Peptide Approaches to Melanoma Vaccines:
Innovations and Challenges

iSTBc/CVC Workshop
Alexandria, VA Nov 10 2005

Craig L. Slingluff, Jr., M.D.
Department of Surgery
Human Immune Therapy Center
University of Virginia, Charlottesville, VA

Supported by: NIH/NCI, Cancer Research Institute, Berlex, philanthropy

Contributors

Human Immune Therapy Ctr

Kim Bullock
Elizabeth Woodson
Scott Boerner
Donna Barnd
Gina Petroni
Mark Smolkin

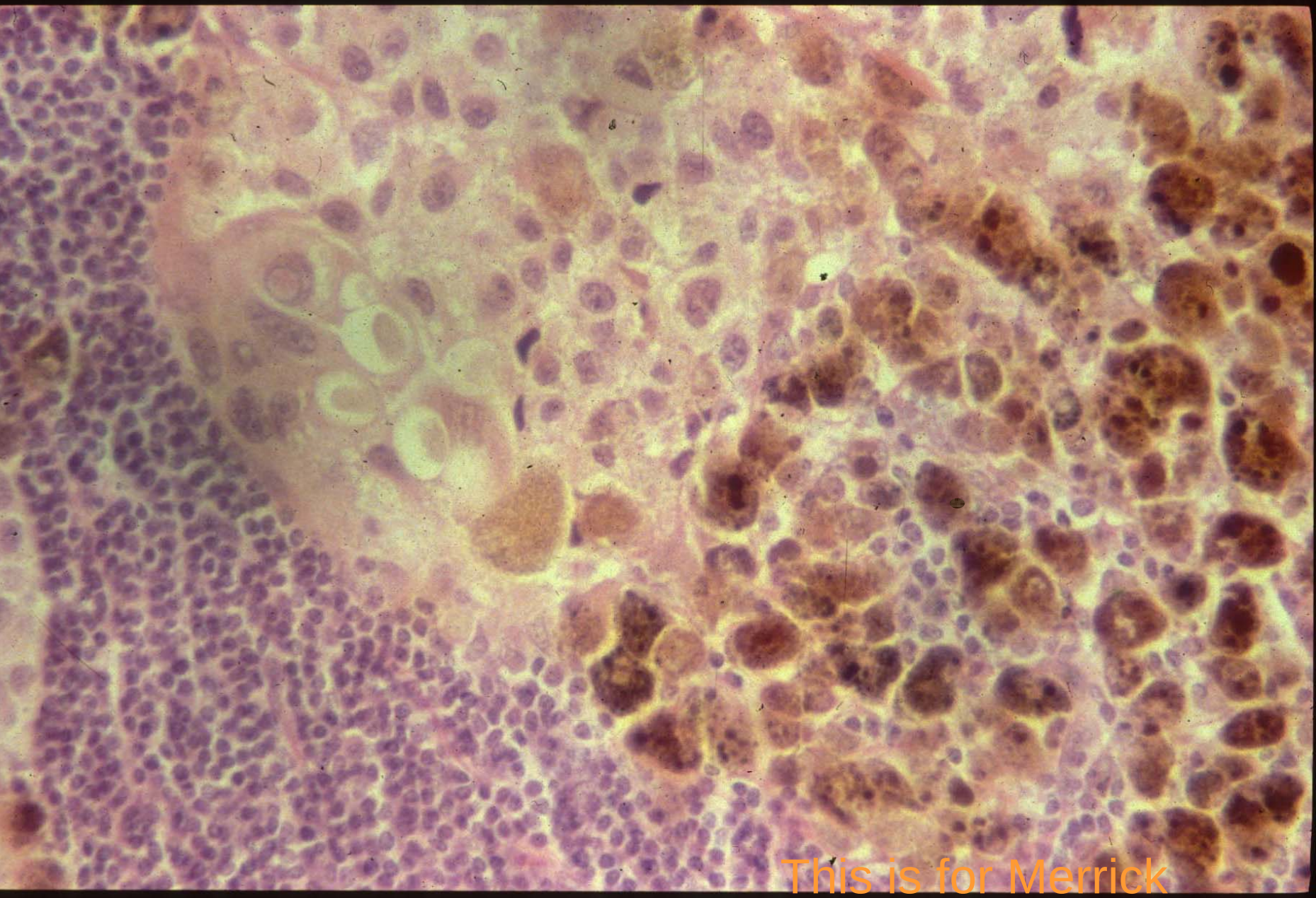
Melanoma Team

William W. Grosh
Geoff Weiss
Patrice Neese
Carmel Nail
Robyn Fink
James Patterson

Laboratory Analyses

Walter Olson
Galina Yamshchikov
Cheryl Murphy
Elizabeth Coleman
Donna Deacon
Courtney Garbee
Jessica Thistlethwaite
Sarah Hibbitts
Naomi Anderson
Chantel McSkimming

Collaborators – MD Anderson (Merrick Ross)
Fox Chase (Naomi Haas, Meg von Mehren),
Pittsburgh (John Kirkwood), Wash Hosp Ctr (Catlett/Boisvert)


This is for Merrick

Do vaccines work?

What is their job?

To induce immune responses…….

……YES they work

To induce clinical responses

……YES they work (3-5%)

Do they work well for clinical outcome?

..... No

Does any other systemic therapy work well for melanoma?

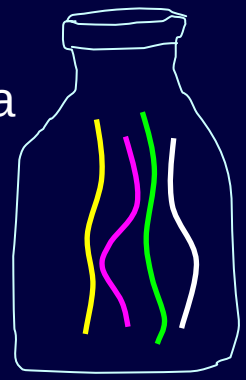
…… No

What is the best cancer vaccine?

- One that hasn’t been developed yet.

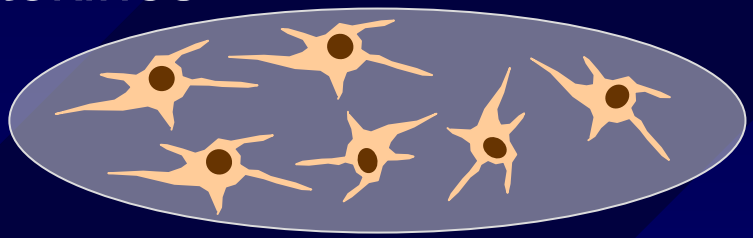
Dendritic cells:
targeting ex vivo and in vivo

