LB224- STC-1010 A NEW THERAPEUTIC VACCINE PROMOTES TUMOR CELL DEATH

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BACKGROUND

- Colorectal cancer (CRC) is the **second leading cause of cancer-related deaths worldwide.**
- Unmet medical need in immunotherapy is high for MSS patients and still present for MSI-H/dMMR patients. Tumor plasticity and treatment resistance are the main drivers of patient's relapse.
- Brenus Pharma develops a **therapeutic cancer vaccine based on Stimulated Tumor Cells** (STC): STC-1010 to educate the immune system to target patient's tumor cells harboring mechanism of relapse.
- This technology has already shown **efficacy in immunocompetent mouse models** and ***in ovo*** **Chorio-allantoic model (CAM).**

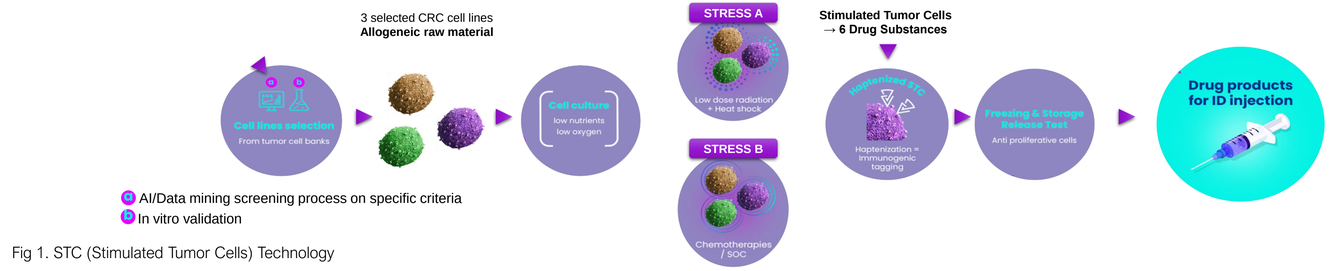


Fig 1. STC (Stimulated Tumor Cells) Technology

METHODS

Starting material : Human PBMC from 2 healthy donors

STEP 0-1 — Mature Dentritic cells — **1 READOUTS** **FACS**: Viability / CD80/ CD86/ CD83/ CD1a /CD209 **HTRF**: IL12/ IL8/ IL10

Monocytes → **4 DAYS** **+/- Media 1 or 2** **+/- STC-1010** → Mature Dentritic cells

Mature Dentritic cells + CD8+ T cells → **10 days Co-culture** → **STEP 2**

2 READOUTS **HTRF**:IFNg **NGS**: TCR-Sequencing

5 days Co-culture → **STEP 3**

Tumor cells : HCT 116, HT29, LS174T

3 READOUTS **Live cell imaging :** Apoptosis rate

Media 1: CD40L
Media 2: CD40L + LPS/INFg

STEP 0 - SELECTION OF ADAPTED MEDIA
STEP 1 - DCs PRIMING
STEP 2 - CD8⁺ ACTIVATION BY DCs
STEP 3 - TUMOR KILLING

Fig 2. Derived Mixed Lymphocyte Reaction
CD8+ T cells priming and apoptosis of CRC cell lines

RESULTS

STEP 1 - DCS PRIMING AND MATURATION AFTER STC-1010

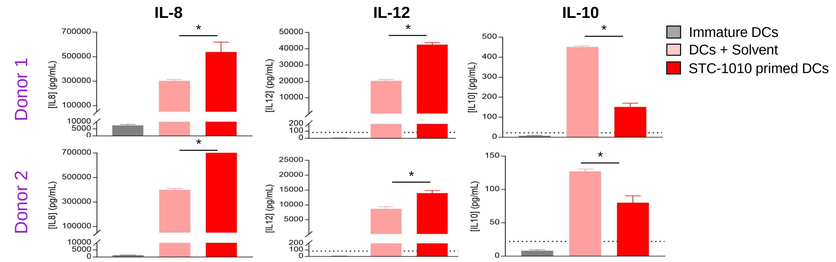


Fig3. Effect of STC-1010 on cytokine released by DC (Media 2)

