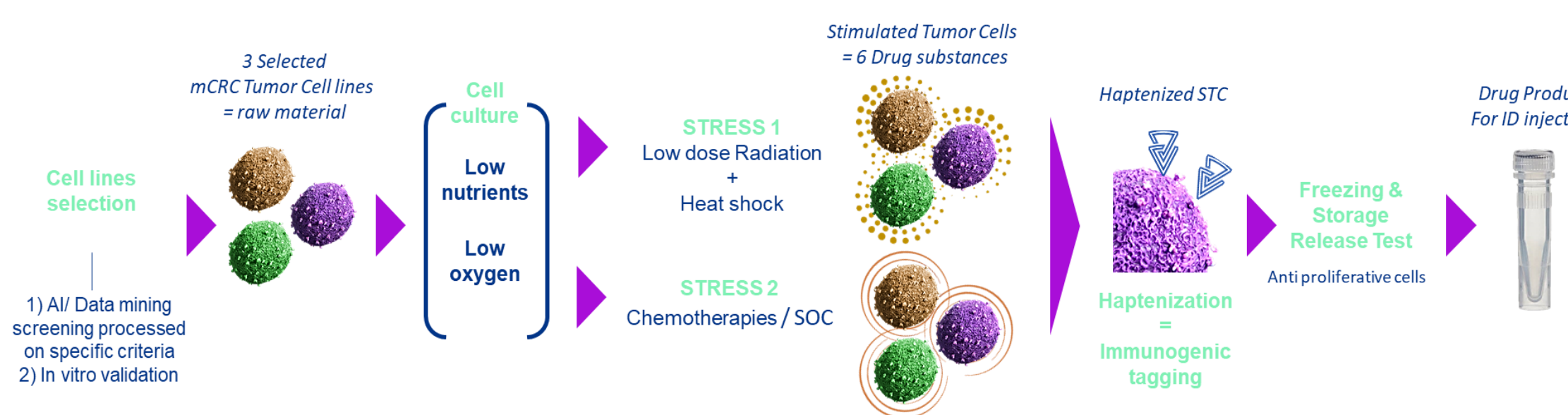


An innovative *in vivo* model for anti-tumor vaccine development: Safety validation and preliminary efficacy evaluation of a new antitumor vaccine STC-1010 on human colorectal adenocarcinoma using the chicken CAM assay

Yan WANG¹, Arnaud PEYRONNIER¹, Benoît PINTEUR², Lionel CHALUS², Corinne TORTORELLI², Paul BRAVETTI², Jean VIALLET¹, François GHIRINGHELLI³
1. Inovotion, La Tronche, France; 2. Brenus Pharma, Issoire, France; 6. Institut Bergonié, Bordeaux, France; 3. Centre Georges François Leclerc, Dijon, France

BACKGROUND

- Colorectal cancer (CRC) is the second-most deadly cancer worldwide.
- Therapeutic resistance to immuno-oncology treatments drives the need for new treatments.
- Stimulated Tumor Cells (STC) vaccine (Brenus Pharma) is composed of selected tumor cell lines, stimulated to overexpress Tumor-Associated Antigens (TAA) or Tumor-Specific Antigens (TSA) and neo-antigens including resistance factors.
- Haptenization of these proteins forms an immunogenic complex, which stimulates the immune system to recognize and target the patient's tumor cells expressing same resistance factors.
- We report *in vivo* the safety validation and the preliminary efficacy evaluation of STC-1010 vaccine, on human CRC adenocarcinoma from HT29 cell using a chorioallantoic membrane (CAM) assay developed by Inovotion in an immune reactive model.



