The biology of chronic myeloid leukaemia

Graduate Program
Course: Biology of cancer
April 2007

Myeloproliferative disorders

- Clonal disorders of haemopoiesis that arise in a haemopoietic stem cell or early progenitor cell.
- They are characterised by the dysregulated production of a particular lineage of mature myeloid cells.
- They exhibit a variable tendency to progress to acute leukaemia.

Myeloproliferative disorders

- Chronic Myeloid Leukaemia
- Polycythaemia
- Idiopathic Thrombocytosis
- Myelofibrosis
Chronic Myeloid Leukaemia (CML)

- CML is classified as clonal myeloproliferative expansion of transformed, primitive haemopoietic progenitor cells.
- It involves myeloid, monocytic, erythroid, megakaryocytic, B-lymphoid and occasionally T-lymphoid lineages.

CML: Clinical manifestations (1)

- Frequency: 15%-20% of cases of leukaemia in adults.
- Annual Incidence: 1-2 cases / 100,000, with a slight male predominance.
- Median age: 45-50 years
- Risk factors: Ionizing radiation.
CML: Clinical manifestations (2)

<table>
<thead>
<tr>
<th>Chronic phase</th>
<th>Advanced phases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median duration</td>
<td>Accelerated phase</td>
</tr>
<tr>
<td>5–6 years</td>
<td>6–9 months</td>
</tr>
</tbody>
</table>


CML: Clinical manifestations (3)

- 20-50% of patients asymptomatic
- Increase of WBCs (shift to the left)
- Splenomegaly ± hepatomegaly or adenopathy
- Fatigue, malaise, weight loss, sweating, abdominal fullness, bleeding
Characteristics of Patients with Chronic Myeloid Leukemia at Presentation

Table 1. Characteristics of Patients with Chronic Myeloid Leukemia at Presentation

<table>
<thead>
<tr>
<th>Clinical Findings</th>
<th>Peripheral Blood Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytosis</td>
<td>Leukocytosis</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Splenomegaly</td>
</tr>
<tr>
<td>Weight loss</td>
<td>Weight loss</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Fatigue</td>
</tr>
</tbody>
</table>

Bone marrow Findings
- Increased size of myeloid cells
- Increased number of megakaryocytes
- Increased number of maturing neutrophils
- Increased number of mature platelets

CML: Bone marrow

CML: Bone marrow
CML: Peripheral Blood Smear

Normal  Chronic phase CML

NORMAL  CML
CML: Molecular Biology

- CML is the first haematological malignancy to be associated with a specific chromosome abnormality, the Ph chromosome.
- It is the hallmark of CML and is found in up to 95% of patients.
- It is also found in 5% of children and 15-30% of adults with acute lymphoid leukaemia and in 2% of patients with newly diagnosed acute myeloid leukaemia.

Cytogenetic Abnormality of CML: the Ph Chromosome

Reciprocal translocation between one #9 and one #22 chromosome forming an extra-long chromosome ("der 9") and the Philadelphia chromosome (Ph1) containing the fused abl-bcr gene. This is a schematic view representing metaphase chromosomes.
Generally, it appears that the Abl protein serves a complex role as a cellular module that integrates signals from various extracellular and intracellular sources and that influences decisions in regard to cell cycle and apoptosis.
Table 2. Interaction of Function Domain of p120^NLS with p60^SHC, C-fms-p60^SHC

<table>
<thead>
<tr>
<th>Domain</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal</td>
<td>T cell activation</td>
</tr>
<tr>
<td>C-terminal</td>
<td>Cytoskeleton/cell membrane</td>
</tr>
</tbody>
</table>

Substrates of BCR-ABL

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shc</td>
<td>Adapter</td>
</tr>
<tr>
<td>Crk</td>
<td>Adapter</td>
</tr>
<tr>
<td>Crkl</td>
<td>Adapter</td>
</tr>
<tr>
<td>Talin</td>
<td>Cytoskeleton/cell membrane</td>
</tr>
<tr>
<td>Paxillin</td>
<td>Cytoskeleton/cell membrane</td>
</tr>
<tr>
<td>FAK</td>
<td>Serine/threonine kinase</td>
</tr>
<tr>
<td>Ras</td>
<td>GDP/GTP exchange factor</td>
</tr>
<tr>
<td>Ras-GAP</td>
<td>GAP activation</td>
</tr>
<tr>
<td>GAP-associated proteins</td>
<td>Ras-GDP exchange factor</td>
</tr>
<tr>
<td>PLC</td>
<td>GTPase activator</td>
</tr>
<tr>
<td>PKC</td>
<td>Serine/threonine kinase</td>
</tr>
<tr>
<td>Raf-1</td>
<td>GTPase activator</td>
</tr>
<tr>
<td>CIK</td>
<td>14-3-3 protein</td>
</tr>
<tr>
<td>Vav</td>
<td>GTPase activator</td>
</tr>
<tr>
<td>HSP27</td>
<td>Heat shock protein</td>
</tr>
</tbody>
</table>

