Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5698–5702, August 1988
Medical Sciences

Diagnosis of chronic myeloid and acute lymphocytic leukemias by detection of leukemia-specific mRNA sequences amplified *in vitro*

(Philadelphia chromosome/BCR-ABL/t(9;22) chromosomal translocation/polymerase chain reaction)

ERNEST S. KAWASAKI*†, STEVEN S. CLARK‡, MAZIE Y. COYNE*, STEPHEN D. SMITH§, RICHARD CHAMPLIN¶, OWEN N. WITTE‡, AND FRANK P. MCCORMICK*

*Department of Molecular Biology, Cetus Corp., Emeryville, CA 94608; ‡Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, CA 90024; §Department of Pediatrics, Children's Hospital at Stanford, Palo Alto, CA 94304; and ¶Department of Medicine, Division of Hematology/Oncology, University of California Center for the Health Sciences, Los Angeles, CA 90024

Communicated by Donald F. Steiner, April 15, 1988

ABSTRACT **The Philadelphia chromosome is present in more than 95% of chronic myeloid leukemia patients and 13% of acute lymphocytic leukemia patients. The Philadelphia translocation, t(9;22), fuses the *BCR* and *ABL* genes resulting in the expression of leukemia-specific, chimeric BCR-ABL messenger RNAs. To facilitate diagnosis of these leukemias, we have developed a method of amplifying and detecting only the unique mRNA sequences, using an extension of the polymerase chain reaction technique. Diagnosis of chronic myeloid and acute lymphocytic leukemias by this procedure is rapid, much more sensitive than existing protocols, and independent of the presence or absence of an identifiable Philadelphia chromosome.**

Chronic myeloid leukemia (CML) was the first disease shown to be consistently associated with a cytogenetic abnormality now known as the Philadelphia (Ph^1) chromosome (1). The abnormality usually involves a reciprocal translocation of chromosomes 9 and 22 (2), and more than 95% of CML patients have this defect. The translocation involves the movement of most of the *ABL* protooncogene (3, 4) on chromosome 9 to the *BCR* or "breakpoint cluster region" gene on chromosome 22 (5). Expression of the fused genes results in a chimeric 8.5-kilobase (kb) mRNA transcript (6) and a large 210-kDa translation product, P210, with increased tyrosine kinase activity relative to the normal ABL protein (7).

The chromosomal abnormalities in acute lymphocytic leukemia (ALL) are more heterogeneous in nature than those found in CML, but a significant proportion of ALL patients carry Ph^1 chromosomes in their leukemic cells. For example, some 6% of children with ALL are Ph^1 chromosome positive (8, 9), and it is the most common chromosomal abnormality in adult ALL, with an incidence of 17–25% (10, 11). Ph^1 chromosomes of CML and ALL patients are indistinguishable by cytogenetics (12). Although studies have shown that the chromosomal breakpoints in ALL can lie outside the *BCR* gene (13, 14), recent complementary DNA cloning results (15–17) indicate that in many cases a more 5′ *BCR* exon is used in the formation of the BCR-ABL mRNA, while the downstream *ABL* sequence remains the same as in CML. With the latest ALL findings, there are now known to exist at least three distinct BCR-ABL mRNAs, two of which are derived from variants of CML and one from ALL. These mRNAs contain one of three different *BCR* exons fused to the same *ABL* exon.

Present means of diagnosing CML and ALL may include some combination of morphological, cytochemical, cytogenetic, immunological, and molecular analyses. Each method has its merits but may be difficult to carry out if only limited clinical samples are available and/or only a small proportion of the cells are leukemic. A more sensitive method would be useful in following the course of disease in patients after bone marrow transplants or in patients in remission or in early stages of relapse. Recently a procedure was described for the enzymatic amplification of genomic DNA sequences *in vitro* (18). We have extended and modified that method to include RNA as the starting template. For CML and ALL diagnosis, a complementary DNA (cDNA) copy of the mRNA from patient blood or bone marrow cells is first synthesized, followed by polymerase chain reaction (PCR) amplification of only the diagnostic BCR-ABL chimeric cDNA sequences. Products of the PCR reaction are then analyzed by hybridization with probes specific for the three known *BCR-ABL* fusion sequences. This new procedure complements existing diagnostic protocols, is much more sensitive, and provides information about the nature of the molecular defect without requiring large clinical samples or resorting to molecular cloning.

MATERIALS AND METHODS

Cells. Peripheral blood was drawn from patients after informed consent and fractionated on Ficoll gradients. K562 is a Ph^1-chromosome-positive cell line (19) and HL-60 is a Ph^1-chromosome-negative myeloid line (20). SUP-B15 is a Ph^1-chromosome-positive line derived from an ALL patient (21), and Jurkat is a Ph^1-chromosome-negative T-cell leukemia line (22).

