Published in final edited form as:

Leuk Res. 2010 February ; 34(2): 210. doi:10.1016/j.leukres.2009.06.007.

Irradiated *Blm*-deficient mice are a highly tumor prone model for analysis of a broad spectrum of hematologic malignancies

Madhuri Warren^{a,b,*}, Yeun-Jun Chung^a, William J. Howat^a, Hannah Kitson^a, Ralph McGinnis^a, Xingpei Hao^c, John McCafferty^a, Torgny N. Fredrickson^c, Allan Bradley^a, and Herbert C. Morse III^{c,**}

Madhuri Warren: mvw@pathologydiagnostics.com; Yeun-Jun Chung: ; William J. Howat: ; Hannah Kitson: ; Ralph McGinnis: ; Xingpei Hao: ; John McCafferty: ; Torgny N. Fredrickson: ; Allan Bradley: ; Herbert C. Morse: hmorse@niaid.nih.gov

^a Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

^b University of Cambridge, Department of Histopathology, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK

^c Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD 20852, USA

Abstract

Mutations in the *BLM* gene cause human Bloom syndrome (BS), an autosomal recessive disorder of growth retardation, immunodeficiency and cancer predisposition. Homozygous null *Blm*^{m3/m3} mice are cancer prone with a 5-fold increased risk of cancer compared with *Blm*^{m3/+} and *Blm*^{+/+} mice. Irradiation of *Blm*^{m3/m3} mice increased the risk to 28-fold. Tumors occurred mainly in the hematopoietic system and were similar to those in BS based on detailed histologic and immunohistochemical analyses. Irradiated *Blm*-deficient mice thus provide a novel model for understanding accelerated malignancies in BS and a new platform for investigating the molecular basis for a wide range of hematopoietic neoplasms.

Keywords

Bloom syndrome; *Blm*-deficient mouse model; Histopathology; Immunohistochemistry; Mouse lymphoma; Mouse leukemia Tissue microarray

*Corresponding author at: Diagnostics Ltd., St. Johns Innovation Centre, Cambridge, CB4 0WS, UK. Tel.: +44 01223 421 334; fax: +44 01223 420 844.

**Corresponding author at: Laboratory of Immunopathology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 5640 Fishers Lane, Twinbrook I, Room 1528, Rockville, MD 20852, USA. Tel.: +1 301 496 6379; fax: +1 301 402 0077.

Conflict of interest statement

The authors declare that they have no competing financial interests.

Contributions. MW is accountable for the conception of the paper, designed and performed the pathology research, collected and analyzed data, is responsible for the integrity of the pathology data and wrote the paper; YJC designed the study and performed the animal research; WJH designed and performed immunohistochemical research and contributed to the writing of the paper; HK collected lab animal data; RMG performed statistical analysis of the data; XH performed immunohistochemistry; JMC is responsible for the integrity of the immunohistochemical data; TNF analyzed the pathology data; AB is accountable for the conception of the animal study, is responsible for the integrity of the animal data, and contributed to the writing of the paper; HCM analyzed the pathology data and contributed to the writing of the paper.

1. Introduction

Human Bloom syndrome (BS) is an extremely rare autosomal recessive genetic disorder characterized by short stature, photosensitive skin rashes, immunodeficiency, reduced fertility, and predisposition to cancer [1,2]. Individuals homozygous for the mutation exhibit frequent B cell non-Hodgkin lymphomas (NHL) and leukemia in their twenties and squamous cell carcinomas of the head and neck in their thirties [3,4]. These tumors are similar to those occurring in the general population but are characterized by an earlier age of onset and the occurrence of multiple cancers in one individual [4].

The BLM protein is a member of the RecQ family of DNA helicases [5] and has an important functional role in the resolution of Holliday junctions that occur during the S phase of the cell cycle [6,7] and prevention of excessive or inappropriate recombination [8,9]. *BLM*-deficient cells attempt to repair certain types of DNA damage by excessive homologous recombination [10], which increases rates of loss of heterozygosity (LOH) resulting in elevated rates of tumorigenesis [11]. In a similar fashion to BS lymphocytes, *Blm*-deficient mouse embryonic stem (ES) cells also show a high rate of mitotic recombination. This feature has been utilized to generate homozygous mutant cells from cells with heterozygous mutations [12,13]. A viable *Blm*-deficient mouse model (*Blm*^{m3/m3}) has been engineered by gene targeting in mouse ES cells [14], providing a useful background for studying tumorigenesis due to elevated rates of mitotic recombination [12,13]. Preliminary investigations of adult *Blm*^{m3/m3} mice monitored for 20 months indicated that the mice are tumor prone, developing lymphomas, carcinomas, and sarcomas [14]; however, a detailed analysis of lymphoma and leukemia development, their histopathologic subtypes, and associated genetic abnormalities has not yet been undertaken. These findings indicated that *Blm*^{m3/m3} mice could provide a useful model for the human disease with opportunities for understanding the genetic mechanisms underlying the variety of neoplasms that characterize this disorder. This prompted our laboratories and others to develop approaches for enhancing the utility of the model through (i) studies of proviral insertional mutagenesis to identify candidate oncogenes and tumor suppressor genes (TSG) and (ii) detailed histologic and immunophenotypic studies of the tumors to examine their relationship to tumors found in BS.

Hematopoietic neoplasms arise spontaneously in a number of strains of mice including those that express endogenous murine leukemia viruses (MuLV) at high levels [15–18]. They can also be induced following exposure to irradiation [19] or chemicals, by exogenous infection with MuLV [20–22], or by genetic manipulation of the mouse genome [23,24]. The tumor spectrum in each case varies and is generally restricted to lymphomas of certain types [24–26]. A prior study utilized proviral insertional mutagenesis screens to identify oncogenic networks operative in *Blm*-deficient strains that develop a high frequency of retrovirus-induced B cell lymphomas [27]. This high-throughput method has been developed for performing forward genetic screens to identify genes involved in pathogenesis of neoplasms in mice expressing high levels of MuLV. It is based on identification of proviral insertion sites that can activate proto oncogenes or inactivate tumor suppressor genes (TSG), a process sometimes referred to as “proviral tagging” [28–30]. Genomic regions that recurrently contain proviral integrations in independent tumors are referred to as common integration sites (CIS) and show substantial overlap with oncogenes or TSG involved in human cancers, indicating the power of this screening strategy [31,32]. As noted above, however, this method tends to identify activating mutations in oncogenes rather than inactivating mutations in TSG. The *Blm*-deficient mouse model, due to the elevated rate of induced mitotic recombination [12], enhances the ability to identify recessive mutations associated with a particular phenotype and therefore may be valuable in identifying tumor suppressor genes involved in the development of hematopoietic neoplasms. Indeed, the fact that mitotic recombination between non-sister chromatids that are heterozygous for a mutation can produce daughter cells that have lost the

wild-type allele and are then homozygous for the mutant allele [33]. This feature was exploited for identifying TSG at CIS in MuLV-expressing mice bearing the *Blm*^{m3/m3} mutation [27].

Because the stains selected for the insertional mutagenesis screen were methodologically strongly biased toward development of B cell lymphomas, it was unclear whether similar oncogenic networks might be involved in the development of the other hematopoietic and solid tumors shown to occur in humans and *Blm*-deficient mice. In addition, the long latencies for tumor development in the original *Blm*^{m3/m3} mutant mice [14] or those generated for the mutagenesis screens [27] greatly complicated the efficient analysis of a large number of animals in various studies including therapeutic efforts. The observations that patient-derived cell lines and cells from *Blm*-deficient mice exhibit genetic instability and have an activated double stranded DNA break response but are radioinsensitive suggested that *Blm* deficiency may be radiomimetic, possibly underlying the nontargeted effects of exposure to ionizing radiation [14,34–36]. We therefore postulated that treatment of *Blm*-deficient mice with ionizing radiation might augment the contribution of *Blm* deficiency to transformation, resulting in the accelerated appearance without a change in the tissue distribution of malignancies.

