



1. BIOLOGY AND PRECLINICAL

DEVELOPMENT OF A NEW 3D EX-VIVO MODEL FOR MULTIPLE MYELOMA**M. Domingos^{1,2}, E. Carneiro², A. Queirós², C. João^{2,3,4}**¹Bioengineering Department, Instituto Superior Técnico, Lisbon, Portugal; ²Myeloma and Lymphoma Research Group, Champalimaud Research Center, Lisbon, Portugal; ³Hemato-Oncology Unit, Champalimaud Clinical Centre, Lisbon, Portugal; ⁴NOVA Medical School, NOVA University, Lisbon, Portugal<https://doi.org/10.3324/haematol.2026.s2.14071>

Introduction. Multiple Myeloma (MM) is a chronic bone marrow (BM) malignancy characterized by marked interpatient heterogeneity and frequent development of drug resistance. Both tumour-intrinsic features and the BM microenvironment contribute to treatment failure, underscoring the need for physiologically relevant ex vivo platforms capable of supporting personalized drug sensitivity profiling. However, existing drug testing models often lack microenvironmental complexity, require high cell input, and show limited scalability for high-throughput applications.

Objectives. To address these challenges, we developed BioMarrow, a miniaturized 3D ex vivo culture platform designed to recapitulate key features of the BM niche while enabling high-throughput drug screening with low cell input.

Methods. Culture conditions were systematically optimized by evaluating cell density and Matrigel-based 3D configurations. Assay protocols for viability, cytotoxicity, and drug sensitivity were subsequently refined for miniaturized, high-throughput formats. The platform was first validated using the MM.1S cell line and then applied to patient-derived BM samples. MM.1S cells and primary BM mononuclear cells were seeded in 384-well plates under either 2D conditions or embedded in growth factor-reduced Matrigel (25-100%) and cultured for 48 hours.

Results. Optimal MM.1S cells growth was achieved at 5,000-10,000 cells per well in 50% Matrigel, yielding signifi-

cantly higher growth rates compared with 2D culture. Drug sensitivity profiling using the following MM treatment regimens: (1) lenalidomide-dexamethasone, (2) bortezomib-dexamethasone, (3) daratumumab, and (4) daratumumab-bortezomib-lenalidomide-dexamethasone, revealed reproducible, dose-dependent responses with measurable IC50 values and robust drug sensitivity scores. Translation to primary BM samples demonstrated that BioMarrow supports short-term ex vivo culture with low cell input (100,000 cells per well) while preserving BM cellular complexity. Across patient samples, malignant plasma cells remained highly viable (>95%) after 48 hours of 3D culture. Spectral flow cytometry enabled identification and quantification of aberrant plasma cells (CD38+/CD138+/CD19-/CD56-/+) and confirmed preservation of clinically relevant plasma cell fractions over time.

Conclusions. BioMarrow is a novel and practical 3D ex vivo culture platform for MM patient-derived bone marrow cells that maintains high malignant plasma cell viability over 48 hours while requiring low cell input. By preserving BM cellular complexity and enabling reproducible, high-throughput drug sensitivity profiling, this system represents a promising functional tool to support personalized therapeutic decision-making in Multiple Myeloma, including the evaluation of combination regimens and immunotherapy-based approaches.