CAM-on-MoLBi: A Rapid and Scalable In Ovo Platform for Translational Cancer Modelling

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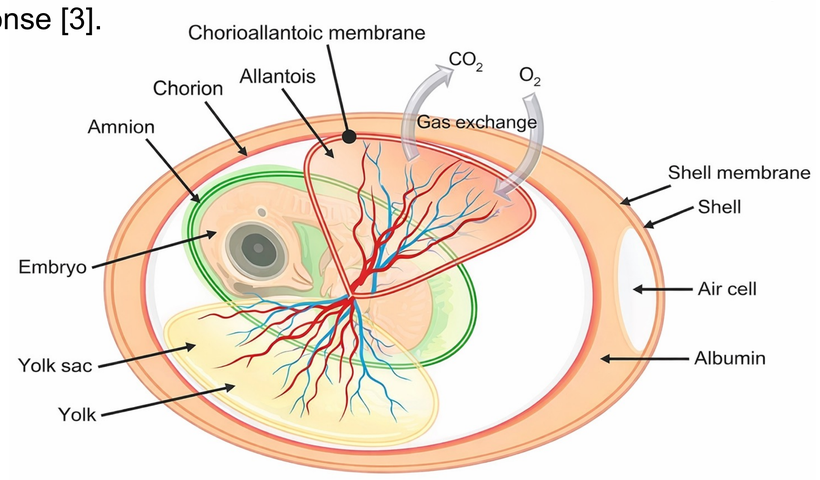
CAM-on-MoLBi provides a rapid, low-cost in vivo bridge between patient tissue and PDX, suitable for molecular and immune interrogation within weeks. The chick chorioallantoic membrane (CAM) assay is a highly vascularised, immunologically permissive, and cost-effective in vivo model for tumour engraftment [1,2]. Traditionally used in developmental biology and angiogenesis research, the CAM model is now emerging as a valuable translational tool for cancer biology. To complement patient-derived xenografts (PDX) and expand the translational capacity of the Monash Live-Biobanking (MoLBi) platform, we have established the CAM model as a rapid and scalable preclinical system to evaluate tumour growth kinetics, vascular recruitment, immune-cell interactions, and therapeutic response [3].

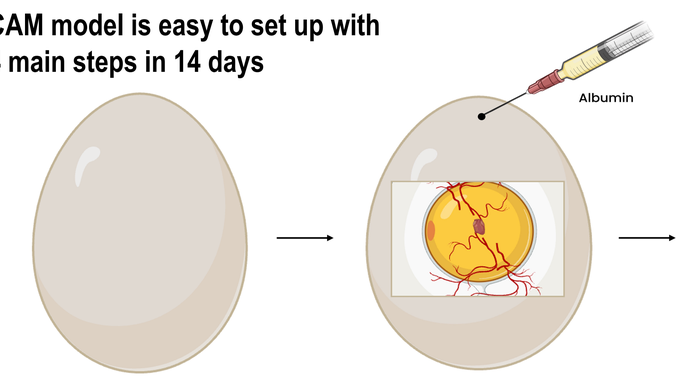


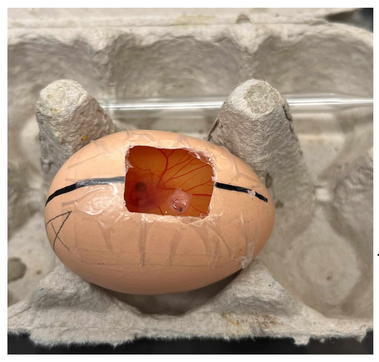
The chick chorioallantoic membrane can supports rapid in vivo engraftment and growth of human tumours.

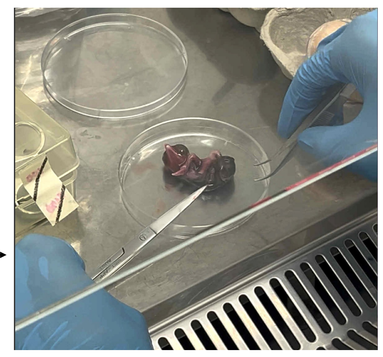
Methods:

Fertilised specific pathogen-free (SPF) chicken eggs were incubated under controlled conditions (37.5 °C, 60–70% humidity), and shell windows were created at embryonic day (ED) 3. Fresh tumour fragments or dissociated cells - primarily glioblastoma (GBM) and renal cell carcinoma - derived from MoLBi biospecimens were grafted onto the CAM at ED 7 near major vascular bifurcations. Co-engraftment experiments were conducted using HLA-matched human $CD8^+$ T cells to examine tumour–immune dynamics. Tumours were monitored for growth by weight and morphological changes and harvested at ED 14–17 for histological and genomic analysis.

CAM model is easy to set up with 4 main steps in 14 days







Day 0
Incubate fertilised eggs in a non-rotating incubator

Cost: farm egg ($2.5/ea.) or SPF-certified egg ($6.5+/ea.)

