Immunophenotypes of Chronic Myelomonocytic Leukemia (CMML) Subtypes by Flow Cytometry

A Comparison of CMML-1 vs CMML-2, Myeloproliferative vs Dysplastic, De Novo vs Therapy-Related, and CMML-Specific Cytogenetic Risk Subtypes

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ABSTRACT

Objectives: *We sought to immunophenotype blasts, monocytes, and granulocytes in chronic myelomonocytic leukemias (CMMLs) and compare CMML subtypes, to identify if significant antigen expression differences existed.*

Methods: *Bone marrow blasts, monocytes, and granulocytes from CMML subgroups (n = 30; World Health Organization types 1/2, proliferative/dysplastic, therapy related/de novo, and low/intermediate/high cytogenetic risk) were immunophenotypically compared by flow cytometry with 10 nonneoplastic control marrows.*

Results: *Aberrancies were present in blasts of 26 (87%) of 30 CMMLs (26 diagnostic; four follow-up) and six (60%) of 10 controls (P = .089), monocytes of 28 (93%) of 30 CMMLs and six (60%) of 10 controls (P = .026), and granulocytes of eight (28%) of 29 CMMLs and zero of 10 controls (P = .166). Underexpression of CD14 and CD15 on monocytes was more common in CMMLs compared with controls (P = .008 and P = .043). Statistical analysis showed no significant difference in antigen expression between the CMML subgroups on blasts or monocytes; granulocytes demonstrated more common HLA-DR expression in CMML-2 vs CMML-1.*

Conclusions: *These findings confirm heterogeneity within CMML subgroups and find no specific qualitative or quantitative findings characteristic of a subgroup.*

Chronic myelomonocytic leukemia (CMML) is a pathologically heterogeneous disease, with overlapping morphologic features of both myelodysplastic syndromes and myeloproliferative neoplasms. Given the similar clinical heterogeneity of this entity, several scoring systems have been recently developed, aiming to prognosticate patients with CMML. These scoring systems have incorporated various laboratory and clinical findings, including WBC, absolute monocyte, absolute lymphocyte, and platelet counts; hemoglobin; presence of immature myeloid cells in the peripheral blood (PB); PB and bone marrow (BM) blast percentage; cytogenetics; *ASXL1* mutations; and RBC transfusion dependence.[1-5] The CMML-specific prognostic scoring system (CPSS), the most commonly used system in clinical practice currently, categorizes patients into low, intermediate 1, intermediate 2, and high-risk categories based on CMML subtypes and RBC transfusion dependence.[2] Overall survival and risk of transformation to acute myeloid leukemia (AML) are predicted in this system by dividing patients with CMML into World Health Organization (WHO) classification subgroups (type 1 and type 2 based on percentage of blasts in the PB and BM), French-American-British (FAB) classification subgroups (myeloproliferative and myelodysplastic based on WBC count), and cytogenetic categories (low, intermediate, and high risk).[2]

When the individual components of the CPSS are further examined, the literature is conflicting. While studies examining PB and BM blast counts appear consistently predictive with few exceptions, supporting classification into WHO subgroups type 1 and type 2,[6-8] separation into distinct

subgroups based on WBC count of less than or greater than 13,000/μL (proliferative and dysplastic types) remains controversial. Several studies have supported the predictive power of WBC counts and found the myeloproliferative variant to be more aggressive, with a higher rate of progression to AML and a lower overall survival.[1-3,5,7,9-16] Others have found no significant difference between the two groups.[4,17-20] Critics of the division have cited the frequent oscillation of the WBC count throughout the course of CMML, rendering the subclassification arbitrary based on the current clinical situation.[21] Similar to the subclassification based on blast counts, when the CMML-specific cytogenetic abnormalities (Spanish cytogenetic risk stratification)[3] are independently examined, there is recognition of the importance of these prognostic indicators, with some debate across studies on categorization of the specific cytogenetic abnormalities.

The literature on flow cytometry (FC) findings in CMML is surprisingly limited. Few studies have reported on various myeloid populations in CMML, and even fewer studies have dissected immunophenotypic findings by CMML subtype. Authors have found immunophenotypic aberrancies in CMMLs at high frequency, with the more common findings, including aberrant CD56 expression on monocytes, expanded CD14 moderate monocyte populations, aberrant CD7 and CD56 expression on blasts, and underexpression of CD45 by blasts.[22-29] Given the sparse flow cytometry data in the literature on CMML generally and the new emphasis on CMML subsets for prognostication in scoring systems, we sought to study the immunophenotypic findings in CMML and compare these findings within prognostically relevant subtypes by comprehensively examining antigen expression patterns by flow cytometry on blasts, monocytes, and granulocytes.

Materials and Methods

Classification of Cases

Thirty CMML cases with FC were identified from the Department of Pathology archives at the Medical College of Wisconsin over a 6-year period. CMML diagnoses were made according to the 2008 WHO classification (S.H.K., A.M.H., or H.O.) and further subclassified into the following prognostic subtypes: WHO types 1 and 2 (CMML-1, CMML-2), proliferative and dysplastic, therapy related and de novo, and CMML-specific cytogenetics subgroups. CMML-1 was defined as 5% or less PB blasts and less than 10% BM blasts; CMML-2 was defined as 5% to 19% PB blasts or 10% to 19% BM blasts.[8] Proliferative and dysplastic CMMLs were defined as WBCs of 13,000/μL or more and less than 13,000/μL, respectively. Therapy-related CMMLs were defined as developing in patients who received previous chemotherapy or radiation. CMMLs were categorized into the following cytogenetic subgroups: low risk, normal karyotype; high risk, trisomy 8, chromosome 7 abnormalities, and complex karyotypes; and intermediate risk, other abnormalities.[3] Additional BM biopsy specimens from patients with blood count abnormalities associated with various nonneoplastic disorders from the same time period were identified as control samples. Institutional review board approval was granted at the Medical College of Wisconsin for this study.

Clinicopathologic Data

CBC, differential, PB and BM blast percentages (based on 100- and 500-cell differentials, respectively), karyotype, and basic demographic data were collected from the BM report and electronic record. Previous therapy was recorded. Indications for BM biopsy and clinical diagnosis were recorded for controls.

