



Jefferson[™]
Kimmel Cancer Center
NCI-designated

Protocol #1304-1226

A Phase I study of Guanylyl Cyclase C (GCC)-Encoding
Replication-Deficient Human Type 5 Recombinant
Adenovirus Vaccine (Ad5-hGCC-PADRE) in Stage I and II
Colon Cancer Patients

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Sponsor: Scott Waldman, M.D., Ph.D.

Colorectal Cancer

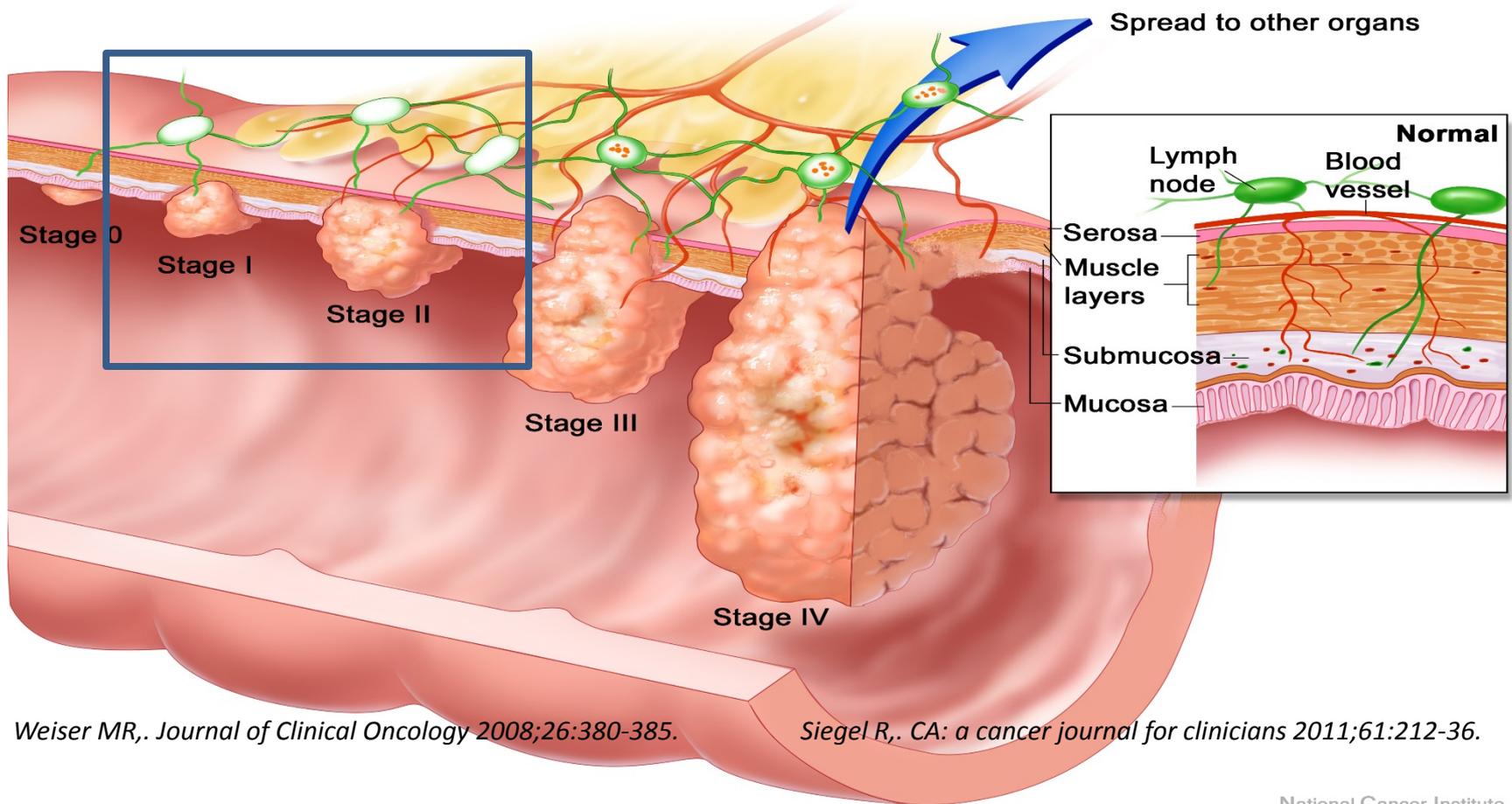
Leading New Cancer Cases and Deaths – 2012 Estimates

| Estimated New Cases* | | Estimated Deaths | |
|--------------------------------------|--------------------------------------|---|--|
| Male | Female | Male | Female |
| Prostate 241,740 (29%) | Breast 226,870 (29%) | Lung & bronchus 87,750 (29%) | Lung & bronchus 72,590 (26%) |
| Lung & bronchus 116,470 (14%) | Lung & bronchus 109,690 (14%) | Prostate 28,170 (9%) | Breast 39,510 (14%) |
| Colon & rectum 73,420 (9%) | Colon & rectum 70,040 (9%) | Colon & rectum 26,470 (9%) | Colon & rectum 25,220 (9%) |
| Urinary bladder 55,600 (7%) | Uterine corpus 47,130 (6%) | Pancreas 18,850 (6%) | Pancreas 18,540 (7%) |
| Melanoma of the skin 44,250 (5%) | Thyroid 43,210 (5%) | Liver & intrahepatic bile duct 13,980 (5%) | Ovary 15,500 (6%) |
| Kidney & renal pelvis 40,250 (5%) | Melanoma of the skin 32,000 (4%) | Leukemia 13,500 (4%) | Leukemia 10,040 (4%) |
| Non-Hodgkin lymphoma 38,160 (4%) | Non-Hodgkin lymphoma 31,970 (4%) | Esophagus 12,040 (4%) | Non-Hodgkin lymphoma 8,620 (3%) |
| Oral cavity & pharynx 28,540 (3%) | Kidney & renal pelvis 24,520 (3%) | Urinary bladder 10,510 (3%) | Uterine corpus 8,010 (3%) |
| Leukemia 26,830 (3%) | Ovary 22,280 (3%) | Non-Hodgkin lymphoma 10,320 (3%) | Liver & intrahepatic bile duct 6,570 (2%) |
| Pancreas 22,090 (3%) | Pancreas 21,830 (3%) | Kidney & renal pelvis 8,650 (3%) | Brain & other nervous system 5,980 (2%) |
| All sites 848,170 (100%) | All sites 790,740 (100%) | All sites 301,820 (100%) | All sites 275,370 (100%) |

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

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Colorectal Cancer



Weiser MR, *Journal of Clinical Oncology* 2008;26:380-385.

Siegel R, *CA: a cancer journal for clinicians* 2011;61:212-36.

