In vivo and in vitro sensitivity of blastic plasmacytoid dendritic cell neoplasms to SL-401, an interleukin-3 receptor targeted biologic agent

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Online supplementary Appendix

Patient’s cells

Data from two BPDCN patients (patients #3 and #10) were previously published\(^1,\)\(^2\). This study was approved by the Besançon local ethic committee (France). Other myeloid and lymphoid leukemic cells were used as controls. This includes: primary leukemic cells from patients suffering from either AML with multilineage dysplasia (n=1), M1 AML (n=2), M0 AML (n=1), or M2 AML (n=2) (These cells were classified in accordance with the French-American-British [FAB] Classification); primary B-ALL (n=2) and T-ALL (n=1) leukemic cells. CD123\(^{\text{pos}}\) [MFI>1000].

Drug and culture

The effects of the following chemotherapy agents were evaluated against BPDCN cells: Erwinia L-asparaginase and asparaginase (Eusapharma, Limonest, France) (10 IU/mL), cytosine arabinoside (0.329 mM), dexamethasone (0.637 mM), methotrexate (9.9 µM), cyclophosphamide (100 µM), vincristine (0.042 µM), and idarubicin (0.158 µM). Drugs were provided by the Pharmacy of the University Hospital of Besançon (Pr Limat) and were tested in concentrations routinely used for in vitro culture\(^3,\)\(^6\)

Cytotoxicity evaluation by flow cytometry

Cytotoxic effects of SL-401 and the various drugs were evaluated using fluorescein isothiocyanate (FITC)-conjugated Annexin V and 7-Amino-Actinomycin D (AV/7AAD kit, Beckman Coulter Immunotech, Miami, FL, USA) staining after gating on the blastic cell population. The following monoclonal antibodies were used to gate BPDCN cells: Horizon V500 (V500)-conjugated CD45 (HI30, BD Biosciences), Horizon V450 (V450)-conjugated CD56 (B156, BD Biosciences), phycoerythrin-cyanin-7 (PE-Cy7)-conjugated CD123 (6H6, Biolegend, Ozyme, Saint-Quentin en Yvelines, France), allophycocyanin (APC)-conjugated BDCA-2 (AC144, Miltenyi Biotec, Paris, France), allophycocyanin-H7 (APC-H7)-conjugated CD4 (SK3, BD Biosciences) and peridinin chlorophyll Protein cyanine 5.5 (PerCP-cy5.5)-conjugated CD3 (SK7, BD Biosciences). When non-BPDCN acute leukemia were tested, leukemic cells were identified by their low CD45 expression and staining with the following antibodies: APC Alexa fluor 750 (APC AF750)-conjugated CD34 (581, Beckman coulter) and APC-conjugated CD117 (104D2, BD Biosciences) for AML; V450-conjugated CD20 (L27, BD Biosciences), APC-conjugated CD19 (J3-119 Beckman Coulter) and APC AF750-
conjugated CD34 expression for B-ALL; APC-conjugated CD5 (L17F12, BD Biosciences) and V450-conjugated CD7 (MT701, BD Biosciences) for T-ALL. In the mouse model APC-conjugated anti-mouse CD45 antibody (30F11, BD Biosciences), and V500-conjugated anti-human CD45, PE-CY7-conjugated anti-human CD123, V450-conjugated anti-human CD56, PerCP-cy5.5-conjugated anti-human CD3, APC-H7-conjugated anti-human CD4, FITC-conjugated anti-human CD34 (BD Biosciences) and PE-conjugated anti-human CD304 (AD5-17F6, Miltenyi Biotec) were used to identify BPDCN human cells.

Cytotoxicity evaluation by MTT assay

BPDCN or control cell lines (CAL-1, GEN2.2, DAUDI, TF/H-Ras) were incubated at 3 x 10^5 cells/mL at 37°C and 5% CO2 with 50 µL of SL-401 at various concentrations. After 48 h, MTT (5 mg/mL) was added for 4 h. Viable cells were detected by measuring formazan concentration using a plate reader at 570 nm (Dynex MRX Revelation, Dynex technologies, Chantilly, VA, USA).

Mice

NOD-SCID IL2Ryc deficient (NSG) mice (The Jackson laboratory, Sacramento, CA, USA), (female and male; age 7±2 weeks, n=16) were inoculated intravenously (i.v.) with 1 x 10^6 GEN2.2 cells diluted in 0.3 mL of phosphate buffered saline (PBS, Fisher scientific, Houston, TX, USA). Animals were monitored weekly. Quantification of blood leukemic cells was determined by flow cytometry and other blood parameters were assessed including: hemoglobin (g/dL), white blood cells (x10^9/L) and platelets (x10^9/L) (XN 10, Sysmex, Norderstedt Germany). As blood parameter controls, we used irradiated mice that did not received GEN2.2 and were no treated with SL-401 (n=3). Mice were examined daily for overall activity and for the presence of tumoral syndrome, and sacrificed whenever appropriate. These procedures were carried out in accordance with the guidelines for animal experimentation according to an approved protocol (protocol 11007R, Veterinary Services for Animal Health & Protection, issued by the Ministry for Agriculture, Paris, France).

Statistical analysis

Group comparisons of normally distributed or non-normally distributed data were performed using the Student t-test or the Mann-Whitney test respectively. The Spearman correlation
A coefficient was used to assess relationships between the level of IL-3Rα and β chain and the viability of cells after SL-401 treatment. Data were presented as the mean ± standard error of the mean (SEM); both minimum and maximum values were also disclosed in square brackets. A p value < 0.05 was considered statistically significant.