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Incidence and characteristics of CD4^{+}/HLA DR^{hi} dendritic cell malignancies

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A B S T R A C T

Background and Objectives. Recent reports suggest that CD4^{+}/CD56^{+}/lineage^{-} hematopoietic neoplasias are aggressive types of malignancies involving lymphoplasmacytoid/DC2 dendritic cells (DC). Here, we report on the incidence of DC malignancies and their clinical, biological, phenotypic and cytogenetic characteristics.

Design and Methods. From a large series of 392 patients with acute myeloblastic leukemia (AML) and 739 with non-Hodgkin's lymphoma (NHL), five cases (three presenting as acute leukemia and two as NHL) showed clinical, morphologic and phenotypic features compatible with a DC malignancy

Results. The overall incidence of DC malignancies among all AML and NHL cases was 0.76% and 0.27%, respectively. At presentation, these patients displayed cutaneous nodules, splenomegaly and lymph node involvement with variable levels of peripheral blood (PB) and/or bone marrow (BM) infiltration in association with anemia and thrombocytopenia. Cytologic studies showed immature appearing blast cells with negative cytochemistry reactions for both myeloperoxidase and esterases. A highly suggestive DC phenotype based on co-expression of CD123^{hi}/HLADR^{+}/lin^{-}/CD56^{+}/CD45^{dim} associated with a germline configuration of both the IgH and TCRγ genes was found in all except one patient who was CD56^{-}. Expression of other markers compatible with a DC origin was found in all cases.

Interpretation and Conclusions. We show that DC-derived malignancies can present as either cutaneous lymphoma or acute leukemia, although their incidence is extremely low (<1%). While most of these DC neoplasias probably correspond to the malignant counterpart of DC2/lymphoplasmacytoid DC, neoplasias of myeloid DC might also exist. Chemotherapy followed by consolidation with ASCT is apparently the most effective strategy for achieving a durable remission in these individuals.

Key words: dendritic cell malignancies, immunophenotype, clinical characteristics.

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Dendritic cells (DC) form discrete subpopulations of highly specialized antigen-presenting cells (APC), with a unique capacity to initiate primary T-lymphocyte responses.[1-3] Despite the unique morphologic and functional properties of mature DC, until the early 1990s little information was available on the characteristics of DC precursors. This was probably due to the fact that DC change their morphology, phenotype and functionality depending on their differentiation and/or activation state;[2,4] the lack of specific DC markers was another limitation to the identification of DC precursors. Recent development of new monoclonal antibodies (MoAb)[5-8] and the use of new MoAb combinations[9,10] together with the demonstration that DC can be cultured *in vitro*[11-13] have greatly facilitated the study of normal DC. Accordingly, it is now well-established that different subsets of circulating DC precursors are constantly found in normal human peripheral blood (PB).[14-16] These include at least two major subpopulations: the so-called lymphoplasmacytoid and the myeloid DC. Despite the fact that knowledge is accumulating on the characteristics of these normal circulating DC precursors, their exact origin and the relationship between the different normal DC subsets still remain unclear.

In 1999 Lucio *et al.*[17] reported on a case of a patient suffering from a CD4^{+}/CD123^{hi}/HLA DR^{hi}/CD56^{-} non-T non-B non-Hodgkin's lymphoma (NHL), whose leukemic cells showed striking similarities to the newly characterized lymphoplasmacytoid subset of DC.[15] These CD4^{+}/CD123^{hi}/HLA DR^{hi}/CD56^{-} cells lacked expression of T-, B-, NK- and myeloid-associated antigens and their T-cell receptor (TCR) and IgH genes displayed a germline configuration. In line with these findings, in 2000, Chaperot *et al.*[18] reported

on a group of 7 patients presenting an unusual form of leukemia that, in common, showed reactivity for HLA DR^{hi}, CD4^{+}, CD56^{+} and CD123^{hi}. The phenotypic and functional properties of these leukemic cells were identical to those of normal lymphoplasmacytoid — or DC2 — DC. A detailed analysis of these patients showed that they had neoplasias corresponding to the CD4^{+}/CD56^{+} hematopoietic malignancies initially described by Brody *et al.* and other groups,[19-24] which are referred to in the WHO classification[25] as blastic natural killer (NK) cell leukemias/lymphomas or as neoplasias of unknown origin.[24] Two further publications of the French GEIL group described the clinico-biological and cytogenetic features of a larger group of such CD4^{+}/CD56^{+} malignancies.[18,26] The clinical behavior of this disease is characterized by extranodal skin involvement, a rapid aggressive clinical course, and evolution towards an overt leukemia. Information on phenotypic markers characteristic of these neoplasias is so far limited, which hampers the definition of an unequivocal phenotypic profile for their diagnosis. Moreover, although they are rare diseases, no information on the exact incidence of these leukemias is provided in the literature. All DC malignancies reported to date belong to the lymphoplasmacytoid DC compartment, while no case with a myeloid-DC origin has been described.

In the present paper, we report on the incidence of DC malignancies in a group of 392 consecutive patients with acute myeloblastic leukemia (AML) and 739 patients with NHL, demonstrating that it is indeed a rare disease (<1%). We provide extensive information on the phenotypic features of leukemic DC from the 5 cases reported here, and compare these features with those of their normal PB counterpart. Furthermore, we describe the clinical and biological features of the disease. Our results suggest that administration of chemotherapy followed by an autologous stem cell transplant (ASCT) might be of benefit for acute DC malignancies.

Design and Methods

Patients

Between November 1998 and January 2002 a total of 1131 patients with NHL (n= 739) and AML (n=392) were referred to the laboratory of Hematology of the University Hospital of Salamanca for immunophenotyping studies. Of these, five patients — four males and one female, mean age 59 years (range 52 to 80 years) — could not be classified as having either a lymphoid or myeloid malignancy, according to the EGIL criteria[27] due to the lack of expression of antigens highly specific for the B-, T-lymphoid as well as the myeloid lineages, despite the fact that the leukemic cells were both CD34^{-} and TdT^{-}. After extensive characterization, these five patients were diagnosed as suffering from DC malignancies. Three out of the five patients were initially suspected to suffer from acute myeloblastic leukemia (AML) — two with AML M0 and one with AML M5a, according to the FAB classification.[28,29] In the remaining two patients, the initial diagnosis was compatible with a B-cell non-Hodgkin's lymphoma (B-NHL) in one case, and an aggressive blastic NK-cell lymphoma/leukemia according to the WHO classification,[25] in the other.

