Multipeptide vaccines for melanoma – Strategies for increasing the breadth of the T cell repertoire

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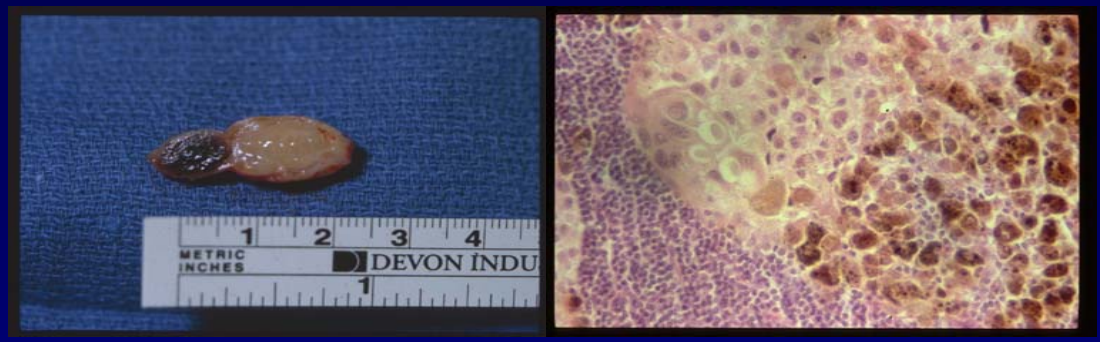
Challenges for Peptide Vaccines



- Heterogeneity of antigen expression
  - Downregulation of melanocytic differentation protein (MDP) expression in metastases
  - Expression of cancer-testis antigens (CTA) in subsets of melanomas, increased in metastases

  → Include peptides from MDPs and CTAs
- Heterogeneity of HLA types in patient populations

  → Target HLA-A1, A2, and A3 (80% of population)

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

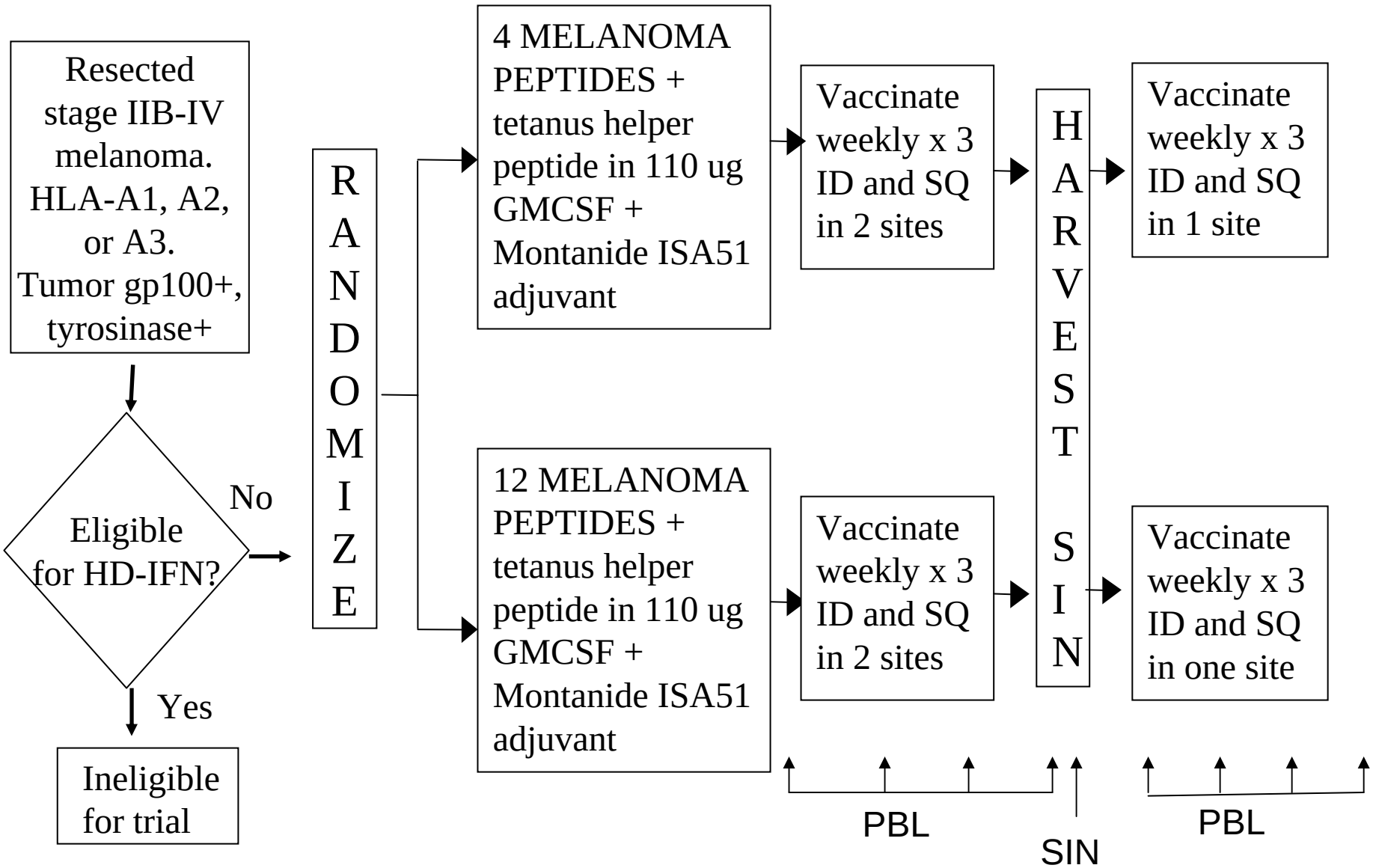
HLA A2

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

HLA-A3

* A31/A3

UVA-Mel 39: Evaluation of Immunogenicity of a Multi-Epitope Vaccine Incorporating Differentiation Proteins and Cancer-Testis Antigens



