

Blastic plasmacytoid dendritic cell neoplasm is dependent on BCL-2 and sensitive to venetoclax

Joan Montero^{1*}, Jason Stephansky^{1*}, Tianyu Cai², Gabriel K. Griffin³, Lucia Cabal-Hierro¹, Katsuhiko Togami¹, Leah J. Hogdal¹, Ilene Galinsky¹, Elizabeth A. Morgan³, Jon C. Aster³, Matthew S. Davids¹, Nicole R. LeBoeuf⁴, Richard M. Stone¹, Marina Konopleva², Naveen Pemmaraju^{2#}, Anthony Letai^{1#}, and Andrew A. Lane^{1,5#}

¹Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

²Department of Leukemia, University of Texas, MD Anderson Cancer Center, Houston, TX

³Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

⁴Department of Dermatology, Center for Cutaneous Oncology, Dana-Farber/Brigham and Women's Cancer Center, Harvard Medical School, Boston, MA

⁵Broad Institute of Harvard and MIT, Cambridge, MA

*These authors contributed equally

#These authors contributed equally

Correspondence

Lead contact:

Andrew Lane, MD, PhD
Dana-Farber Cancer Institute
450 Brookline Ave, Mayer 413
Boston, MA 02215
617-632-4589
andrew_lane@dfci.harvard.edu

Naveen Pemmaraju, MD
University of Texas
MD Anderson Cancer Center
Unit 428, PO Box 301402
Houston, TX 77230
713-792-4956
npemmaraju@mdanderson.org

Anthony Letai, MD, PhD
Dana-Farber Cancer Institute
450 Brookline Ave, Mayer 430
Boston, MA 02215
617-582-7254
anthony_letai@dfci.harvard.edu

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Abstract

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive hematologic malignancy with dismal outcomes for which no standard therapy exists. We found that primary BPDCN cells were dependent on the anti-apoptotic protein BCL-2 and were uniformly sensitive to the BCL-2 inhibitor venetoclax, as measured by direct cytotoxicity, apoptosis assays, and dynamic BH3 profiling. Animals bearing BPDCN patient-derived xenografts had disease responses and improved survival after venetoclax treatment *in vivo*. Finally, we report on two patients with relapsed/refractory BPDCN who received venetoclax off-label and experienced significant disease responses. We propose that venetoclax or other BCL-2 inhibitors undergo expedited clinical evaluation in BPDCN, alone or in combination with other therapies. In addition, these data illustrate an example of precision medicine to predict treatment response using *ex vivo* functional assessment of primary tumor tissue, without requiring a genetic biomarker.

Statement of significance

Therapy for BPDCN is inadequate and survival in patients with the disease is poor. We used primary tumor cell functional profiling to predict BCL-2 antagonist sensitivity as a common feature of BPDCN, and demonstrated *in vivo* clinical activity of venetoclax in patient-derived xenografts and in two patients with relapsed chemotherapy-refractory disease.

Introduction

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive hematologic malignancy that presents with skin nodules and tumors, lymph node and splenic enlargement, central nervous system involvement, circulating leukemia, and/or bone marrow infiltration (1). There is no standard therapy and patients empirically receive chemotherapy regimens based on retrospective case series (2). For patients who respond to chemotherapy, autologous or allogeneic stem cell transplantation may prolong survival (3). However, median survival is only approximately one year, or even shorter in patients who have disseminated disease (1,2).

BPDCN has had many prior names, including CD4+CD56+ hematodermic neoplasm, blastic natural killer cell lymphoma, and agranular CD4+ natural killer cell leukemia, which has complicated study of the disease (1). In 2008, BPDCN was recognized as the malignant counterpart of plasmacytoid dendritic cells (pDCs) (4). However, the pathogenesis underlying the transformation of hematopoietic progenitors to BPDCN remains unclear. Targeted sequencing identified mutated genes in BPDCN that overlap with those observed in other hematologic malignancies, particularly myeloid diseases, including *TET2*, *TP53*, *ASXL1*, and RNA splicing factors (5,6). However, no BPDCN-specific mutations or immediately targetable alterations have been reported from those studies.

A gene expression analysis suggested that BPDCN was similar to acute myeloid leukemia (AML), and identified the anti-apoptotic gene *BCL2* as more highly expressed in BPDCN compared to normal pDCs (7). *BCL2* is also expressed in AML and some AMLs are sensitive to the BCL-2 inhibitor venetoclax (8). Venetoclax has a favorable safety profile and was recently approved by the US Food and Drug Administration (FDA) for use in patients with relapsed chronic lymphocytic leukemia (CLL) (9). Here we demonstrate that BPDCN is dependent on BCL-2, and that venetoclax is active in BPDCN including in two patients with relapsed/refractory

disease. Thus, BCL-2 inhibition could provide a novel therapeutic strategy in this chemotherapy-resistant disease.

Results

We performed immunohistochemistry on BPDCN biopsies from bone marrow or skin, and in all cases we observed prominent BCL-2 staining compared to surrounding normal tissue (Figure 1A, Supplementary Figure 1). We saw similar BCL-2 expression in AML blasts from bone marrow and leukemia cutis (Supplementary Figure 1). These data suggested that, like AML, BPDCN might have some degree of BCL-2 dependence. However, cell death in response to BCL-2 inhibition depends on a complex balance of pro- and anti-apoptotic factors, which makes sensitivity prediction challenging based on expression alone (10).

Therefore, we performed functional mitochondrial profiling and conventional cytotoxicity assays to test BCL-2 inhibition in BPDCN. We first compared a BPDCN cell line, CAL1 (11), to a series of AML cell lines that have a range of sensitivity to BCL-2 inhibition (12). CAL1 cells expressed similar levels of BCL-2 protein to the highest BCL-2 expressing AML cells (Figure 1B), but there was variability in the abundance of other apoptotic pathway proteins. To assess the functional dependence of these cell lines on specific BCL-2 family members, we performed BH3 profiling. This technique exposes mitochondria to peptides that mimic the BH3 domain of pro-apoptotic BCL-2 family members and measures the induced change in mitochondrial permeability, the “point of no return” for apoptotic cell death (13).

