Experimental model systems for preclinical research on Waldenström macroglobulinemia

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ABSTRACT

Waldenström macroglobulinemia (WM) is an incurable low-grade lymphoplasmacytic lymphoma of mature IgM⁺ B-lymphocytes that warrants additional research to increase therapeutic options, enhance quality of life, and improve survival of patients with WM. Here we concluded a miniseries of short reviews on the diagnosis and treatment [1], natural history [2] and putative cell-of-origin of WM [3] with a brief survey of preclinical experimental model systems available for fundamental and translational research studies on this enigmatic neoplasm. The model systems comprise of: ① continuous tumor cell lines, three of which are well authenticated and demonstrated to be derived from the patient’s index tumor; ② human-in-mouse xenografts that rely on immunodeficient laboratory mice, adapted to carry small fragments of implanted human bone, to provide a suitable microenvironment for incoming lymphoma cells; and ③ genetically engineered mouse models (GEMMs) of neoplastic B-cell development, in which WM-like tumors arise spontaneously in the presence of fully functional innate and adaptive immune systems. Because none of the models developed thus far are perfect, additional efforts are required to achieve a better preclinical representation of disease characteristics of WM. To achieve that goal, the active involvement of basic and clinical research experts from China is called for, so novel drugs and immunotherapies for WM will reach clinics sooner, thereby ensuring the future of patients with WM will be brighter.

Keywords: IgM⁺ lymphoplasmacytic lymphoma, human-in-mouse xenografting, genetically engineered mouse model

PAST NEGLECT BUT WELCOME RECENT UPTICK IN PRECLINICAL WM MODEL DEVELOPMENT

Due to low incidence, indolent clinical course and late disease onset among a variety of other reasons, Waldenström macroglobulinemia (WM) has been woefully neglected for many years in terms of preclinical model development. Compared to more prevalent B cell and plasma cell neoplasms, such as chronic lymphocytic leukemia (CLL), diffuse large B cell lymphoma (DLBCL) and multiple myeloma (MM), few cell line-based models and genetically engineered mouse models (GEMMs) are available. The logical consequence of this neglect is the existence of major knowledge gaps in our understanding of the pathophysiology of WM. Closing these gaps warrants a collaborative international research effort of basic, translational and clinical experts from all countries including China, who share an interest in generating and validating faithful experimental model systems of human WM. The laboratory mouse, the workhorse...
of preclinical cancer research, will no-doubt play an important part to that end. Recently, there has been a welcome upswing in WM preclinical model development activities that shall be summarized shortly [4–5]. Preclinical WM research tools ready to be deployed at the time of publication include permanent cell lines for in vitro studies, cell line-based human-in-mouse xenografts for in vivo studies, and GEMMs for studies on de novo development of WM-like tumors that occur spontaneously in an intact mammalian organism harboring fully functional innate and adaptive immune systems.

WM CELL LINES AND XENOTRANSPLANTATION MODELS

Our current understanding of the biology and genetics of WM is based in no small measure on research with continuous tumor cell lines that can be readily propagated in vivo using immunodeficient host mice. In vitro cell culture is also straightforward and provides a convenient and potentially unlimited source of tumor cells growing in suspension culture under standard conditions (37°C, 5% CO₂, commonly used cell culture media). For passaging in vivo, the cells are usually inoculated ectopically in non-autochthonous fashion; i.e., tissue sites that do not correspond to the site where WM normally resides (hematopoietic bone marrow). In most cases, the cells are injected subcutaneously, where they form a clearly demarcated subcutaneous nodule. Permanent human cell lines are by far the most widely used experimental model system for fundamental and translational research on WM, which is due to their low cost, predictable growth pattern in readily available host mice, and easy technical manipulability and scalability. Cell lines that have been developed for preclinical research on WM are listed in Table 1.