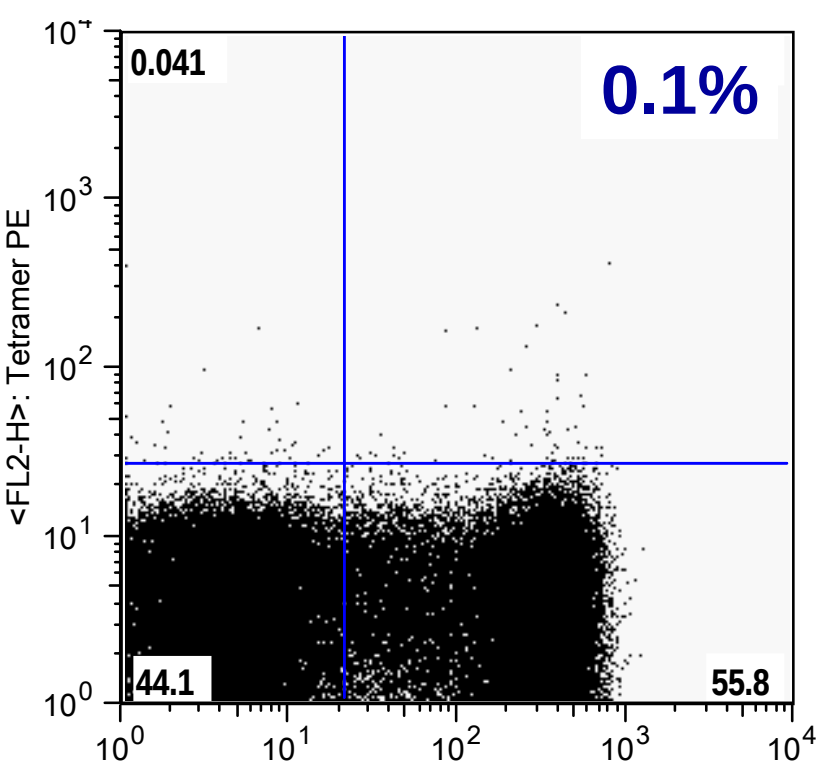
Mel39: Hypotheses

1) A 12-peptide melanoma vaccine will be safe.
2) Each of the 12 peptides in this mixture will be immunogenic.
3) Vaccination with 12 peptides will increase the total CTL reactivity against tumor antigens, when compared to 4 peptides.
4) Addition of 3 peptides competing for binding to the same MHC molecule will not significantly inhibit immunogenicity of an index peptide.

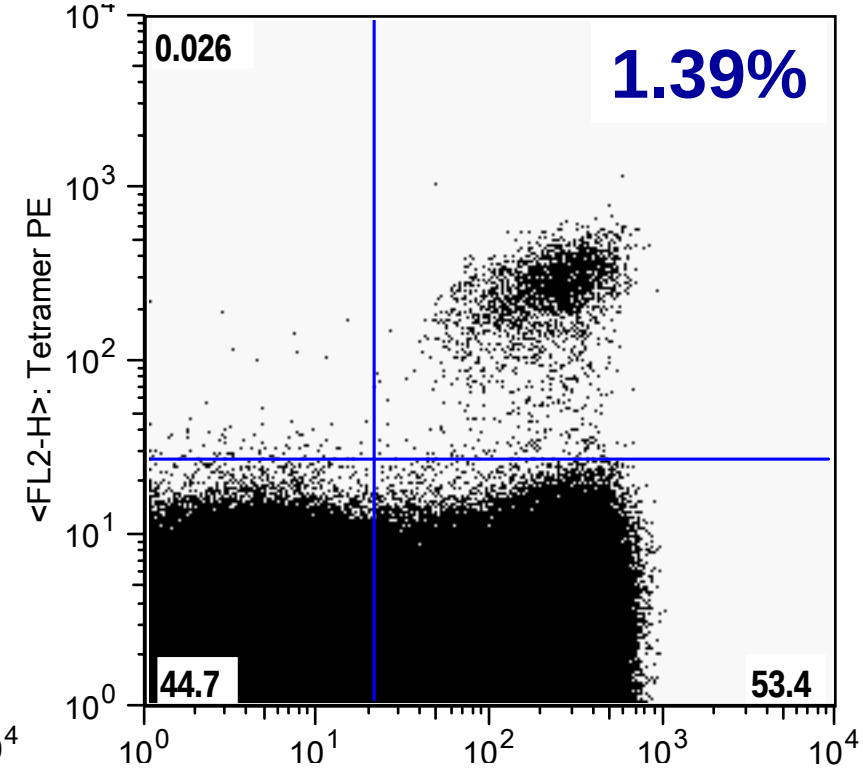
Study Population

	Group A 4 peptide	Group B 12 peptide	Overall
N	26	25	51
Age (median)	49	56	53
Sex (% male)	54%	68%	61%
Stage IIB/C	4	4	8
Stage III	19	17	36
Stage IV	3	4	7
HLA-A1	10	6	16 (31%)
HLA-A2	15	13	28 (55%)
HLA-A3	7	14	21 (41%)

Ex vivo Reactivity to gp100 $_{17\text{-}25}$ (HLA-A3, ALLAVGATK) in 12-peptide vaccine

