

Monitoring Multiantigen Vaccines: State of the Field

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Immune Therapy of Melanoma



Antibody

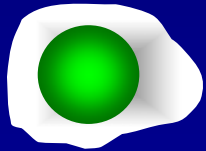


ganglioside



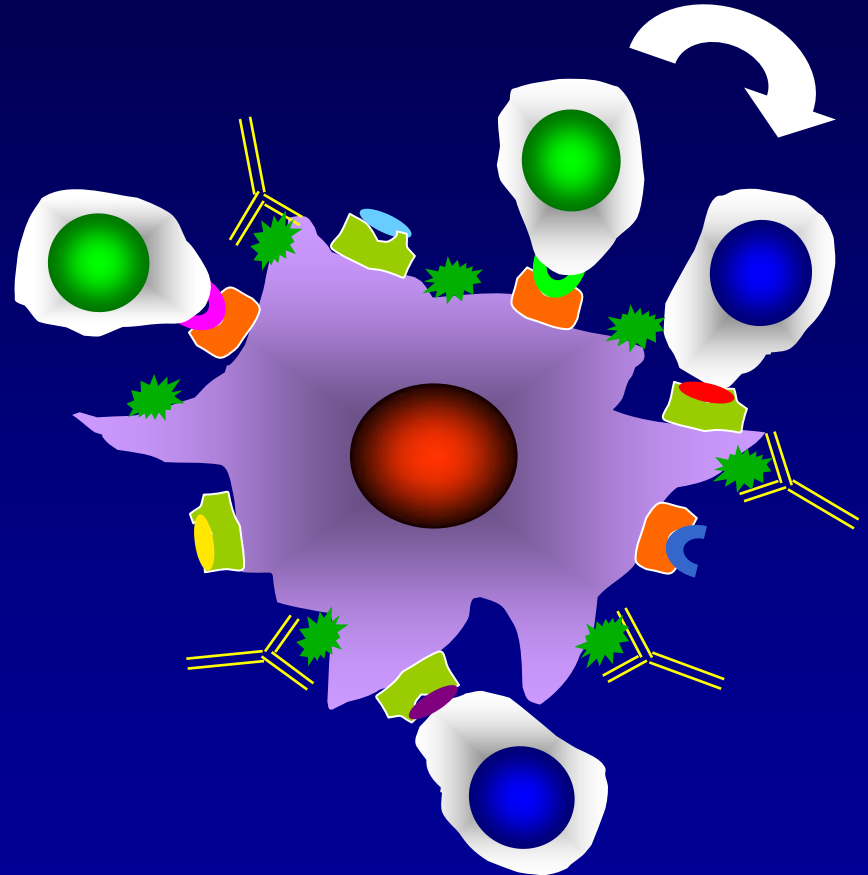
MHC Class I
+ peptide

CD8 cytotoxic T-cells



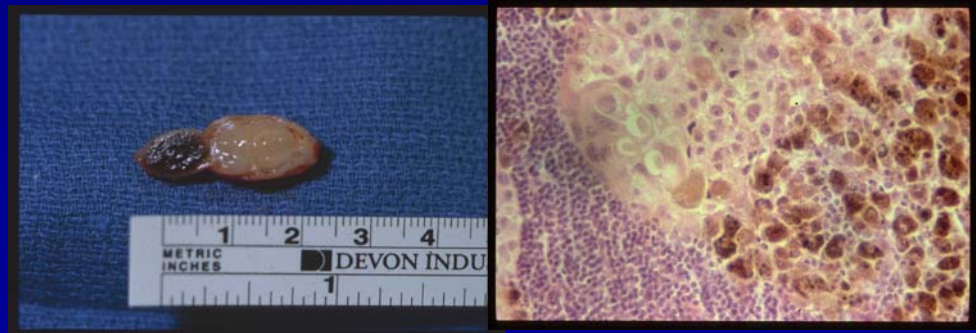
MHC Class II
+ peptide

CD4 helper T-cells



Rationale for Multiantigen Vaccines

- Antigenic heterogeneity is a hallmark of human cancers. Thus, it is unlikely that vaccines containing single antigens will induce immune responses reliably capable of tumor rejection. Thus, there is a growing effort to produce multi-antigen vaccines.



