# EXHIBIT 100

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# Finding Mouse Models of Human Lymphomas and Leukemia's using The Jackson Laboratory Mouse Tumor Biology Database

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## Abstract

Many mouse models have been created to study hematopoietic cancer types. There are over thirty hematopoietic tumor types and subtypes, both human and mouse, with various origins, characteristics and clinical prognoses. Determining the specific type of hematopoietic lesion produced in a mouse model and identifying mouse models that correspond to the human subtypes of these lesions has been a continuing challenge for the scientific community. The Mouse Tumor Biology Database (MTB; http://tumor.informatics.jax.org) is designed to facilitate use of mouse models of human cancer by providing detailed histopathologic and molecular information on lymphoma subtypes, including expertly annotated, on line, whole slide scans, and providing a repository for storing information on and querying these data for specific lymphoma models.

### Keywords

lymphoma; leukemia; mice; Mouse tumor biology database; digital slides; virtual slides

## INTRODUCTION

One of the most important issues in determining the validity of an animal model of human disease is characterizing the condition/lesions associated with that model and identifying the human tumor type these lesions correspond to. Determining the validity of a mouse model of a human disease (i.e., model credentialing) is dependent on properly identifying a tumor type and comparing its characteristics, physical, molecular, immunohistochemical or therapeutic response to the equivalent human tumor type (Nishijo et al., 2009; Olive and Tuveson, 2006; Williams et al., 2008). Tumor subtypes in mice can have very similar

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molecular, histopathological, and immunohistochemical characteristics making identification a complex issue (Ward, 2006; Ward et al., 2012; Ward et al., 1999). Hematopoietic tumors are common types of cancer in humans. Non-Hodgkin Lymphoma and Leukemia's are predicted by the National Cancer Institute to be the 7$^{th}$ and 11$^{th}$ most common tumors diagnosed in 2015; 71,850 and 54,270 (14,620 chronic lymphocytic leukemia (CLL), 6,250 acute lymphocytic leukemia (ALL)) instances respectively, and producing the 6$^{th}$ and 5$^{th}$ most deaths; 19,790 and 24,450 (4,650 CLL, 1,420 ALL) (American Cancer Society, 2015). Hematopoietic lesions, such as lymphomas and leukemias, are a prime example of how difficult this categorization can be. Human lymphoma subtypes cited in scientific literature include Burkitt, Hodgkin, mucosal associated lymphoid tissue (MALT), anaplastic, centroblastic, diffuse large B cell (DLBCL), follicular, histiocytic, immunoblastic, lymphoblastic, lymphocytic, myeloid, non-Hodgkin, plasmablastic, and plasmacytic. Differentiating between these human lymphoma types and subtypes and their relation to similar mouse diseases has been debated for many years (Morse et al., 2002; Morse et al., 2010). For example, the relationship of mouse and human splenic marginal zone lymphomas (MZL) is still unclear as the cells of origin of the human lesion are in question (Morse et al., 2001; Morse et al., 2003). Systematic classification of mouse hematopoietic lesions was first described in 1954 (Dunn, 1954). Subsequent classification systems relied on comparison to established human systems (Fredrickson et al., 1995; Pattengale and Taylor, 1983). Researchers have published a systematic approach to classifying mouse hematopoietic lesions, both non-lymphoid and lymphoid, but detailed, annotated examples of how to apply these methods are not readily available (Kogan et al., 2002; Morse et al., 2002).

Mouse models of human cancers are important tools used in studying human tumors and are the most commonly used model systems. Mouse models are especially valuable due to their ease of use, large number of established models, availability of extensive genomic information, and well developed genetic tools to modify their genome (Carmona-Mora et al., 2009; Chaible et al., 2010; Walrath et al., 2010). The most common mouse lymphomas, B-cell lymphomas, have been compared to human tumors by pathological features and immunohistochemical staining and many "are felt to exhibit enough parallels to suggest they represent the same disease but in different species." (Morse et al., 2003; Morse et al., 2010; Ward, 2006). Mouse models of acute myeloid leukemia (AML) generated with transplanted transgenic fetal liver cells have been shown to mirror clinical responses of human AML cases with similar genetic lesions (Zuber et al., 2009). Mice also are very similar to humans in physiology and genetics. The usefulness of these models, however, is limited by the inbred nature of most models and differences between lesion characteristics in mice and humans (McGonigle and Ruggeri, 2014; Ruggeri et al., 2014). The Mouse Tumor Biology Database (MTB) is a publicly available electronic database of information on mouse models of human tumors (Bult et al., 2015). There are a large number of mouse models that have been developed to investigate the spectrum of lymphomas observed utilizing many different mouse strains and tumor induction mechanisms (Morse et al., 2003; Morse et al., 2010). MTB allows researchers to query these models and has constructed a Mouse Models of Lymphoma resource page that will assist researchers in identifying and characterizing the over 4,000 mouse lymphoma models contained in MTB.

