Therapeutic Effect of Lenalidomide in a Novel Xenograft Mouse Model of Human Blastic NK Cell Lymphoma/Blastic Plasmacytoid Dendritic Cell Neoplasm

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Abstract

Purpose: Blastic natural killer (NK) cell lymphoma/blastic plasmacytoid dendritic cell neoplasm (BNKL) is a rare and aggressive neoplasia characterized by infiltration of blast CD4+CD56+ cells in the skin, the bone marrow, and peripheral blood. Currently, more efforts are required to better define molecular and biological mechanisms associated with this pathology. To the best of our knowledge, no mouse model recapitulated human BNKL so far.

Experimental Design: Primary bone marrow cells from a BNKL patient were injected in nonobese diabetes/severe combined immunodeficient interleukin (IL) 2rγ−/−γ− mice with the intent to generate the first BNKL orthotopic mouse model. Moreover, because of the lack of efficient treatments for BNKL, we treated mice with lenalidomide, an immunomodulatory and antiangiogenic drug.

Results: We generated in mice a fatal disease resembling human BNKL. After lenalidomide treatment, we observed a significant reduction in the number of peripheral blood, bone marrow, and spleen BNKL cells. Tumor reduction parallels with a significant decrease in the number of circulating endothelial and progenitor cells and CD31+ murine endothelial cells. In mice treated with lenalidomide, BNKL levels of active caspase-3 were significantly augmented, thus showing proapoptotic and cytotoxic effects of this drug in vivo. An opposite result was found for proliferating cell nuclear antigen, a proliferation marker.

Conclusions: Our BNKL model might better define the cellular and molecular mechanisms involved in this disease, and lenalidomide might be considered for the future therapy of BNKL patients. Clin Cancer Res; 17(19); 6163–73. ©2011 AACR.
Translational Relevance

We propose, to our knowledge, the first orthotopic mouse model of blastic natural killer cell lymphoma. The development of this model could help with a better understanding of the biology of this malignancy. Moreover, because an effective treatment does not exist so far, we propose a possible treatment strategy with a metronomic administration of immunomodulatory drugs.

WHO European Organization for Research and Treatment of Cancer classification of lymphoma, the previous BNKL terminology was replaced by blastic plasmacytoid dendritic cell neoplasm (10).

BNKL is characterized by a very poor prognosis, and the mean survival is less than 2 years (3–5). So far, most of the reported BNKL cases were treated with conventional polychemotherapy. Complete remission was obtained rapidly, but in most cases, a relapse occurred within a year (4–8).

Because of treatment failure, it is urgent to investigate novel therapies for BNKL. However, no BNKL animal model has been described so far. Different strains of immunodeficient mice have been studied to generate models of hematologic malignancies: nonobese diabetes/severe combined immunodeficient (NOD/SCID) and NOD/SCID–related strains (NOD/SCID B2 null, NSB; NOD/SCID IL-2 rγ null, NSG) have been the most widely used in these studies (11–14). We have generated a BNKL model by injecting human bone marrow cells from a BNKL patient in NSG mice. Human BNKL cells were able to generate in mice a disease that resembles the original characteristics of human tumor. We treated BNKL mice with lenalidomide, an immunomodulatory and antiangiogenic drug acting through multiple mechanisms (15–22), and found a very promising preclinical activity in a prevention experiment. Moreover, lenalidomide administration induces a prolonged survival in a model of BNKL established tumor.

Materials and Methods

Collection of patient cells and normal controls

Bone marrow cells were collected from a patient with newly diagnosed BNKL after obtaining an Internal Review Board approval and informed consent from the patient. Mononuclear cells isolated from the bone marrow were separated by Biocoll Separating Solution (Biochrom AG), suspended in RPMI-1640 (EuroClone) supplemented with 10% FBS (Gibco-BRL), 100 IU/mL of penicillin (EuroClone), 100 µg/mL streptomycin (EuroClone), and 0.3 mg/L 1-glutamine (EuroClone) and frozen viably in FBS plus 10% CryoSure-DMSO (WAK-Chemie Medical GmbH). For some studies, normal NK cells from healthy blood donors were purified (>95% purity) with anti-CD56 beads using a commercially available MACS kit (Miltenyi Biotec).

