Cancer Vaccines: A Primer

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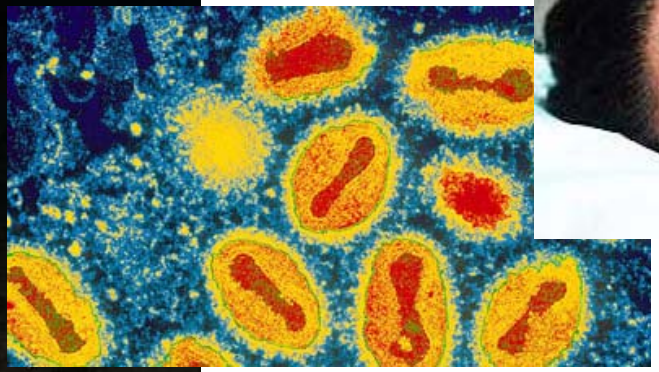
History: terminology

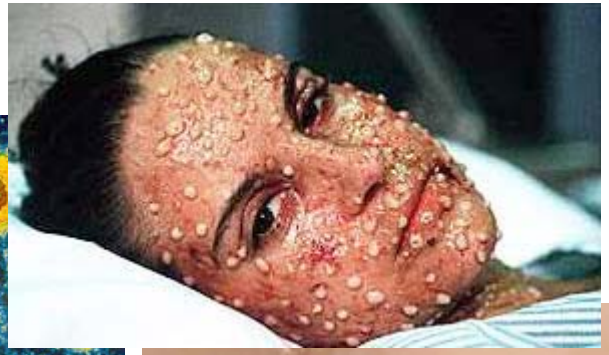
- ***Variolation***- a method of purposefully infecting a person with smallpox virus (*Variola*) in a controlled manner so as to minimize the severity of the infection and to induce immunity against a full blown smallpox infection.

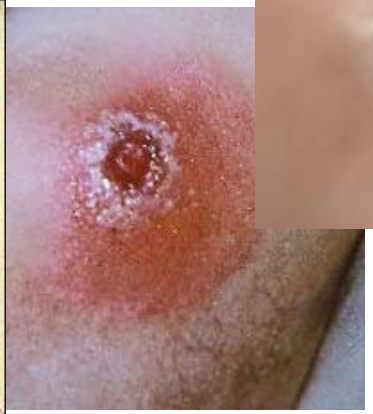
- ***Vaccination-*** the controlled administration of antigenic material (vaccine) to produce immunity to a disease. The word is derived from one of the latin words for cow, "*vacca*." Cowpox virus (vaccinia) is used to vaccinate against smallpox.

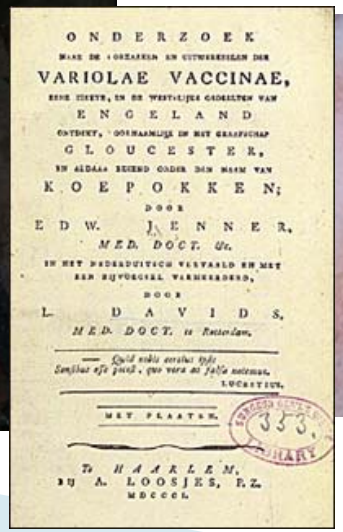
Vaccines

1796: Dr. Edward Jenner - Smallpox vaccine








ONDERZOEK

VARIOLAE VACCINAE,

ENGELAND

GLOUCESTER,

KOEPOKKEN;

EDW. JENNER,

L. DAVIDS,

TE HAARLEM,

Recommended Immunization Schedule for Persons Aged 0 Through 6 Years—United States • 2010

For those who fall behind or start late, see the catch-up schedule

Vaccine ▼ Age ►	Birth	1 month	2 months	4 months	6 months	12 months	15 months	18 months	19–23 months	2–3 years	4–6 years
Hepatitis B[1]	HepB	HepB			HepB						
Rotavirus[2]			RV	RV	RV[2]						
Diphtheria, Tetanus, Pertussis[3]			DTaP	DTaP	DTaP	see footnote[3]	DTaP				DTaP
Haemophilus influenzae type b[4]			Hib	Hib	Hib[4]	Hib					
Pneumococcal[5]			PCV	PCV	PCV	PCV				PPSV	
Inactivated Poliovirus[6]			IPV	IPV	IPV						IPV
Influenza[7]					Influenza (Yearly)						
Measles, Mumps, Rubella[8]						MMR		see footnote[8]			MMR
Varicella[9]						Varicella		see footnote[9]			Varicella
Hepatitis A[10]						HepA (2 doses)				HepA Series	
Meningococcal[11]										MCV	

Range of recommended ages for all children except certain high-risk groups

Range of recommended ages for certain high-risk groups

Recommended Immunization Schedule for Persons Aged 7 Through 18 Years—United States • 2010

For those who fall behind or start late, see the schedule below and the catch-up schedule

Vaccine ▼ Age ►	7–10 years	11–12 years	13–18 years
Tetanus, Diphtheria, Pertussis[1]		Tdap	Tdap
Human Papillomavirus[2]	see footnote 2	HPV (3 doses)	HPV series
Meningococcal[3]	MCV	MCV	MCV
Influenza[4]	Influenza (Yearly)		
Pneumococcal[5]	PPSV		
Hepatitis A[6]	HepA Series		
Hepatitis B[7]	Hep B Series		
Inactivated Poliovirus[8]	IPV Series		
Measles, Mumps, Rubella[9]	MMR Series		
Varicella[10]	Varicella Series		

Range of recommended ages for all children except certain high-risk groups

Range of recommended ages for catch-up immunization

Range of recommended ages for certain high-risk groups

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The Immune System

- **Innate (non-specific) immune system.**
  - Primitive- found in animals as ancient as sea sponges
  - Granulocytes, monocyte-macrophages, natural killer (NK) cells.
  - Complement proteins
  - Non-specifically attack and kill targets.
  - Have no “memory,” thus they confer no long term immunity.

- **Specific immune system- recognizes and targets specific antigens/epitopes and has memory.**

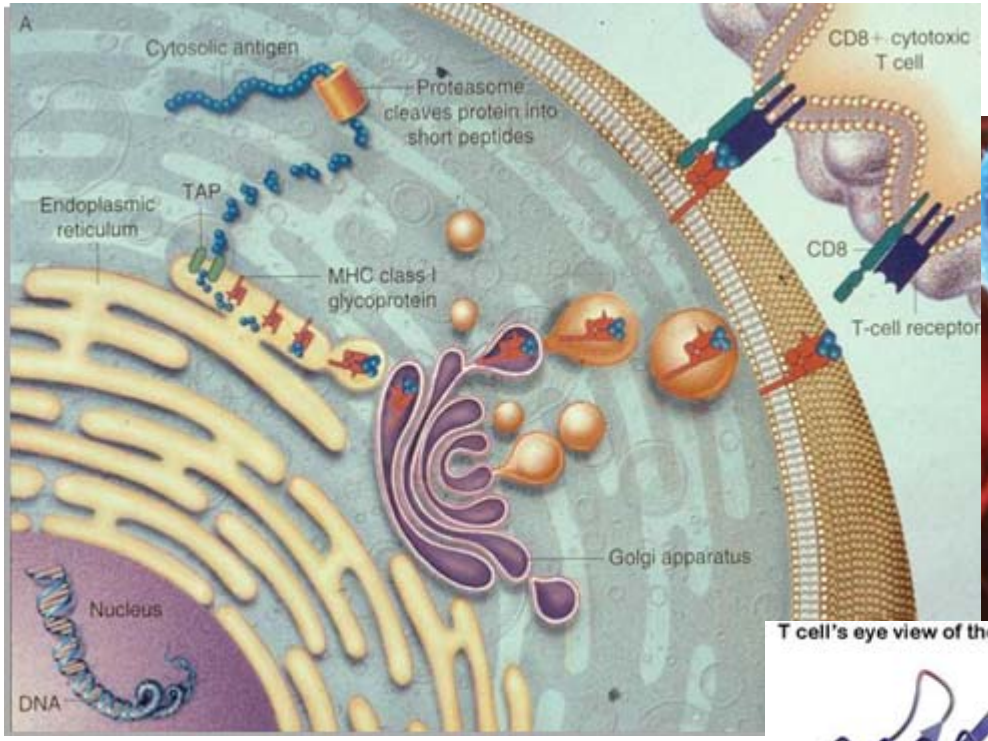
  - **Humoral immune system**- composed of B cells and plasma cells that produce specialized glycoproteins called antibodies that target specific antigens

  - **Cellular immune system**- T cells- immune system regulation and targeted killing.
    - CD4+ T cells: “helper” T cells, T-regulatory cells
    - CD8+ T cells: cytolytic T-lymphocytes (CTL) or “killer” T cells.

