Reviewer’s report

Title: Myeloid antigens in childhood lymphoblastic leukemia: clinical data point to regulation of CD66c distinct from other myeloid antigens

Version: 1 Date: 18 January 2005

Reviewer: Vincent H van der Velden

Reviewer’s report:

General

The authors analyzed 365 precursor-B-ALL patients and show that (1) CD66c (KOR-SA4544) is expressed on 43% of cases, (2) CD66c expression is negatively correlated with the expression of other myeloid antigens (CD13, CD33, and CD65), (3) CD66c expression is stable between diagnosis and relapse, and (4) CD66c expression has no prognostic relevance. In addition, the authors show, in contrast to previously published data, that CD66c is invariably expressed on the cell surface and is not retained in the cytoplasm.

The experiments seem to be performed well and the manuscript is clearly written.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

- The authors performed flowcytometry to determine the intracellular expression of CD66c. It is however not clear how this experiment was exactly performed and what data are shown in figure 2. It now seems that cells were either immediately used for membrane staining with CD66c, or were first permeabilized, followed by (membrane and intracellular) CD66c staining (in combination with other markers for gating?). Theoretically, all samples should at least be on the 45° angle line (or above), but in figure 2 several cases have a lower percentage of CD66c positive cells if CD66c is determined on permeabilized cells as compared to cells with CD66c membrane staining only. The experiment would have been more elegant if, for example, membrane-bound CD66c was first stained using the KOR-SA3544 antibody, followed by permeabilization and subsequent staining with the 9A6 antibody (labeled with a different fluorochrome).

- The authors indicate that CD66c negative cells of heterogeneous populations showed higher CD66c transcript levels than CD66 negative cells from homogeneous populations. However, since the transcript level of CD66c in CD66c positive cells is about 100-times higher, minor contaminations (around 1%) will already result in such seemingly different transcript levels. In my opinion it is more likely that the observed difference is due to such minor contaminations. Furthermore, it would be interesting to see whether there is a correlation between CD66c protein expression levels and CD66c transcript levels, both determined on the total leukemic population.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

- Material and Methods, section ‘Patients’: the authors should indicate in how many patients TEL/AML1, BCR/ABL or MLL/AF4 fusion gene transcripts were detected.
- Although reported before, it may be interesting for the readership of BMC to briefly indicate the correlation between CD66c expression and genotype (presence of particular fusion gene transcript, hyperdiploidy) in the current patient series.
- The authors should not refer to submitted data (reference 26), but should include this in the discussion as 'unpublished results'.
- Legend Figure 1: ‘CD66c positivity excludes positivityâ€’ is not correct, as some cases do express both CD66c and another myeloid antigen.
- Figure 6: Instead of showing the total number of patients in each graph, it would be easier to indicate for each graph the number of patients in the CD66c positive and CD66c negative group. The BCR-ABL positive group is too small for statistical analysis; this figure can be deleted and results can be mentioned briefly in the text. Also results in the MLL-AF4 positive group can be mentioned in the text briefly.

Discretionary Revisions (which the author can choose to ignore)

- CD66c expression is, in many cases, only expressed on a subset of the leukemic blast cells. This implies that, when applied for MRD analysis, CD66c will only detect a part of the leukemic cells. The authors may discuss the use of CD66c for MRD analysis in some more detail in the 'Discussion'.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests