Local, regional and systemic effects of TLR agonists and incomplete Freund's adjuvants for peptide vaccines

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Disclosures

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3M, Clinical trial Research Support

MERCK, Clinical trial Research Support

The rebirth of cancer vaccines

- Immune checkpoint therapy proves the relevance of immune surveillance
- Failures of checkpoint blockade in some patients highlight the need to augment antitumor efficacy with combination immunotherapies, which can include vaccines.
- Technologies for high throughput sequencing of patient tumors have enabled more rapid identification of mutated neoantigens that show promise as ideal antigens for vaccines.

Vaccination with mutated neoantigens
Is there consensus on how to vaccinate?

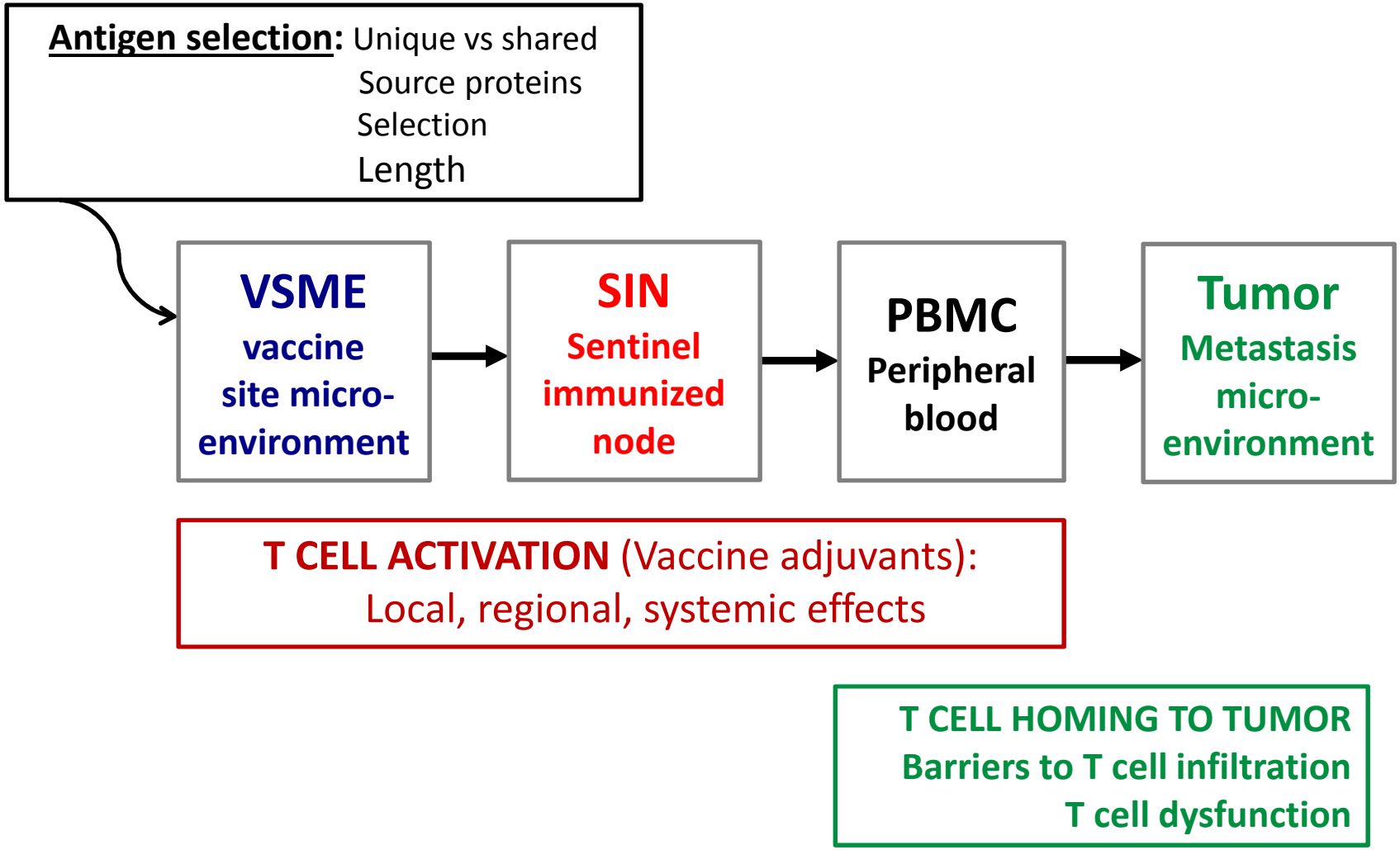
Trial ID	PI (Site)	Mutated neoantigens	Adjuvant
NCT00001703	Khleif, NIH	mutated VHL peptides	IFA
NCT00019006	Khleif, NCI	mutated RAS peptides	DETOX-B
NCT00003959	Stephen Nimer, MSKCC	mutated RAS peptides	GM-CSF
NCT00019331	Barry Gause, Magnuson Ctr	RAS peptides	GMCSF, DETOX-PC, systemic IL2
NCT01885702	Jolanda IM de Vries; Radboud Univ, Netherlands	Mutated neoantigens in MSI-H CRC and germline MMR-gene mutation	Dendritic cells
NCT01970358	Patrick Ott, DFCI	personalized mutated NeoAg	polyICLC

- Wide range of selected adjuvants reflects lack of consensus on the best adjuvant(s) to use, locally or systemically, with these seemingly ideal neoantigens.
- There is a major need to define optimal local and systemic adjuvants, for use with all vaccines.

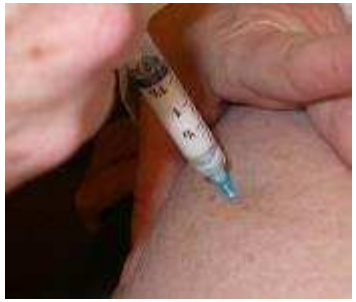
Neo-antigen:
A newly acquired and expressed antigen

- Mutated neo-antigens: Epitopes in normal proteins arising by somatic mutations in cancer cells, not in normal cells.
  - Unique (driver vs passenger)
  - Shared driver (EGFR, KRAS, BRAF)
- Phosphopeptides: expressed by cancer cells as a reflection of their transformed nature (Victor Engelhard and Donald Hunt).
- Cancer-testis antigens (eg: NY-ESO-1)
  - Expressed only in mature spermatids (lack MHC)
- Cancer-oocyte antigen (eg: SAS1B - John Herr)
  - Expressed only in mature oocytes (lack MHC)
- Viral antigens (eg: HPV, HCV, Merkel polyomavirus)

Factors impacting efficacy of cancer vaccines



Antigens for use in cancer vaccines



Crude antigen

Constructs:
protein, DNA, RNA, or viral constructs

Presented on DC/APC or injected directly, or on nanoparticles

Route:
SQ, IM, ID, transdermal, IV

Tumor cells or lysates

Whole protein

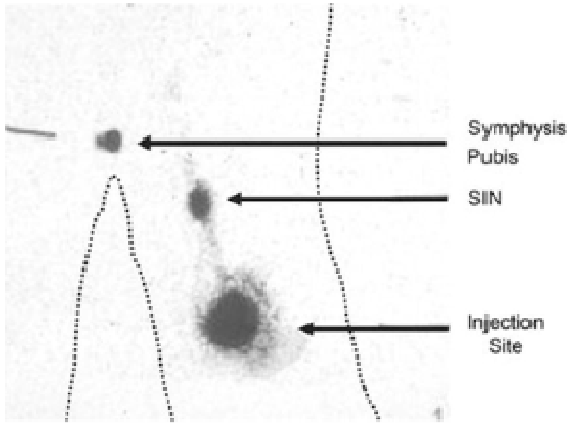
Long peptides

Minimal epitope (short) peptides

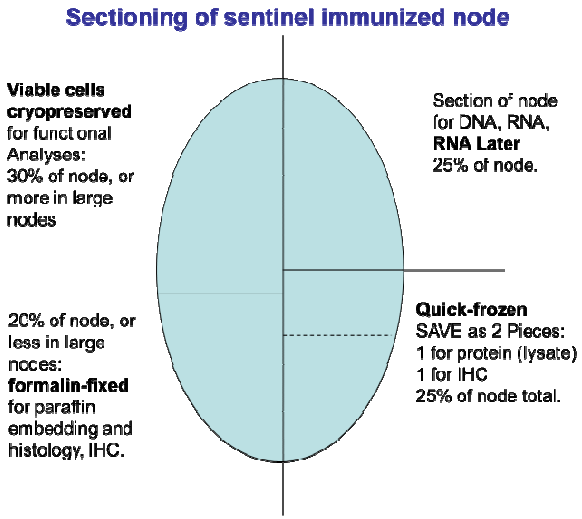
Defined antigen

Excision of the sentinel immunized node (SIN) draining the vaccine site, 1 week after vaccine #3.

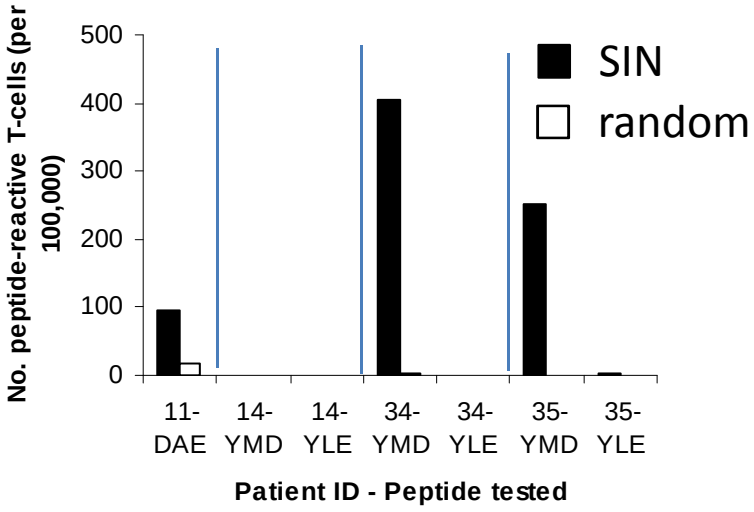
Identification of the sentinel immunized node in the groin draining a cutaneous vaccine site in the left thigh by Tc99-sulfur colloid injected intradermally around the vaccine site. Gamma camera scan demonstrates radioactivity at the injection site, the lymphatic channel draining toward the sentinel immunized node, and the hot spot representing the location of the SIN in the left groin.



