

***In vivo* and *in vitro* sensitivity of Blastic Plasmacytoid Dendritic Cell Neoplasm to SL-401, an interleukin-3 receptor targeted biologic agent**

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Running title

IL-3R-targeted treatment of BPDCN

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Abstract

Blastic plasmacytoid dendritic cell neoplasm is an aggressive malignancy derived from plasmacytoid dendritic cells. Today, no standard of care is accepted to treat blastic plasmacytoid dendritic cell neoplasm, and therapeutic strategies have never been prospectively evaluated. Since blastic plasmacytoid dendritic cell neoplasm cells express high levels of IL-3 receptor α chain (IL3-R α or CD123), antitumor effects of the IL-3R-targeted drug SL-401 against Blastic plasmacytoid dendritic cell neoplasm were evaluated *in vitro* and *in vivo*. Cytotoxicity of SL-401 was assessed in patient-derived blastic plasmacytoid dendritic cell neoplasm cell lines (CAL-1 and GEN2.2) and in primary blastic plasmacytoid dendritic cell neoplasm cells isolated from 12 patients using flow cytometry and an *in vitro* cytotoxicity assay. Cytotoxic effects of SL-401 were compared to several relevant cytotoxic agents. SL-401 exhibited a robust cytotoxicity against blastic plasmacytoid dendritic cell neoplasm cells in a dose-dependent manner. Additionally, the cytotoxic effects of SL-401 were observed at substantially lower concentrations than those achieved in clinical trials to date. Survival of mice inoculated with a blastic plasmacytoid dendritic cell neoplasm cell line and treated with a single cycle of SL-401 was significantly longer than that of untreated controls (median survival, 58 vs. 17 days, $p < 0.001$). These findings indicate that blastic plasmacytoid dendritic cell neoplasm cells are highly sensitive to SL-401, and support the rationale to further evaluate SL-401 in patients suffering from blastic plasmacytoid dendritic cell neoplasm.

Introduction

Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN) is an aggressive neoplasm derived from plasmacytoid dendritic cells (pDC)¹. In 2008, BPDCN was classified by the World Health organization (WHO) as a distinct entity in the group of “acute myeloid leukemia (AML) and related precursor neoplasms”². Although elderly subjects are principally affected, BPDCN can also arise in young adults and children^{3,4}. Approximately 90% of patients exhibit cutaneous lesions at diagnosis, which upon microscopic analysis appear as a dermis infiltrate of immature blastic cells with pDC features^{5,6}. Malignant cells isolated from skin, lymph nodes, bone marrow, spleen and/or other tissues express usually the following markers: interleukin-3 receptor alpha (IL-3R α or CD123), BDCA2 (CD303), BDCA4 (CD304), TCL1 and ILT7⁷⁻⁹.

Currently, there is no consensus regarding the optimal treatment modality of BPDCN. Several treatments, including multi-agent chemotherapy regimens, symptomatic approaches (e.g. local radiation¹⁰), and intensive chemotherapy with allogeneic hematopoietic cell transplantation (AHCT)¹¹⁻¹³, are generally used to treat patients. Although chemotherapy used to treat patients with acute leukemia or lymphoma are often effective at inducing an initial response, the duration of response is typically brief and recurrent disease is generally resistant to chemotherapy. BPDCN patients generally succumb to cytopenias due to tumor infiltration of the bone marrow; median overall survival (OS) has been reported to range from 9 to 32 months irrespective of the initial presentation of the disease¹⁴⁻¹⁶. While longer OS durations have been reported with AHCT, especially in younger patients^{4,11,13,16,17}, many relapses have been observed after AHCT¹³.

