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A National Cancer Institute Comprehensive Cancer Center

Clinical Translation of a Mammaglobin-A DNA Vaccine for Breast Cancer Therapy

William E. Gillanders, M.D.

Recombinant DNA Advisory Committee

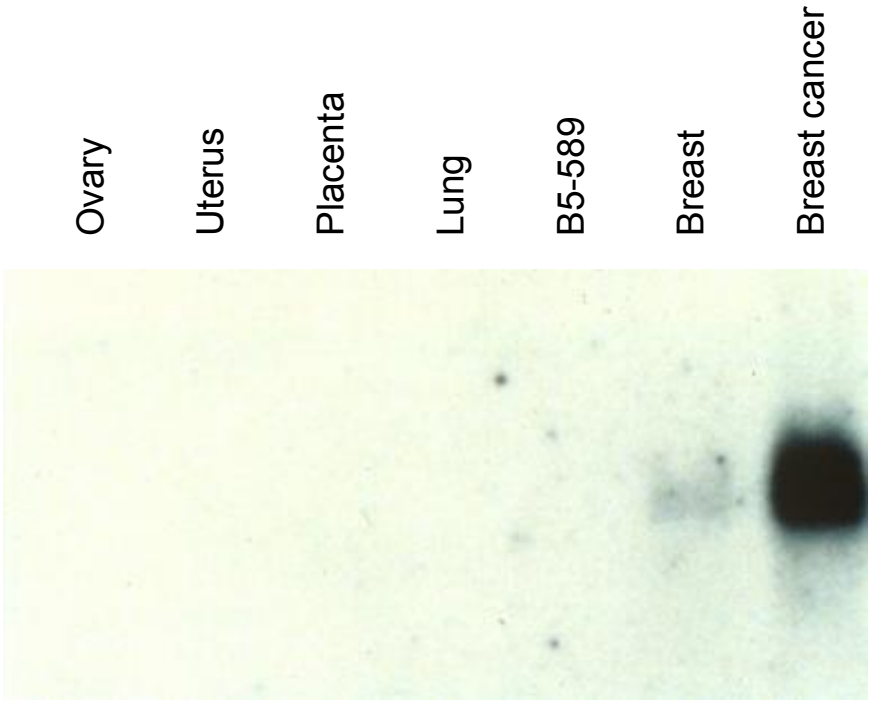
December 14, 2005

Overview

- This is a phase I dose-ranging vaccine safety trial of a mammaglobin-A DNA vaccine

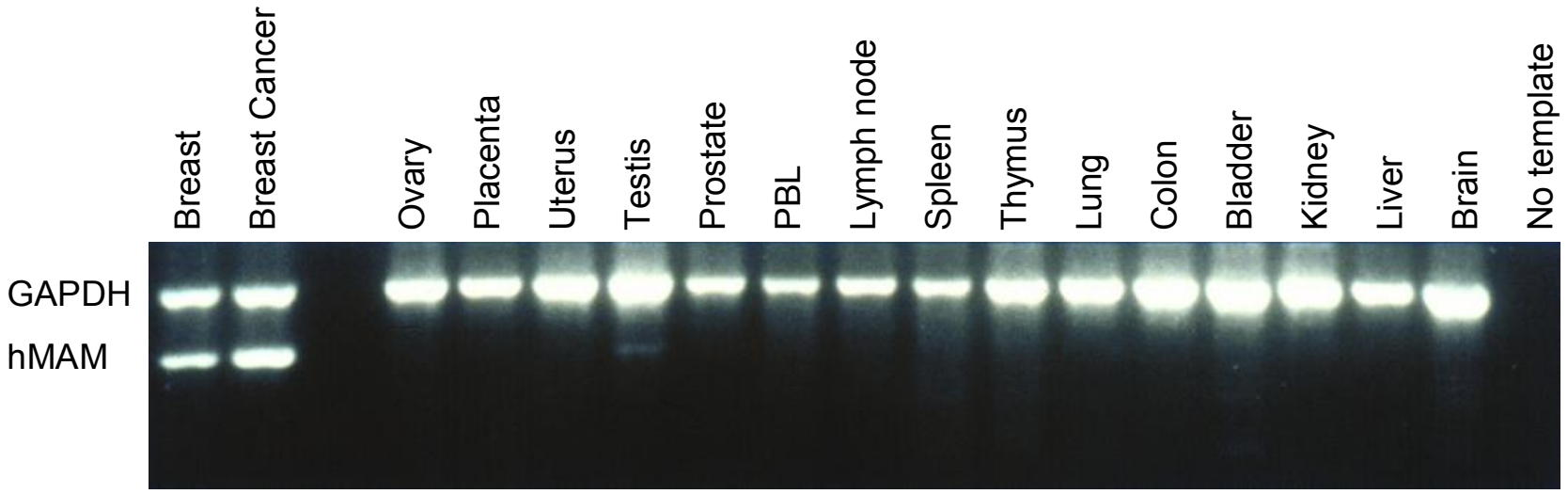
- The broad objective of the study is to identify a safe and immunologically active dose of the mammaglobin-A DNA vaccine that can be used in future studies

