# Size matters: the prognostic implications of large and small deletions of the derivative 9 chromosome in chronic myeloid leukemia

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Deletions of the derivative 9 chromosome (der(9)) are associated with poor prognosis in chronic myeloid leukemia (CML). Several models have been proposed to account for this association. To distinguish between the various models we mapped the deletion in 69 Philadelphia-positive CML patients carrying a der(9) deletion and compared the size of the deletion with the patients' outcome. Our results demonstrate that patients with large deletions had a significantly worse survival than those with small deletions whereas the outcome for patients with small deletions was similar to that of patients lacking a deletion. These results support the tumor suppressor gene model for the pathogenesis of der(9) deletions, argue against alternative models and provide insight into candidate gene location.

Key words: derivative 9 chromosome; large deletions; chronic myeloid leukemia.

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Chronic myeloid leukemia (CML) is characterized by the formation of the *BCR-ABL* fusion gene,[1] usually a direct consequence of a reciprocal translocation between chromosomes 9 and 22 (t(9;22)) to form the Philadelphia (Ph) chromosome.[2] The development of new fluorescence *in situ* hybridization (FISH) techniques[3] identified unexpected deletions adjacent to the translocation breakpoint on the derivative 9 chromosome (der(9)) in 10-15% of CML patients.[4] Subsequent studies demonstrated that CML patients who carry a der(9) deletion progress more rapidly to blast crisis and have a shorter survival than those without a deletion.[4-8] Furthermore, deletion status is independent of, and more powerful than, the Sokal and Hasford scoring systems for predicting prognosis.[5] A number of molecular mechanisms may be responsible for the poor prognosis associated with der(9) deletions. The simplest model invokes loss of one or more tumor suppressor genes important for disease evolution. The biological consequences of a deletion could be a direct effect of haploinsufficiency or be manifested by one or more second hits affecting the remaining normal allele(s).[9] However, other models also require consideration. Firstly, der(9) deletions result in loss of *ABL-BCR* expression although correlation of *ABL-BCR* expression and disease outcome suggest that this mechanism is not sufficient to explain the prognostic significance of deletion status.[8,10,11] Secondly, *BCR-ABL* transcription may be enhanced as a consequence of occult rearrangements on the der(22) chromosome that accompany the der(9) deletions.[8] Thirdly, pre-existing genomic instability, present within the target cell at the time of the Ph translocation, may give rise both to an increased probability of der(9) deletions and also to more rapid disease progression.[9]

Most deletions are large and, if a critical target gene is located distant from the translocation breakpoint, the tumor suppressor gene model predicts that patients with large deletions, which include the critical gene(s), would have a worse prognosis than patients with small deletions. By contrast, the other models would not predict a survival difference between patients carrying large or small der(9) deletions. Detailed FISH mapping to determine deletion size was therefore undertaken and the deletion size correlated with clinical outcome in Ph-positive CML patients carrying a der(9) deletion.

## Design and Methods

### *Patients' samples and FISH*

Fixed cytogenetic preparations from cultured bone marrow samples were obtained from 69 Ph-positive CML patients carrying a der(9) deletion diagnosed between 1990 and 2004. Clinical data for 43 of these patients have previously been reported.[8] Patients were identified as carrying a der(9) deletion using the Vysis Dual Color Dual Fusion Probe (D-FISH)(Figure 1A). FISH was performed as described previously.[4] Patients were treated with standard therapies (interferon-α and hydroxyurea), and 50% received imatinib mesylate. The patients were unselected for stage of disease or type of therapy received.

### Statistical analysis

Survival curves were calculated with the Kaplan-Meier method. Univariate comparisons were performed with the log-rank test. Cox proportional hazards models were used for multivariate analysis. Patients who died in chronic phase for reasons unrelated to CML and bone marrow transplant recipients were censored at the time of death or transplantation. All calculations were performed with S-Plus v6.2 (Insightful Corporation, Seattle, USA).