The α -subunit of the human IL-3 receptor (CD123) is a type I transmembrane glycoprotein belonging to the cytokine receptor superfamily^{18,19}. The IL-3 receptor (IL-3R) is a

heterodimer associating an α chain (CD123) and a β chain (CD131). This chain is shared by IL-3, IL-5, and GM-CSF receptors. SL-401, a novel biologic targeted therapy directed against the IL-3R, is comprised of human recombinant IL-3 joined by an acid-labile group of amino acids to a diphtheria toxin (DT) payload that has been truncated at its receptor binding region²⁰. Since IL-3, the natural ligand for the IL-3R, binds with very high specificity and avidity²¹, SL-401 is able to efficiently and preferentially transport DT to cells that over-express the IL-3R, leading to internalization followed by receptor-mediated endocytosis and localization of SL-401 to early endosomes. After cleavage of the SL-401 DT constituent in the acidic medium of endosomes, DT translocates to the cytosol and binds to ADP-ribosylated elongation factor 2, leading to the protein synthesis blockade and cell death²².

Given the ubiquitous and high expression of IL-3R by BPDCN and the lack of therapies available to treat BPDCN, SL-401 represents an adequate therapeutic to be developed in BPDCN. The present study evaluated the cytotoxicity of SL-401 against patient-derived BPDCN cell lines (CAL-1 and GEN2.2), primary BPDCN cells isolated directly from 12 patients. This was performed *in vitro*, as well as *in vivo* in a murine model of BPDCN. The aim of the study was to provide further support for developing SL-401 in patients suffering from BPDCN.

Methods

Patient's cells, cell lines

Peripheral blood or bone marrow cells were obtained for diagnostic purposes from 12 BPDCN patients (Table 1) from our national network that collects data and cells from cases diagnosed in France since 2004 (authorization number #DC-2008-713). BPDCN diagnosis was performed by histopathology and immunostaining of cutaneous lesions, blood or bone

marrow^{2, 8}. Two established cell lines derived from BPDCN patients were used (GEN 2.2, patent#0215927, Dr Plumas, EFS Rhone-Alpes, Grenoble, France and CAL-1, Dr Maeda, Nagasaki University, Japan) as well as TF/H-Ras (Prof Frankel) and CD123^{neg} [MFI<800] DAUDI cell lines (ACC78, DSMZ Braunschweig, Germany) as positive and negative controls, respectively. Others lymphoid and myeloid leukemic cells used to compare sensitivity to SL-401 are described in the *Online Supplementary Appendix*.

Drug and culture

The SL-401 drug (Stemline Therapeutics, New York, NY, USA) was stored at -80°C and tested at eight concentrations that ranged from 365 pM to 0.08 fM (21 ng/ml to 0.4 ng/ml) in order to cover the concentrations obtained *in vivo* in patients enrolled in clinical trials^{23, 24}. The effects of chemotherapy agents used in acute leukemia were also evaluated against BPDCN cells, (please see the *Online Supplementary Appendix*). BPDCN cells were incubated at 3×10^5 cells/mL in RPMI 1640 glutamax medium (Invitrogen, Cergy Pontoise, France) supplemented with 10% fetal calf serum (Invitrogen), 1% penicillin/streptomycin (PAA Laboratoires, Velizy Villacoublay, France) with or without SL-401 or the relevant drugs under 5% CO₂ for 18 h at 37°C.

Cytotoxicity evaluation by flow cytometry

Flow cytometry was performed using CANTO II cytometer (BD Biosciences, San Jose, CA, USA) and DIVA 6.2 software (BD Biosciences). Cytotoxic effects of SL-401 and the various drugs were evaluated using Annexin-V and 7-Amino Actinomycin D (AV/7AAD) and a panel of different monoclonal antibodies to gate the blastic population described in the *Online Supplementary Appendix*. In the mouse model, anti-mouse and anti human CD45 plus anti-human CD123, CD4, CD56, CD304 were used to identify BPDCN human cells (*Online Supplementary Appendix*). A defined number of calibrated 3-µm latex beads (Flowcount

beads, Beckman Coulter) was added to each sample to obtain the absolute number of circulating BPDCN cells in mice, as previously described²⁵.

Cytotoxicity evaluation by MTT assay

Percentage of viable cells obtained after incubation with or without SL-401 was assessed using the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, Saint Quentin Fallavier, France). Culture and following analysis were detailed in the *Online Supplementary Appendix*.