STEP 2 - CD8⁺ T CELL ACTIVATION AND SPECIFICITY

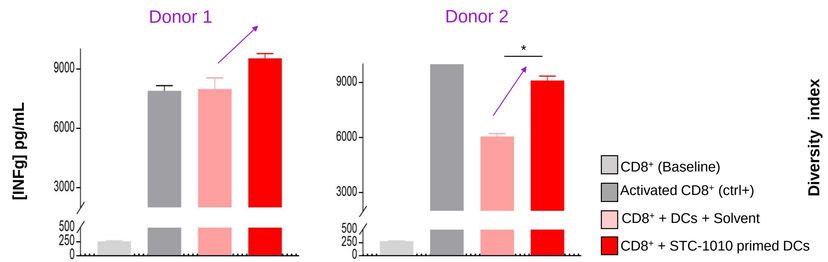


Fig4a. Effects of STC-1010 on the MLR response through IFNg quantification (Media 2)

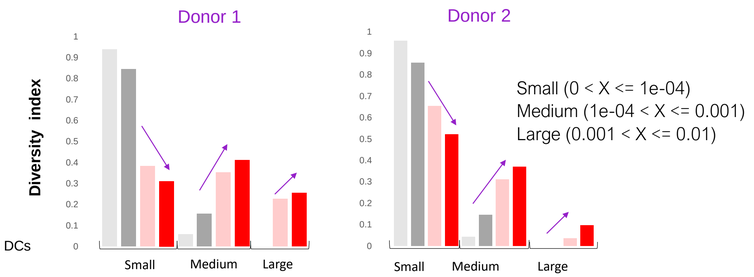


Fig4b. TCRseq: Summary proportions of T cells clonotypes

STEP 3 - TUMOR CELLS KILLING BY CD8⁺ T ACTIVATED WITH STC-1010

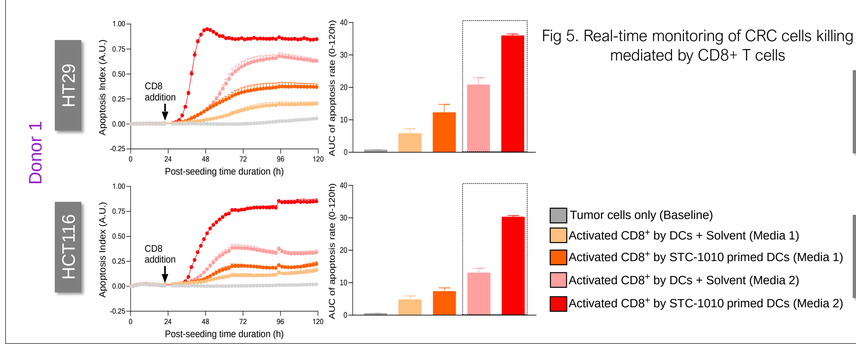
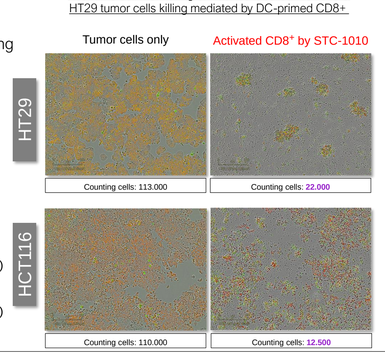


Fig 5. Real-time monitoring of CRC cells killing mediated by CD8+ T cells



- **Dendritic cells (DCs) are activated by the STC-1010 human vaccine**, (increase IL12,IL8, decrease IL10) with no viability issue (*Fig3*)
- **Significative immunological activation** of CD8⁺ T cells, by DCs primed with STC-1010 observed with [INFg] increase (*Fig 4.a*)
- Increase of the clonality index and the number of medium & large CD8⁺ T cells clones, after the education by STC-1010 (TCR sequencing) in favor of an **increased selectivity of specific CD8⁺ T cells** due to STC-1010 (*Fig4.b*)
- **Antitumor effect of** CD8⁺ T cells activated with STC-1010-primed DCs against 2 different human CRC cell lines was observed with massive apoptosis & large reduction of tumor cells viability (90% HCT116 – 80% HT29). Media 2 reproduces better the *ex vivo* immunocompetency (*Fig5*)

CONCLUSION

This ***ex vivo*** **assay confirms the specificity of the immune response induced by Brenus STC approach and validates the strong efficacy on human colorectal tumor cell lines**. This model presents similar data to our previous and different preclinical models and proves the potential of the STC-1010 to be transposed into clinical setting for the treatment of patients with CRC.
This model has the benefit to comply with the FDA's modernization act 2.0 S. 5002.

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