original paper

haematologica 2001; 86:154-161
http://www.haematologica.it/2001_02/0154.htm

Impact of CD133 (AC133) and CD90 expression analysis for acute leukemia immunophenotyping

Christian Wuchter,* Richard Ratei,* Günther Spahn,* Claudia Schoch,° Jochen Harbott,# Susanne Schnittger,° Torsten Haferlach,° Ursula Creutzig,@ Christian Sperling,* Leonid Karawajew,* Wolf-Dieter Ludwig*

*Dept. of Hematology, Oncology and Tumor Immunology, Robert-Rössle-Clinic, Charité, Humboldt-University of Berlin, °Dept. of Internal Medicine III, Ludwig-Maximilians-University of München, #Oncogenetic Laboratory, Children's Hospital, Justus-Liebig-University of Gießen, @Dept. of Hematology-Oncology, University Children's Hospital, Münster, Germany

Background and Objectives. AC133 is a novel monoclonal antibody (Moab) reacting with a population of immature/primitive or granulo-monocytic committed CD34^{+ve} cells. Up to now, only few studies with small numbers of cases have examined AC133 (recently designated CD133) expression in acute leukemia. To determine the value of this Moab for acute leukemia immunophenotyping, we investigated a large series of leukemic cell samples for their reactivity with Moab AC133.

Design and Methods. A total of 298 cell samples from patients with *de novo* acute myeloid leukemia (AML) (n=142), acute lymphoblastic leukemia (ALL) (n=119), CD34^{+ve} biphenotypic acute leukemia (n=13), and CD34^{+ve} CML blast crisis (=BC; 21 myeloid BC/3 lymphoid BC) were investigated by flow cytometry for Moab AC133 reactivity. CD133 expression was compared with CD90(Thy-1) expression, another CD34-associated antigen.

Results. Fifteen (5%) samples expressed CD90, whereas 114 (38%) samples were positive for Moab AC133 (20% cut-off level). No significant differences in CD133 and CD90 expression levels between AML and ALL were observed. In AML, but not ALL, CD133 was more often expressed in CD34^{+ve} cases than in CD34^{-ve} ones (p<0.00001). However, CD133 expression was not restricted to CD34^{+ve} leukemic cells in individual cell samples. All 8 pro-B-ALL cell samples with 11q23-anomalies and MLL (*mixed lineage leukemia*) gene translocations were positive for CD133, whereas only 2 of 9 pro-B-ALL without MLL gene translocations expressed CD133 (p<0.002). In contrast, none of the 5 AML cell samples with a t(9;11) and MLL gene translocation reacted with Moab AC133. CD34^{+ve} CML cells in myeloid BC were less often positive for CD133 than CD34^{+ve} *de novo* AML cells (p <0.0001).

Interpretation and Conclusions. CD133 and CD90 expression analysis is not helpful for lineage determination in acute leukemia immunophenotyping. However, Moab AC133 may be an informative marker for the detection and further characterization of immature AML cells, as well as pro-B-ALL cells with MLL gene translocations, by flow cytometry.

Key words: acute leukemia, CD133 (AC133), CD90, flow cytometry, immunophenotyping

Correspondence: Christian Wuchter, M.D., Robert-Rössle-Clinic, Charité, Humboldt University of Berlin, Lindenberger Weg 80, 13125 Berlin, Germany. Phone: international +0049-30-94171362 - Fax: international +0049-30-94171308 - E-mail: wuchter@rrk-berlin.de

Acute leukemias derive from either malignant, transformed, uncommitted multipotent stem cells or lineage-restricted progenitor cells.[1-3] So far, CD34 is the most commonly used antigen to define immature hematopoietic progenitor cells.[4] In acute leukemia immunophenotyping, CD34 is not lineage-restricted and thus not useful for distinguishing acute myeloid leukemia (AML) from acute lymphoblastic leukemia (ALL).[5] Therefore, new Moabs, recognizing subsets of CD34 leukemic cells with the potential to distinguish uncommitted multipotential stem cells from lineage-restricted progenitor cells are of special interest for acute leukemia immunophenotyping. Moab AC133 reacts with a population of non-committed or granulomonocytic (GM)-committed CD34^{+ve} cells in normal hematopoiesis.[6,7] Recently it was shown that among the CD34^{-ve}CD38^{-ve}Lin^{-ve} subset of human cord blood an extremely rare population of AC133^{+ve}CD7^{-ve} cells is highly enriched for progenitor activity and capable of differentiation into CD34^{+ve} cells.[8] So far, it is not clear which molecule is detected by Moab AC133, recently designated CD133 by the *7th Workshop and Conference on Human Leukocyte Differentiation* (June 2000). The AC133-antigen may be the human homolog of the mouse kidney prominin, which is preferentially found on the apical surface of various murine embryonic and adult epithelia.[9-11] Recent data suggest that CD133 expression also defines a subset of circulating endothelial cells that may play a role in neoangiogenesis.[12]

