Impact of TME-modulation on the efficacy of anticancer agents in a *TUM-CAM* model

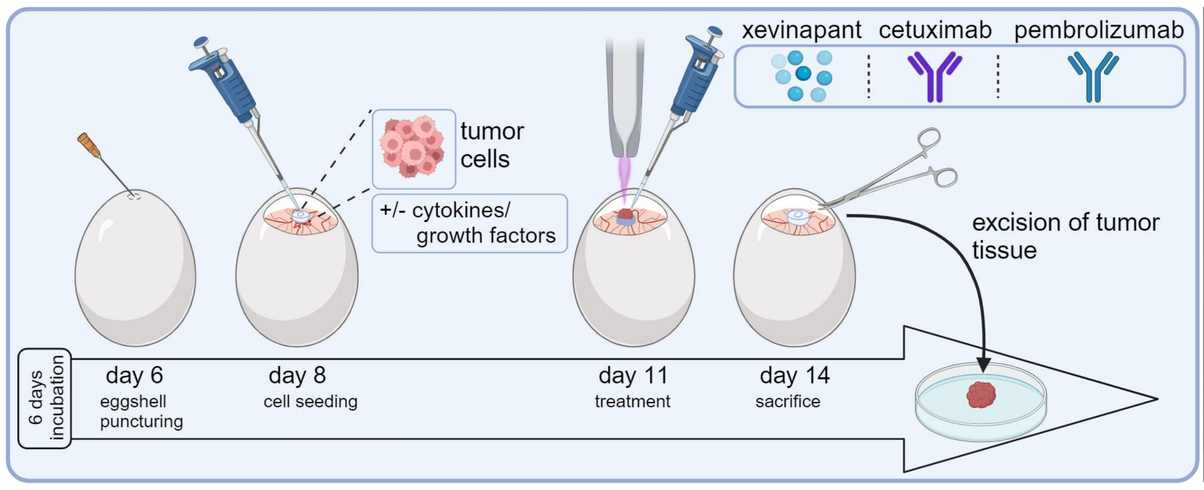
Marvin Schwerdt[1], Julia Berner[1,2], Thomas von Woedtke[1,3], Sander Bekeschus[1,2],
[1]Leibniz Institute for Plasma Science and Technology (INP), Felix-Hausdorff-Straße 2, 17489 Greifswald, Germany
[2]Department of Dermatology and Venereology, Rostock University Medical Center, 18057 Rostock, Germany
[3]Institute for Hygiene and Environmental Medicine, Greifswald University Medicine, 17475 Greifswald, Germany

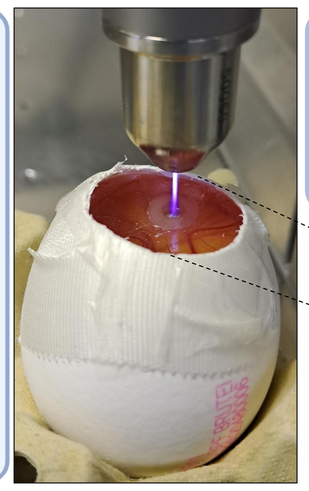
INTRODUCTION

The tumor microenvironment (TME) describes the highly heterogeneous milieu of and around a tumor, which consists of tumor cells, fibroblasts, and different immune cells as well as secretory molecules, extracellular matrix, and blood vessels. The TME plays an essential role for oncogenesis, tumor differentiation, dissemination, and immune evasion. The TME is considered to be the second leading cause for therapeutic failures apart from resistance.

Apparently, common tumor models only partly represent the interactions between tumor cells and the TME (*in vitro* experiments) or are too costly and do not allow high replicate numbers with regard to ethical aspects (*in vivo* experiments). This project addresses this gap by establishment of an *in ovo* tumor model (TUM-CAM) to achieve insights on the interactions between tumor cells and the TME in a clinically relevant setting.

In this project we use the TUM-CAM model to investigate the impact of TME-modulating molecules (VEGF, IL-8, EGF, TGFα) on the anti-cancer treatment success (gas plasma, pembrolizumab, cetuximab, xevinapant) and immunosuppressive capacity of tumor cells, suggesting its prospective use in preclinical drug screenings.