Animals and injection of human cells

NOD/SCID, NOD/SCID B2 null, and NOD/SCID IL-2rγ null were kindly donated by Dr. L. Shultz from The Jackson Laboratory, bred, and housed in the animal facilities at IFOM-IEO campus (Milan, Italy) and at CIMA. All animals, housed in microisolator cages, were used between 6 and 8 weeks of age, and all procedures were carried out in accordance with the guidelines for animal experimentation of our institutions under an approved protocol. For induction of lymphoma in mice, 10 × 10⁶ low-density mononuclear cells from the bone marrow of the BNKL patient were injected intraperitoneally in nonirradiated mice. Secondary transplants were conducted by injecting spleen cells of primary transplanted mice and successively on tertiary and quaternary recipients.

Flow cytometric analyses

Human cell engraftment in the peripheral blood, spleen, and bone marrow was investigated by flow cytometry. Bone marrow and spleen cells were isolated by dissociation of the organs before flow cytometric analysis. Briefly, cells were previously incubated with anti-mouse antibody CD16/CD32 (BD) to block FcRγRII and RIII unspecific signal and then stained with anti-human CD45 (clone 2D1; BD) and anti-mouse CD45 (30-F11; BD). Angiogenic response was measured by flow cytometry as described in detail (23, 24) by enumerating murine circulating endothelial cells (CEC) and progenitors (CEP) in the peripheral blood. To exclude hematopoietic cells, anti-mouse CD45 was used; murine CECs and CEPs were detected using the endothelial murine markers VEGF receptor (VEGFR) 2 (clone Avas12a1; BD) and CD117 (clone 2B8; BD). After incubation of antibodies and red cell lysis, at least 1 × 10⁶ cells per sample were acquired by a FACSCalibur (BD). Seven-aminoactinomycin D (Sigma-Aldrich) was used to enumerate viable, apoptotic, and dead cells (25). After the acquisition, appropriate gating was used to exclude dead cells, platelets, and debris, and analyses were considered as informative when adequate numbers of events (i.e., >50, typically 100–200) were collected in the enumeration gates. Percentages of stained cells were determined and compared with appropriate negative controls defined as nonspecific background staining.

Echography

Spleen’s areas before and after treatment were measured by echography. A Vevo770 microimaging system (Visualsonics Inc.) with a single element probe of center frequency of 30 MHz (RMV 707B) was used for all the examinations. The transducer has a focal depth of 12.7 mm, lateral resolution of 115 µm, and an axial resolution of 55 µm. Precise and repeatable control over the position of the 2-dimensional image plane was obtained with a Vevo Integrated Rail System II.

Mice were anesthetized, and hair was removed from the area of interest to reduce imaging artifacts in the ultrasonic examination. Afterward, mice were placed on a heated table and examined in supine position (slightly
right decubitus) to assess the spleen. Warmed ultrasound gel was placed on the abdomen. All imaging was conducted in 2-dimensional plane (cine loops B mode) of the spleen. Real-time imaging was conducted with a frame rate of 55 MHz with a 10 × 10 mm² yield of view. The organ was evaluated by multiple sagittal scans of the spleen, and the images were measured in duplicate (sagittal and transverse diameter). All the settings were kept constant throughout the experiment, and the echographies were conducted by the same person to reduce the variability.

**RNA isolation, quantitative real-time PCR, and microarray analyses**

Total RNA was isolated from samples with TRIzol (Invitrogen). For microarray analysis, TRIzol followed by a further isolation with RNeasy (Qiagen) was used. Quantitative real-time reverse transcriptase PCR was carried out using standard conditions to quantify mRNA levels of human VEGF-A, IL-6, VEGFR1, VE-cadherin, and CXCL12 mRNA to identify angiogenesis-related genes in BNKL as compared with normal NK cells. For microarray analysis, RNA samples were processed for this hybridization onto Affymetrix chips (Affymetrix Human Gene 1.0 ST). After normalization and transformation of the signal into log₂ values, expression data in BNKL were compared with data from the healthy NK sample and results are shown as fold change. Hybridizations of the samples and bioinformatics analyses were conducted by the Genomics and Bioinformatic core facility at CIMA, University of Navarra.

