Society For Immunotherapy of Cancer (SITC)
Tumor Immunology 101

Augusta, GA

Active Immunization Approaches

Yukai He, MD/PhD
Professor, Department of Medicine
And GRU Cancer Center

Disclosure

- Nothing needs to be declared.

Active immunization Approaches: Vaccines

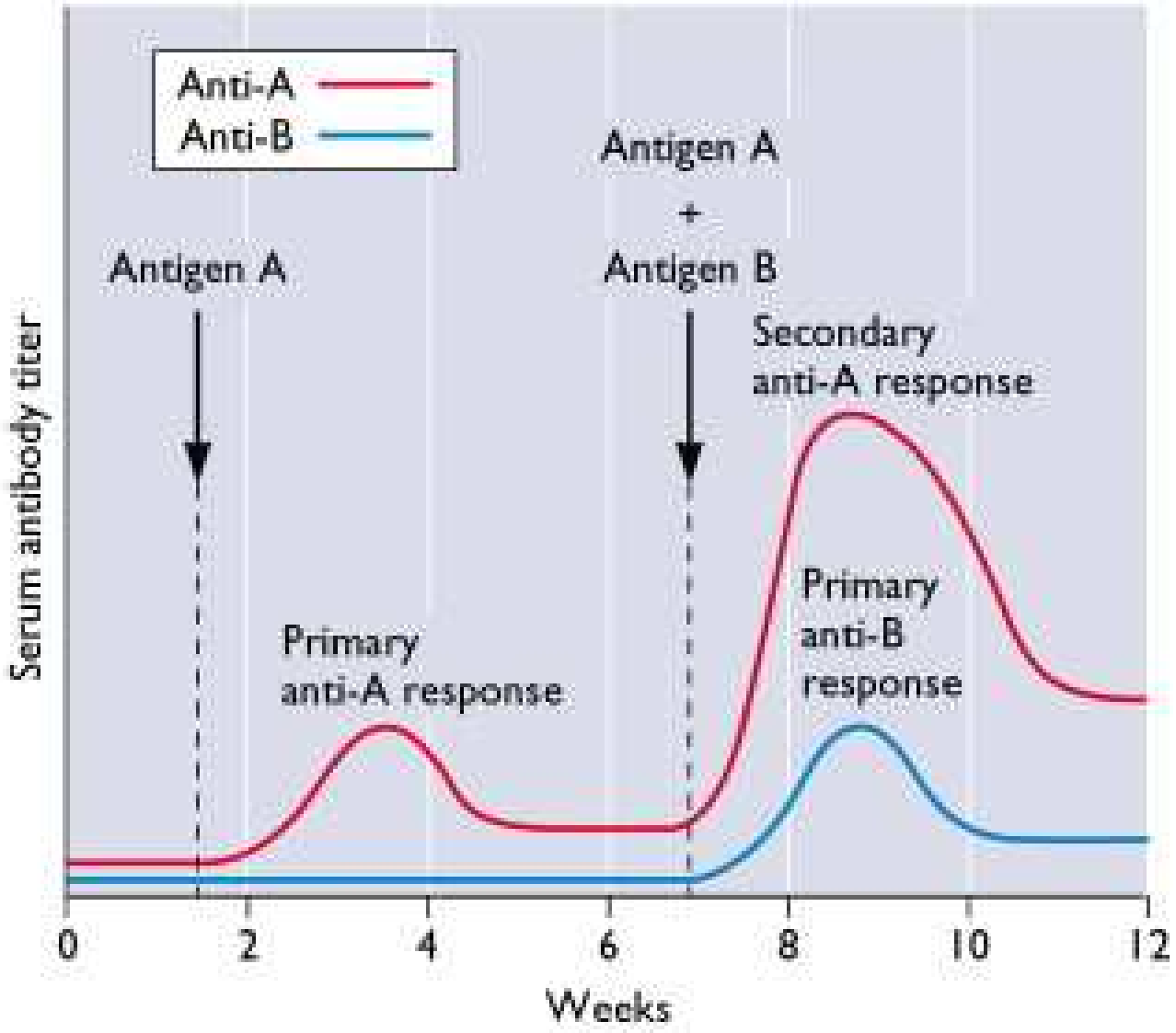
- Vaccines are hailed as one of the greatest medical advances in modern era.
- Immunization is the best strategy to prevent infectious diseases and the most cost-effective public health intervention.
- Today's presentation
  - An overall introduction to vaccine and cancer vaccine
  - Development of liver cancer vaccines

WHY WE NEED IMMUNIZATION?

- Based on clone selection theory, to maintain the immune diversity (T cell epitopes: $>10^{15}$, Ig: 10^{14}) with limited space (total cells in one human body: 10^{13}-10^{14} cells), the immune cell frequency for each epitope is low:
  - CD4: 20-200 (Marc Jenkins, 2008 Immunity
  - CD8: 100-3000
- Thus, the most economic way for the body to fight Ag invasion is to maintain a low number of immune cells for each Ag epitope but which can be expanded when it is required.

The purpose of Immunization

- To expand (quantitatively) selective population of T or B cells that recognize target antigens: to maintain a *Higher Number* of those immune cells that recognize frequently encountered antigens.
- To generate qualitatively enhanced memory immune cells that can better respond to target antigens: *Higher Responsiveness*.


Anti-A
Anti-B
Antigen A
Antigen A
+
Antigen B
Secondary
anti-A response
Primary
anti-A response
Primary
anti-B
response
Serum antibody titer
0
2
4
6
8
10
12
Weeks

Vaccines for infectious Diseases: a little bit of history

- Empirical science from ancient time
  - Ancient China and India
  - Edward Jenner: Smallpox vaccine
  - Louis Pasteur: Anthrax and Rabies vaccines

- <u>B cell based vaccines (humoral immune responses), Immune correlates: neutralizing antibodies</u>

- Technologies:
  - Inactivated microbes
  - Attenuated microbes
  - Subunits:
    - Purified: 1981: Purified HBV surface antigen
    - Recombinant proteins: VLP: 1986 HBV, 2006 HPV

Jenner

Pasteur

Vaccines on the market

- Diphtheria & Tetanus Toxoids & Pertussis
- Meningitis virus or bacteria
- Measles, Mumps, and Rubella Virus
- Poliovirus
- Rabies
- Smallpox
- Rotavirus
- Typhoid
- Yellow Fever
- HBV
- HPV

Jonas Salk: killed virus
Albert Sabin: attenuated, alive

1
Inactivated

Attenuated
2

Subunit
3

T cell vaccines (*Vaccine in development*)

- **Prophylactic Vaccines:**
  - **TB**
  - **HIV**
  - **Malaria**
- **Therapeutic vaccines:**
  - **Chronic HBV infections,**
  - **Tumor**

Vaccines stimulating T cell responses

- Vaccines based on protein are not effective to activate T cell responses
- Peptide vaccines [4]
  - Peptide Epitopes can be identified and used to activate CD4 and CD8 T cells
  - TLR ligands, CD40 activation as adjuvant
  - However, even though immune responses are generated, clinical efficacy is limited.
- Attenuated microbes
- Recombinant genetic vaccines [5]

Gene based vaccines (Genetic vaccines)

Better activation of T cells.

- Attenuated microbes (contain Ag and Ag encoding genes)
- DNA
  - Naked DNA,
  - Gene gun, Electroporation
- Recombinant Viral Vectors:
  - **Vaccinia vector**,
  - **Adenovector,**
  - **Lentivector,**
  - Alpha viral vector,
  - AAV, Sendai Vius

Vaccinia viruses and vectors

- Large DNA virus:
  - 190kb genome (250 proteins),
  - Related to cowpox virus and variola (smallpox)
  - WR, Copenhagen, Dryvax, ACAM2000, MVA, fowlpox
- Viral vectors are replication competent and come from:
  - Attenuated WR
  - MVA
  - Fowpox

Adenovirus and vector

- Mid size virus, 53 serotypes in human, cause respiratory infections, ~30kb genome (20-40 genes)
- Recombinant vector:
  - Replication defective
  - Has been tested in clinical trials

