

# Mimicking the Myeloma Niche: A 3D Bone-Derived Co-Culture System to Selectively Assess Bystander BMSCs and to Perform High-Throughput Drug Screening in Multiple Myeloma

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## Introduction

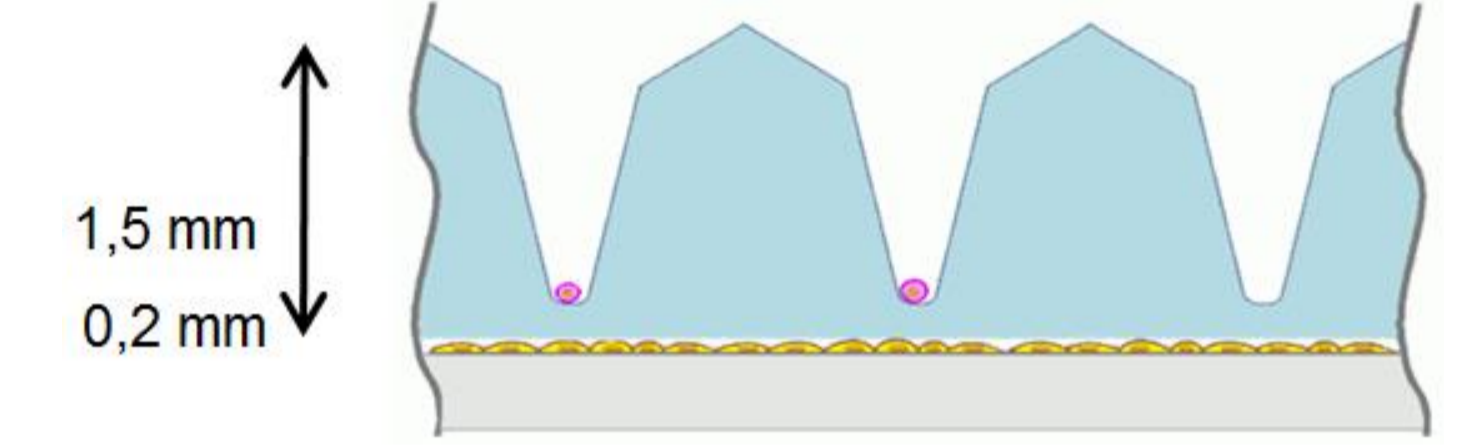
Multiple myeloma (MM) typically presents with widespread involvement of the bone marrow (BM) which indicates the pathognomonic circulation of MM cells passing out into the peripheral blood and reentering the BM via an ongoing process termed homing causing osteolysis as one clinical hallmark of MM patients. Recent findings suggest that binding of MM PCs to the BM niche promotes MM migration, cell growth and drug resistance. The here presented

project focusses on 3 key questions: A) Do MM PCs generally benefit from MM coculture? B) Does growth support differ among certain subsets of BM bystander cells? C) How may we use a better understanding of these BM subsets to develop more authentic in-vitro models to increase the predictability of agents tested in-vitro for successful adoption in the clinic?

## Methods

A novel 3D high-throughput model was used to more closely resemble in vivo MM proliferation as described (Udi. BJH 2013; Zlei. Exp Hematol 2007; Schüler. EOBT 2013). Consisting of an agarose matrix with conical microwells and semipermeable surface allowing diffusion of cytokines (e.g. CXCL12) but not of BMSCs, this model tested MMCL (U266, RPMI-8226 and OPM-2) vs. primary BM pt cells, both with/