Immunotype and Karyotype Analysis. Immunotype analysis of BCR and ABL proteins was done by using ABL-specific antisera (23, 24). The antisera used were: normal rabbit antiserum; rabbit anti-pEX2 antiserum specific for the ABL protein kinase domain; and rabbit anti-pEX5 antiserum specific for the carboxyl-terminal region of ABL. Labeling of proteins was done by the *in vivo* ortho[^{32}P]phosphate method (23) or in the *in vitro* kinase assay (24). Samples were analyzed by $NaDodSO_4$/PAGE on 8% gels followed by autoradiography. Determination of karyotypes was done at the University of California, Los Angeles, cytogenetics laboratory.

RNA Isolation. RNA was isolated from cell lines as described (25, 26). Total cellular RNA was isolated from clinical samples by a guanidinium isothiocyanate solubilization/LiCl precipitation procedure (27). One microgram of cell line RNA was used as template for the amplification reactions (see

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ALL, acute lymphocytic leukemia; CML, chronic myeloid leukemia; PCR, polymerase chain reaction; Ph^1 chromosome, Philadelphia chromosome.

†To whom reprint requests should be addressed at: Cetus Corp., 1400 53rd Street, Emeryville, CA 94608.

below), and 1/10th of the total sample (5 μl of 50) was used from the clinical samples.

RNA from the clinical samples (≈10^7 cells) was not quantitated because analysis by gel electrophoresis indicated that the samples were contaminated with nuclear DNA.

Oligonucleotides Used for Amplification and Detection of BCR-ABL mRNAs. Oligonucleotides were synthesized on a Biosearch (San Rafael, CA) DNA synthesizer, and their sequences are given below. DNA sequences used for the CML studies are found in refs. 28–30. The sequences for ALL DNA are found in refs. 15–17. Fig. 2 shows the approximate location of the oligonucleotides in the three forms of BCR-ABL mRNAs.

CML oligonucleotides were:

CML A, 5′-GGAGCTGCAGATGCTGACCAAC-3′;
CML B, 5′-TCAGACCCTGAGGCTCAAAGTC-3′;
CML C, 5′-GCTGAAGGGCTT^TTGAACTCTGCTTA-3′; and
CML D, 5′-GCTGAAGGGCTT^CTTCCTTATTGATG-3′.

ALL oligonucleotides were:

ALL E, 5′-CGCATGTTCCGGGACAAAAGC-3′;
ALL F, 5′-GGTCATTTTCACTGGGTCCAGC-3′; and
ALL G, 5′-GCTGAAGGGCTT^CTGCGTCTCCAT-3′.

CML A is the 5′ (homologous to the *BCR* sequence) PCR primer, and CML B (homologous to *ABL*) is the 3′ PCR primer (see refs. 18, 31, and 32 for details on amplification methods). CML C is the probe that identifies *BCR* exon 3/*ABL* exon II junction sequences, and CML D will hybridize only to *BCR* exon 2-*ABL* exon II fusions. Enumeration of *BCR* and *ABL* exons is from Refs. 16 and 6, respectively. The junction probes are complementary to the coding sequences; the arrows denote the junction between *BCR* and *ABL* exons. When *BCR* exon 3 is present in the chimeric mRNA, a 200-base-pair (bp) fragment is amplified. A 125-bp fragment is expected when exon 2 of *BCR* is joined to *ABL* exon II. Similarly, ALL E is the 5′ *BCR* PCR primer and ALL F is the 3′ *ABL* primer. ALL G is the junction probe. Amplification of the ALL sequence yields a 307-bp product.

Amplification Method. cDNA was synthesized by using murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) according to the manufacturer's protocol. The 20-μl reaction mixture contained the enzyme buffer as supplied by Bethesda Research Laboratories, various RNA samples, 1 unit of RNasin (Promega Biotec, Madison, WI) per μl, 10 pmol of 3′ PCR primer (see Fig. 2 and above), 1 mM (each) deoxynucleoside triphosphate, and 200 units of reverse transcriptase. The reaction mixture was incubated for 30 min at 37°C. The reaction mixture was then diluted with 80 μl of PCR buffer (50 mM KCl/50 mM Tris chloride/2.5 mM $MgCl_2$/100 μg of bovine serum albumin per ml, pH 8.4), followed by addition of 40 pmol of the 3′ primer, 50 pmol of the 5′ primer and 1 unit of thermostable DNA polymerase from *Thermus aquaticus, Taq* polymerase (32, 33). To prevent evaporation, 150 μl of mineral oil was added, and the reaction was started by denaturing the RNA·cDNA hybrid by heat (95°C) for 20 sec, annealing the primers for 15 sec at 55°C, and then extending the primers at 72°C for 1 min. Heat denaturation started the cycle over again and was repeated 40–50 times by using a programmable heat block designed and manufactured by Cetus (Emeryville, CA). After the final cycle, the temperature was held at 72°C for 10 min to allow reannealing of the amplified products and then was chilled.

