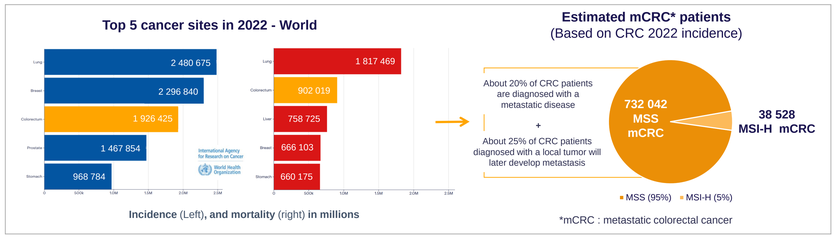
LB383 Preclinical efficacy of an innovative therapeutic cancer vaccine: A new era for immunotherapy

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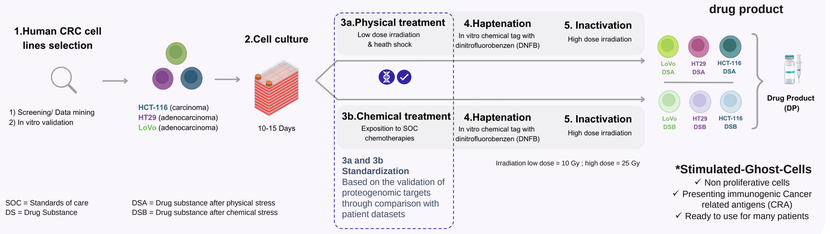
I. BACKGROUND

- **Colorectal cancer** (CRC) **ranks third in terms of incidence. worldwide and is the second leading cause of cancer-related deaths, with ~2 million deaths annualy**
- **Ninety-five percent of patients with metastatic CRC** (mCRC) **present microsatellite stable** (MSS) **tumors. Low mutation burden of MSS tumors and low immune infiltration render standard immunotherapies ineffective for most patients. This is affecting approximately 730,000 patients, still treated with chemotherapy.**



- **To address this unmet medical need, Brenus Pharma has developed "off-the-shelf" vaccines generated with the innovative "Stimulated-*Tumor*-Ghost Cells"** (SGC) **platform. The first candidate, STC-1010, targets CRC. Pre-clinical safety and efficacy has been tested in various CRC models with promising results.**

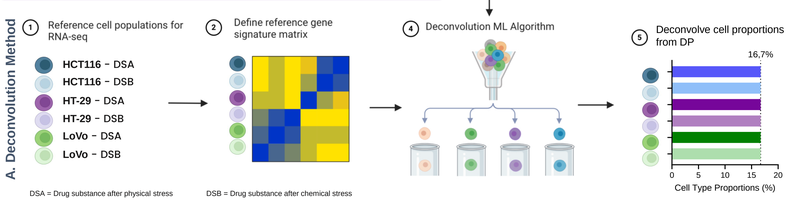
STC-1010 MANUFACTURING PROCESS



II. PRODUCT CHARACTERIZATION

DECONVOLUTION ANALYSIS

The CIBERSORTx algorithm was trained on the DS RNA data to generate a signature specific to each treatment condition and cell line enabling the identification and quantification of all DS within the DP.



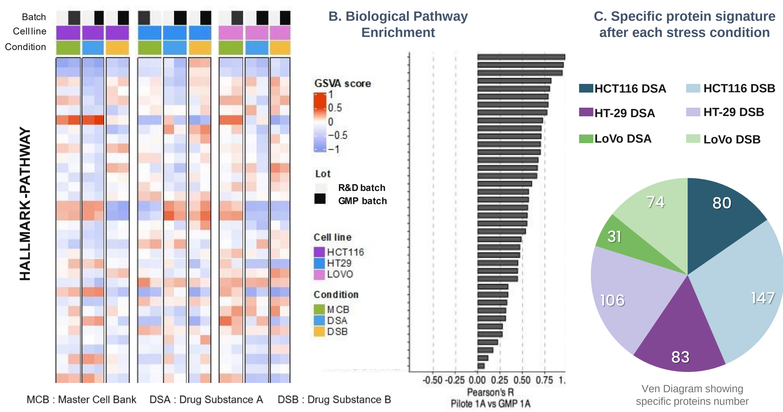


Fig. 1A. Deconvolution analysis of STC-1010 shows DS proportion estimates near 16%, consistent with an equal mixture of the six DS in the DP.

1B. Biological Pathway Enrichment Analysis (GSVA): Using the HALLMARK database, biological pathway enrichment was evaluated across different conditions from R&D and GMP batches. Each cell line displayed distinct pathway activation profiles. Batch Correlation Analysis: across batches confirmed strong reproducibility for key pathways, highlighting consistent activation patterns.