To examine this possibility, we analyzed tumors arising in *Blm*-deficient mice with or without exposure to gamma irradiation. We analyzed the survival pattern, tumor incidence, and tumor spectrum to determine whether this system provides an accurate model of human Bloom syndrome and to assess whether the spectrum of tumors occurring in these mice is biased by *Blm* status or gamma irradiation. Our results revealed that irradiated *Blm*-deficient mice develop a broad spectrum of hematopoietic neoplasms that includes all lineages except megakaryocytic, dendritic, and NK cells, marking this as a particularly useful system for modeling human hematopoietic diseases. We present a detailed morphologic and immunohistochemical assessment of these tumors and validate a diagnostic panel of primary antibodies on tissue arrays of these tumors as a means to facilitate high-throughput immunohistochemical analysis of mouse hematologic malignancies.

2. Materials and methods

2.1. Mice

Blm^{m3/+} mice [14] were bred and housed in specific pathogen-free (SPF) facilities conforming to the Home Office Code of Practice for the Housing and Care of Animals used in Scientific Procedures. The mice were maintained on a mixed 129S7 and C57BL/6^{Tyrc-Brd} background (approximately 25% 129 and 75% C57BL/6^{Tyrc-Brd} contribution). To generate homozygous *Blm*^{m3/m3} mice for this study, heterozygous mice were intercrossed, and homozygous mice were identified by molecular genotyping.

2.2. Irradiation

Four-week $\pm$ 2-day-old mice (409 *Blm*^{m3/m3}, 128 *Blm*^{m3/+}, and 88 *Blm*^{+/+}) were irradiated with a single dose of gamma irradiation of 4 Gy from a cobalt 60 irradiation source. The control group consisted of age- and sex-matched nonirradiated mice of the same genotypes (103 *Blm*^{m3/m3}, 19 *Blm*^{m3/+}, and 15 *Blm*^{+/+}). Each genotype-irradiation status category had an equal or near equal number ($\pm$ 1 mouse) of male and females.

2.3. Histopathology

Mice were necropsied at the onset of symptomatic pathologic lesions. At necropsy, tissues and organs were snap frozen for later analysis of DNA. All internal organs were submitted for histopathology. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E). Lymphoid and non-

lymphoid hematopoietic neoplasms were diagnosed according to consensus Bethesda proposals [37,38], which parallel the revised WHO classifications of human lymphoid neoplasms [39].

2.4. Array manufacture

Tissue cores (0.6 mm) from paraffin donor blocks were embedded into a recipient paraffin wax block using either a manual (MTA-1) or an automated (ATA27) tissue arrayer (Beecher Instruments, Silver Spring, MD). In total, 159 of 165 malignant hematologic lesions were sampled. Two cores were taken from each donor block to provide accurate representation [40]. Tissue array blocks were tempered by heating overnight at 40 °C prior to flattening the array surface with a glass slide and cooling to 4 °C. Sections (4 µm) were cut and mounted on Superfrost Plus glass slides. Adequacy of tumor sampling was assessed on H&E sections of the tissue array with reference to the original tumor block. Antigenicity of array cores was assessed by immunohistochemical staining for laminin (Table 1) and scoring of the cores for intensity and percentage of cells stained.

2.5. Immunohistochemistry

The panel of primary antibodies used is detailed in Table 1.

TSA amplification using a tyramine–DNP system (Ventana, Tucson, AZ) was required for anti-CD5, anti-CD79b, and anti-Bcl-6. Appropriate biotin-conjugated secondary antibodies, cross-absorbed against murine serum, were used according to the host species in which the primary antibody was raised (Jackson Laboratories, West Grove, PA).

Manual immunostaining was performed for PAX5 and F4/80 antibodies. Primary antibody staining for 60 min at room temperature (RT) was followed by washing and biotinylated secondary antibody staining for 30 min at RT. Streptavidin-peroxidase conjugates were applied for 30 min at RT and visualized using 3,3-diaminobenzidine tetrahydrochloride. Antigen retrieval conditions for PAX5 and F4/80 by trypsin digestion and heat-mediated antigen retrieval (HMAR), respectively, are indicated in Table 1.

All other immunohistochemistry was performed on the Ventana Discovery automated stainer. Primary antibody staining for 20 min was followed by washing and biotinylated secondary antibody staining for 8 min. Streptavidin-peroxidase conjugates were applied for 16 min and visualized using 3,3-diaminobenzidine tetrahydrochloride with copper enhancement. All incubations were at 37 °C except antigen retrieval. The latter was performed using either an alkaline protease at 0.02 units/ml for 10 min at 37 °C, HMAR in a Tris/EDTA/borate buffer pH 8.5 for 48 min at 95 °C, or a combination of HMAR for 16 min and 0.03 units/ml protease for 10 min as determined for each antibody (Table 1). All pretreatments were performed on the Ventana Discovery automated stainer using Ventana reagents.

Immunohistochemistry images were captured on an Ariol SL-50 automated scan station (Applied Imaging, Santa Clara, CA) using an Olympus BX61 microscope with UPlan APO 20× magnification lens and a Redlake Megaplug ES4.0/E 2048 × 2048 pixel camera. Composite images were stitched automatically from individual frames. H&E images were captured on a Zeiss Axioskop II with a 40× NeoFluor lens and a 100× oil immersion lens using Axiovision 4.2 software (Imaging Associates, Bicester, UK).

Immunohistochemical staining was assessed on a semiquantitative basis according to percentage of cells stained and intensity of staining, i.e., negative (–), weakly positive (+), moderately positive (++), or strongly positive (+++). Additional assessments were made of the percentage of total tumor cells positive for each marker and the percentage of tumors of each type that were positive for the antigenic marker.

2.6. Statistical analysis

Analyses were carried out with R and SPLUS software packages. Odds ratios and 95% confidence intervals for mortality and tumor development as a function of genotype and radiation status were calculated by logistic regression. The regression model employed was determined by first evaluating the saturated model and removing nonsignificant terms by backward stepwise elimination. Pearson's χ^2 -test was used to test for the equality of two ratios.

3. Results

3.1. Survival data

Over an observation period of 520 days, 217 irradiated mice (187 *Blm*^{m3/m3}, 20 *Blm*^{m3/+}, and 10 *Blm*^{+/+}) and 15 nonirradiated mice (14 *Blm*^{m3/m3} and 1 *Blm*^{+/+}) became moribund and were necropsied (Table 2). Within the group of irradiated mice, 233 primary pathologic lesions were identified, representing a cumulative incidence of 51% (208/409) in *Blm*^{m3/m3} mice, 13% (16/128) in *Blm*^{m3/+} mice, and 10% (9/88) in *Blm*^{+/+} mice. (Here the denominator represents the total number of mice within the group at the outset.) Within the control groups of nonirradiated mice, 17 primary pathologic lesions were identified, yielding a cumulative incidence of 16% (16/103) in *Blm*^{m3/m3} mice and 3% (1/34) in *Blm*^{m3/+} or *Blm*^{+/+} mice.

To quantify the effects of genotype and irradiation on the risk of mortality and tumor development, the odds of death:survival and of death-with-tumor:survival were evaluated by logistic regression. This analysis revealed highly significant effects of gamma irradiation ($P < 10^{-8}$) and of the *Blm*^{m3/m3} genotype ($P < 10^{-8}$); however, there was no significant effect of the *Blm*^{m3/+} genotype on baseline odds ($P = 0.46$), nor were there any significant irradiation-genotype interactions ($P = 0.39$ – 0.83).