Up to now, only a few studies with small numbers of cases have examined CD133 expression in acute leukemia: in one study, CD133 expression was mainly restricted to immature AML FAB subtypes.[13] However, in another study the highest CD133 expression levels were found in myelomonocytic differentiated AML FAB M4/M5 cases.[6] Two other studies revealed no correlation between CD133 expression, FAB subtypes and cytogenetics in AML.[14,15] AC133 reactivity was observed in 56-67% of ALL cell samples examined.[6,15,16] In contrast, another study could not detect CD133 expression in any of 17 ALL samples tested.[13] In AML, some stud-

ies have found that Moab AC133 reacts preferentially with CD34^{+ve} leukemic cells,[13,14,17] although another study found no clear difference in CD133 expression levels between CD34^{+ve} and CD34^{-ve} AML.[15] A recent study revealed disconcordant CD133 expression in patients with AML or myelodysplastic syndrome (MDS) compared with precursor cells from normal marrow or peripheral blood stem cells.[18]

To determine the value of CD133 expression analysis for acute leukemia immunophenotyping on a large number of acute leukemia samples, we investigated 298 cell samples from children and adults with acute leukemia for their reactivity with Moab AC133 by flow cytometry. Furthermore, CD133 expression was compared with CD90(Thy-1) expression, an epitope found on approximately 25% of CD34^{+ve} cells with enhanced proliferative and self-renewal capabilities.[19] Finally, we correlated CD133 and CD90 expression with immunophenotypic features, FAB morphology and cyto-/molecular genetic data of the examined patients.

Design and Methods

Patients and cell samples

We examined either freshly obtained or cryopreserved bone marrow or peripheral blood samples from 298 patients with acute leukemia for their reactivity with Moab AC133[6] and anti-CD90 Moab 5E10.[19] In our series, 110 children with ALL (90 B-lineage ALL, 20 T-lineage ALL), 38 children and 104 adults with *de novo* AML, and 9 adults with ALL (8 B-lineage ALL, 1 T-lineage ALL) were included. Moreover, cell samples from 13 patients (2 adults, 11 children) with biphenotypic acute leukemia (=BAL), and 24 adults with CD34^{+ve} CML blast crisis (=BC; 21 myeloid BC/3 B-lymphoid BC) were examined in this study. The age of the patients examined ranged between 10 months and 78 years (median 51 years) for those with *de novo* AML, between 1 month and 59 years (median 5 years) for those with ALL, between 1 year and 70 years (median 12 years) for BAL, and between 32 years and 83 years (median 53 years) for those in CML blast crisis. Diagnosis of acute leukemia was based on immunologic criteria according to EGIL recommendations[5] and morphologically in accordance with FAB criteria.[20] B-lineage ALL cases were subgrouped into pro-B-ALL, common-ALL, pre-B-ALL, and mature B-ALL, and T-lineage ALL cases into pro-/pre-T-ALL, cortical T-ALL, and mature T-ALL.[5] The diagnosis of BAL was based on the EGIL scoring system.[5]

Leukemia immunophenotyping

Leukemic cells from heparinized bone marrow or peripheral blood samples were isolated by FicoII-Hypaque (Pharmacia, Freiburg, Germany) density gradient centrifugation, and leukemia-associated antigens were detected by a panel of Moabs either by a direct or indirect immunofluorescence assay as previously described.[21,22] Cell samples were analyzed by flow cytometry (FACScan; Becton Dickinson, San Diego, USA) using the Cell-Quest-software program (Becton Dickinson). Cell samples were considered positive for a specific antigen if the antigen was expressed on at least 20% of the leukemic cells/sample (= 20 % cut-off level).[5,22]

Detection of CD133 and CD90 expression in acute leukemia by flow cytometry

Cells were stained by direct immunofluorescence using the phycoerythrin (PE)-conjugated Moab AC133[6] (IgG1; Miltenyi Biotech, Auburn, CA, USA) and the FITC-conjugated anti-CD90 Moab 5E10[19] (IgG1; PharMingen, San Diego, CA, USA) as recommended by the manufacturers. Non-specific binding of Fcγ-receptors was blocked by pre-incubation of the cells with a polyclonal rabbit serum (Gibco BRL, Paisley, UK). At least 10,000 cells per sample were acquired and analyzed by flow cytometry as described above. Freshly obtained cell samples were analyzed by three-color immunophenotyping, using CD90-FITC, CD133-PE and CD34-PECy5 (clone QBend10; Beckman Coulter, Marseille, France). Non-viable cells were excluded by scatter gating. Previously cryopreserved cell samples were analyzed by two-color immunophenotyping using the combinations CD90-FITC/CD34-PE, CD34-FITC/CD133-PE, and CD90/CD133-PE. In each of these samples, non-viable cells were excluded from analysis by propidium iodide co-staining (0.3 μg/mL; Sigma, Deisenhofen, Germany). No differences in CD133 and CD90 expression patterns between freshly obtained and previously cryopreserved cell samples were observed. Cell samples were considered positive for CD90 or CD133 if at least 20% of the leukemic cells/sample specifically stained with Moabs 5E10 or AC133 revealed a higher fluorescence intensity than cells stained with the isotype-matched control antibody (=20% cut-off level).[5,22]

Cytogenetics

Chromosome analyses were performed on metaphases from short-term (24h, 48h) cultures of pretreatment bone marrow and/or peripheral blood cells. Cell cultivation and chromosome preparation were carried out according to standard protocols. G-banding was used and the chromosomes interpreted according to ISCN (1995) nomenclature.[23] Patients with adult AML were grouped into three different risk categories: good: t(8;21), inv(16)/t(16;16), t(15;17); intermediate: normal karyotype, other abnormalities; poor: -5/5q-, -7/7q-, 11q23-abnormalities, inv(3)/t(3;3), t(9;22), t(6;9), 17p-abnormalities, complex aberrant karyotype (three or more numerical or structural abnormalities).