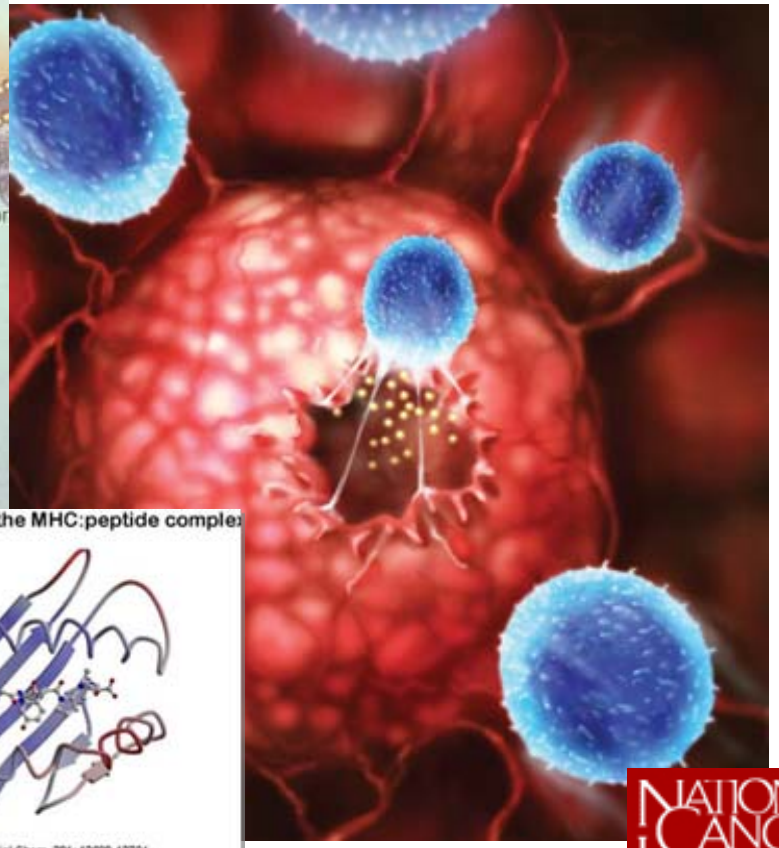
- **Antigen presenting cells (dendritic cells) bridge the innate and specific immune systems.**

Antigen Processing, Presentation, T-cell Activation and Tumor Cell Killing

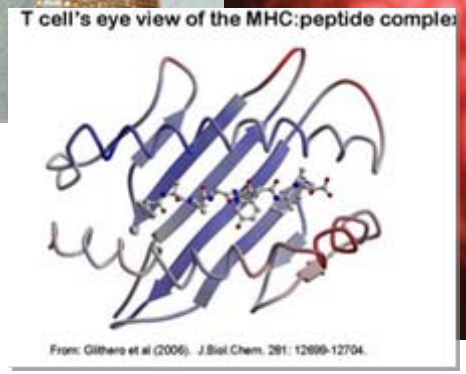
Antigen Processing



Tumor Cell Killing



MHC Antigen Presentation



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Immunotherapy of Cancer

William B. Coley, M.D.
(1862–1936)

- **Non-specific Immunotherapy**
  - Coley's toxin (1893), Bacille Calmette-Guérin (BCG), *C. parvum*
  - Adjuvants
    - Imiquimod (Aldara®)
  - Cytokines, Chemokines, and Growth factors
    - Interferons (α, β, γ) , interleukin-2, -12, -15, G-CSF, etc.

- **Specific Immunotherapy**
  - **Passive immunization**
    - Monoclonal Antibodies- alemtuzumab, cetuximab, rituximab, etc.
    - Immunotoxins- denileukin diftitox (Ontak®), BL22, HA22, LMB-2, Myelotarg®, SS1P
    - Radioimmunotherapy- Bexxar®, Zevalin®, ^{90}Y-daclizumab

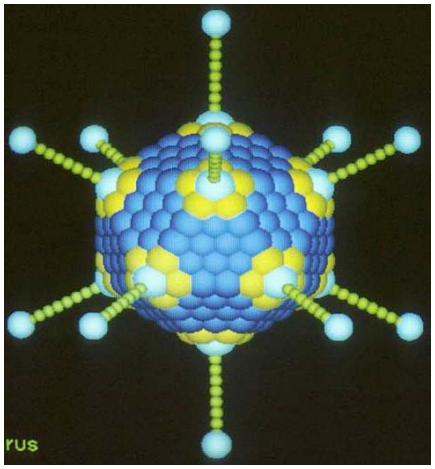
  - **Active immunization**
    - **Cancer Vaccines-** Tumor antigen protein, peptides, dendritic cells, tumor cells, gene transfer, virotherapy, radiofrequency ablation (RFA).
    - Adoptive Immune Transfer
      - T cell therapy
        - Tumor Infiltrating lymphocytes
        - Engineered T cell receptors

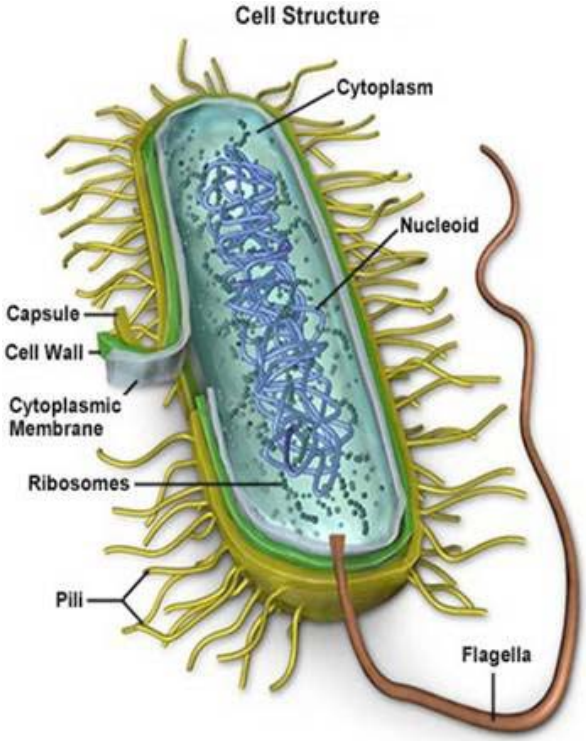
Cancer Vaccines

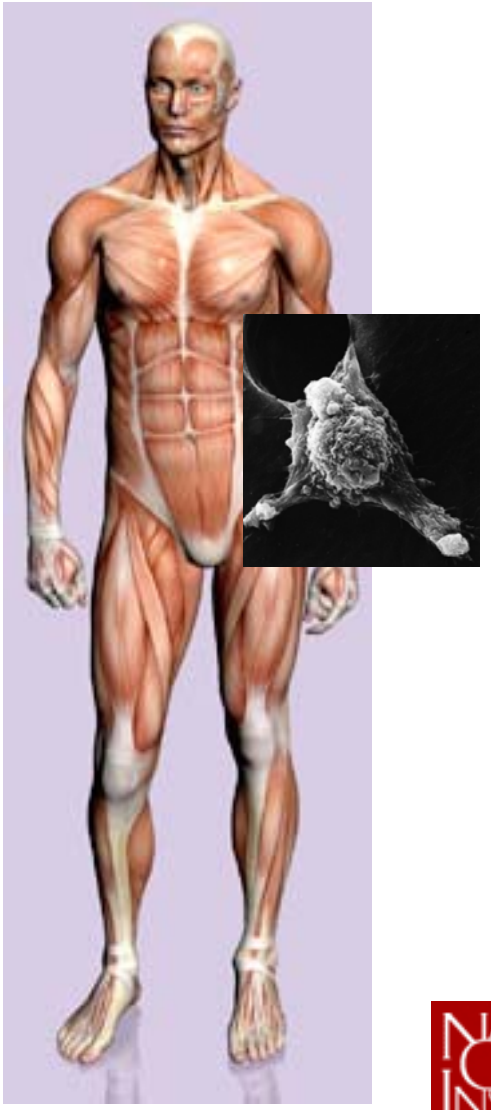
- **Prophylactic Vaccines**- prevent infections that can lead to the development of cancer.
  - Not useful once a cancer is diagnosed:
    - HBV vaccine- prevents chronic hepatitis B → cirrhosis → hepatocellular carcinoma.
    - HPV vaccine- reduces risk of infection with oncogenic HPV genotypes and cervical IEN and invasive cancer.

- **Therapeutic Cancer Vaccines**- used to treat established cancer.
  - **Adjuvant**- used after primary cancer treatment and patient render clinically free of cancer.
    - HER-2/neu vaccine in HER-2/neu+ resected breast cancer.
  - **Therapeutic**- used once cancer is established
    - Provenge® (Sipuleucel-T, Dendreon Corp.)- Prostate cancer

So why is it is difficult to vaccinate against tumors in man?







Mechanisms used by tumors to evade the immune response?

- **Immunological tolerance**- tumors are often recognized by the immune system as "self" and not as foreign, and therefore not rejected.
  - **Central tolerance**- deletion of autoreactive T-cell clones during development.
  - **Peripheral tolerance**- number of mechanisms that act as down-modulators of the immune response.

- **Tumors employ numerous strategies to hide from the immune system.**
  - Decreased MHC class I expression- decreased antigen presentation.
  - Loss of tumor antigens (e.g., CEA, PSA)
  - Loss of co-stimulatory molecules

- **Tumor cells employ numerous strategies to attenuate immune responses.**
  - Tregs (CD4+CD25+FoxP3+), MDSC's (Gr-1+CD11b+)
  - Increased expression of immunosuppressive cytokines and enzymes- Arginase, TGF-β, indoleamine oxidase, IL-10, VEGF.
  - T-cell "exhaustion"- PD-1, PDL-1.

Evidence that cancers are immunogenic

Spontaneous Regression of Cancer

Cancer	No. Cases
Leukemia/Lymphoma	124
Melanoma	69
Renal Cell Carcinoma	68
Neuroblastoma	41
Gastrointestinal Cancer	34
Retinoblastoma	33
Lung & Bronchus	25
Breast Cancer	22
Testicular Cancer	16
Head & Neck Cancer	8
Other Cancers	65
TOTAL	504

