5003 - EFFICACY OF THE STC-1010 A NEW ALLOGENEIC CANCER VACCINE IN DIFFERENT COLORECTAL CANCER MODELS

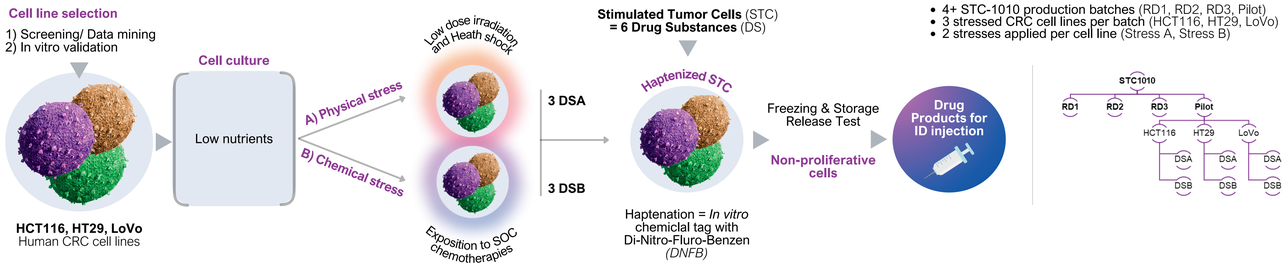
G. Alzeeb,[1] C. Gongora,[2] C. Richard,[3] R. Boidot,[3] T. Fortin,[4] Y. Wang,[5] A. Bessede,[6] B. Pinteur,[1] L. Chalus,[1] C. Tortorelli, P.[1] Bravetti,[1] F. Ghiringhelli [7]

[1]Brenus Pharma, Lyon, France,[2]INSERM U1194, Montpellier, France,[3]CNRS 6302, Dijon, France,[4]Anaquant, Lyon, France,[5]Inovotion, La Tronche, France,[6]Explicyte, Bordeaux, France,[7]Inserm 1231, Dijon, France

INTRODUCTION AND BACKGROUND

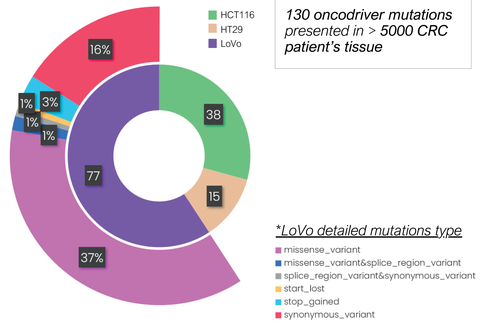
- Colorectal cancer (CRC) is the **seconde leading cause of cancer-related deaths worldwide**, 2M patients were diagnosed in 2022 and nearly **90%** encountered treatment failure.
- Unmet medical need in immunotherapy is high for MSS patients and still present for MSI-H/dMMR patients.
- Tumor plasticity and treatment resistance are the main drivers of patient's relapse.
- Brenus Pharma develops a **therapeutic cancer vaccine based on Stimulated Tumor Cells** (STC) to educate the immune system to target patient's tumor cells harboring mechanism of relapse. The lead vaccine candidate, STC-1010 is targeting CRC.

Fig. 1 STC Technology and STC-1010 batches production



CELL LINE SELECTION COVERING CRC CLINICAL HETEROGENEITY

Fig. 2 Whole exome sequencing (WES) of STC-1010 cell lines:
HCT116, LoVo (MSI-H) and HT29 (MSS), with LoVo detailed mutations type*
130 oncodriver mutations shared with more than 5000 CRC patient's tissue *from* http://coloncanceratlas.org/. Including BRAF, KRAS, TP53...



TRANSCRIPTOMIC SIGNATURE To IDENTIFY AND QUANTIFY DS IN DRUG PRODUCT

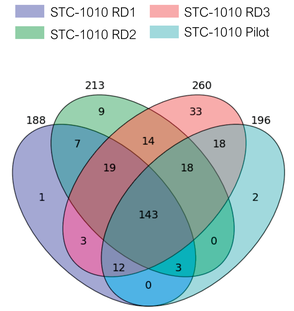
	HCT-116		HT29		LoVo	
%	DSA	DSB	DSA	DSB	DSA	DSB
STC-1010	17,2	19,2	17,4	13,7	17,5	15,0

Fig. 3 RNAseq based repartition of the 6 Drug Substances (= 6 DS*) in DP
Each DS is charaterized with a specific transcriptomic signature and quantified as 16% of total STC product (Deconvolution analysis via Cibersort algorithm : error estimation < 1,5%)
** 3 DS A = Drug substance with physical stimulation*
** 3 DSB = Drug substance with chemical stimulation*

