Expression of CD2 in Acute Promyelocytic Leukemia Correlates With Short Form of PML-RARα Transcripts and Poorer Prognosis

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Key Words: Acute promyelocytic leukemia; Immunophenotype; PML-RARα; RT-PCR; Reverse transcriptase–polymerase chain reaction

DOI: 10.1309/XC8P9M8NKQDT38LB

Abstract

*We studied the immunophenotype of 100 cases of acute promyelocytic leukemia (APL) with cytogenetic evidence of t(15;17)(q22;q21), 72 hypergranular (M3) and 28 microgranular (M3v), and correlated the results with molecular and clinical features. Most neoplasms (75/100 [75%]) had a typical immunophenotype: CD13+CD33+CD34–HLA-DR–. CD64, CD2, CD34, and HLA-DR were expressed in 27% (24/88), 23% (22/94), 21% (21/100), and 9% (9/98), respectively. CD34 expression was restricted to M3v; HLA-DR and CD2 were expressed more often in M3v than in M3 (*P < *.001). PML-RARα fusion transcripts were detected by reverse transcriptase–polymerase chain reaction in all 70 patients assessed. The short form of PML-RARα transcripts was found more frequently in M3v (*P < *.002) and CD2+ APL (*P < *.0001) than in M3 and CD2– APL, respectively. The median follow-up was 128 weeks. CD2+ APL was associated significantly with leukocytosis (*P = *.004), shorter complete remission duration (*P = *.03), and a trend toward shorter overall survival (*P = *.07) than CD2– APL. Overall survival for M3v vs M3 (*P = *.68) and short vs long transcripts (*P = *.21) was not significantly different. Immunophenotyping is useful for predicting the biologic and clinical behavior of APL.*

Acute promyelocytic leukemia (APL) is characterized by a proliferation of neoplastic promyelocytes carrying the t(15;17)(q22;q21). Hypergranular (M3) and microgranular (M3v) APL, as defined by the French-American-British (FAB) classification of acute myeloid leukemia (AML),[1] are the 2 most commonly recognized morphologic variants of APL. M3v accounts for approximately 20% of all cases of APL.[2] The clinical course and prognosis of patients with M3v are reported to be worse than for patients with M3.[3]

Flow cytometric (FC) immunophenotyping is considered a powerful ancillary tool for diagnosing APL. Leukemic promyelocytes characteristically express mature myeloid markers, including CD13 and CD33, and are negative for CD34 and HLA-DR.[4] However, this immunophenotype is not specific for APL. Furthermore, the typical immunophenotype commonly is associated with M3, whereas M3v is more heterogeneous, with variable expression of CD2, CD34, CD56, HLA-DR, and terminal deoxynucleotidyl transferase.[3,5-10]

The t(15;17) results in fusion of the promyelocytic leukemia (*PML*) gene on chromosome 15 and the retinoic acid receptor-α (*RARα*) gene on chromosome 17. Three breakpoint cluster regions (bcr) within the *PML* gene occur: bcr1 (intron 6), bcr2 (exon 6), and bcr3 (intron 3), generating 3 different transcripts, so-called long, variable, and short forms, respectively. In approximately 5% of APL cases, the chimeric gene is composed of RARα and other partners including nucleophosmin (NPM), nuclear mitotic apparatus (NuMA), promyelocytic leukemia zinc finger (PLZF), and signal transducer and activator of transcription 5b (Stat5b) on chromosomes 5q35, 11q13, 11q23, and 17q11, respectively.[11,12]

Correlation between the morphologic features and immunophenotype of leukemic promyelocytes and their

molecular and clinical features has been the subject of several previous studies.[6,13] Data regarding the association between CD2 expression and the short form (bcr3) of PML-RARα transcripts are controversial.[10,14-16] Patients with APL and bcr3, which are found more commonly in M3v,[17] were shown to have shorter remission duration and survival than patients with bcr1.[18] CD2 expression was reported to be associated with a favorable complete remission (CR) rate and event-free survival in a large series of both pediatric and adult patients with APL.[10] Other investigators found that CD34 expression correlated with CD2 positivity, M3v, and bcr3 in 2 smaller study cohorts but disagreed on its correlation with overall survival (OS).[19,20]

We retrospectively reviewed the morphologic features, immunophenotype, and molecular features of 100 cases of APL with cytogenetic evidence of t(15;17)(q22;q21) and correlated these findings with clinical outcome.