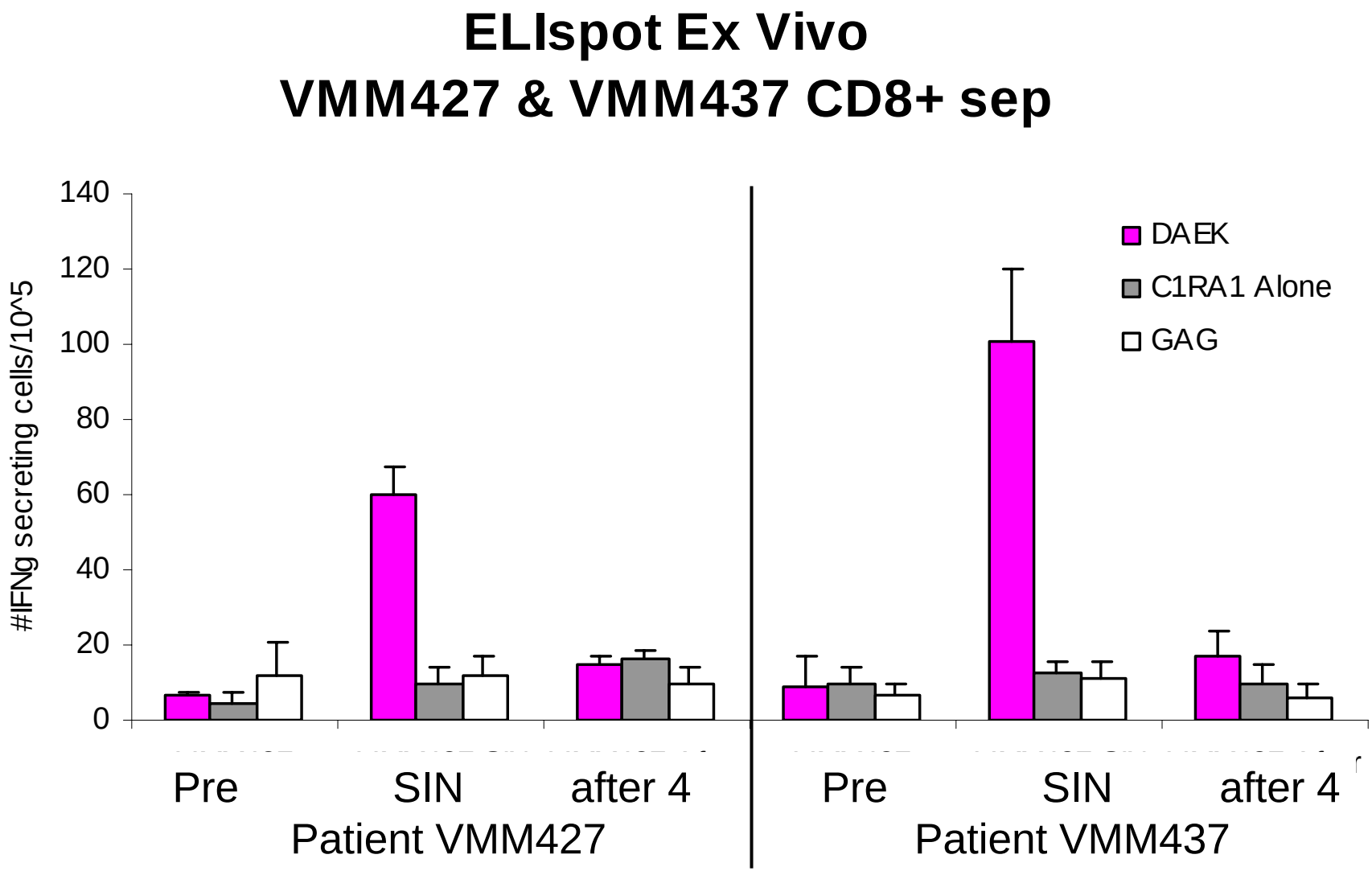


Pre-vaccine



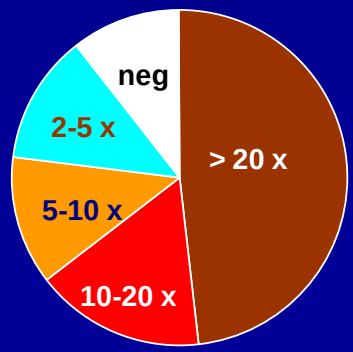
After 4 vaccines

Ex vivo Evaluation of Patient Lymphocytes, ELIspot assay



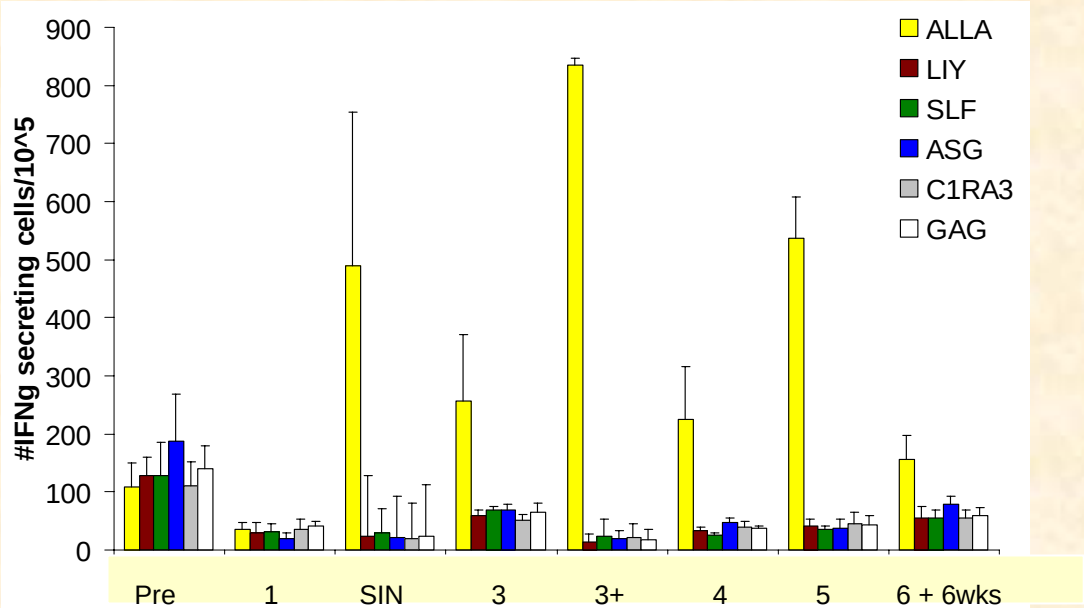
ELIspot Assays on IVS Lymphocytes: Immune Responses against melanoma peptides

