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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Establishment of the Bcwm.2 Cell Line As a BTK-Inhibitor Resistant, BCL2 Inhibitor Sensitive *in Vitro* and *In Vivo* Study Model for Waldenström's Macroglobulinemia

Shirong Liu, MDPhD[1], *Xia Liu, MD*[1], *Kara M Soroko*[2], *Alexa G Canning, BA*[1], *Amanda Kofides*[1], *Choudhury Fabliha Binte Yusuf*[2], *Johany Penailillo*[3], *Nickolas Tsakmaklis, BA*[1], *Christopher J Patterson*[1], *Ruben D. Carrasco, MD PhD*[3], *Zachary R Hunter, PhD*[1], *Prafulla C. Gokhale, PhD*[4], *Shayna R Sarosiek*[1], *Jorge J. Castillo, MD*[1], *Steven P Treon, MDPhDFRCP*[1]

[1] Dana-Farber Cancer Institute, Bing Center for Waldenström Macroglobulinemia, Boston, MA
[2] Dana-Farber Cancer Institute, Experimental Therapeutics Core and Belfer Center for Applied Cancer Science, Boston, MA
[3] Dana-Farber Cancer Institute, Harvard Medical School, Department of Oncologic Pathology, Boston, MA
[4] Experimental Therapeutics Core and Belfer Center for Applied Cancer Science, Dana-Farber Cancer Institute, Boston, MA

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 can play a pivotal role as disease models, contributing to a comprehensive understanding of WM biology and advancing therapeutic strategies. The availability of cell lines for the study of WM remains 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 attained a major response to treatment, and after 2 years progressed. The patient underwent serial bone marrow biopsies, and extensive genomic and transcriptome analysis of his tumor during the study, and the corresponding cell line established at baseline. An in vivo xenograft mouse model of BCWM.2 was also established in NOD SCID mice, and the origin of the xenografted tumor was confirmed by genomic 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 original patient WM cells carried somatic activating mutation in MYD88 (S243N) and shared trisomy in chromosomes 3 and 12, heterozygous deletion of 6q, and amplification of 6p. Notably, a novel mutation in the SRC family member LYN (I297N; T>A), a component of BCR, was identified in BCWM.2 but not the original patient tumor. The I297N mutation found in the regulatory hinge region is predicted to cause activation of LYN. At time of progression on ibrutinib, the dominant clone that emerged in the patient's tumor also carried the I297N mutation. Additionally, mutated genes in BCWM.2 included histone deacetylase HDAC5, tumor suppressor RUNX3, B-cell transcriptional activator SPI1, and AKAP9 (A-Kinase Anchoring Protein 9). Transcriptome analysis showed shared expression patterns between BCWM.2 and patient tumor samples, including diminished gene expression of chromosome 6q. Drug response testing revealed that BCWM.2 cells did not respond to the BTK-inhibitors ibrutinib, zanubrutinib or pirtobrutinib but were sensitive to the BCL2 inhibitor venetoclax. Furthermore, BCWM.2 cells 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. Importantly, follow-up evaluations demonstrated serial increases in human IgM levels in the mouse sera post-engraftment. Moreover, 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, 60% KI67 staining. Whole exome sequencing of engrafted tumors further confirmed the presence of the MYD88 S243N and LYN I297N mutations detected in the cell line and the patient at the time of ibrutinib progression.
Conclusions: BCWM.2 represents a novel, BTK-inhibitor resistant, BCL2 inhibitor sensitive WM cell line that demonstrates MYD88 (S243N) and LYN (I297N) somatic activating mutations, and deletions of 6q. BCWM.2 thereby provides an important

new disease appropriate cell model for the *in vitro* and *in vivo* study of WM, including the development of targeted therapies for this disease.

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