PubMed: 1966-1987

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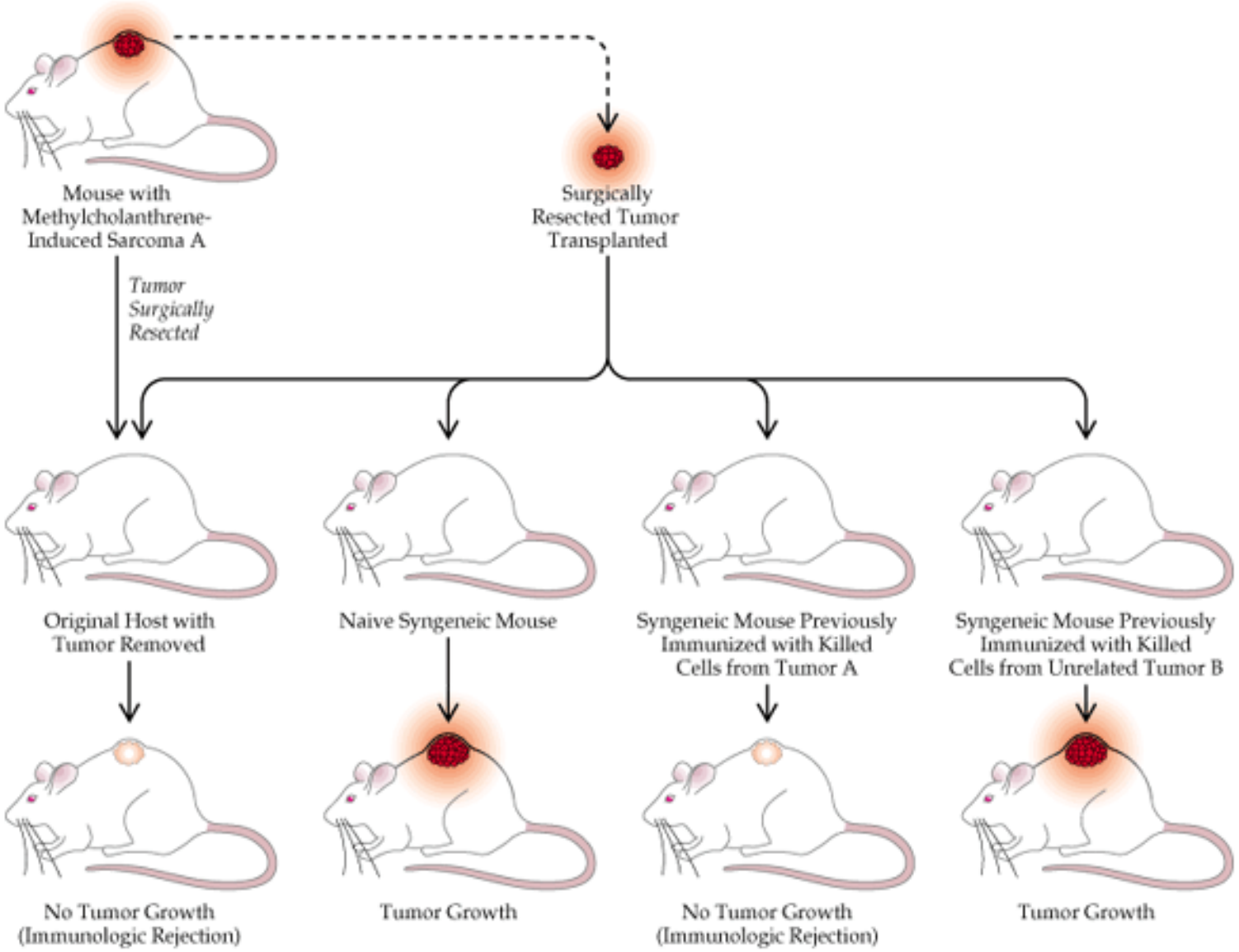
Evidence that we can vaccinate against tumors



JNCI 18:769, 1957

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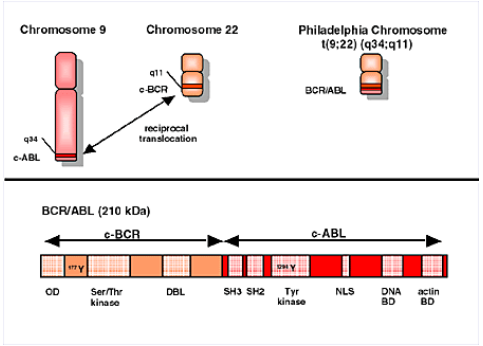
What is needed for a successful anti-cancer vaccine?

- Ideal target:
  - An antigen that is both necessary and sufficient for cellular transformation (e.g., bcr-abl fusion protein).
  - Tumor-specific
  - Expressed at high levels.
  - Accessible to the immune system.



- Effective vaccine- antigen delivery system: vehicle/vector, route and schedule.
  - Capable of inducing strong and lasting immunity.
  - Induces CD8+ cytolytic T-cell responses?

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What do we need for a successful anti-cancer vaccine?

- Accurate methods to monitor the effectiveness of the vaccine.
- Active and safe adjuvants:
  - Enhances immunogenicity of the vaccine.
- Ease of manufacture, reasonable costs, and patient acceptance.

Antigen Targets for Cancer Vaccines

- Tumor specific antigens (TSA)- unique and specific.
  - Unique fusion proteins: CML- bcr-abl, sarcomas- EWS/FLI-1
  - Oncogenic viral proteins:
    - Cervix cancer- HPV E6/E7.
    - Burkitt's lymphoma- EBV EBNA-1.
- Tumor-associated antigens (TAA)
  - Mutated normal proteins: MAGE-1, K-ras, p53
  - Over expressed normal proteins: HER-2/*neu*, EGFR
  - Post-translational antigen modifications: MUC-1 glycosylation.
  - Genes read in alternate reading frames: TARP

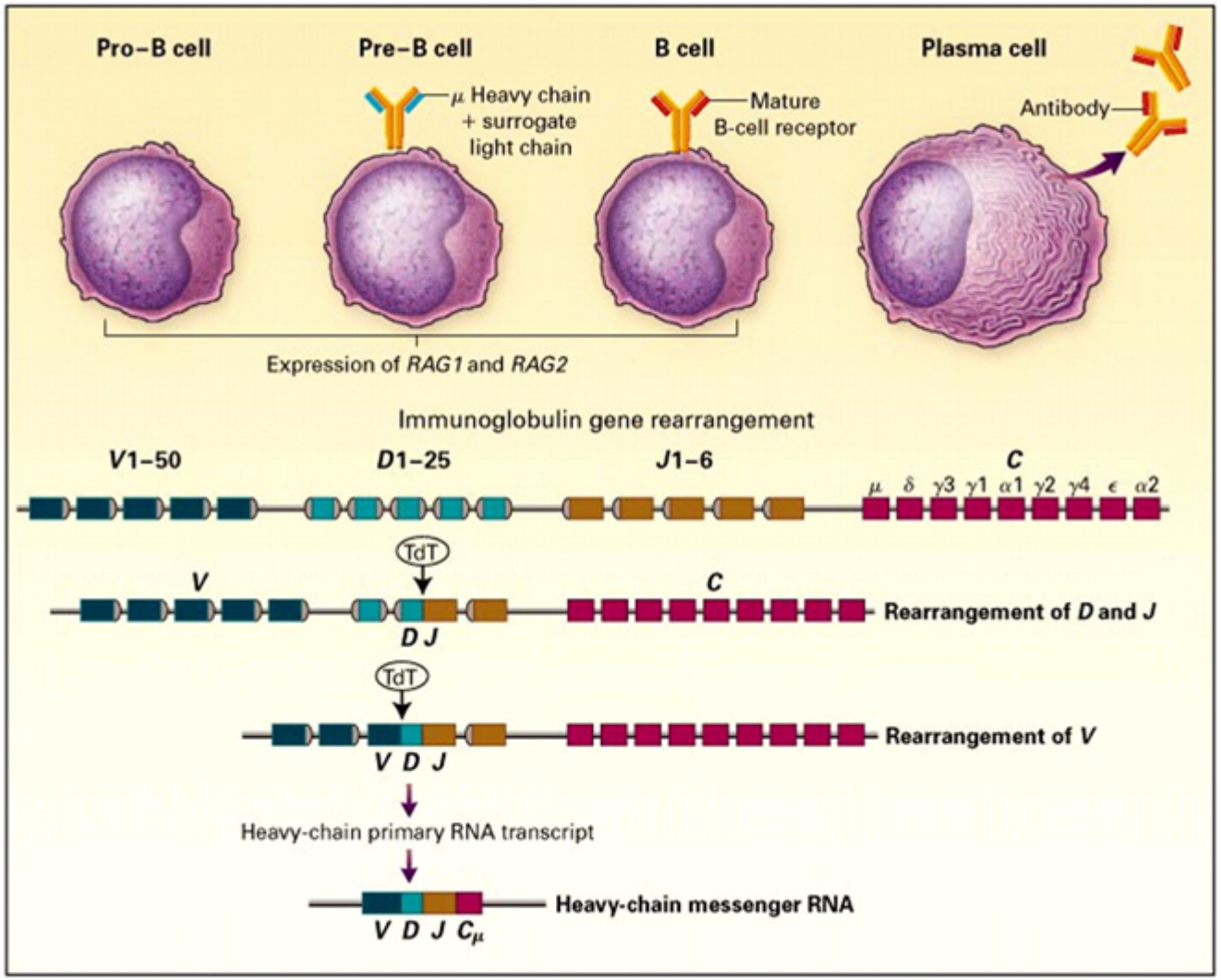
Targets for Tumor Vaccines

Overexpressed Antigens	
Cell Cycle Regulators	
Cyclins D1, E	Breast, colon, head & neck,
mdm-2	Sarcoma, leukemia, glioma
Receptor Tyrosine Kinases	
Epidermal growth factor receptor	Lung, head & neck, glioma
ErbB2 (HER-2/*neu*)	Breast, ovarian, stomach
ErbB3	Breast, colon, pancreas
Fibroblast growth factor receptor I	Glioma, melanoma, pancreas
Insulin-like growth factor receptor II	Breast, lung, ovarian
c-MET	Ovarian, thyroid
Nuclear Oncogenes	
c-, L-, N-myc	Breast, neuroblastoma, SCLC
Anti-apoptotic Proteins	
Telomerase	Stomach
Bcl-2	Non-Hodgkin's lymphoma

Targets for Tumor Vaccines

Mutated Antigens	
Fusion Proteins	**Tumor**
Bcr-abl	Chronic myelocytic leukemia
EWS/FLI1	Sarcomas
BcR idiotype	Follicular lymphoma
Point mutations	
p53	Lung, head & neck, pancreas
K-ras	Lung, pancreas, GI
MAGE-1	Melanoma
Oncogenic viral proteins	
HPV E6/E7	Cervix, oropharyngeal
Oncofetal antigens	
AFP	Hepatocellular, testicular
CEA	
Non-essential tissue specific antigens	
PSA, PSMA, PAP	Prostate

BcR idiotype antigen



Peptide vaccines

- Immunodominant peptides (e.g., gp100, MART-1- melanoma, p53- lung cancer, K-ras- colon cancer).
  - Advantages:
    - Targets a single or small number of specific epitope(s).
    - Easy to manufacture and QA/QC
    - Easy to assay immune responses in CTL or ELISPOT assays.
  - Disadvantages
    - Limited to certain HLA types (e.g., HLA-A2 <50% population)
    - Vaccinating for a limited number of epitopes
    - May require unacceptably frequent vaccination to induce an meaningful immune response.
  - May be combined with other approaches (e.g., peptide loaded dendritric cells).
  - May be modified to enhance MHC affinity (epitope enhancement).

