**Haematology and Blood Transfusion** Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch


# Similar Molecular Alterations Occur in Related Leukemias With and Without the Philadelphia Chromosome

L. M. Wiedemann, K. Karhi, and L. C. Chan

## A. Introduction

The reciprocal translocation between the long arms of chromosomes 9 and 22, t(9:22), results in the Philadelphia (Ph1) chromosome, the karyotypic hallmark of chronic myeloid leukaemia (CML) [1]. The molecular consequences of this translocation have been well characterized, although their contribution to the disease process is less clear. The translocation creates a hybrid transcription unit consisting of the 5′ end of the so-called breakpoint cluster region (*bcr*) gene on ch22q11 and the *c-abl* proto-oncogene on ch9q34 [2]. This new gene is capable of being expressed as a chimeric 8.7 kb mRNA [3] which, when translated, produces a fusion protein (p210) with an enhanced phosphorylating activity [4] compared, in vitro, to the normal *c-abl* protein (p145).

This translocation event can be seen at the DNA level in the *bcr* gene, since it usually occurs within an 5.8 kb region of DNA and can be detected with a specific probe for this region (bcr probe) [2]. Involvement of *c-abl* is more difficult to demonstrate since the break on ch9q34 can occur anywhere within 50 kb or more [5] upstream of the proto-oncogene. We therefore chose to look at the size of the *abl* protein-tyrosine kinase as well as the level of its activity, as an assay for *c-abl* involvement. Using these criteria we analysed two other types of leukaemias (a) Ph1-positive acute lymphoblastic leukaemia, ($Ph1^+$ ALL) and (b) Ph1-negative ($Ph1^-$) CML.

The Ph1 chromosome is present in about 10% of adult ALL and is associated with a poor prognosis. It is not clear, however, whether $Ph1^+$ ALL represents blast crisis of previously undiagnosed $Ph1^+$ CML, whether it is a clinically distinct group, or (what is more likely) a mixture of the two [6].

The $Ph1^-$ CML group, represents 5% of patients diagnosed as CML whose cells do not contain the Ph1 chromosome. However, the need for more accurate assessment of these cases has been demonstrated by Pugh et al. [7], who examined 25 cases originally diagnosed as $Ph1^-$ CML and reclassified all but one as myelodysplastic syndromes including various refractory anaemias, chronic myelomonocytic leukaemia (CMML) and polycythaemia rubra vera.

## B. Results

DNA was isolated and purified from peripheral blood and/or bone marrow leukocytes of samples from patients diagnosed on the basis of clinical and haematological criteria by Prof. D. Galton (Royal Postgraduate Medical School, Hammersmith, London) as $Ph1^+$ CML (1 case), bona fide $Ph1^-$ CML (2 cases), atypical $Ph1^-$ CML (aCML) (2 cases) and $Ph1^-$ CMML (1 case). The DNA was digested with the restriction endonuclease *Bgl*II, fractionated by electrophoresis through a 0.7% agarose gel, and blotted onto nitrocellulose according to the method of Southern [8]. Relevant restriction frag-

Leukaemia Research Fund Centre, Institute of Cancer Research, Fulham Road, London SW3 6JB

