# Vaccination with Two Distinct Vaccine Platforms Targeting the Same Antigen Generates Phenotypically and Functionally Distinct T-cell Populations

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## Abstract #347

Purpose: Studies comparing different vaccine types in a given model are usually performed to identify a superior vaccine for monotherapy. We hypothesized that different vaccine platforms could introduce an antigen to the immune system by both shared and unique mechanisms, and that these diverse platforms might induce distinct antigen specific T-cell populations. Moreover, the vector itself could contribute to the way antigen is processed and effect the generation of T-cell responses. We examined the possibility that two vaccine platforms targeting the same antigen could be used concomitantly.

Experimental Design: We have previously documented the antitumor efficacy of the recombinant poxviral based vaccine regimen of rV/F-CEA/TRICOM in preclinical models, which has provided the rationale for ongoing clinical trials. Recently, we reported antitumor effects upon vaccination with a recombinant *Saccharomyces cerevisiae* (Yeast-CEA) in preclinical models of pancreatic and colon cancers. Here we investigated the antigen-specific immune responses generated by rV/F-CEA/TRICOM vs. Yeast-CEA, and directly compared the two vaccine platforms in terms of CD4 T-cell proliferation, CD8 T-cell activity, and induction of CEA antibody, cytokine response, and T-cell receptor phenotype. After vaccination of CEA transgenic mice with either vaccine platform, T-cell lines were isolated that were specific for two distinct epitopes of the CEA protein. These were used in in-vitro assays to compare properties of T-cells generated from either vaccine platform. Finally, we have examined the administration of these vaccine platforms concomitantly to determine whether it can generate a more polyclonal and functionally diverse T-cell population.

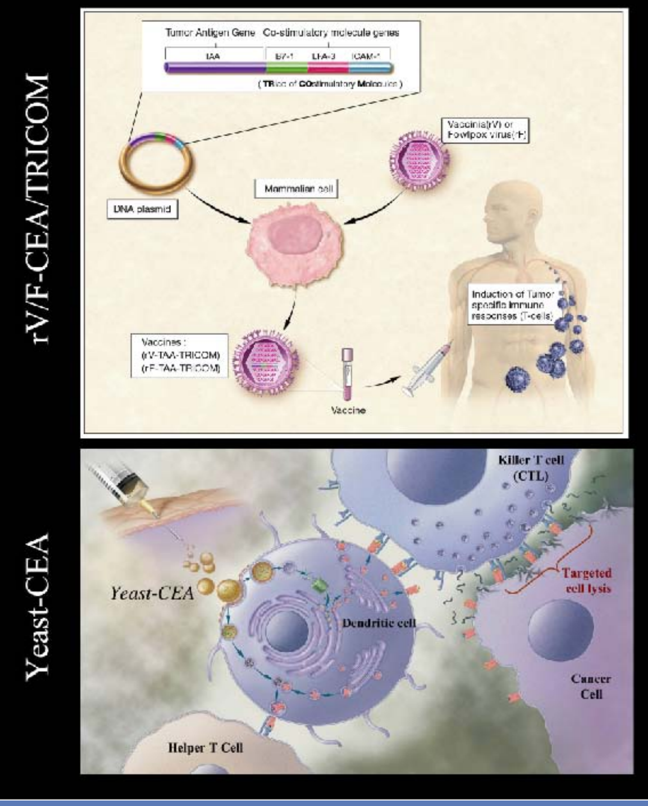
Results: These studies demonstrate for the first time that vaccination with either of the rV/F-CEA/TRICOM or Yeast-CEA vaccine platforms elicited T-cells with both shared and unique TRC TCR profiles and distinct cytokine expression patterns. Concomitant vaccination with both vaccine platforms elicited a significantly higher level of antitumor activity in an orthotopic pulmonary metastasis model.

Conclusions: These findings suggest that rV/F-CEA/TRICOM and Yeast-CEA could be combined to generate a more polyclonal and functionally diverse T-cell population.

