

Development of a murine model for blastoid variant mantle-cell lymphoma

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Blastoid-variant mantle-cell lymphoma (MCL-BV), unlike most B-cell non-Hodgkin lymphomas (NHL-Bs), is refractory to conventional chemotherapy and associated with a very poor prognosis. Development of new therapies has been hampered by the lack of valid animal models. We have developed a novel murine model of MCL-BV by crossing interleukin 14 α (IL-14 α) transgenic mice with c-Myc transgenic mice (double transgenic [DTG]). IL-14 α is a B-cell growth factor that is expressed in a number of high-grade lymphomas, including MCL-BV. Ninety-five percent of IL-14 α transgenic mice develop CD5⁺ large B-cell lymphomas by 18 months of age. Sixty percent of c-Myc transgenic mice develop pre-B-cell lymphomas by 12 months of age. Close to 100% of DTG mice develop an aggressive, rapidly fatal lymphoma at 3 to 4 months of age that is CD5⁺, CD19⁺, CD21⁺, CD23⁺, sIgM⁺. The tumor is found in the blood, bone marrow, liver, spleen, lymph nodes, gastrointestinal tract, and lungs and rarely in the brain, similar to

the involvement seen in human MCL-BV. Immunoglobulin gene rearrangements document the monoclonality of the tumor. Cyclin D1 is highly expressed in these tumors, as it is in MCL-BV. DTG represents a novel model for MCL-BV that should reveal important insights into the pathogenesis of the lymphoma and contribute to the development of new forms of therapy. (Blood. 2007;109:4899-4906)

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Introduction

Non-Hodgkin lymphomas (NHLs) are one of only a small number of human cancers that have been increasing in incidence over the last 3 decades.^{1,2} NHLs are a heterogeneous group of lymphoid tumors, more than 80% of which are derived from the B-lymphoid lineage (NHL-B). Most NHL-Bs are derived from the B2 subset of mature B cells, usually associated with germinal centers (GCs).^{3,4} Two important forms of NHL-B, small lymphocytic lymphoma/chronic lymphocytic lymphoma (SLL/CLL) and mantle-cell lymphoma (MCL), usually express CD5 and appear to be derived from the B1 subset.^{4,6} SLL/CLL and MCL, while sharing CD5⁺, B1 cell lineage characteristics, have quite different cellular, genetic, and clinical characteristics. SLL/CLL is an indolent lymphoma often associated with low-grade B-cell leukemia, which is sensitive to chemotherapy with a relatively good survival.^{7,8} MCL, however, is an aggressive lymphoma that is quite refractory to standard chemotherapy with an overall poor prognosis, although newer therapies have recently improved these patients' outlook.^{9,10}

Clinically, MCL presents with a male predominance in the sixth decade of life, with a mean survival of 40 months. A more aggressive form, the blastoid-variant MCL (MCL-BV), presents at an earlier age and has a mean survival of less than 20 months. Besides involvement of the bone marrow, lymph nodes, and spleen, there is frequently involvement of the colon and lungs. Approximately 25% of patients have a leukemic phase to their disease and 6% of patients develop central nervous system involvement.^{9,11}

Mantle-cell lymphoma is strongly associated with chromosomal abnormalities including the translocation t(11;14) and deletion of 11q22-23.¹²⁻¹⁴ The involvement of the *ATM* and cyclin D1

genes in this disease has been postulated from these abnormalities.¹³ The deletion of 13q14 commonly seen in B-CLL also occurred in 70% of patients with MCL, suggesting overlapping genetic disorders in these 2 diseases.¹⁵ Increased expression of *bcl2* and *c-Myc* is seen in most cases of MCL,^{9,16-20} with the antiapoptotic *bcl2* often increased in expression in many lymphoid malignancies.²¹ Myc proteins participate in oncogenesis in a wide variety of tumors by a number of different mechanisms, including increasing the entry of cells into the cell cycle, regulating the transcription of many relevant genes, and inducing genomic instability.²²⁻²⁶ The NF- κ B family of transcription factors are central mediators in the growth and survival of MCL,²⁷⁻³⁰ consisting of 5 members: c-rel, RelA (p65), RelB, NF- κ B1 (p50/p105), and NF- κ B2 (p52/p100).³¹

Currently, there are no spontaneous animal models for MCL, although xenotransplantation models have been produced in severe combined immunodeficiency (SCID) mice growing human MCL cells.^{32,33} In addition, a candidate MCL model has recently been created in E μ -cyclin D1 transgenic Balb/c mice treated with 3 monthly intraperitoneal injections of the tumor promoter pristane.³⁴

Interleukin 14 was identified in a Burkitt lymphoma cell line and initially called high-molecular-weight B-cell growth factor (HMW-BCGF).³⁵ It acts as a growth factor for germinal center B-lymphocytes, B1 cells, and memory B cells.^{35,36} IL-14 is also a potent growth factor for B-CLL in vitro.³⁷ Two transcripts are derived from the *IL14* gene, which have been designated IL-14 α and IL-14 β . Transgenic mice expressing *IL4A* constructed with the pE μ .SR vector develop autoimmunity and large B-cell lymphomas by 18 months of age.³⁸ The expression of IL-14 α mRNA has been

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identified in high-grade B-cell tumors *in vivo*, and IL-14 antisense oligonucleotides inhibit these tumors *in vitro*.³⁹

We describe here the development of lymphoid malignancies closely resembling the blastoid variant of MCL (MCL-BV) in IL-14 α \times c-Myc double-transgenic mice (DTG), each produced using the vector pE μ SR that directs transgene expression in the B-cell compartment.⁴⁰ The DTG mice develop an aggressive, monoclonal, CD5⁺, CD19⁺, IgM⁺, and cyclin D1⁺ B-cell lymphoma early in life that involves the bone marrow, lymph nodes, spleen, lungs, colon, and, rarely, the central nervous system. Increased expression of bcl-2, ATM, RelA, and NF- κ B2 is noted along with the translocation of the NF- κ B proteins to the lymphoma cell nucleus. This is the first animal model that spontaneously develops lymphoma consistent with human MCL-BV.

