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Specificity of stem cell markers in acute myeloid leukemia

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To the editor

In acute myeloid leukemia (AML), leukemic stem cells (LSCs) are thought to underlay relapses, thereby being associated with poor outcome (1). The lack of cure in a high percentage of AML patients suggests that current treatments do not effectively target LSCs. There is increasing evidence for malignant stem cell specific antigens to be of potential therapeutic use (2). However, the applicability of antibody directed therapy not only depends on efficacy, likely mainly defined by (level of) antigen expression on the LSCs, but also on potential harm, being caused by concomitant expression on non-hematological tissues as well as on normal hematopoietic stem cells (HSCs). In view of the latter we investigated differential expression of putative LSC specific antigens on HSCs, obtained from different clinically relevant sources, to compare with expression on LSCs.

It was shown by Dick and colleagues in an immune-compromised (NOD/SCID) mouse model, that LSCs in AML are harboured in many cases in the CD34+CD38- compartment (3). Later on, an alternative stem cell compartment, defined by high efflux of Hoechst 33342, the so-called side population (SP), was shown to contain both CD34-positive and CD34-negative cells and to be able to initiate AML in NOD/SCID mice (4). However, similar to LSCs, the immunophenotype of HSC is CD34+CD38- while these normal stem cells may have SP character too. This underscores the need for identification of LSC specific, discriminating antigens. Such would allow, both at diagnosis and in follow up, not only to define specific therapeutic targets, but also to accurately quantify both LSC and HSC frequencies for diagnostic purposes. Moreover, it would offer the possibility to purify LSCs and HSCs from the same AML bone marrow (BM) to identify new putative LSC targets. The present paper compares such potencies for several candidate antigens.

Recently, we have shown that both CD34+CD38- and SP LSCs can be discriminated from normal stem cells by combining stem cell associated cell surface markers including C-type lectin-like molecule-1 (CLL-1) and lineage markers such as CD7, CD19 and CD56 (5, 6, 7). Studies by others have shown that other cell surface markers, i.e. CD123 (8), CD96 (9), CD44 (10), CD47 (11), CD25 and CD32 (12), mark LSCs as well and are considered as targets for therapy. Although in general normal BM (NBM) of healthy donors or umbilical cord blood were used as HSC controls, antigen expression on normal stem cells has not been investigated in regenerating bone marrow (RBM). Moreover, no attempt were made to differentiate between LSCs and HSCs in the BM from the same patient at diagnosis and follow up. This is of great importance as we found earlier that antigen expression may be different on

HSCs in RBM compared to those in NBM (5). Moreover, leukemic cells are known to affect the behaviour of normal hematopoietic progenitor cells (13) necessitating to consider the possibility for changes in antigenic profiles of HSCs present in a leukemic environment. The study on HSCs of these three different HSC sources should allow to define the potential risk of antibody therapy at different time points during disease/ treatment and, moreover, to define whether LSCs and HSCs can be distinguished for prognostic purposes for example prediction of relapse. Since efficacy of therapy depends on antigen expression on AML, this was studied first: when comparing antigen expression on CD34+CD38- and SP cells in AML patients at diagnosis, for CD34+CD38- cells the highest percentages were found for CD123, CD44, CD47 and CD33 (Table 1, column 2; examples shown in second row of Figure 1). For SP, CLL-1 expression was higher than CD123 expression and both were lower compared to CD33. When considering expression in terms of fluorescence shifts compared to controls (e.g. control antibody), more detailed differences between the antigens on CD34+CD38- cells became clear: CD44>CD33>CD47>CD123≥CLL-1, CD96 (Table 2A, column2). For SP cells the ranking of CLL-1, CD123 and CD33 (Tabel 2B, column 2) was the same as in Table 1.

