

Self-Organizing Bone Marrow Organoids for the Co-culture of Waldenström Macroglobulinaemia Tumour Cells

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Introduction: The study of Waldenström Macroglobulinemia (WM) is significantly hampered by the lack of model systems that reproduce the physiological environment in which the tumours exist. Although the recent progress made in mouse model development and WM cell lines has shed important light on the disease, there are still significant gaps in our knowledge of WM that would benefit from new models for the disease. In particular, the study of how the tumour microenvironment influences WM biology would be greatly facilitated by new models of the WM TME.

Methods: Here we have set up a model system of the human bone marrow by inducing self-organizing bone marrow organoids (BMOs) derived from induced pluripotent stem cells (iPSCs). We used both non-engineered iPSCs for BMO generation and iPSCs engineered with a stably integrated piggyBac vector for doxycycline-inducible expression of CCL5. The organoids were induced as published¹. First, spheroids were induced with subsequent mesendoderm, vascular- and haematopoietic commitment steps, sprouting, and downstream organoid harvesting and culture in ultra-low adherent 96-well plates. Primary CD19-sorted WM tumour cells were stained with a cell proliferation tracking dye, CellTrace Far Red, and 2-20 thousand cells were seeded into each BMO-containing well in a culture medium containing VEGF-A, VEGFC, FLT3L, FGF2, SCF, EPO, TPO, IL3, IL6, and doxycycline in the case of piggyBAC BMOs, and cultured for 3-14 days with a half media change every 72 hours. Subsequently, the BMOs were enzymatically dissociated and analyzed by flow cytometry to track engraftment and proliferation of WM tumour cells using DAPI dye exclusion to exclude dead cells. Alternatively, the BMOs were fixed for whole-mount immunofluorescence staining and stained for vascular markers as well as anti-CD19 or anti-IgM and analyzed by confocal microscopy.

Results: WM cells showed minimal engraftment into BMOs after 3 days of co-culture, with an increasing proportion of cells engrafting at 7 days, which was further increased by 14 days. Cells cultured in the fully completed media alone also survived but to a much lesser extent. Proliferation tracking further showed progressive dye dilution between 7 and 14 days, with WM cells exhibiting several rounds of cell division within the organoids. In contrast, cells cultured in complete media without BMOs exhibited survival but no dye dilution, indicating the lack of proliferation. Interestingly, unengrafted WM tumour cells co-cultured with the BMOs survived to a greater extent than WM cells in complete media alone and exhibited dye dilution to an intermediate extent compared to cells that engrafted within the organoid or cells in media alone,

indicating that soluble factors excreted by the BMOs might be a strong determinant of proliferation and survival, but that cell-cell interaction within the organoid might enhance the proliferative effect. Finally, the inducible expression of CCL5 did not enhance the proliferation or survival of BMO co-cultured WM cells.

Conclusions: Our study shows the successful culture of WM cells *ex vivo* for an extended period in a BMO model system highly amenable to genetic engineering, thereby establishing a new framework for the study of WM-TME interactions.

1. Olijnik, A.-A. *et al.* Generating human bone marrow organoids for disease modeling and drug discovery. *Nat. Protoc.* **19**, 2117–2146 (2024).



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