**Alpha-Interferon Secreting Blastic Plasmacytoid Dendritic Cells Neoplasm. A Case Report With Histological, Molecular Genetics and Long-Term Tumor Cells Culture Studies**

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**INTRODUCTION**

This disease named blastic plasmacytoid dendritic cell neoplasm (BPDCN) in the last WHO classification of Tumors of Hematopoietic and Lymphoid Tissues is a recently identified disease arising from interferon (IFN)-producing plasmacytoid dendritic cells (PDCs). It is a highly malignant disease, clinically characterized by a striking cutaneous tropism and rapid, lethal dissemination, with or without leukemic phase, within several months. It is a rare disease representing approximately 0.8% of the primary cutaneous lymphomas.

Initially, it was phenotypically defined by the coexpression of CD4 and CD56 antigens in the absence of any common myeloid, T-, B-, NK lymphoid lineage markers. Recent phenotypical advances and availability of new antibodies, working on paraffin sections and recognizing PDC-related antigens (eg, BDCA-2, BCL11a, CD123, CD2AP, and TCL1), now help pathologists to confirm the diagnosis of BPDCN.

Likely due to BPDCN’s rarity, data are limited and variable regarding its ontogeny, its genotype, and the properties of its constituent tumor cells. In this case report, the authors document the results of extensive immunophenotyping, genotyping [karyotype, array-comparative genomic hybridization (CGH), and fluorescent in situ hybridization (FISH)], and long-term tumor cell culture.

**CASE REPORT**

**Clinical Data**

This 82-year-old man presented in November 2007 for the evaluation of a reddish, purpuric cutaneous nodule of the forehead, which appeared several weeks earlier and weakness. At the time of consultation, the nodule measured 3 cm in diameter (Fig. 1A). A complete blood cell count displayed anemia and thrombocytopenia of consultation, the nodule measured 3 cm in diameter (Fig. 1A). A complete blood cell count displayed anemia and thrombocytopenia. During the 5-week interval between the diagnosis and hospitalization, the patient developed, disseminated purpuric lesions (Fig. 1B) and rapidly increasing circulating blast cells. Staging revealed bone marrow involvement but not lymph node enlargement or hepatosplenomegaly. Treatment by mini-CHOP (adriamycine, cyclophosphamide, vincristine, prednisone) was started 1 month after the diagnosis. Disease remission occurred after 3 courses of chemotherapy with both skin lesions and circulating blast cells quickly clearing after the first course of therapy. Five months after the diagnosis, while still in remission, he developed meningoencephalitis and disseminated intravascular coagulation. Seven months after the diagnosis, the disease relapsed and he expired.
Histology and Immunohistochemistry

A snap-frozen skin biopsy, a formalin-fixed paraffin-embedded skin biopsy, and a formalin-fixed paraffin embedded bone marrow biopsy were available for investigation. The routine skin biopsy showed that the tumor infiltrated the dermis and obliterated adnexal structures but spared the epidermis and the subepidermal region (Fig. 2A). There were no features of angiodestruction. Tumor cells were predominantly medium sized (Fig. 2B) with a few large cells and had minimal cytoplasm without granulation on Giemsa stain. Nuclei were irregular, with finely dispersed chromatin, and had several small or medium-sized nucleoli. Mitotic figures were rare. No plasma cells or eosinophils were found. Histochemical butyrate—esterase staining was negative on frozen sections. Histological study of the bone marrow biopsy showed a discrete infiltration by similar appearing tumor cells. Blood smears of the blastic leukemic phase showed lymphoid-appearing undifferentiated blasts with round or slightly irregular eccentric nuclei containing small nucleoli. Nuclei were surrounded by slightly or moderately gray–blue cytoplasm. Occasionally, cytoplasmic microvacuoles could be seen. Histochemical myeloperoxidase and butyrate—esterase stains were both negative.

