

Bethesda proposals for classification of lymphoid neoplasms in mice

SUPPLEMENTARY INFORMATION

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H.C.M.III and J.M.W. assisted in the organization of the pathology meeting and prepared the manuscript with assistance from the other authors listed alphabetically. The publication represents the consensus of the committee that included the authors, Paul K. Pattengale, Robert D. Cardiff, Cory Brayton, James Downing, Hiroshi Hiai, Pier Paolo Pandolfi, and Archibald S. Perkins.

This project was supported in part with funds from the Mouse Models of Human Cancers Consortium, National Cancer Institute, NIH, and NCI contract N01-CO-56000. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the United States government.

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The proposed classification of lymphoid neoplasms presented in the accompanying article¹ provides a starting point for furthering our understanding of spontaneous and induced neoplasms of mice as they relate to human diseases. The authors and the Hematopathology Committee of the Mouse Models of Human Cancer Consortium (MMHCC) have generated a set of guidelines for studying these mouse disorders. They should enhance our depth of understanding of currently recognized diseases as well as serving to facilitate the recognition and careful definition of new diseases. These include approaches to necropsies and the use of histologic, phenotypic, and molecular characterizations of lymphoid tumors for making diagnoses. Broader coverage of mouse models of human cancers can be found at the MMHCC web site, <http://emice.nci.nih.gov>.

SUPPLEMENTARY MATERIAL

Recommended approaches to diagnosing lymphoid neoplasms of mice

Necropsy

Gross observations

Description of clinical signs

Mice developing leukemia/lymphoma can present with a variety of signs. These include mainly enlargement of lymph nodes and spleen but less definitively hunched posture, ruffled fur, labored breathing, paralysis, and ataxia.

Description and characterization of tissue involvement

Different types of lymphomas often have characteristic patterns of tissue involvement visible at the time of necropsy (Table 1) such that careful records are often quite helpful in making final diagnoses.

Involved thymus, spleen, and/or specific lymph nodes should be measured and weighed. Recording body weights permits determination of relative weights of involved tissues. Each type of lymphoma has a characteristic pattern of tissue involvement that can be seen at the time of autopsy. These can be described as follows.

Generalized (systemic): Most superficial and internal lymph nodes and the spleen are enlarged, and often the liver and kidney. This is typical of mice with lymphoblastic lymphomas. In some mice, such as NZB and SJL, generalized lymphadenopathy can occur in the absence of prominent splenic or liver involvement.

Thymic: The earliest gross lesions are enlargement or reduced size of one thymic lobe followed by enlargement of the other lobe. Care must be taken to distinguish and separate the thymus from mediastinal lymph nodes.

Thymic lymphomas spread to thoracic non-lymphoid tissues and mediastinal lymph nodes and subsequently to peripheral nodes, spleen, liver, and other tissues. Mediastinal nodes can be larger than the involved thymus. The enlarged thymus and/or mediastinal nodes can sometimes fill most of the thoracic cavity, causing dyspnea. Thymic lymphomas, often mistakenly referred to as thymomas, are characteristic of AKR and C58 mice between 6 months and a year of age and are very common in many GEM, often at even younger ages.

Splenic: Many lymphomas present as an enlarged spleen with no other tissue involvement. Isolated splenomegaly is characteristic, for example, of splenic marginal zone lymphoma (SMZL) occurring in NFS congenic, NZB, p53 null, and other GEM lines on the B6,129 background. These diseases do not arise in the white pulp but at the margins of follicles, giving the gross appearance of a diffusely enlarged spleen of a pale to dark red or other color with a small or atrophic white pulp. Other types of spontaneous lymphomas in many strains of mice arise in the spleen, beginning as gross enlargement of the white pulp, appearing as a diffusely mottled-white spleen, followed by coalescence. These mice may later present with enlargement of the splenic, mesenteric, and peripheral lymph nodes.

Mesenteric lymph node: Some strains of mice have lymphomas with prominent mesenteric node enlargement, sometimes as the only abnormality. The spleen may also be enlarged but often only modestly. The nodes can be large white masses. SJL in particular but also B6,129 mice and C57BL/10 mice can present with this form of lymphoma.

Gut-associated lymphoid tissue (GALT): Peyer's patches that usually appear as small white dots may be enlarged by lymphoma in isolation or in concert with enlargement of the spleen and lymph nodes. Large intestinal GALT can also be involved. This occurs in B6,129 mice.

Liver: The liver is the most commonly involved non-lymphoid organ, with either diffuse or nodular infiltrates. The liver may be enlarged with no other gross involvement, appearing pale, mottled in color, lobulated, and firm due to infiltration by lymphomas that develop in other tissues. This occurs with NFS.V⁺ and other inbred strains.

Kidney: After the liver, the kidney is the next most common non-lymphoid organ involved by lymphoma with diffuse or nodular infiltration.

Lung: After liver and kidney, the next most common non-lymphoid site of lymphoma infiltration is the lung.

Other tissues: Involvement of ovary, skeletal muscle, and brain occurs at lower frequency than liver, kidney, and lung.

Tissues to be collected for evaluation

The most common errors made in collecting tissues for histologic diagnoses are: i) not taking a sufficiently large piece of tissue; ii) not taking a wide enough range of tissues; iii) placing too much tissue in fixative; and iv) not placing tissue in fixative immediately after necropsy. Samples on the order of 50 mg from liver, kidney, and lung and involved lymphoid tissues permit rapid exposure of the tissues to fixative, ensuring preservation of structure. Bone samples from the sternum and selected long bones can be saved separately in fixative for later decalcification and staining. Samples from seemingly normal nodes, involuted thymuses, and gut-associated lymphoid tissue can also be helpful. Material not placed directly in fixative should be kept on ice for less than 15 minutes before it is fixed. Blood smears for differential counts and evaluations of morphology can add great value, as can manual or machine-based determinations of white blood cell counts, hematocrits, and platelet counts.

Samples for histopathology

For the benefit of investigators not trained in pathology, the following considerations are outlined to aid in providing materials of optimum quality.

Fixation

Formalin: This is a very useful and convenient routine fixative, as no further manipulation of samples is required before embedding. It is also easiest to use for tissue holding and embedding and for routine studies of morphology. An increasing number of antibodies are being used and developed for immunohistochemistry (IHC) of formalin-fixed, paraffin-embedded sections. Optimal results with immunocytochemistry are obtained when the time between fixation and processing is between 24 and 48 hours.