We determined the propensity of each cell line to initiate mitochondrial apoptosis after stimulation with a non-specific pro-death BIM peptide (overall priming), and the relative dependency on three anti-apoptotic BCL-2 family members: BCL-2, BCL-XL, and MCL-1. CAL1 cells were most dependent on BCL-2, as they showed significant priming in response to BAD

stimulation (BCL-2/BCL-XL), but only minor priming after HRK (BCL-XL only) and none after MS1 (MCL-1) stimulation (Figure 1C). In contrast, AML cells were equally or less BCL-2 dependent, and/or were co-dependent on BCL-XL or MCL-1 in addition to BCL-2.

To directly measure responses to pharmacologic BCL-2 inhibition, we treated cells with venetoclax, a BH3 mimetic molecule that displaces pro-apoptotic proteins such as BIM from sequestration by BCL-2, allowing them to initiate mitochondrial permeabilization (14). We performed dynamic BH3 profiling (15), which measures the increase in apoptotic priming induced by incubation with a drug. Dynamic BH3 profiling strongly correlates with eventual induction of apoptotic cell death by the same treatment yet requires only short-term exposure to drug (≤ 4 hours), which is advantageous in analyses of primary cells. By measuring the dose-dependent cytochrome c release induced by BIM peptide we calculated the change in overall apoptotic priming caused by venetoclax pretreatment (“delta priming”). CAL1 cells had an equal or higher delta priming compared with the AML cells tested (Figure 1D).

As predicted by this result, CAL1 cells were equally or more sensitive to venetoclax compared with AML cells in viability assays (Figure 1E). We confirmed that venetoclax induces dose-dependent apoptotic cell death in CAL1 cells by measuring Annexin V and propidium iodide staining following treatment (Supplementary Figure 2A-B). We also noted that dynamic BH3 profiling significantly correlated with Annexin V positivity in response to venetoclax ($P=0.0067$, Figure 1F), supporting the validity of this assay as a functional surrogate of drug sensitivity. The predictions made by BH3 profiling across all cell lines were also confirmed using navitoclax (ABT-263), which inhibits both BCL-2 and BCL-XL, and A-1331852, which targets BCL-XL only (16) (Supplementary Figure 3). Together, these data suggest that BPDCN cells are sensitive to venetoclax, at least in part because they are highly dependent on BCL-2 to inhibit mitochondrial apoptosis.

To test this hypothesis in primary cells, we analyzed patient bone marrow aspirates and skin biopsies involved by BPDCN, as well as BPDCN patient-derived xenografts (PDXs) (17). PDXs displayed pathological characteristics of human BPDCN, including infiltration of bone marrow and spleen by CD4+CD56+CD123+ leukemia cells that were also BCL-2 positive (Supplementary Figure 4). Patient tumor samples and PDXs were subjected to targeted DNA sequencing and represent a variety of genotypes (Supplementary Table 1).

Culturing primary leukemia cells *in vitro* to perform drug treatment assays is challenging. *Ex vivo* cytotoxicity assays with primary BPDCNs suggested a dose-dependent response to venetoclax in some cases (Supplementary Figure 5A-B), but maintaining viability even in vehicle-treated samples for more than 8 hours was not uniformly feasible. Therefore, we performed BH3 profiling in primary BPDCNs similarly to how we had done in cell lines. In addition, we used flow cytometry-based BH3 profiling to measure cytochrome c release in defined subpopulations of interest (18). Using this technique, we were able to selectively assess responses in leukemia cells admixed with normal cells. For comparison, we also tested a randomly selected set of primary AMLs. Baseline BCL-2/BCL-XL dependency was higher in BPDCN compared to AML (69.1% vs 2.1% priming by BAD peptide, $P < 0.0001$ by t-test; Figure 2A). Similarly, when permeabilized cells were directly stimulated with equivalent doses of venetoclax, BPDCNs had a higher level of cytochrome c release than the AMLs tested (57.4% vs 2.62%, $P = 0.0001$; Figure 2B).

Next, we used dynamic BH3 profiling to measure the change in apoptotic priming in response to venetoclax treatment of live cells with intact membranes. All BPDCNs had a significant increase in priming, or a decrease in their apoptotic threshold, after short-term treatment with venetoclax, and BPDCN priming was higher than in AML (59.8% vs 12.1%, $P < 0.0001$; Figure 2C). Normal

bone marrow had significantly less priming than BPDCN in response to the same dose of venetoclax (18.5% vs 59.8%, $P=0.0002$; Figure 2C), suggesting a therapeutic window.

We performed an orthogonal assay for apoptotic cell death by measuring cell surface Annexin V in primary BPDCN and AML after short-term venetoclax treatment. On average, BPDCN had a greater increase in Annexin V positivity than AML (2.25-fold vs 1.2-fold, $P=0.03$; Figure 2D). Annexin V positivity was significantly correlated with the increase in priming measured by dynamic BH3 profiling ($P=0.0088$, Figure 2D). Together, these data suggest that BPDCN is highly BCL-2 dependent and predicts that BPDCN may be sensitive to venetoclax therapy.

To test BPDCN response to BCL-2 inhibition *in vivo* we transplanted two PDXs into recipient animals. When human BPDCN was detectable in blood, we randomized animals to treatment with venetoclax or vehicle for 28 days. After 21 days we sacrificed a subset for pharmacodynamic evaluation. Venetoclax treatment resulted in a reduced burden of human BPDCN cells in peripheral blood, bone marrow, and spleen (Figure 3A-B, Supplementary Figure 6). Histologic and immunohistochemical analyses confirmed decreased BPDCN in tissues, and showed restoration of normal hematopoietic elements in treated animals (Figure 3C). Animals receiving venetoclax had prolonged overall survival compared to vehicle-treated mice (median survival 57 vs 36 days, $P=0.0025$; Figure 3D).