<table>
<thead>
<tr>
<th>Table 1 Continuous tumor cell lines established for preclinical WM research</th>
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<tr>
<td>Cell line</td>
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<tr>
<td>RPCI-WM1[27]</td>
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<td>BCWM.2[14]</td>
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1 Cell lines are presented in chronological order of development. WM, Waldenstrom macroglobulinemia; WSU, Wayne State University, Detroit, Michigan; BC, Bing Center for WM, Dana Farber Cancer Institute, Boston, Massachusetts; RPCI, Roswell Park Cancer Institute, Buffalo, New York.
2 Original report in peer-reviewed scientific literature.
3 Infection with Epstein-Barr virus (EBV). Plus “+” and minus “−” symbols denote presence and absence of virus, respectively.
5 Analysis relied on conventional karyotyping in case of WM1 and later included molecular cytogenetic methods such as spectral karyotyping (SKY) and fluorescence in situ hybridization (FISH).
6 See main text for additional information.
7 Based on evidence available today, tumor cell lines are deemed to be representative of WM or not. One cell line, BCWM.1, is difficult to call because it is unlikely to be derived from the index tumor (discrepancy in Ig light-chain usage) yet carries WM’s hallmark mutation (MYD88[26–29]).
8 Not determined.

In 1982, Finerty et al. reported the establishment of the first WM cell line designated WM1. The investigators obtained B cells from the peripheral blood of a patient with WM at an advanced leukemic stage, and infected the cells in vitro with Epstein-Barr virus (EBV). Infection was confirmed by virtue of determining the expression of the virus-associated nuclear antigen, EBNA, and a continuous cell line was established. EBNA positivity and evidence of both restricted immunoglobulin expression and presence of marker chromosomes indicated that WM1 cells are in fact EBV transformed WM tumor cells [6]. However, because the cell line was generated by EBV infection in vitro, it should not be considered representative of WM. Additionally, the cell line was not widely distributed to independent laboratories and xenograft studies were not performed at that early juncture of preclinical WM research.
The WSU-WM-SCID model is based on a cell line developed at Wayne State University (WSU)\textsuperscript{[7,8]}, where it was used to evaluate the experimental drug XK469\textsuperscript{[9]}. The model constitutes the first attempt to passage WM in laboratory mice using the human-immunodeficiency (SCID) mice provide the nesting ground for tumor cell engraftment (Fig. 1). Later on, this approach was refined using SCID mice that harbored subcutaneous implants of human bone (SCID-hu)\textsuperscript{[10]}--or in case of the more severely immunocompromised non-obese diabetic (NOD) SCID strain-intumescent implants of human bone (NOD-SCID-hu)\textsuperscript{[11]}. In both situations, hematopoietic bone marrow contained in the bone fragments supply the proper tumor microenvironment (TME) for the growth and proliferation of incoming tumor cells and their interaction with normal bystanders, thus mimicking the tumor-TME interaction in patients with WM. However, technical and logistic barriers have prevented the WSU-WM-SCID model from having a significant impact on WM research, including drug discovery and testing. The model was neither extensively used in preclinical trials, nor did it gain acceptance by the pharmacological industry. A major factor for that is the suspected derivation of WSU-WM cells from EBV-transformed B-lymphoblasts\textsuperscript{[12]}, which in conjunction with other shortcomings, led to the conclusion that WSU-WM cells are not representative of WM\textsuperscript{[12]}. Although this outcome has questioned the validity of published results using WSU-WM cells, it has had the welcome effect of defining the level of evidence that must be achieved before a newly developed cell line should be designated representative of WM.