NBM HSC antigen expression is illustrated in representative examples in Figure 1 (first row) and shows important differences: CLL-1 and CD96 have no expression on CD34+CD38- HSC, while part of the progenitors is positive. Low, but clear expression of CD123 was consistently found with expression levels in between those found for CD34+CD38+ CD123-negative and CD34+CD38+ CD123-positive progenitor populations. On the other hand, high expression was found for CD44, CD47 and CD33; this includes both stem and progenitor cells. Table 1 summarizes the results for all experiments performed on NBM CD34+CD38- HSC in terms of percentage positivity (Table 1, column 3). Table 2 shows fluorescence shifts which confirm the results shown in Figure 1 and Table 1 and show more detail as to the level of expression: CD44>CD47>CD33>CD123≥CD96 and CLL-1 (Table 2A, column 4). Essentially similar results were found for the SP compartment: Table 1 (column 6) and Table 2B (column 4).

RBM HSC from patients described in the legends of Table 1, similar to NBM HSC, for CD34+CD38- HSC shows absence of CLL-1 (Table 1, column 4). CD123 and CD33 had high expression, while CD96, CD44, and CD47 were not studied. This was confirmed using fluorescence shifts (Table 2A, column 5). The SP compartment showed a similar order (Table 1, column 7 and Table 2B, column 5).

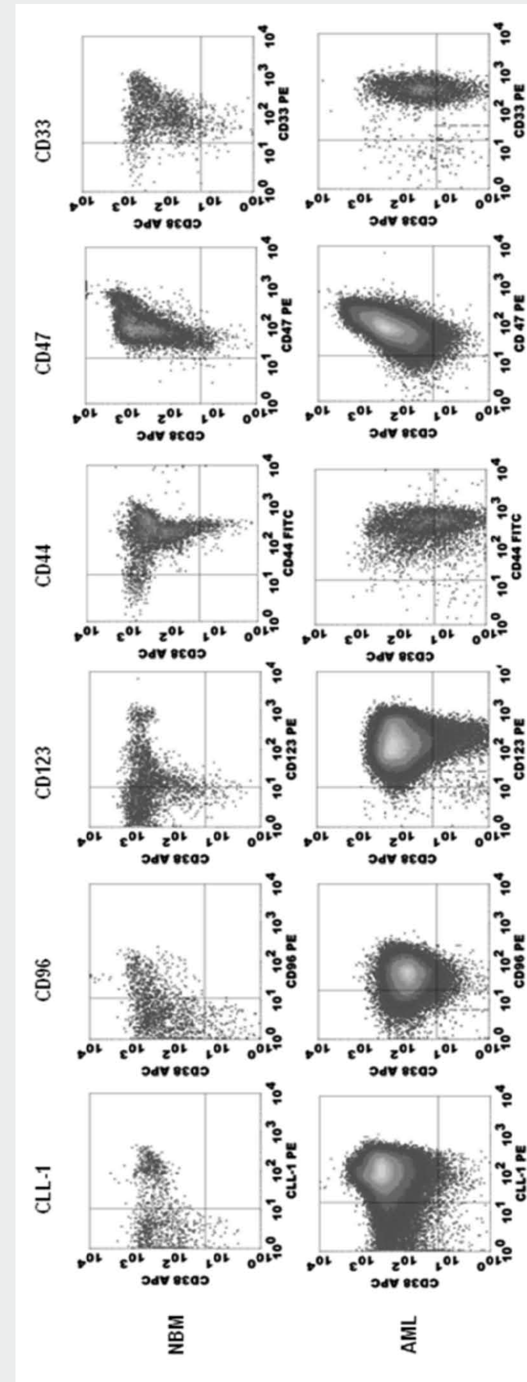

Figure 1 Leukemia associated stem cell marker expression in normal and AML bone marrow

Both for NBM and AML CD45dim CD34-positive cells were gated as described earlier (6). Within the CD34-positive gate, marker expression is plotted against CD38 expression. The first NBM row shows the different patterns of marker expression found: for CLL-1 and CD96, the CD34+CD38- HSCs are marker-negative, while only part of CD34+CD38+ progenitors are positive; for CD123 the CD34+CD38- cells have low expression in between a CD123-negative and a CD123-positive CD34+CD38+ progenitor population. For CD44, CD47 and CD33 both progenitors and stem cells are positive for the marker. The second AML row shows 6 different AML cases with expression on both CD34+CD38+ progenitors and CD34+CD38- LSC. Expression patterns of the markers are not per se representative. Note the small but clear marker-negative/low CD34+CD38- stem cell population for CLL-1 (negative), CD96 (negative), CD123 (low), and CD33 (low). Using additional scatter parameters, these were shown to represent HSC. The blue dashed lines for CD96, CD123 and CD33 indicate that the HSC population should not be defined solely by the quadrants used for the total CD34-positive population: for CD96 that would result in inclusion of part of the CD96-positive LSC population; for CD123 and CD33 it would result in exclusion of part of the CD123low and CD33low HSCs, thereby erroneously attributing the excluded events to the marker-positive LSC compartment.