Node sectioned for 4 preservation conditions within 1-5 minutes at the bedside



Immune response in SIN, not in random node

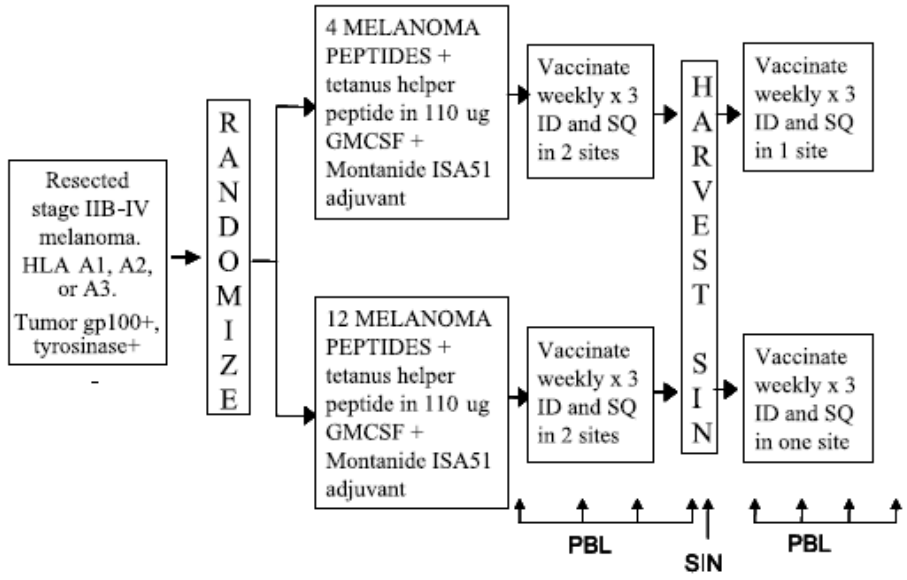


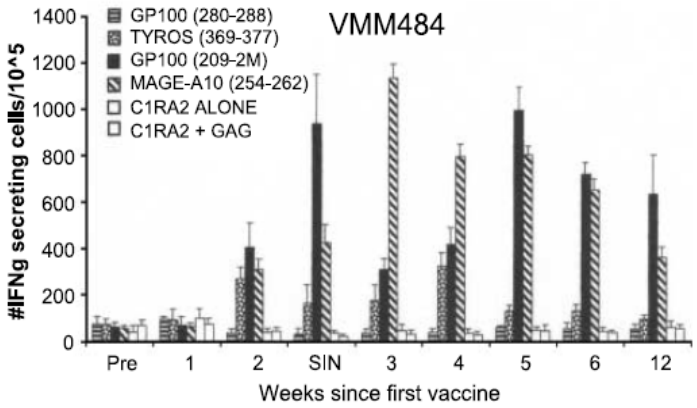
Slingluff, et al. Evaluation of the sentinel immunized node for immune monitoring of cancer vaccines Ann Surg Oncol 2008.

Increasing the number of peptides targeted with a multipeptide vaccine – Mel39

*	DAEKSDICTDEY	tyrosinase	A1
	SSDYVIPIGTY	tyrosinase	
	EADPTGHSY	MAGE-A1	
	EVDPIGHLY	MAGE-A3	
*	YMDGTMSQV	tyrosinase	A2
	IMDQVPFSV	gp100	
*	YLEPGPVTA	gp100	
	GLYDGMEHL	MAGE-A10	
*	ALLAVGATK	gp100	A3
	LIYRRRLMK	gp100	
	SLFRAVITK	MAGE1	
	ASGPGGGAPR**	NYESO1	

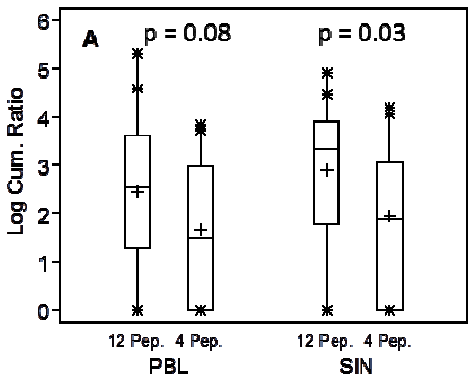
* Index peptides
** A31/A3



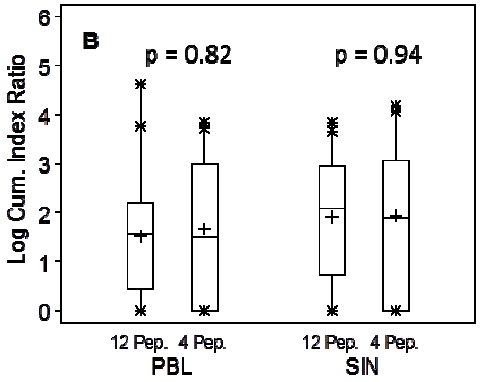


12 peptide vaccine: Reactivity to: tyrosinase (369-377), gp100 (209-2M), MAGE-A10 (254-262)

Immune response (ratio over background)



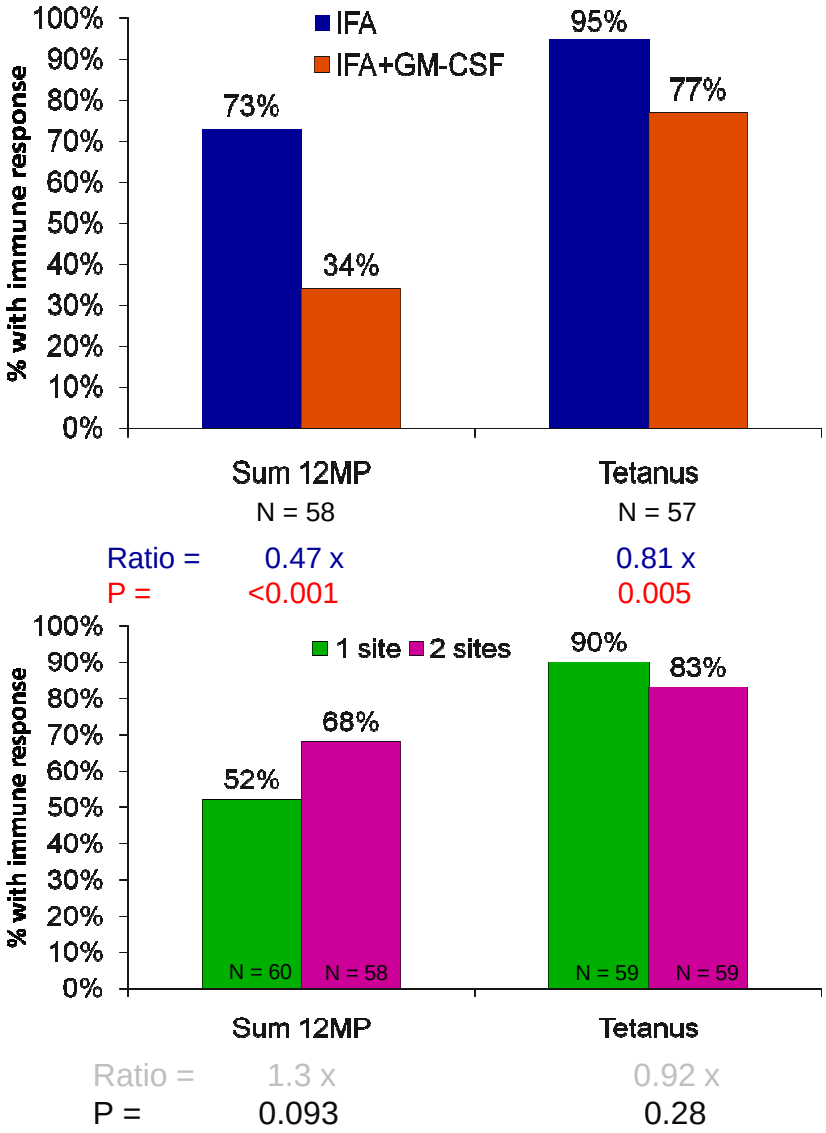
to 12 melanoma peptides



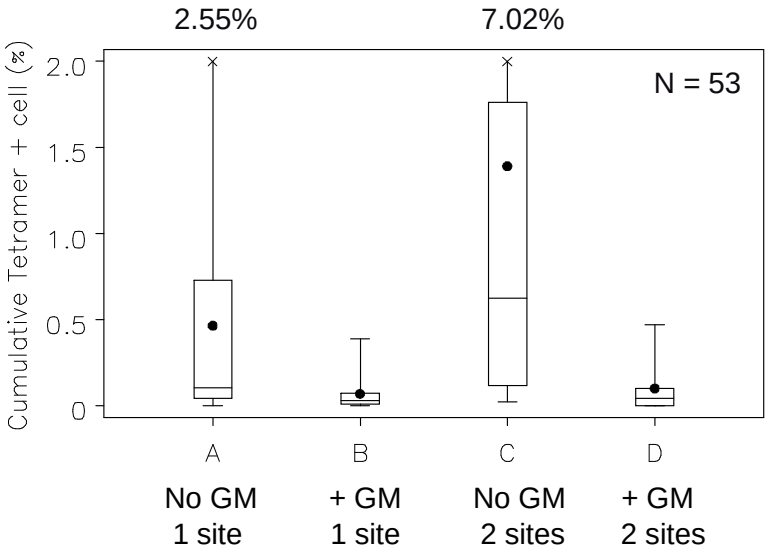
to 4 index melanoma peptides

Randomized trial testing effect of GM-CSF as vaccine adjuvant combined with IFA (Mel43)

Direct ELIspot responses



Tetramer data for HLA-A2 peptides



- Addition of GM-CSF significantly decreases circulating T cell responses to the vaccine.
  - $CD8^+$ T cell responses: ELIspot & tetramer
  - $CD4^+$ T cell responses: ELIspot
- Concurrent paper by Faries … Morton showed negative clinical effect of GM-CSF added to a whole cell vaccine/BCG.
- GMCSF induces MDSC/MSC in melanoma patients when given with HSP vaccine: Filipazzi... Rivoltini, JCO 2007

Slingluff CCR 2009

Mel 41: Vaccination with Peptides to induce CD4+ helper T cells: 6 melanoma helper peptides (6MHP) in IFA (+/- GM-CSF)

Class II-MHC Restricted Melanoma Peptides (6MHP)

Protein (residues)	Allele	Peptide Sequence (14-23 aa)
Tyrosinase $_{56\text{-}70}$	DR4	(A)QNILLSNAPLGPQFP (Topalian)
Tyrosinase $_{388\text{-}406}$	DR15	FLLHHAFVDSIFEQWLQRHRP (Kobayashi)
MelanA $_{51\text{-}73}$	DR4	RNGYRALMDKSLHVGTQCALTRR (Zarour)
MAGE-3 $_{281\text{-}295}$	DR11	TSYVKVLHHMVKISG (Manici)
MAGE-1-3, 6 $_{121\text{-}134}$	DR13	LLKYRAREPVTKAE (Chaux)
gp100 $_{44\text{-}59}$	DR1, DR4	WNRQLYPEWTEAQRLD (Halder/LI)

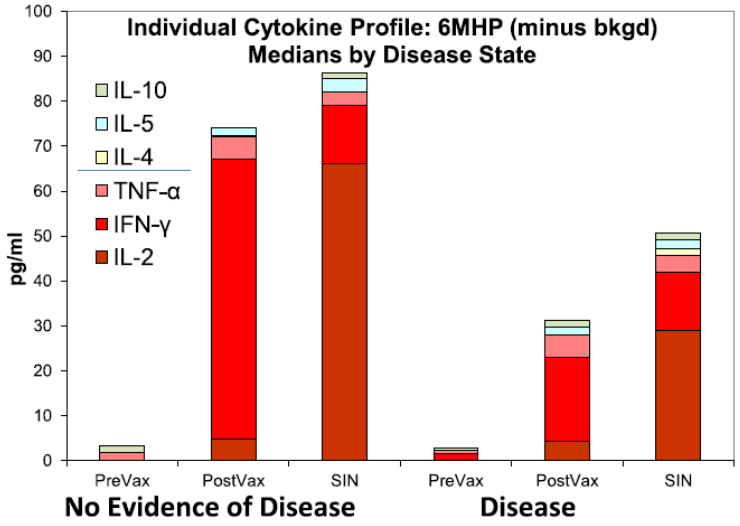
Helper T cell immune responses (n = 39)