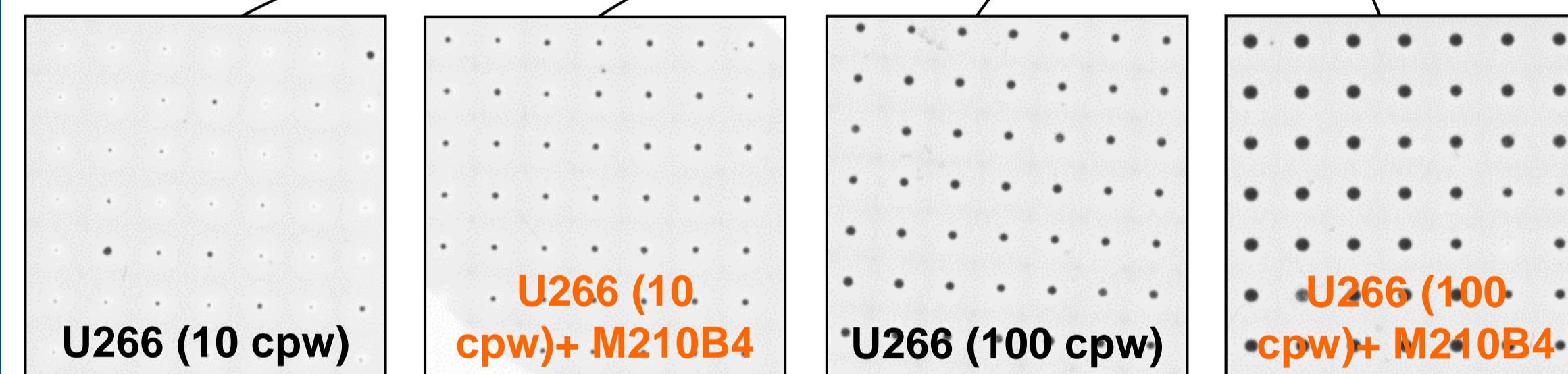
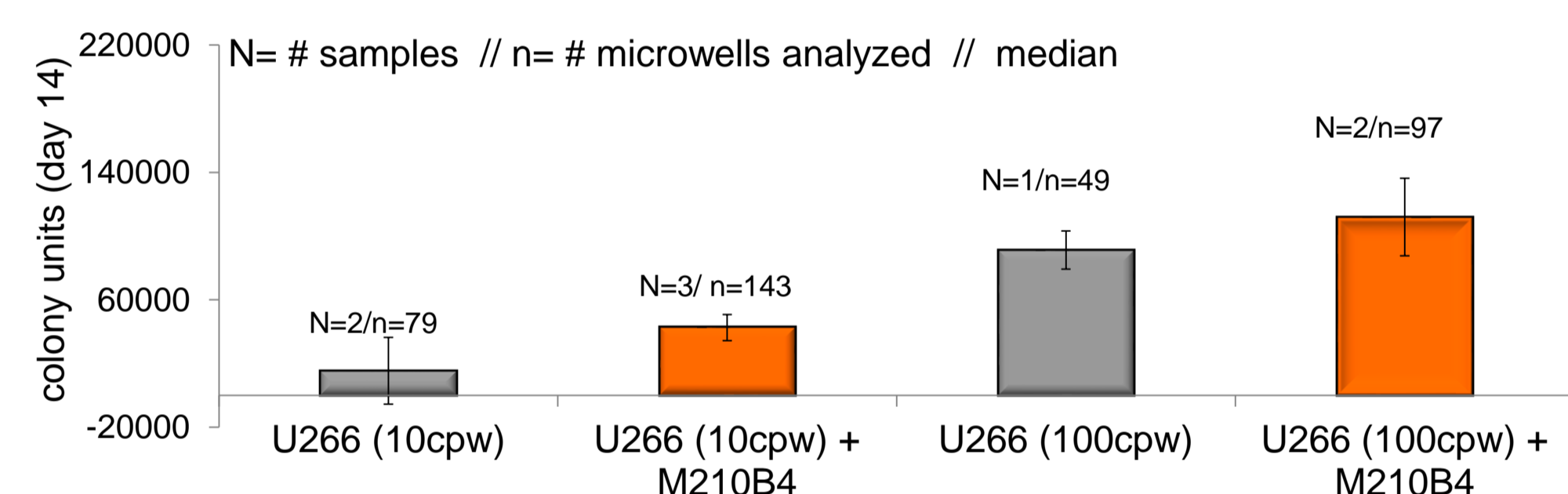
without HS-5 vs. M210B4 stroma support. Analyses included growth kinetics, volumetric analyses (MTT), apoptosis (Annexin/PI), Western Blots (CXCR4, pCXCR4,...), FACS (CD31, CD34, CD45, CXCR4, 12G5, 4G10 abs) and cell cycle analyses. Preliminary niche sorting experiments were performed by the use of an established FACS sorting protocol after sacrifice of C57BL6 mice.



