Advancing Multiple Myeloma Research:Patient Derived 3D Organ-on-a-Chip Models To Reveal Immunological Mechanisms Influencing Cancer Growth and Resistance

Abstract #3970

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INTRODUCTION

Patient derived models are vital to the study of disease. In vitro growth of multiple myeloma (MM) has proven to be challenging due to disease heterogenity and the difficulty in culturing bone marrow. We aimed to address this by developing a novel, patient derived 3D organ-on-a-chip model of MM bone marrow. Proteomic data, generated by our group, from the plasma and bone marrow interstitial fluid (BMIF) of patients undergoing VRd induction therapy, identified key growth factors important in the growth of multiple myeloma. We categorized these growth factors into pro and anti tumor groups and using bone marrow mononuclear cells (BMMC) and donor matched bone marrow stromal cells (BMSC) we developed a complex tumor microenvironment (TME) on-chip using primary human cells. This model allow us to examine the role of differentially expressed growth factors on tumor development, hematopoietic stem cell differentiation and cytokine production in the TME. Growth factors designated as pro tumor, caused increased growth of multiple myeloma as well as increased production of tumor associated cytokines QPCT and TXNDC5 when added to our 3D bone marrow on-a-chip-system.

METHODS

Figure 1

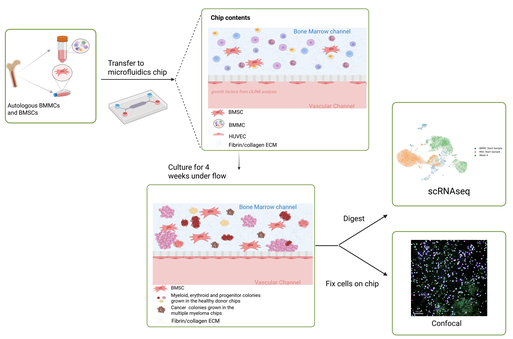

Figure 1 shows the set up of the bone marrow chips

- **Cells:** Cryopreserved BMMCs from patients with MM were obtained from Discovery Life Sciences. BMSCs from the same donor were isolated by culture on plastic and cultured for no more than 3 passages prior to chip initiation. Healthy whole bone marrow was obtained from AllCells and processed over ficoll to get BMMCs. BMSCs from the same donor were isolated as above. Human Umbilical Vein Endothelial cells (HUVEC) from pooled donors were obtained from Lonza at passage 1 and added to the chip between passages 3 and 5
- **Extracellular matrix (ECM):** Both channels of the chip were coated with a collagen/fibronectin layer. The top channel ECM was a fibrin/collagen mix generated on chip by cleaving fibrinogen with thrombin
- **3D culture chips:** The dual-channel, silicon chips used were the S1 model from Emulate. The chips were cultured under continuous flow using the Emulate Zoe module
- **Feed media:** X-Vivo media containing serum and typical cytokines used to culture hematopoietic progenitor cells was used as a base for all culture conditions. Additional cytokines were added to promote the growth of MM.
  - Base media cytokines: IL-3, TPO, EPO, GM-CSF, Flt-3, G-CSF, SCF
- **scRNAseq:** Single-cell RNA sequencing was performed using the Flex kit from 10X Genomics
- **Imaging:** Images were acquired on a Nikon Ti2-E motorized inverted CSU-W1 SoRa Super-Resolution, 7-line Laser Unit, High QE sCMOS, Spinning Disk Confocal microscope using a CFI PLAN APOCHROMAT LAMBDA 20X
- **Data analysis:**
  - Images were analyzed using the Aivia software by Leica
  - scRNAseq data was analyzed using python packages scanpy, scrublet and harmony
  - All other graphs were created using GraphPad Prism