## Rationale

Numerous immunotherapy studies have been reported comparing vaccine platforms that target the same antigen, in terms of their ability to induce immune cell activity and antitumor effects . We have previously documented the antitumor efficacy of the vaccine regimen of recombinant vaccinia (rV) and recombinant fowlpox (rF) viruses containing murine B7-1, ICAM-1, and LFA-3 genes as well as the human CEA gene (rV/F-CEA/TRICOM) in preclinical models. Recently, we reported the antitumor effects of a recombinant *Saccharomyces cerevisiae* (yeast-CEA) vaccine in preclinical models. We have also previously documented the induction of immune response after vaccination with either rV/F-CEA/TRICOM or yeast-CEA. The antitumor effects elicited by either vaccine are mainly attributed to the induction of a CEA-specific T-cell population. This is the first study to concurrently administer two different vaccine vectors targeting the same antigen in an antitumor model. As we had previously shown that the vaccines induce similar levels of $CD4^+$ T-cell proliferation and $CD8^+$ T-cell cytolytic activity, this study focused on comparing the T-cell populations induced by the rV/F-CEA/TRICOM and yeast-CEA vaccines. We hypothesized that due to both vector- and antigen-specific effects, the rV/F-CEA/TRICOM and yeast-CEA vaccines would induce distinct T-cell populations, and that concurrent administration of the two vaccines would thus result in a more diverse T-cell population and enhanced antitumor immunity.

## Vaccine Platforms Targeting the Same Tumor Antigen (CEA)




## Vaccination with rV-CEA/TRICOM or Yeast-CEA Induces Different Serum Cytokine Profiles

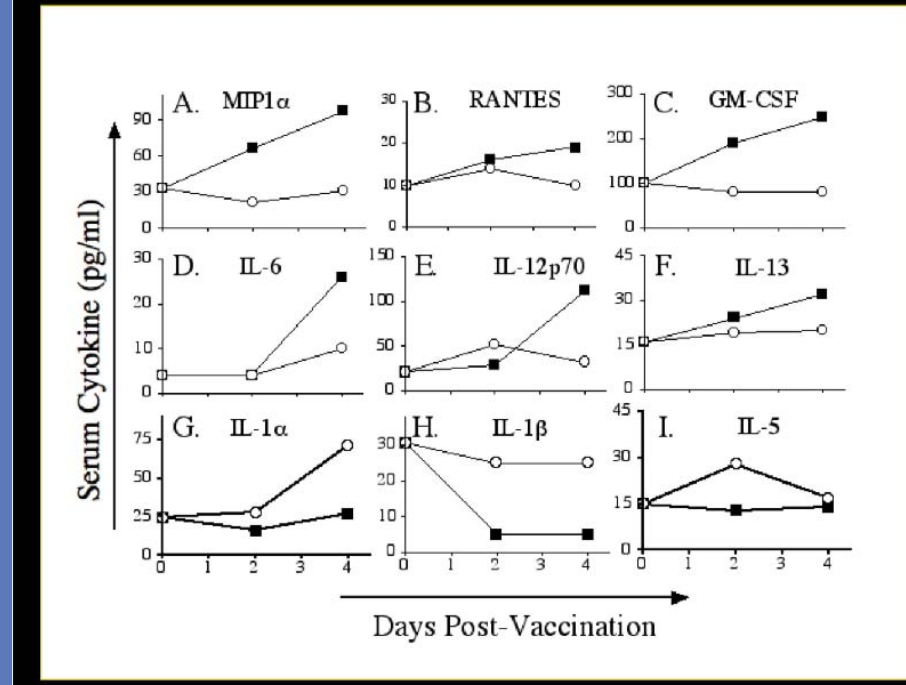



Figure 1.

CEA-Tg mice were vaccinated with rV-CEA/TRICOM (closed squares) or yeast-CEA (open circles). Serum was collected at 0, 2, and 4 days, pooled and analyzed for a panel of cytokines including (A) MIP1α, (B) RANTES, (C) GM-CSF, (D) IL-6, (E) IL-12p70, (F) IL-13, (G) IL-1α, (H) IL-1β and (I) IL-5. Data are presented as pg/ml of cytokine on each day.

## Vaccination with rV/F-CEA/TRICOM or Yeast-CEA Induces Expression of both Shared and Unique Genes Involved in T- and B-cell Activation, Chemokines and Chemokine Receptors, and Cytokines