Paraformaldehyde: This fixative is best for *in situ* hybridization and studies of some cell surface antigens. It must be made up fresh and kept at 4°C.

Bouin's: This fixative is best for revealing nuclear features, quick fixation, and processing, but requires a further step in preparation of samples. Antibodies for IHC that do not work with formalin-fixed tissues may work with sections of materials fixed in Bouin's, especially antibodies directed against cell surface antigens.

Methacarn: This fixative is used for enhancement of immunostaining for many antigens. It requires quick processing and careful attention.

B5: This fixative was previously widely used in veterinary pathology but has fallen out of favor because it contains mercury, thereby raising serious environmental concerns.

Stains

Hematoxylin and eosin (H&E): This is the most widely used general-purpose stain and is familiar to all pathologists.

Giemsa: Giemsa provides excellent definition of both nuclear and cytoplasmic features but has not been widely used for mice. It is useful for staining bone marrow and blood smears.

Wright-Giemsa: This stain is the best for tissue imprints and blood smears.

Methyl green pyronin: This is used to discriminate RNA from DNA in cells. It is particularly useful for indicating high-level immunoglobulin transcription in plasma cells.

Frozen samples for immunohistochemistry, DNA, and RNA

Immunohistochemistry

Freeze small samples in OCT (Sakura Finetek, Torrance, CA) on liquid nitrogen or isopentane and dry ice.

DNA

Immediately freeze 50 mg samples on dry ice or liquid nitrogen and store at -80°C.

RNA

Immediately place 50 mg sample in Trizol (Invitrogen, Carlsbad, CA) or equivalent, place in dry ice, and store at -80°C. Alternatively, freeze on dry ice or liquid nitrogen before storage.

Samples for flow cytometry

Single-cell suspensions are prepared from primary or secondary lymphoid tissues in cold medium used specifically for flow cytometry or phosphate-buffered saline with 1% fetal calf serum. The cells are stained with antibodies to cell surface antigens. The antibodies can be: directly labeled with fluorochromes; labeled with biotin and detected with fluorochrome-conjugated avidin; or unlabeled and detected by incubation with second-step antibodies labeled with fluorochromes. Intracellular staining for antigens can be performed with cells first fixed in paraformaldehyde and then permeabilized with Tween 20 before staining with antibodies. Alternatively, staining for both cell surface and intracellular antigens or paraformaldehyde-sensitive cytoplasmic antigens can be done with cells permeabilized with saponin. Flow cytometry is performed with any of a number of commercially available machines. Viable stained cells are incubated with propidium iodide to permit exclusion of dead cells that take up the dye. Multicolor analyses using antibodies with differing specificities for cell surface antigen, combined with studies of narrow forward-angle light scatter, will almost always reveal tumor cell populations with a sensitivity approaching 5%.

Viable cells

Prepare a sterile single-cell suspension, pellet by centrifugation, and resuspend in 8% dimethylsulfoxide in fetal calf serum. Freeze and store at -80°C.

Serum

Prepared from blood taken in any of a variety of ways.

Cytocentrifuge and touch preparations

Valuable information can be obtained by making cytocentrifuge preparations using viable single-cell suspensions prepared from affected tissues. These can be stained with Wright-Giemsa or used for immunocytochemistry. Similar advantage can be made of touch preps. For this technique, fresh spleen, node, or other tissue is cut with a scalpel or scissors to generate a flat surface that is lightly touched to several points along a microscope slide. Cells transferred to the slide can be treated similarly to cytocentrifuge preparations.

Histologic characterization—definitions

The cell morphologies and characteristics described below depend, in part, on fixative, stain, the process of embedding, and other factors, but are generally applicable to samples fixed with formalin or Bouin's, embedded in paraffin, and stained with H&E.

Cells

Size and shape: Tumor cell size can be compared to the size of non-neoplastic cells within the same tumor or tissues. Tissue histiocytes and phagocytic macrophages are often used for size comparisons with tumor cells. Red cells can also be used. Size range is from small lymphocyte (one and a half times the diameter of a red cell) to histiocyte (up to ten times the diameter of a red cell). Progressive intermediate sizes of lymphoid cells are centrocyte, small lymphocyte, marginal zone B cell and plasma cell, lymphoblast, centroblast and plasmablast, and immunoblast. These relationships are less clear-cut when applied to neoplastic lymphocytes, as cells comprising specific types of lymphoma in different mice can cover a significant size range.

Cytoplasm—quantity and color: On sections stained with H&E, the quantity ranges from scant (a thin rim surrounding the nucleus) to abundant (twice the nuclear

size). For each cell type, more cytoplasm is seen on imprints stained with Wright-Giemsa than sections stained with H&E. Similarly, cells in samples fixed in Bouin's appear larger with more cytoplasm than in material fixed in formalin.

Nucleus—size, chromatin pattern, membrane features, shape: The size of lymphoid cells is usually given in terms of the size of the nucleus relative to that of normal or reactive histiocytes or endothelial cells. Small, medium, and large refer to nuclei smaller than, the same size, or larger than those of histiocytes or endothelial cells. Relative sizes can be accurately quantitated using micrometers or computer-based image analysis programs, although the absolute size will vary with tissue and the specifics of tissue preparation, particularly the fixative employed. The chromatin pattern is variable, characterized as vesicular (open), coarse, or clumped (condensed). Lymphomas phenotypically similar to mature lymphocytes have clumped chromatin, whereas those resembling immature lymphocytes can have various chromatin patterns. The closed-to-open progression is felt to reflect the level of transcription occurring in a cell. Fully mature plasma cells often have cartwheel chromatin patterns associated with chromatin degradation, a feature not associated with plasmablasts. A thickened nuclear membrane can also be seen. Shapes include round, oval, multilobated, angulated, and cleaved.

Nucleolus—number, size, prominence, color, location: The location and number of nucleoli can be associated with specific entities. A single central nucleolus is common in lymphoblastic and plasmablastic tumors, while two nucleoli attached to the nuclear membrane are seen in almost all centroblastic lymphomas. A bar-shaped nucleolus attached to the nuclear membrane on one end is seen in immunoblastic lymphomas. Colors range from dark blue to amphophilic.

