Identification of CD8 T Cell Epitopes on the 2,000 KDa MUC16 (CA125) and Development of Pentamers for Immune Monitoring in Ovarian Cancer Vaccine Trials

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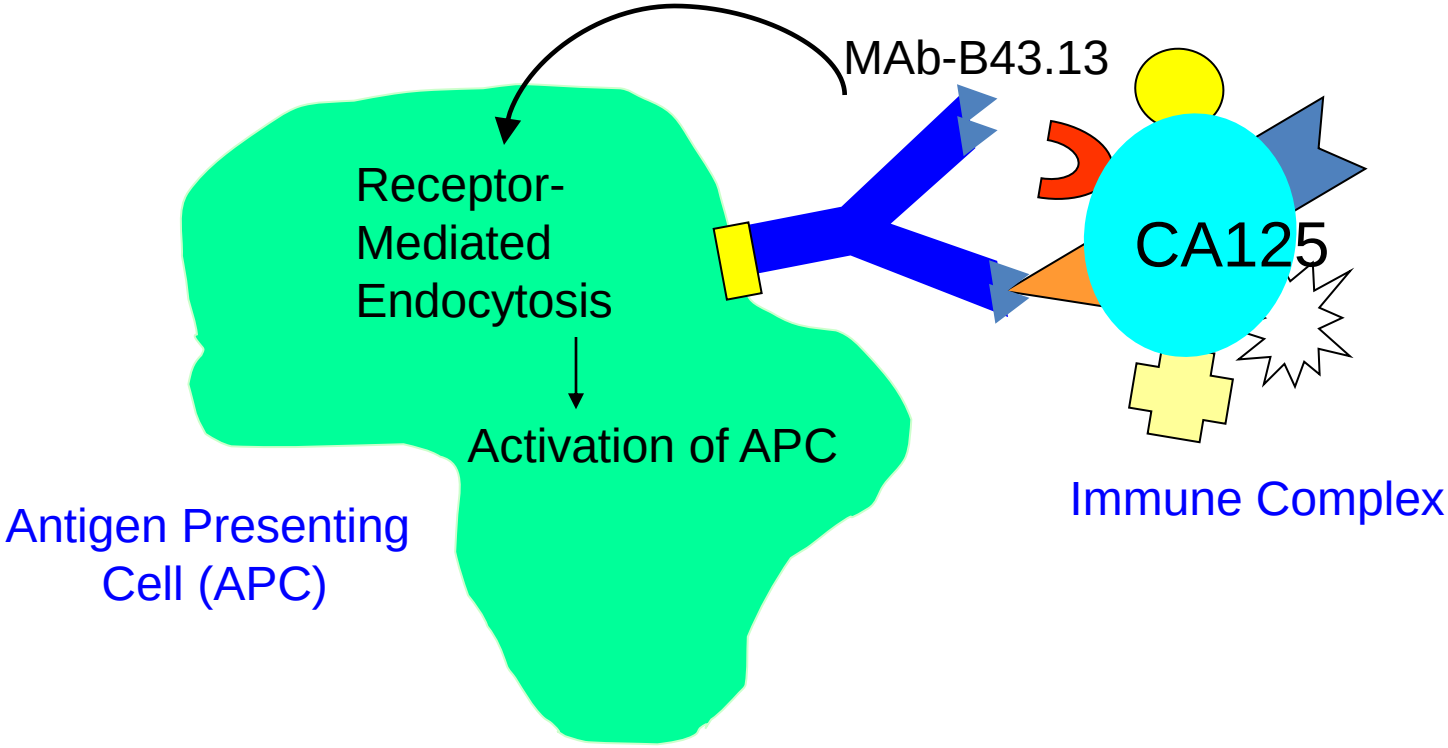
Introduction

- Studies focused on the use of antibodies as immune modulators
- Mechanism of action is believed to involve altered immune processing of the target antigen via immune complex formation
- OvaRex® Mab-B43.13 is in Phase II and III clinical trials for the treatment of ovarian cancer
- Target antigen is CA125 (MUC16)
- Complex and very large gene, only fully sequenced in 2002
- T cell epitopes not identified yet
- Current study aimed at identifying T cell epitopes for:
  - Better characterize the immune processing of CA125 and immune complexes
  - Tools in monitoring T cell responses in patients treated with B43.13
  - Developing potential peptide vaccines

