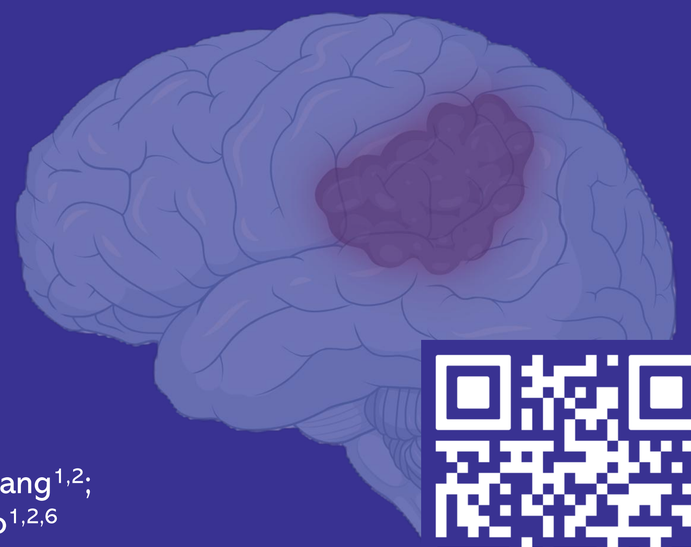
# Establishing a rapid scalable *in ovo* patient-derived chick chorioallantoic membrane (CAM) xenograft platform

## to evaluate PRMT5 inhibition in MTAP-deficient glioblastoma



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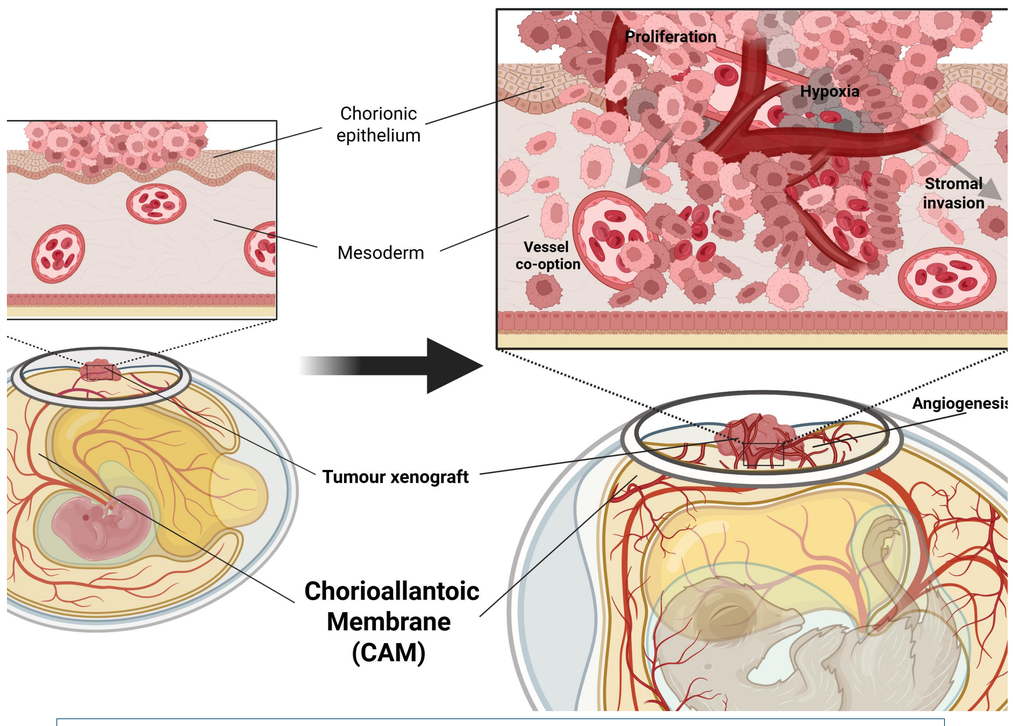
## From Patient to Rapid In Vivo Testing.