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Table 1 Percentage expression of leukemia initiating/stem cell markers in AML, NBM and RBM

	CD34+CD38-			SP		
	AML#	NBM	RBM§	AML	NBM	RBM
Marker	Median (range), n	Median (range), n	Median (range), n	Median (range), n	Median (range), n	Median (range), n
CLL-1	28 (0-86)‡ n=28	0 (0-11) n=10	2 (0-9) n=8	53 (2-100) n=40	0 (0-4) n=12	0 (0-8) n=5
CD96	27 (0-89) n=13	2.1 (0.7-4.2) n=4	ND	ND	NE	ND
CD123	98 (5-100) n=36	14 (0-72) n=9	66 (46-71) n=7	26 (0-100) n=40	27 (1-82) n=12	37 (25-79) n=5
CD44*	100 (98-100) n=25	100 (100-100) n=2	ND	ND	NE	ND
CD47	94 (13-100) n=16	96 (56-100) n=7	ND	ND	NE	ND
CD33	82 (38-99) n=12	82 (10-97) n=9	96 (79-100) n=3	69 (13-96) n=37	72 (38-84) n=7	43 n=1

Abbreviations: AML, acute myeloid leukemia; NBM, normal bone marrow; RBM, regenerating bone marrow; CLL-1, C-type lectin-like molecule-1; SP, side population; ND, not done; NE, not evaluated, Median, median percentage of marker expression; Range, range of percentage marker expression; n, number of samples.
#, AML data for CLL-1 and CD123 were in part from reference 6.
§, regenerating BM was from patients with acute lymphoblastic leukemia, lymphoma and, whenever possible, from AML patients who had no expression of the marker studies on the blasts at diagnosis, thereby assuming, and checking with other markers, that expression of the specific marker studied reflected expression on HSC and not LSC.
‡, % positivity compared to isotype control (for CLL-1, CD44 and CD47), and internal marker-negative cell population (for CD96, CD123 and CD33).
*, CD44 variants 4, 6 and 7 have been measured on the total blast compartment (no specific detection in CD34+CD38- cells) of 25 additional patients with less than 10% of the cases positive (not shown). These variants were therefore not considered to be potential therapeutic targets.
Staining for CD34, CD38, CD45, markers or isotype controls and staining for SP with Hoechst 33342 combined with markers was performed as described previously (5-7). Monoclonal antibody sources: CLL-1-PE (dilution 1:10, 10 μg/ml), see reference 6; CD123-PE (dilution: 1:10), CD47-PE (dilution 1:75) and CD33-PE (dilution 1:20), were all from BD Biosciences (San Jose, CA). Unlabeled CD96 (clone TH111, dilution 1:50) was a kind gift from dr. Gramatzki (Erlangen-N rnberg, Germany); staining with PE-labelled RAM (dilution 1:10) was used for the second step. Directly-labelled (PE) CD96 (clone NK92.39, dilution 1:20) was from BioLegend (Uithoorn, Netherlands). No differences were found between both labelling types. CD44-PE (dilution 1:50) was from eBioscience (Hatfield, UK).

HSC in AML diagnosis BM. This was studied only for fluorescence shifts and results are shown in Table 2A (column 3) for CD34+CD38- HSC and in Table 2B (column 3) for SP HSC. Overall, fluorescence shifts were quite similar to NBM and RBM. The high expression of CD44 and CD47 on HSCs, which was often comparable to the levels seen in the concomitantly present LSCs, makes the separate analyses of HSC inaccurate in most cases. For CD33, which also has high expression levels on LSC (Figure 1, Table 1, columns 2 and 5, Tables 2A and 2B, columns 2), the expression levels on HSC were lower as compared to NBM and RBM (compare Tables 2A and 2B, columns 3 with columns 4 and 5, respectively).