melanoma peptides

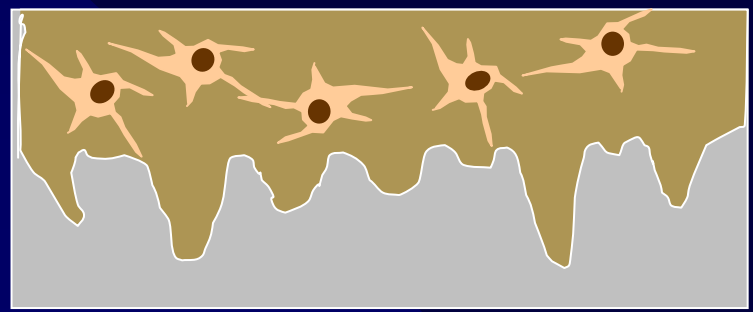


+

cytokines



+ Adjuvant

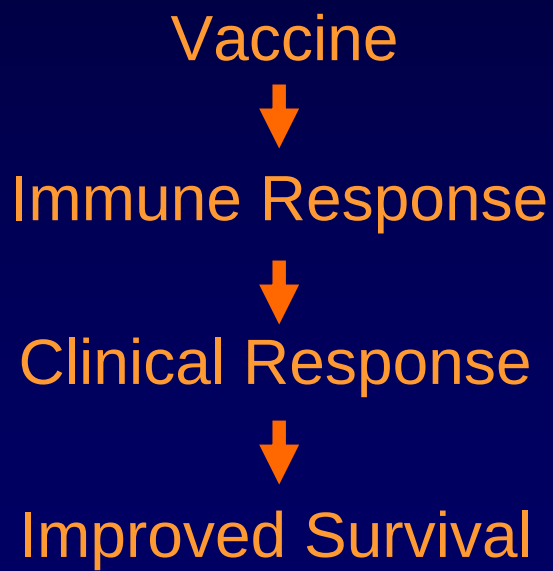


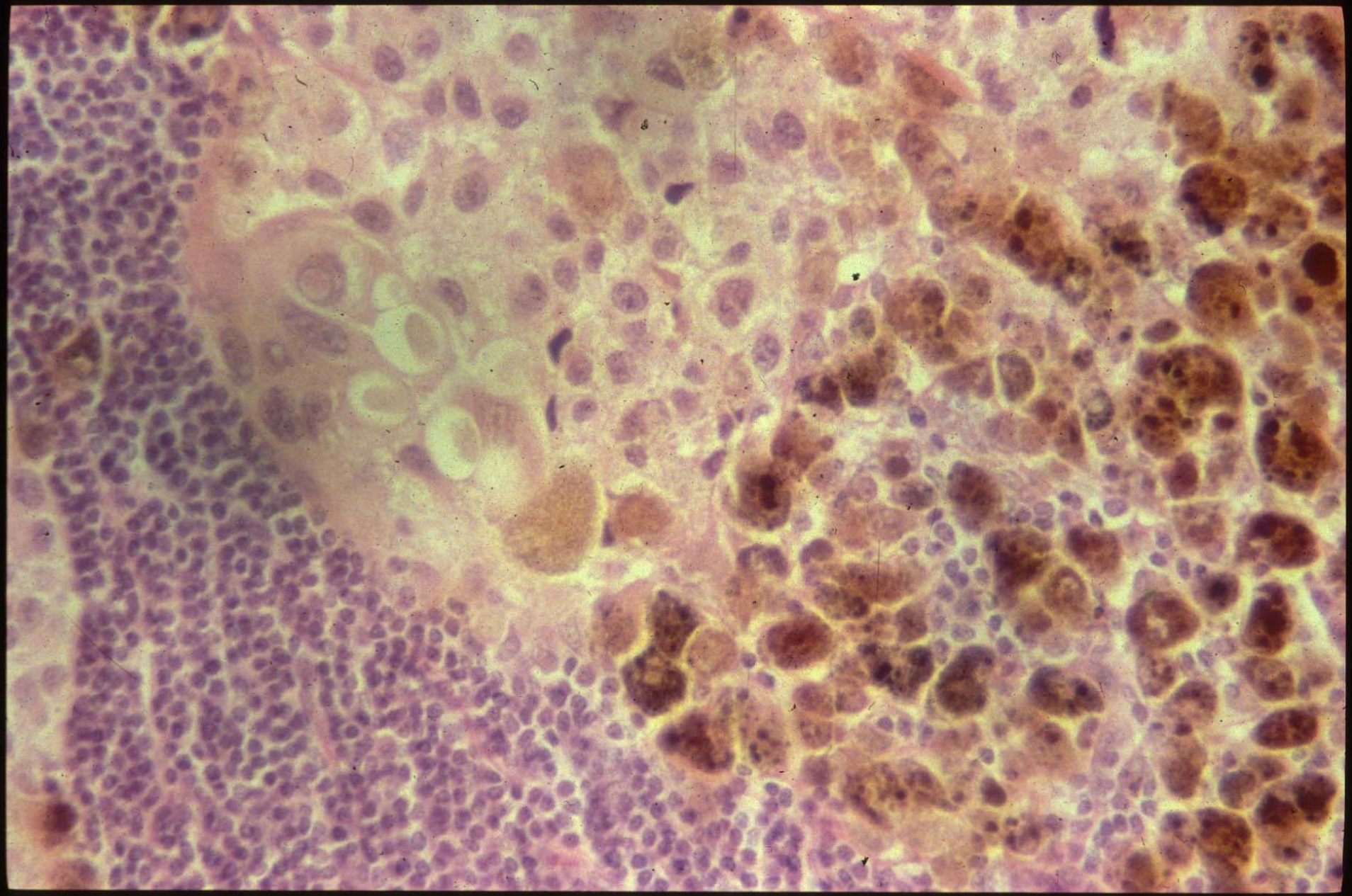
Why study peptide vaccines ?

1. Pure……Avoid tolerizing cellular antigens;
   exclude normal protein, avoid autoimmunity.
2. Processed…..Avoid effects of immunoproteasome.
3. Cheap……Feasible to study without corporate support.
4. Easier……Lower regulatory hurdles
5. Evaluable…....Excellent cancer vaccine model, allowing
   direct evaluation of response to the specific
   immunogen
6. Modifiable..…Create synthetic peptides better than native
   peptides
7. Immunogenic..Induce T cell responses in patients
8. Combinable….Multipeptide vaccines may mimic immune
   effects of whole cell vaccines.