| Vaccination | | Genes involved in T-cell and B-cell activation | Chemokines and chemokine receptors | Cytokines |
|---|---|---|---|---|
| rV/F-CEA/TRICOM / No Treatment | Genes up-regulated ≥ 2-fold | H60, Igbp1b, Il11, Il4 | Inha (3.03), Ltb4r2, Bmp10, Bmp5 | **Il17c, Il17f, Inhba** Fgf10, Gdf2, Gdf5, Gdf8, Ifna2, Ifna4, Ifnb1, Il13, Il17b, Il25, Il19, Ilf10, Ilf5, Ilf6, Ilf8, Il20, Il3, Il9 |
| | Genes down-regulated ≥ 2-fold | Ms4a1, Spp1 (4.00) | | **Il1rn** (4.29) |
| Yeast-CEA / No Treatment | Genes up-regulated ≥ 2-fold | **Rag1** (3.48) H60 (3.25), Igbp1b (3.25), Il11 (3.25), Il4 | **Bdnf, Ccl20, Cmtm2a, Cmtm5, Cxcl15, Gdf5, Ccl17** Inha (4.92), Ltb4r2 (4.92), Bmp10, Bmp5 | **Gdf1** Fgf10, Gdf2, Gdf5, Gdf8, Ifna2, Ifna4, Ifnb1, Il13, Il17b, Il25, Il19, Ilf10, Ilf5, Ilf6, Ilf8, Il20, Il3, Il9 |
| | Genes down-regulated ≥ 2-fold | **Tnfrsf13c** (6.06) Ms4a1 (3.25), Spp1 | **Ccl12** (3.03), **Ccl8**, (3.73) **Ccr9**, **Ccrl2**, **Csf2**, **Cx3cr1**, **Cxcl10**, **Cxcl13**, **Hif1a**, **Inhbb**, **Lif** (3.48), **Gusb**, **Cxcl9** (4.92) | **Gdf3** (4.0), **Il10**, **Csf-2**, **Fasl**, **Tnf**, **Tnfrsf11b**, **Tnfsf15** |

* Genes in bold are up-regulated/down-regulated specifically by splenocytes from mice vaccinated with either rV/F-CEA/TRICOM or yeast-CEA, but not by both. Fold changes ≥ 3-fold are noted in parentheses.

## Distinct TCR Repertoires are Induced from Vaccination with rV/F-CEA/TRICOM or Yeast-CEA

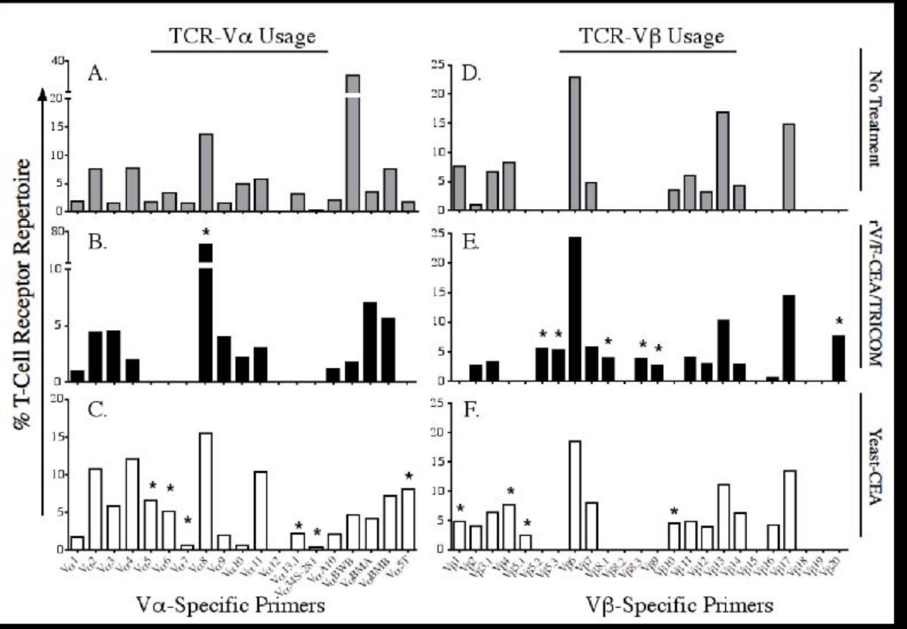



Figure 2.

CEA-Tg mice (n = 5) were vaccinated weekly for four weeks with rV/F-CEA/TRICOM or with yeast-CEA. On day 35, mice were sacrificed, spleens were harvested, and splenocytes were pooled. RNA was extracted from splenocytes and underwent RT-PCR using primers specific for Vα (A-C) or Vβ (D-F) genes. Vα profiles for (A) no treatment, (B) rV/F-CEA/TRICOM, and (C) yeast-CEA are shown. Vβ profiles for (D) no treatment, (E) rV/F-CEA/TRICOM, and (F) yeast-CEA are shown. Each gene was quantified by calculating % of total TCR repertoire. * indicates genes that are uniquely expressed in T-cells from mice vaccinated with one vaccine compared to the other.