## Material and Methods

MTB has constructed a detailed resource page to provide researchers with comprehensive information on how to categorize mouse lymphoma and leukemia models with links to annotated examples. This resource was generated from information provided by JMW and HCM. Data contained in this resource include photomicrographs of routine histopathology, special stains, and, immunohistochemistry with a variety of markers on various mouse lymphoma types and subtypes. Each image is accompanied by detailed annotation. The photomicrographs are linked to whole-slide scan images of the stained slides. The annotations include information on histologic features, immunohistochemistry labeling patterns using multiple diagnostic protein markers, and diagnostic implications of marker staining. MTB also contains information on antibody methods and results under specific conditions in mice and links to the primary information on the antibodies. Antibodies to CD3, CD45R, CD68, and F4/80 were used to label lymphomas included on the Lymphoma Resource page (Rehg et al., 2012).

Lymphomas and leukemias are represented as digital slides with their background information originated from mice on studies with an approved IACUC protocol. Mice were maintained at The Jackson Laboratory or at NCI and NIAID, NIH according to NIH guidelines. Lymphomas and leukemias represented as image files and background information were from four strains of mice obtained from The Jackson Laboratory.

MTB was first made available for the public in 1998 (Begley et al., 2014; Bult et al., 1999; Krupke et al., 2008). MTB provides a publicly available database for the scientific community that collects and integrates information on mouse tumors and provides query forms to identify and analyze specific mouse models. MTB incorporates information on the frequency/incidence, latency, and tissue of origin of mouse tumors and metastases, with all data linked to the original reference. MTB also includes pathology reports, and associated images, background genetics, somatic mutations, and links to tumor-related Comparative Genome Hybridization (CGH), Quantitative Trait Loci (QTL), and gene expression array data from the Gene Expression Omnibus (GEO), and the Array Express (Das and Tan, 2013; Edgar et al., 2002; Hunter and Crawford, 2008; Rustici et al., 2013). MTB enables integrated searches of data from diverse sources through the use of controlled vocabularies and standardized nomenclature. MTB is also the access point for information from The Jackson Laboratory's Patient Derived Xenograft (PDX) Resource (Shultz et al., 2014).

## Results/ Discussion

MTB facilitates user's access to information on mouse models of human lymphomas in two ways, previously established query mechanisms and access through the Lymphoma Resource page. MTB's web interfaces enable a researcher to query for hematologic and other tumor related data in a very comprehensive manner. Queries can be very general for any tumor, more specific for lymphohematopoeitic tumors or very specific for any of the many subtypes (leukemias, lymphomas, etc.), or by tissue of origin (erythrocyte, granulocyte, lymphocyte, etc.). For example, to search for data on lymphoblastic lymphoma models with attached pathology images, users would select the "Advanced Search Form"

listed on the left side of the MTB home page. Researchers can use pull-down menus to select lymphoma-lymphoblastic as tumor type and leukocyte from the organ of origin pull-down and click on the box labeled “Restrict search to entries with associated pathology images”. This search returns a “Results Summary” page listing 10 “Tumor Instances” and shows relevant data, such as strain background and tumor incidence, for all the search results. The “Summary” links on the right open a detailed summary of the selected tumor and associated metastases with links to References, Pathology Records, and any additional notes.