We identified two patients with relapsed/refractory BPDCN who received therapy with venetoclax, prescribed off-label after the recent FDA approval in CLL. Both patients had no alternative therapeutic options and their treating physicians prescribed venetoclax because of the reports of BPDCN responding to “lymphoid-like” leukemia regimens (2) and because of venetoclax’s single-agent activity in AML (8). Patient #1 was an 80-year-old male diagnosed with BPDCN 18 months prior, and had disease involving bone marrow, lymph nodes, and

diffuse cutaneous plaques and tumors. His BPDCN harbored the following mutations: ASXL1 Y591fs*, GNB1 K57E, IDH2 R140W, and NRAS G12D. He previously responded to and then progressed on conventional chemotherapy (doxorubicin, vincristine, and prednisone), an IDH2-targeting agent, and an IL3-receptor targeting agent. Patient #2 was a 73-year-old male diagnosed with BPDCN 15 months prior, with skin, marrow, and widespread nodal involvement. His BPDCN harbored MPL Y591N, TET2 M1456fs*, and TET2 Q1654fs* mutations. He previously received and then relapsed after chemotherapy (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone), an autologous stem cell transplant, an IL3-receptor targeting agent, and decitabine.

We had previously performed dynamic BH3 profiling on biopsies of skin (Patient #1) and bone marrow (Patient #2), which predicted that their disease would respond to venetoclax (BPDCN3 and 6, Figure 2B). Patient #1 received venetoclax orally with a weekly dose escalation of 20 mg, 50 mg, 100 mg, 200 mg, and finally 400 mg per day. He did not have any evidence of tumor lysis syndrome, despite rapid regression of visible tumors. At 4 weeks his disease was restaged, which revealed a marked response in multiple cutaneous sites of involvement (Figure 4A). His modified Severity Weighted Assessment Tool (mSWAT) skin scores decreased from 16.5 to 6.5. He had a decrease in the size of palpable cervical and preauricular lymph nodes, but did not have an appreciable response in his bone marrow at that time. Unfortunately, he died two weeks later with an intracranial hemorrhage before additional response evaluation could be performed. His platelets had been persistently below 10,000/ μ L despite transfusion, predating venetoclax treatment and likely related to bone marrow infiltration by BPDCN.

Patient #2 received a daily dose escalation of venetoclax of 50 mg, 100 mg, 200 mg, to a final dose of 400 mg daily. He did not have any evidence of tumor lysis syndrome nor any other toxicity related to venetoclax. At 4 weeks, his skin disease had substantially improved, and a

PET-CT scan demonstrated a significant decrease in multistation lymphadenopathy and near complete resolution of all areas of FDG-avidity (Figure 4B-C). His bone marrow BPDCN blast count decreased from 85% pre-treatment to 44% after 6 weeks. He remained on venetoclax 400 mg daily for approximately 12 weeks, at which time he experienced disease progression.

Discussion

We have demonstrated that BPDCN is dependent on BCL-2 and is markedly sensitive to BCL-2 inhibition with venetoclax. Amongst the primary leukemias, PDXs, and cell lines we tested, BPDCN was at least as sensitive to BCL-2 inhibition compared with AML. The BPDCN response to venetoclax compares favorably with CLL, a disease that has shown remarkable single agent activity, including in relapsed patients with unfavorable genetics (9). BCL-2 inhibition should be formally evaluated in BPDCN as soon as possible, because there are few therapeutic options for these patients.

The mechanisms underlying BCL-2 dependence in BPDCN are unclear. There are no recurrent DNA copy number changes nor rearrangements reported involving the *BCL2* locus (19,20), although additional analysis of BPDCN genomics may provide more insight into its BCL-2 dependency. Prior studies in AML have suggested that specific somatic mutations may be enriched in venetoclax-sensitive leukemias (8,21). Larger numbers of BPDCN patients treated with venetoclax with complete genetic annotation will be required to make definitive genotype-phenotype correlations in this disease. However, we note that the primary BPDCNs tested here harbored a variety of mutations in genes associated with hematologic malignancies (Supplementary Table 1), and all responded to venetoclax by BH3 profiling. Normal pDCs are selectively depleted *in vivo* by venetoclax compared to conventional dendritic cells (cDCs) and other hematopoietic cell types (22), suggesting that the sensitivity we observed could represent a lineage-specific dependency.

Finally, these data demonstrate precision cancer therapy directed by functional rather than genetic assessment. BH3 profiling served as a biomarker to identify BPDCN as dependent on BCL-2 and likely to respond to venetoclax. The same technique could be used in trials (in BPDCN or other cancers) evaluating venetoclax to determine if drug-resistant cells switch their dependency to another anti-apoptotic protein, which itself might be targetable. Furthermore, microenvironmental signals modulate apoptotic dependencies (22,23), which suggests that correlative studies in clinical trials could analyze BPDCN from distinct anatomical sites to elucidate additional mechanisms of response and resistance. Finally, combination of venetoclax with other agents should also be evaluated in BPDCN, given that BCL-2 inhibition may be synergistic with chemotherapy (24).

Methods

Cell lines

CAL1 cells were obtained from Takahiro Maeda (Nagasaki University) in 2012 (11). AML cell lines were obtained from ATCC or DSMZ between 2008-2014. They were validated by STR profiling in 2014 prior to their use in these experiments and they undergo mycoplasma testing every 6 months.

Patient samples

Primary BPDCN and AML cells were collected from patients who had consented to IRB-approved research protocols for sample analysis from patients with hematologic malignancies. Bone marrow aspirate mononuclear cells from BPDCN and AML patients were purified by Ficoll density centrifugation using standard procedures. Skin biopsies were exposed to an enzymatic digestion solution in 2.5 mL of DMEM/F12 media containing 125 U DNase I (Sigma Aldrich #DN25), 100 U hyaluronidase (Sigma Aldrich #H3506), and 300 U collagenase IV (Gibco #17104-019). The tissue suspension was processed using gentleMACS™ Dissociator (Miltenyl Biotec) using the hTUMOR 1 program. The suspension was then incubated at 37°C for 30 min with constant agitation. Then, dissociation was repeated using the hTUMOR 1 program, and the 30 min incubation was repeated. We then filtered the suspension through a 70 micron filter and cells were centrifuged at 400 x g for 5 min. To lyse residual red blood cells, 100 µL of ice cold water was added to the pellet for 15 sec and then diluted to 50 mL with PBS. Then, cells were centrifuged and resuspended in RPMI media for subsequent analysis.