Patients, materials, and methods

Patient cells were obtained under protocols approved by the institutional review board (IRB) at the M. D. Anderson Cancer Center. Informed consent was provided in accordance with the Declaration of Helsinki.

Mice

C57BL/6 (B6) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in the Laboratory Animal Facility at State University of New York at Buffalo (SUNY Buffalo) in accordance with institutional guidelines. IL-14 α transgenic mice were made as described.³⁸ C-myc transgenic mice were purchased from Charles River Laboratories (Wilmington, MA). Female c.b.-17-ICR/SCID mice at 3 weeks of age were purchased from Taconic Farms (Germantown, NY). The mice were maintained in the M. D. Anderson Cancer Center SCID Mouse Core Facility, which consists of a standard barrier vivarium, under sterile pathogen-free conditions suitable for immune-deficient rodents in accordance with institutional guidelines.

Total RNA purification and semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR)

Dependent on the source of samples, 2 different methods were used to collect total RNA. TRIzol purified total RNA from the whole spleen of mice, according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Total RNA from cells harvested from the spleens of mice was purified with the QIAamp RNA Blood Mini Kit, according to the manufacturer's instructions (Qiagen, Valencia, CA).

RT-PCR was performed as described.³⁸ The following primers were used for cyclin D1, bcl-2, ATM, RelA, RelB, NF- κ B1, NF- κ B2, IL-14 α , c-Myc, PAK, and actin: cyclin D1 forward primer, 5'-AGAAATCCAATGGGGTTGGA; cyclin D1 reverse primer, 5'-CTACGTAGCCTTCAACACAT; Bcl-2 forward primer, 5'-GACTTCGTAGCAGTCATCCT; Bcl-2 reverse primer, 5'-TGCGCATCTTGGCCTTGAGA; ATM forward primer, 5'-GCTGCTTGACTTTGGCACTTAGCA; ATM reverse primer, 5'-ATCAGACTGTCAACACCACACCA; RelA forward primer, 5'-ACCATCAGGGCAGATCTCAAACCA; RelA reverse primer, 5'-ACCGAAGCAGGAGCTATCAACACA; RelB forward primer, 5'-GCGGATTGCGGAATCAACAAGGA; RelB reverse primer, 5'-AGCTGCTCAACTCTCCAAGGACAT; NF- κ B1 forward primer, 5'-TGAA-GCAGCTGACAGAAGACACGA; NF- κ B1 reverse primer, 5'-TGAGTTT-GCGGAAGGATGTCTCCA; NF- κ B2 forward primer, 5'-TGGTGTCATTGAG-CAGATAGCCCA; NF- κ B2 reverse primer, 5'-TTCCTGTATGTGTCCACCAG-GCTT; IL-14 α forward primer, 5'-CACAAGGACCTACAACAGCAGC; IL-14 α reverse primer, 5'-TGATGCTTCTGTGCTCGG; c-Myc forward primer, 5'-GACGCGGGGAGGCTATTCTG; c-Myc reverse primer, 5'-AGGGTGT-GACCGCAACGT; Pak forward primer, 5'-CGCTTGCTTCAAACATCAAA; Pak reverse primer, 5'-TCCCTCATGACCAGGATCTC; actin forward primer, 5'-GTGGGGCGCCCCAGGCACCA; and actin reverse primer, 5'-CTCCTT-AATGTCACGCACGATTTT.

RT-PCR products were separated on 1% agarose gels and visualized by UV light after incorporation of ethidium bromide, as described.⁴¹

Morphologic and immunohistochemical analysis

Lymphoma tumor tissue and mouse organs were harvested from IL-14 α transgenic, c-myc transgenic, and DTG transgenic mice; placed in 10% formalin (Baxter Diagnostics, Issaquah, WA); and embedded in paraffin. Thin sections were prepared on slides and the slides were stained with the HISTOMOUSE-MAX kit (Invitrogen) as instructed by the manufacturer. Antimouse CD5 and B220 antibodies were purchased from BD Pharmingen (Heidelberg, Germany). Anti-cyclin D1 antibody was purchased from Lab Vision (Fremont, CA).

Flow cytometry

Cells collected from spleens, lymph nodes, or tumor tissue of the mice were stained with panels containing antimouse antibody CD19-APC, CD138-PE, CD21-FITC, CD5-PE-Cy5, CD23-PE, IgD-FITC (BD Pharmingen); CD38-PE-Cy5 (eBioscience, San Diego, CA); and IgM-PE (Abcam, Cambridge, United Kingdom) and analyzed on a BD Biosciences FACSCalibur machine and Winlist software (Verity Software House, Topsham, ME).