Tissue involvement

Bone marrow, blood: Tumor cells may be present or absent. Proportions of tumor cells should be recorded. Extensive bone marrow involvement occurs infrequently in mouse lymphomas.

Leukemia vs. lymphoma: In humans, lymphoid leukemia is a neoplastic disease that from its early stages involves the blood and/or hematopoietic tissue of the bone marrow and spleen, while lymphoma is a disease that presents as a solid tumor. The vast majority of lymphoid leukemias in mice, as defined by involvement of the blood, represent "spillover" of lymphoma cells into the blood. These are thus not primary leukemias but rather lymphomas with leukemic phases. Lymphoid leukemias such as those seen in humans occur very rarely, if ever, as spontaneous tumors but do occur with regularity in mice with certain transgenes, such as E μ -MYC, or infected with retroviruses that drive high levels of expression of oncogenes such as v-abl that target B progenitor or pre-B cells. The situation in mice is complicated by the fact that the spleen is a primary hematopoietic organ equivalent in many ways to human bone marrow. Simultaneous involvement of the spleen with bone marrow and blood may thus be the equivalent of bone marrow-based leukemia in humans. In mice, a leukemic phase is seen in about one third of small lymphocytic or lymphoblastic lymphomas.

Immunophenotype

Phenotyping of mouse lymphoid neoplasms can be done using the techniques of immunohistochemistry (IHC) and flow cytometry (FACS). A large panel of antibodies and other reagents is available for use in FACS for determining lineage and differentiation state,² and the list of reagents of use in staining either frozen sections or fixed material using antigen retrieval systems is expanding rapidly.³

The committee recommends that all descriptions of lymphomas include typing by FACS and/or IHC for expression of cell surface and cytoplasmic antigens. It is suggested that routine FACS analyses be developed for antigen-specific receptors (IgM, IgD, IgG, Ig ϵ , Ig δ , TCR α/β , TCR γ/δ), CD3, CD4, CD5, CD8, CD11b, CD19, CD45R(B220), CD90, and CD138. IHC studies for cytoplasmic IgM and CD79a and nuclear TdT are also recommended. The expression of these cell surface and cytoplasmic antigens by the major histologic types of lymphomas described below is given in Table 2. The added value of extensive analyses comes from the ability to identify new patterns of expression that differ from those seen previously. For example, all mature B cell lymphomas that appeared in a mouse model of Burkitt lymphoma were CD5⁻⁴, in contrast to the common CD5⁺ phenotype of most spontaneously occurring B-cell lymphomas.

Several points deserve particular attention. First, the ratio of Ig ϵ - to Ig δ -expressing normal B cells in humans is close to 2:1, providing the opportunity to use light chain restriction as a relatively reliable marker for clonality. In contrast, around 95% of normal mouse B cells express Ig ϵ light chain, so only Ig δ restriction is informative. Second, expression of CD5 is a helpful marker for aiding in the diagnosis of chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) and mantle cell lymphoma in humans. In the mouse, up to 85% of all spontaneous B-lineage lymphomas except plasmacytomas are CD5⁺. Third, up to 40% of spontaneous mouse B-cell lymphomas of several types express CD11b at low levels (H.C.M., unpublished observations). This marker is expressed at high levels on normal mouse myeloid cells as well as on a subset of peritoneal B cells. It should also be recognized that certain markers for normal B-cell differentiation in humans can behave quite differently in the mouse. For example, human CD38⁻ follicular B cells differentiate into CD38⁺ germinal center B cells, whereas CD38 is downregulated on germinal center B cells in the mouse.⁵

Molecular analysis

Gene rearrangements–IgH, TCR

Southern blot hybridization of high-molecular-weight cellular DNA digested with appropriate enzymes utilizing probes capable of identifying rearrangements of immunoglobulin heavy chain (IgH) or light chain (IgL) genes or TCR genes provides one approach to determining lineage and clonality. The rationale for performing these studies is that they can assist in distinguishing polyclonal, non-neoplastic lymphoproliferative diseases from clonal malignant lymphomas. For true lymphomas, they usually give an unequivocal diagnosis of T or B cell origin and sometimes the state of differentiation within each lineage. Table 3 presents the patterns of Ig and TCR gene organization that can be seen by Southern analysis of DNA prepared from normal, reactive, and neoplastic tissue samples.

Gene rearrangements–MuLV

Many strains of inbred mice have germline integrations of endogenous ecotropic MuLV that can be activated to yield infectious virus capable of polyclonal somatic integrations in normal cells and clonal integrations in lymphomas.^{6,7} These can be recognized by digesting DNA with an enzyme that does not cut within the virus and hybridization with an ecotropic virus-specific probe. In such a test, oligoclonal or clonal outgrowths of cells with a newly acquired integration can be revealed by non-germline bands hybridizing with intermediate to high intensity.

Contributions of establishing clonality and transplantability to studies of lymphoid neoplasms

The committee debated extensively the importance of defining clonality in identifying and classifying murine hematopoietic neoplasms. The concept that non-hematopoietic neoplasms arise through a progressive accumulation of genetic and epigenetic changes resulting in clonal proliferation has gained wide acceptance. Most spontaneous and engineered mouse hematopoietic tumors probably undergo a functionally similar series of changes, but it may be possible, through engineering, to introduce genetic changes in many cells sufficient to induce disease, thereby yielding non-clonal tumors.

For normal T and B cells and, as a result, their derivative lymphomas, immune receptor gene rearrangements that occur in the pre-malignant cell are clonal by definition and can be readily detected by standard techniques of molecular biology. In these lymphomas, mono- or oligo-clonal populations are the rule. This is true even in the context of engineered mice with striking polyclonal expansions of cells uniformly bearing defined genetic lesions such as dominantly acting proto-oncogenes or biallelic disruptions of tumor suppressor genes. Indeed, the expectation would be that a mouse engineered to express a combination of genes sufficient for induction of a particular lymphoma type would carry polyclonal tumors. This could prove to be a useful distinction from mice with spontaneous or induced clonal disease. In addition, the identification of clonal disease is an important element in strategies for distinguishing T- and B-cell lymphomas from reactive conditions (Table 4). We propose that demonstrating clonality is very important for the diagnosis and classification of T- and B-cell lymphomas, with the recognition that in the setting of multiple genetically engineered lesions, polyclonal lymphoma may occur.