The consequences of high expression on HSC for specific LSC detection and the possible consequences for specific LSC targeting are illustrated in Figure 2. As argued earlier, the HSC compartment can often be reliably discriminated from LSC using not only marker expression but also flow cytometric scatter parameters: LSC have higher FSC and SSC compared to HSC (Terwijn M, Blood 114:165, 2009). Figure 2 first row illustrates this for an AML case: HSCs (green) and LSCs (red). This AML example also illustrates the heterogeneity of stem marker expression seen in AML: CLL-1, CD123 and CD44 expression is higher on LSCs than on the corresponding HSCs. However, CLL-1 and CD123 expression on corresponding HSC is low/absent, while CD44 expression on HSCs is very high. Further, expression of CD96 and CD33 on HSC is very low, expression on the corresponding LSCs is too low to enable proper discrimination of LSC and HSC. CD47 is clearly present on both LSC and HSC, but overlap is too high for proper discrimination. As a result, proper identification of LSC and HSC in this case is possible only for CLL-1, CD123 and CD44. Other AML cases may have completely different patterns with even opposite results compared to the present example (not shown).

The results shown in Table 2 now allows to define an *in vitro* “therapeutic window” or “prognostic window”, depending on whether the aim is to predict therapeutic usefulness or possibilities to detect and quantify LSCs and HSCs: marker expression on LSC divided by marker expression on HSC from either of the three HSC sources. Table 2A shows this for CD34+CD38- HSC and Table 2B for SP HSC. In Tables 2A and 2B, columns 6, the ratio of expression on LSC versus HSC was calculated for each individual AML case that had identifiable LSCs and HSCs present. In Tables 2A and 2B, columns 7, for each individual AML case the expression on the LSC is shown relative to the median expression on the HSC seen in NBM (from columns 4 in Tables 2A and 2B). Tables 2A and 2B, columns 8 shows a similar approach for RBM, using the median expression values on the HSC shown in columns 5 in Tables 2A and 2B.

Table 2 In vitro therapeutic windows of leukemia initiating/stem cell markers using different HSC sources

A CD34+CD38-

Marker	Fluorescence Index*				In vitro therapeutic window using: ‡		
	LSC in AML	HSC in AML	HSC in NBM	HSC in RBM	HSC in AML	HSC in NBM	HSC in RBM
	A	B	C	D	A/B	A/C	A/D
CLL-1	3.5 (0.8-67) n=39	1.15 (0.9-1.5) n=8	0.9 (0.8-1.5) n=6	1.05 (0.6-2.1) n=5	3.5 (1-100) n=37	3.8 (0.5-45) n=41	3.3 (0.4-40) n=41
CD96	3.4 (1.0-18) n=9	1.0 (0.6-2.5) n=8	1.4 (1.0-3.5) n=8	ND	2.4 (0.9-19) n=9	2.7 (0.8-14) n=9	ND
CD123	5.9 (1.3-63) n=22	3.0 (1.1-4.0) n=10	3.1 (1.3-4.5) n=12	2.8 (1.6-6.5) n=10	2.9 (1.0-16) n=19	2.0 (0.6-21) n=21	2.2 (0.6-22) n=21
CD44	128 (83-243) n=4	NA	73 (14-150) n=7	ND	NA	1.3 (0.9-2.5) n=4	ND
CD47	11 (2.6-29) n=16	NA	12 (1.3-51) n=10	ND	NA	1.1 (0.3-2.1) n=16	ND
CD33	35 (2.5-163) n=12	5.1 (1.0-13) n=11	6.8 (2.4-33) n=11	12 (8.1-22) n=3	6.9 (1.0-50) n=11	2.7 (0.2-13) n=12	3.1 (0.2-14) n=12