## Vaccination with rV/F-CEA/TRICOM or Yeast-CEA Induces Distinct Cytokine Profiles in Response to *in vitro* Stimulation with Two Discrete CEA Epitopes

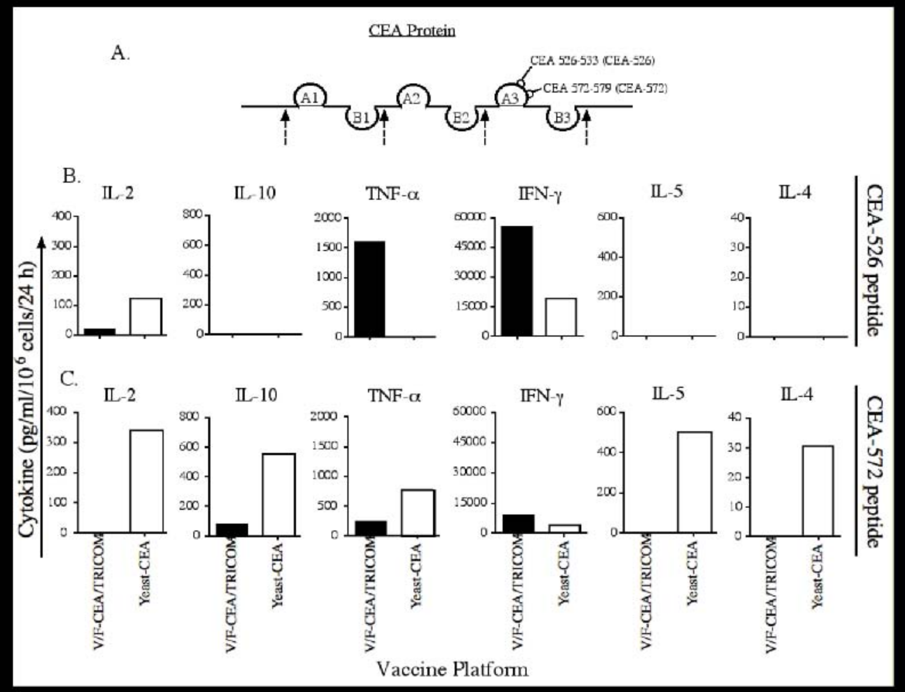



Figure 3.

(A) CEA protein showing the discrete, non-overlapping CEA-526 and CEA-572 epitopes on the A3 loop of domain III. CEA-Tg mice (n = 5) were vaccinated weekly for 4 weeks with rV/F-CEA/TRICOM (solid bars) or with yeast-CEA (open bars). On day 33, mice were sacrificed and spleens pooled and put into bulk cultures with either (B) CEA-526 or (C) CEA-572 epitope for 7 days. IL-2, IL-10, TNF-α, IFN-γ, IL-5 and IL-4 were measured by cytokine bead array (pg/ml/1x10$^6$ cells) after lymphocytes were restimulated for 24 h with CEA-specific peptide or VSVN peptide control.

## T-Cell Lines Specific for the CEA-572 Epitope from Mice Vaccinated with rV/F-CEA/TRICOM or Yeast-CEA have Distinct TCR Vα Profiles

