CD4+ CD56− lineage negative malignancies: a new entity developed from malignant early plasmacytoid dendritic cells

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Background and Objectives. The CD4+ CD56− lineage immunophenotype characterizes rare malignancies, so far considered as arising from the transformation of NK progenitors, and therefore classified as blastic NK-cell leukemia/lymphoma by the WHO committee. Recently it was formally demonstrated that such malignancies do, in fact, develop from plasmacytoid dendritic cells (pDC), according to immunophenotypic and functional criteria. The clinico-biological features of this neoplasm were moreover recently summarized from a large series of 23 patients.

Information Sources. The main symptoms at presentation were cutaneous lesions and bone marrow failure, due to invasion by blastic cells, all of which were EBV negative and agranular. Most patients were initially sensitive to chemotherapy regimens, but they rapidly relapsed and died within 3 years. Only 2 allotransplanted patients were long survivors. Recurrent chromosomal aberrations involving chromosomes 5q, 6q, 12p, 13q, 15q and 9 were described and it was characteristic that these were associated in the same cell. In the present review we compared these findings to those in the literature.

State of the Art and Perspectives. The concordant characteristics led us to confirm that this neoplasm actually represents a new entity, that we propose to rename blastic or blastic NK leukemia/lymphoma. The diagnosis is primarily based on a characteristic immunophenotypic profile i.e. CD4+ CD56− CD3− CD13+ CD33+ CD19+. Complementary analyses assessing the expression of more specific pDC-related markers showed the cells to be HLA-DR+, CD123+, CD11b+, CD45RA+, BDCA-2+ or BDCA-4+. Such complementary investigations are necessary only in the case of an atypical phenotype, in order to confirm a pDC origin and exclude another hematologic disease. This presently regards the expression of CD33 or cytoplasmic CD3e (cyCD3e) and the negativity of CD56.

Key words: plasmacytoid dendritic cells, CD4+CD56−, leukemia, lymphoma, blastic.

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A new classification of all neoplasms of hematopoietic origin has recently been published by the World Health Organization (WHO). This classification combines all available information to define a disease entity, i.e. morphologic, immunophenotypic, genetic and clinical features. This approach has led to the stratification of hematologic malignancies primarily according to their lineage and stage of differentiation. Moreover, a cell of origin is postulated for each neoplasm. Nevertheless, current knowledge is likely to be imperfect, and lineage or nomenclature might be ill assigned in some cases.

In 2002, a series of 23 leukemias with an unusual, but unique, immunophenotype was reported by the French Groupe d’Etude Immunologique des Leucémies (GEIL). Malignant cells were positive for CD4 and CD56, but they belonged neither to the T-lymphoid nor to the myeloid-monocytic lineage. Membrane and cytoplasmatic CD3 were absent, as was CD5. T-cell receptor genes were in germine configuration, and CD13, CD33, CD14, CD64 and MPO were never expressed. Of note, these cases appeared to share many common clinico-biological features. Most patients were elderly. They presented with cutaneous lesions, bone marrow involvement and frequent evolution towards an overt leukemia. The clinical course was always aggressive, leading to death within 3 years despite initial response to therapy. Malignant cells appeared morphologically immature, with an agranular cytoplasm full of microvesicles. Histologic analysis revealed that the cells always infiltrated the dermis but spared the epidermis. In lymph nodes, the cells localized in interfollicular areas (personal observation). Cytogenetic abnormalities were frequent, showing recurrent and mostly complex chromosome aberrations. No association with EBV infection was observed.

Since 1989 a total of 18 articles have reported 58 cases of malignancies showing similar features and the characteristic CD4+CD56−CD3−CD13+CD33+CD19− cell-phenotypic profile. Most often, the articles reported single cases or small series from 2 up to 5 patients. The most important one apart from ours concerned 14 patients. These malignancies have variably been classified as histiocytic lymphoma or histiocytic associated hematologic malignancy, cutaneous agranular CD4+CD56− lymphoma or agranular CD4+CD56− hematodermic neoplasm, or myelo-monocytic precursor cell related lymphoma. The cell of origin was proposed to be an NK-cell precursor, an immature myelo-monocyte precursor, or a mixed NK/myelo-monocyte precursor. These affiliations are very doubtful, since they were based only on the sole
positivity of one or two lineage associated markers (CD56 for the NK cell lineage, CD68 and CD36 for the monocytic lineage), but not on a complete immunophenotype consistent with a normal defined equivalent. In the recent, new WHO classification, these malignancies have been considered as "blastic NK-cell lymphoma," without further demonstration of an NK cell origin. Importantly, our group identified the normal counterpart of these cells as the plasmacytoid dendritic cell or pDC, based on morphologic and functional properties. In 1999 Luccio et al. had already suggested that malignant pDC exist, based on an evocative immunophenotype including CD123 and CD45RA. Unfortunately, CD56 expression was not documented.

