Beyond 2D cultures: Establishment of Lung Cancer organoids and Ex Vivo explant for enhanced preclinical therapeutic screening

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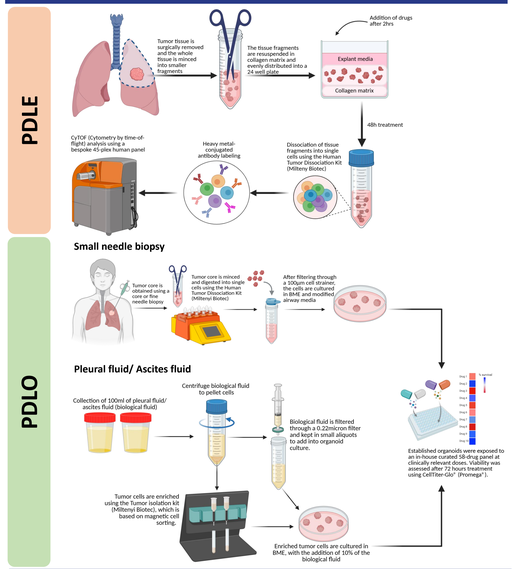
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INTRODUCTION

- Clinical efficacy of immune checkpoint blockade (ICB) and tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer (NSCLC) is limited by heterogeneous patient responses and the emergence of acquired resistance.
- Conventional 2D cultures is unable to recapitulate the spatial and cellular complexity inherent in the tumor microenvironment (TME).
- We have established 2 in vitro models to address these limitations.
 Model 1: Patient-derived lung explant (PDLE)
 Model 2: Patient-derived lung organoids (PDLO)

METHODS



SUMMARY

Our work demonstrates the establishment of robust ex vivo models that effectively recapitulate the spatial and cellular complexity inherent in lung cancer. PDLEs serve as a rapid system to interrogate early immune TME remodelling and investigate synergistic drug effects, while PDLOs provide a scalable model for drug screen and resistance profiling. Collectively, these models provide a mechanistic rationale for clinical treatment strategies and a pathway toward personalized oncology beyond traditional 2D cultures.

FUTURE WORK: **We currently have biobanked 9 PDLE tissues for future perturbation studies and will expand on CyTOF profiling for more samples. With further analysis, the panel will be refined to 10 robust markers for in-house flow cytometry analysis. This allows for early prediction of drug response and real time clinical intervention.**

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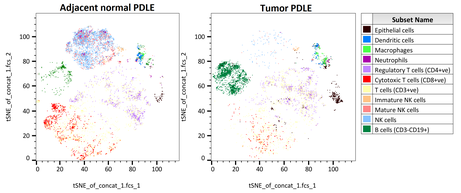
RESULTS

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Model 1: Patient-derived lung explant

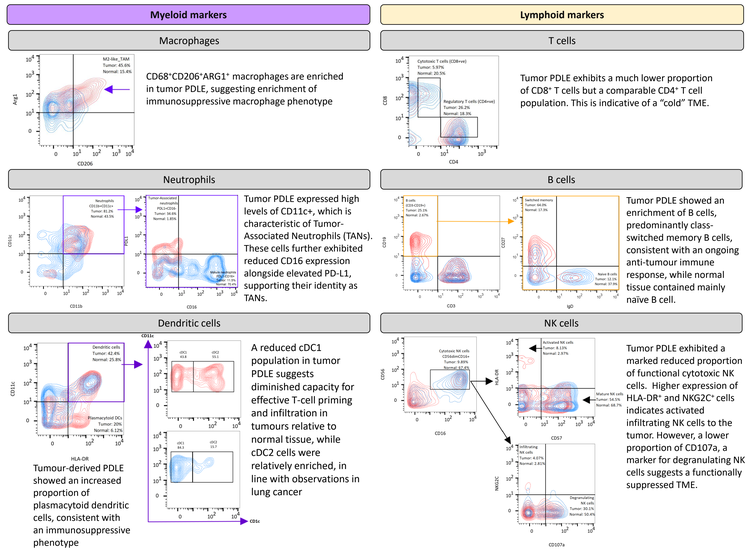
Tumor background: EGFR ex19del, MET amplified treatment naïve
Treatment condition: Osimertinib, crizotinib and combination therapy

(A) t-SNE visualization of single-cell CyTOF data showing major immune cell subsets in paired adjacent normal and tumor PDLE. Differences in distribution reflect tumour-associated remodelling of the immune compartment and demonstrates that the PDLE model captures key features of the TME.

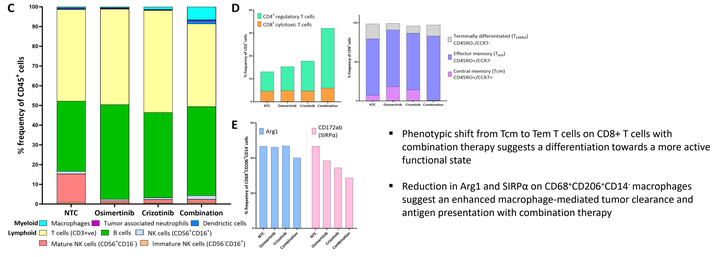


Each point on t-SNE plot represents an individual cell, with clustering based on the expression of the indicated markers. Colors denote annotated cell populations as defined by canonical marker expression.