Analysis of PCR-Amplified Products. Ten microliters of each reaction mixture was run on composite gels containing 3% NuSieve/1% SeaKem agaroses (FMC, Rockland, ME) in Tris/borate/EDTA (TBE) buffer. Gels were stained with ethidium bromide, photographed, and transferred onto Zeta-Probe membranes (Bio-Rad) by using an alkaline transfer protocol (34) or onto nitrocellulose by the method of Southern (35). The membranes were prehybridized for 1 hr at 60°C in 0.75 M NaCl/0.075 M sodium citrate, pH 7.0/20 mM sodium phosphate, pH 7.0/5 mM EDTA, 200 μg of yeast RNA per ml/1% sarkosyl (Sigma). The buffer was removed and replaced with the same containing 1–2 × 10^6 cpm of 5′-end ^{32}P-labeled oligonucleotides per ml. Hybridization was for 5–6 hr at 60°C, and washing was done at 60°C in 0.75 M NaCl/0.075 M sodium citrate/0.1% $NaDodSO_4$. Blots were autoradiographed by using Kodak XAR film and DuPont Quanta III intensifier screens at −70°C. For a size marker, the 123-bp ladder from Bethesda Research Laboratories was used. Note that during the course of this study we found that alkaline transfer onto Zeta-Probe using NuSieve agarose is unreliable.

RESULTS

Immunotype and Karyotype Analyses of Clinical Samples. Leukocytes isolated from four clinical peripheral blood samples were used to evaluate the expression of BCR-ABL mRNA in leukemic cells with the PCR method. Protein and cytogenetic data of three of the patients are shown in Fig. 1. Patient 1 (Fig. 1*B*) was diagnosed as Ph^1 chromosome negative with a myeloproliferative syndrome. Patient 2 (Fig. 1*D*) and patient 3 (Fig. 1*E*) were Ph^1 chromosome positive in an accelerated stage of CML. Patient 4, whose data are not shown, was previously diagnosed as Ph^1 chromosome positive in a chronic stage of CML; protein data could not be obtained because of the poor quality of the cells. Leukemic cells from patients 2, 3, and 4 contained both Ph^1- and 9q-positive chromosomes, suggesting the presence of the usual t(9;22) reciprocal translocation. Patients 2 and 3 (Fig. 1 *D* and *E*) showed the characteristic 210-kDa BCR-ABL fusion protein, while patient 1 (Fig. 1*B*) showed only the normal P145 ABL polypeptide. Fig. 1 *A* and *C* show K562 cell controls that synthesize both P210 and P145 proteins.

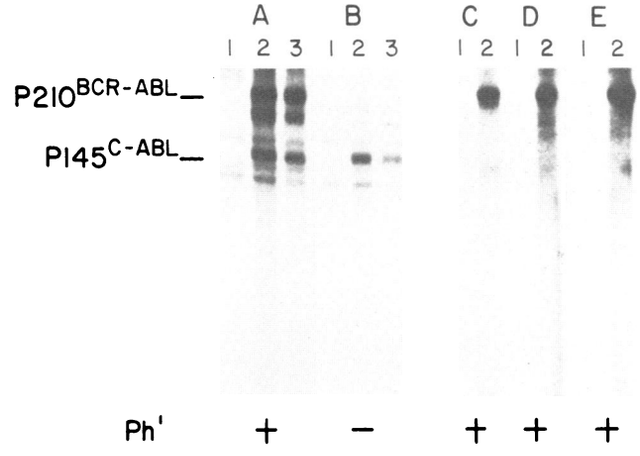

FIG. 1. Immunotype and karyotype analysis of clinical samples. (*A*) K562 cell control. (*B*) Patient 1. (*C*) K562 control. (*D*) Patient 2. (*E*) Patient 3. Antiserum in lanes: 1, normal rabbit; 2, rabbit anti-pEX5 (23); 3, rabbit anti-pEX2 (23). The data in *A* and *B* were obtained by immunoprecipitation of samples labeled *in vivo* with ortho[^{32}P]phosphate (23). The data in *C–E* were obtained by using the *in vitro* kinase assay (24). Samples were analyzed by $NaDodSO_4$/PAGE on 8% gels followed by autoradiography. K562 and patients 2 and 3 are Ph^1 chromosome positive, while patient 1 is Ph^1 chromosome negative. Ph^1 chromosome diagnosis is indicated as + or −. Protein analysis on another sample, from patient 4, was not done because of the poor quality of the cells; this patient was previously karyotyped as Ph^1 chromosome positive.