BCWM.1, developed at the Bing Center (BC) for WM research at the Dana Farber Cancer Institute, Boston, Massachusetts, is a cell line derived from CD19\textsuperscript{+} lymphoplasmacytic bone marrow cells obtained from a patient with newly diagnosed WM\textsuperscript{[13]}. Phenotypic surface markers detected by flow cytometry include CD19, CD20, CD23, CD38, CD138, CD40, CD52, CD70, and CD117. In contrast, CD5, CD10, and CD27 are not expressed. BCWM.1 cells contain cytoplasmic IgM \(\lambda\) and express the survival proteins, APRIL and BAFF, together with their surface receptors, TACI, BCMA, and BAFF-R. Karyotyping and FISH revealed no cytogenetic aberrations. Xenografting using SCID-hu mice is possible\textsuperscript{[13]}. BCWM.1 was used in research that yielded interesting results on tumor proteomics\textsuperscript{[14]}, interaction of CD27 and CD70\textsuperscript{[15]}, AKT signaling\textsuperscript{[16]}, targeted inhibition of NF-\kappaB(nuclear factor kappa B)\textsuperscript{[17]} and experimental treatments\textsuperscript{[18,19]} that included for the first time interleukin 1 receptor associated kinase (IRAK) inhibitors\textsuperscript{[20]}. Importantly, BCWM.1 cells harbor the MYD88\textsuperscript{L265P} hallmark mutation of WM\textsuperscript{[21]}. Nonetheless, similar to WSU-MW described above, uncertainties remained with regard to BCWM.1 being representative of the index tumor; i.e., the primary tumor from which the cell line was derived. BCWM.1 cells express the EBV-encoded latent membrane protein 1 (LMP1), a telltale sign of EBV infection and reason to suspect that BCWM.1 is in fact a lymphoblastoid cell line derived by EBV transformation of a bystander B cell in the patient’s bone marrow\textsuperscript{[12]}. Key information, such as clonotypic \(V_{\mu}\) rearrangements and mutational status of expressed Ig heavy and light chains in BCWM.1, is lacking. Furthermore, BCWM.1 does not have the deletion of the long arm of Chr6 (del 6q) that is frequently seen in WM. There is also a nagging discrepancy in light chain usage: \(\kappa\) in case of the index tumor but \(\lambda\) in case of the cell line. In sum, although BCWM.1 exhibits key biologic properties of WM including the MYD88\textsuperscript{L265P} exchange, its objective authentication as a bona fide model of the disease is relatively weak compared to more recently developed cell lines described as follows\textsuperscript{[22]}

MWCL.1 constitutes a breakthrough with regard to cell line authentication for research on WM because genetic analysis has unambiguously demonstrated derivation from primary tumor cells of the index patient with WM\textsuperscript{[23]}. MWCL.1 cells produce IgM/\(\lambda\) and exhibit an immunophenotype consistent with WM: CD19\textsuperscript{+}CD20\textsuperscript{-}CD27\textsuperscript{-}CD38\textsuperscript{-}CD138\textsuperscript{-}. Like many other continuous human cancer cell lines, MWCL-1 cells are deficient of the p53 tumor suppressor; one copy of the p53-encoding gene, \(TP53\), is deleted, whereas the second copy is mutated. Just like BCWM.1 described above, MWCL-1 harbors the mutant MYD88\textsuperscript{L265P} protein\textsuperscript{[24,25]}. Unlike BCWM.1, MWCL-1’s clonal

![Fig. 1 Xenograft models of WM. See Table 1 for details on three tumor cell lines shown to be derived from index tumors of patients with WM.](Image)
relationship to the index tumor has been confirmed based on a unique DNA fingerprint of 34 single-nucleotide polymorphisms (SNPs) that is fully shared between index tumor and cell line. $\text{V}_h$ gene usage and light chain restriction was also identical between the primary tumor and the cell line. Array comparative genomic hybridization (aCGH) demonstrated additional homologies; however, while del 6q was noted in a small subset of cells at an early stage of cell line establishment, the cytogenetic aberration was lost during subsequent passageing of cells in vitro. The circumstance that MWCL-1 cells are EBV-positive but do not shed the virus, raises the possibility that EBV was mechanistically involved at some point during malignant cell transformation, but this is difficult to prove (or reject) with certainty. There is little doubt that MWCL-1 is representative of WM, and that MWCL-1 xenografts enhance the preclinical tool box for WM research; e.g., on new targeted treatments [20].

