

01 **Chapter 8**  
02 **Mouse Models of Human Mature B-Cell**  
03 **and Plasma Cell Neoplasms**  
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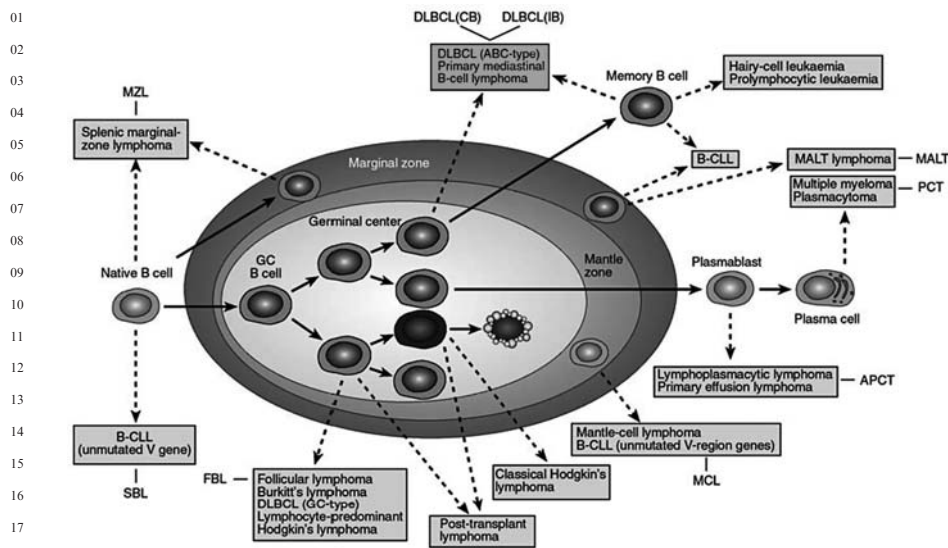
11 **8.1 Introduction**

12  
 13 Developing mouse models that accurately reflect features of human B-cell lineage  
 14 neoplasms has been a daunting but increasingly rewarding task. Studies of  
 15 spontaneous tumors or those induced by chemicals, irradiation, or retroviruses  
 16 performed before the 1980s provided remarkable insights into mechanisms and  
 17 genetics of lymphomagenesis. With the advent of genetic engineering, it became  
 18 possible to rapidly develop and explore new models and to enhance the value  
 19 of established systems. Here, we will review past and present accomplishments in  
 20 modeling mature human B-cell lymphomas and plasmas cell neoplasms (PCN)  
 21 in mice, examine their strengths and limitations, and discuss obstacles that  
 22 must be addressed in future work. These systems have accelerated our ability to  
 23 understand the development of complex disease in vivo and to develop novel  
 24 therapeutic approaches to diseases, many of which are almost uniformly lethal.

26 AQ1

25 The cellular origins of human mature B cell and PCN defined by the consensus  
 26 WHO classification (Jaffe, Harris et al. 2001) are presented diagrammatically  
 27 in Fig. 8.1. The lymphomas reflect various features of normal pregerminal center  
 28 (pre-GC) and post-GC cells including anatomic location, expression patterns  
 29 of differentiation markers, and mutational status of immunoglobulin gene vari-  
 30 able region (IgV) sequences. Recently, further distinctions have been made based  
 31 on the results of microarray gene expression profiling of normal B cells and  
 32 lymphomas.

33 Most types of human B-cell lymphoma are derived from GC or post-GC  
 34 B cells. A number of these are recognized as having close parallels among  
 35 mouse B-lineage tumors classified according to the Bethesda proposals (Morse,  
 36 Anver et al. 2002) including follicular B-cell lymphoma (FBL) and diffuse  
 37 large B-cell lymphomas (DLBCL) as well as plasmacytomas (PCT) (Fig. 8.1).  
 38 Human lymphomas of pre-GC origin, including a subset of chronic lymphocytic  
 39 leukemia (CLL), termed small B-cell lymphoma (SBL) in mice and splenic  
 40 marginal zone lymphoma (SMZL), also have parallels among spontaneous  
 41 and induced tumors of mice. To date, there are no reports of mouse neoplasms  
 42 with significant similarities to human Hodgkin, Burkitt, primary effusion or post-  
 43 transplant lymphomas or to hairy cell or prolymphocytic leukemias. There  
 44 are reports, however, of mouse models of marginal zone lymphomas (MZL) of  
 45



**Fig. 8.1 Cellular origins of human and mouse mature B-cell lymphomas and plasma cell neoplasms.** After undergoing early maturation in the bone marrow, naïve transitional B cells populate peripheral lymphoid tissues where they are recruited into the follicular and marginal zone B-cell subsets. Following interaction with antigen and helper T cells, follicular B cells establish germinal centers. There they undergo rapid clonal expansion and somatic hypermutation in the dark zone and move to the light zone where they undergo class switch recombination and positive selection on antigen-bearing follicular dendritic cells. Selected cells exit the germinal center to form memory B cells or to become plasma cells. Reciprocal chromosomal translocations involving *IgH* or *IgL* gene and a proto-oncogene, characteristic of most non-Hodgkin lymphomas (NHL), are thought to be generated as unfortunate by-products of the several mechanisms involved in *Ig* gene diversification that involves the generation of double-stranded DNA breaks. Some, such as *IgH*-*BCL2* in follicular lymphoma, appear to occur as a consequence of aberrant  $V_HDJ_H$  recombination. Others, located adjacent to rearranged, somatically mutated  $V_HDJ_H$  genes, such as *IgH*-*BCL6* in diffuse large B-cell lymphoma, most likely occur as a consequence of somatic hypermutation. A third group features breakpoint in *IgH* switch regions and appears to reflect aberrant class switch recombination, as in a subset of Burkitt lymphoma. The classes of human lymphomas and the mouse neoplasms most closely related when known are indicated. [Modified from Kuppers (2005).]

mucosa-associated lymphoid tissue (MALT) and mantle cell lymphomas (MCL) as well as lymphoplasmacytic lymphoma, termed anaplastic PCT (APCT) in mice. Systems that help define, explore, and extend these parallels are the subject of this chapter.

An ongoing challenge for developing accurate mouse models of human NHL and PCN is the need to reproduce recurrent somatic mutations in the appropriate B cell to target—pre-GC, GC, or MZ B cell or extrafollicular plasmablast, for example—will be difficult as long as the precise nature and differentiation stage of the human tumor precursors remains unknown. Additional challenges for transgenic (TG) mouse models of NHL

01 and PCN relate to the recapitulation of tumor progression pathways, mechan-  
02 isms of stromal interactions, and responses to drugs used in human patients.  
03 Although mouse models of NHL and PCN might well be expected to pheno-  
04 copy the human tumors, engineering the signaling pathways and responses is  
05 much more difficult than inducing rapid onset neoplasms such as those using  
06 retroviral transfer of classic oncogenes, which have remarkable value in their  
07 own right (Hu, Swerdlow et al. 2006).

08 A variety of TG techniques are now available to address the challenges men-  
09 tioned above. It is possible to introduce into the mouse germ line gain-of-function  
10 mutations such as constitutively expressed oncogenes under control of B cell  
11 or plasma cell-specific enhancers and promoters. Conversely, normal mouse  
12 germ line genes can be replaced with loss-of-function alleles, which may take the  
13 form of classic null alleles (“knock-out” mice) or, of increasing importance, weak-  
14 efficiency alleles that retain some gene activity and thus more closely mimic alleles  
15 segregating in the human population. A more sophisticated method involves  
16 inducible transgenes, which provide for the temporal and spatial control of gene  
17 expression or gene attenuation, thereby circumventing potential developmental  
18 problems including embryonic toxicity and lethality that may be posed by con-  
19 stitutively expressed TG. Adenoviruses (Strair, Sheay et al. 2002) and retroviruses  
20 (Mikkers and Berns 2003) offer additional means for spatial and temporal gene  
21 regulation. A lesser known but very attractive example of the latter strategy takes  
22 advantage of cell type-specific retroviral binding to specific receptors of the  
23 subgroup A avian leucosis virus, affording introduction of several cancer genes  
24 into mouse target cells (Du, Podsypanina et al. 2006). Lentivirus-mediated trans-  
25 genesis (Aronoff and Petersen 2006) represents yet another alternative to conven-  
26 tional technologies for generating the panel of TG mouse strains that may be  
27 required to accurately model human plasma cell tumors.

28 Mouse models of NHL and PCN should not only phenocopy their human  
29 counterparts at the histopathologic and molecular level but also exhibit desir-  
30 able features including high tumor penetrance, short latencies, and predictable  
31 stages of tumor progression. Additional expectations, such as means for mon-  
32 itoring the tumor burden in live animals in a repeatable and reproducible  
33 manner, need also be considered.

34 Barriers to using TG mouse models of human mature B-cell lymphomas and  
35 PCN more widely are several and include the following:

- 36  
37 1. *Problems related to establishment and maintenance of mouse colony.* Differ-  
38 ences in genetic background of mice require costly and time-consuming  
39 backcrosses of individual TG from donor strains to desired recipient strains.  
40 In addition, differences in the microbial floras of mouse colonies frequently  
41 results in quarantine and monitoring of microbiological features and health  
42 status frequently resulting in rederivation of strains under specific pathogen-  
43 free (SPF) conditions in the recipient institution.
- 44 2. *Logistical problems.* There are limited financial and space resources for scaling  
45 up mouse breeding, husbandry, and genotyping to perform statistically robust

01 time course analyses of tumor development and drug testing. In addition,  
02 there are intellectual property issues that seriously affect time, cost, and  
03 mechanisms to negotiate and acquire mouse strains. The complexities of  
04 multiparty agreements and differing priorities of academic and commercial  
05 outfits lend an additional layer of difficulties. Finally, we are faced with  
06 problems related to data acquisition and analysis that include, as one example,  
07 a paucity of methods for high-throughput biomedical imaging and high-  
08 throughput microscopy.

## 11 8.2 Chronic Lymphocytic Leukemia/Small Lymphocytic 12 Lymphoma

### 13 8.2.1 *CLL/Small Lymphocytic Lymphoma in Humans*

14  
15  
16  
17 CLL and SLL are clonal, accumulative diseases of monomorphic, small, round,  
18 slowly proliferating CD5<sup>+</sup> B cells in peripheral blood, bone marrow, and lymph  
19 nodes admixed with smaller numbers of prolymphocytes and para-immunoblasts.  
20 Lymph nodes exhibit a pseudofollicular pattern. The term small lymphocytic  
21 lymphoma (SLL) is reserved for non-leukemic cases with similar tissue involve-  
22 ment and cellular phenotype. SLL is not considered to be the precursor to CLL.  
23 Rather, tissue involvement is thought to be almost always secondary to an  
24 established leukemia. Transformation to clonally related high-grade lymphoma,  
25 usually DLBCL, termed Richter syndrome, occurs in about 5% of cases (Jaffe,  
26 Harris et al. 2001). Interestingly, CLL may be preceded by a condition, tentatively  
27 designated benign monoclonal lymphocytosis (Victor Hoffbrand and Hamblin  
28 2007), that may bear a similar relationship to CLL as monoclonal gammopathy of  
29 undetermined significance (MGUS) does to multiple myeloma (MM) (Kyle,  
30 Therneau et al. 2002).

31 Abnormal karyotypes are found by FISH in ~80% of cases with deletions  
32 of 13q14 (50%) and 12q trisomy (20%) being among the most common; translo-  
33 cation t(14q32) occurs in 5% of cases (Dohner, Stilgenbauer et al. 2000). The  
34 13q14 deletion encompasses the micro-RNA genes, miR-15a, and miR-16-1  
35 (miR15/16) (Calin, Sevignani et al. 2004). Familial aggregations of CLL are not  
36 uncommon. CLL/SLL is usually considered to be incurable with current therapy  
37 with an overall 5-year survival rate of around 50%. Molecular and phenotypic  
38 characterizations, however, have allowed the definition of subsets with clinically  
39 indolent and aggressive forms of the disease (Table 8.1). These are based on  
40 studies of IgV regions, cytogenetics, cell surface phenotype, and gene expression.  
41 Of particular interest is the observation that cases with mutated IgV genes and  
42 particular biases in V gene family utilization, such as V1-69, have a much more  
43 favorable course than cases with unmutated IgV genes and biased utilization of a  
44 separate set of V regions, such as V4-34 (Chiorazzi and Ferrarini 2003). This  
45 suggests that that the ability of BCRs to bind specific antigens might drive

**Table 8.1** Features of indolent and aggressive chronic lymphocytic leukemia (CLL)

Course of disease			
Variable	Indolent	Aggressive	Ref.
IgV regions	Mutated	Not mutated	Chiorazzi and Ferrarini (2003)
IgV preference	V1–69	V4–34	Chiorazzi and Ferrarini (2003)
Chr 11, Chr 17 aberrations	Less common	More common	Dohner, Stilgenbauer et al. (2000)
Chr 13q14 deletion	More common	Less common	Dohner, Stilgenbauer et al. (2000)
CD38 expression	Low	High	Damle, Wasil et al. (1999)
BCL2 expression	Low	High	Faderl, Keating et al. (2002)
ZAP70 expression	Low	High	Crespo, Bosch et al. (2003)
Serum thymidine kinase	Low	High	Hallek, Langenmayer et al. (1999)
Serum CD23	Low	High	Sarfati, Chevret et al. (1996)

expressing cells from a state of normality to the leukemic state. Studies of antigenic specificity have shown these BCRs to be poly- and autoreactive.

## 8.2.2 Mouse Models of CLL/SLL

### 8.2.2.1 Spontaneous Small B-Cell Lymphoma/Leukemia in Mice

Old mice of some strains spontaneously develop a clonal, mature B-cell disease termed SBL with many features reminiscent of CLL in humans (Fredrickson and Harris 2000; Hartley, Chattopadhyay et al. 2000). A predominant population of small lymphocytes with low mitotic activity associated with prolymphocytes and proliferation centers originates in the spleen. Advanced cases may exhibit involvement of lymph nodes, liver, and kidneys. A leukemic phase occurs in about 25% of cases and conversion to high-grade immunoblastic (IBL) lymphoma (Richter syndrome) is sometimes seen (Fredrickson and Harris 2000; Morse, Anver et al. 2002). The few cases that were phenotyped by flow cytometry were CD5<sup>lo</sup>. There is no information on V<sub>H</sub>DJ<sub>H</sub> repertoire for these mice.

### 8.2.2.2 Models of CLL Based on Studies of New Zealand Mice

The first associations relating CD5 expression on B cells, neoplasia and NZB mice, were made in 1981 with the finding that cultured B-lymphoma lines, including several from (BALB/c × NZB)F1 mice, expressed low levels of “Lyt-1” (Lanier, Warner et al. 1981). Later studies demonstrated that a subset of normal spleen cells expressed CD5 with spleens of NZB and (NZB × NZW)F1 mice having the highest frequencies (Manohar, Brown et al. 1982; Hayakawa, Hardy et al. 1983). CD5<sup>+</sup> B cells, now termed B1a cells, were later found to comprise a high proportion of peritoneal B cells and to share a distinctive IgM<sup>hi</sup>CD11b<sup>+</sup>

01 phenotype with another subset of functionally similar peritoneal B cells, now  
02 designated B1b B cells. B1b cells are not found in spleen, and splenic B1a cells  
03 do not express CD11b. Both subsets of peritoneal B1 cells derive from fetal/  
04 neonatal progenitors that are distinct from the B-cell progenitors in adult bone  
05 marrow that give rise to follicular and MZ B2 cells (Hardy 2006).

