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BARNES-JEWISH HOSPITAL • WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

A National Cancer Institute Comprehensive Cancer Center

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

William E. Gillanders, M.D.

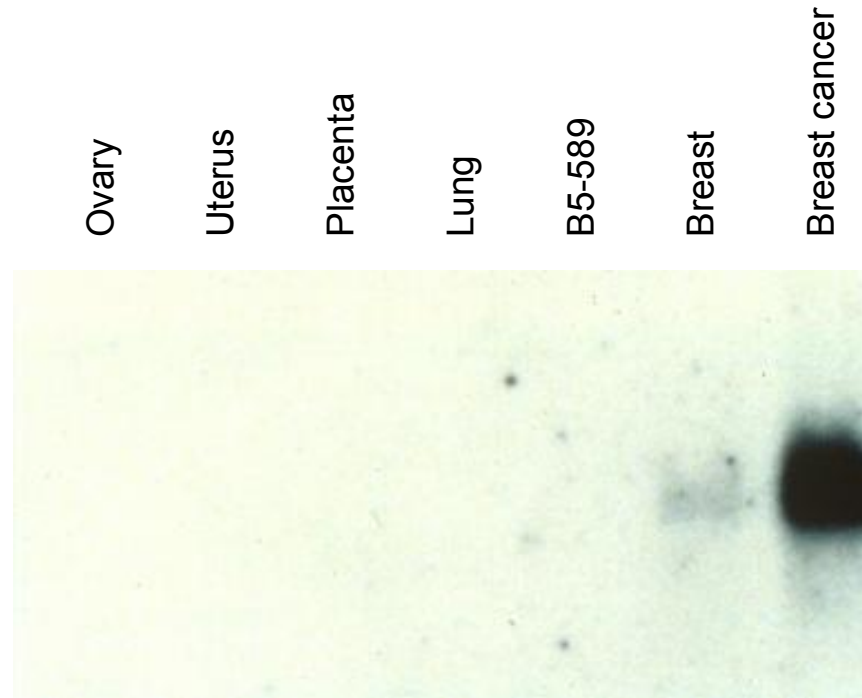
Recombinant DNA Advisory Committee

December 14, 2005

Overview

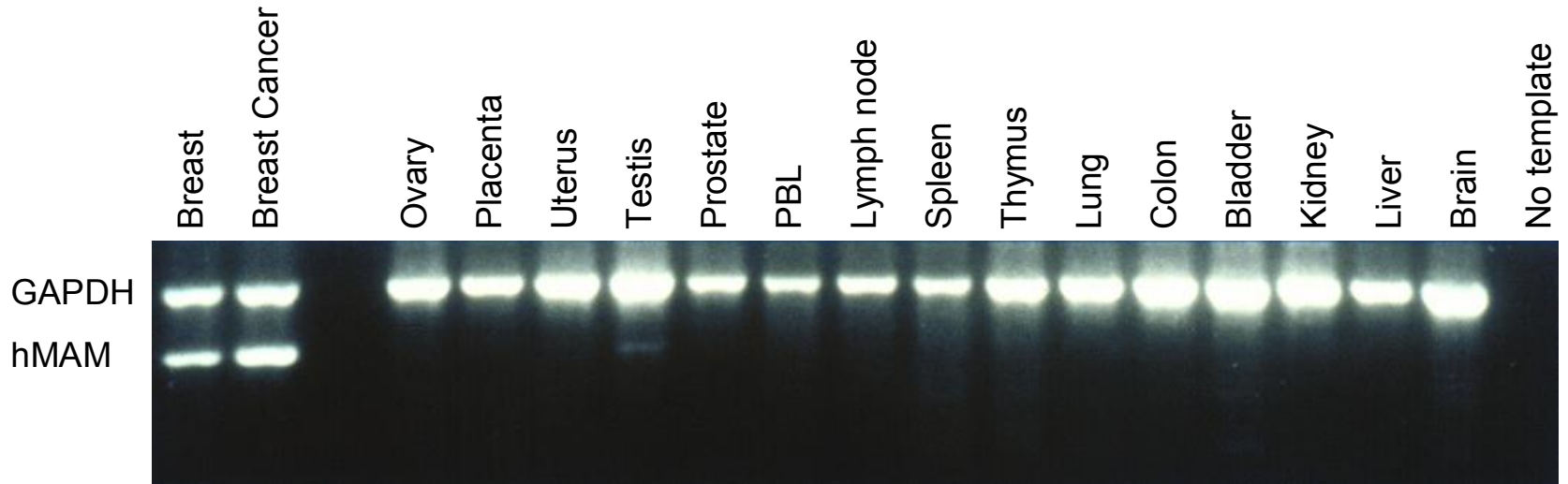
- This is a phase I dose-ranging vaccine safety trial of a mammaglobin-A DNA vaccine
- The broad objective of the study is to identify a safe and immunologically active dose of the mammaglobin-A DNA vaccine that can be used in future studies

Mammaglobin-A Tissue Expression



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

Mammaglobin-A Tissue Expression



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

Mammaglobin-A Tissue Expression

The screenshot displays the PubMed search interface. At the top, the NCBI logo is on the left, and the National Library of Medicine (NLM) logo is on the right. The search bar contains the text 'mammaglobin' and includes buttons for 'Go', 'Clear', and 'Save Search'. Below the search bar, there are tabs for 'Limits', 'Preview/Index', 'History', 'Clipboard', and 'Details'. The 'Display' section shows 'Summary' selected, with 'Show 20' and 'Sort by' options. The results section indicates 'All: 108' items and 'Review: 6'. The first four results are listed, each with a checkbox, a citation link, and a brief description of the article.