Mice

NOD-SCID IL2R γ c deficient (NSG) mice were irradiated (2 Gy) and inoculated intravenously (i.v.) 24 h later with 1×10^6 GEN2.2 cells and treated intraperitoneally (IP) 8 days later with five daily injections of SL-401 (2 μ g/mouse/injection, total experimental dose 100 μ g/kg) or with PBS only (PBS). Mice were monitored weekly for blood cell count and flow cytometry (*Online Supplementary Appendix*).

Statistical analysis

Statistical analyses were performed using Statal software 2.6 (Adscience, Paris, France) (*Online Supplementary Appendix*).

Results

SL-401 is cytotoxic against BPDCN cell lines and BPDCN primary cells.

The viability of the two CD123^{pos} BPDCN cell lines, GEN2.2 and CAL-1, decreased from $62 \pm 6\%$ [44-96] to $5 \pm 2\%$ [0-17] after treatment with SL-401 at the experimental dose 365 pM

(21ng/ml) for 18 hours (n=9, Figure 1A). Moreover the viability of these two BPDCN cell lines decreased in a dose dependent manner (Figure 1B). Similarly, after treatment with SL-401 at the experimental dose of 365 pM (21ng/ml), viability of freshly isolated BPDCN primary cells (n=12) decreased significantly from $50 \pm 4\%$ [31-71] to $10 \pm 1\%$ [3-17]. As expected, the CD123^{neg} (DAUDI) cells were not sensitive to treatment with SL-401 concentrations as high as 365 pM for 18 hours (viability were unchanged from 65% [88-41] to 61% [83-41] untreated and treated with SL-401 respectively, n=3, Figure 1A and B).

SL-401 is cytotoxic against BPDCN primary cells in a concentration-dependent manner.

Viability of primary malignant cells obtained from 12 BPDCN patients treated with SL-401 for 18 or 48 hours decreased in a concentration-dependent manner, as measured by flow cytometry and MTT (Figure 1C and 1D). As expected, CD123^{neg} DAUDI cells were resistant to SL-401 treatment (Figure 1C and 1D). For patient #10, primary BPDCN cells were obtained at both the time of diagnosis and the disease relapse following treatment with chemotherapy (including: CHOP [cyclophosphamide, hydroxydaunorubicine, vincristine, and prednisone], methotrexate, and L asparaginase). Interestingly, BPDCN cells obtained at the disease recurrence were slightly more sensitive to SL-401 than cells obtained at diagnosis (IC_{50} , [6 fM] vs. [79 fM], respectively; $p = 0.049$, Figure 1E). Thus, relapsing cells were still sensitive to SL-401 in a concentration-dependent manner indicating that BPDCN cells retain their sensitivity to SL-401 following treatment with various cytotoxic agents, thereby suggesting a lack of cross-resistance.

SL-401 is more efficient than other tested chemotherapeutic drugs –excepted idarubicine– to kill BPDCN cells.

In order to better appreciate the cytotoxic effect of the SL-401, we also assessed the effects of other chemotherapeutic drugs. Primary malignant cells obtained from 3 BPDCN patients (#7-9 and #11) were significantly more sensitive to SL-401 than a wide variety of cytotoxic agents commonly used for the treatment of hematologic malignancies, including: cytosine arabinoside, cyclophosphamide, vincristine, dexamethasone, methotrexate, Erwinia L-asparaginase, and asparaginase (Figure 2; $p < 0.05$ for all agents). Only the drug idarubicin was found more efficient than SL-401 (viability $< 1\%$, $n = 5$, Figure 2).

BPDCN primary cells are more sensitive to SL-401 than ALL or AML primary cells *in vitro*.

We next assessed the viability after SL-401 treatment of primary malignant cells isolated from three previously untreated patients suffering from ALL and six untreated patients suffering from AML. We compared these results to those achieved following SL-401 treatment of primary BPDCN cells. Viability of ALL and AML cells decreased slightly after treatment with SL-401 (365 pM for 18 hours). We observed an average decrease of 13% in viability for ALL cells ($33 \pm 20\%$, [6-73] to $26 \pm 20\%$, [4-69], $n=3$) and 16% for AML cells ($40 \pm 8\%$, [18-75] to $36 \pm 8\%$, [11-70], $n=6$). BPDCN cells were significantly more sensitive to SL-401 (75% decrease in viable cells ($50 \pm 5\%$, [20-71] to $11 \pm 1\%$, [4-17], $n=11$, $p < 0.001$; Figure 3).