Using MWCL-1 as blueprint, an investigator team at Roswell Park Cancer Institute (RPCI) developed another cell line, RPCI-WM1, deemed to be fully representative of WM. The line was derived from a patient with WM that required treatment [27]. The cells secrete IgM with $\kappa$-light chain restriction identical to the primary tumor. The cells are near diploid (modal chromosome number of 46) and harbor cytogenetic changes including deletion at 6q21, 9p21 (CDKN2A), 13q14 (RB1), and 18q21 (BCL2) that are shared with the index tumor. Amplification of 14q32 (IgH) is also seen shared between cell line and index tumor. The contention of clonal relationship is additionally supported by identical CDR3 length and SNPs, and by matching IgH rearrangements in cell line and index tumor. Another shared feature is the MYD88 L265P exchange, which is heterozygous in both cell line and index tumor. The finding that the cell line maintains most of the cell surface markers detected on parental tumor cells further increased the value of RPCI-WM1 for preclinical WM research [20]. RPCI-WM1 has been recently employed to demonstrate that protein phosphorylation alters Bim-mediated Mcl-1 stabilization in WM [29]. Another study found that the glutamine metabolism affords a therapeutic vulnerability in WM [30].

The most recently developed cell line thought to be fully representative of WM is BCWM.2 [31]. Similar to its counterpart, BCWM.1, it was developed at the Bing Center of the Dana Farber Cancer Institute. The cell line was established from cultured CD19-selected bone marrow cells obtained from a patient with WM. The immunophenotype of BCWM.2 is consistent with WM: CD5, CD10, CD19, CD20, sIgM, CD11c, CD38, and CD138. Whole genome sequencing (WGS) and transcriptional profiling of the cell line revealed a match with the parental tumor, in regard to trisomy 3 and 12 and deletion of the entire arm of 6q. Just like its founding cell clone, BCWM.2 carries an activating heterozygous G-to-A transition mutation in MYD88, that results in a serine-to-asparagine substitution at residue 243 of the MYD88 protein. The same mutation, MYD88S243N, has been previously described in ABC DLBCL (see following chapter for details).

Also found in BCWM.2, but not in the index tumor or in the germline of the patient, was a novel I318N mutation in LYN that is predicted to be activated. In analogy to BCWM.1 and MWCL-1, BCWM.2 cells over-express WM-associated genes including interleukin 17 receptor B (IL17RB), Cdk5 and Abl substrates 1 (CABLES1), Wnt family member 5a (WNT5A), G protein-coupled estrogen receptor 1 (GPER1) and Wnk lysine deficient protein kinase 2 (WNK2).

**GEMMS OF HUMAN WM**

The Mighty Mouse, the most dependable performer in the preclinical WM research arena, enables not only cell line-based xenografting of human WM (described above), but also lends itself to generating WM-like neoplasms that arise spontaneously in immunocompetent hosts—thus recapitulating the multiple-step developmental process that leads to WM in human beings. Among its many advantages, the laboratory mouse affords as the premier mammalian model organism for cancer research due to its close genetic relationship to man. Virtually, all genes in human beings have a homologue with comparable function in mice. Secondly, methods for manipulating the mouse genome—including gene targeting for "knocking" mouse genes "out" and human genes "in"—have been perfected. The latter permits us to "humanize" mice in such a way that human disease manifestations can be more accurately mimicked than ever before. However, the inherent challenges of modeling the complex pathophysiology of WM in laboratory mice should not be underestimated. This is underscored by the circumstance that an accurate mouse model of human WM has not yet been achieved, neither with regard to reproducing the core set of tumor-associated somatic mutations in appropriate target cells (genocopy of human WM), nor with regard to reproducing the main clinicopathological features and laboratory findings of the human disease (phenocopy of human WM). Nonetheless, there is reason to be optimistic that designer models of human WM are feasible. Models of this sort are necessary to assess new biomarkers and prevention and treatment strategies of WM [32].
Additionally, the models are poised to shed light on mechanisms of tumor progression, including the poorly defined transition from premalignancy (IgM MGUS) to frank malignancy (WM). A brief overview of selected mouse models of human WM follows below (Table 2).