06 In spite of their differing origins, B1 cells share many features with MZ B  
07 cells including the ability to act as front-line responders to invading pathogens  
08 from the gut or circulating in the blood (Martin and Kearney 2000, 2001). The  
09 repertoires of both B1 and MZ B cells are also enriched for poly- or autoreactive  
10 antibody specificities. Importantly, the splenomegaly of aging NZB was shown  
11 in some cases to be the result of MZ enlargement, and NZB is the strain in which  
12 splenic MZL was first described (Yumoto 1980).

13 CLL cells were first shown to express CD5 in 1980 (Boumsell, Coppin et al.  
14 1980). The fact that NZB mice were known to have high levels of normal splenic  
15 CD5<sup>+</sup> B cells prompted studies of the possible relationship of these cells to NZB  
16 lymphomas (East 1970). Soon, several laboratories reported the identification  
17 of clonal populations of CD5<sup>+</sup> B cells in aging NZB mice and showed that they  
18 were readily transplanted. In some mice, these lymphomas were associated with  
19 leukemic phases. This suggested that the CD5<sup>+</sup> lymphoma/leukemias of NZB  
20 mice might serve as a model for human CLL (Okada, Takiura et al. 1991;  
21 Phillips, Mehta et al. 1992; Stall, Farinas et al. 1988).

22 The clonal B-cell populations were found to develop in a characteristic  
23 fashion, first appearing in the peritoneal cavity in mice 2–3 months of age;  
24 these peritoneal populations were usually oligoclonal. This was followed by  
25 successive spread to the spleen at 3–5 months, peripheral blood at 5–7 months,  
26 lymph nodes at 7–10 months, and bone marrow at 10–13 months (Stall, Farinas  
27 et al. 1988). The clones identified in spleens or lymph nodes were most often  
28 present among the several clones found in the peritoneum of the same animal.  
29 A similar pattern was seen in and NZW and (NZB × NZW)F1 mice (Stall,  
30 Farinas et al. 1988). Studies of congenic NZB and NZW and F1 mice showed  
31 that animals homozygous for *H-2<sup>Z</sup>* had the highest frequencies of peritoneal  
32 CD5<sup>+</sup> B cells (Okada, Takiura et al. 1991). Other studies of NZB mice and  
33 progeny of crosses with DBA/2 demonstrated that the CD5<sup>+</sup> B cells accumulat-  
34 ing in the spleens of aging mice were hyperdiploid and readily transplantable  
35 (Raveche, Lalor et al. 1988). Interestingly, this group did not identify hyperdi-  
36 ploid cells in the peritoneum of these mice.

37 Changes in expression of the cytokines IL-5 and IL-10 were found to mark-  
38 edly affect the occurrence of CD5<sup>+</sup> lymphomas in NZB and (NZB × NZW)F1  
39 mice. Studies of NZB mice homozygous for a null mutation of IL-10 showed  
40 that expansion of CD5<sup>+</sup> B cell outside the peritoneum and the development of  
41 clonal lymphomas was markedly reduced (Czarneski, Lin et al. 2004). In addi-  
42 tion, (NZB × NZW)F1 mice overexpressing IL-5 from a transgene had greatly  
43 increased populations of CD5<sup>+</sup> B cells and were at increased risk for lymphoma  
44 development (Xiangshu Wen et al. 2004).

01 Two studies have examined the genetic basis for the lymphoma susceptibility  
02 of New Zealand mice. Analyses of backcross progeny from the crosses of (NZW  
03  $\times$  B10.NZW)F1  $\times$  B10.NZW indicated that the frequency of CD5<sup>+</sup> B cells in  
04 peripheral blood was governed by three susceptibility alleles (Hamano, Hirose  
05 et al. 1998). The first, *Bpal-1*, was closely linked to the MHC on Chr 17. This  
06 was consistent with earlier studies associating homozygosity for H-2<sup>z</sup> and  
07 CD5<sup>+</sup> B-cell frequency (Okada, Takiura et al. 1991). The second, *Bpal-2*, was  
08 located toward the centromeric end of Chr 13, and the third, *Bpal-3*, was close  
09 to the centromere on Chr 17 but not linked to the MHC (Hamano, Hirose et al.  
10 1998). The development of leukemia in older mice was associated with elevated  
11 levels of CD5<sup>+</sup> B cells in blood at an early age. Each allele functioned independ-  
12 dently and in an incompletely dominant fashion.

13 A second study examined mice from the cross (NZB  $\times$  DBA/2)F1  $\times$  DBA/2,  
14 a low lymphoma strain (Raveche, Salerno et al. 2007). Lymphoproliferative  
15 disease occurred in 37% of the mice. Histologically, 94% of these cases were  
16 diagnosed as splenic MZL. Occurrence of disease was linked to three loci on  
17 chromosomes 14, 18, and 19. Sequence studies of Chr 14 identified a single base  
18 polymorphism 6 bp downstream of the pre-miR-16-1 sequence. This was asso-  
19 ciated with reduced expression of miR16 in NZB lymphoid cells and a NZB  
20 B-lymphoma cell line. Further studies suggested that miR-16 normally func-  
21 tions in B cells to retard cell cycle progression and promote apoptosis. These  
22 results were very suggestive of an important tie between the lymphomas of NZB  
23 mice and human CLL as the miR15/16 locus is frequently deleted in the  
24 malignant B cells of CLL.

25 The IgH V region sequences of lymphomas occurring in New Zealand mice  
26 were examined for three cases (Mahboudi, Phillips et al. 1992). All lymphomas  
27 expressed unmutated V genes and DFL16.1, a D region gene frequently used by  
28 fetal B cells. No N additions were seen. This pattern resembles that of the  
29 normal fetal/neonatal B-cell repertoire (Feeney 1990).

30 Taken together, these studies indicate that the peritoneal and splenic CD5<sup>+</sup>  
31 B-cell populations of young New Zealand mice are considerably larger than  
32 those of most other strains. Over time, the peritoneal and splenic populations  
33 exhibit oligo- or monoclonal expansions of CD5<sup>+</sup> B cells that can spill into the  
34 blood as leukemia, seeding lymph nodes and other tissue but rarely affecting the  
35 bone marrow. For mice that have clonal populations in both spleen and  
36 peritoneum, the clones are sometimes common to the peritoneal and splenic  
37 B-cell populations, while in others they may be restricted to one site or the other  
38 (Stall, Farina et al. 1988; Okada, Takiura et al. 1991). Numbers of CD5<sup>+</sup> B-cell  
39 numbers in young mice are tied to later development of lymphoma. CD5<sup>+</sup> B-  
40 cell numbers and lymphoma incidence are both under polygenic control but  
41 from genes that appear to differ between NZB and NZW. Little has been done  
42 to characterize Ig mutational state, but the few available sequences are very  
43 much like those of fetal/neonatal B cells.

44 Importantly, the lymphomas that develop in these mice have been diagnosed  
45 almost uniformly as splenic MZL. Pseudofollicles/proliferation centers,

01 prolymphocytes, and para-immunoblasts are not features of the lymphomas of  
02 New Zealand mice, marking them as histologically quite distinct from human  
03 CLL.

### 06 8.2.2.3 The TCL1 TG Mouse Model of CLL

07 The *TCL1* oncogene was initially identified at Chr 14q32.1 as the gene com-  
08 monly activated by translocations or inversions involving T-cell receptor loci  
09 (Fu, Virgilio et al. 1994). The development of T-cell lymphomas in mice with  
10 TCL1 expression driven by the *Lck* promoter in T cells established TCL1 as a  
11 true proto-oncogene (Virgilio, Lazzeri et al. 1998). TCL1 is also expressed in  
12 normal B-lineage cells from the pre-B to follicular B-cell stages and is then  
13 downregulated in GC B cells and extinguished in memory and plasma cells  
14 (Said, Hoyer et al. 2001; Virgilio, Narducci et al. 1994). High levels of expres-  
15 sion have also been seen in a variety of immature and mature B-cell lymphomas  
16 (Teitell, Damore et al. 1999; Narducci, Pescarmona et al. 2000; Teitell 2005).  
17 The oncogenic effect of TCL1 is thought to be mediated, in part, by its inter-  
18 actions with AKT, enhancing its activation and stimulating downstream path-  
19 ways that promote proliferation and survival (Teitell 2005).

20  
21 In 2002, Bichi and her collaborators described features of mice bearing an  
22 E $\mu$ -TCL1 TG (Bichi, Shinton et al. 2002) that included the development of  
23 late onset clonal CD5<sup>+</sup> lymphomas/leukemias. The general picture of this  
24 disease is remarkably similar to that described by Stall et al. for NZB mice  
25 (Stall, Farinas et al. 1988). Beginning around 2 months of age, the mice exhib-  
26 ited an expanded population of CD5<sup>+</sup>CD11b<sup>+</sup> B cells in the peritoneum that  
27 became evident in the spleen at 4-5 months and then the bone marrow at  
28 8 months. Analyses of Ig gene rearrangements in mice older than 7 months  
29 showed that the expanded populations were clonal and that clonal markers  
30 were sometimes shared between the peritoneum and spleen. All mice older than  
31 13 months developed marked splenomegaly, hepatomegaly, lymphadenopathy,  
32 and leukemia with a mean WBC of  $180 \times 10^6$ /ml.

33 The B-cell expansion in mice younger than 7 months was polyclonal with a  
34 repertoire that was like that of normal CD5<sup>+</sup> B cells, including the recurrent use  
35 of specific V<sub>H</sub>V<sub>L</sub> combinations (Bichi, Shinton et al. 2002). The V<sub>H</sub>11 sequences  
36 examined were essentially unmutated with only low levels of N-region additions,  
37 similar to V<sub>H</sub> regions that characterize normal CD5<sup>+</sup> and fetal B cells to varying  
38 extents (Feeney 1990; Li, Hayakawa et al. 1993; Kantor, Merrill et al. 1997).

39 Histologic studies of mice 8 months of age and older showed a progressive  
40 enlargement of the splenic MZ by cells cytologically indistinguishable from  
41 normal MZ B cells. The phenotype of the MZ B cells was atypical, however, as  
42 they were CD21<sup>lo</sup>CD5<sup>lo</sup> rather than the CD21<sup>hi</sup>CD5<sup>-</sup> phenotype of normal MZ  
43 B cells. In older mice, these cells extended into the red pulp in a manner similar  
44 to that seen with MZL in NFS.V<sup>+</sup> mice, but with no cytologic progression  
45 toward high-grade disease. Even cells from leukemic mice were unchanged.

01 More detailed studies of Ig utilization by the malignancies of older mice  
02 (Yan, Albesiano et al. 2006) revealed several important points: (1) sequences of  
03 the expressed  $V_H$  and  $V_L$  genes were identical to or differed minimally from  
04 germline; (2) utilization of  $V_H$  families 1, 11, and 12, D segment families, and  
05 the  $J_H$  segments,  $J_{H1}$ , diverged from that of the normal B-cell repertoire;  
06 (3) the HCDR3 and LCDR3 regions of the clones tended to be longer than  
07 for normal adult B cells and many contained two or more charged amino acids;  
08 (3) the cases exhibited stereotypic  $V_HDJ_H$  rearrangements that resembled  
09 sequences reported previously for certain autoantibodies and antibodies reac-  
10 tive with microbial antigens; and (4) cloned expressed sequences that were poly-  
11 and autoreactive and bound to a variety of polysaccharides. Almost all these  
12 features, except for reactivity with non-protein antigens, are similar to those of  
13 Ig genes from aggressive, unmutated CLL.

14 Other studies have forwarded the  $E\mu$ -TCL1 TG mouse as a tool for pre-  
15 clinical drug testing for human CLL (Johnson, Lucas et al. 2006). Leukemic  
16 mice responded initially to treatment with standard drug used in CLL, fludar-  
17 abine, but then became resistant, similar to human CLL. Heightened expression  
18 of BCL2, MCL1, PDK1, and AKT1 in the transformed lymphocytes suggested  
19 that other potential drug targets were available. The importance of AKT  
20 signaling in this model was examined in studies of the effects of rapamycin,  
21 an mTOR inhibitor, on the survival of mice transplanted with expanded  $CD5^+$   
22 B-cell populations from the TG mice. Treated mice began to die significantly  
23 later than untreated animals although all animals in both groups were dead by  
24  $\sim 200$  days after transplantation (Zanesi, Aqeilan et al. 2006).

#### 25 26 **8.2.2.4 The BCL2/TRAF Model of CLL—the NF $\kappa$ B Connection**

28 CLL and other NHL are characterized by overexpression of BCL2, which con-  
29 tributes to an apoptosis-resistant phenotype. TRAF1 is also overexpressed in a  
30 spectrum of NHL and in CLL is associated with aggressive disease (Zapata,  
31 Krajewska et al. 2000). TG mice overexpressing BCL2 or a dominant negative  
32 form of TRAF2 (TRAF2DN), which mimics the signaling features of TRAF1,  
33 develop expanded populations of B cells with occasional BCL2 TG mice devel-  
34 oping long-latency, low-grade lymphomas (Strasser, Harris et al. 1993). Mice  
35 doubly TG for BCL2 and TRAF2DN were found to die between 6 and 18 months  
36 of age with marked splenomegaly, lymphadenopathy infiltration of non-lymphoid  
37 tissues, and in many cases, ascites and pleural effusions (Zapata, Krajewska et al.  
38 2004). Leukemias WBC counts around  $150 \times 10^6/\text{ml}$  were common. The mice  
39 were diagnosed histologically as having SBL with a leukemic phase, and analyses  
40 of Ig gene organization showed the disease to be clonal. The tumor cells were  
41  $IgM^{hi}CD23^+CD21^{lo}/-CD5^{lo}$ , consistent with an origin from B1a cells (Zapata,  
42 Krajewska et al. 2004). In addition, the cells were slowly proliferative and exhib-  
43 ited increased resistance to apoptosis due to effects of both BCL2 and  
44 TRAF2DN. These combined features were felt to be indicative of a CLL-like  
45 disease.

01 The use of double TG in preclinical drug studies was recently described  
02 (Kress, Martinez-Garcia et al. 2007). First, cells from double TG mice and  
03 humans with CLL were compared for their responses to treatment in vitro with  
04 synthetic triterpenoid derivatives. Both cell types were susceptible to induction  
05 of apoptosis. In addition, tests of the same drugs in double TG mice with  
06 leukemia resulted in marked decreases in WBC counts and reduced tissue  
07 burdens. These results were thought to support the testing of the drugs in  
08 patients with CLL.