Antibodies and Western blotting

Samples for Western blotting were prepared by lysing 10⁶ cells in radioimmunoprecipitation assay buffer (RIPA, Boston Bioproducts, #BP-115) containing 1x protease inhibitor (ThermoFisher, cat. 87786). The antibodies used in were BCL-2 (BD Pharmingen, #551107),

BCL-XL (Cell Signaling Technologies, #2764), MCL-1 (Cell Signaling Technologies, #5453), BIM (Cell Signaling Technologies, #2933), BAX (#2774) and β -Actin (Sigma-Aldrich, #A5441). Western blots were imaged using an ImageQuant LAS-4000 (GE Healthcare, #28-9558-10).

Drug treatment of cell lines

Cell lines cultured in RPMI-1640 with 10% FBS were plated in a 96-well dish at a concentration of 5000 cells per 120 μ L media. Compounds were added to the cells in serial five-fold dilutions. After a 72 hr incubation at 37°C, viability was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, 40 μ L of 5 mg/mL MTT (EMD Millipore, #475989) was added to each well and incubated for 2 hrs, and then 100 μ L MTT lysis buffer was added to each well followed by additional 4 hr incubation. Absorbance values were measured using a SpectraMax M3 plate reader (Molecular Devices) at 570 and 630 nm. Viability curves values were generated using the nonlinear regression (curve fit) function in GraphPad Prism (GraphPad Software, Inc.).

Annexin V flow cytometry

The cells were treated with drug for the indicated times, washed with Annexin V binding buffer (ABB) and stained with 1:100 dilutions of Annexin V-FITC (Biolegend, #640906), CD45-APC (BD Biosciences, #340943) and CD123-PerCP-Cy5.5 (BD Pharmingen, #560904) for 30 min. Samples were washed twice with ABB, propidium iodide (BD Pharmingen, #51-66211E) was added after the last wash, and then cells were analyzed using a Cytoflex flow cytometer (Beckman Coulter, #B53012).

BH3 profiling

Baseline and dynamic BH3 profiling were performed as described (13,15). Dynamic BH3 profiling was performed after 4 hrs of incubation of cell lines or primary leukemia cells in vehicle

or 100 nM venetoclax, unless otherwise specified. We used a flow cytometry-based BH3 profiling to perform the analysis, as previously described (18), using Zombie Aqua Dye (Biolegend, #423101) for viability, CD123-PerCP-Cy5.5 (BD Pharmingen, #560904), CD56-PECy7 (BD Pharmingen, #557747), and cytochrome c-Alexa Fluor 647 (Biolegend, #612310).

Patient derived xenografts

All animal experiments were approved by Institutional Animal Care and Use Committees. BPDCN PDXs were generated as described in the Dana-Farber Cancer Institute Public Repository of Xenografts (PRoXe.org) (17). For the treatment trial, one million cells from two independent PDXs (PDX1 and PDX4) were injected into 16 NSG-SGM3 mice each (NOD-Scid IL2Rgnull-3/GM/SF, NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}Tg(CMV-IL3,CSF2,KITLG)1Eav/MloySzJ, Jackson Labs #013062) each. Mice were monitored weekly for evidence of human BPDCN in the peripheral blood. When peripheral human CD45+CD123+ cells were more than 1% of total white blood cells, animals were randomized to two groups and treatment was started with vehicle or venetoclax 100 mg/kg/daily by oral gavage for 28 days. Three mice in each group were sacrificed at day 21 of treatment for pharmacodynamic assessment. The remaining animals were followed for survival. Both PDXs showed evidence of tumor response. PD and survival analysis for PDX4 is shown in Figure 3, and PDX1 in Supplementary Figure 4. Kaplan-Meier curves were compared using the log-rank test in GraphPad Prism. Immunohistochemistry was performed in the Dana-Farber/Harvard Cancer Center Specialized Histopathology Core Laboratory using standard protocols.

DNA sequencing

Patient samples were sequenced using a 95-gene targeted sequencing panel covering genes recurrently mutated in hematologic malignancies (25).

Venetoclax treatment

Patients with relapsed/refractory BPDCN who had exhausted other treatment options were prescribed venetoclax off-label by their treating physicians after they signed written informed consent. Prescriptions were filled in standard outpatient pharmacies after the FDA approval of venetoclax in 2016. The patients provided written informed consent to IRB-approved protocols for sample collection from patients with hematologic malignancies. They also signed additional specific consents for photographic data to be published, and all studies were in accordance with the Declaration of Helsinki. Patient #1 received oral venetoclax with a weekly dose escalation, starting at 20 mg daily for 7 days, followed by 50 mg daily for 7 days, 100 mg daily for 7 days, 200 mg daily for 7 days, followed by 400 mg daily. He was admitted to the hospital for the first dose initiation and the escalation to 50 mg to monitor for tumor lysis syndrome. Patient #2 received oral venetoclax with a daily dose escalation of 50 mg for one day, 100 mg for one day, 200 mg for one day, and then 400 mg daily. He was admitted to the hospital during the dose escalation to monitor for tumor lysis.

