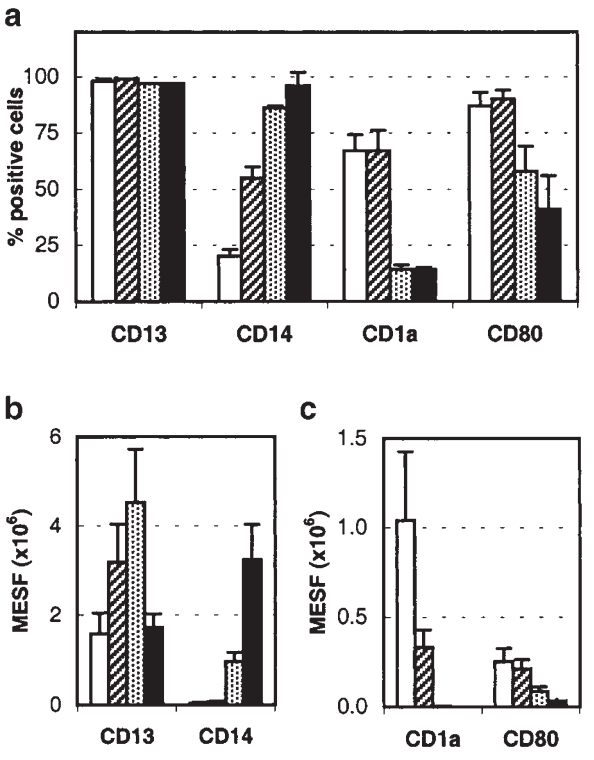

Figure 2 Effect of CD13/APN inhibition on the immunophenotype of the high scatter population obtained from GM-CSF + IL-4-stimulated monocyte cultures. Cells were immunophenotyped as described previously[6] followed by analysis of the high scatter population. (a) Percentage of positive cells and (b, c) fluorescence intensity (expressed as mean equivalents of soluble fluorescence (MESF)[6] of the positive population after a 5 day culture of monocytes in culture fluid only (black bars) or culture fluid supplemented with GM-CSF and IL-4 in the absence (white bars) or presence of bestatin (hatched bars) or leuhistin (dotted bars) (mean ± s.e.m.; $n = 3$).

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Deletion of the multidrug resistance protein 1 (*MRP1*) gene in acute myeloid leukemia patients with inversion 16: expression of *MRP1* homologues

TO THE EDITOR

In the prospective study of Döhner *et al*,[1] it is described that the deletion of the multidrug resistance protein (*MRP1*) gene has no prognostic impact in acute myeloid leukemia (AML) patients with an inversion on chromosome 16. These findings are in agreement with the results we recently described,[2] and might be due to the fact that the expression of *MRP1* homologues can be upregulated in the case of *MRP1* deletion.

MRP1, encoded by the *MRP1* gene located on chromosome 16p13, is a member of the superfamily of ATP-binding cassette (ABC) transporters. MRP1 has been shown to transport a broad range of organic substrates, and the expression of MRP1 results in resistance to different classes of chemotherapeutic agents. The MRP family of proteins consists of at least six family members, MRP1 to MRP6.[3] However, the role of the *MRP1* isoforms is not yet well defined. MRP2, MRP3 and MRP5 have been described to transport chemotherapeutic agents, such as cisplatin, doxorubicin, etoposide and 6-mercaptopurine, but less is known about the transport mechanisms of the proteins MRP4 and MRP6. Another membrane transporter, P-glycoprotein (P-gp) encoded by the *MDR1* gene, has also been shown to transport chemotherapeutic drugs. The simultaneous expression and function of both proteins has been shown to be correlated with poor overall survival in AML.[4]

A subgroup of patients with AML, most commonly patients with the French–American–British (FAB) classification M4Eo, show the chromosomal inversion 16 inv(16)(p13q22), resulting in a fusion of the *MYH11* gene on 16p13 with the *CBFB* gene on 16q22. Deletion of 1 *MRP1* allele has been demonstrated in a subgroup of AML patients with inv(16) and patients with the deletion showed an increased duration of disease-free survival as compared with patients without deletion.[5] However, these findings could not be confirmed in several other studies. In addition the study of Döhner *et al*[1] in 25 AML patients with inv(16) also showed no correlation between *MRP1* deletion and remission duration.