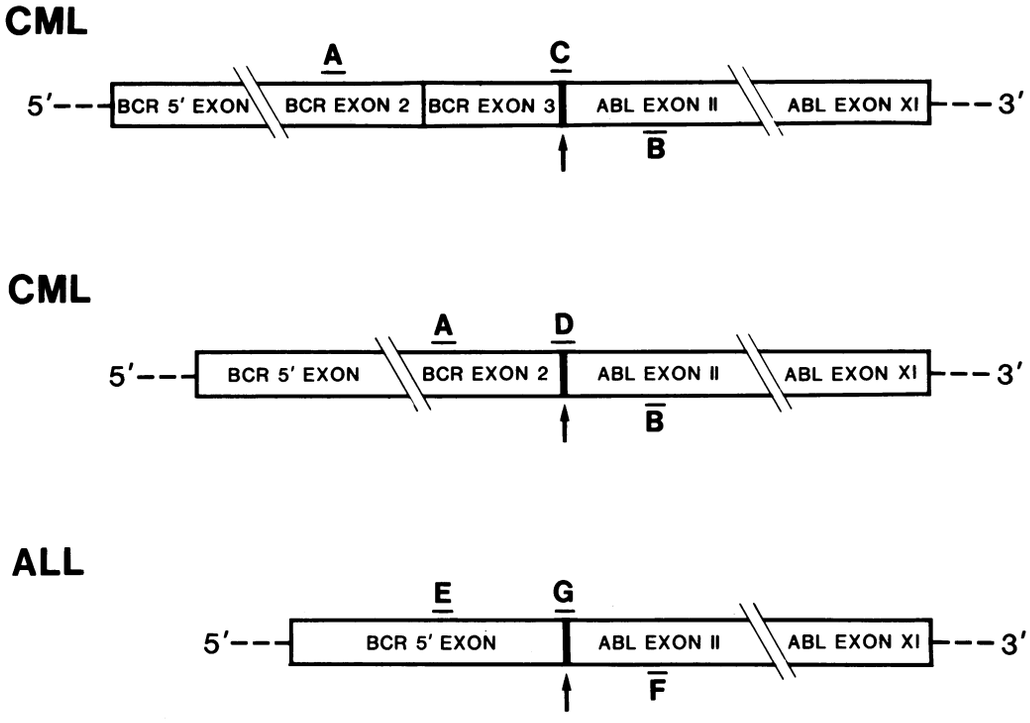

FIG. 2. CML and ALL mRNA structure and location of diagnostic oligonucleotides. A and B represent PCR primers used for amplifying the two known CML *BCR/ABL* junction sequences. C and D are CML probes specific for *BCR* exon 3/*ABL* exon II and *BCR* exon 2/*ABL* exon II junction sequences, respectively. E and F are primers that amplify the ALL *BCR/ABL* junction sequence, and G is the probe specific for the ALL junction. The arrows point to the junction between the *BCR* and *ABL* exons. No number as yet has been assigned to the ALL *BCR* exon. The figure is not drawn to scale.

Polymerase Chain Reaction (PCR) Analysis of Clinical Samples. The four clinical samples were analyzed "blind" by the PCR method. BCR-ABL RNA sequences from the four samples were PCR-amplified by using the CML-specific amplification primers CML A and CML B (see Fig. 2 and *Materials and Methods* for details on PCR primers and probes). The PCR products were analyzed by Southern blot (Fig. 3 *Left*) with oligonucleotide CML C, which hybridizes only to *BCR* exon 3/*ABL* exon II junctions. The amplified DNA from patient 3 (Fig. 3 *Left*, lane 3) and the positive control K562 hybridized to the probe, showing that their *BCR/ABL* junctions are the same. The Ph^1-chromosome-negative HL-60 sample, which did not hybridize to CML C, contains only normal *BCR* and *ABL* sequences and was used as a negative control. The same blot was then stripped of the CML C probe and rehybridized with CML D, which is specific for *BCR* exon 2/*ABL* exon II junctions. Clinical samples 2 and 4 (Fig. 3 *Right*, lanes 2 and 4) both hybridized strongly to this probe, showing that their *BCR/ABL* junctions are the same but are different from those of patient 3 and K562 mRNAs. The sample from patient 1 (lane 1) hybridized to neither probe, consistent with the Ph^1-chromosome-negative cytogenetic diagnosis and the lack of P210 BCR-ABL protein.