Dendritic cells (DC) form a heterogeneous population of rare cells with distinct origins, stages of differentiation, specific functions and migratory profiles. At least 3 different human DC subsets have been identified to date - Langerhans cells, myeloid CD11c+ (or DC1) and plasmacytoid DC (or lymphoid DC, DC2, interferon producing cells) -, which can be distinguished morphologically, immunophenotypically and functionally. All of them are leukocytes, originating from a CD34+ progenitor. At an immature stage, they are involved in the sensing and uptake of different pathogens, and secrete large amounts of pro-inflammatory cytokines. At a mature stage, they are highly specialized antigen-presenting cells (APC) that are essential for the initiation and skewing of immune response through the priming of naive or resting T cells. DC represent a link between innate and adaptive immunity.

Of note, the final outcome of the immune response they trigger might be the induction of tolerance or immunity, depending on their microenvironment. Within this family, pDC represent a distinct and rare cell type that was first described in 1958 by Lennert et al. in the paracortex of reactive human lymph nodes. These cells look like plasma cells both upon optical and ultrastructural examination (eccentric nuclei, basophilic cytoplasm and abundant rough endoplasmic reticulum). They express CD4, CD31, CD36, CD68, CLA, and lack cell lineage markers. Due to the presence of CD4 and their preferential location in T-cell areas of human lymphoid tissues, they were originally referred to as T-associated plasma cells or plasmacytoid T cells. Later, a monocyte origin was favored because of the positivity of CD68 and CD36, and the term plasmacytoid monocyte was increasingly used, until the study by Grouard et al. in 1997, which unconfutably proved that these cells were indeed dendritic cells. pDC have also been clearly identified in normal bone marrow, peripheral blood, thymus and all lymphoid tissues. They originate from CD34+ hematopoietic progenitors, most likely depending on the cytokine FLT3-ligand. They display unique morphologic and functional characteristics as compared to other subtypes of dendritic and hematopoietic cells. However there are still unsolved questions related to their lineage and function in vivo. Regarding their homogeneous biological and clinical features, CD4+ CD56+ lin- malignancies actually fulfill the criterion for the definition of an entity. Since the cell of origin proved to belong to the pDC family, based on well-documented immunophenotypic, morphologic and functional properties, we propose reclassifying this entity as a pDC-related neoplasm.

**Characterization of the malignant cell**

**CD4+56+ lin malignant cells arise from pDC**

To account for the origin of CD4+CD56+ malignant cells, we selected patients on the basis of a homogeneous phenotype, i.e. CD4+ CD56+ CD3- CD5- CD19- CD13- CD33+, and germline TCR genes. Such an immunophenotypic profile is unique in that it does not correspond to any known T, B, NK or myeloid subset at any stage of differentiation. Conversely, it exactly matches that of pDC precursors present in the peripheral blood or lymphoid organs (Table 1). The presence of CD56 was first considered as an important difference. However, it has now been demonstrated that CD56 is actually expressed on a subset of normal pDC. This origin was further confirmed using a more extensive panel of antibodies. Like normal pDC, malignant cells express HLA-DR (23/23) and other molecules associated with antigen presentation, such as CD40 (6/10) or CD86 (8/10). CD32, which is involved in antigen capture via IgG antibodies, is also present in most cases (personal observation). More interestingly, malignant cells exhibit characteristic markers of normal pDC. They are highly positive for CD123 (11/11) and express the RA isoform of CD45 (10/10), as well as the newly described BDC2A or BDC-4 markers (personal results). Conversely, they constantly lack monocyte-derived DC related antigens, such as CD11c, CD11b, CD45RO and only faintly express CD116 (Figure 1).

Normal pDC precursors also contain mRNA for the invariant chain of the pre-T-cell receptor (pTa), for the surrogate light chain, for Spi-B and for granzyme B. Likewise, malignant cells demonstrated the same properties. Not only is the immunophenotype of these cells characteristic of pDC, but so too is their behavior in vitro. In culture with interleukin-3 (IL-3) and CD40-L, or with viruses, they acquire the typical morphology of DC with multiple fine dendrites all around the cells. CD1a, CD1c and CD83 become positive, and molecules involved in T-lymphocyte co-stimulation or antigen presentation are induced or highly up-regulated. Stimulated malignant cells also demonstrate two fundamental properties that unequivocally link them to the plasmacytoid dendritic lineage. In response to
The Influenza virus, they are able to synthesize interferon-α, and at a differentiated stage (i.e., after IL-3 and CD40L stimulation) they are capable of activating naïve cord blood lymphocytes. The latter property is specific to DC and the former is specific to the plasmacytoid subset. Like normal DC, malignant cells could also bias the T-cell repertoire towards a Th1 or Th2 cytokine profile, depending on the activation signal provided. IL-3 and CD40L-activated leukemic pDC induced the generation of Th2, while they induced a Th1 profile in response to virus (Chapérot et al., personal communication).

