Universitätsmedizin Essen
Klinik für Augenheilkunde
Direktor: Prof. Dr. med. Dr. h.c. N.E. Bechrakis, FEBO

DOG 2023
28.9.–1.10.2023
Estrel Berlin

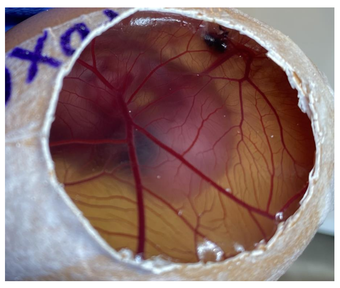
UNIVERSITÄT DUISBURG ESSEN
Offen im Denken

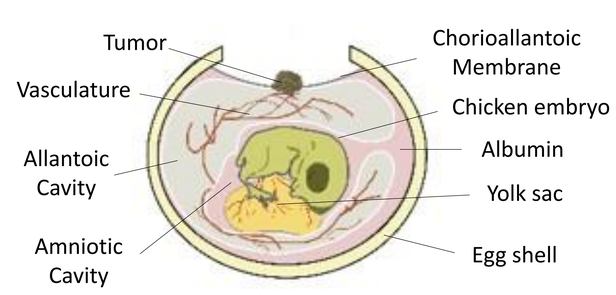
Measurement and imaging modalities for growth and vascularisation in a new patient-derived xenograft model for uveal melanoma based on the chick chorioallantoic membrane assay

T. Tsimpaki[1], R. Anastasova[1], N.E. Bechrakis[1], B. Seitz[2], H. Liu[1], E. Sokolenko[1], S. Dalbah[1], M.M. Krämer[1], U. Berchner-Pfannschmidt[1], M. Fiorentzis[1]
[1] Department of Ophthalmology, Ocular Oncology Research Group, University Hospital Essen, Germany
[2] Department of Opthalmology, Saarland University Medical Center, Homburg, Germany

Background

Uveal melanoma (UM) is the most common primary intraocular tumor in adults. Due to the rarity of UM and its high accompanying metastatic and mortality rate, reliable in vivo models are required for the investigation of the tumor's behavior, its metastatic setting, and the efficacy of novel therapeutic targets.
Patient-derived xenograft (PDX) models offer the possibility of investigating individualized treatments due to the accurate reflection of the complexity of the tumor microenvironment. The chick chorioallantoic membrane (CAM) assay is a highly vascularized extraembryonic membrane that is formed by the partial fusing of the chick's chorion and its allantois during embryonal development and is connected to the embryo through a continuous circulatory system.

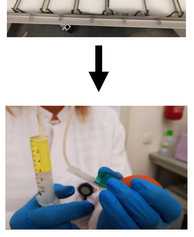




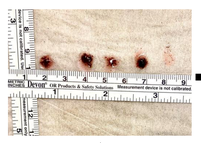
Methods

ED 1-4: Incubation in upright position at 37,7 ◦C and a humidity of 60–70%.

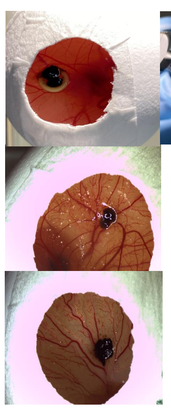
ED 5: Removal of 6-8mL albumin



46 fresh tumor grafts:
- Size, length, weight
- Patient´s molecular pathology

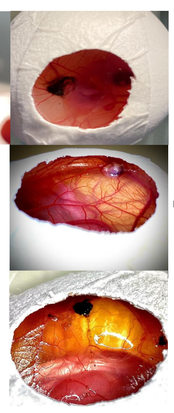


ED 6: Window opening



ED 7: Implantation
- Group 1: Graft+Matrigel +Ring
- Group 2: Graft+Matrigel
- Group 3: Graft alone