Flow cytometry immunophenotyping studies

In all cases, multiparameter flow cytometry immunophenotyping studies were performed on EDTA-anticoagulated bone marrow (BM) and/or peripheral blood (PB) samples. In all cases the analysis was performed on erythrocyte-lysed samples using well-established stain, lyse and then wash procedures.[9] For this purpose a large panel of monoclonal antibodies (MoAb) with 4-color stainings — fluorescein isothiocyanate (FITC)/phycoerythrin (PE)/peridin chlorophyll protein (PerCP) or PE-cyanine 5 (PC5)/allophycocyanine (APC) — were used. The exact specificities of the MoAb reagents used is shown in Table 1. For those combinations in which direct and indirect labeling were used together, the indirect staining was performed first, followed by 3 washing steps in phosphate buffered saline (PBS) and then labeling with the MoAb directly conjugated to fluorochromes, in order to ensure that the second step antibodies used in the indirect fluorescence technique did not bind to the fluorochrome-conjugated MoAb. Combined staining for surface antigens and CD3, CD79a, myeloperoxidase (MPO), lysozyme and bcl2 intracellular markers was performed using the Fix & Perm reagent kit (Caltag Laboratories, San Francisco, CA, USA), strictly following the recommendations of the manufacturer. A FACSCalibur flow cytometer and CellQUEST software (Becton Dickinson Biosciences, San Jose, CA, USA) were used for data acquisition. The Paint-A-Gate PRO software program (Becton Dickinson) was employed for data analysis. In all cases, leukemic cells were specifically identified based on their intense reactivity for the HLA DR antigen, and/or dim CD45 expression together with their intermediate/low light scatter characteristics (FSC/SSC).

Normal circulating PB lymphoplasmacytoid- and myeloid- DC were studied in a group of 67 age- and sex-matched healthy volunteers (41 males and 26 females, mean age 31 years, range 19 to 54 years) after informed consent. The exact strategy used to identify and characterize the two DC subsets in normal PB has already been described in detail.[9,10] Briefly, non-cultured/non-isolated erythrocyte-lysed whole blood samples were stained in all tests with a mixture of MoAb (CD3, CD14, CD19 and CD56) conjugated with the same fluorochrome (either FITC- or PE-conjugated), together

Table 1-1. Immunophenotypic characteristics of normal PB lymphoplasmacytoid (DC2) and myeloid dendritic cells (DC1) as compared to neoplastic dendritic cells.

	Description and/or specificity	*Normal PB DC (n= 67)*		*Patients*				
		DC1	*DC2*	*#1*	*#2*	*#3*	*#4*	*#5*
Antigen-presenting molecules								
HLA ABC[1]	HLA class I	++	+/++	+		+	+	+
HLA DR[2]	HLA class II	+++	++	++	++	++	++	++
HLA DP[3]	HLA class II	++	++	++		++	+++	++
HLA DQ[3]	HLA class II	++	+/++	++		++	++	+dim
β2-MG[1]	β chain of HLA class I			+		+	+dim	+
DC-associated antigens								
CD1a[4]	Cortical tymocytes, DCs, Langerhans cells	–	–	-/+		15%+	–	6%+dim
CD1b/c[5]	Cortical thymocytes, DCs, Langerhans cells	+	–			–	–	–
CD83	Activated lymphocytes, antigen presenting cells	–	–			-/++	20+	12%+
CD85i[5]	ILT-LIR family (ILT2). DCs, monocytes, plasma cells	+	+			+	–	+
CD85j[5]	ILT-LIR family (ILT3). DCs, monocytes, plasma cells	+	+			+	–	+
CD85f[5]	ILT-LIR family (ILT4). DCs, monocytes, plasma cells	–	–			–/+	–	–
CD85a[5]	ILT-LIR family (ILT5). DCs, monocytes, plasma cells	+	–			-	+	–
CD87[3]	Urokinase plasminogen activator receptor	–	–	–		+dim	–/+	+dim
CD206[5]	Macrophage mannose receptor	–	–			+dim	–	–
CD208[5]	DC-LAMP	–	–			+dim	–	–
RFD-1[5]	DCs	+	+			–		–
CMRF-44[5]	DCs (marker of activation status)	–	–			–	+	–
CMRF-56[5]	DCs (marker of activation status)	–	–			–	+	–
L25[5]	DCs	+	+			+		–
BDCA2[6]	Lymphoplasmacytoid DCs	–	+/++					80%+
BDCA3[6]	A subset of myeloid DCs	–/+	–			–	17%+	–
BDCA4[6]	Lymphoplasmacytoid dendritic cells	–	+/++					75%+
Adhesion molecules								
CD11a[7]	α chain of the integrin β2 LFA-1 (lymphocyte function-associated antigen 1 or CD18)	+/++	+	–		+	+	
CD29[8]	Integrin β1 subunit	++	+	+		+dim	+dim	+
CD49d[2]	Integrin α4 chain			+		+	+	+
CD54[7]	Intercellular adhesion molecule-1 (ICAM-1)	+	+	+dim		+	-	+
CD56[2]	Neural cell adhesion molecule (NCAM)	–	–	–		+	++	+
CD58[2]	Lymphocyte function-associated antigen-3 (LFA-3)	–	–	–		+dim	–	–
CD62L[2]	L-selectin	++	+/++	+dim		-/+dim	–	–/+
CD103[9]	Integrin αEβ7 (ligand for E-cadherin)	–	–	–		+dim	–	50%+
CD138[10]	Syndecan-1. Plasma cell marker					–	30%+	–
Co-stimulatory molecules								
CD2[11]	Lymphocyte function-associated antigen-2 (LFA-2) Present on T-cells, thymocytes, most NK cells	++/+++	+	–		–	++	38%++
CD4[2]	Helper/inducer T-cells, thymocytes, monocytes, DCs	+	++	+	+dim	+	+	++
CD5[2]	Mature T-cells, thymocytes, B-cell subset	+/++	–	–		17%+	–	–
CD86[3]	B70/B7.2. Involved in co-stimulation of T-cells	++	–/+			+	63%+	+
Immunoglobulin Fc and complement receptors								
CD11b[2]	Complement receptor 3 (CR3) α chain of the integrin β2 LFA-1 (Lymphocyte function-associated antigen 1 or CD18)	–	–	10%+	+dim	–	–	–
CD11c[2]	CR4. α chain of the integrin β2 LFA-1 (lymphocyte function-associated antigen 1 or CD18)	++/+++	–			4%+	-	30%+
CD32[10]	Fcγ receptor type II (FcγRII)	+	–	+dim		+dim	-	+
CD35[7]	Complement receptor 1 (CR1)	–	–	–/+dim		–	+dim	–
CD55[1]	Protects cells from damage by autologous complement	+/++	+/++	+dim		++	+	+
CD59[1]	Protects cells from damage by autologous complement	+	+	++		++	++	–
CD64[4]	Fcγ receptor type I (FcγRI)	–	–	–		–	++	+dim

