Blastic plasmacytoid dendritic cell neoplasm frequently shows occult central nervous system involvement at diagnosis and benefits from intrathecal therapy

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ABSTRACT

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare aggressive myeloid neoplasm which shows a high rate of central nervous system (CNS) recurrence and overall survival (OS) of <1 year. Despite this, screening for CNS involvement is not routinely performed at diagnosis and intrathecal (IT) prophylaxis is not regularly administered in BPDCN. Here, we prospectively evaluated 13 consecutive BPDCN patients for the presence of CNS involvement by flow cytometry. Despite none of the patients presented with neurological symptoms, occult CNS involvement was detected in 6/10 cases evaluated at diagnosis and 3/3 studied at relapse/progression. BPDCN patients evaluated at diagnosis received IT treatment -either CNS prophylaxis (n = 4) or active therapy (n = 6)- and all but one remain alive (median follow-up of 20 months). In contrast, all three patients assessed at relapse/progression died. The potential benefit of IT treatment administered early at diagnosis on OS and CNS recurrence-free survival of BPDCN was further confirmed in a retrospective cohort of another 23 BPDCN patients. Our results show that BPDCN patients studied at diagnosis frequently display occult CNS involvement; moreover, they also indicate that treatment of occult CNS disease might lead to a dramatically improved outcome of BPDCN.
INTRODUCTION

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare disease -i.e. < 1% acute myeloblastic leukemia cases (AML)- with a historically dismal prognosis. Despite most BPDCN patients treated with different intensive chemotherapy regimens (e.g. CHOP, AML-type and acute lymphoblastic leukemia (ALL)-type regimens) achieve complete remission (CR), the great majority relapse early after CR, with further resistance to therapy and a fatal outcome. Of note, a significant percentage (=50%) [1-3] of BPDCN relapses occur in the central nervous system (CNS), either as isolated CNS relapses or in the context of a systemic relapse. This is in line with the relatively high rate (≈10% of cases) [1-6] of overt CNS involvement (i.e. confirmed by cytology) observed at diagnosis in patients with leukemic variant of BPDCN presenting with neurological symptoms.

Although BPDCN prognosis is extremely poor, patients treated with ALL-type regimens that include CNS prophylaxis [1-3] and/or allogeneic hematopoietic stem cell transplantation (AH SCT) [7, 8] appear to show a better outcome. Altogether, these findings suggest that occult CNS involvement might be already present at diagnosis in a significant fraction of BPDCN patients, and could contribute to explain the relatively high number of CNS recurrences [9].

Here, we prospectively evaluated for the first time the presence of CNS involvement by next generation flow cytometry (NGF) in 13 consecutive BPDCN patients studied at diagnosis (n = 10) or at disease recurrence (n = 3). The impact of CNS involvement and CNS-directed therapy in patient outcome was further validated in a retrospective series of 23 additional BPDCN patients.

RESULTS AND DISCUSSION

At diagnosis, most prospectively analyzed patients showed skin lesions (n=10/13; 77%), peripheral blood (7/13; 54%) and/or bone marrow (9/13; 69%) involvement with normal neurological physical examination (n=13/13; 100%) and a good performance status (ECOG≤0/1: n=12/13; 92%) (Table 1). Despite this, cerebrospinal fluid (CSF) samples positive for tumor plasmacytoid dendritic cells were found in 6/10 (60%) cases studied at diagnosis –median of 11 tumor cells/µl (range: 0.6–47), representing 82% of the total CSF cellularity (range: 68%-95%)- in association with ≥20% bone marrow (BM) infiltration by tumor cells (6/6 vs. 1/4 cases, respectively; p=0.03). Except for the high rate of occult CNS involvement at diagnosis, prospectively analyzed here for the first time, the above described pattern of extranodal disease involvement has been previously reported as typical for BPDCN patients [2, 4, 5].

Regarding the 3 patients referred at relapse (5, 7 and 14 months after diagnosis), none had been screened for CNS disease (nor received CNS therapy) at diagnosis; all 3 had their CSF referred because of the onset of neurological symptoms, consisting of seizure in two patients and the presence of symptoms and signs of intracranial hypertension together with involvement of cranial nerves in the remaining case. They all showed CNS involvement by NGF with ≥50% tumor cells (958, 5 and 46 tumor cells/µl, respectively). These results confirm previous observations about the relatively high rate of overt CNS involvement in BPDCN, particularly at relapse [1-3]; in addition, these results also support the notion that many BPDCN patients may already have occult CNS disease at diagnosis, in the absence of neurological symptoms.