The role of transplant studies in tumor diagnosis was also considered. The importance of these studies in identifying a lymphoid tumor population would appear to be

greater than for non-lymphoid hematopoietic neoplasms (Kogan SC, et al., submitted for publication), in part because of the ease with which clonality of lymphoid tumors can be established. The identification of the same clonal population in a primary tumor and a transplant recipient is felt to clearly define the initial disease as non-reactive and to provide forceful evidence for neoplasia. It is recognized that non-transformed cells can passage with a lymphoma and that the same clones may not always be found in donors and transplants,⁸ probably due to the selective outgrowth of aggressive tumor cells represented at only low levels in the primary host.

Distinguishing reactive from malignant populations of lymphoid cells

Non-malignant proliferations of hematopoietic cells can occur as a result of infection, toxic insult, the presence of non-hematopoietic tumors, and immune dysfunction among other causes. These disturbances can result in enlargement of secondary lymphoid tissues and sometimes the liver, giving the outward appearance of a neoplasm; however, a variety of histologic and molecular features can be used to distinguish reactive from neoplastic disorders of the lymphoid system (Table 4). Histologic analyses of affected tissues may sometimes be sufficient to make the distinction, but the additional strengths provided by the opportunity to perform molecular tests for T- and B-cell clonality emphasize the importance of saving material from affected tissues for analyses of DNA. This is extremely important when lesions very early in lymphoma development are observed, as is often the case of sequential sacrifice studies or the termination of a study. Routine inclusion of liver, lung, kidney, and any gross lesions for fixation at the time of necropsy is critical for ruling out effects of non-malignant processes even in the presence of overt lymphoma. Indolent or early lymphomas are usually not difficult to distinguish histologically from reactive processes, and they have a molecular signature of clonal expansion recognizable as distinct but faint rearranged bands on Southern blot analyses of Ig and TCR gene organization.

Combined modalities for diagnosis

It should be recognized that the starting point (and often the end point for veterinary pathology) for all definitive diagnoses is microscopic examination of fixed tissues for histologic and cytologic features. The most common appearances for T- and B-cell lymphomas and their relation to final diagnoses established through immunophenotypic and molecular studies is shown in Figure 1.

Occurrence of lymphoid neoplasms in mice

Table 5 lists the occurrence of spontaneous lymphomas of different types among inbred, congenic, transgenic, and "knockout" strains of mice as well as mice infected with acutely transforming retroviruses. The list is not meant to be all-inclusive, but was the source of many cases that were important in establishing the proposed classification scheme. It is meant to serve as a starting point for evaluating published descriptions of different tumor types against which to compare lymphomas arising in new model systems. It is hoped that investigators will contact the authors to assist in the generation of a more complete listing, which will be maintained at the MMHCC web site.

Genetic determinants of lymphoid neoplasms

Chromosomal translocations are found in many human hematopoietic neoplasms and are responsible for the activation of proto-oncogenes or the generation of fusion proteins. In many cases, the translocations are tumor specific. It is these genes in particular that are being used in efforts to model human neoplasms in the mouse. Studies of chromosomal anomalies in mice have been more limited, due in large part to difficulties in reliable identification related to their acrocentric nature and similar sizes. Indeed, the only translocations shown to occur at high frequency are those involving Ig gene and MYC in pristane-induced plasmacytomas.⁹ The route to identifying gene alterations contributing to neoplasms in mice expressing MuLV at high levels has been the characterization of common, somatically acquired integration sites for ecotropic and MCF MuLV.⁷ Some of these sites are associated with particular tumor types (Table 6), but it is noteworthy that they usually do not identify the same genes revealed by translocations in human tumors of the same type. In addition, it is only a minority of cases of each histologic type in mice that have the genetic lesions that have been defined. The involvement of different paths in transforming the same cell type in the two species could suggest that these alternative routes are responsible for the differences in phenotype we see, for example, in murine FBL *vs.* human follicular lymphoma. Unraveling the paths in mice could assist in better modeling and treatment for the human tumors.

References

1. Morse HC, Anver MR, Fredrickson TN, et al. Bethesda proposals for classification of lymphoid neoplasms in mice. *Blood*. 2002, accompanying print manuscript.
2. Lai L, Alaverdi N, Maltais L, Morse HC. Mouse cell surface antigens: nomenclature and

- immunophenotyping. *J Immunol.* 1998;160:3861-3868.
3. Cattoretti G, Fei Q. Application of the antigen retrieval technique in experimental pathology: from human to mouse. In: Shi S-R, Gu J, Taylor CR, eds. *Antigen Retrieval Techniques*. Natick, MA: Eaton Publishing; 2000.
 4. Kovalchuk AL, Qi CF, Torrey TA, et al. Burkitt lymphoma in the mouse. *J Exp Med.* 2000;192:1183-1190.
 5. Lund FE, Cockayne DA, Randall TD, Solvason N, Schuber F, Howard MC. CD38: a new paradigm in lymphocyte activation and signal transduction. *Immunol Rev.* 1998;161:79-93.
 6. Rowe WP. Leukemia virus genomes in the chromosomal DNA of the mouse. *Harvey Lect.* 1978;71:173-192.
 7. Rosenberg N, Jolicoeur P. Retroviral pathogenesis. In: *Retroviruses*. Plaview, NY: Cold Spring Harbor Laboratory Press; 1997;475-486.
 8. Tang Y, Chattopadhyay SK, Hartley JW, Fredrickson TN, Morse HC III. Clonal outgrowths of T and B cells in SCID mice reconstituted with cells from mice with MAIDS. *In Vivo.* 1994;8:953-960.
 9. Potter M, Wiener F. Plasmacytomagenesis in mice: model of neoplastic development dependent on chromosomal translocations. *Carcinogenesis.* 1992;13:1681-1697.
 10. Hartley JW, Chattopadhyay SK, Lander MR, et al. Accelerated appearance of multiple B cell lymphoma types in NFS/N mice congenic for ecotropic murine leukemia viruses. *Lab Invest.* 2000;80:159-169.
 11. Morse HC III, Qi CF, Chattopadhyay SK, et al. Combined histologic and molecular features reveal previously unappreciated subsets of lymphoma in AKXD recombinant inbred mice. *Leuk Res.* 2001;25:719-733.
 12. Yamada Y, Matsushiro H, Ogawa MS, et al. Genetic predisposition to pre-B lymphomas in SI Kh strain mice. *Cancer Res.* 1994;54:403-407.