continued on next page

Table 1-1. Immunophenotypic characteristics of normal PB lymphoplasmacytoid (DC2) and myeloid dendritic cells (DC1) as compared to neoplastic dendritic cells (continued from previous page).

	Description and/or specificity	*Normal PB DC (n= 67)*		*Patients*				
		DC1	*DC2*	*#1*	*#2*	*#3*	*#4*	*#5*
Cytokine and chemokine receptors								
CD25[2]	Interleukin 2 receptor α chain (IL2Rα)	–	–	–		–	–	–/+
CD116[3]	GM-CSF receptor α chain	+dim/+	–/+dim	–		+dim	+dim	
CD117[8]	c-kit, stem cell factor (SCF) receptor	–	–	–	35%+	18%+	–	17%+
CD122[7]	Interleukin 2 receptor β chain (IL2Rβ)	–	–	+dim		–	+	–
CD123[3]	Interleukin 3 receptor α chain (IL3Rα)	+/++	+++	+++		+++	+++	+++
CD126[8]	Interleukin 6 receptor α chain (IL6Rα)	+	–/+dim			30%+		
CD127[8]	Interleukin 7 receptor α chain (IL7Rα)	–	–			8%+	–	+dim
CD135[8]	FLT–3	–/+	+dim			+dim	–	
CXCR1[3]	Chemokine receptor	33%+	–			–	+++	–
CXCR2[3]	Chemokine receptor	68%+	+			–	–	9%+
CXCR4 (CD184)[3]	Chemokine receptor (fusin)	57%+	+			+	+	++
CCR5 (CD195)[3]	Chemokine receptor	54%+	+			+	+	+
Myeloid–associated antigens								
CD13[2]	Myeloid cells	++	–	–	–	–	30%+	+
CD33[2]	Myeloid cells	+++	–/+dim	–	–	–	34%+	+
Lymphoid–associated antigens								
cCD3[2]	T–lymphocytes, thymocytes	–	–	–		–	–	–
CD7[2]	T–cells, NK cells	–	–	–	–	8%+	–	5%+
CD8[2]	Cytotoxic T–cells, NK cell subset, cortical thymocytes	–	–	–		–	–	5%+
CD10[11]	Pre–B and B–cell subsets, cortical thymocyte subset, granulocytes	–	–	+dim		20%+	–	–
CD20[2]	B–cells	–	–	–/+		–	–	–
CD22[2]	B–cells	+	++	–		7%+	–	+dim
FMC7[8]	Mature B–cells	–	–	13%+		–	–	–
cCD79a[2]	B–cells	–	–	–	–	–	–	+dim
CD79b[2]	B–cells	–	–	–		–	–	+
Miscellaneous								
CD36[2]	Platelets, monocytes, endothelial cells	+	+	+dim		71%+	+	+
CD38[2]	Broad distribution	++/+++	++	++	+	26%+	–	–
CD40[2]	B lymphocytes, monocytes and DCs					43%+	–	
CD45[4]	Leukocyte common antigen	++	++	+	+dim	+dim	+dim	+dim
CD45RA[2]	Naïve T cells, B cells, NK cells, granulocytes, monocytes	–	+/++	–		+	+	+
CD45RO[2]	Memory/activated T cells, thymocytes	–/+dim#	–	–		–		–
CD71[2]	Transferring receptor. Proliferating cells, red blood cells			+		–/+	+dim	+dim
CD90[3]	CD34+ bone marrow progenitor cells			–		20%+	–	–
CD99[3]	Broad distribution	+	+dim	+		+	+	+dim
cBcl21[2]	Involved in apoptosis regulation			++		++	++	++
7.1[8]	Human counterpart of the mouse NG2 molecule	–	–	++		++	++	++

[1]Cymbus Biociences, Southampton, UK; [2]Becton Dickinson Biosciences, San Jose, CA, USA; [3]PharMingen, San Diego, CA; [4]Caltag Laboratories, San Francisco, CA, USA; [5]7th Leukocyte Differentiation Antigen Workshop DC Section; [6]Miltenyi, Bergisch Gladbach, Germany; [7]CLB, Amsterdan, The Netherlands; [8]Immunotech, Marseille, France; [9]Immunoquality Products, Gröningen, The Nederlands; [10]IMICO, Madrid, Spain; [11]Coulter Corporation, Miami, FL, USA; [12]Dakopatts A/S, Glostrup, Denmark. Abbreviations. HLA: human leukocyte antigens; MG: microglobulin; DCs: dendritic cells; ILT-LIR: immunoglobulin like transcript-leukocyte Ig like receptor; DC-LAMP: dendritic cell-lysosome associated membrane glycoprotein; FLT-3: fms-related tyrosine kinase-3. The following markers were constantly absent in both normal and leukemic cells: BU10, DCGM-4, TPD153, BG6, cCD3, CD14, CD15, CD16, CD19, CD21, CD23, CD24, CD41a, CD42b, CD57, CD61, CD65, CD66b, CD69, CD72, CD80, CD94, CD133, CD154, CD158a, CD235, CD161, NKB1, cMPO, cIgμ, TdT and cLysozyme. #in 50% of samples cells showed dim expression for CD45RO. Symbols: – negative (mean intensity of fluorescence between 0 and 10 in a scale of arbitrary fluorochrome units ranging from 0 to 10^4); + dim: dim positive (mean intensity of fluorescence around 10^1); +: moderate positive (mean intensity of fluorescence of around 10^2); ++: positive; (mean intensity of fluorescence between 10^2 and 10^3); +++: strong positive (mean intensity of fluorescence ≥ 10^3). Mixed symbols indicate variable reactivity, either among cases (intervariation between the controls) or among cells from the same patient.