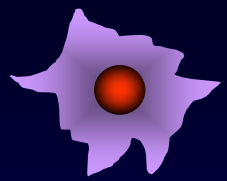
Early concept for vaccine therapy

- Immunologic ignorance
- Tumor antigens as weak antigens

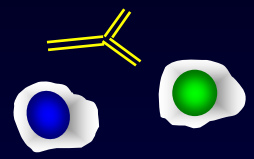




Immunologic pathways in melanoma progression



melanoma



p16 → RAS → BRAF, AKT → MAPK, mTOR

MHC loss → Ag/APM loss → IL-10 → Arginase, IDO

Pre-existing tolerance → Ag-spec Tc → Escape variants

Pre-existing tolerance → T_{reg} → Anergy

Failure of immune rejection
Or immune adaptation

Concept only

Approaches for Immune Therapy of Melanoma

Antibody

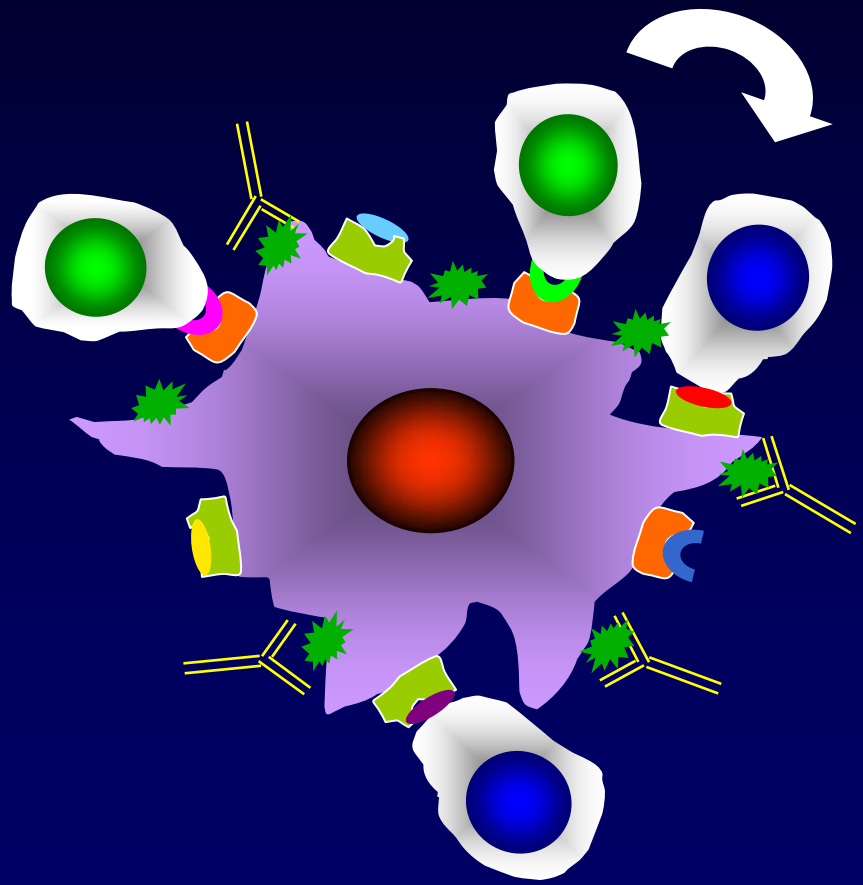
carbohydrates and proteins

* MHC Class I + peptide

CD8 killer T-cells

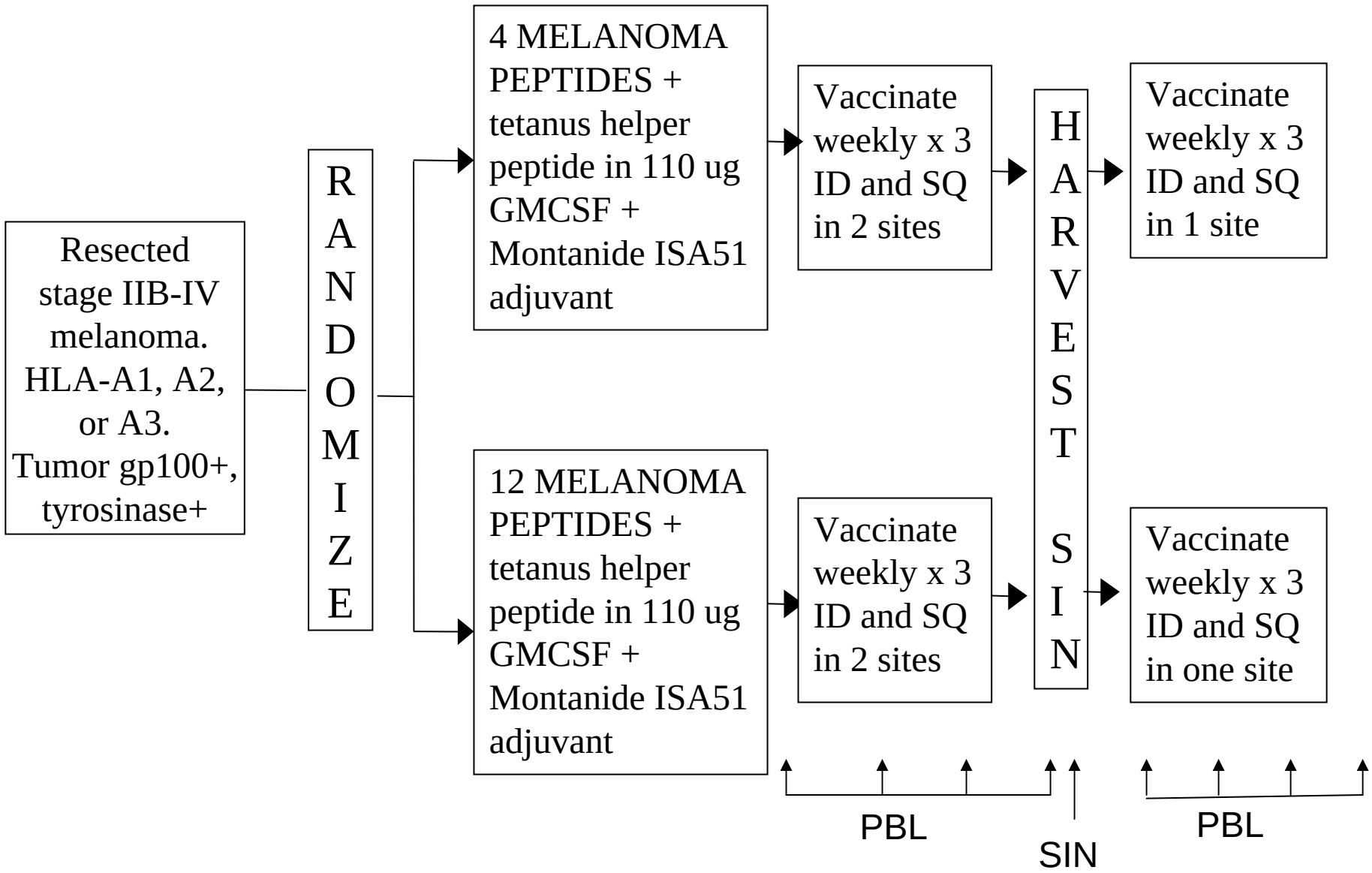
* MHC Class II + peptide

CD4 helper T-cells



Adoptive vs Active Immunotherapy

Typical Regimen for Multipeptide Vaccines – University of Virginia



12-peptide: MDPs and CTAs; 3 index peptides

- DAEKSDICTDEY tyrosinase 240-251S
- SSDYVIPIGTY tyrosinase 146-156
- EADPTGHSY MAGE-A1 161-169
- EVDPIGHLY MAGE-A3 168-176

HLA A1

- YMDGTMSQV tyrosinase 369-376D
- YLEPGPVTA gp100 280-288
- IMDQVPFSV gp100 209-217M
- GLYDGMEHL MAGE-A10 254-262

HLA A2

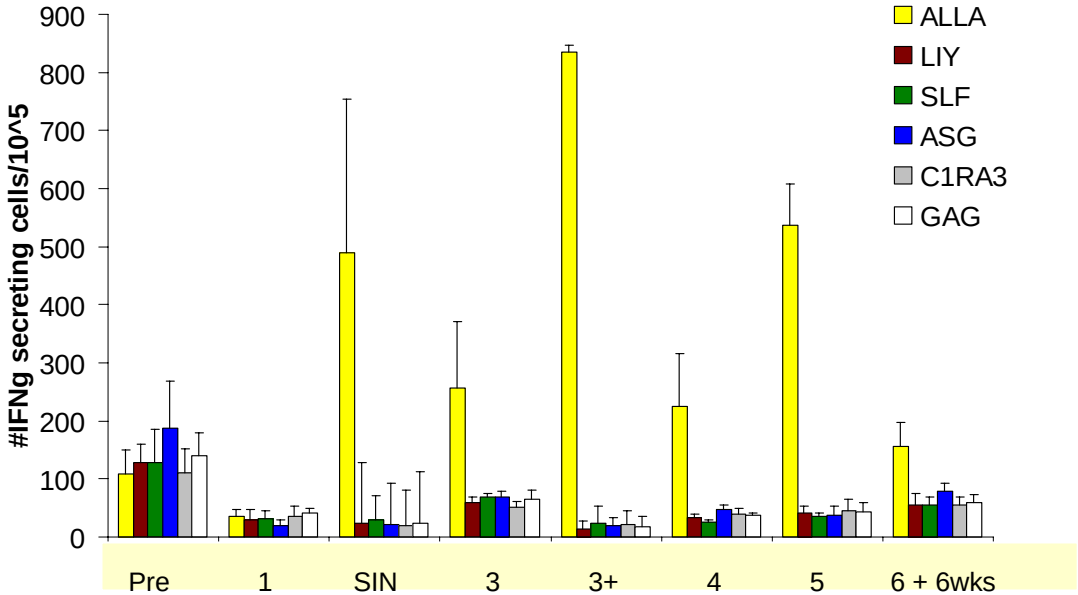
- ALLAVGATK gp100 17 - 25
- LIYRRRLMK gp100 614-622
- SLFRAVITK MAGE-A1 96-104
- ASGPGGGAPR* NY-ESO-1 53-62

HLA-A3

* A31/A3

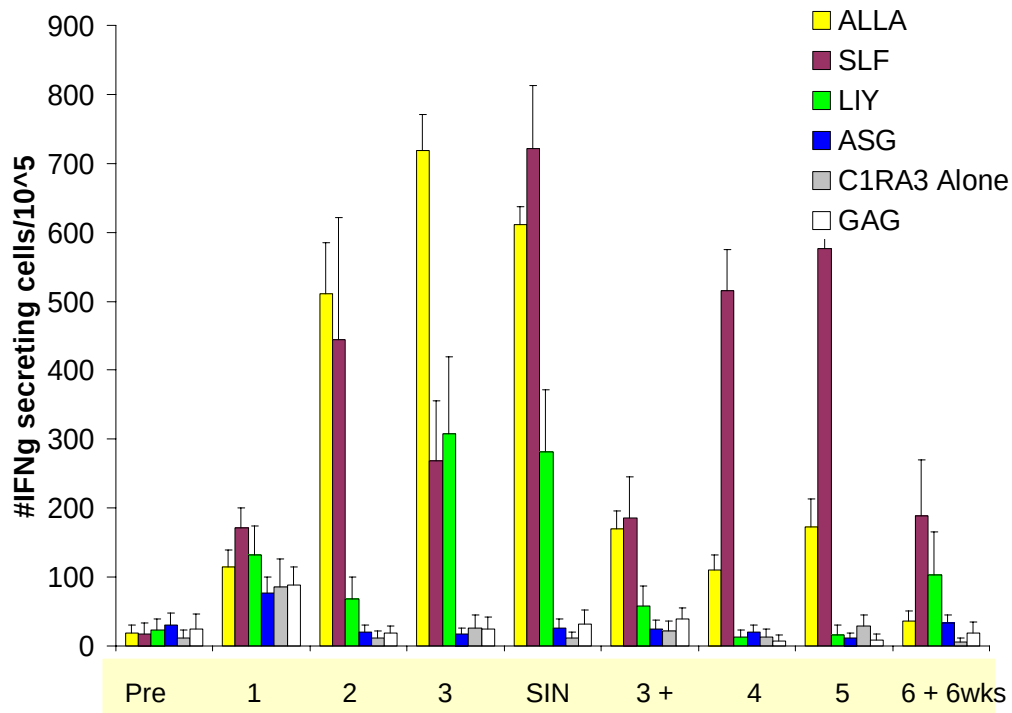
Arm A
(4 peptide mix):