Immunoglobulin gene rearrangements

Ig gene rearrangements for clonal B-cell determination of mouse tumor were performed by high-fidelity PCR using 0.1 mg of DNA extracted from normal C57BL/6J mouse liver, spleen, and transgenic mouse tumors using the DNeasy Tissue kit (Qiagen). The primers for immunoglobulin gene rearrangement in the lymphomas from the IL-14 α transgenic mice were DHL (5'-GGAATTCGTTTTTTGTSAAGGGATCTACTACTGTG), J3 (5'-GTCTAGATTCTCACAAGAGTCCGATAGACCCTGG); and DHR (5'-TTTGTGTGMTGGATATAKCACTGAG), J3 (5'-GTCTAGATTCTCAAG-AGTCCGATAGACCCTGG). PCR was performed as described⁴² with the following protocol: incubation at 95°C for 5 minutes then 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes for 35 cycles, followed by extension at 72°C for 10 minutes. The PCR products were analyzed on 1.2% agarose gel electrophoresis.

Spectral karyotyping

Spectral karyotyping (SKY) was performed as previously described.⁴³ In short, metaphase chromosome spreads were prepared from murine lymphoma cells using standard hypotonic treatment and fixation. They were then denatured with 70% formamide and hybridized with the murine SKY probes (Applied Spectral Imaging, Carlsbad, CA). Chromosomes were isolated by high-resolution flow sorting and tagged with different combinations of digoxigenin, Texas red, Cy5, biotin/streptavidin, and Cy5.5. SKY images were captured using the Spectral Curve/Interferometer, and karyotypes were prepared using SKYView software (Applied Spectral Imaging).

Western blot for human IL-14 α

The Western blot for human IL-14 α in lymphoma cells was performed as previously described.³⁹

NF- κ B translocation

Translocation of NF- κ B to the nucleus was detected using electrophoretic mobility shift assay (EMSA) analysis and Western-blot analysis as previously described.²⁹ Antibodies to NF- κ B proteins were purchased from Santa Cruz Biotech (Santa Cruz, CA).

Oversight

The animal studies were all approved by the Institutional Animal Care and Use Committee, SUNY at Buffalo School of Medicine and Biomedical Sciences. Lymphoma cells were obtained from patients at the M. D. Anderson Cancer Center according to the local IRB.

Microscopy

Images were visualized under an Olympus BX41 microscope (Olympus, Melville, NY) equipped with a 40 \times /0.65 or 40 \times /1.25 oil-immersion

Olympus Uplan F1 objective lens. Permount imaging medium (Fisher Scientific, Pittsburgh, PA) and haematoxylin-eosin stain (H&E stain) were used. Images were captured with an Olympus Q color 5 camera, acquired with Olympus Q Capture software version 2.7, and processed with Adobe Photoshop CS2 software (Adobe Systems, San Jose, CA).

Results

Characteristics of the malignancy in IL-14 α TG \times c-Myc TG (DTG) mice

Since IL-14 is expressed in high-grade B-cell lymphomas, many of which also express c-Myc, we evaluated whether c-Myc could accelerate tumor formation in IL-14 α TG mice.³⁹ Previous studies had demonstrated that 95% of C57BL/6 IL-14 α TG mice develop CD5⁺, CD19⁺, sIgM⁺, cyclin D1-negative large B-cell lymphomas by 16 to 18 months of age.³⁸ We crossed c-Myc transgenic mice, produced with the same vector as the IL-14 α transgenic mice,⁴⁰ with IL-14 α TG mice to obtain double-transgenic mice (DTG). Every DTG mouse is characterized by an initial leukemic phase (Figure 1A) and develops a B-cell malignancy within 4 months, preceding the appearance of dramatic, widespread lymphadenopathy and splenomegaly (Figure 1B). DTG mice at 2 months of age have high peripheral white blood cell counts that are 1.5 to 3 times higher than littermate controls with 10% to 50% leukemic blasts. Lymphoma can readily be demonstrated in the bone marrow (Figure

1C), spleen (Figure 1D), gastrointestinal tract (Figure 1E), and liver (Figure 1F) in all DTG mice. This pattern of organ involvement is characteristically seen in the most aggressive forms of MCL.^{11,12,44,45} The morphology of these tumors closely resembles human MCL-BV, with diffuse structural effacement of lymphoid tissue with large (blastoid) lymphoma cells showing large ovoid or cleft nuclei with a vesicular chromatin pattern and often prominent nucleoli (Figure 1G).⁴⁶ There are often prominent large histiocytes admixed throughout the tumor. To characterize this tumor further and to distinguish it from the tumors of the IL-14 α TG mice and c-myc TG mice, we compared the histology (Figure 2A), expression of cell-surface markers (Figure 2B), and gene-expression profile (Figure 2C) of these 3 tumors. Immunoglobulin gene rearrangements demonstrated that the tumors of the IL-14 α TG mice, the c-myc TG mice, and the DTG mice were all monoclonal in origin (Figure 2F). The histology of tumors from the IL-14 α TG mice was most consistent with large B-cell lymphoma (LBCL), demonstrating atypical large transformed B-lymphoid cells with large vesicular nuclei, slightly basophilic cytoplasm, morphologic variability, and easily found mitotic figures (Figure 2A).³⁸ The histology of the tumors from the c-myc TG mice was most consistent with an aggressive pre-B-cell lymphoma (Figure 2A) and demonstrated relatively monomorphic, medium-sized atypical lymphoid cells with a moderate amount of basophilic cytoplasm, regular oval-to-round nuclei, and easily found mitotic figures.⁴⁰ The histology of the tumors from the DTG mice was very different