Table 2. Clinical and biological characteristics of dendritic cell malignancies.

	Case #1	*Case #2*	*Case #3*	*Case #4*	*Case #5*
Sex	Male	Female	Male	Male	Male
Age (years)	62	52	80	63	71
Reason for consulting	Cutaneous lesions with erythematosis	Constitutional symptoms (anemia/fever)	Cutaneous lesions with erythematosis Constitutional symptoms	Constitutional symptoms (anemia)	Bone pain Cutaneous lesions with erythematosis
Suspected diagnosis	B-NHL	AML M5a	AML M0	AML M0	Aggressive NK-cell leukemia
Adenopathies	Supraclavicular/ axillar	No	No	Mediastinal/ Retroperitoneal	No
Hepatomegaly	No	No	No	No	No
Splenomegaly	No	Yes	No	Yes	Yes
Skin lesions	Yes	No	Yes	No	Yes
WBC ($\times 10^9$/L)	9.2	28	61	4.4	40.3
Hemoglobin (g/L)	100	85	69	65	113
Platelets ($\times 10^9$/L)	42	92	35	59	60
%PB neoplastic cells[1]	10	4	40	8	80
%PB neutrophils	70	90	40	63	20
%PB eosinophils	5	0	0	0	0
%PB lymphocytes	10	3	18	15	0
%PB monocytes	5	3	2	2	0
LDH	5071mg/dL	1370 mg/dL	1058 U/L	558 U/L	433 U/L
%BM neoplastic cells	<5	83	90	58	70
Associated neoplasias	Colon cancer	Breast cancer	No	No	No
TCR genes	Germline	Germline	Germline	Germline	Germline
IgH genes	Germline	Germline	Germline	Germline	Germline
Cytogenetic/FISH	Normal	t(9;11)(p21;q23)[2]	t(11q;23)[3]	Normal[2,3]	Hypodiploid 44,XY,-?[2]
CHT regimen	radiotherapy/ CHOP	IDA+ARAC**	Palliative CHT (cytarabine-thioguanine)	Dauno/ARA-C	Vincristine/Dauno* Prednisone
Response to CHT	CR	CR	PR	CR	CR
Relapse	Yes	No	NA	No	YES
Disease-free survival (months)	6	54	0	6	8
Exitus	Yes	No	Yes	Yes	YES
Overall survival (months)	10	54	2	10	14

*DAUNO: daunorubicin; IDA: idarubicin; ARA-C:cytosine arabinoside; CHOP: cyclophosphamide, hydroxydaunomycin/doxorubicin, oncovin, prednisone; **followed by autologous PB stem cell transplantation; CR:complete response; PR:partial response; CHT: chemotherapy; [1]neoplastic cells by flow cytometry; [2]conventional cytogenetics; [3]FISH ; NA: not appropriate.*

with anti-HLA-DR-PerCP, either CD4-APC or CD33-APC (the sources of the MoAb are shown in Table 1) and the MoAb against the molecule under study. After collecting information on 30,000 events from all the cells in the sample, a second step acquisition procedure was performed and information stored exclusively for those cells included in a HLA-DR$^+$/CD3$^-$, CD14$^-$, CD19$^-$, CD56$^-$ (HLA-DR$^+$/lineage$^-$ cells) live gate. Two clearly different subsets of HLA-DR$^+$/lineage$^-$ cells were identified, showing different reactivity for CD33 and CD123 antigens. One subset, corresponding to lymphoplasmacytoid DC, displayed strong reactivity for CD123 and dim CD33 expression, while the other (myeloid DC) was CD123^{dim+} and CD33$^{strong+}$. An extensive phenotypic characterization of both DC subpopulations has been previously described in detail.[9]

For each antigen analyzed, the intensity of expression was evaluated by the mean fluorescence intensity, expressed in arbitrary relative linear units scaled from 0 to 10^4, and represented by the following five codes: -: negative (mean intensity of fluorescence between 0 and 10 and overlapping with the intensity of fluorescence of the corresponding isotype control); + dim: dim positive (mean intensity of fluorescence of around 10^1); +: moderate positive (mean intensity of fluorescence between 10^1 and 10^2); ++: positive; (mean intensity of fluorescence between 10^2 and 10^3); +++: strong positive (mean intensity of fluorescence $\geq 10^3$).

Immunohistochemistry studies

Skin biopsy tissue sections from two patients who showed skin involvement were stained by a biotin-streptavidin-amplified (B-SA) detection system (Supersensitive Multilink-HRP/DAB; BioGenex, San Ramon, CA, USA). Sections were sequentially incubated with the specific MoAb for 30 min in a humid chamber at room temperature, washed in PBS and incubated for another 20 min with a biotinylated anti-mouse immunoglobulin antibody. Antibody binding was visualized by incubation with a PBS solution containing diaminobenzidine and H_2O_2 and cells were counterstained with Meyer's hematoxylin. Expression of the CD20, κ and λ Ig light chains, CD4, CD45RO, CD45RA, CD123 and HLA DR antigens was assessed in the cases studied.

Analysis of TCR and IgH gene rearrangements

TCR-γ and IgH gene rearrangements were evaluated by using polymerase chain reaction (PCR) and gene scanning techniques. Briefly, high molecular weight DNA was isolated using standard proteinase K digestion, phenol-chloroform extraction and ethanol precipitation; either VDJ or TCRγ clonal rearrangements were studied by PCR using approximately 50 ng of genomic DNA per test. The pair of consensus primers used to amplify VDJ has been previously described;[30] the primers described by Delatesse *et al.*[31] were used to amplify Vγ-Jγ. The PCR product (2 μL) was mixed with 3 μL of deionized formamide, 1 μL of loading buffer (Applied Biosystems, Foster City, CA, USA) and 0.5 μL of the specific molecular marker (GeneScan 500 ROX, Applied Biosystems). The mixture was then warmed for 3 min at 95°C and loaded in a 5% polyacrylamide denaturing gel with urea placed in a 377 DNA automated sequencer (Applied Biosystems). The electrophoresis was maintained for 2.5 hours at 3000 V. The PCR and gene scanning results were analyzed and interpreted as previously described.[30]