Reactivity to Index peptide only

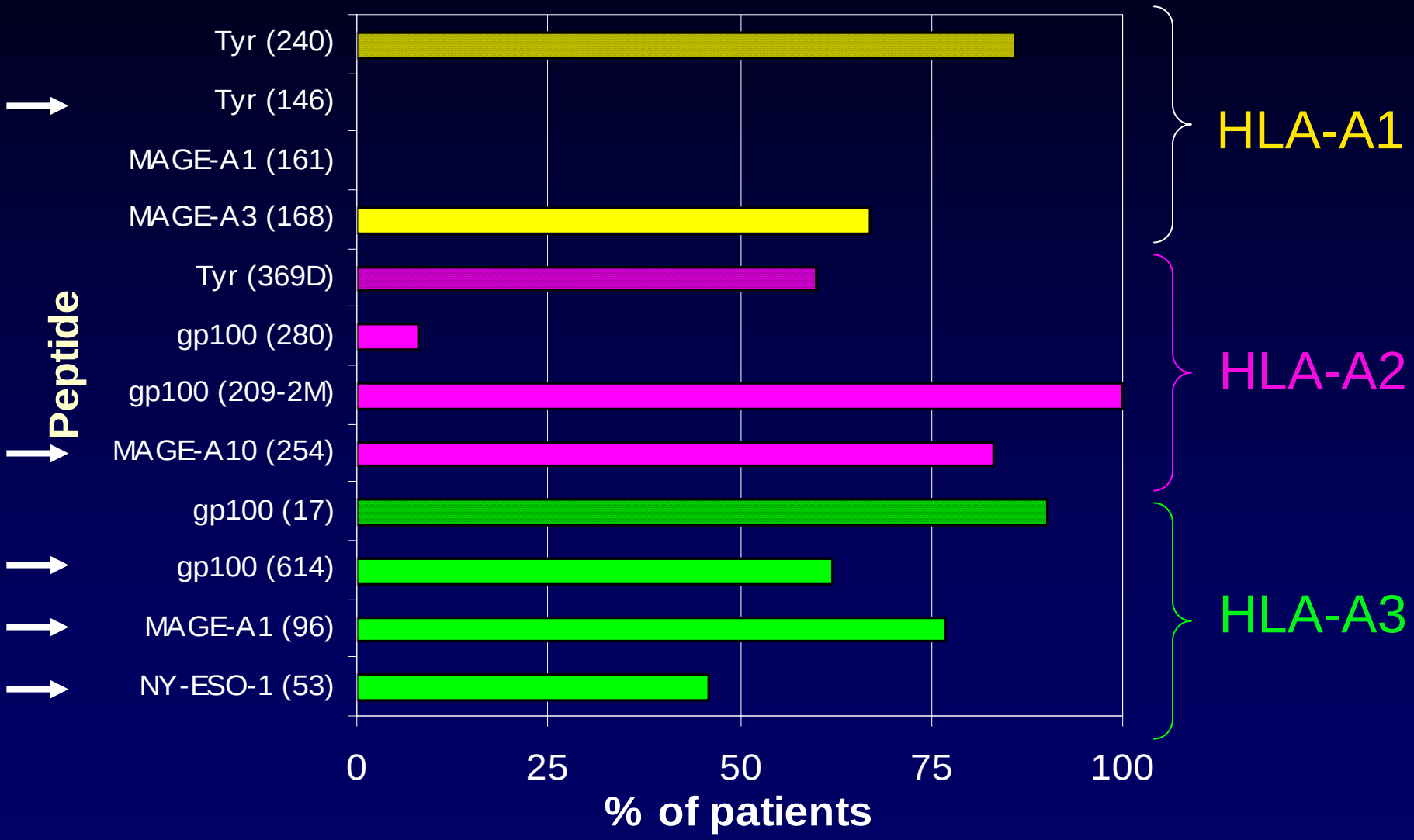


Arm B
(12 peptide mix):