Why lentivector

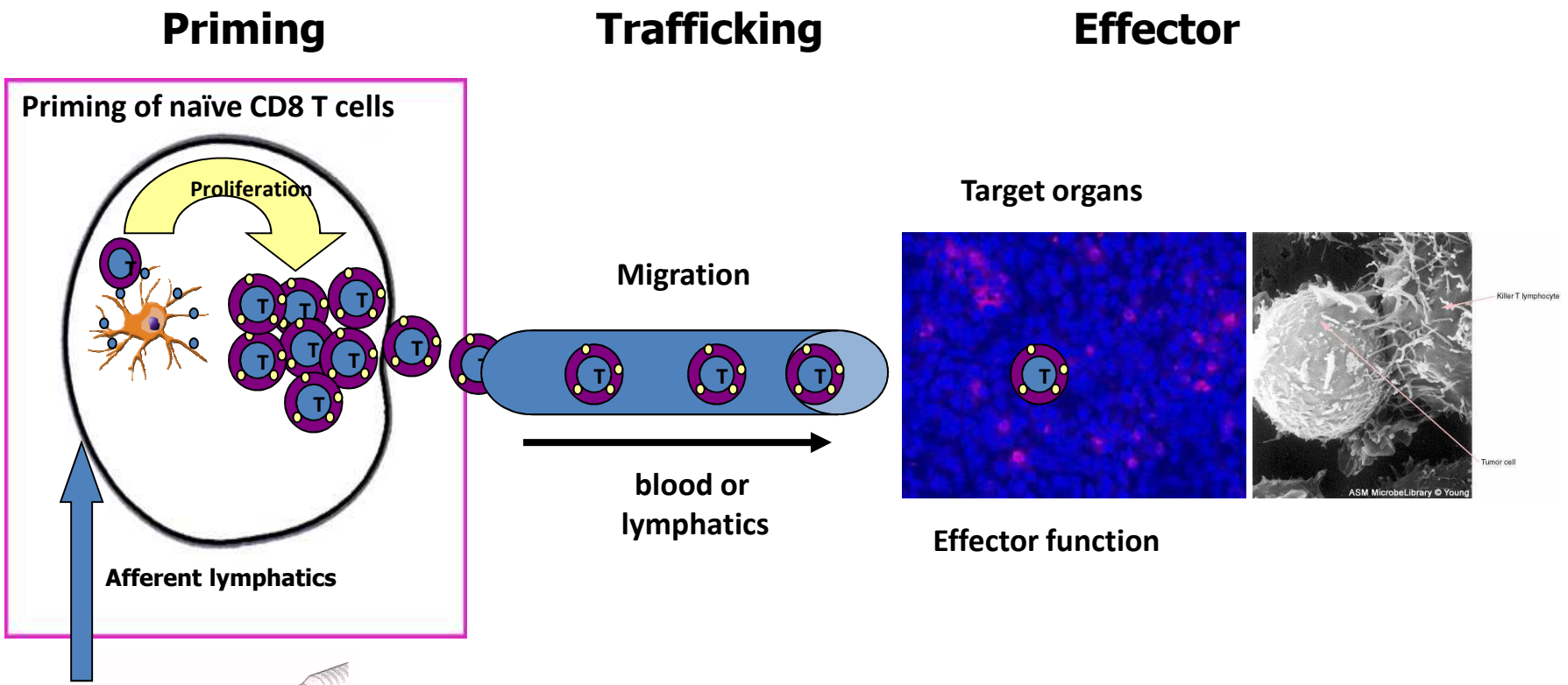
Adenovector

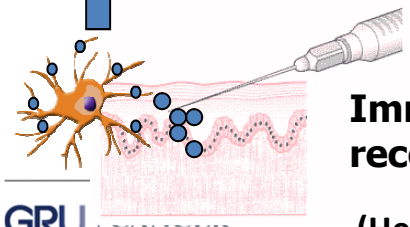
- Pre-existing antivector immunity
- Immune dominant antivector immunity
- Complicated vector: 32kb of human Ad5 sequences

Lentivector

- Low or no immune antivector immunity
- No pre-existing antivector immunity
- Low genotoxicity
- Efficient transduction of non-dividing DCs in vitro and in vivo
- Stimulation of potent T cell immunity
- Ex vivo lentivector transduced cells are in clinical trial

Crucial steps for generating Ag specific immune responses to control diseases





Immunization with recombinant viral vectors

(He et al, Immunity, 2006)

GRU GEORGIA REGENTS UNIVERSITY CANCER CENTER

Cancer Vaccines

- Over 20 years of investigation and clinical trials, only one FDA approved cancer vaccine (Provenge (Dendreon Corporation).
- Potential yet to be realized
- *Possible reasons of ineffective antitumor effect of cancer vaccines:*
  - Insufficient magnitude
  - Low quality of immune responses
  - Suppressive tumor microenvironment
  - Inappropriate setting (prevention vs treatment)

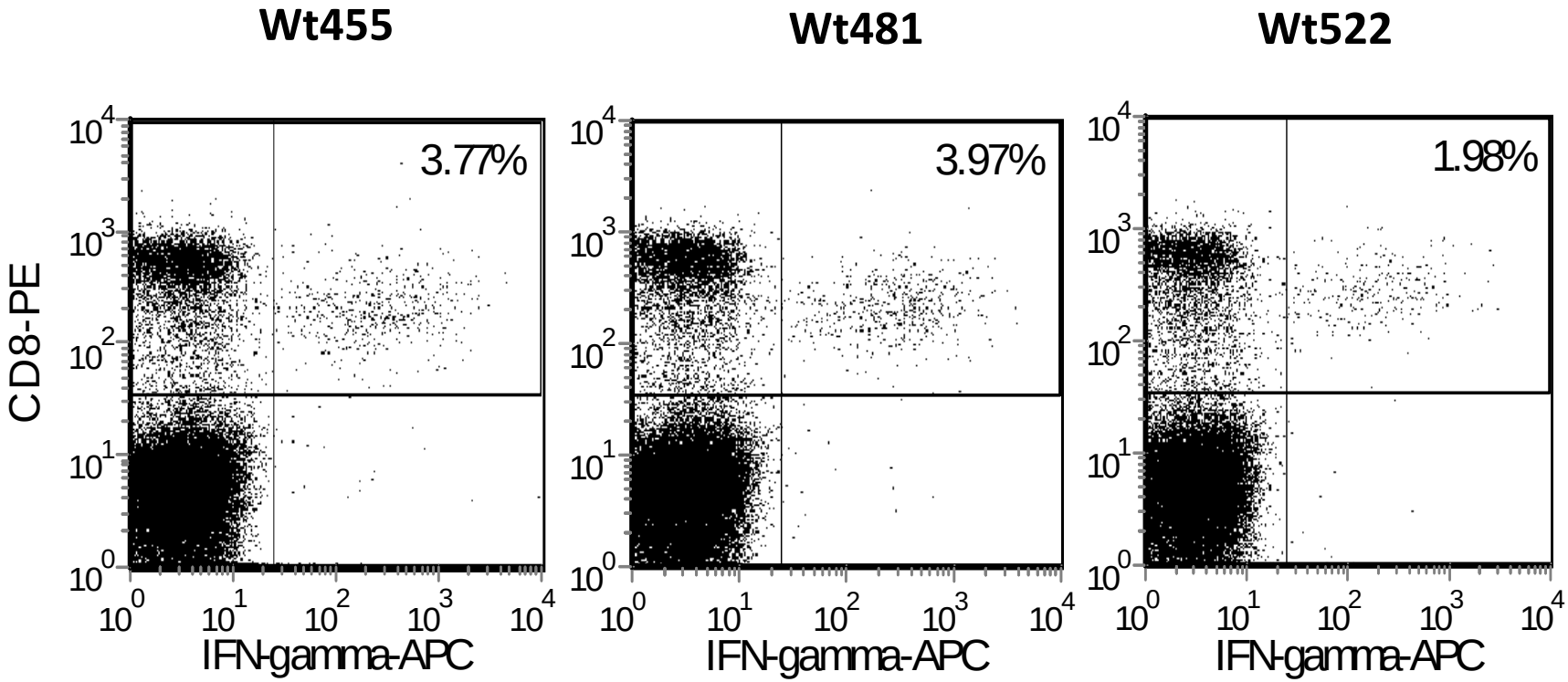
Cancer Vaccines

- Just like vaccines for infectious diseases, cancer vaccines likely will work in prevention setting
  - Prevent de novo cancer development
  - Prevent relapse
- In the therapeutic setting, cancer vaccines, will likely generate antitumor effect only when combined with other established cancer treatment modality including *checkpoint blockade*
  - In order for checkpoint antibody to work, existence of TILs is required.
  - Vaccination increase TILs

Rational Cancer Vaccine Designs: Antigen engineering

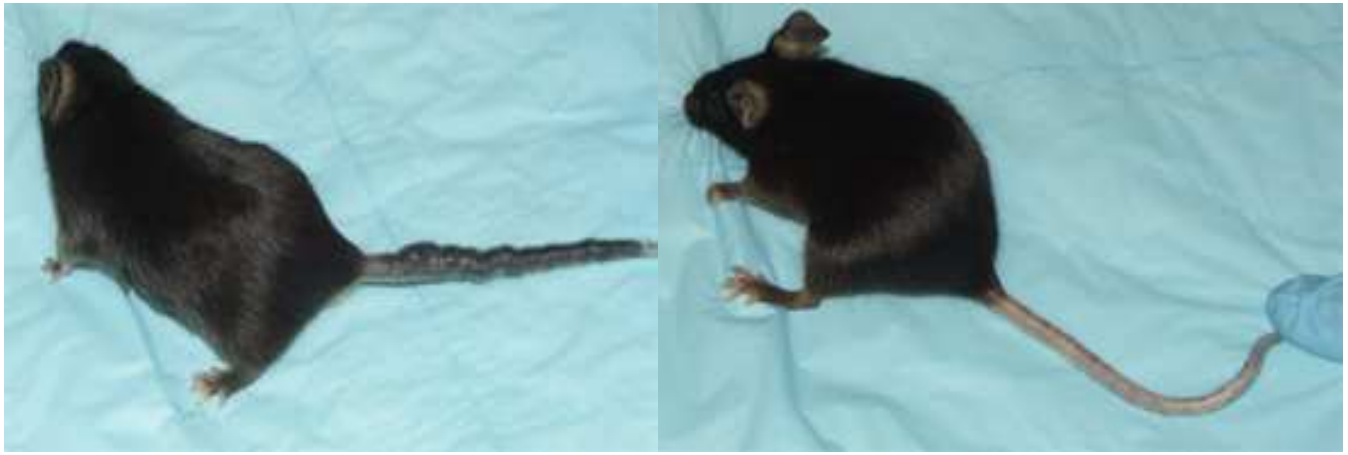
- Two critical parameters for vaccine design:
  - Delivery system:
    - Gene based: viral vector and genetic vaccine are better than protein based vaccines
    - Protein based
  - Antigen engineering
    - Entire Ag, multiple antigens (even whole tumor cells)
    - Epitope optimization (heteroclitic)
    - Virus like particle (VLP)