Reverse transcriptase polymerase chain reaction (RT-PCR)

RT-PCR was performed as previously described.[24,25] Primer sequences used for the detection of specific leukemic fusion transcripts have been described elsewhere (MLL ("mixed lineage leukemia") gene rearrangements,[26] PML/RARα,[27] AML1/ETO,[27] BCR/ABL,[28] TEL/AML1[25]).

Statistical analysis

Differences in the immunophenotypic expression patterns, cyto-/molecular genetic groups and FAB mor-

phology between CD133 and/or CD90 positive and negative cell samples were analyzed using the Chi-squared test (Pearson coefficient). The Kruskal-Wallis test was used to determine differences in CD133 or CD90 expression for the immunophenotypic subtypes in ALL, morphologic FAB subtypes in AML as well as the different CD34^{+ve} acute leukemia subtypes. In individual cases, CD133 and CD90 expression was correlated with the age of the patient examined using bivariate correlation (Spearman coefficient r_S). p values < 0.05 were considered statistically significant. All statistical analyses were done with the SPSS software program (version 9.0).

Results

CD133 and CD90 expression on CD34^{+ve} and CD34^{-ve} acute leukemia cells (Table 1)

In *de novo* AML, 44 (65%) of 68 CD34^{+ve} acute leukemia cell samples expressed CD133, whereas only 9 (12%) of 74 CD34^{-ve} acute leukemia cell samples were CD133 positive (p <0.00001). In ALL, 38 (42%) of 91 CD34^{+ve} acute leukemia cell samples expressed CD133 and 9 (32%) of 28 CD34^{-ve} acute leukemia cell samples were CD133 positive (not significant).

In *de novo* AML, 6 (9%) of 70 CD34^{+ve} acute leukemia cell samples expressed CD90 and 3 (4%) of 78 CD34^{-ve} AML cell samples were CD90 positive (not significant). In ALL, 2 (2%) of 91 CD34^{+ve} ALL cell samples expressed CD90 and none of 28 CD34^{-ve} acute leukemia cell samples were CD90 positive (not significant).

Cell samples of CD34^{+ve} CML in myeloid BC were less often positive for CD133 than were CD34^{+ve} *de novo* AML cell samples [5/21 (24%) CD133 positive cell samples vs. 44/68 (65%); p <0.001]. In contrast, CD90 expression differed, but not significantly, within these two subtypes [4/21 (19%) CD90 positive cell samples vs. 6/68 (9%); not significant]. The percentage of CD34^{+ve} cells/sample of the examined CD34^{+ve} CML and CD34^{+ve} *de novo* AML cell samples did not differ significantly within the two subgroups (CD34^{+ve} CML: 70±22% CD34^{+ve} cells/sample; CD34^{+ve} *de novo* AML: 76±18% CD34^{+ve} cells/sample). CD34^{+ve} BAL cases revealed no specific CD133 and CD90 expression pattern compared with CD34^{+ve} AML or CD34^{+ve} ALL cases (Table 1).

Figure 1 shows representative examples of CD133 and CD90 expression in acute leukemia.

CD133 and CD90 expression in acute leukemia: correlation with immunophenotype, FAB morphology, and age of the patients examined

There was no significant difference in CD133 expression between AML and ALL cell samples using the 20% cut-off level [53/142 (37%) CD133 positive cell samples vs. 47/119 (39%); not significant]. Within ALL, B-lineage ALL cell samples were more often positive for Moab AC133 than were the T-ALL cell samples (Table 2; p <0.01). Neither in B-lineage nor in T-lineage ALL was a correlation between immunophenotypic subtypes and CD133 expression observed (Table 2). In AML, CD133 positive cell samples

Table 1. CD133 and CD90 expression in CD34^{+ve} and CD34^{-ve} (20% cut-off level) acute leukemia subtypes.

	CD34^{+ve} de novo AML (n=68)	*CD34^{-ve} de novo AML (n=74)*	*CD34^{+ve} ALL (n=91)*	*CD34^{-ve} ALL (n=28)*	*CD34^{+ve} BAL (n=13)*	*CD34^{-ve} blast crisis (n=24)*
≥ 20% CD133+ cells/sample	44 (65%)*	9 (12%)	38 (42%)	9 (32%)	6 (46%)	8 (33%)
<20% CD133+ cells/sample	24 (35%)	65 (88%)	53 (58%)	19 (68%)	7 (54%)	16 (67%)
≥ 20% CD90+ cells/sample	6 (9%)	3 (4%)	2 (2%)	0 (0%)	1 (8%)	3 (13%)
<20% CD90+ cells/sample	62 (91%)	71 (96%)	89 (98%)	28 (100%)	12 (92%)	21 (87%)

**Number of cell samples (% of cell samples within the specific leukemia subtype). Abbreviations: BAL, biphenotypic acute leukemia.*

