The Good, the Bad and the Ugly:

Clinical trials which assess vaccine characteristics

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Ideal cancer vaccine trial

1. An informative immune assay
2. Ability to derive data on immune response
3. Toxicity and clinical response/survival data

4. Correlate ability of the assay in #1 to be a surrogate for #3.
5. Problem: #3 not the idea scenario for #2!

What is needed in a vaccine trial?

- Sufficient number of highly avid T cells that are antigen specific
- Ability of the T cells in question to traffic to lymph nodes and sites
- T cells generated must be in a proper state of activation and able to overcome both passive (antigen and MHC down-regulation) and active (Tregs, IL-10, TGF-beta) defences

What is the evidence that immune monitoring has clinical relevance?

- We need to determine if any immune assay correlates with relapse-free or overall survival
- Is there a surrogate endpoint for survival and/or clinical benefit?
- If simple enumeration is not useful, why not?
- Immune monitoring, if it correlates with clinical benefit can help us decide what qualities are important for a therapeutic T cell

Do we have the right assay, in the right type of trial?

- Different immune assays need to be prioritized
- Is there a place for pure immune surrogate trials, in patients without evidence of disease?
- Should we concentrate on patients with measurable disease, or are NED patients OK?
- What clinical endpoints are proper for vaccine trials; survival vs. response vs. stability?

Are we measuring the correct thing, and in the right place?

- Measurement of circulating T cells in PBMC is important, but what about draining nodes and tumor infiltrating T cells?
- Should we be measuring circulating or tumor Treg cells as well as effector cells?
- How important are circulating cytokines, both proinflammatory and suppressive?
- Are NK, NKT or DC relevant as a measure of immunity?
- Should we be measuring cytokine gene polymorphisms and cytokine gene epigenetic modifications and changes after vaccination as a surrogate marker for the ability to immunize?

Case studies in immune monitoring of clinical vaccine trials

- CanVaxin: cell based vaccine with BCG
- Peptide vaccines: melanoma differentiation antigens
- Dendritic cells: pulsed with peptides, lysates and fusion products
- This is not a comprehensive assessment, more a set of instructive examples to assess whether immune assays correlate with clinical benefit

Canvaxin: cellular vaccine

Chung et al JCO 2003 21: 313

- Three melanoma cell lines administered with BCG for two injections, then alone for 6 months total
- Induces antibody responses against a 90 kD tumor associated glycoprotein TA-90
- 54 patients: (-)SNB, all had >4 mm melanoma
- 43 got vaccine, 11 were observed
- DFS and OS correlated with maximal TA-90 IgM response ($p=0.006$ and 0.06) in the vaccine group, but not the observation group
- Non-randomized, but encouraging result

Canvaxin: cellular vaccine

Morton et al Ann Surg 2002 236: 438

- 2602 patients had complete lymphadenectomy in the period 1984-1998; 935 received Canvaxin, and 1667 did not
- Comparison group had no therapy or IFN 1971-1998
- They were matched for 7 co-variates
- Median OS was 49% vs. 37% favoring vaccne
- The authors claim OS was the same in the observed group pre-1985 and post-1985, which disagrees with SWOG data
- Canvaxin correlated with OS p= 0.001; RR death = 0.64
- Justifies a randomized phase III trial, just concluded in over 1100 patients of Canvaxin/BCG vs. BCG alone

CanVaxin: Phase III trials

- Two randomized trials, one ongoing, one just finished in resected stages III and IV melanoma
- A lower than expected rate of events will slow down the final interpretation of the trial; BCG effect?
- Evidence that Canvaxin/BCG may be beneficial:
  - Vaccinated patients have increased DTH to the vaccine, which correlates with survival
  - Vaccinated patients have a reduction in TA-90 IgM levels, which correlates with survival
  - Anti-ganglioside antibodies are induced by CanVaxin
  - T cell responses can be detected to known antigens

