

High Resolution Profiling of a Complex in Vitro 3D Lung Cancer Model to Monitor Resistance to Chemo-Immunotherapy

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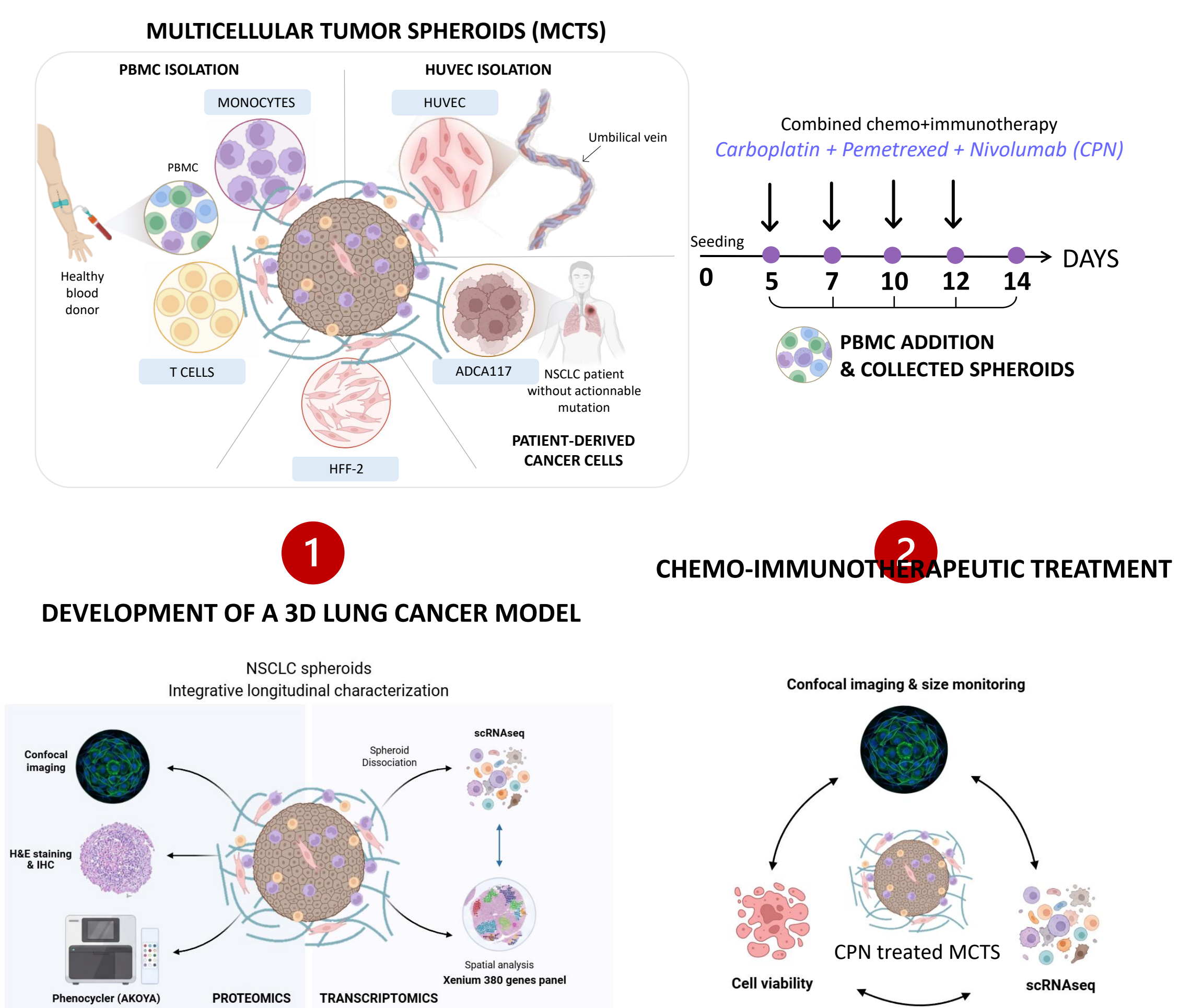
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INTRODUCTION