09 A fascinating story that relates to that of the double TG mice comes from  
10 studies of mice bearing a mutant *NFkB2* gene (Zhang, Wang et al. 2007).  
11 Genomic alterations that result in truncation and constitutive activation of  
12 NFkB2 occur in a variety of human B- and T-cell malignancies (Zhang ,  
13 Lombardi et al. 1994). TG mice developed marked splenomegaly, lymphadeno-  
14 pathology, and infiltration of non-lymphoid tissues and died with clonal B cell  
15 as well as some T-cell lymphomas between 5 and 18 months of age. The tumors  
16 were diagnosed histologically as SBL, but expression of CD5 was not evaluated  
17 and a leukemic phase was not described. The cells were non-proliferative and  
18 exhibited increased resistance to apoptosis. Interestingly, premalignant B cells  
19 and lymphoma exhibited significantly increased levels of TRAF1 and to a lesser  
20 extent TRAF2. TRAF1 was shown to be a direct transcriptional target of  
21 mutant NFkB2. Remarkably, TRAF1-deficient TG mice did not develop lym-  
22 phoproliferation or lymphomas. These findings, together with the studies of the  
23 double TG mice, suggest a common pathway to development of SBL in mice.

#### 24 25 **8.2.2.5 APRIL and CLL?**

26  
27 APRIL (a proliferation inducing TNF ligand), also known as TNFSF13, is a  
28 secreted member of the TNF superfamily expressed by normal T cells, neutro-  
29 phils, and dendritic cells as well as by a variety of cancers. APRIL binds to two  
30 receptors, BCMA and TACI, on the surface of B-lineage cells and is known to  
31 influence plasma cell survival, Ig switching, and the function of B1 B cells  
32 (Cancro 2004; Schneider 2005). Previous studies of human NHL showed that  
33 APRIL was expressed in association with high-grade DLBCL and Burkitt  
34 lymphoma but not with low-grade NHL including mantle cell and MZ lym-  
35 phoma or CLL (Schwaller, Schneider et al. 2007). In contrast, Planelles, Car-  
36 valho-Pinto et al. (2004) found APRIL transcripts to be associated with nearly  
37 50% of the CLL cases tested and demonstrated elevated levels of APRIL in  
38 serum from these patients. To investigate a role for APRIL in CLL, they  
39 generated TG mice with APRIL expressed from T cells.

40 Studies of APRIL TG mice older than 9 months revealed an expanded popula-  
41 tion of peritoneal B1a cells associated with enlargement of the mesenteric LN and  
42 Peyer's patches in about 40% of mice and splenomegaly and extralymphoid  
43 spread in fewer. The cells were non-proliferative but exhibited increased resis-  
44 tance to apoptosis. Comparisons with non-TG mice suggested that APRIL  
45 accelerates the expansion of peritoneal B1a cells seen in NZB and other strains.

01 Unfortunately, no studies were done to evaluate clonality, the mice were not  
02 identified as being leukemic, and none were said to have died of their disease.

#### 04 **8.2.2.6 Conclusions Regarding Mouse Models of CLL**

06 As noted above, SBL, an uncommon spontaneous disease of old mice has many  
07 cytologic and histologic features in common with CLL. For mice suggested as  
08 models for CLL, some cases with histologic features of SBL are among those  
09 seen in *BCL2/TRAF2DN* mice (Zapata, Krajewska et al. 2004) but other  
10 lymphoma classes also develop in these mice, and the occurrence of ascites  
11 and pleural effusions is unusual. The description of the disease of mice carrying  
12 a mutant NFkB TG is like that of SBL, but the cells were not phenotyped or  
13 shown to be clonal. The diseases of NZB and *TCL1* TG mice are histologically  
14 and cytologically quite distinct from SBL.

15 Many features of the malignant B-cell diseases of New Zealand mice and  
16 *TCL1* TG mice are remarkably similar. They originate among oligoclonal  
17 populations of B1a cells in the peritoneum and progressively spread to spleen,  
18 peripheral blood, lymph nodes, and bone marrow. In the spleen, the histologic  
19 appearance is of MZL, but several features weigh against their origin from  
20 normal MZ B cells. First, the surface phenotype— $CD5^{lo}CD21^{lo/-}$ —is not that  
21 of MZ B cells (Martin, Oliver et al. 2001; Bichi, Shinton et al. 2002). Second, the  
22 “MZL” often does not show the cytologic progression to high-grade disease  
23 seen in *NFS.V<sup>+</sup>* congenic mice. Instead, the cytology of cells in spleen, nodes,  
24 and even the blood of leukemic mice may differ little from those of mice with  
25 early expansion of the MZ. Third, MZL of *NFS.V<sup>+</sup>* almost never extend  
26 beyond the spleen. Finally, the  $V_HDJ_H$  sequences of the lymphomas are closer  
27 to those of normal B1a cells than to normal MZ B cells.

28 The process of peritoneal B1a B-cell transformation and subsequent spread  
29 may be accelerated by constitutive expression of *TCL1*, of the TRAF1 mimic,  
30 *TRAF2DN*, plus *BCL2*, and possibly of the mutant NFkB2 upstream of  
31 TRAF1 since CLL features heightened expression of *TCL1*, *BCL2*, and  
32 TRAF1. In this regard, it would be of interest to cross the *TCL1* TG or mutant  
33 NFkB2 mice with NZB. The possible contributions of genetically determined  
34 changes in miR15/16 require confirmation but could relate the NZB disease to  
35 human CLL in yet another way. Caution is suggested by the understanding that  
36 NZW mice are like NZB in the development of clonal B1 populations but do  
37 not share the miR16 polymorphism with NZB.

38 The normal B-cell counterpart of the leukemic cell in CLL is not known but is  
39 clearly an issue of significant import. The candidates under consideration include  
40 resident or recirculating  $CD5^+$  mantle zone B cells, MZ B cells, and lastly, the  
41 unidentified human equivalent of mouse B1a cells. Mantle cells seem unlikely as  
42 they exhibit little auto- or polyreactivity (Herve, Xu et al. 2005) while MZ and B1a  
43 cells of mice have this as a prominent feature (Martin, Oliver et al. 2001). A  
44 rationale for choosing between these cell subsets for one most like CLL is provided  
45 by extensive studies of  $V_HDJ_H$  sequences of purified peritoneal B1 and MZ B cells

01 (Kantor, Merrill et al. 1997; Schelonka, Tanner et al. 2007). Although the conclu-  
02 sions are based solely on analyses of the  $V_H7183$  family, the CDR3s of MZ B cells  
03 were considerably shorter than for other B-cell subsets in spleen and bone marrow,  
04  $D_H$  usage was biased toward DFL3, and  $J_H$  usage toward  $J_H2$ , N-region additions  
05 were fewer than for other B-cell subsets, and they had an increased proportion  
06 with charged amino acids. In contrast, B1a sequences revealed repertoires biased  
07 toward  $V_H1$ ,  $V_H11$  and  $V_H12$ , DSP  $D_H$ , and  $J_H1$  with fewer N-region additions  
08 than B1b or conventional B cells. In addition, CDR3 lengths were similar or  
09 slightly greater than those for B1b and B2 cells. These observations support a  
10 derivation of TCL1 lymphoma/leukemia from peritoneal B1a cells with the great-  
11 est discrepancy being the near-germline sequences of the clonal TCL1 TG popula-  
12 tions. Human CLL might well derive from a parallel population of B1a cells.  
13 Efforts to develop an accelerated model of the NZB or TCL1 TG-based diseases  
14 would provide a superior preclinical model of CLL.  
15  
16

## 17 8.3 Marginal Zone Lymphomas

### 18 8.3.1 MZL in Humans

19  
20  
21  
22 There are three general categories of MZL in humans: extranodal MZL of  
23 MALT lymphoma, nodal MZL, and splenic MZL (Jaffe, Harris et al. 2001).  
24 MALT and nodal MZL are characterized by accumulations of a heterogeneous  
25 population of small B cells infiltrating the marginal zones of reactive B-cell  
26 follicles. These centrocyte-like cells can be associated with occasional centro-  
27 blasts and immunoblasts, and plasmacytoid differentiation is seen in some  
28 cases. In MALT lymphomas, the malignant cells characteristically infiltrate  
29 the epithelium-forming lymphoepithelial structures. Nodal MZL morphologi-  
30 cally resembles lymph node infiltration by MALT but there is no evidence of  
31 extranodal involvement. Patients with MALT often have a history of autoim-  
32 mune conditions, including Hashimoto's thyroiditis and Sjogren's syndrome,  
33 or inflammatory conditions, such as *Helicobacter pylori*-associated chronic  
34 gastritis or ocular infections with *Chlamydia psittaci*.

35 Splenic MZL is a rare disease in which small lymphocytes replace splenic white  
36 pulp GCs, infiltrate the surrounding marginal zones, and expand into the red  
37 pulp. Extension to splenic nodes and the bone marrow is common but involve-  
38 ment of peripheral nodes is not. These tumors may account for a high proportion  
39 of CD5<sup>+</sup> chronic lymphoid leukemias, sometimes featuring villous lymphocytes.

40 Two genes implicated in the development of MALT, *MALT1* and *BCL10*,  
41 were originally identified because of their involvement in recurring chromoso-  
42 mal translocations— $t(11;18)(q21;q21)$  and  $t(14;18)(q32;q21)$  for *MALT1* and  
43  $t(1;14)(p22;q32)$  for *BCL10*—that occur specifically in MALT lymphomas. In  
44 normal B cells, *MALT1* and *BCL10* associate with *CARMA1* downstream of  
45 the BCR to activate NF $\kappa$ B (Thome 2004).

### 01 **8.3.2 Mouse Models of MZL**

#### 02 **8.3.2.1 Spontaneous MZL in Mice**

03  
04 Splenic MZL is the only type of MZL that occurs spontaneously in mice  
05 (Fredrickson, Lennert et al. 1999). The disease, best characterized in NFS.V<sup>+</sup>  
06 mice more than a year of age (Hartley, Chattopadhyay et al. 2000), is a clonal  
07 disorder that initiates with expansion of the MZ by cells cytologically indis-  
08 tinguishable from normal MZ B cells and with almost no mitotic activity. Over  
09 time, these cells begin to finger into the red pulp and exhibit a more open  
10 chromatin pattern with more prominent nucleoli, and mitotic figures are  
11 more readily seen. This can progress to a high-grade lymphoma with a high  
12 mitotic index and cells cytologically indistinguishable from those of DLBCL of  
13 centroblastic (CBL) type. The lymphoma cells compress the white pulp and  
14 force out red pulp elements (Fredrickson, Lennert et al. 1999). The disease is  
15 almost always confined to the spleen but occasionally spreads to the splenic  
16 node and the liver. A leukemia phase is rare. FACS analyses have shown that  
17 the lymphomas cells are CD5<sup>lo</sup>IgM<sup>hi</sup>B220<sup>lo</sup> in the majority of cases.

18 Studies of NFS.V<sup>+</sup> MZL for somatically acquired proviral insertions of eco-  
19 tropic MuLV identified a series of common integration sites (CIS) previously  
20 identified as candidate cancer genes (Shin, Fredrickson et al. 2004). Seven new CIS  
21 unique to MZL were also found including *Gfil*, *Sox4*, and *Stat6* among others.  
22 Heightened expression of *Gfil* distinguished MZL from other classes of B-cell  
23 lymphoma and was characteristic of MZL at all stages of progression suggesting a  
24 role in disease initiation.  
25  
26

#### 27 **8.3.2.2 Mouse Model of MALT**

28 Mice infected with *Helicobacter felis* for 22 months or more developed a chronic  
29 gastritis associated with the development of lymphoid follicles, the appearance  
30 of lymphoepithelial lesions, and glandular destruction (Enno, O'Rourke et al.  
31 1995). The later development of lymphoma was shown to be antigen-dependent  
32 since the incidence and severity of disease was significantly reduced in infected  
33 mice given anti-microbial therapy (Enno, O'Rourke et al. 1998). In addition,  
34 mice immunized against *H. felis* were protected from development of lym-  
35 phoma (Sutton, O'Rourke et al. 2004). None of the lymphoid lesions were  
36 tested for clonality of the expanded B-cell populations, and upregulation of  
37 MALT or BCL10 was not described in reports of expression profiling (Mueller,  
38 O'Rourke et al. 2003).  
39  
40

#### 41 **8.3.2.3 Mouse Models of Splenic MZL**

42  
43 *Aire*-deficient mice replicate autoimmune features of patients with autoimmune  
44 polyendocrine syndrome type I, an inherited autosomal recessive disorder  
45 associated with progressive immune destruction of many tissues (Anderson,

01 Venanzi et al. 2002; Ramsey, Winqvist et al. 2002). More recent studies showed  
02 that mutant mice 15–24 months of age exhibited expansion of the MZ (Hassler,  
03 Ramsey et al. 2006). Analyses of IgH D–J rearrangements revealed an oligo-  
04 clonal pattern suggestive of early MZL. Interestingly, the cells populating the  
05 MZ were CD21<sup>lo</sup> and secreted autoantibodies on transfer, consistent with an  
06 activated phenotype. Unfortunately, expression of CD5 was not examined.  
07 These studies suggest a role for *Aire* as a tumor suppressor gene and stimulation  
08 with autoantigens as possibly contributory to development of MZL.

09 Interestingly, recent studies of mice with TG-induced expression of BCL10  
10 in B cells were found to have significantly expanded populations of splenic MZ  
11 B cells, nuclear BCL10, and constitutive activation of the canonical NFκB  
12 signaling pathway (Stephen Morris, H. Morse, unpublished observations).  
13 Mice older than 18 months have started to develop lymphomas not seen in  
14 control littermates.