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References

1. Pagano L, Valentini CG, Grammatico S, Pulsoni A. Blastic plasmacytoid dendritic cell neoplasm: diagnostic criteria and therapeutical approaches. *British journal of haematology* 2016;174:188-202.
2. Pagano L, Valentini CG, Pulsoni A, Fisogni S, Carluccio P, Mannelli F, et al. Blastic plasmacytoid dendritic cell neoplasm with leukemic presentation: an Italian multicenter study. *Haematologica* 2013;98:239-46.
3. Aoki T, Suzuki R, Kuwatsuka Y, Kako S, Fujimoto K, Taguchi J, et al. Long-term survival following autologous and allogeneic stem cell transplantation for blastic plasmacytoid dendritic cell neoplasm. *Blood* 2015;125:3559-62.
4. Jegalian AG, Facchetti F, Jaffe ES. Plasmacytoid dendritic cells: physiologic roles and pathologic states. *Advances in anatomic pathology* 2009;16:392-404.
5. Menezes J, Acquadro F, Wiseman M, Gomez-Lopez G, Salgado RN, Talavera-Casanas JG, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. *Leukemia* 2014;28:823-9.
6. Stenzinger A, Endris V, Pfarr N, Andrulis M, Johrens K, Klauschen F, et al. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. *Oncotarget* 2014;5:6404-13.
7. Sapienza MR, Fuligni F, Agostinelli C, Tripodo C, Righi S, Laginestra MA, et al. Molecular profiling of blastic plasmacytoid dendritic cell neoplasm reveals a unique pattern and suggests selective sensitivity to NF- κ B pathway inhibition. *Leukemia* 2014;28:1606-16.
8. Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. *Cancer discovery* 2016;6:1106-17.
9. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *The New England journal of medicine* 2016;374:311-22.
10. Del Gaizo Moore V, Letai A. BH3 profiling--measuring integrated function of the mitochondrial apoptotic pathway to predict cell fate decisions. *Cancer letters* 2013;332:202-5.
11. Maeda T, Murata K, Fukushima T, Sugahara K, Tsuruda K, Anami M, et al. A novel plasmacytoid dendritic cell line, CAL-1, established from a patient with blastic natural killer cell lymphoma. *International journal of hematology* 2005;81:148-54.
12. Pan R, Hogdal LJ, Benito JM, Bucci D, Han L, Borthakur G, et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. *Cancer discovery* 2014;4:362-75.
13. Deng J, Carlson N, Takeyama K, Dal Cin P, Shipp M, Letai A. BH3 profiling identifies three distinct classes of apoptotic blocks to predict response to ABT-737 and conventional chemotherapeutic agents. *Cancer cell* 2007;12:171-85.
14. Souers AJ, Levenson JD, Boghaert ER, Ackler SL, Catron ND, Chen J, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature medicine* 2013;19:202-8.

15. Montero J, Sarosiek KA, DeAngelo JD, Maertens O, Ryan J, Ercan D, et al. Drug-induced death signaling strategy rapidly predicts cancer response to chemotherapy. *Cell* 2015;160:977-89.
16. Levenson JD. Chemical parsing: Dissecting cell dependencies with a toolkit of selective BCL-2 family inhibitors. *Molecular & cellular oncology* 2016;3:e1050155.
17. Townsend EC, Murakami MA, Christodoulou A, Christie AL, Koster J, DeSouza TA, et al. The Public Repository of Xenografts Enables Discovery and Randomized Phase II-like Trials in Mice. *Cancer cell* 2016;30:183.
18. Ryan J, Montero J, Rocco J, Letai A. iBH3: simple, fixable BH3 profiling to determine apoptotic priming in primary tissue by flow cytometry. *Biological chemistry* 2016;397:671-8.
19. Leroux D, Mugneret F, Callanan M, Radford-Weiss I, Dastugue N, Feuillard J, et al. CD4(+), CD56(+) DC2 acute leukemia is characterized by recurrent clonal chromosomal changes affecting 6 major targets: a study of 21 cases by the Groupe Francais de Cytogenetique Hematologique. *Blood* 2002;99:4154-9.
20. Lucioni M, Novara F, Fiandrino G, Riboni R, Fanoni D, Arra M, et al. Twenty-one cases of blastic plasmacytoid dendritic cell neoplasm: focus on biallelic locus 9p21.3 deletion. *Blood* 2011;118:4591-4.
21. Chan SM, Thomas D, Corces-Zimmerman MR, Xavy S, Rastogi S, Hong WJ, et al. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nature medicine* 2015;21:178-84.
22. Carrington EM, Zhang JG, Sutherland RM, Vikstrom IB, Brady JL, Soo P, et al. Prosurvival Bcl-2 family members reveal a distinct apoptotic identity between conventional and plasmacytoid dendritic cells. *Proceedings of the National Academy of Sciences of the United States of America* 2015;112:4044-9.
23. Davids MS, Deng J, Wiestner A, Lannutti BJ, Wang L, Wu CJ, et al. Decreased mitochondrial apoptotic priming underlies stroma-mediated treatment resistance in chronic lymphocytic leukemia. *Blood* 2012;120:3501-9.
24. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. *Cancer cell* 2006;10:375-88.
25. Kluk MJ, Lindsley RC, Aster JC, Lindeman NI, Szeto D, Hall D, et al. Validation and Implementation of a Custom Next-Generation Sequencing Clinical Assay for Hematologic Malignancies. *The Journal of molecular diagnostics : JMD* 2016;18:507-15.

Figure Legends

Figure 1. BPDCN cells are dependent on BCL-2 and sensitive to venetoclax *in vitro*.

(A) Representative photomicrograph of a bone marrow biopsy demonstrating involvement with BPDCN by hematoxylin and eosin (H&E) staining and BCL-2 immunohistochemistry are shown. (B) Western blotting in BPDCN (CAL1) and AML (KASUMI1, OCI-AML2, THP1, MOLM13, U937, and TF1) cell lines is shown for the indicated anti- and pro-apoptotic proteins. (C) Baseline BH3 profiling is shown in the indicated cell lines. 'Overall priming' represents cytochrome c release in response to BIM peptide stimulation at four concentrations (0.03 μ M, 0.1 μ M, 0.3 μ M, and 1 μ M); 'BCL-2/BCL-XL dependence,' BAD peptide (10 μ M, 100 μ M); 'BCL-XL dependence,' HRK peptide (100 μ M); and 'MCL-1 dependence,' MS1 peptide (10 μ M). Graphs represent mean \pm SEM of 3 independent experiments. (D) Dynamic BH3 profiling depicting the difference in overall apoptotic priming before and after exposure to 100 nM venetoclax for 4 hours ('delta priming' or ' $\Delta\%$ priming', graphs represent mean \pm SEM of 3 independent experiments, **P<0.01, ***P<0.001 vs CAL1 by t-test). (E) MTT assay results for the indicated cell lines after 72 hours of exposure to the indicated doses of venetoclax. Data points represent mean of 3 replicates \pm SEM, line represents non-linear regression (curve fit). (F) Correlation of 4 hour dynamic BH3 profiling with 24 hour percent cell death by Annexin V / propidium iodide flow cytometry after exposure to 100 nM venetoclax (Spearman r, and P values for correlation are shown).