Lung cancer is the leading cause of cancer related deaths worldwide with non-small cell lung cancer (NSCLC) being the most common types (80-85%). While Immune Checkpoint Inhibitors (ICIs) has revolutionized the treatment of NSCLC, their benefits remain limited due to significant resistance and therapeutic failure. The tumor microenvironment (TME) plays a pivotal role in response to immunotherapies. However, accessing tumors after the first lines of treatment remains challenging. To overcome this limitation, we propose to characterize the influence of the TME on ICIs resistance by developing a complex 3D co-culture model derived from human NSCLC. This model includes patient-derived NSCLC lung tumor cells, human umbilical vein endothelial cells, fibroblasts, as well as monocytes and T lymphocytes isolated from healthy donor blood. We aim to characterize the 3D model at baseline and monitor the influence of TME on response to treatment.

METHODS



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RESULTS

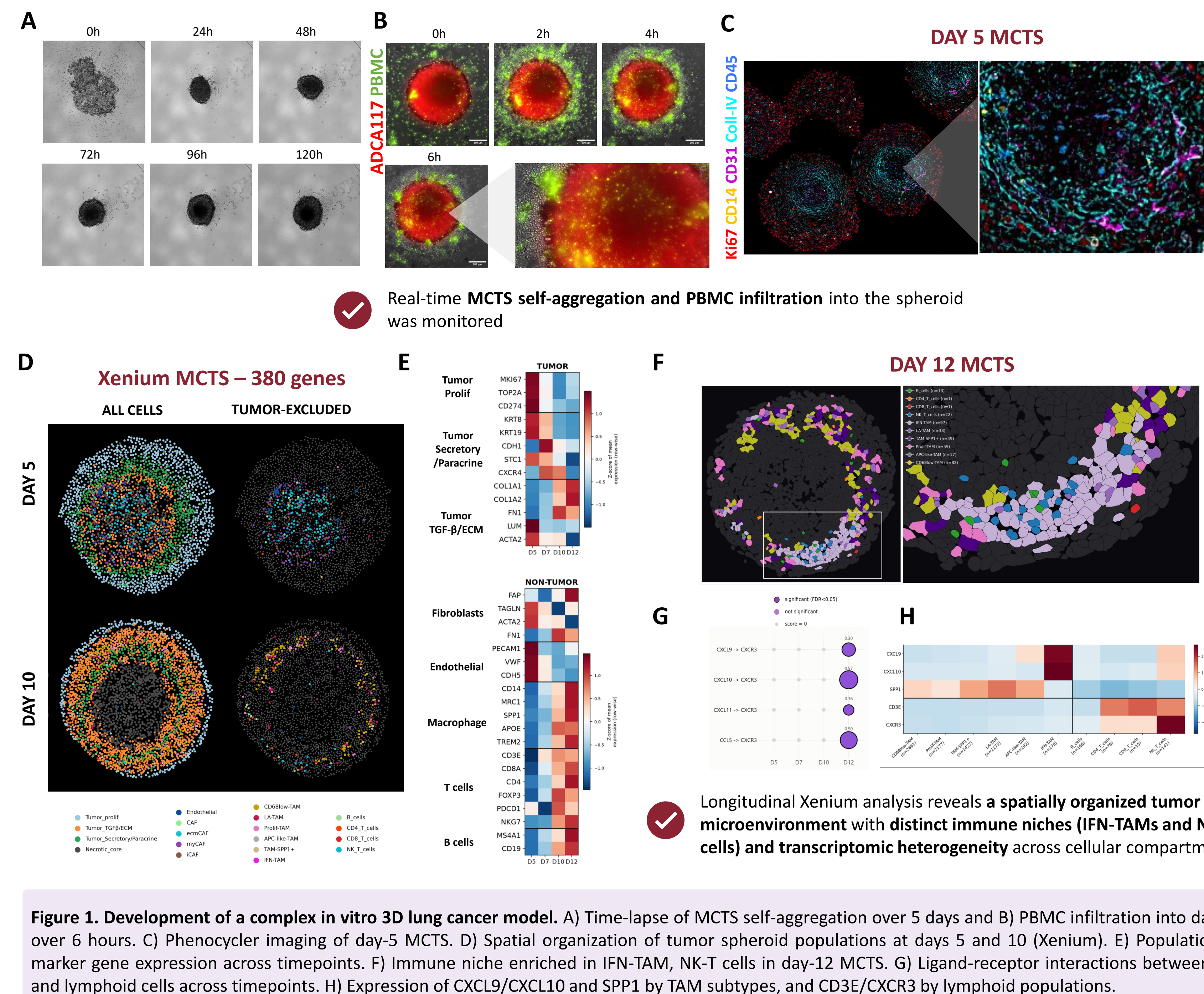


Figure 1. Development of a complex in vitro 3D lung cancer model. A) Time-lapse of MCTS self-aggregation over 5 days and B) PBMC infiltration into day-5 MCTS over 6 hours. C) Phenocycler imaging of day-5 MCTS. D) Spatial organization of tumor spheroid populations at days 5 and 10 (Xenium). E) Population-specific marker gene expression across timepoints. F) Immune niche enriched in IFN-TAM, NK-T cells in day-12 MCTS. G) Ligand-receptor interactions between IFN-TAM and lymphoid cells across timepoints. H) Expression of CXCL9/CXCL10 and SPP1 by TAM subtypes, and CD3E/CXCR3 by lymphoid populations.

