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 (ABL) 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.\(^3,4\) 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.\(^5\) 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

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

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.
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.5,10,11 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 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
case resistance to TKI therapy have been suggested.
These mechanisms of acquired resistance are
predominantly unknown but additional muta-
tions or genomic deletions located “down-
stream” from the BCR-ABL kinase could con-
tribute 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 underly-
ing biology of the tumor and the host: each of
these variables must be factored into initial
treatment decisions, as well as later refine-
ments based on initial response, and several
biological features.

Second generation ATP-competitive bcr-abl
inhibitors

Since point mutation are the major mecha-
nism 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-methylpiper-
azine moiety with alternative binding groups,
pilot to the discovery of a more potent com-
 pound, nilotinib (AMN107, Tasigna; Novartis).20
Nilotinib does not inhibit the activ-
ity 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 ima-
tinib 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 sur-
vival 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 tol-
erated and common adverse events included
grade 3-4 myelosuppression, elevated bilirubin
and lipase levels (Table 1).
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

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).

<table>
<thead>
<tr>
<th>Months on therapy</th>
<th>Dasatinib 400 mg</th>
<th>Nilotinib 800 mg</th>
<th>Imatinib 400 mg</th>
<th>Imatinib 800 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>79%(33)</td>
<td>95%(22)</td>
<td>37%(49%)</td>
<td>62%(202)</td>
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<tr>
<td>6 months</td>
<td>94(32)</td>
<td>100(13)</td>
<td>54(48)</td>
<td>82 (199)</td>
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<tr>
<td>12 months</td>
<td>100(824)</td>
<td>100(11)</td>
<td>65(48)</td>
<td>86(197)</td>
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Figure 1. Rate of complete of hematological, cytogenetic and major molecular remission in CML chronic Phase patients after imatinib failure treated with Nilotinib.
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>
<thead>
<tr>
<th>Table 2.</th>
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<tr>
<td>Response</td>
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<tr>
<td><strong>Hematologic response</strong></td>
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<tr>
<td>Evaluable</td>
</tr>
<tr>
<td>Complete</td>
</tr>
<tr>
<td>Major (complete+No evidence of leukemia)</td>
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<tr>
<td><strong>Cytogenetic response</strong></td>
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<tr>
<td>Evaluable</td>
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<tr>
<td>Complete</td>
</tr>
<tr>
<td>Major</td>
</tr>
<tr>
<td>(complete+partial)</td>
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<tr>
<td><strong>Molecular response</strong></td>
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<tr>
<td>Evaluable</td>
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</table>

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 patients 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.

**References**