MTB contains over 6,300 lymphoma and leukemia records from over 4000 different models covering over thirty lymphoma sub-types and 283 images (Table 1). The listings of lymphomas in Table 1 are the original diagnoses provided by the pathologist or scientist contributing them and may not agree with current established criteria. With today’s classification (Morse et al., 2002; Rehg et al., [Epub ahead of print]), they may be classified differently. The new Lymphoma Resource page allows the user the ability to compare the data for a specific mouse lymphoma model to the histopathology and immunohistochemical labeling patterns listed to help determine the exact lymphoma sub-type. The Lymphoma page presents a consolidated list of all lymphomas/leukemias annotated with whole slide scan data along with references for comparing mouse and human lymphomas. The 26 digital slide cases are diagnosed by the Morse et al. criteria (Morse et al., 2002). This Lymphoma Resource page is enhanced by MTB providing links to whole-slide scans of lymphoma and leukemia examples with comprehensive annotations attached. Figure 1 shows an example of a pathology record for an annotated lymphobastic lymphoma from the lymphoma page that can be used to compare to other lymphoblastic lymphoma models to help assess model validity. The link to the Mouse Lymphoma Resource page is in the menu bar on the left of all MTB web-pages (http://tumor.informatics.jax.org).

The Lymphoma Resource page provides the user with a summary page that provides access to 26 different types of lymphomas, each with detailed annotations from one of the co-authors (JMW) describing the histopathologic patterns and immunohistochemical labeling that can help to identify the lymphoma subtype of a mouse model. The lymphoma page also includes a table listing implications of labeling for the listed antigens/proteins. Case number, strain, diagnosis, antibody details and specific references are included for each lymphoma record. The MTB Immunohistochemistry page includes details for all antibodies used and many others with links to specific web-pages from the provider. References also provide immunohistochemical methods.

## Conclusions

MTB has constructed a lymphoma resource page containing information on mouse models of human lymphomas, query mechanisms for locating lymphoma models, and methodology for systematically classifying them. This resource assists researchers in identifying mouse models of human lymphomas and evaluating the validity (credentialing) of a specific mouse lymphoma model based on consistent pathological and immunohistochemical criteria to identify tumor types. MTB provides a valuable source of high quality, curated information on mouse lymphoma models and a wide-ranging spectrum of additional mouse tumor

models. MTB also provides a venue for direct electronic submission of data from individual scientists and inclusion of data from other database such as images from the Jackson Aging Center and Pathbase (Schofield et al., 2004; Schofield et al., 2010; Sundberg et al., in press). Finally, MTB continues to provide community access to comprehensive data from mouse tumor models and now makes available information to systematically classify lymphomas occurring in mouse models.

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## References

American Cancer Society. American Cancer Society: Cancer Facts and Figures 2015. American Cancer Society; Atlanta, Ga: 2015.

Begley DA, et al. Identifying mouse models for skin cancer using the Mouse Tumor Biology Database. Exp Dermatol. 2014; 23:761–3. [PubMed: 25040013]

Bult CJ, et al. Mouse Tumor Biology (MTB): a database of mouse models for human cancer. Nucleic Acids Res. 2015; 43:D818–24. [PubMed: 25332399]

Bult CJ, et al. Electronic access to mouse tumor data: the Mouse Tumor Biology Database (MTB) project. Nucleic Acids Res. 1999; 27:99–105. [PubMed: 9847151]

Carmona-Mora P, et al. Mouse models of genomic syndromes as tools for understanding the basis of complex traits: an example with the smith-magenis and the potocki-lupski syndromes. Curr Genomics. 2009; 10:259–68. [PubMed: 19949547]

Chaible LM, et al. Genetically modified animals for use in research and biotechnology. Genet Mol Res. 2010; 9:1469–82. [PubMed: 20677136]

Das K, Tan P. Molecular cytogenetics: recent developments and applications in cancer. Clin Genet. 2013; 84:315–25. [PubMed: 23829296]

Dunn TB. Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J Natl Cancer Inst. 1954; 14:1281–433. [PubMed: 13233863]

Edgar R, et al. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002; 30:207–10. [PubMed: 11752295]

Fredrickson TN, et al. Classification of mouse lymphomas. Curr Top Microbiol Immunol. 1995; 194:109–16. [PubMed: 7895485]

Hunter KW, Crawford NP. The future of mouse QTL mapping to diagnose disease in mice in the age of whole-genome association studies. Annu Rev Genet. 2008; 42:131–41. [PubMed: 18759635]

Kogan SC, et al. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. Blood. 2002; 100:238–45. [PubMed: 12070033]