13. Green PL, Kaehler DA, Risser R. Cell transformation and tumor induction by Abelson murine leukemia virus in the absence of helper virus. *Proc Natl Acad Sci USA*. 1987;84:5932-5936.
14. Morse HC, Hartley JW, Fredrickson TN, et al. Recombinant murine retroviruses containing avian v-Myc induce a wide spectrum of neoplasms in newborn mice. *Proc Natl Acad Sci USA*. 1986;83:6868-6872.
15. Adams JM, Harris AW, Pinkert CA, et al. The *c-myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature*. 1985;318:533-538.
16. Gao YJ, Ferguson DO, Xie W, et al. Interplay of p53 and DNA-repair protein Xrcc4 in tumorigenesis, genomic stability and development. *Nature*. 2000;404:897-900.
17. Difilippantonio MJ, Zhu J, Chen HT, et al. DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature*. 2000;404:510-514.
18. Taddesse-Heath L, Chattopadhyay SK, Dillehay DL, et al. Lymphomas and high-level expression of murine leukemia viruses in CFW mice. *J Virol*. 2000;74:6832-6837.
19. Yumoto T, Yoshida Y, Yoshida H, Ando K, Matsui K. Prelymphomatous and lymphomatous changes in splenomegaly of New Zealand Black mice. *Acta Pathol Jpn*. 1980;30:171-186.
20. Fredrickson TN, Lennert K, Chattopadhyay SK, Morse HC, Hartley JW. Splenic marginal zone lymphomas of mice. *Am J Pathol*. 1999;154:805-812.
21. Ward JM, Tadesse-Heath L, Perkins SN, Chattopadhyay SK, Hursting SD, Morse HC. Splenic marginal zone B-cell and thymic T-cell lymphomas in P53-deficient mice. *Lab Invest*. 1999;79:3-14.
22. Repacholi MH, Basten A, Gebiski V, Noonan D, Finnie J, Harris AW. Lymphomas in E μ -Pim1 transgenic mice exposed to pulsed 900 Mhz electromagnetic fields. *Radiation Res*. 1997;147:631-640.
23. Dunn TB. Plasma cell neoplasms beginning in the ileocecal area in strain C3H mice. *J Nat Cancer Inst*. 1957;19:371-391.

24. Clynes R, Wax J, Stanton LW, Smithgill S, Potter M, Marcu KB. Rapid induction of IgM-secreting murine plasmacytomas by pristane and an immunoglobulin heavy-chain promoter enhancer-driven C-Myc/V-Ha-Ras retrovirus. *Proc Natl Acad Sci USA*. 1988;85:6067-6071.
25. Rosenbaum H, Harris AW, Bath ML, et al. An E- λ -V-Abl transgene elicits plasmacytomas in concert with an activated Myc gene. *EMBO J*. 1990;9:897-905.
26. Morse HC III, Riblet R, Asofsky R, Weigert M. Plasmacytomas of the NZB mouse. *J Immunol*. 1978;121:1969-1972.
27. Kovalchuk AL, Kim JS, Park SS, et al. IL-6 transgenic mouse model for extraosseous plasmacytoma. *Proc Natl Acad Sci USA*. 2002;99:1509-1514.
28. Harris AW, Bath ML, Rosenbaum H, McNeill J, Adams JM, Cory S. Lymphoid tumorigenesis by V-Abl and Bcr-V-Abl in transgenic mice. *Curr Topics Microbiol Immunol*. 1990;166:165-173.
29. Lu L-M, Hiai H. Mixed phenotype lymphomas in thymectomized (SL/Kh \times AKR/Ms)F1 mice. *Jpn J Cancer Res*. 1999;90:1218-1223.
30. Cole RK, Furth J. Experimental studies of spontaneous leukemia in mice. *Cancer Res*. 1941;1:957-965.
31. MacDowell EC, Richter MN. Mouse leukemia. IX. The role of heredity in spontaneous cases. *Arch Pathol*. 1935;20:709-724.
32. Moloney JB. Biological studies on a lymphoid leukemia virus extracted from sarcoma S.37. I. Origin and introductory investigations. *J Nat Cancer Inst*. 1960;24:933-951.
33. Hartley JW, Wolford NK, Old LJ, Rowe WP. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc Natl Acad Sci USA*. 1977;74:789-792.
34. Vesselinovitch SD, Rao VK, Mihailovich RN, Rice JM, Lombard LS. Development of broad spectrum of tumors by ethylnitrosourea in mice and the modifying role of age, sex, and strain. *Cancer Res*. 1974;34:2530-2538.
35. Boiato L, Mirvish SS, Berenblum I. The carcinogenic action and metabolism of

- N-hydroxyurethane in newborn mice. *Int J Cancer*. 1966;15:265-269.
36. Stewart M, Terry A, O'Hara M, Cameron E, Onions D, Neil JC. *til-1: a novel proviral locus for Moloney murine leukaemia virus in lymphomas of CD2-myc transgenic mice*. *J Gen Virol*. 1996;77:443-446.
 37. Donehower LA, Harvey M, Slagle BL, et al. *Mice deficient for P53 are developmentally normal but susceptible to spontaneous tumors*. *Nature*. 1992;356:215-221.
 38. Barlow C, Hirotsune S, Paylor R, et al. *Atm-deficient mice: a paradigm of ataxia telangiectasia*. *Cell*. 1996;86:159-171.
 39. Fredrickson TN, Harris AW. *Atlas of Mouse Hematopathology*. Amsterdam: Harwood Academic Publishers; 2000.
 40. Fehniger TA, Suzuki K, Ponnappan A, et al. *Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8⁺ T cells*. *J Exp Med*. 2001;193:219-231.
 41. Baldassarre G, Fedele M, Battista S, et al. *Onset of natural killer cell lymphomas in transgenic mice carrying a truncated Hmgi-C gene by the chronic stimulation of the IL-2 and IL-5 pathway*. *Proc Natl Acad Sci USA*. 2001;98:7970-7975.
 42. Rabbitts TH. *Chromosomal translocations in human Cancer*. *Nature*. 1994;372:143-149.
 43. Willis TG, Dyer MJS. *The role of immunoglobulin translocations in the pathogenesis of B cell malignancies*. *Blood*. 2000;96:818-822.