Because the *Blm*^{m3/+} genotype had no significant effect on mortality or tumor incidence, the data from *Blm*^{m3/+} and *Blm*^{+/+} mice were pooled. The risk of mortality and tumor development in nonirradiated *Blm*^{m3/+} or *Blm*^{+/+} mice was increased approximately 5-fold by gamma irradiation alone or by the *Blm*^{m3/m3} genotype alone. This implies that the *Blm*^{m3/m3} genotype has tumorigenic effects comparable to gamma irradiation. When both tumor-predisposing factors were present (gamma irradiation of *Blm*^{m3/m3} mice), the odds ratio of tumor and death is approximately 28-fold higher than baseline, implying that *Blm*^{m3/m3} and gamma irradiation acted in a multiplicative fashion.

3.2. Tumor spectrum

Of the 233 pathologic lesions analyzed in the irradiated groups, 68 were benign and 165 were malignant (Table 2). The benign lesions consisted of 10 lymphoid hyperplasias, 23 myeloproliferative disorders, 5 hemangiomas, and 30 adenomas affecting various organs (Table 2). The malignant lesions consisted of 93 lymphomas, 7 leukemias, 24 histiocytic sarcomas, 13 sarcomas (soft tissue sarcomas and angiosarcomas), and 28 carcinomas (Table 2). In irradiated *Blm*^{m3/m3} mice, as in patients with Bloom syndrome, hematologic malignancies predominated over nonhematologic malignant lesions with a ratio of 3:1 (124/41).

The most common sarcoma observed was angiosarcoma (69% of all sarcomas). The carcinomas occurred at varying sites, the commonest site being the ovary (32% of all carcinomas). The most frequent site for adenoma formation was the gut (53% of all adenomas) (Table 2). Hematologic malignancies including lymphomas, leukemias, and histiocytic sarcomas (HS) accounted for 75% (124/165) of the neoplasms seen in irradiated *Blm* mice of all genotypes (Table 2). The majority of these lesions were lymphomas (75%), followed by HS (19%) and acute myeloid leukemias (6%).

The lymphomas comprised subtypes reported to have morphologic similarities to their closest human counterparts [37] (Table 3). Within the irradiated *Blm*^{-/-} group, T lymphoblastic lymphoma (TLL) predominated (29%), followed by follicular B cell lymphoma (FBL) (6.6%), centroblastic (CBL) diffuse large B cell lymphoma (DLBCL) (10%), B cell lymphoblastic lymphoma (BLL) (8.2%), DLBCL of immunoblastic subtype (IBL) (10%), plasmacytoma/plasmacytoid lymphoma (PCT) (5.5%), and one case of small B cell lymphoma (SBL) (Table 3).

The leukemias were predominantly acute myeloid leukemia (AML) (4.5%), with one case resembling chronic lymphatic leukemia (CLL) (Table 3). HS was observed frequently (20%), either on a background of myeloproliferation or associated lymphoma (19%) or as a pure population of malignant cells, morphologically resembling histiocytes infiltrating liver and uterus (1 case) (Table 3). No examples were identified of B or T natural killer cell lymphoma, disorders described for mice but with no human counterparts [37], nor were mature T cell neoplasms, large-cell anaplastic T cell lymphoma, or Hodgkin's lymphoma, human neoplastic disorders with no established mouse counterparts. In addition, there were no cases of pure megakaryocytic leukemia, erythroleukemia, or mast cell leukemia, disorders common to humans and mice [38,41].

The 14 nonirradiated *Blm*^{m3/m3} mice died with the following lesions: 1 TLL, 2 FBL, 1 PCT, 1 AML, 3 HS (1 with associated myeloproliferative disease; 1 with associated lymphoma, and 1 “pure” HS); 2 cases of erythrocytoses, and 1 of myeloproliferative disease. The single nonirradiated *Blm*^{+/+} mouse to have died had dermatitis at necropsy. The age at diagnosis for mice belonging to the different diagnostic categories of hematopoietic and nonhematopoietic neoplasms is shown in Fig. 1. Among the lymphoid neoplasms, both TLL and BLL developed with relatively short latencies (219 days on average), whereas the germinal center-derived lymphomas (CBL, IBL, and FBL) and PCT developed with mean latencies centered around 400 days. Early onset TLL followed by later onset B cell lineage tumors mirrors the patterns of disease seen in NFS.V⁺ mice that also develop a wide range of hematopoietic neoplasms [22]. The cases of myeloid origin, HS and AML, developed in a timeframe similar to those of the B cell lineage neoplasms, with an average age at presentation of around 400 days. The mean age at necropsy was 362 days for carcinomas and 331 days for nonhistiocytic sarcomas.

Although our experiment had essentially equal numbers of female and male mice in each genotype-radiation status category, we observed substantially more tumors in females than in males (161:93), a highly significant departure from the expected 1:1 ratio ($P < 2 \times 10^{-5}$). This increased female susceptibility to tumor development was observed in irradiated mice of all genotypes and in nonirradiated *Blm*^{m3/m3} mice.

3.3. Detailed histologic analysis of hematopoietic neoplasms

We performed an extensive histologic classification of lymphomas and leukemias arising in this model using the latest NIAID/NCI (Bethesda classification) of mouse lymphomas [37], which parallels the WHO classification of human lymphomas [39,42] and the companion classification of mouse non-lymphoid neoplasms [38]. TLL was the most frequently identified lymphoma. Cytologically, the tumors were composed of medium-sized, round lymphoblasts with inconspicuous nucleoli and interspersed tingible body macrophages containing apoptotic debris, giving the classical starry sky appearance (Fig. 2a). All TLL involved the thymus, and they often showed spread to other organs including heart, lungs, mediastinal tissues, spleen, kidney, and salivary gland. Involvement of other tissues, including the GI tract, bladder, testes, and CNS, was less common.

BLL were also observed (Fig. 2b). These tumors were either of similar cytologic appearance to the TLL or were composed of medium-sized cells that were slightly more pleiomorphic than

their TLL counterparts, and lacked the classical starry sky appearance. Organ involvement often included ovaries, uterus, mediastinal lymph nodes, salivary gland lymph nodes, lungs, spleen, liver, and kidneys. Four cases of BLL were identified infiltrating the thymus. On purely morphologic grounds, it was not possible to distinguish pre-B lymphoblastic lymphoma from mature B lymphoblastic lymphoma, provisionally reclassified as diffuse high-grade blastic B cell lymphoma/leukemia [43].

The FBL consisted of a characteristic mixture of centrocytes and centroblasts as seen in human follicular cell lymphomas (Fig. 2c); however, unlike their human counterparts, these tumors diffusely involved all organs that they infiltrated, without the formation of follicles. Common sites of tumor involvement included the spleen and mesenteric lymph nodes. By convention, we have called these tumors FBL when 50% or fewer cells were centroblasts or immunoblasts.

The DLBCL were of varied morphology and consisted of two different subsets: CBL and IBL. By convention, these tumors differed from the FBL in that there were more than 50% centroblasts and/or immunoblasts. The CBL consisted of a mixture of centrocytes and much greater numbers of large centroblasts, classically with two nucleoli appended to the nuclear membrane. The organ involvement was similar to that seen in the FBL, although in the spleen the DLBCL tended to expand the white pulp to a greater degree and often infiltrated the red pulp to almost completely efface the normal structure of the spleen. Cells with indistinguishable centroblastic cytology are also characteristic of high-grade splenic marginal zone lymphomas [44] but retain an association with the marginal zone, a feature not seen with the CBL of *Blm^{m3/m3}* mice.