CD123 expression correlates with SL-401 cytotoxicity *in vitro*.

We next compared the relative expression levels of IL-3R α (CD123) and β (CD131) chains in primary malignant BPDCN, AML, and ALL cells, as well as, established BPDCN cell lines to the respective sensitivities to SL-401 (Table 2). Overall, sensitivity of primary BPDCN cells was related to CD123 expression, as demonstrated by the inverse relationship between cell

viability and CD123 expression (Spearman test: $r = -0.58$, $p < 0.012$). The high dependence of pDC lineage cells (including normal or leukemic pDC) to IL-3 may contribute also to the high sensitivity of BPDCN cells to deprivation of IL-3 signaling during SL-401 exposure. Importantly, no such relationship was observed for CD131 (Spearman test: $r = 0.01$, $p < 0.93$). For patient #10, the MFI of CD123 was quite similar both at diagnosis and relapse (5509 vs. 5137 respectively).

Treatment with SL-401 significantly increases the overall survival of NSG mice inoculated with BPDCN cells.

Irradiated NSG mice were inoculated with the GEN2.2 cell line (1×10^6 per mouse) through the tail vein. Eight day post-inoculation, mice were treated by a daily intra-peritoneal injection of SL-401 (2 $\mu\text{g/day}$) for 5 days or by PBS as controls ($n = 8$ mice in 3 independent experiments). In PBS-treated control mice, the mean overall survival (OS) was 17 ± 1 days. Treatment with 5 daily injections of SL-401 significantly increased the OS of mice compared to controls mice (58 ± 2 days; $p < 0.001$; Figures 4A and 4B). Circulating BPDCN cells were identified as human CD45^{pos} , $\text{CD123}^{\text{pos}}$, $\text{BDCA4}^{\text{pos}}$, CD4^{pos} , CD56^{pos} , CD3^{neg} , and CD34^{neg} cells (Figures 4C). Nearly all of the BPDCN cells expressed CD123 when mice developed the BPDCN (Figure 4C). This suggest that one cure of SL-401 (2 $\mu\text{g/day}$ for 5 days) is not sufficient to kill all of the BPDCN cells rather than $\text{CD123}^{\text{neg}}$ BPDCN cells emerge in response to SL-401. In PBS-treated control mice, number of BPDCN cells progressively increased until death, whereas treatment for 5 days with SL-401 successfully reduced circulating BPDCN cells to undetectable levels for 15 ± 3 days after treatment (Figure 4D). We monitored hemoglobin and platelet counts in mice to assess leukemic cell bone marrow involvement. In PBS-treated control mice inoculated with BPDCN, hemoglobin and platelet counts progressively decreased until death. In contrast, in treated mice, these hematological

parameters reached the levels observed in irradiated control mice that were not inoculated with BPDCN cells and not treated (Figure 4E). Regression of cytopenia under treatment indicates an absence, or at least, a lower level of medullar involvement by BPDCN cells in SL-401 treated mice. Overall, SL-401 is efficient *in vivo* to control BPDCN cells.

Discussion

SL-401 is a biologic agent corresponding to IL-3 genetically fused to truncated DT *via* a cleavable linker. This agent induces cytotoxicity by inhibiting ribosomal function, and thereby, inhibiting protein synthesis, a mechanism that is distinct from all other anticancer therapeutics²⁰. SL-401 has been demonstrated to induce a profound cytotoxicity at picomolar and subpicomolar concentrations in AML cell lines²⁶, as well as, in a model of human AML inoculated in immunocompromised mice²⁷. Moreover, SL-401 is cytotoxic *in vivo*, in patients with advanced AML and myelodysplastic syndrome²³, suggesting that SL-401 targets leukemic stem cells (LSCs), as well as, more mature tumor cells. In contrast, SL-401 is not cytotoxic to normal hematopoietic progenitor cells²⁸, which has translated into a paucity of myelosuppression in clinical trials to date^{23, 28-31}.