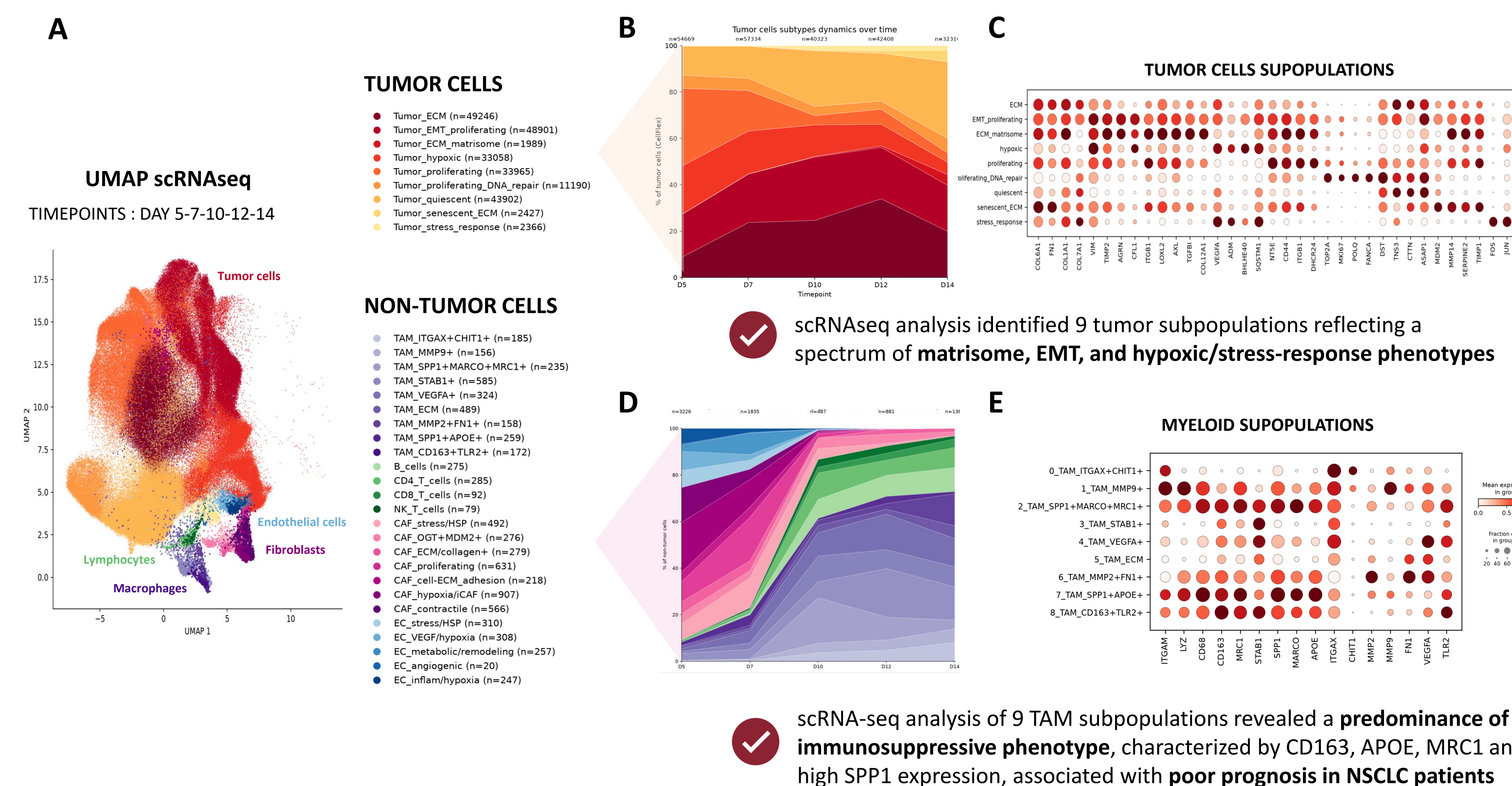


Figure 2. Longitudinal scRNA-seq characterization of complex MCTS. A) UMAP visualization of cell populations from longitudinal single-cell RNA sequencing (days 5-14). B) Dynamics of tumor cell subpopulations over time. C) Dotplot of population-specific marker gene expression across tumor subpopulations. D) Dynamics of non-tumor cell subpopulations over time. E) Dotplot of population-specific marker gene expression across myeloid subpopulations.