The immunohistochemistry was performed on paraffin sections using a Benchmark automate (Ventana Medical System, Illkirch, France) (Table 1). Tumor cells expressed: CD4, CD56, CD68 (dots), CD123 (Fig. 3A), CD303 (BDCA2) (Fig. 3B), TCL1 (Fig. 3C), and CD2AP. Mib-1 labeled 10% of the blastic cells. No tumor cells expressed CD2, CD3, CD5, CD7, CD20, CD33, TIA-1, granzyme B, TdT, myeloperoxidase. Tumor cells also expressed Mx-1 protein (Fig 3D). Mx-1 is a GTPase induced by IFN-I, which plays an important antiviral role in host defense. Immunocytochemistry was also performed on air-dried cytopsin smears of cultured cells with CD4 CD56, CD123, and CD303. Cultured cells also expressed these 4 antigens.

EBER In Situ Hybridization and Polymerase Chain Reaction Studies

Epstein-Barr virus–encoded small RNA in situ hybridization was negative. By polymerase chain reaction (PCR), no clonal T or B-cell genes rearrangements were detected.
Karyotype, Array-CGH, and Fluorescent In Situ hybridization Hybridization

Karyotyping
Cytogenetic analyses of involved bone marrow samples using R and G banding techniques showed normal karyotype.

Comparative Genomic Hybridization
To carry out array-CGH on DNA extracted from snap-frozen skin biopsy and bone marrow samples, we used the IntegraChip genomewide bacterial chromosome (BAC) array of 4898 BAC clones (600-kb median spacing) of IntegraGen (Evry, France). The genomic DNA-labeling Kit (Enzo, Farmingdale, NY) was used to label 1 μg of the reference DNA with Cy3. The labeled products were purified with QiaQuick PCR purification kits (Qiagen GmbH, Hilden, Germany). After the addition of tRNA (48 μg) plus a quantity of 50 times the mass of the labeled DNAs, the products were concentrated by vacuum centrifugation, resuspended in 43 μl of hybridization solution (Ambion, Austin, TX) and deposited on the array under a coverslip. Hybridization in a sealed chamber (Corning, Schiphol-Rijk, The Netherlands) lasted for 66 hours at 55°C and posthybridization treatment was that of a published protocol with 2 IU/mL. Clusters of blastoid cells were obtained immediately. Four clusters (oligoclones) were picked up and cultured separately in a microplate. Long-Term Tumor Cells Culture
Tumor cells were isolated from leukocyte-rich decanted heparinized plasma. They were cultured on feeder-layer (MRC5) at 37°C (5% CO₂) in RPMI completed with 10% MRC5-supernatant (minimal essential medium containing 7.5% fetal calf serum). IL3 at a concentration of 10 ng/mL was added in the medium of culture during the 4 first months of culture. Biological alpha-IFN (IFN-I) activity was assayed in the supernatant of culture at 2 weeks and 24 weeks. IFN-I levels were measured by assessing the reduction of the cytopathic effect of vesicular stomatitis virus–infected Madin-Darby bovine kidney cells as previously described. This method of reference, used in the laboratories of virology, is the most sensible for detection of IFN-I. Results are given in international units per milliliter with a threshold of 2 IU/mL. Dosage was repeated twice at each time. Free RPMI with 10% MRC5-supernatant was used as negative control.

Clusters of blastoid cells were obtained immediately. Four clusters (oligoclones) were picked up and cultured separately in the same way, adding 20% fetal calf serum. Tumor cells were cultured during 6 months through 17 passages. At the fifth month, the growth began to decrease progressively. When stressed by flask shaking or heating, the tumor cells tended to acquire dendritic morphology and stick to the MRC5 layer. After 6 months, the growth was over.

Results of the IFN-I activity showed significant levels. At 2 weeks, the activity was of 50 IU/mL and decreased progressively to 6 IU/mL at 24 weeks (Fig. 5). Negative controls showed 0 IU/mL.

