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CD30 Expression in High-Risk Acute Myeloid Leukemia and Myelodysplastic Syndromes

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Abstract

Background—We assessed for CD30 expression in patients with acute myeloid leukemia (AML) or high-grade myelodysplastic syndrome (MDS) to examine the possibility that anti-CD30 could be targeted therapy in these patients.

Methods—Multicolor flow cytometry immunophenotypic analysis was performed on bone marrow aspirates of 135 AML/MDS patients and peripheral blood (PB) samples in a subset of 33 patients. Immunohistochemistry was performed on BM aspirate clot specimens of 84 patients.

Results—The median patient age was 63 years (range, 13–92). One hundred and two (75%) patients had refractory or recurrent disease, and 68 (50%) had high-risk cytogenetics. Overall, the median percentage of blasts positive for CD30 was 14% (range, 0–91). Using an arbitrary 20% cutoff, 49 (36%) patients were considered to have CD30-expression. Monocytic cells, either mature or immature, were consistently negative for CD30. Therefore, CD30 expression was less in AML with monocytic differentiation ($P=0.006$). Patients with persistent disease who had been actively treated had a higher level of CD30 expression than untreated patients ($p=0.031$). In paired samples, CD30 expression was consistently higher in BM blasts than PB blasts ($p=0.002$). Immunohistochemistry demonstrated CD30 expression by myeloblasts in a subset of patients, but reactivity was generally weaker and focal compared.

Conclusions—CD30 is expressed by myeloblasts in a substantial subset of AML/MDS patients. As the study group was composed mostly of high-risk AML/MDS patients in whom very few treatment options are available, these data raise the possibility that anti-CD30- targeted therapy could be a potential option for this patient group.

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Disclosure of Potential Conflicts of Interest

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Keywords

CD30; acute myeloid leukemia; myelodysplastic syndromes; flow cytometry; immunohistochemistry

Introduction

Acute myeloid leukemia (AML) in elderly ¹, associated with high-risk cytogenetics, therapy-related ², secondary to myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN) responds poorly to conventional therapy. Refractory or relapsed AML is often perceived as an untreatable disease³. New agents, including the nucleoside analogues; inhibitors of FLT3, farnesyl-transferase, histone deacetylase, or DNA methyltransferase; and lenalidomide, administered alone or in combination, provide therapeutic options for these patients who are less likely to benefit from intensive cytotoxic therapy. The long-term survival of patients with AML with unfavorable factors, however, remains unsatisfactory ⁴. Novel antibody-based target therapies⁵, such as anti-CD20 (rituximab)⁶ and anti-CD22-calecheamicin conjugate (inotuzumab ozogamicin)⁷ have shown promising results in adult acute lymphoblastic leukemia. In contrast, in the past 20 years the only antibody-drug conjugate approved for the treatment of patients with refractory/relapsed AML was gemtuzumab ozogamicin which was subsequently withdrawn from the market ⁸.

Brentuximab vedotin, also known as SGN-35 or ADCETRIS®, is an anti-CD30 chimeric antibody conjugated by a protease-cleavable linker to monomethylauristatin E, an agent that disrupts microtubules⁹. Brentuximab vedotin has been shown to induce durable responses in patients with refractory and relapsed Hodgkin lymphoma and relapsed anaplastic large cell lymphoma with well tolerated side effects^{10, 11}. The drug recently received accelerated approval by the United States Food and Drug Administration for patients with relapsed Hodgkin lymphoma and anaplastic large cell lymphoma..

CD30 expression by myeloblasts has not been assessed rigorously in patients with AML and high-grade MDS. In 1993, Masuya and colleagues ¹² studied 10 cases of AML arising from MDS using flow cytometry immunophenotyping and detected surface CD30 expression in one (10%) case; they also used immunohistochemistry to assess CD30 expression in cytospin smears showed cytoplasmic expression in 4 of 9 (44%) cases. By contrast, immunohistochemical analysis for CD30 expression using fixed, paraffin-embedded tissue sections of myeloid sarcoma cases have been generally negative ^{13–15}. Recently, Fathi and colleagues performed immunohistochemistry for CD30 on 26 AML BM samples and found that 13 of 26 cases showed variable CD30 immunoreactivity,, range from 2 to 80% CD30-positive cells (median, 20%)¹⁶.

