An *In Vivo* Therapeutic Antibody Efficacy Evaluation: the Chicken Embryo's CAM-based Assay

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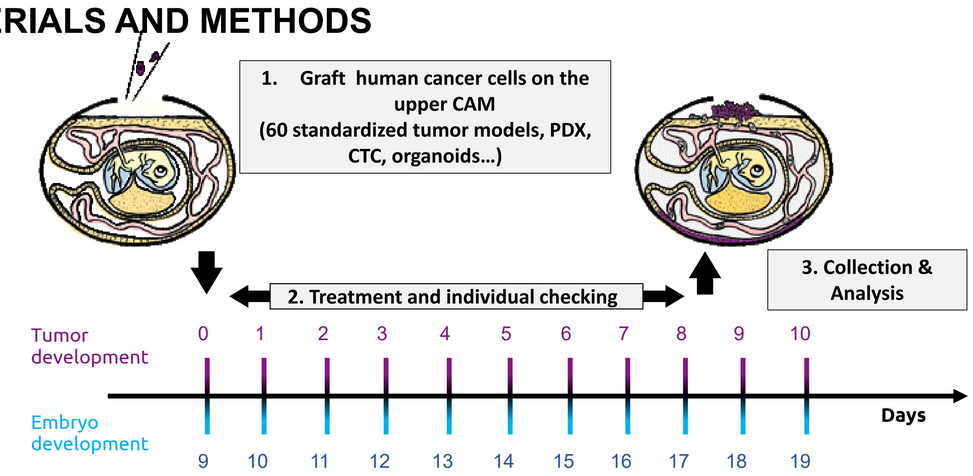
WHICH MOLECULE WILL STAND OUT

INTRODUCTION

For cancer drug development, a pertinent preclinical model is essential for the rapid and efficient transition from preclinical evaluation to clinical progress. Up until now, rodents are the most-often used models for preclinical evaluation. However, their use presents several drawbacks, including ethical constraints (3Rs), time-consuming and costly experiments, and the immunodeficiency of humanized models, which could slow down drug development [1, 2]. Since their introduction, xenografts on the chicken embryo's ChorioAllantoic Membrane (CAM) have proven extremely valuable for *in vivo* studies in cancerology [3]. They are suitable for studying tumor development, angiogenesis, malignant cell dissemination, and for estimating the toxicity and the efficacy of novel therapies [4]. Here, we demonstrate that the CAM-based *in ovo* model, which has an active immune system, is useful for rapidly testing and comparing therapeutic antibodies' efficacy, such as anti-PD1/PDL1 Abs, Antibody Drug Conjugates (ADCs), and others, on tumor growth and metastatic invasion. Our results clearly show the power of the chicken embryo model as an efficient *in vivo* model for testing a large spectrum of cancer drugs.

MATERIALS AND METHODS



Available *in ovo* readouts:

- Drug toxicity (embryo survival rate & malformation tracking)
- Tumor growth (tumor weight)
- Metastatic invasion in the lower CAM (away from the tumor), and in embryonic tissues
- Immune cell infiltration (RT-qPCR analysis of intra-tumoral immune cell biomarkers)
- Angiogenesis
- Xenografted tumor morphology: histological and immunohistochemistry (IHC) analysis
- Transcriptomic analysis of the tumor for exploring mechanisms of action

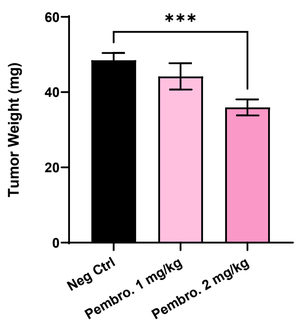
NB: This list is non exhaustive.