National Cancer Institute



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Current Treatment Options

| Standard Treatment Options for Colon Cancer | |
|---|---|
| Stage 0, I and II Colon Cancer | Surgery |
| Stage III Colon Cancer | Surgery Adjuvant chemotherapy |
| Stage IV and Recurrent Colon Cancer | Surgery Chemotherapy and targeted therapy Second-line chemotherapy |
| Liver Metastasis | Surgery Neoadjuvant chemotherapy Local ablation Adjuvant chemotherapy Intra-arterial chemotherapy |

National Cancer Institute: PDQ® Colon Cancer Treatment. Bethesda, MD: National Cancer Institute. Date last modified 02/08/2013. Available at: <http://cancer.gov/cancertopics/pdq/treatment/colon/HealthProfessional>. Accessed 05/09/2013.



Colorectal Cancer Immunotherapy Meta-Analysis

Stage II suspected minimal residual CRC

OS: HR = 0.71, P = 0.09

DFS: HR = 0.66, P = 0.02.

Stage III suspected minimal residual CRC

OS: HR = 0.76, P = 0.02

DFS: HR = 0.81, P = 0.03

Stage IV

CR or PR in 11/656 patients (1.68%)

No serious adverse events have been observed in 2031 patients.

Rao et al. Journal of Translational Medicine 2011, 9:17



Colorectal Cancer-Associated Antigens

Mutant Self Proteins

K-ras p53

Oncofetal / Cancer Testis Antigens

β hCG Gastrin 5T4

Overexpressed Self Antigens

p53 MUC1 SART

Sialyl-Tn Her2/neu ART

Survivin CD55 Ep-CAM

Carcinoembryonic Antigen (CEA)

These antigens may be suboptimal:

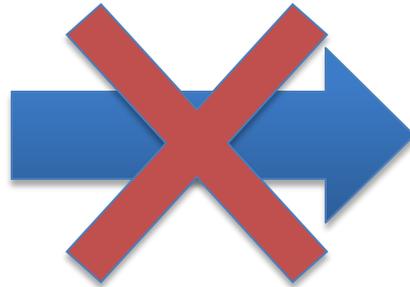
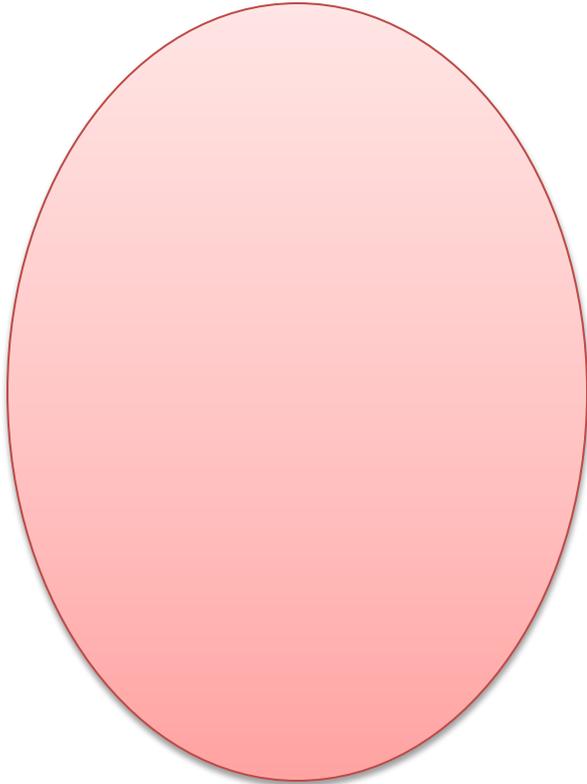
- Not tumor-specific
- Not sufficiently immunogenic
- Not universally-shared among different patients

Adapted from: Dalerba P, et al. Crit Rev Oncol Hemat 2003;46(1):33-57.