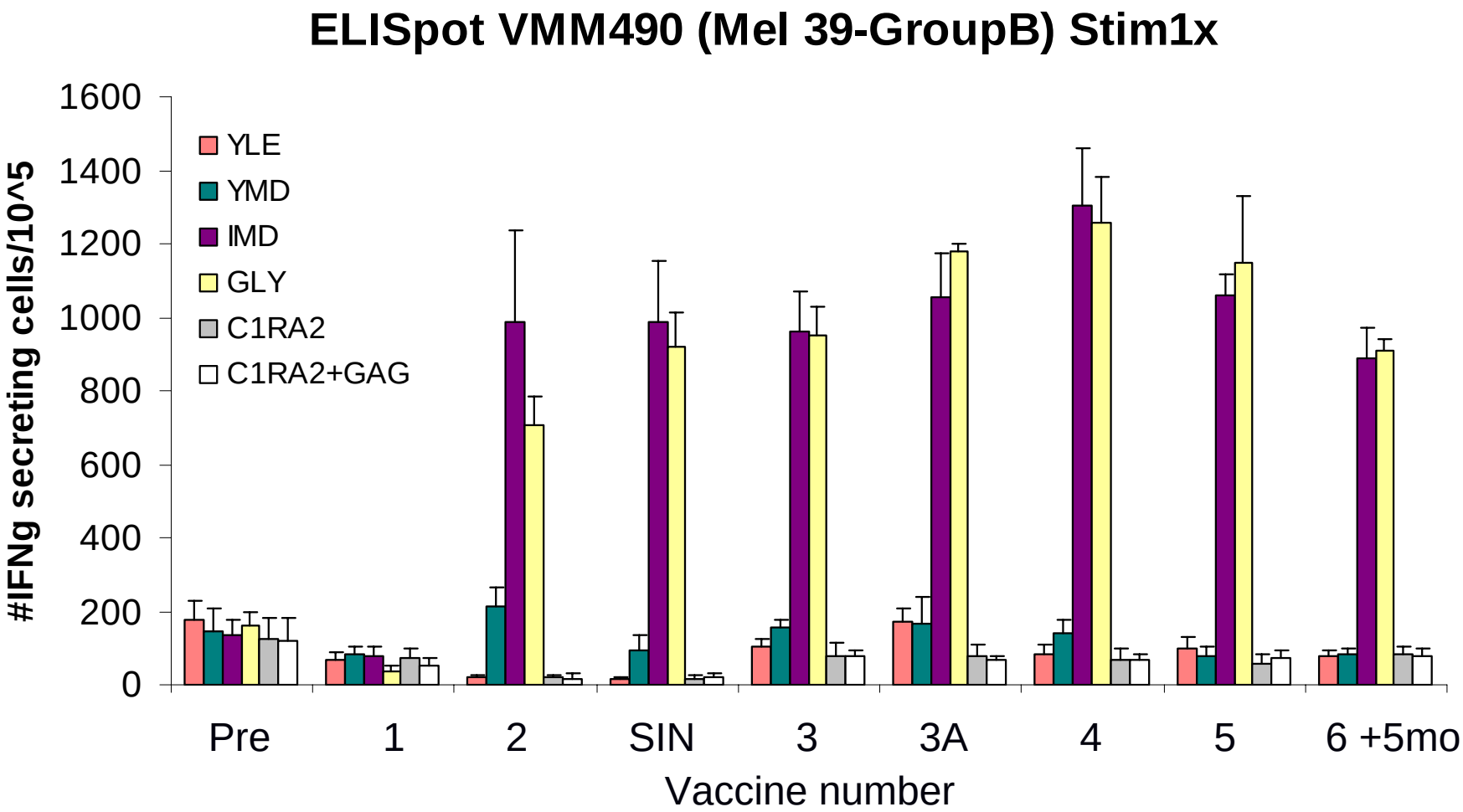
Reactivity to Index peptide + 2 others



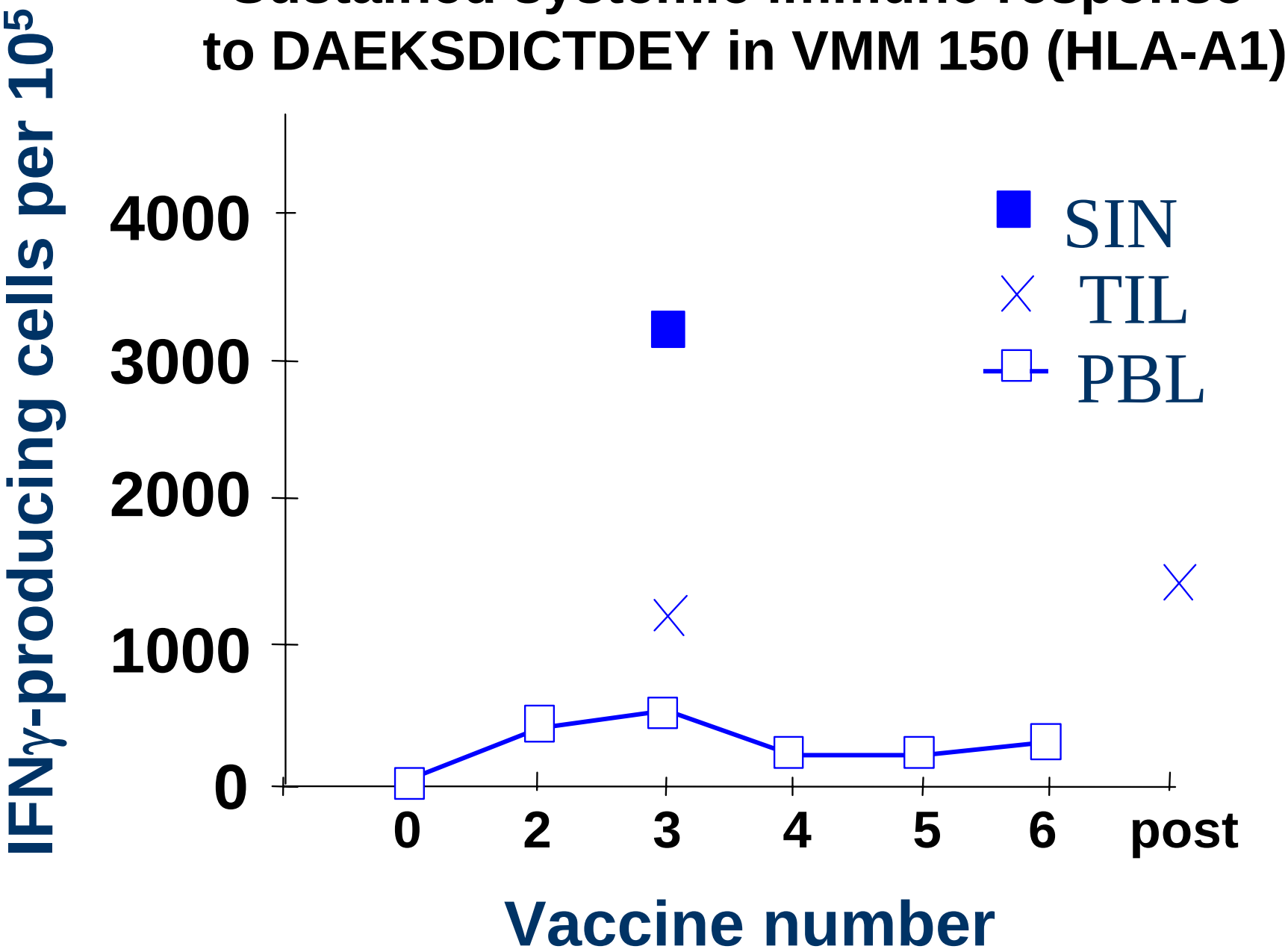
Immunogenicity of the 12 peptides

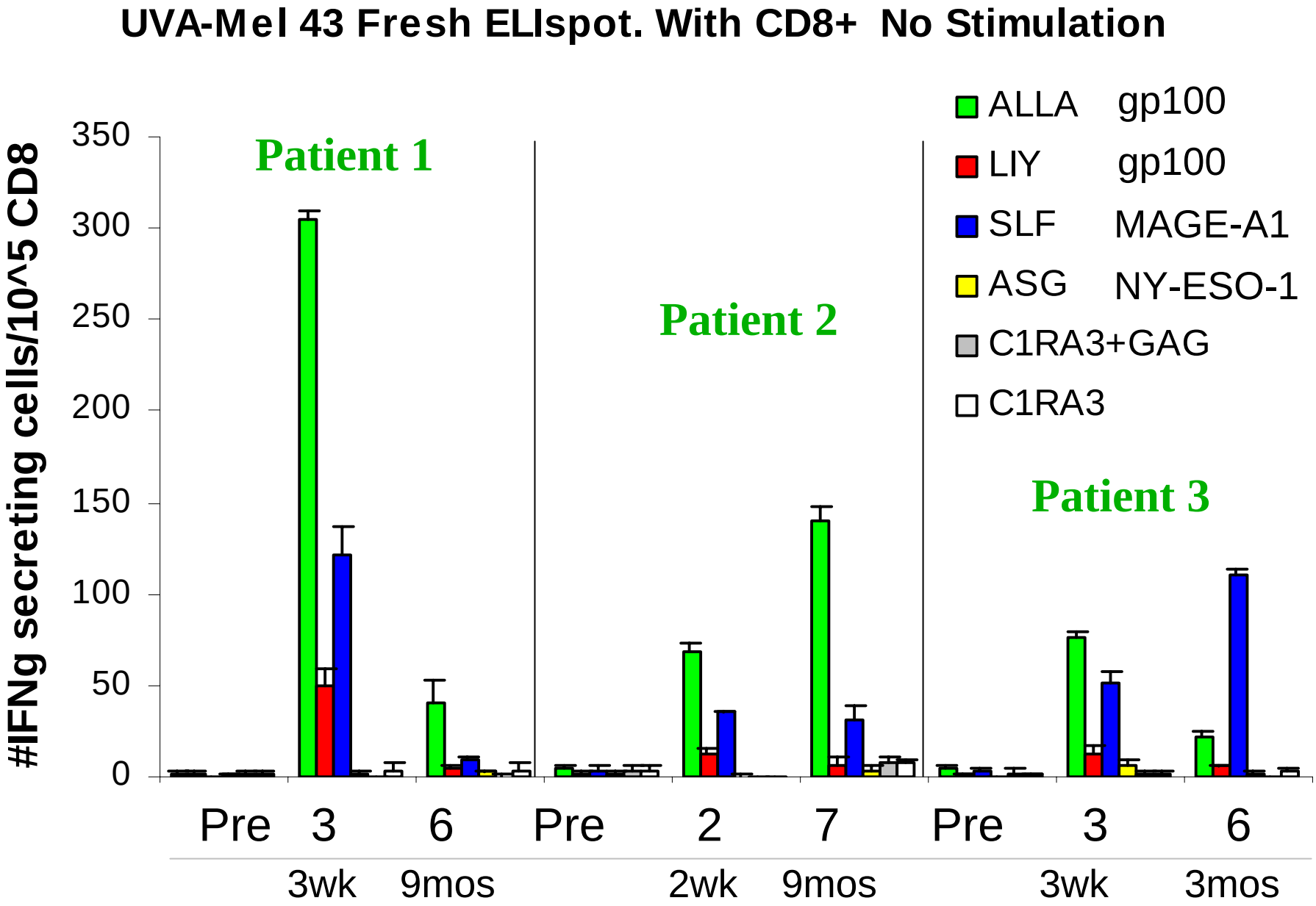


Persistent vaccine-induced responses to GP100-209-2M [IMD] & MAGE-A10 [GLY], transient response to tyrosinase [YMD]



Sustained systemic immune response to DAEKSDICTDEY in VMM 150 (HLA-A1)

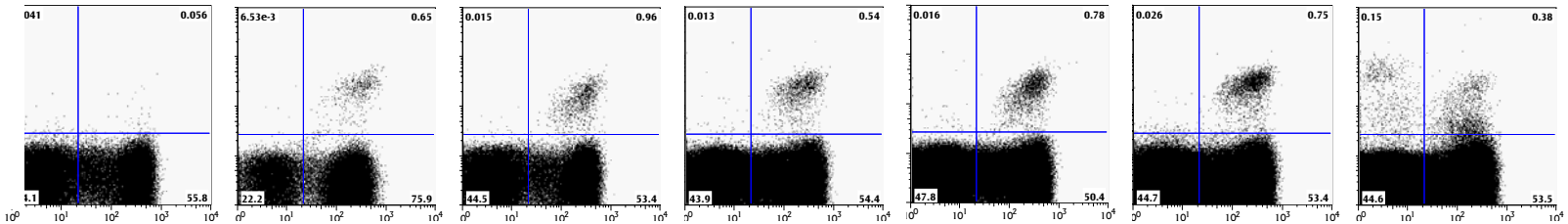



UVA-Mel 43 Fresh ELIspot. With CD8+ No Stimulation
#IFNg secreting cells/10^5 CD8
350
300
250
200
150
100
50
0
Patient 1
Patient 2
Patient 3
ALLA gp100
LIY gp100
SLF MAGE-A1
ASG NY-ESO-1
C1RA3+GAG
C1RA3
Pre
3
6
Pre
2
7
Pre
3
6
3wk
9mos
2wk
9mos
3wk
3mos

T cell responses to HLA-A3 / ALLAVGATK (gp100 $_{17-25}$)

PBL Prevaccine Week 0	SIN 3 vaccines Week 3	PBL 3 vaccines Week 3	PBL 3 vaccines Week 4	PBL 4 vaccines Week 5	PBL 5 vaccines Week 6	PBL 6 vaccines Week 12

Tetramer

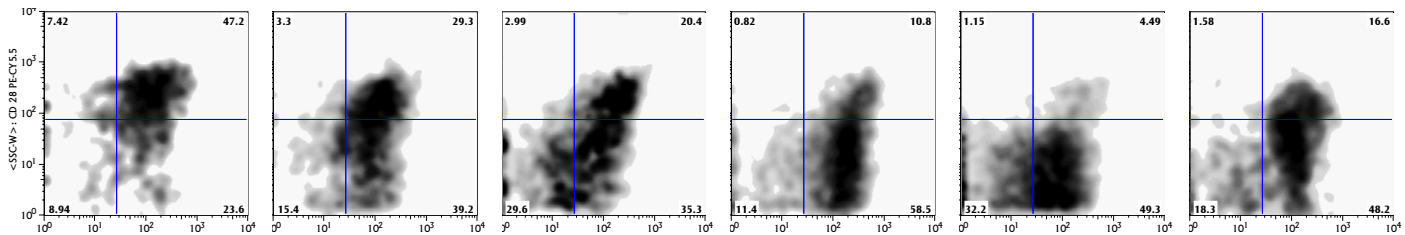


CD8

%tet+/CD8+:	0.10%	0.85%	1.77%	0.98%	1.52%	1.39%	0.71%

Staining of tetramer+ cells for markers of effector or memory phenotype:

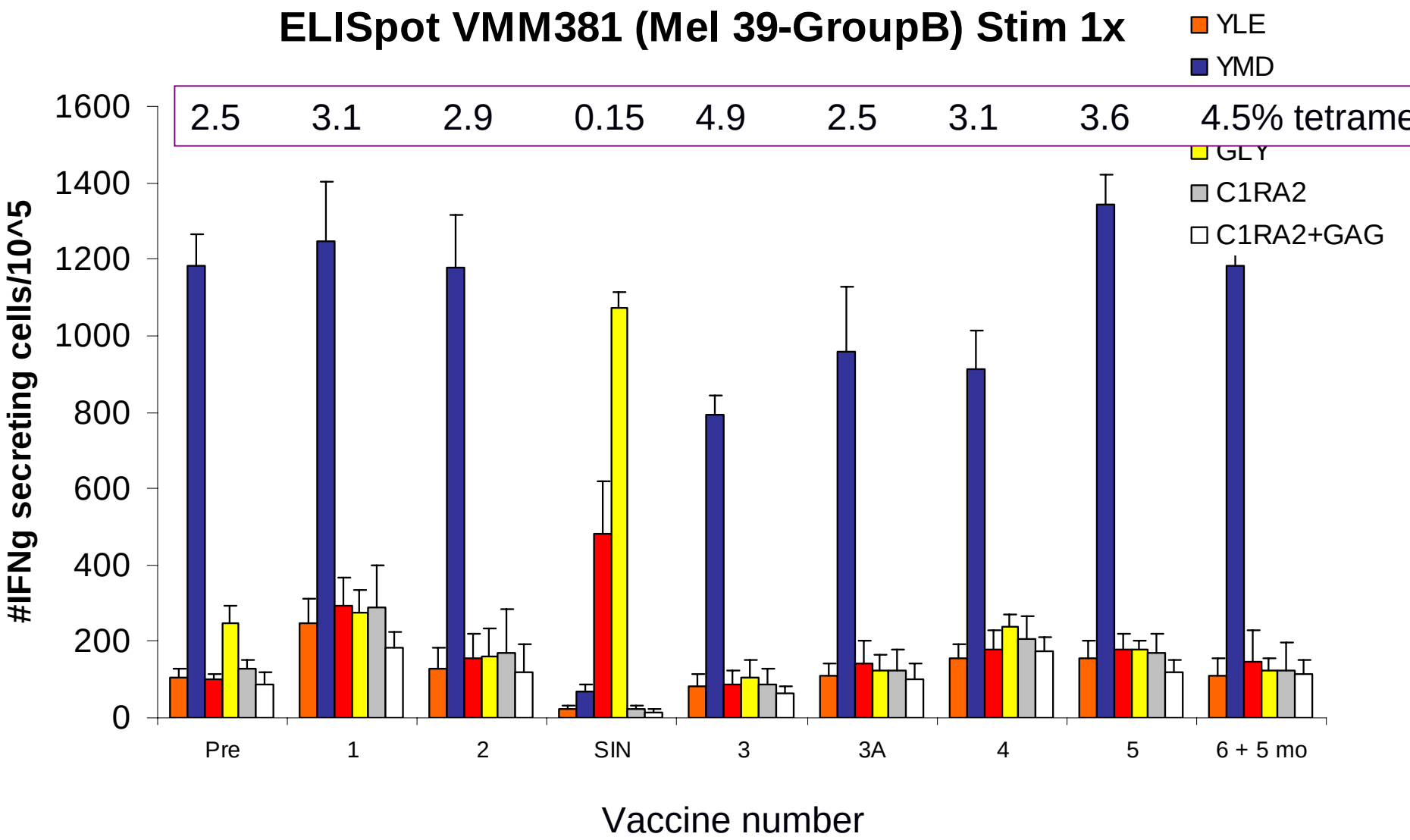
CD28



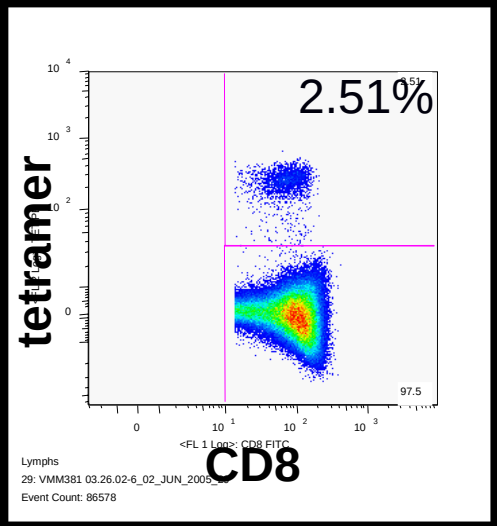
CD45RO

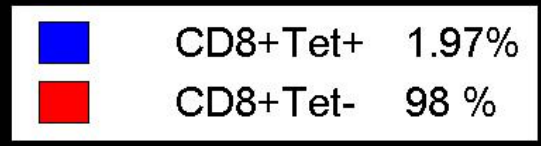
% CD45RO+/CD28+	47.2	29.3	20.4	10.8	4.5	16.6
% CD45RO-/CD28-	8.9	15.4	29.6	11.4	32.2	18.3
% CD28	54.6	32.6	23.4	11.6	5.6	17.2
% CD45RO	70.8	68.5	55.7	69.3	53.8	64.8
% CD62L	3.3	17.3	20.7	19.1	11.9	23.4
% CD27	78.0	58.0	70.4	59.7	38.2	51.5

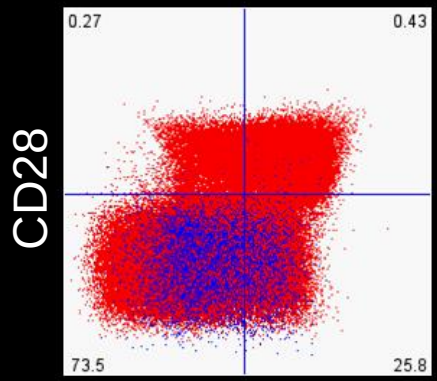
Tumor-induced response to YMD (Tyrosinase)
Transient vaccine-induced response to IMD, GLY

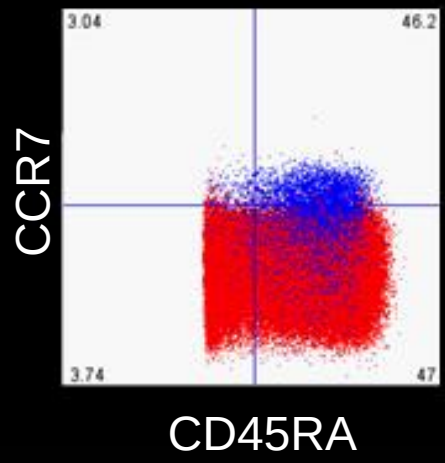


Stable Phenotype of Tumor-Induced CD8+ T cells reactive to Tyr $_{369\text{-}377D}$

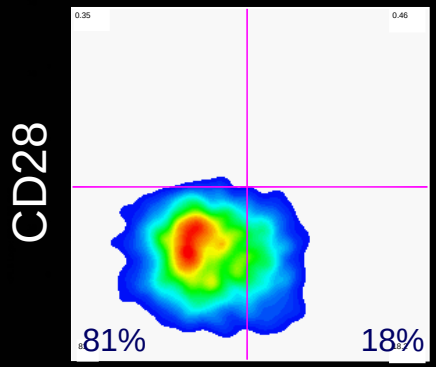


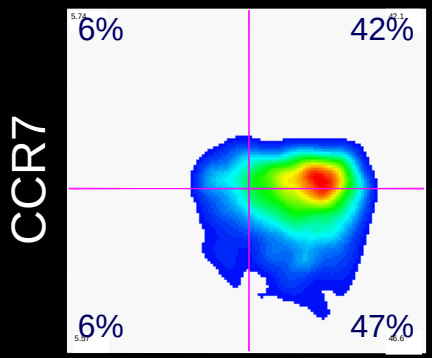






CD27 negative (80%)
CD28 negative
CD45RA positive (90%)
CCR7 pos/neg (50/50)





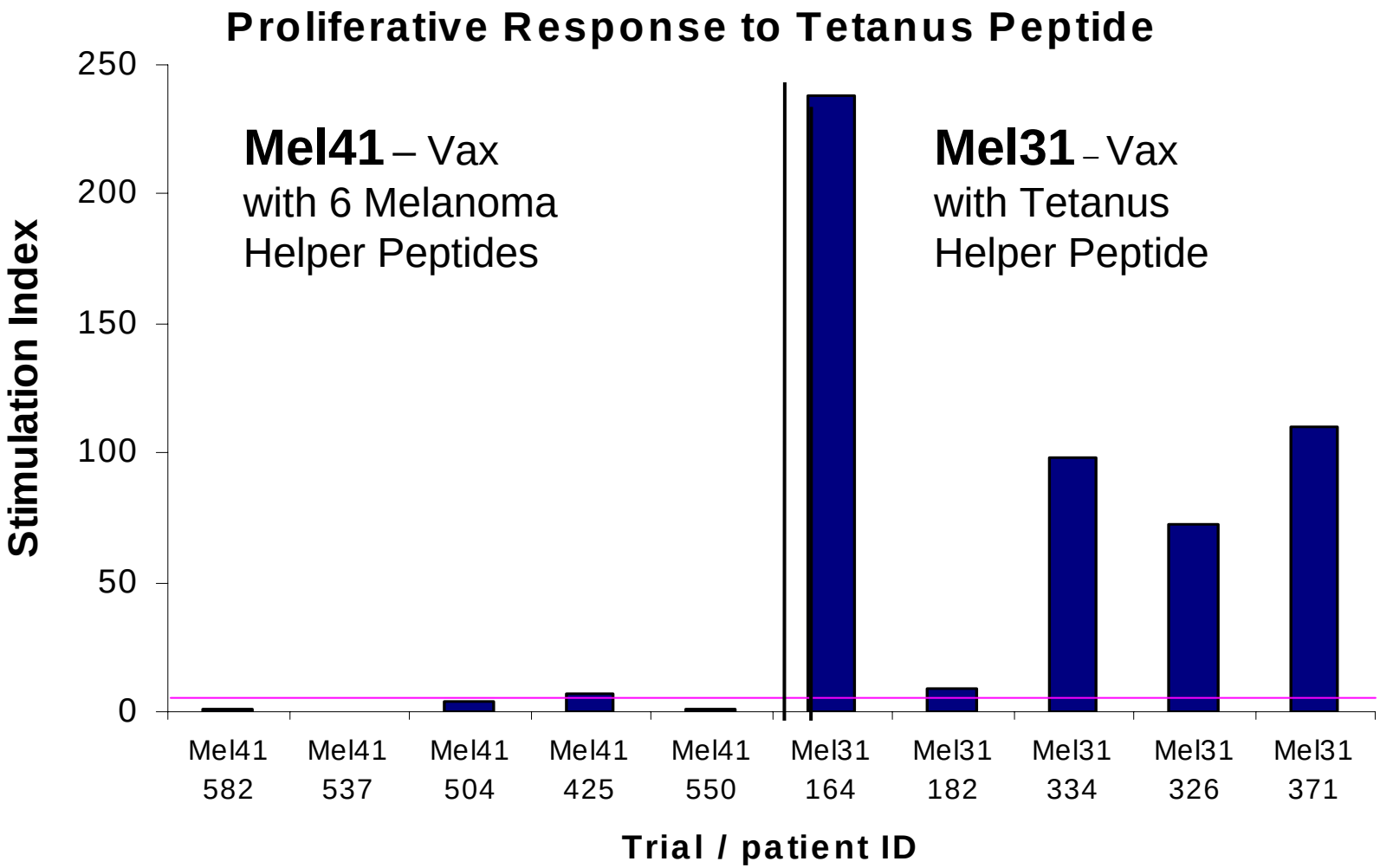
Evaluation over time and in different compartments

- Three patterns of T cell response
  - Persistent vaccine-induced
    - SIN CD28+ CD45RO+
    - PBL progressive loss of CD28 and CD45RO with vaccination
    - PBL 6 weeks after last vaccine, regain CD28, and fewer total tetramer+ cells
    - May mimic acute antigen exposure, conversion to memory
  - Transient vaccine-induced
  - Tumor-induced
    - Response may be limited to PBL
    - Vaccine responses may be limited to SIN
    - CD45RA+, CD27 low, CD28-neg, CCR7 50%+
    - Central memory? Terminally differentiated effectors?
    - Regulatory function?