Krupke DM, et al. The Mouse Tumor Biology database. Nat Rev Cancer. 2008; 8:459–65. [PubMed: 18432250]

McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. Biochem Pharmacol. 2014; 87:162–71. [PubMed: 23954708]

Morse HC 3rd, et al. Bethesda proposals for classification of lymphoid neoplasms in mice. Blood. 2002; 100:246–58. [PubMed: 12070034]

Morse HC 3rd, et al. Cells of the marginal zone--origins, function and neoplasia. Leuk Res. 2001; 25:169–78. [PubMed: 11166833]

Morse HC 3rd, 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]

Morse, HCI., et al. Mouse models of human B lymphoid neoplasms. In: Magrath, I., et al., editors. The Lymphoid Neoplasms. Hodder Arnold; London: 2010. p. 281-292.

Nishijo K, et al. Credentialing a preclinical mouse model of alveolar rhabdomyosarcoma. Cancer Res. 2009; 69:2902–11. [PubMed: 19339268]

Olive KP, Tuveson DA. The use of targeted mouse models for preclinical testing of novel cancer therapeutics. Clin Cancer Res. 2006; 12:5277–87. [PubMed: 17000660]

Pattengale PK, Taylor CR. Experimental models of lymphoproliferative disease. The mouse as a model for human non-Hodgkin's lymphomas and related leukemias. Am J Pathol. 1983; 113:237–65. [PubMed: 6605691]

Rehg JE, et al. The utility of immunohistochemistry for the identification of hematopoietic and lymphoid cells in normal tissues and interpretation of proliferative and inflammatory lesions of mice and rats. Toxicol Pathol. 2012; 40:345–74. [PubMed: 22434870]

Rehg JE, et al. Immunophenotype of spontaneous hematolymphoid tumors occurring in young and aging female CD-1 mice. Toxicol Pathol. 2015 Jul 28. pii: 0192623315587922. [Epub ahead of print].

Ruggeri BA, et al. Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. Biochem Pharmacol. 2014; 87:150–61. [PubMed: 23817077]

Rustici G, et al. ArrayExpress update--trends in database growth and links to data analysis tools. Nucleic Acids Res. 2013; 41:D987–90. [PubMed: 23193272]

Schofield PN, et al. Pathbase: a database of mutant mouse pathology. Nucleic Acids Res. 2004; 32:D512–5. [PubMed: 14681470]

Schofield PN, et al. Pathbase and the MPATH ontology. Community resources for mouse histopathology. Vet Pathol. 2010; 47:1016–20. [PubMed: 20587689]

Shultz LD, et al. Human cancer growth and therapy in immunodeficient mouse models. Mouse Models of Cancer: A Laboratory Manual. Cold Spring Harbor Press Protocols. 2014

Sundberg JP, et al. Strain specific diseases in aging inbred mice. Vet Pathol. in press.

Walrath JC, et al. Genetically engineered mouse models in cancer research. Adv Cancer Res. 2010; 106:113–64. [PubMed: 20399958]

Ward JM. Lymphomas and leukemias in mice. Exp Toxicol Pathol. 2006; 57:377–81. [PubMed: 16713211]

Ward JM, et al. Differentiation of rodent immune and hematopoietic system reactive lesions from neoplasias. Toxicol Pathol. 2012; 40:425–34. [PubMed: 22215512]

Ward JM, et al. Splenic marginal zone B-cell and thymic T-cell lymphomas in p53-deficient mice. Lab Invest. 1999; 79:3–14. [PubMed: 9952106]

Williams PD, et al. Molecular credentialing of rodent bladder carcinogenesis models. Neoplasia. 2008; 10:838–46. [PubMed: 18670642]

Zuber J, et al. Mouse models of human AML accurately predict chemotherapy response. Genes Dev. 2009; 23:877–89. [PubMed: 19339691]

- The Mouse Tumor Biology (MTB)Database curates detailed information on mouse lymphomas.
- MTB provides query mechanisms to access lymphoma and other tumor type data.
- MTB enhances these data by providing 51 Whole-slide scans of 23 lymphomas.
- MTB provides detailed histopathological and immunohistochemical slide annotations.
- New resource to assist investigators at properly defining mouse lymphoma type.