Types of Multiantigen Vaccines

- Multi-peptide vaccines
- Whole cell vaccines
- Heat shock protein vaccines
- Protein vaccines
- RNA vaccines
- DNA vaccines
- Viral vaccines incorporating multiple peptides
- Viral vaccines encoding whole proteins
- Dendritic cell vaccines pulsed with tumor cells or cellular DNA or RNA
- Dendritic cells vaccines pulsed with synthetic protein

Types of Multiantigen Vaccines

From the standpoint of monitoring, these types of vaccines can be considered in three categories:

1. vaccines incorporating multiple defined epitopes (eg peptides)
2. vaccines incorporating multiple defined proteins or genes, from which some epitopes are undefined (eg: protein)
3. vaccines incorporating multiple undefined antigens (eg: cell based vaccines)

Why monitor immune responses to multi-antigen vaccines?

- Immune responses to vaccine antigens do not reliably correlate with clinical outcomes.
- This has led to a suggestion that immune monitoring has no relevance for the success of immunotherapy.

However, there are numerous explanations for failure of effective peripheral immune responses to mediate tumor control:

Tumor-mediated immune dysfunction

- Immune escape by tumor cells.

Thus, effective tumor immunity is **NECESSARY**, but not sufficient, for clinical control of tumor growth (clinical responses or survival)

Why monitor immune responses to vaccines?:

A reasonable analogy is the importance of measuring blood pressure for a patient who has suffered cardiopulmonary arrest. While return of blood pressure in a certain normal range is necessary for survival, the recovery of oxygenation is also required for survival.

Immune Monitoring of Multi-Antigen Vaccines: Current status

- Not standardized
 - Varied antigens
 - Different endpoints
 - Different assays
- Complicated by varied antigen preparations
- Critical to assessment of the relative efficacy of one vaccine approach vs another.

1. Quantification or detection of antigen-reactive T cells

- a) cytotoxic (CD8) T cells
- b) helper (CD4) T cells
- c) responses to defined antigens individually
- d) Responses to allogeneic tumor cells
- e) Responses to autologous tumor cells

ELIspot assays

Tetramer assays

Cytotoxicity assays

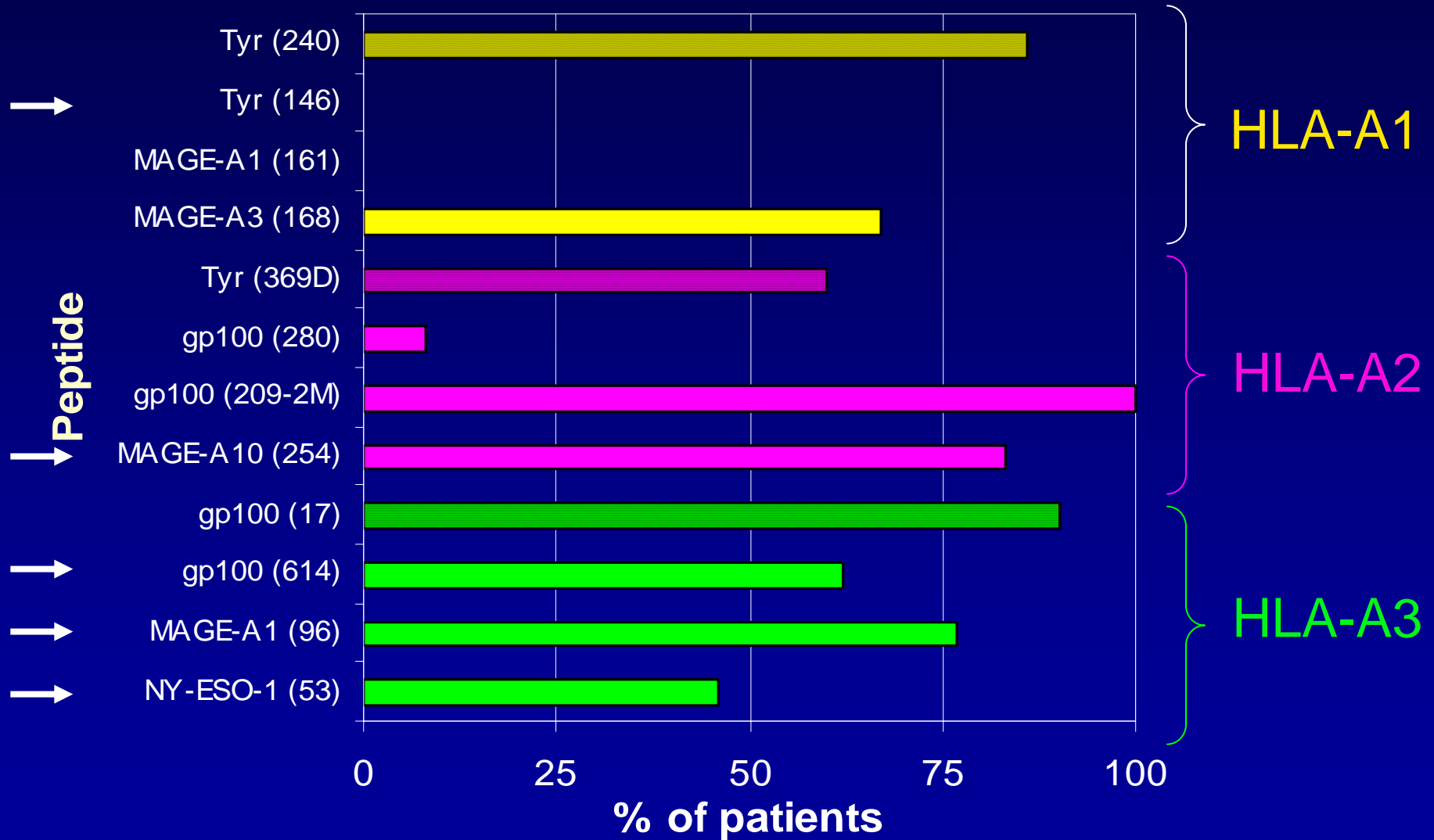
Intracellular cytokine assays (IFN, IL-5)