The present study was performed to evaluate and quantify the effects of SL-401 on various preclinical models of BPDCN, a malignancy that ubiquitously expresses high level of the IL-3R α chain, which is the target of SL-401. The results reported here demonstrate that SL-401 is highly potent against BPDCN cell lines, primary BPDCN blasts obtained from patients. Although AML cell lines and primary AML cells have demonstrated sensitivity to SL-401 with IC₅₀ values in the picomolar range (10^{-12} M)²³ that are lower than plasma concentrations achieved in leukemic patients undergoing treatment with SL-401, BPDCN blasts are more sensitive, with IC₅₀ values in the femtomolar range (10^{-15} M, experimental dose 0 to 21

ng/ml)³². In addition, SL-401 produced a robust antitumor effect in an *in vivo* xenograft model using human BPDCN. This also indicates a potential good therapeutic index, as well as a systemic activity, since mouse survival persists more than 40 days after SL-401 treatment. The high sensitivity of BPDCN to SL-401 and the potential good therapeutic index of this agent likely reflect the high specificity of the IL-3 ligand component of SL-401 for CD123, in addition to the mechanism of action and potency for its DT payload. Since the IL-3 component of SL-401 is bound *via* an amino acid linker to a DT for which the receptor binding site is truncated, free DT is essentially inert from a toxicity standpoint. DT can only be delivered intracellularly following the binding of SL-401 to the IL-3R *via* IL-3 and internalization. Since IL-3R α expression is limited to only a few normal tissues (pDC and basophils) and, in contrast, the receptor is over-expressed by BPDCN cells, SL-401 can potentially confer high therapeutic indexes for patients. Additionally, SL-401 is not a substrate for p-glycoprotein and other efflux pumps, and thus, cannot be excluded from the blastic cells. Moreover, its cytotoxic mechanism, binding to ADP-ribosylated elongation factor 2, thereby uncoupling of protein synthesis blockade, does not overlap with other agents currently used²². IL-3 has been also shown as a critical survival factor for pDC³³. Thus, interference of this pathway by SL-401 may explain the high sensitivity of BPDCN compared with other myeloid and lymphoid leukemic cells.

There is still no current consensus on the best therapeutic approach for BPDCN^{3,12-15,34-38}, and overall, BPDCN remains a chemotherapy resistant disease and may also resist to the Graft-versus-Leukemia effect, since 32 % of patients relapse after AHCT according to a recent study¹³. In that way, targeted or immune-based therapies are alternative strategies to treat this aggressive leukemia³⁹⁻⁴¹. Here, we propose SL-401 as an efficient target-based therapy that is available for clinical trials and has already shown favorable responses in refractory or

relapsed AML or MDS patients, although myeloid blasts express CD123 at a lower level than BPDCN cells²³. Moreover, we published recently data from a phase I/II clinical study involving 11 BPDCN patients. These data show that a single cycle of SL-401 induces 78% of major responses²⁴. Indeed, the way to use SL-401 in BPDCN patients must be discussed in the light of data from literature obtained in BPDCN patients. SL-401 administration can be used to consolidate the first line chemotherapy to reduce the number of relapses, which always occur after chemotherapy treatment. The combination of SL-401 with chemotherapy should make it possible to reduce chemotherapy doses, and consequently, their adverse effects, which are significant in elderly patients with comorbidities (*i.e.*, most of the patients suffering BPDCN). We showed here that idarubicin, at the dose we tested, induces a relevant level of cytotoxic activity *in vitro* whereas cytosine arabinoside does not. This confirms recent data showing that the BPDCN cell line CAL-1 is resistant to cytosine arabinoside⁴¹. Intrathecal injection of SL-401 could also be of interest since there are frequently patient with central nervous system relapse⁴² and the molecular weight of SL-401 (57KD) predicts no diffusion across the brain blood barrier. For patients who undergo AHCT, SL-401 treatment can be used before allograft to minimize the level of minimal residual disease that is the most important prognosis factor in a recent study on allograft BPDCN patients¹³, or as a consolidation treatment after AHCT¹³. To support this later hypothesis, we observed that *in vitro*, the blastic cells from a relapsing BPDCN patient were still sensitive to SL-401-mediated death. Moreover, the IL-3R α chain (CD123) was still expressed on the surface at relapse (CD123 MFI at diagnosis: 5509 *vs.* 5137 at relapse, patient #10). In the mouse model after one course of SL-401, all the BPDCN cells at relapse expressed also CD123. This supports the hypothesis that patients suffering from BPDCN can be treated with SL-401, as first line or second line therapy, and maybe even with several courses of SL-401. Overall, immune-based therapy using SL-401 appears to be an appropriate way to treat BPDCN