A similar number of *Blm^{m3/m3}* DLBCL had a predominantly immunoblastic phenotype (Fig. 2d). The immunoblasts were large cells with single prominent, often eosinophilic bar-shaped nucleoli set in a background of centrocytes and centroblasts. Organ involvement was more widespread than in CBL, often including liver, kidneys, lungs, and salivary gland as well as spleen and lymph nodes.

A certain percentage of FBL and DLBCL, both CBL and IBL, contained a significant component of associated T cells and histiocytes, thereby resembling a subset of histiocyte-rich neoplasms first identified in AKXD recombinant inbred strains that has been designated as histiocyte-associated (HA) B cell lymphomas [17,37]. Cases of HA B cell lymphomas never attain the level of T cell enrichment seen in human T cell-rich, histiocyte-rich lymphomas [45–47], and recent studies have established criteria for distinguishing them from true HS, unpublished observations. This group showed a characteristic pattern of tumor growth consisting of nodules of tumor in spleen, lymph nodes, liver, and often kidneys and lungs. A substantial part of the infiltrate consists of large histiocytes with associated T cells; however, interspersed among these were scanty large malignant centroblasts and immunoblasts.

The few cases of small B cell lymphoma and plasmacytoma were characteristic of tumors previously described in classifications of mouse lymphoid neoplasms [37] (Fig. 2e and f).

Red pulp hyperplasias of either multiple or single lineage were prominent in spleens of irradiated and nonirradiated *Blm*-deficient mice (Table 3; Fig. 3). There was evidence of consistent red pulp hyperplasia in the spleen involving all three lineages (Fig. 3a). An additional finding in some older mice was myeloproliferative disease, most often of the myelomonocytic lineage. Single lineage hyperplasia, predominantly erythroid, was also seen (Fig. 3b). AML was usually well differentiated in type, either myelomonocytic (Fig. 3c), predominantly granulocytic (Fig. 3d), or predominantly monocytic (Fig. 3e). More immature forms of AML were rare (Fig. 3f).

In this study, two distinct groups of HS were identified (Fig. 3). The first group (two cases) (Table 3) consisted of pure HS, a well defined uniform population of histiocytes involving the spleen and infiltrating liver and uterus (Fig. 3g); however, a much larger group of HS was consistently associated with background myeloproliferation and myelofibrosis in the spleen (Table 3; Fig. 3h). These tumors had a much wider pattern of organ distribution, involving not only spleen but sometimes also liver, kidney, salivary gland, and lung. A small minority of cases of what appeared to be true histiocytic malignancies (5/24) was associated with B cell NHL in the splenic white pulp.

3.4. Detailed immunohistochemical analysis of hematopoietic neoplasms

Hematologic tumors identified in the irradiated mice were categorized according to Bethesda classification guidelines [37,38] with the aid of a panel of polyclonal and monoclonal antibodies (Table 1). FACS analysis could not be performed, as only formalin-fixed tumor material was available for this study. A summary of the results is shown in Table 4. Consistently strong staining results were obtained with antibodies against surface IgM, IgG, CD45R (B220), CD3, CD138, PAX5, Mac-2, myeloperoxidase, and lysozyme.

Overall, the immunohistochemical profile of each tumor subtype was like that previously published for tumors of similar morphology [37,38] (Table 4). Following immunohistochemical profiling, BLL could be subdivided into two groups: surface IgM and IgG negative (presumably pre-B cell in origin), and surface IgM or IgG positive (presumably mature B cell in origin) (Table 4). These groups did not correlate with thymic or nonthymic involvement by the tumor. As observed in H&E-stained sections of tumors, 30% of FBL and DLBCL were of T cell-rich, histiocyte-associated type (Table 4). The infiltrate of normal T cells and histiocytes accounted for the significant proportion of CD3-positive cells (T cells) and Mac-2/lysozyme-positive cells (histiocytes) in these tumors (Table 4); however, the malignant centroblasts and immunoblasts were consistently negative for these markers but positive for PAX5 and B220. In addition, 30% of FBL were also associated with a T cell and histiocyte-rich infiltrate (Table 4). Both CBL and IBL were associated with some positivity for BCL6. Plasmacytoid lymphoma/plasmacytoma and IBL were associated with lower levels of PAX5 expression and increased expression of CD138.

Staining for the proliferation marker, Ki-67, was high in the mitotically active TLL and BLL and in the myeloblast component of AML. The myeloid component within AML was also positive for myeloperoxidase. A certain proportion of cells of monocytic lineage within the well-differentiated AML were positive for lysozyme. HS were positive for Mac-2 and lysozyme only (Table 4).

4. Discussion

In this study, we performed a detailed analysis of pathologic lesions, mainly tumors, arising in irradiated *Blm*^{m3/m3} mice, a model for human Bloom syndrome. The incidence and spectrum of tumors observed in mice homozygous for a null allele of *Blm* partly mimics those seen in individuals with Bloom syndrome. As in human Bloom syndrome [4], malignant lesions occurred more frequently than benign lesions, and lymphomas and leukemias predominated over carcinomas and sarcomas by a ratio of 3:1 [1]. Hematopoietic tumors generally occurred with a shorter latency than the carcinomas and sarcomas. We also identified irradiated *Blm*^{m3/m3} mice with multiple concurrent tumors in different organs.

The pattern of organ involvement for the carcinomas and sarcomas occurring in irradiated *Blm*^{m3/m3} mice differed from that in patients with Bloom syndrome. In mice, ovarian carcinomas were most common followed at lower frequencies by tumors of the Harderian gland, gastrointestinal tract and skin. In humans with Bloom syndrome, the large intestine was

the most common site for carcinomas followed by the skin, breast and tongue. Some of this difference may be due to species-specific and strain-specific differences in anatomy and tumor susceptibility [25]. Thus, aging B6;129 mice, genotypically similar to the animals studied in this report, are susceptible to carcinomas of the Harderian gland, lung, liver, and ovary [25, 27].

It is noteworthy that the incidence of tumors in the irradiated *Blm*^{m3/+} (13%) and *Blm*^{+/+} mice (10%) were not significantly different. Humans mutant for *BLM* and mice heterozygous for a different mutant *Blm* allele are reported to show an increased susceptibility to tumor development compared with the general population or *Blm*^{+/+} controls [49,50]. There are two potential reasons why an increased incidence of neoplasms was not observed in the *Blm*^{m3/+} heterozygous mutants described here: (i) the numbers of mice in the *Blm*^{m3/+} and *Blm*^{+/+} groups were not high enough to attain statistical significance for development of a tumor type that is rare and (ii) different mutant *Blm* alleles may have different phenotypic effects. The latter is suggested by the fact that mice homozygous for other alleles designated *Blm*^{m2} [14], *Blm*^{Cin} allele [50] or the *Blm*^{tm1Ches} allele [51] exhibited embryonic lethality; however, this effect could equally well be due to the fact that each of these alleles retained a PGKneo cassette and vector sequences that were absent from the *Blm*^{me3} mice.

It appears that the *Blm*^{m3/m3} genotype and gamma irradiation act synergistically to potentiate tumor development. This may be related to the different roles of gamma irradiation and the *Blm*-deficient genotype in the events involved in initiation and progression to malignancy. It is well established that ionizing radiation induces delayed genetic instability in the clonal offspring of the irradiated cell (reviewed in [52]). This is manifested as chromosomal instability and genetic mutations that contribute to the genesis of TLL and AML in mice [53,54]. Gamma irradiation is known to induce random, large-scale chromosomal deletions [52] that can result in LOH of a tumor suppressor gene. This, combined with the high rate of mitotic recombination due to the *Blm*-null genotype, could accelerate conversion of LOH to bi-allelic loss of the tumor suppressor gene, promoting tumor development. Thus, alone, in combination with gamma irradiation, or in combination with mutations in tumor suppressor genes such as *Apc*, the *Blm*^{m3/m3} genotype exerts an extremely potent influence on tumor development.