Diagram of BCR-ABL signal transduction pathway.
Altered adhesion properties of BCR-ABL-positive cells

- CML progenitor cells exhibit decreased adhesion to BM stroma cells and ECM
- CML progenitor cells express an adhesion-inhibitory variant of β1 integrin that is not found in normal progenitors
- Crkl, one of the most prominent tyrosine-phosphorylated proteins in Bcr-Abl transformed cells, is involved in the regulation of cell motility, and in integrin-mediated cell adhesion by association with other focal adhesion proteins such as paxillin, Fak, p130Cas, and Hef1.
Inhibition of apoptosis in BCR-ABL-positive cells

- Blockade of the release of cytochrome C from the mitochondria and thus the activation of caspases.
- Upregulation of Bcl-2 (via Ras- or PI3K).
- Transcriptional activation of BclxL by Stat5.
- Phosphorylation and therefore inactivation of the pro-apoptotic protein Bad.

CML: CELLULAR BIOLOGY

- Myeloid progenitor cells expand in various stages of maturation, are released prematurely into the peripheral blood and home to extramedullary locations.
- Alterations in the proliferative capacity of immature haemopoietic progenitors and a shift in the balance between self renewal and differentiation.
- Defective adherence of immature haemopoietic progenitors to marrow stromal elements.
- Activation of antiapoptotic pathways.
CML: Clinical manifestations

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Accelerated phase</td>
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<tr>
<td>Median duration 5–6 years</td>
<td>Median duration 6–9 months</td>
</tr>
</tbody>
</table>

MOLECULAR AND CELLULAR EVENTS IN DISEASE TRANSFORMATION (1)

- **Minor cytogenetic changes**
  - Monosomies of chromosomes 7, 17, Y
  - Trisomies of chromosomes 17, 21
  - Translocation t(3;21)(q26;q22)

- **Major cytogenetic changes**
  - Trisomy 8, isochromosome i(17q)

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MOLECULAR AND CELLULAR EVENTS IN DISEASE TRANSFORMATION (2)

- Abnormalities in p53 (17p13)
- RB1 (13q14)
- c-MYC (8q24)
- p16 (9p21)
- RAS
- AML-EVI-1 t(3;21)(q26;q22)
ACUTE TRANSFORMATION

- The rate of response to standard induction chemotherapy for patients in the myeloid blastic phase is approximately 20%, and the rate of complete remission is less than 10%.
- In patients in the lymphoid blastic phase, the rate of response is approximately 50%, but remissions are transient and the median survival is 9 to 12 months.

DIAGNOSIS OF CML (1)

- Cytogenetic analysis
  - Reveals the Ph chromosome in 95% of patients.
  - Valuable for demonstrating additional abnormalities.
  - BM cells are required.
  - The procedure is time-consuming and only 20-25 cells in metaphase are examined per sample.

DIAGNOSIS OF CML (2)

- FISH (Fluorescence In Situ Hybridization)
  - Uses double-color probes for the detection of Ph chromosome.
  - PB cells can be analyzed.
  - Fast technique
Molecular Methods for Detecting \textit{bcr-\textit{abl}} on the Ph Chromosome

- Fluorescence in situ hybridization (FISH)

![Diagram of FISH process]

**Goals of treatment of CML**

- Elimination of the Ph chromosome
  - Complete cytogenetic response
  - Molecular response

- Other therapeutic goals
  - Hematologic response
  - Disappearance of splenomegaly
  - Elimination of symptoms

**TREATMENT OF CML**

- 1860: Arsenic Potassium
- 1902: Irradiation
- 1930: Spleen irradiation
- 1952: Busulphan
- 1966: Hydroxyurea
- 1981: Transplantation
- 1983: Interferon
- 2001: Tyrosine kinase inhibitors
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Results of Allogeneic Bone Marrow Transplantation in Patients with Chronic Myeloid Leukemia in Chronic Phase