**Immunohistochemistry and quantification**

Tissues were fixed in 10% buffered formalin, embedded in paraffin, and sectioned (5 μm in thickness). Slides were stained with hematoxylin and eosin (H&E). For immunohistochemistry, slides were deparaffinized, hydrated through graded alcohols, and incubated for 10 minutes with 3% H₂O₂ in water to quench the endogenous peroxidase activity. An antigen retrieval method was used for detection of the antibodies. Slides were incubated with goat normal serum in TBS (0.01 mol/L Trizma base, 0.1 mol/L NaCl, pH = 7.36) for 30 minutes at room temperature. Dilutions of primary antibodies were as follows: 1:400 for anti-PCNA (proliferating cell nuclear antigen; clone PC10; Dako), 1:200 for caspase-3 (cleaved caspase-3; Asp 175; Cell Signaling), 1:20 for CD31 (clone SZ31; Dianova), and 1:100 for hVEGF (Santa Cruz Biotechnology). Primary antibodies were incubated at 4°C overnight or for 1 hour at room temperature in the case of CD31. Tissues were washed in TBS and incubated with the appropriate secondary antibody. Afterward, slides were incubated for 30 minutes with the EnVision anti-rabbit detection system (Dako). Peroxidase activity was carried out with 3,3′-diaminobenzidine (Dako). Finally, slides were counterstained with hematoxylin, dehydrated, and mounted. For quantifications, 30 random images (>200) per experimental group were captured with a microscope (Leica) equipped with the Analysis software. Positive cells were quantified with ImageJ software (Java image processing program developed by NIH). Measurements are given as relative area occupied by positive signals with respect to the reference area.

**Drug and schedule**

Lenalidomide was generously provided by Celgene Corporation. The drug was prepared fresh daily just before gavage with the concentration of 50 mg/kg/d, dissolved in 0.5% carboxymethylcellulose and 0.25% Tween-80 to avoid drug hydrolysis in the aqueous medium.

**Luminex xMAP technology analysis**

Peripheral blood samples were collected at mice sacrifice. An aliquot was kept to obtain serum, after blood centrifugation at 1,500 rpm for 15 minutes at 4°C. Samples were aliquoted and stored at −80°C. Defrosted serum samples were analyzed with an xMAP multiplex immunoassay technology (Luminex Corp.) to simultaneously quantify different mouse cytokines (IL-6, TNFα, VEGF); a second kit was used to quantify human TNFα. The xMAP technology is based on binding of the proteins of interest to antibodies linked to microspheres onto an internal spectral code specific for each protein. The amount of the bound proteins is determined by a second antibody labeled with a fluorescent dye. Twenty-five microliter of each serum sample was assayed in duplicate in a 96-well microtiter filter plate by Milliplex MAP human (Millipore Co.), according to the manufacturer’s instructions. The potential biomarkers were selected according to previous publications describing the role of these molecules in lenalidomide clinical activity. Measurements were done in a Luminex IS100/200 instrument. Concentrations of each protein were assessed according to standard calibration curves, analyzing the median fluorescent intensity data with the 5-parameter logistic curve-fitting method.

**Statistical analysis**

Statistical differences between groups were examined using Student’s t test for unpaired data for parametric variables, and the Mann–Whitney U test for unpaired nonparametric variables. Normality was analyzed with the Shapiro–Wilks test. Data were analyzed with the SPSS statistical software (version 15.0 for Windows SPSS). Values of P < 0.05 were considered as statistically significant. Time-to-event variables were assayed with the Kaplan–Meier test (GraphPad Prism version 5.00).

**Results**

**Patient clinical diagnosis**

The patient, a male of 52 years of age at the time of diagnosis, was referred to our cancer center because of multiple, palpable, enlarged lymph nodes, a skin lesion near the left nipple, and night sweating. A computer-assisted tomographic scan described a variety of mediastinal, axillary, and abdominally enlarged lymph nodes with diameters ranging from 10 to 25 mm and an enlarged spleen. After the surgical removal of the skin lesion, a...
<table>
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<td>Interleukin 2 receptor, beta</td>
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(Continued on the following page)
diagnosis of BNKL was made according to the histologic and immunophenotypic standards and characteristics described in the WHO classification system (10). At diagnosis, immunophenotypic analysis was CD45+, CD4+, CD56+, CD27+, CD3+, CD14+, CD33+, CD13+, MPO+, and Tdt+ (Supplementary Fig. S1A). This immunophenotypic analysis showed 80% of BNKL blast cells in the bone marrow and 70% in the peripheral blood. Patient karyotype was 44,XY,+der(1)(t(1;21)(q21;q22), t(2;7)(q37:p13), der(9)(t(9;13)(p21;q14),–13,–15,der (15)(t(15;19)(p13;q15),–21 [28]/46,XY [2].