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Items 1 - 20 of 108 Page 1 of 6 Next

1: [De Longueville F, Lacroix M, Barbuto AM, Bertholet V, Gallo D, Larsimont D, Marcq L, Zammatteo N, Boffe S, Leclercq G, Remacle J.](#) Related Articles, Links
Molecular characterization of breast cancer cell lines by a low-density microarray.
Int J Oncol. 2005 Oct;27(4):881-92.
PMID: 16142302 [PubMed - in process]

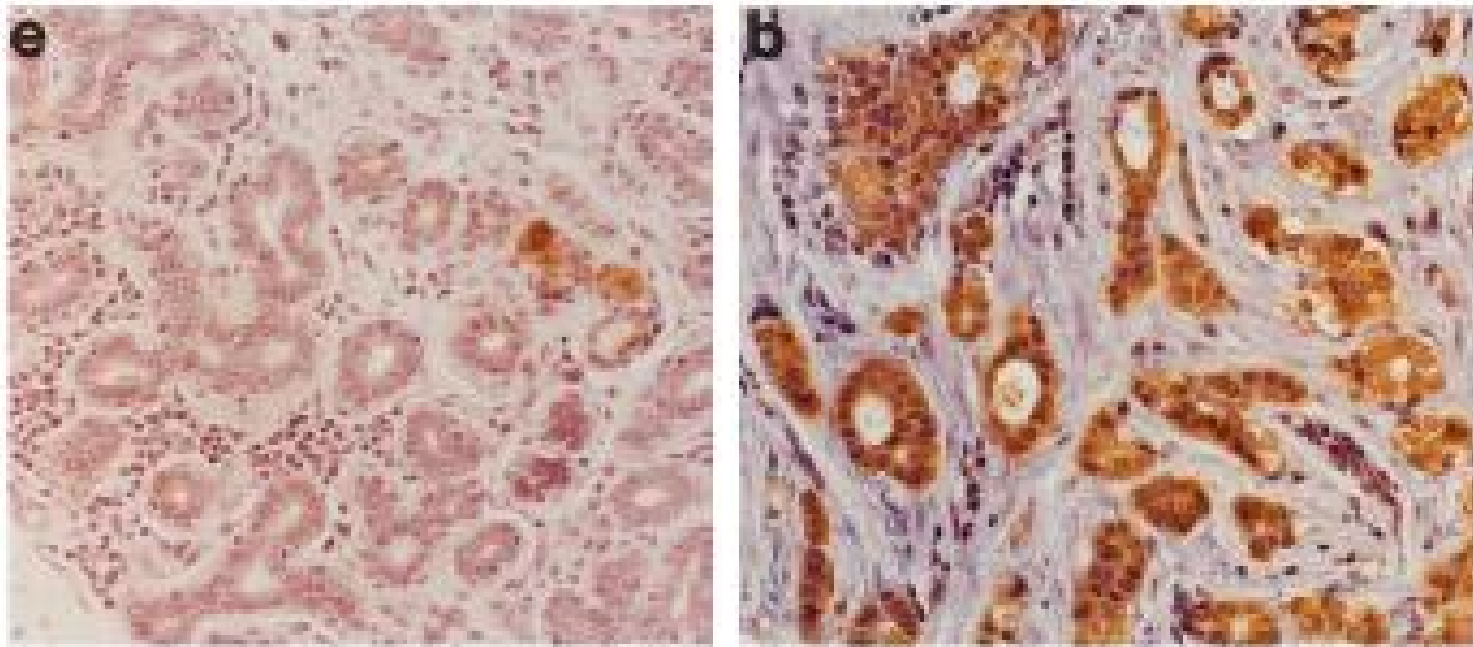
2: [Roncella S, Ferro P, Bacigalupo B, Pronzato P, Tognoni A, Falco E, Gianquinto D, Ansaldo V, Dessanti P, Fais F, Rosai J, Fedeli F.](#) Related Articles, Links
Human mammaglobin mRNA is a reliable molecular marker for detecting occult breast cancer cells in peripheral blood.
J Exp Clin Cancer Res. 2005 Jun;24(2):265-71.
PMID: 16110760 [PubMed - in process]

3: [Sadeghi H, Hitt MM.](#) Related Articles, Links
Transcriptionally targeted adenovirus vectors.
Curr Gene Ther. 2005 Aug;5(4):411-27. Review.
PMID: 16101515 [PubMed - indexed for MEDLINE]

4: [Backus J, Laughlin T, Wang Y, Belly R, White R, Baden J, Justus Min C, Mannie A, Tafra L, Atkins D, Verbanac KM.](#) Related Articles, Links
Identification and characterization of optimal gene expression markers for detection of breast cancer metastasis.
J Mol Diagn. 2005 Aug;7(3):327-36.