Whole protein vaccines

- Uses the entire antigen.
- May be purified from tumor cells, expressed in recombinant systems in vitro, or in a tumor cell lysate.
- Advantages:
  - Multiple epitopes
- Disadvantages
  - Potential for allergic reactions and autoimmunity.
  - Not very successful.

Gene-based antitumor vaccines

- Expresses the antigen gene
- Expression plasmids
  - Easy to manufacture
  - Low levels of expression.
- Recombinant viral vector or plasmid-based gene transfer
  - Adenovirus
  - Pox virus (avipox, vaccinia, etc.)
  - Retrovirus (Molony virus, lentivirus)
  - Others
- Advantages:
  - Express entire antigen (multiple epitopes)
  - Viral proteins may act as adjuvants boosting response.
  - May be combined with other approaches.
  - May express multiple antigens.

Whole Cancer Cell Vaccines

- GVAX (Cell Genysis), Lucanix (NovoRx), HyperAcute (NewLink), etc.
- Autologous vs. Allogeneic
- Unmodified
- Modified
  - Irradiated
    - increases MHC class I expression
    - Safety
  - Gene-modified
    - Express co-stimulatory proteins (B7) or immunostimulatory cytokines
    - Immunogenic proteins

Dendritic Cell Vaccines

- Generated by various methods
  - Most common uses peripheral blood monocytes and GM-CSF
- Advantages:
  - Most powerful antigen presenting cells
  - Can be loaded with target antigen by various means
- Disadvantages:
  - Immature or non-activated DC may be tolerizing
  - Requires specialized facilities- cell processing, flow cytometry

Antigen loading of dendritic cells

- Tumor antigen peptides
- Whole tumor protein
- Tumor protein lysates
- Tumor apoptotic bodies
- mRNA electroporation
- Plasmid DNA transfection
- Viral gene transfer
- Tumor cell–DC fusions

Dendritic cell vaccines

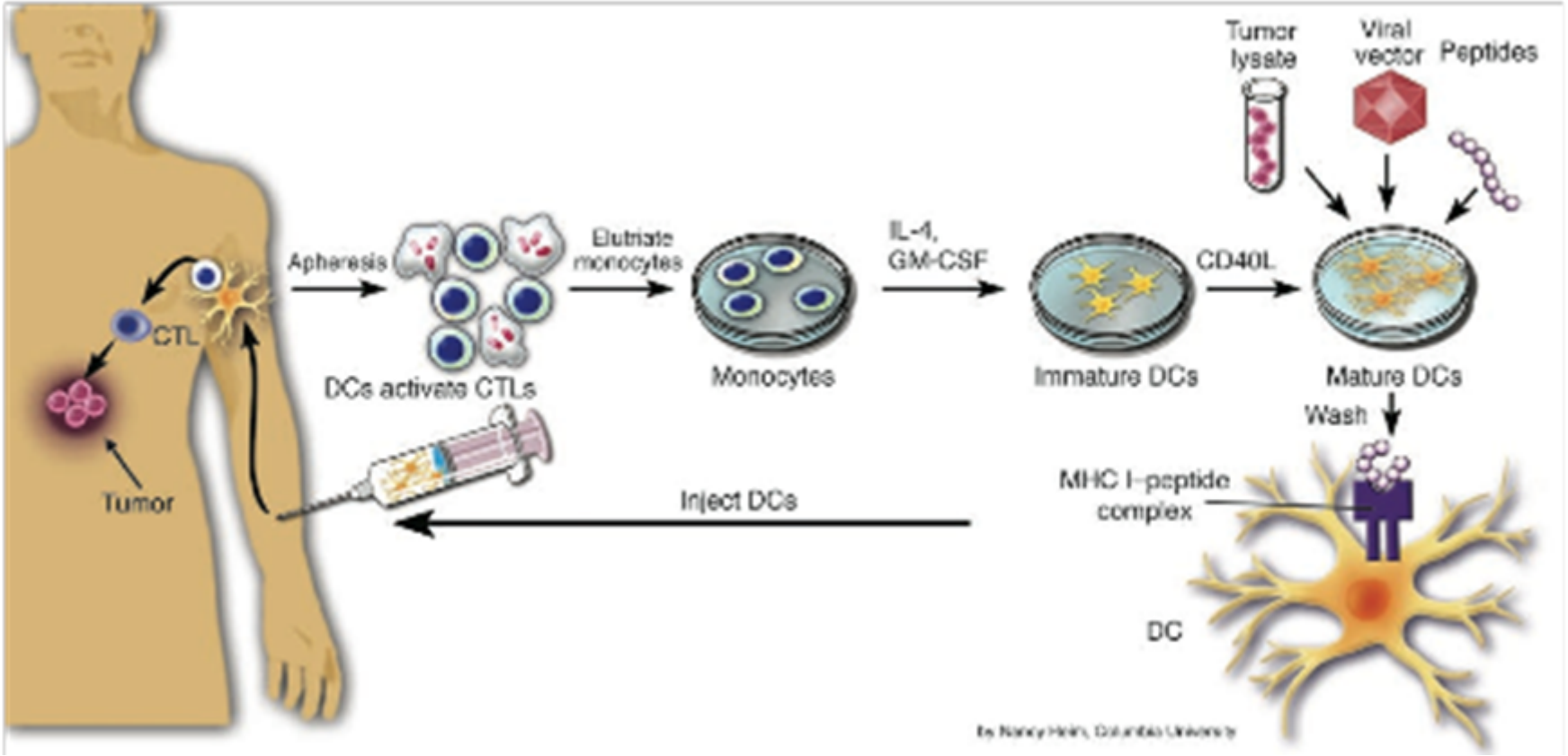
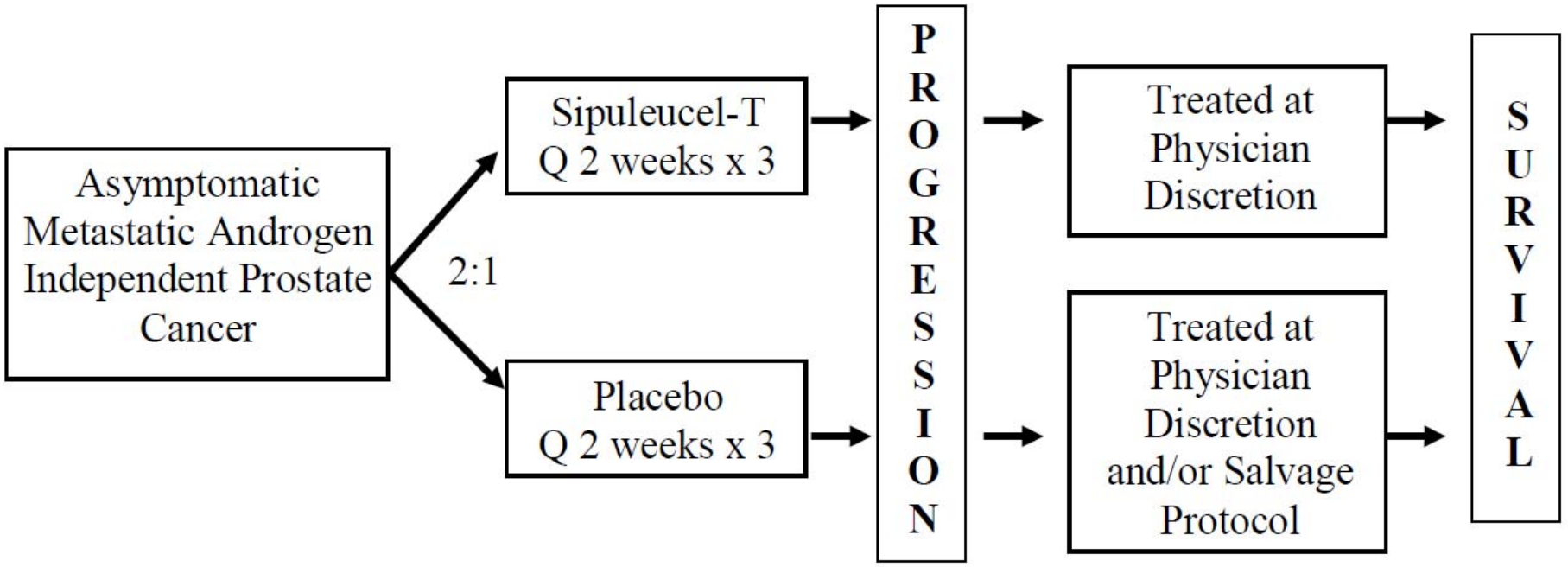