FC

EDTA- or heparin-anticoagulated BM aspirates were prepared using previously described methods[27] on the day of procurement or within 20 hours of procurement. Analysis was performed using four- or eight-color flow cytometry with the following antibodies: anti-CD7, CD10, CD11b, CD13, CD14, CD15, CD20, CD22, CD33, CD34, CD38, CD45, CD56, CD64, CD117, and HLA-DR (Becton Dickinson, Franklin Lakes, NJ) and anti-CD36 (Coulter, Brea, CA). All cases were analyzed on a FACS Calibur (February 2006 to February 2009) or FACS Canto (March 2009-2013) instrument (Becton Dickinson) by one observer (L.A.S.) using Paint-a-Gate software (Becton Dickinson). Populations were identified across tubes using cluster analysis as follows: monocytes, intermediate forward and side scatter, CD45 bright+, and CD34–; granulocytes, moderate to high forward and side scatter, CD15 bright+, CD45 moderately+; lymphocytes, low forward and side scatter and CD45 bright+; hematogones, low forward and side scatter, CD10+, CD22 moderately+, CD38 moderately bright+, HLA-DR+, CD34 subset+, and CD45 moderate to dim+; basophils, tight cluster with intermediate forward scatter, low side scatter, CD38+, CD11b+, CD13 moderately+, CD33 moderately+, CD45 moderate to bright, and CD34–; and erythroids, variable but predominantly low forward and side scatter, CD36+, CD34 predominately–, CD45 negative to dim+, and CD64–. Nonviable cells and debris were removed based on very low forward and side scatter. Blasts were recognized after exclusion of all other populations as cohesive, well-delineated clusters, with consistent light scatter and CD45 expression patterns across multiple tubes. If

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the monocyte population was not distinct by CD45 and forward and side-scatter properties, further antigens were used to define the cluster across tubes. Twelve antigens were assessed on myeloblasts (CD7, CD11b, CD13, CD15, CD33, CD34, CD36, CD38, CD45, CD56, CD117, and HLA-DR), 11 antigens on monocytes (CD11b, CD13, CD14, CD15, CD33, CD36, CD38, CD45, CD56, CD64, and HLA-DR), and two antigens on granulocytes (CD56 and HLA-DR).

Positive antigen expression was defined as at least 20% of the population of interest displaying fluorescence above the isotype control, using an isotype cutoff of 2%. Blast aberrancies were defined as positive antigen expression in an antigen not normally expressed in blasts or a 1/4 log shift in a population compared with previously published data from 20 negative lymphoma staging BMs.[27] Aberrancies in monocytes were defined in one of two ways: either expression of an antigen not found on normal monocytes, using the 20% cutoff, or underexpression or overexpression of a normal monocyte antigen.[24] The underexpression and overexpression were defined as a shift of at least a half a log compared with nonneoplastic monocytes. Expression of CD56 or HLA-DR on granulocytes was considered aberrant. In cases where granulocytes and monocytes were difficult to distinguish on flow plots, the granulocytes were not further described. Neoplasia-specific aberrancies were defined in blasts, monocytes, and granulocytes as those aberrancies seen in neoplastic marrows and not observed in the nonneoplastic controls.

Statistical Analysis

Statistical analysis was performed using Prism software (GraphPad Software, version 5.0c, La Jolla, CA). Mann-Whitney *t* tests were used for continuous variables, and Fisher exact test was used to compare categorical variables. One-way analysis of variance tests were used to compare quantitative data across three groups. Spearman correlation studies were performed for paired results. Statistically significant relationships were defined as *P* values of less than .05.

Results

Patients

Thirty CMMLs (26 diagnostic; four follow-up, posttherapy) were compared with 10 nonneoplastic BMs. Demographic and CBC plus differential data are presented in Table 1. Controls had BMs performed for bicytopenias (n = 4), thrombocytopenia (n = 3), anemia (n = 1), leukopenia (n = 1), and anemia plus leukocytosis (n = 1) for the following clinical diagnoses: immune-mediated cytopenia

Table 1
Patient Demographics, Mean Laboratory Values, and Presence of Aberrancies in Blasts, Monocytes, and Granulocytes for CMMLs and Controls[a]

Characteristic	CMMLs (n = 30)	Nonneoplastic (n = 10)	*P* Value
Sex, %			
Male	77	30	**.018**
Female	23	70	
Age, median (range), y	75 (51-91)	70 (41-74)	.068
Cytogenetics, %			
Normal	77	100	.161
Abnormal	23	0	
WBC count, × 10^3/μL	20 (2.2-176.9)	5.4 (2.5-11.8)	**.003**
Hemoglobin, g/dL	11 (7.6-16.1)	11.6 (9.9-12.3)	.30
Platelet count, × 10^3/μL	179 (31-852)	183 (87-362)	.342
Blood monocytes, %	30 (11-72)	9.8 (3-17)	**<.001**
Blood neutrophils, %	42 (3-77)	66 (43-87)	**<.001**
Blood basophils, %	1.0 (0-4)	0.6 (0-2)	.684
Absolute monocyte count, × 10^3/μL	7.7 (1.1-127.3)	0.48 (0.2-1.2)	**<.001**
Absolute neutrophil count, × 10^3/μL	7.7 (0.07-30)	3.7 (1.5-10.2)	.493
Absolute basophil count, × 10^3/μL	0.25 (0.0-2.2)	0.02 (0.0-0.1)	.147
Blood blast, %	0.48 (0-6)	0 (0)	.244
Bone marrow blast, %	4.1 (0-15.4)	0.96 (0-2)	**.004**
Mean blasts by FC, %	1.2 (0.08-6.5)	0.33 (0.18-0.56)	**.049**
Mean monocytes by FC, %	19 (7.7-51)	3.7 (2.1-6.2)	**<.001**
Mean granulocytes by FC, %	59 (26-86)	69 (53-85)	.059
Cases with blast aberrancies, No. (%)	26 (87)	6 (60)	.089
Cases with monocyte aberrancies, No. (%)	28 (93)	6 (60)	**.026**
Cases with granulocyte aberrancies, No. (%)	8 (28)	0	.166

CMML, chronic myelomonocytic leukemia; FC, flow cytometry.
[a]Values are presented as mean (range) unless otherwise indicated. Bold values represent statistically significant relationships.