Despite BPDCN patients have been found to show already at diagnosis, a rather high rate (=10%) [1-6] of CNS involvement by conventional CSF cytology (e.g. similar to ALL patients), they are neither routinely screened for CNS involvement, nor receive CNS-directed therapy. This is even more striking because CNS relapses have been recurrently reported among BPDCN patients at relapse at higher frequencies than at diagnosis (=50%) [1-3]. Among other reasons, this might be due to the rarity of the disease –and hence the limited experience with these patients-, as well as the inclusion of BPDCN as a myeloid malignancy in the WHO classification [10], where CNS involvement at diagnosis is uncommon (<5% of patients) [11] and therefore, systemic CSF screening is not performed at diagnosis, in the absence of neurological symptoms. Moreover, NGF has not been routinely applied for the diagnostic screening of CNS involvement in BPDCN patients, despite its higher sensitivity over conventional cytology for detecting tumor cells on stabilized CSF samples [12, 13], and the fact that NGF can detect CNS disease before clinical symptoms emerge [14].

Of note, follow-up CSF samples obtained after triple intrathecal therapy (TIT) showed absence of tumor cells in 6/6 CSF+ cases studied at diagnosis, either after one -5/6 cases (83%)- or 4 doses of therapy (6/6 cases; Table 1). At the moment of closing this study, 5/6 cases remained CNS-disease free, in continuous CR; the remaining patient (case#4 in Table 1) had an isolated CNS relapse 6 months after diagnosis. Upon relapse, the patient received intrathecal (IT) chemotherapy, a single dose being sufficient to re-induce CR with NGF-negative CSF; subsequently, he underwent an AH SCT and remains disease-free for >2 years. At the moment of closing this study, only 1/10 patients studied at diagnosis had died in CR (case #5 in Table 1), the other 9 patients remaining alive in CR after a median follow-up of 20 months (range: 6–48 months). In contrast, those three patients studied at disease recurrence suffered disease progression and death, despite IT therapy was administered in 2/3 cases (median overall survival (OS): 7 months) (Table 1). Overall, these findings suggest that BPDCN patients with occult CNS involvement at diagnosis might specifically benefit from
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CNS-directed therapy, particularly if administered (early) at diagnosis; in line with this, early administration of TIT translated into a significantly better outcome and prolonged survival versus cases who did not receive CNS prophylaxis/therapy at diagnosis and presented with CNS-recurrent disease (Figure 1C and 1D).

In order to further validate our results, a series of 23 BPDCN patients previously reported in the literature [1] -and recruited in an earlier time period- was retrospectively analyzed; of note, no major differences were observed between the two cohorts (prospectively vs. retrospectively analyzed patients) in terms of disease features and outcome (Table 1). Five of these 23 patients received IT prophylaxis at diagnosis and 4/23 received CNS-directed therapy at relapse/progression (Table 2). In this independent validation cohort, patients who did receive IT prophylaxis at diagnosis also had prolonged CNS recurrence-free survival (CNS-RFS) (p=0.06) and OS (p=0.03) (Figure 1E and 1F). Univariate analysis of prognostic factors performed in the whole patient cohort (n=36) showed a favorable impact on CNS-RFS and/or OS for children (p>.05; p=0.03), patients receiving ALL-type therapy (p=0.01; p=0.002), AHSCT (p>.05; p=0.003) and IT prophylaxis/treatment at diagnosis (p=0.002; p<.001) (Figure 1G and 1H), the later variable emerging as the only independent (favorable) prognostic factor for CNS-RFS (p=0.02, hazard ratio [HR]=11.2, 95% confidence interval [CI]: 1.4 – 88.8) and OS (p=0.001, HR=7.6, 95% CI: 2.2 – 25.9).

The high rate of CNS involvement found in our study strongly suggests that the CNS could be a persistent blast-cell sanctuary in BPDCN patients with leukemic

ECOG: Eastern cooperative oncology group performance status; BM: Bone marrow; PB: Peripheral blood; CSF: Cerebrospinal fluid; NFG: Next generation flow cytometry; CC: Conventional cytology; IT: Intrathecal; CNS: Central nervous system; AHSCST: Allogeneic hematopoietic stem cell transplantation; M: Male; F: Female; NE: Not evaluated; PD: Progressive disease; CYC & PRED: Cyclophosphamide and prednisone; PD: Progressive disease; CR: Complete remission; SD: Stable disease; Leptom: Leptomeningeal; TIT: Triple intrathecal therapy (methotrexate cytarabine and hydrocortisone); Lip: Liposomal; AraC: cytarabine; HD MTX: High dose methotrexate; NA: Not applicable; NC: No clearance of CSF observed; ALL: Acute lymphoblastic leukemia; NR: Not reported; NS: Not statistically significant. #Due to the confirmed superioritiy of NFG versus CC in detecting occult leptomeningeal disease[12], and the limited volume of available CSF sample, CC evaluation was not systematically performed in parallel *At any time (diagnosis or follow-up).