Application of multicolor flow cytometry immunophenotypic analysis has allowed investigators to assess antigen expression on precisely defined cell populations. This method is ideal to assess CD30 expression by blasts in patients with myeloid neoplasms. We conducted the current study to analyze bone marrow (BM) aspirate and/or peripheral blood (PB) specimens submitted consecutively for routine flow cytometry immunophenotyping. Using an aliquot of these patient samples we used flow cytometry immunophenotyping to

assess for CD30 expression by myeloblasts in AML and MDS. We also performed immunohistochemistry on fixed, paraffin- embedded bone marrow specimens of AML and MDS in a subset of cases.

Materials and Methods

Study Group

The study was conducted in accordance with protocols approved by the Institutional Review Board (IRB) at The University of Texas MD Anderson Cancer Center (MDACC). All patients were evaluated and treated at MDACC. The study was conducted over a 9-month interval, and BM aspirates and/or PB samples of patients with AML, high-grade MDS (refractory anemia with excess blasts; RAEB), and blast phase of MPN were acquired consecutively as part of the routine workup. The diseases were diagnosed and classified based on the 2008 World Health Organization criteria¹⁷.

Many patients in the study group had received various treatments for their underlying diseases. These included imatinib and dasatinib for chronic myelogenous leukemia (CML), hydroxyurea, interferon, anagrelide, JAK2 inhibitor and other experimental therapies for Philadelphia-chromosome negative MPNs, hypomethylating agents for MDS or AML; induction chemotherapy or modified chemotherapy and/or stem cell transplant (SCT) for AML. For comparison, we also assessed non-neoplastic myeloid precursors for CD30 expression in a group of lymphoma patients during the same time interval. This group of patients had been treated for lymphoma with various therapies and underwent BM aspiration and biopsy for lymphoma re-staging. Five of these patients received growth factor support within a week of BM aspiration and biopsy.

Flow Cytometry Immunophenotyping

BM aspirate specimens were collected in EDTA-anticoagulated tubes and processed within 24 hrs of collection. After incubation with monoclonal antibodies for 15 min at 4°C, erythrocytes were lysed with ammonium chloride (PharmLyse™, BD Biosciences, San Diego, CA) at room temperature for 10 min using a standard lyse/wash technique. Samples were acquired on FACSCanto II instruments (BD Biosciences). The antibody combinations used were: CD14-FITC; CD30-PE; CD64-APC; CD117- PE-Cy7; CD34-PerCP-Cy5.5; CD45-V500. The negative control contained CD34-PerCP-Cy5.5 and CD45-V500 with absence of CD30-PE. We compared CD30-PE (Clone HRS4) from Beckman Coulter and CD30-PE (BerH-83) from BD Biosciences side by side in 10 samples. The staining patterns and percentages of positive cells were very similar, but the former provided slightly better fluorescence intensity and therefore was used consistently in this study. The remaining antibodies were purchased from BD Biosciences. In some patients, PB samples positive for circulating blasts were collected along with the BM aspirate samples and analyzed simultaneously. At least 100,000 live events in each sample were acquired. In lymphoma re-staging BM specimens, up to 500,000 live events were acquired to obtain at least 200 events in the myeloblast gate.

Data were analyzed using FCS Express software (De Novo Software, Los Angeles, CA). Nonviable cells, debris and aggregates were excluded based on forward scatter-height/forward scatter-area (FSC-H/FSC-A). Blast populations were identified based primarily on CD45dim/SSC-A, and further defined by CD34 and CD117. Monocytes were identified by CD45dim/SSC-A and further characterized by CD64/CD14. If monocytes were part of the blast population based on morphology and esterase stain, they were included in the calculation of total myeloblasts. CD30 expression was assessed on the entire blast population, and reported as a percentage of positive blasts. Mean fluorescence intensity (MFI) ratio was calculated by comparison with negative control.

In each patient in the study group, BM aspirate smears were prepared and assessed independently. Manual 500 cell counts were performed to determine a blast count, and cytochemical stains for myeloperoxidase and nonspecific esterase were performed and assessed.