Furthermore, leukemic as well as normal pDC secrete various chemokines capable of attracting either Th1 or Th2 cells, depending on the activation used (Bendriss et al., personal communication). All these similarities strengthen the likely pDC origin of CD4+CD56+ leukemic cells. Interestingly, the differentiation of malignant pDC is not blocked in vitro, so they could give rise to functional mature cells under appropriate stimulation (Figure 2).

Another cell of origin was ruled out not only because of a non-relevant immunophenotype, but also by the demonstration that malignant cells were not able to differentiate in vitro into NK cells, B-lymphocytes, myeloid cells or monocytes in standard culture conditions. In the experiment presented in Figure 3, CD4+CD56+ lin- malignant cells exhibited neither bi-potentiality nor multi-potentiality in 21-day culture assays.

**Tumor cell affiliation to the pDC lineage relies on immunophenotypic criteria**

A positive diagnosis is easy nowadays, based on the following profile defined with antibodies usually used in leukemia immunophenotyping, i.e. CD4-CD56-CD3-CD13-CD33-CD19-. Indeed, all the observations selected from the GEIL laboratories on the basis of this simple profile were found to display the characteristic markers of pDC, i.e. HLA-DR+ CD123high CD116low CD45RA+ CD45R0− BDCA-2+ BDCA-4+. They were also found to be negative for all conventional markers of the B or myeloid lineage, i.e. CD20, CD22, CD79α, surface and cytoplasmic immunoglobulins (Ig), CD11c, CD65, CD117, MPO, CD14 and CD64. Only CD36 and CD68 were frequently observed (85% and 92%, respectively). CD7 or CD2 could also be expressed (61% and 9%, respectively), but the TCR genes were always germline. It should be underlined that CD36, CD68, CD7 and CD2 have also been described on normal pDC. Finally, all these cases appeared to be CD34 negative and only one was positive for TdT.

Several observations showing the very same profile have been reported in the literature. Such cases have mostly been referred to as blastoid CD4+CD56+ NK leukemia/lymphoma or agranular CD4+CD56+ hematodermic neoplasms. It is noteworthy that identical cases of agranular CD4+CD56+ malignancies, with the same cytological, histologic and clinical features but with otherwise slight immunophenotypic differences, have also been occasionally described. These differences mainly regarded the expression of CD33 (9 cases), CD32, CD56, CD7 and CD2 have also been described on normal pDC, and finally, all these cases appeared to be CD34 negative and only one was positive for TdT.

### Table 1. Comparison of normal and malignant pDC immunophenotype.

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<th>Specific markers for</th>
<th>Normal pDC</th>
<th>Malignant pDC</th>
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<td>T or NK lymphocytes</td>
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<td>granzyme-B</td>
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<td>perforin</td>
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<td>Myeloid lineage</td>
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<td>CD83</td>
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mb or cy: membrane or cytoplasm; + or -: positive or negative, depending on the case.
M. C. Jacob et al.

eral blood pDC. Moreover, a case of CD4+ CD56+ CD33- CD13- malignancy was indubitably characterized as arising from pDC, with tumor cells exhibiting the characteristic CD123^hi^ CD45RA^- BDCA^-2^- and BDCA^-4^- pDC immunophenotype, and demonstrating the same functional properties in vitro. Expression of CD33 therefore does not exclude the diagnosis of pDC malignancy, and should not be considered as an aberrant marker. However, its presence makes the diagnosis more difficult since other malignancies such as acute myeloid leukemia (AML), mixed myeloid/NK leukemia or Langerhans cell histiocytosis may exhibit the CD4+ CD56+ CD33^- profile. (and personal observations). It has been

![Figure 1. Representative immunophenotype of malignant cells (CD45 low events) by flow cytometry. Open histograms represent isotype match controls, filled histograms represent staining with specific antibodies.](image-url)
Figure 2. Characteristics of CD4+CD56- lin- pDC neoplasm.
reported that these malignancies also have a predilection for skin involvement. Some characteristics may orient the diagnosis, in particular the cell morphology or the negativity of other conventional myeloid markers. However, we believe that it is necessary to study DC specific markers to prove the cell lineage assignment definitively in such cases.

In the EGIL classification of leukemia, the presence of surface or cytoplasmic CD3 is considered as a specific T-cell lineage marker. Depending on the stage of differentiation, pre-T lymphocytes express CD7 in

association with CD2 and finally CD5. CD4 and CD8 are acquired subsequently. Except for rare cases of leukemia involving a very early committed T lymphoid cell, all acute lymphoblastic leukemias of T-cell origin (T-ALL) demonstrate clonal TCRγ gene rearrangement. This is particularly true when pan-T markers are expressed. For the present patients, CD7 was positive in 7/11 cases and CD2 in 1/8 cases, while CD5 was absent in the 9 cases tested. TCRγ genes were in germline configuration in all instances, nearly ruling out a T-cell ori-

Figure 3. CD4+ CD56− lin− malignant cells do not differentiate in vitro into NK or B lymphocytes, nor into myelomonocytic cells.
Overall survival was also poorer, despite intensive care, in patients over 45 years old and only 1 infant and 2 young adults. The literature on pDC malignancies. Thus, these two observations also raise the possibility that CD56 might not be necessary for the definition of this entity. Indeed, most normal pDC do not express CD56.