Readouts:

- tumor weight
- flow cytometry
- *ex ovo* imaging
- angiogenesis
- fluorescence measurement (plate reader + stereomicroscopy)
- analysis of secretion profile

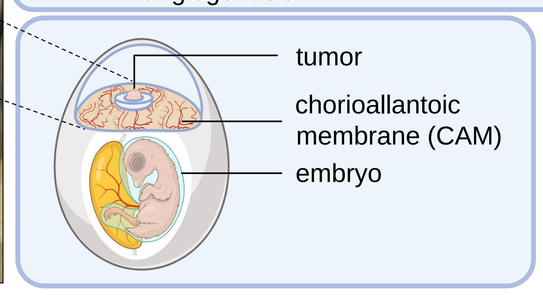
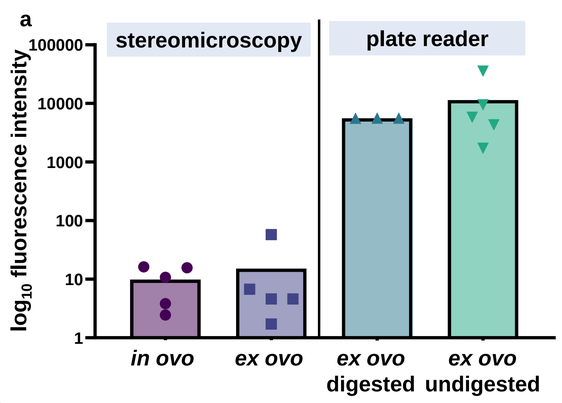


Fig. 1:. Experimental design of the study.

Schematic overview of the TUM-CAM model and top view of the CAM with an engrafted tumor during plasma treatment.

Methods for measuring tumor growth



b

	stereomicroscopy		plate reader	
	in ovo	*ex ovo*	*ex ovo* undigested	*ex ovo* digested
correlation weight/fluorescence	0.1156	0.5803	0.6423	0.6835
standard deviation %	58.97	141.8	108.58	0.27

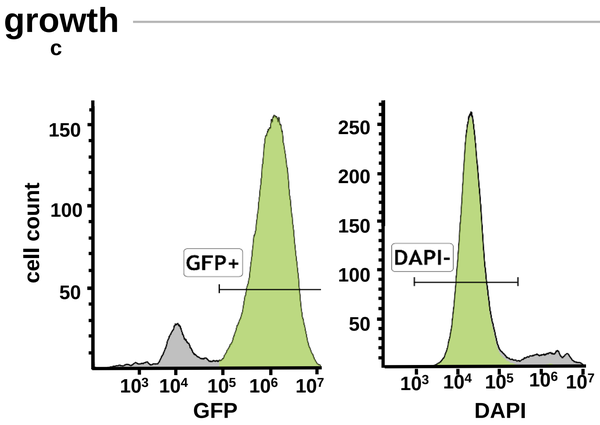


Fig. 2: Comparison of different parameter for GFP-fluorescence-based quantification of tumor growth. (**a**) Fluorescent tumor cells were used in order to quantify tumor growth. Four methods were compared to determine tumor fluorescence intensity and (**b**) checked for their standard deviation and comparability to the measured weight. (**c**) Flow cytometric analysis showed that GFP is an appropriate marker for cell viability, as the GFP$^+$ population was DAPI$^-$ (cell death marker; **d**).

Tissue penetration of topical treatments

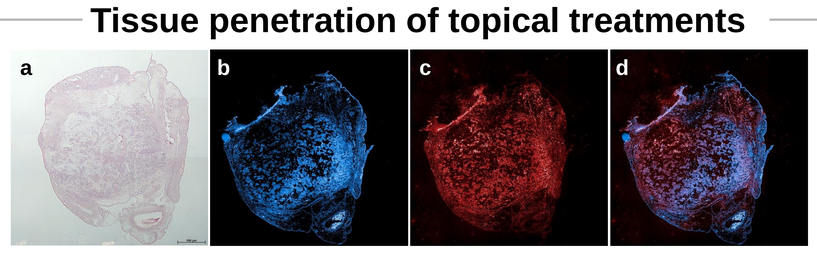


Fig. 3: Histological investigation of drug tissue penetration following topical application. (**a**) HE staining of *in ovo* tumor tissue sections. (**b**) Fluorescence images of DAPI staining, (**c**) BRDU antibody staining and (**d**) an overlay of both fluorescence channels three days following topical application.