## Results

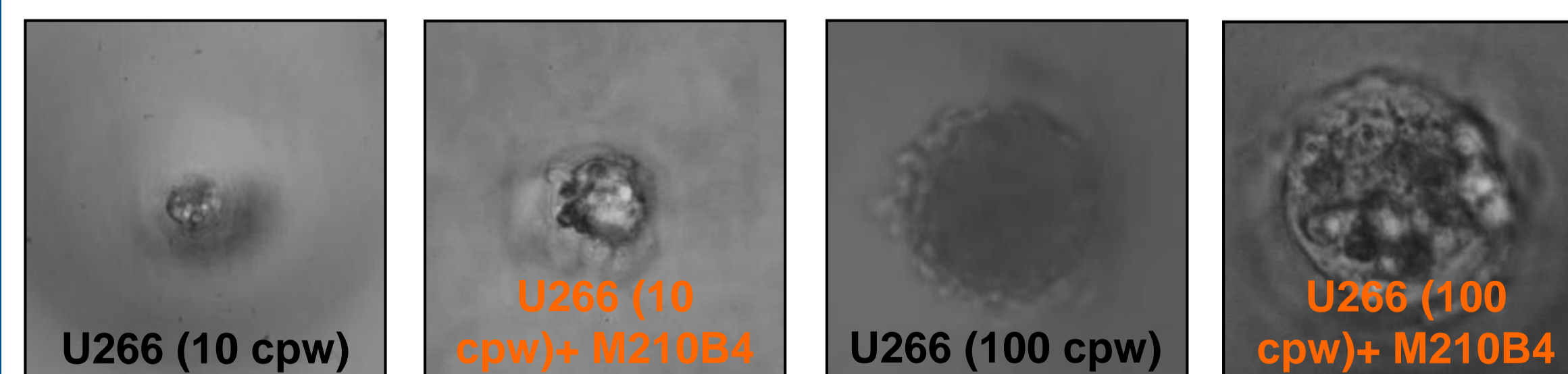
### A. Growth studies

#### (I) M210B4 distance culture promotes MMCL aggregate growth in vitro



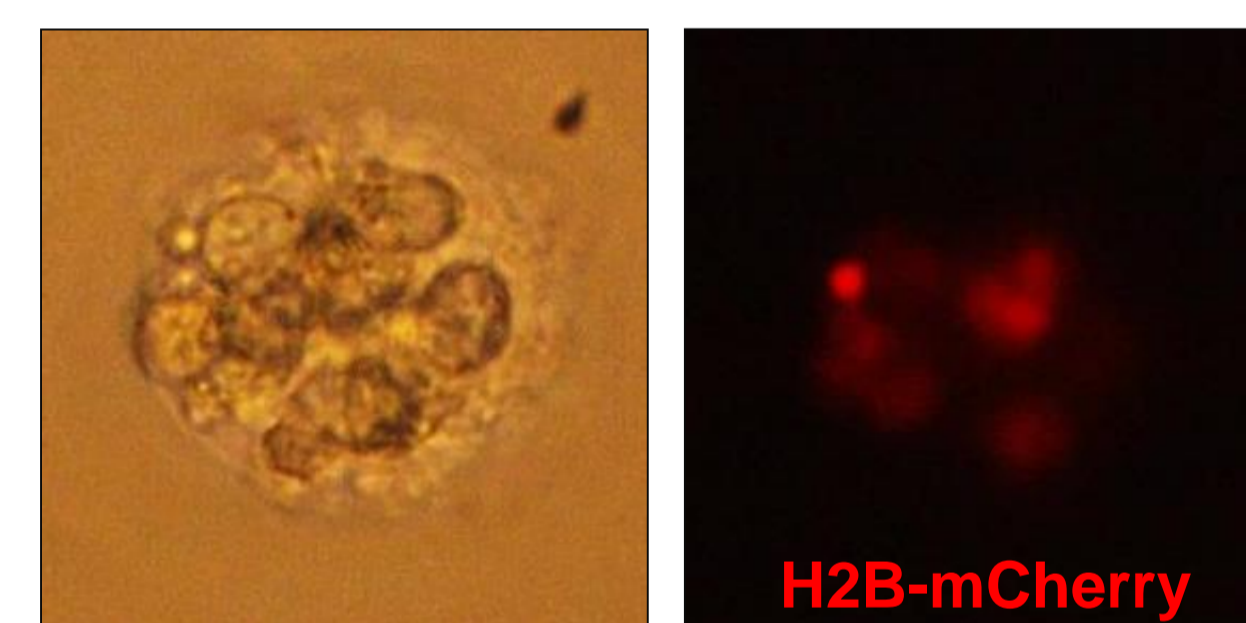
(I) U266 cells were cultured at different concentrations (10 vs. 100 cells per microwell) w and wo M210B4 stroma support for 14d demonstrating a growth advantage when M210B4 cell support was used.