- All Patients (Group A & B) - PBL & SIN lymphocytes sensitized once *in vitro* with 12 Melanoma Peptide Mixture
- ELIspot assay day 14, against all 12 Melanoma peptides relevant to their HLA-type, individually.
- 48 of 51 patients evaluable for T cell response (94%)
- Positive response: > 2 x background, > 30 spots/100,000 cells, > 2x pre-vaccine response.
- Among observed T-cell responses,
  - 86% > 5x, 72% > 10x, 53% > 20x background.
  - Prevaccine responses seen in only 3 cases.



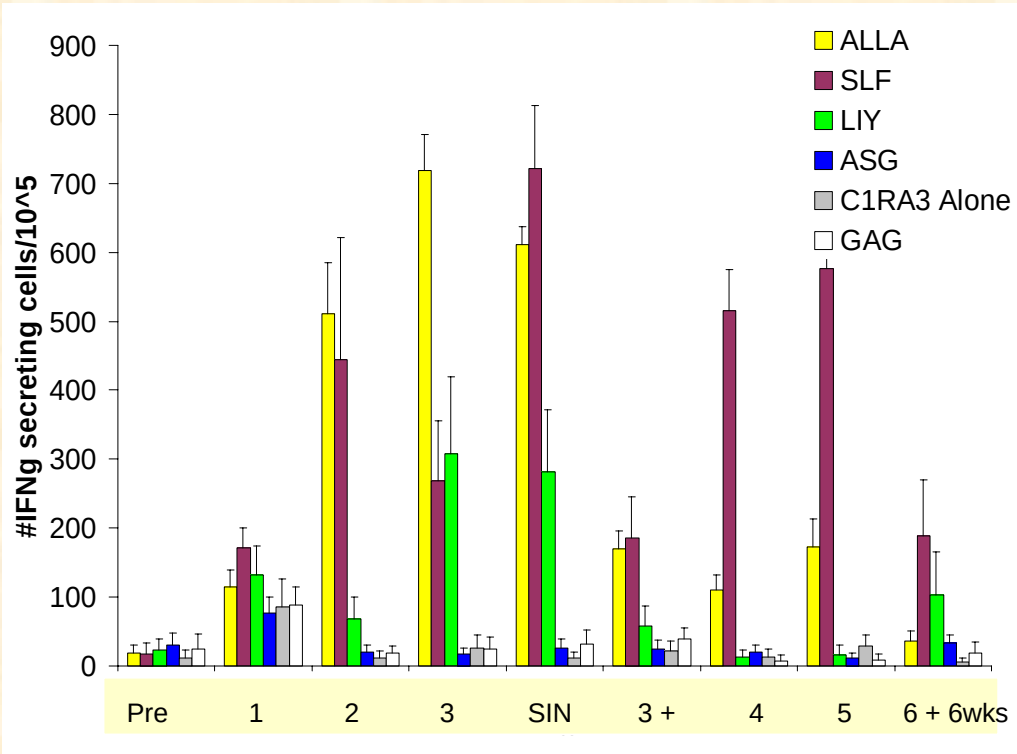
Arm A
(4 peptide mix):