Table 2 not necessarily covers the data shown in Table 1: cohorts may differ.
Abbreviations: LSC, leukemic stem cell; HSC, hematopoietic stem cell; AML, acute myeloid leukemia; NBM, normal bone marrow; RBM, regenerating bone marrow; CLL-1, C-type lectin-like molecule-1; n, number of samples; NA, not analyzable due to overlap of LSC and HSC; ND, not done.
*, Mean fluorescence intensity (MFI) of population of interest was divided by MFI of isotype control (and/or internal negative control): ratio. To define separately the LSC and HSC present in the AML BM, differential antigen expression between LSC and HSC (5-11) was combined with differences in scatter properties (Terwijn M, Blood 114:165, 2009). A: MFI ratio for LSC in diagnosis BM; B: MFI ratio for HSC in diagnosis BM; C: MFI ratio for HSC in NBM; D: MFI ratio for HSC in RBM. All values are median values with ranges in between brackets.
‡, A/B = therapeutic window : MFI ratio LSC/MFI ratio HSC present in diagnosis BM;
A/C = therapeutic window MFI ratio LSC/median MFI ratio HSC present in NBM;
A/D = therapeutic window MFI ratio LSC/median MFI ratio HSC present in RBM.
In column A/B each LSC value has a corresponding HSC value resulting in a A/B value for each AML case (with a median and a range). In A/C and A/D there is no corresponding HSC value, but only a median value in NBM and RBM; therefore for each marker the individual LSC values in column 2 were divided by median value of the NBM (column 4) or RBM (column 5).

Table 2 In vitro therapeutic windows of leukemia initiating/stem cell markers using different HSC sources

B Side Population

Marker	Fluorescence Index*				In vitro therapeutic window using:‡		
	LSC in AML	HSC in AML	HSC in NBM	HSC in RBM	HSC in AML	HSC in NBM	HSC in RBM
	A	B	C	D	A/B	A/C	A/D
CLL-1	3.8 (1.3-14) n=40	1.7 (1-4.7) n=25	1.0 (1.0-1.2) n=12	1.0 (1.0-1.1) n=5	2.1 (1.0-7.7) n=25	3.8 (1.3-14) n=40	3.8 (1.3-14) n=40
CD96	ND	ND	2.4 (1.5-4.9) n=6	ND	ND	ND	ND
CD123	3.0 (1.1-10) n=37	1.4 (1.0-6.3) n=25	2.0 (0.9-15) n=17	1.2 (1.0-5.2) n=5	2.0 (1.0-5.7) n=25	1.5 (0.6-5.0) n=37	2.5 (0.9-8.3) n=37
CD44	ND	ND	16 (8.3-30) n=3	ND	ND	ND	ND
CD47	ND	ND	5.2 (3.2-17.5) n=5	ND	ND	ND	ND
CD33	6.7 (1.2-42) n=37	3.1 (1.0-24) n=22	5.2 (1.1-15.5) n=11	4.2	1.9 (1.1-5.6) n=22	1.3 (0.2-8.1) n=37	1.6 (0.3-10) n=37

Table 2 not necessarily covers the data shown in Table 1: cohorts may differ.
Abbreviations: LSC, leukemic stem cell; HSC, hematopoietic stem cell; AML, acute myeloid leukemia; NBM, normal bone marrow; RBM, regenerating bone marrow; CLL-1, C-type lectin-like molecule-1; n, number of samples; NA, not analyzable due to overlap of LSC and HSC; ND, not done.
*, Mean fluorescence intensity (MFI) of population of interest was divided by MFI of isotype control (and/or internal negative control): ratio. To define separately the LSC and HSC present in the AML BM, differential antigen expression between LSC and HSC (5-11) was combined with differences in scatter properties (Terwijn M, Blood 114:165, 2009). A: MFI ratio for LSC in diagnosis BM; B: MFI ratio for HSC in diagnosis BM; C: MFI ratio for HSC in NBM; D: MFI ratio for HSC in RBM. All values are median values with ranges in between brackets.
‡, A/B = therapeutic window : MFI ratio LSC/MFI ratio HSC present in diagnosis BM;
A/C = therapeutic window MFI ratio LSC/median MFI ratio HSC present in NBM;
A/D = therapeutic window MFI ratio LSC/median MFI ratio HSC present in RBM.
In column A/B each LSC value has a corresponding HSC value resulting in a A/B value for each AML case (with a median and a range). In A/C and A/D there is no corresponding HSC value, but only a median value in NBM and RBM; therefore for each marker the individual LSC values in column 2 were divided by median value of the NBM (column 4) or RBM (column 5).