To define molecular alterations that occurred in this neoplasm, a microarray analysis was conducted to compare gene expression profiles of BNKL with healthy NK cells. Using 2-fold change criteria, a total number of 3,075 genes (for bone marrow) and 2,799 genes (for peripheral blood) were found in comparison with normal NK cells, thus showing numerous differences in the transcriptomes between these samples. However, comparison of transcripts in both malignant samples (peripheral blood and bone marrow) revealed a very similar profile (with variation in only 233 genes among them). Many of the genetic alterations found in the malignant samples were related to cell cycle, growth factors, TNF and IFN cell regulation, apoptosis, immunoglobulins, angiogenesis, and lymphocyte-related cell signaling. According to the gene expression results, TGF-β and JAK/STAT pathways seemed to be profoundly altered in this neoplasm. A brief list of relevant deregulated genes was selected on the basis of their possible involvement in BNKL and is shown in Table 1.

To further measure the angiogenic profile of BNKL, quantitative real-time PCR analyses for angiogenesis-related genes was carried out. Results showed that VEGF-A, IL-6, VEGFR1, VE-cadherin, and CXCL12 mRNA were overexpressed in bone marrow BNKL cells compared with normal CD56+ cells purified from healthy blood donors (Supplementary Fig. S1C).

The patient has received several lines of chemotherapy including cyclophosphamide/adriamycin/vincristine/prednisone (CHOP); etoposide/solumedrol/high-dose cytarabine/platinum (ESHAP); and an autologous stem cell transplant after BCNU/etoposide/Ara-C/melphalan (BEAM). After failure of these therapies, the patient is currently undergoing a stem cell transplant from an unrelated donor after reduced intensity conditioning.

### Development of a xenograft mouse model of human BNKL

Two months after BNKL cell injection, the number of human BNKL cells circulating in the murine peripheral blood begun to rise above the detection limit of the flow cytometry procedure used (0.1%). The phenotype and morphology of human cells in NSG mice overlapped with that observed in the patient (Fig. 1A–C). Day 100, BNKL-related symptoms were observed (animals were hunchbacked and ruffled coat), and mice began to die. At necropsy, mice had splenomegaly (Fig. 1D) and enlarged lymph nodes. Tumor cell invasion of mouse tissues, such as bone marrow (Fig. 1C, bottom), spleen (Fig. 1D, right), and lymph nodes (Fig. 1E), can be appreciated by immunohistochemistry. This underlines again the typical tropism of lymphoma cells to spleen, as diagnosed in the original patient. BNKL cells were found by flow cytometry in peripheral blood (11%), bone marrow (35%), and spleen (68%).

In secondary and tertiary NSG mice transplants, obtained by injection of a cell suspension of mice spleen, the BNKL engraftment kinetic and the time of symptom onset and death were not significantly different (Supplementary Fig. S2A and B).

On day 100, BNKL-related symptoms and lymphoma cells were observed in 70% (7 of 10), 67% (4 of 6), and 100% (13 of 13) of NS, NSB, and NSG mice, respectively. These data suggest, as previously shown in acute myelog-
енous leukemia models (24), that NSG mice are more prone to the engraftment of human hematologic malignancies than other NOD/SCID–related strains. It is relevant to mention that culture with different types of feeder layer (e.g., irradiated bone marrow or human kidney follicular dendritic cell line; ref. 26) or conditioned supernatant obtained by bladder carcinoma cell line 5637 (27) was unable to sustain the growth of these cells. Therefore, our orthotopic mouse model is a unique in vivo model to study this disease.

**Therapeutic effect of lenalidomide in the human BNKL mouse model**

So far, lenalidomide has been used to treat hematologic neoplastic diseases such as multiple myeloma, myelodysplastic syndromes (MDS), and lymphoma (15, 16, 28, 29), with some encouraging evidence of possible clinical activity in colorectal carcinoma (30). We therefore decided to test this drug in our model of human BNKL. Two weeks after the primary cell injection, mice were divided in 2 groups: one group was treated daily for 3 months by gavage with lenalidomide (Lena group) and other group was administered with vehicle, a solution of carboxymethylcellulose/Tween-80 used to dissolve the drug (control group). To monitor engraftment during treatment, we conducted successive blood collections from the tail vein and observed the presence of human neoplastic BNKL cells by flow cytometry. As Figure 2A shows, human engraftment was significantly reduced in the treated group compared with the control group. The low levels of engraftment present before treatment in Lena group decreased during treatment (Fig. 2A). In agreement with these data, also spleen echographies (taken at day 83 after BNKL cell injection and after 69 days of treatment) revealed a significant reduction in spleen areas of Lena group compared with control and healthy groups (Fig. 2B).