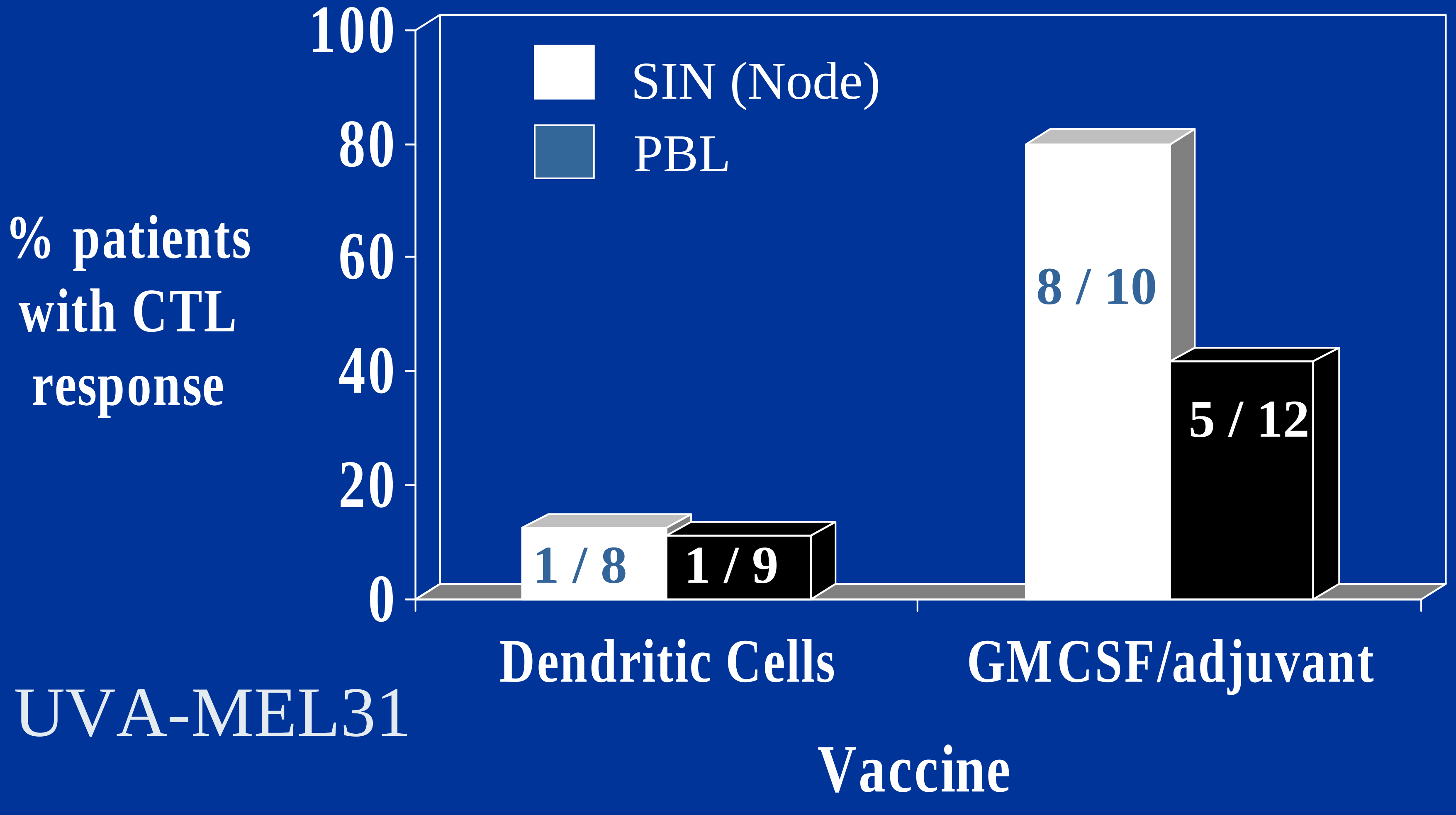
Peptide vaccine for resected melanoma: Walker et al *Clin Can Res* 2004

- 35 patients received gp100 209-217 (210M) with Montanide ISA 51
- Tetramer staining shows median of 0.36% post-vaccine (0.05 to 8.9%)
- Cells were CCR7(-) CD45RA (+) or (-) > suggesting effector or effector-memory type
- Virtually all cells expressed gamma interferon after *ex vivo* expansion

Peptide vs. DC vaccine for stage IV melanoma: Slingluff et al *J Clin Oncol* 2004

- 26 patients, stage IV melanoma, 13 each randomly allocated to receive peptides with Montanide/GM-CSF or pulsed onto DC
- Higher overall immune response with restimulated ELISPOT in peptide arm p<.02
- Vitiligo seen in 2 peptide but no DC patient
- 4 SD + PR in the peptide arm, versus 2 SD + PR in the DC arm
- Immune response appeared to correlate with PR/SD

Evaluation of CTL Responses to Vaccination with GMCSF-in-Adjuvant or DC+peptide in Patients with Substantial Tumor Burden (Stage IV)