STC (Stimulated Tumor Cells) Technology

METHODS

- The aim of this study is the *in vivo* evaluation of the tolerability and the efficacy of ST-1010 for activating the antitumoral immune response against human colorectal adenocarcinoma initiated from the HT29 cell line in a chorioallantoic membrane (CAM).
- This study is carried out in 2 steps:
 - 1) The chicken embryo's immune system is stimulated via two injections of STC-1010 (or the Negative Control) at Embryo Development Day (EDD) 11 and EDD13. At EDD18, the chicken peripheral blood mononuclear cells (PBMCs) are collected. Three batches of chicken PBMCs are generated for further treatment with the embryos grafted with HT29.

The activation of PBMCs is evaluated basing on IL-2 and IL-12 secretion as measured by ELISA.

- 2) After purification, PBMCs are used as anti-tumor reagents to treat chicken embryos xenografted with HT29 cells at EDD11, EDD13 and EDD16, respectively.

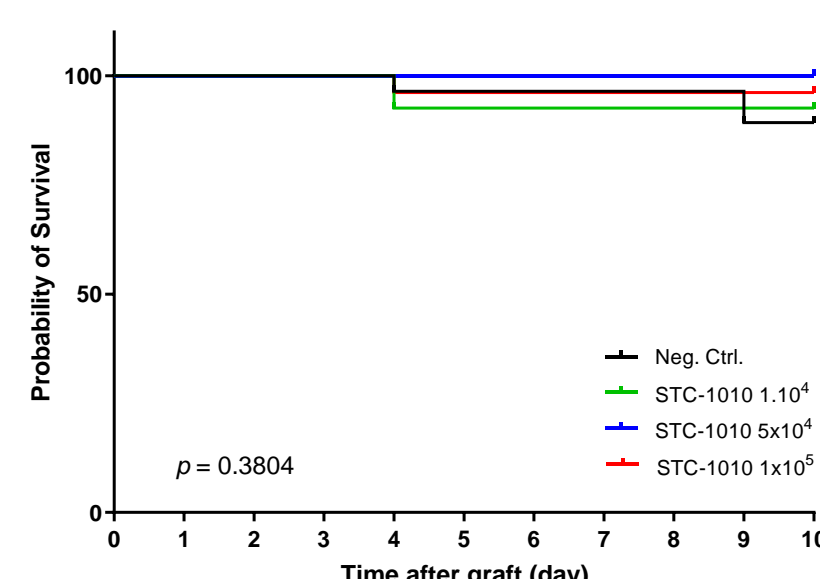
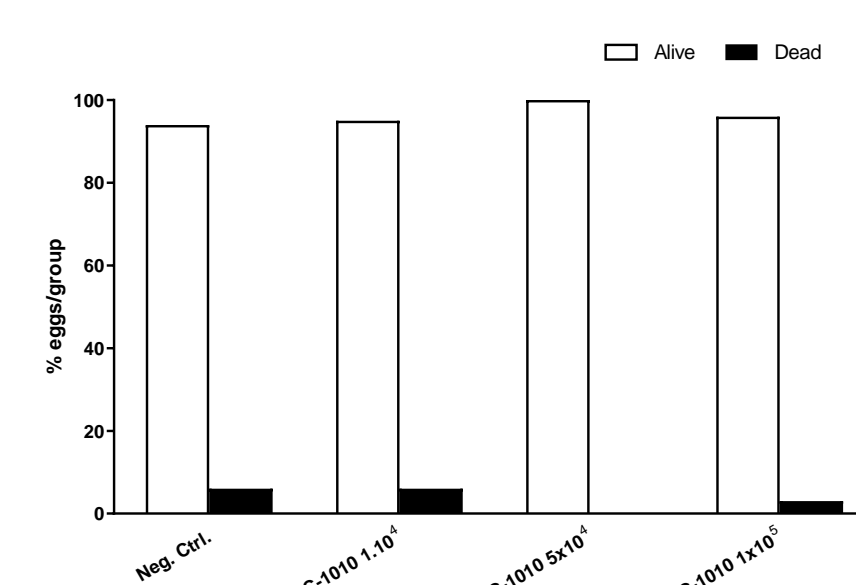
At EDD18, i.e., 9 days post-graft, the *in ovo* anti-tumor efficacy is evaluated via tumor weight, metastatic invasion (qPCR analysis of human Alu sequences in the lower CAM), quantification of tumor-infiltrating biomarkers (CD8, CD4, IFN- γ , Perforin and TNF α) and histological & IHC analyses.