In the literature, 16 other patients with blasts CD4+ CD56+ cyCD3− leukemia/lymphoma, but with absence of information about CD33, have also been reported. Thus a total of about 96 cases with typical or marginal immunophenotypes have been described.

**CD4+ CD56+ lin pDC are arrested at an early stage of maturation**

Blom et al. have proposed a developmental pathway of pre-pDC from CD34+ DA4+ stem cells, and described four main stages using flow cytometry: CD4+ CD56+ CD123− early progenitors, CD34+ CD45RA+ CD123− late progenitors, CD34+ CD45RA− CD123+ pre-pDC, and finally CD34+ CD45RA+ CD123++ pre-pDC. Both pre- and pre-pDC exhibit the typical morphology of plasma cells and secrete large amounts of IFN-α.

CD4+ CD56+ lin malignant cells had a low expression of CD45 in all cases as do blasts from other hematologic malignancies, and were positive for CD34 or TdT in several cases. The cytological features of pDC malignancies, best described by Feuillard et al., have essentially been observed on Giemsa stains from bone marrow aspirates. Malignant DC morphology appeared to be close to that of either myeloid or lymphoid blasts, depending on the case. However, pDC blasts appeared somewhat more mature than typical blasts in most instances. Mitoses could be observed in some cases. The malignant population was homogeneous or not, composed of cells of either small, intermediate or large size. Nuclei were regular or not, chromatin was lacy or loose, but never condensed, and nucleoli were frequently observed in a variable number of cells. Of note, the most important characteristics of pDC blasts appeared to be in the cytoplasm, which was rather abundant in nearly all cases, gray-blue or faintly basophilic, never granular, but heterogeneous. Characteristically, but not specifically, numerous microvacuoles could be observed in most instances. In typical cases, they organized like necklace pearls beneath the inner cell membrane. They could correspond to the pinocytosis vacuoles described in normal DC. Another characteristic was the association with cytoplasmic expansions resembling pseudopodia. Such expansions have also been observed on normal pDC in culture with IL-3. Malignant cells from early pDC neoplasms did not look like plasma cells, as did normal pDC isolated from blood or secondary lymphoid organs. Ultrastructural examination showed no rough endoplasmic reticulum (personal results).

According to the previous maturation sequence, it could be proposed that pDC malignant cells are arrested in their maturation close to the stage of...
pro-pDC. It should be remembered that plasmacytoid T-cell lymphoma might also develop from the malignant transformation of pDC. These tumor cells exhibit features that are similar to those of normal pDC: they look like plasma cells under light microscopy and ultrastructural examination, express CD4 and CD68 without CD3 or Ig chains, and localize in the T-cell areas of lymph nodes. Since plasmacytoid T cells or plasmacytoid monocytes unequivocally relate to plasmacytoid dendritic cells, neoplasia arising from these cells should be considered of pDC origin. Given their plasma cell morphology and localization, it could be postulated that malignant cells are arrested at the pDC precursor stage of maturation. Only 12 cases have been reported in the world literature.\(^8\),\(^100\)-\(^108\) They were considered as constituting a defined entity.

Ultimately, two different neoplasias appear to arise from the malignant transformation of pDC, which probably represent two subsets or two stages of maturation. In plasmacytoid T-cell lymphoma, the normal equivalent is plausibly at the precursor step, whereas it is more immature in the newly described entity, hence the proposal of its designation as early pDC.

**Which origin for malignant CD4\(^+\) CD56\(^-\) lin DC: lymphoid or myeloid?**

The origin of normal pDC is still under debate.\(^57\)