#### (II) M210B4 distance culture promotes MMCL (U266, L363) aggregate growth in vitro



(II) Liquid overlay technique allows cluster formation of primary MM specimen leading to more reliable propagation monitorable throughout the time of culture (=21 days).

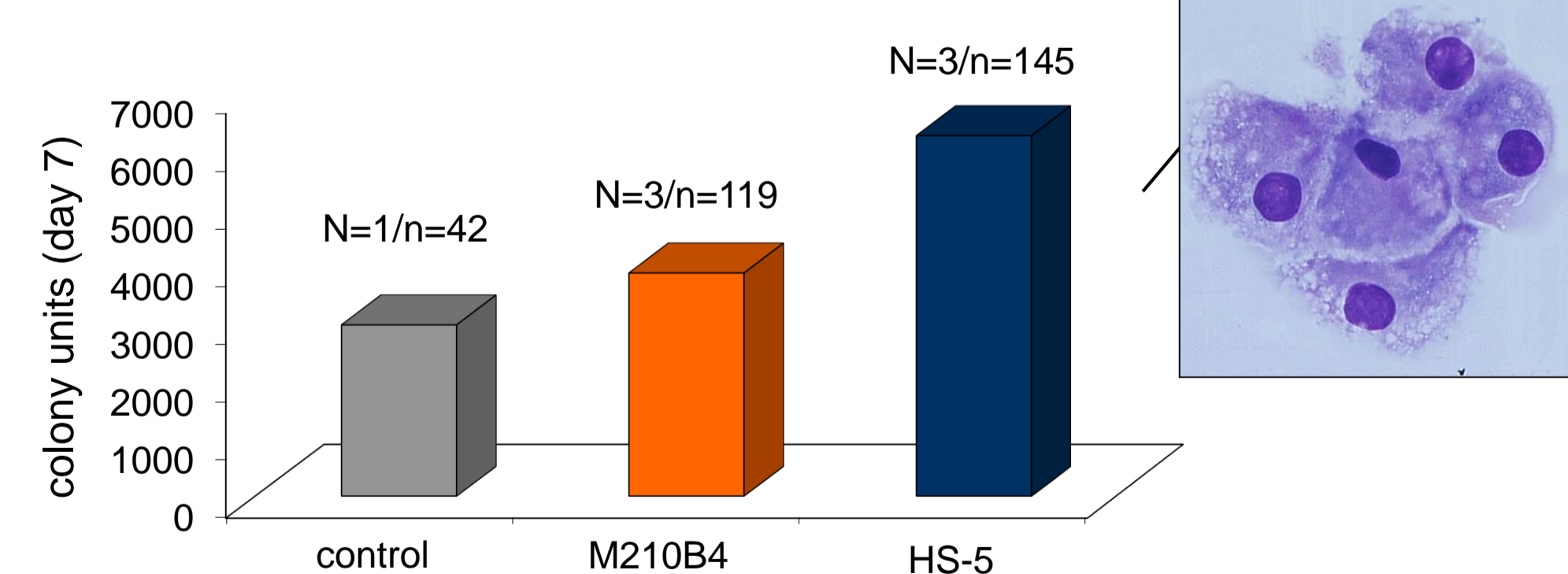
#### (III) Detection of induced apoptosis