Sensitivity of the PCR Assay. To determine the sensitivity of the assay, a dilution experiment was conducted. K562 cytoplasmic RNA was diluted in steps from 1 to 6 orders of magnitude by using 10 μg of yeast RNA per ml as the diluent

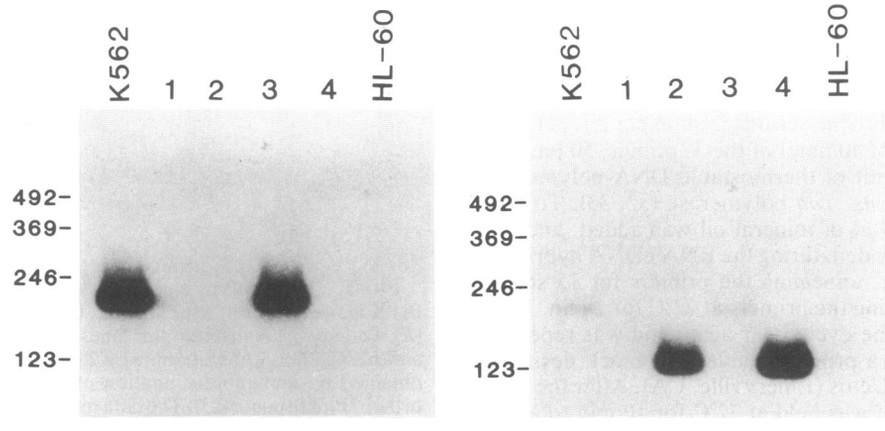

FIG. 3. PCR analysis of clinical samples. (*Left*) Blot probed with CML C, the BCR exon 3/ABL exon II-junction probe (see Fig. 2). (*Right*) Same blot stripped of probe and reprobed with CML D, the BCR exon 2/ABL exon II-junction probe. Lanes: K562, the Ph^1-chromosome-positive cell control; HL-60, Ph^1-chromosome-negative cell control; 1–4, samples from patients 1–4. Patients 2–4 were cytogenetically diagnosed as Ph^1 chromosome positive, whereas patient 1 was Ph^1 negative. Samples were amplified by using CML A and B, and 1/10th of the amplification products were analyzed on agarose gels, transferred to nylon membranes, and probed. K562 and HL-60 RNAs (1 μg) were used as templates, and 5 μl from 50-μl patient samples was used for the clinical samples. The marker is a 123-bp DNA ladder from Bethesda Research Laboratories.

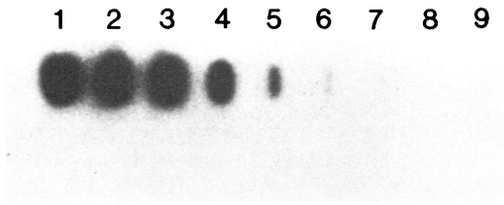

FIG. 4. Sensitivity of PCR assay. Slots: 1, K562 RNA; 2–7, dilutions of K562 RNA by 1–6 orders of magnitude, respectively; 8 and 9, buffer and Jurkat mRNA-negative controls, respectively. One microgram of K562 total cytoplasmic RNA was used as the initial sample (slot 1); consecutive 1:9 dilutions were made with yeast RNA at 10 μg/ml as diluent. Samples were amplified by using primers CML A and B as described in Fig. 3. One-tenth of the reaction mixture was bound to nitrocellulose by using a Schleicher & Schuell slot-blot apparatus. Probing was done with CML C as in Fig. 3. Jurkat is a Ph^1-chromosome-negative T-cell control RNA.

and carrier for the reverse transcriptase–PCR reaction. The PCR products were analyzed (Fig. 4) in a slot-blot format by probing with the CML A oligonucleotide, which is specific for the *BCR* exon 3/*ABL* exon II junction sequence. Slots 2 through 7 in Fig. 4 are order-of-magnitude dilutions of PCR products in slot 1. Slots 8 and 9 are negative controls consisting of yeast RNA diluent and Jurkat cell mRNA, respectively. With the undiluted RNA sample being 1 μg (slot 1), the final dilution represents 1 pg of K562 RNA (slot 7). A positive hybridization signal was easily detectable at the 5-order-of-magnitude dilution (slot 6). There was 10 pg of K562 RNA at this dilution, but since only 1/10th of the reaction mixture was actually analyzed, the signal was derived from the amplified product of 1 pg of total cytoplasmic RNA.