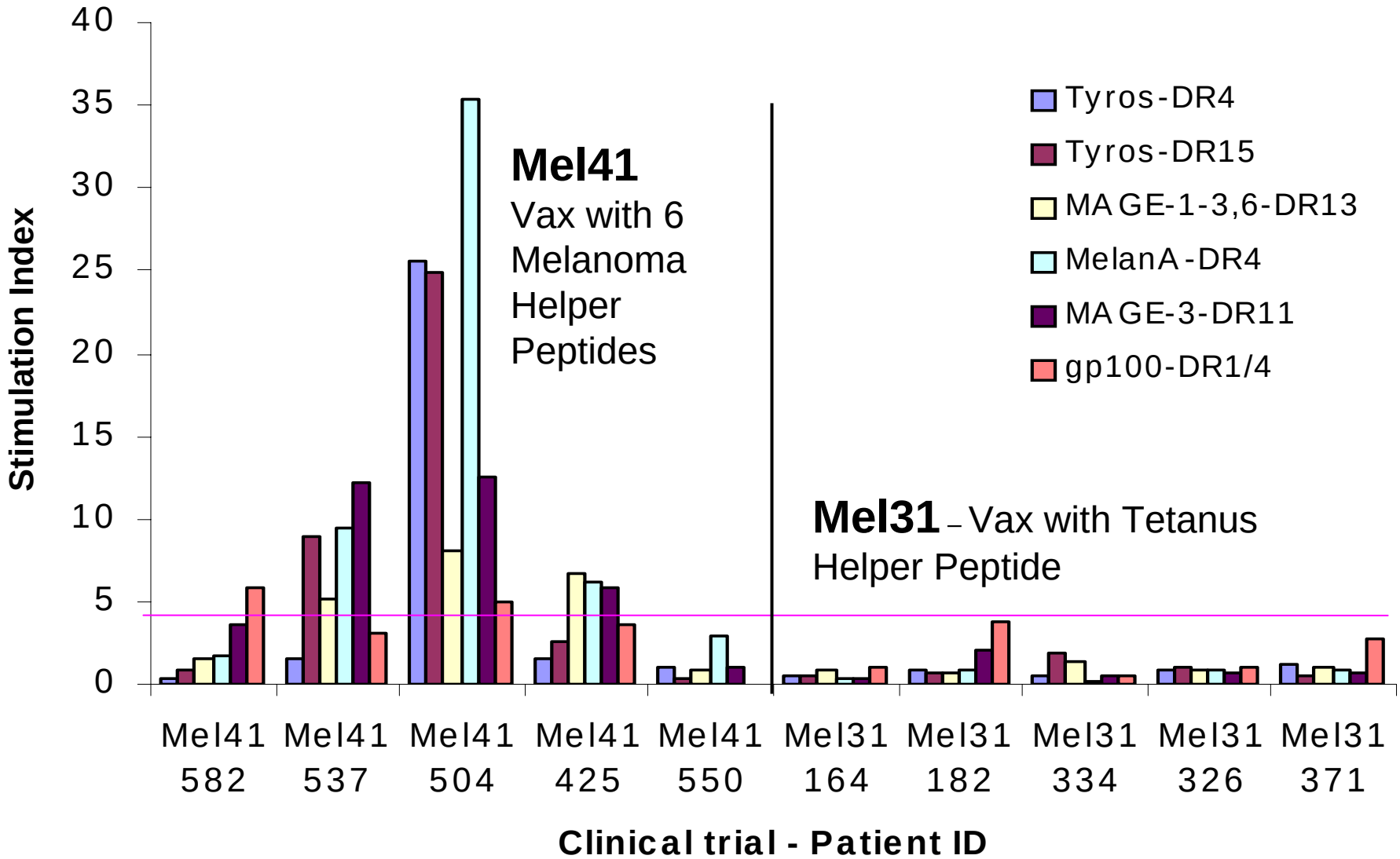
Class II-MHC Restricted Melanoma Peptides (6)

Protein (residues)	Allele	Peptide Sequence
Tyrosinase $_{56\text{-}70}$	DR4	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)

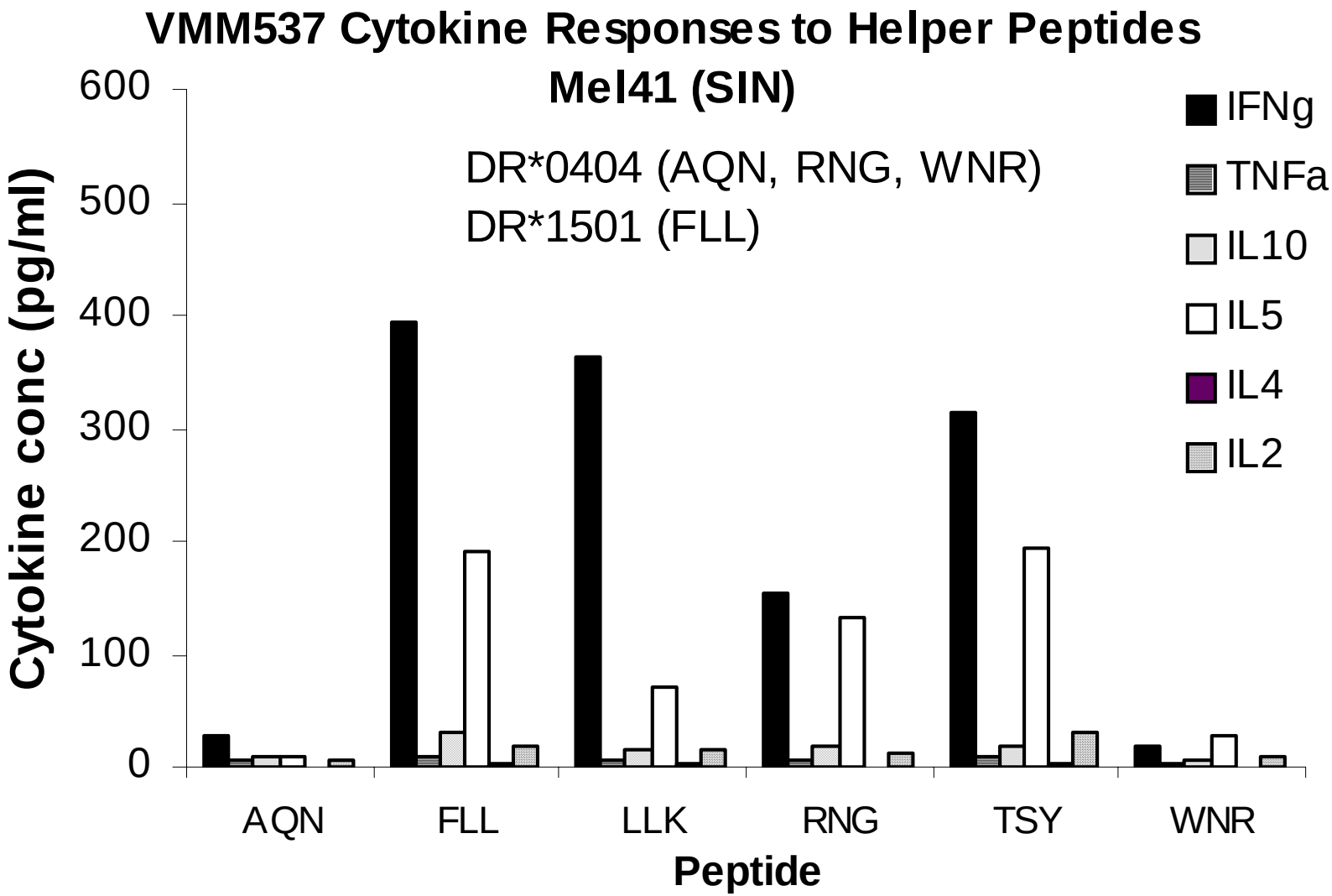
T cell proliferative response to tetanus peptide