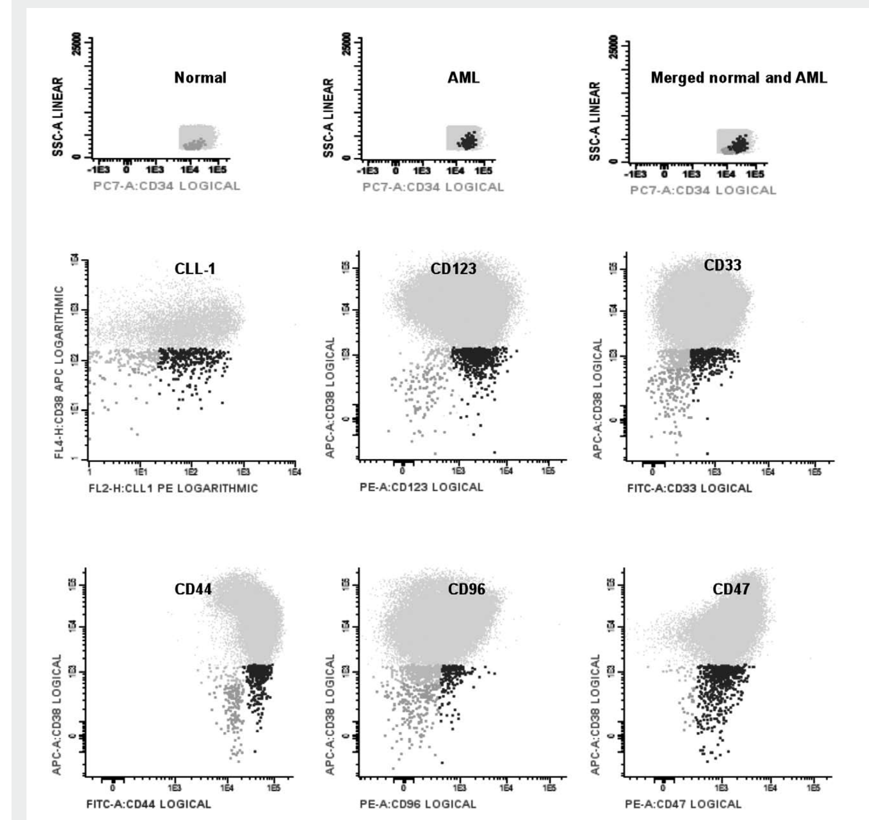
These *in vitro* “therapeutic window” values for CLL-1 were somewhat higher than for CD96 and CD123. For CD33 in CD34+CD38- HSC the therapeutic window values using NBM and RBM as HSC source, were similar to CLL-1, CD96 (only NBM studied) and CD123 (columns 7 and 8, respectively), but remarkably HSC in the AML BM itself had lower CD33 expression compared to NBM and RBM, resulting in a high therapeutic index for CD33 (6.9, Table 2A, column 6). For CD44 and CD47, however, the therapeutic window in the CD34+CD38- stem cell compartment was calculate only for NBM and was very low (Table 2A, column 7). For unknown reasons, for CLL-1, CD123 and CD33 in SP the index was lower compared to CD34+CD38-.

In part of the experiments two markers were compared with each other in order to estimate whether these are overlapping or not, which is important for therapeutic and prognostic use. Usefulness was arbitrary defined as having a therapeutic window of >3 (the therapeutic window defined as in column 6 in Table 2A). In a series comparing CLL-1 and CD123, CLL-1 showed therapeutic window >3 in 10/19 cases and CD123 in 7/19 cases, with 5/19 overlapping and 7/19 being single positive. With 12/19 single or double thus being positive, still in 7/19 patients both markers would not be highly suitable for therapeutic or prognostic use.

In conclusion, for specific detection of both LSC and HSC, marker expression on the LSC should be quite different from marker expression on the HSC, without a need for complete absence of marker expression on the HSCs. CLL-1, CD96, CD123 and CD33 are the best in this respect. CD44 and CD47 in general lack such specificity.