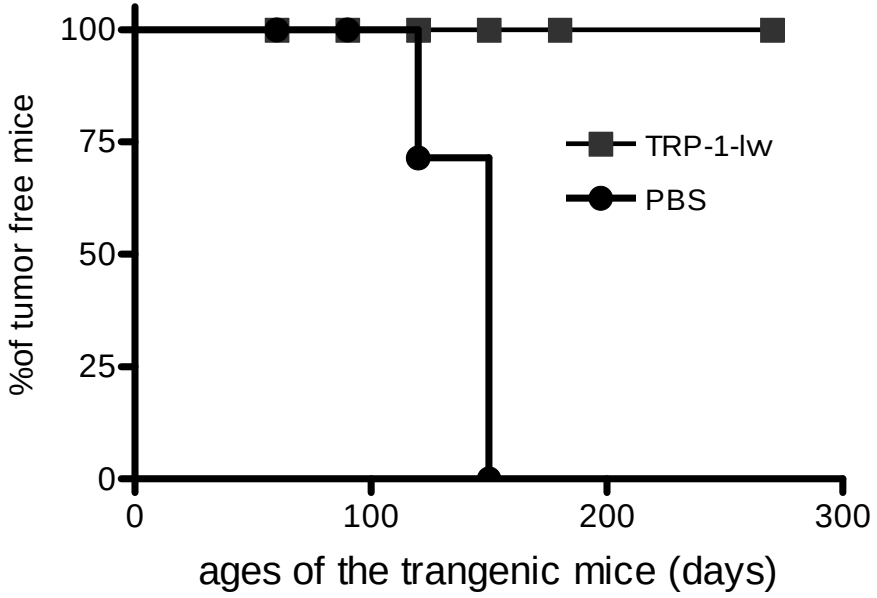
Melanoma specific CD8 responses by lentivector encoding the entire epitope-optimzed TRP1 gene

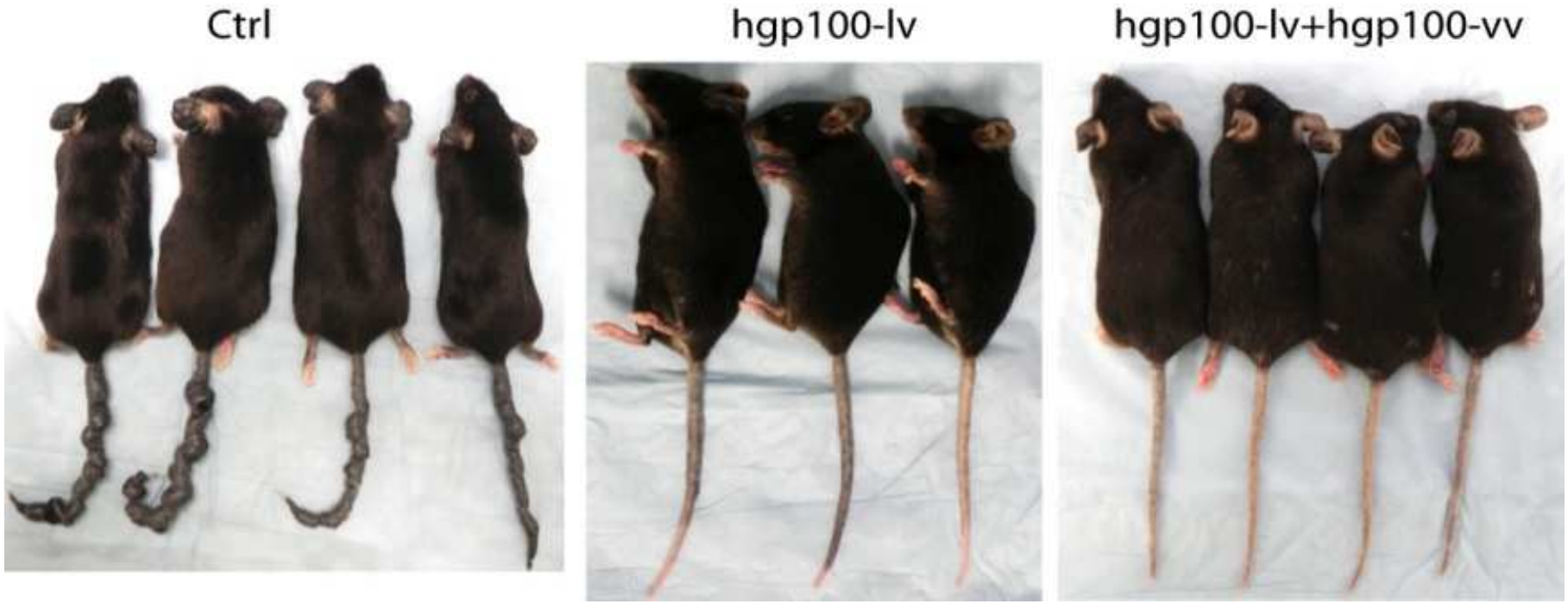


Liu et al, 2009 JI

Ctrl mouse **TRP1-lvv immunized mouse**

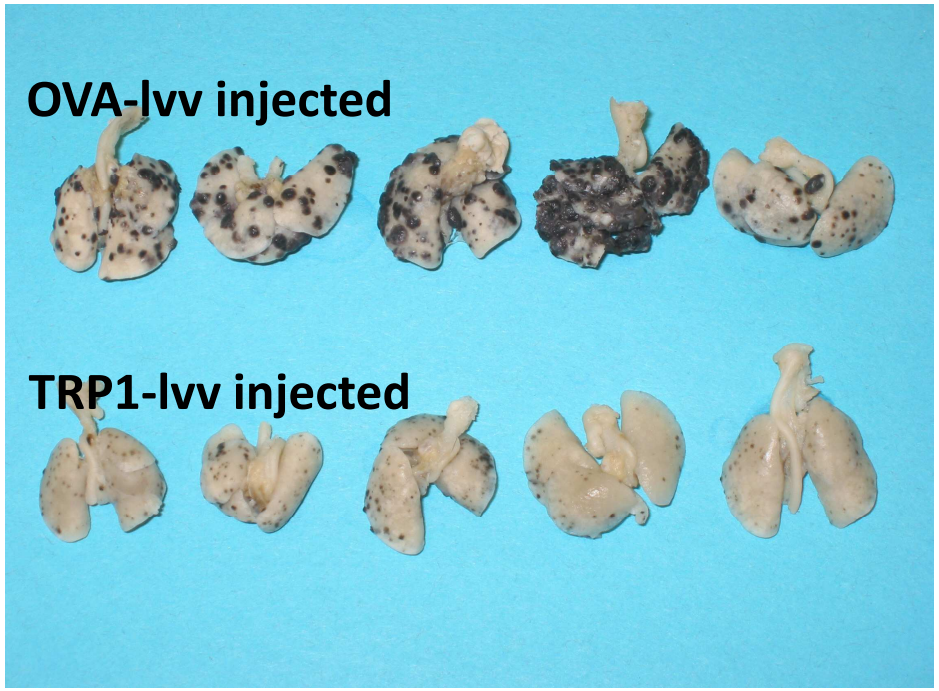


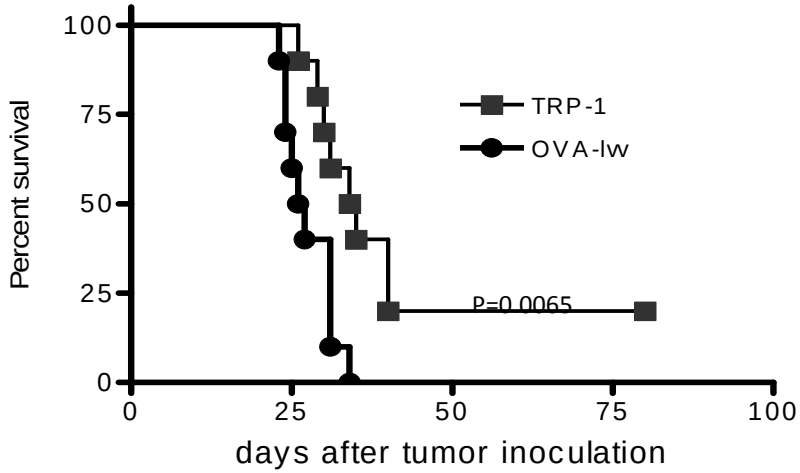


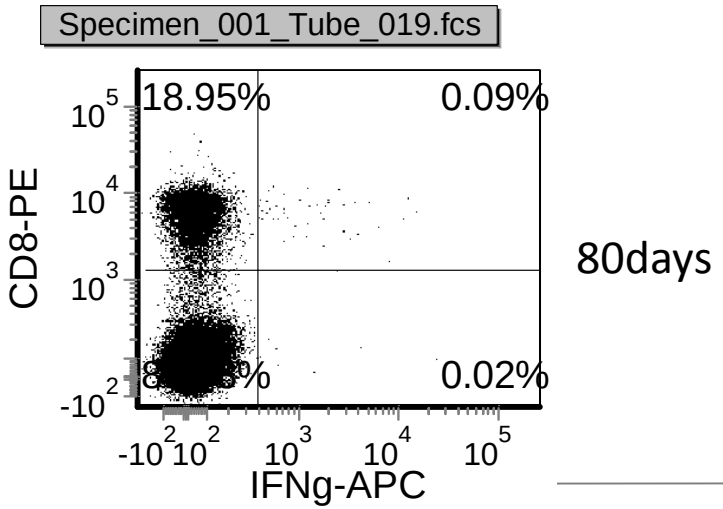


Xiao et al, 2011

Reduce B16 lung metastasis and prolong mouse survival







Preventive cancer vaccine trials

- **Vaccine Therapy in Treating Patients with Newly Diagnosed Advanced Colon Polyps (**NCI-2014-01080, HHSN261201200042I, N01-CN-2012-00042, NCT0213492, 2013) to prevent colon cancer
- **Phase II Trial of Combination Immunotherapy With NeuVax and Trastuzumab in High-risk HER2+ Breast Cancer Patients (2014-0443**
  NCI-2015-00033, NCT02297698) To prevent recurrence.
- **Combination Immunotherapy With Herceptin and the HER2 Vaccine NeuVax (**NCI-2015-00026, 1137008 / 20130058, NCT01570036) to prevent relapse
- **More**

