

Title: Developing in vitro 3D bone marrow models for preclinical drug development in Waldenström Macroglobulinemia

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Abstract IWWM (420/500 words):

Increasing evidence suggests that the Waldenström macroglobulinemia (WM) bone marrow (BM) microenvironment contributes to WM disease progression and treatment resistance. However, existing in vitro models rely predominantly on 2D cell line systems, which insufficiently capture this complexity. We recently developed a 3D chronic lymphocytic leukemia (CLL) model which enables high-throughput drug screening using patient-derived material in a physiologically relevant lymph node (LN) microenvironment¹, and has since been adopted in the field as well as in the context of alternative LN-dominant B cell malignancies^{2,3}. Here, we aim to develop a physiologically relevant 3D BM model that faithfully recapitulates the WM clone within its complex BM niche, to exploit this as a platform for preclinical drug development, including the use of patient-derived material.

In this pilot study, we compared different platforms to assess the effects of microenvironmental signals on WM cell survival, proliferation, and drug resistance. To this end, MWCL-1 and BCWM.1 cell lines were cultured either in conventional 2D cultures, in a 3D spheroid model using non-adherent plates, which facilitate self-organization of cells into structures that enable direct cell-cell interactions, or within β -tricalcium phosphate bone scaffolds that incorporate a structural bone component for WM cells to adhere to (Figure 1).

Both WM cell lines showed high expression of anti-apoptotic Bcl-2 family proteins alongside strong resistance to BH3 mimetics (targeting either Bcl-2, Bcl-XL, or Mcl-1). Combination of multiple BH3 mimetics significantly increased apoptosis in WM cell lines, and combination of any BH3 mimetic with the endoplasmic reticulum stress-inducer thapsigargin resulted in drug synergy mediated by induction of the pro-apoptotic protein Bim.

Whereas coculture with either HS-5 or 3T40L stromal cells effectively induced drug resistance in primary chronic lymphocytic leukemia cells, stromal support did not affect drug sensitivity in WM cell lines in standard 2D cocultures. In contrast, WM cells cultured in spheroids displayed increased venetoclax resistance, which was further promoted in bone scaffolds (Figure 2). Notably, bone scaffolds enabled long-term survival of WM cells and significantly promoted proliferation even in the absence of soluble stimuli, revealing distinct subpopulations in which WM cells located deeper inside the scaffolds displayed higher levels of proliferation.

These pilot results underscore the importance of a structural 3D BM architecture in the in vitro functionality of WM cells and their sensitivity to drugs. They also support the further development and optimization of a 3D BM model for in vitro studies focusing on WM biology and therapeutic responses. Such a model would enable the integration of diverse microenvironmental components and therapeutic agents in a fully controlled in vitro setting.

¹Haselager, M. V. et al. In Vitro 3D Spheroid Culture System Displays Sustained T Cell-dependent CLL Proliferation and Survival. *HemaSphere* **7**, e938 (2023).

²Dobaño-López et al., Patient-derived follicular lymphoma spheroids recapitulate lymph node signaling and immune profile uncovering galectin-9 as a novel immunotherapeutic target, *Blood Cancer J.* **14**, 75 (2024).

³Araujo-Ayala et al., A novel patient-derived 3D model recapitulates mantle cell lymphoma lymph node signaling, immune profile and in vivo ibrutinib responses, *Leukemia* **37**, 1311-1323 (2023).

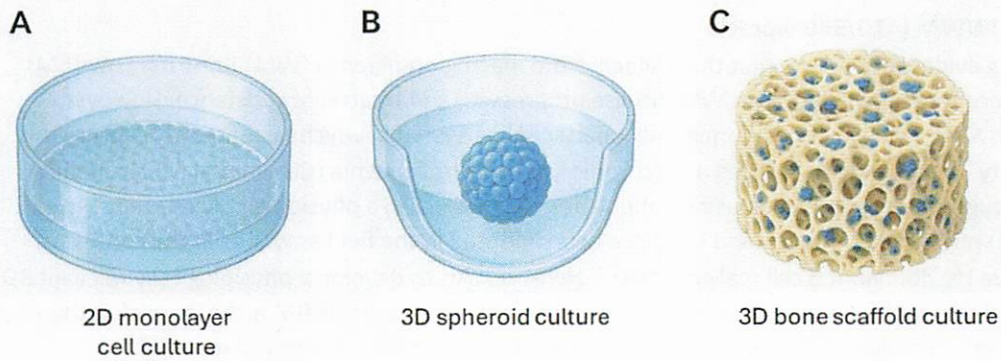


Figure 1: Schematic overview of the different culture platforms tested in this study.

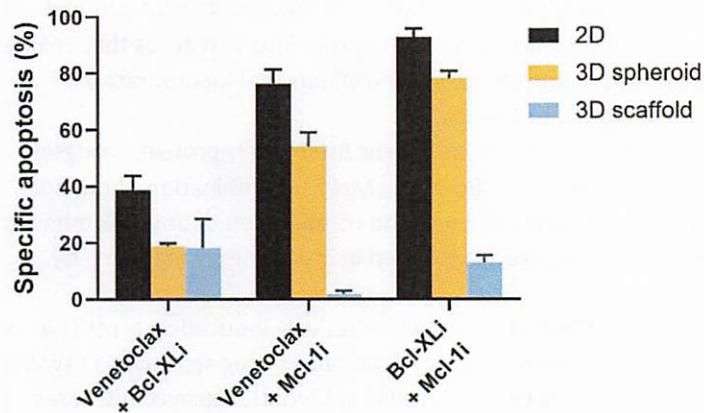


Figure 2: Specific cell death of MWCL-1 cells across 2D, 3D spheroid or 3D scaffold conditions following 48 hours of treatment with indicated BH3 mimetics. Error bars represent the mean $\pm$ SEM (n=3).

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