RESULTS

In Ovo Safety

- STC-1010 at three doses:

- 1.10⁴
- 5.10⁴
- 1.10⁵



Immune System Activation

- Significant increase of IL-12 secretion (p=0.0003) in peripheral blood after the *in ovo* administration of SCT-1010.

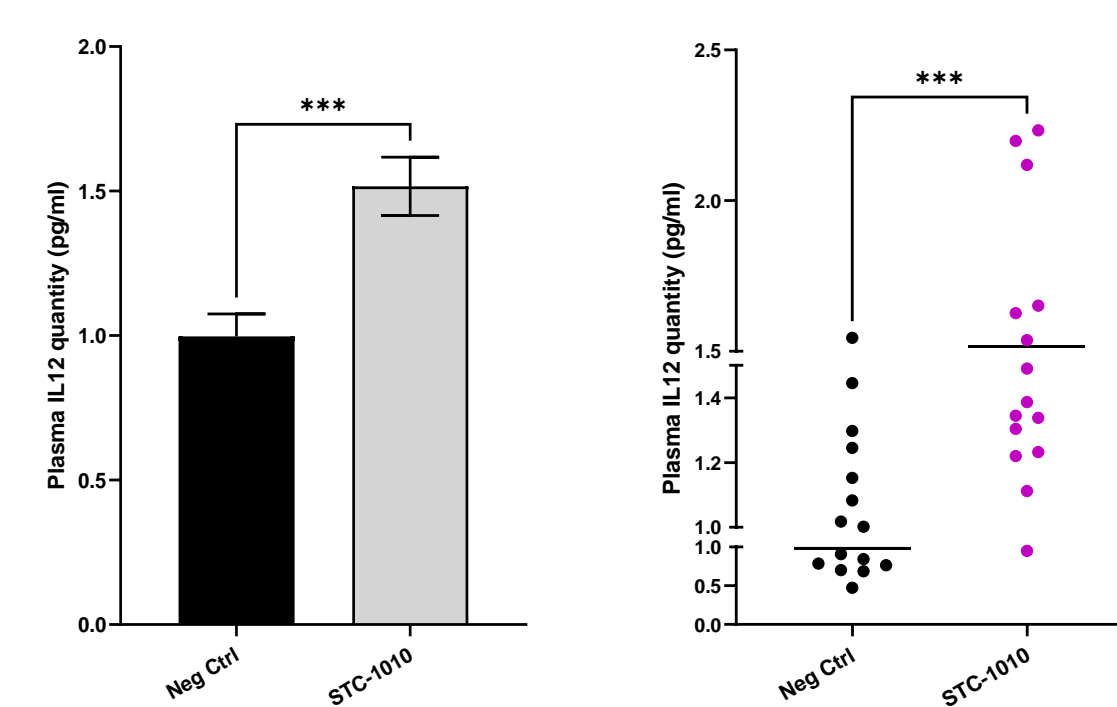
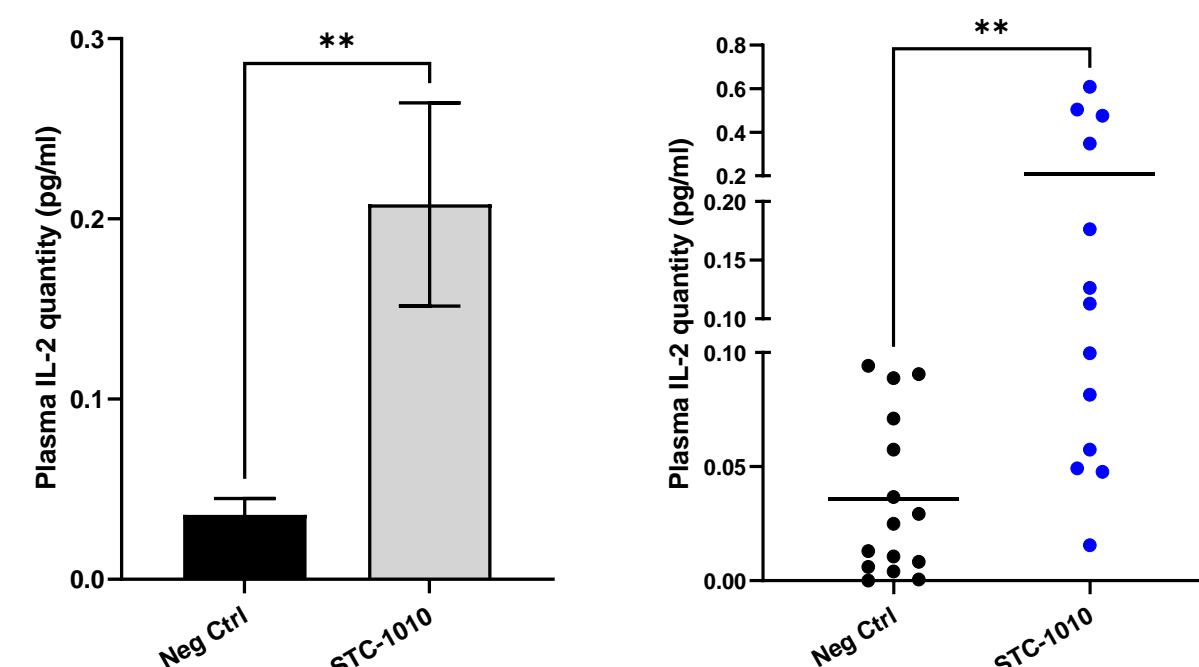