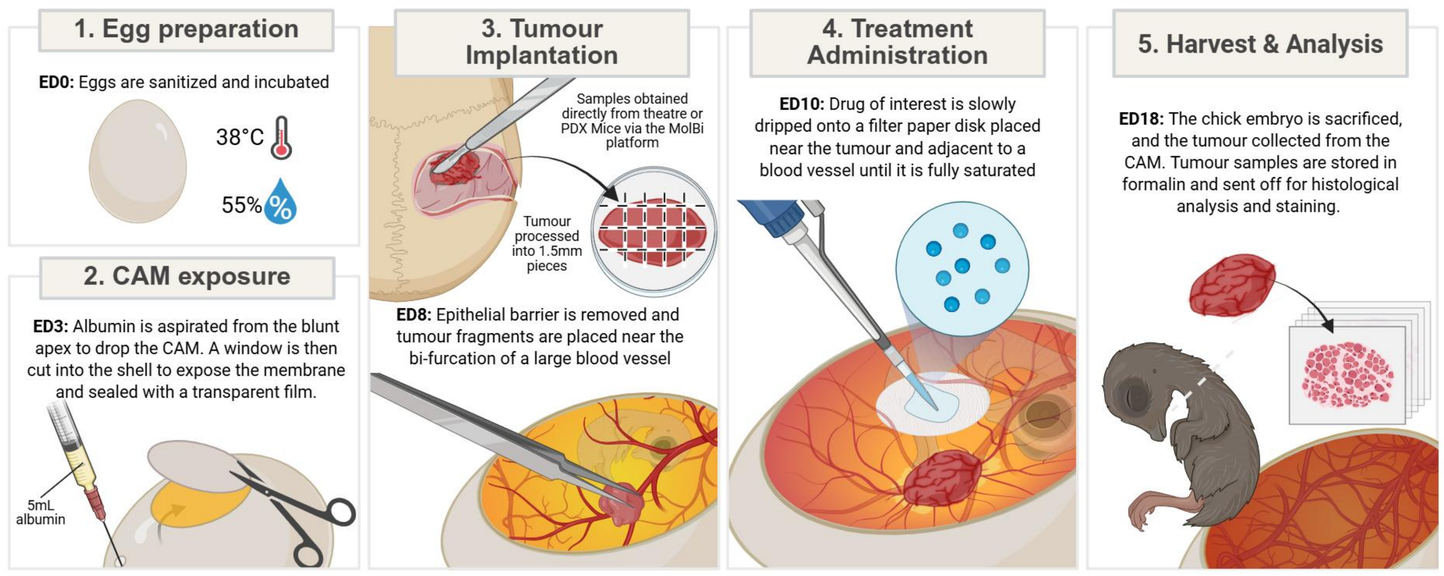
- **Glioblastoma (GBM)** urgently needs rapid, **clinically relevant** models to guide therapeutic decisions.
- **Current gold standard: Patient-derived xenograft (PDX) mouse models**
  - **Months** to grow
  - Low throughput and **expensive**
- Our approach: **CAM–MoLBi**
  - The highly vascularized chick chorioallantoic membrane (CAM) supports rapid engraftment and growth of patient-derived GBM
  - Enables assessment of **tumor growth, vascularisation and treatment response** using both fresh and cryopreserved tumor tissue.
  - **Functional assessment** of individual patient tumors within **weeks**, providing an **early readout of therapeutic response**
  - **Faster, scalable and** complementary to longer-term mouse PDX studies