Immunohistochemistry

Routinely fixed, paraffin-embedded sections of BM aspirate clot specimens (without decalcification process) were assessed by immunohistochemistry for CD30 (BerH2, Dako, Ventana Benchmark) following heat-induced epitope retrieval. In some cases, immunohistochemistry was performed on BM biopsy specimens in parallel with the BM aspirate clot specimen. The BM biopsy specimens were fixed in formalin, decalcified in 10% formic acid for 3 hrs at 50°C in a microwave prior to paraffin embedding. These studies were performed in an automated immunostainer (Ventana Medical Systems) according to the manufacturer's instructions with appropriate positive and negative controls. The assessment of CD30 expression was based on the percentage of positive cells and intensity of the reactivity estimated on blasts. We used isotype controls as negative controls. A case of CD30-positive anaplastic large cell lymphoma, was used as a positive control. The intensity of CD30 expression by blasts was evaluated using as a reference values established on a classical Hodgkin lymphoma cell line (Seattle Genetics) and graded as weak/dim, moderate and strong/bright.

Cytogenetic Analysis

Conventional cytogenetic analysis was performed on 24-hour and 48-hour cultures of BM aspirate specimens. At least 20 metaphases or all available metaphases were examined. The International System for Human Cytogenetic Nomenclature was used to describe abnormal clones. Cytogenetics risk score was categorized as "good", "intermediate" or "poor" according to the revised UK Medical Research Council (UKMRC) criteria for AML¹⁸ and the International Prognostic Scoring System (IPSS) for MDS¹⁹; and the International Prognostic System for primary myelofibrosis (PMF)²⁰. As these prognostic scoring systems are unique for diseases included in this study, we simply grouped the cases as "poor risk" and "good/intermediate risk".

Molecular Analysis

NPM1 mutations were detected using primers designed to amplify mutational hotspots spanning codons 956–971 of exon 12, followed by capillary electrophoresis as described

previously ²¹. The *FLT3* internal tandem duplication (*FLT3*-ITD) and tyrosine kinase domain codon 835/836 point mutations (*FLT3*-D835) were detected by a fluorescent-based multiplex PCR assay followed by capillary electrophoresis.²² For *FLT3*-D835 point mutation analysis, PCR products were digested with *EcoRV* before capillary electrophoresis. *K-RAS* and *N-RAS* mutations at codons 12, 13 and 61 were tested using PCR followed by pyrosequencing as described previously ²³. Mutations in exons 8 and 17 of the *KIT* gene were detected using Sanger sequencing ²¹.

Statistics

The Fisher's exact test or chi-square was used for comparison of categorical variables and the t test or one-way analysis of variance (ANOVA) was applied for numerical comparisons. A p value of <0.05 was considered to be statistically significant.

RESULTS

Study Cohort

The patients included in this study are shown in Table 1. There were 80 men and 55 women, with a median age of 63 years (13–92, only 1 patient <18 years old). These cases were categorized as de novo AML, primary MDS, or therapy-related (t) AML/MDS, and blast crisis of MPN (including blast crisis of CML). Thirty-three patients were newly diagnosed, 68 patients had primary refractory disease, and 34 patients had disease recurrence (8 patients recurred after stem cell transplant). In the subgroup of 33 newly diagnosed patients, 14 patients had been treated for preexisting MDS or MPN and 19 AML patients had not received any therapy. The median blast count assessed by manual counting was 30% (range, 3–96). Cytogenetic results were available in 133/135 patients. A total of 129 patients could be categorized into prognostic groups using various systems, and these included 7 good risk, 57 intermediate risk and 65 poor risk using the UKMRC system for AML ¹⁸, IPSS for MDS ¹⁹, and the IPSS for PMF ²⁰. Four patients had non-PMF MPN and could not be fit into a prognostic group.

The 31 patients who underwent BM aspiration and biopsy for lymphoma staging had a median age of 59 years (range, 23 to 79) with 22 males and 9 females. These patients had no evidence of MDS, MPN or any other stem cell neoplasm. Four of these BM specimens were positive for lymphoma.