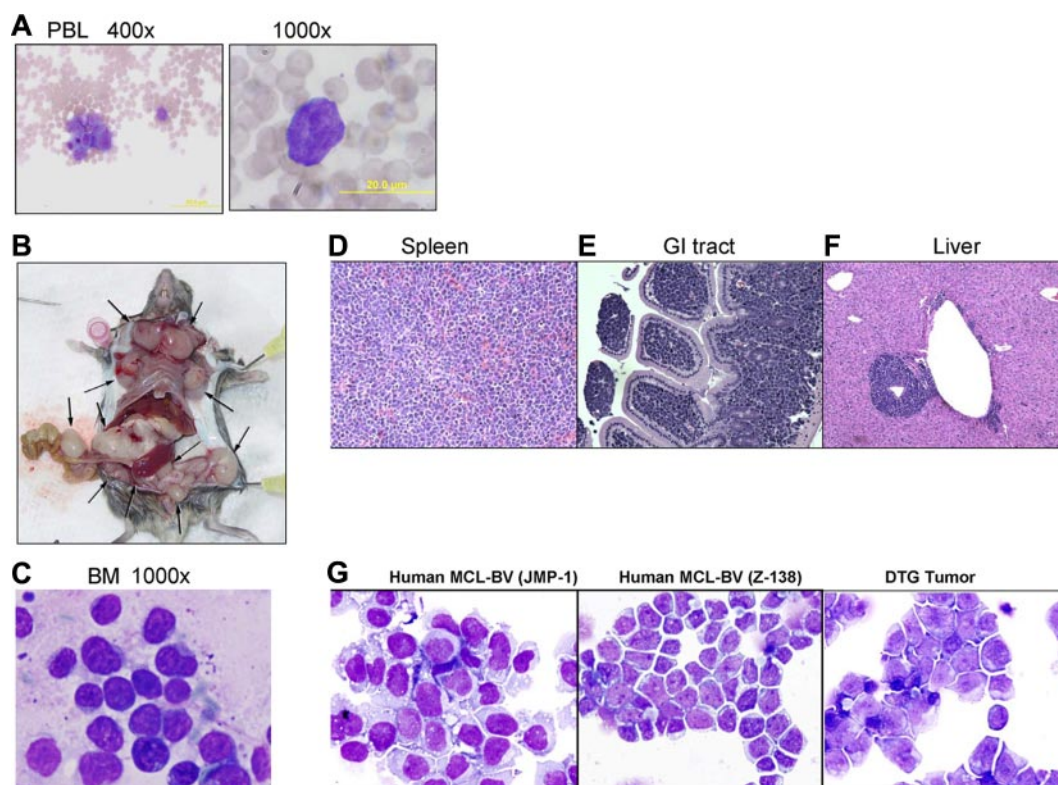


Figure 1. Clinical appearance of tumors in DTG mice. (A) Peripheral-blood smear from a 3-week-old DTG mouse showing leukemic atypical large blastic lymphoid cells morphologically similar to lymphomas subsequently arising in peripheral lymphoid tissues (original magnifications: left, $\times 400$, right, $\times 1000$). (B) Gross necropsy photo of DTG mouse, showing widespread and extensive peripheral lymphadenopathy indicative of lymphomatous involvement as histologically shown in panel D. Arrows indicate lymph nodes. (C) Bone marrow from a 3-month-old DTG mouse showing large "blastoid" lymphoma cells (original magnification, $\times 1000$). (D) Histopathologic H&E-stained paraffin section of the spleen showing diffuse effacement of an enlarged lymph node diffusely replaced with large "blastoid" lymphoma cells with admixed large "pink" histiocytes characteristic of MCL-BV (original magnification, $\times 400$). (E) Lymphomatous involvement in the gastrointestinal tract showing diffuse involvement of the lamina propria and submucosa (original magnification, $\times 400$). (F) Liver involvement in DTG mice, showing perivascular infiltrates of lymphoma cells (original magnification, $\times 400$). (G) Comparison of DTG lymphoma cell line with 2 representative MCL-BV cell lines from patients showing morphologic similarity between human MCL-BV and the DTG murine model (H&E; original magnification, $\times 1000$).

