Guidelines for Assessment and Monitoring of patients with CML

It is recommended that monitoring of patients with CML combine analysis of bone marrow and peripheral blood, using traditional cytogenetics, FISH, Q-PCR, and RT-PCR. Mutation analysis of the kinase domain of BCR-ABL may also be indicated (see 4 below).

To assist the laboratory, please provide the following relevant clinical information on the request form:

- State if there is a confirmed diagnosis of CML
- State if patient is on imatinib 400 mg (or dose if increased), or alternative tyrosine kinase inhibitor.
- State if patient is post-transplant (with transplantation date)
- State patient’s BCR-ABL transcript type if known.

1. At Diagnosis of Chronic Phase Disease and before initiation of treatment

Before initiation of treatment, every new CML patient for whom treatment with imatinib or similar agents, or transplantation is being considered, should have peripheral blood Q-PCR, RT-PCR, and standard bone marrow cytogenetics, together with FISH with dual probes for BCR and ABL.

a. Molecular Analysis:
RT-PCR on peripheral blood (or bone marrow) determines the transcript type(s) while Q-PCR on peripheral blood determines the baseline values for subsequent comparisons during patient monitoring and treatment. Note: Baseline values may also be calculated by laboratory mean or taken to be 100% if compliant with international standardisation.1

b. Cytogenetics:
Traditional chromosome analysis of bone marrow is important to identify unusual translocations or additional cytogenetic abnormalities. FISH analysis may detect ‘silent’ BCR-ABL rearrangements and deletion in the derivative 9q that occurs in 15% of cases and which may have prognostic significance.

2. Follow-Up: Post-Allogeneic Stem Cell Transplantation

a. Molecular Analysis:
Peripheral blood from patients should be monitored 3-monthly by Q-PCR until a MMR has been achieved.2 Six-monthly monitoring should continue for 5 years post-transplant, followed by 6–12 monthly monitoring thereafter. Following a CMR by Q-PCR, the laboratory will confirm molecular negativity by nested RT-PCR.3,4

b. Cytogenetics:
Standard cytogenetic bone marrow analysis should be requested for all patients at 6 months post-SCT. Thereafter cytogenetics are not required for patients who have achieved a MMR as determined

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**Definitions**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>RT-PCR</td>
<td>reverse-transcription PCR</td>
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<tr>
<td>Q-PCR</td>
<td>quantitative PCR</td>
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<tr>
<td>Major Cytogenetic Response (MCR)</td>
<td>1 log reduction in BCR-ABL transcripts. Associated with ≤35% Ph⁻ metaphases.</td>
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<tr>
<td>Complete Cytogenetic Response (CCR)</td>
<td>2 log reduction in BCR-ABL transcripts.</td>
</tr>
<tr>
<td>Major Molecular Response (MMR)</td>
<td>3 log reduction in BCR-ABL transcripts. Patients who achieve a ≥3 log reduction have a very low probability of disease progression.</td>
</tr>
<tr>
<td>Complete Molecular Response (CMR)</td>
<td>No detectable BCR-ABL transcripts by Q-PCR.</td>
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1 Baseline BCR-ABL/BCR ratios and ratios determined prior to patient achieving a MMR are reliable only with absolute BCR transcript levels >30,000. Reports will indicate if BCR levels are below this threshold.
2 Although technically very sensitive, continued molecular monitoring by RT-PCR is not considered best-practice because it does not quantify the number of target cells screened.
3 Molecular negativity appears more durable after allogeneic stem cell transplantation than after ‘successful’ treatment with imatinib.
4 To become Q-PCR negative requires a ≥4.5 log reduction in BCR-ABL transcripts and occurs in 3-6% of imatinib treated patients.

Canterbury Health Laboratories

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3. Follow-Up: Patients that have commenced imatinib therapy

a. Molecular Analysis:
   Three-monthly peripheral blood Q-PCR monitoring. Patients that have a >2-fold increase in transcript level ratios over a ratio of 0.01% BCR-ABL/BCR should have monitoring advanced from 3-monthly to 1-monthly intervals. If a >2-fold increase is not confirmed by a repeat test 1 month later, continue with monthly monitoring. If a >2-fold increase is confirmed by a repeat test 1 month later then proceed with mutation analysis.

b. Cytogenetics:
   Standard cytogenetics on bone marrow at 6 months to confirm Ph⁺ status, then again at 12 months. Thereafter annually, or earlier if disease progression is suspected.

c. Indications for increased imatinib dose (ALLG):
   Increased imatinib dose is considered in patients who lose a MMR and in patients with a sub-optimal response to imatinib 400 mg:
   - with no MCR at 3 months
   - with no CCR at 6 months
   - with no MMR at 12 months
   Following increase, one to three-monthly monitoring should continue and if no improvement or rising transcript levels 3 months post-dose increase, consider alternative therapy i.e. allograft (no achievement of CCR) or a second generation tyrosine kinase inhibitor (no achievement of CCR or in CCR but no MMR beyond 12 months).

4. Indications for Mutation Analysis
   The lab will endeavour to advise the requesting service if an indication for mutation analysis arises during monitoring. Indications include:
   - Advanced stage patients, every 3 to 6 months.
   - Patients on imatinib that have a <1-log reduction, or <MCR at 6 months and/or <CCR at 12 months.
   - Patients who, after starting on imatinib, have a confirmed loss of response i.e. a 2-5 fold increase in BCR-ABL levels.

5. References
   - www.spirit-cml.org
   - Radich J. American Society of Hematology Education Program Book (2002); 111-135.
   - Optimising the Management of Chronic Myeloid Leukaemia (CML). Novartis Oncology.
   - On behalf of the ALLG myeloproliferative disease subcommittee.
   - Optimising the Management of Chronic Myeloid Leukaemia (CML). Glivec (imatinib mesylate) Data Sheet, Novatis New Zealand Limited 14.08.06.

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5 Bone marrow cytogenetic analysis should be considered if dysplasia or unexplained cytopenia occurs.

6 2-fold increases are usually not reliable with transcript level ratios <0.01%.

7 Bone marrow cytogenetics should not be abandoned completely as the possible onset of dysplasia or clonal changes in Ph⁺ cells may go undetected by Q-PCR. Dual-labelled FISH should be carried out on relapse samples.

8 Australasian Leukaemia and Lymphoma Trials Group

9 Data from Adelaide indicates that those with a CCR by 3 months are likely to achieve a MMR after 24 months of imatinib. Most patients (51%) with <CCR at 6 months still achieve a MMR, but those that fail to have a MCR at 6 months have a very low probability of achieving a MMR and do not do so well on imatinib.

10 The Adelaide group found a strong association between a >2-fold increase in BCR-ABL level and detection of mutations. Others define more stringent criteria for triggering mutation analysis (i.e. at least a 5-fold increase in the BCR-ABL/BCR ratio, confirmed by more than one test). Increasing transcript levels are more likely to be associated with KD mutations in patients who never reach a MMR.