At sacrifice, the first macroscopic difference we observed, in the lenalidomide-treated mice, was a reduction of the

![Figure 1.](https://example.com/figure1.png)

**Figure 1.** Development of a xenograft mouse model of human BNKL. A, flow cytometric analysis of the BNKL patient at the time of diagnosis. Bone marrow cells are evaluated for CD45 (adenomatous polyposis coli), CD4 (fluorescein isothiocyanate), and CD56 (phycoerythrin). B, representative dot plots of mouse bone marrow. BNKL cells are conserving the original CD45 CD4 CD56 phenotype. C, representative natural killer lymphoma morphology at the time of diagnosis stained with May-Grünwald Giemsa (top, x400), and H&E mouse bone marrow cells (bottom, x400). D, representative images of the enlarged spleen (right) found in NSG mouse injected with BNKL human cells, compared with the healthy control (HC) spleen (left), 100 days after injection, with respective representative spleen histologic slides stained with H&E show spleen involvement in tumor-bearing mice compared with healthy controls (x200). E, representative enlarged lymph node found in BNKL mice (x400, x200). SSC, side scatter.
splenomegaly with respect to the control group (Fig. 2C). As observed in H&E staining, spleens of treated mice did not show any alterations and are comparable with healthy control spleens (Figs. 1D and 2C and D). Lenalidomide improved significantly the survival ($P = 0.0002$) of treated mice compared with control mice that died by day 100 (Fig. 3C). We evaluated peripheral blood, bone marrow, and spleen BNKL cell engraftment by flow cytometry (Fig. 3A). BNKL cells were below the detection limit in peripheral blood, bone marrow, and spleen in the Lena group, whereas the mean of the percentages of BNKL engraftment in control mice spleen was about 68%. Moreover, because NK cells express TNFα and lenalidomide inhibits TNFα production (15), we decided to evaluate serum values; in the Lena group, cytokine levels were significantly decreased with respect to the control group ($P = 0.046$; Fig. 3B). As it occurs for hTNFα, we observed the same behavior for hVEGF, highly expressed by BNKL cells, and reduced after treatment (Fig. 3D).

Although lenalidomide has numerous putative mechanisms of action which in particular involve the immune system (15, 16–20), it also exerts a demonstrated antiangiogenic effect as well (15–17, 18). To evaluate the antiangiogenic effect in our model, levels of CECs and CEPs were quantified at mice sacrifice. A significant decrease was found in CECs and CEPs levels in treated mice compared with untreated ones (Fig. 4A). Moreover, immunohistochemical (IHC) analysis of spleens and bone marrow of CD31+ mouse endothelial cells showed significant increased levels in the control and healthy groups compared with the Lena group (Fig. 4B and Supplementary Fig. S2C). Because VEGF and IL-6 are molecules directly involved in angiogenesis, we decided to evaluate their levels in serum samples collected at mice sacrifice. We obtained significant differences between control and tumor-bearing mice for IL-6 ($P = 0.049$) and VEGF ($P = 0.03$). However, IL-6 and VEGF levels were similar between treated and untreated groups (Fig. 4C).

It has been described that lenalidomide might have a direct cytotoxic activity against tumor cells (15). In this regard, immunohistochemistry revealed that levels of active caspase-3 were significantly augmented in Lena group spleens compared with healthy spleen and control group (untreated tumor-bearing mice). This finding shows the in vivo proapoptotic effect of lenalidomide (Fig. 5A). Moreover, an opposite result was found for PCNA, a marker of proliferation (Fig. 5B), thus indicating that treatment might inhibit cell proliferation and cause cell-cycle arrest.

To verify that lenalidomide was not only able to prevent tumor development but also able to reduce engraftment in established tumors, we carried out another experiment starting the treatment 2 months after BNKL cell injection.
Mice survival was significantly increased in treated mice compared with tumor-bearing untreated animals ($P = 0.0004$; Fig. 6A). When all the untreated mice were sacrificed, evaluation of human engraftment showed a significant reduction in the Lena group compared with control group (Fig. 6B).