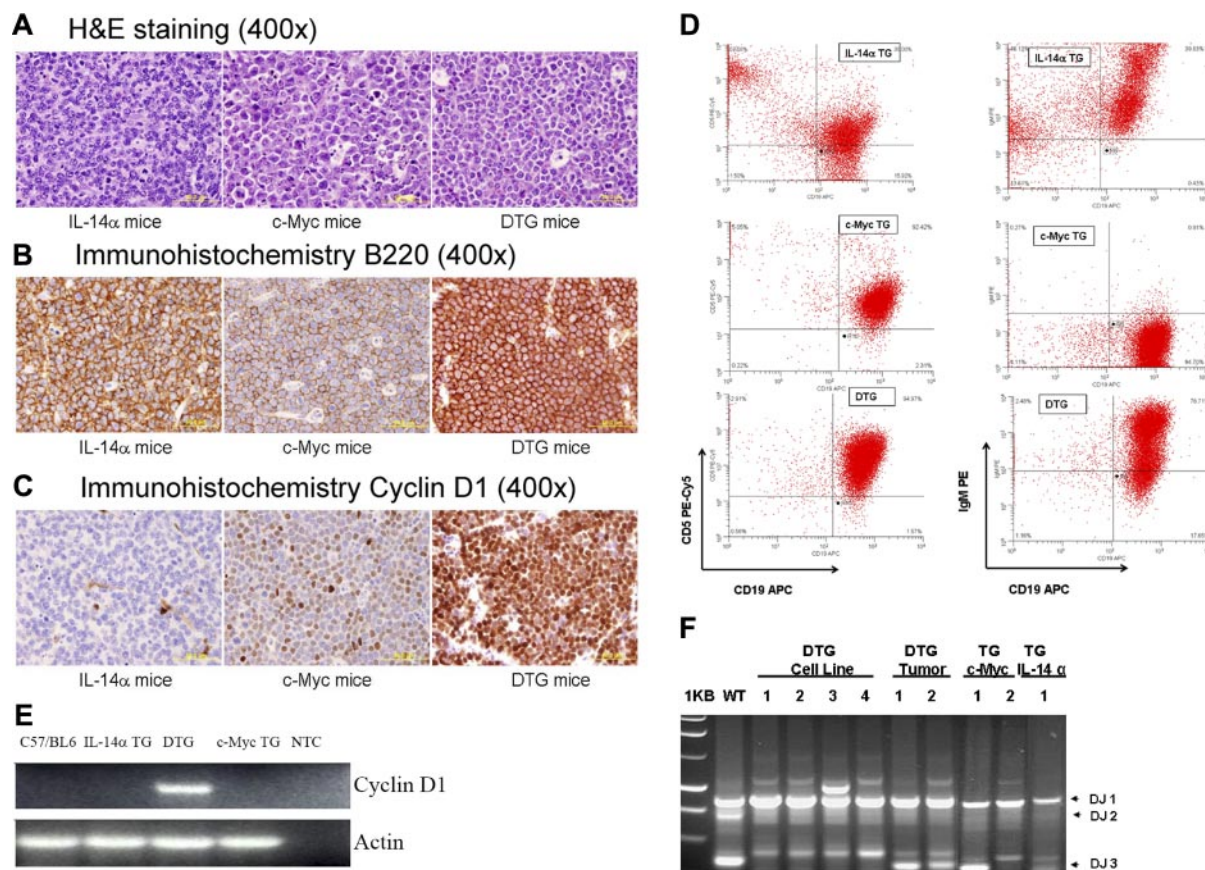


Figure 2. Histopathologic and immunohistochemical characteristics of the DTG lymphomas in relation to the B-cell lymphomas in parental IL-14α TG and c-myc TG mice. (A) H&E-stained paraffin sections (original magnification, $\times 400$). (B) Sections stained with anti-B220 (original magnification, $\times 400$). (C) Sections stained with anti-cyclin D (original magnification, $\times 400$). DTG tumor staining is more uniform and intense than that of the c-myc TG tumor. IL-14α TG tumor is negative. (D) Flow cytometry of tumors from IL-14α TG, c-myc TG, and DTG mice showing CD19/CD5 and CD19/sIgM staining. (E) Semiquantitative RT-PCR of C57/BL6 spleen and tumors from IL-14α TG, DTG, or c-myc TG mice analyzed for cyclin D1 and actin, as described in "Patients, materials, and methods." (F) Immunoglobulin gene rearrangements performed on tumors from IL-14α TG, c-myc TG, and DTG mice showing monoclonality of all of these tumors, as described in "Patients, materials, and methods."

from the tumors of the IL-14α TG mice or c-myc TG mice (Figures 1 and 2A). The tumors of the IL-14α TG mice, c-myc TG mice, and DTG mice all expressed CD5 (Figure 2D) and CD19 (Figure 2B,D).³⁸ The tumors of the IL-14α TG and DTG mice expressed sIgM, but the tumors from the c-myc TG mice did not, as would be expected of a pre-B-cell tumor (Figure 2D).⁴⁰ None of these tumors expressed CD21, CD23, or sIgD (Figures S1-S2, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). The cell-surface phenotype of the DTG tumors is consistent with MCL-BV.^{9,11,47,48}

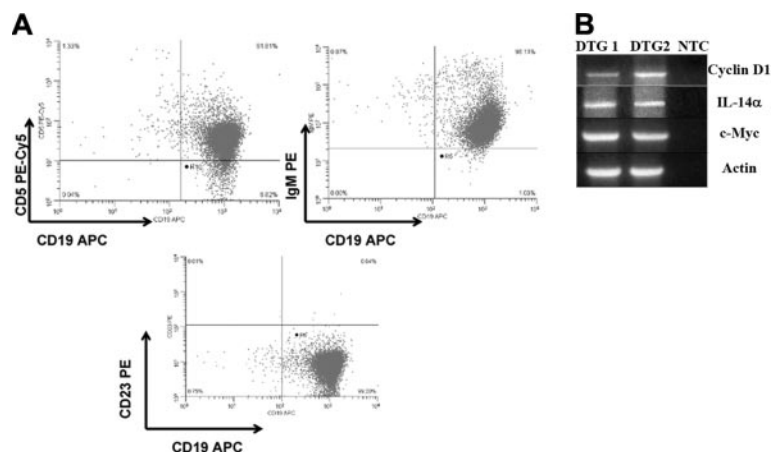
One of the characteristics of MCL-BV is the expression of bcl-1/cyclin D1.^{5,9,49} We evaluated cyclin D1 expression in the tumors of the IL-14α TG, c-myc TG, and DTG mice. The tumors of the DTG mice express high levels of cyclin D1 at both the RNA (Figure 2E) and protein level (Figure 2C), whereas the tumors of IL-14α TG mice do not. A small percentage of the tumor cells from the c-myc TG mice express cyclin D1 at a low level, as visualized by immunohistochemistry (Figure 2C). The DTG lymphomas are distinct from the lymphoid tumors of either the IL-14α TG mice or c-myc TG mice, from which the DTG mice were derived. Several lymphoma cell lines have been developed from the tumors of the DTG mice that retain the characteristics of the primary tumors, with expression of CD5, CD19, sIgM, and cyclin D1 (Figure 3). Early in vitro-explanted cultures initially showed mixtures of lymphoid tumor cells with large macrophage-like cells, as in the