RESULTS

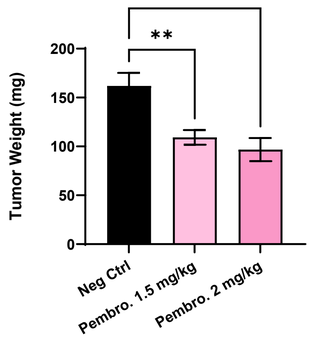
Immune Checkpoint Inhibitors

Anti-PD1

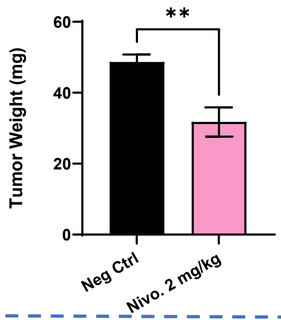
Pembrolizumab on MDA-MB-231 Breast Cancer
Tumor Growth



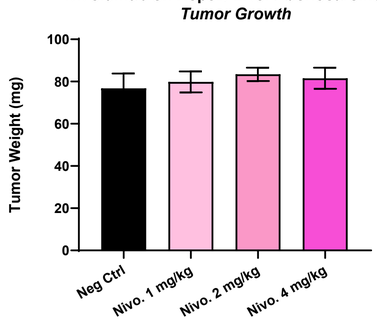
Pembrolizumab on H460 Lung Cancer
Tumor Growth



Nivolumab on MDA-MB-231 Breast Cancer
Tumor Growth

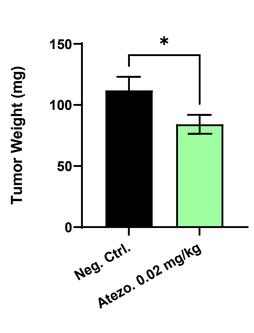


Nivolumab on Hep3B Liver Adenocarcinoma
Tumor Growth

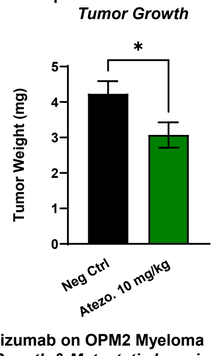


Anti-PDL1

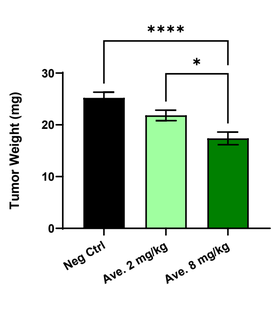
Atezolizumab on H460 Lung Cancer
Tumor Growth



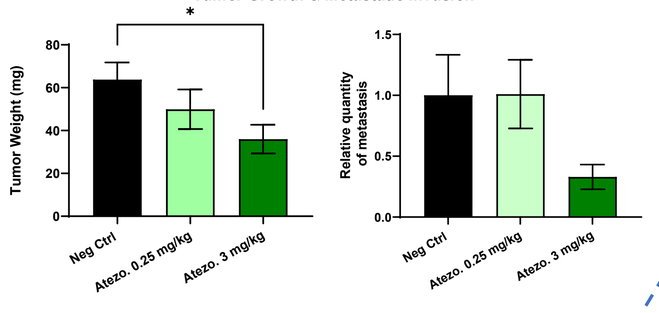
Atezolizumab on SNU182 Hepatocellular Carcinoma
Tumor Growth



Avelumab on A375 Melanoma
Tumor Growth

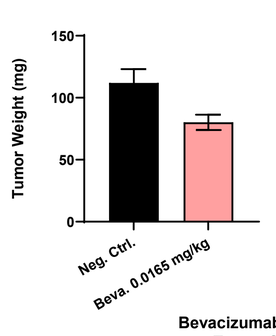


Atezolizumab on OPM2 Myeloma
Tumor Growth & Metastatic Invasion

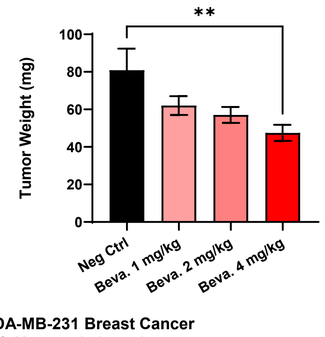


Anti-VEGF

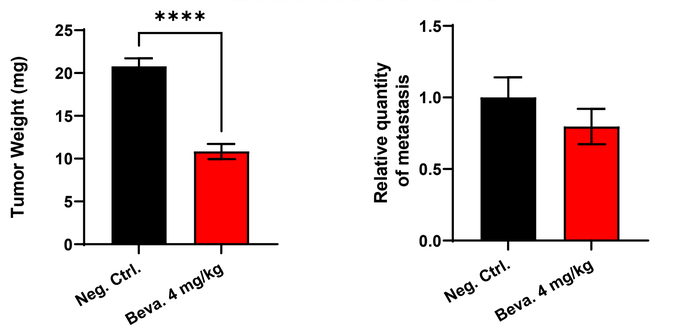
Bevacizumab on H460 Lung Cancer
Tumor Growth