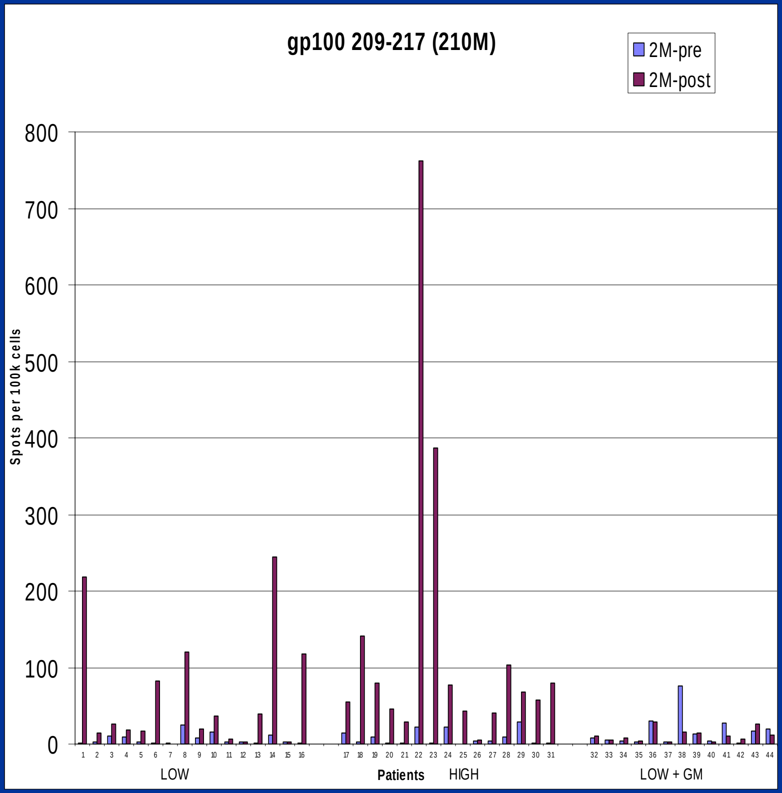


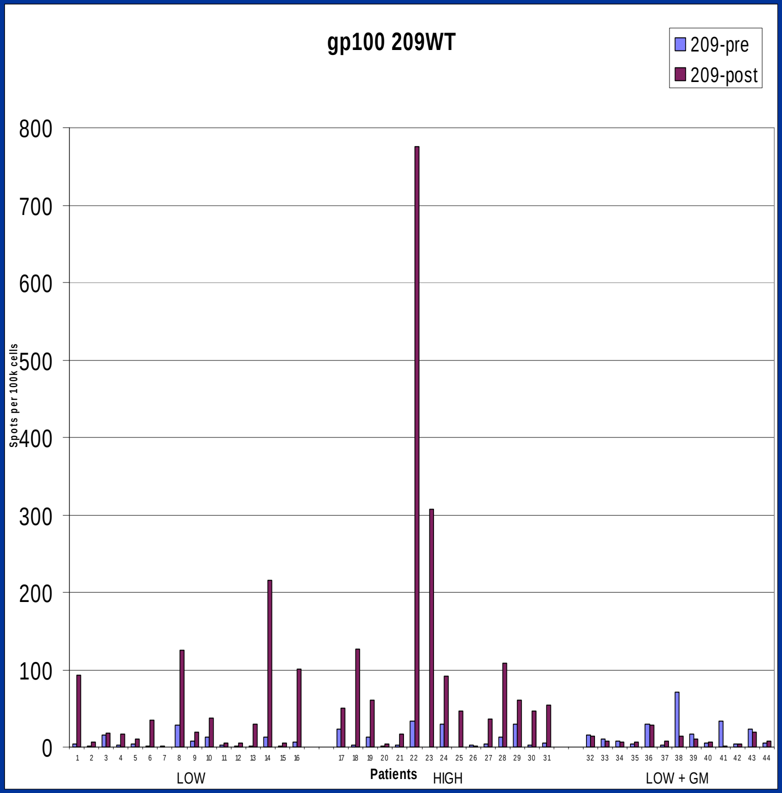
UVA-MEL31

Peptide vaccines for melanoma: Clinical data

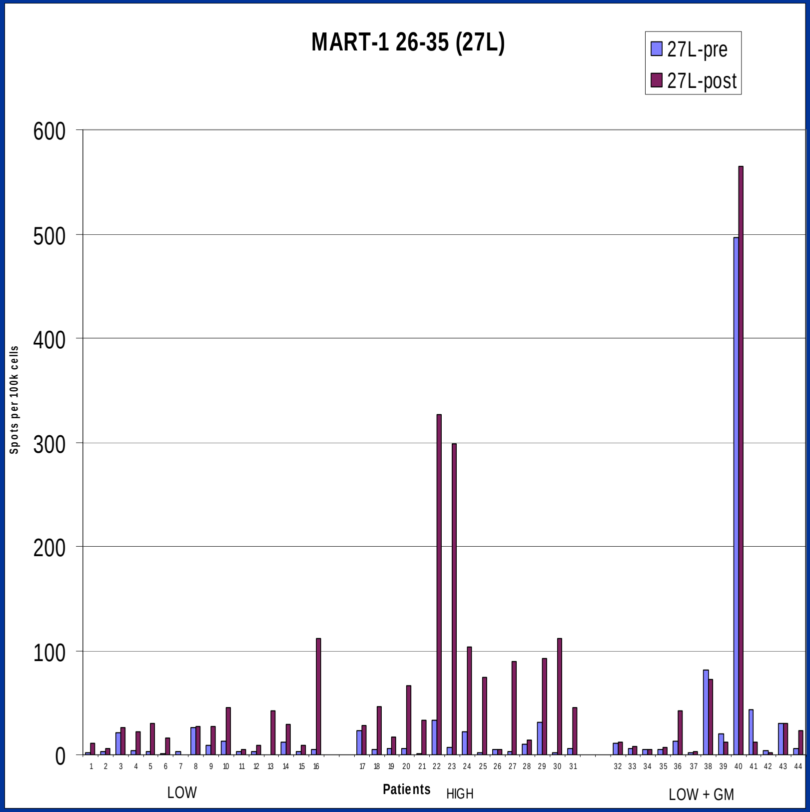
- gp100/tyrosinase/IFA+IL-12 trial for resected stage III/IV patients: 26 with stage III, 22 with stage IV disease; median relapse-free survival 20 months, median survival greater than 57 months, 85% had augmented immunity to gp100 by tetramer staining, with increase from 0.03 to 0.08% IL-12 vs. no IL-12 Lee et al *J Clin Oncol* 2001
- In an ongoing trial, <u>three</u> peptides with IFA were used to vaccinate stage III/IV resected patients with low dose IL-12/alum, low dose IL-12+GM-CSF or high dose IL-12/alum.

