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CD33 is frequently expressed in cases of myelodysplastic syndrome and chronic myelomonocytic leukemia with elevated blast count

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CD33 is a transmembrane protein that belongs to the Siglecs (sialic-acid-binding immunoglobulin-like lectins) family[1]. In normal individuals, expression of CD33 is associated with myeloid maturation and is present on multipotent myeloid precursors and maturing monocytes and granulocytes, but is also found on some lymphoid cells [2–4]. The majority of cases of acute myeloid leukemia (AML) also express CD33 and there have been several attempts to develop monoclonal antibody-based therapies that target this molecule[5]. Gemtuzumab-ozogamicin, an antibody-drug conjugate (ADC), was approved initially by the FDA in 2000 after demonstrating activity as a single agent in patients with relapsed/refractory AML. It was later withdrawn after a phase III trial reported no survival benefit with increased rates of toxicity and early mortality [6,7]. However, a recent meta-analysis of 5 clinical trials combining gemtuzumab with chemotherapy for frontline treatment reported that use of gemtuzumab significantly decreased relapse and improved survival, particularly in patients with intermediate and favorable risk AML[8]. CD33-targeted therapy has not been as widely studied in myelodysplastic syndromes (MDS) and chronic myelomonocytic leukemia (CMML), although several smaller trials suggest gemtuzumab has some activity in these diseases [9–14]. The intensity of CD33-expression (as measured by the number of CD33 molecules per cell) on the CD34-positive blast-population has previously been reported to be lower in patients with MDS and CMML in comparison with AML [15].

We aimed to determine the expression of CD33 in patients with MDS and elevated blast count (≥ 5%). We searched a database of clinical trials conducted at our center for patients with high risk MDS and CMML to identify cases. We limited our search between January 2000 and September 2014 and included only untreated patients with flow-cytometry for quantification of CD33 expression performed on a bone marrow aspirate sample prior to starting treatment. We also identified AML patients treated over the same time period to serve as a comparator group for CD33 expression. At our center, the diagnosis of AML, MDS and CMML has been made according to WHO classification systems over this time period, and bone marrow samples with ≥ 20% blasts of the total nucleated cells are

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considered to have AML[16–18]. The blast population from AML and MDS patients was analyzed using flow cytometry to quantify CD33 expression. Myeloblasts were identified based on dim CD45 expression and low side scatter. CD33 expression on the blast population was measured using an isotype-matched non-reactive antibody or autofluorescence to set a negative control threshold. Representative CD33-positive and CD33-negative cases of MDS are shown in Figure 1. We calculated the mean percentage of CD33-positive myeloblasts for AML, CMML and MDS and compared the results between groups using an unpaired student's t-test.

We identified 2295 patients with AML, 25 patients with CMML and 174 patients with MDS in our database in the specified time period, where flow-cytometry measuring CD33 was performed prior to starting treatment. Mean bone marrow blast count by morphology for patients with MDS and CMML was 13.3% (SD 3.1%, Range 5–19%) and 14% (SD 2.5%, Range 10–18%) respectively. Most patients with MDS had refractory anemia with excess blasts (RAEB)-2 (n=159) and only a small number had RAEB-1 (n=15). All patients with CMML had 10% blasts plus promonocytes by morphology and were classified as CMML-2.

The mean percentage of CD33-positivity of the myeloblast population was 80.5% (95% CI 79.5–81.6%) in AML, 81.8% (95% CI 71.7–91.8%) in CMML and 75% (95% CI 71.5 – 78.7%) in MDS. Mean CD33 expression according to the French-American-British (FAB) classification system is shown in Table 1. This was significantly lower in MDS in comparison to AML ($p=0.005$) although not in comparison to CMML ($p=0.2$). Only 8 (4%) patients with CMML or MDS had a mean percentage of CD33-positive blasts less than 20%, which is shown in figure 2. In patients with MDS and CMML, CD33 was expressed across cytogenetic risk groups as defined by IPSS-R criteria (data not shown), although the number of patients in some categories was small making statistical comparison impractical. For MDS cases, mean CD34 expression was significantly higher in CD33-negative (<20%) cases (77% vs. 59%, $p=0.002$), although this comparison is based on a small number of CD33-negative cases (n=7). There was no significant difference in CD117 expression between CD33-negative and CD33-positive cases (71% vs. 66%, $p=0.39$). For AML cases, both mean CD34 (73.5% vs. 48.3%, $p<0.001$) and mean CD117 (75.0% vs. 63.8%, $p<0.001$) expression were significantly higher in CD33-negative cases.