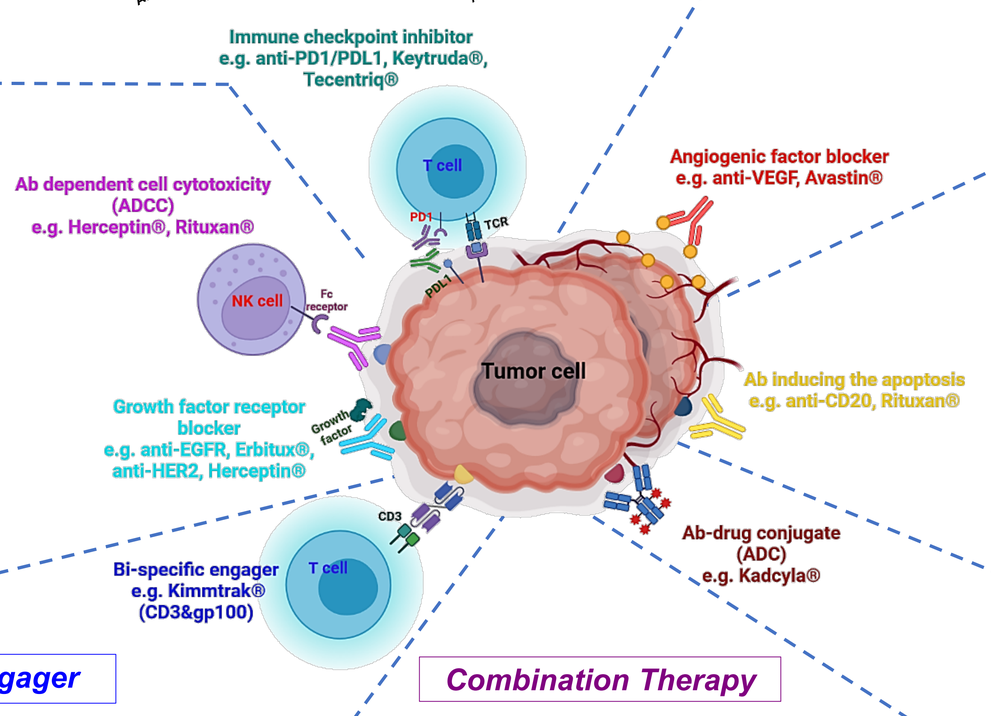


Bevacizumab on Hep3B Liver Adenocarcinoma
Tumor Growth



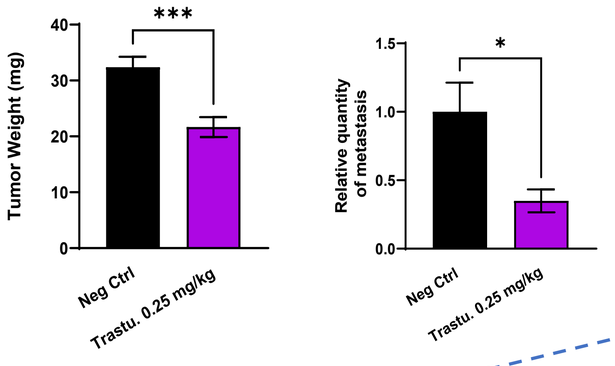
Bevacizumab on MDA-MB-231 Breast Cancer
Tumor Growth & Metastatic Invasion