## Results and Discussion

Der(9) deletions in 69 CML patients were mapped using six BAC/PACs, three on either side of the Ph translocation breakpoint (Figure 1A). Fifty-three patients had deletions of both chromosome 9 and 22 regions, 13 patients carried a deletion of chromosome 9 only and three patients carried a chromosome 22 deletion only. The deletions were of variable sizes (33 patients with deletions <1.5 Mb; 36 patients with deletions >1.5 Mb) and, with the exception of two patients (patients I and III; Figure 1B), all were adjacent to and spanned the translocation breakpoint on the der(9). Four patients (patients I, II, III and IV; Figure 1B) showed a non-contiguous deletion pattern (although all were shown to carry a der(9) deletion using the Vysis D-FISH probe), possibly reflecting an inversion event at the time of the Ph translocation. In order to assess the possible biological significance of the size and pattern of the deletion, survival analysis was performed. Survival data were available for 66 of the 69 patients, with a median follow-up of 35 months (range 1-117 months). Patients carrying a deletion of any two probes or fewer (n=17; median survival, not reached) survived significantly longer than patients with larger deletions, defined as deletion of any three probes or more (n=49; median survival, 60 months; hazard ratio (HR), 2.8; 95% confidence interval (CI), 1.2-6.7; $p$=0.02). In addition, our data provide some insight into the location of a tumor suppressor gene. Starting with deletions of only one probe adjacent to the breakpoint, we performed stepwise increments in deletion pattern and size in order to identify the maximum deleted region that did not confer a worse prognosis. Patients who carried a deletion restricted to the region encompassed by probes B, C and/or D (a region of approximately 1.4 Mb) (n=18; Figure 1A) had a significantly better prognosis than those patients whose deletion extended beyond this region (centromeric of probe B and/or telomeric of probe D)(n=48; HR, 2.8; 95% CI, 1.2-6.8; $p$=0.02; Figure 2). Furthermore, the Kaplan-Meier survival curve for those patients with small deletions, encompassing probes B, C and/or D only, is no different from that for patients without a deletion (Figure 2) suggesting that one or more putative tumor suppressor genes lie outside this 1.4 Mb region.

To ensure that the difference in survival between patients with small and large deletions was not due to confounding factors, we performed multivariate analysis with age, sex and imatinib treatment as additional

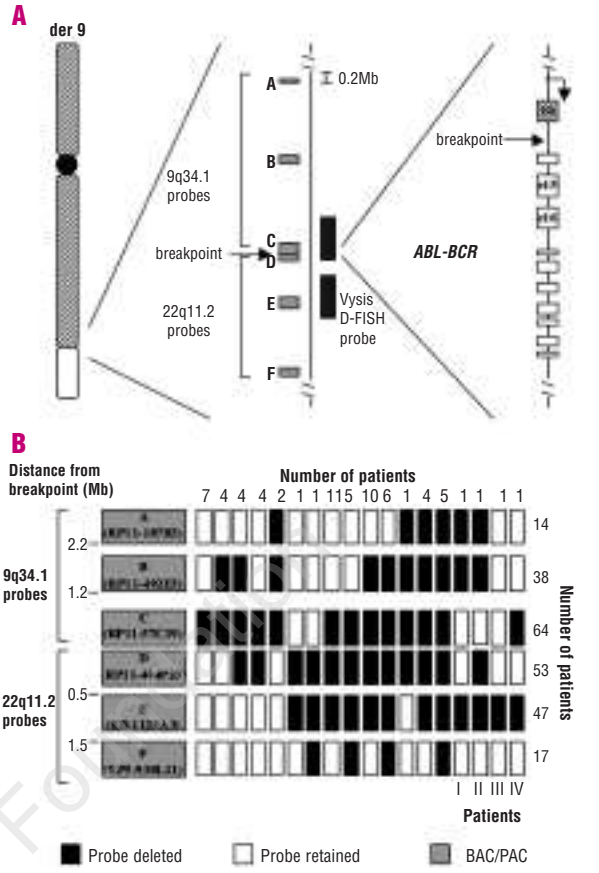


**Figure 1. Summary of FISH mapping of der(9) deletions. (A) Five bacterial artificial chromosomes (BAC) and one P1-derived artificial chromosome (PAC) were obtained from The Wellcome Trust Sanger Institute. Probes were chosen to span an approximately 4 Mb region across the derivative 9 (der(9)) translocation breakpoint in order to coincide with the area covered by FISH probes utilized by Sinclair *et al.*[4] The genomic map also shows the position of the Vysis D-FISH probe; probe A, RP11-187B3; probe B, RP11-492E3; probe C, RP11-57C19; probe D, RP11-444P20; probe E, KB-1125A3; and probe F, RP5-930L11. (B) FISH mapping was performed on the cohort of 69 Ph-positive CML patients, as previously described.[4] The 3' ends of probes A and B are 2.2 Mb and 1.2 Mb, respectively, from the breakpoint. The 5' ends of probes E and F are 0.5 Mb and 1.5 Mb, respectively, from the breakpoint. Probes C and D lie immediately adjacent to the breakpoint. The total number of patients with each particular deletion pattern is shown along the top of the chart and the total number of patients with any one probe deleted is shown on the right. Patients I, II, III and IV represent individuals with a non-contiguous deletion pattern.**

variables. The effect of large deletions (involving probes A, E or F) remained an independent predictor of poor survival ($p$=0.02) after correction for these factors. Specifically, patients with large deletions had poorer survival independent of whether they received imatinib or not.