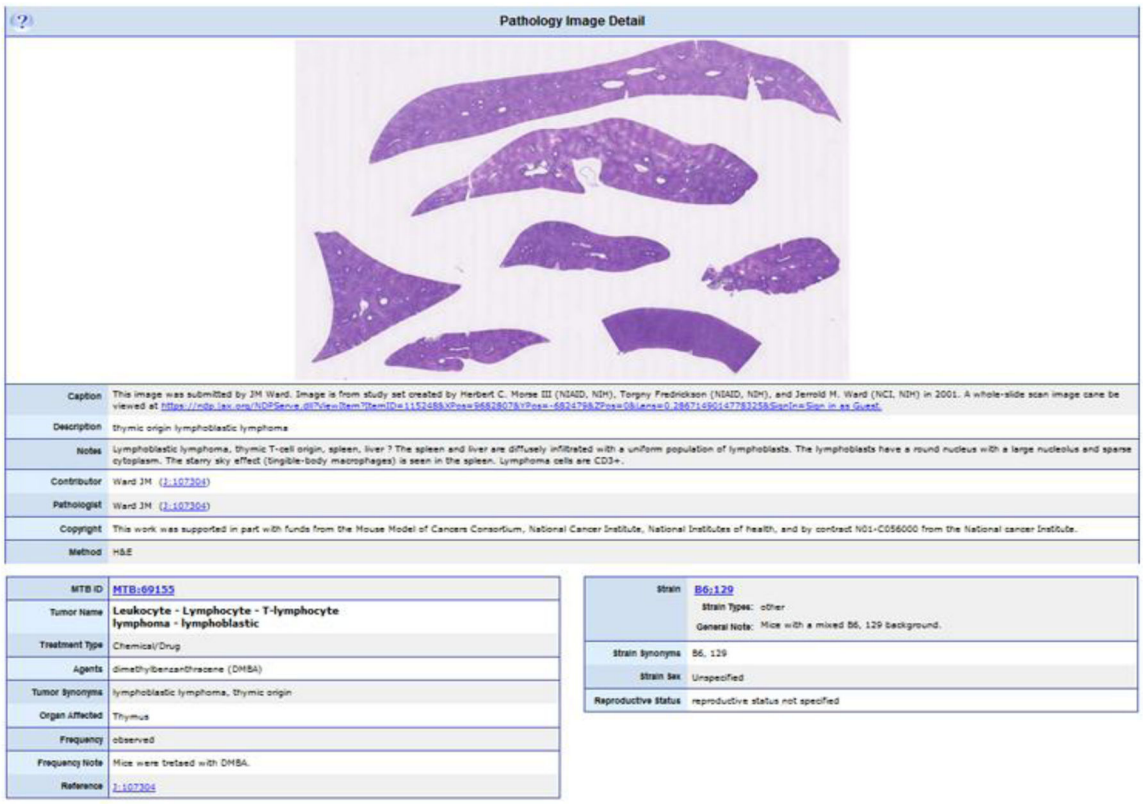


**Figure 1.**

Whole- Slide Scan image from MTB. This is a Pathology Image Detail for a lymphobastic lymphoma. The Link in the caption connects to a whole slide scan of the image.

**Table 1**

Lymphoma subtypes in MTB. A partial list of the types of lymphomas and leukemias in MTB with the number of Tumor Records and Pathology Images for each type. Tumor types with * have annotated whole slide scans.

| Lymphoma/Leukemia Type | Number of Tumor Frequencies | Number of Images |
|---|---|---|
| Lymphoma (All subtypes)* | 4868 | 282 |
| Lymphoma-diffuse large B-cell (DLBCL)* | 117 | 3 |
| Lymphoma-follicular* | 75 | 20 |
| Lymphoma-histiocytic or histiocytic sarcoma* | 11 | 2 |
| Lymphoma-immunoblastic* | 13 | 1 |
| Lymphoma-lymphoblastic* | 266 | 18 |
| Lymphoma-lymphocytic | 74 | 8 |
| Lymphoma-Not Otherwise Specified (NOS) | 97 | 27 |
| Lymphoma-mixed | 22 | 5 |
| Lymphoma-plasmacytoid | 6 | 5 |
| Lymphoma-plasma cell* | 5 | 1 |
| Leukemia* | 1311 | 5 |
| Leukemia-myeloid* | 163 | 4 |
| Leukemia-erythroid* | 35 | 1 |