Numerous data presently favor a lymphoid origin. First, apart from the low expression of CD33, normal and malignant pDC are devoid of classical myeloid-associated antigens. Conversely, these cells may express lymphoid-related antigens, such as CD2, CD5, CD7, granzyme B, Spi-B or TdT. Normal and malignant pDC also express several lymphoid-restricted transcripts, among which specific transcripts for the invariant chain of the pre-T receptor (pT\(\alpha\)), \(\lambda\)-like chain of Ig V-preB and Spi-B. Interestingly, a subtractive hybridization technique against monocyte-derived DC revealed that 92 out of 650 sequences analyzed were B-cell transcripts, including Ig \(\lambda\) and \(\kappa\) light chain, the surrogate \(\kappa\) light chain and the pentamer IgM and dimer IgA joining component J chain. Thus, the high number of Ig-related genes found in pDC suggests a common precursor with the early steps of B-lymphocyte development. Also in favor of a lymphoid origin is the demonstration that DC, T-, B- and NK lymphocytes, but not myeloid cells could differentiate from bone marrow CD34\(^+\) progenitors.\(^109\),\(^110\) Another indication is the finding that thymic CD34\(^+\) progenitors display DC and NK potentials.\(^111\)-\(^113\) However, DC differentiated from lymphoid CD34\(^+\) progenitors in these studies are still poorly characterized, and it is not known at present whether they relate to myeloid DC or pDC, or whether they represent an original subset. The most important argument in favor of a lymphoid origin is the demonstration that two transcriptional inhibitors, ld2 and ld3, are able to block the development of pDC, B and T cells, but not that of myeloid lineage cells.\(^21\) Moreover pDC do not express the macrophage colony-stimulating factor (M-CSF) receptor and do not differentiate into macrophages upon incubation in vitro with M-CSF.\(^25\),\(^26\)

Data arguing for a myeloid origin are scarcer. They arise from the work by Olweus et al., who demonstrated that CD123\(^{high}\) DC could be generated from a M-CSF\(^-\)CD34\(^+\) progenitor.\(^114\) Otherwise, CD13, CD33, and CD11c can be acquired by both normal and malignant pDC upon in vitro culture.\(^25\)

The observation of such mixed lymphoid and myeloid features led Galibert et al. to postulate that pDC in fact represent a composite group of both myeloid and lymphoid early committed cells that are characterized by their ability to differentiate in vitro into DC.\(^115\)

Interestingly, myeloid disorders have been observed in both malignant entities arising from pDC. In our series of 23 cases from the GEIL,\(^2\) one patient suffered from chronic myelomonocytic leukemia (CMML), another had a history of myelodysplastic syndrome (MDS), and 3 others demonstrated myelodysplastic features on the bone-marrow Giemsa stain. In the literature, 2 cases of acute myeloid leukemia (AML) of the FAB subtype M4 were diagnosed during the course of the disease, and MDS occurred in another patient.\(^14\) More surprisingly, myeloid disorders were systematically observed in plasmacytoid T-cell lymphomas and were part of the definition of the entity. They occurred either before the diagnosis of lymphoma, at the same time, or during the follow-up. They consisted in MDS, AML, CMML or chronic myelogenous leukemia (CML).\(^100\)-\(^108\) Whenever searched for, the Philadelphia chromosome was not found. Likewise, an increase in myeloid- and lymphoid-related DC has recently been demonstrated in AML, which exhibited the same chromosomal abnormalities as myeloid blasts, thus confirming a common origin.\(^62\)

The mainly lymphoid characteristics of pDC and the association of both neoplasms with myeloid disorders led us suppose that the malignant process involves a common lymphoid and myeloid progenitor at a very early stage of differentiation. This is the same reasoning as for CML. In the chronic phase of this pathology the clone is myeloid, but about 10% of the blast crisis cells are B or more rarely T lymphoblasts. In such cases, a common lymphoid and myeloid progenitor has formally been involved by the demonstration of the Philadelphia chromosome expression in all subsets.\(^116\),\(^117\)

**Clinical and biological features**

**Patients**

The clinical characteristics of patients reported in the literature were similar whatever the phenotype
of the tumor cells, i.e. typical (59 cases), CD33+ (9 cases) or undetermined (16 cases), cyCD33+ (11 cases) or CD56− (1 case).

Patients from eastern as well as from western countries have been described. Two Africans were also identified. Although the relevance of race has infrequently been documented in the literature, this malignancy probably does not have the same predilection for Asians as true NK and CTL lymphoma. Both sexes are involved, but with a large imbalance in favor of men (sex ratio: 3/1). Essentially elderly people are affected, but the disease may develop at all ages of life. A review of the literature showed that about 67% of the patients were over the age of 50 years (mean: 70 years, max: 89 years), whereas 7% were adults between 36 and 50 years, and 18% were young people between 18 and 35 years. The cases of 4 children (6, 8, 8 and 14 years old), and 3 infants (3, 6 and 7 months old) have been documented. The malignant cell immunophenotype was typical in all 4 young children. CD33 and CD94 were expressed in one infant, and cyCD3 in another. For the last infant, cyCD3 was negative and the results for CD13 and CD33 were lacking. Similarly, plasmacytoid T-cell lymphoma affects mostly elderly people (10 cases over the age of 50 years), and occa-
tionally young adults (one 22-year old man) or children (one 6-year old child).

Of note, EBV-related antigens were studied in most of the 96 cases and found to be negative except in 2 cases, which contrasts with the findings in the majority of tumors of NK origin. Furthermore, no relation could be observed with other lymphotropic viruses such as HIV, HBV, HCV, HHV8, HHV6, CMV and HTLV-1 or 2.