#### 15 16 17 **8.3.2.4 Conclusions Regarding Mouse Models of MZL**

18 Spontaneous splenic MZL in mice has many similarities to splenic MZL in  
19 humans, but the latency for disease of greater than a year, the occurrence with  
20 other classes of B-cell lymphoma in NFS.V<sup>+</sup> mice, and the lack of demonstrable  
21 involvement of BCL10 or MALT1 make the model impractical for preclinical  
22 studies. The *Aire*-deficient mouse model of splenic MZL also suffers from  
23 long latency and low penetrance. *Helicobacter*-associated gastric lesions similar  
24 to those of gastric MALT never appears to evolve to clonal disease but appears  
25 to be useful for understanding the role of antigenic drive in early disease. The  
26 fact that there is no unequivocal evidence in mice for MZ B cells other than  
27 those in spleen may be responsible for the lack of models for nodal MZL.  
28 Mouse disorders with similarities to Sjogren's syndrome or Hashimoto's thy-  
29 roiditis exhibit B-cell infiltrates of affected tissues but have never shown pro-  
30 gression to clonal disease.  
31  
32  
33

### 34 **8.4 Mantle Cell Lymphoma**

#### 35 **8.4.1 MCL in Humans**

36  
37 Human MCL is a mature B-cell neoplasm of small to medium-sized  
38 lymphocytes with irregular/cleaved nuclear contours that resemble centrocytes.  
39 Synonyms are morphologically descriptive and include intermediate or poorly  
40 differentiated lymphocytic lymphoma-diffuse or nodular type centrocytic  
41 (mantle cell) lymphoma, and malignant lymphoma diffuses small cleaved  
42 cell type. Human MCL typically involves lymph nodes and less frequently  
43 the spleen, bone marrow, and GI tract and is an intermediate to aggressive,  
44 usually incurable lesion with large cell blastoid variants of ominous prognosis  
45

AQ5 01 (Jaffe 2001). Until recently, there were no spontaneous or genetic mouse models  
02 of MCL, precluding development of a MMHCC classification although two  
03 MCL xenotransplant models have been reported (Bryant, Pham et al. 2000;  
04 M'Kacher, Farace et al. 2003). A t(11;14)(q13;32) between *IGH* and *CYCLIN*  
05 *D* loci is the hallmark aberration of human MCL, with dysregulated expression  
06 of the cyclin CCND1 protein (Williams, Westermann et al. 1990; Williams,  
07 Swerdlow et al. 1993). However, *Eμ-cyclin D1* TG mice have usual B-cell devel-  
08 opment and fail to develop tumors (Bodrug, Warner et al. 1994), indicating that  
09 cyclin D1 dysregulation may be necessary but not sufficient for developing  
10 a mouse model of MCL. By FACS or IHC, most human MCL are  
11 IgM<sup>+</sup>IgD<sup>+/-</sup>CCND1<sup>+</sup>BCL-2<sup>+</sup>CD10<sup>-</sup>BCL6<sup>-</sup>CD23<sup>-</sup>CD43<sup>+</sup>FMC-7<sup>+</sup> (Jaffe 2001).  
12 The cell(s) of origin are unknown although most cases show unmutated *IG*  
13 genes, suggesting a naïve or extrafollicular precursor B-cell type.  
14  
15

## 16 **8.4.2 Mouse Models of MCL**

### 17 **8.4.2.1 MCL in Genetically Engineered Mice**

18  
19 Intrapерitoneal injection of the tumor promoter pristane (2,6,10,14-tetramethyl-  
20 pentadecane) for 3 months into *Eμ-CCND1* TG mice >9 months of age resulted  
21 in a diffusely infiltrative, clonal, IgM<sup>+</sup>CD5<sup>+</sup>CD20<sup>+</sup>CD23<sup>-</sup> B-cell lymphoma  
22 expressing the cyclin D1 transgene with intermediately sized, cleaved B cells  
23 reminiscent of MCL (Smith, Joshi et al. 2006). CD5 may represent an activation  
24 rather than differentiation marker in mouse B-cell tumors, and additional  
25 studies of tumor transplantability, aggression, and cell of origin are required to  
26 determine this model's resemblance to human MCL. Crossing *Eμ-IL-14α* with  
27 *Eμ-Myc* TG mice results in a disseminated, blastoid variant of MCL (MCL-BV)  
28 in almost 100% of mice by 3–4 months of age (Ford, Shen et al. 2007). Tumor cells  
29 are transplantable into SCID mice and show sIgM<sup>+</sup>CD5<sup>+</sup>CD19<sup>+</sup>CD21<sup>-</sup>CD23<sup>-</sup>  
30 by flow cytometry, with increased expression of endogenous CCND1, BCL2,  
31 ATM, RelA, and NF-κB2, and clonal Ig gene rearrangements, providing  
32 several molecular features that are observed in human MCL. However, the  
33 histology of these tumors is that of diffuse high-grade blastic B-cell lymphoma,  
34 frequently seen as a spontaneous disease in many strains of mice as well as in  
35 some genetically engineered strains (see below).  
36  
37  
38

## 39 **8.5 Follicular B-Cell Lymphoma**

### 40 **8.5.1 FBL in Humans**

41  
42 Follicular lymphoma in humans (FBL in mice) is mature B-cell lymphoma of  
43 GC origin comprised of a mixture of centrocytes and centroblasts with at least a  
44 partially follicular pattern (Jaffe, Harris et al. 2001). The cells are embedded in a  
45

01 dense network of follicular dendritic cells. The disease appears to originate in  
02 lymph nodes, but spleen bone marrow and occasional blood involvement are  
03 not uncommon. The cells are usually IgM<sup>+</sup>CD5<sup>-</sup> and, like normal GC B cells,  
04 express BCL6 and have mutated IgV region sequences. Almost all express  
05 BCL2, usually as the result of t(14;18)(q32;p21) translocations that bring the  
06 BCL2 gene under the control of IgL regulatory sequences (Tsujimoto, Finger  
07 et al. 1984; Hockenbery, Nunez et al. 1990). Morphologic transformation to  
08 aggressive DLBCL is common and is typically the cause of death. FBL makes  
09 up about 35% of adult NHL in the United States.

## 12 **8.5.2 Mouse Models of FBL**

### 14 **8.5.2.1 Spontaneous FBL in Mice**

15 FBL is a mature B-cell tumor characterized by a varying mixture of neoplastic  
16 centrocytes and centroblasts. Synonyms include follicular lymphoma, follicular  
17 center cell lymphoma mixed, CBL/centrocytic lymphoma, reticulum cell sar-  
18 coma type B, and lymphoma-pleomorphic. FBL is typically a low-grade lesion  
19 that resembles human follicular lymphoma and is the most frequent B-cell  
20 tumor of aging mice in many inbred strains, including NFS.V<sup>+</sup>, CFW, and  
21 AKXD RI strains (Morse, McCarty et al. 2003). Distinct from human follicular  
22 lymphoma, spontaneous FBL is not associated with *Bcl2* gene rearrangements  
23 (Morse, Anver et al. 2002). Splenomegaly and variable enlargements of mesen-  
24 teric lymph nodes and Peyer's patches are typically seen. Histologic examina-  
25 tion reveals white pulp expansions that appear as white nodules and coalesce  
26 with advancing disease. Centroblasts and centrocytes are the main cell types  
27 present, with small follicular B cells pushed to the periphery and the T-cell zone  
28 reduced or eliminated. Blast cells should be less than 50% to distinguish FBL  
29 from diffuse high-grade blastic B-cell lymphoma/leukemia (DLBCL or DBLL).  
30 By FACS, most cases are IgM<sup>+</sup>IgD<sup>-</sup>CD5<sup>du</sup>CD45R(B220)<sup>lo/+</sup>. By IHC, cen-  
31 troblasts and centrocytes are both IgM<sup>+</sup>CD45R(B220)<sup>+</sup>CD19<sup>+</sup>. Tumor cells  
32 are mono- or oligoclonal for Ig gene rearrangements, with the presumed cells of  
33 origin being GC centrocytes and centroblasts.

### 36 **8.5.2.2 FBL in Genetically Engineered Mice**

37 Two independent lines of *Eμ-BCL2* TG mice develop follicular hyperplasia but  
38 rarely develop FBL and then with greatly prolonged latencies (McDonnell and  
39 Korsmeyer 1991; Strasser, Harris et al. 1993). In contrast, *VavP-Bcl2* TG mice  
40 that do not succumb to autoimmune disease develop follicular hyperplasia,  
41 followed by FBL at 10–18 months of age in up to 50% of mice. The disease is  
42 characterized by PCNA-positive, class-switched neoplastic B cells containing  
43 mutated IgV region genes (Egle, Harris et al. 2004). GC expansion and lympho-  
44 magenesis depended upon concurrent expansion of BCL2 transgene-expressing  
45

01 CD4<sup>+</sup> T cells, suggesting that microenvironmental support was required for  
02 FBL development.

03 *E $\mu$ -Pim1* TG mice develop pre-T-lymphoblastic lymphoma (pre-T-LBL) at  
04 7–10 months of age. Mice unaffected by pre-T-LBL demonstrating probable  
05 FBL and multiple subtypes of DLBCL at older ages using pre-Bethesda nomen-  
06 clature criteria (van Lohuizen, Verbeek et al. 1989; Repacholi, Basten et al.  
07 1997). A construct using the flanking regulatory elements of human *MCLI*  
08 caused lymphoma in 65% of TG mice by 24 months, with about 20% of cases  
09 diagnosed as FBL under pre-Bethesda criteria (Zhou, Levy et al. 2001).

10 *E $\mu$ -B29-TCL1* TG mice, encoding the AKT co-activator TCL1 oncoprotein,  
11 develop a spectrum of mature GC and non-GC B-cell lymphomas, with rela-  
12 tively rare FBL and more common DLBCL and DBLL generation (Hoyer,  
13 French et al. 2002; Shen, Ferguson et al. 2006). Similar to *VavP-Bcl2* TG mice,  
14 *E $\mu$ -B29-TCL1* TG mice require concurrent *TCL1*-mediated CD4<sup>+</sup> T-cell  
15 expansion to transform GC B cells, because mice with only B-lineage *TCL1*  
16 transgene expression develop mainly a model of the aggressive form of B-CLL  
17 (Bichi, Shinton et al. 2002).

18 Knock-in of a human *MLL-AF4* fusion gene into the mouse *Mll* locus  
19 produces predominantly FBL with clonal sIgM<sup>+</sup>B220<sup>+</sup>Pax5<sup>+</sup>Bcl6<sup>+</sup>CD19<sup>+</sup>  
20 tumor cells arising from follicular centers following a mixed myeloid/lymphoid  
21 hyperplasia (Chen, Li et al. 2006). *MLL-AF4* tumor cells metastasized widely  
22 without a leukemic phase and are transplantable. Knock-out of the *Ing1* gene,  
23 which encodes a nuclear PHD finger-containing protein not yet associated  
24 with human lymphoid malignancies, results in 20% of mice developing lym-  
25 phoma. Tumors originate in the spleen and contain a mixed population of  
26 B220<sup>+</sup> cells that histologically resemble centroblasts and centrocytes to suggest  
27 FBL (Kichina, Zeremski et al. 2006). However, additional marker studies,  
28 evaluation of *Ig* mutation status, and clonality and transplantation studies  
29 are required to confirm this diagnosis and to exclude a robust follicular hyper-  
30 plasia instead of malignancy (Kichina, Zeremski et al. 2006).

### 33 8.5.2.3 Conclusions Regarding Mouse Models of FBL

34 Spontaneous FBL in mice and many of the disorders of TG mice models have a  
35 number of histologic and particularly cytologic features in common with the  
36 human disease. However, the true follicular pattern seen in humans is absent in  
37 all these cases including those with extensive lymph node involvement. None of  
38 the models exhibit chromosomal translocations affecting the *Bcl2* locus, and the  
39 *VavP-Bcl-2* TG is the only one with constitute *Bcl2* expression. It may be  
40 important that the major breakpoint region (MBR) in the human *BCL2* locus  
41 bears is markedly different to the same general region in the mouse locus. These  
42 differences may preclude chromosomal rearrangements in the mouse. This  
43 possibility is being tested by knocking in 2 kb around the human MBR into  
44 the mouse *Bcl2* locus.  
45

## 8.6 Diffuse Large B-Cell Lymphoma

### 8.6.1 DLBCL in Humans

DLBCL is characterized by a diffuse proliferation of large neoplastic B cells with nuclear size that exceeds that of normal histiocytes. A number of cytologic variants have been described including CBL, IBL, T-cell/histiocyte rich, plasmablastic, and anaplastic with CBL being the most common. Distinction among these variants suffers from poor interobserver reproducibility, and subsets have not been reliably tied to prognosis. Consequently, a designation simply as DLBCL is felt by pathologists to be most appropriate (Jaffe, Harris et al. 2001). The tumor cells express pan-B-cell markers and surface or cytoplasmic Ig, and about 10% are CD5<sup>+</sup>. Nuclear BCL6 is expressed in almost all cases, and IgV region genes are mutated. Gene expression profiling by one group using microarrays delineated two major subsets that are related to cell of origin as activated B-cell-like and GC B-cell-like (Alizadeh, Eisen et al. 2000). The distinctions have prognostic significance as the prognosis for patients in the GC B-cell-like subset is considerably better than that of patients with the activated B-cell type. However, array-based studies of DLBCL by another group did not reproduce these associations, defining instead three discreet subgroups designated “oxidative phosphorylation”, “B-cell receptor/proliferation,” and “host response” (Monti, Savage et al. 2005).

### 8.6.2 Mouse Models of DLBCL

#### 8.6.2.1 Spontaneous DLBCL in Mice

DLBCL is an aggressive mature B-cell malignancy that demonstrates a diffuse proliferation of tumor cells with large nuclei and distinct cytologic features. Characteristic DLBCL variants occur spontaneously in aging mice and are classified as CBL, IBL, histiocyte-associated (HA), and primary mediastinal (PM, thymic) subtypes (Morse, Anver et al. 2002). CBL, IBL, and HA variants are common in NFS.V<sup>+</sup> mice and usually arise with splenomegaly or lymphadenopathy (Hartley, Chattopadhyay et al. 2000), whereas PM shows mainly thymic enlargement and has been seen only in mice infected with a unique replication-defective retrovirus (Morse, Anver et al. 2002).

#### 8.6.2.2 Spontaneous Variants of DLBCL in Mice

##### DLBCL–CBL Variant

CBL synonyms include large cleaved follicular center cell lymphoma and CBL lymphoma. About 12% of spontaneous lymphomas in NFS.V<sup>+</sup> and 17% in CFW mice are CBL, whereas CBL was not detected in AKXD RI lymphomas

01 and is not common in other inbred strains (Morse, McCarty et al. 2003).  
02 Histologically, the splenic white pulp is greatly expanded by tumor cells with  
03 round nuclei, often with one or two prominent nucleoli, basophilic cytoplasm,  
04 and numerous mitoses. These cells are admixed with varying amounts of  
05 smaller centrocytes. A diagnosis is made when >70% of the cells are blasts.  
06 When the proportion of centrocytes to blasts ranges from 40–70% and the  
07 ratio varies in different microscopic fields, a distinction between DLBCL and  
08 FBL is difficult although CBL may more completely destroy the usual GC  
09 architecture than FBL at advanced stages. CBL frequently infiltrates the  
10 lung, liver, and kidney and, less frequently, the bone marrow. By FACS,  
11 most cases are IgM<sup>+</sup> or IgG<sup>+</sup>, B220<sup>+</sup>CD5<sup>low</sup>CD19<sup>+</sup>. By IHC, they are usually  
12 BCL6<sup>+</sup>PAX5<sup>+</sup>IRF8<sup>+</sup>PU.1<sup>+</sup>CD138<sup>-</sup>XBP1<sup>-</sup>Blimp1<sup>-</sup>. Tumors are clonal for Ig  
13 gene rearrangements, and oligonucleotide expression microarrays have shown  
14 no clear differences between follicular or diffuse CBL subtypes, suggesting that  
15 these subtypes may represent earlier and later stages of progression (Morse,  
16 unpublished results). By expression microarray analysis, CBL is readily distin-  
17 guished from the CBL form of MZL and is similar to the CBL variant of human  
18 DLBCL.