CD30 Expression

The median blast count of AML/MDS cases enumerated by flow cytometry immunophenotyping was 26% (range, 2–92) and this blast count highly correlated with the manual blast count ($r=0.875$, $p<0.001$). Overall, the median percentage of CD30-positive blasts was 14% with a range from 0 to 91%. The mean fluorescence intensity (MFI) of CD30 expression (compared with control) was 1.56 with a range from 0.1 to 26.9. Using a traditional 20% cutoff, 49 of 135 (36%) patients had AML/MDS positive for CD30 and the median MFI in these 49 cases was 3.6 (range, 1.6 to 29.6). Figure 1 illustrates an AML case positive for CD30 expression, and the typical expression pattern of CD30 expression which

is heterogeneous with a spectrum of cells with dim, moderate, and bright CD30 expression. The MFI over control in this case is 9.8.

CD30 Expression is Absent in Monocytic Cells

There was significantly lower CD30 expression observed in myeloid neoplasms with monocytic differentiation. In 20 patients in whom blasts were monoblastic or monocytic or with a substantial monocytic component, in other words French-American-British classification M5a/M5b or M4, CD30 expression was often completely absent in monocytic cells. In a subset of FAB M4 cases, CD30 was expressed in CD34+CD117+ myeloblasts. The levels of CD30 expression in CD34+CD117+ myeloblasts in FAB-M4 cases were similar to CD34+CD117+ myeloblasts in other types of AML, but the case number of M4 were too low to perform a statistical comparison. Overall, in the FAB M4 and M5 groups, a median of 2% cells (range, 0–33%) were positive for CD30; using a 20% cutoff 2 (10%) cases were CD30-positive. These results are significantly lower than the results for non-monocytic AML cases in this study (Table 2).

Higher CD30 Expression in Refractory/Relapse AML Patients

In the AML group, 19 patients presented de novo and had never received therapy and 68 patients had primary refractory disease undergoing various treatments. The latter subgroup showed a significantly higher level of CD30 expression than the de novo subgroup (Table 2). This comparison remained significant when monocytic leukemia cases were excluded (data not shown). CD30 expression was higher in the poor cytogenetic risk group (complex with frequent chromosome 5 and 3q abnormalities), only when CD30 was expressed as % of blasts overall but not significant when compared by the number of cases meeting the 20% cutoff of CD30 expression. Notably, many patients with a high cytogenetic risk score had either refractory or recurrent disease.

CD30 Expression and *NPM1*, *FLT3* or *RAS* Mutations

CD30 expression did not correlate with gene mutation status. *NPM1* gene mutations were detected in 6/86 (7%) cases with <20% CD30 expression, and 8/49 (16%) cases with ≥20% CD30 expression ($p=0.139$). Notably, 11 *NPM1*+ cases also had *FLT3* mutations (10 *FLT3 ITD* and 1 *FLT3 D835*); and another 3 had concurrent *IDH1/IDH2* mutations. *FLT3 ITD* was detected in 14/86 (16%) cases with <20% CD30 expression; and 9/49 (18%) cases with ≥20% CD30 expression ($p=0.814$). *RAS* mutations were detected in 11/86 (13%) cases with <20% CD30 expression, and 10/49 (20%) cases with ≥20% CD30 expression ($p=0.323$).

CD30 Expression in Normal Regenerating Bone Marrow CD34+ Cells

In lymphoma restaging BM specimens, the median blast count was 1% (range, 0–2%) assessed by manual count and 0.5% (range, 0.2–1.7%) assessed by flow cytometry immunophenotyping. With a median of 970 CD34+CD117+ events analyzed (range, 298–2,792), CD30 expression on normal/regenerating myeloblasts was 26% (range, 11–68) overall, and ≥20% in 22/31 (71%) cases. CD30 expression was significantly higher and more frequent in normal/regenerating than in the AML/MDS group ($p<0.001$). Non-neoplastic myeloblasts showed CD30 expression in a subset of cells in almost all patients,

whereas myeloblasts in patients with AML/MDS were either positive or completely negative for CD30 expression. In contrast, CD34+ hematogones (normal precursor B cells) in the BM showed very few cells with CD30 expression (Figure 2A and 2B).

CD30 Expression by Blasts in Peripheral Blood versus Bone Marrow

In 33 patients, PB samples containing circulating blasts (median 16%, range 2–91%) were analyzed parallel with BM aspirate samples by flow cytometry immunophenotyping. Although CD30 expression showed a high correlation between PB and BM blasts ($r=0.92$, $p<0.001$), BM blasts consistently showed a higher number of CD30-positive cells with brighter intensity (Table 1) (MFI) ($p=0.054$).