- 57% in PBMC
- 81% in node + PBMC

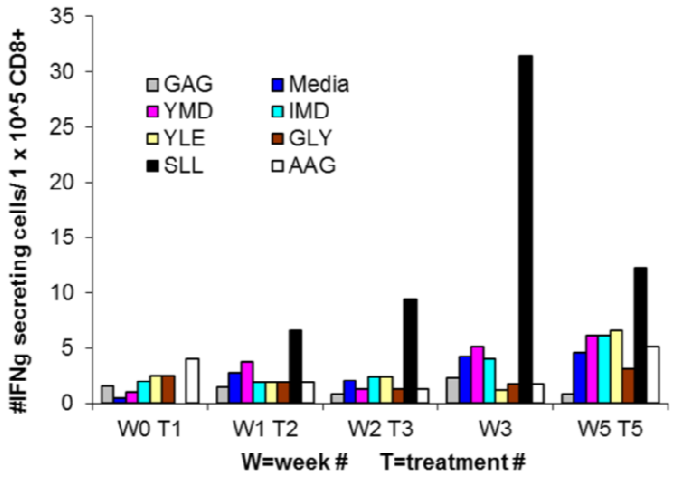
Clinical responses (n = 17 measurable):

- PR (2/17, 12%) Duration 1, 3.9+ years
- SD (2/17, 12%) Duration 1.8, 7 years
- Durable PR or SD (24%) ***J Clin Oncol 2008***

Th1 dominant responses to 6MHP. ***Dillon PM. JITC 2014***

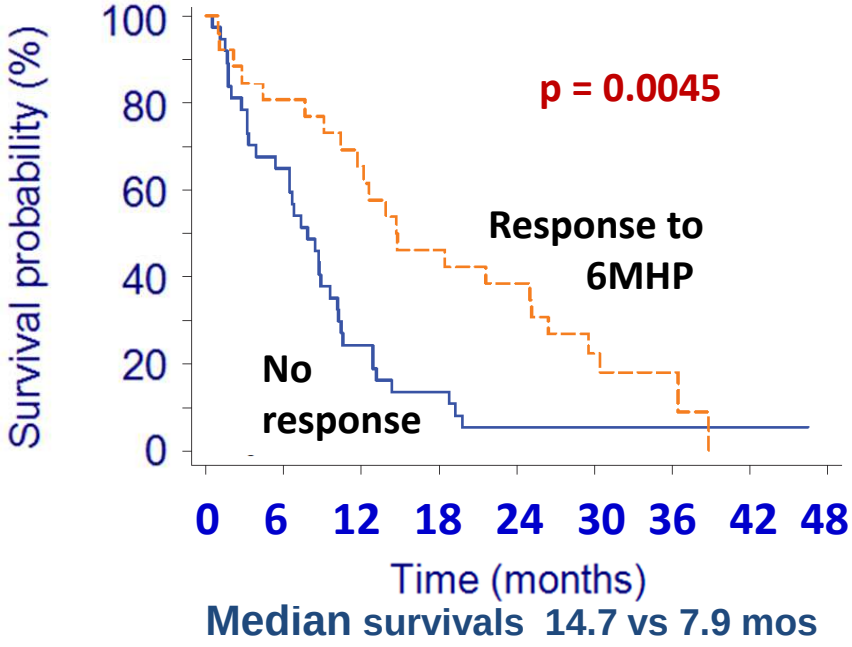


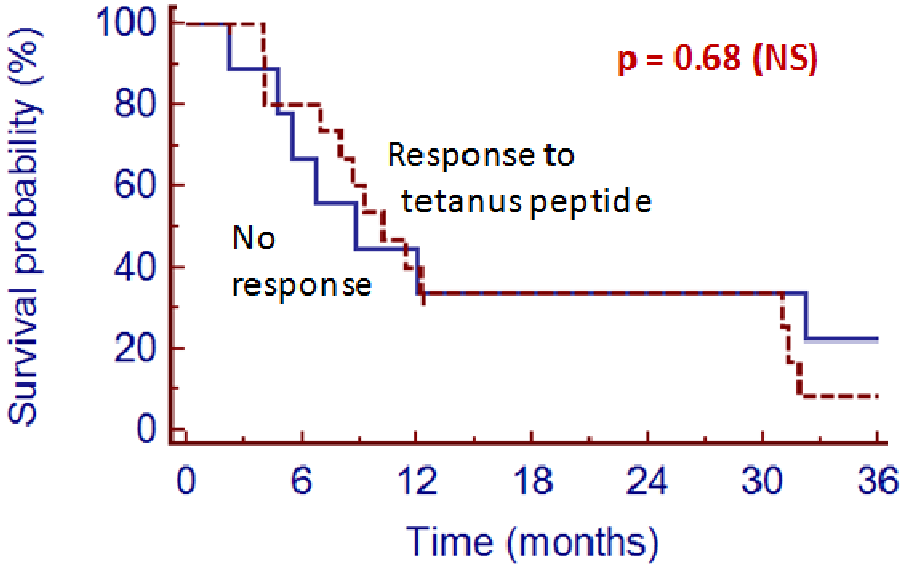
Epitope spreading to CD8+ cell epitopes in 5/11 patients (45%) tested ***Hu, Y. CII 2014***



Associations of immune response to 6MHP with survival

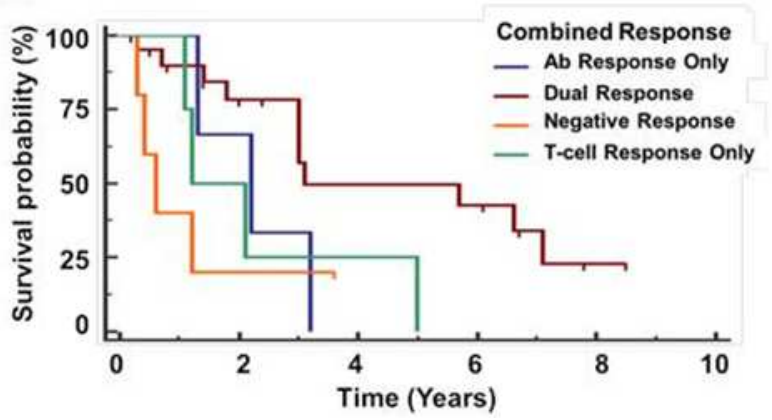
ECOG 1602 Landmark analysis (2 months) of overall survival associated with CD4+ T cell response to 6MHP, but not to tetanus peptide (CCR 2013)



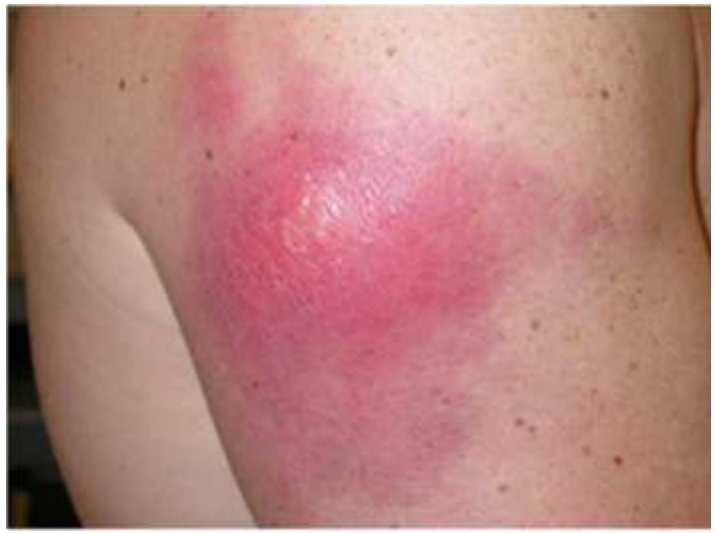


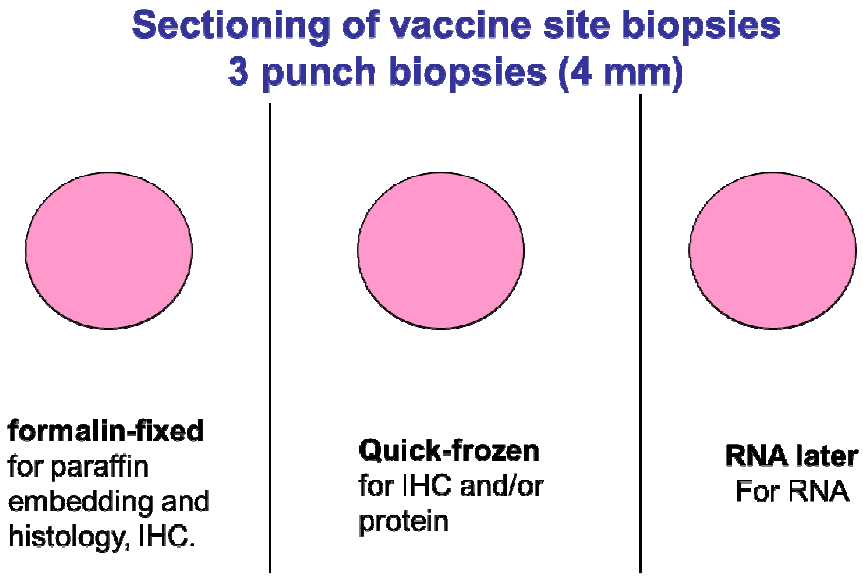
Mel41 trial: Improved overall survival associated with (i) early Ab responses (week 5-8, p=0.009), (ii) $CD4^+$ T-cell responses (p=0.03), an d (iii) Ab + $CD4^+$ T cell responses , compared to T-cell response alone (p=0.0264)

Reed CM in preparation

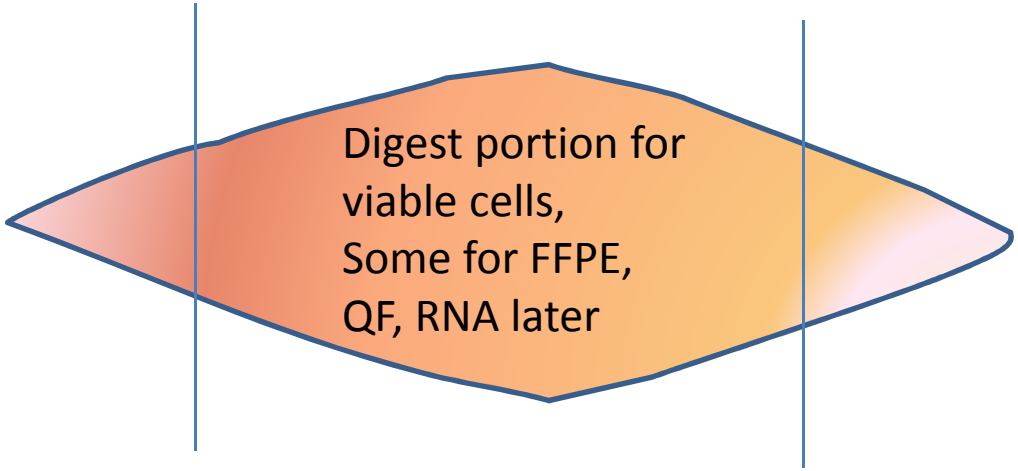


Biopsies to test for T cell recruitment and function/dysfunction in the vaccine site microenvironment (VSME)