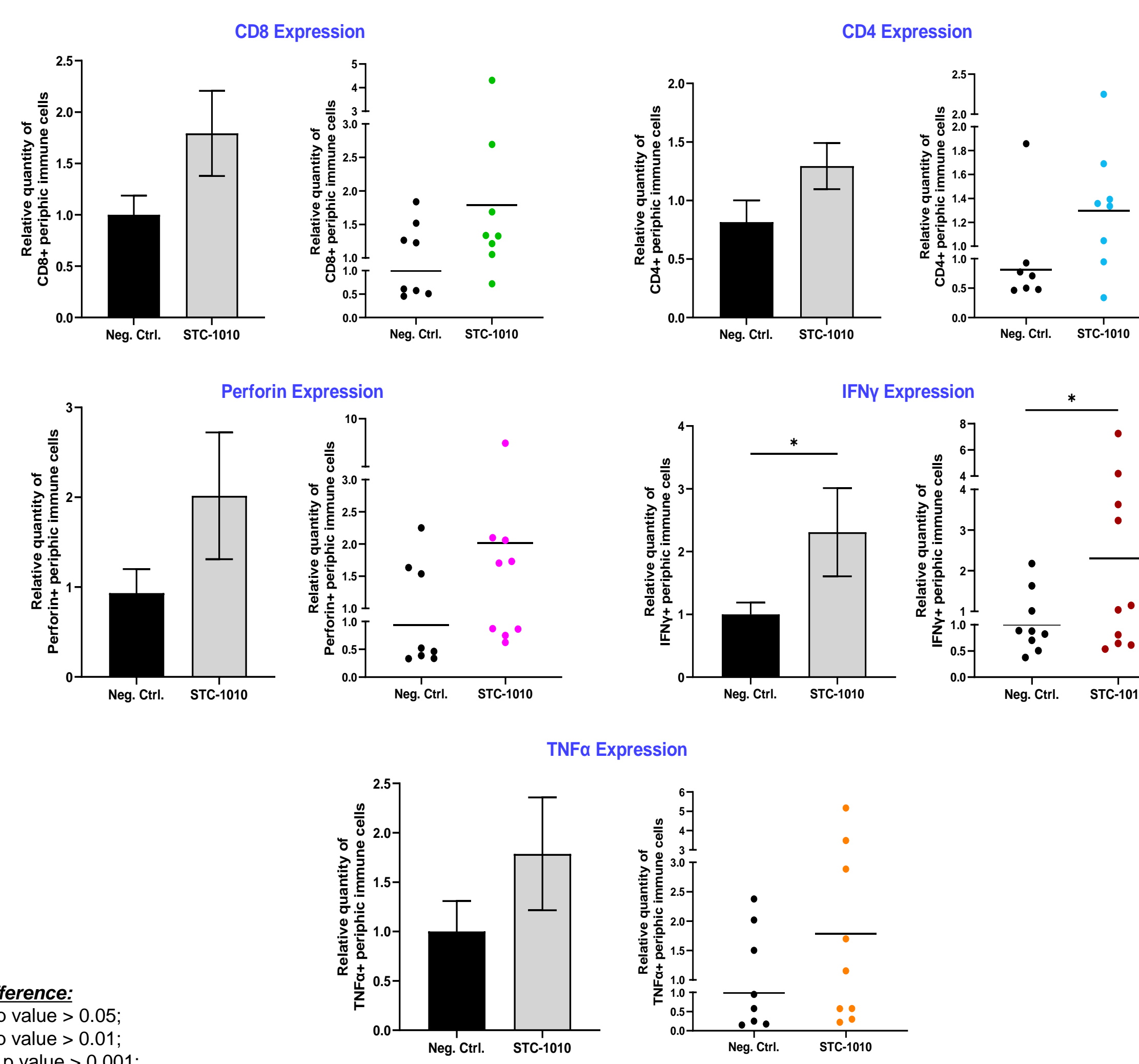
Fig. 3 Effect of STC-1010 on cytokine released (peripheral blood)