Materials and Methods

Case Selection

We searched the cytogenetic database of M.D. Anderson Cancer Center, Houston, TX, and identified cases with the t(15;17)(q22;q21) found in bone marrow (BM) aspirate material between August 1992 and April 2003. Methods for the cytogenetic analysis have been described before.[21] We then retrieved all available corresponding BM material, immunophenotypic data, and reverse transcriptase–polymerase chain reaction (RT-PCR) data for the PML-RARα fusion transcript type.

Morphologic Evaluation

For each case, BM aspirate smears stained with Wright-Giemsa and studied cytochemically for myeloperoxidase were reviewed independently by 2 hematopathologists (P.L. and S.H.) to ensure objective classification. Each case was classified according to the guidelines of the FAB classification.

Flow Cytometry Immunophenotypic Methods

We performed 3- or 4-color FC analysis on BM aspirate samples collected in EDTA. After incubation of cells with monoclonal antibodies for 10 minutes at 4°C, the RBCs were lysed with NH_4Cl for 10 minutes, followed by 2 washing steps using phosphate-buffered saline solution. The cells were resuspended and fixed with 1% paraformaldehyde. The panel of antibodies for analyzing AML has evolved over the years, but generally included the following mouse monoclonal antibodies specific for CD45 (peridinin chlorophyll protein [PerCP]), CD34 (fluorescein isothiocyanate [FITC]-conjugated), CD33 (FITC or phycoerythrin [PE]), CD13 (PE), CD14 (allophycocyanin [APC]), CD19 (FITC or APC), CD117 (FITC or PE), CD64 (PE), CD10 (FITC), CD7 (FITC), CD3 (APC), and CD2 (FITC). All antibodies were from BD Biosciences (San Jose, CA), and analysis was performed using FACScan or FACSCaliber cytometers (BD). For each antibody, negative staining levels were set by comparison with an isotype-matched control. For this study, a marker was considered positive if expressed by more than 20% of the analyzed events.

RT-PCR for PML-RARα

RNA extraction and RT-PCR were performed on BM aspirate samples as described.[22,23] The primers and biotin-labeled probe were purchased from Invitrogen (Houston, TX) and Synthegen (Houston, TX). The amplified product (complementary DNA) was detected by using ethidium bromide gel electrophoresis followed by hybridization to a specific chemiluminescent-labeled probe to confirm the identity of the amplicon. To ensure the reliability of the RT-PCR assay, an uninvolved segment of the *RARα* gene (163 base pairs [bp]) was coamplified as an internal control to rule out false-negative results due to reverse transcription, PCR failure, or poor RNA preparation. All samples were analyzed in duplicate, in conjunction with negative (HL60) and positive (NB4) cell line controls. The sensitivity of the test was determined to be 1 in 10^5 cells.

Follow-up

The CR and OS were analyzed by using Cox model multivariate analysis. The analysis excluded 2 pediatric patients treated with pediatric protocols and 14 patients with relapsed disease at the time of first evaluation at our institution.

Results

There were 47 males and 53 females with a median age of 47 years (range, 3-77 years). The leukemia samples assessed were obtained at the time of initial evaluation in 86 cases (86%) and at the time of relapse in the remaining 14 (14%). All adult patients and 2 pediatric patients received all-*trans*-retinoic acid with or without other chemotherapeutic agents.

Morphologic Findings

Seventy-two cases were classified as M3 and the remaining 28 as M3v. The leukemic cells in M3 typically had abundant, large, azurophilic granules that often obscured the nuclei. The leukemic cells in M3v typically had bilobed or reniform nuclei with inconspicuous cytoplasmic granules **Image 1**. Both variants showed intense reactivity with myeloperoxidase by cytochemical staining.