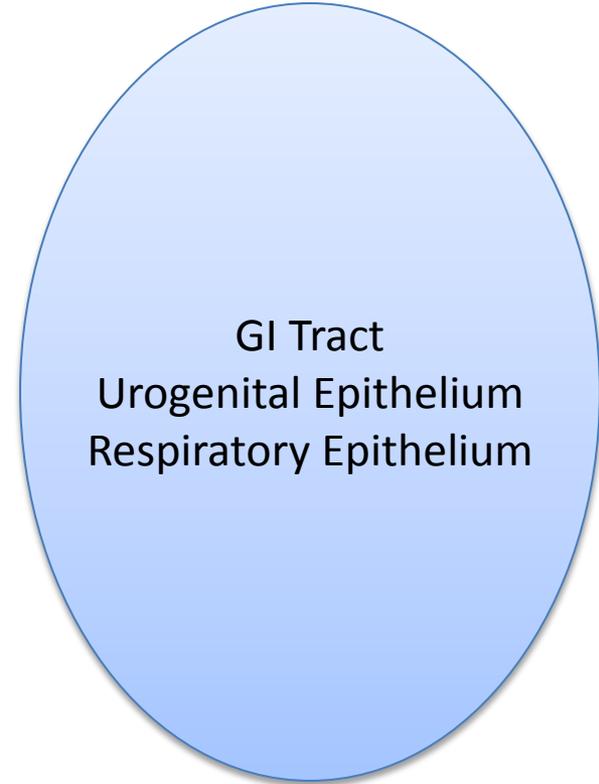


Immune Compartmentalization

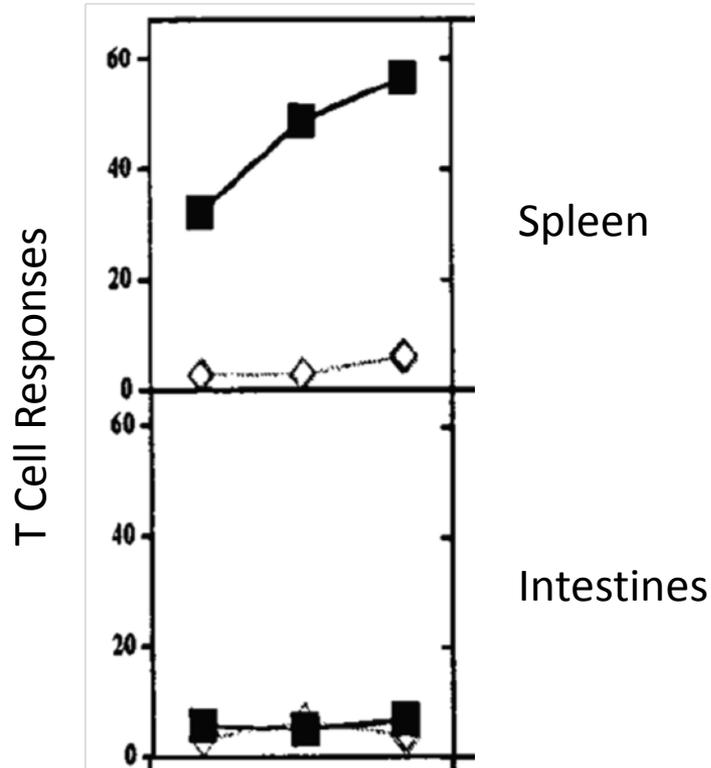
Systemic Compartment



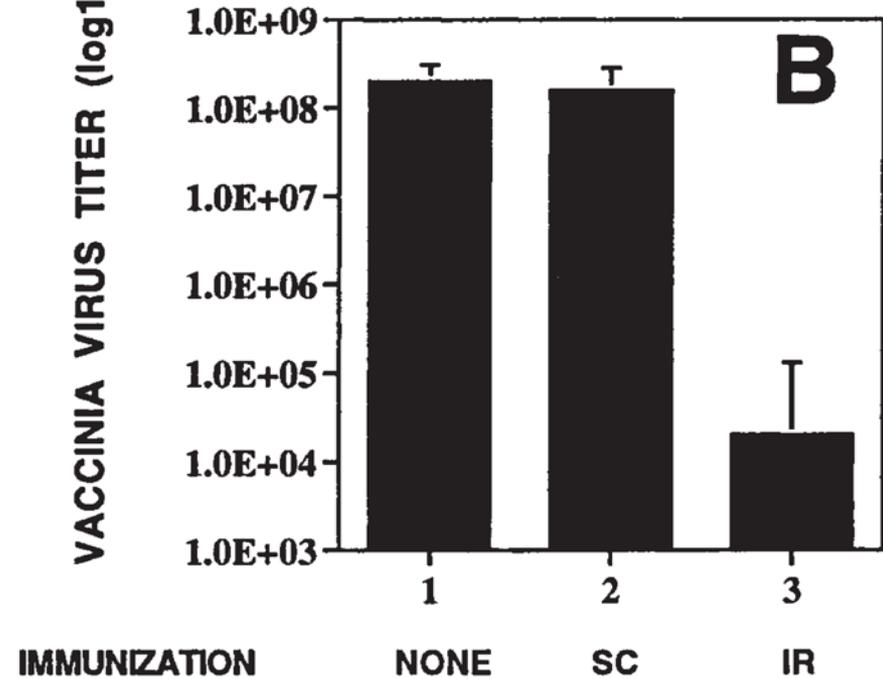
Mucosal Compartment



Systemic
Vaccin~



VACCINIA VIRUS TITER (log10)

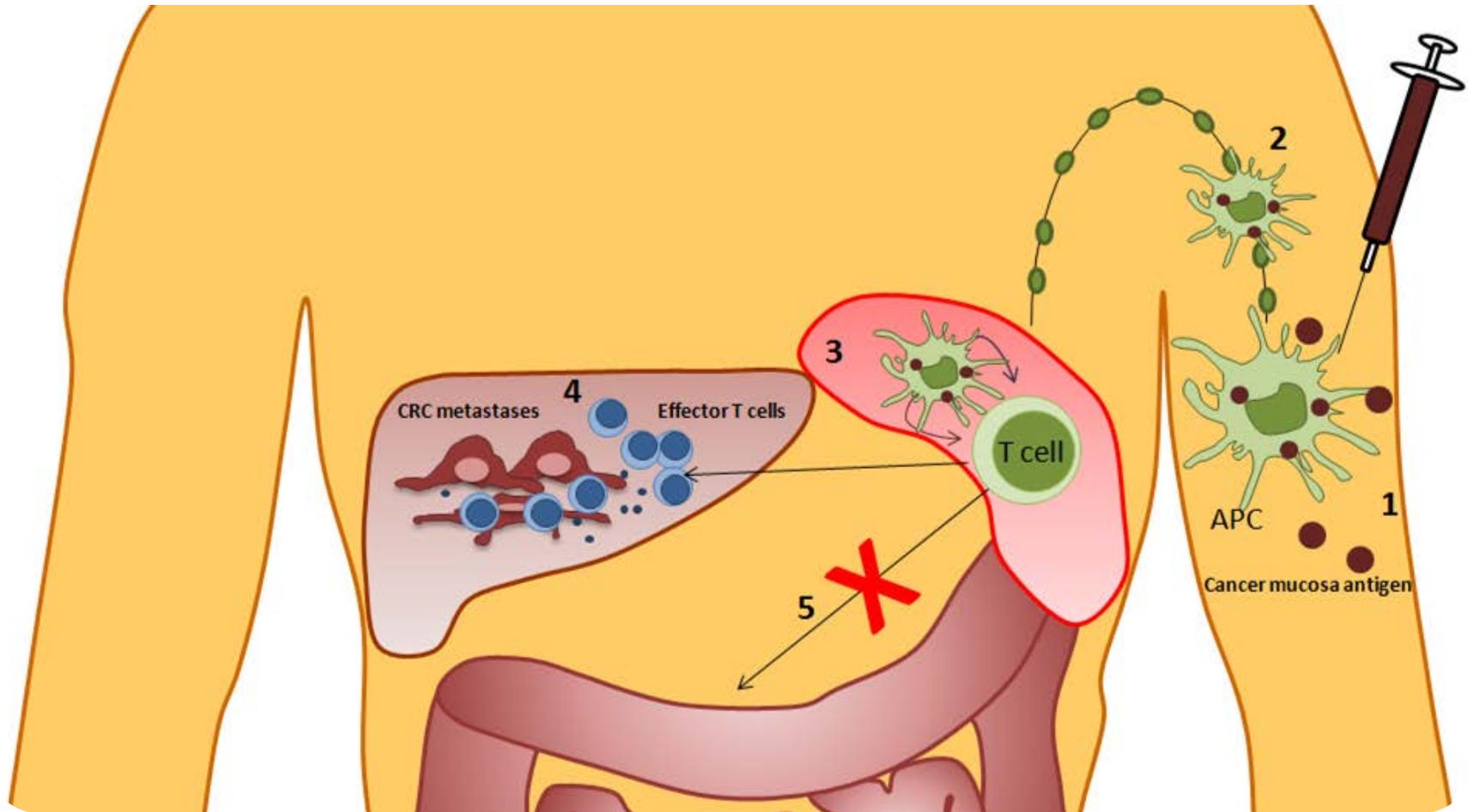


Belyakov, I.M. et al. 1998. Mucosal immunization with HIV-1 peptide vaccine induces mucosal and systemic cytotoxic T lymphocytes and protective immunity in mice against intrarectal recombinant HIV-vaccinia challenge. *PNAS* 95:1709-1714.

Belyakov, I.M., et al. 1998. The Importance of Local Mucosal HIV-Specific CD8+ Cytotoxic T Lymphocytes for Resistance to Mucosal Viral Transmission in Mice and Enhancement of Resistance by Local Administration of IL-12. *J. Clin. Invest.* 102:2072-2081.