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(n = 2), anemia of chronic disease (n = 1), anemia of renal failure (n = 2), medication-induced cytopenias (n = 2), congestive splenomegaly (n = 1), and unknown (n = 2). The following statistically significant relationships were identified in the CBC and PB and BM differential counts between the CMMLs and controls: male predominance in the CMMLs ($P = .018$), higher WBC counts in CMMLs ($P = .003$), higher relative blood neutrophils in controls ($P < .001$), higher relative and absolute monocyte counts in the blood in CMMLs ($P < .001$), and higher BM blast counts in CMMLs ($P = .004$).

FC Findings of CMMLs vs Controls

By FC, blasts averaged 1.2% of events in CMMLs and 0.33% in controls ($P = .049$), monocytes averaged 19% in CMMLs vs 3.7% in controls ($P < .001$), and granulocytes averaged 59% in CMMLs compared with 69% in controls ($P = .059$) (Table 1). Blast percentages derived by morphology and FC correlated in the CMMLs ($P = .05$, $R = 0.360$) but not in the controls ($P = .492$, $R = 0.249$). Blast aberrancies were present in 26 (87%) of 30 CMMLs compared with six (60%) of 10 nonneoplastic BMs ($P = .089$) and ranged from zero to five (mean, 2.3) in CMMLs and zero to two (mean, 0.9; $P = .015$) for nonneoplastic marrows. Monocyte aberrancies were present in 28 (93%) of 30 CMMLs compared with six (60%) of 10 nonneoplastic BMs ($P = .026$) and ranged from zero to six (mean, 2.5) for CMMLs and zero to two (mean, 0.8; $P < .001$) for nonneoplastic marrows. Granulocyte aberrancies were present in eight (28%) of 29 CMMLs and were not observed in nonneoplastic BMs ($P = .166$).

Aberrancies in blasts, monocytes, and granulocytes for CMMLs and controls are displayed in **Table 2**. In controls, the most common blast aberrancies included underexpression of CD33 (4/10; 40%) and overexpression of HLA-DR (2/10; 20%), as well as monocytes with aberrant expression of CD56 (5/10; 50%) and underexpression of HLA-DR (2/10; 20%); no granulocyte aberrancies were observed. The most common blast aberrancies identified in CMMLs in descending order included underexpression of CD33 (16/30; 53%), overexpression of CD117 (9/30; 30%), overexpression of CD13 (7/30; 23%), and underexpression of CD38 or CD45 (5/30; 17% each). Aberrant expression of CD7, CD11b, and CD56; increased expression of CD13, CD33, and CD34; and diminished expression of CD38, CD45, and HLA-DR on blasts were present in CMMLs but not the nonneoplastic controls **Image 1**. CD13, CD14, CD15, CD33, CD36, CD45, and CD64 were underexpressed on monocytes in some CMML cases but not in the nonneoplastic cases; however, only CD14 and CD15 underexpression was statistically significant **Image 1B**. Granulocytes expressed CD56 in five (18%) of 28 CMMLs but not in nonneoplastic BMs **Image 1C**. HLA-DR was aberrantly expressed on granulocytes in three (11%) of 28 CMMLs but not in nonneoplastic BMs.

Table 2
Immunophenotypic Aberrancies in CMMLs vs Controls

Aberrancies	CMMLs (n = 30), No. (%)	Controls (n = 10), No. (%)	*P* Value[a]
Blasts			
CD7+	3 (10)	0	.560
CD11b+	2 (7)	0	1.0
Bright CD13	7 (23)	0	.161
↓ CD13	1 (3)	1 (10)	.442
Bright CD33	2 (7)	0	1.0
↓ CD33	16 (53)	4 (40)	.716
Bright CD34	4 (13)	0	.556
Bright CD38	3 (10)	0	.560
↓ CD38	5 (17)	0	.306
↓ CD45	5 (17)	0	.306
CD56+	3 (10)	0	.560
Bright CD117	9 (30)	1 (10)	.401
Bright HLA-DR	2 (7)	2 (20)	.256
↓ HLA-DR	3 (10)	0	.560
Monocytes			
↓ CD13	4 (13)	0	.556
↓ CD14	14 (47)	0	**.008**
↓ CD15	10 (33)	0	**.043**
↓ CD33	1 (3)	0	1.0
↓ CD36	1 (3)	0	1.0
↓ CD45	5 (17)	0	.306
CD56+	21 (70)	5 (50)	.278
↓ CD64	5 (17)	0	.306
↓ HLA-DR	9 (30)	2 (20)	.688
Granulocytes	(n = 28)		
CD56+	5 (18)	0	.298
HLA-DR+	3 (11)	0	.552

CMML, chronic myelomonocytic leukemia; +, positive; ↓, decrease.
[a]Bold values represent statistically significant relationships.

WHO Subtypes

Twenty-six (87%) cases were classified as CMML-1 and four (13%) cases as CMML-2. There was no significant difference between CMML-1 and CMML-2 for age, sex, and presence of cytogenetic abnormalities. The percentage of PB and BM blasts differed between the CMML-1 cases (median, 0% and 2.4%, respectively), CMML-2 cases (1.8% and 11.5%), and controls (0% and 0.96%; $P < .001$ each). There was no correlation between the morphologic and FC blast counts in these groups.

FC findings for these subgroups are presented in **Table 3**. The most common blast aberrancies in CMML-1 included decreased CD33 (14/26; 54%) and increased CD117 (7/26; 27%) and CD13 expression (5/26; 19%), whereas the most common blast aberrancies present in CMML-2 were increased CD13 and CD117, as well as decreased CD33, CD38, and CD45, each observed in two (50%) of four cases. Neoplasia-specific blast aberrancies were more common in CMML-2