Mammaglobin-A Tissue Expression



Watson MA et al, ***Cancer Research*** 1996; 56:860

Mammaglobin-A Tissue Expression



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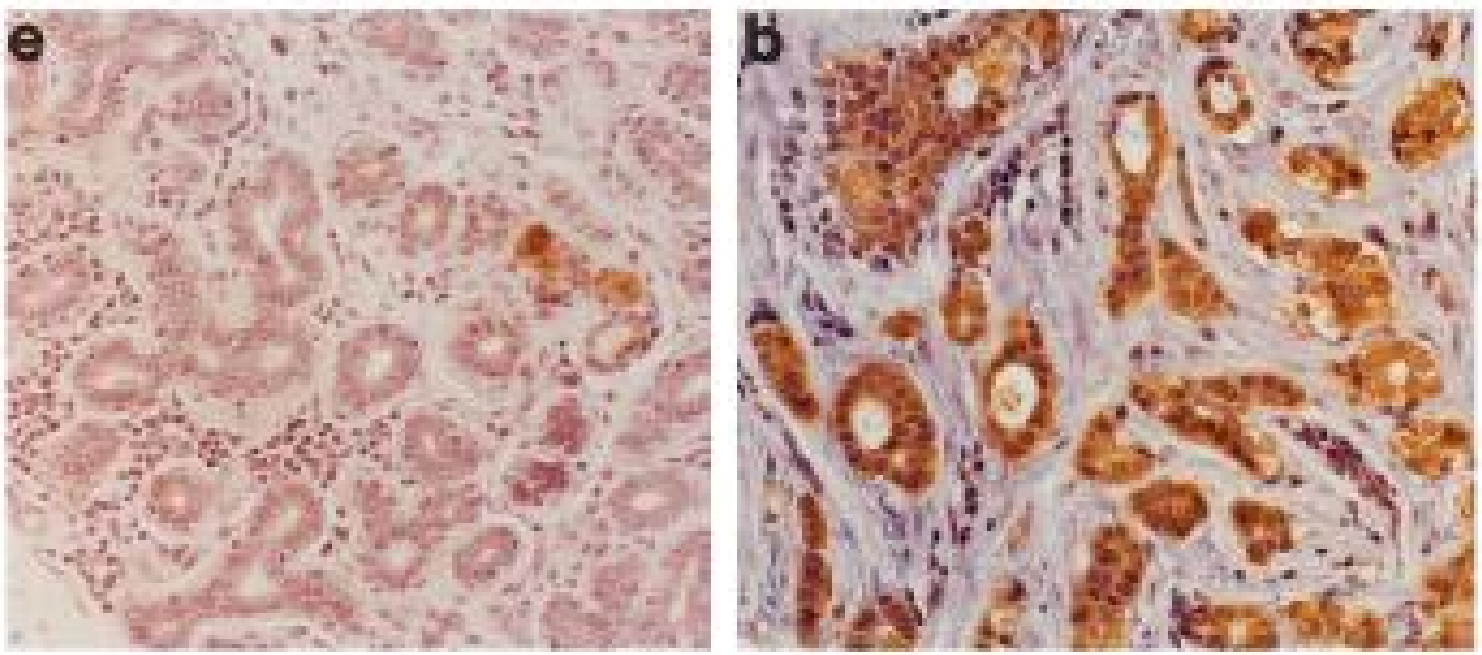

Watson MA et al, ***Cancer Research*** 1999; 59:3028

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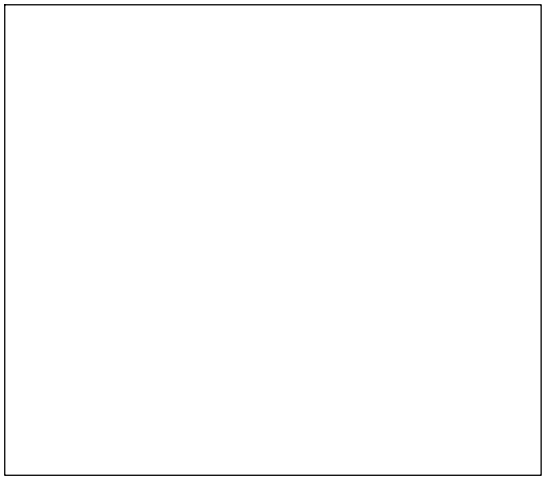
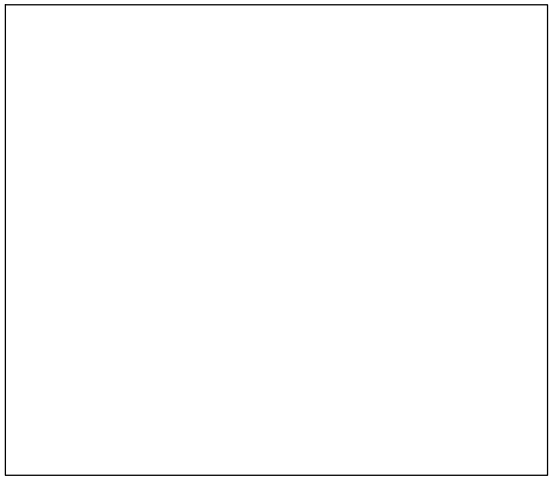
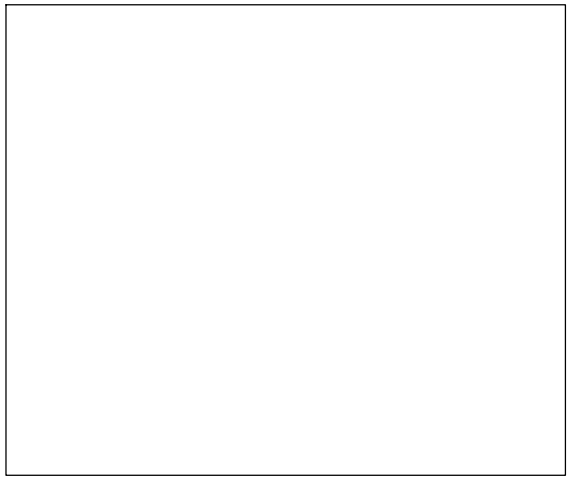