DOI: 10.1309/XC8P9M8NKQDT38LB

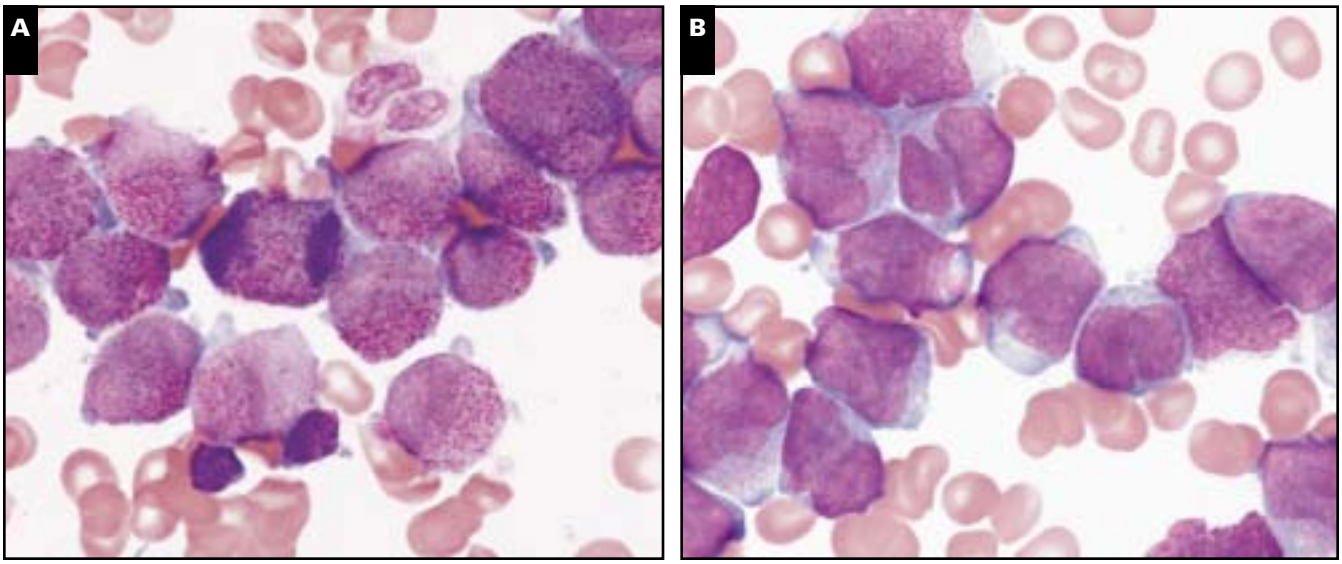

Image 1 **A**, Hypergranular (M3) acute promyelocytic leukemia (APL) (Wright-Giemsa, ×100). **B**, Microgranular (M3v) APL (Wright-Giemsa, ×100).

Immunophenotypic Findings

The expression patterns of selected markers studied by FC analysis and their correlation with morphologic variants are summarized in **Table 1**.

On side scatter vs CD45, M3 leukemic cells displayed primarily high side scatter. In contrast, M3v leukemic cells displayed less scatter and generally fell in an area closer to blasts **Image 2A**. CD13 and CD33 were expressed consistently by both variants, with the exception of 1 case of M3 that was CD33–. CD34 and HLA-DR were expressed in 21 (21%) of 100 and 9 (9%) of 98 cases, respectively. CD34 was expressed in 7 (78%) of 9 cases that expressed HLA-DR. Although not present in every M3v case, CD34 was positive exclusively in M3v cases **Image 2B**. CD2 was expressed in 22 (23%) of 94 cases overall and was coexpressed in 11 (52%) of 21 CD34+ APL cases. Unlike CD34, CD2 expression was not restricted to M3v, but was significantly more common in M3v than in M3 cases ($P < .001$; χ^2 test). CD64 was expressed in 24 (27%) of 88 cases and distributed equally between the M3v and M3 cases ($P = .1$; χ^2 test). APL with CD64 expression displayed a light scatter pattern indistinguishable from that of AML with monocytic differentiation; however, coexpression with CD14 was not detected.

