipase levels (Table 1).

Table 1. Comparison of rate of hematological response in early CP CML patients treated upfront with Nilotinib, Dasatinib or 400 mg/d or 800 mg/d with imatinib. (data from Jorge Cortes *et al.*, MD Anderson Cancer Center, ASH 2007).

	Percent with CCyR (no. of patients evaluable)			
Months on therapy	*Dasatinib*	*Nilotinib*	*Imatinib 400 mg*	*Imatinib 800 mg*
3 months	79%(33)	95%(22)	37%(49%)	62%(202)
6 months	94(32)	100(13)	54(48)	82 (199)
12 moths	100(824)	100(11)	65(48)	86(197)

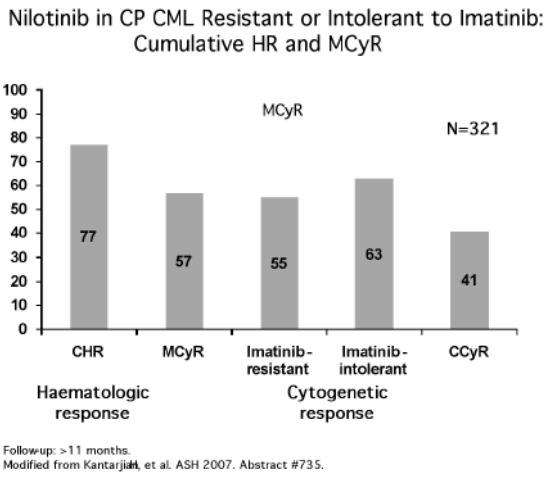

Figure 1. Rate of complete of hematological, cytogenetic and major molecular remission in CML chronic Phase patients after imatinib failure treated with Nilotinib.

Nilotinib is now in phase 2 investigational trial as frontline therapy in early CP CML (Figure 1).

Dual Src-family kinase/Abl kinase inhibitors

Dasatinib

Dasatinib (BMS-354825, Sprycel; Bristol-Myers Squibb) is a multitargeted kinase inhibitor of Bcr-Abl, SFK, ephrin receptor

kinases, PDGFR and Kit. Dasatinib is more potent than imatinib. It is effective against the imatinib-resistant active conformation of the kinase domain and it inhibits the proliferation and kinase activity of wild type and mostly of Bcr-Abl mutant cell lines. However, as nilotinib it is ineffective against the BCR-ABL T315I mutant. Phase II clinical trials of dasatinib in imatinib-resistant and -intolerant CML have established its efficacy, and the hematologic and cytogenetic responses are summarized in Figure 2. Data presented at the ASH meeting 2007, show that responses are durable in chronic phase (CP) patients with 59% and 49% achieving a major and complete cytogenetic response respectively, after a median follow-up of 15.2 months. Dasatinib is well tolerated with only rare grade 3-4 myelosuppression in the advance phases. Resistance to dasatinib is also an emerging problem mostly due to emerging and selection of pre-existing T315I or T317 mutant.[13]

Bosutinib

Bosutinib (SKI-606; Wyeth) is an orally available, src/Abl kinase inhibitor with minimal activity against PDGFR and c-Kit. An open-label study in pts with Ph+ AP (Accelerated Phase) or BP (Blast Phase) CML and ALL (Acute Lymphoblastic Leukemia) who failed prior imatinib ± other TKI therapy, is currently ongoing. It has potent antiproliferative activity against imatinib-sensitive and -resistant Bcr-Abl-positive cell lines, including the Y253F, E255K and D276G mutants: still and again it is ineffective on T315I mutant. Despite Bosutinib inhibit the proliferation of CML progenitors, it is only slow effective in inducing apoptosis or to eliminate the primitive, quiescent population. Early results from phase II studies have demonstrated its efficacy and are summarized in Table 2. Data for 72 pts (32 AP CML, 23 BP CML and 17 Ph+ ALL) was reported by Gambacorti-Passerini *et al.*[21]

Table 2.

Response	*Patients Exposed to Imatinibonly*		*Patients Exposed to Imatinib and other TKIs*	
	Patients, n (%)		*Patients, n (%)*	
	AP	*BP+ALL*	*AP*	*BP+ALL*
Hematologic response				
Evaluable	8	13	9	14
Complete	4(50)	2(15)	1(11)	1(7)
Major (complete+ No evidence of leukemia)	7(88)	2(15)	1(11)	1(7)
Cytogenetic response				
Evaluable	9	11	6	10
Complete	2(22)	1(9)	0	2(20)
Major (complete+partial)	4(44)	2(18)	0	2(20)
Molecular response				
Evaluable	8	14	7	15

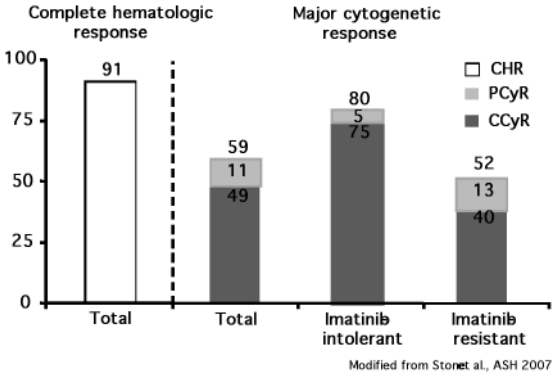

Figure 2. Rate of complete of hematological, cytogenetic and major molecular remission in CML chronic Phase patients after inmatinib failure treated with Dasatinib 70 mg/BID.

With a median follow-up of 6.1 wks, 18% achieved CHR and with a follow-up of 7.6 wks, 22% achieved a major cytogenetic response. 27/53 (51%) evaluable patients had 13 different Bcr/Abl mutations including 5 patients with T315I. Complete hematologic response occurred in 1/3 (33%) patients with P-loop, 2/15 (13%) with non-P-loop, and in 5/18 (28%) patients with no mutation. MCyR

occurred in 1/2 (50%) of patints with P-loop, 3/12 (18%) with non-P-loop, and in 4/10 (40%) with no mutation.

Only patients harboring T315I were consistently resistant to bosutinib. Bosutinib was also effective in CP patients previously treated with dasatinib or nilotinib with 38% achieving a complete haematologic response (CHR) and 25% a major cytogenetic response (MCyR). Bosutinib has a more favorable toxicity profile than Dasatinib with adverse events related to gastrointestinal toxicity and grade 3-4 myelosuppression only in the advanced phases. The most common treatment emergent adverse events (TEAEs) were diarrhea (61%), nausea (43%) and vomiting (38%); usually grade 1/2, manageable, and resolved after 3-4 weeks. Grade 3-4 hematologic laboratory abnormalities included thrombocytopenia (71%), neutropenia (46%) and anemia (32%).

Conclusions

In summary, new genes and new mechanism of resistance to imatinib have been identified in CML and ALL Ph$^+$ leukemia patients that may significantly contribute to the refinement of risk classification in Ph$^+$ acute lymphoblastic leukemia and CML and which may be further developed as diagnostic and therapeutic targets. These new findings highlight specific recognizable differences between Ph$^+$ ALL and CML and suggest that recurrent gene copy number losses affecting B-cell differentiation are universal in Ph$^+$ ALL. The aggressive nature of these BCR-ABL-induced malignancies calls for treatment by potent second generation tyrosine kinase inhibitors that are anticipated to more efficaciously prevent the emergence of mutant clones.

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