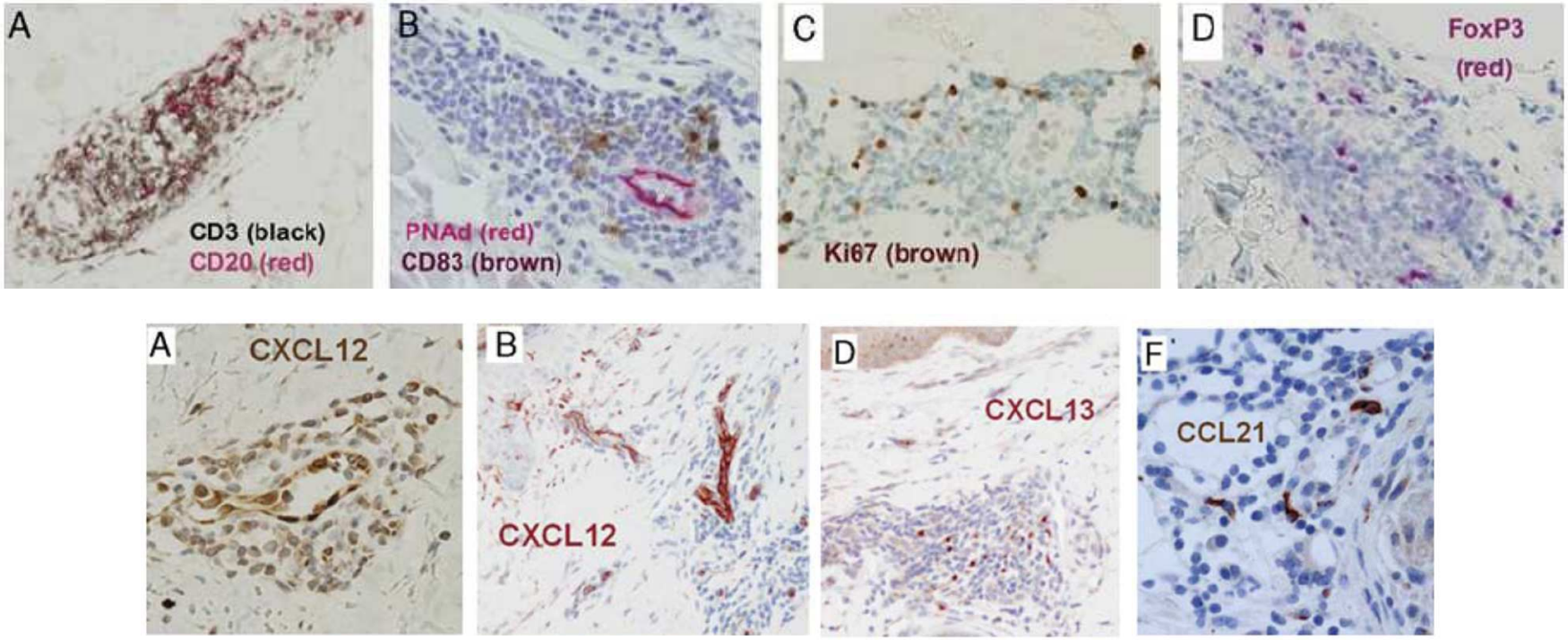




1 x 3 cm
or 2 x 6 cm
full thickness
excision



Lymphoid aggregates after repeated vaccination with multipeptide vaccine in IFA, without GM-CSF (Mel44), 2 weeks after the 3rd vaccine.

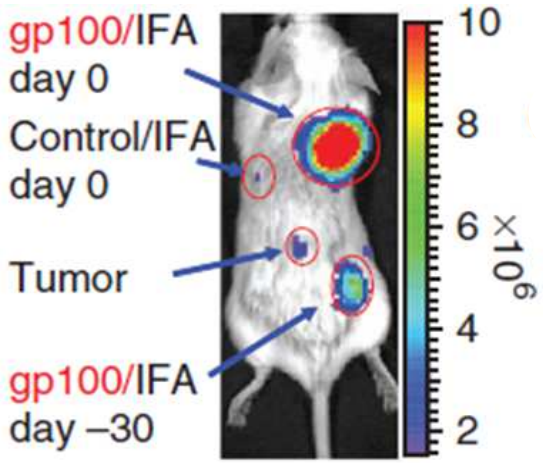


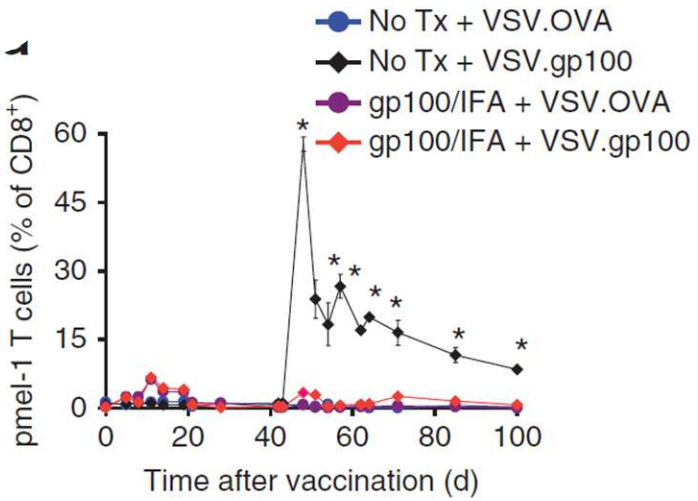
Induction of lymphoid aggregates resembling tertiary lymphoid structures (TLS), with PNAd+ endothelium and mature DC (CD83) in T cell areas, and clustering of B cells. T cell proliferation (CD4+Ki67+ and CD8+Ki67+) counterbalanced by T regs (CD4+ FoxP3+), with chemokines CXCL12,13, CCL21.

Harris RC. J Immunother 2012

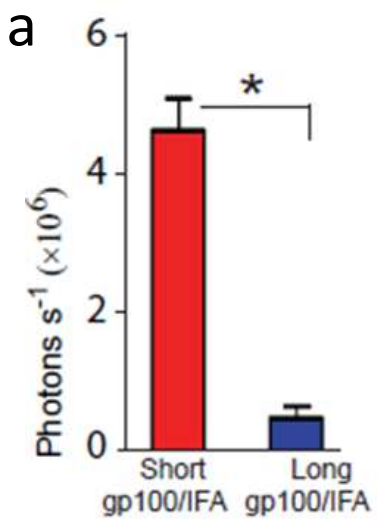
gp100-reactive T cells home preferentially to vaccine site with gp100/incomplete Freund's adjuvant (IFA), rather than to tumor

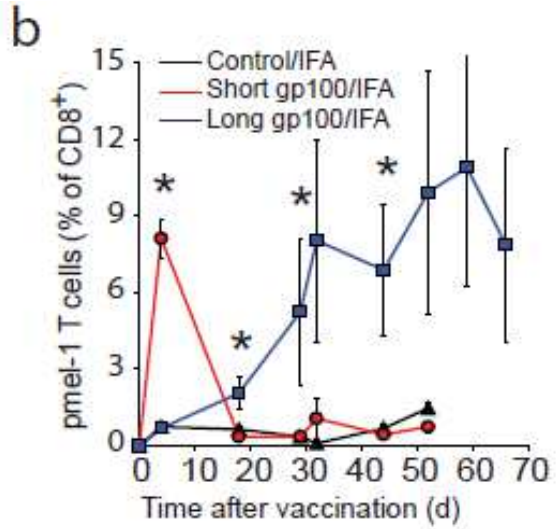
Short peptide plus IFA induces T cell retention at the VSME and depletion from circulation.

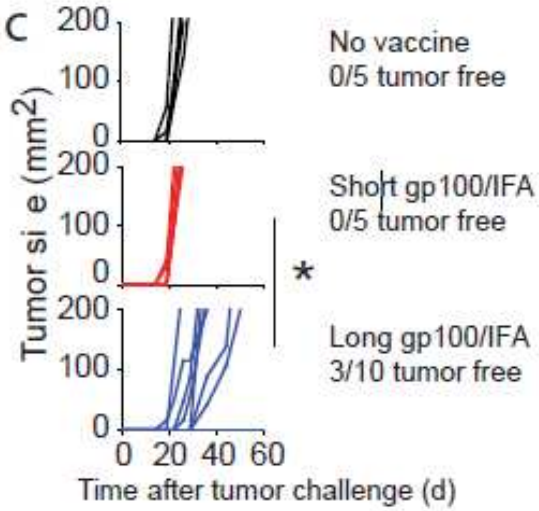




Long peptides in IFA induce durable immune responses and tumor control.







Hailemaichael….Overwijk, Nat Med 2013 (MDACC) S.Fig 15)

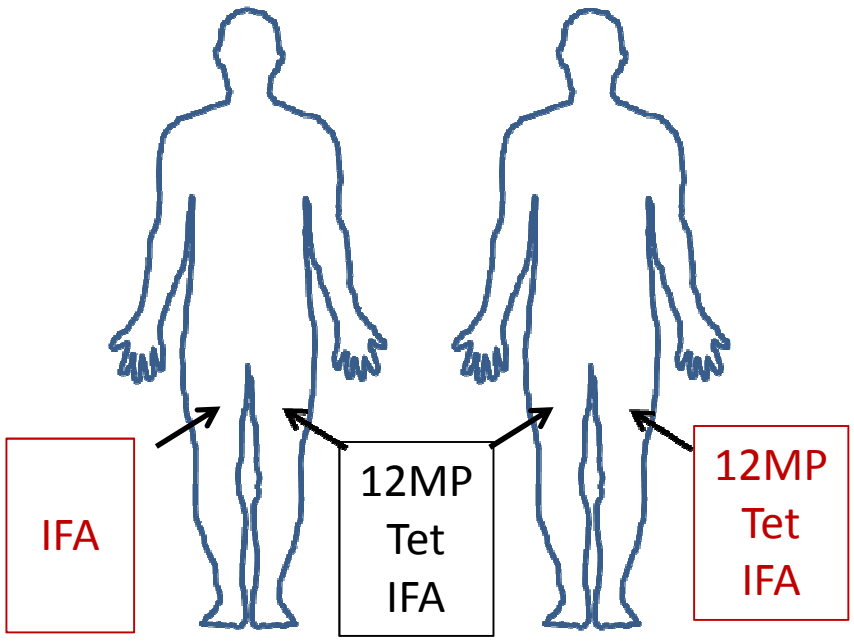
Mel48: A multipeptide vaccine in melanoma patients with evaluation of the injection site microenvironment

Group 1

Vaccines:
Wks 0, 1, 2, 4, 5, 6

Biopsy replicate site:

Subgroup	Week
A	0
B	1
C	3
D	6
E	12



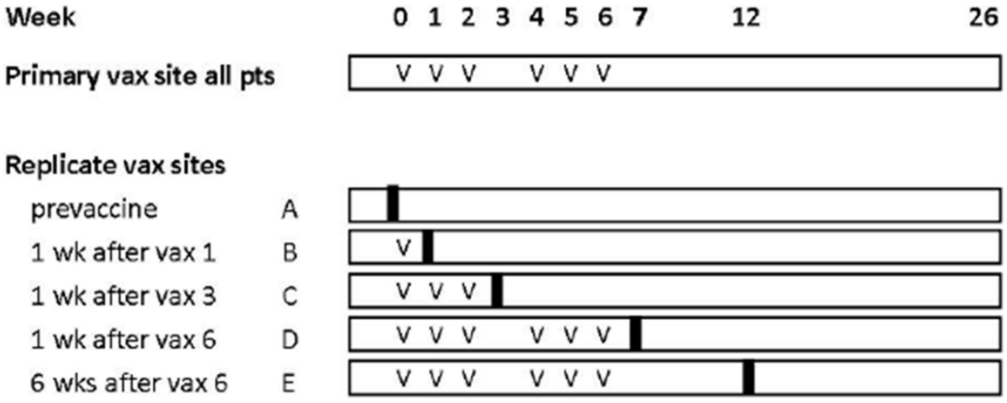
Group 2

Vaccines:
Wks 0, 1, 2, 4, 5, 6

Biopsy replicate site:

Subgroup	Week
A	0
B	1
C	3
D	6
E	12

Skin biopsies
2 x 6 cm excision
Flow, IHC, QF,
RNA later

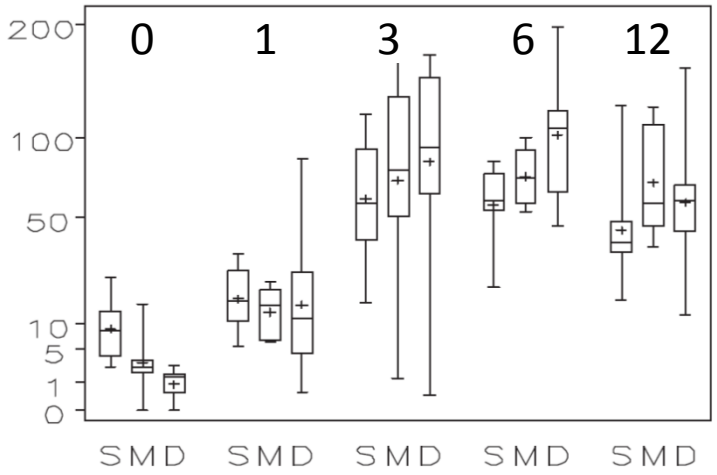


Schaefer.
J Transl Med
2012

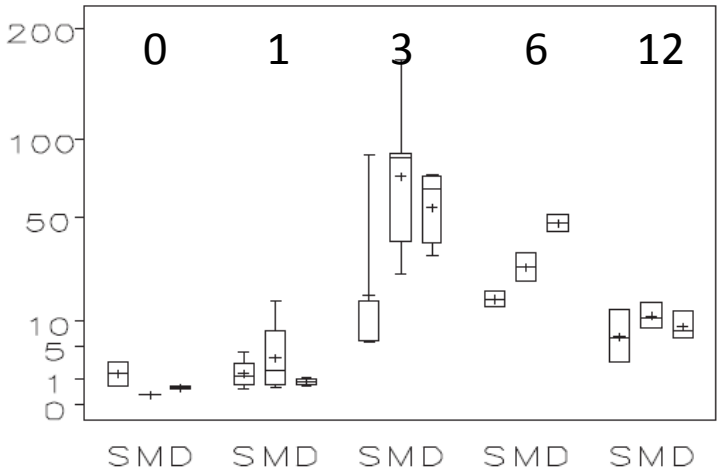
Cellular infiltrates in VSME (with IFA)

Cells per HPF

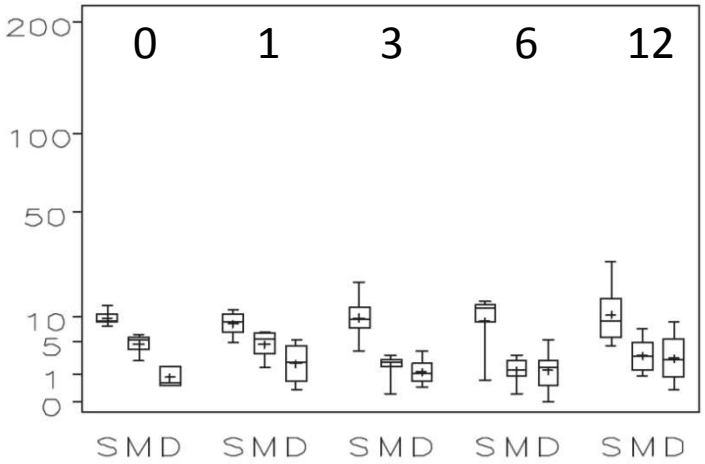
CD3



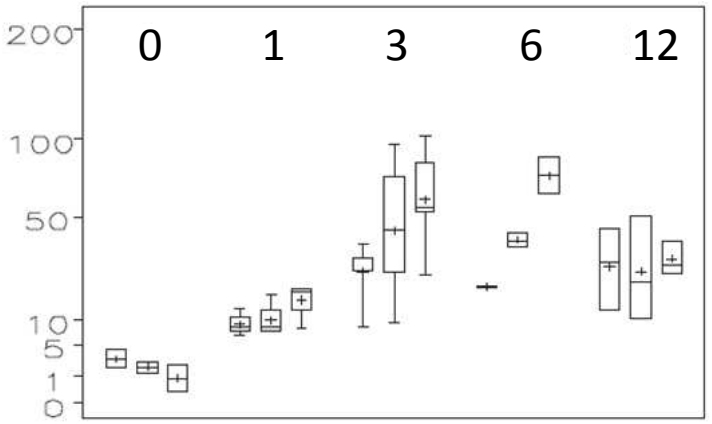
T-bet (Th1)



CD83



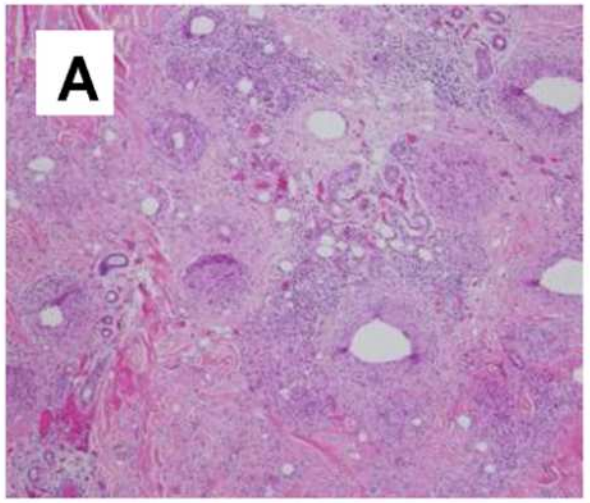
GATA-3 (Th2)

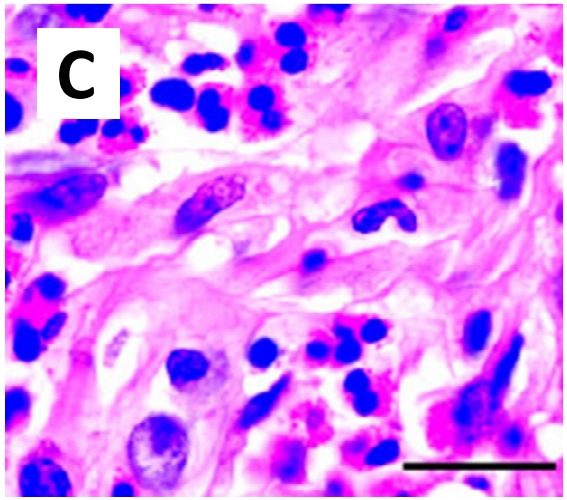


GATA3/T-bet ratio:

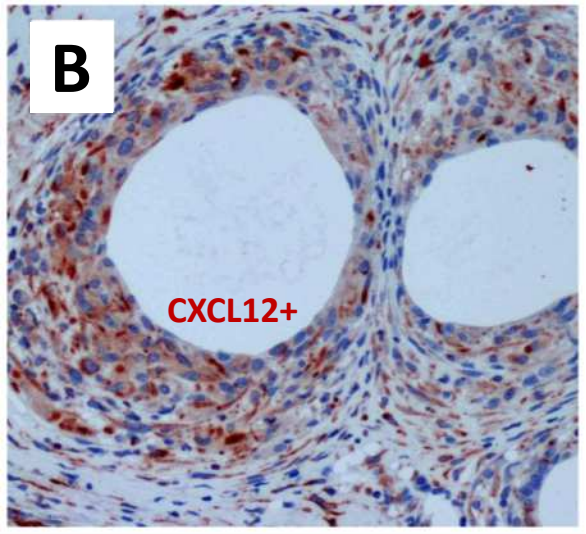
2.8 6.6 0.9 1.8 2.9

Vaccine Site Micro-Environment (VSME)

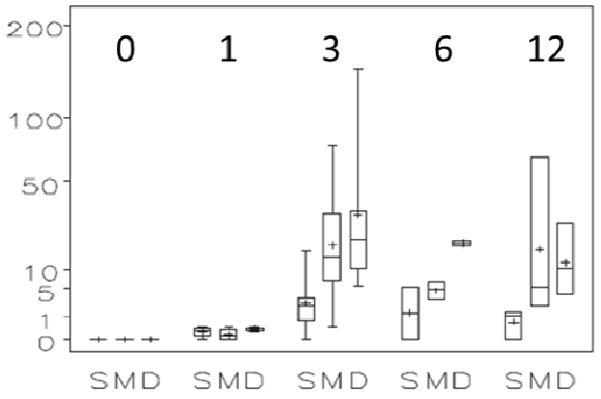




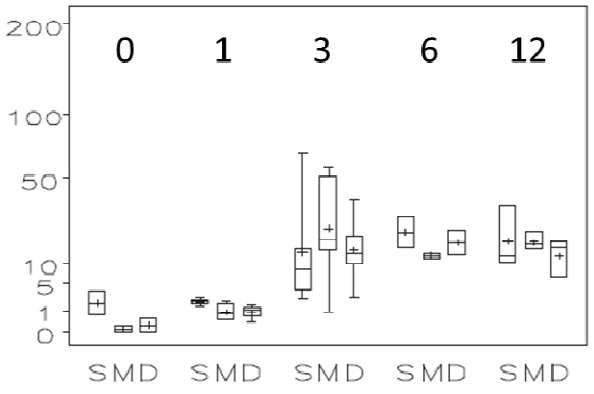
Late formation of granulomas; accumulation of eosinophils, FoxP3+ T regs