**Clinical presentation**

Clinical features were similar, regardless of the presence or not of CD33 and cyCD3 (Figure 4).

A typical disease presentation consists in isolated cutaneous lesions at the time of diagnosis, followed by disseminated tumor localizations within a few months. Exceptionally, the skin has remained the sole anatomical site involved for more than 6 months. Only Brody et al. reported a case with a history of multiple cutaneous nodules present for more than 15 years. The diagnosis of primary cutaneous lymphoma was proposed for these patients. Other patients presented immediately with disseminated disease, involving the bone marrow and peripheral blood, nodal and extra-nodal sites: the skin was involved in almost all cases. These malignancies were diagnosed as leukemia. Intermediate presentations were also frequently observed. Apart from the tumor syndrome, patients were otherwise in good health, with no systemic symptoms. Fatigue, weight loss or fever have rarely been reported, which contrasts with the symptoms of plasmacytoid T-cell lymphoma. Furthermore, no sign of immunodeficiency or autoimmunity could be suspected from the literature. Thus malignant DC seemed to be silent regarding such functions in vivo. Of note, the clinical course was aggressive, with the outcome being frequently and rapidly fatal.

Skin involvement was the most constant symptom of the disease, affecting more than 90% of the patients. It was noted at presentation, and represented the main initial reason for seeking medical advice. In several cases only one cutaneous lesion was described. More often, however, the lesions were multiple, either grouped in a defined region or disseminated. Almost all territories could be involved – scalp, face, trunk, arms and legs. The size of the tumors ranged from a few millimeters up to more than 10 cm, and they had varied appearances. Skin lesions were described as plaques, papules, bruise tumefactions or subcutaneous nodules. They could be erythematous, hyperpigmented, reddish, bluish, bright red, purpuric, erosive or even necrotic, sometimes with crusts, depending on the case. One unique patient presented an unusual purpura, looking like a black ring, of the lower eyelid. In some instances, the clinical aspect was evocative of angiosarcoma, or mycosis fungoides or Kaposi’s sarcoma.

Palpable or deep lymph nodes were involved in about half of the cases at presentation. This nodal involvement could be localized or disseminated. Extra-nodal lymphoid organs were also often invaded, e.g. spleen (25%), liver (16 %) or tonsils (4 cases), as well as mucosa-associated lymphoid tissues i.e. nasopharynx (8 cases), gum (2 cases), ocular conjunctiva (2 cases) or bronchial mucosa (1 case), either at presentation or during follow-up. Bone marrow involvement was also very frequent, either at presentation (52%) or otherwise rapidly developing during the course of the disease (35%). Only a few patients had a long survival without proven bone marrow localization. Most often malignant cells could be observed simultaneously in the peripheral blood at diagnosis (36%) or during follow-up (20 %), and in several observations these were consistent with an overt leukemia. It should be pointed out that bone marrow and circulating malignant cells exhibited the immunophenotype of pDC, except for two cases of M4-AML. This is very different from the situation in plasmacytoid T-cell lymphoma, in which leukemic cells were always myeloid cells (except one case of null acute leukemia). Cases with initial skin involvement and further development of leukemia are reminiscent of chloroma. These tumors consist in tissue localization of malignant monoblastic cells that secondly evolve as AML. It is noteworthy that the diagnosis of chloroma is highly exceptional, whereas cutaneous lesions in early pDC leukemia are rather constant.

Other sites were rarer, but varied i.e. lung (2 cases), kidney (2 cases), muscle (2 cases), bone (3 cases), lachrymal glandular and anterior chamber of the eye (3 cases), female reproductive tract (1 case), heart (1 case) or central nervous system (CNS) (11 cases). In the single patient on whom a post-mortem examination was performed, multiorgan involvement was found, showing that malignant cells might develop widespread localizations.

Inflamed skin is a normal site for pDC. They have been identified in this site according to their plasma cell morphology, or expression of CD123high CD11c-immunophenotype. Curiously, plasmacytoid T-cell lymphomas, which are possibly blocked at this precise stage of maturation, infrequently invade the skin. Conversely, early pDC malignancies, which are more immature, demonstrate an almost systematic tropism for cutaneous tissue. Of note, skin lesions have often been associated with the presence of CD56 on malignant cells from various diseases, such as NK-cell lymphoma or myeloid leukemia. However the implication of this adhesion molecule has never been understood. At present, increasing knowledge about cell trafficking is emerging, and numerous cellular adhesion molecules and chemokine receptors, such as CCR3, CCR4, CCR6, CCR10 and CLA, have been implicated in skin homing. Their expression and functionality on normal and malignant pDC are presently under

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investigation. Normal CD34+ progenitors for pDC have been described in the bone marrow, thymus, cord blood and fetal liver, where differentiated DC have been observed in the thymus, secondary lymphoid organs and peripheral blood, where they acquired the morphology of plasma cells. They could also be present in the skin, CNS and bronchoalveolar fluid in the case of immunological conflict. In tissues, they are identified with difficulty, in the absence of specific positive markers. At present, it remains unknown whether the various malignant DC localizations are due to some indefinite oncogenic events, or just mimic undiscovered routes of their normal equivalent.