1C. Specific protein signature justify the interest in using three cell lines and two treatment conditions in the specificity and complementarity of their effects in a final product.

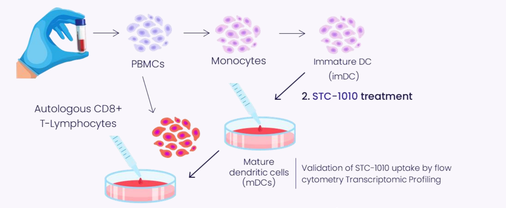
✓ **Characterization ensures manufacturing control and helps in understanding the mechanism of action**

III. STC-1010 INDUCES AN ANTI-TUMORAL IMMUNE RESPONSE

✓ **Both *ex vivo* and *in ovo* assays confirm STC-1010's potency to induce an anti-tumoral immune response**

EX-VIVO IMMUNE REACTION ASSAY METHOD :

- **Monocytes are differentiated into monocyte-derived dendritic cells** (mDCs) **and treated with STC-1010.**
- **The uptake of STC-1010 antigens by mDCs was confirmed by detecting haptenated proteins within the mDCs.**
- **Activated CD8+ T cells are then tested for their cytotoxicity in real-time on CRC cell line** (HCT116).



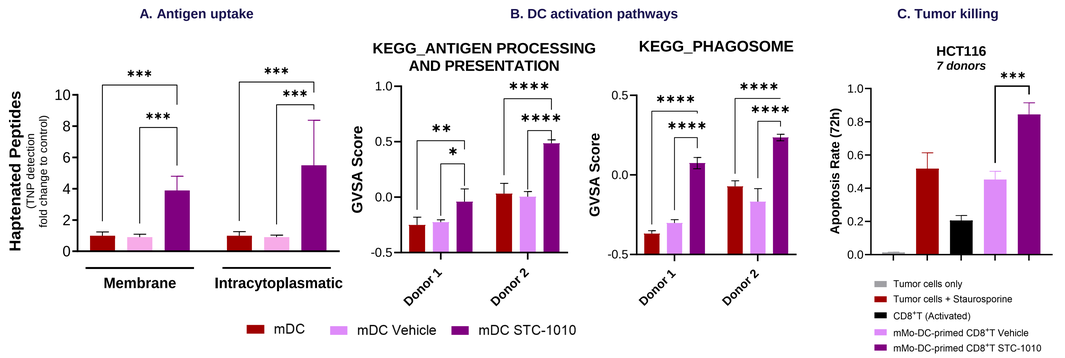


Fig. 3A. Haptenated proteins were detected within primed mDCs, indicating that STC-1010 was successfully taken up and presented by mDCs..

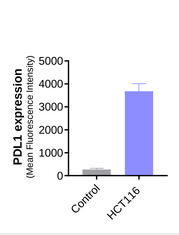
3B. STC-1010–primed mDCs display stronger activation of antigen processing, presentation, and phagosome pathways vs. control.

3C. mDC-STC-1010-activated CD8+ T cells significantly induce apoptosis in HCT116 tumor cells.

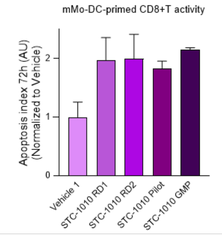
3D. PD-L1 expression in HCT116 cells suggests this model is suitable for evaluating STC-1010 in combination with anti-PD-1/PD-L1 therapy.

3E. STC-1010 consistently demonstrated tumor-killing activity across four independent batches, from R&D to GMP-clinical production.

D. Checkpoints expression on target cells

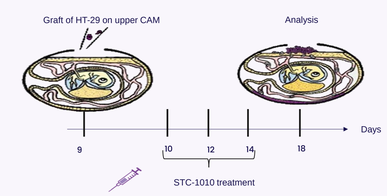


E. Inter-batch reproducibility



✓ **STC-1010 significantly increases colon cancer cell apoptosis with a robust batch-to-batch reproducibility via immune cell activation**