DISCUSSION
Since the first description of the entity in 1999, significant improvements have been made in the knowledge of the disease leading to the recognition of a specific tumor entity likely originating from PDC (or its precursors/stem cell). Clinically, BPDCN is a well-defined hematological tumor characterized by strong skin tropism, whose presence is heralded in most patients by a skin tumor that rapidly spreads to involve lymph nodes and/or bone marrow and/or peripheral blood. BPDCN’s distinct immunophenotype, CD4+, CD56+, PDC markers+, and the skin sample and additional losses on chromosomes 2 and 5: del (2) (p11.2) (2.7 Mb), del (2) (p16.1) (4.3 Mb), del (2) (p21p22.1) (5.3 Mb), del (2) (p24.1) (2.8 Mb), del (5) (q23.2) (3 Mb), del (5) (q31.3q33.1) (7.5 Mb), and del (5) (q33.2q33.3) (7 Mb).

Fluorescent In Situ Hybridization
FISH analysis was performed by using bacterial artificial chromosome (BACs) probes of the region for chromosomes 2p, 5q, 13q using and commercial probes for 12p (ETV6, DAKO) and 17p (TP53, Vysis) according to the manufacturer’s instructions. For bone marrow, metaphase spreads were hybridized overnight at 37°C with labeled. After posthybridization washes, the chromosomes were counterstained with DAPI (Vectorshied, Vector, Burlingame, CA). Cell images were captured using the Isis Digital Imaging System (Metasystem, Altussheim, Germany).

FISH analysis on bone marrow samples confirmed all the deletions with specific probes for chromosomal regions 2p, 5q, 12p (ETV6), and 13q. However, the 17p (TP53) deletion was not detected. Furthermore, ETV6 (red and green) and CEP 12 (aqua) FISH probes showed loss of 12p region in 80% of interphase nuclei, either monoallelic (50%) or bi-allelic (30%) (Fig. 4). FISH was also performed on snap-frozen skin sample sections, exhibiting the same deletions and matched the distribution of monoallelic and biallelic deletion of ETV6.

Long-Term Tumor Cells Culture
Tumor cells were isolated from leukocyte-rich decanted heparinized plasma. They were cultured on feeder-layer (MRC5) at 37°C (5% CO₂) in RPMI completed with 10% MRC5-supernatant (minimal essential medium containing 7.5% fetal calf serum). IL3 at a concentration of 10 ng/mL was added in the medium of culture during the 4 first months of culture. Biological alpha-IFN (IFN-I) activity was assayed in the supernatant of culture at 2 weeks and 24 weeks. IFN-I levels were measured by assessing the reduction of the cytopathic effect of vesicular stomatitis virus–infected Madin-Darby bovine kidney cells as previously described. This method of reference, used in the laboratories of virology, is the most sensible for detection of IFN-I. Results are given in international units per milliliter with a threshold of 2 IU/mL. Dosage was repeated twice at each time. Free RPMI with 10% MRC5-supernatant was used as negative control.

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negative pan B-, pan T-cell, and myelo-monocytic-cell markers, sets it apart from other hematologic malignancies.

Based on clinical and phenotypical findings, the case presented here is a typical case of BPDCN. The disease began with a skin nodule that rapidly disseminated to other cutaneous surfaces, bone marrow, and blood. The pattern of skin involvement, the tumor cell cytology, and its immunophenotype are all characteristic of BPDCN. Moreover, tumor cells expressed the PDC markers CD303 (BDCA2), CD123, CD2AP, and TCL1, which is a reference panel for the diagnosis of BPDCN.