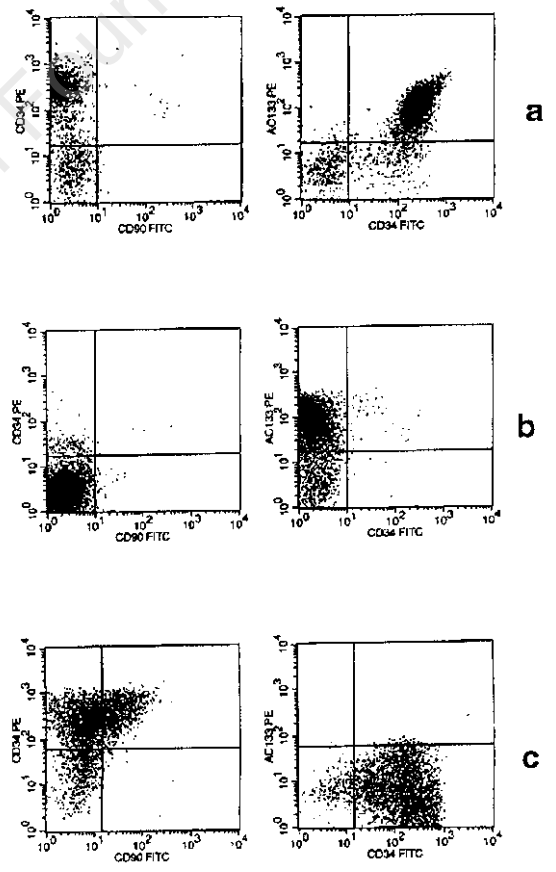

Figure 1. Representative examples of CD133 and CD90 expression analysis in acute leukemia by flow cytometry as described in Design and Methods. a) AML FAB M1 (CD34 95%/CD133 91%/CD90 0%); b) Pro-B-ALL with t(4;11) CD34 2%/CD133 93%/CD90 0%) c) CML in myeloid blast crisis (CD34 90%/CD133 0%/CD90 27%).

Table 2. CD133 and CD90 expression (20% cut-off level) in ALL: correlation with immunophenotypic subtypes.

	CD133 positive	*p*	*CD90 positive*	*p*
B-lineage ALL	44/98 (45%)[a]	<0.01	1/98 (1%)	ns
T-lineage ALL	3/21 (14%)		1/21 (5%)	
B-lineage ALL subtypes				
pro-B-ALL	10/17	ns	0/17	ns
c-ALL	26/54		1/54	
pre-B-ALL	8/26		0/26	
mature B-ALL	0/1		0/1	
T-lineage ALL subtypes				
pro-/pre-T-ALL	1/8	ns	1/8	ns
cortical T-ALL	2/9		0/9	
mature T-ALL	0/4		0/4	
My positive ALL	24/56 (43%)	ns	2/56 (4%)	ns
My negative ALL	23/63 (37%)		0/63 (0%)	

*Abbreviations: ns, not significant; My, co-expression of at least one myeloid antigen (CD13, CD15, CD33, CD65s); *positive cell samples/cell samples examined (positive cell samples in %).*

Table 4. FAB morphology of CD133 and/or CD90 positive AML cases (20%-cut-off level) compared with CD133 and/or CD90 negative AML cases.

	CD133 positive AML	*CD133 negative AML*	*p*	*CD90 positive AML*	*CD90 negative AML*	*p*
FAB total	41 (36%)*	72 (64%)		6 (5%)	107 (95%)	
FAB M0	6 (86%)	1 (14%)	<0.005	1 (14%)	6 (86%)	ns
FAB M1-7	35 (33%)	71 (67%)		5 (5%)	101 (95%)	
FAB M0	6	1	<0.002	1	6	ns
FAB M1	12	13		3	22	
FAB M2	15	17		0	32	
FAB M3	0	8		1	7	
FAB M4	7	14		1	20	
FAB M5	1	16		0	17	
FAB M6	0	2		0	2	
FAB M7	0	1		0	1	

**Positive cell samples/cell samples examined (positive cell samples in %). Abbreviation: ns, not significant.*

Table 3.Immunophenotype of CD133 and/or CD90 positive *de novo* AML cases.

