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Micro-Organospheres (MOS) retain patient tumor microenvironment for precision immuno-oncology

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Background

Current patient-derived organoid (PDO) models are largely devoid of immune components. We developed a precision microfluidic and membrane platform to generate patient-derived micro-organospheres (MOS) that retain tumor-resident immune and stromal components for personalized immuno-oncology (IO) assays.

Methods

MOS were generated from lung, kidney, and colorectal cancer patients. The composition and function of patient tumor-resident immune cells in MOS were characterized by flow cytometry, single-cell RNA-seq, antibody staining, and TCR-seq. High-content and longitudinal imaging with AI analyses were used to quantify tumor cell death and immune cell dynamics inside MOS in response to IO therapies including checkpoint inhibitors, T cell bispecific antibodies, and adoptive tumor infiltrating lymphocyte (TIL) therapies, followed by single-cell analyses.

Results

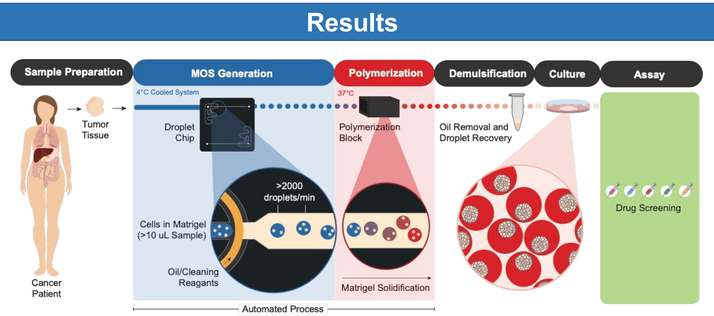

Figure 1. Scheme of patient tumor MOS generation and drug screening.

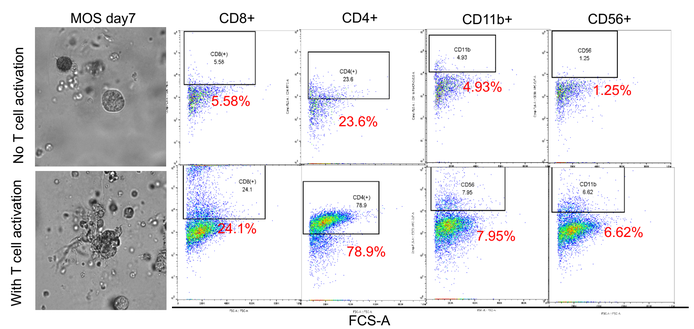

Figure 2. Resident immune cells encapsulated in MOS are viable and responsive to immune stimulation.

Results

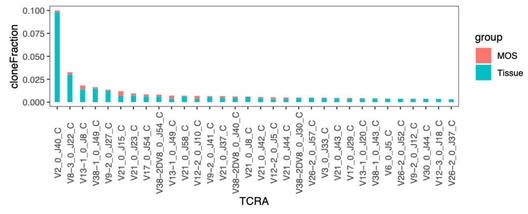

Figure 3. MOS preserves majority of TCR subclones as in primary tumor tissue. TCR seq was performed on CRC tumor tissue and derived MOS (day7).

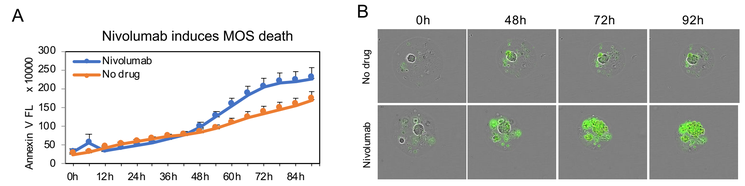

Figure 4. Immune cells preserved in MOS are responsive to immunotherapy. (A) Nivolumab induced significant cytotoxicity in tumorspheres within MOS. Incucyte images were taken every 2 hours for 4 days, and Annexin V Green dye was added to indicate apoptosis. (B) Representative images from Incucyte demonstrated Nivolumab induces cell apoptosis within MOS.