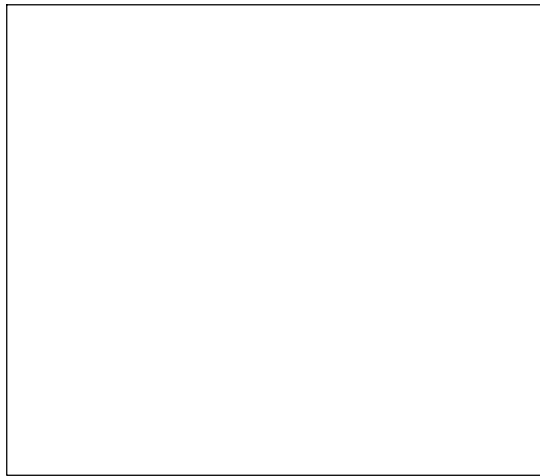
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Mammaglobin-A Tissue Expression

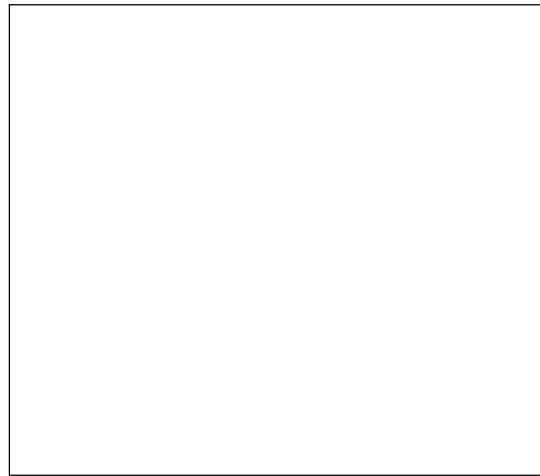


Watson MA et al, *Cancer Research* 1999; 59:3028

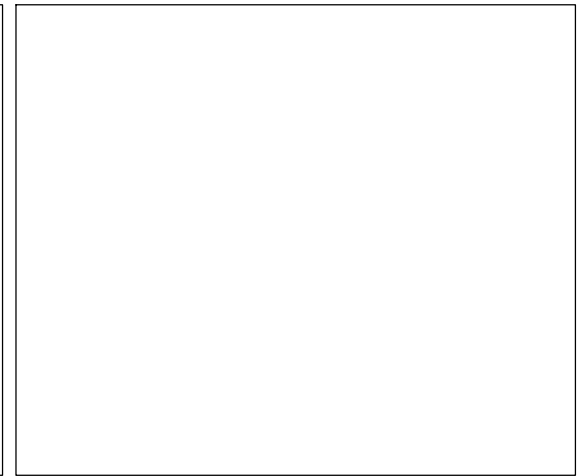
Mammaglobin-A Tissue Expression



Ductal carcinoma in situ



Well differentiated ductal carcinoma



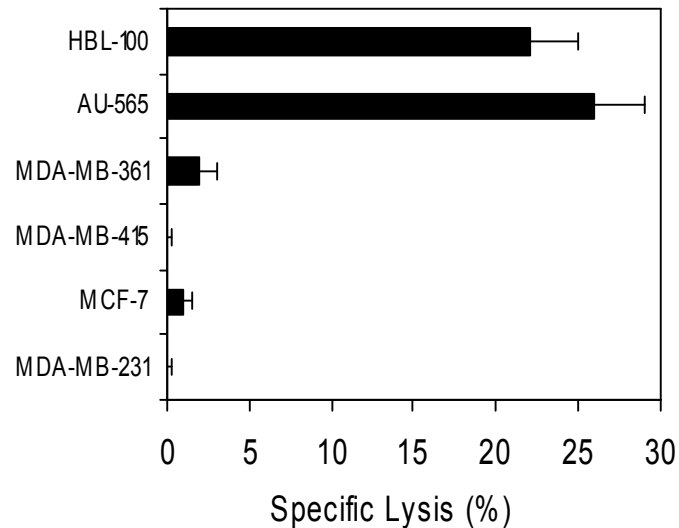
Poorly differentiated ductal carcinoma

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