Table 2 Mouse models in which WM-like tumors occur

<table>
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<tr>
<th>Mouse model</th>
<th>Key features 1</th>
<th>First report 2</th>
<th>Genetic background 3</th>
<th>Useful insight for modeling human WM in laboratory mice</th>
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<tr>
<td>NSF/V 4</td>
<td>Congenic for ecotropic MuLV induction loci</td>
<td>Fredrickson et al. JNCI 1984 568</td>
<td>NSF</td>
<td>Insertional mutagens may accelerate lymphoma development</td>
</tr>
<tr>
<td>Gld/Lpr</td>
<td>Fas (Gld) or FasL (Lpr) deficiency</td>
<td>Davidson et al. J Exp Med 1996 572</td>
<td>BALB/c 4</td>
<td>Autoreactive B cell may be cell of origin for WM</td>
</tr>
<tr>
<td>p53 5-Null</td>
<td>p53 deficiency in mature B lymphocytes</td>
<td>Gostissa et al. PNAS 2013 573</td>
<td>Mixed</td>
<td>Naive B cell may be cell of origin for lymphoma development</td>
</tr>
<tr>
<td>BCL2 5 IL6 5 AID 5</td>
<td>Oncogene collaboration in AID-deficient B cells 5</td>
<td>Tompkins et al. BCJ 2016 575</td>
<td>BALB/c</td>
<td>Loss of AID blocks lymphoma development at the IgM stage</td>
</tr>
<tr>
<td>Mdy 5 D6 5</td>
<td>Recapitulation of human MYD88 5 allele in WM</td>
<td>Knittel et al. Blood 2016 574</td>
<td>C57BL/6</td>
<td>Humanizing mice with hallmark mutations of WM is feasible</td>
</tr>
</tbody>
</table>

1 Genetic changes that drive neoplastic B lymphocyte development.
2 Original reports, all published in high-impact cancer journals.
3 Genetic background of mouse strain. BALB/c is a member of the Castle’s family of mice. NSF belongs to the Swiss mice family. C57BL/6 is at the center of C57-related strains. See genealogy of inbred mice at the website of The Jackson Laboratory, Bar Harbor, Maine for additional information.
4 Strain C is uniquely susceptible to late-stage B-cell and plasma cell tumors due to a complex genetic trait that includes hypomorphic (weak-efficiency) alleles of genes encoding the cell cycle inhibitor p16 and the FKB12 rapamycin-associated protein Frap. Also part of this trait is deficiency of Mndal, an interferon-inducible gene.
5 In human WM, IL-6 has long been recognized as a major growth, differentiation and survival factor. In mice, IL-6 is firmly linked to inflammation-induced plasmacytoma (PCT) that develops in a tissue site rich in IL-6. Evidence for the involvement of IL-6 in inflammation-dependent PCT includes studies in BALB/c mice demonstrating that tumor growth is enhanced by exogenous IL-6 but inhibited by antibodies to IL-6 or its receptor. Furthermore, BALB/c mice homozygous for a null allele of IL6 are resistant to inflammation-induced PCT and Myc/Raf retrovirus-induced PCT, whereas mice carrying the IL6 transgene used here (IL6+) developed PCT spontaneously. Enforced expression of the BCL-2 (denoted BCL2+ in the table), a death repressor, increases the apoptotic threshold for tumor precursors because BCL-2 blocks many death signals during normal B-cell development, cooperates with oncogenes in vitro to promote mitogen-independent cell survival; and accelerates oncogene-driven B-lymphoma in vivo. BCL2 overexpression keeps B cells alive that would normally be eliminated due to developmental problems; auto-reactivity, dysfunctional BCR, or illegitimate genetic rearrangements. Because AID deficient mice (denoted AID- in the table) only harbor pre-switch IgM + B cells, tumor induction results in IgM neoplasms - as shown with AID "knockout" mice that carried IL6 or Bclx transgenes, also seen. A comparison of mice of the same genetic background, except for the presence of V+ or absence of V- of functional ecotropic virus genomes, demonstrated that V+ lymphomas developed at about half the rate compared to V- lymphomas. In concordance with that, V+ mice developed mostly low-grade tumors with extended latent periods. In V+ mice, clonal outgrowth, defined by Ig gene rearrangements, was associated with the acquisition of somatic proviral integrations, suggesting that--although generation of B cell clones can be virus independent--ecotropic viruses facilitate the rate with which (pre) malignant cell clones are generated and able to progress to frank lymphomas. With regard to modeling human WM in transgenic mice, the findings suggested that inducible insertional mutagens that can be unleashed in B cells may be useful engineering tools. The Sleeping Beauty transposon may be particularly effective to that end. Another strain of mice that exhibits proclivity to

