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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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 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 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. 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, the prognostic use-
shown that progression-free (encoding a widely expressed compo-
and/or the new probe on the
4-8
the low death rate in these studies re-
duces
Moreover, using surrogates
5,8
Our data demonstrate that deletions restricted
in length)(n=18) have a significantly better prognosis than have
passing probes B, C and/or D; a region of approximately 1.4 Mb
large deletions. Patients who carry a small deletion (only encom-
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
They also emphasize the importance of
assessing deletion status and potentially deletion size in
current D-FISH probe sets (e.g. Vysis D-FISH system)
be able to distinguish between the two sub-
groups. 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 signi-
ificance of der(9) deletions is the presence of a tumor sup-
pressor 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 500
genes. 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.

NF performed the FISH, FISH analysis, and wrote the manu-
script; PJC performed the statistical analysis and acquired clinical
data; BJPH designed the FISH mapping strategy, acquired cytoge-
netic material and supervised the research; SS performed FISH
analysis; EJB assisted with FISH and supervised the research;
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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