RESULTS

Figure 2

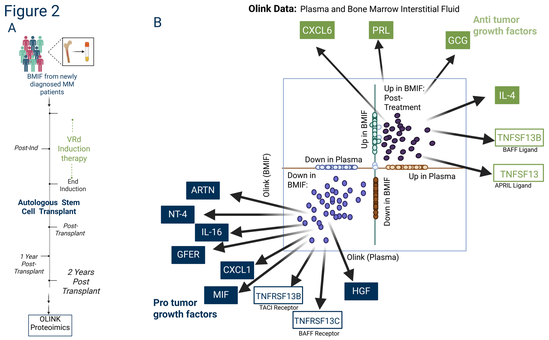

Figure 2: Choosing the growth factors
2A shows the study trajectory of patients going through VRd therapy as part of the longitudinal multiple myeloma study (abstract #4540)
2B shows the GFs chosen for this project based on the Olink data from the study

Figure 3

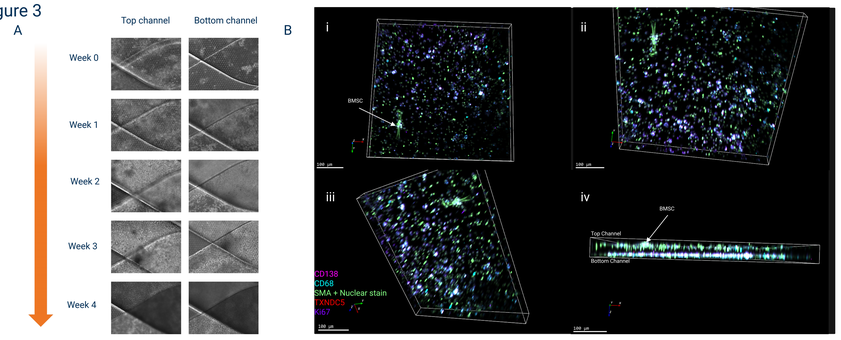

Figure 3: Bone marrow on a chip - how does it grow?
3A shows the cell growth of a healthy bone marrow-on-a-chip over four weeks

3B shows a 3D confocal image of a healthy bone marrow on-a-chip.

Figure 4

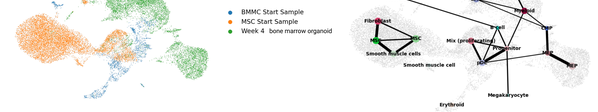

Figure 4: Did we grow healthy bone marrow? Verification by scRNSseq

4A shows the organoid clustering separately to the BMSC and BMMC start samples with some overlap. The network graph shows the relationships between the start sample cells and those grown in culture with thicker lines showing stronger connections.

4B looks more closely at the cells grown in the bone marrow chip. The cells are predominantly myeloid, erythroid and megakaryocyte lineage.

4C illustrates the differentiation of hematopoietic stem cells (HSCs) into various blood cell lineages. The media used in this assay selected for the myeloid/erythroid lineage.

Figure 4 cont.

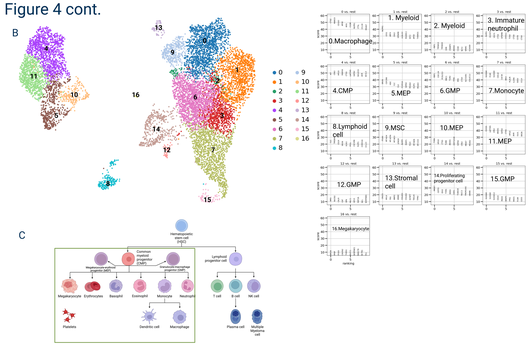

Figure 5

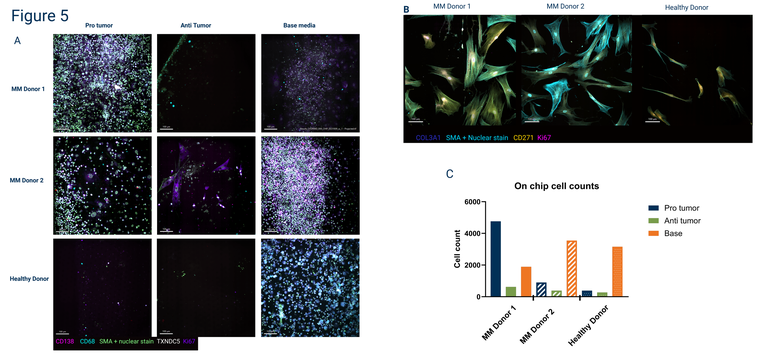

Figure 5: Multiple myeloma on a chip - image based analysis
5A shows max projection images of the multiple myeloma chips under each culture condition. Images shown are of a representative area in the chip. Cells are stained for CD68, Ki67 , TXNDC5 and smooth muscle actin as well as a nuclear stain.