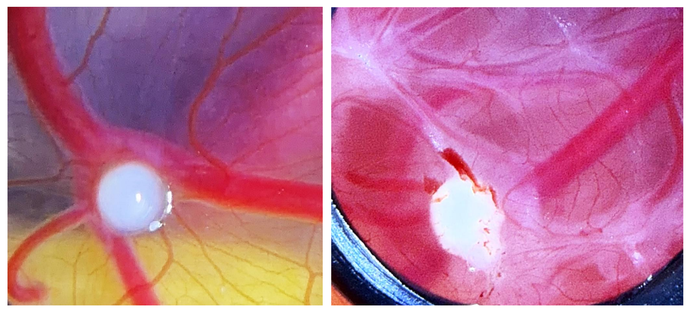

**Patient tumour → MoLBi → CAM → functional therapeutic testing within weeks**

## Building the CAM-on-MoLBi Platform

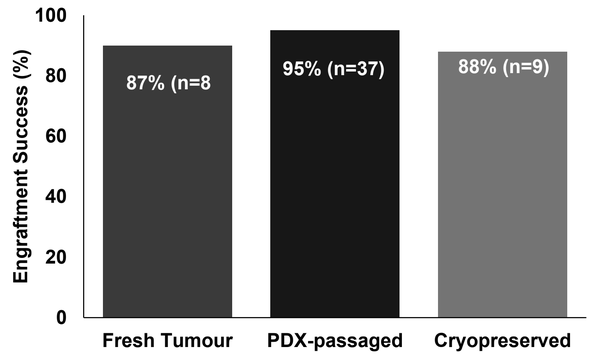
- Patient-derived MTAP-deficient GBM specimens from the Monash Live Biobanking (MoLBi) program were dissected into 1-2 mm fragments and implanted onto the chorioallantoic membrane (CAM) of fertilised specific pathogen free (SPF) chicken embryos at embryonic day (ED) 8. Xenografts were serially imaged to monitor engraftment and tumour growth.
- To investigate the model's capacity to assess drug response, engrafted tumours received cisplatin (400–1000 µM; 10 µL) or vehicle control at ED15. Treatment response was assessed by change in relative tumour area, with embryo viability monitored throughout. Tumours were harvested at ED18 for endpoint and histological assessment.




## High-Fidelity Engraftment



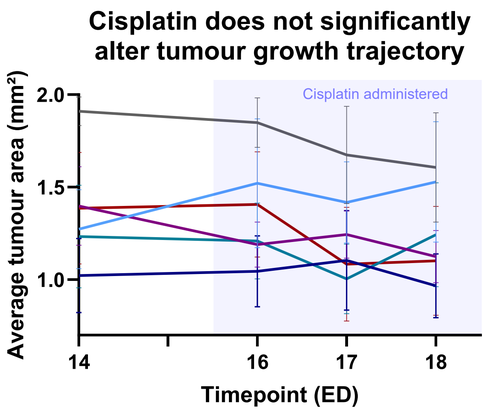
**The CAM model supports tumour survival with high engraftment rates**



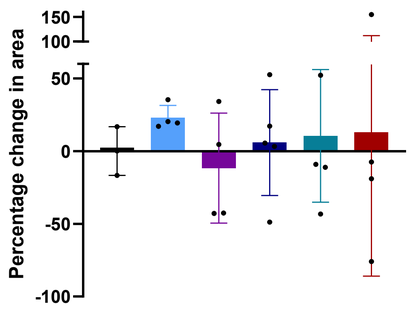

The CAM model achieves **robust engraftment success** (87–95%) across fresh, PDX-passaged, and cryopreserved tumor tissues.

## Functional drug-response data within weeks

**Cisplatin does not significantly alter tumour growth trajectory**



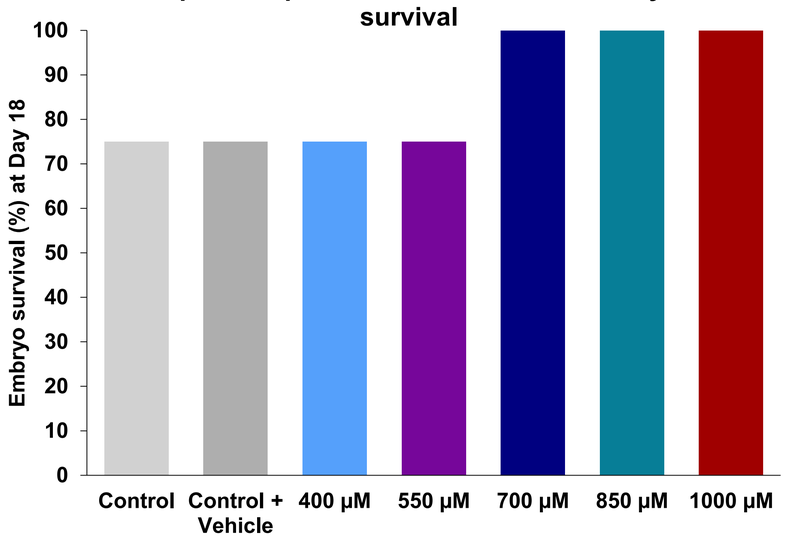

**Cisplatin does not significantly alter final tumor graft area**




Both **longitudinal tracking** and **endpoint percentage analysis** confirm that cisplatin administration does not significantly alter tumour growth trajectories or final graft area.