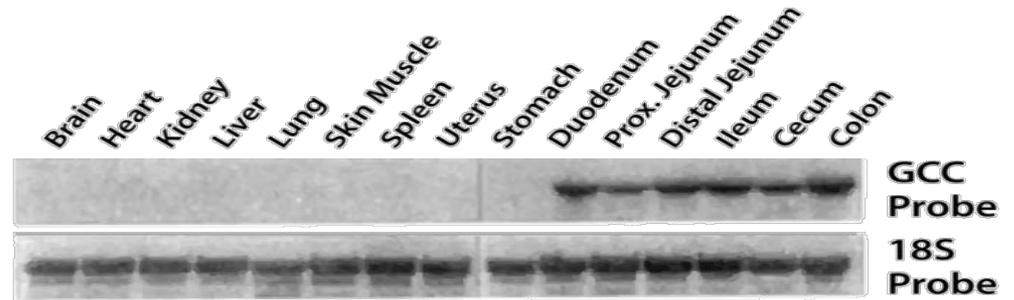
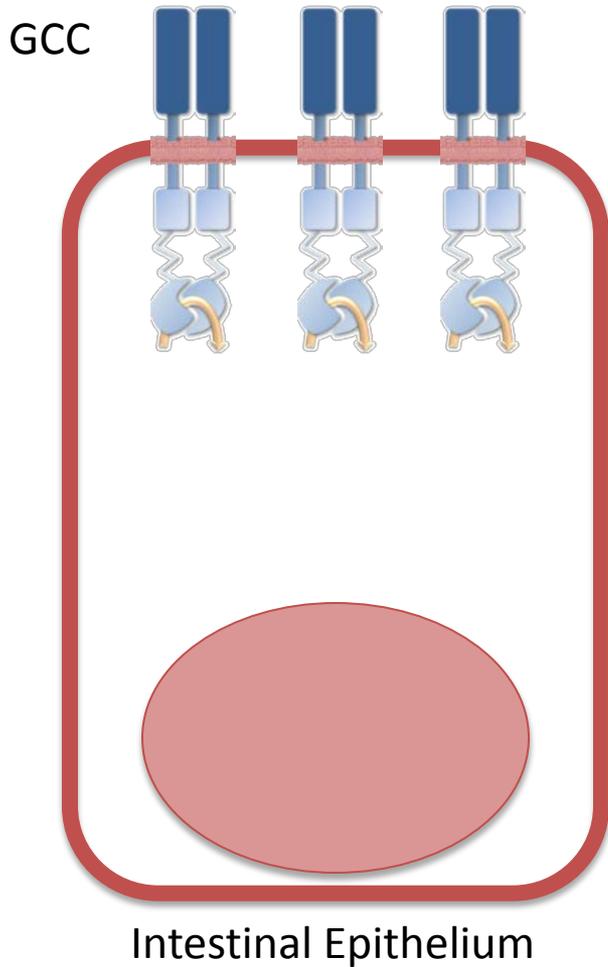


Intestinal Antigen-Targeted Immunotherapy

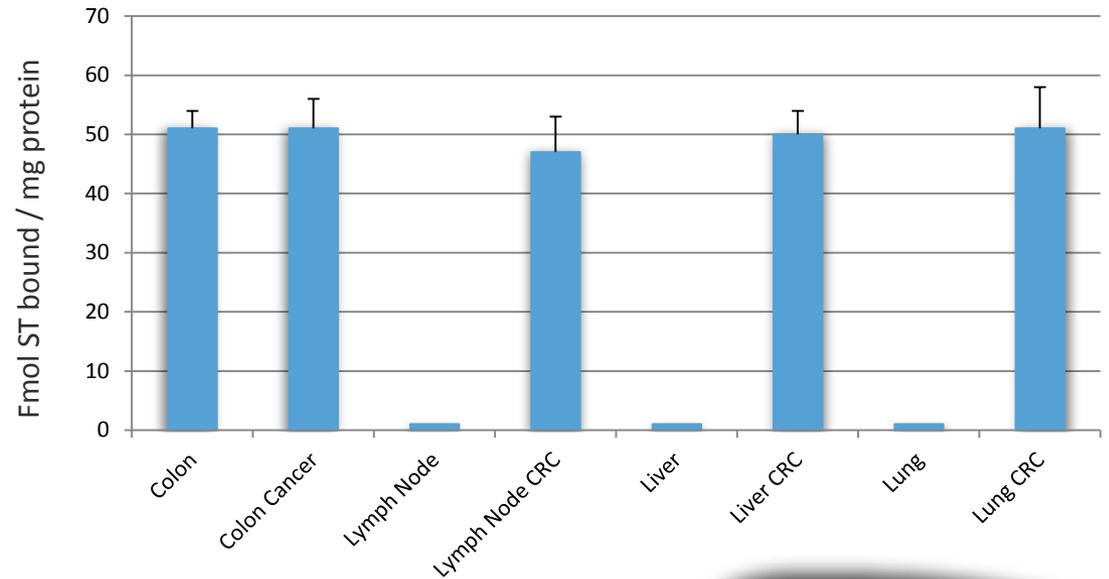


Snook AE, et al. Cancer Mucosa Antigens as a Novel Immunotherapeutic Class of Tumor-associated Antigen. Clin Pharmacol Ther 2007;82: 734-9.

Guanylyl Cyclase C (GCC)