Summary

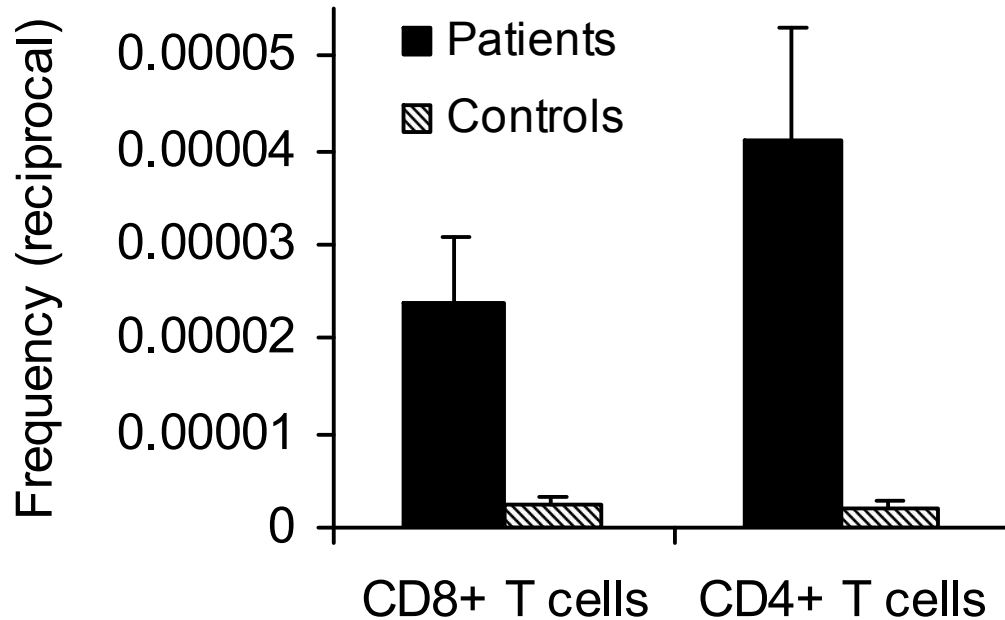
- Mammaglobin-A is expressed almost exclusively in normal breast epithelium and breast cancer
- Mammaglobin-A is overexpressed in up to 80% of primary and metastatic breast cancers
- Mammaglobin-A overexpression appears to be consistent in all stages of breast cancer

Presentation of Mammaglobin-A



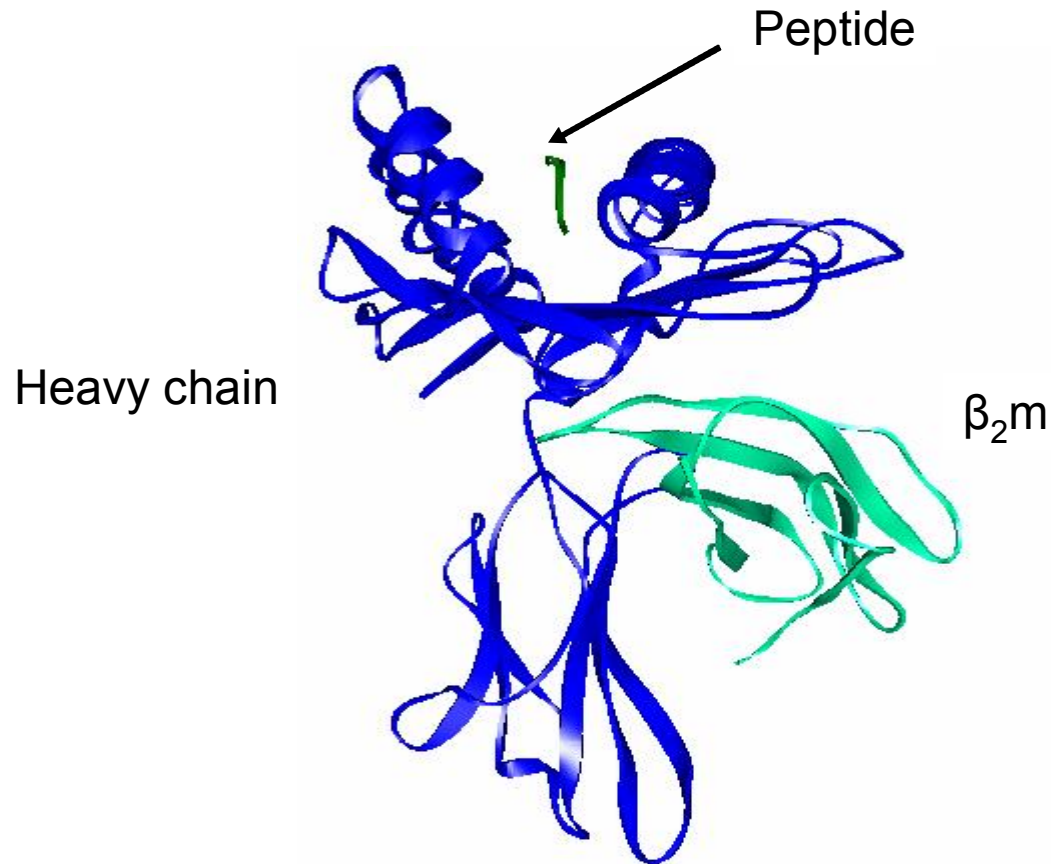
Jaramillo A et al, *International Journal of Cancer*
2002; 102:499