(III) Generated RPMI8226 expressing a red chromatin marker (H2B-mCherry) and green fluorescent protein-tagged cytochrome c-GFP to determine early apoptosis w vs. wo BSMC stroma support

#### (IV) A suitable model for propagating BM MM pt samples + comparing BSMC/ cytokine supportive effects for pt specimens

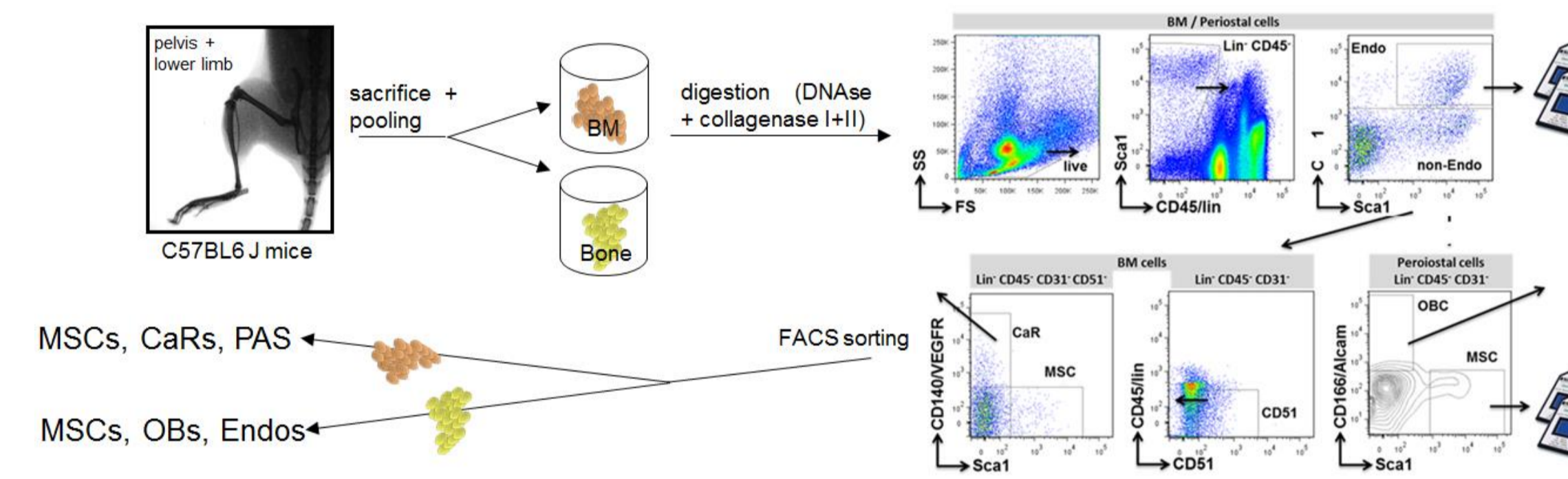
(IV) Pt specimen were cultured with and without M210B4 demonstrating a growth advantage with vs. without M210B4 after 7d which was even more distinct when human HS-5 cells instead of murine M210B4 cells were used.



#	Sex	Age	MM-type	MM disease status	D&S	BM infiltration (CD38+)
#1	♀	78	IgA kappa MM	initial diagnosis	IIIA	15%
#2	♂	71	IgG lambda MM	initial diagnosis	IIIA	70%
#3	♀	75	IgA lambda MM	3rd relapse after 6x VCD, VD, RD, 6x VBDD	IIIA	90%
#4	♂	70	IgA lambda MM	initial diagnosis	IIA	60%