Ductal carcinoma in situ



Well differentiated ductal carcinoma



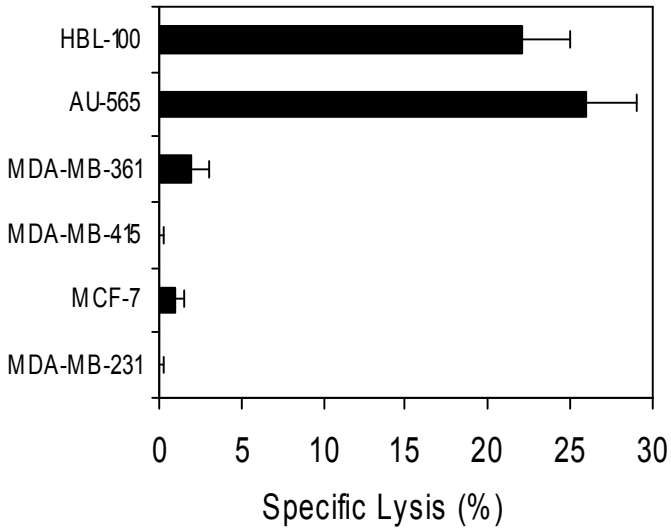
Poorly differentiated ductal carcinoma

Watson MA et al, ***Cancer Research*** 1999; 59:3028

Summary

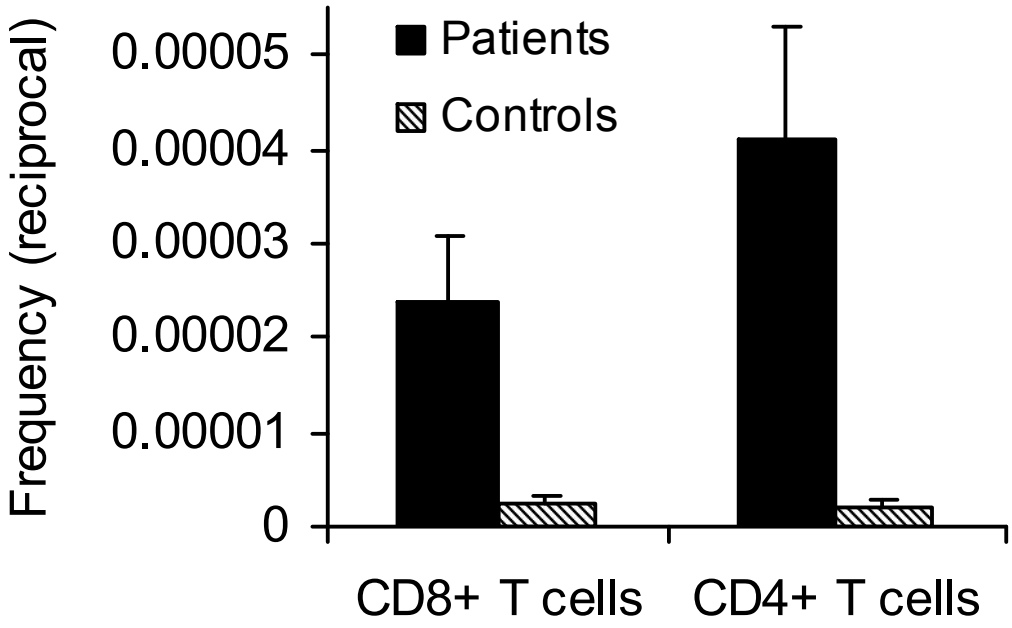
- Mammaglobin-A is expressed almost exclusively in normal breast epithelium and breast cancer
- Mammaglobin-A is overexpressed in up to 80% of primary and metastatic breast cancers
- Mammaglobin-A overexpression appears to be consistent in all stages of breast cancer

