

**Association of Molecular Pathology  
USCAP Companion Meeting  
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***"Molecular Monitoring Strategies to Track Response to BCR-ABL Kinase Inhibitors in CML"***

Chronic myelogenous leukemia (CML) remains the success story in tumor molecular diagnostics because it fits the targeted therapy paradigm nearly perfectly:

- CML (and Ph+ ALL) are defined by the presence of the bcr-abl fusion transcript/protein.
- Imatinib (Gleevec/Glivec/STI571) is a widely used small molecule inhibitor that competes for the ATP-binding pocket of the abl kinase and thus (rather) selectively blocks proliferation of the CML clone.<sup>1</sup>
- Monotherapy with imatinib is the standard therapy for CML worldwide.<sup>2-4</sup>
- However, other (curative) therapies are available, including IFN +/- cytarabine, marrow transplantation, other bcr-abl inhibitors, and combination therapies.<sup>5</sup>
- Primary or secondary resistance to imatinib requires dose escalation<sup>6</sup> or a change in therapy.

**The goals of molecular monitoring in CML thus include:**

- Making the diagnosis (i.e. differentiating CML from other MPDs).
- Minimal residual disease monitoring.
- Monitoring for imatinib resistance.
- Predicting therapeutic response to new agents once resistance develops.

**Where is the evidence that molecular monitoring of CML response to imatinib is clinically important?**

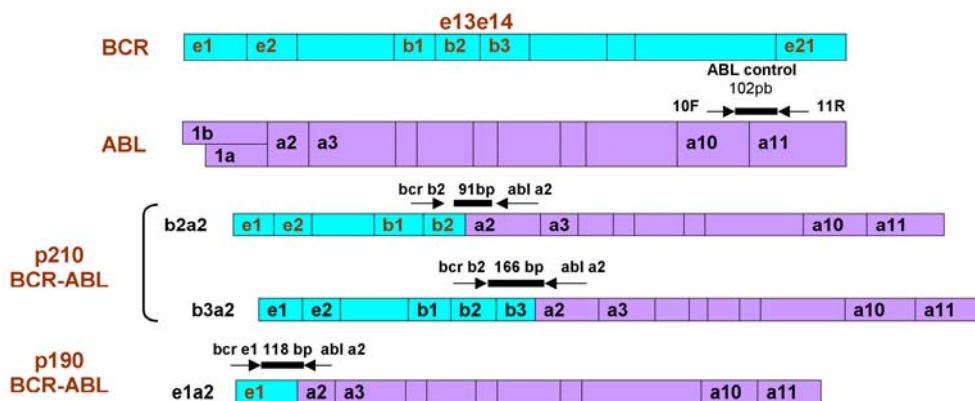
*The IRIS Trial (1998-2005):*

- 1,106 patients with newly diagnosed CML, randomized to 400 mg imatinib qD vs. IFNalpha and cytarabine (The International Randomized Study of Interferon and STI571 Study).<sup>3</sup>
- Imatinib was associated with higher response rates (74% predicted complete cytogenetic response (CCR) at 18 months) than IFNalpha/cytarabine (14.5% predicted CCR) and was more easily tolerated. Failure to achieve CCR for either group was strongly predictive of a shorter progressive-free survival.

- Failure to achieve 3-log or 4-log fold reductions in bcr-abl transcript levels within 6 months, as assessed by quantitative reverse transcription PCR (RT-qPCR), provided additional risk stratification.<sup>7</sup>

*The bcr-abl RT-qPCR assay at M.D. Anderson Cancer Center:*<sup>8,9</sup>

- 10 ml PB or 3 ml BM aspirate as input for RNA extraction/cDNA synthesis.
- Single tube TaqMan-based assay covering the b2a2, b3a2 and e1a2 transcripts.
- Normalization to total abl transcript levels (fusion + normal abl).
- “1;100,000” lower limit of detection.
- Post-PCR sizing to detect transcript type.
- Samples run in duplicate.



### The molecular testing community is moving towards more calibrated and reproducible assays for bcr-abl RT-qPCR

- Assessment of accuracy requires development of universal standards
  - Favored approach is dissemination of a (readily available/commercial) bcr-abl standard that can be used to calibrate each lab’s bcr-abl/control ratio at several dilutions (analogous to the INR in coagulation).
- Establishing the minimal analytical sensitivity required for clinical use
  - Minimum sensitivity would be in the range of 4-log reduction from baseline (i.e. the MMR established in the IRIS trial).
  - Sensitivity controls should be included in every run
- Monitoring precision by tracking run-to-run variability
  - Guidelines on when to reject or repeat testing (e.g. less than 2-4-fold variation in replicates down to the level of MMR).
- Establishing analytical specificity
  - How to assess low-level “false-positives”, including use of post-PCR sizing or a qualitative (nested) assay.