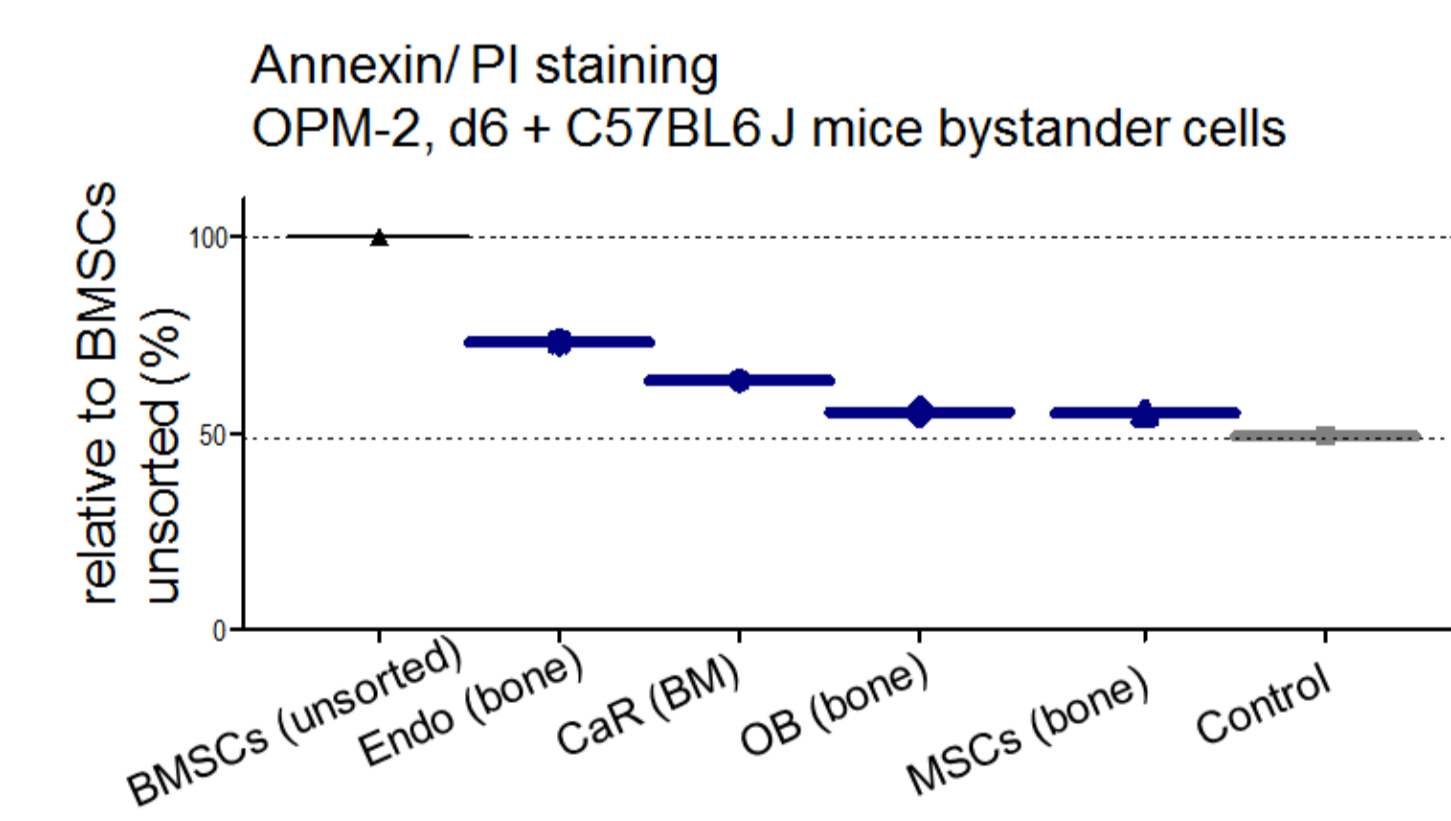
### B. Niche modelling

#### (V) Test platform design

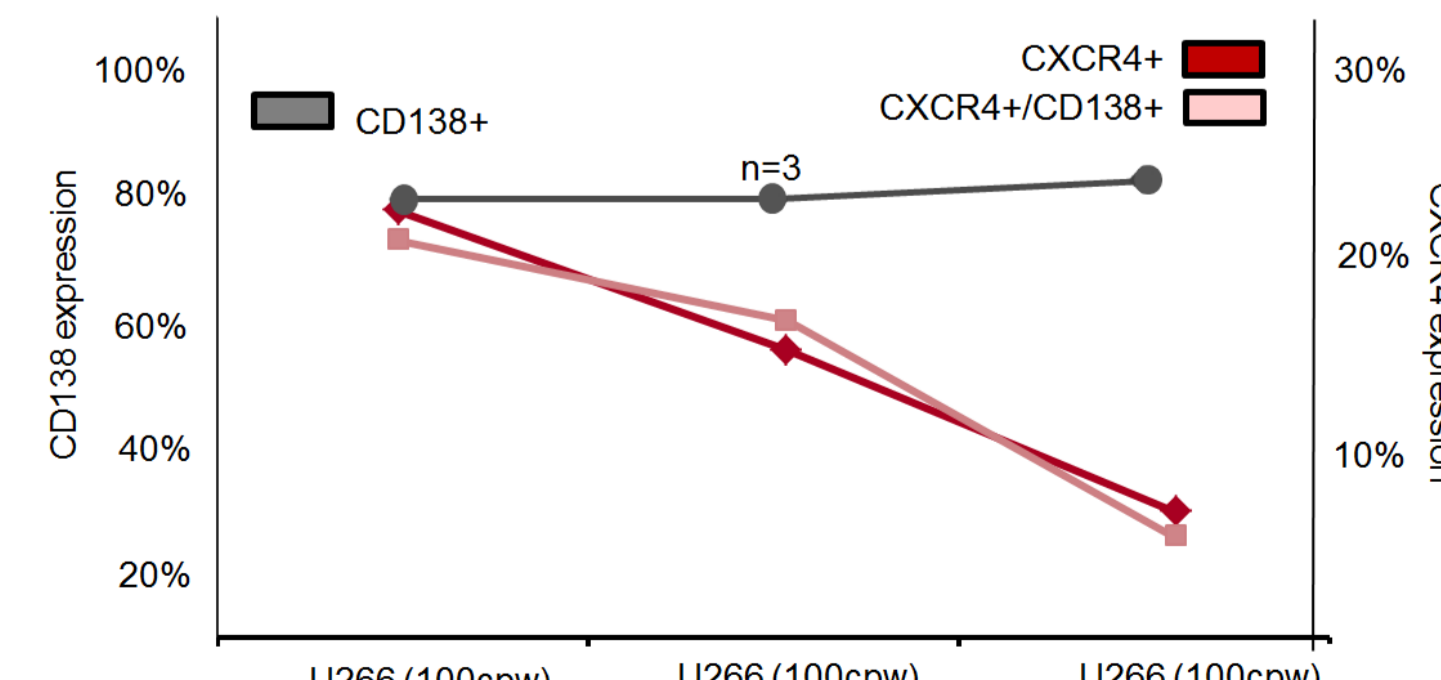


#### (VI) Assessing growth support of BM bystander cells

(VI) C57BL6 derived BM and bone bystander cell subsets provide different anti-apoptotic effect on OPM-2 after 6d of coculture with endothelial cells being