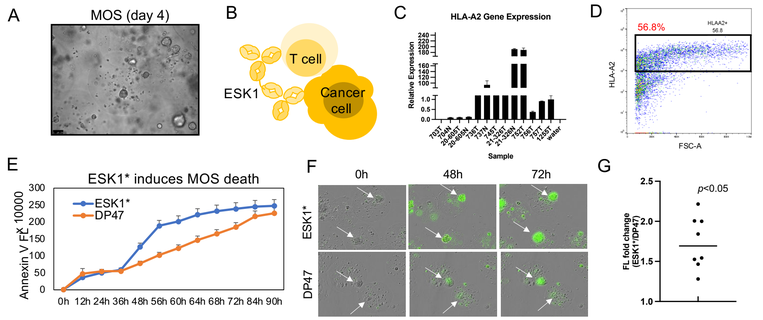

Figure 5. Immune cells preserved in MOS are responsive to ESK1* immunotherapy. (A) Established MOS (day 4) derived from lung tumor tissue. (B) Animation of how ESK1* TCB drug induces CTL-mediated killing in MOS. (C) HLA-A2 gene expression in lung tumor tissues. (D) HLA-A2 expression detected by flow cytometry in established MOS derived from lung tumor tissue. (E) ESK1* induced higher apoptosis signal (indicated by Annexin V signal) in MOS. (F) Representative images of apoptosis induced by ESK1* treatment. (G) ESK1* induced killing of lung cancer MOS in all eight lung cases (p<0.005).

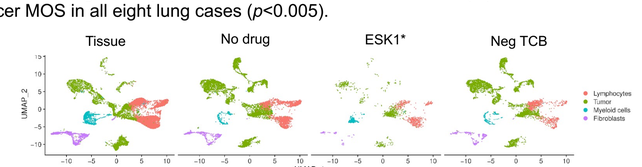

Figure 6. UMAPs of cells from primary lung tumor tissue and derived MOS with and without treatments.

Results

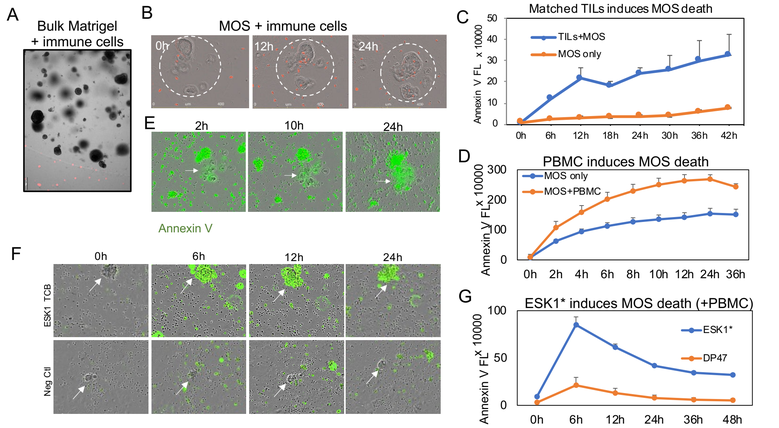

Figure 7. A MOS potency assay for T-Cell therapies. (A) TILs cannot penetrate traditional Matrigel. (B) TILs can penetrate MOS and adhere to tumor cells. Immune cells were stained with Cytolight Red dye before images taken using Incucyte. (C) Increased killing indicated by Annexin V was observed in MOS treated with autologous TILs. (D) Representative images of MOS killing by TILs in MOS (indicated by Annexin V dye). (E) Activated PBMCs induce MOS killing (indicated by Annexin V Green dye). (F) Representative images of induced death of ESK1* treated MOS combined with PBMCs. White arrows indicating lung cancer tumorspheres within MOS. Compared to ESK1*, the negative control TCB, DP47, did not induce significant apoptosis of tumorspheres within MOS. (G) ESK1* enhanced PBMC-induced tumor cell killing compared to the DP47 (CD3 only TCB).

Conclusion

MOS provide a rapid and scalable personalized platform for developing and testing IO therapies such as checkpoint inhibitors, bispecific antibodies, and T cell therapies on patient tumor models that still retain the original tumor microenvironments.

Future Directions

- High throughput IO drug candidate screening
- Designing clinical trials

References

1. Yuki K, Cheng N, Nakano M, Kuo CJ. Organoid models of tumor immunology. *Trends Immunol*. 41, 652-664 (2020).
2. Drost, J. & Clevers, H. Organoids in cancer research. *Nat Rev Cancer* **18**, 407-418 (2018).
3. Dao, T., Pankov, D., et al. (2015). Therapeutic bispecific T-cell engager antibody targeting the intracellular oncoprotein WT1. Nature biotechnology 33, 1079-1086.