PCR Analysis of a Ph^1-Chromosome-Positive ALL Cell Line. To test the PCR amplification method in the case of ALL, RNA was isolated from SUP-B15, a cell line derived from a Ph^1-chromosome-positive ALL patient (21). This cell line has been shown to express a P185 BCR-ABL protein and a 7-kb BCR-ABL transcript (15, 24). ALL-specific BCR-ABL RNA sequences were amplified with PCR oligonucleotide primers ALL E and F (see Fig. 2 and *Materials and Methods*), and the products were analyzed by probing a Southern gel with the ALL G oligonucleotide (Fig. 5). The same downstream oligonucleotide used for CML (CML B) could have been used for ALL, but a different one was used in ALL to make a larger PCR product; also this keeps the CML primers separate from ALL primers, which helps to prevent cross-contamination. In this case, K562 and patients 1 through 4 were used as negative controls. Only the amplification reaction from SUP-B15 RNA gave a strong hybridization signal in this experiment, demonstrating that Ph^1-chromosome-positive ALL BCR-ABL mRNA sequences can easily be distinguished from Ph^1-chromosome-positive CML BCR-ABL mRNA by this method.

DISCUSSION

The advent of PCR technology (18, 32) has greatly facilitated the analysis of small nucleic acid samples. As little as 1 ng of human genomic DNA can be used to amplify and detect allelic sequence variations (36). We have modified this approach to include mRNA as the initial template in the amplification scheme. The mRNA is first converted to single-stranded cDNA, which can then be used for enzymatic amplification of sequences between specific primers by using a thermostable DNA polymerase (32, 33). Diagnostic PCR products are then easily detected by hybridization to amplification-specific oligonucleotides. By this approach we have successfully analyzed four clinical samples (Fig. 3), three of

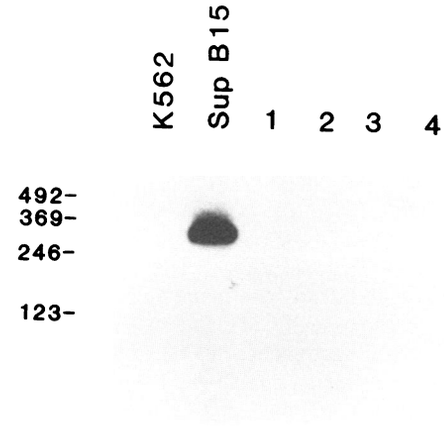

FIG. 5. PCR analysis of ALL RNA. Samples in lanes: K562, the negative control; SUP-B15, the ALL Ph^1-chromosome-positive cell line (21); 1–4, patients 1–4. One microgram of K562 and SUP-B15 RNAs were used in the PCR reactions. Five micrograms of 50-μg clinical samples was used. PCR primers ALL E and ALL F were used for amplification, and ALL G was used for hybridization analysis. Patients 1–4 are the same as in Fig. 3. Other details are as in Fig. 3.

which were previously diagnosed as Ph^1-chromosome-positive CML. Besides concurring with the earlier diagnoses, the analysis provided information that was not known prior to this study. Two of the patients (patients 2 and 4) expressed BCR-ABL mRNA resulting from a fusion of *BCR* exon 2 with *ABL* exon II, while patient 3 had the product resulting from the fusion of *BCR* exon 3 with *ABL* exon II. Normal diagnostic procedures cannot distinguish between these two types of BCR-ABL mRNA structures. While the influence of the two *BCR* exons on the progression or severity of CML is not known, routine use of the RNA-PCR technique should make any such difference far easier to detect.

We also have shown that this amplification protocol can be used to distinguish between the BCR-ABL transcripts present in Ph^1-chromosome-positive ALL cells versus CML cells (Fig. 5). Since the treatment and prognosis of ALL are somewhat dependent on the type of chromosomal abnormality present (9), a facile method of diagnosis would help greatly in this area.

The sensitivity of this method is higher than existing protocols for CML diagnosis. A $1:10^5$ dilution of 1 μg of RNA from the Ph^1-positive K562 cell line still provided an easily detectable signal in this assay (Fig. 4, slot 6). One microgram of RNA is roughly equivalent to the amount contained in the cytoplasm of 100,000 K562 cells (10 pg of cytoplasmic RNA per cell). Thus, the $1:10^5$ dilution contains the RNA from about one K562 cell. Since just 1/10th of the reaction mixture was used for analysis, the positive signal represents the amplified product of less than one cell equivalent. This result demonstrates that diagnosis is feasible even when the leukemic cells are present in extremely small numbers. A similar finding was reported for amplification studies of human DNA from follicular lymphoma cells (37). The sensitivity of this method was further demonstrated when we were able to correctly identify BCR-ABL messages in a CML sample (patient 4, Fig. 3 *Right*) that proved refractile to the *in vitro* kinase assay (24). To estimate the limits of detection of the kinase assay, K562 cells were serially diluted with Ph^1-chromosome-negative HL-60 cells and tested for kinase activity. In this mixing experiment, detection of the P210 *BCR-ABL*-encoded kinase enzyme required the presence of about 10^4 K562 cells (S.S.C. and O.N.W., unpublished observations). As described above, the P210 BCR-ABL mRNA can be detected by amplifying the RNA from the equivalent of one cell. Thus, the RNA-PCR method is more

than 10^3 times more sensitive than the kinase assay in detecting the expression of the *BCR-ABL* gene.