For therapeutic applications the absence of marker expression seen for CLL-1 and CD96 under all conditions of disease/treatment, may be advantageous in terms of toxicity for normal hematopoiesis. However, it can not be excluded that the therapeutic window for CD123 and CD33 still allows selective efficacy for LSCs. The remarkably high therapeutic window of CD33 at diagnosis, hypothetically suggests that Mylotarg may be useful in an upfront setting. Whether or not antibody therapy will be possible for CD44 and CD47 may depend on how differences in marker expression between HSC and LSC as well as absolute levels on HSC translate into differences in processes important for cell kill such as endocytosis or signalling (11). Lastly, huge differences in therapeutic index may exist between patients (shown in between brackets in Table 2). Since expression of the antigens studied only partly overlap, the ultimate choice for therapy as well as for prognostic use should be based on a comparison of the different antigens for each new diagnosis case. Eradicating the leukemia initiating cell with antibody-based therapy in part of the cases likely will demand a combination of antibodies.

Figure 2 Heterogeneity of leukemia associated markers in an AML patient



Both for NBM and AML CD45dim CD34-positive cells were gated as described earlier (6). Within the CD34-positive gate, marker expression is plotted against CD38 expression. The first row shows the presence of the CD34+CD38- marker-negative (green) and marker-positive (red) cells, as well as the merged populations, in an FSC/SSC plot. These represent HSCs and LSCs, respectively. HSC have lower FSC/SSC compared to LSC. The second and third row shows the distribution of putative LSC markers in the CD34+CD38- stem cell compartment of this patient. HSC are negative/low for CLL-1, CD123, CD33 and CD96 and positive for CD44 and CD47. LSC have higher expression than HSC for CLL-1 and CD123, but also for CD44, whereas for CD33, CD96 and CD47 expression on LSC is only slightly higher than HSC.

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Reference List

1. van Rhenen A, Feller N, Kelder A, et al.: High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin Cancer Res 2005;11:6520–6527.
2. Krause DS and Van Etten RA. Right on target: eradicating leukemic stem cells. Trends in Molecular Medicine 2007;13:470-481.
3. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730-737.
4. Wulf GG, Wang RY, Kuehnle I, Weidner D, Marini F, Brenner MK, Andreeff M, Goodell MA. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. Blood 2001;98:1166-1173.
5. van Rhenen A, Moshaver B, Kelder A, Feller N, Nieuwint AW, Zweegman S, Ossenkoppele GJ, Schuurhuis GJ. Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. Leukemia 2007;21:1700-1707.
6. van Rhenen, A., van Dongen GAMS, Kelder A, Rombouts EJ, Feller N, Moshaver B, Stigter-van Walsum M, Zweegman S, Ossenkoppele GJ, Schuurhuis GJ. CLL-1. The novel AML stem cell associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. Blood 2007;110:2659-2666.
7. Moshaver B, van Rhenen A, Kelder A, van der Pol M, Terwijn M, Bachas C, Westra AH, Ossenkoppele GJ, Zweegman S, Schuurhuis GJ. Identification of a small subpopulation of candidate leukemia-initiating cells in the side population of patients with acute myeloid leukemia. Stem Cells 2008;26:3059-3067.
8. Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, Meyerrose T, Rossi R, Grimes B, Rizzieri DA, Luger SM, Phillips GL. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia 2000;14:1777-1784.
9. Hosen N, Park CY, Tatsumi N, Oji Y, Sugiyama H, Gramatzki M, Krensky AM, Weissman IL. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A 2007;104:11008-11013.
10. Jin L, Hope KJ, Zhai Q, Smadja-Joffe F, Dick JE. Targeting of CD44 eradicates human acute myeloid leukemic stem cells. Nat Med 2006;12:1167-1174.
11. Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD Jr, van Rooijen N, Weissman IL. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell 2009;138:286-299.
12. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, Uchida N, Suzuki N, Sone A, Najima Y, Ozawa H, Wake A, Taniguchi S, Shultz LD, Ohara O, Ishikawa F. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. Sci Transl Med 2010;2:17ra9.
13. Colmone A, Amorim M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells. Science 2008;322:1861-1865.

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