This model is highly advantageous for generating hematologic malignancies for further genetic analysis. The spectrum of lymphomas and leukemias seen in this model is unique for a GEMM mouse, in that lymphomas and leukemia of almost all lineages are represented excepting dendritic, NK, and megakaryocytic neoplasms. In addition, this tumor spectrum is much broader than that seen in aging B6;129 mice, which develop predominantly FBL and HS [27]. This probably reflects the ubiquitous tissue expression of *Blm* and its essential role in all differentiated cell types.

Immunohistochemical analysis provided a powerful tool for firmly establishing diagnoses for these hematologic lesions. In particular, we have been able to subtype BLL into pre-B and mature B cell in origin and to identify FBL and DLBCL with a significant T cell and histiocytic infiltrate. An additional immunomarker that would be useful in this differentiation would be TdT. Identification of tumor-associated benign cell infiltrates in T cell-rich, histiocyte-associated FBL and DLBCL will likely be of importance in the interpretation of the genetic analyses of these tumors.

Together our studies indicate that irradiated *Blm*^{m3/3} provide a novel model for Bloom syndrome that recapitulates a number of important features of the human disease. These include (i) the development of both hematopoietic and non-hematopoietic neoplasms with a spectrum of malignancies within each subset; (ii) the tendency for lymphoid neoplasms to appear earlier in life than carcinomas; and (iii) the development of more than one neoplasm in a single mouse.

These commonalities suggest that studies of the tumors of irradiated and untreated *Blm*^{m3/m3} mice by comparative genomic hybridization and spectral karyotyping could provide important insights into gross chromosomal anomalies and finer genetic changes that contribute to the pathogenesis of many cancers that plague individuals bearing the *BLM* mutation. The potential of these mice for identifying the contributions of TSG inactivation to transformation has already been established [14,27].

Further studies should also be directed at examining the acquired and innate immune responses of the mutant mice, as Bloom syndrome patients are reportedly immunodeficient [55]. This could contribute to impaired cancer surveillance as a component of increased tumor susceptibility. Indeed, studies of mice selectively deficient for *BLM* in B cells and T cells have highlighted the importance of *BLM* genome surveillance for the development and function of these lymphocyte subsets [56,57]. Developing better mouse models of human diseases requires more than our ability to simply manipulate the genome to establish a parallel genotype.

Acknowledgments

This work was supported by the Wellcome Trust and the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

We thank C. Brandt for assistance with mouse necropsies and genotyping, and B. Haynes and the Sanger Atlas of Protein Expression group for technical assistance with histology and immunohistochemistry. Professors Jim Lupski and Ming-Qing Du provided useful comments on the manuscript. NIAID intramural editor B. R. Marshall assisted in preparation of the manuscript.

References

1. German J. Bloom syndrome: a Mendelian prototype of somatic mutational disease. *Medicine* (Baltimore) 1993;72:393–406. [PubMed: 8231788]
2. Bloom D. Congenital telangiectatic erythema resembling lupus erythematosus in dwarfs; probably a syndrome entity. *AMA Am J Dis Child* 1954;88:754–8. [PubMed: 13206391]
3. Sawitsky A, Bloom D, German J. Chromosomal breakage and acute leukemia in congenital telangiectatic erythema and stunted growth. *Ann Intern Med* 1966;65:487–95. [PubMed: 5911744]
4. German J. Bloom's syndrome. XX. The first 100 cancers. *Cancer Genet Cytogenet* 1997;93:100–6. [PubMed: 9062585]
5. Ellis NA, Groden J, Ye TZ, et al. The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 1995;83:655–66. [PubMed: 7585968]
6. Karow JK, Constantinou A, Li JL, West SC, Hickson ID. The Bloom's syndrome gene product promotes branch migration of Holliday junctions. *Proc Natl Acad Sci USA* 2000;97:6504–8. [PubMed: 10823897]
7. Wu L, Hickson ID. The Bloom's syndrome helicase suppresses crossing over during homologous recombination. *Nature* 2003;426:8704.
8. Chakraverty RK, Hickson ID. Defending genome integrity during DNA replication: a proposed role for RecQ family helicases. *Bioessays* 1999;21:286–94. [PubMed: 10377891]
9. Imamura O, Campbell JL. The human Bloom syndrome gene suppresses the DNA replication and repair defects of yeast *dna2* mutants. *Proc Natl Acad Sci USA* 2003;100:8193–8. [PubMed: 12826610]
10. Onclercq-Delic R, Calsou P, Delteil C, Salles B, Papadopoulos D, Amor-Gueret M. Possible anti-recombinogenic role of Bloom's syndrome helicase in double-strand break processing. *Nucleic Acids Res* 2003;31:6272–82. [PubMed: 14576316]
11. Hickson ID. RecQ helicases: caretakers of the genome. *Nat Rev Cancer* 2003;3:169–78. [PubMed: 12612652]
12. Guo G, Wang W, Bradley A. Mismatch repair genes identified using genetic screens in *Blm*-deficient embryonic stem cells. *Nature* 2004;429:891–5. [PubMed: 15215866]
13. Yusa K, Horie K, Kondoh G, et al. Genome-wide phenotype analysis in ES cells by regulated disruption of Bloom's syndrome gene. *Nature* 2004;429:896–9. [PubMed: 15215867]