The diagnosis of CML and ALL by amplification and detection of leukemia-specific mRNA sequences has some advantages over other molecular methods. (*i*) Standard Southern gel analysis requires about 5% of the cells to be leukemic for the detection of deletions and/or translocations in genomic DNA (38). (*ii*) PCR amplification of coding sequences in genomic DNA requires knowledge of the exon–intron structure of the gene, since interruptions of the coding sequence with large introns can make amplification difficult or impossible. In the case of CML and ALL, not only is there a problem of intron sequences, but there is also the added difficulty imposed by the variable *BCR-ABL* gene structure found from patient to patient. In addition, the breakpoints that occur in the first intron of the *ABL* gene can arise in a region of up to 200 kb in length (39). Thus, use of amplification methods to analyze Ph^1-chromosome-type translocations in these leukemias requires that mRNA be used as the starting template. (*iii*) In a sense, the RNA template is already amplified because expression of most genes results in many copies of their mRNAs per cell. The BCR-ABL mRNA can be identified without amplification by using a sensitive RNase A assay (40), but large numbers of leukemic cells are required for this method and, thus, will not be easy to use routinely. (*iv*) Finally, the PCR method is applicable to the study of any type of RNA found in the cell, whether it is of normal or abnormal origin. Its use will most certainly include the diagnosis of other cancers and many infectious diseases as well as general studies of the molecular biology of the cell. As proof, while this manuscript was in preparation, two studies were reported in which amplification of RNA sequences was used (*i*) to determine the mechanism for tissue-specific processing of an apolipoprotein mRNA (41) and (*ii*) to facilitate cloning of *HLA-DQ* sequences (42).

We acknowledge the Cetus DNA Synthesis Group for numerous oligonucleotides; D. Gelfand and S. Stoffel for providing pure *Taq* polymerase; R. Saiki, A. Wang, and D. Gelfand for helpful discussions on RNA-PCR techniques; the Cetus Instrumentation Group for the PCR machine; and the Cetus Graphics Group for figures. S.S.C. is a Special Fellow of the Leukemia Society of America, and O.N.W. is an Investigator of the Howard Hughes Medical Institute.