Conventional karyotyping and fluorescence in situ hybridization (FISH) studies

Cytogenetic studies were performed on heparin-anticoagulated PB and/or BM samples that had been cultured for 2 hours at 37°C in the presence of 100 μL of an isotonic buffer containing 10 μg/mL of colcemid or for 24 hours in either the absence of any stimuli or after stimulation with stem cell factor (SCF) and/or granulomonocytic-colony stimulating factor (GM-CSF); for these latter samples 100 μL of 10 μg/mL colcemid were added 2 hours prior to harvesting the cells. Cultured cells were fixed in Carnoy's medium – methanol/acetic acid at a ratio of 3/1 (vol/vol) – according to conventional cytogenetic protocols. Fixed cells were dropped onto ethanol/ether cleaned slides and metaphases were analyzed for G-banding; at least 20 metaphases were studied for each case.

Abnormalities involving the *MLL* gene at 11q23 were systematically explored using conventional interphase FISH techniques[32] on PB and/or BM cells which were fixed in methanol/acetic acid 1:1 (vol:vol) and placed onto slides as described above.

Results

Incidence and clinical presentation of DC malignancies

From all cases referred to our laboratory as having either NHL (n=739) or AML (n=392), the $CD34^-/TdT^-$ blast cell lineage could not be assigned in only five cases. In these patients, neoplastic cells showed morphologic, cytochemical, phenotypic and molecular characteristics compatible with a DC origin (Tables 1 and 2 and Figures 1, 2 and 3), two presenting as a cutaneous lymphoma, and three as acute leukemia. Accordingly, the overall incidence of DC neoplasias among the cases that presented as either NHL or AML was 0.27% and 0.76%, respectively.

Although two of our cases displayed a more lymphomatous presentation while the other three resembled a myeloid leukemia, no clear differences regarding immunophenotype and cytogenetics were observed between the two subsets. In fact, they should be considered as a single cohort. Table 2 shows the clinico-biological disease characteristics from each of the five patients analyzed. As indicated in this table, the reason for consultation was related to the development of constitutional symptoms (n= 2), the appearance of skin lesions (n= 2) or both (n= 1). Physical examination at diagnosis revealed the presence of splenomegaly in two out of the five cases and lymph node involvement in another two patients (supraclavicular and axillary lymphadenopathy in one of them; enlarged mediastinal and retroperitoneal lymph nodes in the other). As mentioned above, extensive skin involvement consistent with disseminated purpuric lesions of the dermis was found in three out of the five patients.

Laboratory studies revealed the presence of both anemia and thrombocytopenia in all patients. Three patients presented with a WBC > 25×10^9/L at diagnosis and variable levels of PB infiltration by leukemic cells (range: 8% to 63% of the total nucleated cells) were observed in all cases. At diagnosis, lactate dehydrogenase (LDH) serum levels were increased in all except one patient (case #5). Two out of the five patients had a previous history of neoplasia: colorectal carcinoma in one (case #1) and a breast cancer in the other (case #2).

Characterization of the neoplastic cells

From the morphologic point of view, both BM and PB

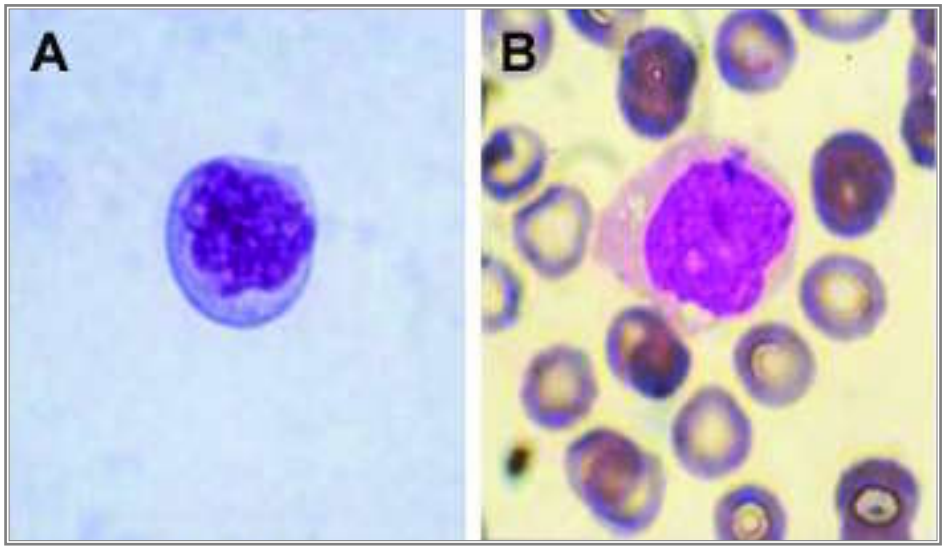

Figure 1. Morphologic characteristics of normal PB lymphoplasmacytoid DC after immunomagnetic isolation (using the BDCA4-Cell Isolation Kit, Miltenyi Biotec, Bergish Gladbach, Germany) (panel A); a PB-derived malignant DC from one of the patients studied (case 3) is shown in panel B (May-Grünwald-Giemsa, ×1000).

neoplastic cells from three of the patients showed a minimally differentiated non-lymphoid blastic appearance which was compatible with the diagnosis of AML M0 in two cases and M5a in another. The other two cases showed large cells with a blastic lymphomatous morphology characterized by the presence of one or more prominent nucleoli and a basophilic cytoplasm in the absence of granules. In these two latter patients skin biopsy studies were performed and the histologic diagnosis was documented as large diffuse high grade B-cell NHL ($CD20^+$ by immunohistochemistry) and aggressive NK cell lymphoblastic lymphoma ($CD56^+$, $CD4^+$, $HLADR^+$, $CD3^-$, $CD20^-$, κ^-, λ^- by immunohistochemistry). Figure 1 illustrates the morphology of malignant cells present in the PB of one of the patients studied (case #3) and normal PB lymphoplasmacytoid DC for a comparison.

Conventional cytochemistry revealed the absence of myeloperoxidase (MPO) and α-naphthyl-acetate-esterase (α-NAE) in all cases.