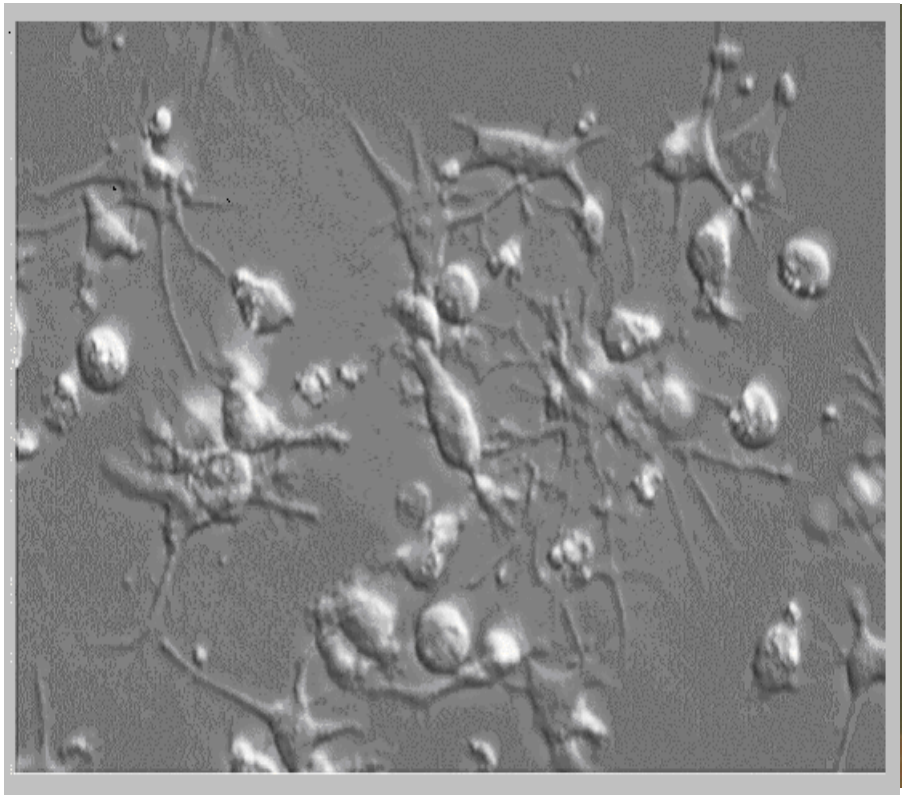
B43.13-CA125 Complexes Are Internalized by Antigen-Presenting Cells via Receptors



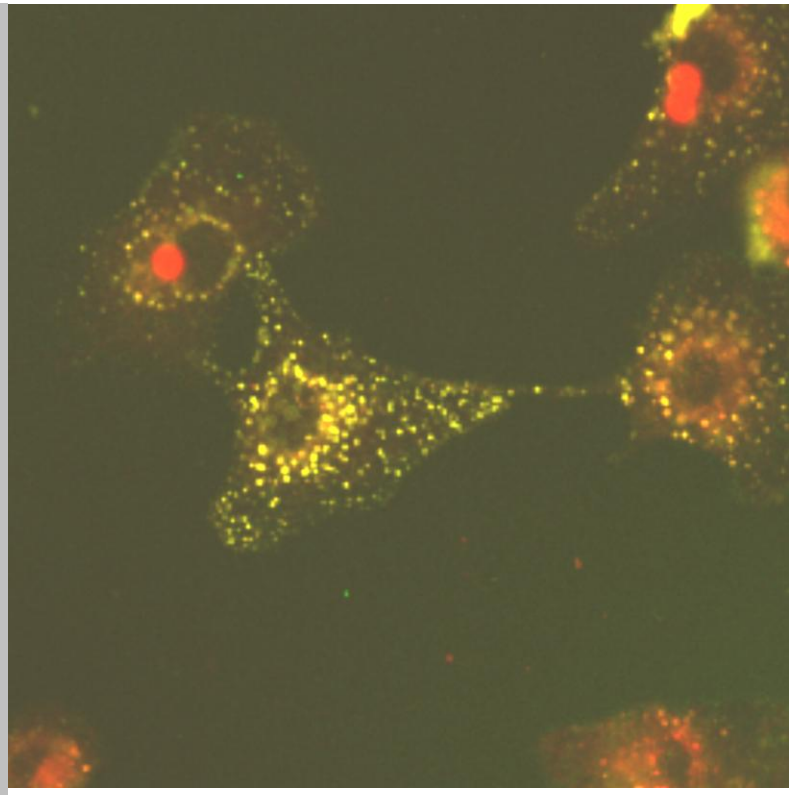
Complex is taken up by antigen presenting cells, e.g. dendritic cells, mainly via CD64 and CD32 as well as the MMR

In Vitro Studies with Human Dendritic Cells

Confocal microscopy shows internalization of IC



Human Dendritic Cells in Culture



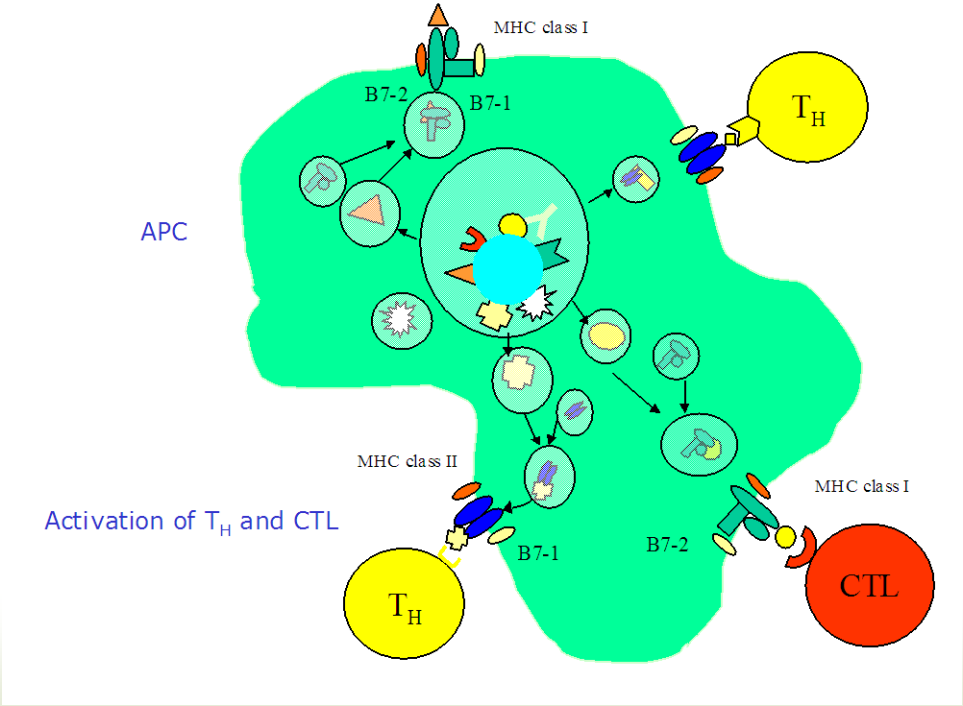
CA125-B43.13 Complex

CA125 conjugated with FITC (green)
pre-incubated with MAb-B43.13-Cy3 (red)
added to day 5 DC for 30 min. before fixation; yellow: co-localized complex