**PHENOCOPY MODEL**

WM-like tumors that arise spontaneously in genetically engineered mice have been described on several occasions in peer-reviewed cancer literature. An early example is NSF/V+, where mice are congenic for ecotropic murine leukemia virus (MuLV) proviral genes that comprise a complete functional ecotropic virus genome, express the virus at high titer, and undergo spontaneous lymphoma development at high frequencies. A study of more than seven hundred NSF/V+ lymphomas, which relied on histopathology, immunophenotyping, analysis of immunoglobulin heavy chain or T cell receptor beta chain rearrangements, and determination of somatic viral integration sites, showed that 90% of the lymphomas tested were of the B cell lineage. Low-grade tumors included small lymphocytic, follicular, and splenic marginal zone lymphomas, while high-grade tumors comprised diffuse large-cell lymphomas of the centroblastic and immunoblastic types. Lymphoblastic lymphoma was also seen. A comparison of mice of the same genetic background, except for the presence (V+) or absence (V-) of functional ecotropic virus genomes, demonstrated that V+ lymphomas developed at about half the rate compared to V- lymphomas. In concordance with that, V+ mice developed mostly low-grade tumors with extended latent periods. In V+ mice, clonal outgrowth, defined by Ig gene rearrangements, was associated with the acquisition of somatic proviral integrations, suggesting that--although generation of B cell clones can be virus independent--ecotropic viruses facilitate the rate with which (pre) malignant cell clones are generated and able to progress to frank lymphomas. With regard to modeling human WM in transgenic mice, the findings suggested that inducible insertional mutagens that can be unleashed in B cells may be useful engineering tools. The Sleeping Beauty transposon may be particularly effective to that end. Another strain of mice that exhibits proclivity to
IgM⁺ B-cell lymphoma with lymphoplasmacytic features is BALB/c deficient in Fas signaling⁶¹,⁶². Germline mutations in Fas (TNF receptor superfamily member 6) and FasL (Fas ligand) lead to T cell hyperplasia and systemic autoimmunity, but also greatly increase the risk of neoplastic B cell development. B lymphomas found in FasL mutant Gld mice were usually Ig isotype switched, secreted Ig, and were plasmacytoid in appearance. However, IgM⁺ WM-like tumors also occurred at low frequencies. Genomic and molecular genetic analyses showed that the majority of plasmacytoid lymphomas derived from antigen-experienced autoreactive B cells, which produced antinuclear antibody or rheumatoid factor and exhibited the skewed IgV gene repertoire and Ig gene rearrangement pattern, were associated with these specificities. Transcriptional profiling placed the lymphomas at an early stage in plasma cell differentiation, clearly distinguishing them from several other types of B-cell neoplasia. The findings implicated autoreactive B cells as tumor precursors and established a strong link between systemic autoimmunity and B-cell neoplasia. Additionally, the results suggested that pre-switch IgM⁺ tumors may be more abundant in lymphoma-bearing mice when isotype switching (CSR) in B lymphocytes is blocked by pharmacologic or genetic means.

