Neo-antigen based EpiGVAX vaccine initiates anti-tumor immunity in colorectal cancer

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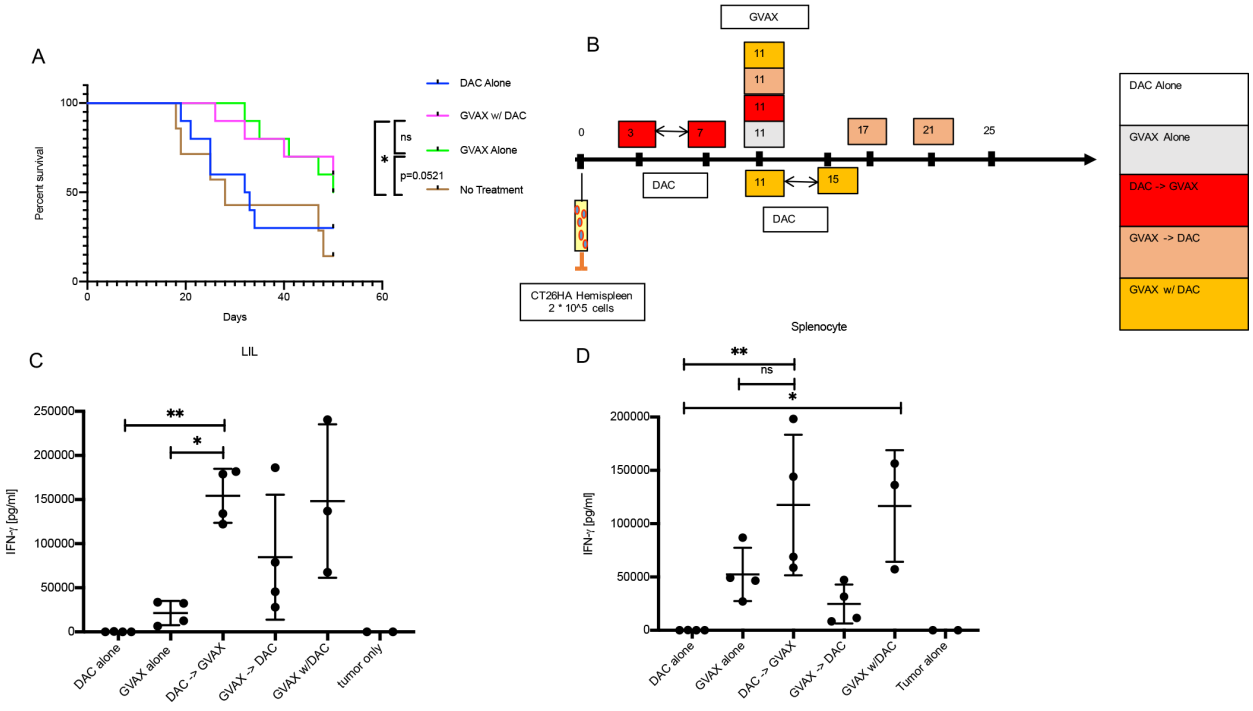
Supplement Materials:

Methods:

Mouse IFN-γ enzyme-linked immunosorbent assays (ELISA)

CD8+T cells were isolated from tumor-bearing mice liver and spleen and co-cultured with autologous CT26-HA tumor cells irradiated with 50 Gy for 18 hours in AIMV medium (Thermo Fisher Scientific) at 37°C incubator with 5% CO_2. Mouse IFN-γ enzyme-linked immunosorbent assay (ELISA) Ready-Set-Go (eBioscience) was then conducted using the supernatant following the manufacturer's protocol.



Supplement Figure 1: Addition of Decitabine (DAC) to GVAX therapy did not improve its anti-tumor efficacy in a liver metastatic colorectal murine model. GVAX+Pre-DAC regimen was adopted for futher optimization. (A) 2×10^5 CT26 tumor cells were inoculated via a hemispleen surgery on day 0, mice were treated with GVAX at day 11, and DAC was dosed from day 11-15 for 5 days. Mice were followed up to 50 days for survival analysis. DAC alone (*n*=10), Cy+DAC w/DAC (*n*=10 mice), Cy+DAC (*n*=10 mice), No treatment (*n*=7 mice). Data represents one representative experiment that was repeated twice. Survival statistical analysis was done by using log-rank test adjusted by multiple testing. **p<0.01; *p < 0.05; ns, not significant. **(B)** Treatment schema to test the dosing schedules of DAC combined with GVAX in three

different ways, mice were sacrificed at day 25. **(C) (D)** Mice were sacrificed on day 14 post-surgery. IFN-γ ELISA was performed on both isolated liver infiltrating lymphocytes and splenocytes after co-culturing with irradiated CT26-HA cells for 72 hs. N=4 for all treatment groups except N=2 for tumor only group, N=3 for GVAX/DAC group. Multiple comparions of unpaired one-way ANOVA test with Tukey p-value adjustment was used for statistical analysis. **$p < 0.01$; *$p < 0.05$; ns, non-signifiant.

Table S1. Day 50 Interim Analysis of Mouse Survival

Treatment Groups	Survival % at Day 50	*P* value (versus GVAX w/DAC) by two-sided Chi-Square test
DAC Alone (n=10)	30	ns
GVAX w/DAC (n=10)	70	
GVAX Alone (n=10)	60	ns
No treatment (n=7)	10	**

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Table S2. % Mice Develop Tumor at Day 25 Post Surgery

Treatment Groups (n=5)	% Mice with visble tumor (liver mets, peritoneal implants)	*P* value (versus DAC -> GVAX) by two-sided Chi-Square test
Tumor Alone	100	****
DAC Alone	60	****
GVAX Alone	0	ns
DAC -> GVAX	0	
GVAX -> DAC	80	****
GVAX w/DAC	20	ns

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