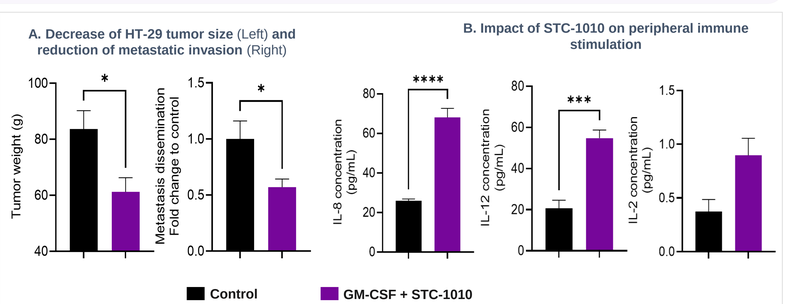
IN OVO ASSAY CAM* MODEL METHOD (*Chorioallantoic Membrane) :



- **Chicken embryos were grafted with HT-29 CRC cells onto the CAM on day 9 and treated at day 10/12/14 with vehicle or STC-1010** (1.10^5 cells) **combined with GM-CSF** (200ng) **before analysis at day 18.**
- **The antitumor effect of STC-1010 was evaluated by assessing tumor growth and metastasis dissemination.**
- **The immune activation was monitored by measuring the secretion of peripheral cytokines.**

Fig. 4A. STC-1010 promotes an anti-tumor response by decreasing tumor growth (p=0,01) **and metastasis dissemination in the HT-29 CAM model.** (p=0,04) **p<0,05 Unpaired-t-test*

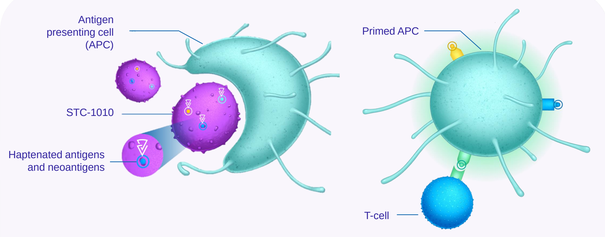
4B. STC-1010, in combination with GM-CSF, induces an immunoactivation by increasing secretion of IL-8 (p<0.0001) **and IL-12** (p=0.0001) **reflecting an induced immune stimulant response.**



✓ **STC-1010 significantly induces an anti-tumoral response associated with peripheral immunoactivation**

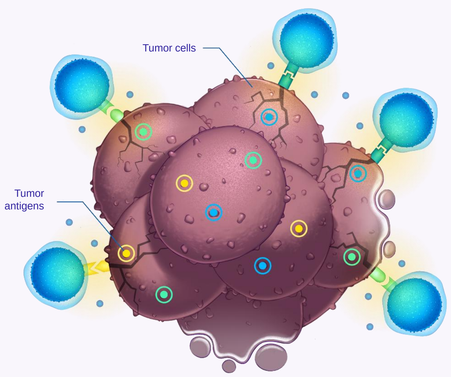
IV. STC-1010 *IN VIVO* MECHANISM OF ACTION

✓ **STC-1010 presenting haptenated cancer-related antigens to the immune system for effective tumor recognition and destruction**



I/ Phagocytosis of STC-1010 by APCs under the derm

II/ T-cells education by matured APCs and their expansion



III/ T-cells (CD8+) recognize evolving tumor cells for tumor killing

CONCLUSIONS

STRESS INDUCES SPECIFIC SIGNATURES

- Deconvolution analysis enable the identification and quantification of all DS within the DP based on specific signature.
- RNAseq analysis shows stress signature by activating similar biological pathways across batches, confirming manufacturing reproducibility.
- Proteomic analysis confirms previous *in vivo* data [1] showing the interest in using 3 cell lines and 2 stress conditions.

STC-1010 INDUCES AN ANTI-TUMORAL IMMUNE RESPONSE

- STC-1010 antigens are efficiently uptaken and presented by DCs, enabling the activation of $CD8^+$ T cells that significantly enhance CRC cell apoptosis. This immune response was consistently reproduced across four batches, demonstrating the standardization of STC-1010 manufacturing.
- In the HT-29 CAM model, STC-1010 reduces tumor size, prevents metastasis, and increases peripheral immune stimulant cytokines.

STC-1010 AS A POTENT IMMUNOTHERAPY CANDIDATE IN CRC

- Based on these preclinical findings, the clinical efficacy of STC-1010 will be evaluated in a multi-center phase I/II trial for non-resectable metastatic and advanced CRC patients – Q2 2025.
- This trial aims to evaluate the safety and preliminary effectiveness of STC-1010 in combination with immunostimulants and mFOLFOX6, with or without bevacizumab, in MSS and MSI-H CRC patients.

Source (1) : Alzeeb, et al., Front. Oncol., 2024 https://doi.org/10.3389/fonc.2024.1427428

AACR American Association for Cancer Research ANNUAL MEETING 2025 CHICAGO

Brenus PHARMA

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