A third example of a genetically modified strain of mice prone to WM-like tumors emerged when Gostissa et al. used Cre/Lox technology to inactivate the tumor suppressor p53 in mature B lymphocytes. The rationale for making these mice was derived from a large body of evidence indicating that: p53 exerts a central role in protecting cells from oncogenic transformation; the gene encoding p53, TP53, is mutated in a large number of human cancers; and germ-line inactivation of p53 in mice confers strong predisposition to early-onset thymic lymphoma and thus inadvertently prevents studies on neoplastic development in other cell lineages such as B lymphocytes. The investigator team showed that inactivation of p53 specifically in mature B lymphocytes resulted in the development of IgM⁺ peripheral B-cell lymphomas that arose from naïve B cells that had not undergone Ig heavy-chain gene class switching (CSR), or somatic hypermutation of expressed Ig heavy and light chains. Surprisingly, naïve B cells served as tumor precursors, even when mice were subjected to immunization protocols that induced vigorous Th-dependent humeral immune responses including active germinal centers, the tissue site in which CSR and V(D)J hypermutation take place and most human B-cell tumors originate. The results demonstrated that deletion of p53 suffices to trigger the malignant transformation of mature B cells and that p53-deficient lymphomas provide a valuable mouse model for human IgM⁺ B-lineage tumors, such as splenic marginal zone B-cell lymphoma and WM. The findings also suggested that p53 deficiency may be a useful engineering tool for designing dedicated mouse models of human WM.

In the authors’ laboratory, WM-like tumors have been repeatedly observed in mice that carried either a widely expressed human IL6⁶⁶ or B-cell specific BCL2⁶⁴ transgene. IL6 encodes the proinflammatory cytokine interleukin 6 (IL-6) and BCL2 encodes the synonymous survival-enhancing oncoprotein. However, the great majority of these mice developed “switched” IgG⁺ or IgA⁺ B cell and plasma cell tumors with long latency and incomplete penetrance. To favor IgM⁺ neoplasms in this setting, we initiated a new research project aimed at reproducing key features of human WM in compound transgenic IL6/BCL2/AIDnull mice. These mice carried both transgenes, IL6 and BCL2, on the genetic background of AID (activation-induced cytidine deaminase) deficiency. The rationale for this approach was oncogene collaboration; i.e., IL6 and BCL2 are key players in the natural history of WM that collaborate effectively in neoplastic B-cell development in mice, and thus were likely to increase tumor incidence and shorten tumor onset relative to single transgenic mice. Secondly, AID deficiency was expected to block IL6/BCL2-driven tumor development at the IgM⁺ stage. This is because AID is essential for CSR. In accordance with these expectations, Tompkins et al. found that IL6/BCL2/AIDnull mice developed, with full genetic penetrance (100% tumor incidence) and short latency (93 days median survival), a severe IgM⁺ lymphoproliferative disorder that recapitulated important features of human WM. However, the mouse model also exhibited shortcomings, such as low serum IgM levels, histopathological changes not seen in patients with WM, and difficulties to propagate apparent tumors in host mice. This indicated that further refinement of this approach is required to achieve better correlation with disease characteristics of WM.