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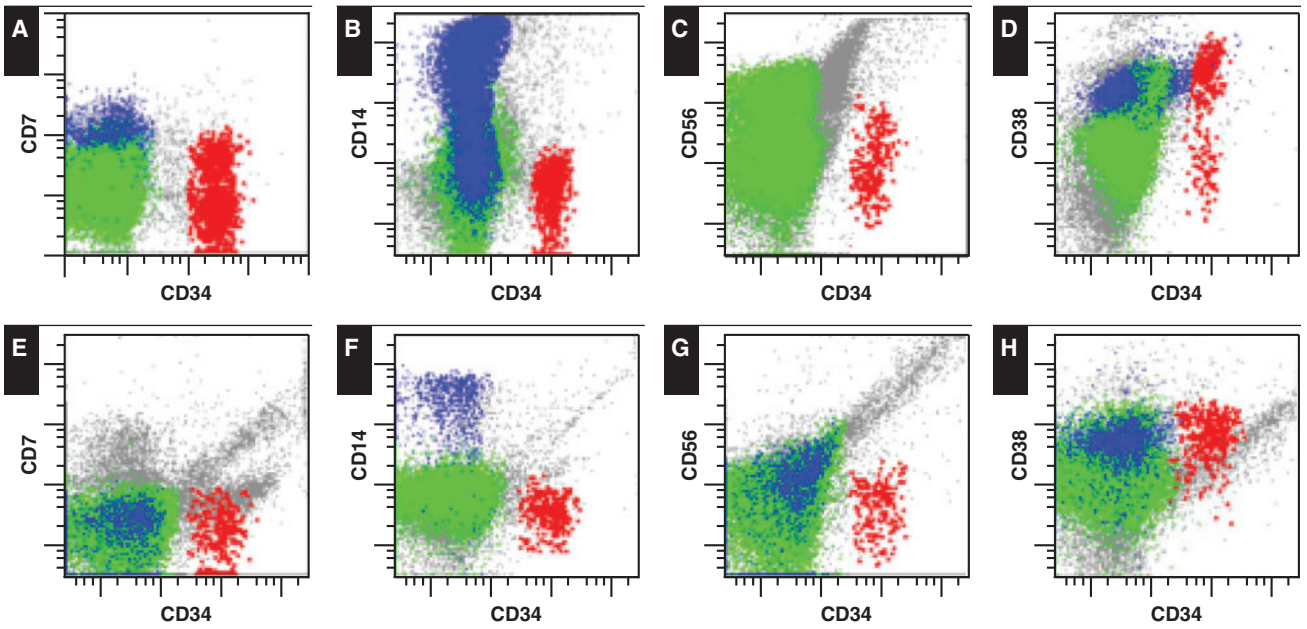

Image 1 Neoplasia-specific immunophenotypic aberrancies on chronic myelomonocytic leukemias (CMMLs) (**A-D**) compared with nonneoplastic bone marrows (**E-H**). Aberrant expression of CD7 on blasts (**A**), diminished CD14 on monocytes (**B**), aberrant CD56 on blasts and granulocytes (**C**), and aberrant CD38 expression on blasts (**D**) in CMMLs but not nonneoplastic bone marrows. Red, blasts; green, granulocytes; blue, monocytes.

than in CMML-1, including decreased CD38 and CD45 expression (50% vs 23% for CD38 and 50% vs 12% for CD45) and CD7 expression (25% vs 8%), with the exception of CD34 bright+ and CD56+ blasts, which were observed only in CMML-1 cases (15% and 12%, respectively). The most common aberrancies in monocytes in CMML-1 included CD56 positivity (20/26; 77%), decreased CD14 (12/26; 46%), and decreased CD15 and HLA-DR expression (8/26; 31% each). CD14 underexpression was observed equally on monocytes in CMML-1 and CMML-2. CD56+ granulocytes were observed only in CMML-1, and HLA-DR+ granulocytes were more commonly present in CMML-2 cases (2/4 [50%] vs 1/25 [4%] of CMML-1 cases; $P = .039$) **Image 2**. Granulocyte HLA-DR expression was the only statistically significant difference in antigen expression between CMML-1 and CMML-2; there were no significant differences in blasts or monocytes.

Proliferative and Dysplastic CMMLs

The CMMLs were subclassified into 10 proliferative and 20 dysplastic cases. Statistically significant differences were observed between proliferative CMMLs, dysplastic CMMLs, and controls for WBC count ($P < .0001$) and absolute monocyte counts ($P < .0001$). Absolute neutrophil counts were higher in proliferative CMMLs vs dysplastic CMMLs and controls ($P = .0001$), and PB blast percentage was higher in proliferative vs dysplastic CMMLs ($P = .04$). There was no significant difference between the proliferative and dysplastic CMMLs for age, sex, and presence of cytogenetic abnormalities. For unclear reasons, blast counts by morphology and FC showed a correlation within the proliferative group ($P = .007$, $R = 0.799$) but not the dysplastic group ($P = .338$, $R = 0.226$).

FC findings for these subgroups are presented in **Table 4**. The most common blast aberrancies observed in proliferative CMMLs were increased CD13 and CD117 and decreased CD45 and CD33 (each present in 30%), while the most common aberrancies present in dysplastic CMMLs were decreased CD33 (13/20; 65%), increased CD117 (6/20; 30%) and increased CD13 and CD34 (4/20; 20%). Blast aberrancies in CD11b, CD34, and CD56 were observed in dysplastic CMMLs but not in proliferative CMMLs **Image 3**. The blast neoplasia-specific aberrancy of CD56 expression was present in dysplastic but not proliferative cases, with CD7 and decreased CD38 expression seen comparably across these groups. The most common monocyte aberrancies detected in both proliferative and dysplastic CMMLs included CD56 positivity (8/10 [80%] and 13/20 [65%], respectively) (Images 3B and 3E) and decreased CD14 expression (5/10 [50%] and 9/20 [45%], respectively). Abnormal CD33 and CD36 expression was observed in monocytes of proliferative CMMLs (1/10 each) but not dysplastic CMMLs. Aberrant expression of HLA-DR on granulocytes was present in dysplastic CMMLs (3/21) but not proliferative CMMLs. Despite

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Table 3
Flow Cytometric Findings and Aberrancies for CMML-1 and CMML-2[a]

Characteristic	CMML-1 (n = 26)	CMML-2 (n = 4)	*P* Value
Blasts			
% by FC, mean (range)	0.73 (0.08-4.2)	4.2 (1.1-6.5)	**<.001**
No. of aberrancies, mean (range)	2 (0-5)	3 (1-4)	.257
CD7+	2 (8)	1 (25)	.360
CD11b+	2 (8)	0	1.0
Bright CD13	5 (19)	2 (50)	.284
↓ CD13	1 (4)	0	.225
Bright CD33	2 (8)	0	1.0
↓ CD33	14 (54)	2 (50)	1.0
Bright CD34	4 (15)	0	1.0
↓ CD38	6 (23)	2 (50)	.284
↓ CD45	3 (12)	2 (50)	.119
CD56+	3 (12)	0	1.0
Bright CD117	7 (27)	2 (50)	.563
HLA-DR	4 (15)	1 (25)	.538
Monocytes			
% by FC, mean (range)	19 (7.7-51)	17 (8.9-36)	.738
No. of aberrancies, mean (range)	2.5 (0-6)	1.5 (0-3)	.206
↓ CD13	4 (15)	0	1.0
↓ CD14	12 (46)	2 (50)	1.0
↓ CD15	8 (31)	2 (50)	.584
↓ CD33	1 (4)	0	1.0
↓ CD36	1 (4)	0	1.0
↓ CD45	5 (19)	0	1.0
CD56+	20 (77)	1 (25)	.069
↓ CD64	5 (19)	0	1.0
↓ HLA-DR	8 (31)	1 (25)	1.0
Granulocytes	(n = 25)	(n = 4)	
CD56+	5 (20)	0	1.0
HLA-DR+	1 (4)	2 (50)	**.039**