Reactivity to Index peptide only

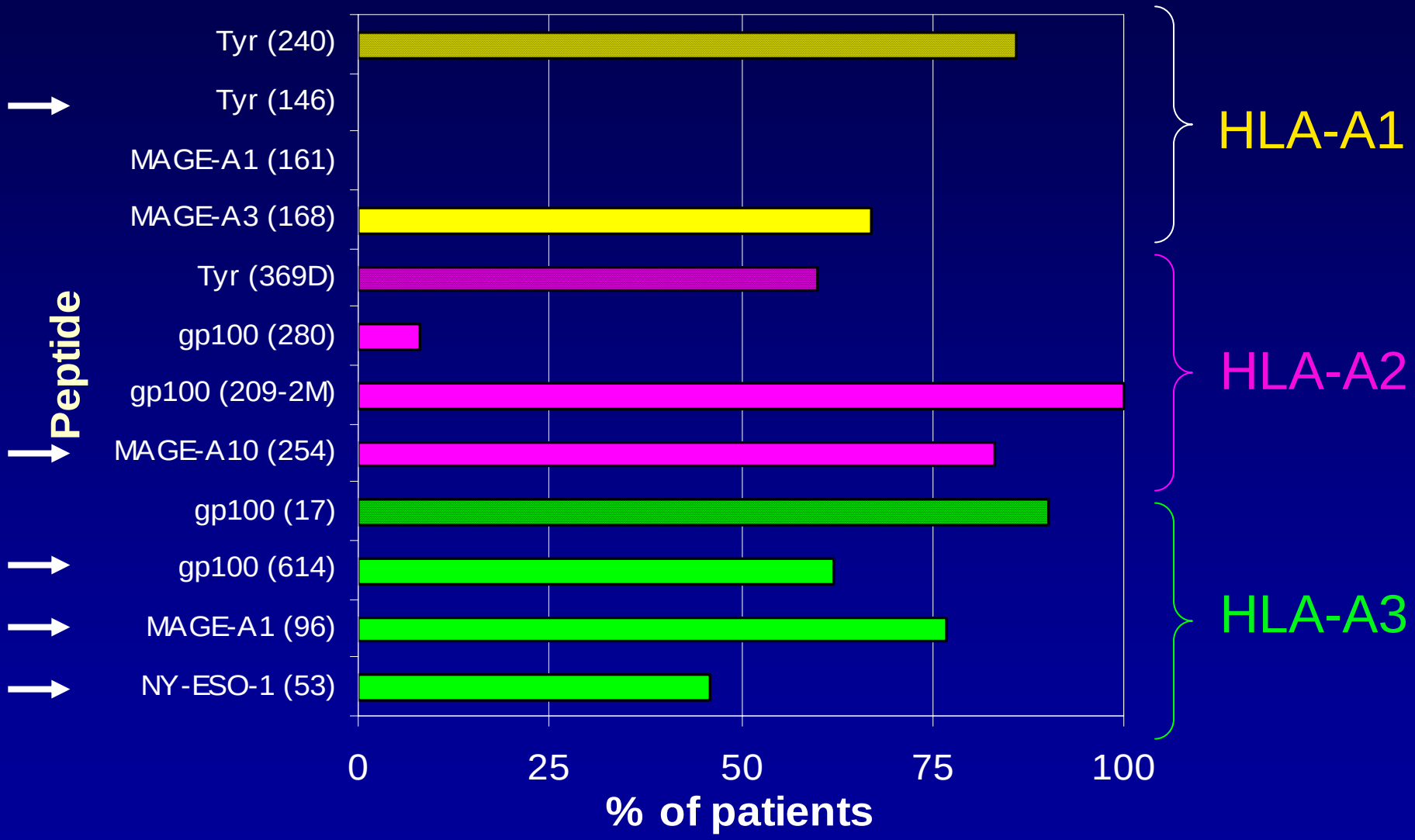


Arm B
(12 peptide mix):