14. Luo G, Santoro IM, McDaniel LD, Nishijima I, Mills M, Youssoufian H, et al. Cancer predisposition caused by elevated mitotic recombination in Bloom mice. *Nat Genet* 2000;26:424–9. [PubMed: 11101838]
15. Mucenski ML, Taylor BA, Jenkins NA, Copeland NG. AKXD recombinant inbred strains: models for studying the molecular genetic basis of murine lymphomas. *Mol Cell Biol* 1986;6:4236–43. [PubMed: 3025647]
16. Morse HC III, Qi CF, Chattopadhyay SK, Hori T, Taddesse-Heath L, Ozato K, et al. Combined histologic and molecular features reveal previously unappreciated subsets of lymphoma in AKXD recombinant inbred mice. *Leuk Res* 2001;25:719–33. [PubMed: 11397479]
17. Taddesse-Heath L, Chattopadhyay SK, Dillehay DL, Lander MR, Nagashfar Z, Morse HC III, et al. Lymphomas and high-level expression of murine leukemia viruses in CFW mice. *J Virol* 2000;74:6832–7. [PubMed: 10888622]
18. Bedigian HG, Johnson DA, Jenkins NA, Copeland NG, Evans R. Spontaneous and induced leukemias of myeloid origin in recombinant inbred BXH mice. *J Virol* 1984;51:586–94. [PubMed: 6088784]
19. Boulton E, Cleary H, Plumb M. Myeloid, B and T lymphoid and mixed lineage thymic lymphomas in the irradiated mouse. *Carcinogenesis* 2002;23:1079–85. [PubMed: 12082032]
20. Cloyd MW, Hartley JW, Rowe WP. Genetic study of lymphoma induction by AKR mink cell focus-inducing virus in AKR × NFS crosses. *J Exp Med* 1981;154:450–7. [PubMed: 7264563]
21. Hartley JW, Chattopadhyay SK, Lander MR, Taddesse-Heath L, Naghashfar Z, Morse HC III, et al. Accelerated appearance of multiple B cell lymphoma types in NFS/N mice congenic for ecotropic murine leukemia viruses. *Lab Invest* 2000;80:159–69. [PubMed: 10701686]
22. Mucenski ML, Taylor BA, Copeland NG, Jenkins NA. Characterization of somatically acquired ecotropic and mink cell focus-forming viruses in lymphomas of AKXD recombinant inbred mice. *J Virol* 1987;61:2929–33. [PubMed: 3039180]
23. Morse HC III, McCarty T, Qi CF, Torrey TA, Naghashfar Z, Chattopadhyay SK, et al. B lymphoid neoplasms of mice: characteristics of naturally occurring and engineered diseases and relationships to human disorders. *Adv Immunol* 2003;81:97–121. [PubMed: 14711054]
24. Taddesse-Heath L.; Morse, HC, III. Lymphoma in genetically engineered mice. In: Ward, JM.; Mahler, JF.; Maronpot, RR.; Sundberg, JP.; Fredrickson, RM., editors. *Pathology of genetically engineered mice*. Ames, IA: Iowa State University Press; 2000. p. 365–81.
25. Pattengale, PK. Tumors of the lymphohematopoietic system. In: Turusov, VS.; Mohr, U., editors. *Pathology of tumors in laboratory animals, vol. II. Tumors of the mouse. 2*. Lyons, France; IARC: 1994. p. 651–70.
26. Haines DC, Chattopadhyay S, Ward JM. Pathology of aging B6;129 mice. *Toxicol Pathol* 2001;29:653–61. [PubMed: 11794381]
27. Suzuki T, Minehata K, Akagi K, Jenkins NA, Copeland NG. Tumor suppressor gene identification using retroviral insertional mutagenesis in Blm-deficient mice. *EMBO J* 2006;25:3422–31. [PubMed: 16858412]
28. Mikkers H, Allen J, Knipscheer P, Romeijn L, Hart A, Vink E, et al. High-throughput retroviral tagging to identify components of specific signaling pathways in cancer. *Nat Genet* 2002;32:153–9. [PubMed: 12185366]
29. Lund AH, Turner G, Trubetskoy A, Verhoeven E, Weintjens E, Hulsman D, et al. Genome-wide retroviral insertional tagging of genes involved in cancer in Cdkn2a-deficient mice. *Nat Genet* 2002;32:160–5. [PubMed: 12185367]
30. Suzuki T, Shen H, Akagi K, Morse HC, Malley JD, Naiman DQ, et al. New genes involved in cancer identified by retroviral tagging. *Nat Genet* 2002;32:166–74. [PubMed: 12185365]
31. Neil JC, Cameron ER. Retroviral insertion sites and cancer: fountain of all knowledge? *Cancer Cell* 2002;2:253–5. [PubMed: 12398888]
32. Kool J, Berns A. High-throughput insertional mutagenesis screens in mice to identify oncogenic networks. *Nat Rev Cancer* 2009;9:389–99. [PubMed: 19461666]
33. Adams DJ, Bradley A. Induced mitotic recombination: a switch in time. *Nat Genet* 2002;30:6–7. [PubMed: 11753379]

34. Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation? *Oncogene* 2003;22:7094–9. [PubMed: 14557815]
35. Gravel S, Chapman JR, Magill C, Jackson SP. DNA helicases Sgs1 and BLM promote DNA double-strand break resection. *Genes Dev* 2008;22:2767–72. [PubMed: 18923075]
36. Horowitz DP, Topaloglu O, Zhang Y, Bunz F. Deficiency of Bloom syndrome helicase activity is radiomimetic. *Cancer Biol Ther* 2008;7:1783–6.
37. Morse HC III, Anver MR, Fredrickson TN, Haines DC, Harris AW, Harris NL, et al. Bethesda proposals for classification of lymphoid neoplasms in mice. *Blood* 2002;100:246–58. [PubMed: 12070034]
38. Kogan SC, Ward JM, Anver MR, Berman JJ, Brayton C, Cardiff RD, et al. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. *Blood* 2002;100:238–45. [PubMed: 12070033]
39. Swerdlow, SH.; Campo, E.; Harris, NL.; Jaffe, ES.; Pileri, SA.; Stein, H.; Thiele, J.; Vardiman, JW., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4. Lyon, France: International Agency for Research on Cancer; 2008. p. 439
40. Kononen J, Bubendorf L, Kallioniemi A, Bärklund M, Schraml P, Leighton S, et al. Tissue microarrays for high throughput molecular profiling of tumor specimens. *Nat Med* 1998;4:844–7. [PubMed: 9662379]
41. Hao X, Shin MS, Zhou JX, Lee CH, Qi CF, Naghashfar Z, et al. Histologic and molecular characterizations of megakaryocytic leukemia in mice. *Leuk Res* 2006;30:397–406. [PubMed: 16219351]
42. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood* 2008;112:4384–99. [PubMed: 19029456]
43. Janz, S.; Morse, HC., III; Teitell, MA. Mouse models of human mature B cell and plasma cell neoplasms. In: Li, S., editor. Mouse models of human blood cancer. New York: Springer; 2008. p. 179-225.
44. Fredrickson TN, Lennert K, Chattopadhyay SK, Morse HC III, Hartley JW. Splenic marginal zone lymphomas of mice. *Am J Pathol* 1999;154:805–12. [PubMed: 10079258]
45. Achten R, Verhoef G, Vanuytsel L, De Wolf-Peeters C. Histiocyte-rich, T-cell-rich B-cell lymphoma: a distinct diffuse large B-cell lymphoma subtype showing characteristic morphologic and immunophenotypic features. *Histopathology* 2002;40:31–5. [PubMed: 11903596]
46. Lim MS, Beaty M, Sorbara L, Cheng RZ, Pittaluga S, Raffeld M, et al. T cell/histiocyte-rich large B cell lymphoma: a heterogeneous entity with derivation from germinal center B cells. *Am J Surg Pathol* 2002;26:1458–66. [PubMed: 12409722]
47. Fraga M, Sánchez-Verde L, Forteza J, García-Rivero A, Piris MA. T-cell/histiocyte-rich large B-cell lymphoma is a disseminated aggressive neoplasm: differential diagnosis from Hodgkin's lymphoma. *Histopathology* 2002;41:216–29. [PubMed: 12207783]
49. Gruber SB, Ellis NA, Scott KK, Almog R, Kolachana P, Bonner JD, et al. BLM heterozygosity and the risk of colorectal cancer. *Science* 2002;297:2013. [PubMed: 12242432]
50. Goss KH, Risinger MA, Kordich JJ, Sanz MM, Straughen JE, Slovek LE, et al. Enhanced tumor formation in mice heterozygous for BLM mutation. *Science* 2002;297:2051–3. [PubMed: 12242442]
51. Chester N, Kuo F, Kozak C, O'Hara CD, Leder P. Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. *Genes Dev* 1998;12:3382–93. [PubMed: 9808625]
52. Iyer R, Lehnert BE. Effects of ionizing radiation in targeted and nontargeted cells. *Arch Biochem Biophys* 2000;376:14–25. [PubMed: 10729186]
53. Grosovsky AJ, Parks KK, Giver CR, Nelson SL. Clonal analysis of delayed karyotypic abnormalities and gene mutations in radiation-induced genetic instability. *Mol Cell Biol* 1996;16:6252–62. [PubMed: 8887655]
54. Little JB. Genomic instability and radiation. *J Radiol Prot* 2003;23:173–81. [PubMed: 12875549]
55. Kondo N, Motoyoshi F, Mori S, Kuwabara N, Orii T, German J. Long-term study of the immunodeficiency of Bloom's syndrome. *Acta Paediatr* 1992;81:86–90. [PubMed: 1600313]

56. Babbe H, Chester N, Leder P, Reizis B. The Bloom's syndrome helicase is critical for development and function of the $\alpha\beta$ T-cell lineage. *Mol Cell Biol* 2007;27:1947–59. [PubMed: 17210642]
57. Babbe H, McMenamin J, Hobeika E, Wang J, Rodig SJ, Reth M, et al. Genomic instability resulting from Blm deficiency compromises development, maintenance, and function of the B cell lineage. *J Immunol* 2008;182:347–60. [PubMed: 19109166]

Fig. 1.