Mammaglobin-A-reactive T cells

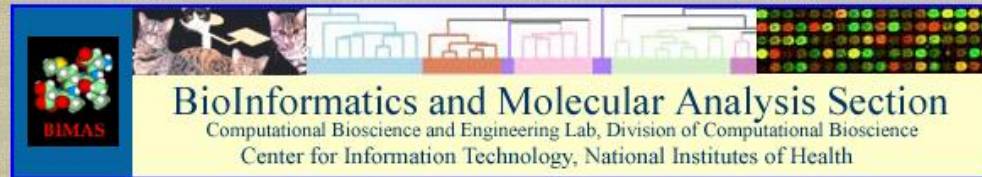


Jaramillo A et al, *International Journal of Cancer* 2002; 102:499

MHC Class I trimeric structure



Predicting mammaglobin-A epitopes



HLA Peptide Binding Predictions

Function: Rank potential 8-mer, 9-mer, or 10-mer peptides based on a predicted half-time of dissociation to HLA class I molecules. The [analysis](#) is based on coefficient tables deduced from the published literature by Dr. Kenneth Parker, Applied Biosystems (email: parkerkc@appliedbiosystems.com). Another web site for predicting which peptides bind to MHC molecules is [SYFPEITHI](#), developed by Hans-Georg Rammensee's lab.

Analysis Options:

HLA molecule	n-mers
A_0201	9
A_0205	
A24	
A3	
A68.1	

Results Limited by: Explicit Number Predicted $T_{(1/2)} \geq$

Please enter or paste an AA sequence to analyze (most [formats](#) accepted):

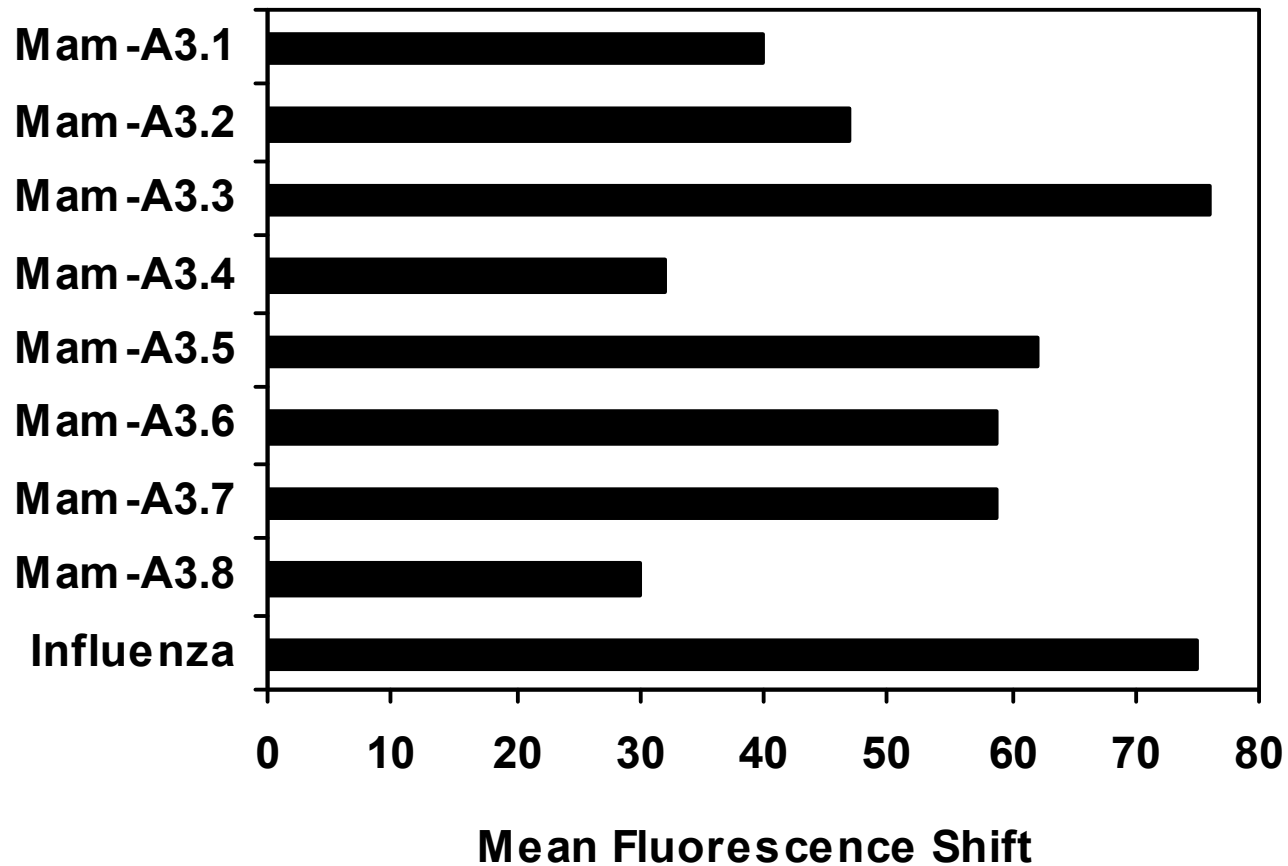
Mammaglobin HLA-A3 peptides

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

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

Membrane Stabilization Assay



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

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

Patients	HLA-A3 peptides								Influenza
	A3.1	A3.2	A3.3	A3.4	A3.5	A3.6	A3.7	A3.8	
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