histopathologic sections, that in time evolved into cultures of predominantly MCL-BV cells.

Cytogenetic studies and spectral karyotyping on cultured DTG/MCL cells

Because MCL-BV is strongly associated with chromosomal abnormalities including the translocation t(11;14) and deletion of 11q22-23,¹²⁻¹⁴ we undertook preliminary cytogenetic analysis and karyotyping of tumor cells from several of the DTG lymphoma cell lines. The chromosomal range on the DTG lymphoma cell lines studied was 37-41 and the modal number was 40. Figure 4 shows spectral karyotyping of a representative DTG cell line of 4 studied. Additional cytogenetic data are shown in Figures S1-S2. These studies revealed a complex karyotype with multiple chromosomal abnormalities in the DTG lymphoma cells. Chromosomal abnormalities seen in all the preparations include trisomy 15 and a 3:10 translocation. Other chromosomal abnormalities are seen variably in the different tumor cell lines. We did not identify a syntenic equivalent chromosomal t(11;14) translocation in any of the cell lines studied so far. The chromosome 3 abnormality could potentially involve *ATM*, a gene frequently implicated in the pathophysiology of MCL, particularly MCL-BV,^{13,42,50-52} and *PAK*, a gene involved with increased transcription and translation of cyclin D1.⁵³

Figure 3. Characteristics of tumor cell lines derived from DTG mice. (A) Flow cytometry evaluating CD19/CD5, CD19/sIgM, and CD19/CD23. (B) Semiquantitative RT-PCR evaluating cyclin D1, IL-14 α , and c-Myc, as described in "Patients, materials, and methods."



Additional molecular studies on the DTG/MCL murine lymphomas

Several potential oncogenes are frequently expressed in MCL-BV in addition to cyclin D1, particularly in MCL-BV. We have demonstrated that *IL14A* is expressed in several human MCL-BV cell lines, as well as primary MCL-BV cells (Figure 5A). The *MYC* gene is frequently expressed in MCL-BV and represented in our model through the expression of the pE μ SR c-myc transgene.^{9,19,33,51,54} Bcl-2 or its antiapoptotic family members is often expressed in MCL and is expressed in our animal model as well (Figure 5B).^{17,55} Recent studies have suggested that various NF- κ B proteins are constitutively activated in MCL-BV and are important for the growth of these tumors.^{29,56-58} We have demonstrated that the mRNA for the NF- κ B family members RelA and NF- κ B2 are overexpressed and constitutively activated in the tumors from the DTG mice but not from the IL-14 α TG or c-myc TG mice (Figure 5C). To demonstrate that NF κ B is constitutively active in the DTG tumors, we also demonstrated nuclear translocation of these proteins by EMSA analysis and Western blotting in the DTG lymphoma cells (Figure 5D-E).⁵⁸⁻⁶⁰ Because of the translocation involving chromosome 3 and the potential involvement of *PAK* in regulation of cyclin D1, we evaluated the expression of *PAK* in the DTG tumors. Figure 5B demonstrated that *PAK* is overexpressed in DTG tumors and

weakly expressed in IL-14 α TG tumors but is not expressed in c-myc TG tumors.

Transplantation of DTG lymphoma cells into immune-deficient SCID mice

Although the DTG lymphoma appears to display all of the requisite characteristics of an aggressive NHL-B, it was important to determine if the DTG lymphoma cells could be successfully transplanted. For these studies, DTG lymphoma cells from 1 of the recently established DTG cell lines were adoptively transferred into immune-deficient c.b.17 SCID mice, as previously described.³² However, while most human lymphoma cells (including MCL) required xenotransplantation at doses of 10×10^6 to 20×10^6 or greater cells per mouse, DTG tumor cells transplanted at this dose repeatedly killed the SCID mice in 2 to 3 days. The maximum number of DTG tumor cells that could be successfully transplanted into a SCID mouse, without killing the mouse, is 10×10^6 , and we have successfully transplanted some DTG tumors at 5×10^6 cells/mouse. Lymphoid tumors appeared within 2 weeks after transplantation and arose primarily within the peritoneal cavity, attached to mesentery and external to the bowel (Figure 6A-B). Immunohistochemistry of the tumors growing in the SCID mice expressed B220, CD5, and cyclin D1, identical to the original DTG lymphoma (Figure 6C).