Presentation of Mammaglobin-A



Jaramillo A et al, ***International Journal of Cancer*** 2002; 102:499

Mammaglobin-A-reactive T cells



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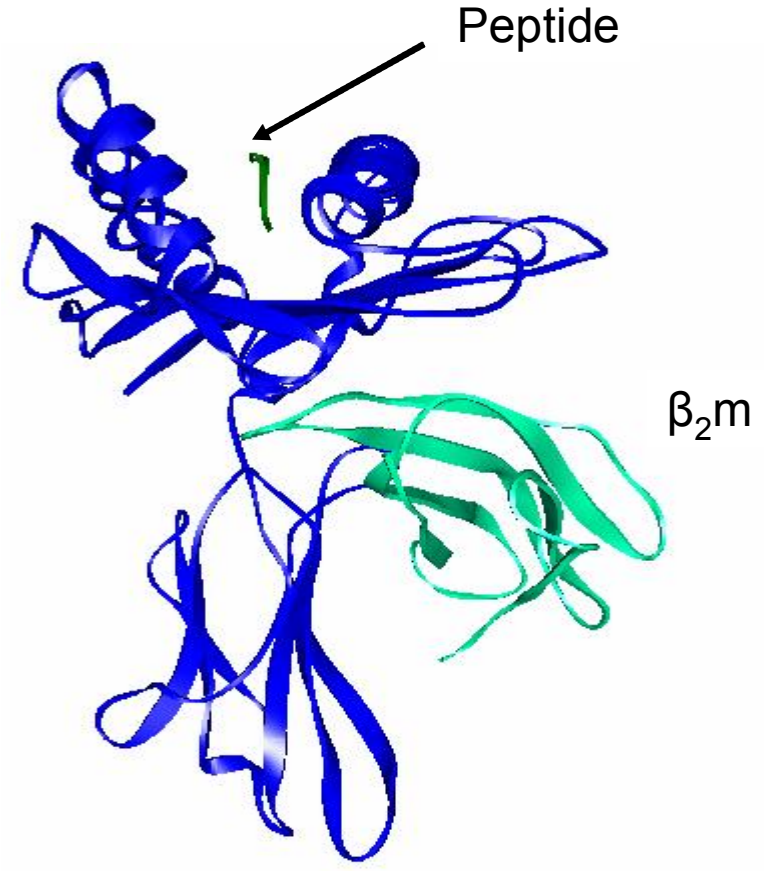
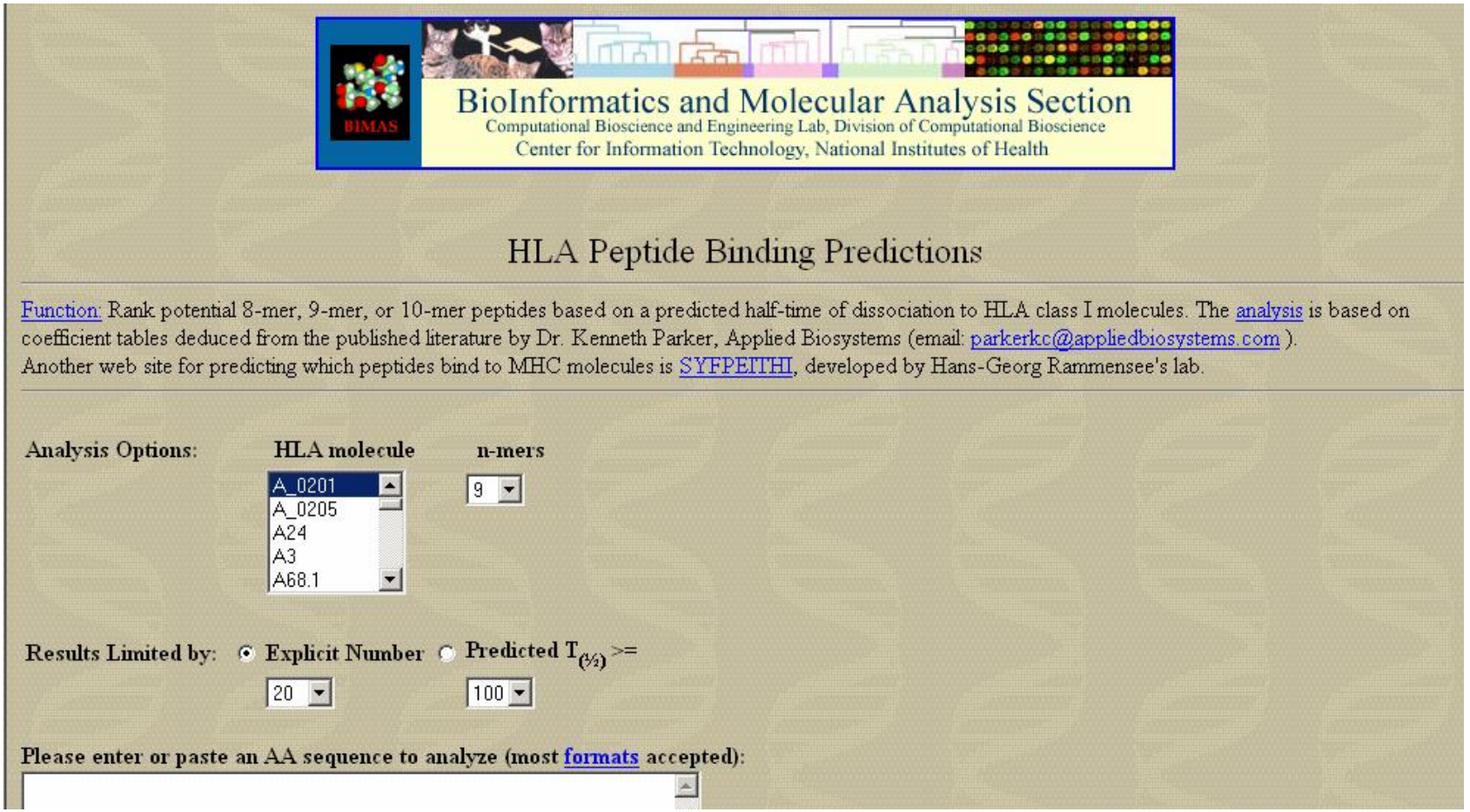
MHC Class I trimeric structure



Predicting mammaglobin-A epitopes



Mammaglobin HLA-A3 peptides

HLA-A3-Binding Peptides Derived from Mammaglobin-A

Peptide	Amino Acid Position	Peptide Sequence	HLA-A3-binding score
Mam-A3.1	23-31	PLLENVISK	27.00
Mam-A3.2	31-39	KTINPQVSK	6.75
Mam-A3.3	02-10	KLLMVLMLA	4.05
Mam-A3.4	55-63	TTNAIDELK	1.50
Mam-A3.5	04-12	LMVLMLAAL	1.35
Mam-A3.6	66-74	FLNQTDETL	0.60
Mam-A3.7	07-15	LMLAALSQH	0.45
Mam-A3.8	58-66	AIDELKECF	0.30