ED 8: Ring removal

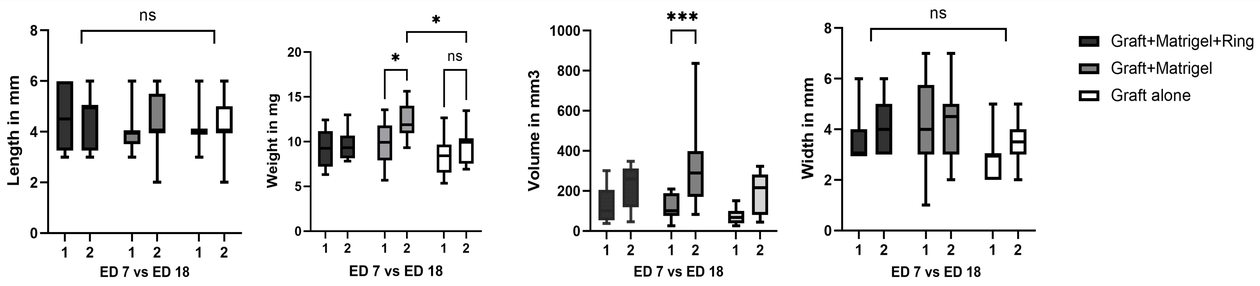


ED 18:
- Tumor growth and extension: various ultrasound modalities, optical coherence tomography (OCT), infrared imaging, Image J
- Angiogenesis: color doppler, optical coherence angiography, fluorescein angiography

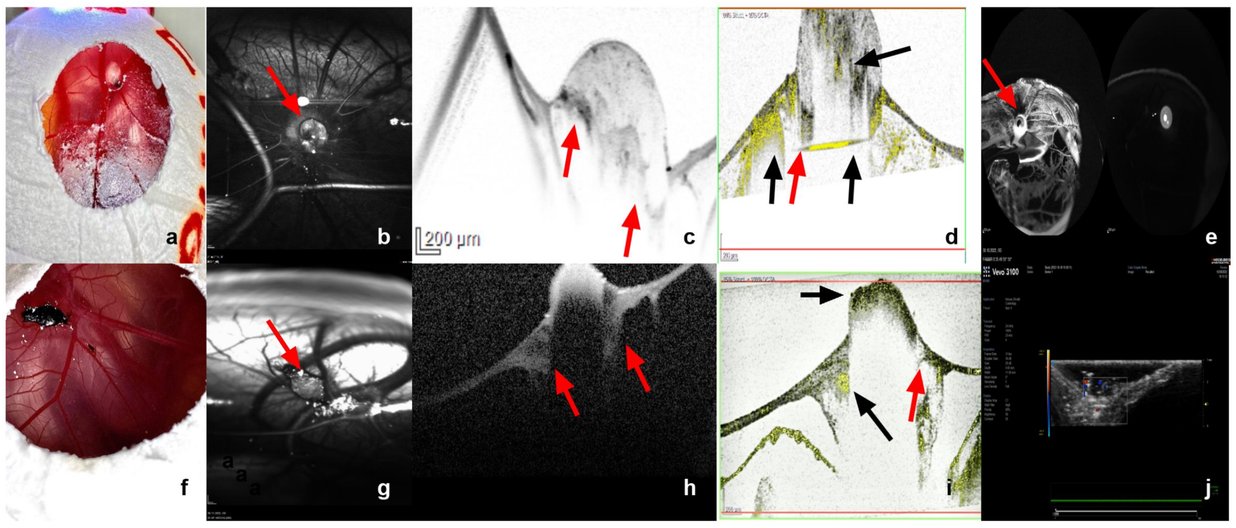
Signs of migration, invasion, extravasation

ED 18:
Harvesting of tumor grafts, lower CAM, liver, lung, eyes, brain:
- H&E Staining
- Ki67, BrdU, LCA, CD31, DAPI, Mel-Mix