Genetic data are limited for this malignancy. Karyotype often shows complex aberrations, but specific abnormalities have not been identified. However, 6 major recurrent chromosomal targets are recognized, namely, 5q (72%), 12p (64%), 13q (64%), 6q (50%), 15q (43%), and 9 (28%). A first array-CGH and gene expression profiling study conducted on 5 patients from Dijkman et al demonstrated recurrent deletion of regions on 4q34, 9p13-p11, 9q12-q34, and 13q12-q31, resulting in the loss of tumor suppressor genes such as RB1 and LATS2. Jardin et al performed high-resolution array-CGH and quantitative multiplex polymerase chain reaction of short fluorescent fragments (QMPSF) on 9 BPDCN cases, showing losses of multiple genes involved in the G1/S transition. Especially common were deletions of chromosomes 9 (harboring the CDKN2A and CDKN2B genes), 13q (containing the RB1 locus), 17p (containing TP53), or 12p (including the tumor suppressor genes CDKN1B and ETV6). In our case, the karyotype did not show any chromosomal rearrangement, but array-CGH profile demonstrated deletions, in accordance to what should be expected from the previous studies cited above, particularly for chromosomes 5, 6, 12, and 13 (without RB1 deletion). However, contrarily to Jardin et al, Wiesner et al, Lucioni et al we did not detect any abnormalities on chromosome 9 and chromosome 17 (P53 gene) by array-CGH and FISH analysis. In their recent study, Lucioni et al showed that the 9p21.3 deletion (p16-INK4a gene) had a worse prognosis. Although our patient did not demonstrate this deletion, he still died very quickly, 7 months after the diagnosis.

FIGURE 3. Immunohistochemistry. The lesional cells strongly express CD123 (A), CD303 (B), TCL1 (C, nuclear and cytoplasmic staining), Mx-1 Protein (D).
In our patient, the CGH study showed a discrepancy between skin and bone marrow with additional genomic abnormalities found in bone marrow involving chromosomes 2 and 5. The acquisition of additional genetic defects in the disseminated disease may reflect tumor progression and a high degree of genomic (chromosomal) instability. This finding may support the hypothesis, previously discussed,\(^4\) that BPDCN could be a primary skin disease, which secondary involves other organs and blood.

The CGH analysis showed, on skin and bone marrow, 12p12.1p13.31 region deletion, which contains CDKN1B and ETV6 tumor suppressor genes. In hematological malignancies, alterations of ETV6 gene are frequently observed.\(^{14}\) One allele is involved in a translocation resulting in the formation of a new fusion gene, whereas the second allele is lost.\(^{16}\) However, in BPDCN, translocations involving ETV6 have not yet been reported by karyotyping.\(^{14}\) These concomitant translocations and deletions led to the suggestion that ETV6 may act as a tumor suppressor. However, the oncogenic relevance seems to be related to the formation of fusion genes, not deletions.\(^{14}\) ETV6 gene acts as a transcriptional repressor, and the relevance of the ETV6 loss alone is at present unclear.

We cultured in vitro tumor cells during 6 months. The main goal of this culture was to look for IFN-I production by tumor cells. We could demonstrate by biological dosage that the tumor cells in the supernatant of culture secreted a high amount of IFN-I. The concentration of IFN-I decreased in parallel to the impoverishment of the culture cell. Maeda et al.\(^{17}\) published in 2004 a cell line named CAL-1. They showed that CAL-1 cells were able to secrete large amount of alpha tumor necrosis factor, but little IFN-I after stimulation by TLR9 ligand of CPG ODNs. However, they utilized a commercially available enzyme-linked immunosorbent assay kit, instead of the assay used in this report. Interestingly, they also showed that CAL-1 cells could transform into mature dendritic cells, which we also observed in our cultured cells. Heat shock and mechanical stress from culture flask shaking triggered growth of long dendrites, which had a strong tendency to stick to the bottom of the flask like dendritic cells. This capability of tumor to mature into dendritic cells was also noticed in a short culture by Chaperot et al.\(^{18}\) Inducing maturation of blast cells in vivo might be a key for the treatment of the disease as it could be for other hematologic malignancies such as acute promyelocytic leukemia (AML3).\(^{19}\)

**CONCLUSIONS**

The data collected in this case report provides additional biological and genotypic data on tumor cells of BPDCN. This study confirms the capability of tumor cells to secrete IFN-I, demonstrated by the biological IFN-I activity of cultured cells and immunohistochemical expression of Mx-1 protein. Although a common genetic profile involving chromosomes 5, 6, 9, 12, 13, and 15 has been identified, no specific genetic marker has been demonstrated that is specific to BPDCN. Nonetheless, our finding of ETV6 gene deletion deserves further investigation as a putative BPDCN marker.

**REFERENCES**


