P3.391: Development and optimisation of a patient-derived organoid T cell co-culture model to study tumour-immune interactions in pleural malignancies

Lucy Franzon[1], Zivile Useckaite[1], Ashleigh Hocking[1], Yazad Irani[1], Lauren Mortimer[1], Arya Layla Arul[1], Giles Best[1], Sarita Prabhakaran[1], Sonja Klebe[1, 2]
[1]*Flinders University, College of Medicine and Public Health, Flinders Health and Medical Research Institute, Adelaide, South Australia,* [2]*SA Pathology, Adelaide, South Australia*
Correspondence: *fran0606@flinders.edu.au*

IASLC 2026 World Conference on Lung Cancer
SEPTEMBER 12 – 15, 2026
SEOUL, REPUBLIC OF KOREA

INTRODUCTION

Current Challenge: Despite advances in immunotherapy, pleural malignancies remain associated with poor prognosis and treatment response cannot currently be predicted using existing biomarkers.
Unmet Need: There is a critical need for physiologically relevant preclinical models that accurately capture tumour–immune interactions within the pleural tumour microenvironment. Current patient-derived organoid (PDO) models inadequately incorporate immune and stromal components, limiting their translational value.
Aim: This study developed and optimised a pleural effusion- and tissue-derived PDO–T cell co-culture platform and evaluated the impact of stromal-derived factors on tumour metabolism to establish a robust model for functional immunotherapy research.

METHODS

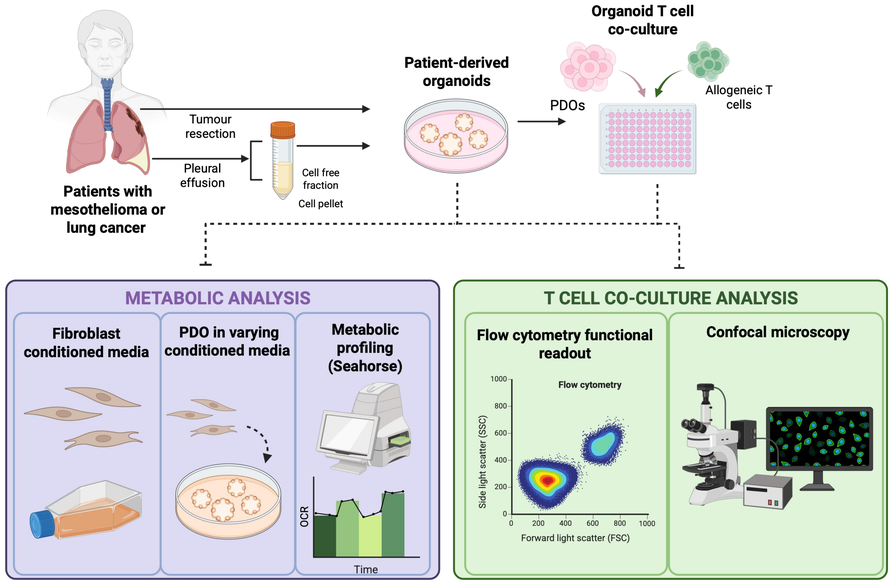


Figure 1: Experimental workflow for the development and optimisation of a PDO–T cell co-culture model. PDOs were established from pleural effusions or lung tissue collected from patients with mesothelioma or lung cancer. PDOs were co-cultured with activated allogeneic T cells and assessed using flow cytometry and confocal microscopy. To investigate the influence of the tumour microenvironment, PDOs were cultured in cancer associated fibroblast-conditioned media (FCM) and analysed using an Agilent Seahorse XF Analyzer extracellular flux assays to evaluate metabolic changes.

REFERENCES & ACKNOWLEDGEMENTS

This research is supported by a Medical Research Future Fund grant, Icare Discovery grant and a Tour de Cure grant. We acknowledge the Anatomical Pathology Staff (SA Pathology, Flinders Medical Centre) for support in cytology assistance. We thank the patients and families for their samples and contribution.

RESULTS

	FCM 100%	FCM 80% / Media 20%	FCM 50% / Media 50%	FCM 20% / Media 80%	FCM 10% / Media 90%	Media 100%
Lung cancer tissue PDO	✓	✓	✓	✓	✓	✓
Lung cancer pleural effusion PDO	✗	✗	✗	?	✓	✗
Mesothelioma pleural effusion PDO	✗	✗	✗	✗	✓	✗

LEGEND	
✓	**Excellent (Metabolically Robust)** media was characterised by stable baseline readings, strong ATP coupling efficiency and a high Spare Respiratory Capacity (SRC). Cells were healthy, unstressed and highly adaptable to energetic demands.
?	**Moderate (Metabolically Constrained)** characterised by adequate basal respiration but a reduced SRC and slight baseline drift. Cells were surviving and functioning but lack the metabolic flexibility to handle acute physiological stress.
✗	**Unsuitable (Toxic or Exhaustive)** characterised by a depleted SRC (cells are operating at maximum capacity just to survive at baseline), high proton leak (uncoupled mitochondria), or declining baselines indicating starvation, severe stress, or apoptosis.