Immunohistochemistry

Immunohistochemical analysis for CD30 was performed using fixed, paraffin-embedded sections of BM aspirate clot specimens and the results were compared with CD30 expression assessed by flow cytometry immunophenotyping (Table 3). As could be directly observed, CD30 reactivity in blasts was membranous and cytoplasmic. Although CD30 reactivity detected by immunohistochemistry correlated with the flow cytometry immunophenotyping results, CD30 intensity and the percentage of positive cells were generally weaker/dimmer by immunohistochemistry (Figure 3). In 29 cases, immunohistochemistry was performed on both BM biopsy (decalcified) and aspirate clot (non-decalcified) specimens. In 10 cases in which myeloblasts in the aspirate clot specimen were positive for CD30, in 9 cases CD30 expression in biopsy specimens was either negative or only focally positive; 1 case showed comparable results in both types of specimens. These results indicate that BM aspirate clot without decalcification provides significantly better immunohistochemical results than bone marrow core biopsy after decalcification.

Discussion

The CD30 antigen, also known as Ki-1, is a member of the tumor necrosis factor (TNF) receptor superfamily. In physiologic states, CD30 expression is restricted to subpopulations of activated B- and T-cells²⁴, and upregulation of CD30 and CD30L is linked to autoimmune and inflammatory diseases²⁵. In neoplastic diseases, CD30 is best known for its being expressed in classical Hodgkin lymphomas and anaplastic large cell lymphoma (ALCL). Additionally, CD30 is also expressed by embryonal carcinoma, a subset of diffuse large B-cell lymphomas and peripheral T-cell lymphomas²⁶, and in aggressive forms of systemic mastocytosis^{27, 28}. To our knowledge, relatively little data for CD30 expression in myeloid neoplasms are available with few cases studied^{12–15}.

In this study, we show that CD30 is expressed by myeloblasts (CD34+ and/or CD117+) in a substantial number of cases of AML, either de novo or arising from MDS or MPN, as well as in high-grade MDS. In contrast, CD30 is often absent in monocytic blasts or background maturing/mature monocytes; therefore, AML, either monocytic or myelomonocytic, has a much less CD30 expression. These results are in contrast with CD30 ligand (CD30L), a marker frequently observed in AML, that is preferentially expressed in AML with

monocytic differentiation^{29, 30}. We also show that CD30 expression is more frequent in AML patients who were refractory to various ongoing therapies as compared with de novo AML patients with no treatment. Patients with AML with a poor cytogenetic risk score, many refractory to therapy, also exhibited greater CD30 expression overall as shown by the median percentage of positive cells, but not by using a 20% cutoff. CD30 expression did not correlate with *FLT3 ITD*, *D835*, *NPM1* or *RAS* mutation status or a specific karyotypic abnormality. These findings suggest that CD30 expression by myeloblasts may be upregulated by therapy. In accord with this hypothesis, we studied lymphoma re-staging BM specimens and observed a median of 27% (11–68) CD34+CD117+ myeloblasts positive for CD30, but not in other BM cells including monocytes. These findings suggest that CD34+ myeloid precursors, similarly to B- and T-cells, may constitutively express low levels of CD30 which can be up-regulated upon activation and induction by various stimuli. Although this study did not address the mechanisms of CD30 expression by myeloblasts, the staining pattern, intensity and heterogeneity of CD30 expression in AML/MDS contrasts with the uniform CD30 expression pattern that is characteristic of the Reed-Sternberg and Hodgkin cells of classical Hodgkin lymphoma as well as the neoplastic cells of ALCL. We therefore suspect that the mechanisms of CD30 expression in myeloblasts differ from those in classical Hodgkin lymphoma and ALCL in which constitutive activation of the ERK1/2-MAPK pathway plays a central role in overexpression of JunB and CD30^{31, 32}.

