**Background**

Metastatic colorectal cancer (mCRC) is a major cause of death. Unmet medical need in immunotherapy is high for MSS patients and still present for MSI-H/dMMR patients. STC-1010 (Brenus Pharma) therapeutic vaccine is developed by tumor cells stimulation to induce overexpression of tumor associated antigens and neoantigens to mimic mCRC resistant cancer cells. The aim is to educate the immune system to target patient’s tumor cells harboring the same resistance factors. We report efficacy results of three (3 CL-S) cell lines S=stimulated by irradiation and heat shock versus three cell lines (6 CL-S) physically and chemically stimulated (irradiation, heat shock and chemotherapies), both haptenized (H) and administrated with immunostimulant (IS=cyclophosphamide and mGM-CSF) associated w/o to standard chemotherapies FOLFOX or FOLFIRI.

**Methods**

Female C57BL6 mice were subcutaneously grafted with $1.10^6$ MC38 tumor cells. 7 groups (15 mice/group) were allocated to: Control, FOLFOX, FOLFIRI (intra-peritoneal injection to D5, D8 and D11 post-tumour injection), 3 CL-SH, 6 CL-SH, 6 CL-SH + FOLFOX and 6 CL-SH + FOLFIRI groups. Subcutaneous vaccine injections (3CL-SH or 6 CL-SH) were associated to IS once a week for 3 weeks. Overall survival (OS) and tumor growth (TG) were recorded until 1600 mm$^3$ or tumor necrosis. We conducted automated immunohistochemical analysis (HALO IndicaLabs software) on 5 tumor groups (n=35) to evaluate the correlation between response and immune population (number of cells/mm$^2$) including: CD3, CD4, CD8, FOXP3 T cells and M1/M2 macrophages.

**Results**

At Day16, all groups treated by 6CL-SH had a significant reduction of the mean tumor volume compared to the control group (p=0.0011), as well as for 6CL-SH+ FOLFIRI versus FOLFIRI alone (p=0.0024).

The necrotic tumors in the 3CL-SH, 6CL-SH and 6CL-SH +FOLFIRI groups are denser (weight/volume) than the control group. Tumors treated by 6CL-SH+FOLFIRI were also denser than the FOLFIRI ones (p=0.0052).

Grafted mice treated by FOLFOX alone had dramatic weight loss and some had to be sacrificed.

HALO analysis showed that adding 6CL-SH to FOLFOX increase CD8 infiltration in comparison with FOLFOX alone (> 200 cells/mm$^3$) and a recruitment of immune cells within the tumor. Among treated groups, M1/M2 ratio >7 was main criteria correlated with a long survival.

**Conclusions**

This third preclinical study confirms efficacy and safety of Brenus STC vaccine stimulated and haptenized alone or with standard chemotherapies associated to immunostimulant. This significant anticancer effect in mice could be explained by mobilization of CD3, CD8, CD4 T cells within the tumors and oriented M1 macrophage immune responses.