CD antigen	*CD133 positive AML*	*CD133 negative AML*	*p*	*CD90 positive AML*	*CD90 negative AML*	*p*
CD34	44/53 (83%)[a]	24/89 (27%)	<0.00001	6/9 (67%)	62/133 (47%)	ns
CD117	50/53 (94%)	51/88 (58%)	<0.00001	9/9 (100%)	92/132 (70%)	=0.05
TdT	20/53 (38%)	5/89 (6%)	<0.00001	3/9 (33%)	22/133 (17%)	ns
HLA-DR	47/53 (89%)	59/89 (66%)	<0.004	6/9 (67%)	100/133 (75%)	ns
CD7	23/53 (43%)	21/89 (24%)	<0.02	3/9 (33%)	41/133 (31%)	ns
CD2	10/51 (20%)	9/87 (10%)	ns	2/9 (22%)	17/129 (13%)	ns
CD19	5/52 (10%)	9/88 (10%)	ns	0/9 (0%)	14/131 (11%)	ns
CD13	50/53 (94%)	70/89 (79%)	<0.02	6/9 (67%)	114/133 (86%)	ns
CD33	43/53 (81%)	87/89 (98%)	<0.0006	7/9 (78%)	123/133 (92%)	ns
MPO	34/53 (64%)	65/89 (73%)	ns	4/9 (44%)	95/133 (71%)	ns
CD65s	25/53 (47%)	56/89 (63%)	ns	2/9 (22%)	79/133 (59%)	<0.03
CD15	22/52 (42%)	50/89 (56%)	ns	0/9 (0%)	72/132 (55%)	<0.002
CD64	29/52 (56%)	67/89 (75%)	<0.02	3/9 (33%)	93/132 (70%)	<0.03
CD14	2/52 (4%)	19/89 (21%)	<0.005	0/9 (0%)	21/132 (16%)	ns
CD4	9/50 (18%)	32/89 (36%)	<0.03	3/8 (38%)	38/131 (29%)	ns
CD36	11/48 (23%)	33/87 (38%)	ns	2/9 (22%)	42/126 (33%)	ns
LF	13/52 (25%)	30/87 (34%)	ns	2/9 (22%)	41/130 (32%)	ns
CD56	10/51 (20%)	32/88 (36%)	<0.04	4/9 (44%)	38/130 (29%)	ns
CD41	3/52 (6%)	8/89 (9%)	ns	3/9 (33%)	8/132 (6%)	<0.004
CD61	4/52 (8%)	8/87 (9%)	ns	3/9 (33%)	9/130 (7%)	<0.007
CD133	–	–	–	6/9 (67%)	47/133 (35%)	ns
CD90	6/53 (11%)	3/89 (3%)	ns	–	–	–

[a] positive cell samples/cell samples examined (positive cell samples in %). Abbreviations: MPO, myeloperoxidase ; LF, lactoferrin ; ns, not significant.

showed a more immature immunophenotype (Table 3) and were more often of the immature FAB M0 subtype (Table 4) compared with CD133 negative AML cell samples. In individual ALL cases, there was a weak inverse correlation between CD133 expression (% of positive cells) and the age of the patient (r_S = -0.29; $p < 0.002$; n=119), whereas in *de novo* AML, no significant correlation between CD133 expression and the age of the patients was observed (data not shown). The increased number of TdT positive AML and rather low number of myeloperoxidase (MPO) positive AML cases in this study (Table 3) is most likely due to the flow-cytometric based detection of these antigens in our laboratory compared to the previously used conventional fluorescence light microscopy technique.

CD90 expression differed, but not significantly, between AML and ALL cell samples [9/142 (6%) CD90 positive cell samples vs. 2/119 (2%)]. There was also no significant difference in CD90 expression between B-lineage ALL and T-ALL cell samples (Table 2). Neither in B-lineage nor in T-lineage ALL was a correlation between immunophenotypic subtypes and CD90 expression observed (Table 2). In AML, CD90 positive cell samples showed an immunophenotype with partial megakaryocytic differentiation (CD41, CD61) (Table 3). No significant differences in CD90 expression between the morphologic FAB subtypes were observed (Table 4). Neither in *de novo* AML nor in ALL was any significant correlation between CD90 expression and the age of the patients observed.

Correlation of CD133 and CD90 expression with cyto-/molecular genetic data

All 8 pro-B-ALL cell samples with 11q23-anomalies and MLL (mixed lineage leukemia) gene translocations [t(4;11) n=7, t(11;19) n=1] were positive for CD133, whereas only 2 of 9 pro-B-ALL without MLL gene translocations expressed CD133 (p <0.002). In contrast, none of the 5 AML cell samples with a t(9;11) and MLL gene translocation reacted with Moab AC133. In B-lineage ALL, CD133 expression did not distinguish leukemic cells with t(12;21) and TEL/AML1 fusion transcript (n=9) or leukemic cells with t(9;22) and BCR/ABL fusion transcript (n=7) from the respective negative cases (data not shown). In AML, none of 7 leukemic cell samples with t(15;17) and PML/RARα fusion transcript reacted with Moab AC133, whereas 36 (41%) of 88 AML cases with normal karyotypes or other abnormalities were CD133 positive (p <0.04). AML cells with t(8;21) and AML1/ETO fusion transcript (n=3) showed no special CD133 expression pattern (data not shown). In adult *de novo* AML, CD133 expression did not differ significantly between cytogenetic risk groups (good: n=6, intermediate: n=46, high: n= 18; cytogenetic risk groups were defined as shown in Design and Methods) (data not shown). For CD90 expression, no significant correlations with specific cyto-/molecular genetic anomalies were observed, either in AML or in ALL (data not shown).

Discussion

In this study, we investigated the expression of the stem cell-related markers CD133 (AC133) and CD90 on leukemic cell samples from 298 children and adults with acute leukemia and determined its diagnostic value for acute leukemia immunophenotyping. In normal hematopoiesis, CD133 expression is thought to be restricted to $CD34^{+ve}$ progenitor cells.[6,7] However, our study demonstrates, in line with previous studies, that CD133 expression is not restricted to $CD34^{+ve}$ leukemia cells within individual cell samples in our series, neither in AML nor in ALL.[13-17] Recently, it was shown that among the $CD34^{-ve}$ $CD38^{-ve}Lin^{-ve}$ subset of human cord blood an extremely rare population of $AC133^{+ve}CD7^{-ve}$ cells is highly enriched for progenitor activity and capable of differentiation into $CD34^{+ve}$ cells.[8] The observed CD34-independent CD133 expression of leukemic cells might represent the malignant counterpart of this very small physiologic subset rather than an aberrant CD133 expression on more mature $CD34^{-ve}$ leukemic cells. Thus, a CD133 positive phenotype in $CD34^{-ve}$ acute leukemia should be considered with caution as a possible aberrant phenotype for minimal residual disease (MRD) monitoring by flow cytometry, both in AML and ALL.