Table 1
Antitumor vaccines in clinical trials

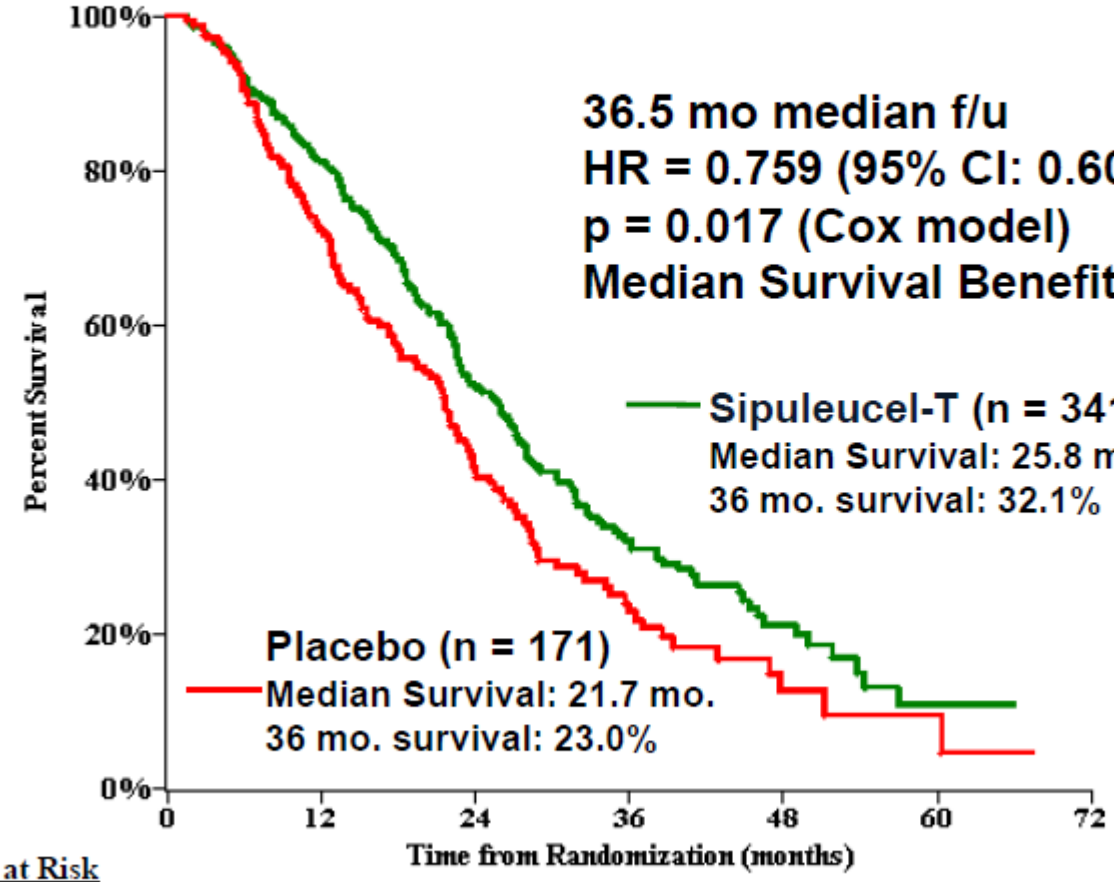
Vaccine	Advantages	Disadvantages
Whole tumor cell	1. Studied extensively 2. Can be processed to enhance antigen presentation (e.g., irradiated tumor cells or tumor lysates); 3. Can be administered with adjuvants (e.g., BCG, KLH, viruses, etc.); 4. Likely to express the relevant tumor antigens; 5. Antigens need not be defined	1. Requires availability of autologous tumor or an allogeneic cell line sharing the relevant tumor antigens; 2. Poor ability to stimulate immune responses; 3. Few responses and little benefit reported when used adjuvantly in randomized clinical trials
Gene-modified tumor cells	1. Likely to express the relevant tumor antigens; 2. Antigens need not be defined; 3. Often engineered to coexpress immunostimulatory molecules and cytokines (e.g., GM-CSF, IL-2); 4. Use of allogeneic tumor cell lines and fibroblasts are under investigation as an approach to accelerate vaccine production; 5. Some immunological and clinical responses reported	1. Requires availability of autologous tumor or an allogeneic cell line expressing the relevant tumor antigens; 2. Weak antigen presentation by many tumors; 3. Long manufacturing time; 4. Need for ex vivo cell culture; 5. Cost, time, and labor intensive
Plasmid (naked) DNA	1. Constructed to express the relevant tumor antigen; 2. Easy to produce and stable; 3. Can be administered as a direct injection or biolistically ("gene gun")	1. Requires detailed knowledge of the antigen DNA sequence; 2. Low immunological potency for self (tumor) antigens; 3. Response may be Th2 skewed; 4. High doses of plasmid DNA are required to generate immune responses
Peptides	1. Can limit immune response to epitopes distinct from the wild type (e.g., point mutations or breakpoint-fusion genes); 2. Epitopes can be enhanced; 3. Easy to produce and stable; 4. Can be combined as cocktails of peptides; 5. Some immunological and clinical responses reported	1. Requires knowledge of the specific epitope; 2. Immunogenicity restricted to a limited number of MHC molecules; 3. Usually requires the addition of an adjuvant for immunogenicity
Viral gene transfer vectors	1. Engineered to express the relevant tumor antigen; 2. Can be engineered to coexpress immunostimulatory molecules and cytokines; 3. Wide variety of available vectors (e.g., adenovirus, pox viruses, lentiviruses, etc.); 4. Some cellular immune responses reported	1. Immunodominance of viral antigens over tumor antigens; 2. Weak antitumor responses seen with most viral vectors; 3. Preexisting immunity against viral vectors may attenuate the antitumor response; 4. Risk of toxicity with "live" viruses
Antigen-modified DCs	1. Use of powerful APCs; 2. Techniques available to generate large numbers of clinical grade DCs; 3. Target antigens may be defined or uncharacterized; 4. Multiple antigen loading techniques (e.g., peptide, lysates, whole protein, RNA transfection, viral vectors, etc.) are available; 5. Some immunological and clinical responses reported	1. Need for ex vivo cell culture; 2. Cost, time, and labor intensive; 3. Optimal technique for antigen loading remains undefined; 4. Possibility of tolerization by immature DCs; 5. Lack of criteria for standardization of final product

BCG, bacille Calmette-Guérin; KLH, keyhole limpet hemocyanin.

Phase III clinical trial of Provenge™ (Sipuleucel-T)



IMPACT Overall Survival
Final Analysis (349 events)



No. at Risk						
Sipuleucel-T	341	274	142	56	18	3
Placebo	171	123	59	22	5	2

How should we administer cancer vaccines for the best effect?

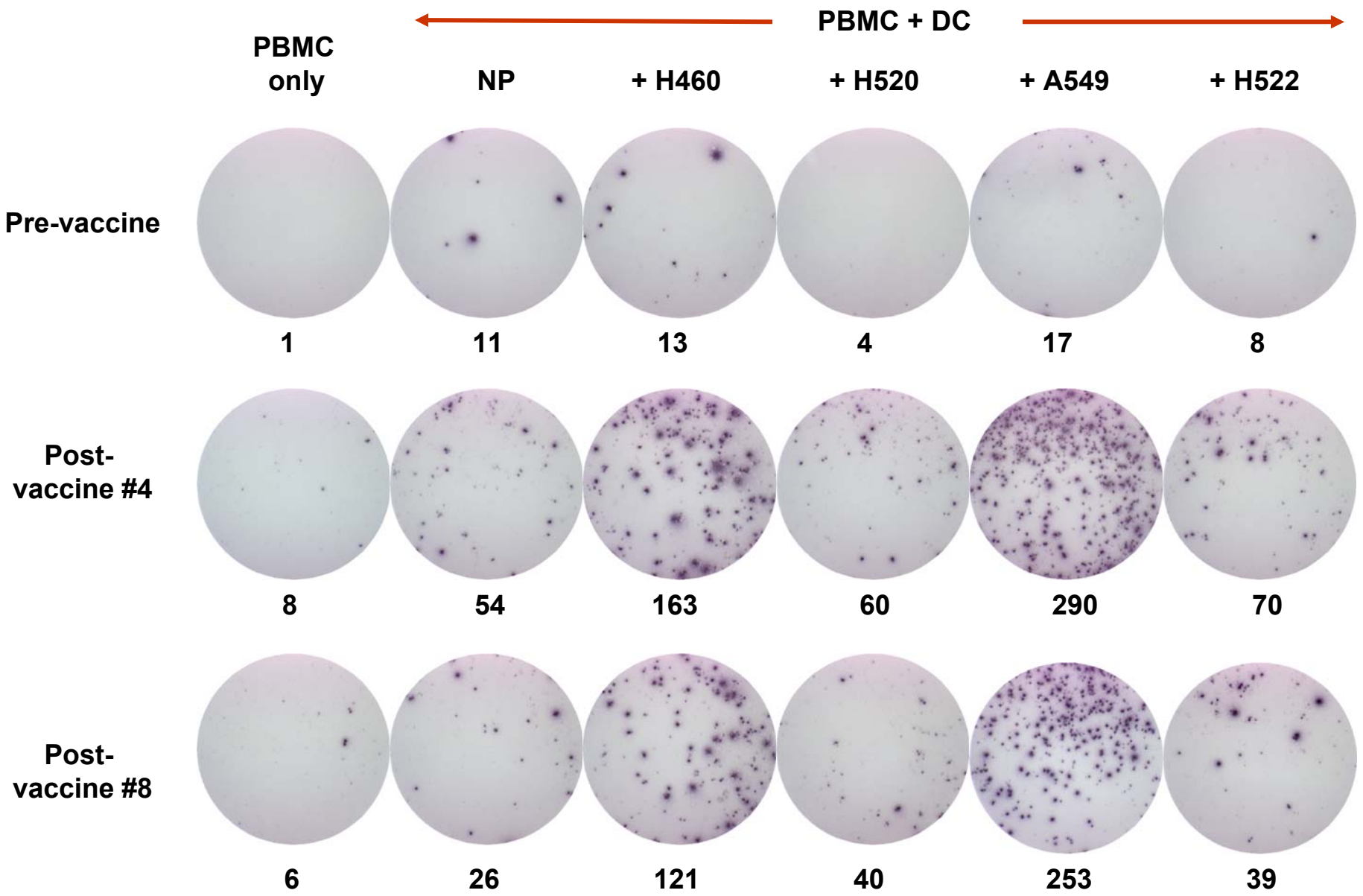
- Route of vaccination
  - Intramuscular
  - Intradermal
  - Subcutaneous
  - Intravenous
  - Intraperitoneal
  - Intralymph node
  - Intratumoral
  - oral

- Best schedule of vaccination- not really known and may vary with the vaccine.