Hematologic findings

In the series of Feuillard et al., 91% of the patients displayed cytopenia, which was symptomatic of bone marrow failure. Thrombocytopenia was observed in 78% of the cases, anemia in 34% and neutropenia in 34%. Cytopenia has also been reported in the literature, but the results are too inconsistently recorded to allow statistical treatment. Leukocytosis existed in 22% of the cases at diagnosis, but such a result might be biased by the kind of recruitment in hematologic laboratories. In these cases, malignant cells represented from 1% to more than 90% of mononuclear cells.

Histologic findings (Figure 4)

Malignant cell infiltration in cutaneous lesions or in lymph nodes has been widely described in the literature and showed the same common features. In agreement with cytological observations, the cells could be small, intermediate or large size, and the cytoplasm was agranular in almost all cases. Malignant cells looked like blasts in most instances, and did not resemble NK cells from nasal or nasal-type lymphomas, which are at a mature stage of differentiation with cytoplasmic azurophilic granula-
tions. As witnessed by Ki67 expression, proliferation of the malignant cells varied between cases. In cutaneous lesions, the cells diffusely invaded the dermis. They sometimes reached the subcutaneous fatty tissue, but never the epidermis. They only exceptionally displayed an angiocentric growth pattern, or an association with vascular damage. Red blood cells could sometimes be observed in the lesions, but inflammatory cells were not present. Of note, normal pDC also localize in the dermis.

In lymph nodes, malignant DC always accumulate in T-cell areas, like their normal counterparts.

Cytogenetic characteristics

The largest cytogenetic investigations available to date on pDC malignancies involved 20 patients, who were studied by Leroux et al., using conventional cytogenetic and 24-color fluorescent in situ hybridization. The clonal nature of the DC expansion was confirmed in all the cases with karyotypic abnormalities (66% of the patients). In most tumors the karyotype was complex, showing a mean of 6.8 anomalies per clone. Characteristically, imbalance in chromosomal material tended to predominate over gene-specific rearrangements. Indeed, of 14 cases with chromosomal aberrations, 9 were hypodiploid-hypotetraploid, 3 were pseudo diploid, and 2 were hyperdiploid. Recurrent abnormalities were described, and these affected 6 major chromosomal targets, i.e. 5q21 or 5q34 (72%), 12p13 (64%), 13q13-21 (64%), 6q23-qter (50%), monosomy 15p (43%) or 9 (28%) (Figure 2). Interestingly, these abnormalities are shared by hematologic malignan-
cies of lymphoid or myeloid origin. No single anomaly could be considered specific for the entity of pDC malignancies: however, their accumulation in the same cell was a characteristic feature.

To date, cytogenetic analysis has been performed in only 23 other patients in the literature. Normal karyotypes were also observed, as were similar complex chromosomal aberrations.

Response to therapy and prognosis

Excellent initial chemosensitivity, but early relapses and a rapidly fatal outcome characterize pDC neoplasms. Indeed, complete remission could easily be obtained for most patients, but relapse always occurred rapidly, despite many different protocols of intensive chemotherapy and/or radiotherapy. Several remissions could be induced, sometimes 3 or 4, but death always occurred within a few months. This was highlighted in the series by Feuillard et al., in which the median disease-free survival was 9 months (range: 3 up to 18 months), and only 52% of the patients were alive at one year and 25% at 2 years of follow-up. It is worth noting that some isolated cases did not have such a drastic outcome. Two patients exhibited isolated cutaneous nodules for about 15 years and 2 years. Three other patients were long survivors. The first case was an 8-year old boy with disseminated disease and overt leukemia. He was treated according to the Brazilian protocol for ALL/93 and has been in remission for 7 years. The other two patients were 6 and 29 years old. They also presented with disseminated disease including bone marrow involvement. Both received an allogenic bone marrow transplant at first complete remission and have remained free from disease for 8 and 6 years, respectively. A total of 6 other patients have also been treated with allogenic bone marrow transplant. Four patients achieved complete remission, but the follow-up did not exceed 17 months; these four patients were a 29-year old man with a typical immunophenotype and disseminated disease, a 7-month old boy, CD33+, disseminated disease, a 29-year old man, CD33 result missing, only cutaneous diffuse lesions; and a 24-year old...
woman with a typical immunophenotype and disseminated disease. Both remaining patients had recurrence after bone marrow transplantation and died after 38 and 39 months of disease evolution. These two patients were young adults, 28 and 35 years old, with a typical immunophenotype and disseminated disease.\textsuperscript{2,13} On account of disease aggressiveness, 4 patients were tentatively treated by intensification and subsequently autologous peripheral stem cell transplantation. Long-lasting complete remission was obtained in 3 cases,\textsuperscript{12,88,69} and early relapse occurred in one case.\textsuperscript{10} Unfortunately, follow-up was less than 1 year in 3 cases, and it is thus not possible to determine whether such a treatment is superior to conventional chemotherapy.