1. Nowell, P. C. & Hungerford, D. A. (1960) *Science* **132,** 1497–1499.
2. Rowley, J. D. (1973) *Nature (London)* **243,** 290–291.
3. de Klein, A., van Kessel, A. G., Grosveld, G., Bartram, C. R., Hagemeijer, A., Bootsma, D., Spurr, N. K., Heisterkamp, N., Groffen, J. & Stephenson, J. R. (1982) *Nature (London)* **300,** 765–767.
4. Heisterkamp, N., Stephenson, J. R., Groffen, J., Hansen, P. F., de Klein, A., Bartram, C. R. & Grosveld, G. (1983) *Nature (London)* **306,** 239–242.
5. Groffen, J., Stephenson, J. R., Heisterkamp, N., de Klein, A., Bartram, C. R. & Grosveld, G. (1984) *Cell* **36,** 93–99.
6. Shtivelman, E., Lifshitz, B., Gale, R. P. & Canaani, E. (1985) *Nature (London)* **315,** 550–554.
7. Konopka, J. B., Watanabe, S. M. & Witte, O. N. (1984) *Cell* **37,** 1035–1042.
8. Williams, D. L., Look, A. T., Melvin, S. L., Roberson, P. K., Dahl, G., Flake, T. & Stass, S. (1984) *Cell* **36,** 101–109.
9. Bloomfield, C. D., Goldman, A. I., Alimena, G., Berger, R., Borgstrom, G. H., Brandt, L., Catovsky, D., de la Chapelle, A., Dewald, G. W., Garson, O. M., Garwicz, S., Golomb, H. M., Hossfeld, D. K., Lawler, S. D., Mitelman, F., Nilsson, P., Pierre, R. V., Philip, P., Prigogina, E., Rowley, J. D., Sakurai, M., Sandberg, A. A., Secker Walker, L. M., Tricot, G., Van Den Berghe, H., Van Orshoven, A., Vuopio, P. & Whang-Peng, J. (1986) *Blood* **67,** 415–420.
10. LeBeau, M. M. & Rowley, J. D. (1984) *Cancer Surv.* **3,** 271–279.
11. Champlin, R. E. & Golde, D. W. (1985) *Blood* **65,** 1039–1047.
12. Catovsky, D. (1979) *Br. J. Haematol.* **42,** 493–498.
13. Erickson, J., Griffen, C. A., ar-Rushdi, A., Valtieri, M., Hoxie, J., Finan, J., Emanuel, B. S., Rovera, G., Nowell, P. C. & Croce, C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1807–1811.
14. de Klein, A., Hagemeijer, A., Bartram, C. R., Houwen, R., Hoefsloot, L., Carbonell, F., Chan, L., Barnett, M., Greaves, M., Kleihauer, E., Heisterkamp, N., Groffen, J. & Grosveld, G. (1986) *Blood* **68,** 1369–1375.
15. Clark, S. S., McLaughlin, J., Timmons, M., Pendergast, A. M., Ben-Neriah, Y., Dow, L. W., Crist, W., Rovera, G., Smith, S. D. & Witte, O. N. (1988) *Science* **239,** 775–777.
16. Hermans, A., Heisterkamp, N., von Lindern, M., van Baal, S., Meijer, D., van der Plas, D., Wiedemann, L. M., Groffen, J., Bootsma, D. & Grosveld, G. (1987) *Cell* **51,** 33–40.
17. Fainstein, E., Marcelle, C., Rosner, A., Canaani, E., Gale, R. P., Dreazen, O., Smith, S. D. & Croce, C. M. (1987) *Nature (London)* **330,** 386–388.
18. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1354.
19. Lozzio, C. G. & Lozzio, B. B. (1975) *Blood* **45,** 321–334.
20. Collins, S. J., Gallo, R. C. & Gallagher, R. E. (1977) *Nature (London)* **270,** 347–349.
21. Naumovski, L., Morgan, R., Hecht, F., Link, M. P., Glader, B. E. & Smith, S. D. (1988) *Cancer Res.*, in press.
22. Hansen, J. A., Martin, P. J. & Nowinski, R. C. (1980) *Immunogenetics* **10,** 247–252.
23. Konopka, J. B., Watanabe, S. M. & Witte, O. N. (1984) *Cell* **37,** 1035–1042.
24. Clark, S. S., McLaughlin, J., Crist, W. M., Champlin, R. & Witte, O. N. (1987) *Science* **235,** 85–88.
25. Kawasaki, E. S., Ladner, M. B., Wang, A. M., Van Arsdell, J., Warren, M. K., Coyne, M. Y., Schweickart, V.L., Lee, M.-T., Wilson, K. J., Boosman, A., Stanley, E. R., Ralph, P. & Mark, D. (1985) *Science* **235,** 85–88.
26. Schwartz, R. C., Sonenshein, G. E., Bothwell, A. & Gefter, M. L. (1981) *J. Immunol.* **126,** 2104–2108.
27. Cathala, G., Savouret, J.-F., Mendez, B., West, B. L., Karin, M., Martial, J. A. & Baxter, J. D. (1983) *DNA* **2,** 329–335.
28. Heisterkamp, N., Stam, K., Groffen, J., de Klein, A. & Grosveld, G. (1985) *Nature (London)* **315,** 758–761.
29. Grosveld, G., Verwoerd, T., van Agthoven, T., de Klein, A., Ramchandran, K. L., Heisterkamp, N., Stam, K. & Groffen, J. (1986) *Mol. Cell. Biol.* **6,** 607–616.
30. Shtivelman, E., Lifshitz, B., Gale, R. P., Roe, B. A. & Canaani, E. (1986) *Cell* **47,** 277–284.
31. Mullis, K. B. & Faloona, F. A. (1987) *Methods Enzymol.* **155,** 335–350.
32. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239,** 487–491.
33. Chien, A., Edgar, D. B. & Trela, J. M. (1976) *J. Bacteriol.* **127,** 1550–1557.
34. Reed, K. C. & Mann, D. A. (1985) *Nucleic Acids Res.* **13,** 7207–7221.
35. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
36. Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986) *Nature (London)* **324,** 163–166.
37. Lee, M.-S., Chang, K. S., Cabanillas, F., Freireich, E. J., Trujillo, J. M. & Stass, S. A. (1987) *Science* **237,** 175–178.
38. Yoffe, G., Blick, M., Kantarjian, H., Spitzer, G., Gutterman, J. & Talpaz, M. (1987) *Blood* **69,** 961–963.
39. Bernards, A., Rubin, C. M., Westbrook, C. A., Paskind, M. & Baltimore, D. (1987) *Mol. Cell. Biol.* **7,** 3231–3236.
40. Shtivelman, E., Gale, R. P., Dreazen, O., Berrebi, A., Zaizov, R., Kubonishi, I., Miyoshi, I. & Canaani, E. (1987) *Blood* **69,** 971–973.
41. Powell, L. M., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J. & Scott, J. (1987) *Cell* **50,** 831–840.
42. Todd, J. A., Bell, J. I. & McDevitt, H. O. (1987) *Nature (London)* **329,** 599–604.