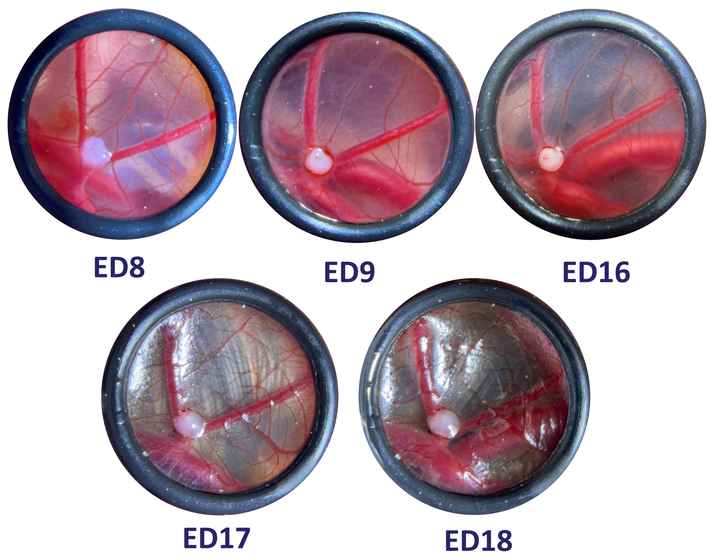
## Robust Platform for Drug Screening

**Cisplatin exposure does not reduce embryo survival**




- Embryos exposed to the canonical chemotherapeutic cisplatin (400–1000 µM) over 18 days showed **no significant decrease in viability** compared to vehicle and untreated controls.
- The established model **successfully tolerates** administration of highly cytotoxic compounds without background embryo death.

## Revolutionizing Biobanking




**Cryopreserved** patient-derived GBM tissue was successfully implanted and engrafted in the CAM.

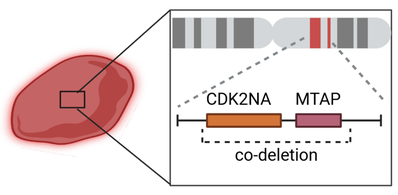
- Demonstrates the feasibility of using **biobanked tumour tissue** for CAM-based functional testing
  - **Overcomes the logistical constraints** of fresh tissue
  - Samples can be collected directly from surgery to be tested **retrospectively**.

## Ongoing in vivo efficacy trials using the CAM platform enable investigation of promising novel treatments

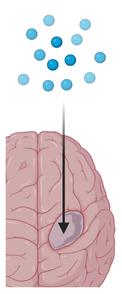
### PEP08: a next-generation PRMT5 inhibitor targeting MTAP-deficient tumors

**MTAP (methylthioadenosine phosphorylase)** is frequently **lost in GBM**

- Leads to the **accumulation** of its substrate **Methylthioadenosine (MTA)**
- Causes an **increased dependence** of tumour cells on PRMT5, an enzyme involved in gene regulation and RNA processing.
- This creates a **promising therapeutic vulnerability** in MTAP-deficient tumours.




**MTAP loss**
↓
**PRMT5 dependency**
↓
**PEP08**
*MTA-cooperative PRMT5 inhibition*
↓
**Selective tumour cell inhibition**



**PEP08: A next-generation PRMT5 inhibitor**

- **Preferentially** inhibits PRMT5 in MTAP-deficient tumour cells while **limiting effects** on cells that retain MTAP.
- Crucially, **previous PRMT5 inhibitors have failed clinically** because reductionist in vitro models **cannot recapitulate** the complex metabolic and stromal dynamics of GBM, highlighting the need for physiologically relevant models to evaluate emerging agents
- Our CAM platform provides a **rapid in vivo system** to investigate PEP08 in patient-derived MTAP-deficient GBM.

## Translational Conclusions

- The CAM platform provides a **rapid and scalable in vivo system for evaluating patient-derived MTAP-deficient GBM** and accelerating therapeutic translation
- By leveraging the **MoLBi biobank**, fresh and PDX-passaged tumour tissue can be rapidly engrafted and assessed while retaining key tumour characteristics
- This platform will enable investigation of **emerging therapies** such as PEP08, supporting the identification of promising treatment strategies for MTAP-deficient GBM

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