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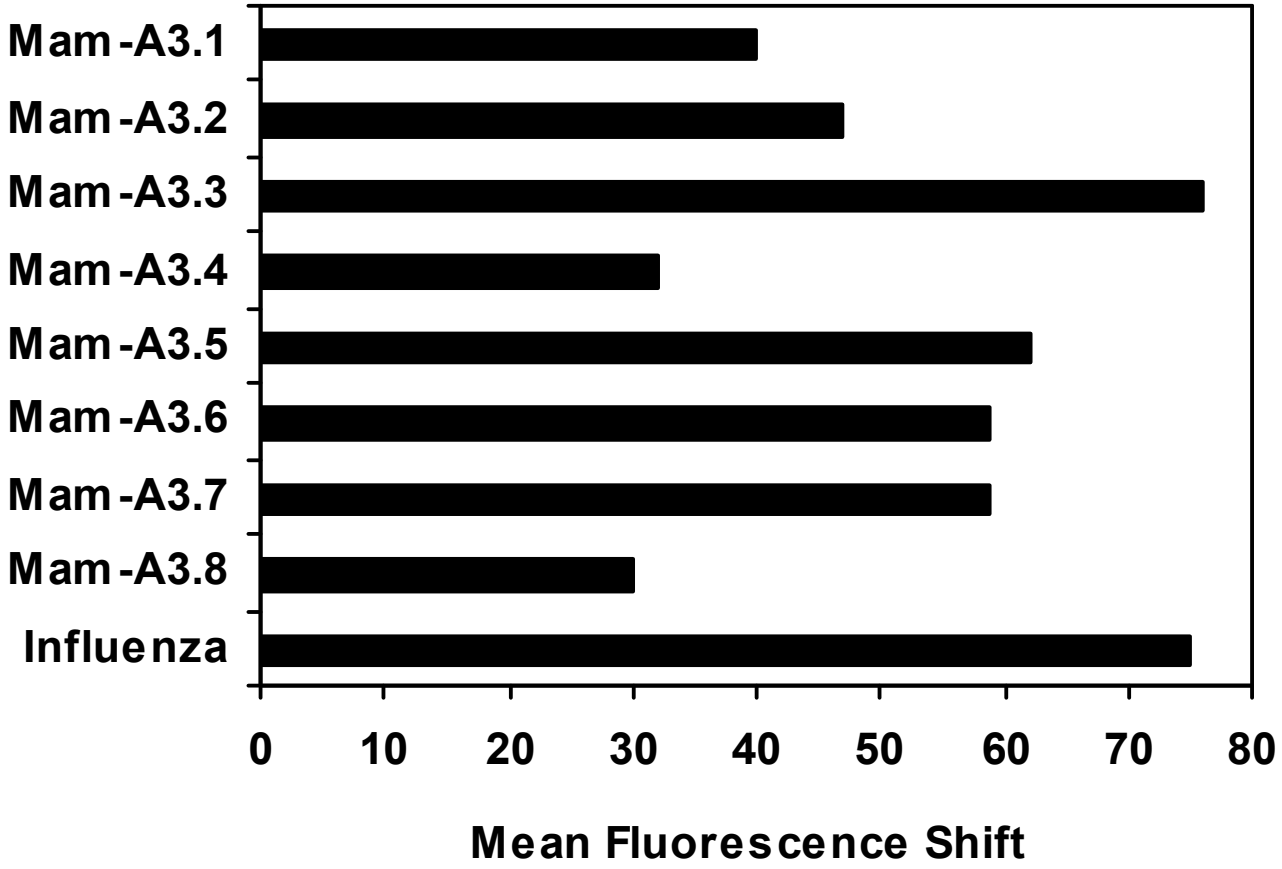
Membrane Stabilization Assay



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Mammaglobin-A-reactive T cells

Frequency of CD8 T cells reactive to mammaglobin-A-derived peptides in the peripheral blood of HLA-A3 breast cancer patients

	HLA-A3 peptides								
Patients	A3.1	A3.2	A3.3	A3.4	A3.5	A3.6	A3.7	A3.8	Influenza
1	33	0	0	156	0	0	0	0	127
2	0	0	40	33	0	0	0	23	93
3	0	0	829	183	0	0	7	80	123
4	20	0	57	0	0	3	0	0	103
5	23	0	0	20	0	0	0	0	89
Controls									
1	0	0	3	0	0	0	0	3	83
2	0	0	0	0	0	0	0	0	96
3	0	0	7	3	0	7	0	0	109
4	3	0	0	0	0	0	0	0	76