RT-PCR Findings

The PML-RARα gene fusion transcript type was analyzed in 70 cases, and the results are summarized in **Table 2**. Translocation involving bcr3 of the *PML* gene (intron 3) yielded a 220-bp product, the short form, whereas involvement of bcr1 (intron 6) yielded a product that was 474 nucleotides longer, the long form. Alternative splicing of the long form yielded 3 different sizes of messenger RNA products, 291, 550, and 695 bp, respectively. Involvement of bcr2 generated a variable form of messenger RNA product.

The long and short forms were detected in 36 (51%) and 33 (47%) of 70 cases, respectively. Only 1 case had the variable form. Further analysis showed that the short form was significantly more common in M3v (15/19 [79%]) than in M3 (18/50 [36%]) ($P < .002$; χ^2 test) and more frequent in CD2+ cases (15/17 [88%]) than in CD2– cases (18/51 [35%]) ($P < .0001$; χ^2 test).

Table 1
Expression of Selected Markers and Comparison Between M3 and M3v*

	CD13	CD33	CD117	CD64	CD34	CD2	HLA-DR
Total	100/100 (100)	98/100 (98)	60/91 (66)	24/88 (27)	21/100 (21)	22/94 (23)	9/98 (9)
M3	72/72 (100)	70/72 (97)	41/65 (63)	15/66 (23)	0/72 (0)	8/69 (12)	1/72 (1)
M3v	28/28 (100)	28/28 (100)	19/26 (73)	9/22 (41)	21/28 (75)	14/25 (56)	8/26 (31)
$P^\dagger$	1.0	1.0	.45	.1	<.001	<.001	<.001

* Data are given as number positive/number tested (percentage).
† Generated by using the Fisher exact and χ^2 tests.