patients. New approaches based on immunomodulators⁴¹ or on demethylating agents⁴⁰ must be further evaluated and compared –or associated– with SL-401.

In conclusion, we demonstrate that clinical grade SL-401, which specifically targets IL-3R efficiently kills primary BPDCN cells in culture and significantly improves OS of mice inoculated with BPDCN receiving a single cycle of SL-401. This provides a strong rationale for the use of SL-401 in the treatment of patients suffering from BPDCN. As BPDCN is a rare leukemia subset, an international clinical trial using SL-401 should now be conducted to prospectively validate these results.

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Table 1: Characteristics of the 12 patients suffering from Blastic Plasmacytoid Dendritic Cell neoplasm (BPDCN) who provide primary BPDCN cells.

	Age/ gender	Immunophenotype (flow cytometry)	Karyotype	Cutaneous lesions	Pathology diagnosis	Sites of disease involvement
#1	75/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ My ⁻ B ⁻ CD7 ⁺ (others T)	45,XY,-5 [3], 46XY [10]	no	NA	LN, BM blood, spleen
#2	59/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ CD33 ⁺ (others My) B ⁻ CD7 ⁺ (others T)	46,XY,del(6q)(q16q25),der(7)t(7;12)(p11;q1?3),?del(7)(p21)-8x2,-9x2,-12x2,+mar [4], 46XY [11]	yes	BPDCN	LN, BM skin
#3	81/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ CD33 ⁺ (others My) CD22 ⁺ (others B) CD2 ⁺ (others T)	46XY	yes	BPDCN	LN, BM, skin, blood
#4	69/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ CD33 ⁺ (others My) CD22 ⁺ (others B) CD7 ⁺ (others T)	46XY	yes	BPDCN	skin, BM
#5	63/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ My ⁻ B ⁻ T	44,X,-Y,-13[3]/44,sl,i(7)(q10)[15]/45,X Y,-15[3]	yes	BPDCN	LN, BM skin blood, spleen
#6	56/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ CD33 ⁺ CD117 ⁺ (others My) CD22 ⁺ (others B) CD7 ⁺ (others T)	NA	yes	NA	BM, skin, blood
#7	33/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ My ⁻ B ⁻ T	44,X,Y,13[3]/44,sl,i(7)(q10)[15]/45,XY,-15[3]	yes	BPDCN	BM, skin, blood
#8	70/F	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ My ⁻ B ⁻ T	46,XX i(7q)	yes	BPDCN	LN, BM, skin, blood, spleen
#9	72/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ My ⁻ B ⁻ CD2 ⁺ CD7 ⁺ (others T)	NA	yes	NA	LN, BM, skin, blood
#10	63/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ My ⁻ B ⁻ CD2 ⁺ CD7 ⁺ (others T)	46XY	yes	BPDCN	BM, skin, blood
#11	58/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ CD33 ⁺ (others My) B ⁻ T	46XY	yes	BPDCN	LN, BM, skin, spleen
#12	80/M	CD4 ⁺ CD56 ⁺ CD123 ⁺⁺ CD303 ⁺ CD304 ⁺ TCL1 ⁺ ILT7 ⁺ My ⁻ B ⁻ CD7 ⁺ (others T)	43-44,XY,der(2)t(2;?) (q23;?),der(4)t(4;?) (q35;?),5,der(7)t(7;?) (q31;?),-9,-10,-13,-15,-16,del(17)(p11),+1-4mar[17];82 83,idemx2[12];46,XY	yes	BPDCN	LN, BM, skin, blood, spleen