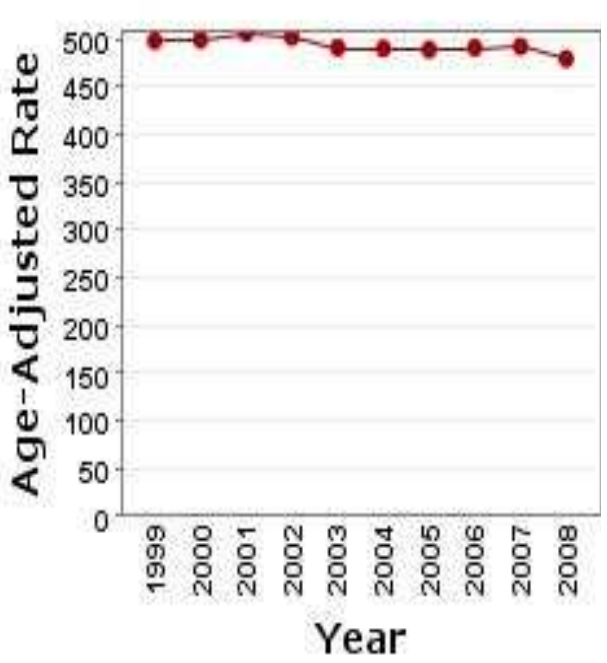
Liver Cancer Vaccine:
Animal model and Proof of principle

Liver Cancer and hepatocellular carcinoma

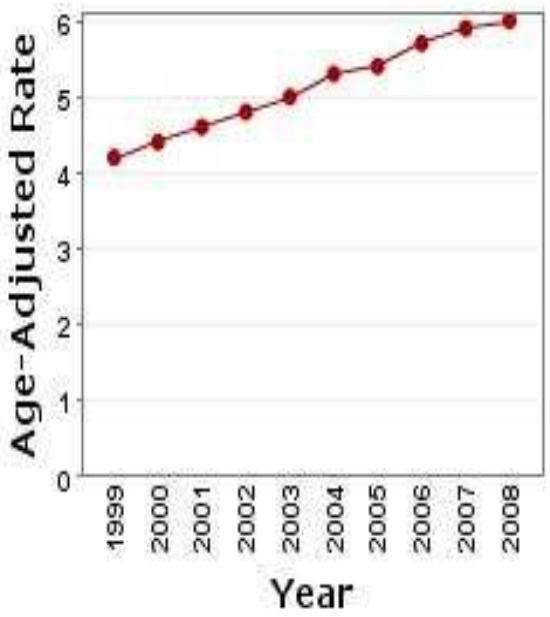
- 5^{th} most common cancer and 3^{rd} most common cause of cancer death
- 750,000 new cases and 690,00 death each years in the world, >80% of them are HCC, half of them are in China.
- In US, the number of HCC has tripled in last 20yrs to 24,500 new cases in 2013---Increasing