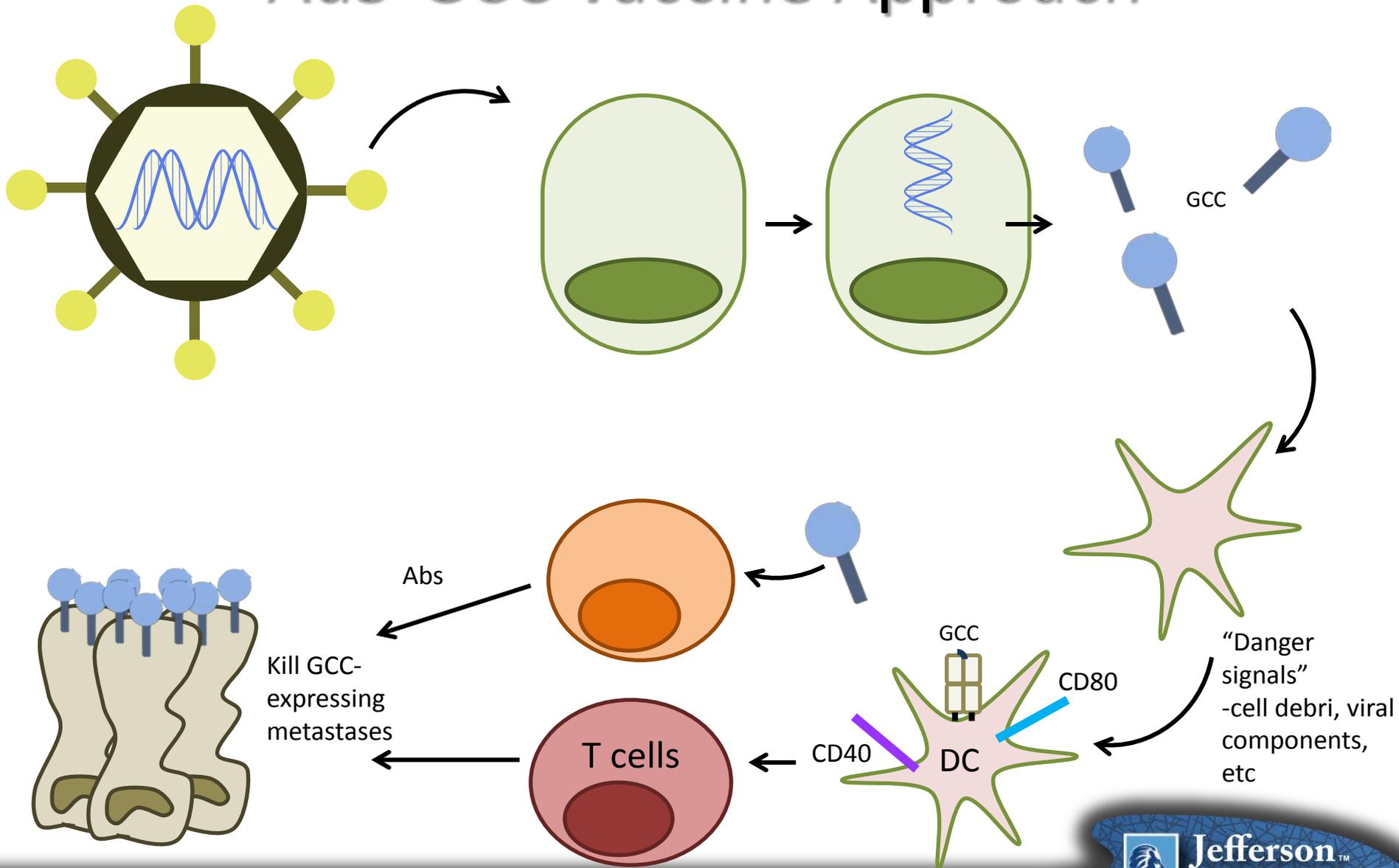


Swensen et al. 1996. Biochemical and Biophysical Research Communications, 225: 1009-1014



Adapted from Carrithers, et al (1996) *Dis Colon Rectum* 39:131

Ad5-GCC Vaccine Approach



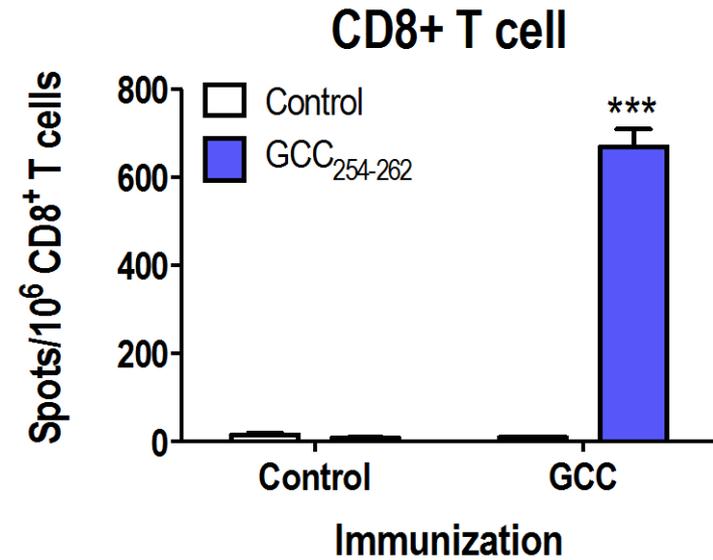
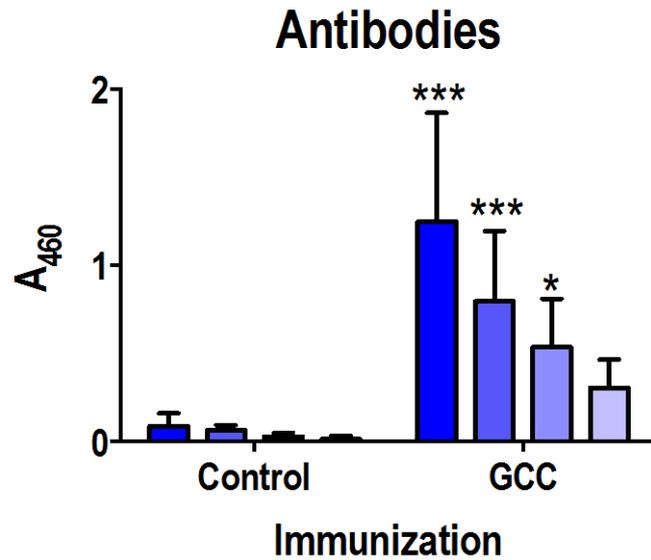
Animal Models