In this study, we also investigated several issues related to CD30 detection. We observed that CD30 expression by BM blasts strongly correlated with PB blasts, but the intensity of CD30 expression by BM blasts was generally brighter. Similar findings have been described previously for a number of AML markers studied by flow cytometry immunophenotyping³³, reflecting different biological effects in the BM and peripheral blood compartments. CD30 expression by myeloblasts was heterogeneous and exhibited a spectrum in positive cases, and the intensity of expression was generally not high (MFI ratio of 3.6). This low intensity of CD30 expression likely contributed to the dim and focal CD30 reactivity observed by immunohistochemical analysis of BM specimens. In addition, several cases positive by flow cytometry immunophenotyping had a low blast count (<10%), and immunohistochemical assessment for CD30 expression by myeloblasts was technically difficult in such cases. Overall, immunohistochemistry for CD30 detection is generally much weaker and often focal as compared with flow cytometry immunophenotyping. The vagaries of flow cytometry immunophenotyping versus immunohistochemistry may explain some of discrepancies between this study and the report by Fathi and colleagues. In their study, the authors defined any immunoreactivity detected by immunohistochemistry as positive and they observed that CD30+ cases had a higher frequency of *FLT3* mutation¹⁶. In our series, we could not confirm this correlation. In this study, our comparison of results generated using the Ber-H83, HRS4 and Ber-H2 antibodies (different clones) showed a generally good correlation, although there were some differences in the intensity of staining. Bone marrow biopsy specimens using our decalcification procedure, which in general does not impair CD30 detection in classical Hodgkin lymphoma and ALCL, is inadequate to assess CD30 expression by myeloblasts by immunohistochemistry. This suggests that the CD30 antigen is partially destroyed by the decalcification procedure, affecting dim CD30

expression as observed in many AML/MDS cases, but decalcification does not affect detection of bright CD30 expression as observed in Hodgkin lymphoma and ALCL.

In summary, we report substantial CD30 expression by myeloblasts in patients with non-monocytic AML and high-grade MDS. CD30 is also more frequently expressed by myeloblasts in patients with refractory AML being actively treated compared with de novo, untreated AML. Based on our comparison of different techniques and specimens, we suggest that flow cytometry immunophenotypic analysis performed on BM aspirate specimens is the optimal approach to assess CD30 expression. These results suggest that CD30 could be a target for therapy using anti-CD30 antibodies in a subset of patients with non-monocytic AML and in high-grade MDS patients.

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Clinical Practice Points

Brentuximab vedotin, an anti-CD30 chimeric antibody conjugated with monomethylauristatin E, has been shown to induce durable responses in patients with refractory/relapsed Hodgkin lymphoma and anaplastic large cell lymphoma with well tolerated side effects. Acute myeloid leukemia (AML) in elderly, secondary to MDS or MPN responds poorly to conventional therapy. Refractory or relapsed AML is often perceived as untreatable, and target therapy may provide potential benefit for these patients. CD30 expression in myeloblasts has not been rigorously assessed in patients with AML and high-grade MDS. In this study, using multicolor flow cytometry immunophenotypic analysis, we investigated CD30 expression in 135 AML/MDS patients including 68 (50%) with high-risk cytogenetics and 102 patients had refractory/relapse disease. We detected CD30 expression in a median of 14% (range, 0–91) blasts. Using an arbitrary 20% cutoff, 49 (36%) AML/MDS were considered positive for CD30. Patients with persistent disease who had been actively treated had a higher level of CD30 expression than untreated patients. CD30 expression was consistently higher in bone marrow blasts than peripheral blasts. Immunohistochemistry demonstrated CD30 expression in a subset of patients, but the reactivity was generally weaker and focal compared with flow cytometry immunophenotyping. We conclude that CD30 is expressed by myeloblasts in a substantial subset of cases of AML/MDS, and flow cytometry is the better method than immunohistochemistry. As the study group composed mostly of high-risk AML/MDS patients with very few treatment options left, these data raise the possibility that anti-CD30- targeted therapy could be an option for this patient group.

Figure 1.

CD30 expression on myeloblasts in a case of acute myeloid leukemia studied by flow cytometry immunophenotypic analysis (gated on CD45 dim "blast" region"). A. Negative control; B. CD30 expression; C. CD30 mean fluorescence Intensity over negative control (MFI ratio 9.8); D. Monocytes (CD14 bright+) in the same bone marrow are negative for CD30.

Figure 2.