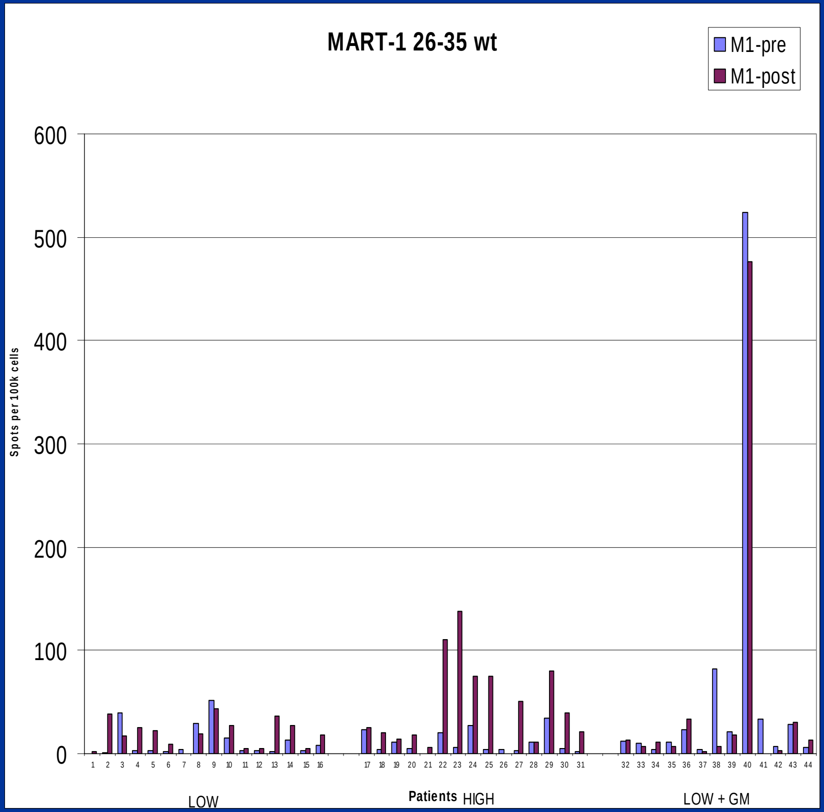
Reactivity to melanoma antigen gp100: are higher doses of IL-12 with alum a superior adjuvant?





Reactivity to melanoma antigen MART-1: are higher doses of IL-12 with alum a superior adjuvant?





Conclusions: Peptide vaccines with Montanide, alum and IL-12

- ELISPOT responses greater for both gp100 and MART-1/Melan-A heteroclitic and wild type in high dose IL-12 than either low dose group, p values ranging from 0.04 to 0.005
- WT immune responses equal to heterclitic
- More deaths (3 versus 1) and more relapses (10 vs. 4) in low dose groups than high dose group; correlation seen with immune response and time to relapse

Fowlpox gp100 vaccine: no correlation of immunity with response

- Three consecutive trials were done with 7, 14 and 16 pts who received a fowlpox-native gp100, fowlpox modified gp100, and folwpox –gp100 minigene (ER targeted)
- Rosenberg et al *Clin Can Res* 2003
- Responses to gp100 seen in 0/7, 10/14 and 12/16 patients respectively
- Restimulation assays done for cytokine release
- No correlation of assays with response and benefit
- The group immunized with the fowlpox gp100 minigene later received IL-2 with a 50% response rate

Class II peptide-pulsed DC Schuler-Thurner et al J Exp Med 2002

- Five biweekly SC vaccinations with peptide pulsed mature DC; only 16 received all DC
- Good responses seen to MAGE-3 243-258 by fresh *ex vivo* ELISPOT, and to KLH
- No clear correlation of immune response with clinical response; 1 CR with very low immunity seen, also 7 stable disease patients with no clear pattern of immunity

hTERT peptide-pulsed DC induce functional T cell responses

- Four of seven patients immunized with hTERT peptide/KLH pulsed DC demonstrated an immune response
- The only objective response in a breast cancer patient was associated with a potent CD8 T cell response Vonderheide et al *Clin Can Res* 2004
- The same hTERT I540 peptide with Montanide did not induce immune responses with CD8 T cells that recognized native cell lines; 0 responses were observed Parkhurst et al *Clin Can Res* 2004