Proof-of-Concept in Mice

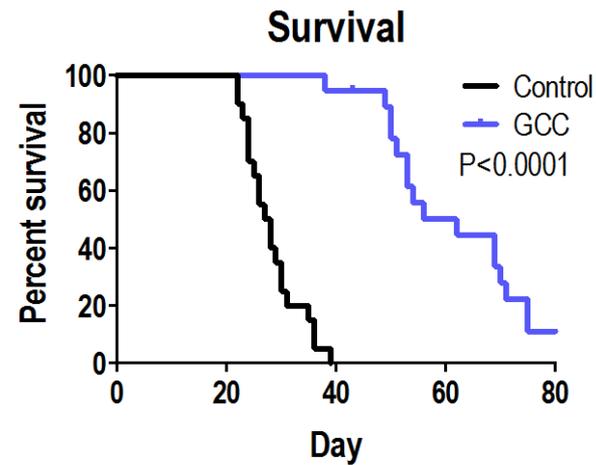
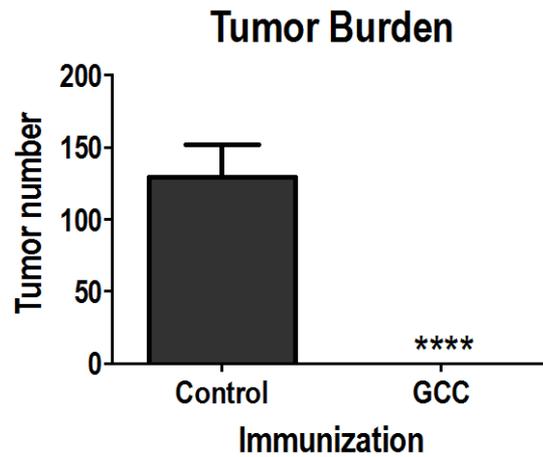
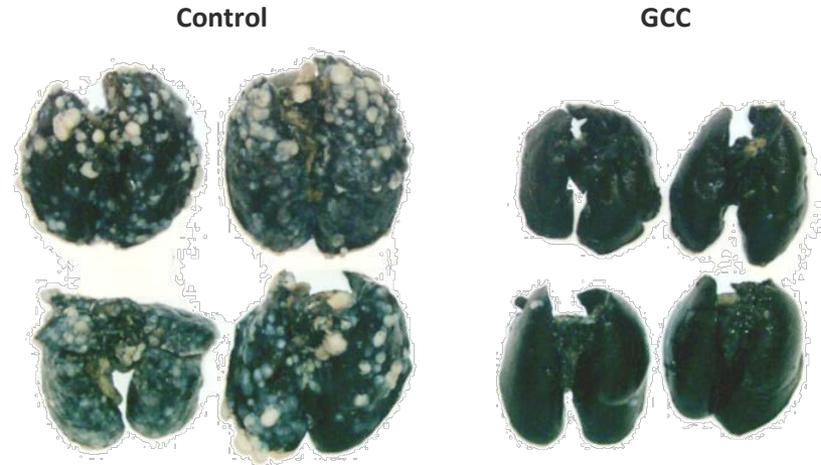
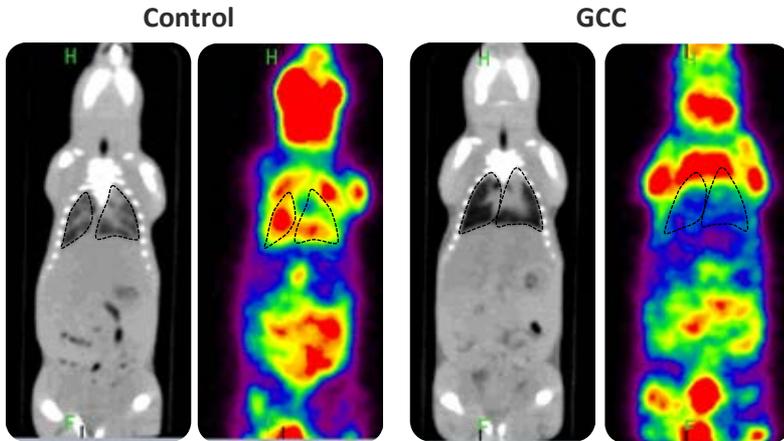
Ad5-GCC-T helper epitope



