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A Novel Waldenström's Macroglobulinemia Cell Line BCWM.2 Carrying a Rare MYD88 Mutation (p.Ser243Asn) with Unique Mutations and Suitability for In Vivo Xenograft Murine Model

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Background: Waldenström's macroglobulinemia (WM) is an indolent B-cell lymphoproliferative disorder characterized by bone marrow (BM) infiltration with lymphoplasmacytic lymphoma and monoclonal immunoglobulin M (IgM) production. Mutations in MYD88 are present in 95-97% of WM patients. Deletions involving chromosome 6q (del6q) are found in up to 50% of WM patients and include genes that regulate MYD88, BCL2, and apoptotic signaling. Cell lines play a pivotal role as disease models, contributing to a comprehensive understanding of WM biology and advancing therapeutic strategies. However, the availability of WM cell lines is limited, and none demonstrate del6q.

Patient and Methods: We developed and characterized a novel cell line (BCWM.2) from long-term cultures of $CD19^+$ selected BM lymphoplasmacytic cells from a symptomatic, treatment-naive WM patient who then received ibrutinib monotherapy on a clinical trial. The patient achieved a major response to treatment but progressed after two years. Serial bone marrow biopsies and extensive genomic and transcriptome analyses of the patient's tumor were performed during the study, and the corresponding cell line was established at baseline. Whole Genome Sequencing (WGS) was performed on BCWM.2 and three other established WM cell lines: BCWM.1, MWCL-1, and RPCI-WM1 cells. An in vivo xenograft mouse model of BCWM.2 was also developed in NOD SCID mice, and the origin of the xenografted tumor was confirmed by whole exome sequencing and immunohistochemistry.

Results: BCWM.2 cells exhibited morphologic and immunophenotypic characteristics resembling lymphoplasmacytic cells and demonstrated robust propagation when co-cultured with HS-5 stromal cells in IMDM medium supplemented with 20% FBS. Flow cytometric analysis revealed that BCWM.2 exhibited an immunophenotype consistent with the source WM patient. Whole genome sequencing demonstrated that both BCWM.2 cells and the original patient WM cells carried a somatic activating mutation in MYD88 (S243N) and shared trisomy in chromosomes 3 and 12, heterozygous deletion of 6q, and amplification of 6p. Notable mutations identified in this cell line, as compared with the other three established cell lines (BCWM.1, MWCL-1, RPCI-WM1), are listed in **Table 1**.

BCWM.2 cells are readily engrafted in NOD-SCID mice by direct subcutaneous injection of 2 x 10^6 cells/mouse. Tumor growth (~500 mm^3) was observed in the mice four weeks post-inoculation. Follow-up evaluations demonstrated serial increases in human IgM levels in the mouse sera post-engraftment (**Figure 1A and B**). BCWM.2 engraftment was confirmed by immunohistochemistry, with tumors exhibiting intermediate lymphoid cell morphology with plasmacytoid differentiation, CD20 and MUM1 expression, clonality for lambda light chain and IgM heavy chain, and 60% KI67 staining (**Figure 1C**). Whole exome sequencing of engrafted tumors confirmed the presence of the MYD88 and LYN mutations detected in the cell line and the patient.

Conclusions: We established a novel WM cell line with unique MYD88 (S243N), LYN (I297N), and SPI1 (Q227E) somatic mutations, among others, and deletions of 6q. Xenograft of BCWM.2 in NOD-SCID mice by direct subcutaneous injection establishes an in vivo model for WM. This cell line provides a new disease model for the in vitro and in vivo study of WM, including the development of targeted therapies for this disease.

Table 1. Unique mutations identified in BCWM.2 cells as compared with 3 established WM cell lines (BCWM.1, MWCL-1, RPCI-WM1)

Symbol	Location	Allele	Protein Position	Amino Acid
MYD88	3:38182292-38182292	G/A	251	S/N
LYN	8:56879436-56879436	T/A	297	I/N
AKAP9	7:91646405-91646405	C/T	1276	R/*
HDAC5	17:42165957-42165957	T/G	236	H/P
RUNX3	1:25254083-25254083	C/A	141	V/L
SPI1	11:47376915-47376915	G/C	227	Q/E

Figure 1

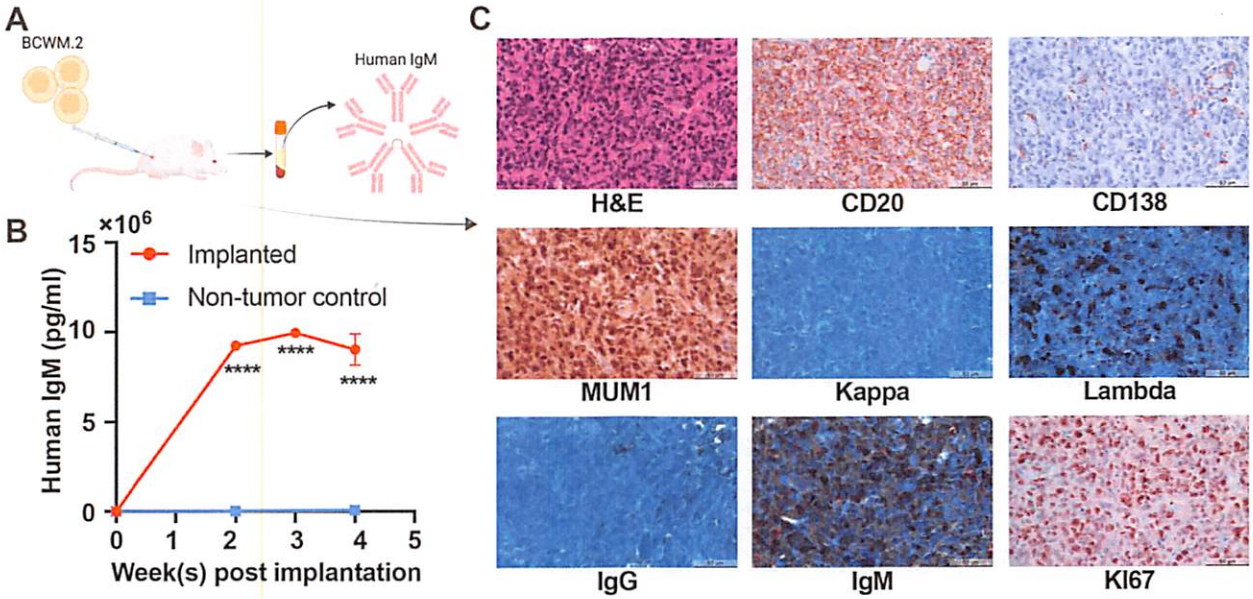

Figure 1. *In vivo* establishment of BCWM.2 tumor in NOD-SCID mice. (A) Schematic representation of establishing BCWM.2 xenograft mouse model and collecting specimens for human IgM evaluation in serum. (B) Human IgM levels in the serum of the xenografted mice were monitored once a week by ELISA. (C) Representative slides of pathological evaluation of the tumor mass from the implanted mice by H&E and immunohistochemical staining for human CD20, CD138, MUM1, kappa, lambda, IgG, IgM, and Ki67.