How do we assess the effects of our vaccination?

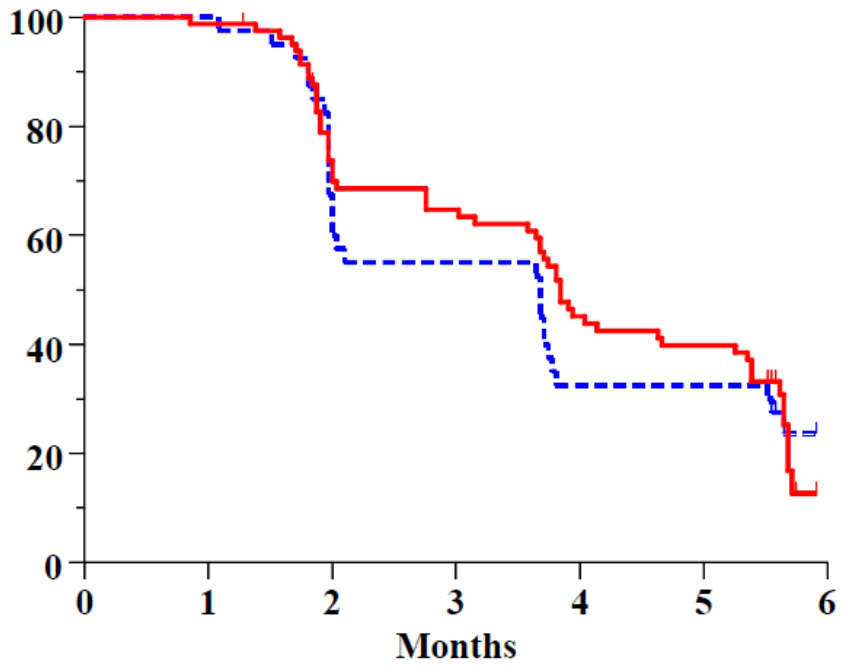
- Antibody titers?
- Cytolytic T-cell (CTL) assays?
- ELISPOT assays?
- Tetramer assays?
- Response rate (tumor regression- RECIST)?
- Progression-free survival?
- Over-all survival?
- Other?

ELISPOT ASSAY- Count the spots

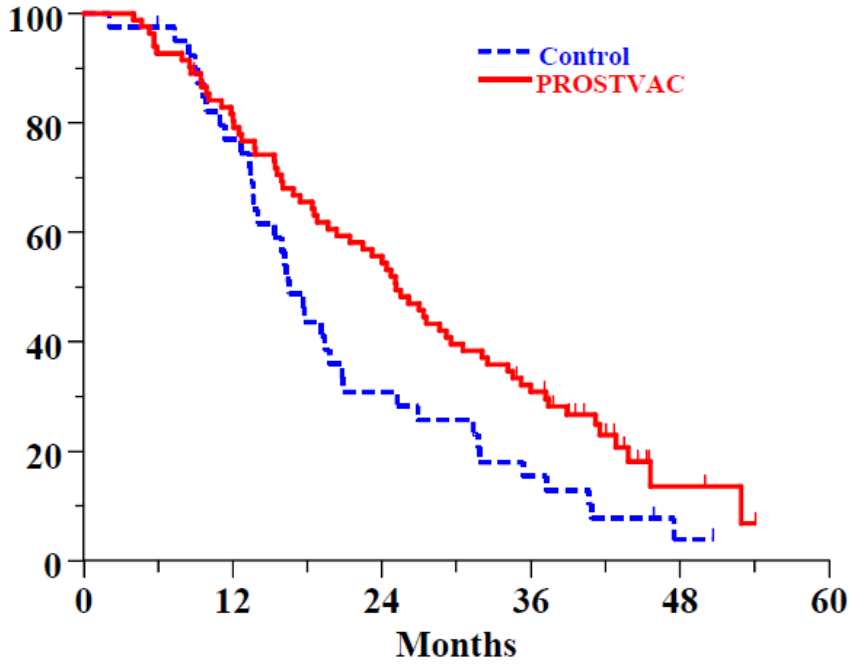


Prostvac-VF Randomized Phase 2

Progression vs Overall Survival (N=122)



Time to Progression



Overall Survival

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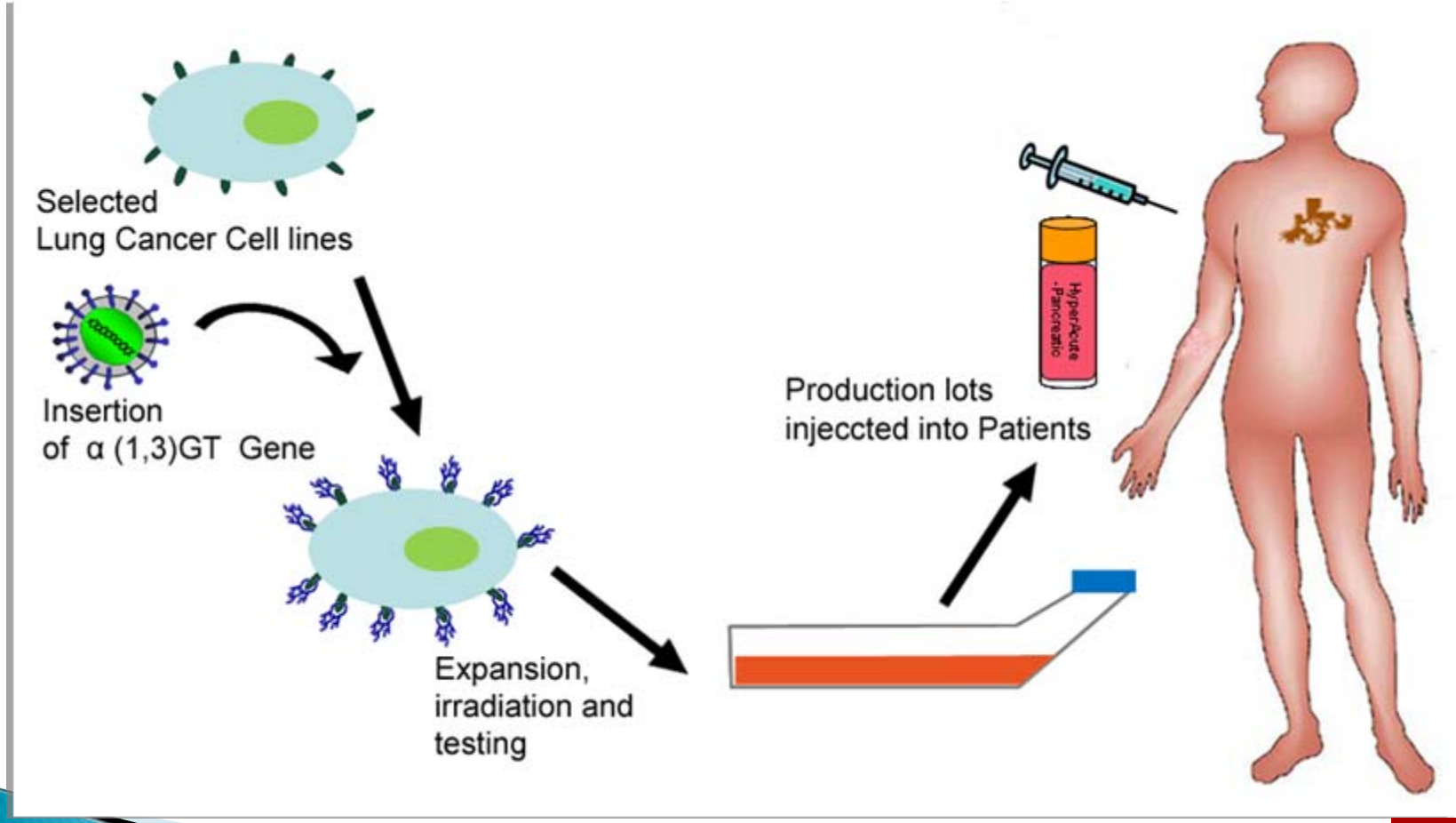
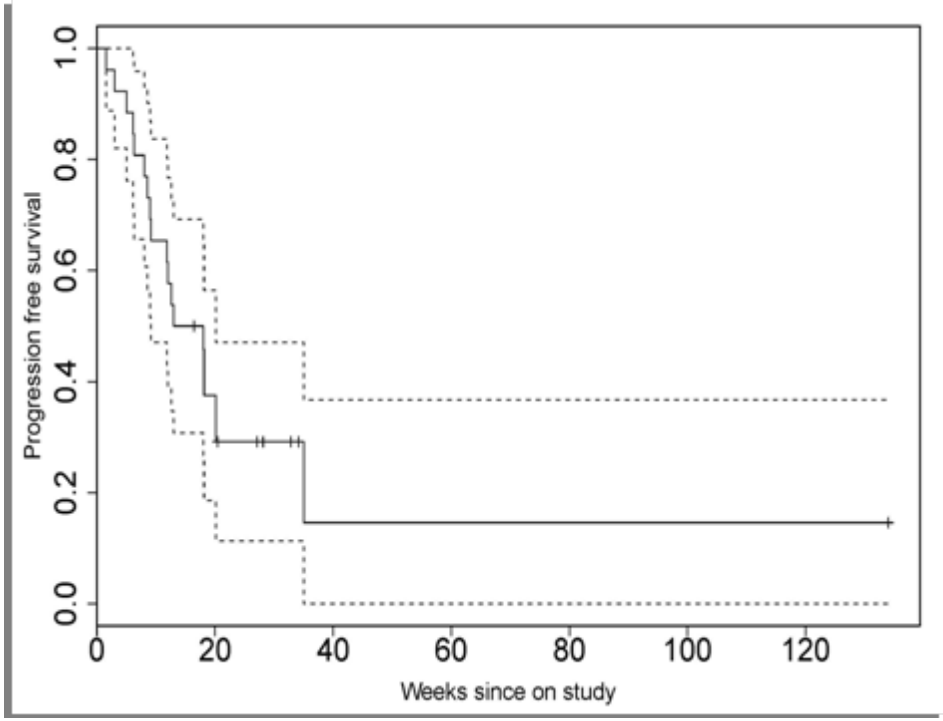
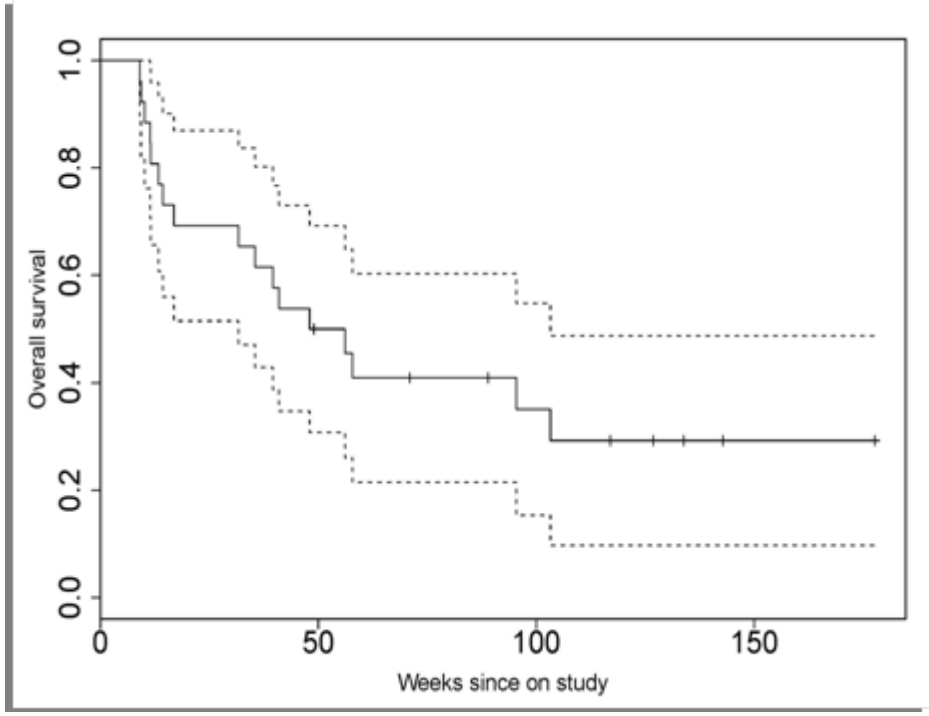
HyperAcute Lung Cancer Vaccine