The trend of US cancer incidence rate

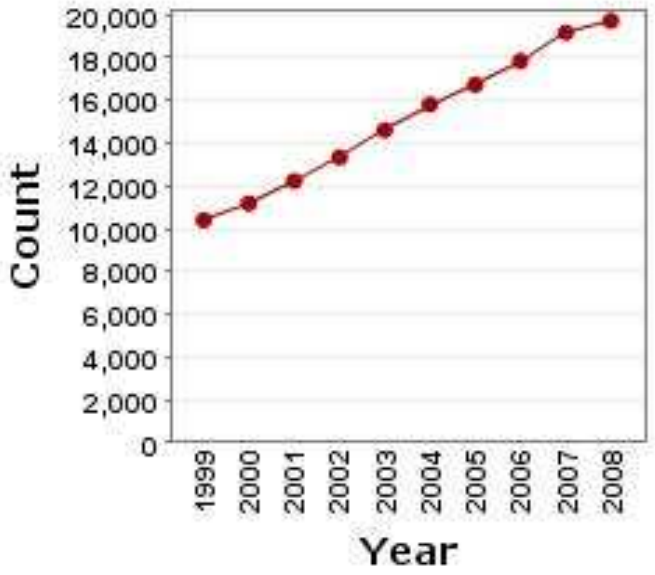
All Cancer



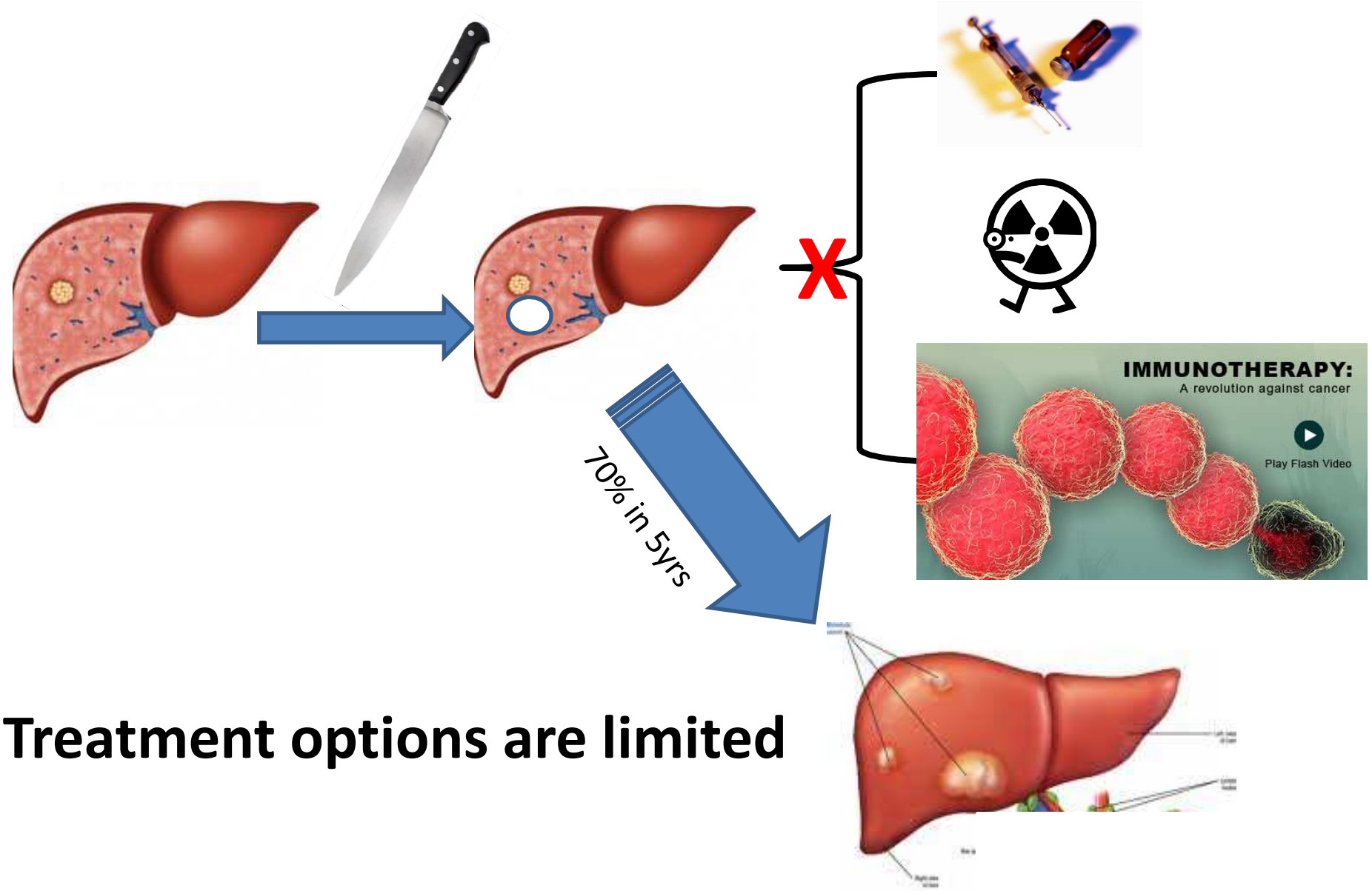
Liver Cancer



The number of HCC
is doubled in 10 years

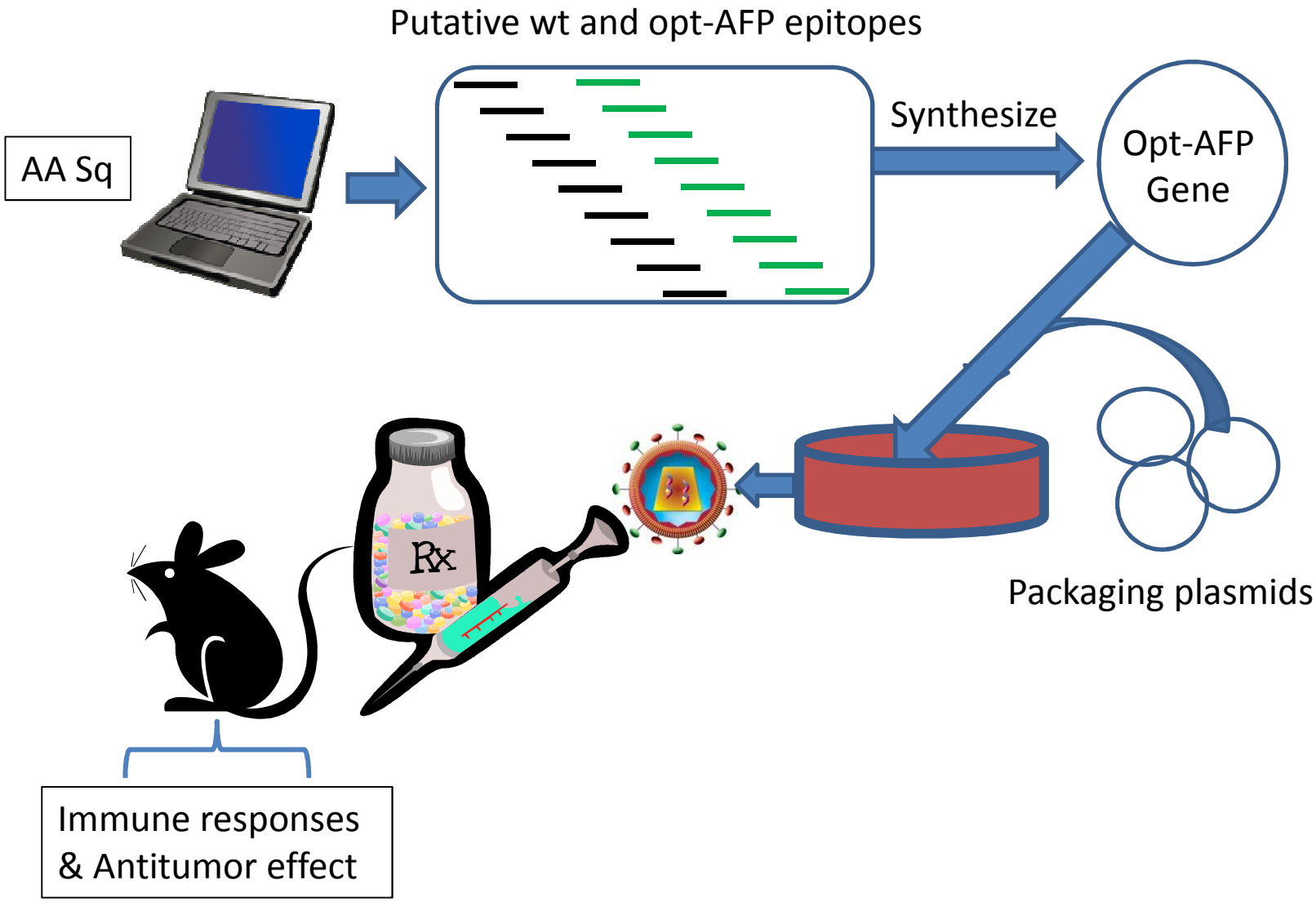


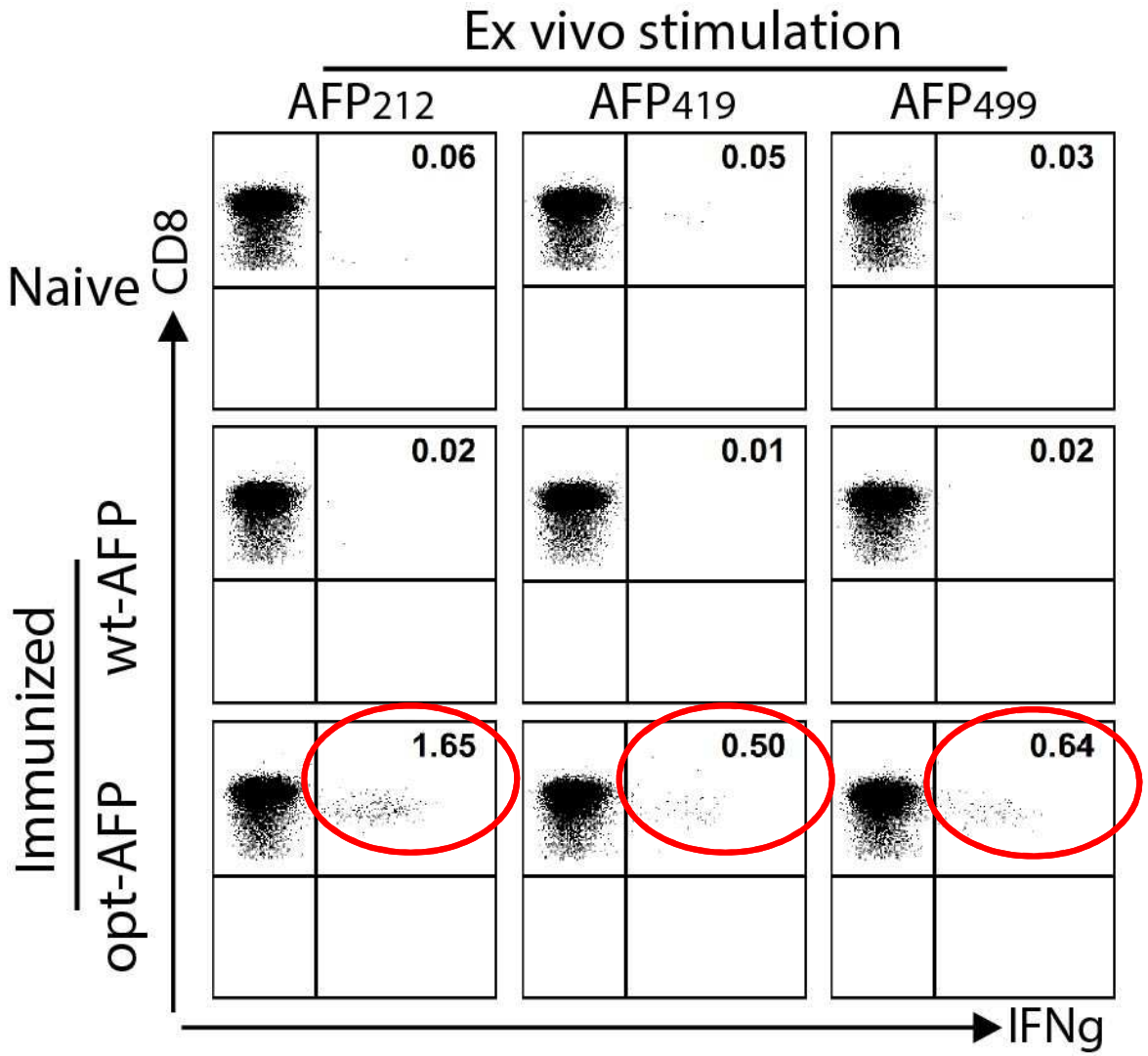
CDC: http://wonder.cdc.gov/cancer.html