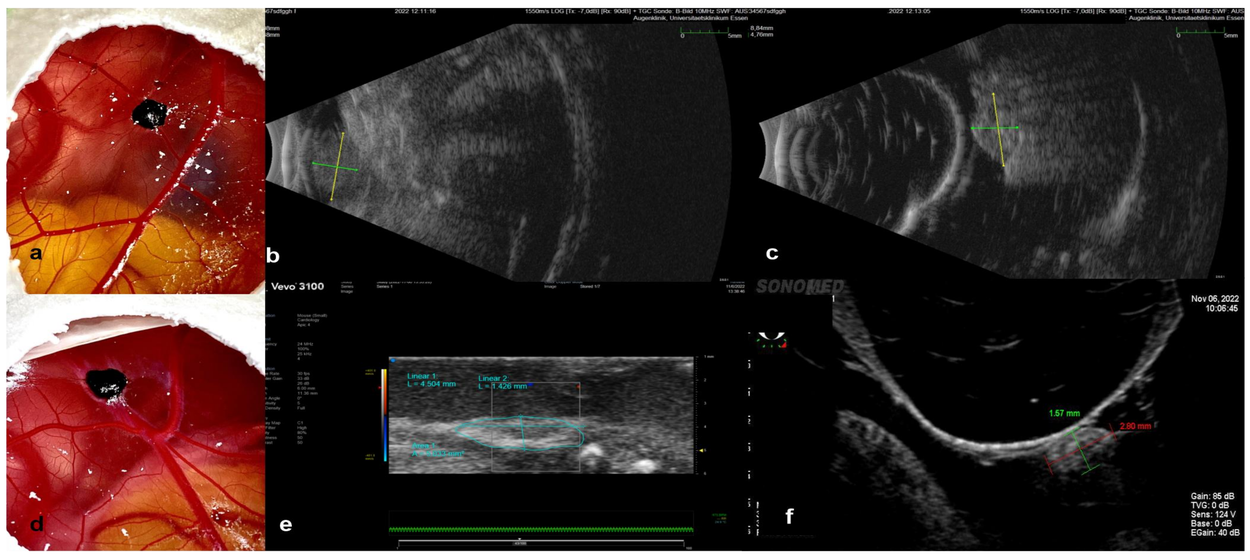
Results



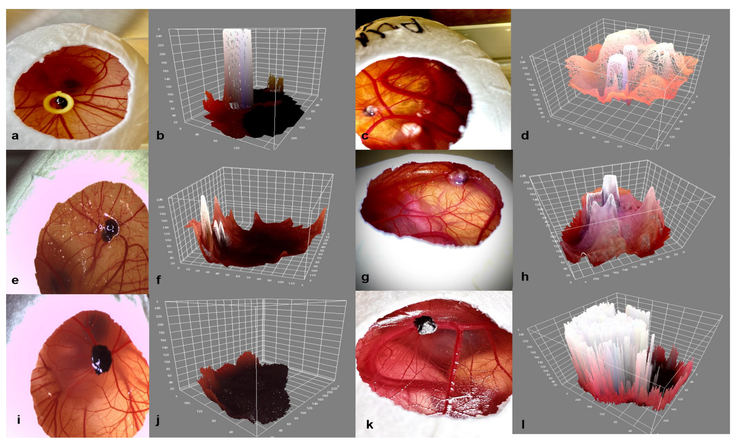
For the characterization of the implanted uveal melanoma patient-derived grafts the length, width, volume and weight between ED 7 and ED 18 as well as among the implantation groups were compared.