Targeting multiple peptides and multiple MHC

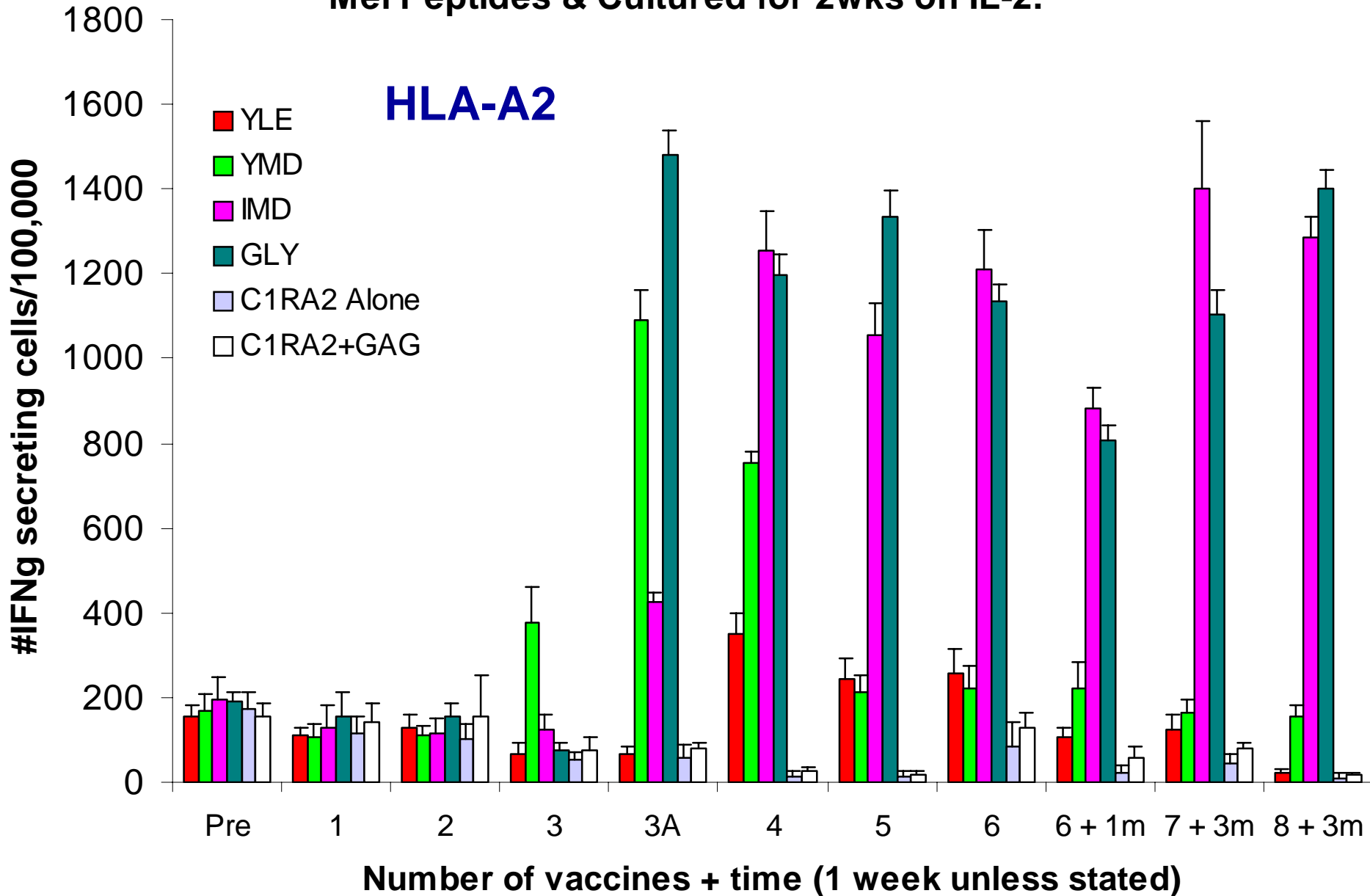
• DAEKSDICTDEY	tyrosinase	240-251S	A1
• SSDYVIPIGTY	tyrosinase	146-156	
• EADPTGHSY	MAGE-A1	161-169	
• EVDPIGHLY	MAGE-A3	168-176	
• YMDGTMSQV	tyrosinase	369-376D	A2
• YLEPGPVTA	gp100	280-288	
• IMDQVPFSV	gp100	209-217M	
• GLYDGMEHL	MAGE-A10	254-262	
• ALLAVGATK	gp100	17 - 25	A3
• LIYRRRLMK	gp100	614-622	
• SLFRAVITK	MAGE-A1	96-104	
• ASGPGGGGAPR*	NY-ESO-1	53-62	