The main technical change in assessment of CD33-expression made at our center during the study period was a switch from 4-color panels on FACSCalibur instruments, to 7-color panels on FACSCantos instruments on July 11, 2011. In both cases we used the same monoclonal antibody, CD33 conjugated to phycoerythrin (PE). To assess possible technical differences in CD33 measurement we compared mean CD33 expression in AML patients before (n=1846) and after (n=449) this switch and there was no difference in expression (mean CD33 expression 80.7% vs. 79.9%, $p=0.56$) suggesting this change did not significantly impact measurement.

Our findings suggest that CD33 is expressed on myeloblasts in most cases of MDS and CMML, although this is significantly lower in MDS in comparison to AML. The optimal level of CD33-expression for treatment with an ADC has not been identified from clinical

studies using gemtuzumab, although pre-clinical data suggest that the cell-surface density of CD33 correlates with cytotoxicity [19]. In a post-hoc analysis of clinical trials of gemtuzumab in elapsed AML, CD33 expression level was significantly higher in responders in comparison to non-responders [20]. However, in multivariate analysis CD33 expression was not significantly associated with response when controlling for p-glycoprotein expression, which was found to inversely correlate with CD33 expression. These trials only included patients with relatively high CD33 expression (>80% of myeloid blasts), and it is possible that a relationship between CD33 expression and response would be evident if patients with lower expression levels were also included. Despite this finding, we expect that some degree of CD33 expression is required for ADC activity, and our findings provide a rationale for investigating therapies targeting CD33 in MDS and CMML. Several novel CD33-targeted therapies are currently under development. SGN-CD33a is second generation ADC that links a DNA damaging agent (pyrrolobenzodiazepine) to an anti-CD33 monoclonal antibody. The interim results of a phase II clinical trial were recently reported and this agent appears to have activity in patients with relapsed/refractory AML, with an overall response rate of 42% (n=16/38) [21]. Bispecific T-cell engaging (BiTE) antibodies and chimeric antigen-receptor (CAR) T cells targeting CD33 have also demonstrated activity in AML pre-clinical studies [22,23]. Treatment options in high-risk MDS and CMML remain limited and hypomethylating agents and allogeneic stem-cell transplant are the primary treatment options outside of a clinical trial. Investigating CD33-targeted therapies in MDS and CMML patients with a high blast count is justified based on the relatively high frequency of expression of this target.

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Figure 1.

Representative (a) CD33-positive case and (b) CD33-negative case of MDS. Scatter plots show expression of CD33 on myeloblasts, which were identified by gating on dim CD45/low side-scatter population.

Figure 2.
Histogram showing percentage of blasts positive for CD33 in MDS and CMML cases

Table 1

Mean CD33 expression in AML, MDS and CMML according to FAB subtype

FAB classification	N	Mean CD33 expression	SD
<i>AML</i>			
M0	134	64.8	32.1
M1	242	76.3	28.8
M2	473	76.7	28.7
M3	176	91.0	16.8
M4	362	90.0	16.1
M5	194	94.2	12.1
M6	85	74.4	27.0
M7	17	73.5	32.0
Unclassified	361	76.9	26.9
<i>MDS</i>			
RAEB-1	15	78.3	22.4
RAEB-2	159	74.8	24.2
RAEB-T	251	76.5	25.2
<i>CMML</i>	25	81.8	24.4
Total	2494	80.2	25.8

Abbreviations: FAB – French-American-British; RAEB-1 – Refractory anemia with excess blasts-1; RAEB-2 – Refractory anemia with excess blasts-2; RAEB-T – Refractory anemia with excess blasts in transformation; SD – standard deviation.