Following interferon therapy, patients with a der(9) deletion have a shorter survival compared to patients without a deletion.[5,7,12] The survival disadvantage appears to reflect a shorter duration of chronic phase and earlier disease progression. Since initial studies of der(9) deletions were based on patients diagnosed prior to the widespread use of imatinib,[4-8] the prognostic use-

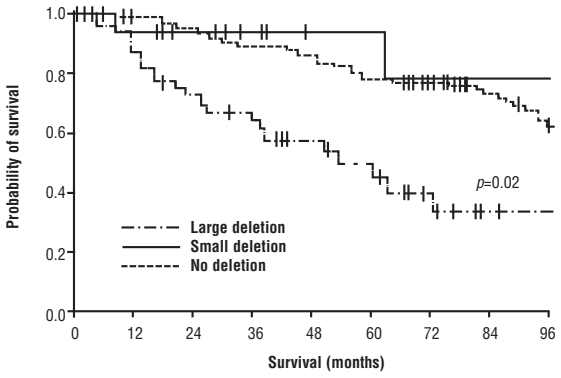


**Figure 2.** Kaplan-Meier survival curves of patients with small and large deletions. Patients who carry a small deletion (only encompassing probes B, C and/or D; a region of approximately 1.4 Mb in length)(n=18) have a significantly better prognosis than have patients with a large deletion (involving probes A, E and/or F) (n=48). The Kaplan-Meier curve for patients not carrying a deletion is taken from Huntly *et al*.[8] (n=132) and virtually overlies the Kaplan-Meier curve for the *good prognosis* group. The cohort of patients without a der(9) deletion was assessed using either the Vysis D-FISH or Oncor D-FISH *BCR-ABL* detection system.[5,8] The proportion of patients that received imatinib was similar within each group (non-deleted group, 40%; deleted group 50%).

fulness of the der(9) deletion in patients treated with imatinib remains unclear. Two studies in imatinib-treated patients have reported no significant survival difference between patients with and without deletions.[13,14] However, the median follow-up was short in both cohorts. Given that imatinib prolongs progression-free survival,[15] the low death rate in these studies reduces the probability of detecting any survival difference. For example, the median follow-up of 352 patients analyzed by Quintas-Cardama *et al*. was only 28 months.[14] We performed power calculations showing that this study had only a 25-30% chance of detecting a survival difference of the same magnitude as found in patients not treated with imatinib.[16] Moreover, using surrogates for survival, Huntly *et al*.[13] showed that progression-free survival following initiation of imatinib was significantly shorter for patients carrying deletions. Furthermore, hematologic and major cytogenetic response rates were significantly lower in patients carrying a deletion than in those without a deletion. These data suggest that deletion status may well influence disease progression in patients on imatinib but longer follow-up data are required. They also emphasize the importance of assessing deletion status and potentially deletion size in the context of large ongoing clinical trials.

Deletion studies performed to date used a number of different probe systems although all demonstrated a poorer prognosis for patients with a der(9) deletion.[4-8] However, none of the probe systems utilized would be able to discriminate between patients carrying a larger deletion (poor prognosis) and those carrying a smaller deletion (good prognosis) as defined in this paper. Inclusion of a third probe equivalent to probe A into the current D-FISH probe sets (e.g. Vysis D-FISH system) would be able to distinguish between the two subgroups. Our data would predict that patients with a deletion of either *BCR* and/or the new probe on the der(9) would have a poorer prognosis. By contrast, patients showing retention of both the new probe and *BCR*, but with deletion of *ABL*, would be predicted to have a good prognosis.

In addition to the clinical implications of the results presented here, our findings provide insight into the biology of CML progression. Our results demonstrate that the most likely model for the prognostic significance of der(9) deletions is the presence of a tumor suppressor gene, since other models (e.g. loss of *ABL-BCR* expression or general genomic instability) would not predict any difference in survival between patients with small and large deletions. Existing data do not allow us to ascertain whether the putative tumor suppressor gene(s) reside(s) on chromosome 9, 22 or both. Deletions can be large (up to 25 Mb) and both regions are gene rich, between them containing at least 300 genes.[9] Our data demonstrate that deletions restricted to probes B, C and/or D (encompassing a 1.4 Mb region adjacent to and spanning the translocation breakpoint) do not confer a worse prognosis, and therefore suggest that candidate tumor suppressor gene(s) will be outside this region. Mutation analysis of one such putative tumor suppressor gene located between probes D and E, *hSNF5/INI1* (encoding a widely expressed component of the SWI/SNF chromatin remodeling complex) revealed no mutations in 31 CML patients analyzed.[17]