CD30 expression on CD34⁺ cells in a regenerating bone marrow. A. All the events shown here are CD34⁺ cells, which include myeloid precursors (CD117⁺, upper) and hematogones (normal bone marrow precursor B cells, CD117⁻, lower). The CD34⁺CD117⁺ myeloid precursors show substantial CD30 expression; whereas, CD34⁺ hematogones are mostly negative for CD30. B. CD30 mean fluorescence Intensity (MFI) over negative control. Hematogones (arrow) overlapping with negative control; and myeloid precursors (arrow head) with a MFI ratio of 18.1.

Figure 3.

Bone marrow aspirate clot of a case of acute myeloid leukemia. Left: Hematoxylin & Eosin (H&E) showing the presence of many myeloblasts; Right. immunohistochemical stain showing membranous and cytoplasmic CD30 reactivity in myeloblasts.

Table 1

Patient cohort

Patients	N=135
Age (year old) (median, range)	63(13–92)
Gender (Male/Female)	80/55
Blasts in bone marrow by counting (%)	30(3–96)
AML (primary)	N=63 (47%)
Therapy-related-MDS/AML	N=22 (16%)
MDS, MPN, Secondary AML	N=50 (37%)
• RAEB-1/RAEB-2	N=14(10%)
• AML arising from MDS or MDS/MPN	N=22 (16%)
• Blast phase of MPN or AML from MPN	N=14 (10%)
> CML blast crisis	N=6
Newly diagnosed	N= 33(24%)
Relapse */Refractory	N=102 (76%)
Cytogenetics **	
• Good/ <u>intermediate risk</u>	N= 64
• Poor <u>risk</u>	N= 65
• Not applicable or not available	N=6
Mutations	
• <i>FLT3</i>	N=30
• <i>NPM1</i>	N=15
• <i>RAS</i>	N=21
• *** Others	N=28

Abbreviation: AML: acute myeloid leukemia; MDS: myelodysplastic syndromes; MPN: myeloproliferative neoplasm; RAEB: refractory anemia with excess blasts; CML: chronic myelogenous leukemia.

* 8 relapsed post stem cell transplant;

** Refer to Text for classification systems used;

*** including *KIT*, *IDH1/IDH2HRM*, *CEBPA*

Table 2

Surface CD30 expression measured by multicolor flow cytometry immunophenotyping

	<i>Number of cases</i>	<i>Blasts count by morphology</i>	<i>CD30% of blasts</i>	<i>Cases with 20% CD30 expression</i>	<i>* p (p1/p2)</i>
Regenerating bone marrow (BM)	N=31	0.5(0.2–1.7%)	26(11–68)	22(71%)	<0.001/0.021
Acute myeloid leukemia/MDS (BM)	N=135	30 (3–96%)	14(0–91)	49 (36%)	
Acute myeloid leukemia (AML) paired samples					
• Bone marrow	N=33	40(4–96%)	16(0–91%)	12/33 (36%)	0.002/0.045
• Blood		16(2–91%)	10(0–78%)	5/33 (15%)	
Blasts with/without Monocytic differentiation					
• Yes	N=20	45(10–70)	2(0–33%)	2/20(10%)	0.006/0.010
• No	N=115	28(3–96)	17(0–91%)	47/115(41%)	
Treatment status of AML cases					
• No treatment	N=19	46(6–95)	2(0–46%)	3/19 (16%)	0.031/0.031
• Refractory	N=68	22(2–96)	17(0–91%)	31/68 (46%)	
Cytogenetics**					
• Good	7	16(6–31)	1(0–64)	2/7 (29%)	0.033/0.276
• Intermediate	57	31(5–95)	8(0–91)	18/57 (32%)	
• Poor	65	38(3–96)	18(0–87)	27/65 (42%)	

* CD30 expression compared as % of positive cells overall (P1), as well as cases with 20% expression (P2)

Correlation of Immunohistochemistry performed on non-decalcified bone marrow aspirate clot and flow cytometry immunophenotypic analysis

Table 3

Immunohistochemistry						
CD30 by Flow cytometry analysis	Cases (n)	Negative	Weak	Moderate	Strong	
	0–5%	12	12	0	0	0
	5–20%	11	6	4	1	0
	20–50%	17	6	9	2	0
	50%	13	3	2	5	3