#### 20 DLBCL–IBL Variant

21  
22 IBL lymphoma is the synonym ascribed to IBL. About 8% of spontaneous  
23 lymphomas in NFS.V<sup>+</sup> and 4% in CFW mice are IBL, whereas IBL was not  
24 detected in AKXD RI lymphomas (Morse, McCarty et al. 2003). Histologi-  
25 cally, IBLs are highly aggressive and demonstrate large, round nuclei having  
26 dispersed chromatin and prominent nucleoli, abundant cytoplasm, and a high  
27 mitotic rate. A “starry sky” pattern may be seen with increased apoptotic  
28 cells. Tumor cells are clonal for Ig gene rearrangements and often admixed  
29 with centroblasts and centrocytes, which may reflect an origin from a FBL or  
30 post-GC immunoblast. By FACS, IBLs typically are sIgM<sup>low</sup>B220<sup>dull</sup> and by  
31 IHC most cases are BCL6<sup>+</sup>PAX5<sup>+</sup>IRF8<sup>+</sup>PU.1<sup>-</sup>XBP1<sup>-</sup>IRF4<sup>-</sup>Blimp1<sup>-</sup>.

#### 33 DLBCL–HA variant

34  
35 DLBCL–HA (histiocyte associated) is the acronym of HA variant. About 20%  
36 of spontaneous lymphomas in AKXD RI (and 1% in NFS.V<sup>+</sup>) strain mice are  
37 HA, and HA is not common in other frequently used inbred strains (Fredrick-  
38 son and Harris 2000; Morse, McCarty et al. 2003). Histologically, all mice show  
39 splenomegaly with a marked expansion of histiocytes (macrophages) that may  
40 obscure malignant B cells. Histiocytes may occupy the entire white pulp,  
41 obliterating the PALS and destroying the usual follicular architecture, thereby  
42 pushing B cells to the periphery. Malignant B cells usually have features of FBL  
43 or CBL although rare cases show tumor cells with features seen in MZL, SBL,  
44 or IBL. Lymphadenopathy is seen in half the cases, and HA may involve the  
45 liver early on. The pattern of tumor growth is mainly nodular rather than

01 diffuse, and this lesion resembles human histiocyte/T-cell-rich DLBCL (Jaffe  
02 2001). It may be difficult to distinguish DLBCL–HA from histiocytic sarcoma.  
03 By IHC, the histiocytes of HA are usually EMR1 (F4/80)<sup>+</sup>LGALS (Mac-2)<sup>+</sup>,  
04 whereas the malignant B cells express markers consistent with their origin and  
05 are typically Pax5<sup>+</sup>. A clonal *Ig* gene rearrangement with PAX5<sup>+</sup> cells in a  
06 histologic picture dominated by histiocytes is diagnostic, with the main differ-  
07 ential diagnosis being histiocytic sarcoma. The presumed cell of origin for the  
08 malignant B-cell component is usually a GC or post-GC B cell, whereas tissue  
09 macrophages comprise the non-malignant histiocytic component.

### 11 8.6.2.3 DLBCL in Genetically Engineered Mice

12 DLBCL may arise de novo or by aggressive transformation of FBL and  
13 possibly SMZL. Ionizing radiation causes an increased frequency of tumors  
14 with histologic features of pre-T-LBL, FBL, and DLBCL–CBL in *Eμ–Pim-1*  
15 TG mice (Repacholi, Basten et al. 1997). Targeted deletion of *Riz1*, encoding a  
16 Rb-binding zinc finger protein, results in 37% of null and 19% of heterozygous  
17 mice developing clonal, B220<sup>+</sup> B-cell lymphomas with histologic features of  
18 DLBCL–CBL by 18–22 months of age (Steele-Perkins, Fang et al. 2001).  
19 *H2-L<sup>d</sup>-Il6* TG mice develop PCT between 6 and 19 months of age, frequently  
20 with co-existing FBL or DLBCL–CBL (Kovalchuk, Kim et al. 2002). By IHC,  
21 the lymphomas are IgM<sup>+</sup>B220<sup>+</sup>CD19<sup>+</sup> and several contained t(12;15) *IgH/*  
22 *Myc* gene rearrangements.

23 *Eμ-B29–TCL1* TG mice develop clonal IgM<sup>+</sup>B220<sup>+</sup>CD5<sup>low</sup>BCL6<sup>+</sup> DLBCL,  
24 most often HA and occasionally CBL or IBL subtypes with somatically  
25 mutated *Ig* genes and widespread dissemination (Hoyer, French et al. 2002).  
26 Equally frequent DBLL and rare FBL, SMZL, and T-PLL are also formed in this  
27 model (Hoyer, French et al. 2002; Dawson, Hong et al. 2007). B-cell lymphomas  
28 were eliminated by crossing the *TCL1* TG with an *OCA-B* null mouse that fare  
29 incapable of developing GC structures (Shen, Ferguson et al. 2006).

30 Knock-out of the proapoptotic BH3-only *Bad* gene results in 20% of mice  
31 developing clonal sIgM<sup>+</sup> or sIgG<sup>+</sup>, B220<sup>+</sup>CD19<sup>+</sup>CD43<sup>–</sup>BCL6<sup>+</sup> DLBCL of  
32 unclear subtype by 18–24 months of age (Ranger, Zha et al. 2003). Knock-in of  
33 the murine *Bcl6* gene into the *IgH* locus results in increased GC formation in  
34 spleens of non-immunized mice, followed successively by a benign lymphopro-  
35 liferative disorder with expanded white pulp and then the development of  
36 DLBCL and FBL between 13 and 20 months of age (Cattoretti, Pasqualucci  
37 et al. 2005). Tumors were clonal and IgM<sup>+</sup>IgD<sup>+</sup>B220<sup>+</sup>CD43<sup>–</sup>CD138<sup>–</sup> with vari-  
38 able Mum1/IRF4 staining by IHC. They contained mutated *IgV* region genes  
39 and frequent trisomy of Chrs 13 and 15. More recent studies showed that mice  
40 bearing the *Bcl6* knock-in do not develop DLBCL when crossed onto an AID-  
41 deficient background (Pasqualucci and Dalla-Favera, unpublished observations)

42 Knock-in of the mouse *Myc* gene 5' of the *Eμ* intronic enhancer results in  
43 clonal IgM<sup>+</sup>B220<sup>+</sup>CD19<sup>+</sup>Bcl6<sup>+</sup> DLBCL of unclear subtype developing between  
44 6 and 21 months of age, along with FBL, DBLL, and PCT formation (Park, Kim  
45

**Table 8.2.** Mouse models of human germinal center (GC) tumors<sup>1</sup>

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
Transfer of human MCL	Peritoneal cavity	MCL	SCID	None	Xenotransplant of leukemic phase MCL cells	Bryant, Pham et al. (2000)
Transfer of human MCL	Lymphoid system	MCL [BV?]	NOD/SCID	None	Xenotransplant of peritoneal MCL cells	M'Kacher, Farace et al. (2003)
de novo	Peritoneal cavity, lymphoid system, metastases	MCL	C57BL/6	<i>Eμ-cyclin D1 TG</i>	Age dependent (>9-months), dependent on peritoneal inflammation (pristane)	Smith, Joshi et al. (2006)
de novo	Lymphoid system, metastases	MCL-BV	C57BL/6	<i>Eμ-IL-14α X, Eμ-Myc DTG</i>	Initial leukemia phase with blasts, lymphoma by 3-4 months in 100% DTG mice	Ford, Shen et al. (2007)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	<i>YavP-Bcl2 TG</i>	Other tumors include PCT, LBL, HS	Egle, Harris et al. (2004)
de novo	Lymphoid system, metastases	FBL, DLBCL -CBL	C57BL/6	<i>Eμ-Pim1 TG</i>	Other tumors include pre-T-LBL, HS	van Lohuizen, Verbeek et al. (1989) and Repacholi, Basten et al. (1997)
de novo	Lymphoid system, metastases	FBL, DLBCL	C57BL/6	<i>gMCL1 TG</i>		Zhou, Levy et al. (2001)

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Table 8.2. (continued)

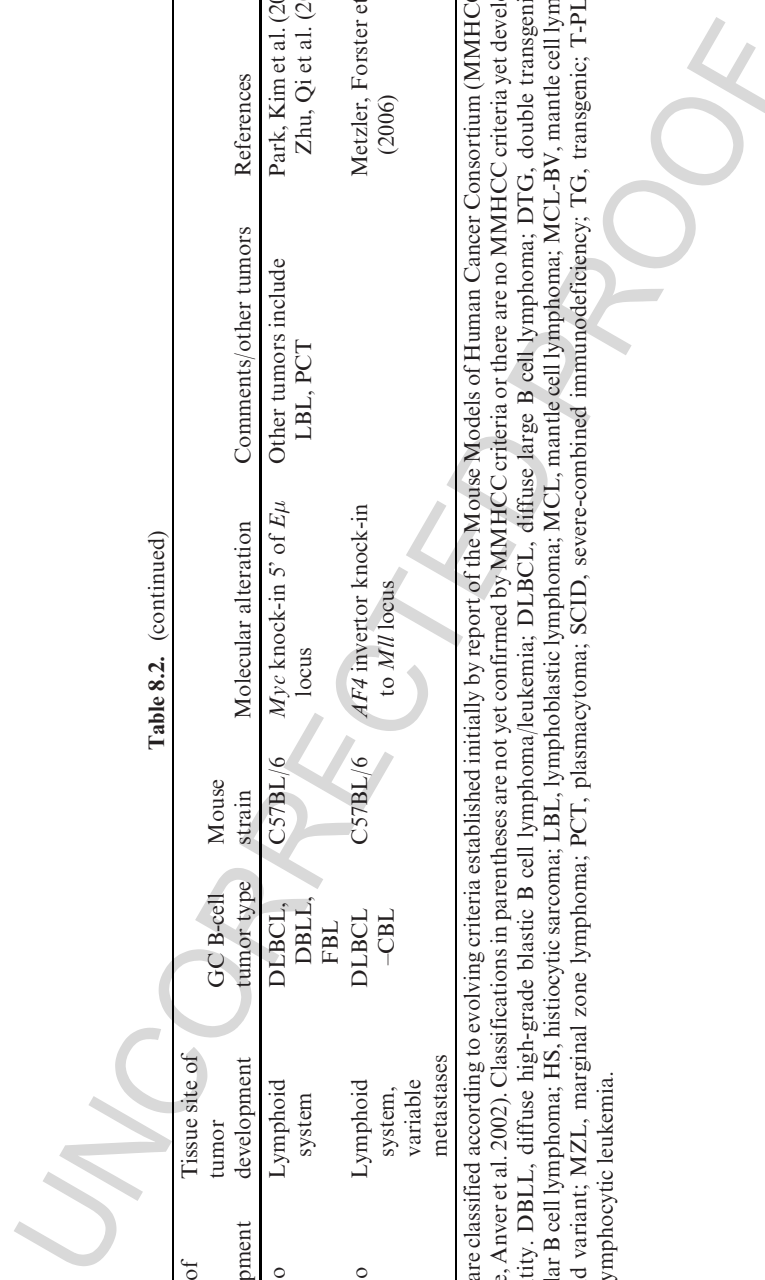
Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system, metastases	FBL, DLBCL, DBLL	C57BL/6 × C3H	<i>Erl-B29-TCL1</i> TG	Other tumors include MZL, T-PLL	Hoyer, French et al. (2002); Shen, Ferguson et al. (2006); and Dawson, Hong et al. (2007)
de novo	Lymphoid system, metastases	FBL	C57BL/6 × FVB	<i>MLL-AF4</i> knock-in	Rare erythroid or myeloid leukemia	Chen, Li et al. (2006)
de novo	Lymphoid system, metastases	FBL	C57BL/6	<i>Ing1</i> knock-out		Kichina, Zeremski et al. (2006)
de novo	Lymphoid system, metastases	DLBCL-CBL	C57BL/6 × 129Sv	<i>Riz1</i> knock-out	Diverse non-lymphoid tumors	Steele-Perkins, Fang et al. (2001)
de novo	Lymphoid system	FBL, DLBCL-CBL	BALB/c	<i>H2-L<sup>d</sup>-Ilg</i> TG	Occasional <i>IgH/Myc</i> gene rearrangements, co-existent PCT	Kovalchuk, Kim et al. (2002)
de novo	Lymphoid system, occasional metastases	DLBCL	C57BL/6 or 129/SvJ	<i>Bad</i> knock-out		Ranger, Zha et al. (2003)
de novo	Spleen, occasional lymph nodes	DLBCL, FBL	C57BL/6	<i>Bcl6</i> knock-in to <i>IgH</i> locus	Trisomy 13 and 15 common	Cattoretti, Pasqualucci et al. (2005)

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**Table 8.2.** (continued)

Mode of tumor development	Tissue site of tumor development	GC B-cell tumor type	Mouse strain	Molecular alteration	Comments/other tumors	References
de novo	Lymphoid system	DLBCL, DBLL, FBL	C57BL/6	<i>Myc</i> knock-in 5' of <i>Eμ</i> locus	Other tumors include LBL, PCT	Park, Kim et al. (2005) and Zhu, Qi et al. (2005)
de novo	Lymphoid system, variable metastases	DLBCL -CBL	C57BL/6	<i>AF4</i> invertor knock-in to <i>Mll</i> locus		Metzler, Forster et al. (2006)

<sup>1</sup>Mice are classified according to evolving criteria established initially by report of the Mouse Models of Human Cancer Consortium (MMHCC) study (Morse, Anver et al. 2002). Classifications in parentheses are not yet confirmed by MMHCC criteria or there are no MMHCC criteria yet developed for this entity. DBLL, diffuse high-grade blastoid B cell lymphoma/leukemia; DLBCL, diffuse large B cell lymphoma; DTG, double transgenic; FBL, follicular B cell lymphoma; HS, histiocytic sarcoma; LBL, lymphoblastic lymphoma; MCL, mantle cell lymphoma; MCL-BV, mantle cell lymphoma-blastoid variant; MZL, marginal zone lymphoma; PCT, plasmacytoma; SCID, severe-combined immunodeficiency; TG, transgenic; T-PLL/CLL, T-prolymphocytic leukemia.



01 et al. 2005; Zhu, Qi et al. 2005). Using an “invertor” conditional knock-in  
02 strategy to bypass embryonic lethality, a Cre-generated *Mil-*AF4** fusion gene in  
03 the endogenous *Mil* locus results in IgM<sup>+</sup>B220<sup>+</sup> CBL with clonal *Ig* gene  
04 rearrangements in 60% of cases (Metzler, Forster et al. 2006). Microarray  
05 profiling of tumor cells shows strong expression of *Pax5* and *Ebf* and variable  
06 expression of *Bcl2* and *Bcl6* differentiation markers (Metzler, Forster et al. 2006).  
07 The CBL tumor cells were transplantable into *Rag1*-deficient recipient mice.

