**Understanding the biology of neuroblastoma and testing new targeted therapies using a novel in vivo model: the chick embryo**

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Introduction : Neuroblastoma (NB) is the most common extra-cranial solid tumour of childhood, with 50% mortality in high-risk cases. Successful treatment requires therapies that block metastasis and promote differentiation. We use the chick embryo as a preclinical model to test new therapies.

Study design : Experimentation in chick embryos of this age does not require Home Office licence. Fluorescently labelled NB cells, representing different genotypes, were placed onto the chorio-amniotic membrane of the embryo. Tumours are allowed to develop and retinoic acid (ATRA), CDK4/6 and CDK1 inhibitor therapies were injected into the allantoic sac. The ability of drugs to reduce metastasis was determined by dissecting the tumours. Tumours were assessed by their morphology and expression of proliferation markers (Ki67).

Pilot data : Preliminary testing showed all therapies reduced the number of NB tumours formed. Confocal microscopy revealed that cells treated with retinoic acid exhibited neurite process development and were therefore mor- phologically more differentiated. Ki67 staining of cells indicated that CDK1-i treated cells had significantly lower levels of active proliferation compared to control (p = 0.01).

Forward plan: Rigorous testing of new chemotherapeutic agents is essential prior to clinical trials in children. Mouse models are expensive and have a low throughput. We have developed the chick embryo as a validated and inexpensive model suitable for high throughput screening in which biochemical markers and tumour morphology can be assessed. Future plans involve testing a range of drugs and introducing combination therapies. Live imaging will allow us to further understand the effects of the pharmotherapeutics on NB cells.

Take-home message : High grade neuroblastoma remains a difficult to treat condition with a mortality rate of over 50%. Screening of novel drugs in the chick embryo model is a fast and cost-effective method of understand- ing metastatic pathways of neuroblastoma and elucidating the impact of new chemotherapeutics.