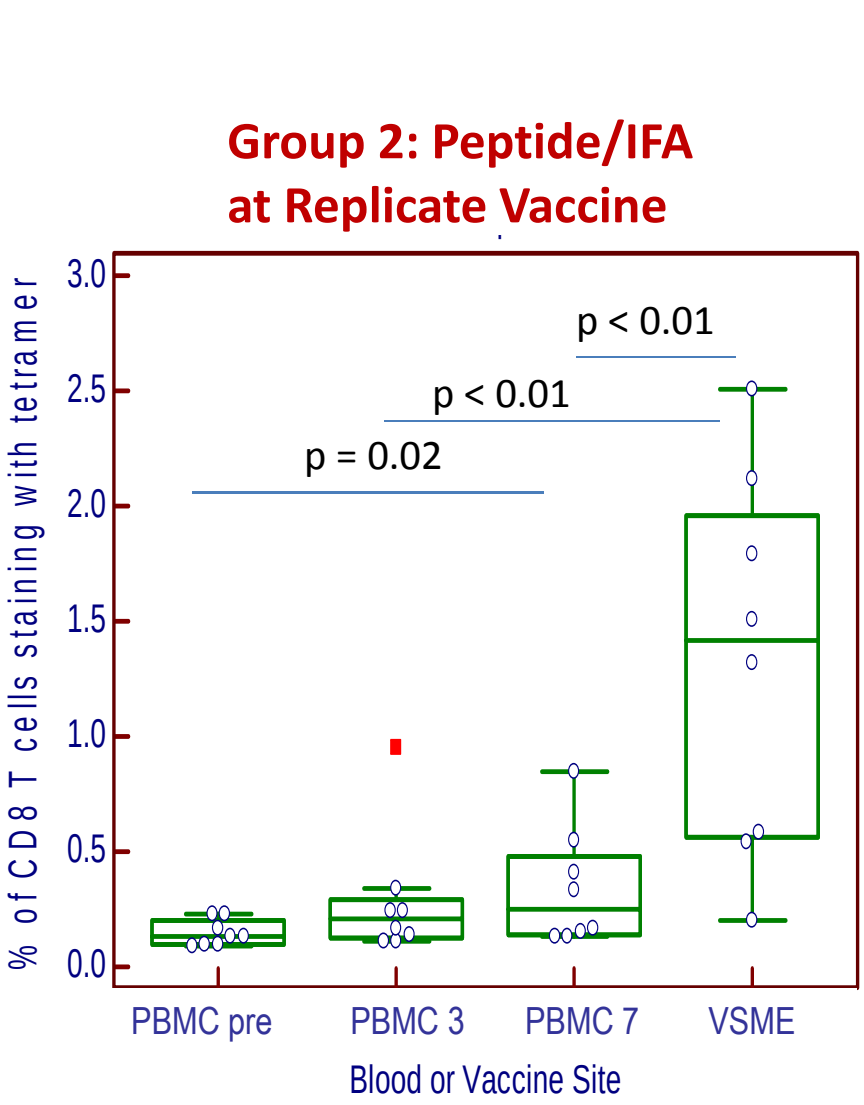
Eosinophils

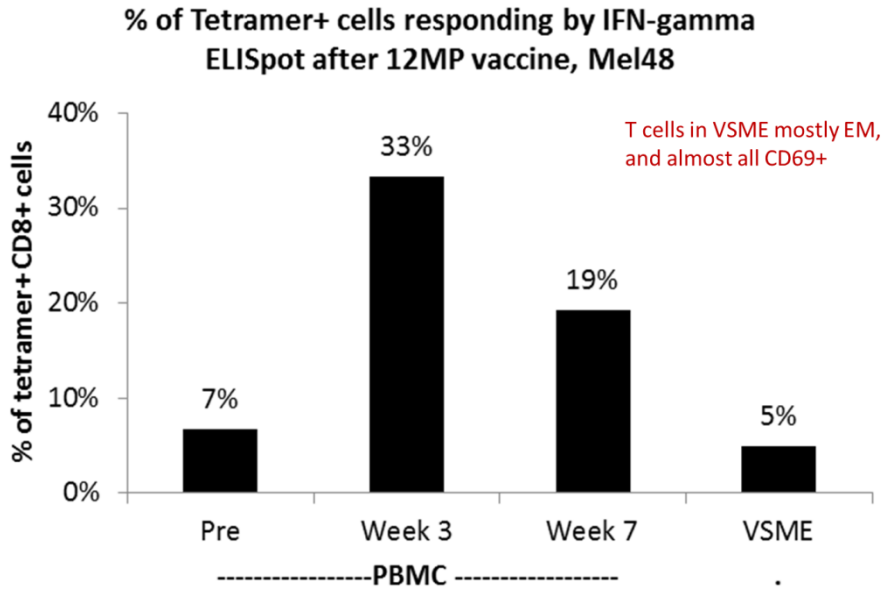


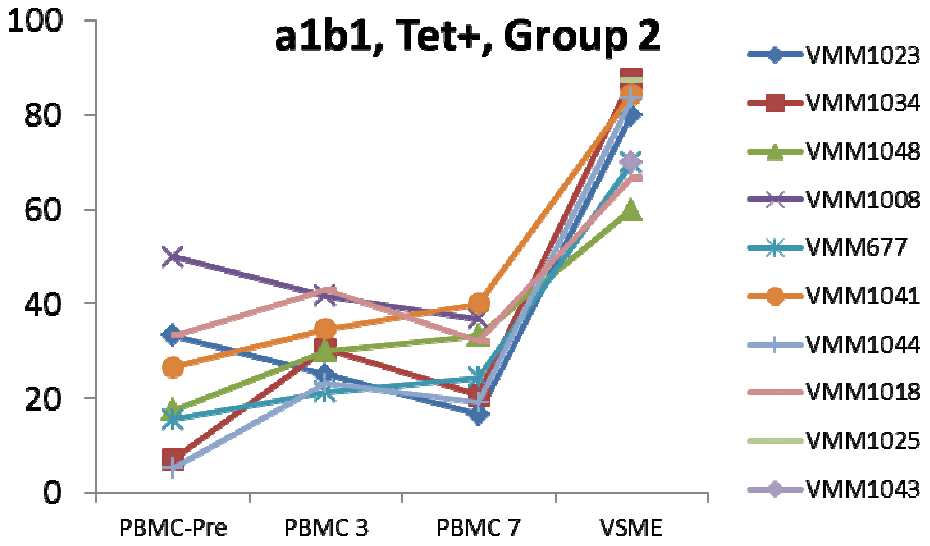
FoxP3



Tetramer+ CD8 T cells accumulate in the VSME, but are largely dysfunctional, and express high levels of retention integrins



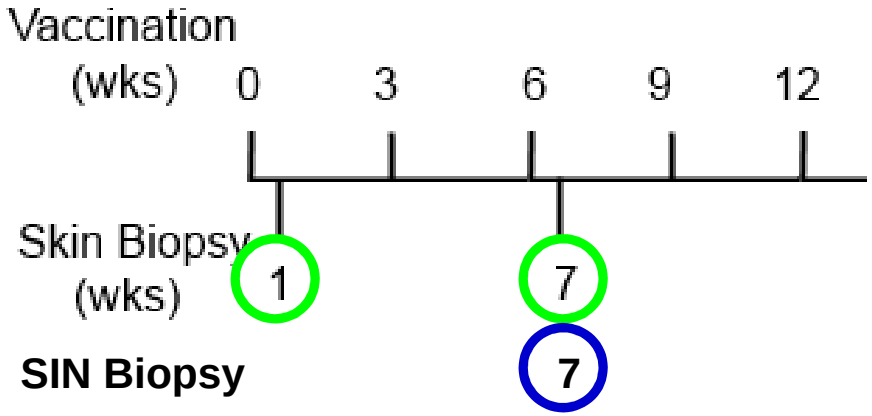




Mel 55: Clinical Trial to evaluate effects of recMAGE-A3 + AS15 Immunotherapeutic at the vaccine site microenvironment

AS15: TLR4 agonist (MPLA), TLR9 agonist (CpG), saponin QS-21, liposomal

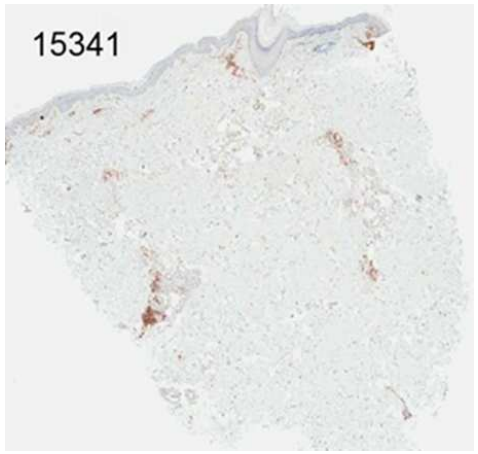
Study arm	Adjuvant	Injection site	Abbreviation	N (eligible)
A	AS15	intramuscular	M15	12
B	AS15	intradermal/subcutaneous	C15	12
Total				24

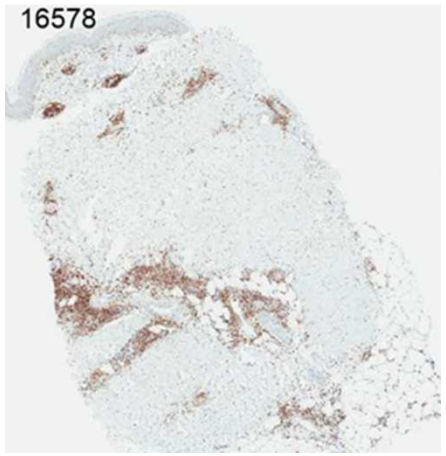


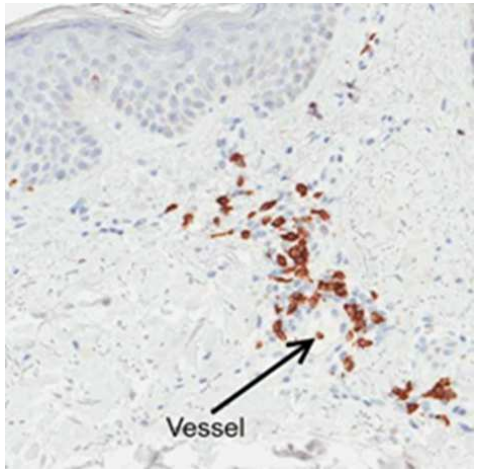
- Resected stage IIB-IV melanoma, MAGE-A3+ tumors

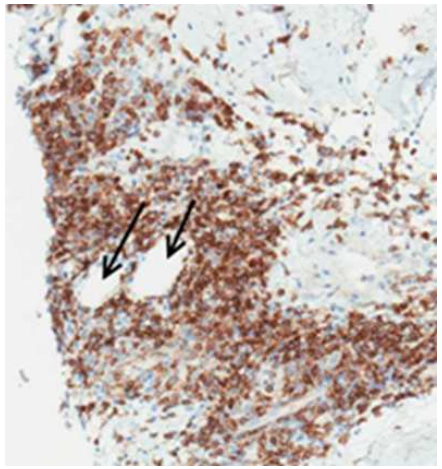
Perivascular lymphoid infiltrates induced by AS15 ASCI, with peripheral node addressin (PNAd) expression