SUPPLEMENTARY MATERIAL

Table 1. Tissue involvement by lymphoid mouse neoplasms^a

Lymphoma lineage	Tissue/Organ										
	Bone marrow	Spleen	Thymus	Thoracic LN	Abdominal LN ^b	Peripheral LN	Mesenteric LN	Liver ^c	Lung ^c	Kidney ^c	GALT
B cell											
Pre-B LL	+++	++	-	+++	+++	+++	+++	+++	++	++	+
SBL	-	+++	-	+	+	+	+	+	+	+	+/-
SMZL	-	+++	-	-	+ ^d	-	-	-	-	-	-
FBL	-	+++	-	+	++	+	++	+	+	+	+/-
DLCL(CB)	-	+++	-	++	++	++	+++	+	+	+	+
DLCL(IB)	-	+++	-	++	++	++	+++	++	+	+	+
DLCL(HA)	-	+	-	+	+	+	+	+	+	+	+
DLCL(PM)	-	-	+	(+)	-	-	-	-	-	-	-
BL	-	++	(+)	+++	+++	+++	+++	++	++	++	+
BLL	-	++	-	+++	+++	+++	+++	++	++	++	+
PCT	-	++	-	(+)	+	+	+	+	+	+	+
PCTA	-	++	-	+	+	+	+	+	+	+	+
BNKL	-	+	-	-	+	+	+	+	NA	NA	+

SUPPLEMENTARY MATERIAL

Table 1. Tissue involvement by lymphoid mouse neoplasms^a

	Tissue/Organ											
T cell												
Pre-T LL	-	++	+++	++	+++	+++	+++	++	++	++	+	
T LL	-	++	+++	++	+++	+++	+++	++	++	++	+	
TNKL	+++	+++	-	++	+++	+++	+++	+++	++	++	+	

^a Abbreviations: Pre-B LL, precursor B cell lymphoblastic lymphoma; SBL, small B cell lymphoma; FBL, follicular B cell lymphoma; DLCL, diffuse large B cell lymphoma; CB, centroblastic; IB, immunoblastic; HA, histiocyte-associated; PM, primary mediastinal (thymic) B cell lymphoma; BL, Burkitt lymphoma; BLL, Burkitt-like lymphoma; PCT, plasmacytoma; PCTA, anaplastic plasmacytoma; BNKL, B-NK cell lymphoma; pre-T LL, precursor T cell lymphoblastic lymphoma; TLL, T cell lymphoblastic lymphoma; TNKL, T-NK cell lymphoma; GALT, gut-associated lymphoid tissue including Peyer's patches; NA, information not available.

^b Includes splenic, renal, and lumbar nodes.

^c Involvement by lymphoblastic and BLL is characteristically perivascular.

^d Splenic node only.

SUPPLEMENTARY MATERIAL

Table 2. Expression of cell surface and cytoplasmic antigens by mouse lymphomas^a

Assay ^b Marker	Lymphoma type ^c									
	PreB	SBL	FBL	MZL	DLBCL	BL	PCT	PreT	T	
F,I IgM	-	+	+	+	+/-	+	(+)	-	-	
F,I IgD	-	-	-	-	-	-/+	-	-	-	
F,I Igê	-	+/-	+/-	+/-	+/-	+	(+)	-	-	
F,I Igë	-	-/+	-/+	-/+	-/+	-	(+)	-	-	
F CD19	+	+	+	+	+	+	+/-	-	-	
F,I B220	+	+	+	+	+	+	+/-	-	-	
F CD5	(+)/-	(+)/-	(+)/-	(+)/-	(+)/-	-	-	+	+	
F CD11b	-/+	-/+	-/+	-/+	-/+	-	-	-	-	
F,I CD138	-	-	-	-	-	-	+	-	+	
F TCRá/â	-	-	-	-	-	-	-	+	+	
F TCRã/ä	-	-	-	-	-	-	-	+	+	
F,I CD3	-	-	-	-	-	-	-	+	+	
F,I CD4	-	-	-	-	-	-	-	+	+	
F,I CD8	-	-	-	-	-	-	-	+	+	
F CD90	-	-	-	-	-	-	-	+	+	
I nucTdT	+	-	-	-	-	-	-	+	-	
I cytIgM ^d	+	-	-	-	-	-	+ ^e	-	-	
I cytCD79a ^d	+	-	-	-	-	-	-	-	-	

^a +, positive; -, negative; +/-, positive more often than negative; -/+, negative more often than positive; (+), low-level expression.

^b F, FACS; I, immunocytochemistry.

^c Lymphoma abbreviations: PreB, precursor B cell; SBL, small B cell; FBL, follicular B cell; MZL, splenic marginal zone; DLBCL, diffuse large cell; PCT, plasmacytoma; PreT, precursor T cell; T, mature T cell.

^d cyt, cytoplasmic staining; nuc, nuclear staining.

^e May be positive for any Ig class.

SUPPLEMENTARY MATERIAL

Table 3. Antigen receptor gene status in normal, reactive, and neoplastic diseases of the mouse lymphoid system^a

Tissue status	IgH	Ig ϵ	Ig δ	TCR α	TCR β
Normal non-lymphoid	G	G	G	G	G
Normal lymphoid	PR	PR	PR	PR	PR
Reactive	PR	PR	PR	PR	PR
Indolent lymphoma ^b	G/R	G/R	G/R	G/R	G/R
Immature B cell lymphoma	R	G/R	G	G	G
Mature B cell lymphoma	R	R*	R*	G/IR	G/IR
T cell lymphoma	G/IR	G	G	R	R

^a Abbreviations: G, germline for both alleles; R, functionally rearranged for one allele. Second allele may be rearranged but nonfunctional; R*, if rearranged for Ig ϵ , Ig δ is usually germline. If rearranged for functional Ig δ , Ig ϵ is often nonfunctionally rearranged; G/IR, incomplete rearrangement (e.g., DJ) for one allele; PR, polyclonal rearrangements. Seen as smears or indistinct bands on Southern blots.