Patients were grouped according to their CSF status at diagnosis. Results in the summary groups are expressed as number of cases from all cases within the group (for categorical variables) or as median (for continuous variables) and minimum and maximum values.

All the patients had a normal neurological physical examination at diagnosis. 10 were screened for parenchymal involvement with a negative result in all these cases. All but case #7 received IT treatment at some point of the disease but none CNS radiotherapy.

Table 1: BPDCN patients included in the prospective cohort (n=13): Disease features at diagnosis and follow-up including patient outcome

| Patient | Source | Age | Sex | BM | PB | CNS | TIT | PP | IT | CNS-RFS | OS | CR | PD |
|---------|--------|-----|-----|----|----|-----|-----|----|----|--------|----|-----|----|
| #1      |        | 50  | M   |    |    |     |     |    |    |        |    |     |    |
| #2      |        | 12  | F   |    |    |     |     |    |    |        |    |     |    |
| #3      |        | 17  | M   |    |    |     |     |    |    |        |    |     |    |
| #4      |        | 22  | M   |    |    |     |     |    |    |        |    |     |    |
| #5      |        | 18  | M   |    |    |     |     |    |    |        |    |     |    |
| #6      |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #7      |        | 18  | M   |    |    |     |     |    |    |        |    |     |    |
| #8      |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #9      |        | 19  | M   |    |    |     |     |    |    |        |    |     |    |
| #10     |        | 21  | M   |    |    |     |     |    |    |        |    |     |    |
| #11     |        | 21  | M   |    |    |     |     |    |    |        |    |     |    |
| #12     |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #13     |        | 20  | M   |    |    |     |     |    |    |        |    |     |    |

Table 2: BPDCN patients included in the prospective cohort (n=13): Disease features at diagnosis and follow-up including patient outcome

| Patient | Source | Age | Sex | BM | PB | CNS | TIT | PP | IT | CNS-RFS | OS | CR | PD |
|---------|--------|-----|-----|----|----|-----|-----|----|----|--------|----|-----|----|
| #1      |        | 50  | M   |    |    |     |     |    |    |        |    |     |    |
| #2      |        | 12  | F   |    |    |     |     |    |    |        |    |     |    |
| #3      |        | 17  | M   |    |    |     |     |    |    |        |    |     |    |
| #4      |        | 22  | M   |    |    |     |     |    |    |        |    |     |    |
| #5      |        | 18  | M   |    |    |     |     |    |    |        |    |     |    |
| #6      |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #7      |        | 18  | M   |    |    |     |     |    |    |        |    |     |    |
| #8      |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #9      |        | 19  | M   |    |    |     |     |    |    |        |    |     |    |
| #10     |        | 21  | M   |    |    |     |     |    |    |        |    |     |    |
| #11     |        | 21  | M   |    |    |     |     |    |    |        |    |     |    |
| #12     |        | 20  | F   |    |    |     |     |    |    |        |    |     |    |
| #13     |        | 20  | M   |    |    |     |     |    |    |        |    |     |    |

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Figure 1: Prognostic impact of (occult) CSF involvement and administration of IT therapy at diagnosis in BPDCN patients. CNS-RFS (panels A, C, E and G) and OS (panels B, D, F and H) curves are separately shown for the prospective cohort (panels A, B, C and D), the validation cohort (panels E and F) and the combined (prospective and validation) cohort (panels G and H).
presentation, due to the limited power of cytostatic drugs to cross the blood-brain barrier into the CSF and brain parenchyma [15]. This reservoir of leukemic cells may also contribute to BM/systemic disease recurrence. Longer survival rates observed in AH SCT BPDCN patients [7, 8], irrespective of IT medication administration, might be explained by the benefit of the antileukemic effect of donor allogeneic cells [16], able to circulate systemically and cross the blood-brain barrier. Unfortunately, AH SCT is not exempt from relapses (~1/3 of patients) [7, 8], it cannot overcome the poor disease status beyond the first CR [7], and toxicity-related mortality is high [17]. Additionally, many patients are elderly or unfit subjects to undergo such an intensive therapeutic approach.