Table 1: Bioenergetic optimisation of PDO co-culture media. Seahorse XF analysis assessed PDO metabolism across varying ratios of standard and fibroblast-conditioned media. Red indicates unsuitable, orange acceptable, and green optimal bioenergetic performance.

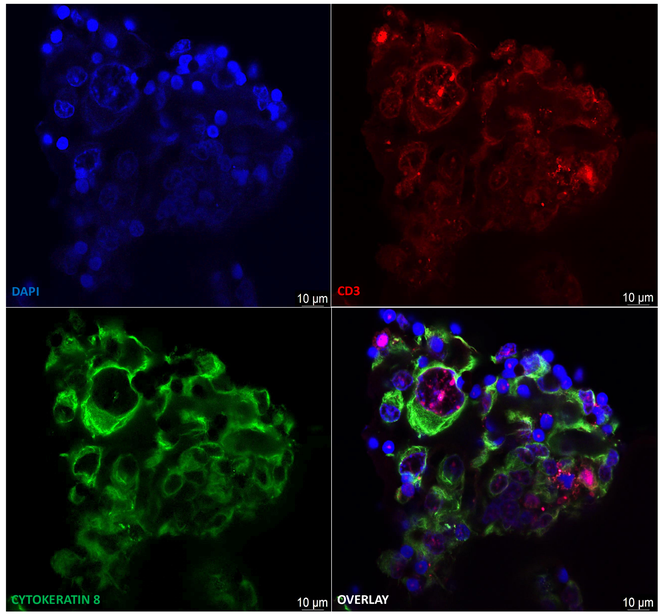


Figure 2: Representative immunofluorescence images of PDO–T-cell co-culture. Nuclei were labelled with DAPI (blue), T cells with CD3 (red) and tumour organoids with cytokeratin 8 (green). The merged image demonstrates direct spatial interaction between T cells and tumour organoids. Scale bars = 10 µm.

DISCUSSION AND CONCLUSION

- Experimental conditions strongly influenced PDO behaviour, highlighting the importance of optimising culture format, media composition and co-culture conditions.
- FCM altered tumour metabolism, demonstrating that stromal-derived factors significantly influence tumour-cell function and should be incorporated when modelling the tumour microenvironment.
- **Take-home message:** Co-cultures using both pleural effusion- and tissue-derived PDOs provide a clinically relevant model for investigating tumour–immune interactions and evaluating responses to immunotherapy.
- Optimisation of PDO-immune co-culture conditions is essential, as changes in culture format, media composition and microenvironmental factors could directly influence assay outcomes and biological interpretation.
- **Future direction:** Validation in larger patient cohorts, together with comparison against clinical treatment outcomes, could determine whether PDO–T cell co-cultures can predict immunotherapy response and support personalised treatment decisions.

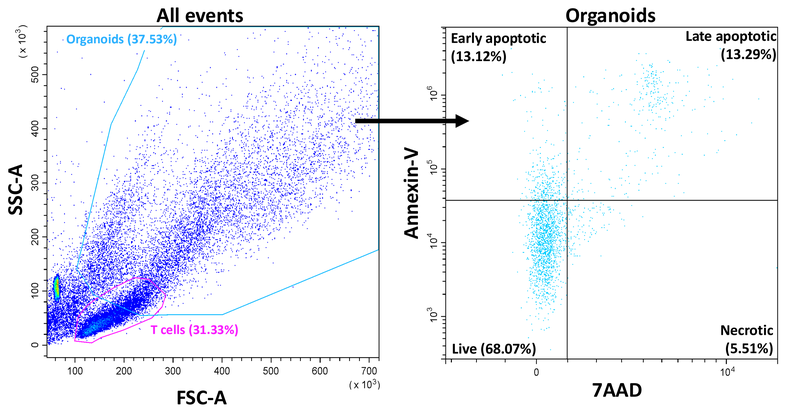


Figure 3: Representative flow cytometry gating strategy for assessment of organoid viability following PDO–T-cell co-culture. Organoid singlets were identified, T cells were excluded and organoid viability was assessed using Annexin V and 7-AAD staining.