Peptide-pulsed CD34+ derived DC

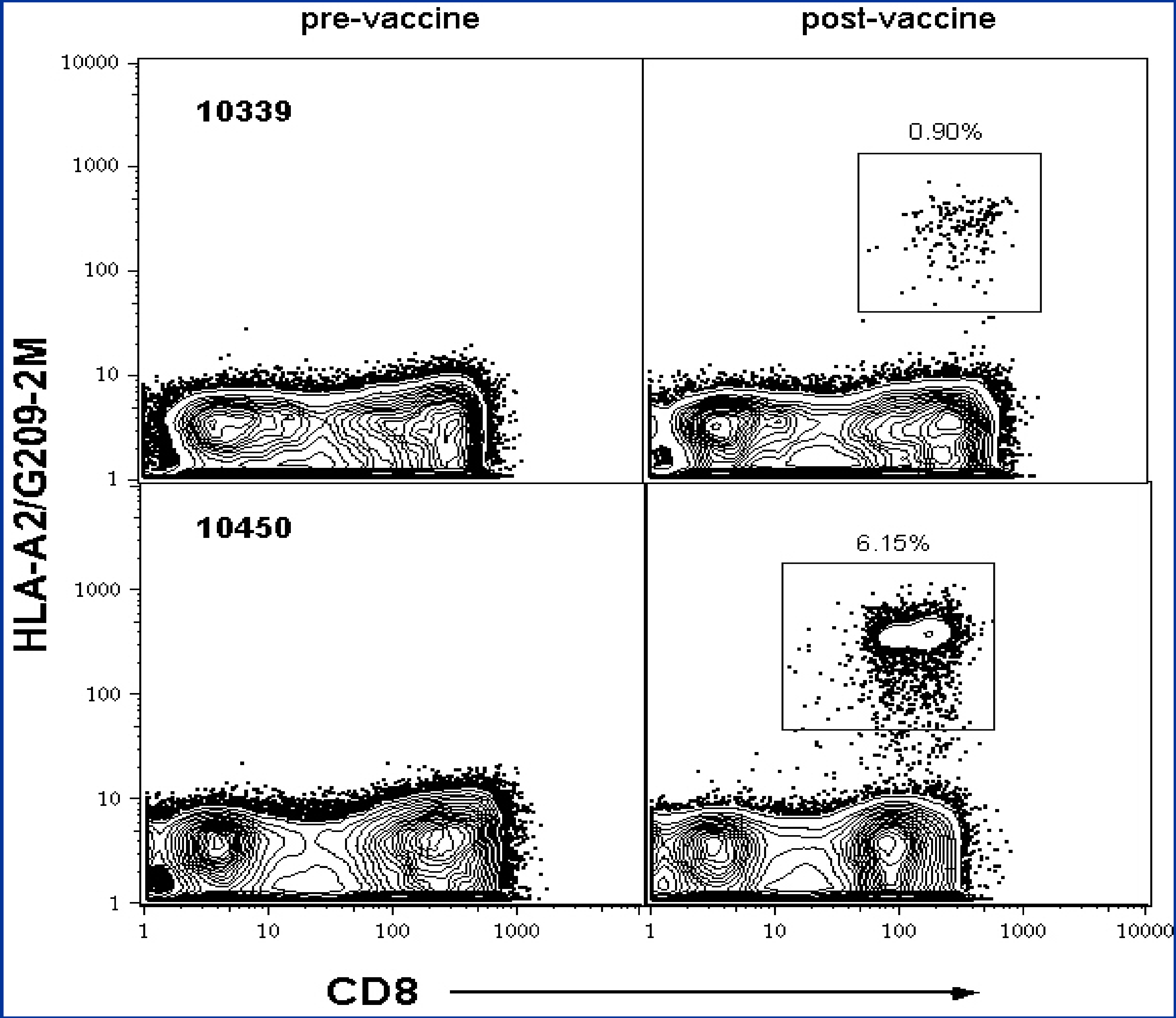
- 18 patients were treated with multiple melanoma peptide-pulsed DC generated from CD34+ progenitor cells
- 16/18 responded by ELISPOT to *ex vivo* or restimulated cells
- 6/7 pts with response to 2 peptides or less progressed, versus only 1 of 9 with an immune response with $p=0.02$; the authors felt that response correlated with benefit
- Follow-up suggests that survival does correlate with immune response to more than 2 antigens
- Palucka et al *Cancer Res* 2001

CEA peptide-pulsed flt3L derived DC: immune response correlation

- Patients were treated with heteroclitic CEA peptide-pulsed DC after flt3L treatment
- 2 clinical responses of 12 seen
- Correlation of clinical response with CD8 tetramer-specific immune response to CEA
- Fong et al *P.N.A.S.* 2001

Immune Assays for tumor specific T cells: strengths and weaknesses

- Choice of surrogate assay in important to guide future development
- ELISPOT methodology is limited in its reliability, flexibility and reproducibility, but is today's choice
- Flow assays can be standardized and easily controlled, but are not functional assays
- New tetramer assay generates functional CD8 T cell data; it is based on staining with CD107a, a lysosomal membrane protein, to denote lytic T cells
- Tetramer array in development yields quantitative data on T cell phenotype and function


pre-vaccine
post-vaccine
10339
0.90%
10450
6.15%
HLA-A2/G209-2M
10000
1000
100
10
1
CD8