Age distribution for the various types of hematologic malignancies, carcinomas, and sarcomas seen in irradiated *Blm*^{+/+}, *Blm*^{m3/+}, and *Blm*^{m3/m3} mice. Each data point represents the age at necropsy in the mouse with the lesion specified. The number of cases represents the total number of cases of each lesion identified at necropsy in irradiated *Blm*^{+/+}, *Blm*^{m3/+}, and *Blm*^{m3/m3} mice in the tumor watched up to 520 days. The vertical oval represents the mean age of tumor incidence.

Fig. 2.

Histologic features of lymphomas identified in irradiated *Blm*^{+/+}, *Blm*^{m3/+}, and *Blm*^{m3/m3} mice (all H&E, 400× original magnification). (a) T lymphoblastic lymphoma (TLL) composed of medium-sized lymphoid blasts with inconspicuous nucleoli. Mitotic activity is high, resulting in a starry sky appearance. (b) B lymphoblastic lymphoma (BLL) composed of medium-sized lymphoid blasts with inconspicuous nucleoli. Some BLL lacked the starry sky appearance. (c) Follicular B cell lymphoma (FBL) composed of a mixed population of small, cleaved centrocytes and larger centroblasts with multiple prominent peripheral nucleoli. Less than 50% of the tumor cell population is centroblasts. (d) Diffuse large B cell lymphoma, immunoblastic (IBL). More than 50% of the cells are immunoblasts/centroblasts. Note the prominent large bar-shaped eosinophilic nucleoli within the large immunoblasts. Often this lymphoma was also associated with a starry sky appearance due to the high mitotic activity of the immunoblasts.

(e) Small B cell lymphoma (SBL). This low-grade lymphoma is composed of small B lymphocytes similar to the small lymphocytes in the human counterpart lymphoma, CLL. (f) Plasmacytoma/plasmacytoid lymphoma, composed of small- to medium-sized cells with plasmacytoid nuclei and abundant cytoplasm. All scale bars represent 20 μm .

Fig. 3. Histologic features of non-lymphoid hematologic lesions in irradiated *Blm*^{+/+}, *Blm*^{m3/+}, and *Blm*^{m3/m3} mice (all H&E, 400× original magnification). (a) Red pulp hyperplasia, a common finding in the spleen of irradiated *Blm*^{m3/m3} mice. There is evidence of tri-lineage proliferation, namely small islands of densely hyperchromatic erythroblasts, surrounded by larger myeloblasts, immature myelocytes, ring forms, and granulocytes, plus occasional large megakaryocytes (bottom right). Red pulp sinusoids are patent and contain mature red blood cells. (b) Erythroid and myeloid hyperplasia. Red pulp sinusoids are collapsed, and cords are filled with sheets of erythroid precursors. There is additional myeloid hyperplasia. No megakaryocytes are visible in this field. (c) Acute myeloid leukemia, well differentiated,

myelomonocytic. There is an excess of immature myeloblasts, with some differentiation, infiltrating and expanding the portal tracts and sinusoids of the liver. The destruction of surrounding hepatocytes also differentiates this from benign extramedullary hematopoiesis in the liver. (d) Acute myeloid leukemia, well differentiated, predominantly granulocytic. The infiltrate contains many ring forms as well as immature myeloblasts and destroys the underlying structure of the renal cortex. (e) Acute myeloid leukemia, well differentiated, predominantly monocytic. The infiltrate contains many large cells with reniform nuclei of monocytic lineage. (f) Acute myeloid leukemia, undifferentiated, infiltrating the red pulp cords and sinusoids of the spleen. At least 90% of the non-lymphoid, non-erythroid cells are immature blasts. (g) Pure histiocytic sarcoma infiltrating the liver sinusoids. The infiltrate is relatively monomorphic and is composed of well-differentiated histiocytes. (h) Histiocytic sarcoma associated with myeloproliferation. Malignant histiocytic infiltration is seen in the top left of the field, adjacent to myeloid proliferation in the red pulp. All scale bars represent 20 μm .

Table 1

Primary antibodies and antigen retrieval conditions employed in immunostaining (TSA amplification: tyramine-DNP system; Ventana, Tucson, AZ).

Antigen	Primary antibody				Antigen retrieval			
	Manufacturer	Clone	Species isotype	Dilution	Incubation time and temperature	TSA amplification	Buffer	Time and temperature
CD45R (B220)	R&D Systems	RA3-6B2	Rat IgG2Ak	5.0 µg/ml	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
CD5	BD Pharmingen	53-7.3	Rat IgG2Ak	10 µg/ml	20 min 37 °C	Yes	Alkaline protease 0.02 units/ml	10 min 37 °C
CD138	BD Pharmingen	281-2	Rat mono IgG1	10 µg/ml	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
Mac-2	Cedarlane Laboratories	M3/38	Rat IgG2Ak	10 µg/ml	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
Ki-67	DAKO Cytomation	MIB5	Mouse IgG1	1:50	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
Lysozyme	DAKO Cytomation	EC3.2.1.17	Rabbit polyclonal	1:100	20 min 37 °C	No	Tris/EDTA/borate pH 8; then alkaline protease 0.03 units/ml	16 min 95 °C 10 min 37 °C
Myeloperoxidase	DAKO Cytomation	A0398	Rabbit polyclonal	1:100	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
CD3	DAKO Cytomation	A0452	Rabbit polyclonal	1:100	20 min 37 °C	No	Tris/EDTA/borate pH 8	48 min 95 °C
Bcl-6	SantaCruz Biotechnology	N-3	Rabbit polyclonal	10 µg/ml	20 min 37 °C	Yes	Tris/EDTA/borate pH 8	48 min 95 °C
CD79	Southern Biochemicals	HM79-12	Rat IgG2Ak	10 µg/ml	20 min 37 °C	Yes	Alkaline protease 0.02 units/ml	10 min 37 °C
Laminin	Sigma	L9393	Rabbit polyclonal	1:100	20 min 37 °C	No	Tris/EDTA/borate pH 8, then alkaline protease 0.03 units/ml	16 min 95 °C 10 min 37 °C

Table 2

Frequency of different tumor types in irradiated $Blm^{-/-}$, $Blm^{+/-}$, and $Blm^{+/+}$ mice.

Tumor/lesion	$Blm^{-/-}$ mice (n = 409)			$Blm^{+/-}$ mice (n = 128)			$Blm^{+/+}$ mice (n = 88)		
	No. of cases	% of all tumors in group (n = 208)	Mean age at death ^a (days)	No. of cases	% of all tumors in group (n = 16)	Mean age at death ^a (days)	No. of cases	% of all tumors in group (n = 9)	Mean age at death ^a (days)
Malignant lesions									
Lymphomas and leukemias									
All lymphomas	81	39.0%	322	8	50.0%	307	4	44.4%	209
All leukemias	7	3.4%	400	—	—	—	—	—	—
Histiocytic sarcoma	22	10.6%	394	1	6.3%	368	1	11.1%	378
Sarcomas									
Angiosarcoma	9	4.3%	308	—	—	—	—	—	—
Soft-tissue sarcoma	3	1.4%	355	1	6.3%	391	—	—	—
Carcinomas									
Ovary	9	4.3%	372	—	—	—	—	—	—
Liver	2	1.0%	326	—	—	—	—	—	—
Mammary	1	0.5%	239	—	—	—	—	—	—
Gastrointestinal tract	3	1.4%	377	—	—	—	—	—	—
Bladder	1	0.5%	388	—	—	—	—	—	—
Uterus	1	0.5%	255	—	—	—	—	—	—
Skin	3	1.4%	381	—	—	—	—	—	—
Harderian gland	4	1.9%	359	—	—	—	—	—	—
Thyroid	1	0.5%	460	—	—	—	—	—	—
CNS metastatic	—	—	—	—	—	—	1	11.1%	345
Odontogenic	—	—	—	1	6.3%	274	—	—	—
Skin adnexal CA	1	0.5%	—	—	—	—	—	—	—
Benign lesions									
Lymphoid hyperplasias	6	2.9%	376	3	18.8%	401	1	11.1%	362
Myeloproliferation—all lineages	17	8.2%	400	—	—	—	—	—	—
Myeloproliferation—single lineage	6	2.9%	354	—	—	—	—	—	—