#### 09 **8.6.2.4 Conclusions Regarding Mouse Models of DLBCL**

11 The last several years have been marked by striking progress in the generation  
12 and validation of mouse DLBCL as shown by studies of *E $\mu$ -B29-TCL1* TG  
13 and *Bcl6* knock-in mice. The lymphomas of these mice share histologic features  
14 with human DLBCL, carry mutated IgV regions, and are strikingly dependent  
15 on normally functioning GC for their development. They provide novel and  
16 important *in vivo* settings for furthering our understanding of the roles played  
17 by TCL1 and BCL6 in normal B-cell biology and lymphomagenesis.

### 21 **8.7 Diffuse High-Grade Blastic B-cell Lymphoma/Leukemia 22 (DBLL) in Mice**

24 The human equivalent or parallel to this disorder is currently not known.  
25 DBLL is a highly aggressive lymphoma of medium-sized B cells that exhibit a  
26 high mitotic rate, extensive apoptosis, and sometimes a leukemic phase. Syno-  
27 nyms include lymphoblastic lymphoma, Burkitt and Burkitt-like lymphoma,  
28 and DLBCL of lymphoblastic lymphoma subtype [DLBCL(LL)]. About 20%  
29 of spontaneous lymphomas in NFS.V<sup>+</sup> and ~30% in AKXD RI strains and  
30 CFW mice are DBLL, but DBLL is not common in other frequently used  
31 inbred strains (Morse, McCarty et al. 2003). Cases present with lymphad-  
32 enopathy, variable involvement of the spleen, and sometimes thymus, with  
33 frequent non-hematopoietic organ dissemination. Affected tissues show uni-  
34 form-appearing lymphoblasts with little cytoplasm, dispersed chromatin, and  
35 indistinct nucleoli. Histologic sections show many mitotic figures typically with  
36 large numbers of tingible body macrophages ingesting apoptotic cells, leading  
37 to a “starry sky” appearance. Infiltration of the deep cortex in lymph nodes  
38 progresses to replacement of normal cells and growth outside the capsule  
39 into the fat. When involved, the spleen shows diffuse infiltration of both the  
40 red and the white pulp. Perivascular and peribronchial infiltrates of the lungs  
41 and periportal liver infiltrates are common. Histologically and cytologically,  
42 these mature B-cell neoplasms are indistinguishable from precursor T-cell  
43 lymphoblastic lymphomas that lack thymic involvement and precursor B-cell  
44 lymphoblastic lymphomas. Analyses of *Ig* and *TCR* gene organization and  
45 IHC studies provide definitive distinctions among these disorders.

01 There is a spectrum of IHC phenotypes for DBLL ranging from patterns  
02 similar to immature or transitional B cells ( $IgM^+IgD^-C1QR1(AA4.1)^+$ ) to that  
03 of GC-experienced B cells that are *Ig* class-switched with *IgV* region mutations.  
04 DBLL are clonal for *Ig* gene rearrangements. Structural rearrangements of  
05 cellular genes, mostly due to proviral insertions, are seen from pooled studies  
06 of NFS.V<sup>+</sup> (Hartley, Chattopadhyay et al. 2000) and AKXD RI (Morse, Qi  
07 et al. 2001) lymphomas for *Zfp521* (*Evi3*) (11.9%), *Pim1* (5.6%), *Evi1* (4.8%),  
08 and *Myc* (0.8%). Lymphomas of  $\lambda$ -*MYC* were characterized by chromosomal  
09 instability and frequent biallelic deletions of *Cdkn2a* (p16) (Kovalchuk, Qi  
10 et al. 2000). Lymphomas of *E $\mu$ -Myc* TG mice had frequent changes in the  
11 p19<sup>ARF</sup>-MDM2-p53 tumor suppressor axis (Park, Kim et al. 2005). Immature  
12 or transitional B cells are the presumed cells of origin for *E $\mu$ -Myc*, *IgH/c-myc*  
13 *YAC*, *E $\mu$ IgH/c-myc YAC*, and  $\lambda$ -*MYC* TG mice. Probable GC or early post-  
14 GC B cells are the cells of origin for those with features similar to the DBLL of  
15 *E $\mu$ -B29-TCL1* TG mice.

### 18 **8.7.1 DBLL in Genetically Engineered Mice**

19  
20 An immature or transitional cell immunophenotype is characteristic of most  
21 lymphomas of *E $\mu$ -Myc*, *IgH/c-Myc YAC*, *E $\mu$ IgH/c-Myc YAC*, and  $\lambda$ -*MYC*  
22 TG mice. More mature immunophenotypes occur in spontaneous DBLL  
23 of NFS.V<sup>+</sup> mice and some lymphomas of *E $\mu$ -B29-TCL1* TG and *E $\mu$ -Myc*  
24 knock-in mice, along with many other genetically engineered mice. B-lineage  
25 lymphomas with lymphoblastic cytology but distinct from precursor  
26 B-lymphoblastic neoplasms are seen at low to high frequency in many strains  
27 of genetically engineered mice and a number of conventional inbred strains. The  
28 lymphomas of  $\lambda$ -*MYC* TG mice were originally designated Burkitt lymphoma  
29 (Morse, Anver et al. 2002), but *Ig* genes are not mutated and tumor cells have an  
30 immunophenotype of transitional or immature B cells indicating that they  
31 differ from human Burkitt cases. A change in nomenclature is clearly war-  
32 ranted. Mouse cases with similar histology and cytology occurring in mice  
33 other than the  $\lambda$ -*MYC* TGs were previously designated Burkitt-like (Morse,  
34 Anver et al. 2002). The findings that these tumors rarely have structural altera-  
35 tions in *Myc* and do not overexpress *Myc* distinguish them from human  
36 Burkitt-like lymphomas (Jaffe 2001).

## 39 **8.8 Plasma Cell Neoplasms**

### 42 **8.8.1 Human PCN Including Multiple Myeloma**

43  
44 The evaluation of present mouse models of human PCN, and attempts to devise  
45 improved models, should be guided by insights into the natural history of PCN

01 development in human beings (Mitsiades, Mitsiades et al. 2007) and the bio-  
02 logic and molecular genetic features of frank, untreated PCN at the time of  
03 clinical presentation (Carrasco, Tonon et al. 2006). In analogy to cancer devel-  
04 opment in general (Hanahan and Weinberg 2000), human PCN including MM  
05 are thought to be initiated by somatic mutations in oncogenes and/or tumor  
06 suppressor genes, followed by the stepwise accumulation of genetic and epige-  
07 netic alterations that comprise tumor progression events (Bergsagel and Kuehl  
08 2005; Kuehl and Bergsagel 2005). The later changes alter the phenotype of the  
09 incipient tumor cell as well as its interactions with the local microenvironment  
10 until fully malignant transformation has occurred. As the acronym suggests,  
11 PCN comprise a spectrum of malignancies that share the rather uniform  
12 histopathology of the aberrant, neoplastic plasma cell. However, despite their  
13 morphologic similarities, PCN demonstrate a great deal of diversity at the  
14 molecular level associated with major differences in epidemiology, clinical  
15 behavior, and treatment options.

16 The classification of PCN in the World Health Organization (WHO) nomen-  
17 clature includes the following:

- 18 1. *Plasma cell myeloma*. This is a bone marrow-based PCN, usually multifocal,  
19 thus commonly referred to as MM. MM is incurable with a survival rate of  
20 ~40% at 5 years after diagnosis. MM is defined by monoclonal Ig protein  
21 (M spike) in serum, bone destruction, hypercalcemia, and anemia. The stan-  
22 dard of care includes low- and high-dose chemotherapy, bone marrow trans-  
23 plantation, and novel drugs. Tumor variants include non-secretory myeloma  
24 (no serum monoclonal Ig), indolent myeloma, smoldering myeloma, and  
25 plasma cell leukemia.
- 26 2. *PCT*. A solitary, localized, monoclonal PCN that grows either in bone  
27 (solitary bone PCT) or soft tissue (solitary extraosseous or extramedullary  
28 PCT). PCT is rare but curable with moderate-dose radiotherapy as the  
29 preferred treatment. The most common pattern of relapse is systemic, indi-  
30 cating progression to MM.
- 31 3. *Ig deposition diseases*. Primary amyloidosis and systemic IgL chain and IgH  
32 chain deposition diseases.
- 33 4. *Osteosclerotic myeloma (POEMS syndrome)*. POEMS, defined by polyneuro-  
34 pathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin  
35 changes is very rare.
- 36 5. *Heavy chain diseases*. Distinguished according to the isotype ( $\gamma$ ,  $\mu$ , and  $\alpha$ ) of  
37 the monoclonal IgH chain produced by neoplastic plasma cells.

38  
39 Among human PCN, MM is by far the most important and well-studied  
40 disease. MM is a neoplasm of mature post-GC, Ig-secreting, isotype-switched  
41 plasma cells (Zojer, Ludwig et al. 2003) that accumulate in the bone marrow  
42 and cause bone destruction (Roodman 2006). Recent molecular and cytoge-  
43 netic studies have shown that MM is remarkably a heterogeneous disease  
44 that can be divided into a number of distinct categories based on global  
45 gene expression profiles, detection of reciprocal chromosomal translocations

01 that recombine Ig loci with oncogenes, and ploidy status of tumor cells (Zhan,  
02 Hardin et al. 2002; Shaughnessy and Barlogie 2003). Depending on the criteria  
03 applied by different laboratories, it is possible to distinguish 5–8 subcategories  
04 of MM. Regardless of how these differences are resolved, the present molecular  
05 and cytogenetic subdivision of MM is already of clinical relevance because  
06 it predicts significant differences in the prognosis and response to therapy of  
07 MM patients, no matter whether treatment relies on standard, high-dose, or  
08 novel therapies (Mulligan, Mitsiades et al. 2007).

09 No definitive cause of MM has been identified. Genetic risk factors include  
10 gender (male > female), race (the incidence in African Americans in the United  
11 States is twice that of US whites), and age (median age at diagnosis is ~70  
12 years). Familial clustering points to a hereditary predisposition consistent with  
13 an autosomal-dominant mode of inheritance, but tumor susceptibility alleles  
14 or “MM genes” have not been identified. Ionizing radiation is thought to be  
15 the strongest environmental risk factor for MM, but definitive studies have  
16 not been described. Although the evidence is not conclusive, there are reports  
17 of associations between MM and occupational exposure to various metals  
18 (nickel), chemical compounds (aromatic hydrocarbons, silicone, and petro-  
19 chemical agents), pesticides and animal viruses (farming), protracted infections  
20 that can lead to sustained B-cell activation by microbial antigens (*H. pylori*,  
21 HHV8), acquired immunodeficiency syndromes, such as HIV/AIDS, that result  
22 in reduced immune surveillance by T cells, and autoimmune diseases, such as  
23 rheumatoid arthritis.

24 The neoplastic cell in MM appears to derive from an antigen-experienced  
25 isotype-switched post-GC B-lymphocyte that has undergone somatic hypermuta-  
26 tion of the expressed *IgH* and *IgL* genes. Pathogenic factors implicated in  
27 MM include cytogenetic and molecular genetic alterations that result in the  
28 deregulated expression of oncogenes, such as *CCND1* (encoding cyclin D1),  
29 *FGFR3* (fibroblast growth factor receptor 3), and *WHSC1* (Wolf–Hirschhorn  
30 syndrome candidate 1; also known as *MMSET* or MM SET domain containing  
31 protein type III). The interaction of tumor cells with the bone marrow micro-  
32 environment is of crucial importance, as it leads to the production of cytokines  
33 including IL-6, IGF-1, VEGF, SDF-1 $\alpha$ , TNF- $\alpha$ , and TGF- $\beta$ .

34 The subgroups of MM are presently distinguished based on recurrent *IGH*  
35 translocations, ploidy status of tumor cells, and global gene expression pat-  
36 terns. Hyperdiploid tumors (40% of cases) contain 48–75 chromosomes and are  
37 characterized by multiple trisomies of Chr 3, 5, 7, 9, 11, 15, 19, and 21. Non-  
38 hyperdiploid tumors (nearly 50% of cases) carry one of seven recurrent chro-  
39 mosomal translocations that recombine *IGH* at 14q32 with seven different  
40 oncogenes. These translocations are thought to be very early if not the initiating  
41 oncogenic events and are caused by errors in Ig switch recombination or somatic  
42 hypermutation during the GC reaction. They fall into one of three groups.

- 43
- 44 1. D-type cyclins: *CCND1* (cyclin D1) at 11q13 in 15% of cases, *CCND2* (cyclin  
45 D2) at 12p13 in <15 of cases, and *CCND3* (cyclin D3) at 6p21 in 2% of cases.

- 01 2. MAF family genes: *MAF* (c-Maf) at 16q23 in 5% of cases, *MAFB* (Maf B) at  
 02 20q12 in 2% of cases, and *MAFA* (Maf A) at 8q24.3 in 1% of cases.  
 03 3. MMSET/FGFR3: *MMSET* (formally designated *WHSC1*) and *FGFR3* at  
 04 4p16 in 15% of cases.

05 Tumor progression events include chromosomal translocations that affect  
 06 the *MYC* gene at 8q24 (15% of primary MM, 45% of advanced MM, and  
 07 >90% of MM-derived cell lines) but do not involve aberrant isotype switching  
 08 or somatic hypermutation and exhibit a similar prevalence in hyperdiploid and  
 09 non-hyperdiploid tumors. They also include activating mutations of *NRAS*,  
 10 *KRAS*, or *FGFR3*; amplifications of 1q; deletions of 13q and p53; constitutive  
 11 activation of NFκB—e.g., via inactivation of TRAF3, constitutive overexpres-  
 12 sion of NIK (NFκB inducing kinase), or activation of NFκB2—perturbation  
 13 of the RB pathway—e.g., via methylation of the p16<sup>INK4a</sup> promoter—and  
 14 deletion of p18<sup>INK4c</sup>.

15 MM is preceded in a sizable fraction of cases by a premalignant disorder that  
 16 is characterized by the abnormal persistence, sometimes for decades, of a clone  
 17 of Ig-producing plasma cells that are lodged in the bone marrow without  
 18 causing loss osteoporosis or osteolytic lesions. This disorder is referred to as  
 19 AQ7 MGUS (Rajkumar, Lacy et al. 2007). MGUS is defined by a monoclonal serum  
 20 Ig of <30 g/l, the presence of 10% or fewer plasma cells in the bone marrow, the  
 21 absence of anemia and lytic bone lesions, and the absence of hypercalcemia and  
 22 renal insufficiency related to the clonal plasma cell proliferation. The preva-  
 23 lence of MGUS in elderly patients is ~5%. The progression from MGUS to  
 24 MM occurs at a slow but remarkably steady rate of 1% per year. The etiology of  
 25 MGUS is not known, but epidemiologic evidence points to age, gender, and  
 26 race as risk factors. The pathogenesis of MGUS is poorly understood. Approxi-  
 27 mately 50% of MGUS cell clones carry chromosomal translocations that  
 28 rearrange *IGH* at 14q32 with oncogenes on one of five partner chromosomes  
 29 also identified in MM: *CCND1*, *CCND3*, *FGFR3* and *MMSET*, *MAF*, and  
 30 *MAFB*. These translocations are thought to play an important role in the  
 31 initiation of MM. Approximately 40% of the plasma cell clones in MGUS  
 32 smoldering MM and frank MM are hyperdiploid. This consistency suggests  
 33 that hyperdiploid MM originates from hyperdiploid MGUS. Likewise, dele-  
 34 tions of Chr 13q, which have an adverse prognostic association in MM, are  
 35 found in similar frequencies in MGUS and MM, indicating a direct precursor–  
 36 product relationship of 13q<sup>−</sup> preneoplastic and neoplastic states. Empirical  
 37 observations of this kind suggest that MGUS occurs as distinct molecular  
 38 subtypes, which lead, in turn, to different forms of MM.