CMML, chronic myelomonocytic leukemia; CMML-1, chronic myelomonocytic leukemia type 1; CMML-2, chronic myelomonocytic leukemia type 2; FC, flow cytometry; +, positive; ↓, decrease.
[a]Values are presented as number (%) unless otherwise indicated. Bold values represent statistically significant relationships.

these observations, statistical analysis showed no significant difference in individual antigen expression between these two CMML subgroups on blasts, monocytes, or granulocytes.

Therapy-Related and De Novo CMMLs

There were seven therapy-related CMMLs (t-CMMLs) and 23 de novo CMMLs (dn-CMMLs). Mean BM blasts by morphology were 3.3% in t-CMMLs and 4.4% in dn-CMMLs compared with 0.96% in controls ($P = .041$) but were not statistically significant between the CMMLs ($P = .542$). There were no statistically significant relationships identified across CBCs, PB blast percentage, or blast, monocyte, and granulocyte percentage by FC. Blast counts by morphology and FC did correlate within the dn-CMMLs ($P = .03$, $R = 0.453$) but not the t-CMMLs ($P = .963$, $R = 0.019$).

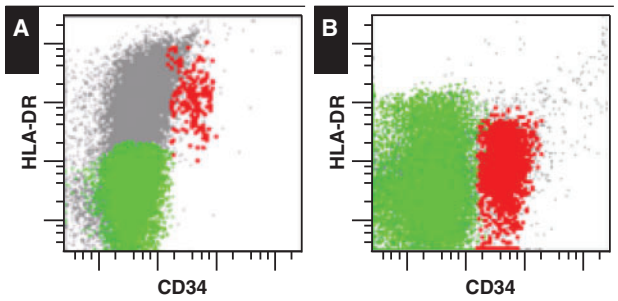

Image 2 HLA-DR expression in granulocytes. Chronic myelomonocytic leukemia type 2 (CMML-2) cases were more likely to show HLA-DR+ granulocytes. **A**, Chronic myelomonocytic leukemia type 1 with granulocytes showing lack of HLA-DR expression (gray events consisting of monocytes and lymphocytes). **B**, CMML-2 with HLA-DR+ granulocytes and blasts showing HLA-DR downregulation. Red, blasts; green, granulocytes.

The FC findings are shown in **Table 5**. The most common blast aberrancy in both dn-CMMLs and t-CMMLs was decreased CD33 expression (13/23 [57%] and 3/7 [43%], respectively). Overexpression of CD117 by blasts was observed in dn-CMMLs (8/23; 35%) but not in t-CMMLs. CD7 and CD11b positivity; underexpression of CD13, CD38, and CD45; and overexpression of CD33 and CD34 were also seen only in dn-CMMLs. The neoplastic-specific blast aberrancies were mostly present in dn-CMMLs (CD7 positivity and decreased CD38 and CD45), but CD56 positivity was present rarely in blasts of both types. The most common monocyte aberrancies in dn-CMMLs and t-CMMLs were CD56 expression (16/23 [70%] and 5/7 [71%], respectively) and decreased CD14 expression (11/23 [48%] and 3/7 [43%], respectively). CD56 expression on granulocytes was observed in both subtypes, while HLA-DR expression was observed only in dn-CMMLs. Despite these observations, statistical analysis showed no significant difference in individual antigen expression between these two CMML subgroups on blasts, monocytes, or granulocytes.

CMML-Specific Cytogenetics Risk Groups

The CMMLs were separated into 23 low-risk subtypes (all normal karyotypes), six intermediate-risk subtypes (two each of trisomy 13 and 12p deletion and one each of an additional 21q and inversion 3q), and one high-risk subtype (trisomy 8). For statistical purposes, the intermediate- and high-risk subtypes were combined (int/high). Blasts in the PB and BM averaged 0.28% and 3.5% in low-risk CMMLs and 1.1% and 6.1% in int/high-risk CMMLs, respectively, compared with 0% and 0.9% in controls ($P = .004$ and

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Table 4
Flow Cytometric Findings for Proliferative and Dysplastic CMMLs[a]

Aberrancies	Proliferative (n = 10)	Dysplastic (n = 20)	*P* Value
Blasts			
% by FC, mean (range)	0.97 (0.08-6.5)	0.98 (0.09-6.3)	.973
No. of aberrancies, mean (range)	1.8 (0-4)	2.4 (0-5)	.335
CD7+	1 (10)	2 (10)	1.0
CD11b+	0	2 (10)	.540
Bright CD13	3 (30)	4 (20)	.657
↓ CD13	0	1 (5)	1.0
Bright CD33	2 (20)	0	.103
↓ CD33	3 (30)	13 (65)	.122
Bright CD34	0	4 (20)	.272
Bright CD38	0	3 (15)	.532
↓ CD38	2 (20)	3 (15)	1.0
↓ CD45	3 (30)	2 (10)	.300
CD56+	0	3 (15)	.532
Bright CD117	3 (30)	6 (30)	1.0
Bright HLA-DR	0	2 (10)	.540
↓ HLA-DR	1 (10)	2 (10)	1.0
Monocytes			
% by FC, mean (range)	12 (2.1-51)	18 (7.7-37)	.121
No. of aberrancies, mean (range)	3.2 (1-6)	2.2 (0-5)	.070
↓ CD13	2 (20)	2 (10)	.584
↓ CD14	5 (50)	9 (45)	1.0
↓ CD15	3 (30)	7 (35)	1.0
↓ CD33	1 (10)	0	.333
↓ CD36	1 (10)	0	.333
↓ CD45	3 (30)	2 (20)	.300
CD56+	8 (80)	13 (65)	.675
↓ CD64	2 (20)	3 (15)	1.0
↓ HLA-DR	4 (40)	5 (25)	.431
Granulocytes	(n = 9)	(n = 19)	
CD56+	2 (22)	3 (16)	1.0
HLA-DR+	0	3 (16)	.530

CMML, chronic myelomonocytic leukemia; FC, flow cytometry; +, positive; ↓, decrease.
[a]Values are presented as number (%) unless otherwise indicated.