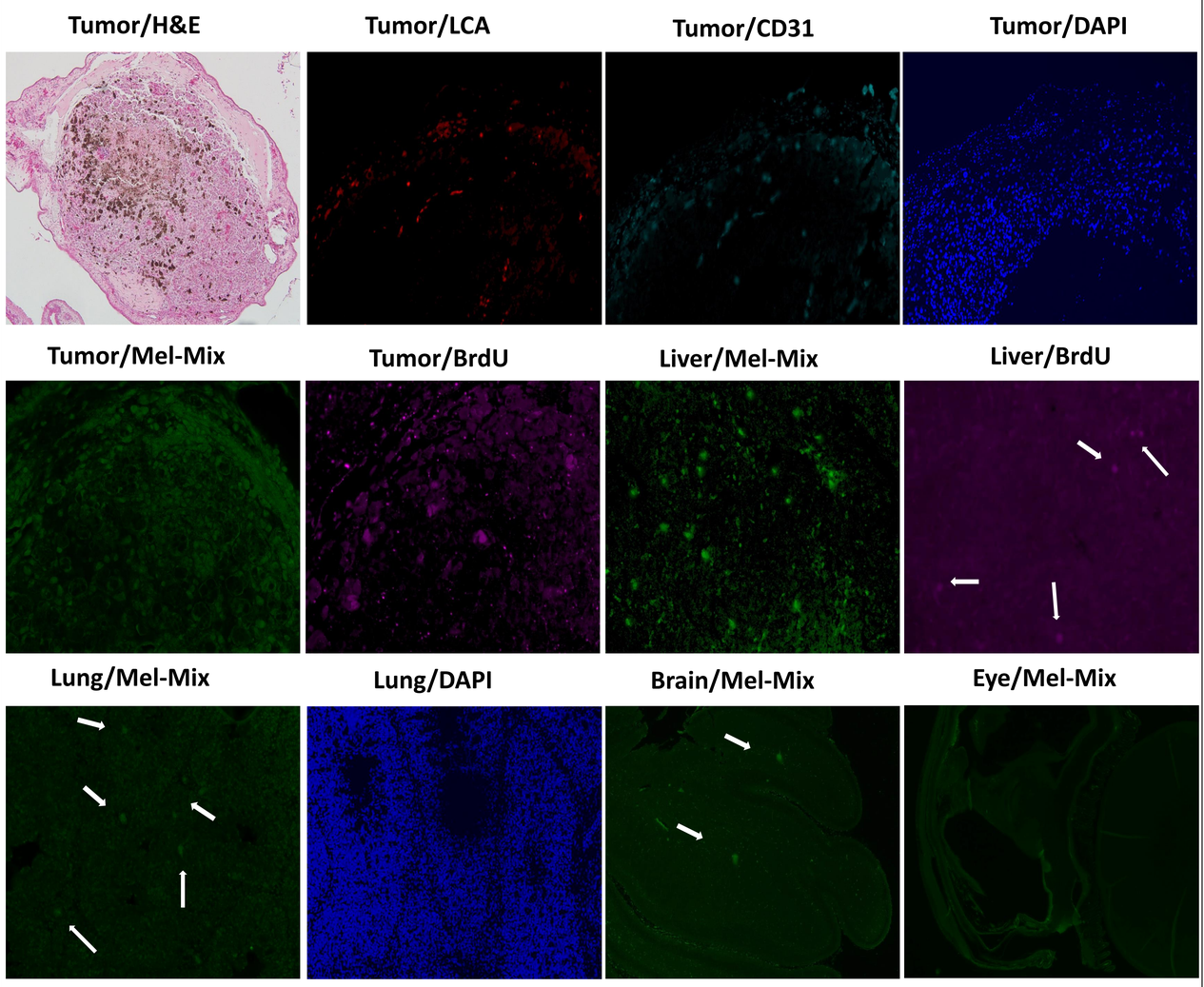
Infrared imaging, OCT and OCT–angiography, fluorescein angiography, as well as color Doppler sonography as monitoring tools for tumor growth and vascularization. a-e: Graft with Matrigel, f-j: Graft alone



Size measurement of the tumor grafts with (a-c) and without (d-f) Matrigel via B-Scan ophthalmic ultrasound with the 10 MHz probe without (b) and with a fingerstall filled with water (c) as well as via ultra-high frequency ultrasound (e) and ultrasound biomicroscopy (f).



The imaging analysis software Image J was used for the calculation of the cross-sectional area, Feret´s diameter as a mass distribution parameter and mean gray value as an indicator of tumor density as well as for the three-dimensional visualization of the tumor grafts.



Expression of markers for melanoma (Melan A, HMB45), angiogenesis (CD31, LCA) and proliferation/viability (BrdU, DAPI) in the grafts. Positive fluorescent staining in liver (premarked BrdU melanoma graft cells, Mel-Mix). Less intense staining with Mel-Mix in lung. Weak signal in brain and non-specific staining in the eye globe.

Summary & Conclusion

We demonstrated the successful establishment of a novel UM-PDX model based on the CAM assay, analyzing different implantation and monitoring techniques. Despite its limitations, especially the short observation period, the chick CAM assay represents a valuable cost- and time-efficient in vivo system, that does not require an approval by an animal experimentation committee and can potentially be used as an intermediate step for the preliminary testing of new drug agents prior to animal experimentation. Incongruences in tumor environments and immune properties are minimized with PDX models, while the high efficiency of PDX engraftment on CAM and the increasing interest in the research community highlight the necessity for precise in vivo monitoring instruments for the evaluation of tumor growth and vascularization in the CAM. Moreover, real-time imaging with multiple comparable modalities expands the applicability of the CAM assay as a PDX model in experimental oncology.

* This research was funded **by Dr. Rolf M. Schwiete Stiftung** and **by Willy Robert Pitzer Stiftung**.
* The ethics committee of the medical faculty of the University Duisburg–Essen approved the study with the number **21-9959-BO**. The research was performed in accordance with the Declaration of Helsinki and with relevant local guidelines and regulations.