39 Evidence suggests that MGUS and MM develop along one of two distinct  
 40 pathways that result in either non-hyperdiploid tumors that usually carry one  
 41 of the seven recurrent *IGH* translocations or hyperdiploid tumors that usually  
 42 are not associated with *IGH* translocations. Despite enormous progress in the  
 43 past decade in our understanding of MM pathogenesis, many important ques-  
 44 tions remain. What are the molecular and microenvironmental mechanisms  
 45

01 that drive the transition from MGUS to MM? How does hyperdiploidy con-  
02 tribute to plasma cell transformation? What genetic lesions underlie recurrent  
03 cytogenetic changes, such as gain of chromosome 1q or loss of chromosome  
04 13q? Mouse models of human PCN may help to provide answers to these and  
05 other important questions.

06  
07  
08 **8.8.2 Established and Newly Emerging Mouse Models**  
09 **of Human PCN**  
10

11 The presently available mouse models of human PCN can be divided into de  
12 novo and transplantation models. Although none of the genetically engineered  
13 strains recapitulate all features of a particular human PCN, several strains have  
14 emerged as useful platforms for mechanistic and therapeutic studies of altera-  
15 tions in signaling pathway found in human PCN (e.g., IL-6, Abl, and Myc).  
16 Furthermore, although the succession of oncogenic processes responsible for  
17 tumor development that occurs de novo does not completely match those in  
18 humans, the incipient tumor cells in mice interact with their microenvironment  
19 (immune cells, vascular and lymphatic networks, and extracellular matrix) in  
20 ways that mirror the interactions of neoplastic plasma cells in humans with their  
21 specific tissue microenvironment. Similarly, mouse models that have been devel-  
22 oped to permit the outgrowth of fully transformed transplanted tumor cells  
23 in vivo are not suitable for studying mechanisms of tumor development. None-  
24 theless, they are highly valuable for many other purposes including preclinical  
25 drug testing. Table 8.3 shows mouse models of human PCN in chronological  
26 order of development, beginning with strains in which tumor development occurs  
27 de novo, followed by models that rely on tumor cell transfer.

28  
29 The first mouse model of de novo PCN, peritoneal PCT in strain BALB/c,  
30 was discovered 50 years ago by Dr. Michael Potter of the National Cancer  
31 Institute and has been progressively developed and refined by him over the  
32 last half century. Salient features of this model include dependency on chronic  
33 inflammation (usually induced by intraperitoneal application of pristane),  
34 genetic background (BALB/cAnPt is highly susceptible, NZB and BALB/cJ  
35 are weakly susceptible, and all other tested strains including DBA/2, C57BL/6,  
36 CBA/J, C3H, and 129 are solidly resistant), maintenance of mice in an antigen-  
37 rich conventional facility (SPF mice are refractory to tumor development), and  
38 the acquisition of *Myc*-deregulating chromosomal translocation in early tumor  
39 precursors. The penetrance of peritoneal PCT at 65% is incomplete and the  
40 average latency of 220 days is long. However, tumor development can be greatly  
41 accelerated by infection of mice with retrovirus expressing any of the series  
42 of oncogenes alone or in combination—Abelson-*(v-abl)*, RIM (*v-Ha-Ras* and  
43  $E\mu$ -*c-Myc*), J3V1 (*v-Raf1* and *v-myc*), and ABL-MYC (*v-abl* and human *MYC*).

44 Unfortunately, this model of PCN has been largely dismissed by the mye-  
45 loma community as artificial and irrelevant for human MM, mainly due to

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**Table 8.3.** Mouse models of human plasma cell neoplasms (PCN) including extraosseous plasmacytoma (PCT) and multiple myeloma (MM)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Peritoneal cavity	PCT	BALB/c	None	High impact on immunology and cancer research Dependent on peritoneal inflammation (pristane)	Potter (2003) Potter and MacCardle (1964) and Anderson and Potter (1969) Potter and Wiener (1992) and Janz (2006)
de novo	Bone marrow	MM	C57BL/Ka	None	Dependent on <i>Myc</i> translocation Accelerated by retroviruses, such as A-MuLV, RIM (c-myc + v-ras), J3V1 (v-myc + v-raf), and ABL-MYC	Potter, Sklar et al. (1973), Ohno, Migita et al. (1984), Clynes, Wax et al. (1988), Troppmair, Huleihel et al. (1988), Weissinger, Mischak et al. (1991) Radl, Croese et al. (1988), and Radl, Van Arkel et al. (1996)
de novo	Lymphoid system	PCT	BALB/c	Eμ-v-abl	Spontaneous tumors Impractical due to long latency and low incidence Source of transplantable 5T tumors (see below) No <i>Myc</i> translocations	Rosenbaum, Harris et al. (1990)
de novo	Lymphoid system, GALT	PCT	BALB/c	H2-L <sup>d</sup> -IL-6	Mice develop peritoneal tumors upon treatment with pristane Mice develop peritoneal tumors upon treatment with pristane	Kovalchuk, Kim et al. (2002)

Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
de novo	Lymphoid system	PCT	BALB/c	E $\mu$ -Bcl-2	Mice develop peritoneal tumors upon treatment with pristane	Silva, Kovalchuk et al. (2003)
de novo	Bone marrow, lymphoid system	MM > PCT	C57BL/6 and BALB/c	NPM-ALK	Transgene targeted to T lymphocytes	Chiarle, Gong et al. (2003)
de novo	Peritoneal cavity, lymphoid system, and bone marrow	PCT > MM	Mixed BALB/c	iMyc	Mice develop peritoneal tumors upon treatment with pristane	Park, Kim et al. (2005), Park, Shaffer et al. (2005), and Kim, Han et al. (2006)
de novo	Lymphoid system, bone marrow	PCT > MM	Mixed	iMyc + Bcl-X <sub>L</sub>	3'KE-Bcl-X <sub>L</sub> transgene	Cheung, Kim et al. (2004)
de novo	Lymphoid system, bone marrow	PCT > MM	FVB/N	Bcl-X <sub>L</sub>	Accelerated tumorigenesis upon crossing in the E $\mu$ /c-Myc transgene	Linden, Kirchhof et al. (2004) and Adams et al. (1985)
de novo	Bone marrow and lymphoid system	MM > PCT	C57BL/6	Bcl-X <sub>L</sub> + virus		Linden, Kirchhof et al. (2005)
de novo	Bone marrow	MM > PCT	C57BL/6	Xbp1		Carrasco, Sukhdeo et al. (2007)
de novo	Not yet reported	Not yet reported	Not yet reported	TVA/TVB	Unpublished	F. Asimakopoulos and H.E. Varmus <sup>1</sup>
de novo	Bone marrow	MM	Not yet reported	Kappa*Myc	Unpublished	M. Chesi, A.K. Stewart, and P.L. Bergsagel et al. <sup>1</sup>

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Table 8.3. (continued)

Mode of tumor development	Tissue site of tumor development	Tumor type	Mouse strain	Transgene	Comment	Reference
Transfer of mouse MM	Bone marrow	MM	C57BL/6	None	Relies on 5T tumor cells homing to mouse bone, extensively studied	Vanderkerken, Goes et al. (1996)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM cells homing to human fetal bone implanted s.c. in mice, well established	Yaccoby, Barlogie et al. (1998)
Transfer of human MM	Bone marrow	MM	SCID-hu	None	Human MM bone cores implanted i.m. in mice	Campbell, Manyak et al. (2006)
Transfer of human MM	Bone marrow	MM	NOD/SCID-hu	None	Immunodeficiency more pronounced than SCID	Pilarski, Hipperson et al. (2000) and Huang, Tien et al. (2004)
Transfer of human MM	Bone marrow	MM	NOG = NOD/SCID/gammac(null)	None	Immunodeficiency more pronounced than NOD/SCID	Miyakawa, Ohnishi et al. (2004)
Transfer of human MM	Bone marrow	MM	SCID-rab	None	Human MM cells homing to rabbit fetal bone implanted s.c. in mice	Yata and Yaccoby (2004)

<sup>1</sup>Unpublished. Presented at the XI International Workshop on Multiple Myeloma, Kos Island, Greece, June 2007. NPM, nucleophosmin; PCT, plasmacytomas; SCID, severe-combined immunodeficiency.

01 the lack of bone marrow involvement. The dismissal may be premature, as  
02 certain properties of peritoneal PCT may be of great relevance for human  
03 MM. For example, peritoneal PCT formation is profoundly inhibited by anti-  
04 inflammatory agents, such as corticosteroids, which play an important role in  
05 the standard therapy of human MM. BALB/cAnPt mice deficient in IL-6 or  
06 treated with the cyclooxygenase inhibitors, indomethacin and sulindac, are also  
07 resistant to tumor induction. This suggests an intriguing parallel to the postu-  
08 lated tumor-promoting role of chronic inflammatory processes in the patho-  
09 genesis of human MM. Just as MM is preceded by MGUS, peritoneal PCT is  
10 preceded by a well defined and easily studied preneoplastic lesion, namely foci  
11 of aberrant plasma cells that reside in the inflammatory granulomas of the  
12 peritoneum where they can persist for months. Plasma cell foci of this sort may  
13 provide a good experimental opportunity to elucidate the enigmatic transition  
14 from preneoplastic to neoplastic plasma cell growth in a genetically defined and  
15 environmentally controlled study.

16 The 5T mouse myeloma of strain C57BL/KaLwRij was developed more  
17 than a quarter century ago by Dr. Jiri Radl (Radl 1981; Radl, Croese et al.  
18 1988). In a survey of 2-year-old C57BL/Ka mice, he estimated that 0.5% of the  
19 mice developed aggressive PCN originating in the bone marrow. These tumors,  
20 designated as 5T myelomas, produced copious amounts of monoclonal Ig,  
21 were readily transplanted when injected intravenously into syngeneic mice, and,  
22 importantly, produced osteolytic lesions in recipient animals. Two serially trans-  
23 planted neoplasms, called 5T2 and 5T33, are now in common use and are widely  
24 considered as the only mouse model that accurately recapitulates key properties  
25 of human MM [reviewed in Vanderkerken, Asosingh et al. (2003)]. 5T2 and 5T33  
26 offer the unique advantage of testing new strategies for the treatment of MM in  
27 a neoplastic plasma cell that resides in the appropriate microenvironment of  
28 the bone marrow in an immunocompetent host. The 5T33 system has recently  
29 been adapted to tissue culture in presence of an adherent layer of stromal cells,  
30 further enhancing the suitability of this preclinical model system for drug testing.  
31 Another enhancement is provided by the continuous improvement of bioimaging  
32 methods that allow a more accurate evaluation of myeloma bone disease and  
33 tumor burden than was previously possible. Relevant methods include micro-CT  
34 of whole bone or bone explants and imaging techniques that detect 5T cells  
35 labeled with fluorochromes (GFP), luciferases (bioluminescence), or sodium  
36 iodide symporter (SPECT) with great sensitivity in vivo. Unlike BALB/c PCT,  
37 5T myeloma does not harbor a chromosomal *c-Myc* translocation. This defines  
38 another intriguing parallel to human MM, in which *MYC* translocations, if they  
39 occur at all, appear as so-called secondary translocations involved in tumor  
40 progression.

41 The classic model of BALB/c PCT has been refined and accelerated by the  
42 development of a number of TGs that target oncogenes to the B-cell lineage.  
43 Among these are the H2-L<sup>d</sup>-hu-IL-6, E $\mu$ -Bcl-2, iMyc, and E $\mu$ -v-abl transgenes.  
44 The Bcl2 and v-abl TG takes advantage of the intronic IgH enhancer, E $\mu$ , to  
45 enforce the expression of the target genes in B-lineage cells. PCT incidence in

01 E $\mu$ -v-abl mice approaches 100%, does not require treatment with inflam-  
02 matory agents, is independent of genetic background, and has been reported  
03 to extensively involve the bone marrow. Some E $\mu$ -v-abl mice were shown to  
04 present with only bone marrow PCT, developing hind limb paralysis as the  
05 result of tumor growth in vertebral marrow cavities. If further modification of  
06 this mouse were to succeed in reproducing this primary bone marrow manifes-  
07 tation of PCN growth more consistently, strain E $\mu$ -v-abl may evolve into a true  
08 counterpart of human MM.

09 Among many attempts to express the plasma cell growth, differentiation and  
10 survival factor, IL-6, in the B cell and other cell lineages of TG mice, the H2-L<sup>d</sup>-  
11 hu-IL-6 TG has emerged as the most promising for studying PCN. Deregulated  
12 expression of IL-6 in young TG mice causes progressive plasma cell hyperplasia  
13 in lymphoid tissues, hypergammaglobulinemia, kidney damage, and a histolo-  
14 gic picture that resembles human multicentric Castleman's disease (Kovalchuk,  
15 Kishimoto et al. 2000). The transition from plasma cell hyperplasia to neoplasia  
16 occurs in older mice, usually in enlarged lymph nodes of the gut-associated  
17 lymphoid tissue, GALT (Kovalchuk, Kim et al. 2002). Bone marrow infiltra-  
18 tion in mice bearing advanced tumors is often extensive. This mouse model may  
19 be useful for elucidating the molecular and cellular mechanisms of IL-6-driven  
20 plasma cell neoplasia and to test new treatments that target the IL-6 receptor  
21 (Yoshio-Hoshino, Adachi et al. 2007) or downstream elements of the IL-6  
22 signaling pathway (Bhutani, Pathak et al. 2007; Hausherr, Tavares et al. 2007;  
23 Loffler, Brocke-Heidrich et al. 2007).

24 The E $\mu$ SV-Bcl-2-22 TG contains a human *BCL2* cDNA driven by E $\mu$   
25 (Strasser, Whittingham et al. 1991). Transfer of the TG from PCT-resistant  
26 C57BL/6 mice onto the PCT-susceptible BALB/c genetic background resulted  
27 in a 24-fold increase in tumor incidence and a two-fold reduction in tumor  
28 latency. Similar to their IL-6 TG counterparts, *BCL2* TG PCT harbor *Myc*-  
29 deregulating T(12;15) translocations (Silva, Kovalchuk et al. 2003). Accelerated  
30 plasmacytomagenesis in strain E $\mu$ -Bcl-2 may facilitate the design and testing  
31 of *BCL2* inhibition strategies of potential relevance to *BCL2*-overexpressing  
32 human PCN, such as Waldenström's macroglobulinemia and MM (Kline,  
33 Rajkumar et al. 2007).