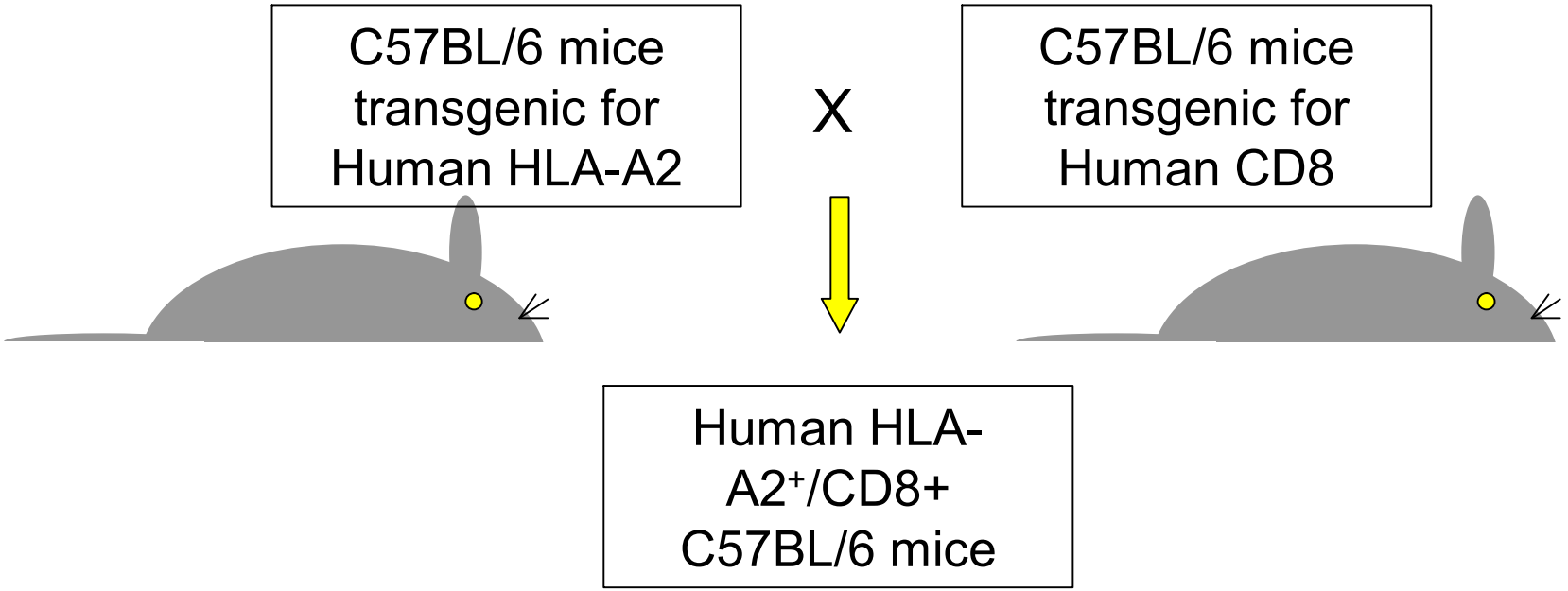
Summary

- CD8 and CD4 T cells specific for mammaglobin-A can be generated *in vitro* from the peripheral blood of breast cancer patients confirming that the immune system can recognize this antigen
- Analyses of peripheral blood from breast cancer patients confirm that breast cancer patients have higher frequencies of mammaglobin-A-reactive T cells

Advantages of DNA Vaccination

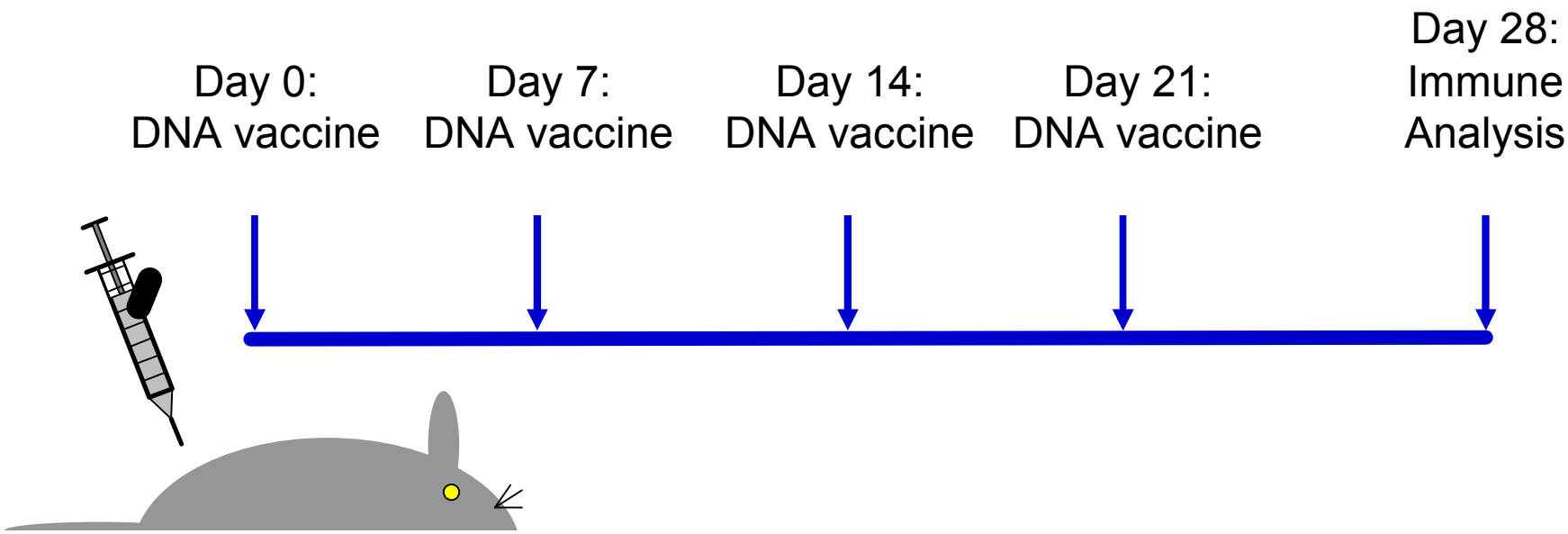
- Safety
- Generic manufacture with high purity and stability relative to protein vaccines
- Cost advantage
- Vaccination with full-length cDNA avoids the requirement of MHC restriction

Humanized Mouse Model



- Expression of HLA-A2 on all tissues
- Tissue-specific expression of human CD8 on $CD8^+$ T cells
- Facilitates recognition of human MHC by mouse $CD8^+$ T cells