$P = .006$, respectively). No other statistically significant relationships were identified for sex, age, or other CBC findings. The monocyte percentage by FC was the only finding statistically significant across the two subtypes ($P = .016$). There was no correlation between the morphologic and FC blast counts in these groups.

The FC findings are presented in **Table 6**. The most common blast aberrancy observed in both subtypes was underexpression of CD33 (11/23 [48%] low-risk CMMLs and 4/7 [57%] int/high-risk CMMLs). The single high-risk case had no blast aberrancies. The neoplasia-specific blast aberrancies, CD7 and CD56 expression and decreased CD38 and CD45 expression, were observed in both subtypes with similar frequency. The most common monocyte aberrancies present in both subtypes included CD56 expression (16/23 [70%] and 5/7 [71%]) and underexpression of CD14 (10/23 [43%] and 4/7 [57%]). Abnormalities of CD13, CD33, and CD45 were present in the monocytes of low-risk CMMLs but not the int/high-risk CMMLs. Granulocytes showed expression of CD56 and HLA-DR in four and three low risk-CMMLs, respectively, but only one int/high-risk CMML had CD56+ granulocytes. No significant difference in individual antigen expression was demonstrated between these two CMML subgroups on blasts, monocytes, or granulocytes.

Discussion

CMML is a heterogeneous hematologic malignancy with both myeloproliferative and myelodysplastic features. Updated prognostic scoring systems have emerged recently to provide risk stratification for this clinically diverse disease. One of the more commonly used scoring systems, the CMML-specific prognostic scoring system, incorporates multiple subtypes of CMML into its algorithm, including WHO types, FAB types, and cytogenetic subcategories. Since few data exist on FC findings in CMML in general, we sought to study a well-characterized cohort of CMMLs immunophenotypically with an emphasis on subtype analysis. We hypothesized that differences in antigen expression patterns on blasts, monocytes, and/or granulocytes may be characteristic of certain subtypes and therefore lend biologic support for division into such categories and further support incorporation of these subtypes into scoring systems.

In our study, we compared our CMML cohort with 10 nonneoplastic control marrows obtained for various blood count abnormalities. Not surprisingly, our CMML cohort had predictably different WBC counts, relative and absolute monocyte counts, BM blast counts by morphology and FC, and mean marrow monocyte percentage by FC. The groups appear age matched and showed similar hemoglobin levels and platelet counts but were not sex matched.

We found immunophenotypic aberrancies on blasts in the majority of CMMLs (87%). This finding appears consistent with previously published reports in which robust panels were employed, including one of our own and a large cohort reported by Shen et al.[29] These aberrancies were not neoplasia specific, however, as many were present in our control cohort. In fact, 60% of our controls had blast aberrancies compared with our previously published normal blast immunophenotypes.[27] The high percentage of blast aberrancies in our control cohort is discrepant from that reported by Shen et al,[29] wherein 25% of their control cases demonstrated blast aberrancies. This discrepancy is likely related to our relatively common identification of CD13 and CD33 underexpression in nonneoplastic blasts (present in 5/10 controls), which was not studied/reported by Shen et al. Given the high percentage of blast abnormalities in the CMML and control

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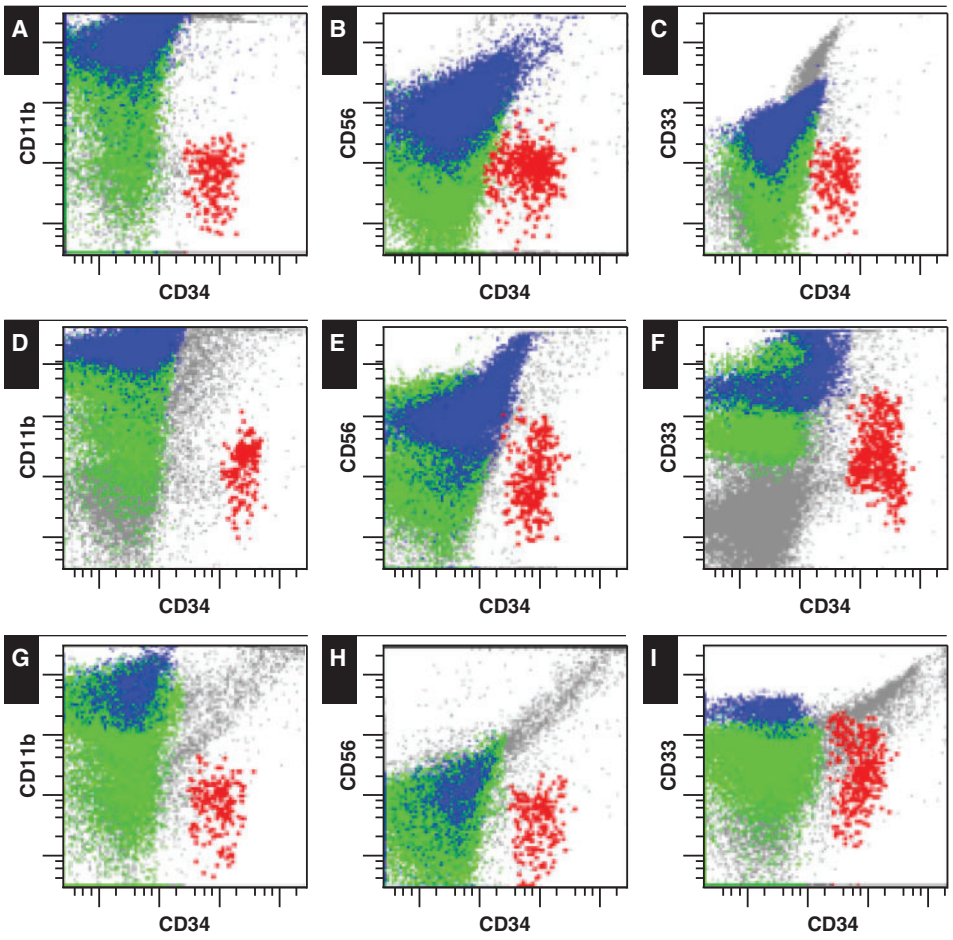