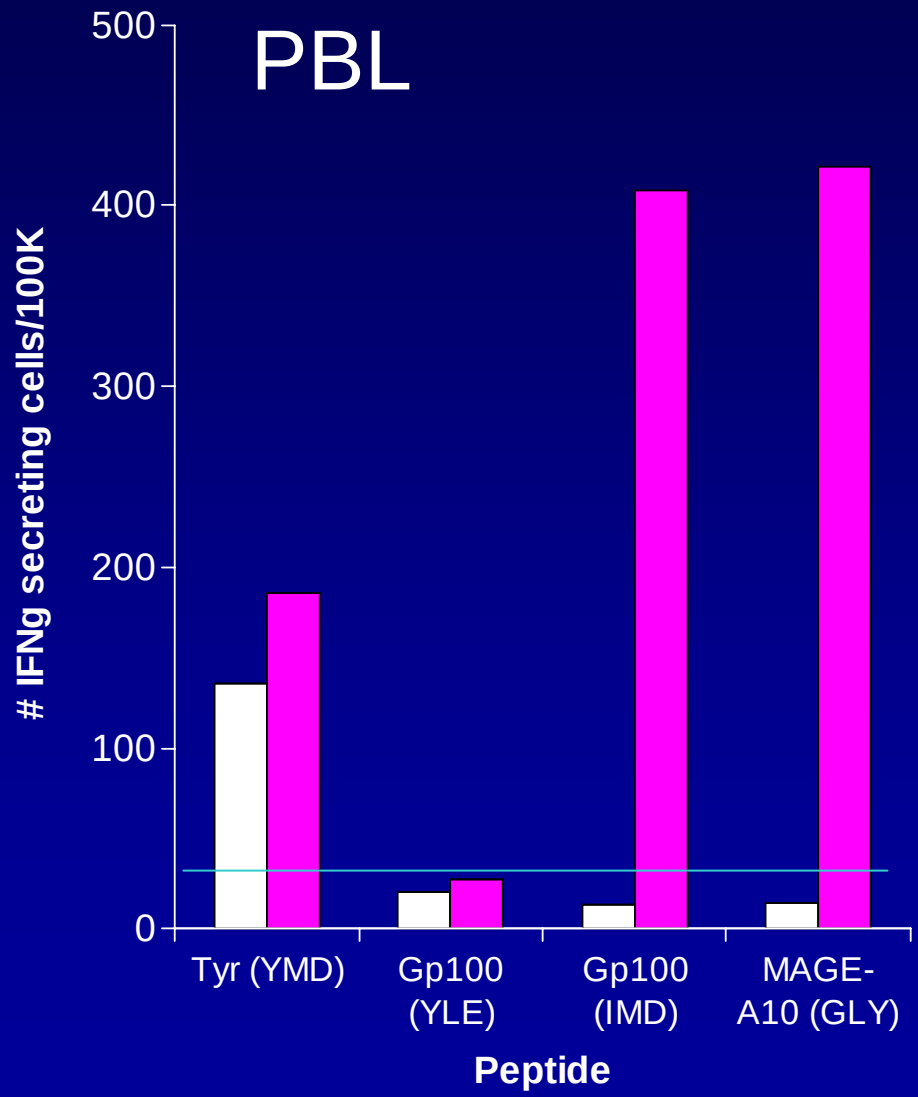
Reactivity to Index peptide + 2 others

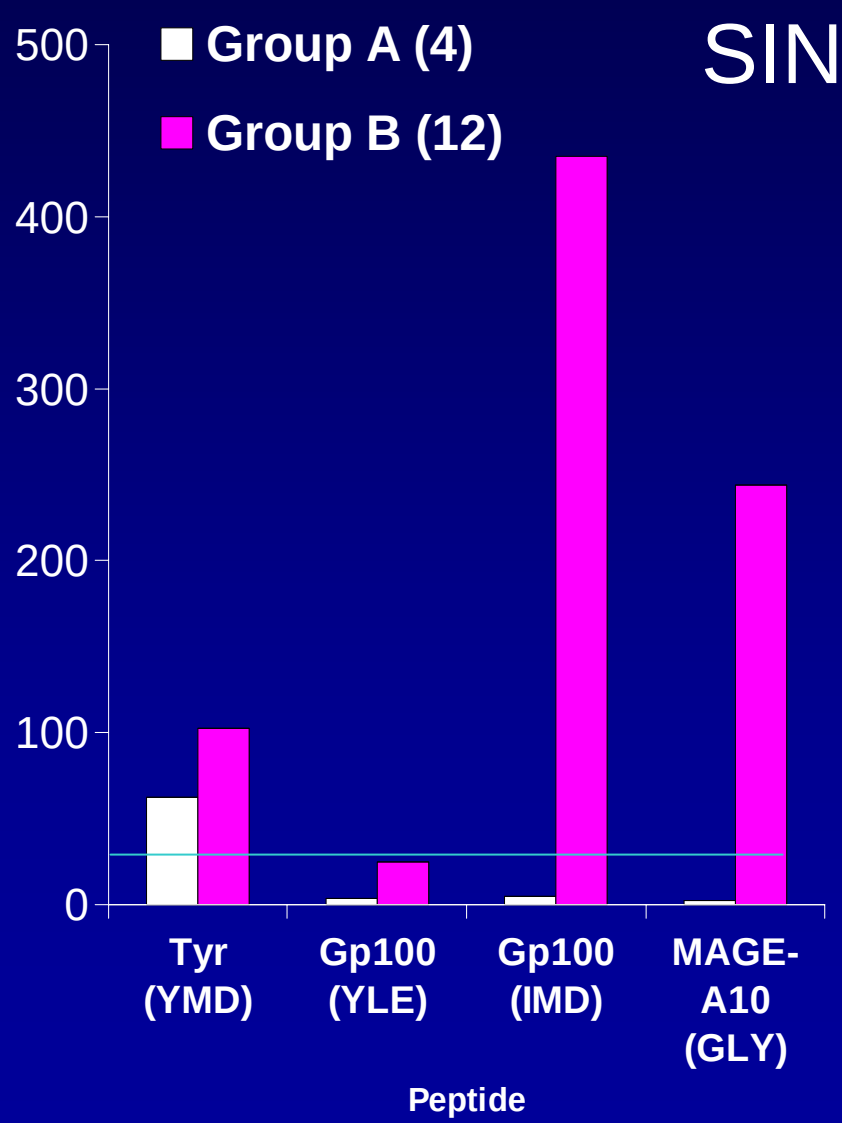


Immunogenicity of the 12 peptides



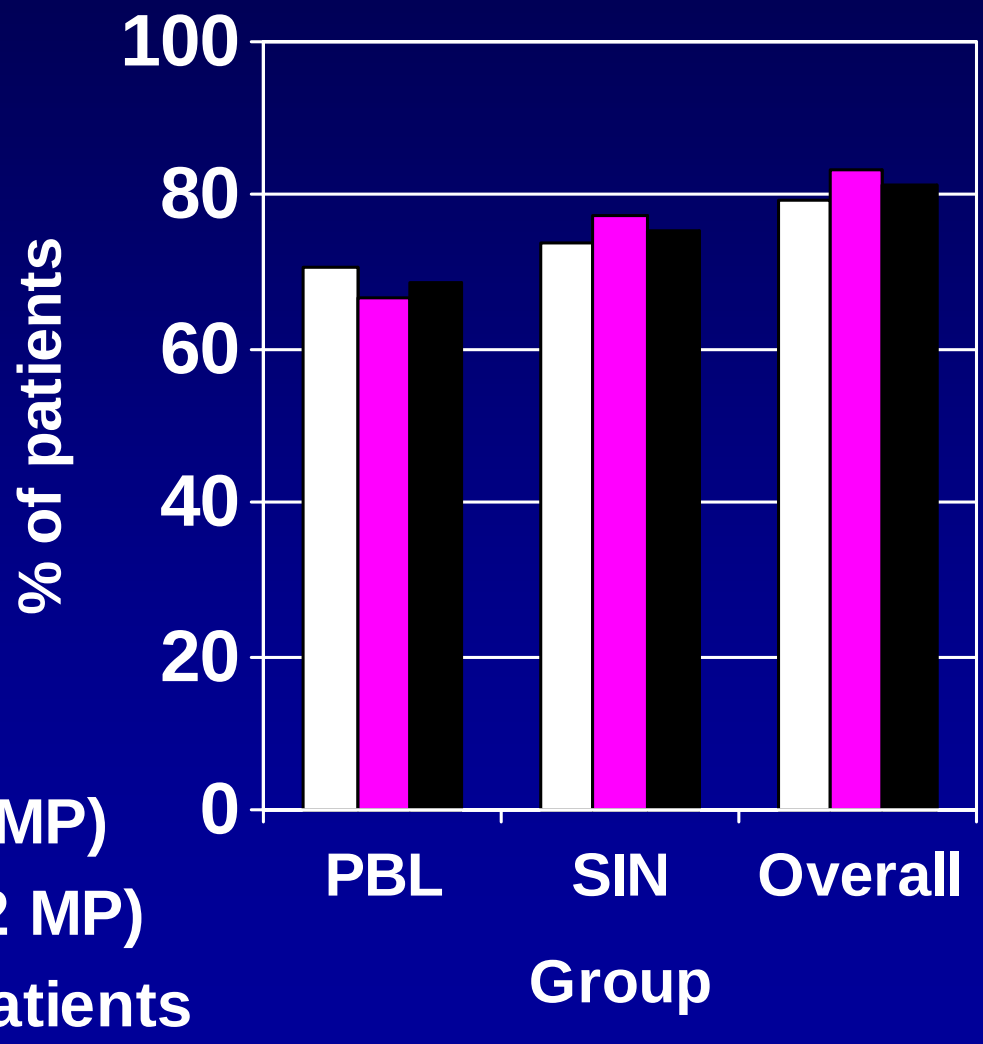
Max PBL and SIN responses (mean) by group restricted by HLA-A2