In AML but not ALL, Moab AC133 reacted preferentially with $CD34^{+ve}$ leukemic cells. Moreover, CD133 expression was correlated with an immature immunophenotype and an immature FAB subtype in AML. As previously described by Yin *et al.*[7] for GM-committed normal progenitor cells, we also observed a decreasing reactivity of Moab AC133 with increasing levels of CD64 in individual AML cell samples (r_S= -0.4 (n=141), p <0.001), indicating a downregulation of CD133 with monocytic differentiation. Thus, Moab AC133 may be an informative marker for the detection and further characterization of immature AML cells by flow cytometry.

In colony assays *in vitro*, normal CD133 positive CD34 cells predominantly showed differentiation along the granulomonocytic differentiation pathway, suggesting that AC133 positivity defines a subset of GM-committed progenitor cells.[6,7] It was, therefore, intriguing to speculate that Moab AC133 reactivity may be helpful in distinguishing between immature AML and ALL cells, as was shown for CD117 expression analysis.[29] However, in line with previous studies, neither CD133 nor CD90 expression was restricted to AML samples in this series.[15,17] Our results clearly underline that neither CD133 nor CD90 expression analysis is feasible for lineage distinction between AML and ALL cell samples.

In our series, CD90 was significantly less often expressed on acute leukemia cells than was CD133 (p <0.001). In previous studies, 2-42% of AML cell samples examined had at least 5% CD90 positive cells/sample.[30–33] We observed 16 (11%) of 142 AML cell samples with ≥5% CD90 positive cells/sample. In one study, CD90 expression was restricted to $CD34^{+ve}$ AML cells,[31] however, in other studies CD90 expression levels were similar in $CD34^{+ve}$ and $CD34^{-ve}$ AML samples.[30,32] We found a preferential expression of CD90 in immature AML,

although this difference in CD90 expression between CD34^{+ve} and CD34^{-ve} AML was less marked than the observed difference in CD133 expression. In previous studies, 0-26% of ALL cell samples examined revealed ≥5% CD90 positive cells/sample.[30,33] We observed 13 (11%) of 119 ALL cell samples with ≥5% CD90 positive cells/sample. In one study, 32 (97%) of 33 CD34^{+ve} ALL were dimly positive for CD90, whereas all 10 CD34^{-ve} ALL cases were negative for CD90.[34] However, in accordance with Campos *et al*., we could not detect any correlation between CD34 and CD90 expression in ALL.[32] Thus, CD90 expression in *de novo* acute leukemia is generally low and not restricted to CD34^{+ve} leukemic cells. Higher CD90 expression levels, as detected by Moab 5E10, may be observed by using the PE- instead of FITC-conjugated antibody.[19] However, a study investigating CD90 expression levels in 52 AML cases using the PE-conjugated Moab 5E10 found only one patient with more than 5% CD90 positive leukemic cells.[30]

CD133 expression differed markedly between AML and ALL cells with 11q23-anomalies and MLL gene translocations. All 8 pro-B-ALL cases with MLL gene translocations were positive for CD133, whereas all 5 AML FAB M4/5 cases with t(9;11) were negative for CD133. The MLL gene on chromosome 11, band q23, is usually disrupted in 11q23 translocations and is thought to play an important role in leukemogenesis as it has been implicated in fusions with more than 25 other genes in both ALL and AML.[35-37] Our findings indicate that in AML cells with MLL gene translocations, CD133 expression is downregulated during monocytic differentiation, whereas CD133 expression in pro-B-ALL cells with MLL gene translocations might reflect the rather immature stem cell-related phenotype of these ALL cell samples with 11q23-anomalies.[36] However, Moab AC133 is not useful for the specific detection of leukemic cells with MLL gene translocations in ALL, as CD133 was also expressed in common-/pre-B-ALL cases without 11q23-anomalies.

A previous study observed a significantly higher CD90 expression in B-lineage ALL cases with cytogenetic anomalies compared with in those with a normal karyotype.[38] In contrast, we could not detect any correlation between cyto-/molecular genetic aberrations and CD90 expression in our series, either in ALL or in AML.

Biphenotypic acute leukemias (BAL) are a relatively newly defined leukemia entity characterized by the co-expression of differentiation markers of different lineages.[5,39] So far, only a few features of the cell biology of this possibly heterogenous leukemia subgroup have been described: BAL more often express CD34 as well as the multidrug-resistance (MDR)-related P-glycoprotein than do AML and ALL.[40] BAL often reveal a t(9;22) in cytogenetic studies[41] and seem to be associated with a poor prognosis.[42] It is unclear whether these leukemias arise from an immature multipotent stem cell with the potential to differentiate into several lineages.[41,42] Our results show that the expression analysis of CD133 and CD90 in BAL cell samples does not further distinguish this entity from other leukemia subtypes.