Regarding the results of these different therapeutic strategies, allogeneic stem cell transplantation was the only therapy that led to a possible cure of the disease. Thus, given the aggressiveness of this neoplasm, such treatment should be proposed whenever possible. Aggressive chemotherapy, specific for either lymphoid or myeloid malignancies, did not lead to prolonged survival, except in one infant, and the effectiveness of intensification followed by autologous stem cell transplantation has not yet been proven. Based on the immunocompetent nature of the malignant cells, an alternative therapy with non-myeloablative stem cell transplantation might be proposed, especially for older patients. Interestingly, protocols including differentiating agents for DC might also be tested, since malignant cells from the pathology are not blocked in vitro towards end lineage cells. Of note, neuromeningeal prophylaxis should be administered to all patients, given the frequency of CNS relapse.

**Conclusions**

CD4\textsuperscript{−}CD56\textsuperscript{+} lin\textsuperscript{−} malignancies are rare: only 96 putative cases have been reported in the world literature. Immunophenotypic, morphologic, cytogenetic and clinical findings revealed many common features corresponding to a well-defined clinicopathological entity. Furthermore, pDC at an early stage of differentiation were identified as the normal counterparts of the tumor cells. Relative to the plasmacytoid T-cell lymphomas, which also develop from the malignant transformation of pDC, possibly at the precursor stage of maturation, we propose a reclassification of this entity, previously referred to as blastic NK-cell lymphoma in the WHO classification, as early pDC leukemia/lymphoma. Obviously, this entity differs from CD56 positive NK lymphoma, in particular of nasal and nasal-type. Malignant cells demonstrated a blastic morphology without azurophilic granulations, no association with EBV, no angiodestruction, no cytoplasmic perforin, and a unique immunophenotype associating CD4 and CD56 in the absence of other NK-associated antigens, in most instances.

Malignancies involving tumors of accessory dendritic cells and histiocytes are very rare. They have been recently referenced and classified by the International Lymphoma Study group.\textsuperscript{134} They consist in histiocytic sarcoma, Langerhans’ cells tumors, follicular dendritic cell tumor/sarcoma and interdigitating dendritic cell tumor/sarcoma. It should be underlined that follicular dendritic cells are not hematopoietic cells, in contrast to all the other tumors, and interact not with T lymphocytes, but with B lymphocytes. One difficulty about such cellular expansions is to determine whether they are clonal or not, and whether represent tumor or reactive populations.\textsuperscript{58,105} Of note, plasmacytoid T-cell lymphoma and early pDC leukemia/lymphoma should be added to this group. Like most of these tumors, early pDC leukemia/lymphomas are high-grade malignancies with an aggressive course and short survival. Only follicular dendritic cell tumor/sarcoma and interdigitating dendritic cell tumor/sarcoma follow an indolent behavior in most instances.\textsuperscript{113,135} APC-related tumors display varied clinical presentation with mostly nodal or extra-nodal involvement. Of note, cutaneous lesions and bone marrow infiltration could be observed in all groups, but were a quite constant localization only in early pDC malignancies. Early pDC malignancies should be recognized very promptly by physicians because of the very poor prognosis of patients with these diseases and the need for aggressive therapeutic protocols. Allogeneic stem cell transplantation has proven to be the sole possibly curative strategy so far. However, it is not clear at present whether it works because of an intensified anti-tumor regimen, or as a consequence of an immune-induced reaction.

The diagnosis of early pDC malignancy may be suspected from a set of converging features from the clinical presentation, cytologic, histologic, cytogenetic and molecular findings. However, since none of these characteristics is specific, the final diagnosis relies on a compatible immunophenotype. The clues to diagnosis are CD4 and CD56 positivity associated with CD3, CD13, CD33 and CD14 negativity. So far all malignant subsets exhibiting such a phenotype have been revealed to be pDC. Malignant cells expressing CD33 or cyCD3e, but also lacking CD56 could possibly belong to this entity too. In these cases, specific markers, such as CD123\textsuperscript{high}, CD45RA, BDCA-2, or BDCA-4 should be identified to demstrate this affiliation. Indeed, such neoplasms must be differentiated from AML, mixed myeloid/NK leukemia, T-ALL or NK-related leukemia and lymphoma, which may express very similar phenotypes. Finally, since malignant and normal pDC have always exhibited the same properties so far, these tumor cells represent an inestimable source of pDC for in vitro investigations.
CD4+ CD56+ lin- pDC neoplasm

References


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