Day 3
Remove 4-5ml of albumin and cut a window open to access the CAM

Cover with a piece of transparent semi-permeable membrane

Day 7
Break the membrane surface tension using a pipette tip to allow engraftment of tumour – preferably near bi-furcation of large blood vessel

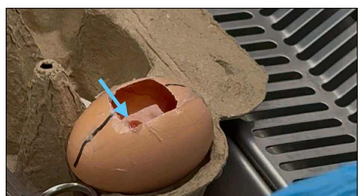
Drugs or immune cells can be also be administer – on a filter paper as reservoir

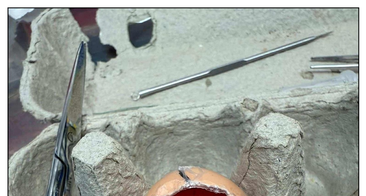
Day 14
Harvest tumour and embryo

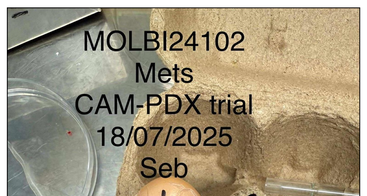
Incubation condition: Humidity: 38%
Temperature: 38 Celsius

Sequential CAM engraftment attempts: lessons learnt

- **Initial engraftment attempt** using fresh MoLBi glioblastoma tissue established feasibility of CAM windowing and tumour placement, but highlighted variability in tumour adherence and early vascular integration.
- **Protocol refinements** focused on optimisation of shell window size, tumour fragment handling, and placement near major vascular bifurcations to improve graft stability.
- **Second engraftment attempt** demonstrated improved tumour attachment, visible vascular recruitment, and reproducible short-term tumour maintenance on the CAM.
- **Key lessons learnt** included the importance of gentle tumour handling, precise graft positioning, and consistent windowing technique, supporting CAM as a learnable and scalable translational platform.







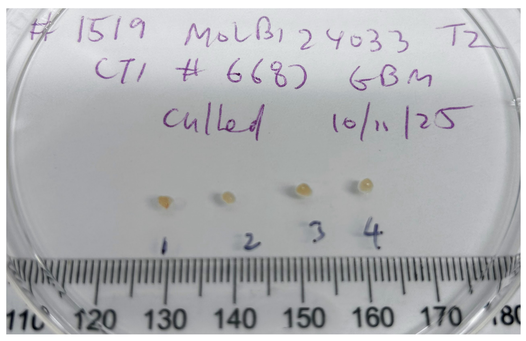
MoLBi24101
31 yr old male
Left temporal IDH wild type high-grade glioma

Tumour weight increased from 20 mg to 40 mg (double in weight)

MoLBi24102
63 yr old male
T2 paraspinal metastatic clear cell renal carcinoma
Engrafted along with human CD8+ T cells

CAM engraftment and tumour harvest (MoLBi24033)

Successful tumour engraftment was achieved in **4/4 CAM assays (n = 4)** using fresh glioblastoma tissue, with rapid vascular integration. Tumours demonstrated short-term in vivo growth, enabling reproducible macroscopic harvest of discrete nodules within days. Histology confirmed preservation of tumour architecture, supporting downstream molecular analyses.

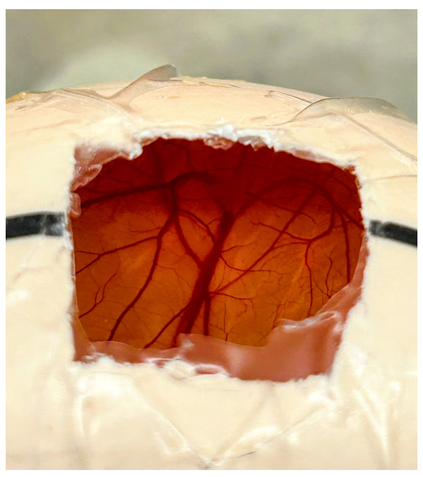


Tumour fragments prior to implantation

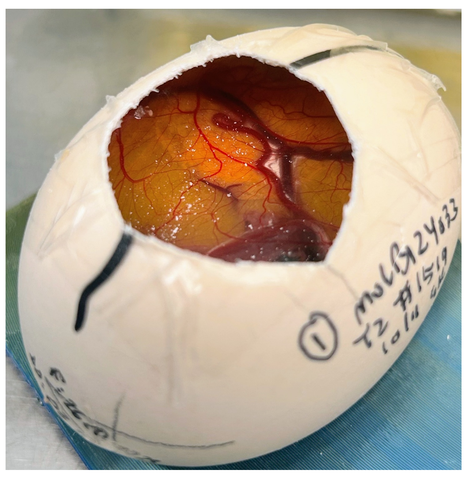
Tumour summary: MoLBi24033 is a primary GBM from an 80-year-old man with TERT promoter mutation c-124C>T, IDH-wildtype, BRAF-negative. He underwent resection in February 2025 and is receiving radiotherapy alone due to comorbidities. The T1 PDX tumour is now large and will be harvested on 18th August