G/R, rearranged bands of low intensity and often multiple appear without major loss in the density of the germline band. The multiple bands are suggestive of oligoclonality, and relative retention of the germline band indicates that the clones comprise a minor proportion of the total cell population.¹⁰

^b The term indolent is used to suggest the presence of either a slowly growing lymphoma or one that has yet to expand to the point that it can be recognized as a monoclonal population of cells.

SUPPLEMENTARY MATERIAL

Table 4. Distinguishing Lymphoma from reactive hyperplasia in mice

Parameter	Lymphoma	Reactive hyperplasia
Clonality	Clonal Ig or TCR rearrangements as indicated by strong non-germline bands in Southern blots	Polyclonal Ig or TCR rearrangements with no prominent new, non-germline bands
Architecture		
Spleen	Loss of normal architecture; enlarged white pulp; involving one, a few, or all white-pulp areas; eventual spillage into red pulp	Maintains normal architecture; enlarged white pulp with formation of secondary follicles, usually uniformly; red pulp reactive; cause/etiology for reactive lesion may be found (inflammation, infection, tumor, ulcer, etc.)
Lymph nodes	Loss of normal architecture; loss of germinal centers; infiltration by pleomorphic or monomorphic population of lymphoid cells in cortex, medulla, and capsule	Maintains normal architecture; hyperplastic germinal centers in cortex; plasmacytosis in medulla and/or paracortical hyperplasia; usually no capsular involvement
Phenotype (FACS)	Cells have increased light scatter, distinguishing them from cells of normal size with low scatter; expression of surface antigens tends to be sharply modal and often different from the range for	Light scatter ranges from normal to moderately increased; cells express surface antigens within the normal range

SUPPLEMENTARY MATERIAL

Table 4. Distinguishing lymphoma from reactive hyperplasia in mice

Parameter	Lymphoma normal cells	Reactive hyperplasia
Cytology	Monomorphic populations of lymphocytes, especially when immature lymphocytes; mitotic figures vary and may be numerous in some types of lymphoma	Plasma cells, plasma cell precursors, germinal center hyperplastic blasts, paracortical mixed population; few mitotic figures
Dissemination	Monomorphic tumor cells may be found in various tissues and blood; there may be secondary blood effects such as anemia, thrombocytopenia (rare)	May have reactive foci in non-lymphoid tissues composed of small lymphocytes with few mitotic figures; blood may show leukocytosis

SUPPLEMENTARY MATERIAL

Table 5. Occurrence of lymphomas in mice

Classification	Occurrence	Mice affected
Pre-BLL	Spontaneous	AKXD ¹¹ SL/Kh ¹²
	Retrovirus	v-abl ¹³ v-myc + raf ¹⁴
	Transgenic	E \bar{i} -myc ¹⁵
	Knockout	p53 ^{-/-} + XRCC4 ^{-/-} ¹⁶ p53 ^{-/-} + Ku80 ^{-/-} ¹⁷
SBL	Spontaneous	NFS.V ⁺¹⁰ CFW ¹⁸
SMZL	Spontaneous	NZB ¹⁹ NFS.V ⁺²⁰ AKXD ¹¹
	Knockout	p53 ^{-/-} ²¹
FBL	Spontaneous	Many inbred strains; NFS.V ⁺¹⁰ AKXD ¹¹ CFW ¹⁸
	Transgenic	E \bar{i} -Pim1 ²²
DLBCL(CB)	Spontaneous	NFS.V ⁺¹⁰ CFW ¹⁸
DLBCL(IB)	Spontaneous	NFS.V ⁺¹⁰ CFW ¹⁸
DLBCL(HA)	Spontaneous	NFS.V ⁺¹⁰ AKXD ¹¹
DLDC(LPM)	Retrovirus	BM5def in C57BL/6 mice (Morse HC III, et al., unpublished)
BL	Transgenic	\bar{e} -MYC ⁴
BLL	Spontaneous	NFS.V ⁺¹⁰ CFW ¹⁸
PCT	Spontaneous	Some inbred strains ²³
	Retrovirus	myc + v-Hras ²⁴ myc + v-abl ²⁵
	Induced	Pristane in BALB/c ⁹ or NZB ²⁶ Pristane + v-abl ¹³
PCT-E	Transgenic	IL-6 ²⁷
PCT-A	Transgenic	E \bar{i} -v-abl ²⁸

SUPPLEMENTARY MATERIAL

Table 5. Occurrence of lymphomas in mice

Classification	Occurrence	Mice affected
BNKL	Manipulated	Thymectomized (SL/Kh × AKR/Ms)F ₁ ²⁹
Pre-TLL	Spontaneous	AKR ³⁰ C58 ³¹ NFS.V ⁺ ¹⁰ AKXD ¹¹
	Retrovirus	Moloney ³² class I MCF viruses ³³
	Chemical	ENU ³⁴ urethane ³⁵
	Transgenic	CD2-Myc ³⁶
	Knockout	p53 ^{-/-} ³⁷ ATM ^{-/-} ³⁸
STL	Spontaneous	NFS.V ⁺ ³⁹
TNKL	Transgenic	IL-15 ^{40,41}
TLCA	Spontaneous	NFS.V ⁺ ³⁹

SUPPLEMENTARY MATERIAL

Table 6. Genetic abnormalities in human and spontaneous mouse lymphoma/leukemia^a