HyperAcute Lung Cancer Vaccine



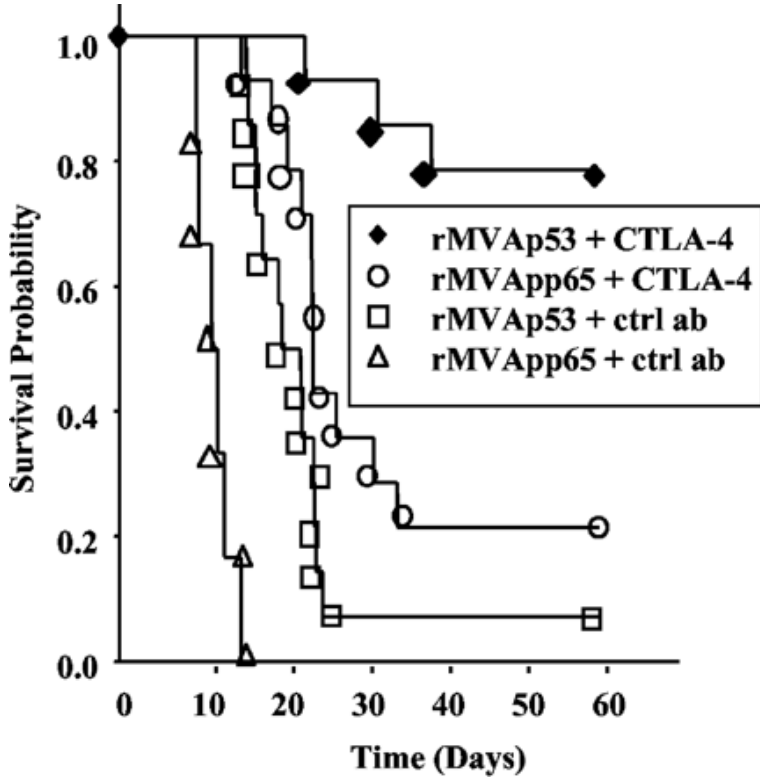


Negative immune system regulators

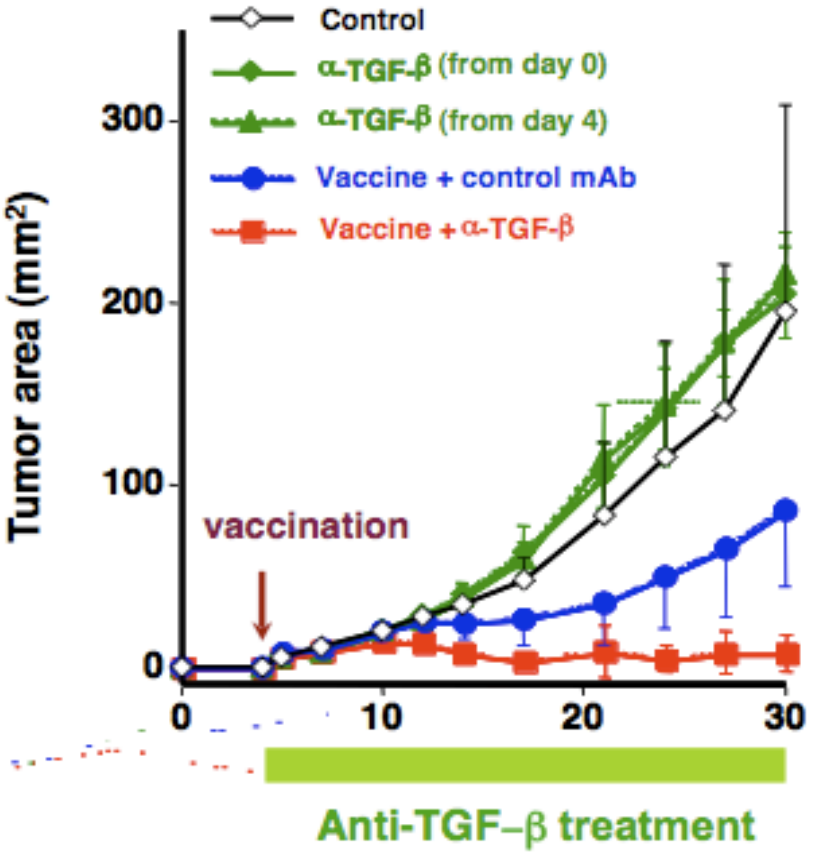
- T-regulatory cells (Tregs)
- Cytotoxic lymphocyte antigen-4 (CTLA-4)
- Transforming growth factor-beta (TGF-β)
- Interleukin-10
- Programmed death-1 (PD-1)/PDL-1
- Indolamineoxidase (IDO)
- Arginase

Effect of negative immune regulatory checkpoint blockade on anti-tumor vaccination