VSME biopsies 1 week after the first vaccine

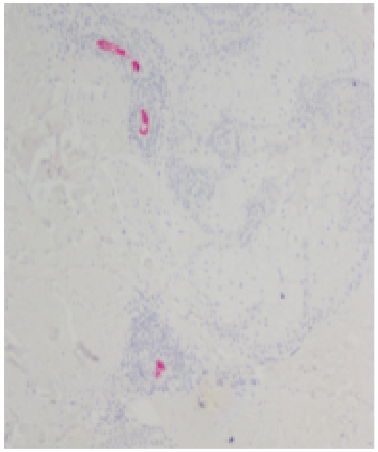


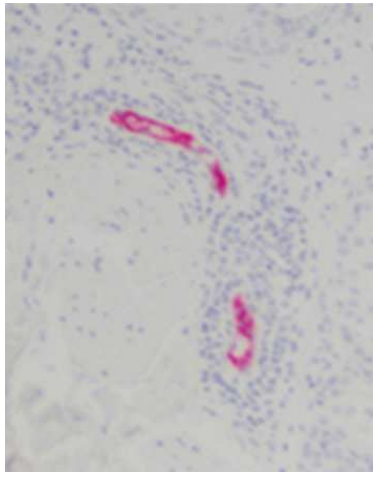






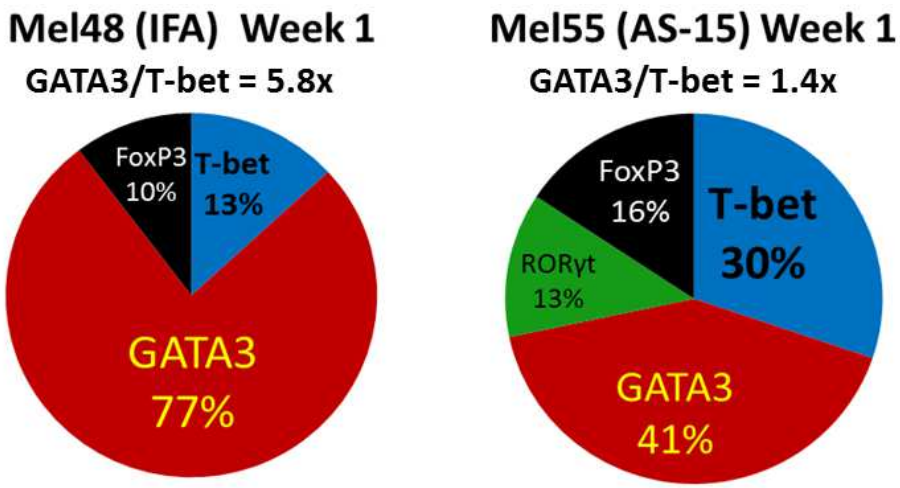
PNAd^{+} vessels

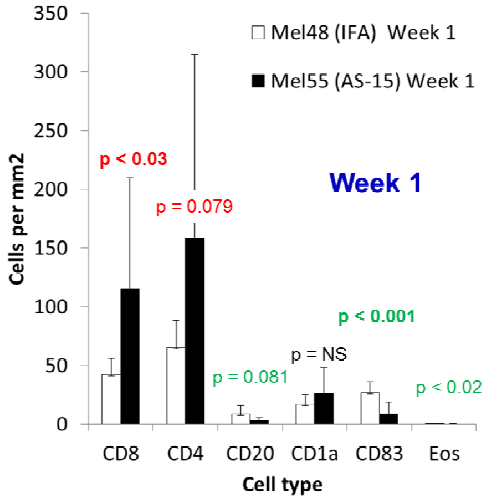


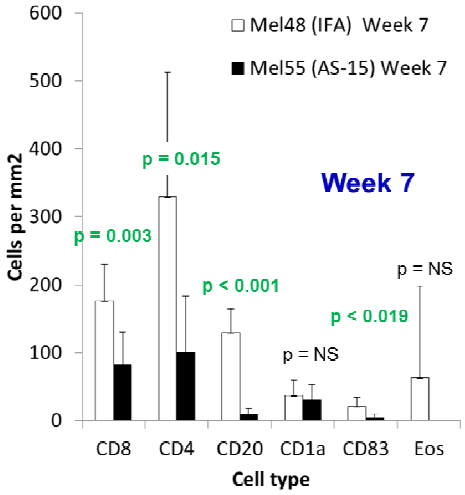


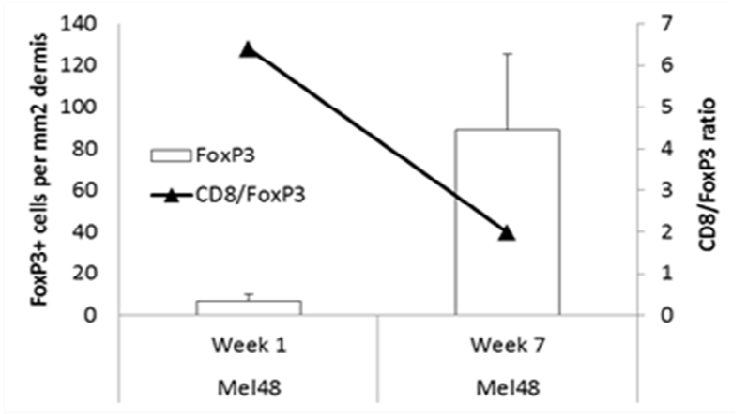
***PNA-d* antibody stains HEV-like vessels**

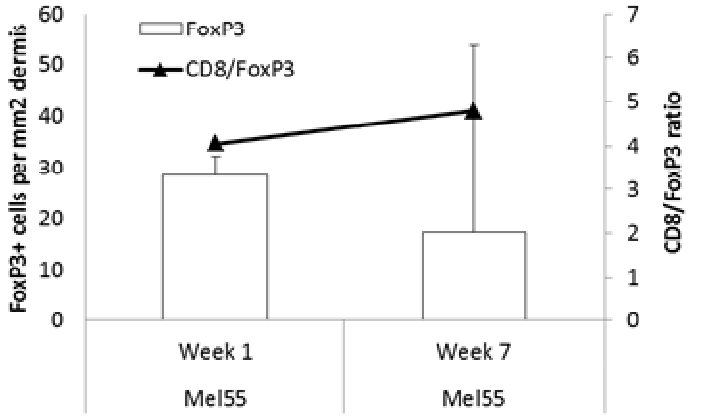
MAGE-A3/AS15 immunotherapeutic induces a 4x more favorable Th1/Th2 balance in the VSME early, than vaccine with IFA



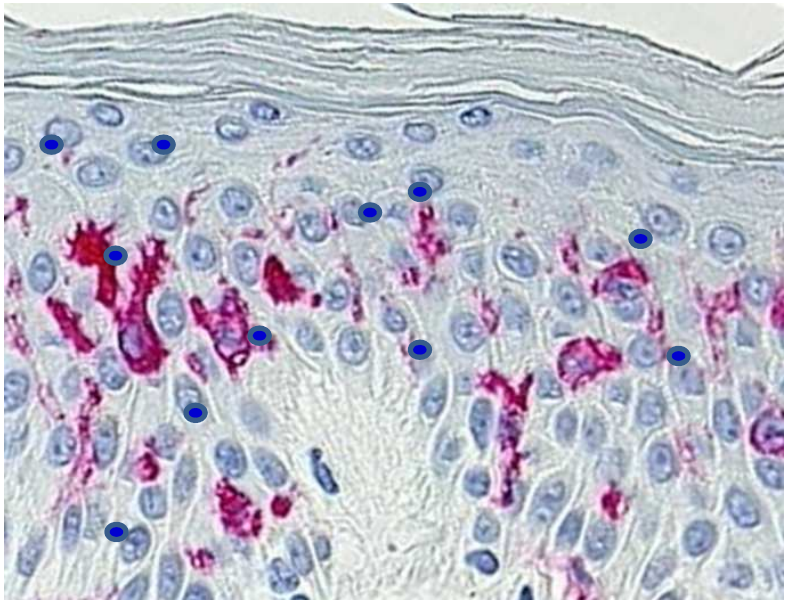


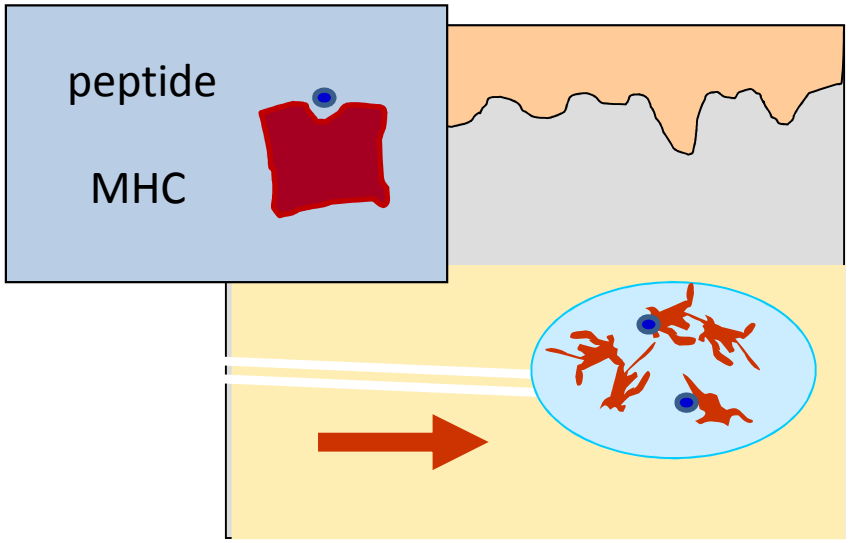


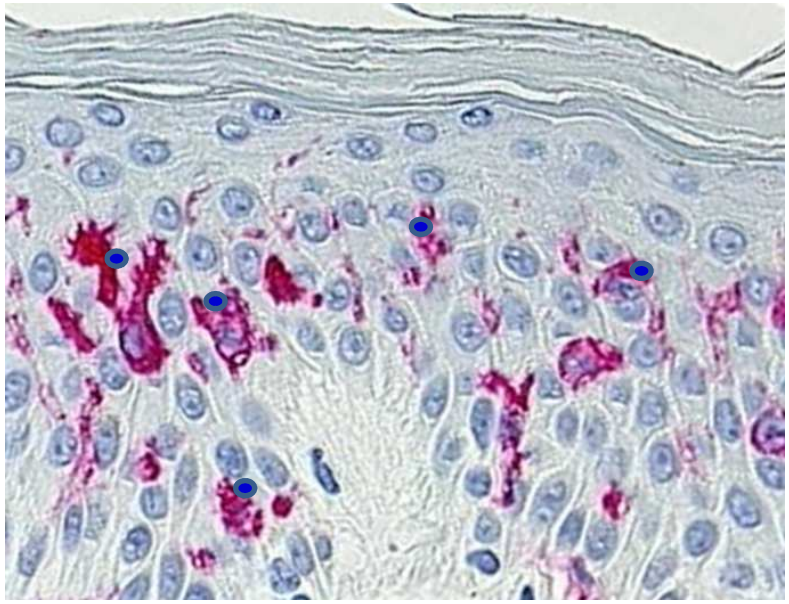


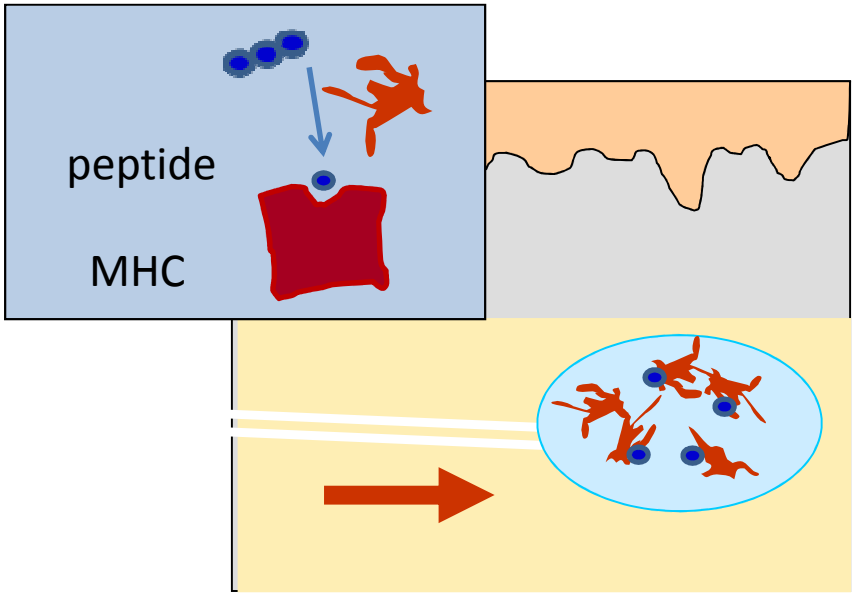


- Th1/Th2 ratio 4x more favorable.
- Higher FoxP3 early.
- Better CD8/FoxP3 ratio wk 7.
- Higher early T cell accumulation.
- Much lower immune cell retention