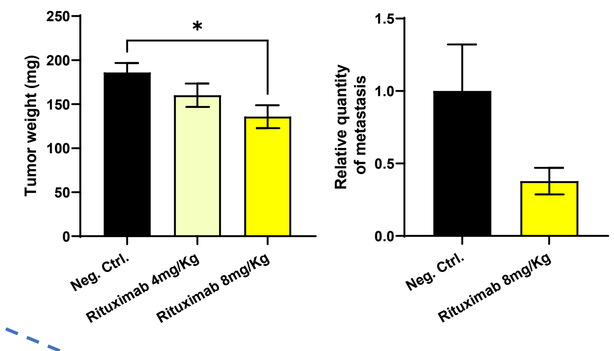
Anti-HER-2

Trastuzumab on N87 Gastric Carcinoma
Tumor Growth & Metastatic Invasion



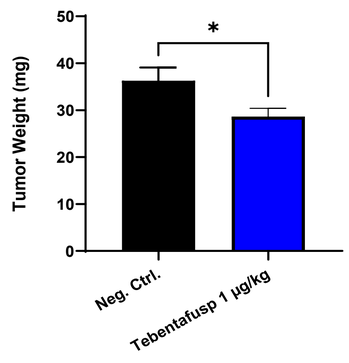
Anti-CD20

Rituximab on Daudi (Burkitt Lymphoma)
Tumor Growth & Metastatic Invasion



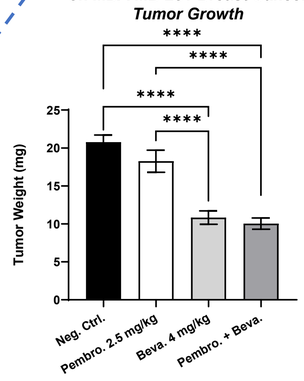
Bi-Specific Engager

Tebentafusp on 92.1 Uveal Melanoma
Tumor Growth

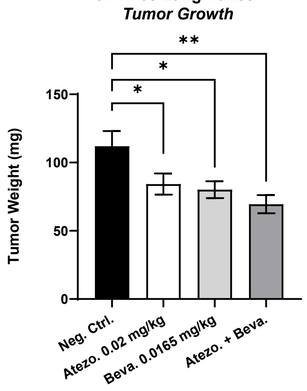


Combination Therapy

Pembrolizumab + Bevacizumab on MDA-MB-231 Breast Cancer
Tumor Growth

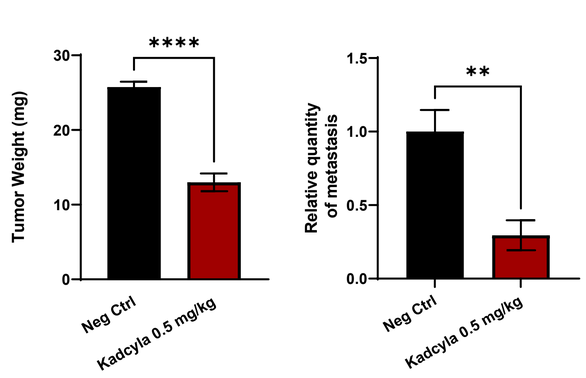


Bevacizumab + Atezolizumab on H460 Lung Cancer
Tumor Growth



ADC

Trastuzumab emtansine (Kadcyla®) on NCI-N87 Gastric Carcinoma
Tumor Growth & Metastatic Invasion



Statistical difference:

- No stars: p value > 0.05;
- * : 0.05 ≥ p value > 0.01;
- ** : 0.01 ≥ p value > 0.001;
- *** : 0.001 ≥ p value ≥ 0.0001;
- **** : 0.0001 ≥ p value.

CONCLUSIONS

The chicken embryo is an immune competent, *in vivo* model, and a robust immune response can be induced *in ovo* [5]. Our results show that the CAM model is suitable for testing therapeutic antibodies, immune therapies and ADCs *in vivo*. The presence of an active immune system allows the characterization of antibodies' effects on tumor cells, the tumor microenvironment, and the entire organism. These findings demonstrate both the pertinent use of this model for testing therapeutic antibodies, and also its high potential for testing more cancer drugs on a large spectrum, as well for validating combination regimens. Furthermore, many additional analyses can also be considered using the CAM assay, including neo-angiogenesis analysis, metastatic invasion, transcriptomics, and many others. More generally, the chicken embryo model has shown many strong advantages over classical models, including the simplicity of egg management and handling, cost-effectiveness, time efficiency, and 3Rs compliance (it is not considered to be an animal model). All these benefits illustrate that the chicken embryo model is a viable, alternative *in vivo* model, which is fast and reliable for use in cancer drug discovery.

References

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