Age (years)/Gender ; Results of phenotypic Analysis performed on blood or bone marrow samples (Flow Cytometry) ; Karyotype ; Presence of Cutaneous Lesions ; Histopathological Diagnosis. My: myeloid markers (including Myeloperoxidase, CD13, CD33, CD117, CD15, CD65, CD14, CD64); T: T lymphoid markers (including membrane CD3, intracytoplasmic CD3, CD7, CD5, CD2, CD8); B: B lymphoid markers (including intracytoplasmic CD79a, intracytoplasmic CD22, intracytoplasmic Ig Mu chains, CD19, CD20, CD22, Surface immunoglobulin). +: positive expression, ++: high expression, -: absence of expression. NA: Not available, LN: lymph nodes, BM: bone marrow

Table 2: Expression of IL-3R α and β chains on primary malignant cells from patients suffering from BPDCN, acute myeloid leukemia (AML) cells, acute lymphoid leukemia (ALL) cells and cell lines –as assessed by mean fluorescence intensity (MFI)– was compared with the viability after treatment with SL-401.

Cells	Mean fluorescence intensity (MFI)		Viability (%)	IC50
	IL-3R α (CD123)	IL-3R β (CD131)	(365 pM)	(pM)
#1	6340	2214	29 [•]	0.06
#2	10189	2352	38 [•]	0.083
#3	9095	2060	17*	0.006
#4	10746	2320	17*	NA
#7	6165	3276	27*	NA
#9	4439	2477	29*	NA
#10 diagnosis	5509	3433	6 \pm 1 [°]	0.006
#10 relapse	5137	1068	5 \pm 1.3 [#]	0.075
AML 1	4557	2073	83 [•]	NA
AML 1	2073	1870	93 [•]	NA
AML MLD	6941	3003	100 [•]	NA
AML 2	3700	1473	93 [•]	NA
AML 2	1068	2749	93 [•]	NA
ALL-T	3049	2761	94 [•]	NA
ALL-B	2399	2890	87 [•]	NA
GEN2.2	11336	5378	0,45*	0.006
CAL-1	6406	629	12,5*	0.05
TF/hras	1478	1174	75*	0.06

IL-3R α and β chains (CD123 and CD131) expression –measured by flow cytometry– in BPDCN cells from patient (# 1-4, 7, 9 and 10), or in different AML, ALL and cell lines (results represent the mean of 2 determinations). Percentage of viability after culture with SL-401 (experimental dose 365 pM= 21ng/ml) in one experiment (•), mean \pm SEM of 2 (*), 3 (°) or 6 (#) independent evaluations and IC50 are indicated. AML MLD: AML with multi lineage dysplasia; NA: not available.

Figure Legend

Figure 1: Sensitivity of BPDCN cells to SL-401-mediated death.

(A) A representative experiment is shown. Upper panels: dot plots showing the staining of Annexin V (AV FITC) and 7-AAD, on the x- and y-axes, respectively, as assessed by flow cytometry (FC), on IL-3R non-expressing DAUDI cells (negative control) and CAL-1 cells (established BPDCN patient-derived cell line) after treatment with SL-401 (365 pM) for 18 hours; lower panels: primary blasts from BPDCN patient #1 after treatment with SL-401 (365 pM, right hand side panel) for 18 hours or no drug treatment (no SL-401, left hand side panel). The percentages indicated in each dot plot represent viable cells (AV⁻/7-AAD⁻ cells). (B) The percent viability (mean \pm SEM) of the BPDCN cell lines CAL-1 (n=3) and GEN 2.2 (n=6) after treatment with SL-401 at different concentrations reaching from 0.08 fM to 365 pM (0-21 ng/ml) for 18 hours. The DAUDI cell line was used as negative control. (C) Each gray line represents the percentage viability of primary blasts (AV⁻/7-AAD⁻ cells) isolated from different BPDCN patients (#1-12) according to different SL-401 concentrations (from 0.08 fM to 365 pM = 0-21 ng/ml) for 18 hours. The black line represents the mean of BPDCN patients samples as a function of SL-401 concentration and the DAUDI cell line was used as negative control. (D) Viability assessed using the MTT assay: the percentage viability of primary blasts from BPDCN patients #3 and #4 and of CAL-1 and GEN 2.2 BPDCN cell lines is dependent on SL-401 concentrations. The DAUDI cell line was used as negative control and is insensitive to SL-401-mediated death whatever the concentration used. Cells were treated with SL-401 for 48 hours. The values represent one experiment for patient's samples or 3 independent experiments for cell lines. (E) Percentage (mean \pm SEM) of viable primary BPDCN cells (AV⁻/7-AAD⁻ cells) from patient #10 at diagnosis (n = 3) and at relapse (n=6) after incubation for 18h with different concentrations of SL-401 or without any drug, as assessed using annexin V and 7-AAD staining and flow cytometry (FC). Untreated cells were considered as 100 % viable ($p = 0.049$).