Routine multiparameter flow cytometry immunophenotypic analysis of PB and/or BM cells (Table 1), revealed the presence of a population of $CD34^-$ cells which expressed the CD45 antigen at abnormally low levels in all cases. Moreover, these cells were constantly negative for $cCD3^-$, $cCD79a^-$, $cMPO^-$, clysozyme$^-$, $CD14^-$, $CD15^-$, $CD65^-$, $CD66b^-$ and $CD19^-$, whereas they were $CD4^+$ and showed high reactivity for the interleukin-3 receptor α-chain ($CD123^{hi}$) and $HLADR^{hi}$ antigens. Other HLA class I and class II molecules were also present in all cases tested. Expression of CD117 was found in a relatively small proportion of all leukemic cells from three of the five patients and co-expression of CD117, CD13 and CD33 was only observed in one individual (case #5).

Interestingly, expression of different lymphoid-associated markers was detected at variable percentages in three cases (Table 1). In turn, all lymphoid-associated markers tested were negative in the other two cases, and no clear phenotypic support was found for the initially suspected diagnosis (AML *versus* NHL). Molecular studies showed a germline configuration for the TCRγ and IgH genes in all cases, further excluding that the malignant cells were of T- or B-lymphoid origin.

Expression of DC-associated markers, apart from $CD123^{hi}$ and HLA class II molecules, was detected in all cases in which these markers were looked for. While some of these DC-associated molecules were present in at least part of the blasts from all cases tested – CD86, CD83, CD87 – others showed a different pattern of reactivity in each of the patients analyzed (Table 1). In this sense, it should be noted that, in comparison to the other cases, case #4 showed a clearly different pattern of reactivity for the DC-associated molecules analyzed ($CD85a^-$, $CD85j^-$, $CD85i^-$, $CMRF44^+$, $CMRF56^-$, and $BDCA3^+$), together with high expression of the CXCR1 chemokine receptor, a phenotype that may be consistent with a myeloid-DC origin. Interestingly, this case also showed a different pattern of expression for both the $CD64^{++}$ and $CD32^-$ IgG receptors, $CD35^+$, $CD138^{-/+}$, $CD54^-$ and $CD62L^-$. The most characteristic phenotypic patterns displayed by malignant dendritic cells are displayed in Figure 2. All cases tested were $CD36^+$, $bcl2^{++}$ and 7.1^{++}, while reactivity for CD56 was detected in all but one patient (case #1, which corresponds to a case reported previously by Lucio *et al.* in 1999).[7] The pattern of expression of the other surface antigens tested is shown in Table 1.

Conventional cytogenetic analysis showed that two patients had a normal 46XY karyotype in the absence of 11q23 abnormalities by FISH (cases #1 and 4). Of the other three cases, two showed 11q23 abnormalities consisting of a t(9;11)(p21;q23) translocation (case #2) and an 11q23 deletion (case #3) as detected by con-

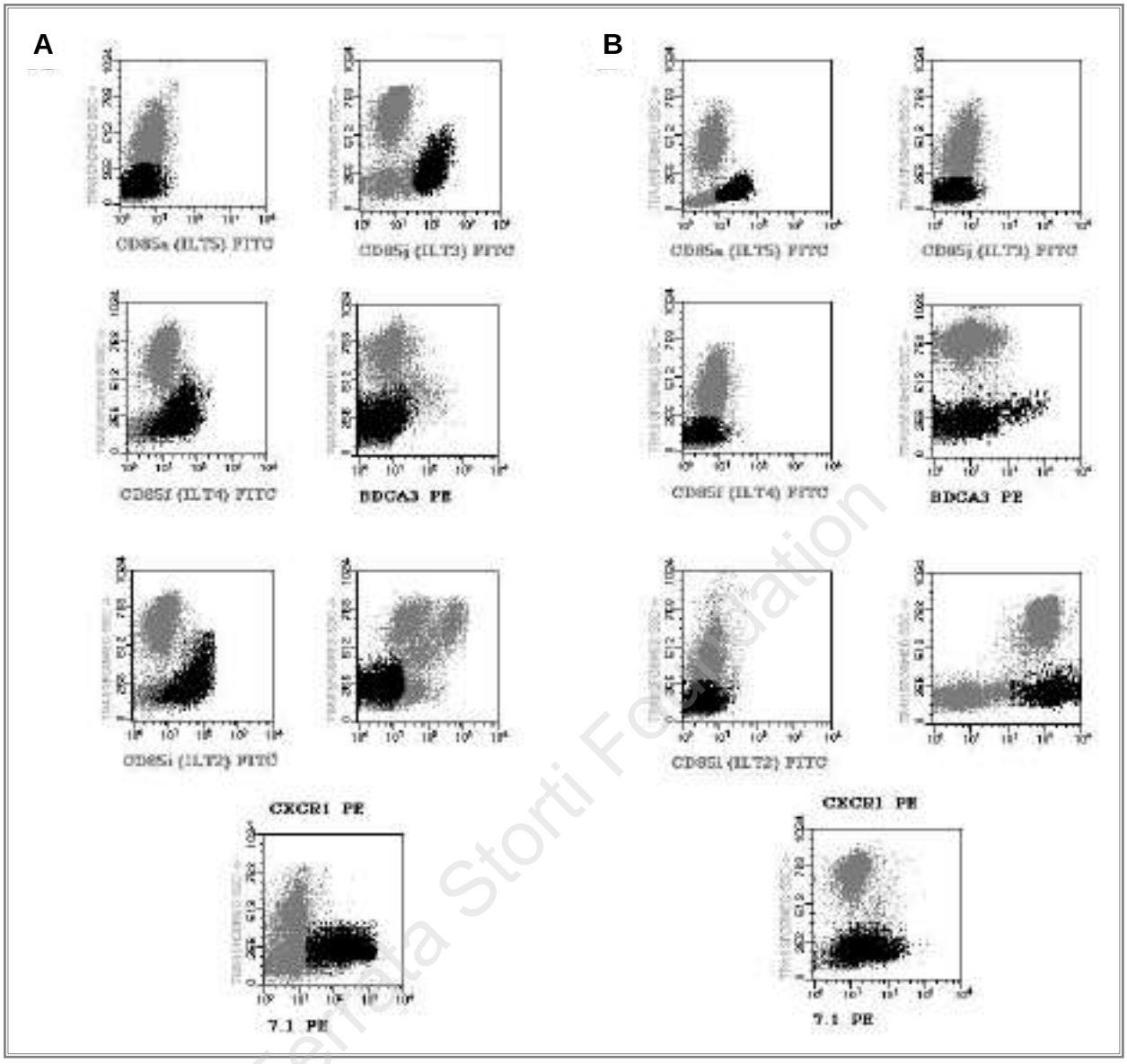

Figure 2. Representative bivariate dot plots showing the major differences in the phenotypic patterns of malignant lymphoplasmacytoid (panel A) and myeloid (panel B) DC.

ventional karyotyping and confirmed by both interphase and metaphase FISH; the fifth case showed a hypodiploid karyotype.