<table>
<thead>
<tr>
<th>Study and Type of Donor</th>
<th>No. of Transplants</th>
<th>Patients</th>
<th>Survival Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-identical sibling donor</td>
<td>229</td>
<td>3</td>
<td>57</td>
</tr>
<tr>
<td>10/10 HLA match</td>
<td>259</td>
<td>0</td>
<td>94</td>
</tr>
<tr>
<td>UIIM and ABO/AATA</td>
<td>280</td>
<td>7</td>
<td>78</td>
</tr>
<tr>
<td>HLA-identical unrelated donor</td>
<td>591</td>
<td>2</td>
<td>48</td>
</tr>
<tr>
<td>10/10 HLA match</td>
<td>148</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>HLA-identical unrelated</td>
<td>40</td>
<td>2</td>
<td>85</td>
</tr>
<tr>
<td>To date as of Aug 1997</td>
<td>19a</td>
<td>5</td>
<td>77</td>
</tr>
</tbody>
</table>

*Heidelberg, Germany; International Bone Marrow Transplant Registry; CIBMTR Registry Group for Marrow and Marrow Transplants; NMDP National Marrow Donor Program, and SNF not available.
*Study was performed at the Fred Hutchinson Cancer Center in Seattle.

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**Mechanisms of action of IFNα**

- Immunomodulatory, antiproliferative, antiogenic activities
- Indirectly influences the survival of CML cells
- Restoration of defective cytoadhesion of CML cells to BM microenvironment
- Augmentation of the cytotoxicity of NK cells
- Inhibition of cytokines through paracrine loops
TREATMENT OF CML

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Protein kinases as therapeutic targets

- Protein kinases are enzymes that transfer phosphate from ATP to specific amino acids on substrate proteins.
- The phosphorylation process leads to activation of signal transduction pathways which have critical role in a variety of biologic processes including cell growth, differentiation, and death.
- Protein kinases are composed of two subfamilies, the protein serine-threonine kinases and the protein tyrosine kinases.

Glivec / Gleevec
Imatinib Mesylate

- A selective tyrosine kinase inhibitor of
  - KIT
  - Bcr-Abl
  - PDGFRα/β

Class: Phenylaminopyrimidines

Imatinib is a specific and potent inhibitor of a small family of tyrosine kinases, including Bcr-Abl.

Imatinib acts specifically by blocking the binding site for ATP in the Abl kinase, thereby preventing the phosphorylation of tyrosine residues on substrate proteins. By inhibiting phosphorylation, imatinib prevents the activation of signal transduction pathways that induce the leukemic transformation to CML.

Imatinib is not entirely selective for the Bcr-Abl tyrosine kinase; it also inhibits the receptor tyrosine kinases for platelet-derived growth factor (PDGF) and stem cell factor (SCF), Kit, and inhibits PDGF and SCF-mediated cellular events.
International Randomized trial of Interferon/Ara-C versus STI571 (IRIS)

1106 CML-CP patients enrolled from June 2000 to January 2001

Randomization

IFN-α + Ara-C

Crossover

matinib

397 pts (72%) remain on first-line treatment

338 (65%)

14 (3%) still on first-line IFN

Progression defined as:
- Increasing WBC count
- Loss of MCR or CHR
- Accelerated phase or blast crisis
- Death during treatment

Kaplan-Meier Estimate of the Time to a Major Cytogenetic Response

Outcome with Frontline Imatinib Therapy: 54-Month Follow-up of the International Randomized Interferon versus STI571 Trial

Table 1. Outcome with Frontline Imatinib Therapy: 54-Month Follow-up of the International Randomized Interferon versus STI571 Trial

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimated Rates for Patients Receiving Frontline Imatinib, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete hematologic response</td>
<td>97</td>
</tr>
<tr>
<td>Major cytogenetic response</td>
<td>88</td>
</tr>
<tr>
<td>Complete cytogenetic response</td>
<td>82</td>
</tr>
<tr>
<td>Estimated 5-year progression-free survival</td>
<td>84</td>
</tr>
<tr>
<td>Intermittent 5-year survival without</td>
<td></td>
</tr>
<tr>
<td>progression to accelerated or blastic phase</td>
<td></td>
</tr>
</tbody>
</table>

* Reference 43.