DOI: 10.1309/XC8P9M8NKQDT38LB

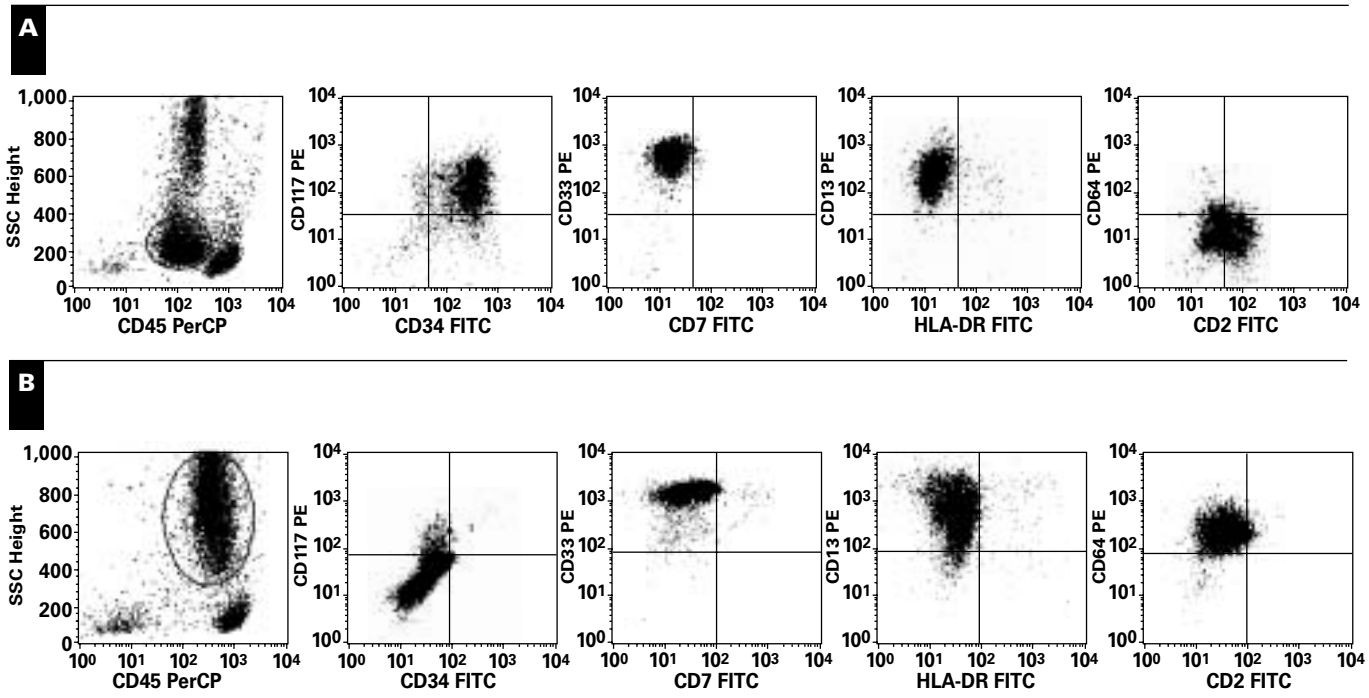

Image 2 **A**, The blasts of M3v fall in an area close to blasts of a more immature type of acute myeloid leukemia (AML) on side scatter vs CD45. CD34 expression is associated exclusively with M3v. In addition to expression of myeloid markers CD33, CD13, and CD117, CD2 also is expressed dimly in this case. **B**, M3 leukemic cells display high side scatter compared with the low side scatter of M3v. The scatter pattern and expression profile (CD34–CD33+CD13+CD64+) simulates AML with monocytic differentiation. Absence of HLA-DR expression in this case was helpful for making the distinction. FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; SSC, side scatter.

Table 2
PML-RARα Gene Fusion Transcripts Analyzed by Reverse Transcriptase–Polymerase Chain Reaction*

	M3	M3v	CD2–	CD2+
Short (bcr3)	18	15	18	15
Long (bcr1)	32	4	33	2
Variant	1	0	1	0
Total	51	19	52	17

* For M3 vs M3v, $P = .002$; for CD2– vs CD2+, $P = .0001$. The P values were generated by using the Fisher exact and χ^2 tests and evaluated the distribution of bcr3 and bcr1 between M3 and M3v and between CD2– and CD2+ acute promyelocytic leukemia.

Follow-up

The median follow-up period was 128 weeks (range, 6-504 weeks). There was no significant difference in OS in M3v vs M3 cases ($P = .68$; χ^2 test) or in cases with short vs long fusion transcripts ($P = .21$; χ^2 test). Cox multivariate analysis showed that patients with CD2+ APL had a significantly shorter median duration of CR ($P = .03$) and a trend toward shorter OS than patients with CD2– APL ($P = .07$) **Figure 1**. The median duration of CR in the CD2– group was 92.5 weeks (range, 0-322 weeks) compared with 46 weeks (range, 0-250 weeks) in the CD2+ group. CD2 expression also was associated significantly with leukocytosis ($P = .004$; Wilcoxon rank sum test) **Figure 2**. The median total WBC count for patients with CD2+ APL was 23,650/μL (23.7×10^9/L; range, 900-142,200/μL [0.9-142.2 $\times 10^9$/L]) compared with 3,150/μL (3.2×10^9/L; range, 400-165,000/μL [0.4-165.0 $\times 10^9$/L]) for patients with CD2– APL. Multivariate analysis also demonstrated that an elevated WBC count was a significant negative prognostic indicator for OS, with a relative risk of 1.81 ($P < .0001$). The expression of CD34 and HLA-DR did not correlate with CR or OS.

Discussion

Accurate and rapid diagnosis of APL is critical for the initiation of effective and specific therapy. This is made possible by recognizing the characteristic morphologic features and typical immunophenotype of APL, with confirmation by molecular and cytogenetic testing for the presence of t(15;17). The diagnosis becomes more difficult when APL with documented t(15;17) falls into the morphologic spectrum of other subtypes of AML in the FAB classification[7,8,20,24-27] or when a case of AML with an immunophenotype characteristic of APL has no detectable molecular or cytogenetic evidence of the t(15;17).[28]