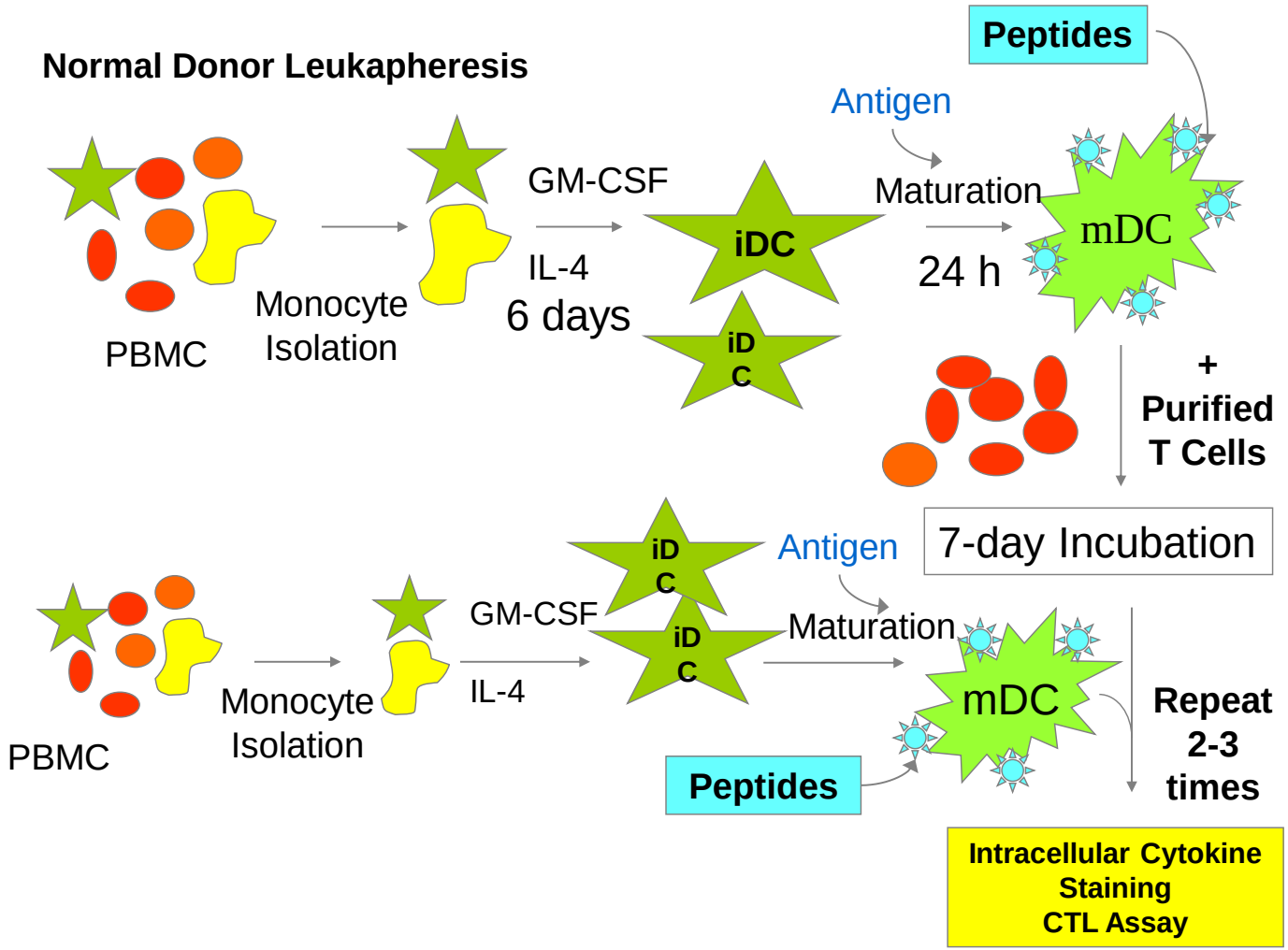
Antibody Enhances Immune Processing

MAb enhances processing and T cell activation:

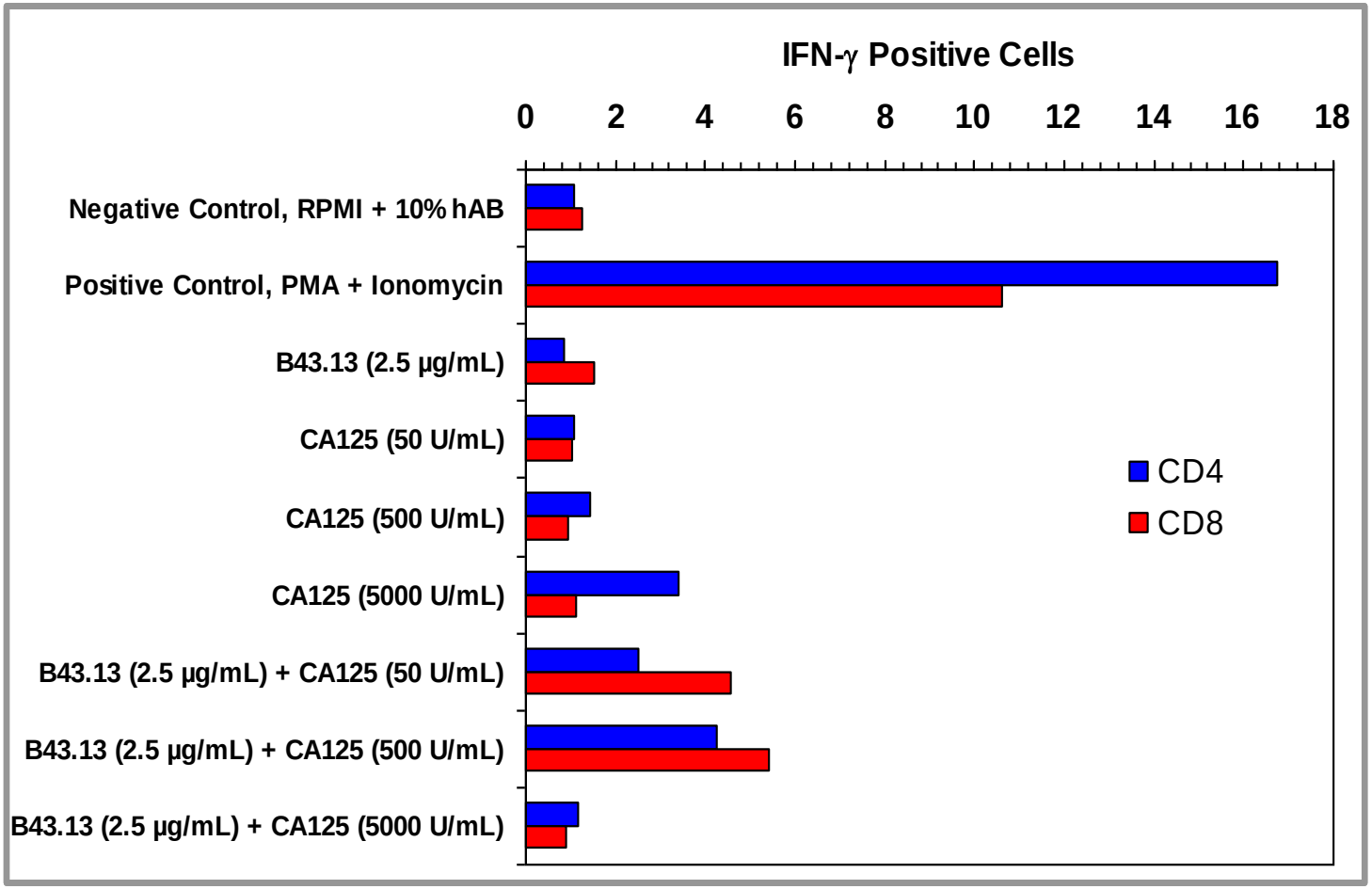
- Increasing uptake
- Cross-presenting on HLA Class I and II
- Activating T helper cells and CTL
- Stimulating immune function of foreign antibody