Recently, SL-401, a novel targeted therapy directed to the interleukin-3 receptor, has shown positive results on BPDCN patients with skin confined disease, whereas long-term benefit has not yet been observed in patients with the leukemic variant/phase of the disease [18]. No comparison can be established in terms of CNS relapses between both approaches (SL-401 vs. IT prophylaxis) since patients showing CNS involvement are excluded from the study (NCT02113982) and sites of relapse/progression after SL-401 administration have not been detailed [18, 19]. Nonetheless, evaluation of IT injection of this targeted therapy will be of great interest, particularly in CNS relapses.

To the best of our knowledge, this is the first study reporting a strikingly high frequency of occult CNS involvement at diagnosis in BPDCN patients, and the potential benefit of CNS-directed therapy to reverse the poor outcome of BPDCN patients. Indirectly, our results also suggest that occult CNS disease might contribute to explain the higher frequency of recurrences (e.g. CNS) observed in these patients, despite their high CR rates. Further prospective studies on larger BPDCN patient cohorts studied at diagnosis are necessary to confirm our findings.

Table 2: BPDCN patients included in the validation cohort (n=23): Disease features at diagnosis and follow-up including patient outcome

| Patient code | Gender | Age | BM% of blast cells | PB% of blast cells | CSF involvement at diagnosis | LYMPHOCYTE CHIMEROLOGY | Response to chemotherapy | CNS involvement | BM involvement | PB involvement | CNS prophylaxis administration at diagnosis (n=15) |
|--------------|--------|-----|-------------------|-------------------|-----------------------------|------------------------|------------------------|-----------------|---------------|--------------|--------------------------------------------------|
| #4R          | F      | 64  | 60                | 8                 | NE                          | AML type                | CR                     | Yes             | No            | Yes          | No, No, No EXITUS PD 10                      |
| #8R          | M      | 70  | Yes              | 0                 | NE                          | AML type                | CR                     | Yes             | No            | No           | No, No, EXITUS PD 13                         |
| #9R          | M      | 70  | Yes              | 80                | NE                          | ALL type                | CR                     | Yes             | No            | Yes          | No, No, EXITUS PD 14                         |
| #10R         | M      | 10  | Yes              | 60                | NE                          | ALL type                | CR                     | Yes             | No            | No           | No, No, EXITUS PD 11                         |
| #11R         | F      | 34  | No               | 0                 | NE                          | AML type                | CR                     | No              | No           | No           | No, No, No EXITUS PD 7                        |
| #12R         | M      | 81  | Yes              | 88                | NE                          | AML type                | CR                     | Yes             | No            | No           | No, No, EXITUS PD 8                          |
| #13R         | M      | 65  | Yes              | 70                | NE                          | ALL type                | ED                     | No              | No           | No           | No, No, No EXITUS PD 6                      |
| #14R         | M      | 70  | No               | 65                | NE                          | AML type                | ED                     | No              | No           | No           | No, No, No EXITUS PD 7                        |
| #15R         | M      | 69  | Yes              | 62                | NE                          | AML type                | CR                     | Yes             | No            | No           | No, No, EXITUS PD 20                         |
| #16R         | M      | 71  | Yes              | 60                | NE                          | AML type                | ED                     | No              | No           | No           | No, No, EXITUS PD 4                          |
| #18R         | M      | 69  | Yes              | 62                | NE                          | ALL type                | CR                     | No              | No           | No           | No, No, EXITUS PD 22                         |
| #19R         | M      | 70  | Yes              | 60                | NE                          | ALL type                | CR                     | No              | No           | No           | No, No, EXITUS PD 22                         |
| #20R         | F      | 58  | Yes              | 60                | NE                          | AML type                | ED                     | No              | No           | No           | No, No, EXITUS PD 3                          |
| #21R         | M      | 82  | Yes              | 60                | NE                          | AML type                | CR                     | No              | No           | No           | No, No, EXITUS PD 71                         |
| #22R         | M      | 42  | No               | 60                | NE                          | AML type                | CR                     | Yes             | No            | No           | No, No, EXITUS PD 70                         |
| #23R         | F      | 45  | No               | 75                | NE                          | AML type                | ED                     | No              | No           | No           | No, No, EXITUS PD 21                         |
| SU0100       | F      | 67  | 12/18            | 7                 | NE                          | 3/18                    | ALL type               | 12/18           | 9/12          | 0/9          | CNS PD 7 (1/14)                                |
| SU0101       | F      | 58  | 12/18            | 7                 | NE                          | 3/18                    | ALL type               | 12/18           | 9/12          | 0/9          | CNS PD 7 (1/14)                                |
| SU0102       | F      | 31  | 2/15             | 7                 | NE                          | 3/18                    | ALL type               | 12/18           | 9/12          | 0/9          | CNS PD 7 (1/14)                                |