Humanized Mouse Model



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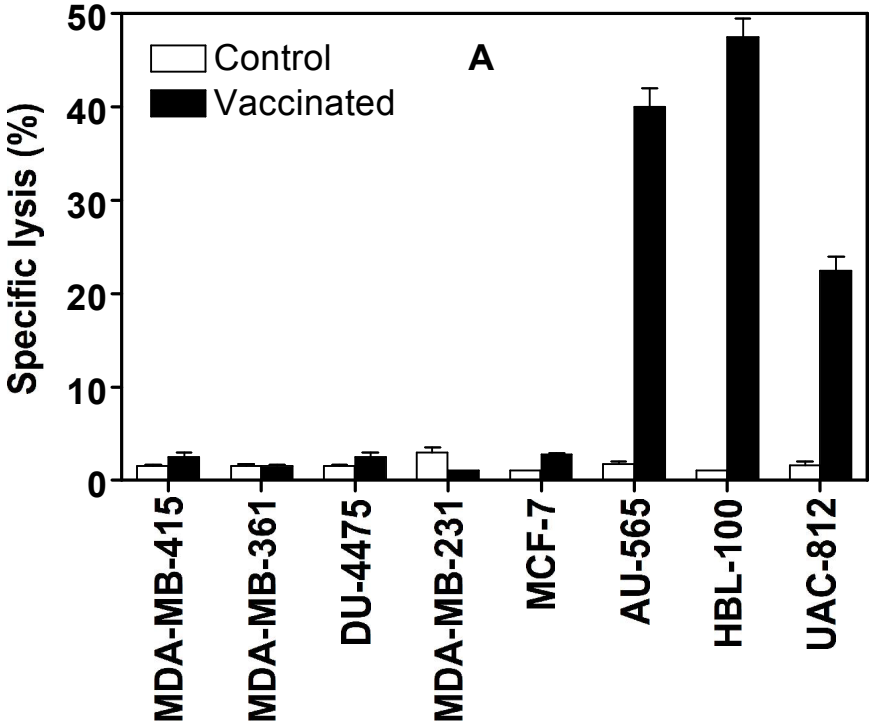


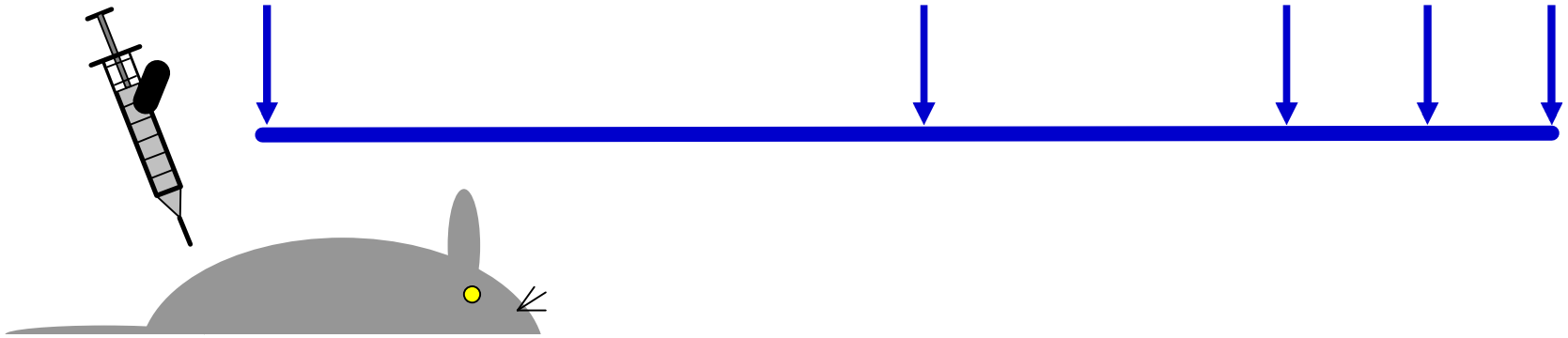
Narayanan et al, ***Journal of the National Cancer Institute*** 2004; 96:1388

Breast Cancer Xenograft Model

Day 0: Tumor Challenge HBL-100 or MDA-231 cells resuspended in basement membrane

Day 14: Adoptive transfer of 4 x 10^7 spleen cells from vaccinated mice

Tumor size measured by calipers weekly



Breast Cancer Xenograft Model

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Phase I Clinical Trial

- This is a phase I dose-ranging vaccine safety trial of a mammaglobin-A DNA vaccine

- The broad objective of the study is to identify a safe and immunologically active dose of the mammaglobin-A DNA vaccine that can be used in future studies

Objectives

- Evaluate the safety of the mammaglobin-A DNA vaccine

- Assess the *in vivo* immune response induced by the mammaglobin-A DNA vaccine by evaluation of the CD8, CD4 and Treg immune responses

Objectives

- Assess the impact of mammaglobin-A DNA vaccination on breast cancer tumor markers, including circulating breast cancer cells

- Evaluate enrolled patients for time to disease progression following vaccination with the mammaglobin-A DNA vaccine

Patient Selection

- Patients with stage IV breast cancer are eligible for enrollment

- Eligible patients will have metastatic breast cancer that has been stable for at least 28 days after chemotherapy, or on hormonal therapy

Dose Escalation

- This trial is a dose-ranging study of four doses of the mammaglobin-A DNA vaccine

- Four groups of at least three patients will be vaccinated with mammaglobin-A DNA delivered intramuscularly at four different dose levels (150 µg, 500 µg, 1500 µg, 5 mg) every three weeks for four injections

Dose Escalation

- Dose escalation will only occur when the final patient at the prior dose level has safely completed all four injections and no dose-limiting toxicity (DLT) has been noted in more than one patient at the final post-vaccination visit

Immune Monitoring

- ELISPOT assays, intracellular cytokine expression analyses using multi-parameter flow cytometry, and peptide MHC tetramer analyses will be used to assess the antigen-specific T-cell response to the mammaglobin-A DNA vaccine