Figure 2. BH3 profiling predicts venetoclax sensitivity in primary BPDCNs.

(A) Basal BH3 profiling showing cytochrome c release in response to 100 μ M BAD peptide stimulation (normalized to DMSO control) in primary BPDCNs from patients and from PDXs, AMLs, and normal bone marrow (NBM). (B) Basal BH3 profiling in response to 1 μ M venetoclax, as in (A). (C) Dynamic BH3 profiling depicting the difference in overall apoptotic

priming before and after exposure to 100 nM venetoclax for 4 hours. (D) Correlation of dynamic BH3 profiling delta priming with percent cell death by Annexin V / propidium iodide flow cytometry (Spearman r , and P values for correlation are shown).

Figure 3. Animals bearing BPDCN patient-derived xenografts experience clinical response and prolonged survival after venetoclax treatment.

(A) Pharmacodynamic (PD) assessment of spleen size and weight in six recipients of a representative PDX after 21 days of continuous treatment with vehicle ($n=3$) or venetoclax 100 mg/kg/daily ($n=3$). (B) PD assessment of percent human CD45+CD123+ cells in the peripheral blood (PB), spleen, and bone marrow (BM) of six animals bearing PDX4 after 21 days of treatment with vehicle ($n=3$) or venetoclax ($n=3$). (C) Bone marrow and splenic tissue stained with H&E and for human CD45 in animals bearing a BPDCN PDX after 21 days of treatment with vehicle or venetoclax. (D) Kaplan-Meier curves comparing overall survival of mice bearing PDX4 that received vehicle or venetoclax daily for days 10-37, as indicated by the gray bar (curves compared by log-rank test, $n=5$ mice/treatment arm).

Figure 4. Venetoclax is active in patients with BPDCN.

(A) Photographs of skin and gingival tumors in Patient #1 pre-treatment and after 4 weeks of venetoclax. (B) Photographs of Patient #2 after 5 days and 25 days of treatment with venetoclax. Photographs in panels A and B published with permission of the patients, given at the time the photo was taken. (C) PET-CT scan images from Patient #2 pre-treatment and after 4 weeks, showing a representative cross section in the neck/cervical spine, and whole body images with near complete resolution of FDG-avid lymphadenopathy in bilateral cervical and axillary lymph nodes (indicated by blue arrows), mediastinum, abdomen, pelvis, and inguinal regions after treatment.