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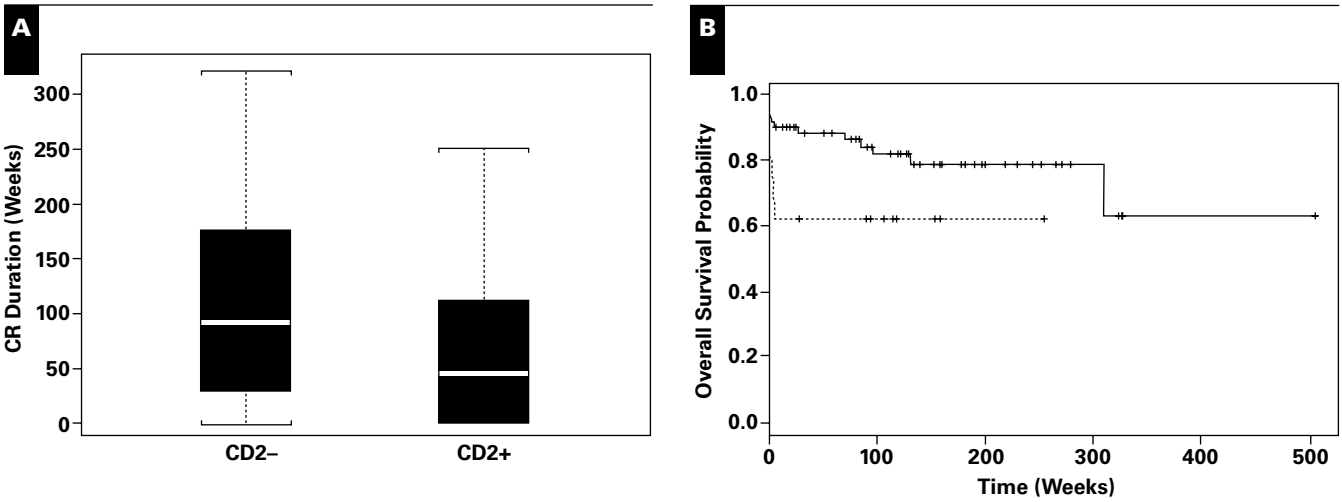

Figure 1 **A**, Box plot comparing duration of complete remission (CR) between cases of CD2+ and CD2– acute promyelocytic leukemia (APL). $P = .03$. **B**, Overall survival in cases of CD2+ (dotted line) vs CD2– (solid line) APL. $P = .07$.

We examined the morphologic features and immunophenotype of BM aspirate samples from 100 cases of APL with cytogenetic evidence of the t(15;17) and correlated the results with molecular findings and clinical outcome. We found that 75% of APL cases had an immunophenotypic profile characterized by CD13+CD33+CD34–HLA-DR–. In the remaining 25%, CD2, CD34, and HLA-DR were expressed variably, most often in M3v. CD34 was expressed consistently in M3v, with rare exceptions, and, thus, reliably distinguished M3v from M3. APL with CD64 expression might simulate AML with monocytic differentiation morphologically and immunophenotypically. Recognition of these variations is essential for preventing misdiagnosis in clinical practice.

We also studied the correlation of immunophenotype with clinical outcome. Patients with CD2+ APL had a significantly shorter duration of CR and a trend toward shorter OS than patients with CD2– APL. These results are in contrast with the favorable outcome in a group of pediatric and adult patients with CD2+ APL described by Guglielmi and colleagues.[10] CD2 is detected more frequently in cases of APL, approximately 25%, than in other FAB subtypes of AML, approximately 5%.[29] The CD2 antigen is a 50-kd transmembrane glycoprotein that belongs to the immunoglobulin gene superfamily and is encoded by a gene mapped to 1p13.[30] CD2 functions as an adhesion molecule through binding to CD15, CD58, and CD59. It also mediates T-cell activation through signal transduction. Although the exact function of CD2 expression in leukemic promyelocytes is unknown, CD2 expression is correlated significantly with leukocytosis, a key negative prognostic indicator demonstrated in the present study and in others.[19,22]

Previous data regarding the association between CD2 and short (bcr3) PML-RARα transcripts are controversial.[10,14-16]

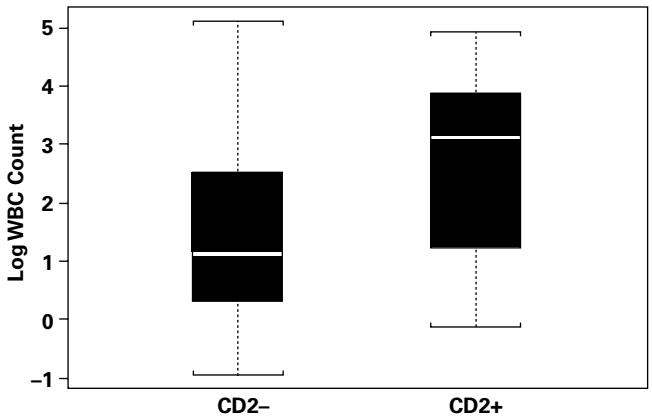

Figure 2 Box plot comparing log scale of WBC counts between cases of CD2+ and CD2– acute promyelocytic leukemia.