It is assumed that CD34^{+ve} blast crisis (BC) in CML arises from an immature malignant cell clone with multipotent stem cell characteristics.[43] In our series, cell samples of CD34^{+ve} CML with myeloid BC expressed CD133 less often than did cell samples of CD34^{+ve} *de novo* AML. Therefore, the expression pattern of CD133 may further define the phenotype of the malignant transformed CML origin cells as CD133^{low} compared with the higher CD133 expression in the malignant transformed *de novo* AML cells. However, CD133 expression in CML can not distinguish leukemic cells with or without the t(9;22), as this cytogenetic anomaly was detected in both CD133 positive and negative CML cells.[44]

We conclude that CD133 and CD90 expression in acute leukemia is neither lineage-specific nor restricted to CD34^{+ve} leukemic cells. Therefore, these markers are not useful for lineage determination in acute leukemia immunophenotyping.[45] However, Moab AC133 might be an informative antibody for the detection and further characterization of immature AML cells as well as pro-B-ALL cells with MLL gene translocations by flow cytometry.

Contributions and Acknowledgments

CW, LK and WDL were responsible for the design of this study and for the interpretation as well as publication of the experimental data. RR, GS and CS did the immunophenotypic characterization of the examined AML and ALL cases and participated in the statistical analysis of the experimental findings. CS and SS performed the cytogenetic and molecular studies in AML and JH in ALL. TH and UC were responsible for the classification of the examined AML cases according to morphologic FAB subtypes. All above mentioned contributors were involved in the revision of the manuscript and approved the final version to be submitted.

We would like to thank MM, KL and GC for their excellent technical assistance. The cell samples included in this study were sent from various hospitals in Germany participating in the ongoing ALL-BFM (co-ordinators: M. Schrappe and H. Riehm, Hannover), GMALL (co-ordinator: D. Hoelzer, Frankfurt), AML-BFM (co-ordinators: U. Creutzig and J. Ritter, Münster) and AML-CG (co-ordinators: T. Büchner and W. Berdel, Münster; W.Hiddemann, München; B.Wörmann, Braunschweig) trials. We would like to thank the co-ordinators of the above studies for their continuous support as well as all clinicians providing cell samples for our investigations.

Funding

This work was supported in part by a grant from the "Deutsche José Carreras Leukämie Stiftung" (JCLS 1998/NAT-3 to CW).

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external

referees and by Dr. Alberto Orfao, who acted as as Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Orfao and the Editors. Manuscript received September 25, 2000; accepted December 27, 2000.

Potential implications for clinical practice

AC133-antigen and CD90 expression analysis is not helpful in distinguishing acute myeloid and lymphoblastic leukemias by flow cytometry. AC133-antigen expression analysis might be useful in further characterization of immature AML cells and pro-B-ALL with 11q23-gene rearrangements.