Figure 1

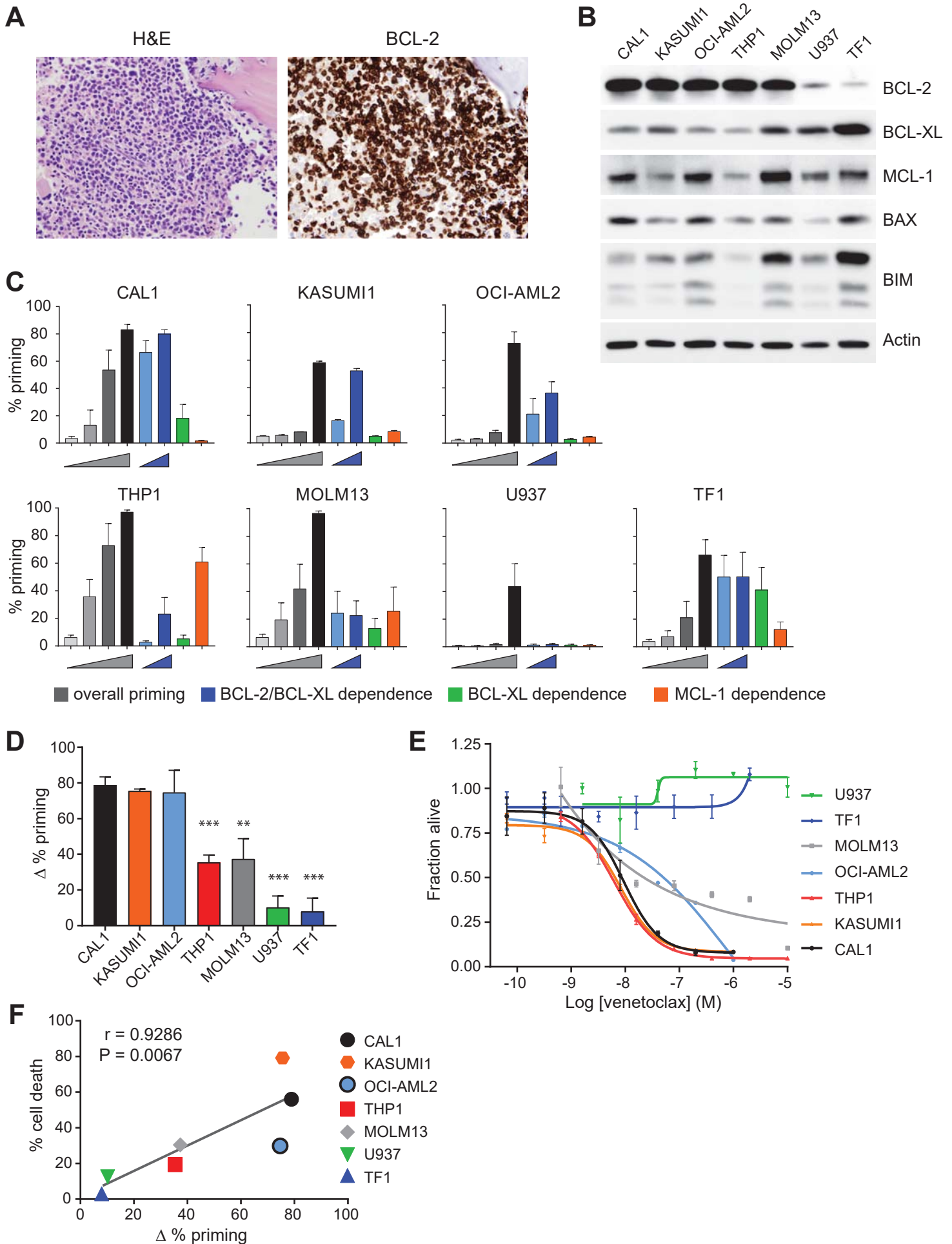


Figure 2

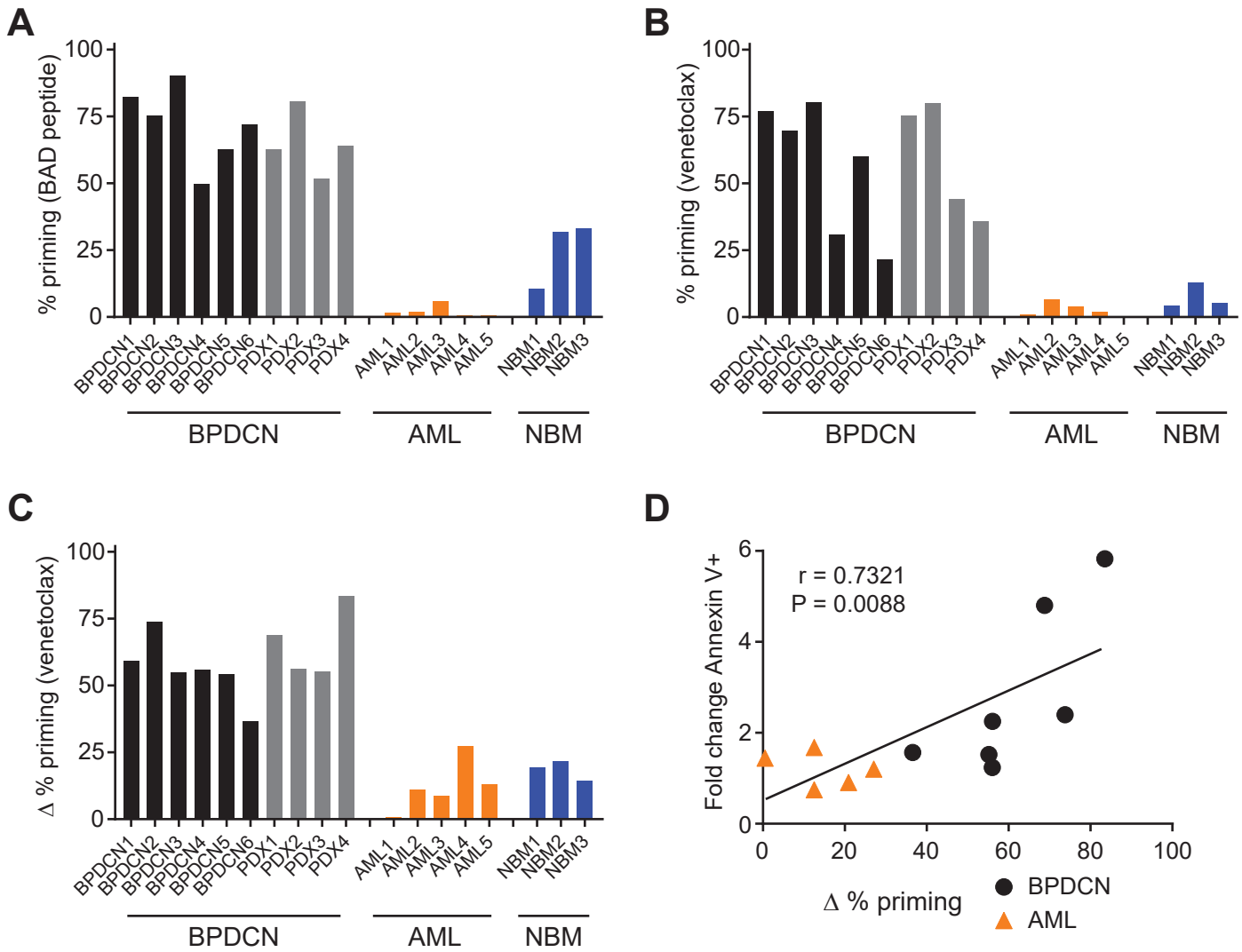


Figure 3

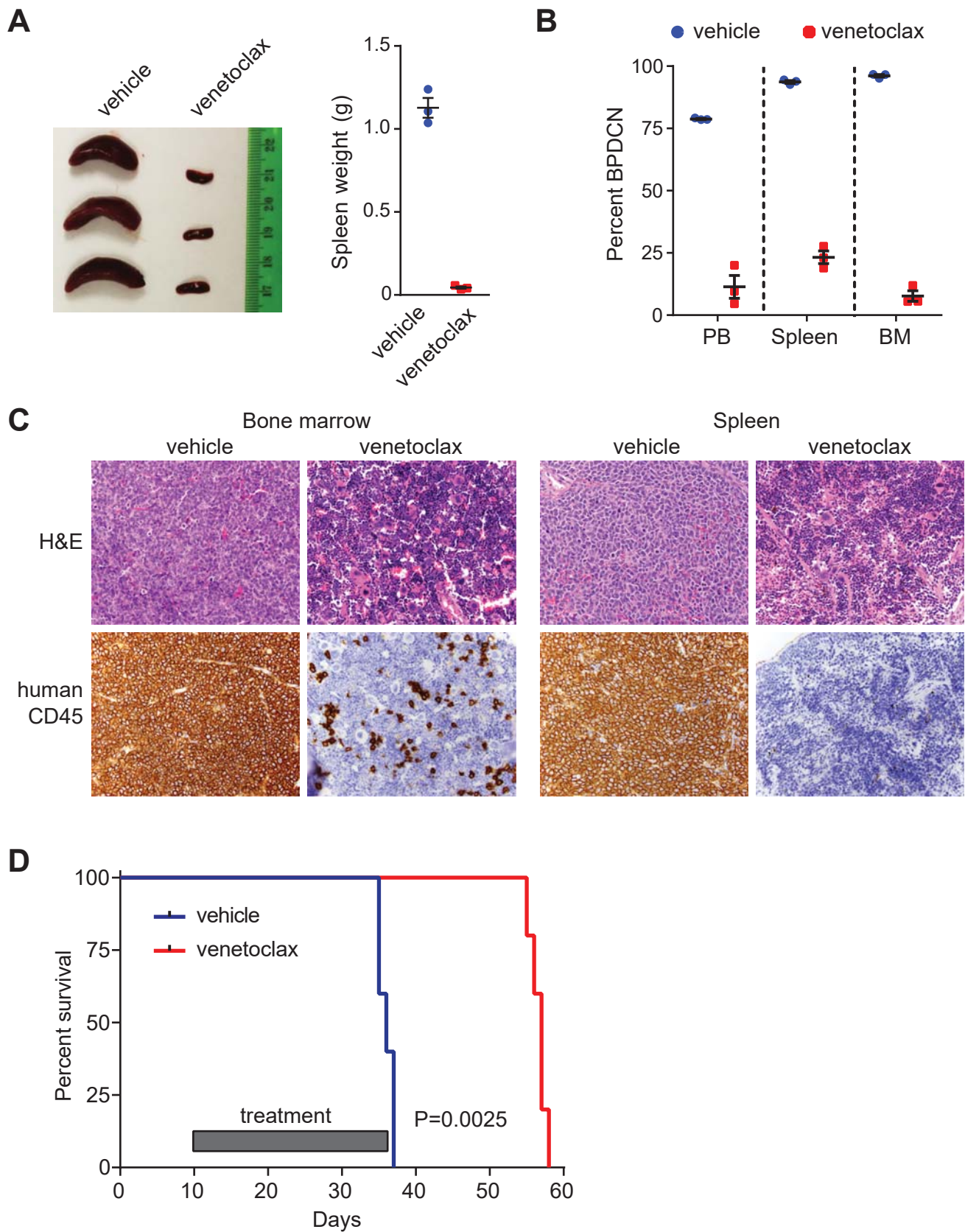
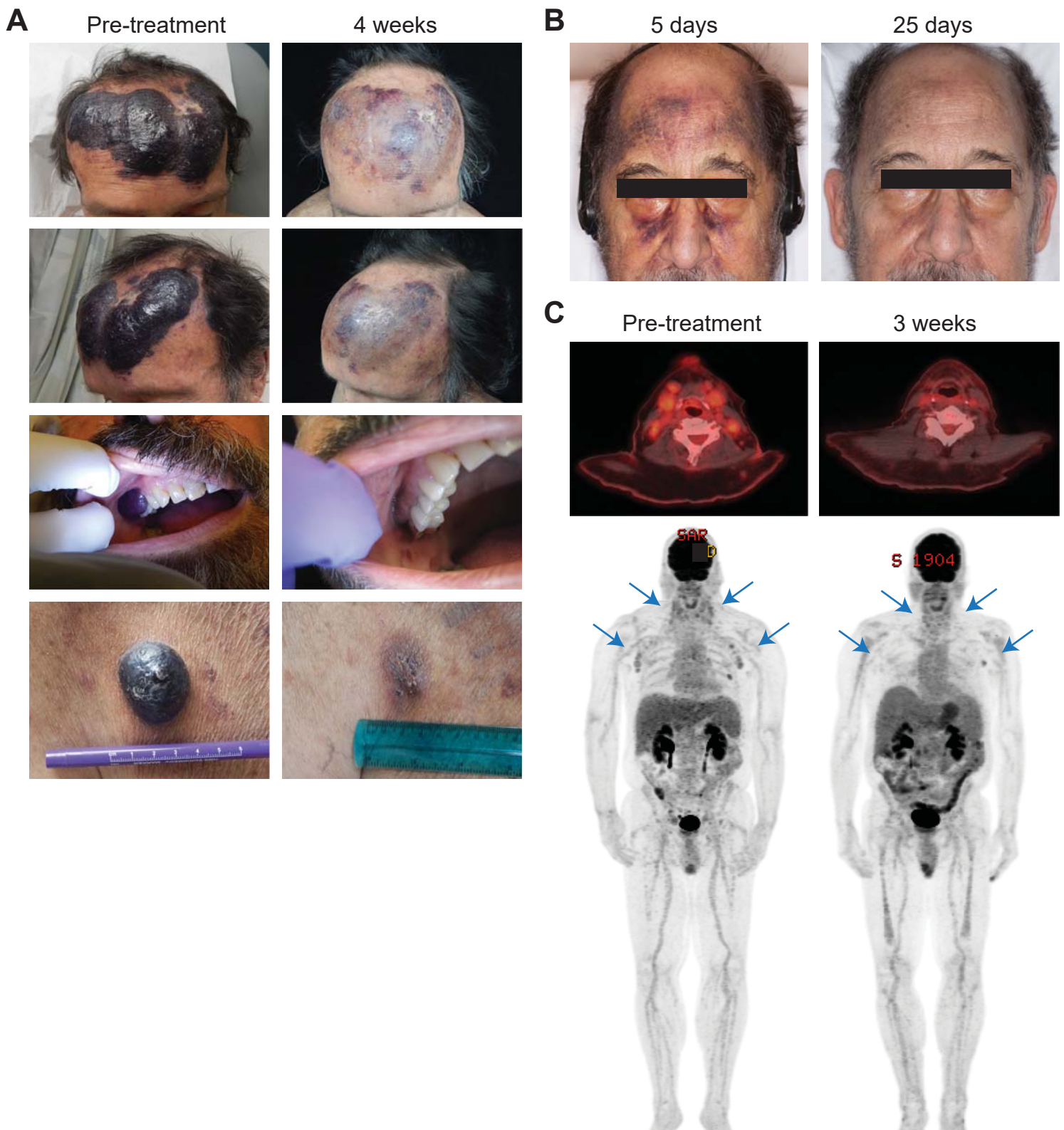


Figure 4



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Blastic Plasmacytoid Dendritic Cell Neoplasm is Dependent on BCL-2 and Sensitive to Venetoclax

Joan Montero, Jason Stephansky, Tianyu Cai, et al.

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