- Significant increase of IL-2 (p=0.0033) secretion in peripheral blood after the *in ovo* administration of SCT-1010.

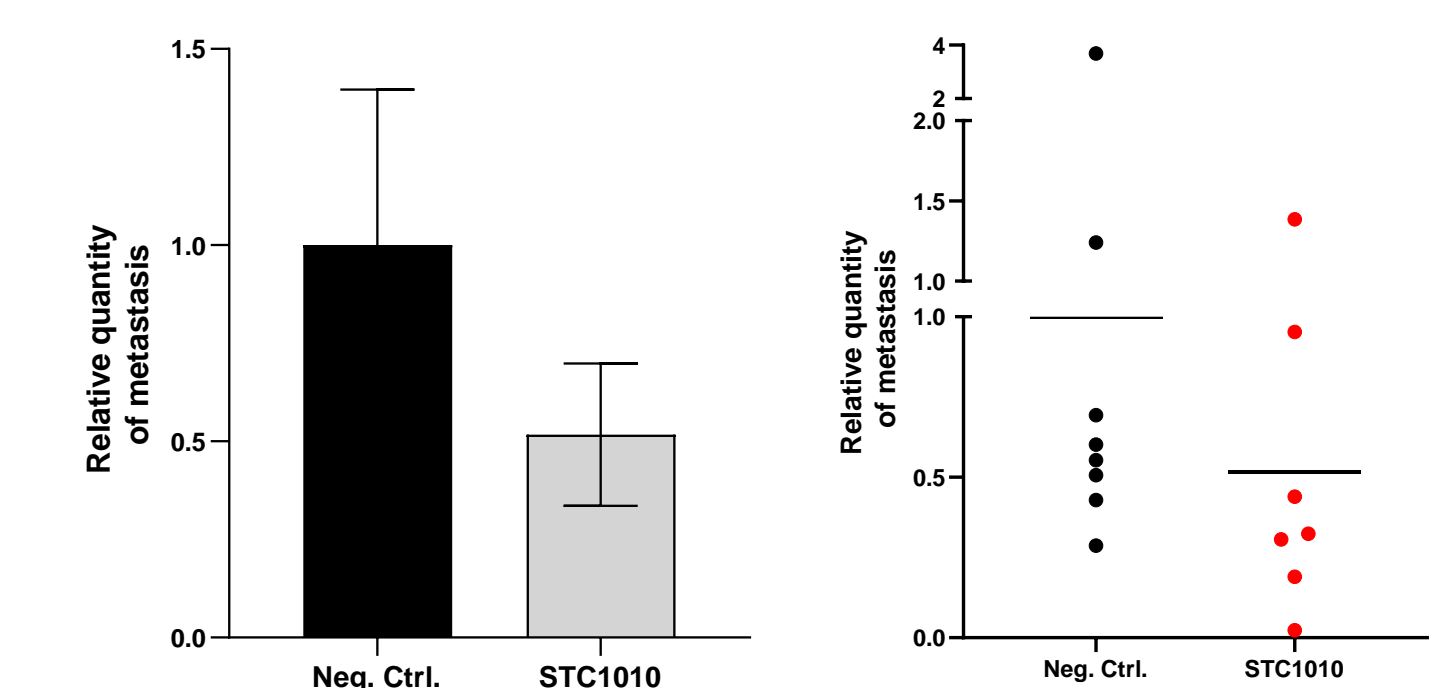


Statistical difference:
- No stars: p value > 0.05;