Patients' outcome

As shown in Table 2, the type of treatment given varied according to the initial diagnosis (AML versus NHL) and age. The 80-year old patient received only palliative treatment while the other four patients were treated with either AML- or NHL-oriented therapy and achieved morphologic complete remission, independently of the treatment protocol used. Despite this, relapses occurred in three of these four patients at month 6, 6 and 8 after starting therapy; all three relapses occurred in patients who had been treated with chemotherapy alone. These three patients died after relapse despite rescue therapy with IVMP-16 or vincristine plus daunorubicin and prednisone.

The fourth patient who received intensive chemotherapy – idarubicin in combination with cytosine arabinoside (ARA-C) followed by an autologous stem cell transplant (ASCT) – at the time of this report, remains in continuous remission 54 months after diagnosis with detectable levels of minimal residual disease by phenotyping (0.08% of all BM cells) in the last follow-up study.

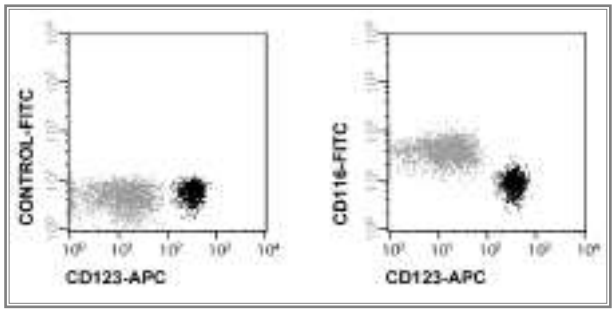

Figure 3. Representative bivariate dot plots showing the different pattern of expression of CD116 (GM-CSF receptor) and CD123 (IL-3 receptor alpha chain) in peripheral blood DC1/myeloid dendritic cells (gray dots) and DC2/lymphoplasmacytoid dendritic cells (black events) from a healthy adult subject.

Due to his age, the fifth case (case #3) was treated with anti-neoplastic drugs (cytarabine and thioguanine) with a palliative aim. Although the number of blast cells in the PB initially dropped in this patient, skin lesions persisted and the patient died four months after diagnosis, due to disease progression.

Discussion

During the last decade several descriptions have been published of an unusual malignancy of $CD4^+/CD56^+$ hematopoietic cells.[19-24] These neoplasias were initially postulated to correspond to NK-cell leukemias/lymphomas. Although they were considered as such in the most recent WHO classification,[25] the exact origin of these neoplasias remains unknown.[24] Recently, Chaperot *et al.*[18] clearly showed that such malignancies of $CD4^+/CD56^+$ cells correspond to the neoplastic counterpart of DC2/lymphoplasmacytoid DC. In fact, the most characteristic phenotypic features of malignancies of DC precursors which have been reported so far rely on the co-expression of CD4 and CD56 together with high levels of HLA DR and CD123 in the absence of reactivity for lineage specific markers.[18,26] This phenotype was found in all except one of the cases reported here, who did not show reactivity for CD56; we have already described this case.[17] Normal DC2/lymphoplasmacytoid DC have been reported to co-express HLA DR^{hi}, $CD123^{hi}$ and $CD4^+$ and they are mostly $CD56^-$,[15] as found in our previously published case. However, low expression of CD56 has recently been reported in a minor subset of cells within both the lymphoplasmacytoid and myeloid DC compartments;[33] in addition, CD56 expression has been shown to be up-regulated in lymphoplasmacytoid DC after treatment with FLT3 ligand.[34] CD56 expression by neoplastic cells is eventually found in other hematologic malignancies, such as AML[35] and multiple myeloma (MM).[36,37] It has been suggested that CD56 expression in AML is related to specific cytogenetic features such as the t(8;21) translocation and 11q23 abnormalities, being co-expressed with 7.1 in these latter cases.[38,39] In the present study, all four cases tested showed strong expression for 7.1 (the homolog of the mouse NG2 molecule), a marker which is absent in normal circulating blood DC. Interestingly, 11q23 abnormalities were detected in only two cases. To the best of our knowledge, this is the first report in which co-expression of CD56 and 7.1 in association or not with 11q23 abnormalities has been reported in DC malignancies.

Apart from CD56, none of the other NK-cell associated markers studied here was positive on the DC. Since CD56 is expressed by normal and/or pathologic B, T and myeloid cells as well as by the NK-cells,[36-41] it cannot be considered as an NK-cell specific antigen.

We were particularly interested in performing a comprehensive immunophenotypic analysis that could contribute to establishing phenotypic profiles for DC malignancies to be used in routine diagnostic screening. Apart from reactivity for CD4, HLA DR and CD123, our results show high expression of both the HLA DP and HLA DQ class II molecules, together with variable positivity for the CD1a, CD83, CD86, CD87, CD85a, CD85f, CD85j, CD85i, BDCA2, BDCA3 and BDCA4 DC-associated antigens. A careful analysis of the reactivity for these markers in the five patients showed that similar phenotypic patterns were found for the majority of cases, but patient #4 displayed a phenotypic pattern for these markers that was unique. In this patient, in addition to strong HLA class II and CD4 expression, blast cells showed reactivity for markers classically associated with the monocytic lineage ($CD13^+/CD33^+/CD36^+/CD64^+$); however, these cells expressed DC-specific molecules, such as BDCA-3, CMRF-44 and CMRF-56, a phenotype that has not been reported on normal monocytes.[42,43] This fact, together with the absence in the malignant cells of other monocytic lineage-specific antigens which appear at the earliest stages of the monocytic maturation, such as α-naphthyl-acetate-esterase and cytoplasmic lysozyme strongly suggests that blast cells from case #4 more likely correspond to DC with aberrant CD64 expression than to cells from monocytic lineage. It is noteworthy that patient #4 was the only patient showing reactivity for BDCA3, a newly identified DC marker whose expression has been associated with DC1/myeloid DC.[7] These malignant DC also showed reactivity for the DC-associated markers CD85a, CMRF44 and CMRF56. Although normal PB myeloid DC from healthy controls, but not lymphoplasmacytoid DC, also expressed CD85a, they were constantly negative for both CMRF44 and CMRF56. It should be noted that pos-