In Vitro Antigen-Presentation Assay



B43.13-CA125 Complexes Increase Cellular Responses with CD8+ (CTL) Phenotype

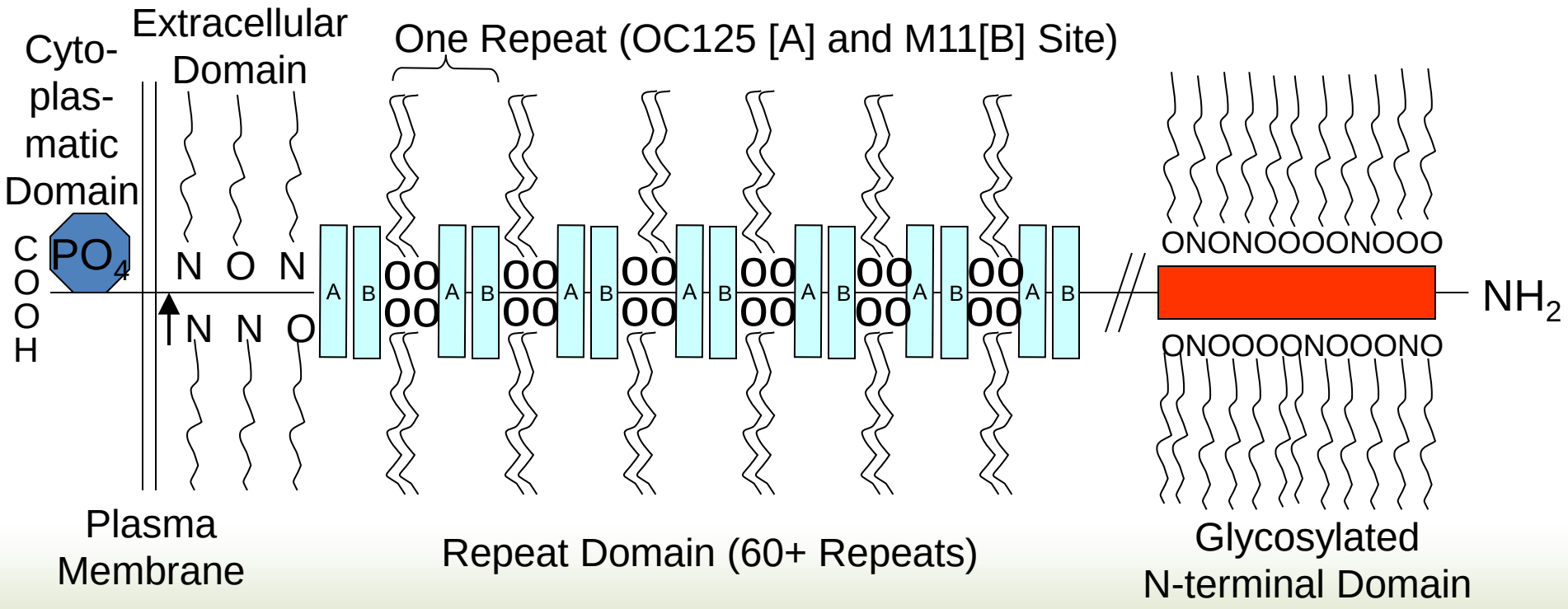


Two rounds of *in vitro* stimulation

MUC16 (CA125) Antigen

Fully Sequenced and Partially Cloned Only in 2002

- Huge, highly-glycosylated transmembrane protein, MW >2.5 Mio Da w/o sugars, >5 Mio Da glycosylated
  - ~12,000 aa amino terminal domain
  - ~10,000 aa of 60+ repeat domains of 156 aa
  - Short cytoplasmatic tail with tyrosine phosphorylation site
- Enzymatically cleaved into extracellular matrix – tumor marker



Examples of MUC16 Repeats

Every 156 AA repeat is slightly altered and contains different putative HLA-A*0201 (in bold) and other allele epitopes

FKNTSV**GLLYSGCRLTLLRPEKDGA**ATGVDAI**C**TH**RLDPKSPGL**NRE**QLYWE**
LSKLTNDIEELGPY**TLDRNSLYV**NGFTHQSSVSTTSTPGTSTVDRLTSGT
PSSLSSPTIMAAGPLLVPTLNFTITNLQYEEDMRRTGSRKFNTMES**VLQG**
LLKPL 1840

FKSTSVGPLYSG**C**RLT**LLRPEKRGA**ATGVDTI**C**TH**RLDPLNPGL**DRE**QLYWE**
LSKLTRGIIELGPY**LLDRGSLYV**NGFTHRTSVPTTSTPGTSTVDLGTSGT
PFSLPSPAXXXPLLXPFTXNXTITNLXXXXXMXXPGSRKFNTTER**VLQTL**
LGPL 5272

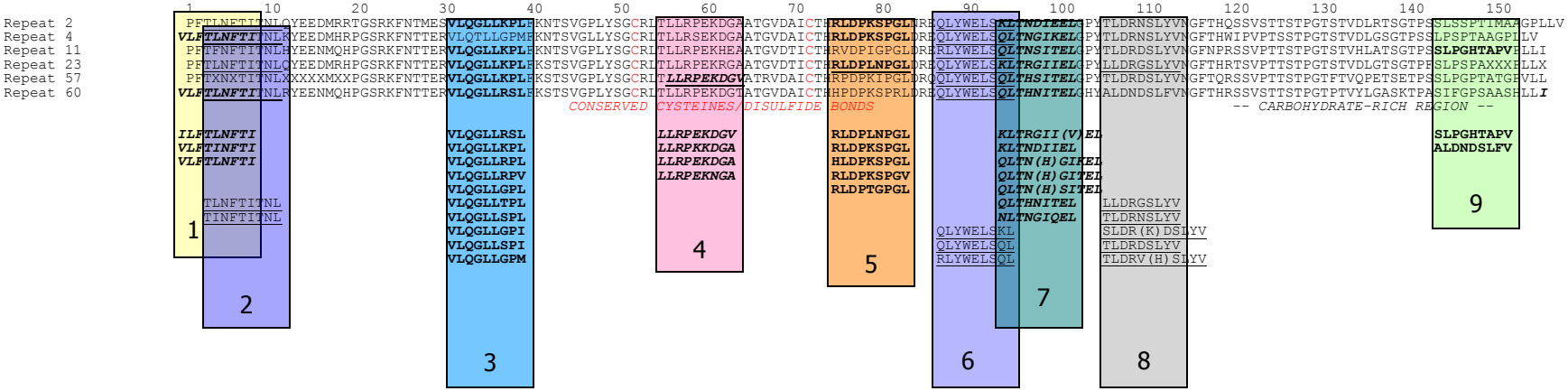
FKNTSVGPLYSG**C**RLT**LLRPKKDGA**ATKVDAI**C**TY**RPDPKSPGL**DRE**QLYWE**
LSQLTHSITELGPY**TLDRDSLYV**NGFTQRSSVPTTSIPGTPTVDLGTSGT
PVSKPGPSAASPLL**VLFTLNFTI**TNLRYEENMQHPGSRKFNTTER**VLQGL**
LRSL 10887

FKSTSVGPLYSG**C**RLTLLRPEKDGTATGVDAI**C**THHPDPKSPRLDRE**QLYWE**
LSQLTHNITELGHY**ALDNDSLFV**NGFTHRSSVSTTSTPGTPTVYLGASKT
PASIFGPSAASHLL**ILFTLNFTITNL**RYEENMWGSRKFNTTER**VLQGLLR**
PL 11042

Conserved cysteines
High ST areas with likely glycosylation
Putative T cell epitopes (using algorithsms such as MAPPP, BIMAS, Propred-I and SYFPEITHI)