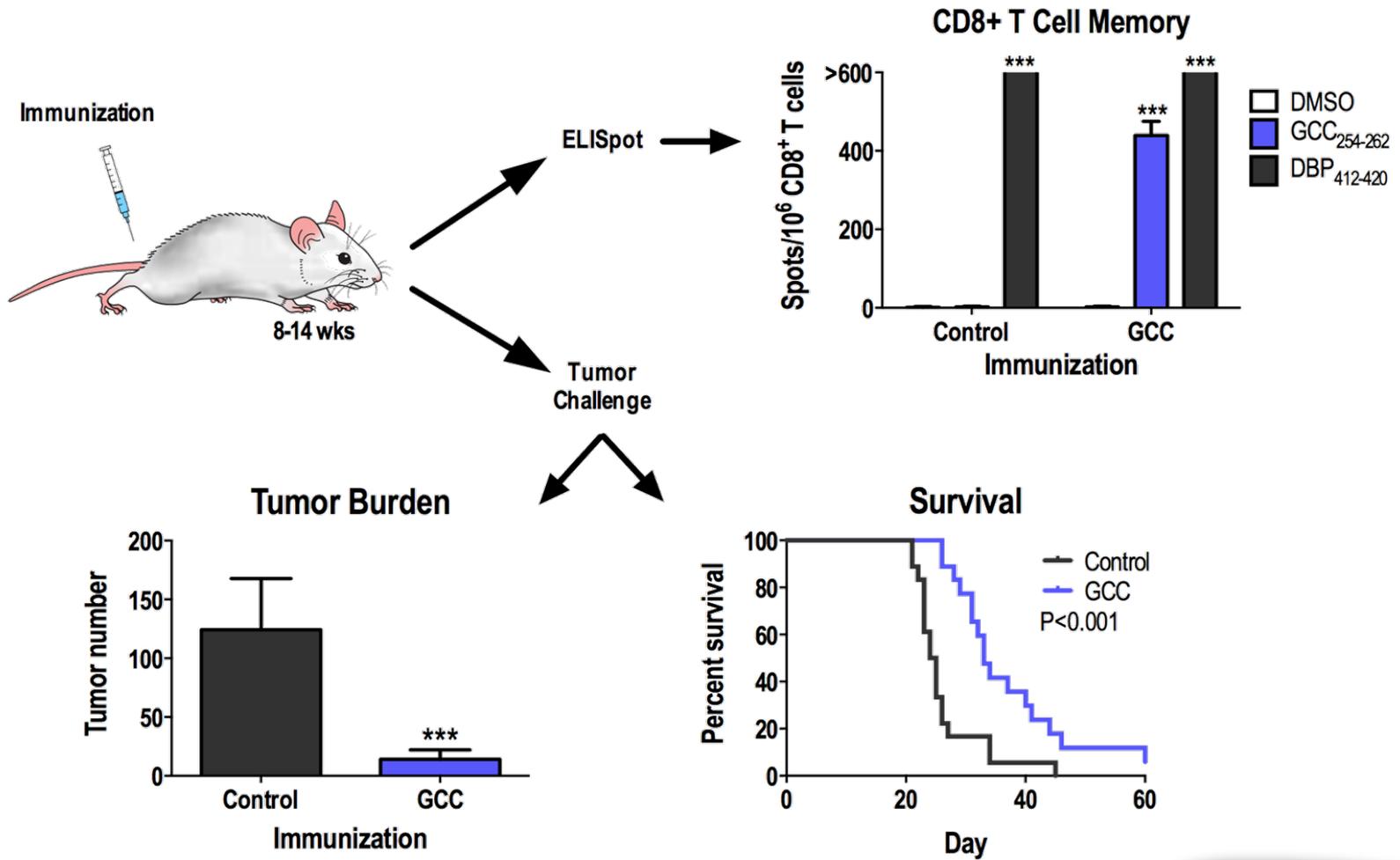
Immune Effectors



Anti-Tumor Immunity



Long-Lasting Immunity



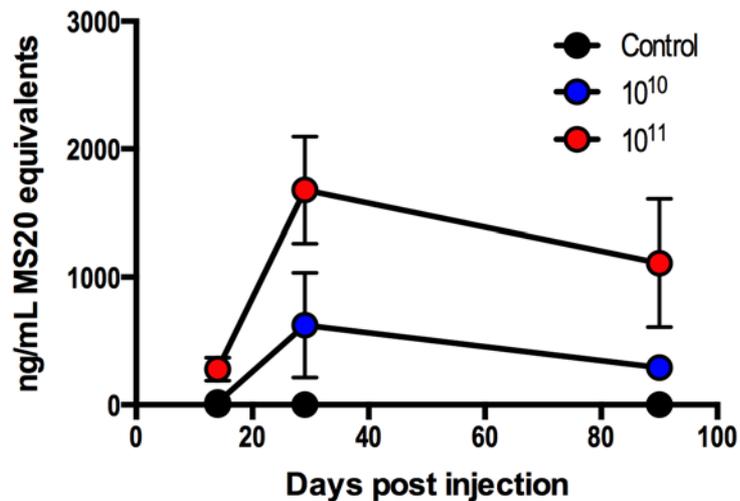
GLP Ad5-mGCC-PADRE Mouse Studies

| GROUP # | ARTICLE | DOSE | # OF ANIMALS (MALE/ FEMALE) | IN-LIFE DURATION (DAYS) | | | SAMPLE COLLECTION AT TERMINATION |
|---------|----------------|----------------------|-----------------------------|-------------------------|--------|--------|--|
| | | | | 14 | 30 ± 1 | 90 ± 1 | |
| 1 | Control | 0 | Immunogenicity | 5M/5F | 5M/5F | 5M/5F | Blood for hematology, clinical chemistry; Tissues for histology. |
| | | | Biodistribution | 5M/5F | 5M/5F | 5M/5F | Blood for ELISA; Spleen for ELISpot. |
| | | | Safety | 5M/5F | 5M/5F | 5M/5F | Blood for PCR; Tissues for PCR. |
| 2 | Ad5-mGCC-PADRE | ~10 ¹⁰ vp | Immunogenicity | 5M/5F | 5M/5F | 5M/5F | Blood for hematology, clinical chemistry; Tissues for histology. |
| | | | Safety | 5M/5F | 5M/5F | 5M/5F | Blood for ELISA; Spleen for ELISpot. |
| 3 | Ad5-mGCC-PADRE | 10 ¹¹ vp | Immunogenicity | 5M/5F | 5M/5F | 5M/5F | Blood for hematology, clinical chemistry; Tissues for histology. |
| | | | Biodistribution | 5M/5F | 5M/5F | 5M/5F | Blood for ELISA; Spleen for ELISpot. |
| | | | Safety | 5M/5F | 5M/5F | 5M/5F | Blood for PCR; Tissues for PCR. |

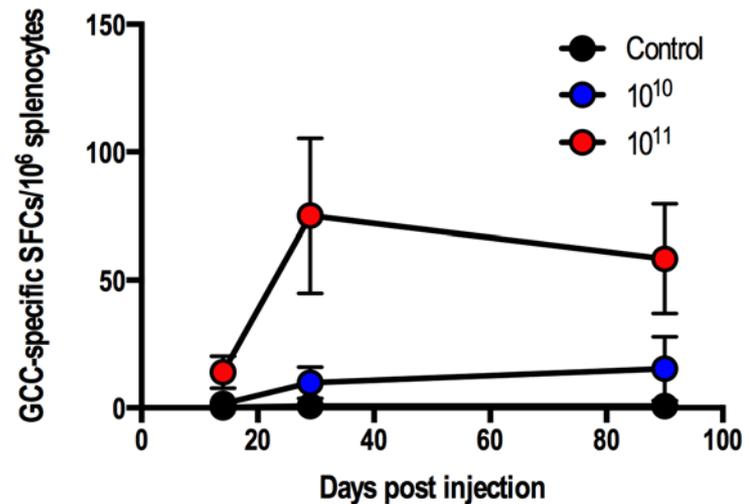


Immunogenicity Summary

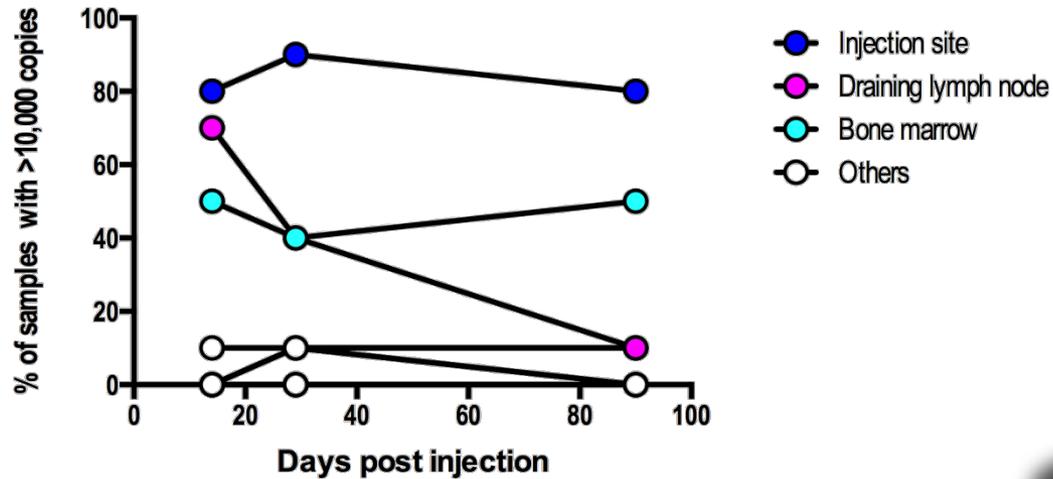
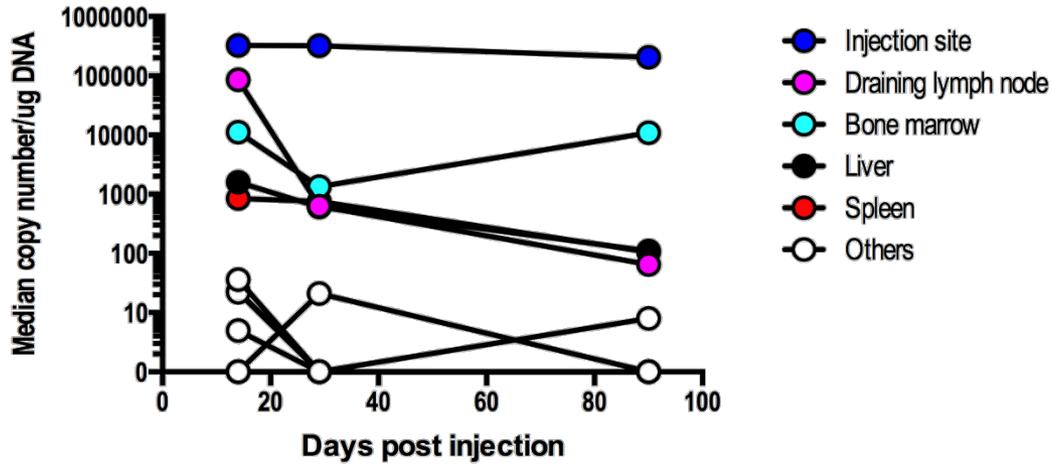
GCC-Specific Antibody Responses



