949- INOVATIVE IN VIVO (IN OVO) CAM MODEL TO PREDICT EFFICACY AND MODE OF ACTION OF A NEW ANTITUMOR VACCINE STC-1010 ON HUMAN COLORECTAL ADENOCARCINOMA

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BACKGROUND

- Colorectal cancer (CRC) is the second-most deadly cancer worldwide.
- Therapeutic resistance to standard of care (SOC) and immuno-oncology drives the need for new treatments.
- Stimulated tumor cells (STC) vaccine (Brenus Pharma) is composed of selected tumor cell lines, overexpressing tumor-associated (TAA), tumor-specific antigens (TSA) and neoantigens linked with this SOC resistance.
- The haptenization process, makes these proteins of "resistance factors" immunogenic inducing a strong immune response to the patient's tumor cells expressing the same resistance factors.
- We report *in ovo* results of STC-1010 vaccine, on human CRC adenocarcinoma from HT29 cell using chorioallantoic membrane (CAM) assay developed by Inovotion in immune reactive model.

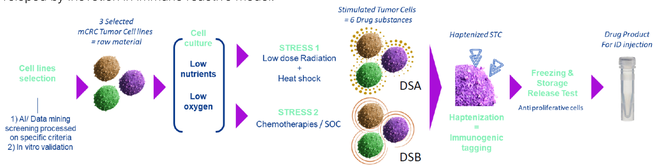

Fig 1. STC (Stimulated Tumor Cells) Technology

METHODS

- The aim of this study is to evaluate in ovo the efficacy of ST-1010 to activate the antitumoral immune response against grafted human colorectal adenocarcinoma initiated from HT29 cell line in CAM model compared to vehicle as negative control.
- This study was carried out in 2 steps:

1°) Generation of activated / immunocompetent chicken PBMC (peripheral blood mononuclear cells) following the injection of STC-1010 at Embryo Development Day (EDD) 11 and EDD13. At EDD18, the chicken PBMCs were collected. Three batches of activated PPMCs are generated and also three negative control with injection of vehicle instead of STC-1010.

2°) Assessment of the antitumoral immune response : the collected PBMCs in step one are injected at EDD 11, EDD 13 and EDD16 in chicken embryos xenografted with HT29 cells.

- Activation of PBMCs was evaluated by IL2 and IL12 secretion quantified by ELISA.
- Anti-tumor efficacy at EDD18 was evaluated by tumor weight, metastatic invasion (qPCR analysis of human Alu sequence in lower CAM) and quantification of tumor-infiltrating by CD8, CD4, IFN-gamma, Perforin and TNF-alpha.

RESULTS

- Compared to negative control, STC-1010 vaccine induced:

- Significant increase of IL-12 (+52%, p=0.0003) and IL-2 (+482%, p=0.0033) secretions in peripheric blood during the generation of all three batches of PBMCs.
- A significant expression of IFN-gamma in tumor (+130,83%, p=0.0185)
- A tendency to increase infiltrating of immune cells: CD4+ (+79,2%), CD8+ (+29,4%) and their secretions: Perforin (+105,5%) and TNFα (+78,63%)
- A significant increase of tumor necrosis (tumor pathologist scoring, p = 0.0267)
- A tendency of metastasis regression (-49%)
- No embryonic toxicity/mortality (daily evaluation of embryonic viability).

CAM Model and processing sequence of the study

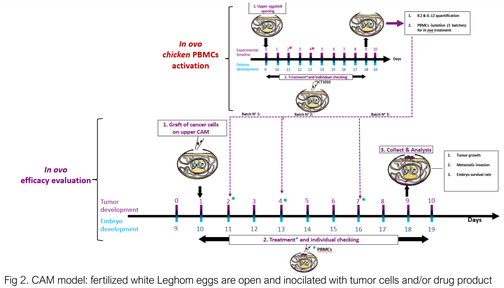

Fig 2. CAM model: fertilized white Leghorn eggs are open and inoculated with tumor cells and/or drug product

Analysis of cytokines secretions: IL-12, IL-2 and IFN-γ

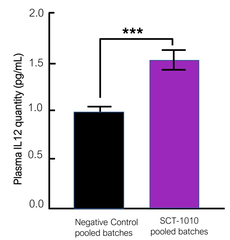

Fig 3. IL 12 quantification in pooled batches

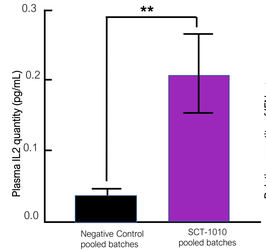

Fig 4. IL 2 quantification in pooled batches

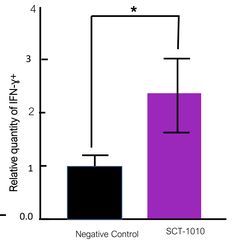

Fig 5. Relative quantity of IFNγ secreted by peripheric immune cells

Metastatic Invasion

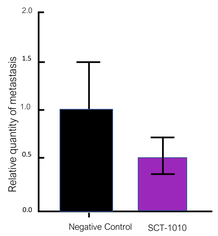

Fig 6. Quantitative evaluation of metastatic invasion

Inflammation and Necrosis

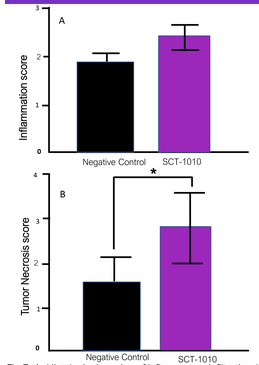

Fig 7. A. Histological scoring of inflammatory infiltration (0-3)
B. Histological scoring of tumor necrosis (0-5)
by semi-quantitative scoring by pathologist

Pictures of Immunohistochemical (IHC, X 20)

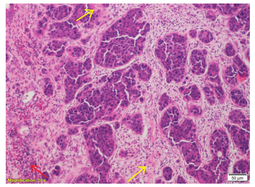

Fig 8. Photo of Tumor after STC-1010 treatment with Inflammation (red arrow) and necrosis (yellow arrows) Hematoxylin & Eosine, x10

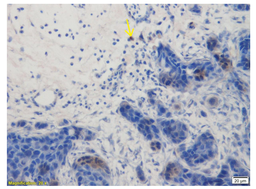

Fig 9. Tumor after STC-1010 treatment with CD8+ infiltration IHC (yellow arrows), x20

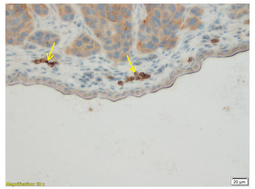

Fig 10. Tumor after STC-1010 treatment with CD4+ infiltration IHC (yellow arrows), x20

CONCLUSIONS

This *in ovo* study confirms efficacy of the STC-1010 observed in previous CRC syngeneic models and gives more insight about STC mechanism of action with the activation and maturation of dendritic cells, induction of CD8+ and LTh1 against tumor as the main driver of the response, all without toxicity. Inovotion's CAM model could be used for indication screening and as a pre-proof-of-concept before syngeneic model study.

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