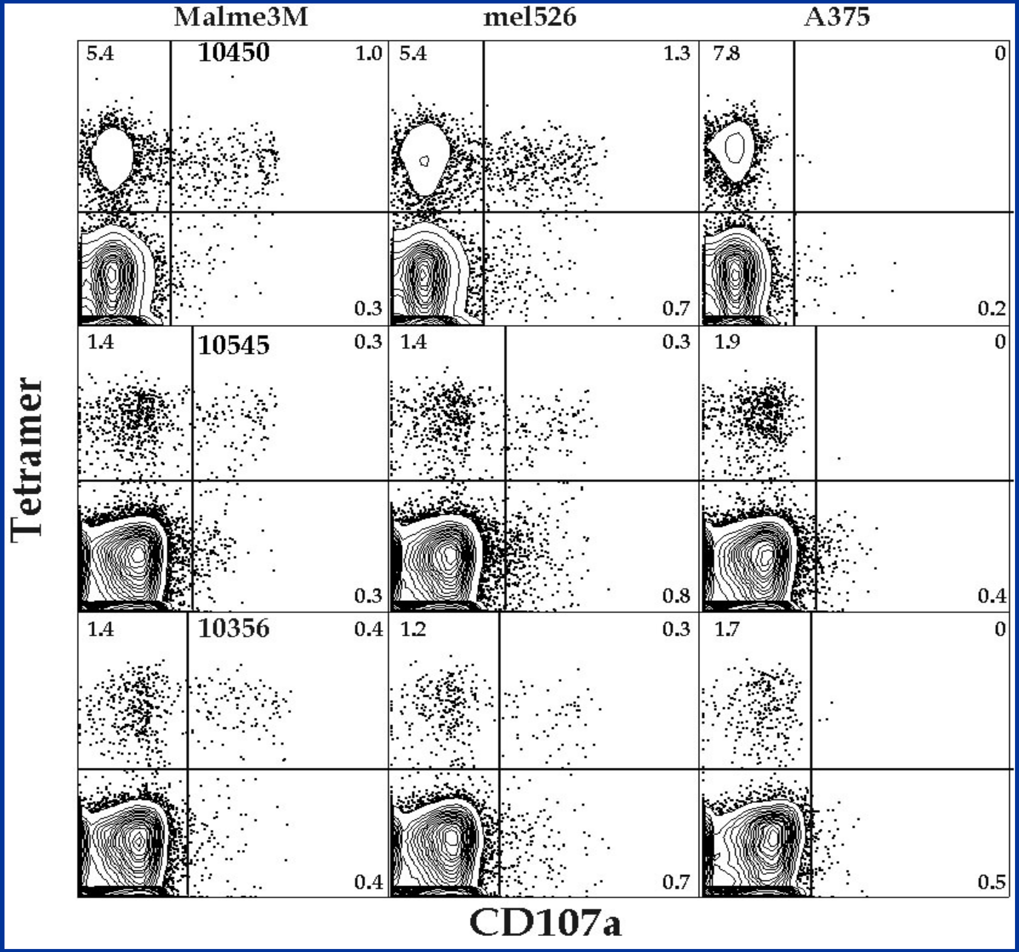
High avidity T cell clones are CD107a positive Lee et al Nat Med 2003

- CD8 T cell clones were raised from gp100 peptide-vaccinated melanoma patients
- Most were low avidity and did not recognize tumor cells or APC pulsed with low peptide concentration; some were high avidity but all bound gp100 tetramer
- The high avidity clones were lytic, recognized tumor cells and expressed CD107a

Tetramer+ CD8 high avidity T cell clones are CD107a positive and recognize tumor cells

Average % cytotoxicity		Functional avidity (M)
CD107+	**CD107-**	**CD107+**
45	-2	10^{-12}
13	-3	10^{-11}
42	-3	10^{-11}
35	-2	10^{-11}
46	-5	10^{-12}
31	-5	10^{-11}
35.3	-3.3	

CD107a/tetramer flow assay: high avidity T cells recognize tumor cells

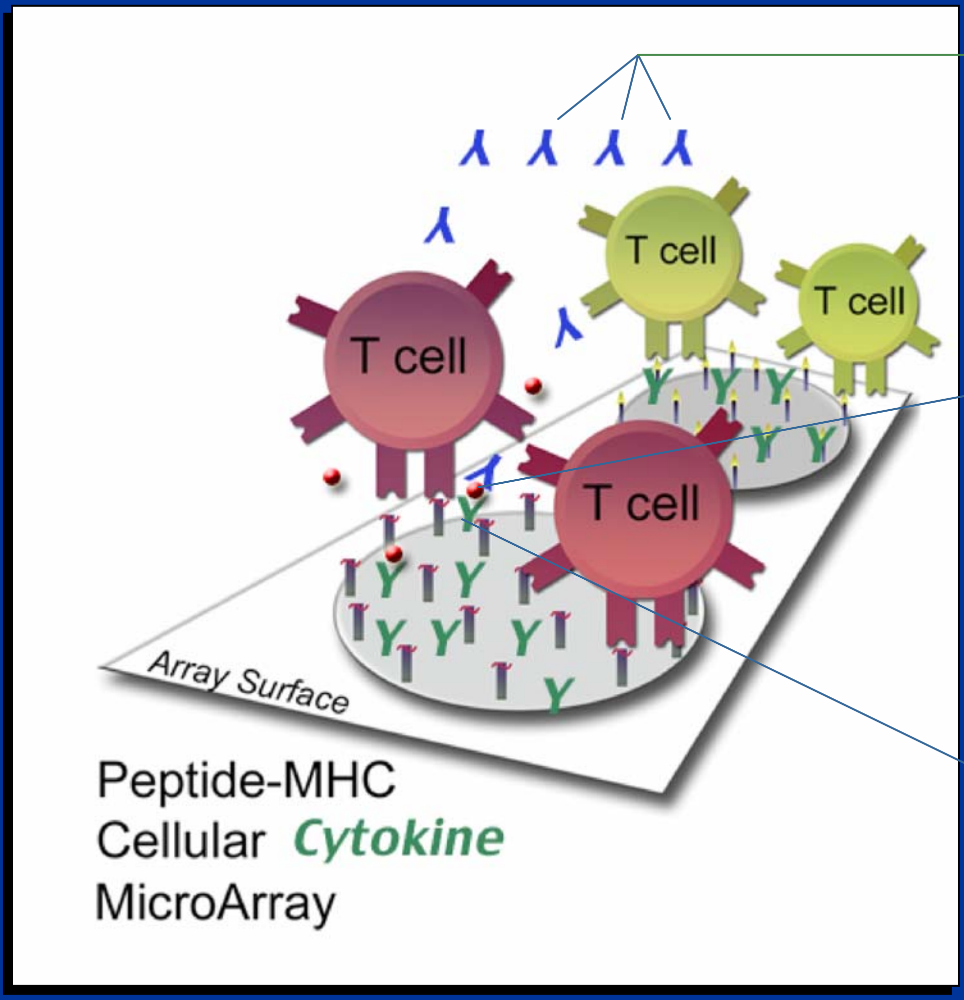


Functional status of TAA specific immune response: endogenous* vs. vaccine induced T cells:

		% functional response			
Patient ID	TAA	T2-peptide	mel526	Malme-3M	A-375
422	G209-2M	98.2	27.2	23.8	0.5
476	G209-2M	99.6	32.5	27.8	2.6
132*	G209-2M	99.2	87	82.2	2.7
517	M26	86	29.3	28.3	6.4
520	M26	93	28.5	25.1	2.8
461*	M26	95.3	54.9	36.8	3

			% functional response		
Patient ID	TAA	% tetramer	mel 526	Malme 3M	A-375
713	M26	0.49	18.2	13	6.1
721	M26	0.19	49.6	45.2	4.8
721	G209-2M	0.23	20.8	24.9	5.2
735	M26	0.21	30.6	42.1	1.8
735	G209-2M	2.7	32.6	35	3
722	G209-2M	0.19	37.4	21.5	6.1

MHC-Cytokine Arrays - Cytokine Sandwich Assays



Secondary cytokine
Detection antibody
Conjugated to a flurophore

Co-spotted Cytokine
Capture antibody

Cytokine secreted by
T cell after recognition of
Peptide/MHC

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T Cell Functional Profile

Capture Probes: αCD8, gp100 209/A2, MART1 25/A2, CMVpp65/A2, αCD3/αCD28

Cytokine Detector Probes:

IL4	IFNγ	IL12
IL5	TNFα	IL15
IL10	GranzymeB	VEGF
IL13	GM-CSF	VEGF-D
TGFβ	IL1b	
IL2	IL6	
IL7	No Co-Spot	

IL4	Regulatory cytokine
IL5	Regulatory cytokine
IL10	Immunosuppressive
IL13	Regulatory cytokine
TGFβ	Growth factor (*f*)
IL2	Stimulatory cytokine
IL7	Cytokine growth *f*
IFNγ	Stimulatory pleiotropic *f*
TNFα	Stimulatory pleiotropic *f*
GranzymeB	Mediator of CTL killing, apoptotic *f*
GM-CSF	Hematologic growth *f*
IL1b	Inflammatory cytokine
IL6	Stimulatory cytokine
IL12	Stimulatory cytokine
IL15	Stimulatory cytokine
VEGF	Angiogenic *f*
VEGF-D	Angiogenic/lymphogenic

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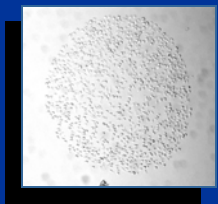
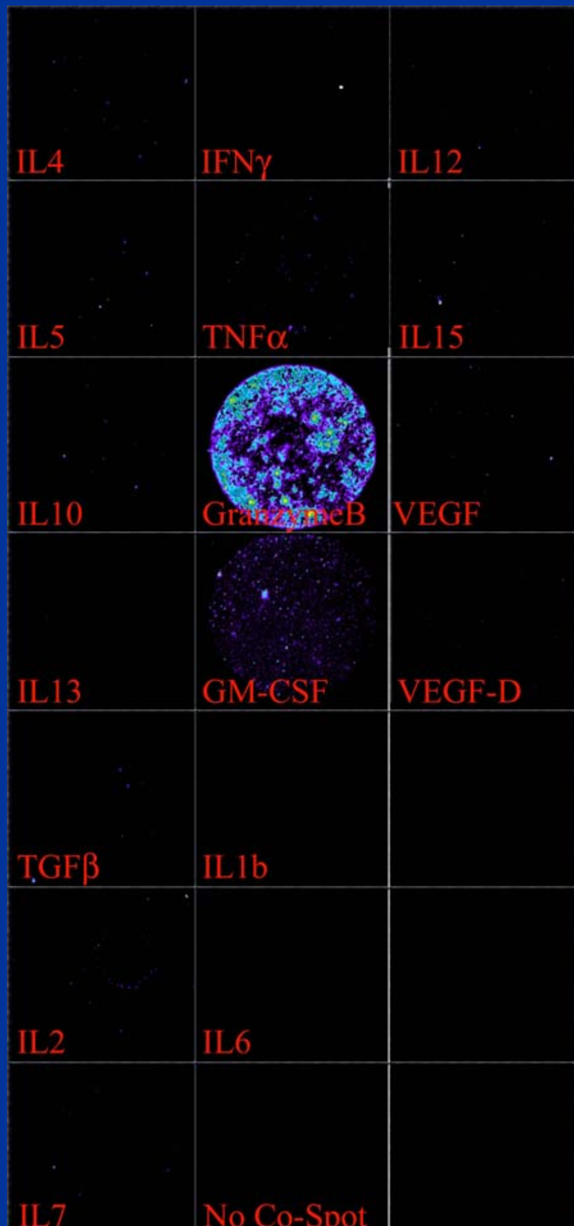
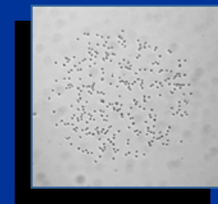
αCD8 Co-Spots