Human disease ^b	Genetic change	Gene involved	Mouse disease	Genetic change	Gene involved
B-ALL/LBL	t(9;22) t(4;11) t(1;19) t(12;21)	<i>BCR/ABL</i> <i>AF4/MLL</i> <i>PEX/E2A</i> <i>TEL/AML1</i>	Pre-B LL	MuLV-IM ^c	<i>Evi3</i>
SLL/CLL	+12 Del 13q14 Del 11q22-23		SBL	Not described	
SMZL	LOH 7q21-32		SMZL	Not described	
FL	t(14;18)	<i>BCL2</i>	FBL	Not described	
DLCL	t(3;14) t(14;18) t(8;14)	<i>BCL6</i> <i>BCL2</i> <i>MYC</i>	DLCL	T(6;15)	<i>Bcl6</i>
DLCL(PM)	Not described		DLCL(PM)	Not described	
BL	t(8;14)	<i>MYC</i>	BL	Not described	
BLL	t(8;14)	<i>MYC</i>	BLL	MuLV-IM MuLV-IM	<i>Pim1</i> <i>Evi3</i>
PCT (MM)	t(11;14) t(4;14) t(6;14) t(14;16)	<i>CCND1</i> <i>FGFR3</i> <i>IRF4</i> <i>C-MAF</i>	PCT	T(12;15)	<i>Myc</i>
T-ALL/LBL	t(8;14) t(1;14) t(11;14) t(7;19)	<i>MYC</i> <i>TAL1</i> <i>RBTN1</i> <i>LYL1</i>	Pre-T LL	MuLV-IM MuLV-IM MuLV-IM	<i>Myc</i> <i>Mycn</i> <i>Pim1</i>

^a The human and mouse diseases are listed as closest counterparts. The genetic changes for human neoplasms are primarily from Rabbitts⁴² and Willis and Dyer.⁴³ Not all observations are included. For mouse lymphomas, the data are from ref. 11.

^b Abbreviations: B-ALL/LBL, B-cell acute lymphoblastic leukemia/lymphoblastic lymphoma; SLL/CLL, small

lymphocytic lymphoma/chronic lymphocytic leukemia; DLCL(PM), primary mediastinal (thymic) diffuse large cell lymphoma; MM, multiple myeloma; T-ALL/LBL, T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma.

^c MuLV-IM, murine leukemia virus insertional mutagenesis at common integration sites.

Figure legends

Figure 1. Histologic and phenotypic characterizations of mouse lymphomas. Light microscopic evaluations permit identification of lymphomas that can be designated as small lymphocytic (SLL), lymphoblastic (LL), immunoblastic (IBL), splenic marginal zone (SMZL), and plasmacytoma (PCT). The proportions of centroblasts can be used to subcategorize lymphoma with mixed centroblastic/centrocytic (CB/CC) into follicular B cell lymphoma (FBL) and diffuse large B cell lymphoma of centroblastic type [DLCL(CB)]. The proportions of immunoblasts and plasmablast-like cells can be used to distinguish anaplastic PCTs from others. Immunophenotypic features defined by flow cytometry or immunocytochemistry using markers that distinguish precursor cells (TdT^+) from mature B cells (sIg^+) and T-lineage cells ($CD3^+$) can be used to establish diagnoses of small B cell lymphoma (SBL), small T-cell lymphoma (STL), precursor T-cell lymphoblastic lymphoma (pre-T LL), precursor B-cell lymphoblastic lymphoma (pre-B LL), and Burkitt type tumors. Classic Burkitt lymphoma (BL) is characterized by deregulation of MYC by translocation or transgenesis along with characteristic cytology and phenotype. Cytologically and phenotypically similar but smaller cells without MYC deregulation comprise Burkitt-like tumors (BLL).

Histologic and Phenotypic Characterizations of Mouse Lymphomas

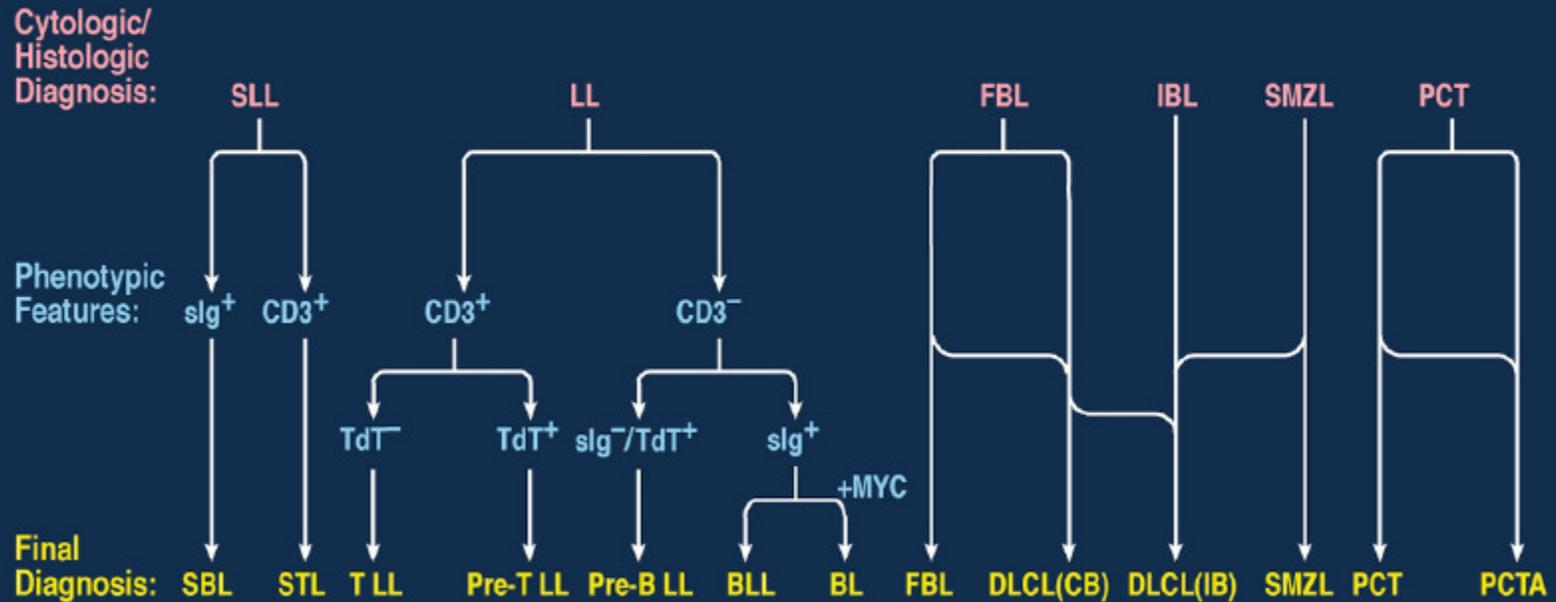


Figure 1