Treatment options are limited

Creating immunogenic AFP to break immune tolerance

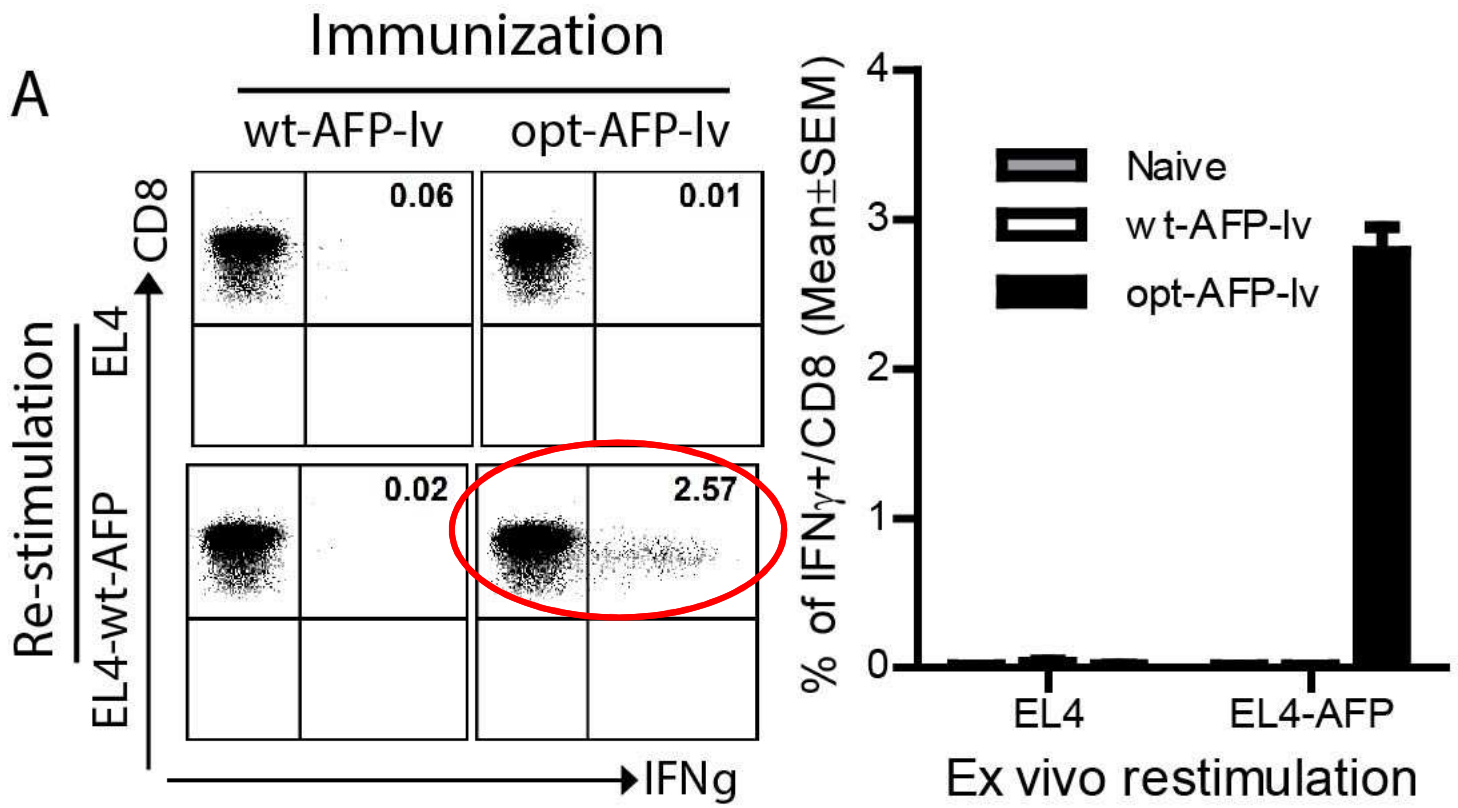




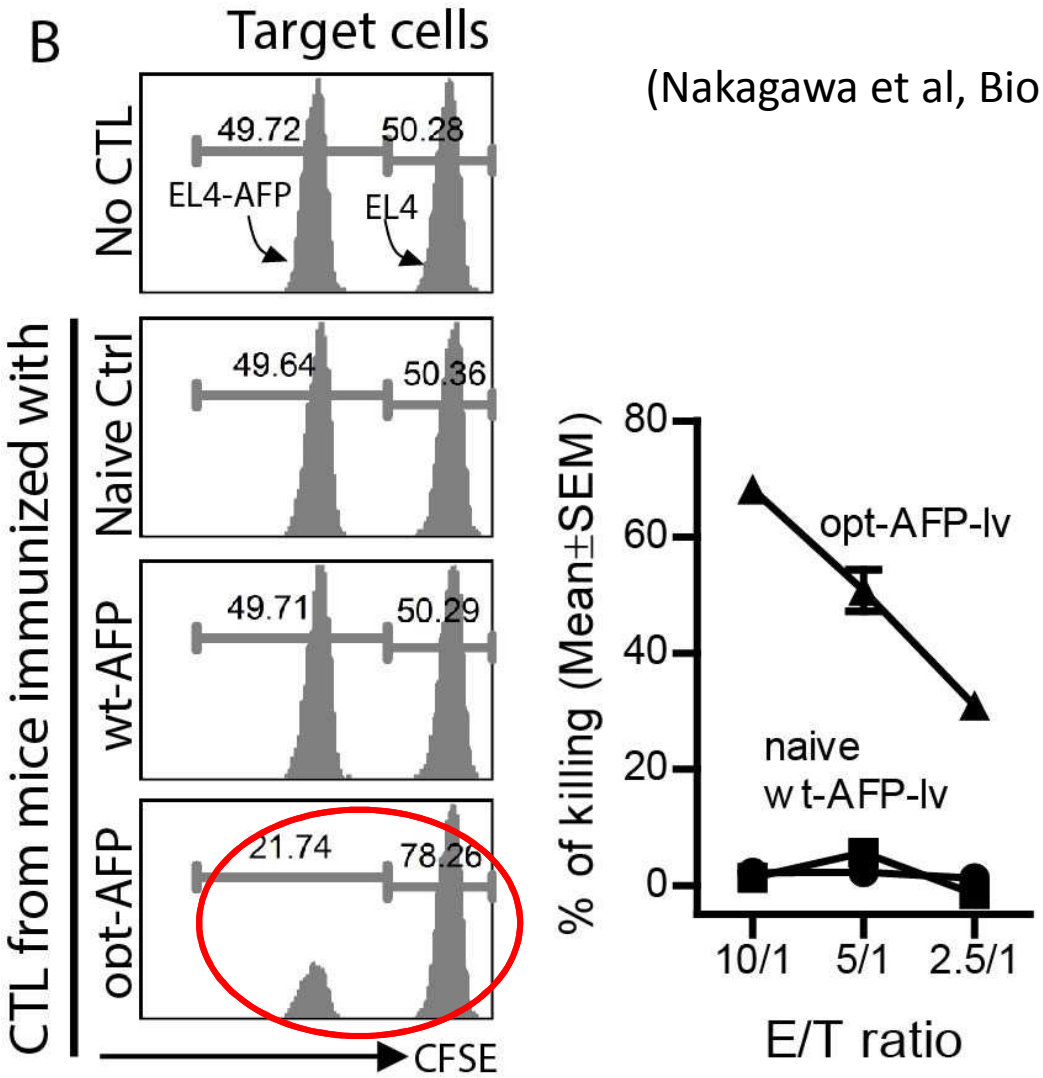
Current Vaccine

Our Vaccine

Epitope-optimization is required to break immune tolerance and to activate AFP-specific CD8 T cells