MUC16 (CA125) Antigen

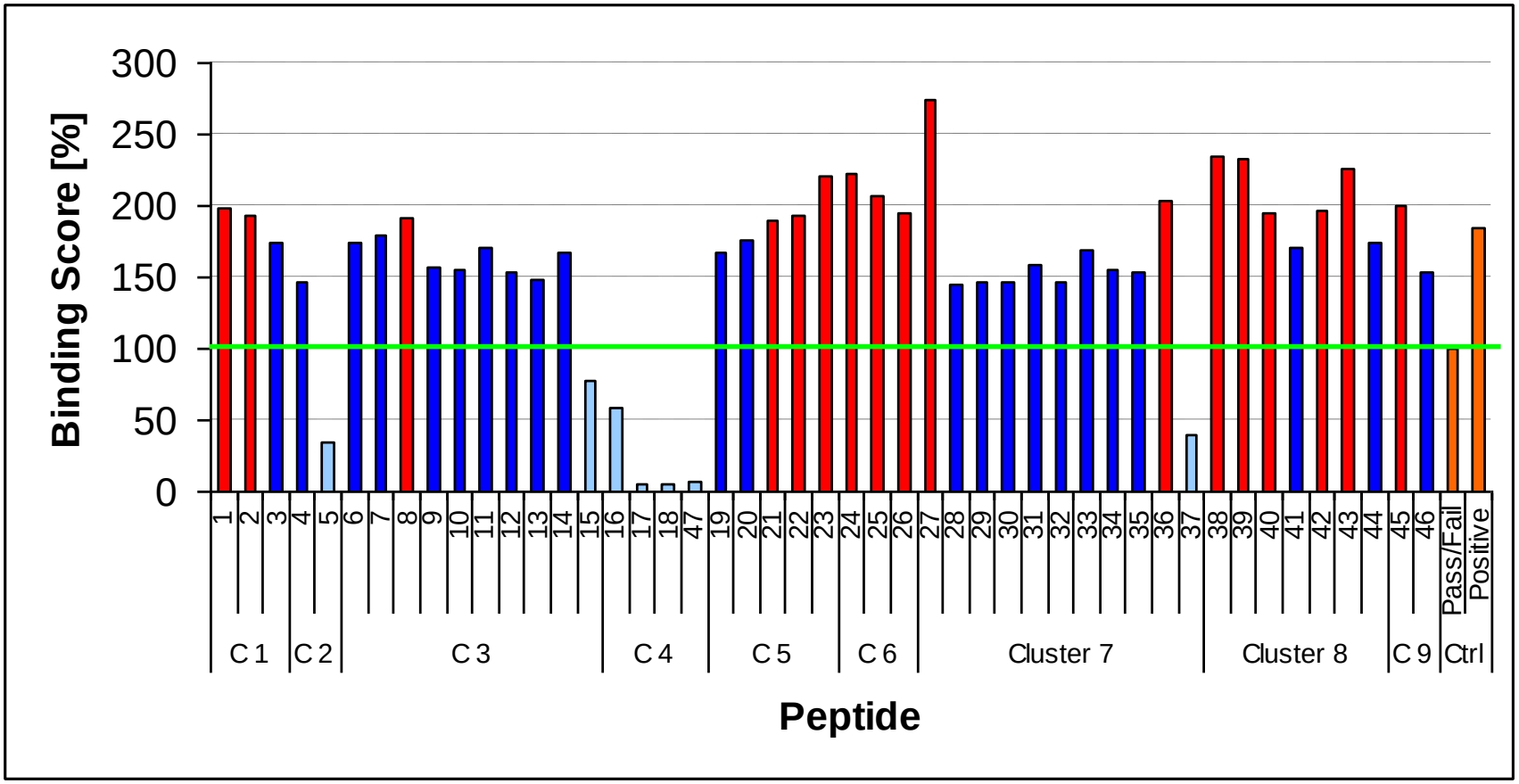
Potential HLA-A*0201 Binding Peptides Fall into Clusters



- Molecule too large to screen overlapping peptides
- Initial focus on HLA-A*0201 and repeat section – higher likelihood of peptides being presented
- Tandem repeat section of CA125 (*O'Brien TJ et al., Tumor Biology 2001; 22:348-366; and Tumor Biology 2002; 23:154-169*) was screened with 4 algorithms (MAPPP, BIMAS, Propred-I and SYFPEITHI) for putative HLA-A*0201 binding peptides and proteasome cleavage sites.
- Nine regions (Clusters 1-9) within the repeat section with >40 potential strong HLA-A*0201 peptides were identified. Those peptides were submitted to ProImmune for synthesis and affinity testing.
- Some peptides are unique to 1 or 2 repeats, others are abundant