34 TG mice, designated iMyc, contain a His<sub>6</sub>-tagged mouse c-Myc cDNA,  
35 Myc<sub>His</sub>, inserted head-to-head into different sites of the mouse *IgH* locus in  
36 ways that mimic the Myc-activating T(12;15) translocations of BALB/c PCT.  
37 A strain carrying the iMyc TG just 5' of E $\mu$  is the most thoroughly characterized  
38 to date (Park, Kim et al. 2005). In analogy to the experience with the IL-6 and  
39 Bcl-2 TG mentioned above, the transfer of the iMyc TG onto BALB/c rendered  
40 the mice hyper-susceptible to inflammation-induced peritoneal PCT (Park,  
41 Shaffer et al. 2005; Kim, Han et al. 2006). As expected, the PCT overexpressed  
42 Myc<sub>His</sub>, produced monoclonal Ig, and exhibited a unique plasma cell signature  
43 upon gene expression profiling on mouse lymphochip.

44 A somewhat surprising observation was made in mice harboring a T-cell-  
45 targeted fusion gene joining nucleophosmin (NPM) and anaplastic lymphoma

01 kinase (ALK). Predictably, these mice developed T-cell lymphomas; however,  
02 20% developed PCN instead of T-cell lymphomas. The PCN arose in peripheral  
03 lymphoid tissues or the bone marrow. In the latter case, tumor growth resulted  
04 in peripheral neuropathy and hind leg paralysis. NPM-ALK TG mice are  
05 currently used primarily for studies on the T-cell neoplasm, anaplastic large  
06 cell lymphoma (Amin and Lai 2007). Further modification of this strain for  
07 modeling human MM has not been attempted.

08 With several newly developed TG models at their disposal, researchers began  
09 to generate double TG mice in efforts to develop robust models of human PCN.  
10 Other modifications, such as infecting TG mice with oncogenic virus or chang-  
11 ing the genetic background, were also pursued to further accelerate tumor  
12 development and/or shift the tumor pattern from B-cell lymphoma to PCN.  
13 PCN formation is dramatically accelerated in double TG mice that carry the  
14 H2-L<sup>d</sup>-hu-IL-6 TG and a Bcl-2 TG (Janz, unpublished finding), a Bcl-X<sub>L</sub> TG  
15 (Fang, Mueller et al. 1996; Potter 2003) (Janz, unpublished finding) or iMyc TG  
16 (Janz, unpublished finding). The same findings were obtained in iMyc/Bcl-2  
17 double TG mice (Janz, unpublished finding). An interesting alternative  
18 approach involved the infection of Bcl-X<sub>L</sub> TG mice with ABL-MYC virus  
19 (Linden, Kirchhof et al. 2005). This resulted in a unique model of MM that  
20 recently was acknowledged by a panel of MM experts as holding promise  
21 for the validation of new therapeutics (Dalton and Anderson 2006). Additional  
22 research is warranted to better characterize double TG and virally accelerated  
23 mouse models of human PCN before recommendations can be made as to  
24 which model may be most suitable for elucidating specific aspects of the human  
25 disease.

26 Recent studies have shown that X-box-binding protein-1 (XBP1), a differ-  
27 entiation and unfolded protein/ergoplasmatic reticulum stress response factor  
28 essential for normal plasma cell development in mice, may also be implicated in  
29 human PCN. This prompted the development of a new model of human MM  
30 that relies on enforced expression of Xbp-1s ORF in the B-cell lineage under  
31 the control of the IgV<sub>H</sub> promoter and E<sub>μ</sub> enhancer. The mice are prone to a  
32 MGUS-like disorder followed by a type of PCN with many similarities to MM.  
33 Like all other TG strains susceptible to PCN, E<sub>μ</sub>-Xbp1 TG also develop  
34 extraosseous PCN, either together with the MM-like tumors or on their own.  
35 Nonetheless, a number of features indicate that the E<sub>μ</sub>-Xbp1 mice offer an  
36 attractive model of human MM. Among other applications, they may be useful  
37 to uncover the elusive genetic changes responsible for the transition of MGUS  
38 to MM.

39 A fresh, unorthodox approach to recapitulating the natural history of MM  
40 in a relevant cellular and physiologic milieu is a mouse model system that  
41 enables the delivery of stochastic, sequential, somatic mutations to precisely  
42 defined plasma cell precursors in vivo. Asimakopoulos and Varmus used BAC  
43 TG technology to express two distinct types of avian leukosis virus (ALV)  
44 receptors, TVA and TVB, in the expanding centroblasts of the GC dark zone  
45 and the committed plasmablasts of the light zone. Mouse cells are refractory to

01 infection by retroviruses of the ALV family unless they ectopically express the  
02 cognate avian-derived receptors. To that end, TG mice were genetically developed  
03 AQ9 that express TVA driven by regulatory elements of *Mybl1* (*A-Myb*), a  
04 transcription factor expressed in dividing blasts of the GC dark zone, and TVB  
05 under control of Blimp-1, a master regulator of plasma cell differentiation. As a  
06 result, the mice express TVA in dividing follicular B cells and TVB in cells of the  
07 GC light zone, extrafollicular plasma cells, and mature plasma cells in the bone  
08 marrow. Viral vectors have been engineered to carry dominant oncogenes or  
09 various inactivators of tumor suppressor genes, permitting the introduction of  
10 sequential oncogenic lesions in putative precursors of PCN.

11 Chesi, Bergsagel, and associates reported recently on a new mouse model of  
12 MM designated VK\*MYC. This model is based on the VK\*MYC TG that  
13 contains an inactive, non-coding human *c-MYC* gene under the transcriptional  
14 control of the V $\kappa$  promoter. TG MYC is activated sporadically in GC B cells  
15 undergoing somatic hypermutation. This approach has two potential benefits.  
16 First, in contrast to all previously generated MYC TG, expression of the  
17 VK\*MYC TG occurs only at the GC stage of B-cell development. This circum-  
18 vents the unwanted transformation of less mature B-lineage cells that occurs  
19 when Myc is expressed earlier in differentiation. Second, B cells in which MYC  
20 becomes activated are likely to participate in an ongoing T-cell-dependent  
21 immune response, because they were part of a GC reaction. This further  
22 restricts the pool of MYC target cells to those that define the postulated MM  
23 precursor pool in humans. Evidence indicates that virtually all V $\kappa$ \*MYC mice  
24 develop MGUS-like disease by 50 weeks of age. Plasma cells are fully differ-  
25 entiated (CD19<sup>-</sup>CD138<sup>+</sup>), have a very low proliferation index, and are found  
26 exclusively in the bone marrow. V $\kappa$ \*MYC mice also develop anemia, bone  
27 disease with low trabecular density as well as sporadic lytic bone lesions and  
28 hind limb paralysis. Similar to the Xbp1 TG mice, 30% of the V $\kappa$ \*MYC mice  
29 exhibit extramedullary disease. Importantly, VK\*MYC mice responded to  
30 drugs known to be active against MM while demonstrating no response to  
31 drugs with little or no clinical activity. These features indicate that strain  
32 VK\*MYC will be useful in the study of MM biology and the development of  
33 new pharmacological and immunological therapies.

### 34 35 36 37 **8.8.3 Xenograft Models of Human Myeloma in Mice**

38  
39 In contrast to the models of de novo PCN formation in mice, xenograft models  
40 use MM cells or cell lines that are transplanted into SCID-Hu, NOD/SCID, or  
41 SCID-Rab mice. These systems offer the unique ability to test therapeutics in  
42 vivo against true MM (Tassone, Neri et al. 2005). The SCID-Hu mouse model  
43 that employs fetal bone permits studies on the interaction of MM cells with the  
44 microenvironment of human bone marrow. The NOD/SCID has been adapted  
45 to myeloma cells labeled with green fluorescent protein. Intravenous injection

01 of these cells creates a model in which diffuse PCN dissemination can be  
02 visualized using whole-body, real-time fluorescence imaging to reproducibly  
03 quantify tumor burden. This allows serial, noninvasive monitoring of drug  
04 treatment. The SCID-Rab model avoids the ethical concerns about the use of  
05 human fetal bone in the SCID-Hu model by using rabbit bones. This model  
06 supports the growth of MM cells in a non-myelomatous, non-human, and non-  
07 fetal microenvironment. Although xenograft models are the current work-  
08 horses in preclinical testing of efficacy and mechanism of action of novel  
09 myeloma drugs, the xenograft implants have their own severe limitations,  
10 including the lack of an intact immune system, inability to model premalignant  
11 neoplastic stages, and imperfections in recapitulating the interactions between  
12 myeloma cells and surrounding stroma.

13 The SCID-hu mouse, which was originally developed for studies on human  
14 hematopoiesis in mice (Shultz, Ishikawa et al. 2007), has been adapted by  
15 Dr. J. Epstein, Y. Yaccoby, and their associates to investigate human MM cells  
16 in their native microenvironment of the human bone marrow (Yaccoby, Barlogie  
17 et al. 1998). In this system, myeloma growth is restricted to and dependent on  
18 human bone marrow and leads to osteolytic lesions in the transplanted human  
19 bone. The SCID-hu model contributed to our understanding of myeloma biol-  
20 ogy by demonstrating that myeloma alters the balanced expression of the osteo-  
21 clast differentiation factor, RANKL/OPG, in the bone marrow (Pearse, Sordillo  
22 et al. 2001), depends on osteoclasts for growth and survival (Yaccoby, Pearse  
23 et al. 2002), relies, in part, on IL-6 to avoid programmed cell death (Yaccoby,  
24 Pearse et al. 2002), abolishes osteoblasts in the course of tumor progression  
25 (Yaccoby, Wezeman et al. 2006), and uses the serine phosphatase, fibroblast  
26 activation protein, to interact with stromal cells (Ge, Zhan et al. 2006). Import-  
27 antly, the SCID-hu model was instrumental in showing that the anti-myeloma  
28 activity is dependent on its metabolism by liver microsomes (Yaccoby, Wezeman  
29 et al. 2006).

30 Progress in research on humanized mice (Shultz, Ishikawa et al. 2007) led to  
31 additional modifications of the SCID-hu model, such as NOD/SCID-hu  
32 (Huang, Tien et al. 2004), NOG (NOD/SCID/ $\gamma_c^{\text{null}}$ ) (Miyakawa, Ohnishi  
33 et al. 2004), and SCID-rab (Yata and Yaccoby 2004), which permit the engraft-  
34 ment of human myeloma cells in endogenous human bone or implanted rabbit  
35 bone, respectively. The addition of the NOD genetic background to SCID mice  
36 enhances the immunodeficiency conferred by the SCID background, resulting  
37 in lack of B and T cells, lack of circulating complement, defective macrophage  
38 function, and low natural killer cell activity. The  $\gamma_c^{\text{null}}$  phenotype causes the  
39 complete loss of natural killer cells. Just like the original SCID-hu model, the  
40 newer models enhance our understanding of myeloma biology and are useful for  
41 evaluating novel drugs and drug candidates in a preclinical setting. The utility of  
42 the SCID-rab system to evaluate effects of antibody to DKK1, of bortezomib,  
43 and of bone anabolic agents on bone remodeling and myeloma growth illustrates  
44 this point (Yaccoby, Ling et al. 2007).  
45

### 8.8.4 Conclusions Regarding Mouse Models of Human PCN

Recent progress in the design and development of genetically engineered mouse models of human PCN has resulted in two categories of experimental models systems. In the first or de novo category, PCN arise either spontaneously or are induced in inbred or TG mice. Tumor development occurs in predictable stages and is preceded by the expansion of premalignant plasma cells resembling benign monoclonal gammopathy (BMG), MGUS, Castleman's disease, or similar non-malignant human plasma cell disorders. The mouse models in this category are indispensable for mechanistic studies of plasma cell transformation and the design and testing of strategies for tumor prevention. The greatest weakness among these models is their failure to recapitulate the bone marrow manifestations of tumor growth that are typical of MM. Two recent models offer a glimmer of hope along this line (Cheung, Kim et al. 2004; Linden, Kirchhof et al. 2005; Boylan, Gosse et al. 2007; Carrasco, Sukhdeo et al. 2007). In the second or transplantation-based category, fully transformed plasma cells of mouse origin (5T) or human origin (primary MM cells and myeloma cell lines) are transferred into syngeneic, immunocompetent mice (5T) or immunodeficient SCID mice (xenotransplant system) that frequently harbor human or rabbit bone as a nesting ground for the incoming plasma cells. The mouse models in this category have made and continue to make important scientific contributions to the preclinical assessment of myeloma therapeutics and our understanding of myeloma bone disease. Nonetheless, technical and logistic barriers have prevented these models from having a significant economic effect on the process of anti-myeloma drug discovery. They are not extensively used in preclinical trials and have not gained wide acceptance in industry. In addition, transplantation models are not useful for studies on tumor development and prevention.

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UNCORRECTED PROOF

## Chapter 8

Query No.	Page No.	Line No.	Query
AQ1	180	26	For the citation "Jaffe, Harris et al. 2001," please clarify whether the year is "2001a", "2001b", "2001c," or "2001d."
AQ2	184	9	Damle, Wasil et al. (1999), Faderl, Keating et al. (2002), Crespo, Bosch et al. (2003), Hallek, Langenmayer et al. (1999) and Sarfati, Chevret et al. (1996) are not listed in the reference list. Please provide.
AQ3	185	44	The citation "Xiangshu Wen (2004)" has been changed to "Xiangshu Wen et al. (2004)" so as to match that given in the reference list. Please clarify whether the change is OK.
AQ4	191	1	The citation "Robert L. Schelonka (2007)" has been changed to "Schelonka, Tanner et al. (2007)" so as to match that given in the reference list. Please clarify whether the change is OK.
AQ5	194	1	The citation "Jaffe (2001)" has not been listed in the reference list. Please check.
AQ6	200	Table	Please provide the citation for Table 8.2.
AQ7	207	19	In the sentence "MM is preceded in a sizable fraction of cases by a premalignant disorder that is characterized," please clarify whether the text "without causing loss osteoporosis or osteolytic lesions" is OK.
AQ8	208	09	The subsection levels "8.8.2 Modeling Human PCN Including MM in Mice: First principles" and "8.8.3 Opportunities and Barriers to Using Mice as Preclinical Models of PCN" present under the "Contents" headings seem to be missing in the text. Please advice.
AQ9	215	3	The sentence "To that end, TG mice were made that express TVA driven by regulatory elements" has been changed to "To that end, TG mice were genetically developed that express TVA driven by regulatory elements." Please clarify whether the change is OK.
AQ10	217	36	The reference "Anderson and Potter (1969)" has not been cited in the text. Please check.
AQ11	217	45	The repeated reference entry "Bichi and Shinton et al. (2002)" has been deleted. Please check.
AQ12	219	21	The repeated reference entry "Hartley, Chattopadhyay et al. (2000)" has been deleted. Please check.
AQ13	220	25	In the reference "Kuehl and Bergsagel (2005)," please provide the volume number.
AQ14	220	35	The repeated reference entry "Linden, Kirchhof et al. (2005)" has been deleted. Please check.