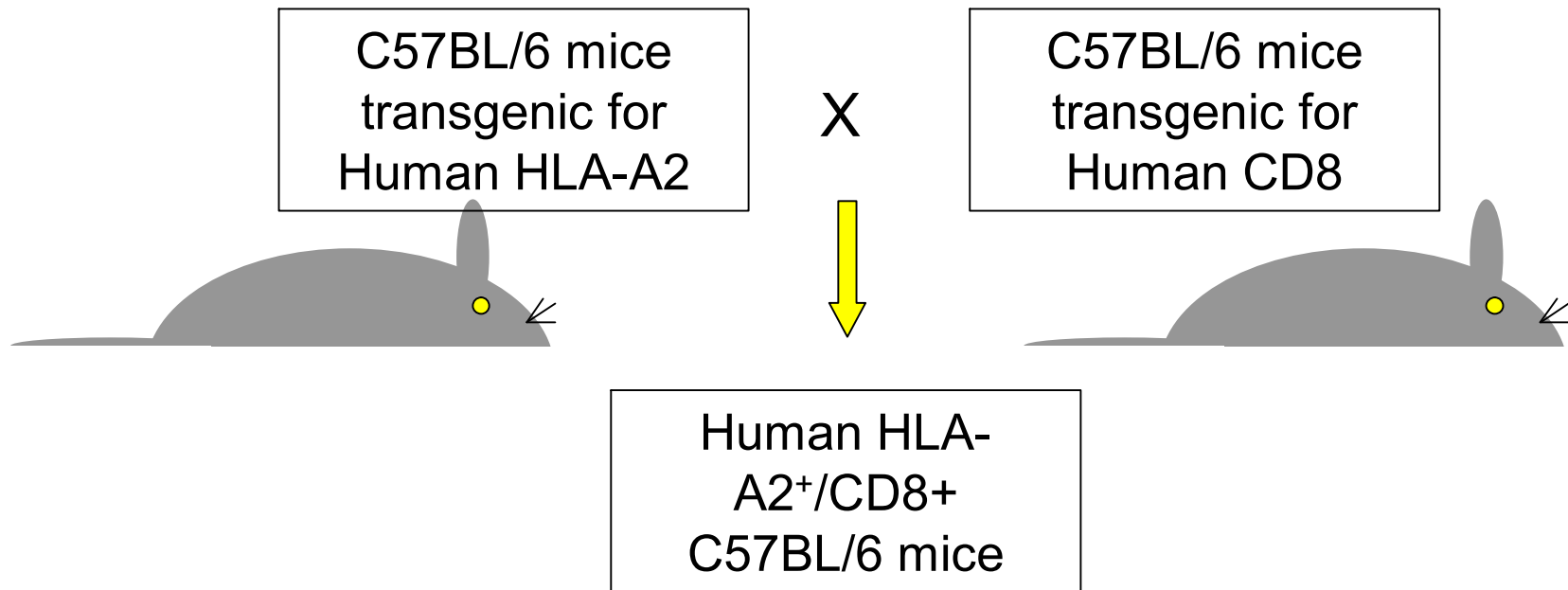
Summary

- 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

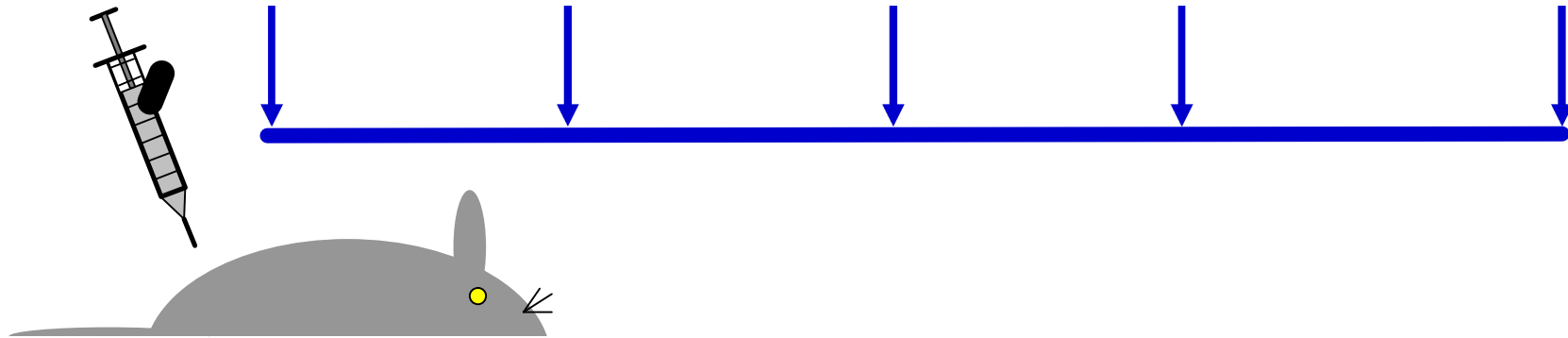
Humanized Mouse Model



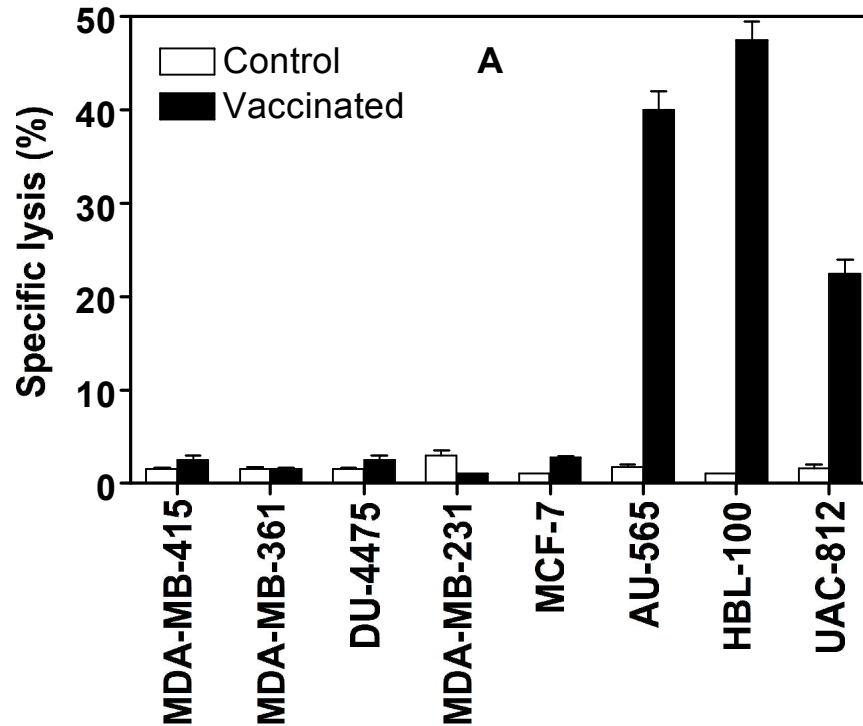
- Expression of HLA-A2 on all tissues
- Tissue-specific expression of human CD8 on CD8⁺ T cells