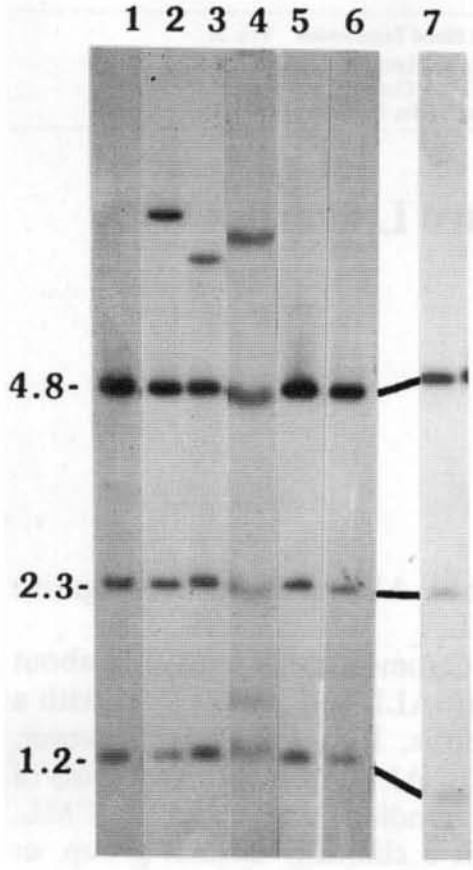
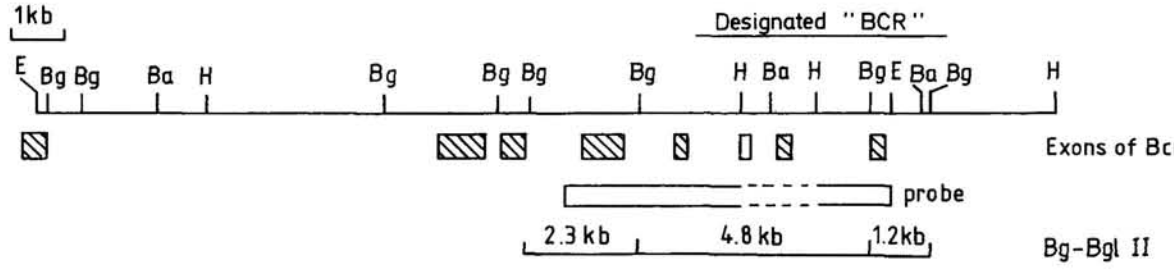



**Fig. 1.** DNA analysis of the breakpoint cluster region (*bcr*) on chromosome 22. The restriction map of the region of chromosome 22 is illustrated in the *lower portion* as well as the probe used for hybridization analysis for rearrangement (kindly supplied by Dr. J. Groffen of Oncogene Sciences, USA). The *dashed region* of the probe was deleted due to the presence of repetitive sequences. The normal size of the *Bgl*II fragments detected by this probe are delineated. The known positions of the bcr exons are also shown. Bg, *Bgl*II; Ba, *Bam*HI; E, *Eco*RI; and H, *Hin*dIII. *Bgl*II digested DNA from cells with the normal *bcr* configuration, Bri-7, *lane 1;* Ph1$^+$, CML, *lane 2;* Ph1$^-$ CML-1, *lane 3;* Ph1$^-$ CML-2, *lane 4;* Ph1$^-$ aCML-1, *lane 5;* Ph1$^-$ CMML, *lane 6,* and Ph1$^-$ aCML-2, *lane 7,* were hybridized to the *bcr* probe and autoradiographed. The normal bcr bands are labelled in kb.




ments were identified by hybridization with the bcr probe (kindly supplied by Dr. J. Groffen, Oncogene Science, USA) and visualized using Kodak XAR-5 film (Fig. 1).

In germline DNA from cells without a Ph1 chromosome, the bcr probe reveals three bands reflecting the normal restriction fragment sizes of the nonrearranged chromosome 22 region (Fig. 1, lane 1). When a translocation occurs in Ph1$^+$ CML, one or two new bands are detected by the probe, (i.e., Fig. 1, lane 2) depending on the position of the break and the retention of the 3′ as well as 5′ portion of the translocation products (deletion of the 3′ region of bcr appears not to be uncommon [6]).

When the DNA from the six patients was analysed, new bands were detected in the Ph1$^+$ CML (Fig. 1, lane 2) as well as two bona fide Ph1$^-$ CML (Fig. 1, lanes 3 and 4), suggesting that the DNA is rearranged in the *bcr* locus in the DNA from these leukaemias. DNA samples from the two aCML and the CMML did not give rise to additional bands (Fig. 1, lanes 5–7). This information shows an interesting correlation with the diagnosis based solely on clinical and haematological features and confirms the conclusion reached in a recent report [9] although our data on aCML is in conflict with a study by Ganesan et al. [10]. DNA from the cells from the bona fide Ph1$^-$ CML patients, although karyotypically apparently normal, appear to have a "masked" Ph1 chromosome as analysed by bcr rearrangement and will be refered to as bcr-positive.