**GENOCOPY**

One way to refine the phenocopy models of human WM described above might be the recreation of important WM genotypes in transgenic mice. Knittel et al. recently accomplished just that by virtue of reproducing the MYD88L²OµP mutation in C57BL/6 mice. The mutation was discovered in DLBCL as a highly recurrent, oncogenic, gain-of-function mutation that substitutes a leucine (L) residue at position 265
of MYD88 with a proline (P) residue. DLBCL is the most common non-Hodgkin’s lymphoma in the United States, and occurs in two major subtypes derived from different cells of origin: a germinal center B cell in the case of GCB-DLBCL, and a plasmablast (activated B cell) in the case of ABC-DLBCL. Combination chemotherapy resulted in an ~80% 3-year survival for patients with GCB-DLBCL, but achieved only 45% survival for patients with ABC-DLBCL. A well-established feature of ABC-DLBCL is the constitutive activation of the classical NF-κB pathway, which in ~40% of cases is accomplished by somatic (acquired) mutations in MYD88, an adaptor protein involved in cellular signal transduction pathways that govern pattern recognition, inflammation, innate and adaptive immune responses, and malignant cell transformation.

MYD88 links TIRs, which are members of the Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) superfamily, with downstream effector hubs that regulate, in addition to NF-κB (Fig. 2), JAK-STAT (Janus kinase–signal transducer and activator of transcription), MAPK (mitogen-activated protein kinases) and type-I IFN (interferons binding to the interferon–receptor) signaling. Subsequent to its discovery in ABC-DLBCL, the MYD88[626/626P] mutation has also been found in the great majority of patients with WM (nearly 100%) and a fraction of patients with primary central nervous system lymphoma (~35%), splenic marginal zone lymphoma (~15%), gastric mucosa associated lymphoid tissue lymphoma (~9%) and chronic lymphocytic leukemia (~3%).

Knittel et al. expressed the orthologous mouse allele of human MYD88[626/626P], Myd88[625/625P], in transgenic mice. This was accomplished by Cre recombinase–induced activation of a MYD88[625/625P] “knock-in” gene inserted into the germ-line Myd88 locus using homologous recombination (gene targeting) in embryonic stem cells. Three different Cre drivers (AID, CD19, and CD22) were employed to target MYD88[625/625P] expression specifically in B cells. Regardless, transgenic mice from all experimental groups developed lymphoma that shared most phenotypes. Practical limitations relating to tumor incidence (low) and tumor onset (long) were readily overcome by co-expression of the Myc oncogene. An advance of great relevance for WM concerned the collaboration of MYD88[625/625P] and deregulated expression of BCL2 (B-cell leukemia 2) in lymphoma development. Thus, combining the MYD88[625/625P] allele with a newly developed, inducible BCL2 allele in a double-transgenic strain of mice resulted in tumor development with full penetrance (100% tumor incidence) and short onset. BCL2/ MYD88[625/625P] may be useful for preclinical trials of experimental WM drugs including BCL2 inhibitors, such as venetoclax (ABT-199) and navitoclax (ABT-263), and newly emerging BTK (Bruton’s tyrosine kinase) inhibitors following in the footsteps of ibrutinib. Future efforts may include small-molecule IRAK4 and TAK1 inhibitors currently in the preclinical pipeline, or combination therapies that target BCL2, MAPK or JAK-STAT in addition to MYD88-NF-κB signaling.

**KEY POINTS AND FUTURE DIRECTIONS**

Preclinical model systems of WM available at this juncture include continuous tumor cell lines suitable for human-in-mouse xenografting and GEMMs of neoplastic B-cell development, in which WM-like tumors
arise spontaneously in immunocompetent hosts. Future research should be aimed at developing additional cell lines to better represent the genetic heterogeneity of WM, including ethnic and racial differences; e.g., Han Chinese or African American patients with WM vs. Caucasian patients. Another important aim is the design of accurate transgenic mouse models of WM in which lymphoplasmacytic lymphoma develops with short onset (tumor latency), high genetic penetrance (tumor incidence) and predictable tumor pattern (hematopoietic bone marrow manifestation). Lastly, in contrast to many solid cancers, patient-derived xenograft (PDX) models of WM have not yet been developed. However, establishing such models is very desirable because PDXs circumvent potential artifacts of in vitro cell culture, stably retain molecular, genetic and histopathologic features of the originating tumor, and thus provide currently the only practical approach for accommodating the vast inter-patient and intra-tumor heterogeneity inherent to human cancer[74].

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