In keeping with the observation reported by Claxton and colleagues,[14] our results confirmed a strong positive correlation between these 2 variables ($P < .0001$; Fisher exact test). The trend toward shorter OS in patients with CD2+ APL did not seem to be attributable to the predominance of short (bcr3) fusion transcript in this group of patients. There was no difference in OS between patients with APL with short (bcr3) or long (bcr1) fusion transcripts ($P = .68$; Fisher exact test).

M3v is a heterogeneous group that frequently expresses CD2, CD34, and HLA-DR and commonly is associated with short (bcr3) PML-RARα fusion transcripts. CD2 expression is correlated with a worse prognosis, with shorter CR and OS. A comprehensive approach with emphasis on combined morphologic, immunophenotypic, cytogenetic, and molecular analysis is not only critical for accurate and rapid diagnosis of APL but is also useful for predicting biologic and clinical behavior.

DOI: 10.1309/XC8P9M8NKQDT38LB

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References

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:620-625.
2. Avvisati G, Lo Coco F, Mandelli F. Acute promyelocytic leukemia: clinical and morphologic features and prognostic factors. *Semin Hematol*. 2001;38:4-12.
3. Rovelli A, Biondi A, Cantu Rajnoldi A, et al. Microgranular variant of acute promyelocytic leukemia in children. *J Clin Oncol*. 1992;10:1413-1418.
4. Paietta E, Andersen J, Gallagher R, et al. The immunophenotype of acute promyelocytic leukemia (APL): an ECOG study. *Leukemia*. 1994;8:1108-1112.
5. Exner M, Thalhammer R, Kapiotis S, et al. The "typical" immunophenotype of acute promyelocytic leukemia (APL-M3): does it prove true for the M3-variant? *Cytometry*. 2000;42:106-109.
6. Sainty D, Liso V, Cantu-Rajnoldi A, et al, on behalf of Group Français d'Hématologie Cellulaire, Group Français de Cytogénétique Hématologique, UK Cancer Cytogenetics Group, and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies." A new morphologic classification system for acute promyelocytic leukemia distinguishes cases with underlying *PLZF/RARA* gene rearrangements. *Blood*. 2000;96:1287-1296.
7. Foley R, Soamboonsrup P, Kouroukis T, et al. PML/RAR alpha APL with undifferentiated morphology and stem cell immunophenotype. *Leukemia*. 1998;12:1492-1493.
8. Stasi R, Bruno A, Venditti A, et al. A microgranular variant of acute promyelocytic leukemia with atypical morpho-cytochemical features and an early myeloid immunophenotype. *Leuk Res*. 1997;21:575-580.
9. Piedras J, Lopez-Karpovitch X, Cardenas R. Light scatter and immunophenotypic characteristics of blast cells in typical acute promyelocytic leukemia and its variant. *Cytometry*. 1998;32:286-290.
10. Guglielmi C, Martelli MP, Diverio D, et al. Immunophenotype of adult and childhood acute promyelocytic leukaemia: correlation with morphology, type of *PML* gene breakpoint and clinical outcome: a cooperative Italian study on 196 cases. *Br J Haematol*. 1998;102:1035-1041.
11. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood*. 1999;93:3167-3215.
12. Faretta M, Di Croce L, Pelicci PG. Effects of the acute myeloid leukemia–associated fusion proteins on nuclear architecture. *Semin Hematol*. 2001;38:42-53.
13. Orfao A, Chillon MC, Bortoluci AM, et al. The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARalpha gene rearrangements. *Haematologica*. 1999;84:405-412.
14. Claxton DF, Reading CL, Nagarajan L, et al. Correlation of CD2 expression with *PML* gene breakpoints in patients with acute promyelocytic leukemia. *Blood*. 1992;80:582-586.