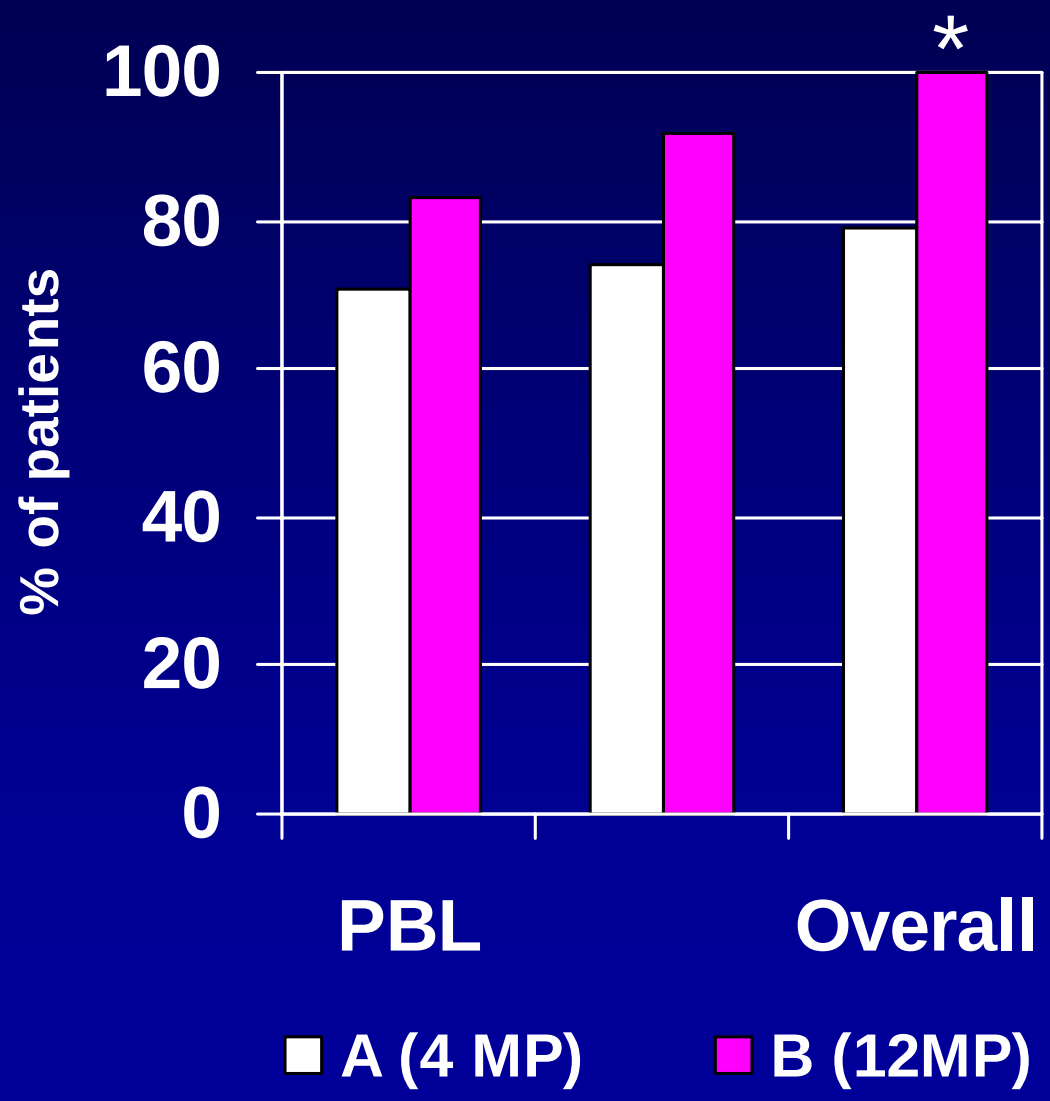
Immune Response to Index Peptides Unchanged between groups

- T cell response to at least one index peptide in the PBL, SIN or either
- By treatment group (n = 24 per group; 48 overall)
- P values (Chi-sq):
  - PBL 0.75
  - SIN 0.79
  - Either 0.71