To exclude the possibility of polymorphism associated with *Bgl*II in a published

report of Ph1$^-$ CML [11], several enzymes were used to confirm the rearrangement of bcr in the DNA from bona fide CML patients and the lack of rearrangement in the bcr$^-$ group.

### Detection of the Abl-Kinase Activity

Mononuclear cells from selected samples were isolated on Ficoll-Hypaque (acute or blast crisis cases) or Percoll density gradients (chronic cases) and analysed by a modification of the in vitro autophosphorylation assay of the abl protein tyrosine kinase [2]. Briefly, 4–10 million cells were lysed in the presence of protease inhibitors and an antiserum raised against a synthetic peptide which was derived from the abl protein tyrosine kinase sequence (a kind gift of Syd Raytner, NIMR, London), was added in the presence or absence of the peptide antigen.

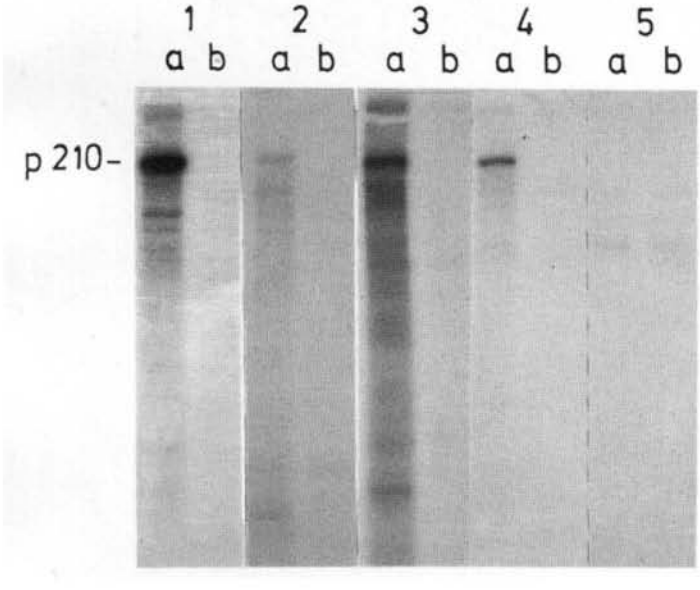



**Fig. 2.** Analysis of samples for bcr-abl protein tyrosine kinase. Blast cells were purified on density step gradients, *abl*-related proteins were isolated from the lysed cells in the presence (*lanes b*) or absence (*lanes a*) of the synthetic peptide used to generate the antiserum. Proteins were allowed to autophosphorylate in the presence of $\gamma$-$^{32}$P-ATP and were analysed by PAGE. The autoradiographic results shown were from Nalm-1 cells, *lanes 1;* Ph1$^+$ CML-myeloid blast crisis, *lanes 2;* Ph1$^-$ CML-2, *lanes 3;* Ph1$^+$ ALL, *lanes 4,* and Ph1$^-$ ALL, *lanes 5*. The position of the p210 protein tyrosine kinase as determined from markers, is indicated.

The lysate was clarified after 15 min and added to Sepharose 4B-CL-protein-A beads. The beads were collected and washed and the bound material was allowed to autophosphorylate in the presence of $\gamma$-$^{32}$P-ATP. The products were analysed by SDS-polyacrylamide gel electrophoresis.