- Facilitates recognition of human MHC by mouse CD8⁺ T cells

Humanized Mouse Model

Day 0: DNA vaccine Day 7: DNA vaccine Day 14: DNA vaccine Day 21: DNA vaccine Day 28: Immune Analysis



Humanized Mouse Model



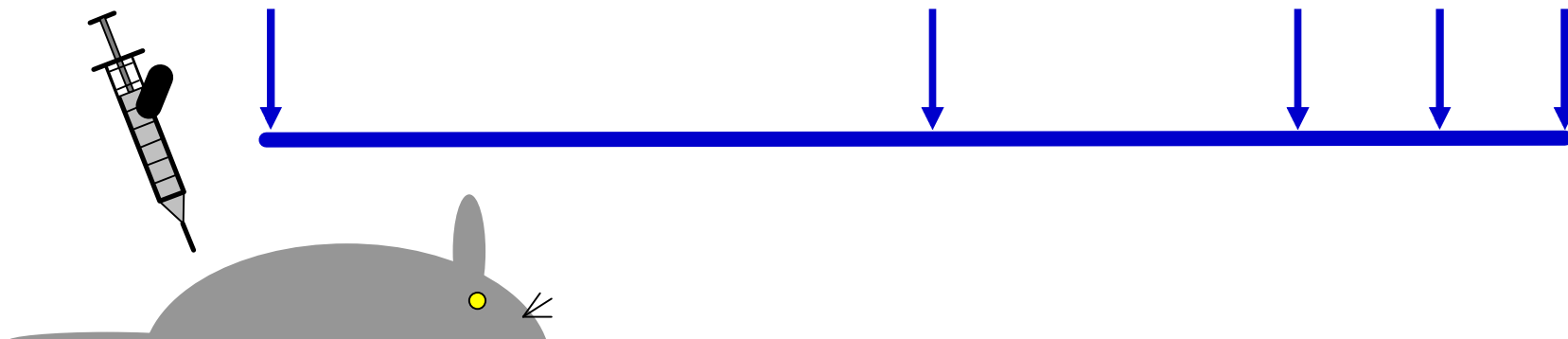
Narayanan et al, *Journal of the National Cancer Institute*
2004; 96:1388