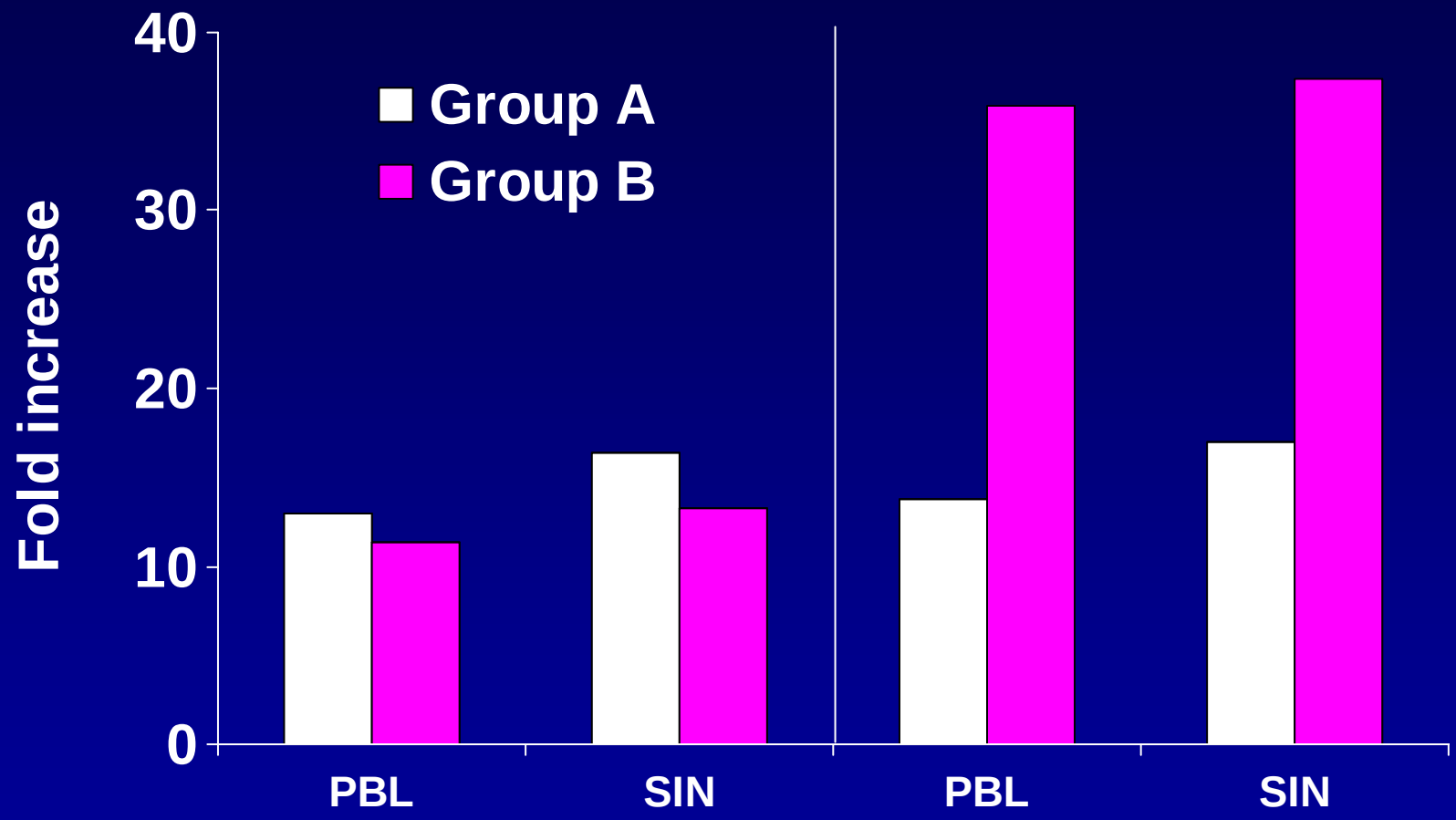
Immune Response to any Peptide increased in Group B (12 peptide)

- T cell response to at least one peptide in the PBL, SIN or either
- By treatment group (n = 24 per group)
- P values (Chi-sq):
  - PBL 0.30
  - SIN 0.11
  - Overall < 0.02 *
- Response to ~0.9 (A) vs ~2.8 (B) peptides



Cumulative responses to All Peptides (mean)

Ratio # IFNγ-secreting cells/ background



Ratio =	0.88 x	0.81 x	2.59 x	2.20 X
P =	0.58	0.80	0.093	0.042

Findings

- Vaccination with this 12 peptide mixture is immunogenic in 100% of melanoma patients tested
- Individually, 10 of the 12 peptides are immunogenic.
- Non-mutated peptide antigens can be reliably immunogenic.
- At least 4 peptides not previously tested in humans are immunogenic, expanding peptides available for clinical trials in HLA-A2+ and HLA-A3+ patients (A2: MAGE-A10$_{254\text{-}262}$ (Huang); A3: gp100 $_{614\text{-}622}$ (Kawakami), MAGE-A1 $_{96\text{-}104}$ (Chaux), NY-ESO-1 $_{53\text{-}62}$ (Wang))
- More peptides restricted by HLA-A1 are needed for clinical investigation.

Findings

- The 12-peptide vaccine mixture induces multivalent T-cell immune responses simultaneously, resulting in a 2-2.5x increase in the cumulative T cell response compared to a 4-peptide vaccine.
- Peptide competition for MHC does not appear to limit immunogenicity when 4 peptides of varied affinity for the MHC are administered in a single emulsion.
- Vaccines with multiple peptides can incorporate those peptides in single preparations of peptide mixtures.
- Ongoing and future studies will evaluate mixtures of peptides for helper T cells given in combination with this mixture of 12 Class I MHC restricted peptides.

Contributors

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