*NF performed the FISH, FISH analysis, and wrote the manuscript; PJC performed the statistical analysis and acquired clinical data; AJB designed the FISH mapping strategy, acquired cytogenetic material and supervised the research; SS performed FISH analysis; EJB assisted with FISH and supervised the research; BJPH acquired the cytogenetic material, clinical data and identified CML patients with der(9) deletions by FISH; ARG co-ordinated and directed the research. All authors reviewed and contributed to the manuscript. The authors declare that they have no potential conflicts of interest.*

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## References


1. Bartram CR, de Klein A, Hagemeijer A, Van Agthoven T, Van Kessel AG, Bootsma D, et al. Translocation of the c-abl oncogene correlates with the presence of a Philadelphia chromosome in chronic myelotic leukemia. Nature 1983;306:277-80.
2. Rowley DJ. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giesma staining. Nature 1973;243:290-3.
3. Sinclair PB, Green AR, Grace C, Nacheva EP. Improved sensitivity of BCR-ABL detection: a triple-probe three-color fluorescence in situ hybridization system. Blood 1997;90: 1395-402.
4. Sinclair PB, Nacheva EP, Leversha M, Telford N, Chang J, Reid A, et al. Large deletions at the t(9;22) breakpoint are common and may identify a poor-prognosis subgroup of patients with chronic myeloid leukemia. Blood 2000;95:738-44.
5. Huntly BJP, Reid AG, Bench AJ, Campbell LJ, Telford N, Shepherd P, et al. Deletions of the derivative chromosome 9 occur at the time of the Philadelphia translocation and provide a powerful and independent prognostic indicator in chronic myeloid leukemia. Blood 2001;98:1732-8.
6. Kolomietz E, Al-Maghradi J, Brennan S, Karaskova J, Minkin S, Lipton J, et al. Primary chromosomal rearrangements of leukemia are frequently accompanied by extensive submicroscopic deletions and may lead to altered prognosis. Blood 2001;97: 3581-8.
7. Cohen N, Rozenfeld-Granot G, Hardan I, Brok-Simoni F, Amariglio N, Rechavi G, et al. Subgroup of patients with Philadelphia-positive chronic myelogenous leukemia characterized by a deletion of 9q proximal to ABL gene: expression profiling, resistance to interferon therapy, and poor prognosis. Cancer Gen Cytogen 2001; 128:114-9.
8. Huntly BJP, Bench AJ, Delabesse E, Reid AG, Li J, Scott MA, et al. Derivative chromosome 9 deletions in chronic myeloid leukemia: poor prognosis is not associated with loss of ABL-BCR expression, elevated BCR-ABL levels, or karyotypic instability. Blood 2002;99:4547-53.
9. Huntly BJP, Bench A, Green AR. Double jeopardy from a single translocation: deletions of the derivative chromosome 9 in chronic myeloid leukemia. Blood 2003;102:1160-8.
10. Loncarevic I, Romer J, Starke H, Heller A, Bleck C, Ziegler M, et al. Heterogenic molecular basis for loss of ABL1-BCR transcription: deletions in der(9)t(9;22) and variants of standard t(9;22) in BCR-ABL1-positive chronic myeloid leukemia. Genes Chromosomes Cancer 2002;34:193-200.
11. de la Fuente J, Merx K, Steer J, Muller M, Szydlo RM, Maywald O, et al. ABL-BCR expression does not correlate with deletions of the derivative chromosome 9 or survival in chronic myeloid leukemia. Blood 2001;98: 2879-80.
12. Storlazzi CT, Specchia G, Anelli L, Albano F, Pastore D, Zagaria A, et al. Breakpoint characterization of der(9) deletions in chronic myeloid leukemia patients. Genes, Chromosomes and Cancer 2002; 3:271-6.
13. Huntly BJP, Guilhot F, Reid AG, Vassiliou G, Hennig E, Franke C, et al. Imatinib improves but may not fully reverse the poor prognosis of CML patients with derivative chromosome 9 deletions. Blood 2003;102:2205-12.
14. Quintas-Cardama A, Katarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, et al. Imatinib mesylate therapy may overcome the poor prognostic significance of deletions of derivative chromosome 9 in patients with chronic myelogenous leukemia. Blood 2005;105:2281-6.
15. O'Brien SG, Guilhot F, Larson R, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukaemia. N Engl J Med 2003; 348:994-1004.
16. Schoenfeld DA, Richter JR. Nomograms for calculating the number of patients needed for a clinical trial with survival as an endpoint. Biometrics 1982;38:163-70.
17. Grand F, Kulkarni S, Chase A, Goldman JM, Gordon M, Cross NCP. Frequent deletion of hSNF5/INI1, a component of the SWI/SNF complex, in chronic myeloid leukemia. Cancer Res 1999;59: 3870-4.