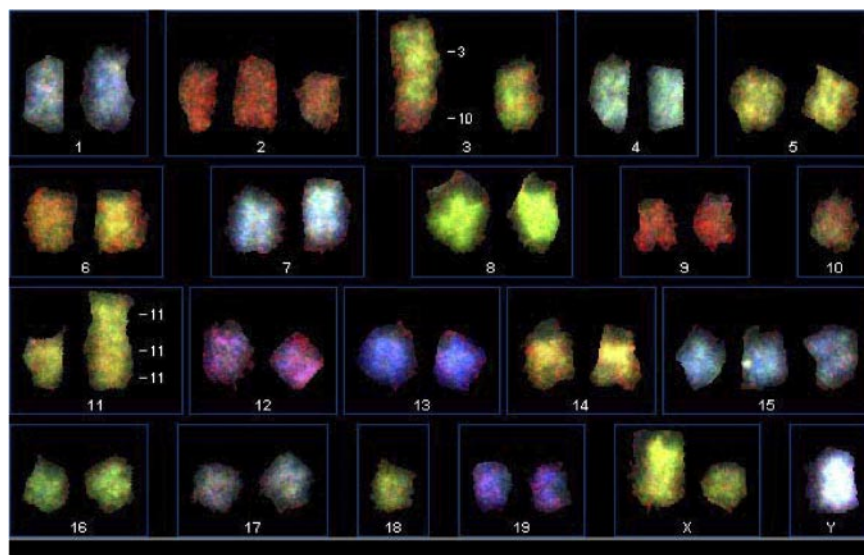


Figure 4. Spectral karyotyping (SKY) of a DTG cell line. SKY was performed as outlined in "Patients, materials, and methods." A 3:10 translocation, trisomy 15, and 11:11 translocation were observed.

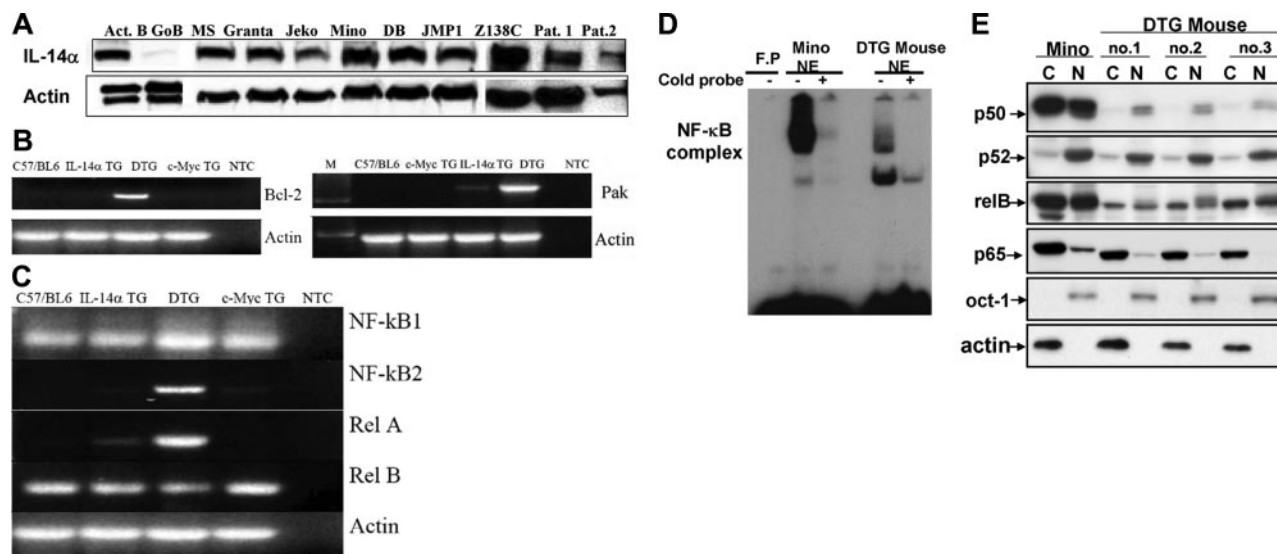


Figure 5. Gene expression in MCL-BV. (A) Demonstration of IL-14 α expression in MCL-BV cell lines and fresh tumors from various patients, performed as described in "Patients, materials, and methods." (B) Semiquantitative RT-PCR analysis of bcl-2 and Pak expression in spleen from C57BL/6 mice, and tumors from IL-14 α TG, DTG, and c-Myc TG mice, performed as described in "Patients, materials, and methods." NTC indicates negative control (no primers). (C) Expression of NF- κ B components in the lymphoma of a DTG mouse. Total RNA purified from the indicated mouse was analyzed for NF- κ B1, NF- κ B2, p65, and RelB by semiquantitative RT-PCR. (D) Translocation of NF- κ B to the nucleus in DTG lymphoma cells. EMSA analysis was performed as described in "Patients, materials, and methods" using nuclear extracts purified from Mino cells or DTG lymphoma cells. FP indicates free probe. (E) Cytoplasmic and nuclear extracts were purified from Mino cells or 3 different cell lines derived from the lymphoma of DTG mouse and analyzed for NF- κ B components by Western blotting as described in "Patients, materials, and methods."