REVEAL™ Assay to Determine On-Rates

A*0201 Binding Assay

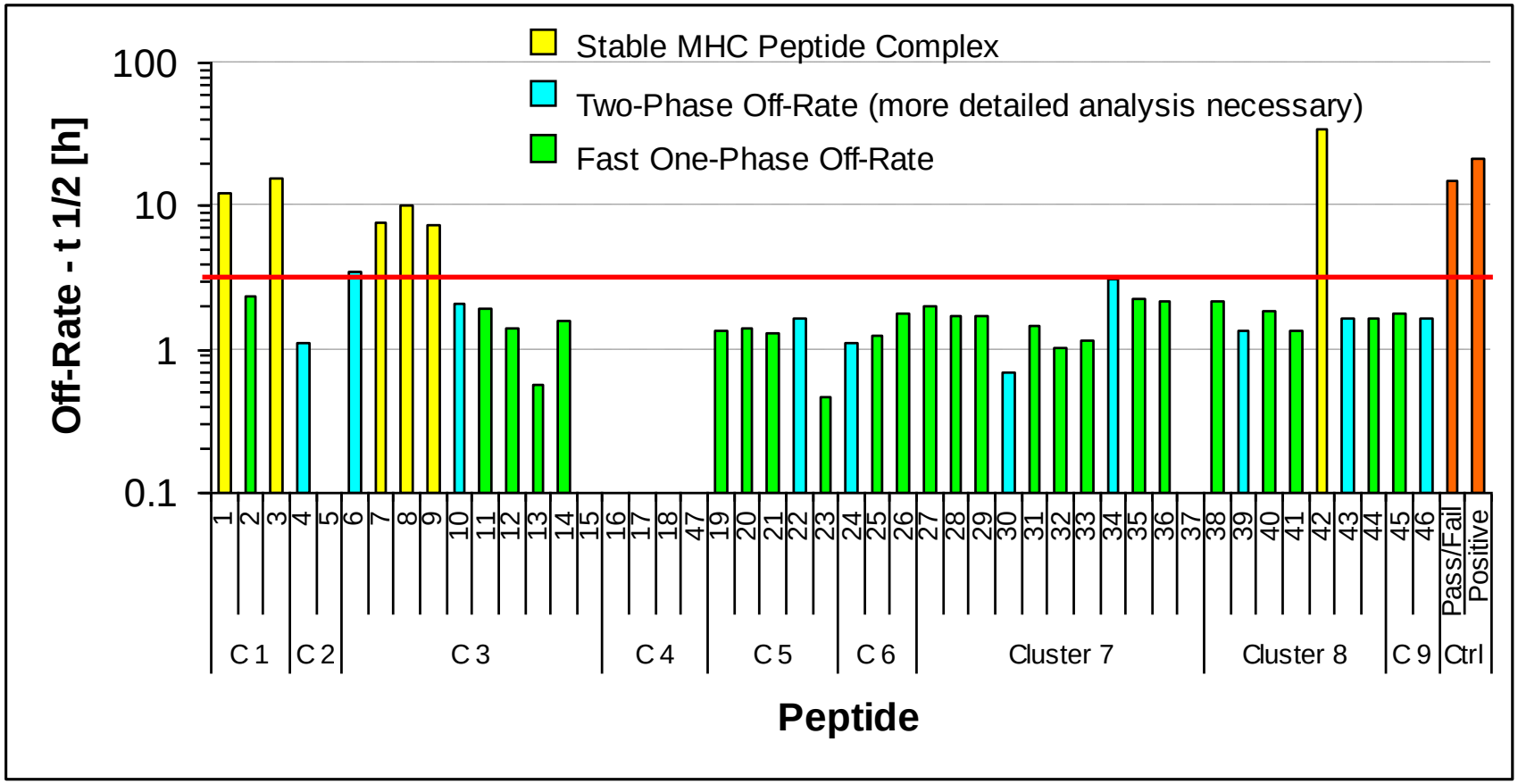


- Light blue peptides: failed to form complex with HLA-A*0201
- Dark blue peptides: formed complexes with HLA-A*0201
- Red peptides: high on-rates, better than positive control

} Pentamers could be generated

REVEAL™ Assay to Determine Off-Rates

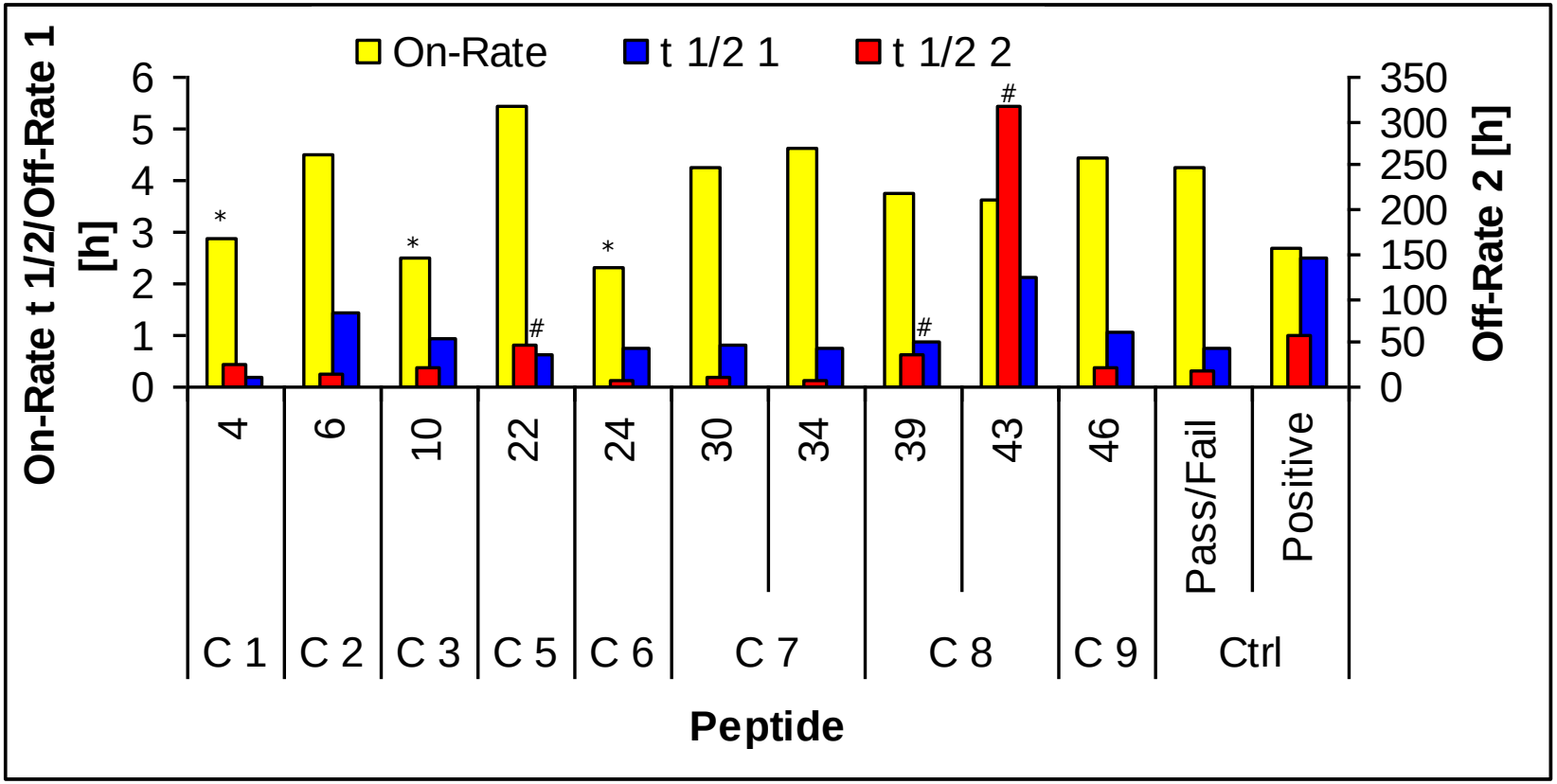
'Quick Check'