TME modulation

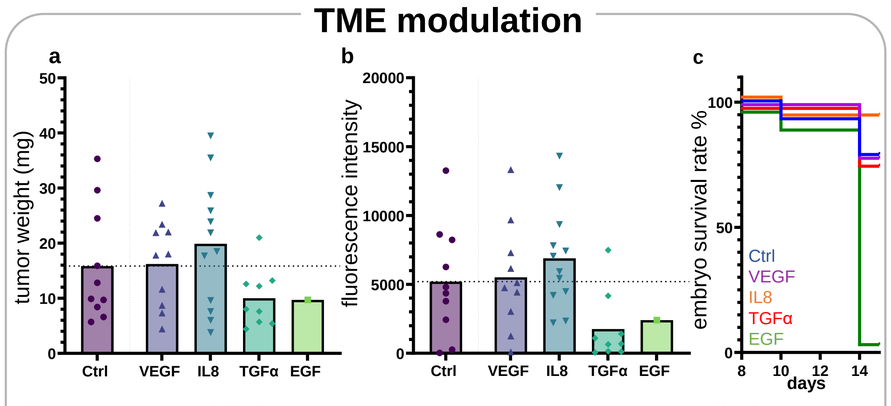


Fig. 4: (**a**) Impact of TME-modulating substances on tumor weight and (**b**) fluorescence intensity of tumors. (**c**) Embryo survival rate following application of TME-modulating substances, revealing high toxicity of EGF.

Conclusion and outlook

- GFP-fluorescence intensity is a suitable marker for tumor growth evaluation
- topical application of drugs ensures total penetration of the whole tumor tissue
- VEGF, as a TME modulator, showed the smallest impact on tumor growth
- VEGF application created an immunosuppressive TME, which reduced the inflammatory profile in response to treatment
- TME modulation had a varying impact on treatment efficacy and survival rate of the embryos

The TUM-CAM model represents a promising model to further elucidate the relation between the TME and antitumor treatment.

Effects of VEGF application on treatment efficacy

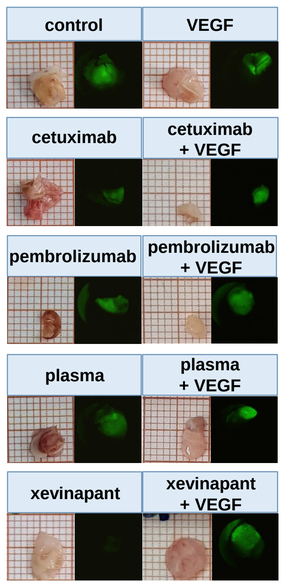


Fig.5: Representative bright field and GFP fluorescence images of excised tumors.