- *: 0.05 \geq p value > 0.01;
- **: 0.01 \geq p value > 0.001;
- ***: 0.001 \geq p value \geq 0.0001.

Tumor Immune Cell Infiltration

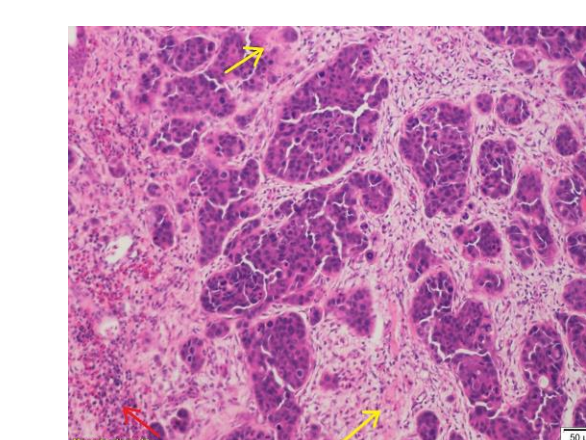
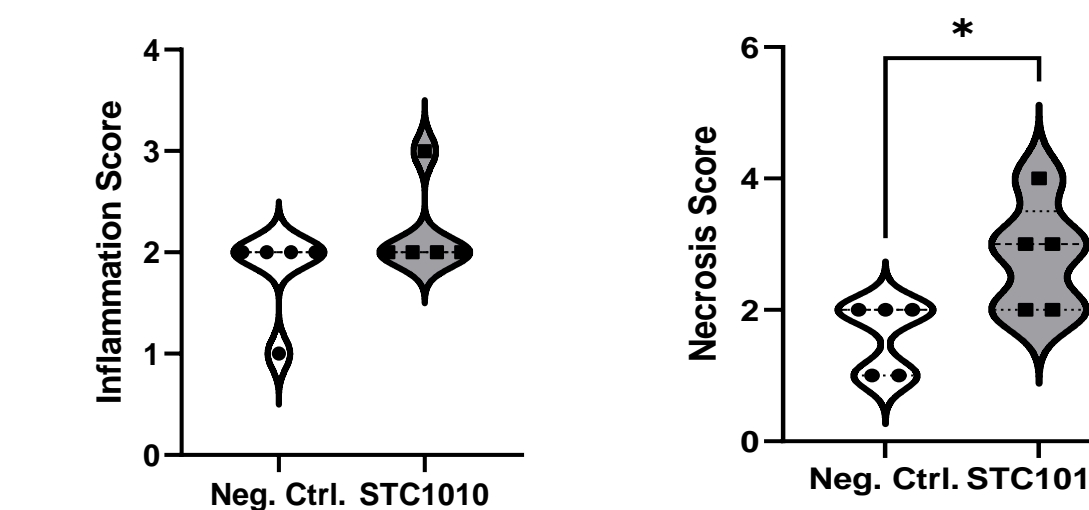