itivity for CMRF44 and CMFR56 has only been observed in normal DC after overnight activation,[33,43] these markers not being expressed on resting freshly obtained normal PB DC. Therefore, the presence of these molecules on the surface of fresh blast cells from case #4 could reflect a state of *in vivo* activation. Such phenotypic features of case #4 are different from those found for normal BDCA2+/BDCA4+ DC2/lymphoplasmacytoid DC, which are BDCA3−, CD85a−, CMRF44− and CMRF56−, as also found for the malignant cells in cases #3 and #5. Although this phenotypic profile could support a DC1/myeloid DC origin for case #4, a DC2/lymphoplasmacytoid DC origin cannot be ruled out, since these cells also expressed CD123hi and CD45RA, and they were CD11c−, which are considered to be characteristic features of DC2/lymphoplasmacytoid DC. Differences were found between case #4 and the other patients as regards expression of the CD32 and CD64 IgG receptors, as well as the CXCR1 chemokine receptor. Interestingly, case #4 did not have skin involvement, but both splenomegaly and lymphoadenopathy were present, further supporting the notion that different patterns of expression of chemokine receptors might be associated with different homing of neoplastic DC precursors.

In the remaining four cases (cases #1, 2, 3 and 5), reactivity for different markers, other than those directly related to the DC lineage, was found. The expression of lymphoid-associated markers in cases #1, 3 and 5, as well as co-expression of CD117 and the myeloid antigens CD13 and CD33 in case #5 were particularly interesting. Despite co-expression of lymphoid and myeloid-associated antigens, case #5 did not score more than 2 points for either the myeloid or for the lymphoid lineage, when the EGIL criteria were followed strictly:[27] CD117 was found to be present in <20% blast cells, and no cytoplasmic, but surface CD22 expression was found. Moreover, it should also be taken into account that CD33, CD13 and CD117 are present during the earliest stages of hematopoiesis[35] and that MPO, considered to be the most specific marker for the myeloid lineage, was negative in this case. In turn, normal human PB lymphoplasmacytoid DC have recently been shown to express several lymphoid-associated markers, such as CD2, CD4, CD7 and CD22, as well as the myeloid-associated marker CD33 in the absence of MPO.[9] In addition, most blast cells from this case, apart from showing positivity for the referred myeloid- and lymphoid-associated markers, also expressed BDCA2 and BDCA4. These latter two markers have been shown to be specifically and exclusively expressed on lymphoplasmacytoid DC in non-cultured blood and bone marrow,[42] further supporting our belief that the malignant cells from case #5 were of lymphoplasmacytoid DC origin. Although in cases #1, 2 and 3 neither BDCA2 nor BDCA4 was tested, and certainly in cases #1 and 2 few DC-associated markers were analyzed, all these three cases displayed typical phenotypic patterns similar to those of lymphoplasmacytoid DC for the remaining markers tested, including strong expression of HLA-I, HLA-II and CD123, and positivity for CD4 in the absence of reactivity for lineage-specific markers; molecular data further supported their non-T and non-B lymphoid origin, since in all these cases TCRγ and IgH genes displayed a germline configuration. Recently, Trimoreau *et al.* reported that, as on normal DC2 dendritic cells, co-expression of high levels of CD123 and CD45RA when CD116 and CD45RO are both absent or only dimly expressed can be considered as a profile of DC2 malignancy.[43] Although these markers were not all systematically tested in our series, in line with these findings, we found that this phenotypic profile was displayed by tumor cells from cases #1 (with the exception of CD45RA, which was negative in this case), case #3 and #5, further supporting their DC2/lymphoplasmacytoid DC origin.

Although few studies have so far analyzed the clinical and biological characteristics of lymphoplasmacytoid DC malignancies, there is general agreement than the disease has an aggressive clinical course.[26,44,45] Accordingly, in most cases patients show skin involvement in the presence or absence of organomegalies and cytopenias. In our patients, infiltration of PB and/or BM was typically found already at diagnosis and increased during disease evolution. Previous reports indicate that CD4+/CD56+ lymphoplasmacytoid DC malignancies have an aggressive clinical evolution and a poor outcome, despite morphologic and clinical remission being achieved in most cases.[18,26] In line with these observations, all four cases reported here who were treated with intensive chemotherapy, achieved complete remission. Despite this, three of them relapsed soon after achieving the remission, while the other patient has remained in continuous complete remission for 54 months. Interestingly, while the three patients who relapsed were treated with chemotherapy alone, the patient with continuing complete remission had received standard AML-oriented chemotherapy followed by an ASCT. These results suggest that, in these patients, consolidation therapy with ASCT, once CR has been achieved, might be more effective than the use of chemotherapy alone, in accordance with the results reported by Feuillard *et al.*[26] In any case, our study seems to confirm the high remission and relapse rates with conventional chemotherapy regimens and supports the suggestion by Feuillard *et al.*[26] that consolidation with an allogeneic or autologous transplant might be the treatment of choice, particularly if applied immediately after remission.

In summary, in the present study we show that DC-derived malignancies can present as a cutaneous malig-

nancy or as acute leukemia, and that their incidence is extremely low (<1%). Although most of these DC malignancies probably correspond to the neoplastic counterpart of DC2/lymphoplasmacytoid DC, neoplasias of myeloid DC might also exist. Despite the low number of cases studied, our results also support the notion that conventional chemotherapy alone is associated with a high remission rate but early relapses in these individuals.

Contributions. All authors gave substantial contributions to the conception and design of the study, and to acquisition and analysis of data. The final manuscript was written by AO and critically revised and approved by all the remaining authors. The authors reported no conflict of interest.

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