Lysates from a cell line Nalm-1 which bears the Ph1 chromosome and in which bcr is rearranged, were used as positive controls. The addition of the peptide 4 completely eliminated the precipitation and subsequent autophosphorylation of the p210 in Nalm-1 demonstrating specificity of the anti-abl antiserum for this protein (Fig. 2, lane 1 b). The p210 activity was observed in all samples that had been shown to rearrange in the *bcr* locus. These included a Ph1$^+$ CML in myeloid blast crisis (Fig. 2, lane 2) the Ph1$^-$ CML-2 (Fig. 2, lane 3) and a Ph1$^+$ ALL (Fig. 2, lane 4) that had previously been shown to rearrange in the *bcr* locus, (see [6], patient L8), while we were unable to detect any *abl*-related products in Ph1$^-$, bcr$^-$ samples of aCML (data not shown) or in a Ph1$^-$, bcr$^-$ All (Fig. 2, lane 5).

## C. Conclusion

We have shown the importance of the protein kinase assay as a complementary study to bcr analysis in leukaemias that are related to Ph1$^+$ CML. We find that: (a) Ph1$^-$ bona fide CML and Ph1$^+$ CML appear to be the same disease as measured at the DNA level by bcr rearrangement and at the protein level as measured by the p210 abl protein tyrosine kinase assay (b) Ph1$^+$ ALLs which rearrange in bcr also express the p210 kinase activity. Since the protein kinase assay is quick (<36 h) and requires only 5 million enriched blast cells, it could be used as an alternative to DNA analysis for identification of bcr-positive samples in clinical specimens.

*Acknowledgements.* We thank the following people who have helped in this study: Dr. D. Bain (St. Mary's Hospital, London), Dr. D. Bevan (St. George's Hospital, London), Dr. A. Lister (St. Bartholomew's Hospital, London), Dr. A. Parker (Royal Infirmary, Edinburgh), Prof. A. Jacobs (University of Wales, Cardiff), and Dr. R. Powles (Royal Marsden Hospital, Sutton) for providing

clinical samples. Prof. D. Galton (Royal Postgraduate Medical School, London) for reviewing the haematological slides; Dr. J. Groffen (Oncogene Sciences, USA) for bcr probe; Drs. G. Foulkes, S. Raytner (NIMR, London) and O. Witte (UCLA, USA) for anti-abl antibodies and Ms. G. Parkins for typing of the manuscript. We also thank Prof. M.F. Greaves for helpful discussion and the Leukaemia Research Fund of Great Britain for funding of this project.

## References

1. Nowell PC, Hungerford DA (1960) Science 132:1947
2. Groffen J, Stephenson JR, Heisterkamp N, Klein A de, Bartram CR, Grosveld G (1984) Cell 36:93
3. Stam K, Heisterkamp N, Grosveld G, Klein A de, Verma RS, Coleman M, Dosik H, Groffen J (1985) New Engl J Med 313:1429
4. Konopka JB, Watanabe SM, Singer JW, Collins SJ, Witte ON (1985) Proc Natl Acad Sci (USA) 82:1810
5. Grosveld G, Verwoerd T, Agthoven T van, Klein A de, Ramachandran KI, Heisterkamp N, Stam R, Groffen J (1986) Mol Cell Biol 6:607
6. Klein A de, Hagemeijer A, Bartram CR, Houwen R, Hoefsloot L, Carbonell F, Chan L, Barnett M, Greaves MF, Kleihauer E, Heisterkamp N, Groffen J, Grosveld G. Blood (1986)6:1369
7. Pugh WC, Pearson M, Vardiman JW, Rowley JD (1985) Br J Haematol 60:457
8. Southern EM (1975) J Mol Biol 98:503
9. Morris CM, Reeve AE, Fitzgerald PH, Hollings PE, Beard MEJ, Heaton DC (1986) Nature (London) 320:281
10. Ganesan TS, Rassol F, Guo A-P, Young BD, Galton DAG, Goldman JM (In this volume)
11. Bartram CR, Carbonell F (1986) Cancer Genet Cytogenet 21:183
12. Konopka JB, Witte ON (1985) Mol Cell Biol 5:3116