Tumor/lesion	<i>Blm</i> ^{-/-} mice (<i>n</i> = 409)			<i>Blm</i> ^{+/-} mice (<i>n</i> = 128)			<i>Blm</i> ^{+/+} mice (<i>n</i> = 88)		
	No. of cases	% of all tumors in group (<i>n</i> = 208)	Mean age at death ^a (days)	No. of cases	% of all tumors in group (<i>n</i> = 16)	Mean age at death ^a (days)	No. of cases	% of all tumors in group (<i>n</i> = 9)	Mean age at death ^a (days)
Hemangioma	5	2.4%	400	-	-	-	-	-	-
Adenomas									
Gastrointestinal tract	15	7.2%	366	-	-	-	1	11.1%	466
Ovary	1	0.5%	344	-	-	-	-	-	-
Harderian	3	1.4%	418	1	6.3%	441	-	-	-
Liver	2	1.0%	483	-	-	-	1	11.1%	378
Lung	1	0.5%	447	1	6.3%	445	-	-	-
Skin dysplasias/Bowen's	4	1.9%	356	-	-	-	-	-	-
Necropsy, no abnormality found	3	1.4%	369	4	25.0%	392	2	22.2%	377
Died before necropsy	12	5.1%	378	-	-	-	-	-	-
Total lesions	208			16			9		
Total no. of deaths	187	N/A	334	20	N/A	342	10	N/A	409

^a Age of death after irradiation equals age at death minus 28 ± 2 days.

Table 3

Frequency of different malignant lymphomas and leukemias in irradiated $Blm^{-/-}$, $Blm^{+/-}$, and $Blm^{+/+}$ mice.

Tumor/lesion	$Blm^{-/-}$ mice ($n = 409$)			$Blm^{+/-}$ mice ($n = 88$)			$Blm^{+/+}$ mice ($n = 103$)		
	No. of cases	% of all tumors in this group ($n = 110$)	Mean age at death ^a (days)	No. of cases	% of all tumors in this group ($n = 9$)	Mean age at death ^a (days)	No. of cases	% of all tumors in this group ($n = 5$)	Mean age at death ^a (days)
All lymphomas	81	73.4%	322	8	88.9%	307	4	80.0%	209
T-LL	32	29.0%	202	3	33.3%	247	4	80.0%	209
B-LL	9	8.2%	250	1	11.1%	208	—	—	—
DLBCL-CB (CBL)	11	10.0%	375	1	11.1%	391	—	—	—
DLBCL-IB (IBL)	11	10.0%	416	—	—	—	—	—	—
FBL	7	6.6%	430	2	22.2%	405	—	—	—
SBL	1	0.9%	317	—	—	—	—	—	—
PCT	6	5.5%	407	1	11.1%	N/A	—	—	—
Unclassifiable	4	3.6%	213	—	—	—	—	—	—
All leukemias	7	6.6%	400	—	—	—	—	—	—
AML undifferentiated	1	0.9%	515	—	—	—	—	—	—
AML well differentiated	4	3.6%	366	—	—	—	—	—	—
CLL	1	0.9%	317	—	—	—	—	—	—
AML unclassifiable	1	0.9%	507	—	—	—	—	—	—
All histiocytic sarcomas	22	20.0%	394	1	11.1%	368	1	20.0%	378
HS with myeloproliferation	16	14.5%	400	1	11.1%	368	—	—	—
Pure HS	1	0.9%	364	—	—	—	1	20.0%	378
HS with lymphoma	5	4.5%	382	—	—	—	—	—	—
Total lesions	110			9			5		
Total no. of deaths	187	N/A	334	20	N/A	342	10	N/A	409

^a Age of death after irradiation equals age at death minus 28 ± 2 days.

Table 4

Expression of antigens in hematologic malignancies occurring in irradiated *Blm*^{-/-} mice as assessed by immunohistochemical staining.

Marker	Pre-B-LL	Mature B-LL	SBL	FBL	CBL	IBL	PCT	T-LL	AML	HS
IgM	-	++ to +++ 90%	+++ 100%	+++ 100%	+ to +++ 80%	+++ 80%	++ 100%	-	-	-
IgD	-	-	-	-	-	Occasional cells +	-	-	-	-
IgG	-	+ to +++ 90%	++ 100%	+++ 100%	++ to +++ 80%	++ to +++ 80%	+ 100%	-	-	-
B220 (CD45R)	++ to +++ 100%	+ to +++ 100%	+++ 90%	+++ to +++ 100%	+++ to +++ 100%	+ to +++ 100%	+++ 100%	-	-	-
CD5	-	-	-	+ 50%*	Occasional cells +	-	-	+ 18% [†]	-	-
CD3	-	-	-	++ (in 30% cells) 30% [†]	+++ (in 30% cells) 30% [†]	+++ (in 30% cells) 30% [†]	-	+++ 100%	-	-
CD79	-	-	-	+ 50% [†]	+ 30% [†]	+ 50% [†]	++ (in 20% cells) 20% [†]	-	-	-
CD138	-	-	-	-	-	+ (in <10% cells) 50% [†]	++ (in 20% cells) 20% [†]	-	-	-
Bd-6	-	+++ (in 30% cells) 30% [†]	-	Occasional cells +	+ (in 10% cells) 100%	+ (in 30% cells) 20% [†]	-	-	-	-
Pax-5	+++ 100%	+++ 100%	+++ 100%	+++ 100%	+++ 80%	+++ 85%	+++ (in 50% cells) 100%	-	-	-
Mac-2	-	-	-	++ to +++ (in 10% cells) 100%	+++ (in 10% cells) 80% [†]	+++ (in <30% cells) 80% [†]	-	-	-	+++ (in 50-100% cells) 100%
MPO	-	-	-	-	-	-	-	-	+++ 100%	-
Lysozyme	-	-	-	+++ (in 10% cells) 50% [†]	+++ (in 10% cells) 30% [†]	++ to +++ (in <30% cells) 80% [†]	-	-	+++ (in <30% cells) 40% [†]	+ to ++ (in 50-100% cells) 78% [†]

Marker	Pre-B-LL	Mature B-LL	SBL	FBL	CBL	IBL	PCT	T-LL	AML	HS
Ki-67	+ (in 50% cells) * 100%	+ (in 50% cells) * 67% †	–	–	–	+ 40% †	–	+ (in 70% cells) * 730% †	+++ (in 20% cells) * 100%	–

Pre-B-LL, immature pre-B cell lymphoblastic lymphoma; mature B-LL, mature B cell (surface IgM⁺) lymphoblastic lymphoma; SBL, small B cell lymphoma; FBL, follicular B cell lymphoma; CBL, diffuse large B cell lymphoma (DLBCL-CB)-centroblastic; IBL, DLBCL-IB (immunoblastic); PCT, plasmacytoma; T-LL, T lymphoblastic lymphoma; AML, acute myeloid leukemia; HS, histiocytic sarcoma; MPO, myeloperoxidase.

–, negative staining; +, weak staining; ++, moderate intensity staining; +++, strong staining;

* only a proportion of cells in the sample stain positive for the antigenic marker;

† only a percentage of total tumor cases sampled stain positive for the antigenic marker.