* A31/A3

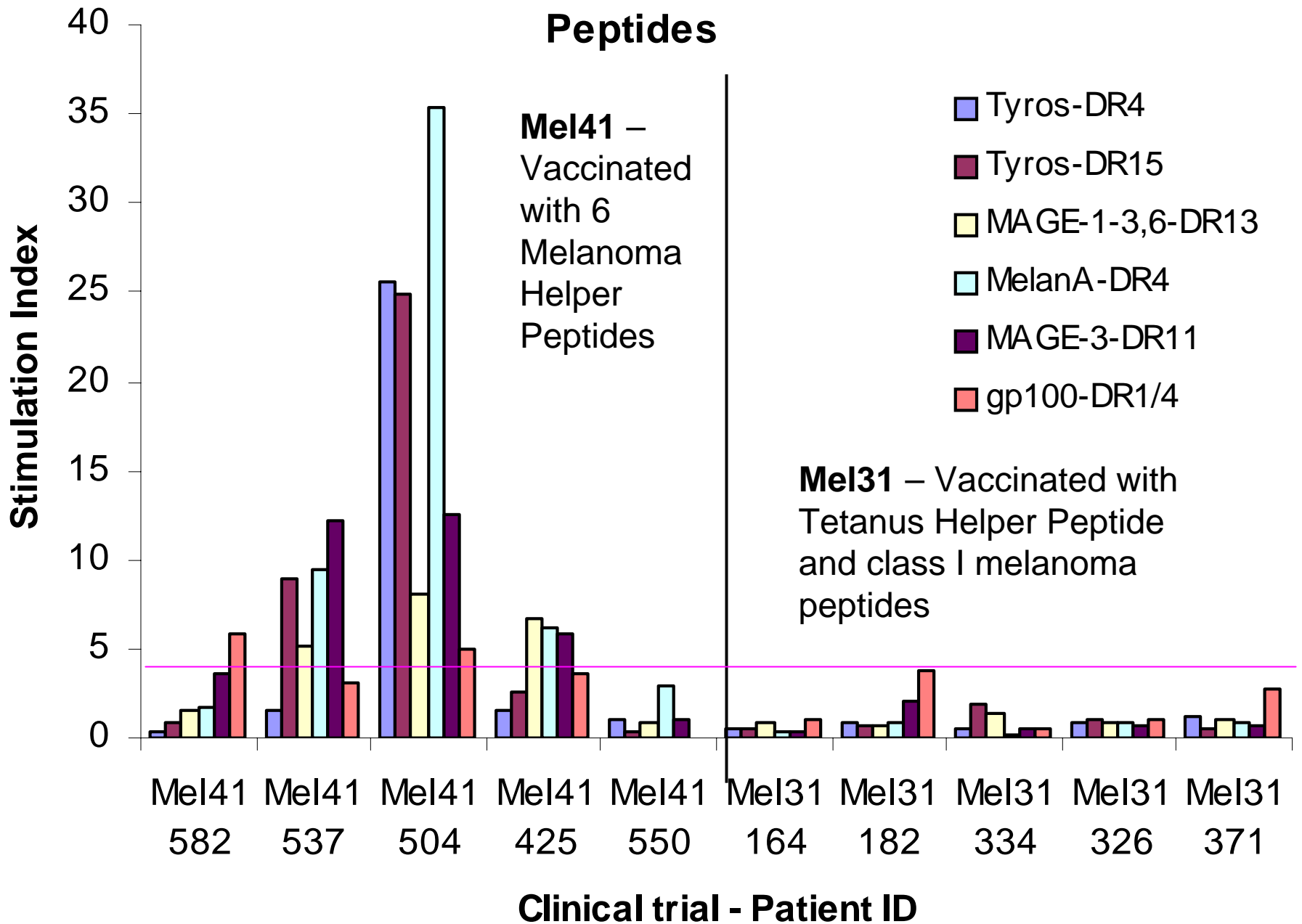
Immunogenicity of the 12 peptides



**ELISPOT 9.13.2004 VMM526 (Mel43-GroupA) PBL Stim 1x w/12
Mel Peptides & Cultured for 2wks on IL-2.**



Proliferative Response to 6 Melanoma Helper Peptides



A critical question

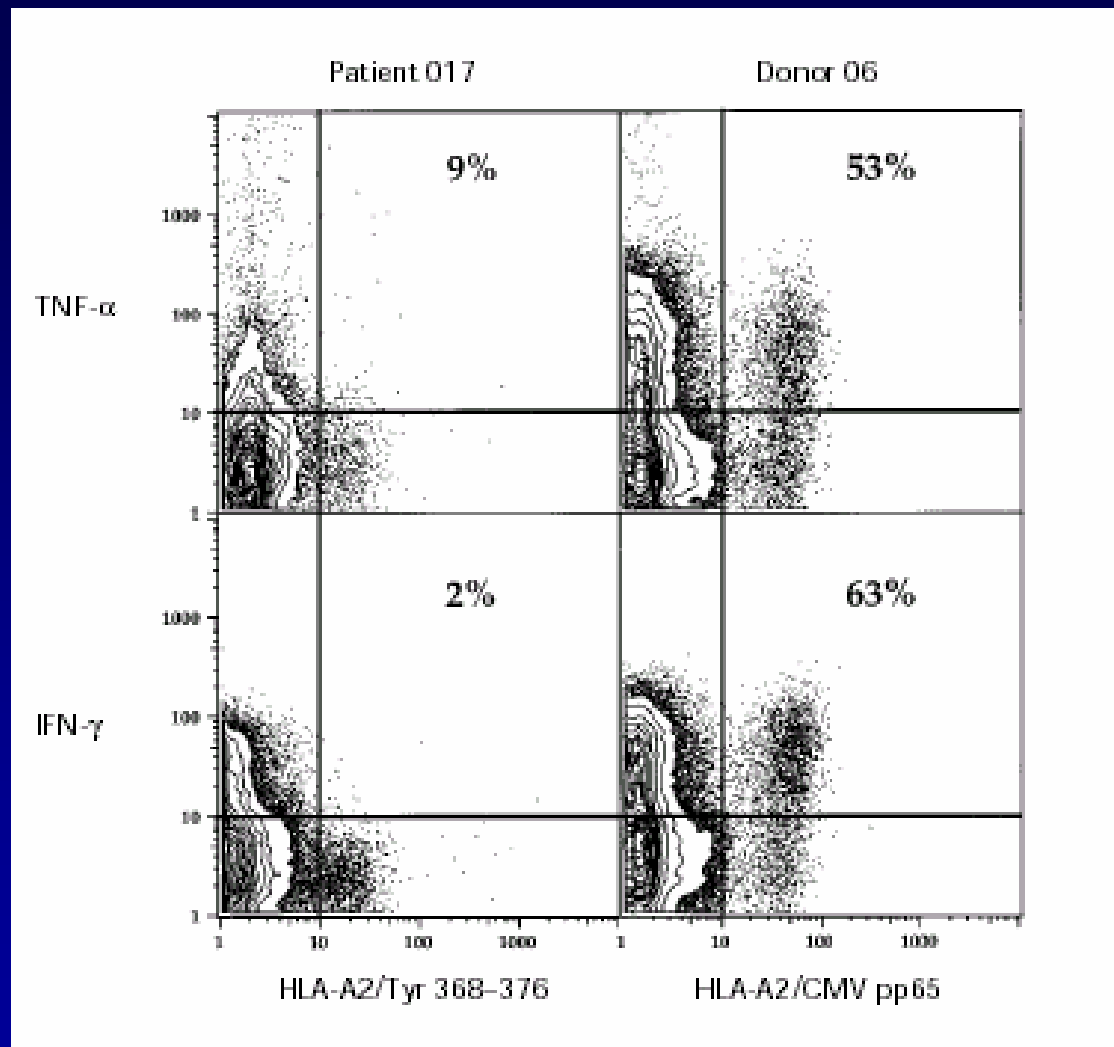
- is why antigen-specific T cells may be expanded without clinical tumor regression in many cases.
- Thus, it is critical to define, over time, the activation status, and phenotype (effector, effector memory, central memory) and cytolytic function of CD8 T cells responding to Class I MHC associated peptides.
- Direct assessment of cytotoxicity of tumor cells naturally expressing antigen (with appropriate controls) is the most definitive measure of effector function.
- Simpler and higher throughput measures may include the finding of perforin and granzyme B in antigen positive T cells.

2. Function of antigen-specific T cells

Ex vivo

- a. cytotoxicity
 - i. against antigen-pulsed targets
 - ii. against tumor cells naturally expressing antigen
 - iii. against autologous tumor cells
- b. effector or memory phenotype
- c. Staining for markers of cytolytic function or effector function:
 - granzyme B, perforin, CD107a
- d. cytokine profile of helper T cells responding to antigen
- e. Regulatory T cells