Metastatic Invasion



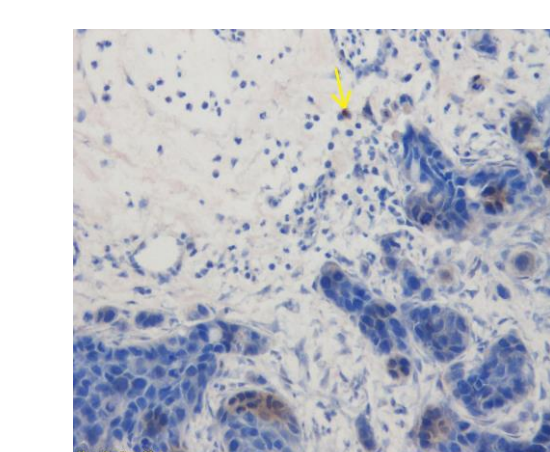
Histological & IHC Analysis

- Inflammation & Necrosis

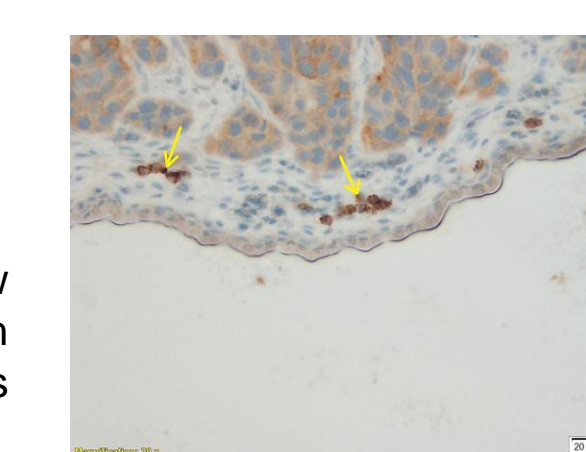


Representative photograph: H&E staining of tumor treated with STC-1010 activated PBMCs (objective magnification x10). Inflammation (red arrow) and necrosis (yellow arrows).

- IHC observation of tumor immune cell infiltration



Representative Photograph: CD8+ T cell infiltration (yellow arrows) in tumor treated with STC-1010 activated PBMCs (objective magnification x20).



Representative photograph: CD4+ T cell infiltration (yellow arrows) in tumors treated with STC-1010 activated PBMCs (objective magnification x20).

CONCLUSIONS

Since their introduction, xenografts on the chicken embryo's ChorioAllantoic Membrane (CAM) have proven extremely valuable for *in vivo* studies in cancerology. In this work, the results obtained *in ovo* allow us to confirm the anti-tumor efficacy of the STC-1010 vaccine previously observed in CRC syngeneic mouse models, and to give more insight about the mechanisms of action of this technology, comprising the activation and maturation of dendritic cells, the induction of different types of T cells (CD8+ T, CD4+ Th, and others) against tumor as the main drivers of the response, without inducing toxicity. Inovotion's CAM assay is suitable for studying tumor development, angiogenesis, malignant cell dissemination, and for estimating the toxicity and the efficacy of novel therapies. It is a viable alternative *in vivo* model for testing different cancer drugs on a large spectrum.