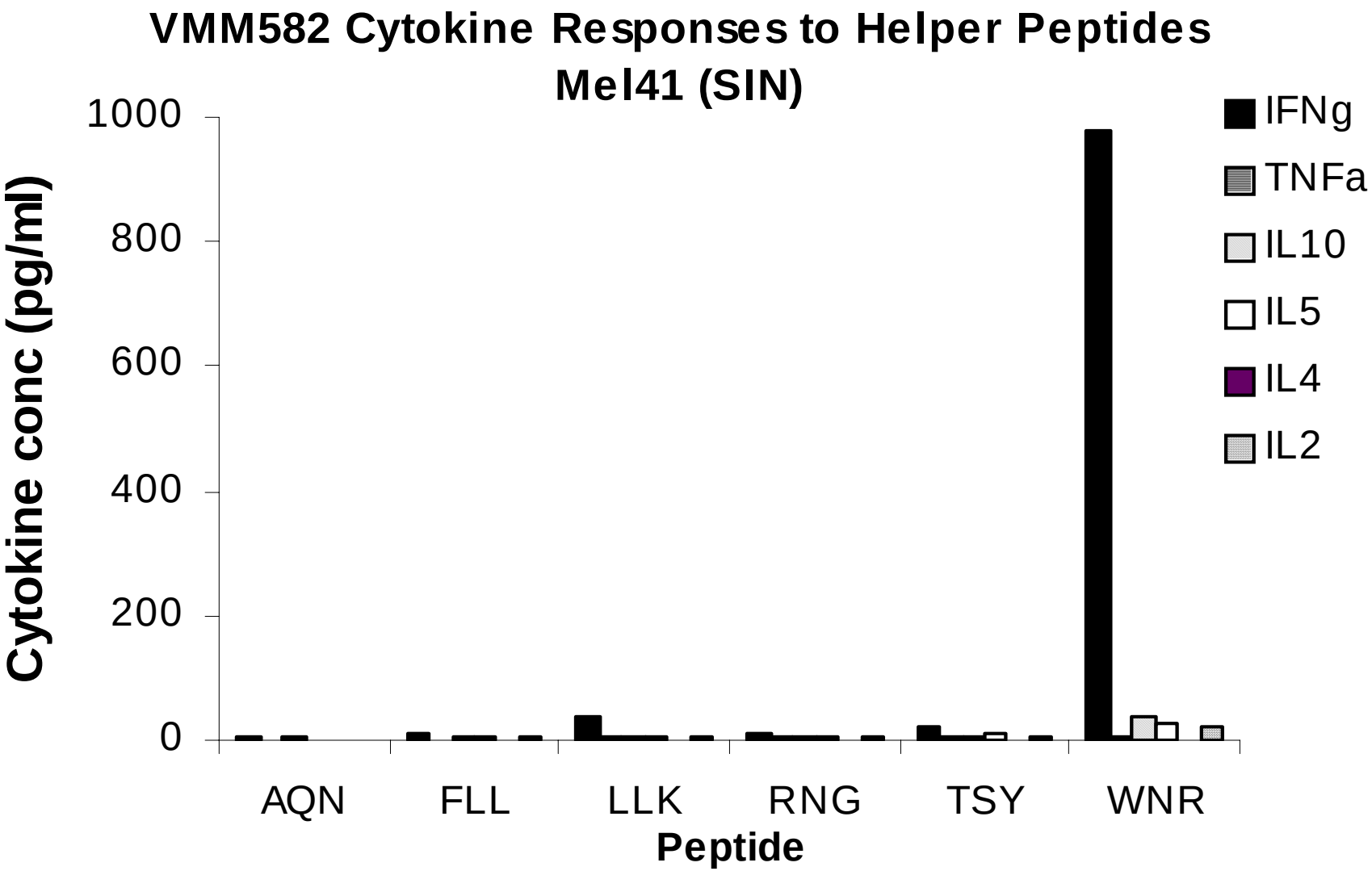
T cell proliferative response to 6 melanoma helper peptides



Cytokine release to melanoma helper peptides



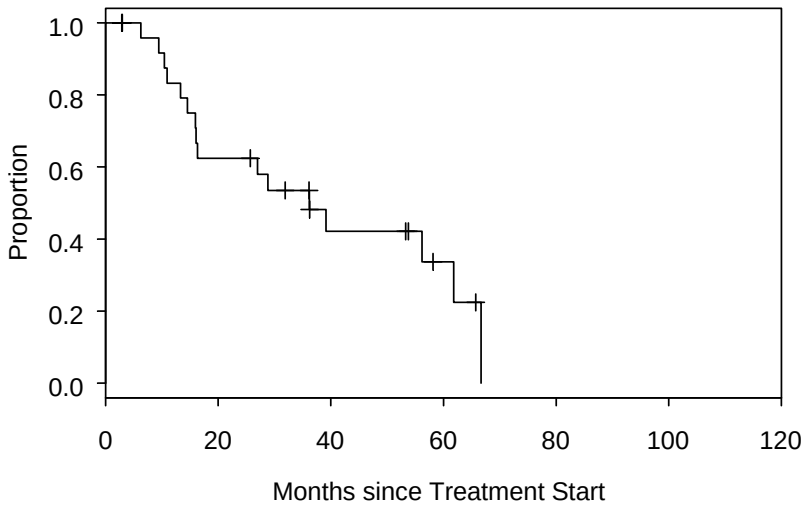
Cytokine release to melanoma helper peptides



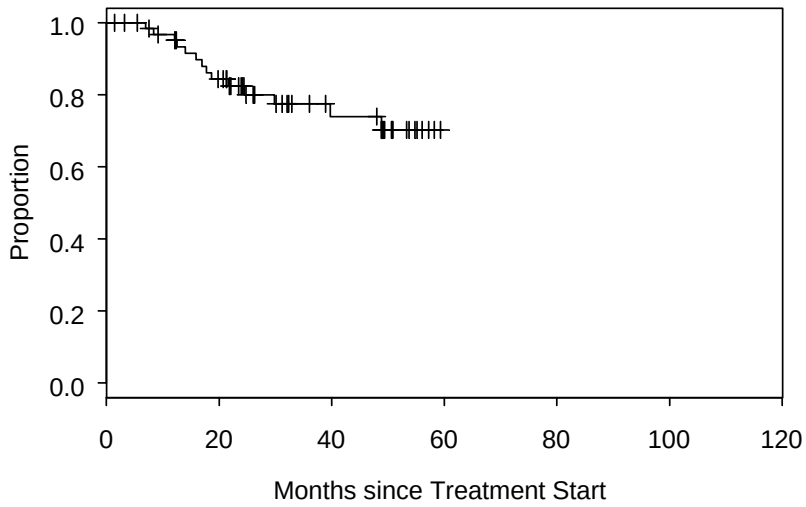
Vaccines with poor T cell response

Vaccines with good T cell response

Stage III Mel 32 and 37

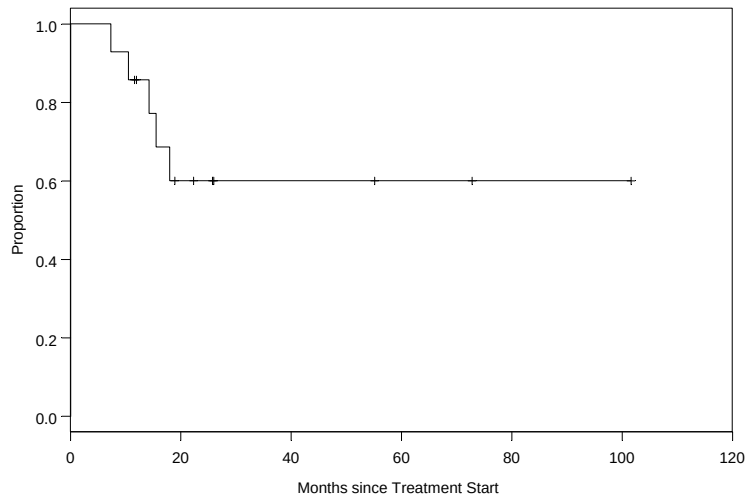


Stage III Mel 36 and 39



Survival of Stage IV Melanoma patients – Peptide vaccine trials

Stage IV Mel 16, 36 and 39



Clinical trials evaluating effects of helper peptides (6 MHP) on CTL response (to 12MP) and clinical outcome

- UVA-Mel44
  - (UVA, MD Anderson, Fox Chase)
  - Resected stages IIB-IV (n = 168)
  - 12 MP + tetanus peptide vs 12 MP + 6 MHP
  - Pretreatment with cytoxan (or not)
- ECOG 1602
  - Advanced stage IV melanoma (n = 176)
  - 4 arms: (i) 12 MP, (ii) 12 MP + tetanus peptide, (iii) 12 MP + 6 MHP, (iv) 6 MHP

Summary

- The lesion in the immune response to cancer is much more complex than simply a weak immune response to defined antigens.
- Given the complexity of the host:tumor relationship and the layers of regulatory control and immune escape mechanisms mediated by melanoma cells, it is remarkable that single interventions with vaccines have led to objective clinical responses in any patients.
- Thus, single digit response rates, though disappointing results for melanoma therapy, are an encouraging proof of principle for T-cell directed cancer vaccines.
- Furthermore, clinical data with peptide vaccines suggest the possibility of clinical benefit
- These results should serve as a call to take a closer look at immune regulatory processes and principles, and to develop more comprehensive and multi-agent approaches to modulate the host: tumor relationship.