Clinical characteristics and treatment outcome of pediatric patients with chronic myeloid leukemia

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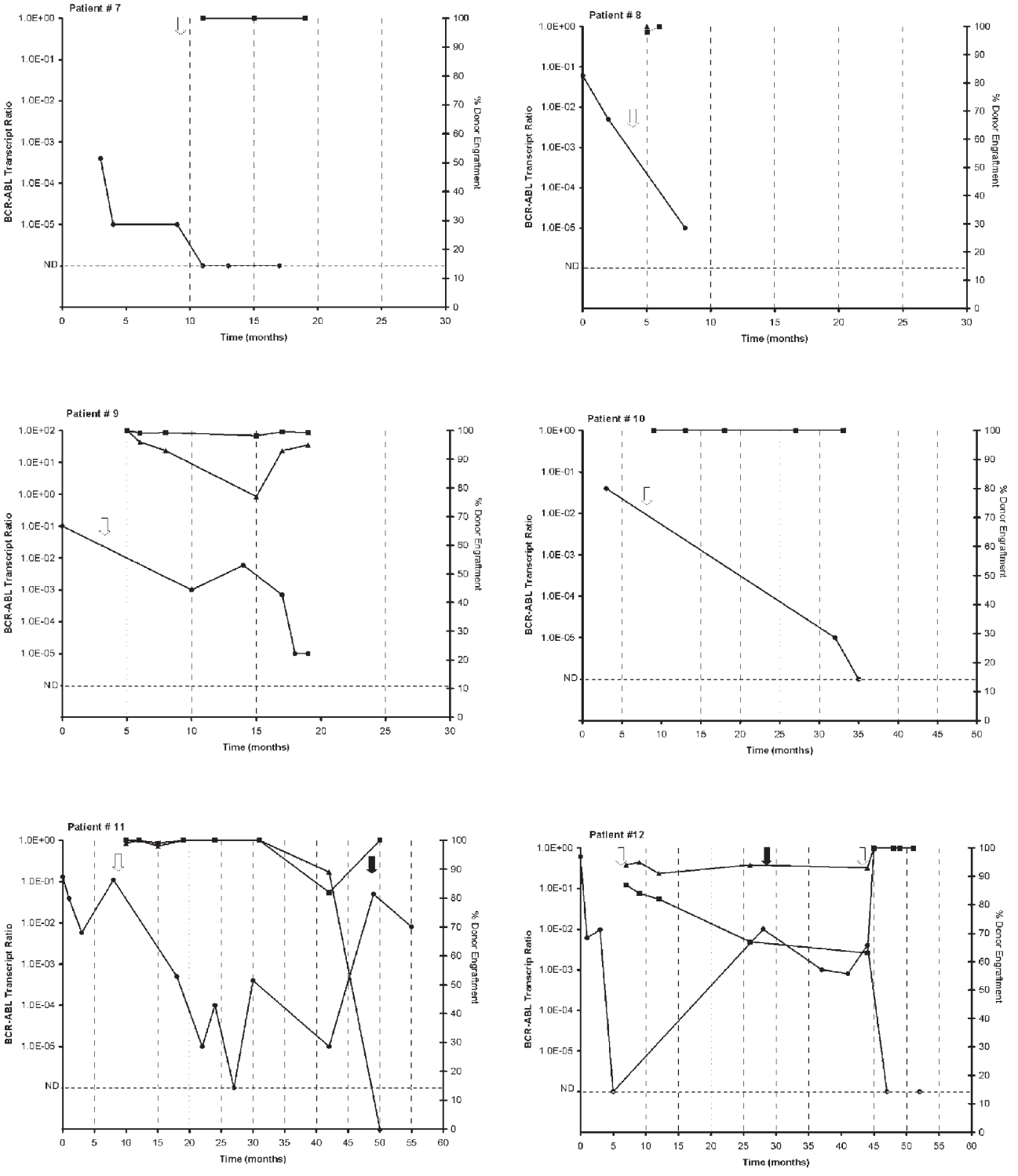
Online Supplementary Appendix

Diagnostic process and molecular analysis methodology for detection and quantification of the BCR/ABL *gene rearrangement.*

The diagnosis for the 12 patients was made on evaluation of the bone marrow aspirate and trephine biopsy. Bone marrow samples were subjected to routine Hematoxylin-Eosin (H&E) and immunohistochemical staining in addition to flow-cytometry to determine the immunophenotypes of the cells. While this was suggestive of the diagnosis, confirmation of Ph+ chronic myeloid leukemia was made only after availability of cytogenetic and/or molecular genetic studies that showed the presence of the characteristic t(9;22)(q34;q11) translocation involving the *BCR* and *ABL* genes.

Molecular analysis for *BCR/ABL* gene rearrangement was performed on extracted RNA which is subjected to real-time reverse transcription-polymerase chain reaction (RT-PCR). The generated cDNA is amplified with forward primer located in the BCR exon b2 to combine with the reverse primer in the *ABL* exon a4. The fluorescent hybridization probe pair is located in the *ABL* exon a3. The location of primers and hybridization probes also allows the amplification and detection of the b2a2 and b3a2 fusion transcripts. The amount of fluorescence resulting from the two probes is proportional to the amount of PCR product. The method uses 5 standards of 10^{-1} to10^{-4} dilution of k562 RNA in normal RNA which is diluted fresh and undergoes RT in each run. The limit of detection is 1/10000 and any result <1/10000 undergoes nested PCR before being considered negative. The sensitivity of the nested PCR is expected to be at the 10^{-5}-10^{-6} level.



Online Supplementary Figure S1. BCR-ABL transcript ratio depicted on a logarithmic scale against time for all the patients. Figure includes the graphs for patients #7-12, who underwent SCT. These graphs also show the values of the percentage engraftment for the myeloid and lymphoid lineages as determined by STR analysis against time and their relationship with the BCR-ABL ratios. BCR-ABL values —●—; STR for myeloid lineage —■— and lymphoid lineage —▲—; timepoint for SCT is shown by the open arrow and time of relapse depicted by the black arrow.