15. Biondi A, Luciano A, Bassan R, et al. CD2 expression in acute promyelocytic leukemia is associated with microgranular morphology (FAB M3v) but not with any *PML* gene breakpoint. *Leukemia*. 1995;9:1461-1466.
16. Maslak P, Miller WH Jr, Heller G, et al. CD2 expression and PML/RAR-alpha transcripts in acute promyelocytic leukemia [letter]. *Blood*. 1993;81:1666.
17. Biondi A, Rambaldi A, Pandolfi PP, et al. Molecular monitoring of the myl/retinoic acid receptor-alpha fusion gene in acute promyelocytic leukemia by polymerase chain reaction. *Blood*. 1992;80:492-497.
18. Sucic M, Zadro R, Burazer B, et al. Acute promyelocytic leukemia M3: cytomorphologic, immunophenotypic, cytogenetic, and molecular variants. *J Hematother Stem Cell Res*. 2002;11:941-950.
19. Foley R, Soamboonsrup P, Carter RF, et al. CD34-positive acute promyelocytic leukemia is associated with leukocytosis, microgranular/hypogranular morphology, expression of CD2 and bcr3 isoform. *Am J Hematol*. 2001;67:34-41.
20. Neame PB, Soamboonsrup P, Leber B, et al. Morphology of acute promyelocytic leukemia with cytogenetic or molecular evidence for the diagnosis: characterization of additional microgranular variants. *Am J Hematol*. 1997;56:131-142.
21. Mansoor A, Medeiros LJ, Weber DM, et al. Cytogenetic findings in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia: chromosomal abnormalities are associated with the polymorphous subtype and an aggressive clinical course. *Am J Clin Pathol*. 2001;116:543-549.
22. Burnett AK, Grimwade D, Solomon E, et al. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-*trans* retinoic acid: result of the Randomized MRC Trial. *Blood*. 1999;93:4131-4143.
23. Gallagher RE, Willman CL, Slack JL, et al. Association of PML-RAR alpha fusion mRNA type with pretreatment hematologic characteristics but not treatment outcome in acute promyelocytic leukemia: an intergroup molecular study. *Blood*. 1997;90:1656-1663.
24. Allford S, Grimwade D, Langabeer S, et al. Identification of the t(15;17) in AML FAB types other than M3: evaluation of the role of molecular screening for the PML/RARalpha rearrangement in newly diagnosed AML. The Medical Research Council (MRC) Adult Leukaemia Working Party. *Br J Haematol*. 1999;105:198-207.
25. Fenu S, Carmini D, Mancini F, et al. Acute myeloid leukemias M2 potentially misdiagnosed as M3 variant French-American-Britain (FAB) subtype: a transitional form? *Leuk Lymphoma*. 1995;18(suppl 1):49-55.
26. Stavroyianni N, Yataganas X, Abazis D, et al. Acute promyelocytic leukemia relapsing into FAB-M2 acute myeloid leukemia with trisomy 8. *Cancer Genet Cytogenet*. 2000;117:82-83.
27. Virchis A, Massey E, Butler T, et al. Acute myeloblastic leukaemias of FAB types M6 and M4, with cryptic PML/RARalpha fusion gene formation, relapsing as acute promyelocytic leukaemia M3. *Br J Haematol*. 2001;114:551-556.
28. Scott AA, Head DR, Kopecky KJ, et al. HLA-DR–, CD33+, CD56+, CD16– myeloid/natural killer cell acute leukemia: a previously unrecognized form of acute leukemia potentially misdiagnosed as French-American-British acute myeloid leukemia-M3. *Blood*. 1994;84:244-255.
29. Reading CL, Estey EH, Huh YO, et al. Expression of unusual immunophenotype combinations in acute myelogenous leukemia. *Blood*. 1993;81:3083-3090.
30. Sewell WA, Palmer RW, Spurr NK, et al. The human *LFA-3* gene is located at the same chromosome band as the gene for its receptor CD2. *Immunogenetics*. 1988;28:278-282.

DOI: 10.1309/XC8P9M8NKQDT38LB