Estimated Percentages of All Study Patients in Each Group with a Reduction from Baseline in BCR-ABL Transcript Levels of at Least 3 Log, 2 Log, or Less Than 2 Log after 12 Months

<table>
<thead>
<tr>
<th>Reduction of ≥ 3 log</th>
<th>Reduction of 2 log</th>
<th>Reduction of &lt; 2 log</th>
<th>No complete cytogenetic remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon</td>
<td>39</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Interferon + imatinib</td>
<td>33</td>
<td>34</td>
<td>9</td>
</tr>
</tbody>
</table>

Degree (%) of BCR-ABL log reduction in 124 CCyR pts

<table>
<thead>
<tr>
<th>Year</th>
<th>Reduction of ≥ 4 log</th>
<th>3 - &lt; 4 log</th>
<th>2 - &lt; 3 log</th>
<th>&lt; 2 log</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>22</td>
<td>31</td>
<td>20</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Year 4</td>
<td>41</td>
<td>39</td>
<td>38</td>
<td>12</td>
<td>4</td>
</tr>
</tbody>
</table>

Approach to the Treatment of Patients with CML in Chronic Phase

Algorithm for Treating Chronic Myeloid Leukemia in Patients under the Age of 60 Years

Potential Mechanisms of Resistance to Imatinib
Mechanisms for resistance to Imatinib

- Overexpression and amplification of the BCR-ABL gene locus.
- BCR-ABL gene mutations.
- Activation of BCR-ABL–independent pathways, such as members of the Src kinase family.
- Binding of imatinib to serum -1 acid glycoprotein.
- Increased drug efflux through the multidrug resistance gene.

BCR-ABL characterized mutants associated with clinical resistance to imatinib


Goldman, J. M. et al. NEJM 2003;349:1451
**Table 2. Spectrum of Tyrosine Kinase Inhibition for Imatinib and Novel Compounds***

<table>
<thead>
<tr>
<th>Drug</th>
<th>Kinase-Inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>BCR-ABL, c-KIT, and PDGFR</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>BCR-ABL, 5c family kinases, c-KIT, epidermal receptor kinases, and PDGFR</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>BCR-ABL, c-KIT, and PDGFR</td>
</tr>
<tr>
<td>SKI-606</td>
<td>BCR-ABL and 5c family kinases</td>
</tr>
<tr>
<td>VX-809</td>
<td>BCR-ABL, Aurora kinases, and FGR3 kinase</td>
</tr>
<tr>
<td>EBR-795</td>
<td>BCR-ABL, p38 MAP kinase</td>
</tr>
<tr>
<td>ORIO12380</td>
<td>BCR-ABL and Lyn kinase</td>
</tr>
<tr>
<td>Adaphitin</td>
<td>BCR-ABL and other tyrosine kinases</td>
</tr>
</tbody>
</table>

*PDGF = platelet-derived growth factor β-receptor; MAP = mitogen-activated protein.

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**Initial Phase II Data for Second-line Therapy with Dasatinib after Imatinib Failure***

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**ALTERNATIVE STRATEGIES TO TARGET ABL-BCR**

- **BCR-ABL**
  - Hsp 90
  - 27-AAG
- **RAS**
  - RAF
  - MEK
  - ERK
- **PI3K**
  - AKT
  - mTOR
  - Rapamycin

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Summary (1)

- The characteristic genetic abnormality of CML, the Ph chr is present in the BM cells of more than 90% of patients with CML and in 15% to 30% of adult patients with ALL.
- It results from a reciprocal chromosomal translocation between the long arms of chromosomes 9 and 22.
- This process fuses the ABL gene on chr 9 with the BCR gene on chr 22, generating an oncogene that encodes the BCR-ABL protein, a constitutively active, cytoplasmic form of ABL kinase.
- The activity of this fusion protein is no longer under the regulatory control mechanisms for ABL and induces malignant disease by activating multiple cytoplasmic and nuclear signal transduction pathways that influence the growth and survival of hematopoietic cells.

Summary (2)

- The targets for BCR-ABL include members of the Ras, phosphatidylinositol-3 kinase (PI3K)/Akt, and Jak/Stat signaling pathways, which regulate cell proliferation and apoptosis.
- BCR-ABL abrogates cell dependence on external growth factors and alters the cell adhesion properties by modulating expression and activation of focal adhesion kinase and associated proteins.
- The kinase also has diverse effects on the DNA repair which may promote additional chromosomal alterations and mutations involved in the progression of the disease and may play a role in the aggressive nature of late-stage CML.
- The Src family kinases may play an important role in late-stage disease, functioning downstream and upstream of BCR-ABL and in pathways that are independent of BCR-ABL.

Imatinib Targets the Cause of CML

- Imatinib selectively inhibits BCR-ABL by occupying the ABL domain adenosine triphosphate–binding site, it maintains the protein in an inactive conformation, thereby inhibiting its tyrosine kinase activity.
A simplified illustration of BCR-ABL and Src family kinase involvement in oncogenic signaling pathways.