Breast Cancer Xenograft Model

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

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

Tumor size
measured by
calipers weekly



Breast Cancer Xenograft Model

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

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

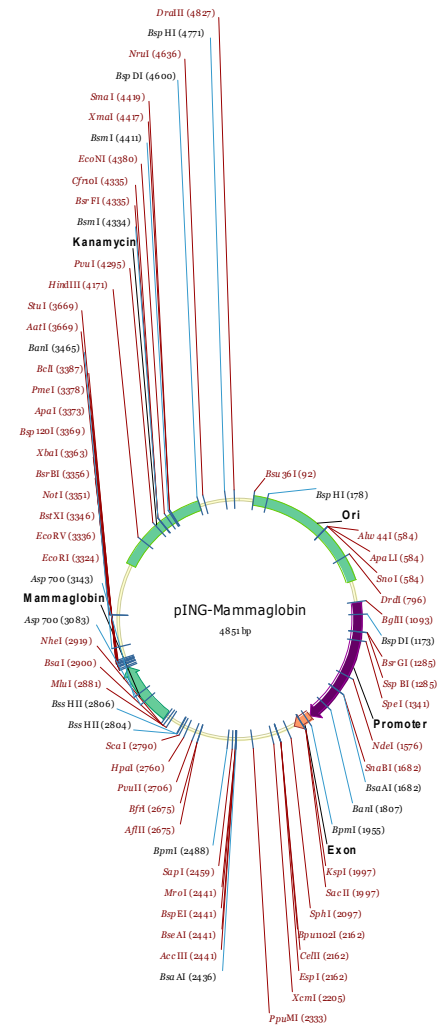
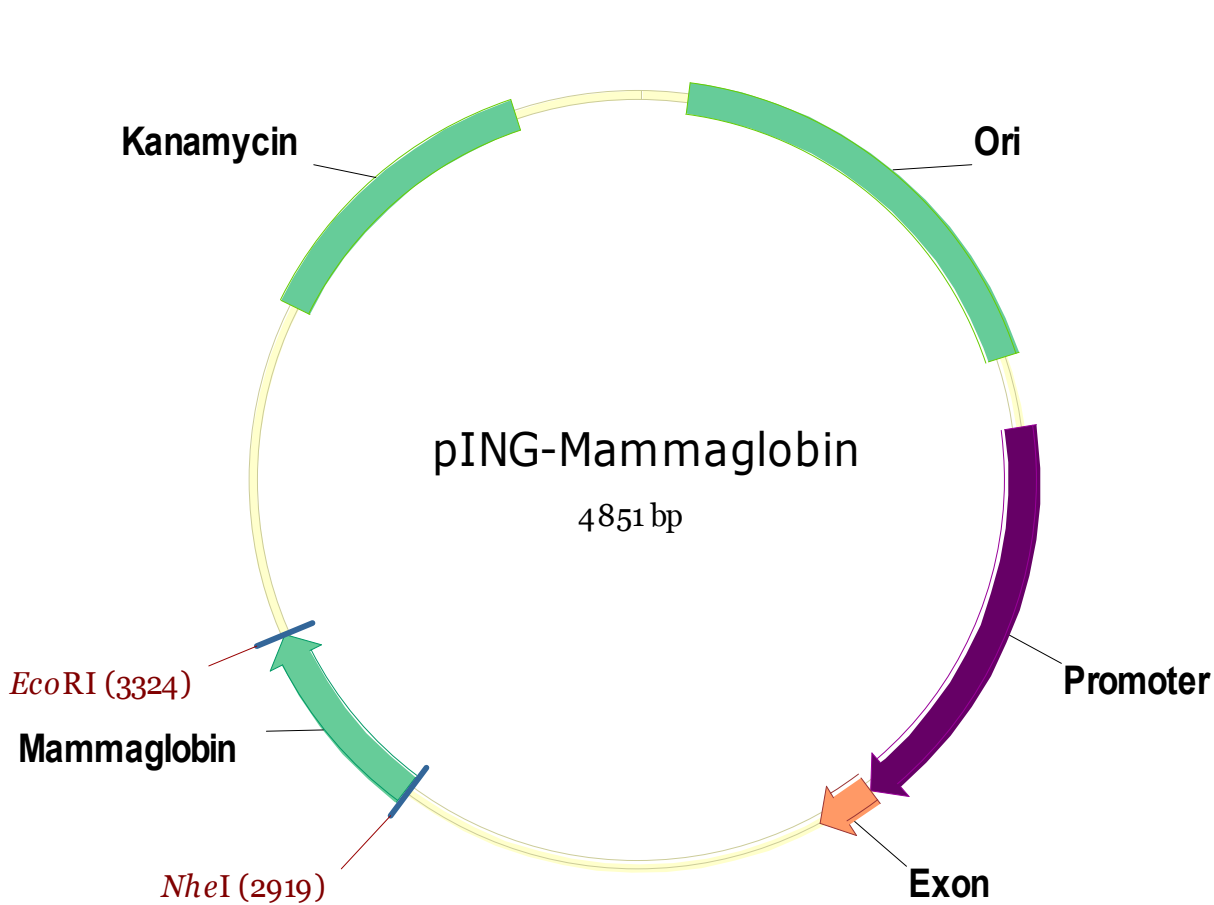
Safety

- Safety defined as the absence of severe 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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