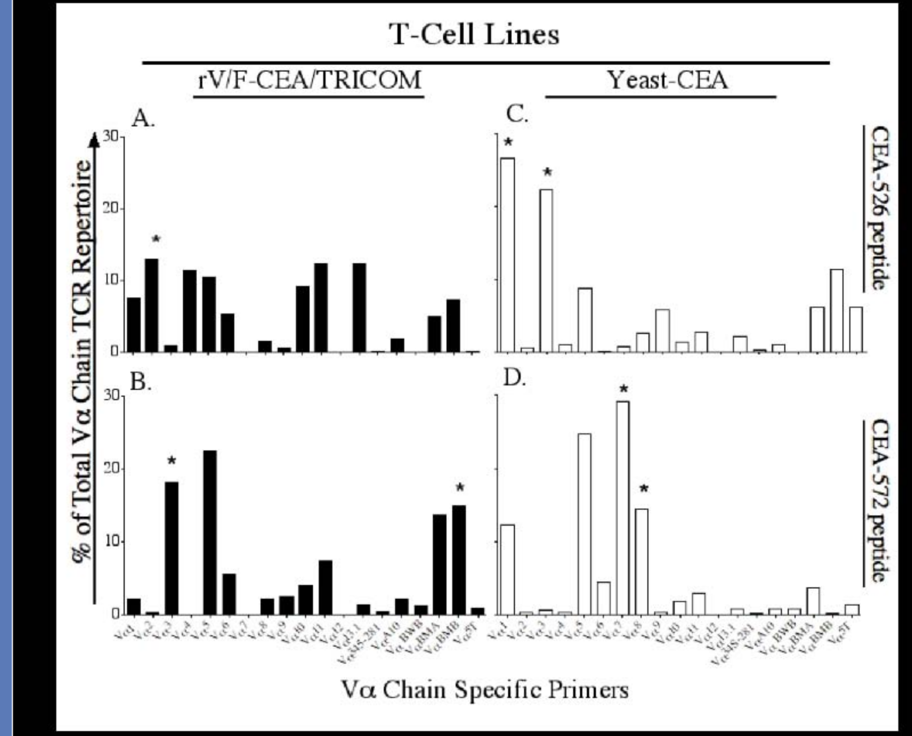



Figure 4.

Vα TCR repertoires of rV/F-CEA/TRICOM T-cell lines (black bars) maintained in the presence of (A) CEA-526 peptide and (B) CEA-572 peptide. Vα TCR repertoires of yeast-CEA T-cell lines (white bars) maintained in the presence of (C) CEA-526 peptide and (D) CEA-572 peptide. Results are expressed as % of total Vα chain TCR repertoire. T cells were maintained in culture for 18 weeks before TCR repertoire analysis was performed. * indicates genes that are uniquely expressed in T cells from mice vaccinated with one vaccine compared to the other.

## CEA Epitope-specific T-Cell Lines Generated from Mice Vaccinated with rV/F-CEA/TRICOM or Yeast-CEA have Similar Levels of Lytic Activity but Unique Avidity

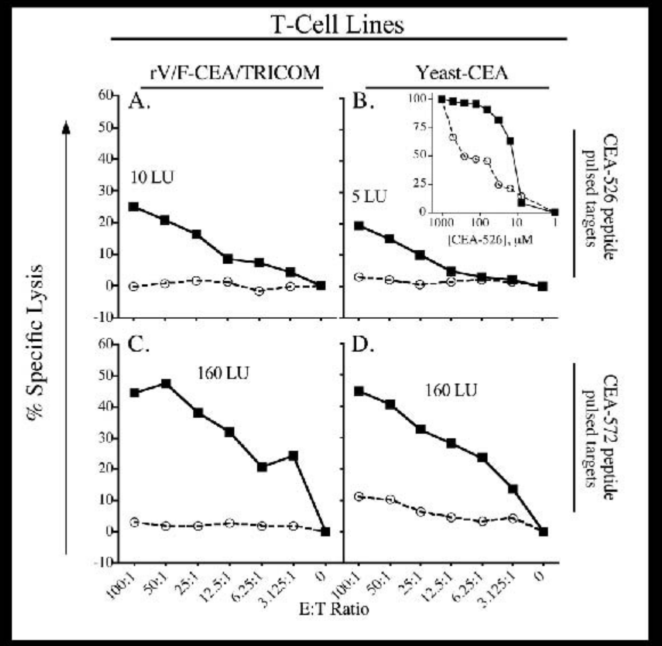



Figure 5.

T-cell lines generated from rV/F-CEA/TRICOM vaccination and specific for (A) CEA-526 peptide and (C) CEA-572 peptide and T-cell lines generated from yeast-CEA vaccination and specific for (B) CEA-526 peptide and (D) CEA-572 peptide were used in CTL assays with $^{111}$In-labled EL-4 targets pulsed with CEA-572 or CEA-526 peptides (solid squares connected by a solid line), and VSVNP (negative control, open circles connected by a dotted line). The % specific lysis was calculated. To determine T-cell avidity, (B, inset) CEA-526-specific T-cell lines from rV/F-CEA/TRICOM (closed squares) and yeast-CEA (open circles) were incubated with $^{111}$In-labeled EL-4 cells in the presence of CEA-526 (or HIV-gag control) peptide; the avidity of each T-cell population was defined as the negative log of the peptide concentration that resulted in 50% maximal target lysis.