the most beneficial and bone-derived MSCs being the less beneficial co-agent for OPM-2. To note, albei different impact on growth support, no single subset seems to be as efficient as the sum of unsorted BMSCs (CaRs=CXCL12-abundant reticular cells).



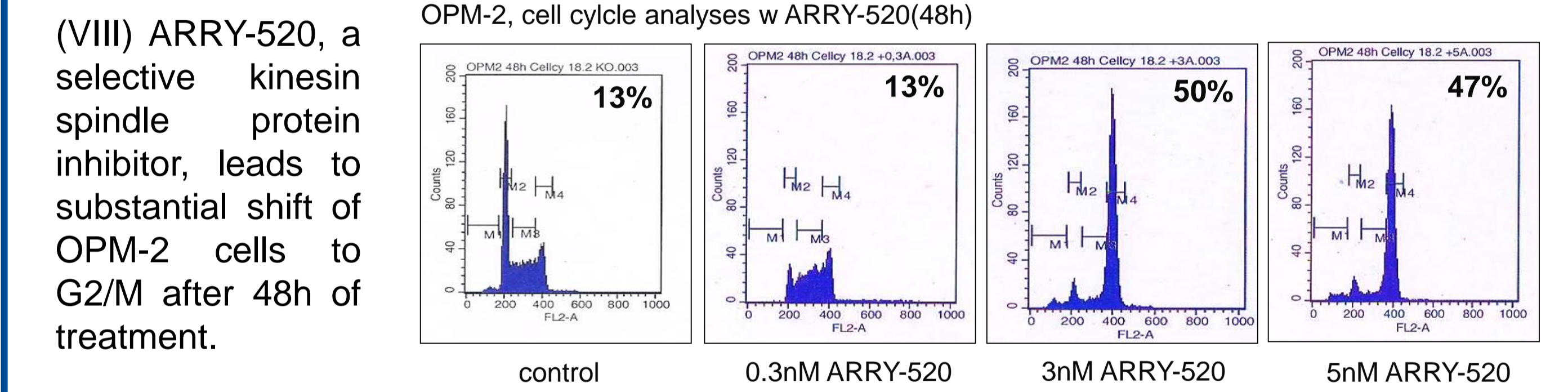
#### (VII) Dynamic regulation of CXCL12/CXCR4-axis