BATCH TO BATCH REPRODUCIBILITY

Fig 4. 143 Cancer-Related Proteins (CRP) identified from the Human Atlas protein's database (incl. patient's biopsies) are shared with STC-1010 proteome (Mass spectometry proteomic analysis) in all 4 STC-1010 batches.

https://www.proteinatlas.org/

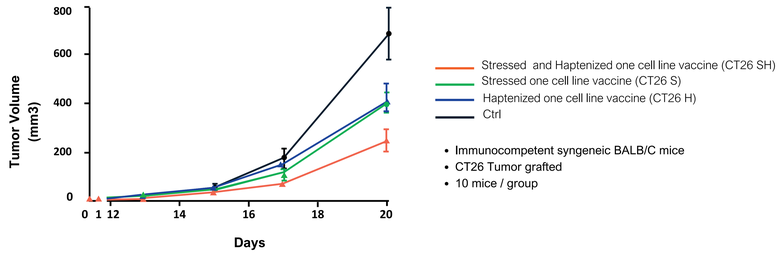


STC PLATFORM BIOPRODUCTION AND CHARACTERIZATION

- Our unique and patented STC platform **mimics patient's relapsing conditions**, *in vitro*, to **educate efficently the patient's immune system against mechanims of relapse** through a **scalable CMC** to benefit to a wide range of patients *(Fig 1)*
- Wole exome sequencing confirms that STC-1010 **allogeneic cell line selection covers the clinical CRC heterogeneity** (MSS/MSI-H) including the main **oncodriver mutations shared with CRC patients**. *(Fig 2)*
- Transcriptomic analysis demonstrates that each physical and chemical stimulation modified gene expression which **confirms the rational of using 3 cell lines with 2 stresses to provide a robust coverage of CRC heterogeneity.** *(Fig 3)*
- *143 Cancer-Related Protein (CRP) from the Atlas proteins' database were identified in 4 batches of STC-1010* ***including CRC-specific proteins and Tumor-Associated Antigens linked to tumor plasticity and resistances.*** *(Fig. 4)*

IN VIVO VALIDATION OF STC PLATFORM EFFICACY

We translated STC technology to produce murine surrogate with 1 cell line vaccine (CT26 CRC cell)
or 3 cell lines vaccine = mSTC-1010 (CT26, LTPA, CMT93)



HAPTENATION PROVIDES AN OPTIMAL IMMUNOGENICITY IN COMBINATIION TO STRESS

Fig. 5 Observation of strong decrease of tumor growth with immunogenic and stimulated vaccine (=Stressed + Haptenized) vs only stressed or Haptenized vaccine.

SIGNIFICANT INCREASE SURVIVAL OF TUMOR-BEARING MICE TREATED WITH mSTC-1010

- Immunocompetent syngeneic BALB/C mice
- CT26 Tumor grafted

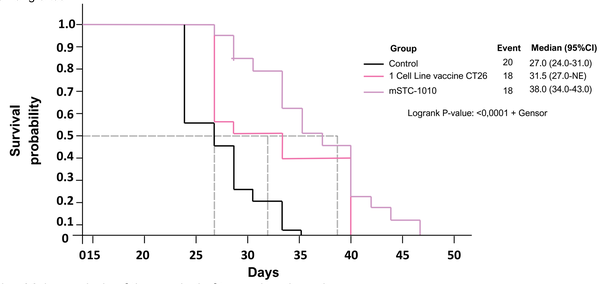


Fig. 6 Kaplan-Meier analysis of the survival of tumor-bearing mice.
Survival analyses indicated that the mSTC-1010 group had the best survival rate compared to the control group (p<0,0001, log-rank test) and the 1 cell line vaccinated group (p-value=0,0023, log-rank test). Log-rank test comparing all groups with each other, which shows the significance of one of the groups on this parameter log-rank test p-value < 0,0001. NE: not evaluable, the bounds of confidence indices (CI) cannot be calculated.

SIGNIFICANT EFFECT OF mSTC-1010 ON IMMUNE CHECKPOINT RESISTANT CRC TUMOR MODEL

- Immunocompetent syngenic C57BL mice
- MC38- antiPD1 Resistant Tumor grafted with its tumor micro environment (TME)
- 15 mice per group