Anticipated Toxicity

- Based on experience with DNA vaccines in phase I clinical trials we expect toxicity to be limited to grade 1 vaccine site reactions

- Other reactions that have been described include hyperglycemia, hypoalbuminemia, myalgia, chills, allergic rhinitis, cough, headache and pruritis

Safety

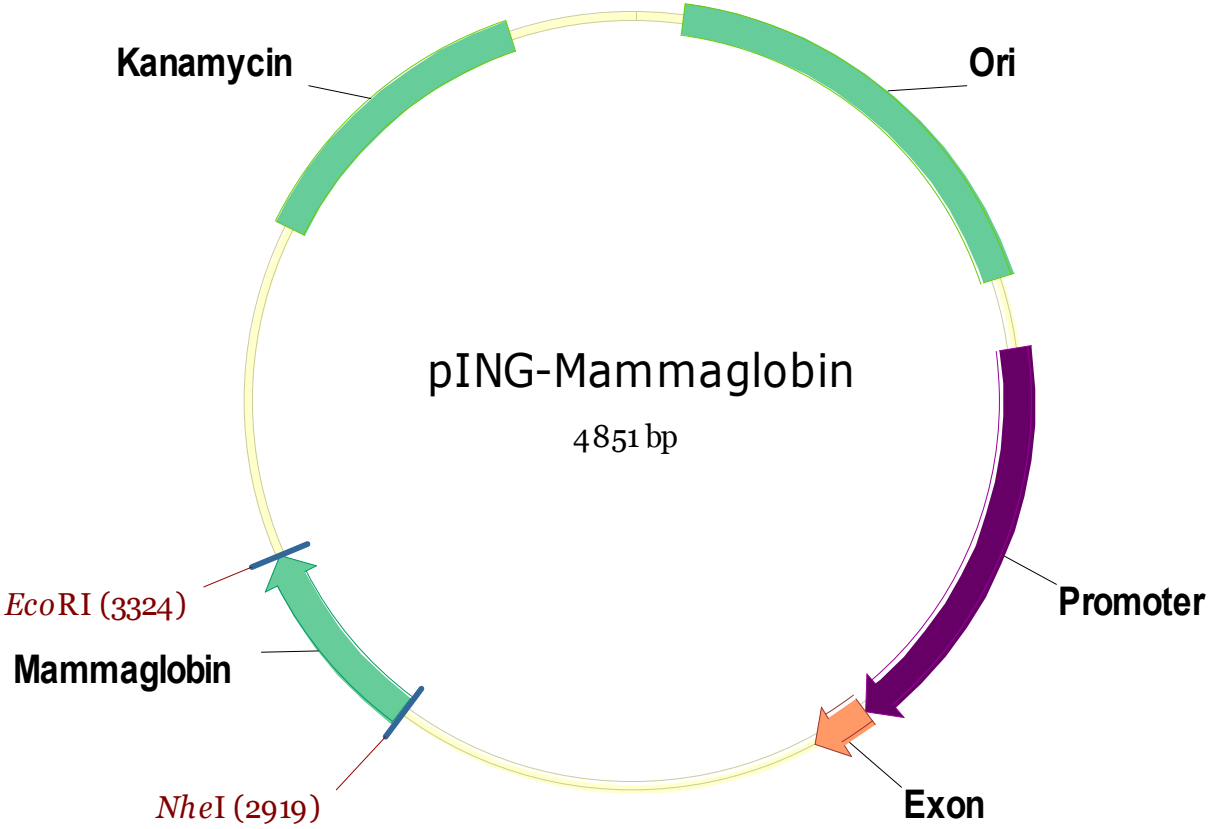
- Safety defined as the absence of sever toxicity (grade 3 or greater) using the National Institutes of Health Common Toxicity Criteria

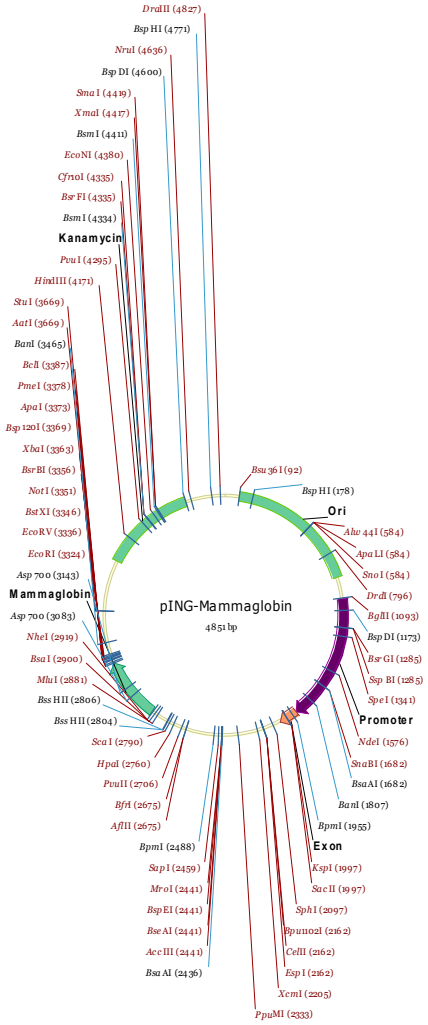
Optimal Dose

- The dose of mammaglobin-A DNA vaccine that is associated with the maximum immune response will be considered optimal

- If there is no clear difference in the immune response to two or more doses of mammaglobin-A DNA vaccine, the lowest dose of mammaglobin-A DNA vaccine that is associated with the maximum immune response will be considered optimal

pING-mammaglobin-A vector





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