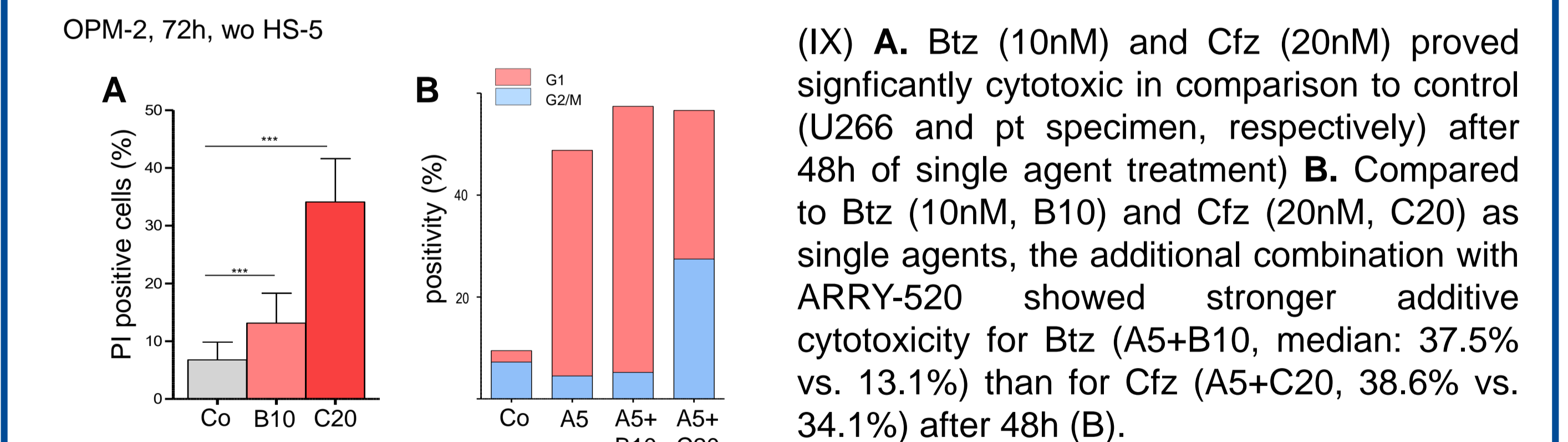
(VII) CXCR4 expression in BMSCs distant co-culture (U266, 7 days); CXCR4 expression decreases if cells were cocultured with M210B4, but is even further decreased if human HS-5 was present (both BMSCs secreting CXCL12)

### C. In vitro drug screening

#### (VIII) Single agent activity of ARRY-520 on G2/M arrest



#### (IX) ARRY-520 increases apoptosis alone and in combination w Btz > Czf



## Conclusions

More complex, 3D bone-derived high-throughput in vitro models are urgently needed to better predict the potency of preclinically tested agents and to better estimate the likelihood of their later clinical adoption into phase I-II trials. With this work, we provide an innovative model which reflects the BM microenvironment as a crucial predictor for in-vivo sensitivity as shown for ARRY-520. This ex-vivo approach helps to better incorporate MM growth support by bone and BM-derived bystander cells and thus depicts a valid tool to better characterize the role of the BM niche in myeloma.