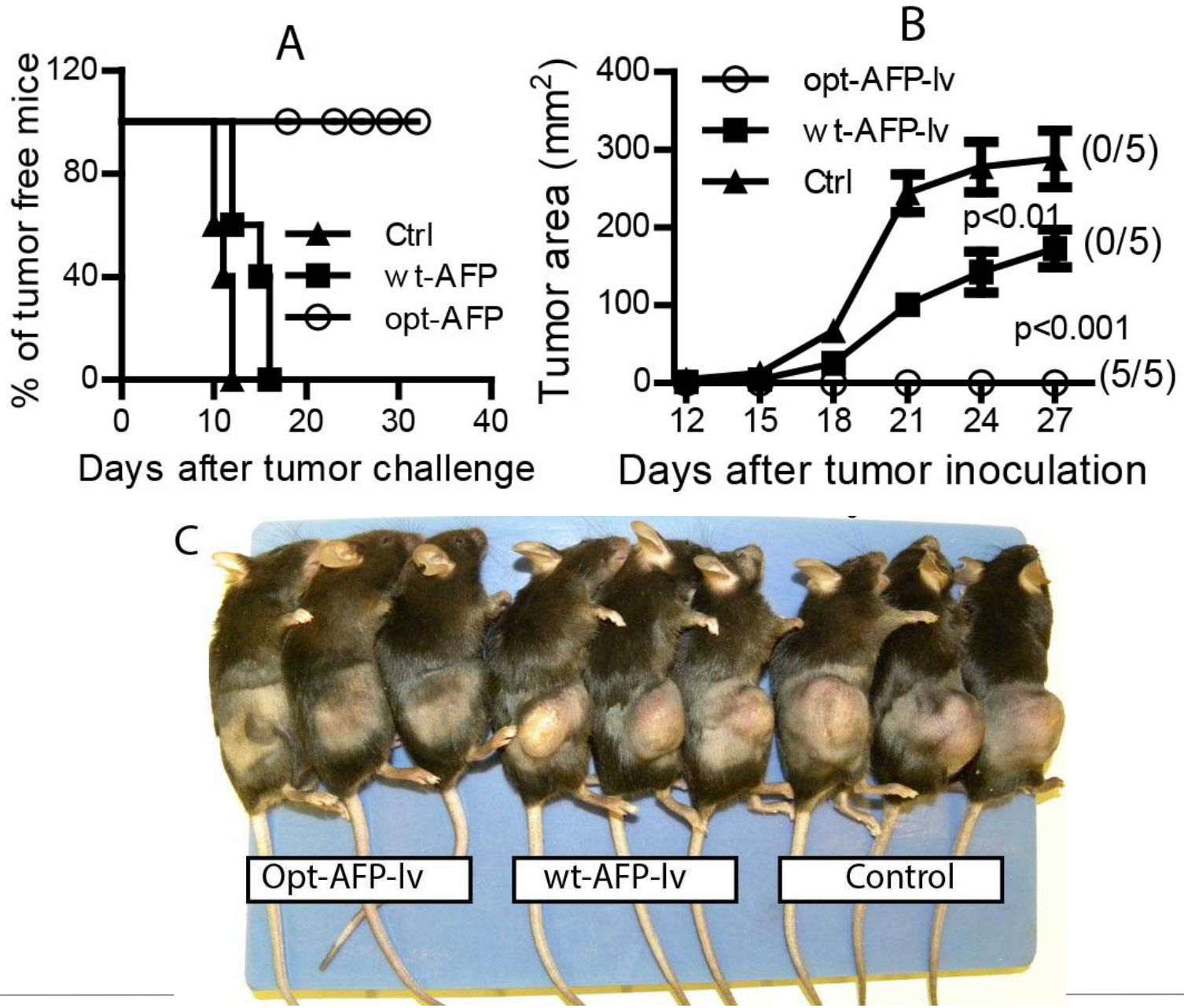


CD8 T cells activated by opt-AFP recognize AFP+ tumor cells

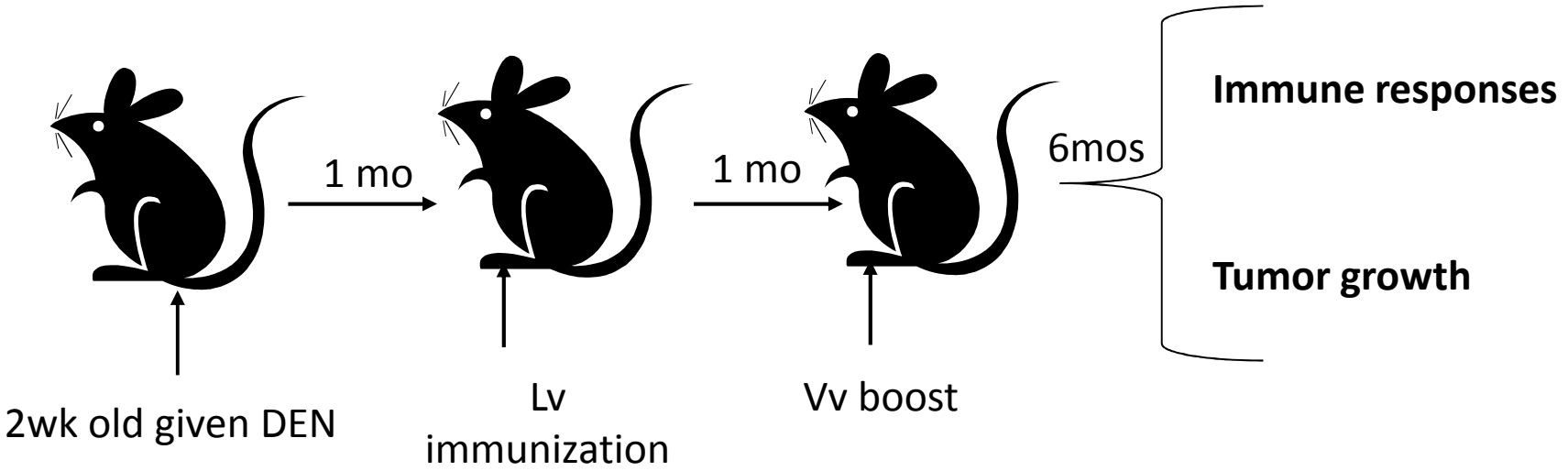


(Nakagawa et al, Biomed Res 2011;32:159-166)

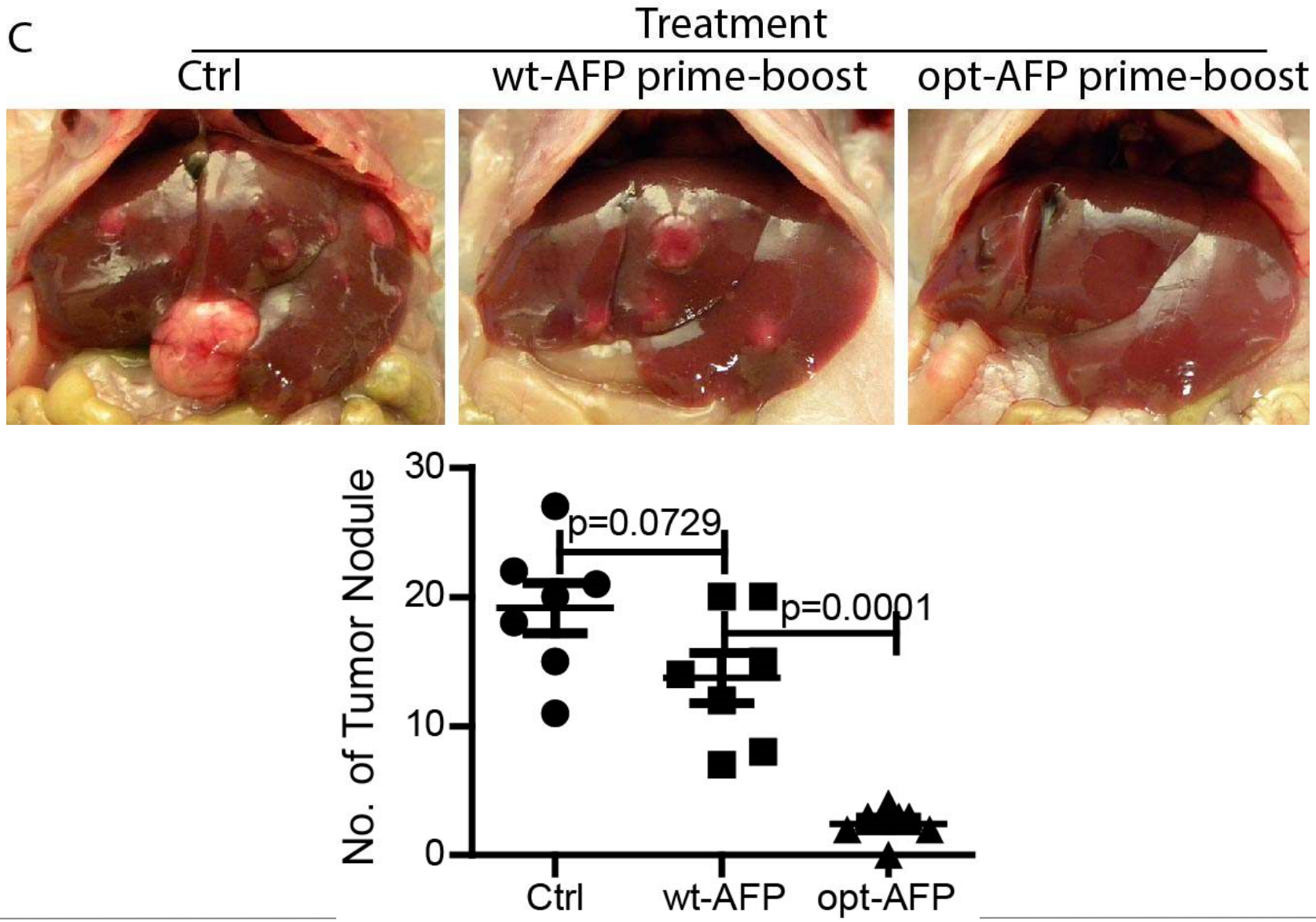
CD8 T cells specifically kill AFP+ tumor cells

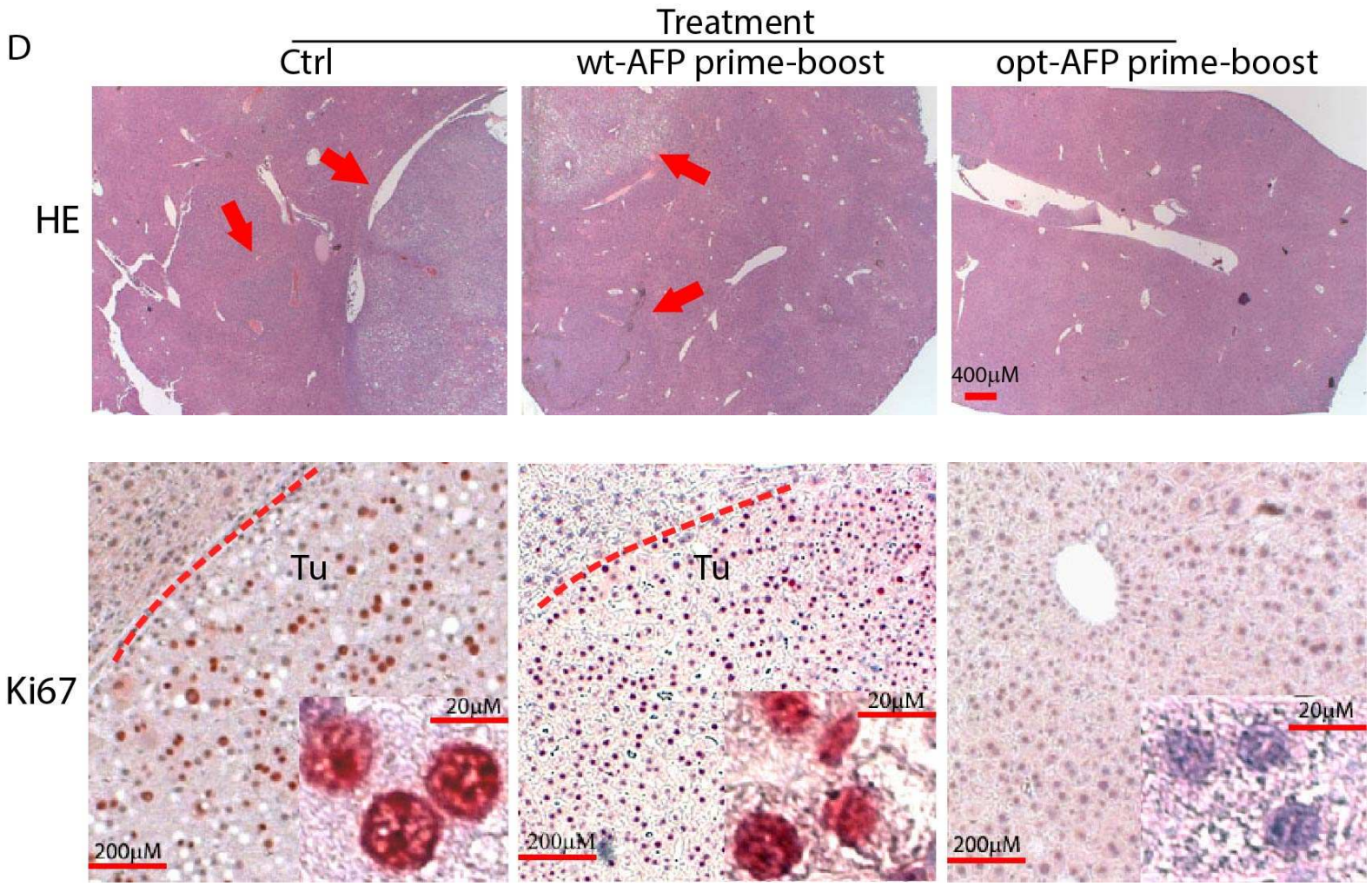

A
% of tumor free mice
120
80
40
0
0
10
20
30
40
Days after tumor challenge
Ctrl
wt-AFP
opt-AFP
B
Tumor area (mm2)
400
300
200
100
0
12
15
18
21
24
27
Days after tumor inoculation
opt-AFP-lv
wt-AFP-lv
Ctrl
p<0.01
p<0.001
(0/5)
(0/5)
(5/5)
C
Opt-AFP-lv
wt-AFP-lv
Control