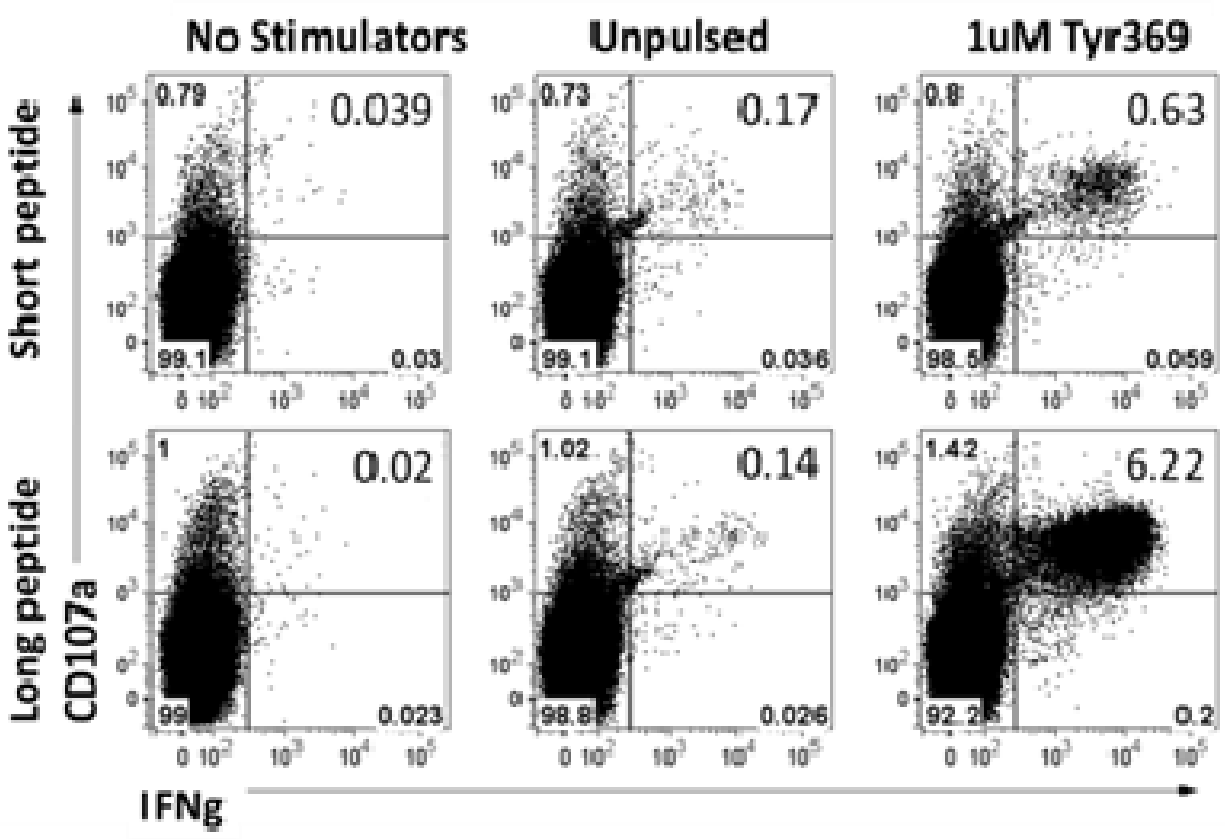
http://www.dermpath.de/cd1a.jpg

Long peptides for multipeptide vaccine (LPV7)

HLA restriction & protein source for short peptides		Amino acid sequences for short and long peptides	Residues for long peptides
A1	Tyrosinase $_{240\text{-}251S}$	FTIPYWDWR DAEKSDICTDEY MGGQHPTN	231-259 (29)
A2	Tyrosinase $_{369\text{-}377}$	SMHNALHI YMDGTMSQV QGSANDPIFLLHH	361-390 (30)
	gp100 $_{209\text{-}217\text{-}2M}$	VPLAHSSSAFT IMDQVPFSV SVSQLRALDG	198-227 (30)
	MAGE-A10 $_{254\text{-}262}$	VIWEALNMM GLYDGMEHL IYGEPRKLLTQD	245-274 (30)
A3	gp100 $_{17\text{-}25}$	LLHLAVIG ALLAVGATK VPRNQDWLGVSRQL	9-39 (31)
	MAGE-A1 $_{96\text{-}104}$	SREEEGPSTSCILE SLFRAVITK KVADLVG	82-111 (30)
B35/51	NY-ESO-1 $_{94\text{-}102}$	GARGPESRLLEFYLA MPFATPMEA ELARRS	79-108 (30)

Peptides selected for inclusion of known immunogenic epitope, polar residues (solubility), avoidance of cysteine residues, N-terminal glutamine or glutamic acid.

Enhanced immunogenicity of long vs short peptide (tyrosinase/HLA-A2), in TLR adjuvant



data from Rebecca Obeng & Vic Engelhard

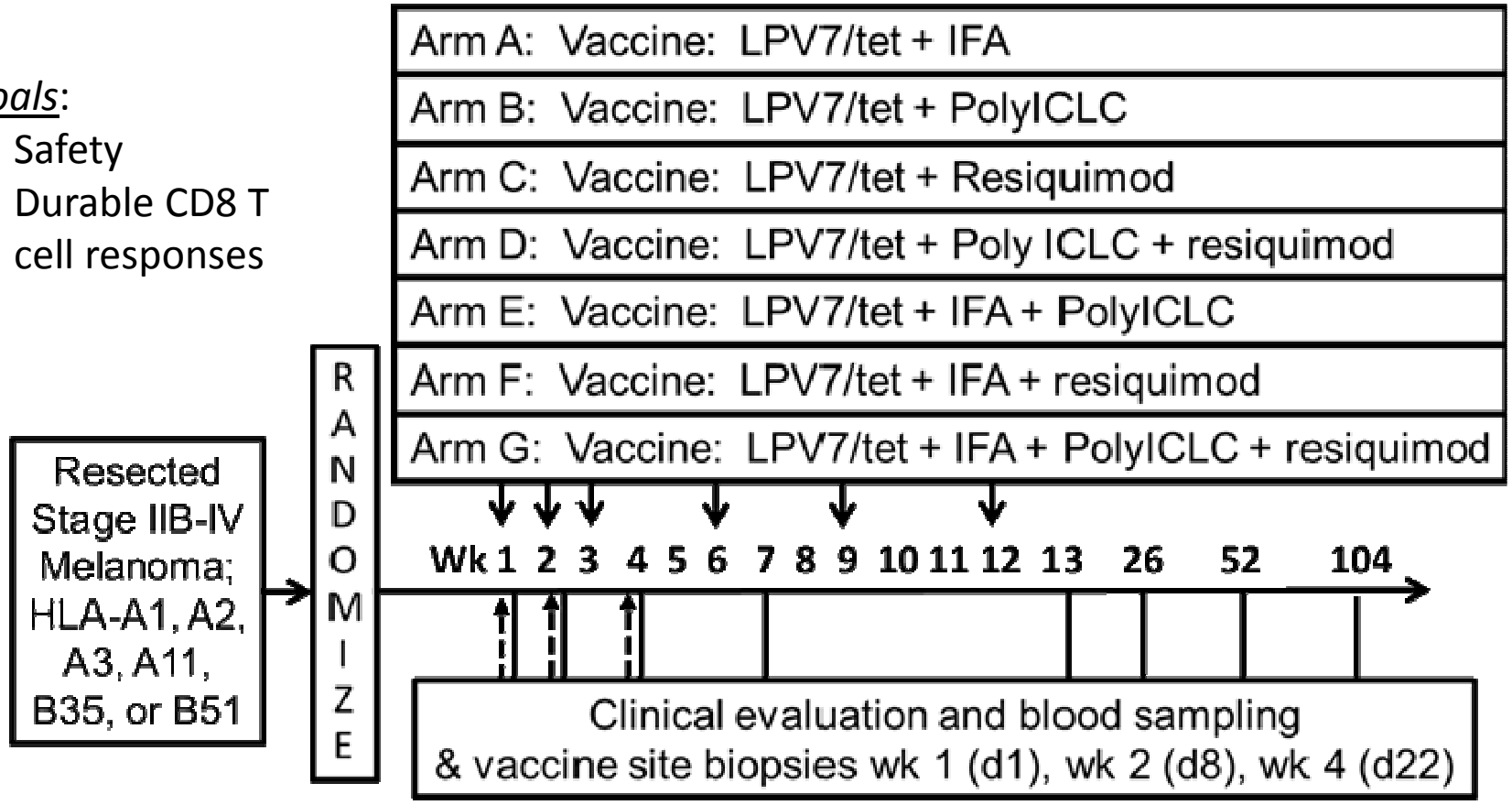
Albino HLA-A2 Tg mice were immunized with 100ug of the short or long tyrosinase peptide, 50ug FGK45 (anti-CD40) and 100ug CpG. Mice were revaccinated 6d later. CD8 T cells were enriched from the spleens and lymph nodes 5d after the 2nd vaccine. T cell response was evaluated by IFNg production and CD107a expression ex vivo.

Mel60 Protocol schema

University of Virginia & M.D. Anderson Cancer Center

Goals:

- Safety
- Durable CD8 T cell responses



Patients with resected high-risk melanoma will be randomized among 6 study arms. Each will be vaccinated wks 1, 2, 3, 6, 9, and 12 in an extremity (50% id/ 50% sc) with LPV7 plus tetanus helper peptide. PolyICLC will be injected at the vaccine site (1 mg). Resiquimod will be administered topically at the vaccine site, 112.5 mcg. Blood will be drawn wks 1, 2, 4, 7, 13, 26, 52, 104. Vaccine sites will be biopsied prevaccine (wk 1), and weeks 2 and 4.

Summary

- Cancer vaccines, including peptide vaccines, are weakly active clinically in melanoma and other cancers.
- Multipeptide vaccines can be administered safely with high rates of immune response.
- The role of GM-CSF as an adjuvant needs further scrutiny.
- Human data corroborate murine findings of T cell recruitment, retention to IFA vaccines, as possible explanation of transient immune responses.
- Improved outcomes may be anticipated with combination therapies to test optimized antigens, newer adjuvants including TLR agonists and CD40 agonists.

Future directions

- Optimized adjuvants
  - TLR agonists, role of IFA, cytokines
- Optimized antigen combinations
  - Neoantigens (Mutated antigens, Phosphopeptides, Cancer:testis, cancer:oocyte antigens, viral antigens)
  - Long peptides
  - Define role of helper peptides
- Define biologic effects of adjuvants on VSME and SIN to enable small trials to guide vaccine formulations.
- Modulate the tumor microenvironment
  - Combinations with intratumoral therapies
  - Combinations with systemic therapies

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Immune cell infiltrates in Vaccine Sites

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