(B) Density plot overlays of key myeloid and lymphoid markers in adjacent normal (BLUE) and tumor (RED) PDLE. Data demonstrates that the PDLE model captures key features of the TME under near-physiological conditions



(C) Stacked barplot for both myeloid and lymphoid cell fraction across treatment group. Further analysis of the T cell (D) and macrophage population (E) suggests a shift from an immunosuppressive phenotype to a more permissive environment for T cell activation and macrophage mediated tumor clearance with combination therapy.



- Phenotypic shift from Tcm to Tem T cells on CD8+ T cells with combination therapy suggests a differentiation towards a more active functional state
- Reduction in Arg1 and SIRPα on CD68+CD206+CD14- macrophages suggest an enhanced macrophage-mediated tumor clearance and antigen presentation with combination therapy

Model 2: Patient-derived lung organoids (Small needle biopsy)

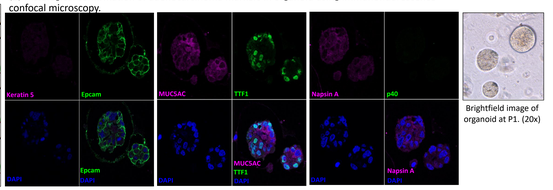
Tumor profile: EGFR ex19del (L747_S752del), TP53 D208V, Adenocarcinoma, EGFR TKI resistant

Patient tumor profiled with panel-based sequencing was positive for EGFR Ex19del (L747_S752del). Sanger sequencing of PDO demonstrated consistent homozygous EGFR ex19del at multiple passages, confirming stable retention of mutant tumor cells (A). Adenocarcinoma markers (TTF1 and Napsin A) was confirmed using immunofluorescence confocal microscopy (B).

(A) Sanger sequencing at P3, P5 and P9



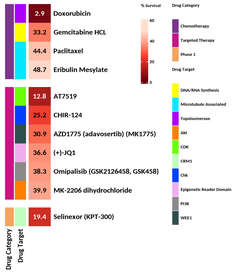
(B) Histological confirmation of adenocarcinoma markers in organoid using immunofluorescence confocal microscopy.



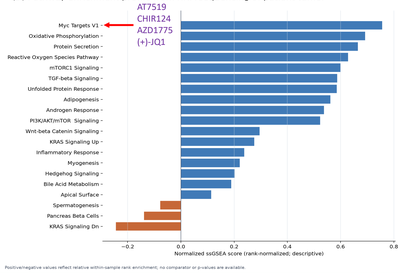
Brightfield image of organoid at P1. (20x)

The organoids was treated with an in-house curated 58 drug screen panel at clinically relevant doses. Results shows sensitivity to chemotherapy drugs, cell cycle checkpoint inhibitors, PI3K and Akt inhibitors (C). This is in concordance with the pathway enrichment profile of the patient tumor which shows an enrichment in Myc targets, indicative of hyper-proliferation and abnormal checkpoint controls (D).

(C) Drug screen



(D) Pathway enrichment profile from RNA sequencing of patient tumor



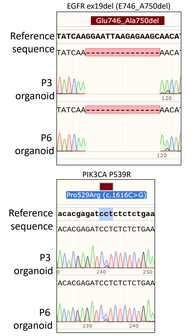
Positive/negative values reflect relative within-sample rank enrichment; no comparator or p-values are available.

Model 2: Patient-derived lung organoids (Ascites fluid)

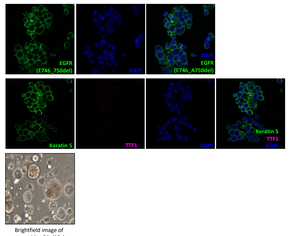
Tumor profile: EGFR ex19del (E746_A750del), PIK3CA P529R, TP53 R249T, Squamous cell carcinoma (SCC), EGFR TKI resistant

Patient tumor profiled with panel-based sequencing was positive for EGFR Ex19del (E746_A750del). Sanger sequencing of PDO demonstrated consistent homozygous EGFR ex19del and heterozygous PIK3CA P529R mutation at multiple passages, confirming stable retention of mutant tumor cells (E). SCC marker (Keratin 5) and the EGFR ex19del was confirmed using immunofluorescence confocal microscopy and the organoid is negative for adenocarcinoma marker (TTF1) (F). Organoids were treated with an in-house curated 58 drug panel screen and results reveals sensitivity to chemotherapy, checkpoint inhibitor and PI3K inhibitors.

(E) Sanger sequencing of organoids at P3 and P6

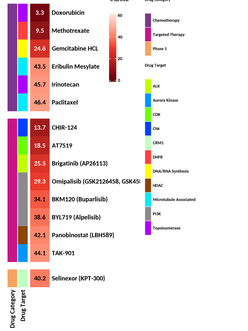


(F) Histological confirmation of SCC marker and EGFR ex19del in organoid using immunofluorescence confocal microscopy.



Brightfield image of organoid at P1. (10x)

(G) Drug screen identifies sensitivity to chemotherapy, checkpoint inhibitors and PI3K inhibitors.



Average turn around time from collection of tumor tissue or biological fluids to drug screen is about 12 weeks. Drug screening results will complement molecular profiling as an integrated readout for Molecular Tumor Board review.