Discussion

In these studies, we demonstrate the production of a murine lymphoma model that recapitulates clinical, histologic, and genetic features of MCL-BV, a morphologic variant of classical MCL. Transgenic mice expressing oncogenes known to be up-regulated

in MCL, such as cyclin D1, *Myc*, and *Bcl2*, have produced lymphomas resembling various NHL-B histotypes, but none of these resembled MCL.^{17,40,61-66} We evaluated lymphomagenesis in IL-14 α transgenic mice because IL-14 was originally identified in a Burkitt lymphoma cell line, induces proliferation of B cells, enhances expression of bcl-2, and shows constitutive expression in a wide range of B-cell malignancies.^{35,39} The IL-14 α transgenic mice develop a lymphoma in older mice resembling human large B-cell lymphoma.³⁸ To further analyze the role of IL-14 in lymphomagenesis, we bred the IL-14 α transgenic mice with c-Myc transgenic mice, since c-Myc is dysregulated in Burkitt and other lymphomas⁶⁷ and c-Myc facilitates the development of cancer without necessarily dictating the phenotype of the tumor.^{63,64,66,68-71} The rapid onset and the high rate of penetrance (close to 100%) of MCL-BV in the DTG mice suggest that IL-14 α is playing a critical role in the development of these tumors.

The DTG/MCL-BV murine model closely resembles the human counterpart except for the lack of the syntenic equivalent translocation t(11;14) involving the cyclin D1 gene. Interestingly, MCL has been described in a small number of patients without the t(11;14) translocation. In all of these cases, cyclin D1 was not highly expressed and utilization of other D cyclins was implicated in the pathophysiology of the tumors.^{72,73} In the tumors of DTG mice, however, cyclin D1 is overexpressed (Figures 2C, 2E, 3B, 6C), so another mechanism(s) for cyclin D1 dysregulation besides t(11;14) must be present. The expression of cyclin D1 is complex and may require constitutive nuclear expression to induce B-cell lymphoma. A number of genes have been implicated in the regulation of cyclin D1 transcription and translation, including *PAK*, which may be involved in the 3:10 translocation identified by SKY (Figure 4) and is overexpressed in the DTG tumors (Figure 5B).⁷⁴⁻⁷⁶

Recent studies have suggested that besides cyclin D1, both *NF κ B* and *ATM* are critical for MCL.^{5,11,29,57} A characteristic deletion 11q22-q23 found in MCL involves *ATM*.^{5,13} The t(3:10) translocation identified in the DTG tumors may involve *ATM* as well (Figure 4). The levels of *ATM* expression were not, however, found to be significantly different

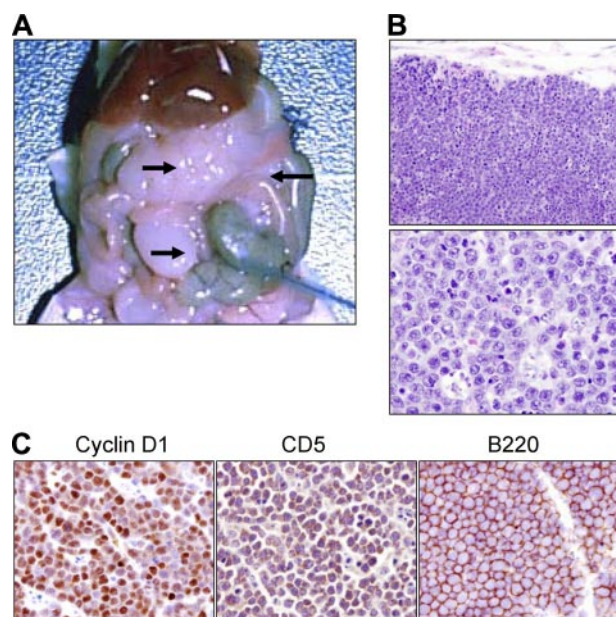


Figure 6. Transplantation of DTG lymphoma cells into immune-deficient SCID mice. (A) Necropsy of a SCID mouse that received a transplant of cells from a cell line derived from a DTG mouse lymphoma (arrows). (B) H&E paraffin sections of the SCID peritoneal tumor bearing the DTG tumor cells from low (top panel; original magnification, $\times 100$) to high magnification (bottom panel; original magnification, $\times 400$). (C) Immunohistochemical analysis of the transplanted lymphoma from a SCID mouse showing the expression of cyclin D1, CD5, and B220 (original magnification, $\times 400$).

among normal spleen and tumors from c-Myc TG, IL-14 α TG, and DTG mice (data not shown). In contrast, significant elevations of RelA and NF- κ B2 mRNA were identified in the DTG tumors (Figure 5C). NF κ B was constitutively activated and expressed in the nucleus of these tumors, as has been described in many NHL-B lymphomas, suggesting that it was active in the disease process (Figure 5D-E).

While closely reproducing many features of MCL-BV, the tumors of the DTG mice provide a novel model to dissect the molecular events leading to this type of malignancy. In addition, this murine model suggests that MCL-BV may arise de novo in bone marrow (BM), rather than only as a progression from classic (small-cell) MCL. Since MCL does not occur spontaneously in mice, the DTG mice provide a unique opportunity to understand the development of the MCL-BV phenotype in the murine lymphoid lineage. How IL-14 and c-Myc interact to produce this phenotype may provide important clues to the pathogenesis of MCL-BV. The DTG mice provide an important model to test therapeutic agents for MCL-BV. Many questions remain to be answered including what cells are these tumors derived from and why these tumors are so widespread in the body at clinical presentation. It is important to determine why these tumors are so biologically and clinically aggressive and so refractory to therapies that have been effective in other forms of non-Hodgkin lymphoma.

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Authorship

Contribution: R.J.F. designed research and wrote the paper; L.S., Y.C.L.-L., and L.V.P. performed research and wrote the paper; A.M., H.-J.Z., A.T.T., C.Z., and L.H. performed research; J.K.C. analyzed data; and J.L.A. designed research, analyzed data, and wrote the paper.

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