**Discussion**

BNKL is a malignancy with an aggressive clinical course and no effective clinical options. We developed what, to the best of our knowledge, should be considered as the first preclinical orthotopic model of human BNKL. Tumor cells were able to grow in NSG mice and consistently replicate a pathology that reflects immunophenotypically and histologically the original cells injected. Human cells found in 3 mouse compartments (peripheral blood, bone marrow, and spleen) had a BNKL phenotype. Moreover, we observed involvement of lymph nodes and splenomegaly, which are typical characteristics found in BNKL patients at diagnosis. The course of the pathology in mice was aggressive: After injection, BNKL cells could be found often at 2 months, lymphoma manifestations occurred rapidly within the third month and mice did not survive more than 100 days. Our study also shows for the first time (to our knowledge) a transcriptomic analysis of BNKL, which may serve to better understand the disease and to identify potential future targets for therapy. Many genes have been found deregulated in this neoplasm in comparison with healthy NK cells. Of note, genes related to activation of TGF-β, JAK/STAT, and TNF/IFN signaling cascades seem to play a role in this malignancy. Interestingly, topoisomerase II-a (Tpo2a), a therapeutic target for many solid and hematologic tumors is highly increased in BNKL.

We could reproduce this model not only by injecting primary cells derived from the patient but also by injecting human cells obtained by mouse spleen dissociation. We
did not observe any particular changes in BNKL growth kinetics in secondary and tertiary recipients, nor differences in cell antigen expression and tumor aggressiveness. Moreover, it is relevant to mention the lack of ability of BNKL cells to grow in vitro under different conditions; they are able to replicate only in vivo. This represents a limitation of the model that hinders molecular characterization of the cells and testing antitumor compounds in vitro. Our
orthotopic model is, therefore, a unique in vivo model to study this disease.

We were also able to show that preclinical BNKL treatment with lenalidomide results in an effective antitumor activity. Lenalidomide exerts antitumor activity through multiple pathways (15), and this drug is currently used in a variety of neoplastic diseases. In our BNKL model, we saw a significant difference in tumor engraftment between control and Lena groups in a prevention experiment. Once mice were sacrificed, none of the 3 compartments analyzed (peripheral blood, bone marrow, and spleen) in treated mice were positive for the presence of BNKL cells. Because of the lack of a mature immune system in NSG mice, NK deficiency, and lack of γ/δ of IL-2, we could affirm that the action of lenalidomide in our model does not involve these mechanisms. It is likely that impairment of angiogenesis is playing a role in this model. Indeed, thalidomide and its derivatives, such as lenalidomide, reduce levels of angiogenic factors such as VEGF and basic fibroblast growth factor. The antiangiogenic effect of thalidomide has been shown in a variety of xenograft models (31–33). In keeping with these results, we have found a significant reduction in the number of CECs and CEPs and murine endothelial cells. However, our current model cannot rule out that the reduction in angiogenic cells was simply due to a lower (or absent) tumor growth. Because lenalidomide has been shown to alter cytokine production (34), we measured hTNFα serum levels and observed downregulation after treatment in our model (Fig. 3B). Moreover, we measured IL-6 and VEGF serum levels and obtained significant differences between control and tumor-bearing mice. However, we did not find a decrease in either IL-6 or VEGF levels after administration of lenalidomide, despite the sharp decrease in tumor growth and vascularization. It was previously shown that antiangiogenic drugs may sustain the expression of VEGF and other angiogenic factors due to drug-induced hypoxia (35). It is possible then that lenalidomide is causing a similar effect in this model.

Interestingly, in mice treated with lenalidomide, levels of active caspase-3 were significantly augmented, thus showing proapoptotic and cytotoxic effects of this drug in vivo. Lenalidomide has also been shown to inhibit proliferation in Burkitt's lymphoma cell lines and MDS cells (15). In agreement with these findings, we observed a decrease in PCNA expression, suggesting a possible induction of cell-cycle arrest.

In summary, we have developed the first preclinical model for BNKL, a rare and aggressive neoplastic disease with no effective treatment. Our model will serve to better define the tumor biology and the molecular mechanisms involved in this pathology. Administration of lenalidomide may offer a therapeutic option for BNKL patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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21.*********************************************************
Therapeutic Effect of Lenalidomide in a Novel Xenograft Mouse Model of Human Blastic NK Cell Lymphoma/Blastic Plasmacytoid Dendritic Cell Neoplasm

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