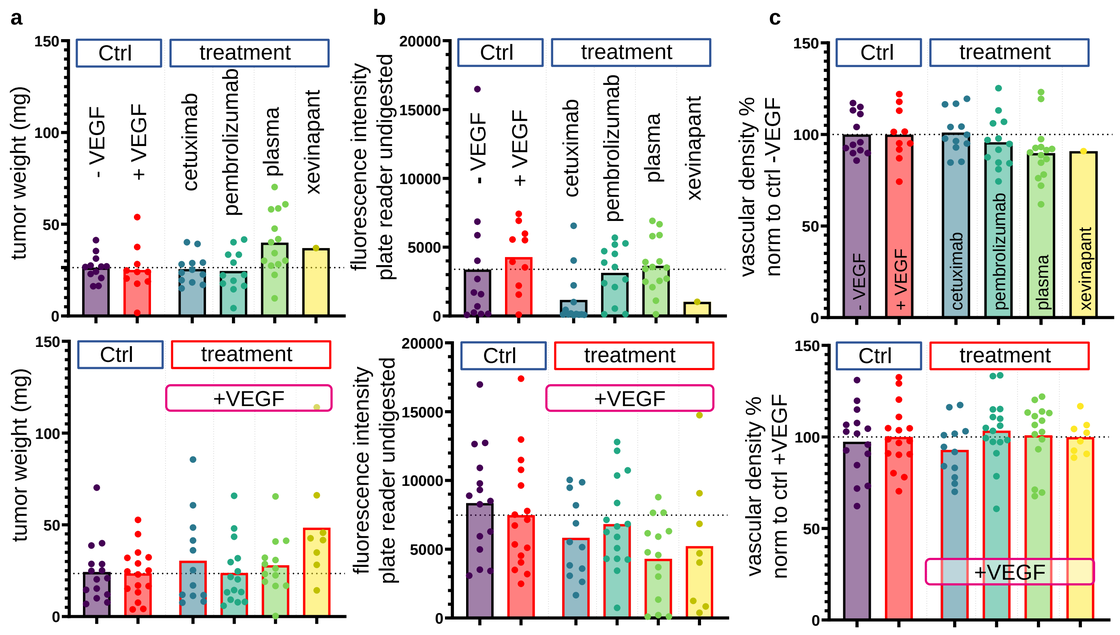


Fig. 6: Effect of TME-modulation mediated by VEGF application on treatment efficacy *in ovo*. (**a-b**) Evaluation of tumor weight (**a**) and fluorescence intensity (**b**) five days following treatment pointed to a strong impact of VEGF on the tumor-toxic potential of cetuximab. (**c**) Calculation of the vascularized area on the CAM using ImageJ showed no major difference in the blood vessel density. Red borders indicate treatments with TME-modulation.

Cytokine analysis

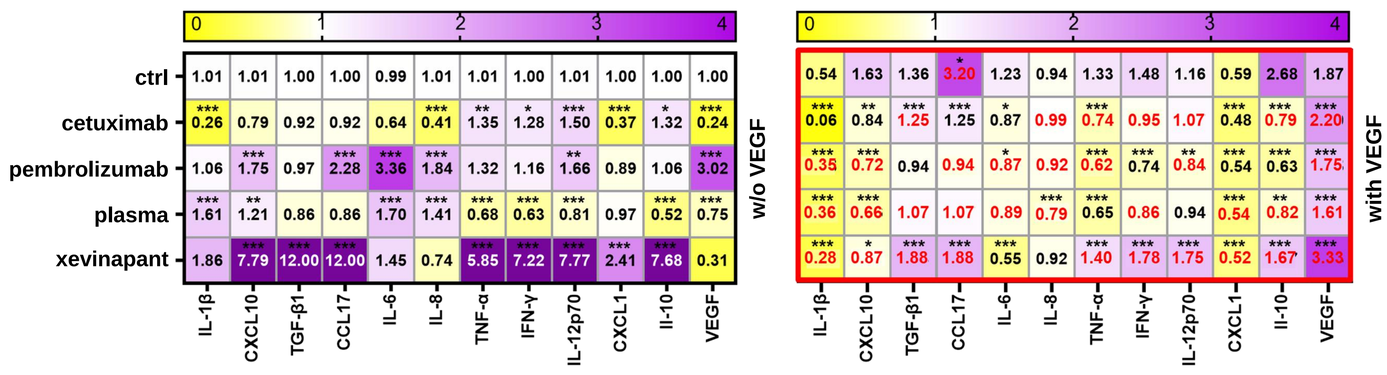


Fig. 7: An increased inflammatory response was detected after treatment using secretion profile analysis. VEGF application significantly diminished the release of pro-inflammatory molecules, promoting an immunosuppressive microenvironment. Red values indicate a significant change between treatment without and with TME modulation. Significant differences in the secretion of cytokines are as follow: p<0.001 = ***; p<0.01 = **; p<0.05 = *.

Kontakt:
Prof. Dr. Sander Bekeschus

sander.bekeschus@inp-greifswald.de
sander.bekeschus@med.uni-rostock.de

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