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In our study[2] we determined MRP activity with a flow cytometric assay[6] in the context of *MRP1* deletion in 11 inv(16) AML patients. A correlation was observed between MRP activity and the occurrence of *MRP1* deletions ($r = 0.91$, P 0.01), suggesting an important role for MRP1. However, we observed an up-regulation of the *MRP1* homologues *MRP2* and *MRP6*, especially in those patient samples with one *MRP1* deletion. In addition we observed a high activity of P-gp in one of the AML patients with *MRP1* deletion. No association was found between *MRP1* deletion and clinical outcome in the AML patients. In conclusion, it seems likely that in the case of a deletion of a transporter gene, the function might be compensated for by other transporter proteins, which might also affect the clinical outcome in inv(16) AML patients.

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Is HLA-DR4 or the HLA-DRB1*0402 allele associated with decreased risk for CML?

TO THE EDITOR

In a study involving a large number of CML patients, Posthuma *et al*[1] recently reported that there was a significantly reduced frequency of HLA-DR4 positivity in CML compared with a very large control population matched by country of origin and ethnicity. None of the other HLA-DR specificities tested showed such an association, with the exception of HLA-DR3, the effect of which was attributed to its linkage disequilibrium with a class I protective specificity, HLA-B8. They speculated that lack of protection by HLA-DR specificities other than DR4 may be due to their inability to bind and present antigenic peptides derived from bcr/abl fusion proteins, specifically b3/a2 peptides. However, previously published results of competition assays assessing the binding capacity of b3/a2 peptides to purified HLA-DR molecules do not support this proposition.[2] Thus, of nine different alleles tested, we found that only HLA-DR11 strongly bound b3/a2 peptides. This finding is consistent with an earlier report on DR11 anchor motifs.[3] In T cell sensitization assays using healthy donors, three of five DR11+ donors showed evidence of responses to the peptides, whereas none of four DR4+ donors responded. However, the DR4+ donors tested were all HLA-DRB1*0401+, leaving open the possibility that one or more of the other DR4 family alleles might be able to bind and present b3/a2. Indeed, the competition assays had indicated weak binding to the 0402 allele, but not to 0401, 0403 or 0404. Furthermore, binding predictions for b3/a2-fusion peptides indicate a relatively weak putative binding to DRB1*0401 compared with stronger binding to DRB1*0101 (see http://www.uni-tuebingen.de/uni/kxi/), which is in line with the aforementioned *in vitro* binding studies. It would therefore be extremely interesting to know whether the protective effect of HLA-DR4 described by Posthuma *et al* is attributable to the presence of the DRB1*0402 allele. As the proportion of DR4+ persons carrying the 0402 specificity in whites is only around 20%, this would represent a highly significant effect for this subset of individuals. A survey of CML patients using more sophisticated HLA typing would be valuable in identifying whether protection is limited to this one particular DR4 family member. Evidence from other investigators for b3/a2 presentation by different DR4 alleles is sparse. One report mentioned an inability of 0404+ donors to respond to b3/a2, consistent with our binding data.[4] The same group also noted heterogeneous responses amongst 0401+ donors, with three failing to respond.[4] There have also been reports of HLA-DR1[5] and DR15[6] presentation, alleles which we found bound b3/a2 peptide measurably but extremely weakly.[2] However, there appear to be no data at all on b3/a2 presentation by DRB1*0402. Therefore, in the light of these considerations and the new data of Posthuma *et al*,[1] it would be extremely useful to investigate presentation by this allele, and to establish whether individuals carrying this allele are indeed resistant to CML.

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