GCC-Specific T cell Responses



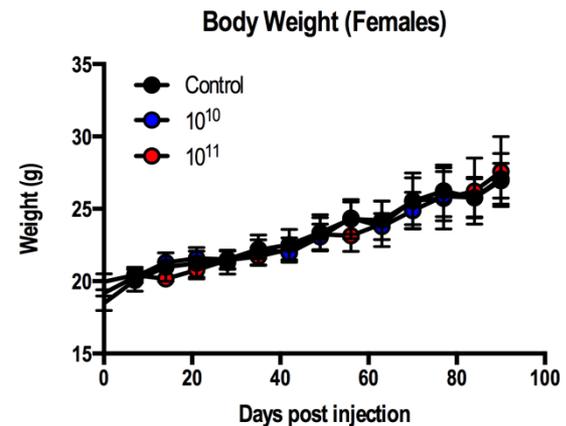
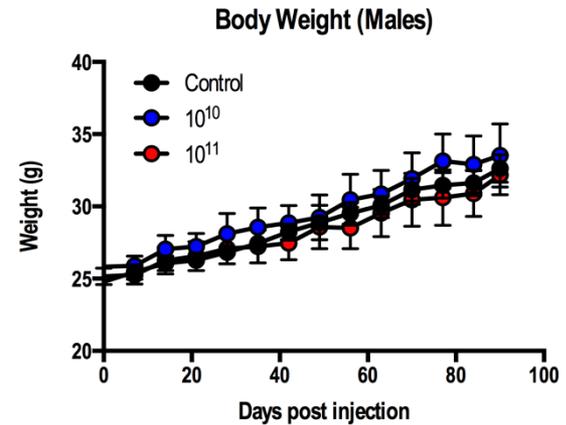
Biodistribution Summary



Safety

No observed differences in any measure:

- Mortality and Clinical Observations
- Body Weight
- Feed Consumption
- Hematology
- Clinical Chemistry
- Necropsy
- Organ Weights
- Histopathology



Summary

- GCC-targeted Ad5 vaccines:
 - Distribute primarily to injection site, DLN and BM
 - Induce GCC-specific immune responses
 - Produce antitumor immunity
 - Well-tolerated
 - NO intestinal autoimmunity or other toxicities



Clinical Trial

Protocol Title:

A Phase I Study of Guanylyl Cyclase C (GCC)-Encoding Replication-Deficient Human Type 5 Recombinant Adenovirus Vaccine (Ad5-hGCC-PADRE) in Stage I and II Colon Cancer Patients

Study Population:

Patients with stage I or stage II (pN0) colon cancer. Two racial cohorts (Black, n=22 vs. White, n=22).



Investigational Product

Vaccine Construct (Ad5-hGCC-PADRE)

- Replication-deficient human type 5 recombinant adenovirus (Ad5)
- Truncated human GCC₁₋₄₃₀ construct (hGCC extracellular domain)
- CD4+ T helper cell epitope (PADRE)

Dose

- A single dose of 10^{11} viral particles

Route

- Intramuscular injection to deltoid



Study Objectives

Primary Objectives:

- To determine the safety, tolerability and toxicity of Ad5-hGCC-PADRE
- To determine whether Ad5-hGCC-PADRE induces an antibody response to GCC at 1 month following vaccination



Study Objectives

Secondary Objectives:

- T cell response to GCC at 1 month following vaccination
- Antibody and/or T cell responses to GCC that persists at 3 month and 6 month following vaccination
- Relationship of antibody and/or T cell responses to GCC with:
 - Occult lymph node metastases quantified by GCC RT-qPCR
 - Race
 - Time to recurrence or survival



Inclusion/Exclusion Criteria

Major Inclusion Criteria

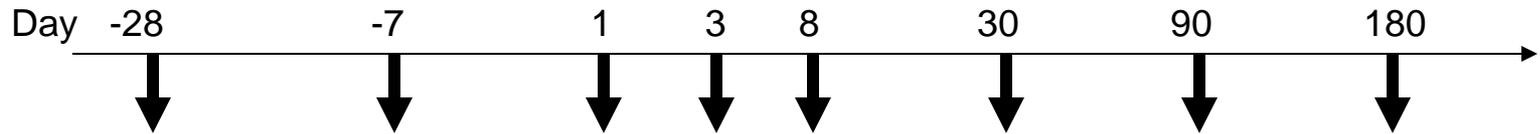
- Black or White patients
- Stage I or stage II (pN0) colon cancer
- Immune competence
- Lymph node specimens available for quantification of occult metastases
- Maximum of 24 months since surgery
- No clinical or laboratory evidence of local or systemic recurrence of colon cancer at entry to the study

Major Exclusion Criteria

- Rectal Cancer
- Medically-proven inflammatory bowel disease
- Prior chemotherapy, radiotherapy, immunotherapy, or other investigational medications for colon cancer



Study Design



**Black or White
Colon Cancer Patient:
Stage I/II, pN0**

**Screening/
Consent**
**Anergy
Skin Test**

**Toxicity
Assessment**

Immunological Assessment



Endpoints

Safety

- Common Terminology Criteria for Adverse Events (CTCAE version 4.0)
- Participant self-assessment questionnaires

Immunological Response

- Antibody response to GCC (ELISA)
- T cell response to GCC (ELISpot)
- PADRE and vector-specific responses

Survival

- Time to recurrence and overall survival



Analytical Design of the Trial

- Simon 2-stage design to estimate cohort size
 - Presumed 10% response is too low (p_0)
 - Presumed 40% response is worthy of continued study (p_1)
 - 90% power
 - 2.5% Type-1 Error (α)
 - Cohort size: $N=22$
- Bayesian continual monitoring
 - Continuous monitoring of safety and efficacy
 - Toxicity $>10\%$ is unacceptably high
 - Efficacy $<10\%$ is unacceptably low
- Initial cohort of 22 patients (mixed race)
- Expanded to 2 cohorts, one White, one Black
 - $n=22$ each cohort



Analysis of Primary Endpoints: Toxicity

- **Toxicity:** Grade 3 or 4 non-laboratory AEs defined by CTCAE v4.0 occurring anytime during the 6-month evaluation period
- Toxicity >10% is unacceptably high

| | | | | | | | |
|----------------|---|------|-------|-------|-------|-------|-------|
| No. toxicities | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| No. subjects | 5 | 6-11 | 12-17 | 18-23 | 24-30 | 31-37 | 38-44 |



Analysis of Primary Endpoints: Immunogenicity

- **Immunogenicity:** antibody response, as measured by ELISA assay
 - Validated human GCC-specific ELISA assay
- A response will be considered positive if the antibody level for a patient is significantly increased at 1 month post-vaccination compared to the pre-vaccine baseline.

| No. responses | No. subjects observed |
|---------------|-----------------------|
| 0 | 10 |
| 1 | 22-27 |
| 2 | 28-44 |



Summary

- Unmet medical need to prevent recurrent disease in pN0 colorectal cancer patients.
- A phase I study in stage I/II (pN0) colon cancer patients will be initiated upon regulatory approval in 2013.
- The results of this study will be utilized to design future phase II and III studies.