Image 3 Proliferative (**A-C**) and dysplastic (**D-F**) chronic myelomonocytic leukemias (CMMLs) vs controls (**G-I**). CD56+ monocytes were common in both subgroups (**B**, **E**). Unique aberrancies in the dysplastic subgroup included CD11b+ blasts (**D**), CD56+ blasts (**E**), and bright CD34 expression on blasts (**F**).

cohorts, it is not surprising that the presence of blast aberrancies between the study population and controls did not meet statistical significance. Nonetheless, there was a clear significant difference between the mean numbers of blast aberrancies in the neoplastic vs nonneoplastic cases, with the CMMLs showing an average of three times the aberrancies of the control group. Increased blast aberrancies by FC, therefore, supports a CMML diagnosis in cases with appropriate morphologic findings.

The literature is surprisingly devoid of published immunophenotypic characteristics of blasts in CMML. The most commonly identified blast aberrancies in our CMML cohort included underexpression of CD33 and overexpression of CD13 and CD117, some of which were observed in both CMMLs and controls, as previously mentioned. Similarly, Shen et al[29] identified increased CD13 and/or CD33 and CD117 in CMML blasts. In addition, these authors found frequent overexpression of CD123 in blasts, which was not an antigen studied in our series. In contrast to findings from Subira et al,[28] in which CD7 expression on blasts was observed in 61% of CMMLs, our cohort demonstrated CD7+ blasts in only 10% of cases. Neoplasia-specific blast aberrancies identified in our study included expression of CD7, CD11b, and CD56; underexpression of HLA-DR, CD38, and CD45; and overexpression of CD13, CD33, CD34, and CD38. Many of these findings have been described across myeloid disorders by various authors and are not unique to CMMLs.[27,30-32] While FC is not required for a CMML diagnosis, identification of such blast aberrancies lends support to a CMML diagnosis, in the presence of persistent monocytosis and cases with either no overt BM dysplasia or suboptimal marrow preparations.

In our study, monocyte aberrancies were present in the majority of CMMLs (94%), which is consistent with findings by several other investigators who have comprehensively studied monocyte antigen expression in this disease.[24,25,29] Aberrancies were also present in the monocytes of 60% of our nonneoplastic cases, which is similar to the 55% observed in the reactive monocytosis controls used in the study by Xu et al[24] (using similar aberrancy

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Table 5
Flow Cytometric Findings for De Novo and Therapy-Related CMMLs[a]

Aberrancies	De Novo (n = 23)	Therapy Related (n = 7)	*P* Value
Blasts			
% by FC, mean (range)	1.5 (0.08-6.5)	0.31 (0.09-0.53)	.116
No. of aberrancies, mean (range)	2.3 (0-5)	1.4 (0-3)	.167
CD7+	3 (13)	0	1.0
CD11b+	2 (9)	0	1.0
Bright CD13	4 (17)	3 (43)	.345
↓ CD13	1 (4)	0	1.0
Bright CD33	2 (9)	0	.392
↓ CD33	13 (57)	3 (43)	.675
Bright CD34	4 (17)	0	.548
Bright CD38	2 (9)	1 (14)	.638
↓ CD38	5 (22)	0	.304
↓ CD45	5 (22)	0	.304
CD56+	2 (9)	1 (14)	1.0
Bright CD117	8 (35)	0	.393
↓ CD117	0	1 (14)	.233
Bright HLA-DR	2 (9)	0	1.0
↓ HLA-DR	2 (9)	1 (14)	1.0
Monocytes			
% by FC, mean (range)	17 (7.7-36)	24 (10-51)	.173
No. of aberrancies, mean (range)	2.3 (0-5)	2.6 (1-6)	.672
↓ CD13	3 (13)	1 (14)	1.0
↓ CD14	11 (48)	3 (43)	1.0
↓ CD15	8 (35)	2 (29)	1.0
↓ CD33	1 (4)	0	1.0
↓ CD36	0	1 (14)	.233
CD56+	16 (70)	5 (71)	1.0
↓ CD64	3 (13)	2 (29)	.565
Bright HLA-DR	1 (4)	0	1.0
↓ HLA-DR	6 (26)	3 (43)	.640
Granulocytes	(n = 21)	(n = 7)	
CD56+	3 (14)	2 (29)	.565
HLA-DR+	3 (14)	0	1.0

CMML, chronic myelomonocytic leukemia; FC, flow cytometry; +, positive; ↓, decrease.
[a]Values are presented as number (%) unless otherwise indicated.

Table 6
Flow Cytometric Findings for Cytogenetics Risk Subgroups of CMMLs[a]

Aberrancies	Low Risk (n = 23)	Intermediate/ High Risk (n = 7)	*P* Value
Blasts			
% by FC, mean (range)	0.88 (0.08-6.5)	2.2 (0.28-6.5)	.065
No. of aberrancies, mean (range)	1.9 (0-5)	2.4 (0-6)	.086
CD7+	2 (9)	1 (14)	1.0
CD11b+	2 (9)	0	1.0
Bright CD13	4 (17)	3 (43)	.306
↓ CD13	1 (4)	0	1.0
Bright CD33	2 (9)	1 (14)	1.0
↓ CD33	11 (48)	4 (57)	1.0
Bright CD34	3 (13)	1 (14)	1.0
Bright CD38	3 (13)	0	1.0
↓ CD38	2 (9)	3 (43)	.068
↓ CD45	3 (13)	2 (29)	.565
CD56+	2 (9)	1 (9)	1.0
Bright CD117	6 (26)	3 (43)	.640
Bright HLA-DR	1 (4)	1 (14)	.418
↓ HLA-DR	2 (9)	1 (14)	1.0
Monocytes			
% by FC, mean (range)	22 (7.9-51)	11 (7.7-77)	**.016**
No. of aberrancies, mean (range)	3 (0-5)	2.1 (1-3)	.644
↓ CD13	4 (17)	0	.548
↓ CD14	10 (43)	4 (57)	.675
↓ CD15	6 (26)	4 (57)	.181
↓ CD33	1 (4)	0	1.0
↓ CD36	1 (4)	0	1.0
↓ CD45	5 (22)	0	.304
CD56+	16 (70)	5 (71)	1.0
↓ CD64	4 (17)	1 (14)	1.0
↓ HLA-DR	9 (39)	1 (14)	.372
Granulocytes	(n = 21)	(n = 7)	
CD56+	4 (14)	1 (14)	1.0
HLA-DR+	3 (14)	0	1.0