| BM: Bone marrow; PB: Peripheral blood; CSF: Cerebrospinal fluid; IT: Intrathecal; CNS: Central nervous System; AH SCT: Allogeneic hematopoietic stem cell transplantation; M: Male; F: Female; NE: Not evaluated; ALL: Acute Lymphoblastic Leukemia; NHL: Non-Hodgkin Lymphoma; AML: Acute Myeloblastic Leukemia; CR: Complete remission; ED: Early death; SD: Stable disease; NR: Not reported; TIT: Triple intrathecal therapy (Methotrexate cytarabine and hydrocortisone); AraC: Cytarabine; MTX: Metothrexate; Dex: Dexamethasone; PD: Progressive disease; TreatTox: Treatment toxicity; NS: Not statistically significant.

Results in the summary groups are expressed as number of cases from all cases within the group or as median. *At any time (diagnosis or follow-up) #CNS IT prophylaxis administration at diagnosis versus not.
MATERIALS AND METHODS

Ethics statement

The study was approved by the Ethics Committee of the Cancer Research Center, and performed following the Declaration of Helsinki. Each participant gave his/her informed consent prior entering the study.

Patients and samples

Forty-one CSF samples from 13 consecutive BPDCN patients (11 males and 2 females; median age: 67 years, range: 11-79 years) were evaluated for the presence of CNS involvement by NGF. Cases were evaluated at diagnosis \( n = 10 \) or at relapse/progression \( n = 3 \) and subsequently, after IT therapy. Diagnosis of BPDCN was based on previously reported recommendations \[10\], including expression of markers claimed to be mandatory to allocate blast cells to the plasmacytoid dendritic cell lineage \[20\], by either flow cytometry or immunohistochemistry of peripheral blood specimens, BM samples or cutaneous lesions. The 10 patients studied at diagnosis received high-risk ALL-type treatment, based on the Spanish PETHEMA protocols (NCT01358201, NCT00853008 and \[21\])(\( n = 9 \)) or HyperCVAD therapy \( n = 1 \), including one dose of TIT as CNS prophylaxis at each treatment phase (Table 1). For CSF-positive cases, additional IT treatment was given -TIT every 72h \( n = 4 \) or liposomal cytarabine (50mg) every two weeks \( n = 2 \)- until two consecutive CSF-negative samples were obtained. In turn, patients with recurrent disease \( n = 3 \) received AML-type or NHL-type (CHOP) therapy (Table 1).

In order to validate the impact of CNS involvement and CNS-directed therapy on patient outcome, an independent validation cohort of 23 BPDCN treated with ALL- \( n = 7 \), AML- \( n = 8 \) or NHL-type \( n = 8 \) therapeutic regimens was retrospectively analyzed \[1\]. The intrathecal treatment administered to these patients is detailed in Table 2.

Multiparameter NGF immunophenotypic studies were performed on Transfix™-stabilized CSF samples (Cytomark, Buckingham, UK) following the EuroFlow panels and protocols \[12, 22\]. Data analysis was performed with the Infinicyt software (Cytognos, Salamanca, Spain). The lower cut-off for CNS involvement was defined as a cluster \( \geq 10 \) events with the appropriate phenotype, based on a 10-parameter tube \( \geq 0.001 \) cells/\muL).

Statistical analyses

The Mann-Whitney U (for continuous variables) and the \( \chi^2 \) tests (for categorical variables) were used to determine the statistical significance of differences observed between groups (PASW 19 statistical software, IBM SPSS Statistics, IBM, Armonk, NY, USA). OS and CNS-RFS curves were plotted according to the method of Kaplan-Meier and compared using the (one-sided) log-rank test. Those variables showing prognostic value in the univariate analysis were also evaluated by multivariate analysis using a Cox stepwise regression model. Statistically significance was set at \( p \) values \(< .05\).

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest to disclose.

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This paper has been accepted based in part on peer-review conducted by another journal and the authors’ response and revisions as well as expedited peer-review in Oncotarget.

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