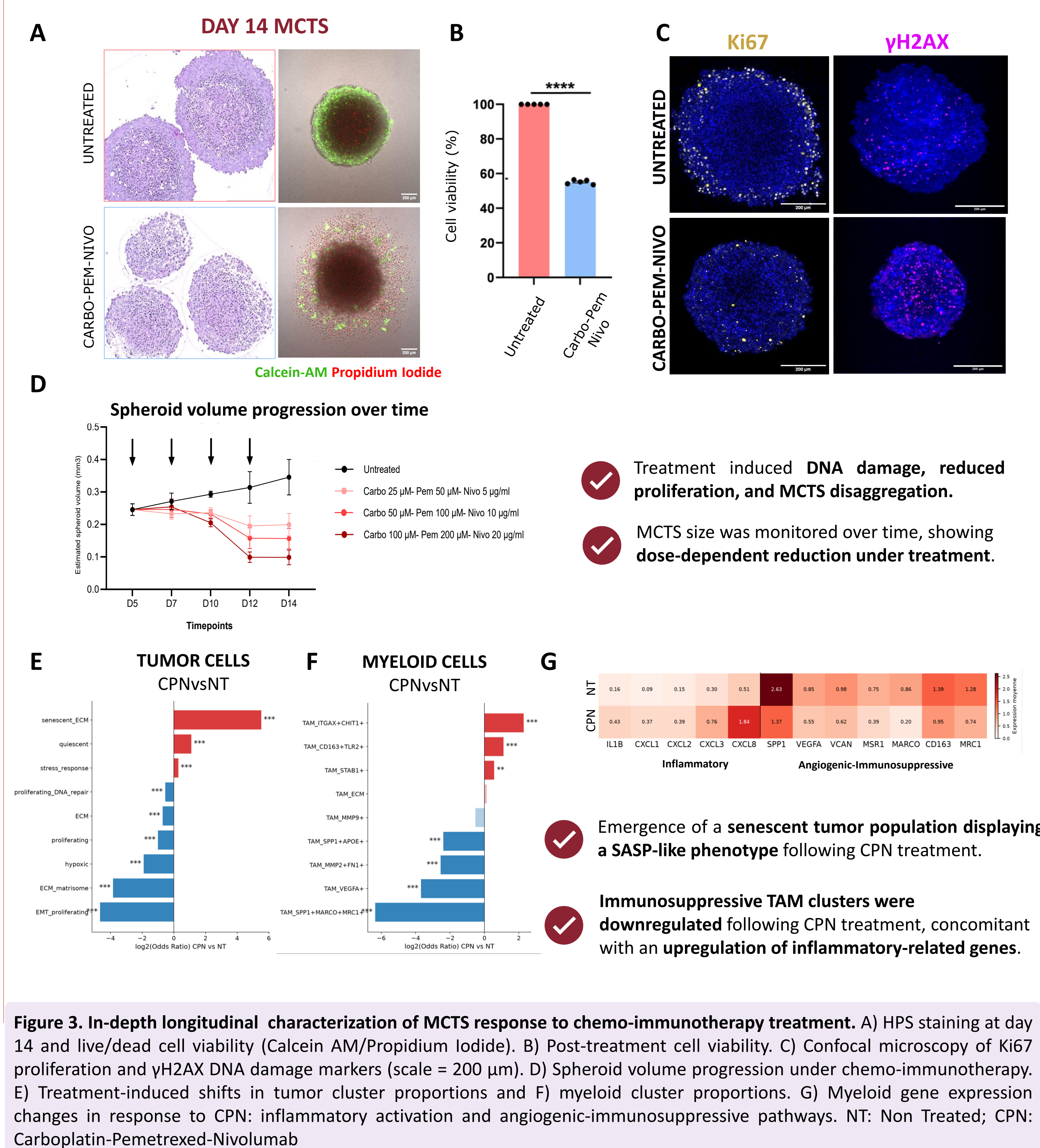


Figure 3. In-depth longitudinal characterization of MCTS response to chemo-immunotherapy treatment. A) HPS staining at day 14 and live/dead cell viability (Calcein AM/Propidium Iodide). B) Post-treatment cell viability. C) Confocal microscopy of Ki67 proliferation and γH2AX DNA damage markers (scale = 200 μm). D) Spheroid volume progression under chemo-immunotherapy. E) Treatment-induced shifts in tumor cluster proportions and F) myeloid cluster proportions. G) Myeloid gene expression changes in response to CPN: inflammatory activation and angiogenic-immunosuppressive pathways. NT: Non Treated; CPN: Carboplatin-Pemetrexed-Nivolumab

CONCLUSION

- We developed an advanced NSCLC-derived 3D co-culture model, progressively enriched with PBMCs, with increased myeloid infiltration over time, to mimic the recruitment of immune populations within the TME.
- In-depth longitudinal characterization uncovers a spatially structured tumor microenvironment featuring distinct stromal and immune niches alongside marked subpopulation heterogeneity within MCTS, establishing it as a powerful tool for assessing treatment effects on both tumor and TME cells.
- Chemo-immunotherapy revealed increased DNA and structural damage within MCTS, driving reduced mesenchymal properties of tumor subpopulations. CPN treatment also downregulated immunosuppressive TAM clusters while upregulating inflammatory-related genes.
- This work opens perspectives for the declination of this responsive model, into a "cold" model with high macrophage infiltration and resistance to treatment. These models will enable the identification of new targets and biomarkers associated with NSCLC, crucial to establish new strategies against chemo-ICIs resistance.