5B shows the BMSCs isolated from each donor in culture. The cells isolated from the MM donors are primarily cancer associated fibroblasts expressing both CD271 and SMA. The cells isolated from the healthy donor are far smaller and appear to retain a more MSC like characteristic

The cells in the chip images were segmented in Aivia based on their nucleus and relative size and then counted in Aivia. The cell counts from each image are shown in figure 5C.

Figure 6

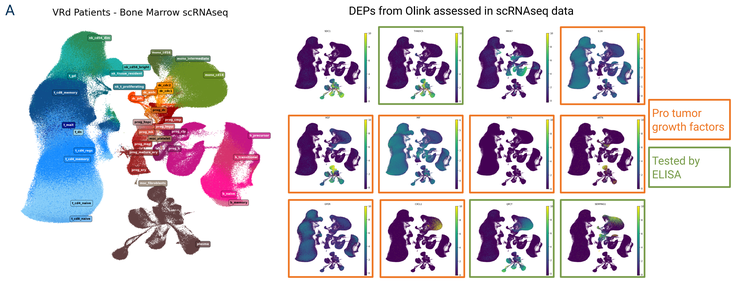

Figure 6 cont.

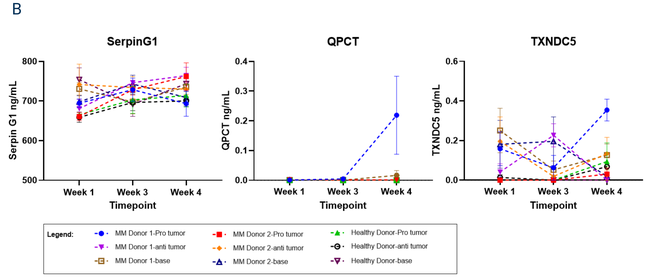

Figure 6: Multiple myeloma on a chip - supernatant analysis
6A shows the scRNAseq data of patients enrolled in the longitudinal study (abstract#4540) undergoing VRd therapy. The image on the left shows the expression of differentially expressed proteins (DEPs) from the Olink data in scRNAseq data. Some of these proteins were chosen as growth factors and some were looked for in the culture supernatant.

6B: Supernatant collected during the MM bone marrow on a chip culture was analysed by ELISA for Serpin G1, QPCT and TXNDC5. Serpin G1 levels were seen to be relatively high and uniform across all chips. TXNDC5 and QPCT showed an increase over time in MM donor 1 - pro tumor.

CONCLUSION

The primary cell types grown in our healthy 3D bone marrow-on-a-chip model are myeloid, megakaryocyte and erythroid lineage hematopoetic progenitor cells. The chip allows for an extended culture time and more mature cells such as macrophages and neutrophils are seen after four weeks in culture.
Although multiple myeloma donors showed different levels of growth on chip, both donors showed increased cell numbers in the pro tumor growth condition compared to the anti tumor condition. Just one MM donor showed any growth in the negative condition and this included significantly higher growth of BMSC cells. Serpin G1 levels across all chips were similarly high suggesting myeloid growth in all chips tested. Imaging results show CD68+ myeloid cells present in the MM chips though not to the same extent as in the healthy chip. MM donor 1 responded extremely well to the pro-tumor growth condition showing increased cell growth of TXNDC5+ cells as well as increased levels of QPCT and TXNDC5 in the supernatant. The pro tumor condition is a good candidate for long term culture of multiple myeloma cells. Our 3D system will allow for hypothesis testing and confirmation of results found in the longitudinal MM study.

ACKNOWLEDGEMENTS

Chou, D.B., Frismantas, V., Milton, Y. *et al.* On-chip recapitulation of clinical bone marrow toxicities and patient-specific pathophysiology. *Nat Biomed Eng* **4**, 394–406 (2020). https://doi.org/10.1038/s41551-019-0495-z

Images and poster were created with BioRender.com

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