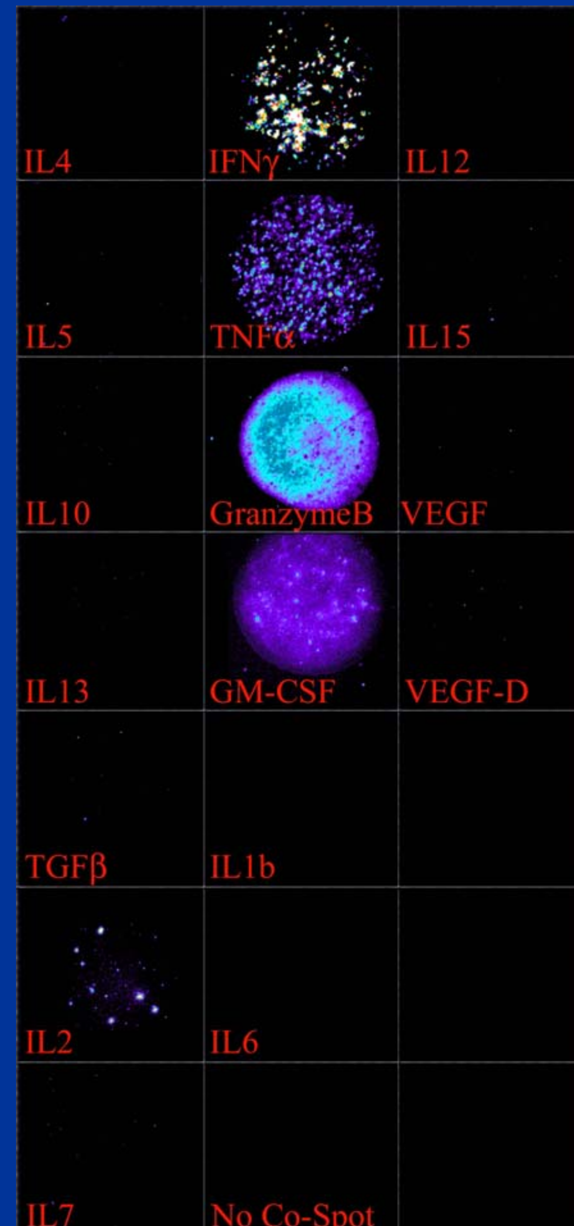
αCD8
brightfield



MART1 / A2 Co-Spots



MART1 / A2
brightfield



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Functional T Cell Responses to Peptide Vaccines

Pt	ID	Vax	Adj	Stage	Outcome	IFNγ	TNFα	GranzB	IL-2	TGFb	IL1b	IL6	GMCSF
1	68w	3 pep	High IL12	IV	Alive, 13m	■■	■	■■	■	■	■	■	■
2	76m	3 pep	High IL12	IV	Alive, 13m	■■■■	■■■	■	■■■■	■	■■	■■■	■
3	72m	3 pep	High IL12	III	Recur m8/deceased	■■■■	■	■■	■■■	■■	■	■	■
4	65w	3 pep	Low IL12	III	Relapsed m11, resected	■■■	■	■■	■■■	■■	■	■	■
5	52w	3 pep	Low IL12	III	Alive, 16m	■■■	■■	■	■■■	■■■	■■	■■	■■
6	74m	3 pep	Low IL12	III	Alive, 16m	■■	■	■	■■■	■■	■	■	■■■
7	37m	3 pep	Montanide	III	Alive, 16m	■■■	■■	■	■	■	■	■	■
8	42m	2 pep	GMCSF	III	Alive, 5yrs	■■■	■■	■	■	■	■	■	■
9	51m	2 pep	GMCSF	III	Alive, 5yrs	■■■	■	■	■■	■	■	■	■
G	clone		no		-	■■■■	■■■■	■■■■	■■■■	■	■	■	■■■■
M	clone		no		-	■	■	■	■	■	■	■	■

<u>Key</u>

■
■■ # of Blocks
■■■ Denotes gp100
■■■■ Specific Activity

Chen, DS 2004

Overview

- Analysis of T cell specificity and function
- Single cell resolution
- High throughput

- Few peptide-specific T cells are responsive
- Different vaccination strategies result in different functional profiles
- Interferon-γ and TNF-α discordance correlates with poor outcomes
- IL-1b and IL-6 secretion is associated with good outcomes
- Representation of complex cellular interplay

Conclusions and Lessons Learned

- Immune monitoring is more rigorously and carefully done and ex vivo tetramer and ELISPOT assays are more widespread than when we last met in 2001
- More evidence on the correlation between immune response and clinical benefit seen, but most trials have failed to show any correlation
- State of the art <u>functional</u> *ex vivo* assays are necessary, and new assays and arrays are likely to be useful
- Immune response assays provide feedback on optimal vaccine development and mechanistic understanding
- High avidity, long lasting T cells capable of recognizing antigen on tumor cells are needed
- We need to think outside the box on the development of new surrogate assays of immunity in cancer