CMML, chronic myelomonocytic leukemia; FC, flow cytometry; +, positive; ↓, decrease.
[a]Values are presented as number (%) unless otherwise indicated. Bold value represents statistically significant relationship.

definitions and analysis techniques), but contrasts with the 37% and 40% monocyte aberrancies observed in the nonneoplastic cytopenia control cohorts used by Shen et al[29] and Sojitra et al,[25] respectively. While our control cohort closely matches the cytopenia inclusion criteria and hematologic values for the Shen et al[29] study, the discrepant monocyte aberrancies in controls across studies is likely related to the antigens evaluated and the respective definitions of aberrancies in the studies. Notably, we identified CD56 expression on monocytes, which is well recognized in PB and BM of reactive states[22-24,33,34] in 50% of controls by using a more than 20% antigen positivity definition compared with an isotype control. Shen et al[29] noted similar CD56 expression on monocytes, but the expression was of lower intensity in controls and therefore the authors adjusted their positivity threshold to increase specificity for neoplasia. Similarly, Sojitra et al[25] identified no CD56 expression in the monocytes of controls by using a positivity definition of ½ log shift from normal monocytes.

On average, we found that the monocytes of CMML had a higher number of aberrancies (three times) than controls, confirming the findings of Xu et al[24] and Shen et al.[29] The most common monocyte aberrancies included CD56 expression in 70% of CMMLs, underexpression of CD14 in approximately 50% of our CMMLs, and underexpression of CD15 and HLA-DR in one-third of CMMLs, with underexpression of CD14 and CD15 reaching statistical significance in differentiating CMMLs and controls. Xu et al[24] reported CD56 expression and underexpression of HLA-DR as the most common aberrancy in monocytes in similar percentages to our study, as well as expanded CD14 moderate populations, which would have been considered underexpression of CD14

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in our study; however, these authors also identified these abnormalities in their reactive cohort. Notably, our finding of CD15 underexpression in some CMML monocyte populations has not been previously described. It is important to state that while we found several neoplasia-specific monocyte aberrancies, our controls were not reactive marrow monocytoses, so our findings lack true specificity in that regard.

Granulocyte aberrancies were observed in 10% to 20% of our CMMLs and none of our controls. Similar to our study, Shen et al[29] described approximately 10% CD56 positivity in the granulocytes of CMMLs and no aberrant expression in the controls. Interestingly, HLA-DR expression on granulocytes, seen in 11% of our CMMLs, has not been studied or described by other authors and appears to represent a neoplasia-specific finding. We chose to limit our analysis of granulocytes to CD56 and HLA-DR expression using 20% positivity thresholds. While other authors have used low side scatter (indicating hypogranularity) and abnormal CD11b/CD13/CD16 maturation curves as supportive evidence of neoplasia across myeloid disorders,[31,35] we find these interpretations subjective and less reproducible and therefore did not include them in our analyses.

Following our total CMML cohort analysis, we divided up the cohort into subgroups. Very few studies have done such a subgroup analysis immunophenotypically, with only two studies citing data comparing only the proliferative and dysplastic subgroups.[28,29] As expected given the blast count definitions for WHO types, the mean blast percentages by morphology and FC were different between CMML-1 and CMML-2. For unclear reasons, the mean monocyte percentage by FC was different between the low-risk and intermediate/high-risk CMMLs, with higher monocytes in the low-risk group. There were no statistically significant relationships between mean blast, monocyte, or granulocyte percentages in any other subtype analysis.

No statistically significant relationship was identified between the mean numbers of blast, monocyte, or granulocyte aberrancies in any of the subgroup comparisons. Interestingly, the theoretically more aggressive clinical subgroups (CMML-2, proliferative, therapy related, and int/high-risk cytogenetics) did not show more immunophenotypic aberrancies in the myeloid populations, compared to their less aggressive counterparts. All of the CMML-2 cases had at least one blast aberrancy, while the proliferative, therapy-related, and int/high-risk subgroups had at least one monocyte aberrancy; the other comparative subgroups had cases with no blast or monocyte aberrancies, respectively.

Across all the subgroup immunophenotypic comparisons, only one statistically significant relationship was identified: HLA-DR expression on granulocytes was more commonly observed in CMML-1 compared with CMML-2. We observed this finding in 50% of our CMML-2 cases and only one of 26 CMML-1 cases, perhaps suggesting a biologic difference between the most mature myeloid elements in these subgroups. To our knowledge, our study is the first to report HLA-DR expression in CMMLs; no other CMML flow cytometry studies have examined this expression pattern. The significance of this finding and its prevalence in CMML-2 requires further investigation, as our cohort contained few CMML-2 cases (n = 4). Notably, HLA-DR expression has been described on granulocytes in the peripheral blood of patients treated with granulocyte-macrophage colony stimulating factor and myelodysplastic syndromes.[30,36,37]

No specific antigen expression patterns were observed across the proliferative and dysplastic groups or the therapy-related vs de novo groups. This confirms the findings of Shen et al[29] and Subira et al,[28] with respect to the proliferative and dysplastic cohorts. To our knowledge, this is the first report describing or comparing the immunophenotypic profiles of therapy-related CMMLs.[38]

In conclusion, our study adds to the limited CMML flow cytometry data in the literature and is the only comprehensive CMML immunophenotypic subgroup analysis. As expected, we found more aberrancies in blasts and monocytes in our CMMLs compared with our controls and have described several neoplasia-specific aberrancies in blasts. There were no statistically significant differences in individual antigen expression patterns across our subgroups for blasts or monocytes, with HLA-DR expression on granulocytes as the only significant aberrancy identified between CMML-1 and CMML-2. Interestingly, there was no qualitative difference in aberrancies within the myeloid populations between the more clinically aggressive subgroup and its corresponding less aggressive partner. These data suggest that within the CMML diagnosis, there exists significant heterogeneity in the immunophenotypic profile of the myeloid lineage with no specific qualitative or quantitative findings characteristic of a subgroup.

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