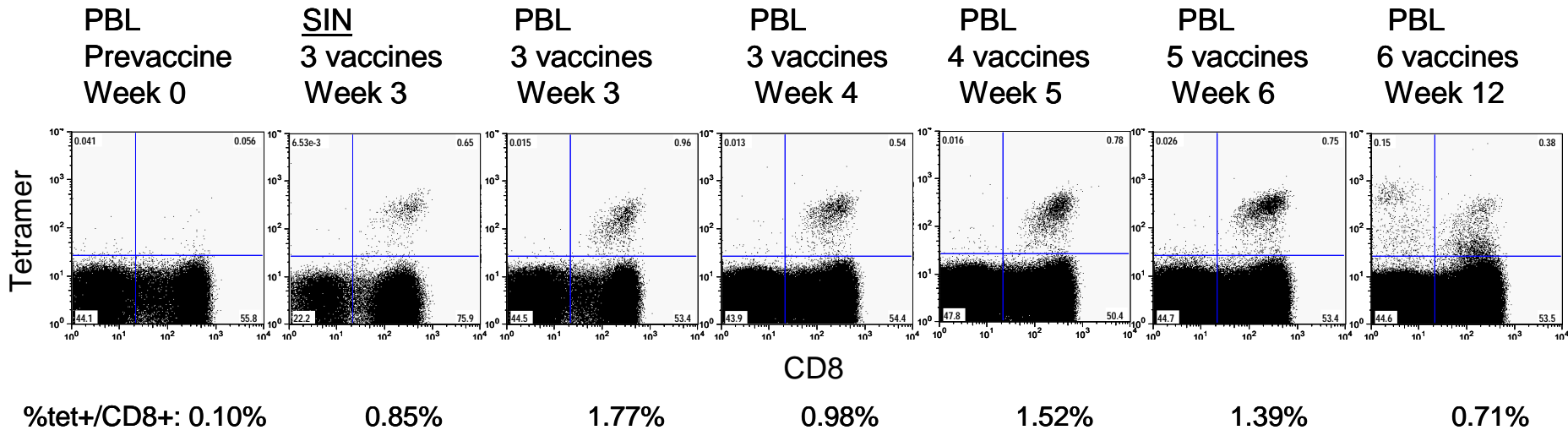
2. Function of antigen-specific T cells



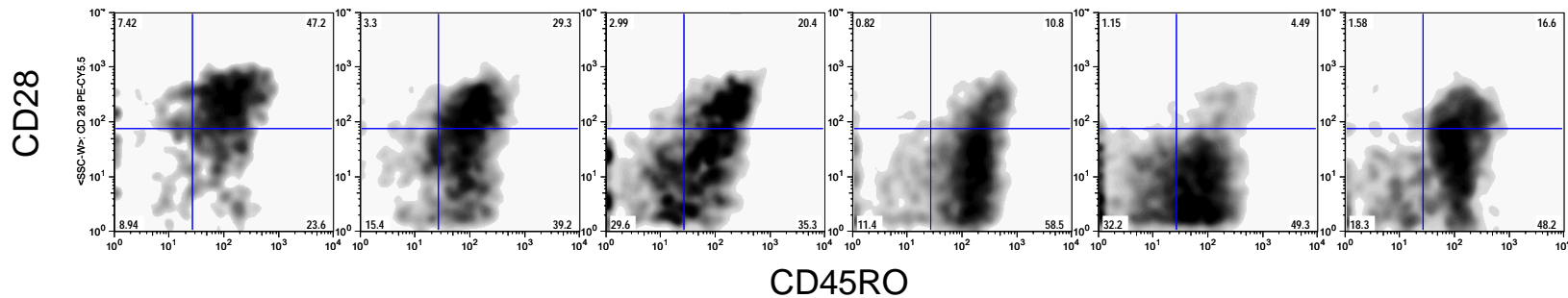
ICC after 6h with PMA+ioomycin

PP Lee. Nat Med 5:677-685, 1999

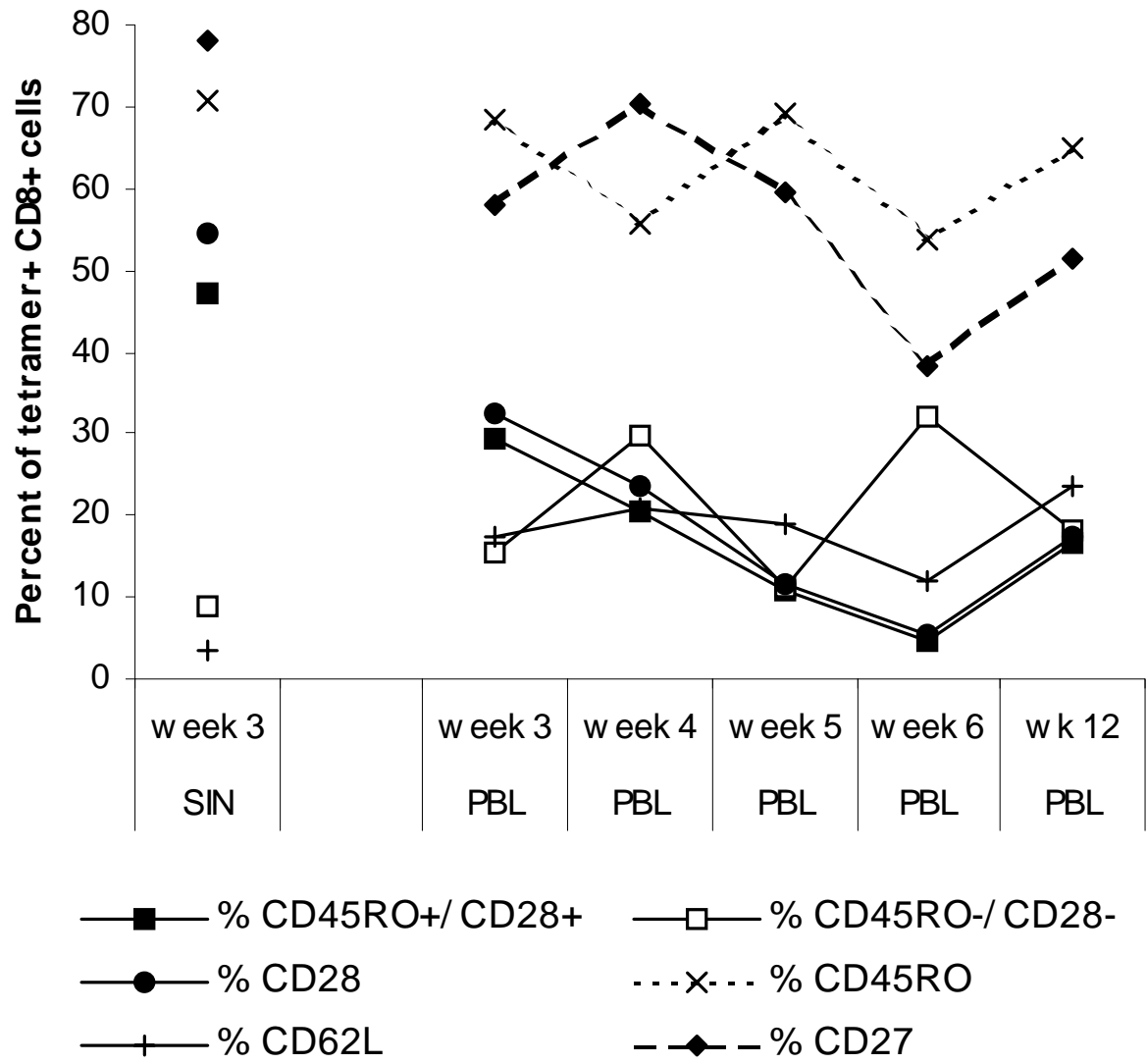
Reactivity to gp100₁₇₋₂₅ (HLA-A3, ALLAVGATK) in 12-peptide vaccine



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



Changing T cell phenotype, reactive to gp100₁₇₋₂₅ (HLA-A3, ALLAVGATK), in 12-peptide vaccine



3. delayed-type hypersensitivity reactions to antigen

- For protein, cellular extracts, and cell-based antigen sources, DTH responses can be expected using standard techniques.
- Standardized criteria for a positive DTH response are needed.
- This is an important area that needs to be developed and validated systematically.

4. activation status of T cells infiltrating tumor

- a. measurable tumor in patients with advanced disease
- b. recurrent tumor in patients vaccinated in the adjuvant setting
- Little work has addressed the T cell response measurable in the tumor. Because T cell infiltration into tumor may be inhibited by the tumor microenvironment, and T cell function can likewise be inhibited, it is critical to begin to design experiments where some assessment of T cell function in the tumors is routinely included.

5. antigen expression by recurrent or progressive tumor

- a. MHC/ β 2m
- b. Tumor-associated antigens
- c. Antigen-processing machinery

Immune escape may be an important measure of the response by tumor to a pre-existing immune response or to an immune response induced by vaccination. Thus, in patients with tumor progression, biopsies should be done to assess the persistence or loss of MHC, tumor antigens, or antigen-processing machinery.

Pressing Research Questions

1. What questions can be answered by immune monitoring of multiantigen vaccines?
2. Is immune monitoring needed for vaccine trials using complex antigens?
3. If so, what immunologic endpoints should be measured? For CD8, CD4, antibody responses?
4. Is it possible to standardize immune monitoring so that results with various trials can be compared?
5. When is the best time to assess immune responses?
6. What are the correct lymphoid compartments to evaluate for monitoring immune responses?

Topics to be covered within the scope of the Immune Monitoring Workshop.

It is suggested that discussion focus on:

- 1) minimum standard set of endpoints to measure
- 2) assays to use for assessing those endpoints
- 3) timing of endpoints
- 4) compartments to evaluate
- 5) extra endpoints that are desirable to assess when feasible