Figure 2: Sensitivity of primary BPDCN cells to SL-401 and other relevant chemotherapeutic agents.

The mean percentages of viable cells (AV⁻/7-AAD⁻ cells) from 3 patients # 7-9-11 after treatment with with SL-401 (365 pM), Erwinia L-asparaginase (L-ASP, 10 IU/mL), asparaginase (ASP, 10IU/ml), methotrexate (MTX, 9.9 μ M), cyclophosphamide (CYC, 100 μ M), cytosine arabinoside (CYT, 0.329 mM), dexamethasone (DEX, 0.637 mM), vincristine (VIN, 0.0242 μ M) and idarubicin (IDA, 0.158 μ M). Untreated cells were considered as 100% viable. Histograms represent the mean \pm SEM of 3 independent experiments ($*p < 0.05$ between SL-401 and all other drugs pairwise except IDA).

Figure 3: Comparison of sensitivity of primary blasts from patients with BPDCN, acute lymphoid leukemia (ALL), and acute myeloid leukemia (AML) to SL-401-mediated death.

Leukemia blasts sampled from three patients with ALL (2 B-ALL and 1 T-ALL) and six patients with AML (1 case of AML with multilineage dysplasia, 2 cases of M1 AML, 1 case of M0 AML, and 2 cases of M2 AML) and blasts from 11 BPDCN patients (#1-10 and 12) were cultured with or without SL-401 (365 pM) for 18 hours. The viability, as assessed by flow cytometry (AV⁺/7-AAD⁻ cells). The bars represent the mean \pm SEM of the percentage viable blastic cells, as evaluated by untreated cells were considered 100% viable $**p < 0.001$.

Figure 4: *In vivo* efficacy of SL-401 in a NSG mouse model inoculated with BPDCN cells.

NSG mice were irradiated with 2 Grays and then inoculated IV with 1×10^6 GEN2.2 BPDCN cells on day 0. **(A)** Overall survival (OS) of BPDCN inoculated-mice treated with SL-401 (solid line; n=4) or with PBS (dotted line; n = 3). Treatment with SL-401 (2 μ g/mouse intraperitoneally, experimental dose 100 μ g/kg) performed daily for 5 days, was begun on day 7 (pink barr). OS from one representative experiments out of 3 is shown, $p = 0.3$. **(B)** Mean OS of BPDCN inoculated-mice treated with SL-401 (n=8) or PBS (n=8) from 3 independent experiments, $**p < 0.001$. **(C)** one exemple of the immunostaining of circulating peripheral blood mononuclear cells performed at day 53 prior to sacrifice. Murine (blue) and human GEN 2.2 (red) cells are distinguishable due to specific CD45 antibody expression. Human GEN2.2 BPDCN cells express CD123, BDCA4, CD4, CD56, but not CD34 or CD3. **(D)** BPDCN cell count values in the blood of a mouse following treatment with SL-401 (dotted line, black circles) or with PBS (blue solid line, blue square). **(E)** Means of Hemoglobin, as well as white blood cell, and platelet count values in the blood of mice following treatment with SL-401(n=4) or saline control (n=3). The gray line represents blood parameter values in irradiated mouse that were not treated with SL-401. †: means that mice die.