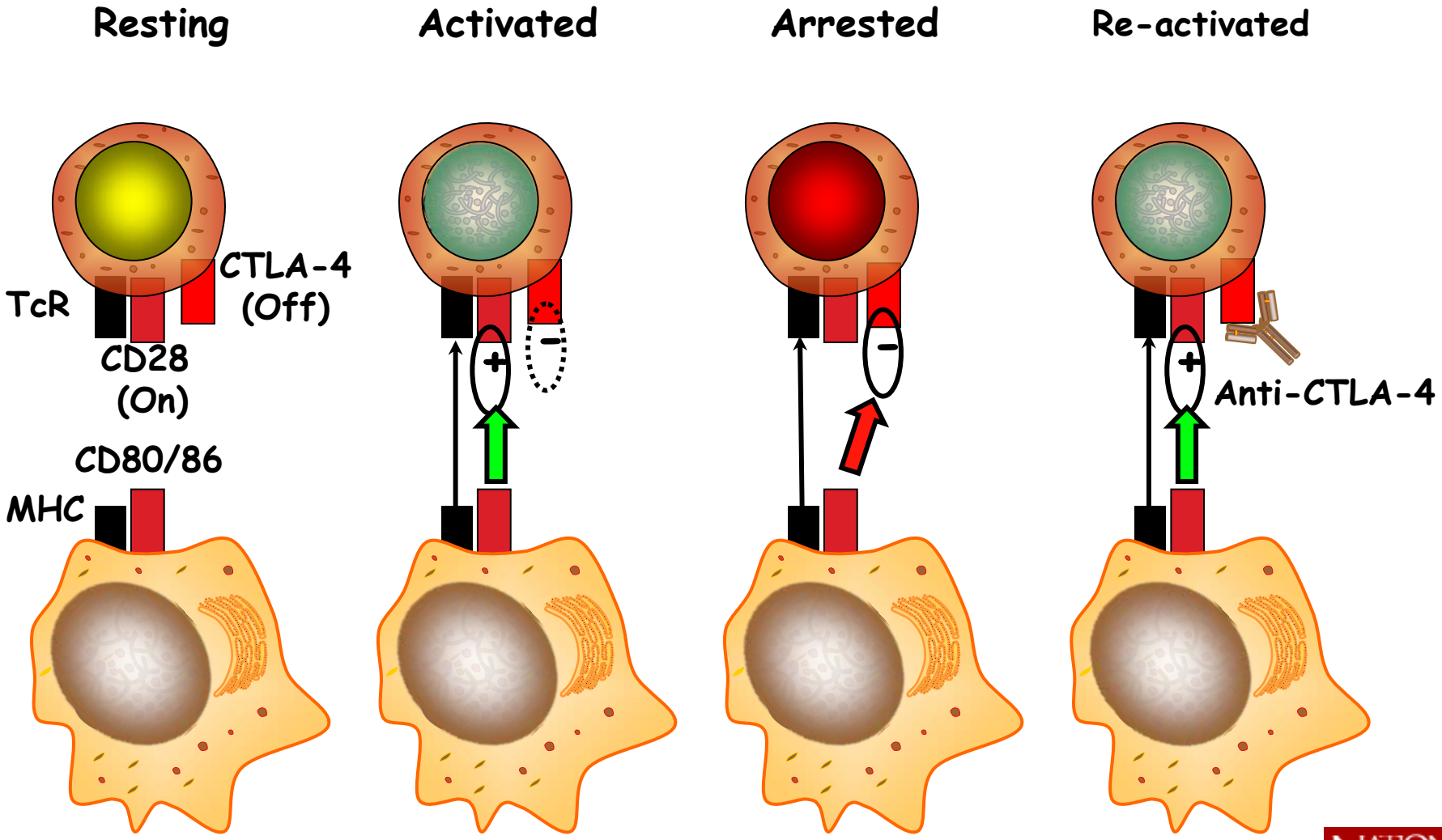
Anti-CTLA-4



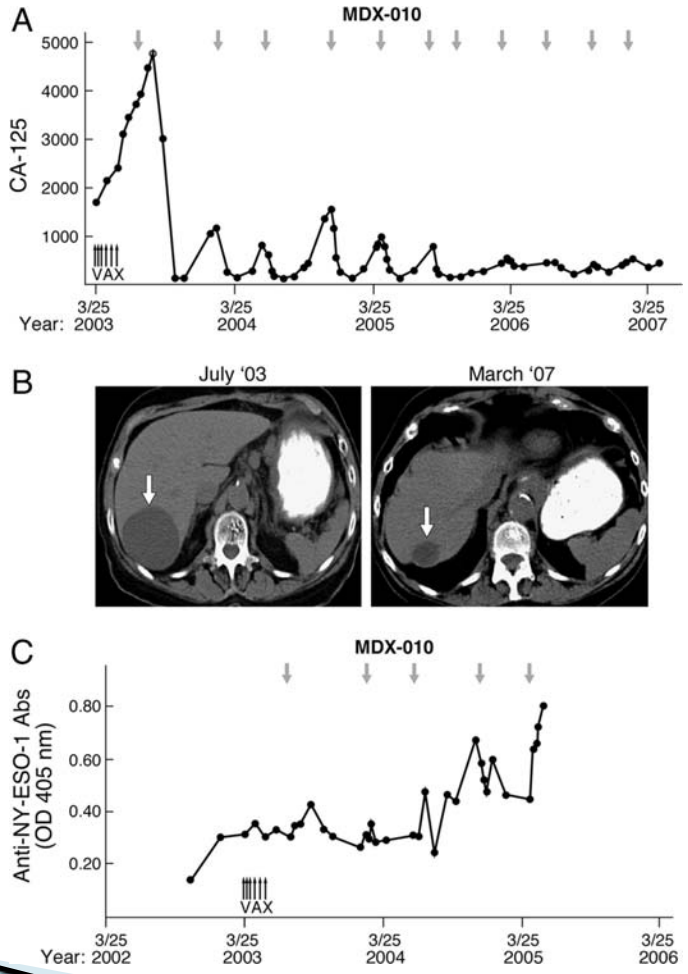
Anti-TGF-β

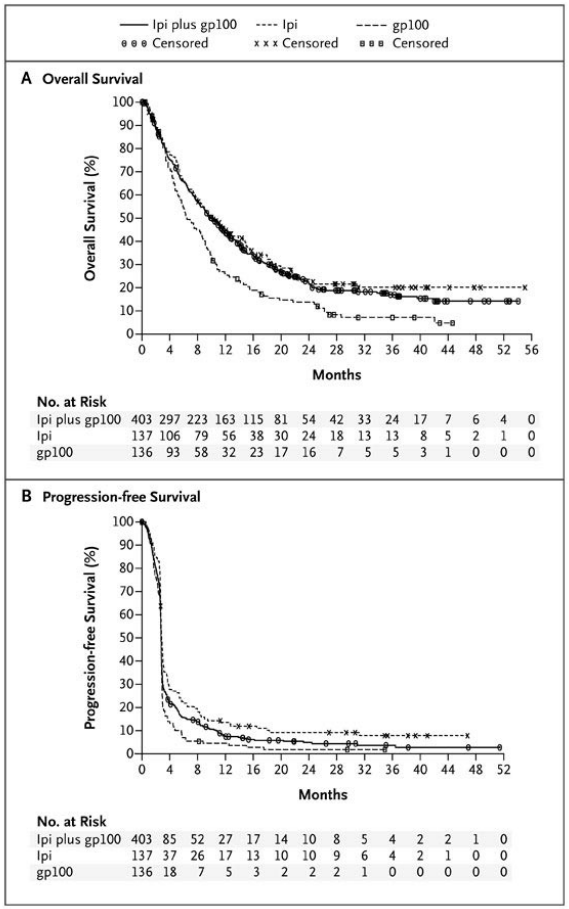


CTLA-4 turns off T-cells



Clinical effect of ipilumumab (anti-CTLA-4) on anti-tumor vaccination





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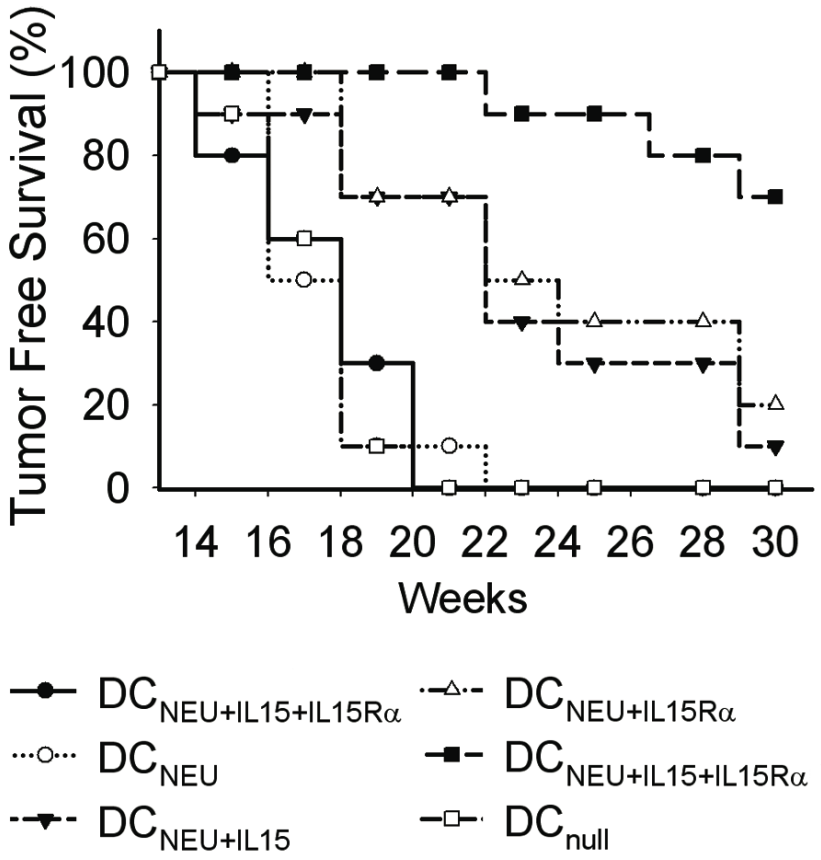
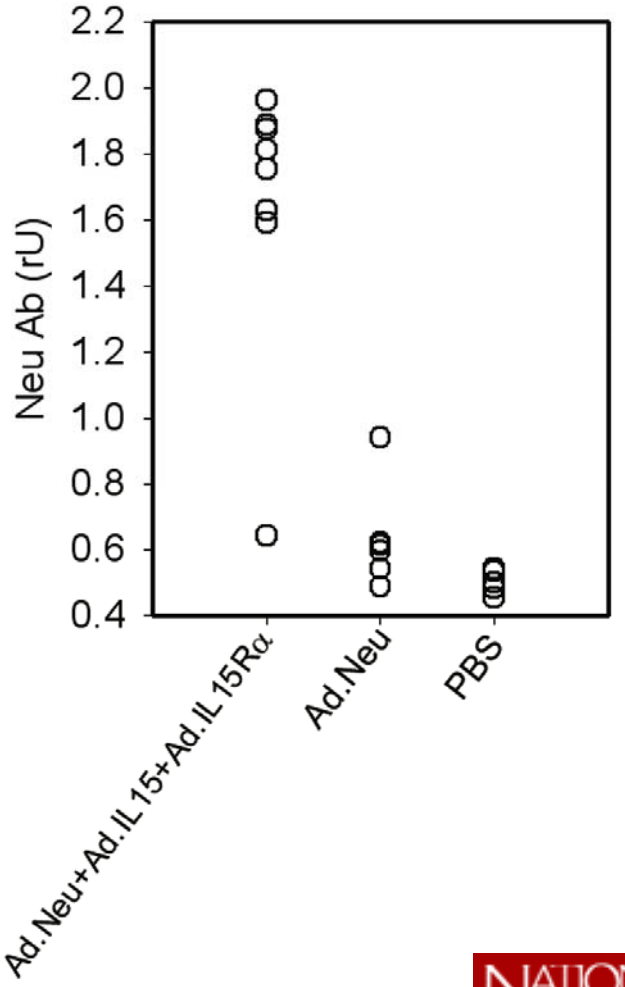
Effect of interleukin-15 on Neu (HER-2/neu) vaccine efficacy in breast cancer





Steel, JC, et al, Cancer Res. 2010

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So where do we go from here?

- It is doubtful that anti-cancer vaccines themselves can be greatly improved upon.

- The future focus will likely be on enhancing the vaccines' activity:
  - Improved administration schedules
  - Negative immune checkpoint blockade
  - Addition of immunostimulatory cytokines/chemokines.
  - Improved immunomonitoring
  - Patient selection.