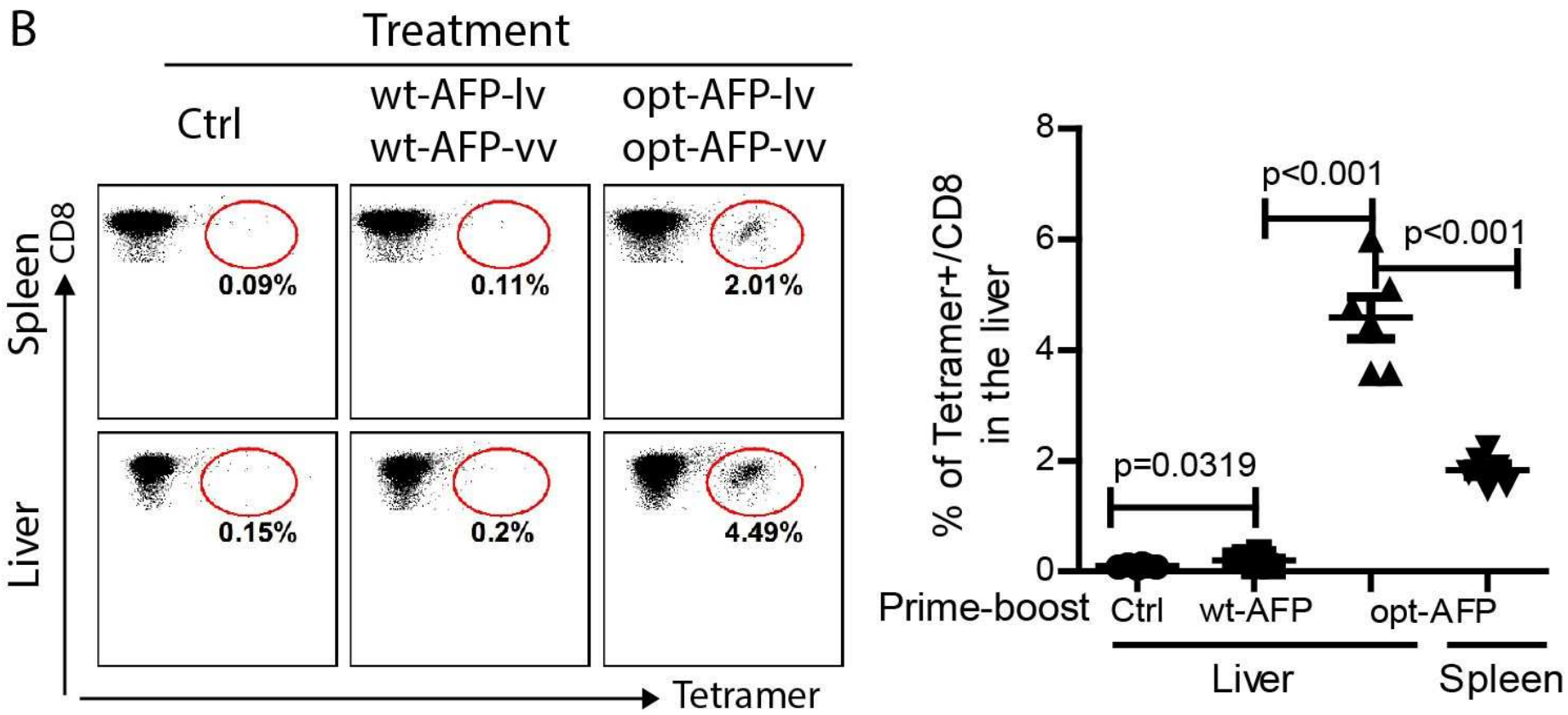
To prevent Carcinogen-induced autochthonous HCC in mice

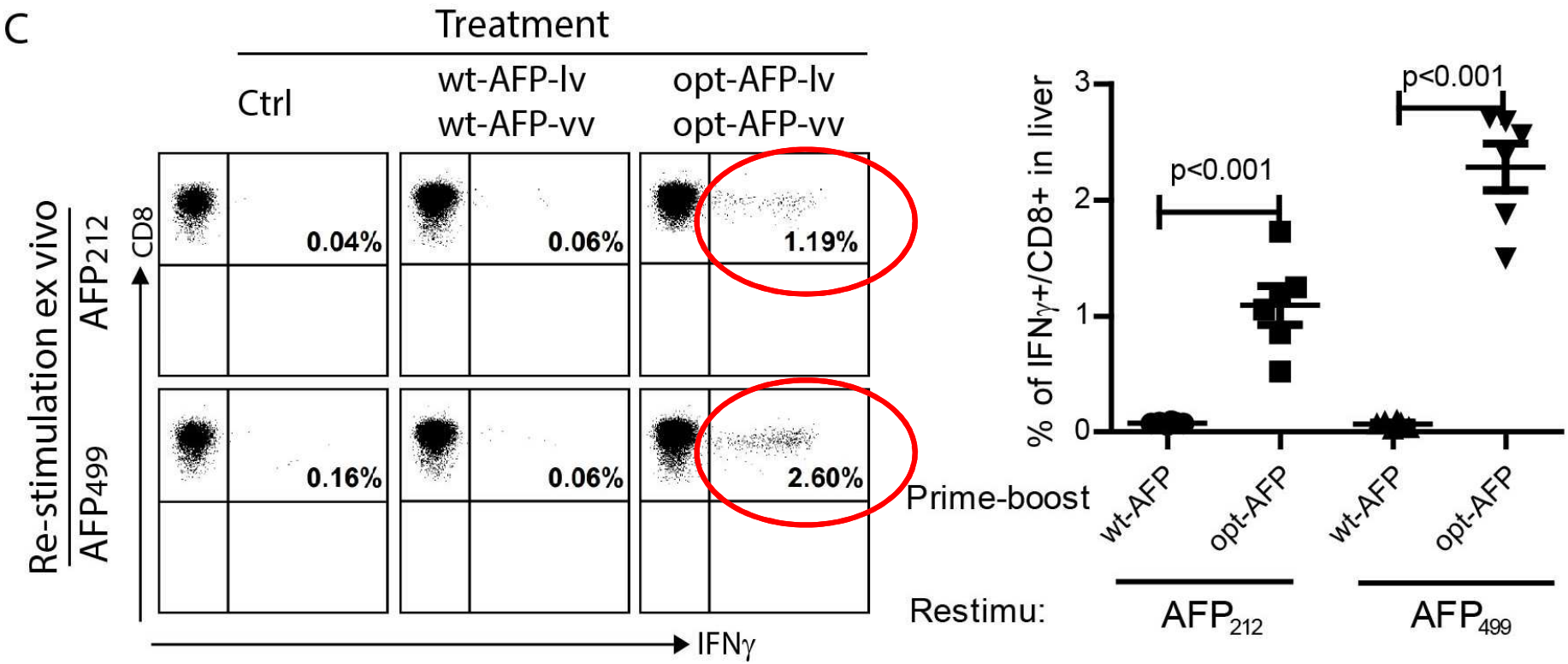


C


Treatment
Ctrl
wt-AFP prime-boost
opt-AFP prime-boost
No. of Tumor Nodule
30
20
10
0
p=0.0729
p=0.0001
Ctrl
wt-AFP
opt-AFP


D
Treatment
Ctrl
wt-AFP prime-boost
opt-AFP prime-boost
HE
400μM
Ki67
Tu
Tu
20μM
20μM
20μM
200μM
200μM
200μM


B
Treatment
Ctrl
wt-AFP-lv
wt-AFP-vv
opt-AFP-lv
opt-AFP-vv
Spleen
Liver
CD8
Tetramer
0.09%
0.11%
2.01%
0.15%
0.2%
4.49%
% of Tetramer+/CD8 in the liver
8
6
4
2
0
p<0.001
p<0.001
p=0.0319
Prime-boost
Ctrl
wt-AFP
opt-AFP
Liver
Spleen


C
Treatment
Ctrl
wt-AFP-lv
wt-AFP-vv
opt-AFP-lv
opt-AFP-vv
Re-stimulation ex vivo
AFP212
AFP499
CD8
IFNγ
0.04%
0.06%
1.19%
0.16%
0.06%
2.60%
% of IFNγ+/CD8+ in liver
p<0.001
p<0.001
Prime-boost
wt-AFP
opt-AFP
wt-AFP
opt-AFP
Restimu:
AFP212
AFP499

Summary

- Analogy to prevention of infectious diseases, cancer vaccines are likely effective in preventing cancer relapse or de novo development.
  - Animal models prove this hypothesis, and preventive cancer vaccines are in development and in trials
- In therapeutic setting, the antitumor effect of the checkpoint blockade antibodies require the presence of immune effector T cells in the tumor lesion.
  - Cancer vaccine will increase the TILs
- *Possible reasons of ineffective antitumor effect by current cancer vaccines:*
  - Insufficient magnitude: better vaccine design
  - Low quality of immune responses: high quality immune cells against mutated antigens.
  - Suppressive tumor microenvironment: combining with checkpoint blockade
  - Inappropriate setting (prevention vs treatment): prevention setting

Thank you

Enjoy Augusta!