## Mechanisms of therapy resistance in CML

*Related to overcoming imatinib blockade (i.e. bcr-abl dependent):*

- Point mutations in bcr-abl kinase domain (KD).<sup>10,11</sup>
- Amplification of the bcr-abl locus.<sup>12</sup>
- Complex rearrangements of bcr-abl transcript producing altered bcr-abl proteins.
- Altered gene regulation/ inhibitor feedback loops.

*Related to bypassing imatinib (i.e. bcr-abl independent mechanisms):*

- Activation of others kinases besides bcr-abl (signal bypass).<sup>13</sup>
- Clonal evolution, particularly p53 loss secondary to isochromosome 17q<sup>12</sup> and acquisition of AML-associated translocations involving the core binding factors.<sup>14</sup>

## **New bcr-abl kinase inhibitors have shown activity against imatinib-resistant CML<sup>15</sup>**

- AMN-107 (Novartis)
  - Activation state-independent bcr-abl inhibitor, in contrast to imatinib.<sup>16</sup>
  - Similar kinase specificity to imatinib (including activity against PDGFR and kit/CD117 tyrosine kinases).<sup>17</sup>
  - In vitro, AMN-107 can block bcr-abl kinase activity in unmutated bcr-abl and in bcr-abl with a number of imatinib-associated KD mutations (but not T315I).<sup>18,19</sup>
  - In vivo, the range of response of AMN-107 is similar but not identical to predicted range.
- Dasatinib/BMS-354825 (Bristol-Myers Squibb)
  - Dual SRC/ABL kinase inhibitor with expanded kinase specificity (e.g. LYN).<sup>20</sup>
  - Might work by overcoming KD mutations in bcr-abl<sup>18</sup> or alternatively by blocking other growth regulatory kinases.<sup>21</sup>
  - bcr-abl KD mutations that arise following dasatinib treatment include novel sites not seen with imatinib.<sup>22</sup>

## **Developing disease-specific algorithms that can applied over the clinical course of CML disease**

*M.D. Anderson Cancer Center molecular monitoring algorithm for CML:*

- RT-qPCR
  - PB used whenever possible
  - During routine therapy: q3 months.
  - During change in therapy: q6 weeks, or per trial design
- Monitoring for bcr-abl mutations is done by assessing the entire KD (codons 220-500) by bidirectional Sanger sequencing
  - At time of initial visit for imatinib-resistant disease.
  - If complete cytogenetic response (CCR) is not obtained by 6-12 months.
  - If CCR is lost at any point during therapy (excluding inability to take imatinib).

- If 10-fold rise in bcr-abl transcripts levels as determined by qPCR (independent of cytogenetic findings).
- Following shifts in therapy from imatinib to another agent at 6 weeks and again at 3 months.
- Rescreen of KD mutation-negative cases at 1-year if still disease is still imatinib-resistant.
- Use of quantitative mutations screening methods (e.g. ASO-qPCR or Pyrosequencing using primers for mutational hotspots) may be useful in high-intensity screening in the post-therapy-shift interval.
- Imatinib resistance mediated by bcr-abl amplification is assessed by karyotyping and FISH analysis at time of bone marrow aspirate (i.e. presence of extra Ph).

*Other approaches for monitoring for KD mutations:*

- Screen for KD mutations for any case with >2X fold elevation in bcr-abl qPCR levels (Adelaide Australia group)<sup>23</sup>
- High-throughput screening of CML cases at regular intervals on imatinib using DHPLC/Wave methodology: confirm suspected KD mutations with definitive sequencing
  - May detect multiple preexisting, low-level KD mutations that will be difficult to interpret clinically.<sup>24</sup>

**Two major initiatives for worldwide harmonization in bcr-abl molecular testing**

- CAP survey covering minimal residual monitoring (coming Spring 2006)
- International consensus conference (October 25-26<sup>th</sup>, 2005, NIH, Bethesda): “Molecular monitoring of CML”
  - Organized by John Goldman
  - 53 participants including representation from AMP, CAP, Pharma and biotech industry
  - Goals: consensus document on how to test (qPCR, KD mutations) and how often (clinical algorithms)

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