References

1. Fialkow PJ, Singer JW, Raskind WH, et al. Clonal development, stem-cell differentiation, and clinical remissions in acute nonlymphocytic leukemia. N Engl J Med 1987; 317:468-73.
2. Greaves MF. Stem cell origins of leukaemia and curability. Br J Cancer 1993; 67:413-23.
3. Russell NH. Biology of acute leukaemia. Lancet 1997; 349:118-22.
4. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. Blood 1996; 87:1-13.
5. Bené MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia 1995; 9:1783-6.
6. Miraglia S, Godfrey W, Yin AH, et al. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. Blood 1997; 90: 5013-21.
7. Yin AH, Miraglia S, Zanjani ED, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997; 90:5002-12.
8. Gallacher L, Murdoch B, Wu DM, Karanu FN, Keeney M, Bhatia M. Isolation and characterization of human CD34(-) Lin(-) and CD34(+)Lin(-) hematopoietic stem cells using cell surface markers AC133 and CD7. Blood 2000; 95:2813-20.
9. Corbeil D, Roper K, Weigmann A, Huttner WB. AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? Blood 1998; 91:2625-6.
10. Miraglia S, Godfrey W, Buck D. A response to AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? Blood 1998; 91:4390-1.
11. Corbeil D, Roper K, Hellwig A, et al. The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. J Biol Chem 2000; 275:5512-20.
12. Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000; 95:952-8.
13. Snell V, Jackson E, Buck D, Andreef M. Expression of the AC133 antigen in leukemic and normal progenitors. Blood 1998; 92 (Suppl 1):119a.
14. Horn PA, Tesch H, Staib P, Kube D, Diehl V, Voliotis D. Expression of AC133, a novel hematopoietic precursor antigen, on acute myeloid leukemia cells. Blood 1999; 93:1435-7.
15. Bühring HJ, Seiffert M, Marxer A, et al. AC133 antigen expression is not restricted to acute myeloid leukemia blasts but is also found on acute lymphoid leukemia blasts and on a subset of CD34+ B-cell precursors. Blood 1999; 94:832-3.
16. Baersch G, Baumann M, Ritter J, Jürgens H, Vormoor J. Expression of AC133 and CD117 on candidate normal stem cell populations in childhood B-cell precursor acute lymphoblastic leukaemia. Br J Haematol 1999; 107:572-80.
17. Kratz Albers K, Zühlsdorf M, Leo R, Berdel WL, Büchner T, Serve H. Expression of a AC133, a novel stem cell marker, on human leukemic blasts lacking CD34-antigen and on a human CD34+ leukemic line:MUTZ-2. Blood 1998; 92:4485-7.
18. Green CL, Loken M, Buck D, Deeg HJ. Discordant expression of AC133 and AC141 in patients with myelodysplastic syndrome (MDS) and acute myelogeneous leukemia (AML). Leukemia 2000; 14:770-2.
19. Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of Thy-1 on human hematopoietic progenitor cells. J Exp Med 1993; 177:1331-42.
20. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976; 33:451-8.
21. Creutzig U, Harbott J, Sperling C, et al. Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. Blood 1995; 86:3097-108.
22. Ludwig WD, Rieder H, Bartram CR, et al. Immunophenotypic and genotypic features, clinical characteristics, and treatment outcome of adult pro-B acute lymphoblastic leukemia: results of the German multicenter trials GMALL 03/87 and 04/89. Blood 1998; 92:1898-909.
23. Mitelman F. ISCN 1995, Guidelines for Cancer Cytogenetics. Supplement to: An International System for Human Cytogenetic Nomenclature. Karger, Basel; 1995.
24. Schnittger S, Wörmann B, Hiddemann W, Griesinger F. Partial tandem duplications of the MLL gene are detectable in peripheral blood and bone marrow of nearly all healthy donors. Blood 1998; 92:1728-34.
25. Harbott J, Viehmann S, Borkhardt A, Henze G, Lampert F. Incidence of TEL/AML1 fusion gene analyzed consecutively in children with acute lymphoblastic leukemia in relapse. Blood 1997; 90:4933-7.
26. Repp R, Borkhardt A, Haupt E, et al. Detection of four different 11q23 chromosomal abnormalities by multiplex-PCR and fluorescence-based automatic DNA-fragment analysis. Leukemia 1995; 9:210-5.
27. Evans P, Jack A, Short M, et al. A single tube nested RT-PCR for detecting the common myeloid specific chromosomal translocations. Leukemia 1995; 9:1285-6.
28. Schlieben S, Borkhardt A, Reinisch I, et al. Incidence and clinical outcome of children with BCR/ABL-positive acute lymphoblastic leukemia (ALL). A prospective RT-PCR study based on 673 patients enrolled in the German pediatric multicenter therapy trials ALL-BFM-90 and CoALL-05-92. Leukemia 1996; 10:957-63.
29. Bené MC, Bernier M, Casasnovas RO, et al. The reliability and specificity of c-kit for the diagnosis of acute myeloid leukemias and undifferentiated leukemias. The European Group for the Immunological Classification of Leukemias (EGIL). Blood 1998; 92:596-9.
30. Blair A, Hogge DE, Ailles LE, Lansdorp PM, Sutherland HJ. Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. Blood 1997; 89:3104-12.
31. Kozii R, Wilson J, Persichetti J, Phelps V, Ball SE, Ball ED. Thy-1 expression on blast cells from adult patients with acute myeloid leukemia. Leuk Res 1997; 21:381-5.
32. Campos L, Guyotat D. Expression of Thy-1 antigen (CDw90) on adult acute leukemia blast cells. Blood 1996;

87:413-4.
33. Holden JT, Geller RB, Farhi DC, et al. Characterization of Thy-1 (CDw90) expression in CD34+ acute leukemia. Blood 1995; 86:60-5.
34. Lamkin T, Brooks J, Annett G, Roberts W, Weinberg K. Immunophenotypic differences between putative hematopoietic stem cells and childhood B-cell precursor acute lymphoblastic leukemia cells. Leukemia 1994; 8:1871-8.
35. Look AT. Oncogenic transcription factors in the human acute leukemias. Science 1997; 278:1059-64.
36. Rubnitz JE, Behm FG, Downing JR. 11q23 rearrangements in acute leukemia. Leukemia 1996; 10:74-82.
37. Cimino G, Rapanotti MC, Sprovieri T, Elia L. ALL1 gene alterations in acute leukemia: biological and clinical aspects. Haematologica 1998; 83:350-7.
38. Takahashi T, Mizutani M, Miwa H, et al. Frequent expression of human Thy-1 antigen on pre-B cell acute lymphoblastic leukemia with t(9;22). Int J Hematol 1998; 67:369-78.
39. Matutes E, Morilla R, Farahat N, et al. Definition of acute biphenotypic leukemia. Haematologica. 1997; 82:64-6.
40. Legrand O, Perrot JY, Simonin G, et al. Adult biphenotypic acute leukaemia: an entity with poor prognosis which is related to unfavourable cytogenetics and P-glycoprotein over-expression. Br J Haematol 1998; 100:147-55.
41. Carbonell F, Swansbury J, Min T, et al. Cytogenetic findings in acute biphenotypic leukaemia. Leukemia 1996; 10:1283-7.
42. Killick S, Matutes E, Powles RL, et al. Outcome of biphenotypic acute leukemia. Haematologica 1999; 84:699-706.
43. Thijsen S, Schuurhuis G, van Oostveen J, Ossenkoppele G. Chronic myeloid leukemia from basics to bedside. Leukemia 1999; 13:1646-74.
44. Waller CF, Martens UM, Lange W. Philadelphia chromosome-positive cells are equally distributed in AC133+ and AC133- fractions of CD34+ peripheral blood progenitor cells from patients with CML. Leukemia 1999; 13:1466-7.
45. Bene MC, Bernier M, Castoldi G, et al. Impact of immunophenotyping on management of acute leukemias. Haematologica 1999; 84:1024-34.