## CEA Epitope-specific T-Cell Lines Generated from Mice Vaccinated with rV/F-CEA/TRICOM or Yeast-CEA have Differential Activity Against Tumor Cells

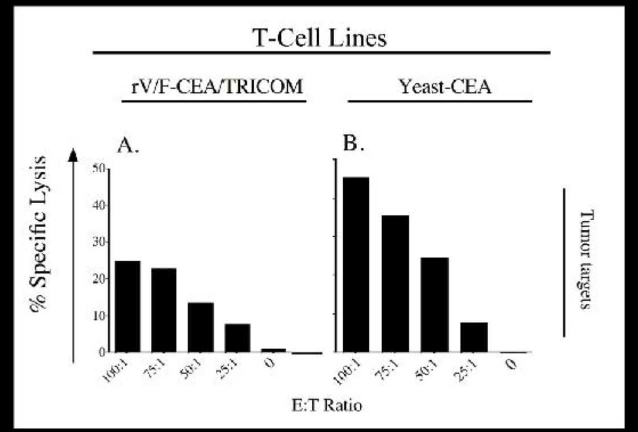



Figure 6.

T-cell lines specific for CEA-572 epitope, generated from mice vaccinated with rV/F-CEA/TRICOM (A) or yeast-CEA (B), were also used in cytolytic T-cell assays with $^{111}$In-labeled LL2-CEA.

## Combining rV/F-CEA/TRICOM and Yeast-CEA Vaccines in a Pulmonary Metastasis Model Increases Antitumor Immunity

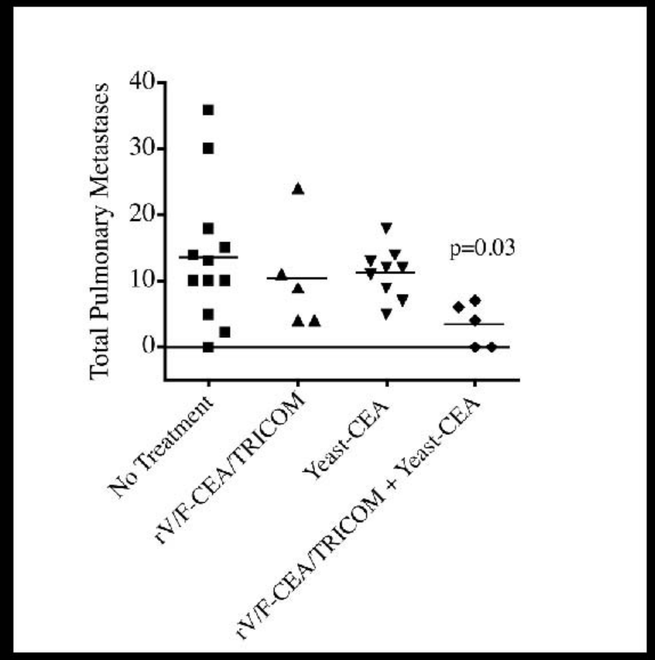



Figure 7.

CEA-Tg mice were injected i.v. with LL2-CEA tumor cells. Mice were vaccinated weekly begining on day 4 with rV/F-CEA/TRICOM (n = 5), yeast-CEA (n = 9), or rV-CEA/TRICOM and yeast-CEA (n = 5); a control group (n = 12) received no treatment. rV/F-CEA/TRICOM was administered s.c. on the dorsal right flank. Yeast-CEA was administered s.c. 1 YU/site at 4 distal sites. On day 45, mice were sacrificed and lungs were harvested, stained, and fixed. (A) Data represent the number of lung metastases per mouse. The bar indicates the average number of metastases per mouse. p = 0.03 comparing untreated mice with the rV/F-CEA/TRICOM and yeast-CEA combination group.

## Conclusions

- rV/F-CEA/TRICOM and yeast-CEA induce shared and unique cytokine response, gene expression, and T-cell receptor phenotype
- Analysis of cytokine expression showed that rV/F-CEA/TRICOM induces a Tc1 $CD8^+$ T-cell population, while yeast-CEA can drive both Tc1- and Tc2-type $CD8^+$ T cells, and indicated that rV-CEA/TRICOM produces a Th1 response, while yeast-CEA induces a mixed Th1/Th2 response in $CD4^+$ T cells
- These studies show for the first time that:
  - These two vaccine platforms, both of which target the same antigen, induce distinct T-cell populations
  - Induction of these T-cell populations is both vector- and antigen-specific
  - Two vaccines can be combined concurrently in an antitumor model to improve antitumor efficacy