1180 LISTERIA-BASED IMMUNOTHERAPY SCULPTS CD8$^+$ T CELL RESPONSE IN THE TUMOR MICROENVIRONMENT TO CONTROL RENAL CELL CARCINOMA

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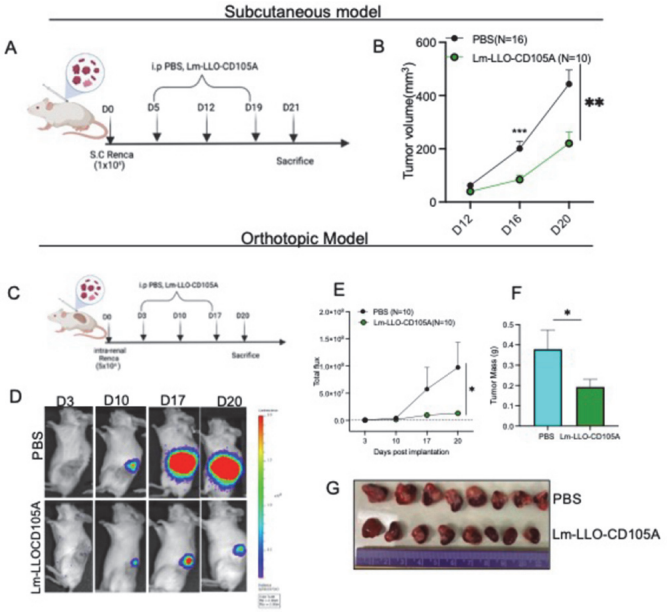
Background Agents that modulate the tumor microenvironment (TME) to promote anti-tumor effects are desirable for the therapy of solid tumors. Recombinant Listeria-monocytogenes vaccine consisting of the fusion protein of truncated Listeriolysin O and tumor-associated antigen (TAA) is an established strategy to improve antigen-specific T cell response for cancer immunotherapy. CD105 is a co-receptor for the TGF-beta signaling cascade. Due to its elevated expression in many cancer types, including renal cell carcinoma (RCC), CD105 is characterized as a TAA of therapeutic potential. Here we evaluated the efficacy and immunomodulatory effects of a Listeria-based vaccine encoding CD105 (Lm-LLO-CD105A) for the therapy of renal cell carcinoma (RCC) in a murine model.

Methods Murine RCC cell line (Renca) cells were implanted subcutaneously or orthotopically into male Balb/c mice. Subsequently, mice were vaccinated with PBS, Lm-LLO-CD105A, or Control Lm weekly for three weeks, and tumor progression was routinely monitored. Splenocytes and subcutaneous and orthotopic tumor tissues were immunophenotyped by flow cytometry. Lymphocyte depletion experiments were carried out in subcutaneous and orthotopic models to evaluate the immune subset responsible for the anti-tumoral effect of the recombinant Listeria-based vaccine.

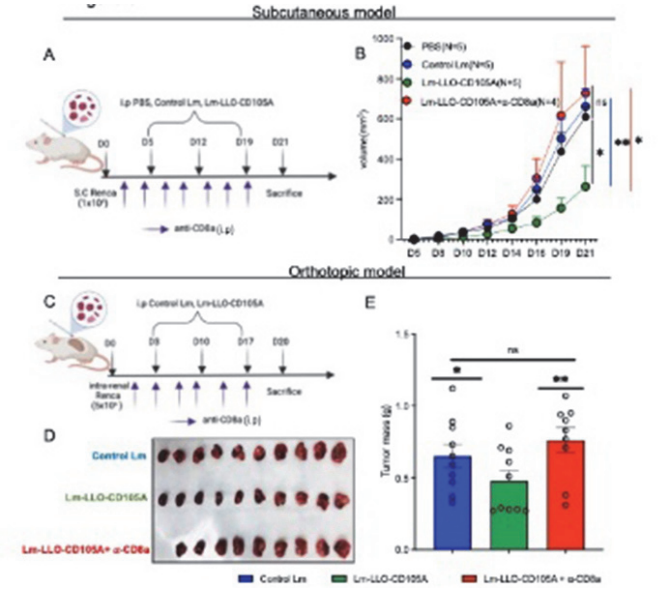
Results In both models, vaccination with Lm-LLO-CD105A led to significant control of tumor growth compared to placebo and Control Lm (figure 1). This antigen-specific vaccine promoted tumor control by significantly improving the infiltration of effector CD8$^+$ T and CD4$^+$T cells into the TME. The CD8$^+$ T cells are polyfunctional and characterized by profound production of inflammatory cytokines, including IFN-g, IL-2, and TNF-a, but the functionality of the CD4$^+$ T cells was not as robust. Similarly, the systemic cytotoxic response demarcated by IFN-g $^+$IL-2 $^+$ TNF -a $^+$ producing splenic CD8$^+$ T cells was highly potent upon Lm-LLO-CD105A vaccination. Further, in both subcutaneous and orthotopic models, the TME was polarized from an immunosuppressive phenotype to an inflamed phenotype characterized by a reduction in the CD4$^+$ Foxp3$^+$Treg population and a reduced population of MDSCs (CD11b$^+$Gr1$^+$) in the kidney TME. Not surprisingly, the antitumor effect was completely abrogated when Lm-LLO-CD105A was utilized in the presence of a CD8$^+$ T cell-depleting antibody (figure 2)

Conclusions Lm-LLO-CD105A effectively controlled subcutaneous and orthotopic kidney tumors. This efficacy depended on the profound infiltration of cytokine-producing CD8$^+$ T cells and the reduction of suppressive immune subsets in the TME. Altogether our data suggest that tumor targeting with a Listeria-based approach is an effective strategy to modulate the TME for effective control of renal cell carcinoma.

Ethics Approval All studies involving animals were carried out in accordance with ethical standards of the Texas Tech University Health Sciences Center under the IACUC protocol number 17018



Abstract 1180 Figure 1 Lm-LLO-CD105 controls RCC tumor growth (A) vaccination schedule for subcutaneous tumor challenge (B) longitudinal tumor growth curve of subcutaneous tumor challenge (C) vaccination schedule for orthotopic tumor challenge (D) representative bioluminescence imaging of kidney tumor burden (E) graphical representation of longitudinal luciferase flux (F) tumor mass of diseased kidney (G) image of final kidney tumor burden. Number of animals are depicted in the plots. Data were analysed using unpaired t-test. *P < 0.05, **P < 0.01, ***P< 0.001. All error bars are shown as mean ± SEM.



Abstract 1180 Figure 2 CD8+ T cells are responsible for the therapeutic efficacy
Experimental schedule for subcutaneous CD8+ depletion studies (B) longitudinal tumor growth curve (C) experimental schedule for orthotopic CD8+ depletion studies (D) Representative image of diseased kidney (E) final kidney mass. Number of mice for A-B is depicted in the plots. Number of animals for (C-E) Control Lm=10, Lm-LLO-CD105A =10, Lm-LLO-CD105A+α-CD8a=9 Data were analyzed using unpaired t-test for subcutaneous model (B) and Mann Whitney U test for

orthotopic model (E). *P < 0.05, **P < 0.01, ***P< 0.001. All error bars are shown as mean ± SEM.

http://dx.doi.org/10.1136/jitc-2022-SITC2022.1180