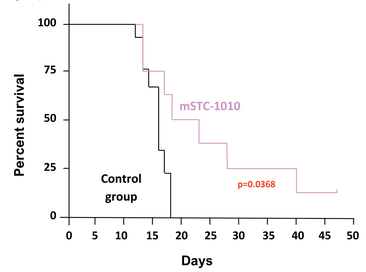
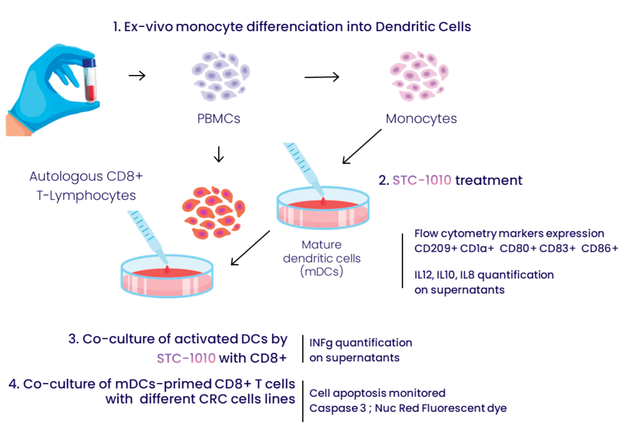


Fig. 7 Kaplan-Meier analysis of the survival of tumor-bearing mice
Significant improvement of the survival of mice bearing MC38- anti-PD-1 Resistant tumor treated by mSTC-1010 vs control (Log-rank Mantel-Cox test, p-value=0,0368).

- In vivo models confirm the efficacy of the STC platform within its different production steps, highlighting that a **three-cell line approach (mSTC-1010) improves survival of treated tumor-baring mice** over single-cell line vaccine *(Fig. 6)*, and **haptenation significantly enhances stressed cells vaccine anti-tumor efficacy** *(Fig. 5)*.
- STC Platform also **demonstrates significant anti-tumor activity in immune checkpoint resistant CRC in vivo model** *(Fig. 7)*
- In total, **175 mice were treated with the vaccine with no safety issues reported** (data not presented).

EX-VIVO IMMUNE RESPONSE ACTIVATION AND INDUCTION OF TUMOR KILLING

Fig. 8 Illustrating method of Ex-vivo model
Immune response activation and induction of CRC human cell lines Killing



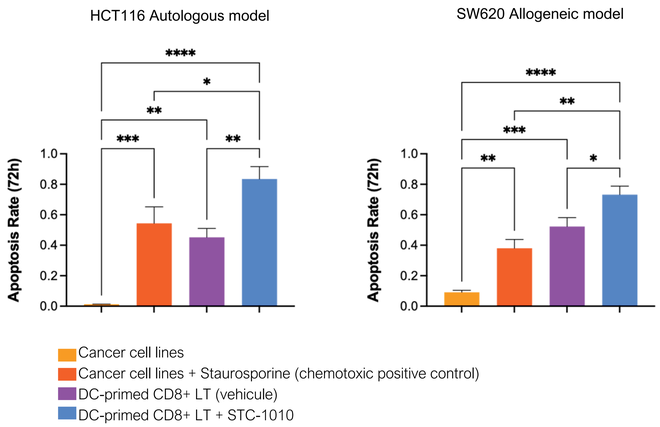


Fig 9. Significant anti-tumor effect of STC-1010 against different CRC cell lines
Tested on 6 donors for HCT116 (+50% apotosis) and on 4 donros for SW620 (+30% apotosis)

STC-1010 INDUCES MASSIVE APOTOSIS OF HUMAN CRC CELL LINES (ALLOGENEIC MODEL)

BATCH TO BATCH REPRODUCIBILITY

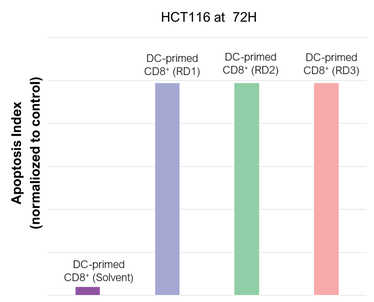


Fig 10. Apotosis index of HCT116 tumor cells induced by STC-1010 (RD1, RD2, RD3) *vs* Control at 72h

- *Ex-vivo* model, from different donors, mimicking human conditions confirms a **significant anti-tumor efficacy of human STC-1010 against CRC cell lines** in an allogeneic model demonstrating a powerfull immune induction. *(Fig. 8,9)*
- STC-1010 induces a robust anti-tumor efficacy performed via CD8+ LT apotosis induction against CRC tumor with a perfect **batch to batch reproducibility** *(Fig. 10)*

CONCLUSION

- **STC vaccine was tested on different models** (*in-vivo, ex-vivo*) **and confirms that the 3 cell lines, stimulated and haptenized vaccine is the best option to safely and efficently educate the patient's immune system against resistant tumor cells.**
- **A robust STC batch to batch reproducibility has been validated with multi-omic characterization and with ex-vivo induction of tumor apoptosis.**
- **The good tolerability and reproducible efficiency of the STC-1010 vaccine in these different pre-clinical models allow to plan a first-in-human phase I/II clinical trial for metastatic CRC patients.**

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Contact : galzeeb@brenus-pharma.com

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