HLA typing:
- *A02:01:01:01, A02:01:01:01*
- *B18:01:01:02, B51:01:01*
- *C07:01:01:01, C07:01:01:01*

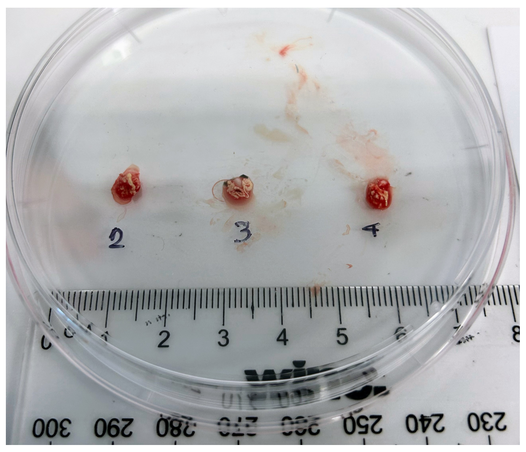
Tumour weight increased consistently from implantation to harvest across all CAM replicates (n = 4).



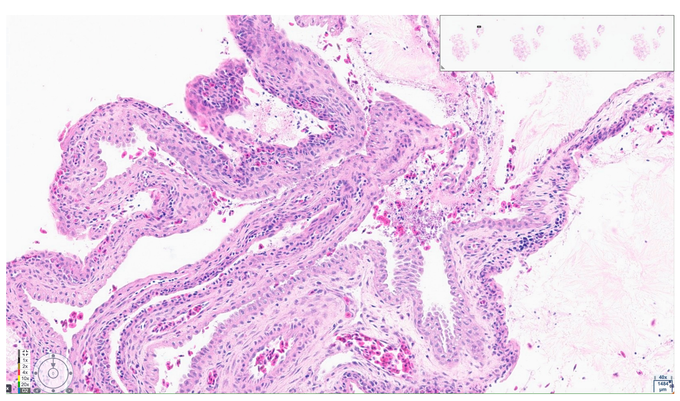
Tumour at implantation on the chorioallantoic membrane



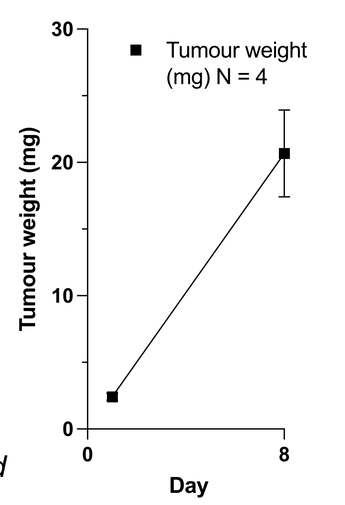
Tumour expansion and vascular recruitment on the CAM on ED8



Discrete tumour nodules recovered at harvest following CAM growth



Representative H&E-stained section of CAM-grown tumour showing preserved architecture and vascular-associated viable tumour tissue.



Key findings and next steps:

1. **Feasibility established:** Fresh patient-derived glioblastoma tissue can be reproducibly engrafted, grown, and harvested on the CAM, with preserved tumour architecture and measurable short-term growth.
2. **Rapid translational readout:** The CAM model enables in vivo tumour assessment within days, providing a fast and cost-effective complement to murine PDX models within the MoLBi platform.
3. **Platform integration:** CAM-grown tumours are suitable for downstream histological and molecular analyses and can be integrated with existing MoLBi genomic and immunopeptidomic pipelines.
4. **Future plans:** Ongoing work will focus on scaling to additional tumour types, bidirectional passaging between CAM and PDX models, and incorporation of immune co-engraftment and therapeutic testing to support precision oncology studies.

References: (1) Chu PY, Koh AP, Antony J, Huang RY. Applications of the Chick Chorioallantoic Membrane as an Alternative Model for Cancer Studies. Cells Tissues Organs. 2022;211(2):222-237. doi: 10.1159/000513039. Epub 2021 Mar 29. PMID: 33780951; PMCID: PMC9153341, (2) Fischer D, Fluegen G, Garcia P, Ghaffari-Tabrizi-Wizsy N, Gribaldo L, Huang RY, Rasche V, Ribatti D, Rousset X, Pinto MT, Viallet J, Wang Y, Schneider-Stock R. The CAM Model-Q&A with Experts. Cancers (Basel). 2022 Dec 28;15(1):191. doi: 10.3390/cancers15010191. PMID: 36612187; PMCID: PMC9818221, (3) https://www.monash.edu/medicine/scs/research-facilities/monash-live-biobanking-molbi-platform