Flow cytometric detection of BCR-ABL fusion proteins in leukemia patients via an immunobead assay

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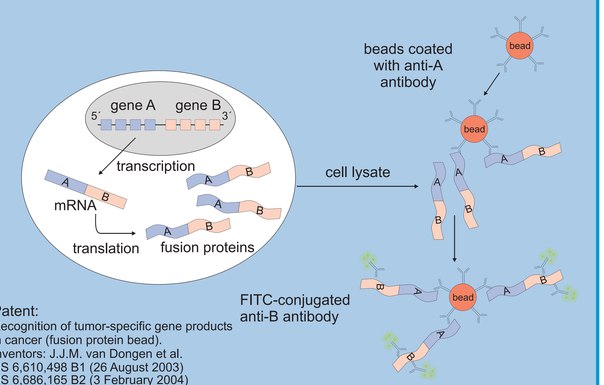
INTRODUCTION

The *BCR-ABL* fusion gene results from the translocation t(9;22). It is the hallmark of chronic myeloid leukemia (CML) and is present in a poor-risk subgroup of precursor B cell acute lymphoblastic leukemia (ALL), which represents 25-30% of adult ALL and 3-5% of childhood ALL. Consequently the detection of the *BCR-ABL* aberration is of utmost importance for diagnosis and classification of leukemia patients and can also be used as marker for monitoring of BCR-ABL$^+$ leukemias to evaluate treatment effectiveness.
So far, the *BCR-ABL* aberration has been detected by cytogenetics, FISH or PCR, all techniques that are time consuming and require special facilities.

We developed a simple flow cytometric bead assay for detection of the BCR-ABL fusion protein in cell lysates, using a bead-bound catching antibody against one side of the fusion protein and a fluorochrome-conjugated detection antibody against the other side of the fusion protein (see Figure 1).



Patent:
Recognition of tumor-specific gene products in cancer (fusion protein bead).
Inventors: J.J.M. van Dongen et al.
US 6,610,498 B1 (26 August 2003)
US 6,686,165 B2 (3 February 2004)

Figure 1. Principle of the flow cytometric immunobead assay for fusion protein detection. The target cells are lysed to release the fusion proteins. The bead-bound catching antibody recognizes one side of the fusion proteins and the fluorochrome-conjugated detection antibody recognizes the other side of the fusion proteins.

RESULTS

1. Detection of all variants of the BCR-ABL fusion protein.
The anti-BCR antibody was developed against a non-homologous region of ~80 amino acids, encoded by exon 1, which allowed us to detect all known BCR-ABL variants, including p190, p210, and p230, as demonstrated by analysis of a series of well-defined cell lines. The assay appeared to be specific and sufficiently sensitive to detect BCR-ABL proteins in lysates of leukocyte samples of leukemia patients at diagnosis.

2. Protease inhibition needed in cell samples with many mature myeloid cells.
Protein stability problems were encountered when cell samples contained high frequencies of mature myeloid cells with high levels of protease activity, such as CML cells and granulocyte fractions. This problem could be significantly reduced by adding protease inhibitors to several steps of the immunobead assay.

3. Development and testing of standardized Research Use Only (RUO) kit.
The immunobead assay was further developed and standardized by BD Biosciences into the Cytometric Bead Array (CBA) assay (BCR-ABL Protein Kit; BD Biosciences, San Jose, CA) for Research Use Only (RUO). The BCR-ABL immunobead kit for BCR-ABL fusion protein detection was evaluated in parallel to PCR analysis for BCR-ABL transcipt in 9 diagnostic laboratories of the EuroFlow Consortium on a series of 145 freshly collected samples from newly diagnosed (n=134) or relapsed (n=11) leukemia patients: 19 CML patients, 78 precursor-B-ALL patients, 18 T-ALL patients, 27 acute myeloid leukemia (AML) patients, and 3 patients with other hematological proliferations (see Table). In addition, 72 healthy controls were tested with the immunobead assay.

4. High specificity of BCR-ABL immunobead assay.
High concordance (100%; 145/145) was obtained between BCR-ABL PCR results and the BCR-ABL immunobead results:
- 17/78 precursor-B-ALL were BCR-ABL positive in both assays (mainly adults)
- 19/19 CML were BCR-ABL positive in both assays (with very weak immunobead signals in one CML case)
- 0/48 of other acute leukemias or proliferations were BCR-ABL positive

5. Two subgroups of pateints according to levels of BCR-ABL expression.
According to the Mean Fluorescence Intensity (MFI) values, two main groups of BCR-ABL positive patient samples were seen: high level positivity with MFI values 1,000 and low level positivity with MFI values 135 but <1,000. Negative samples were defined as MFI values <135 (see Figure 2). Different MFI values were seen in precursor-B-ALL and CML:
- Precursor-B-ALL: 88% (15/17) high level positivity
- CML: 84% (16/19) low level positivity (true low expression or remaining protease activity?)

Samples tested	*BCR-ABL* PCR assay			BCR-ABL RUO immunobead assay		
	negative	p190	p210	negative*	low positivity*	high positivity*
CML (n=19)	0	1	18	0	16	3
Precursor-B-ALL						
- childhood (n=50)	49	1	0	49	0	1
- adult (n=28)	12	13	3	12	2	14
T-ALL (n=18)	18	0	0	18	0	0
AML (n=27)	27	0	0	27	0	0
Other hematological proliferations (n=3)	3	0	0	3	0	0
Healthy controls (n=72)	NT			72	0	0

* negative: MFI value <135; low positivity: MFI value 135 and <1,000; high positivity: 1,000

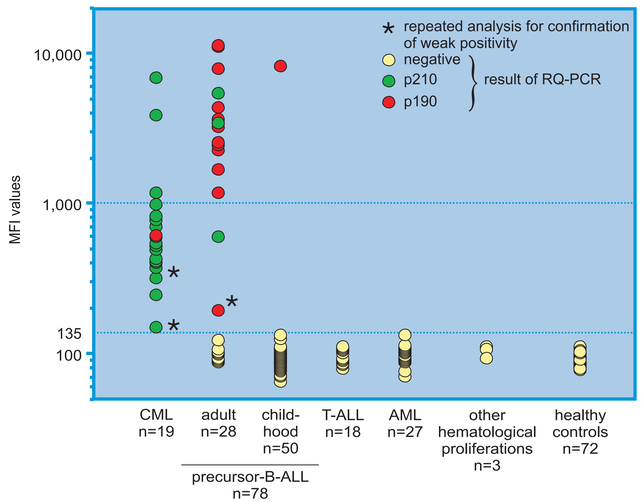


Figure 2. Results of the flow cytometric BCR-ABL RUO immunobead assay.

CONCLUSIONS

We conclude that the flow cytometric immunobead assay is a fast and easy technique for specific detection of BCR-ABL proteins in leukemic cells.

1. The main advantages of the immunobead assay are:
   - not dependent of the breakpoint position in the BCR gene;
   - does not need special laboratory facilities other then a routine flow cytometer;
   - provides results within several hours;
   - can be run in parallel to routine immunophenotyping (no extra technician time needed).

2. Since differentially labeled beads allow multiplexing, it will be possible to develop single tube assays for combined evaluation of multiple different fusion proteins, e.g. per disease category.

3. Consequently, the flow cytometric immunobead assay can contribute to fast and easy diagnosis and classification of leukemias with fusion genes.

4. If sufficient sensitivity can be reached, also monitoring becomes possible.