- Stable MHC-peptide complexes in clusters 1 (#1, #3), 3 (#7, #8, #9) and 8 (#42)

REVEAL™ Detailed Affinity Assays

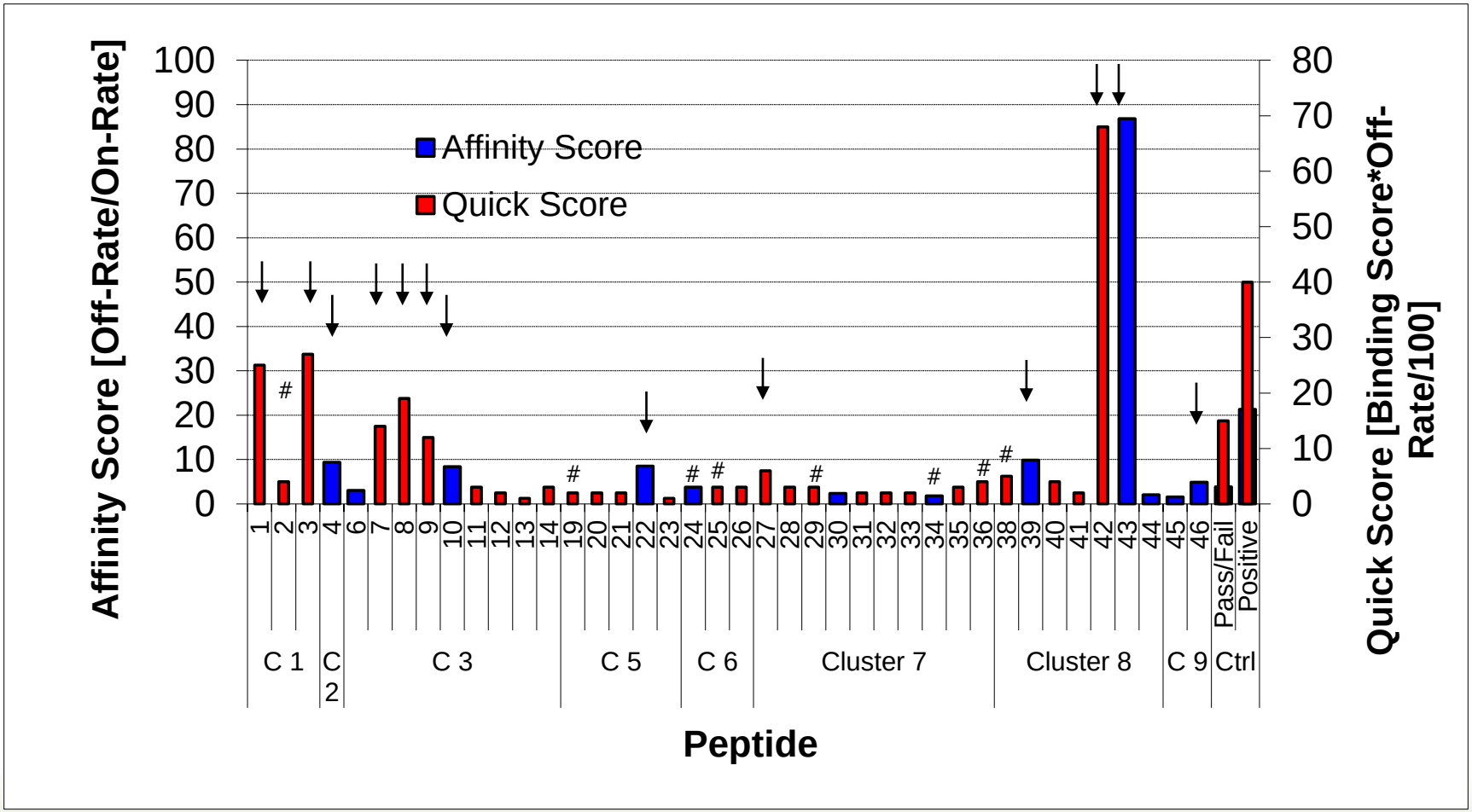
Performed for peptides with 2-phase off-rates



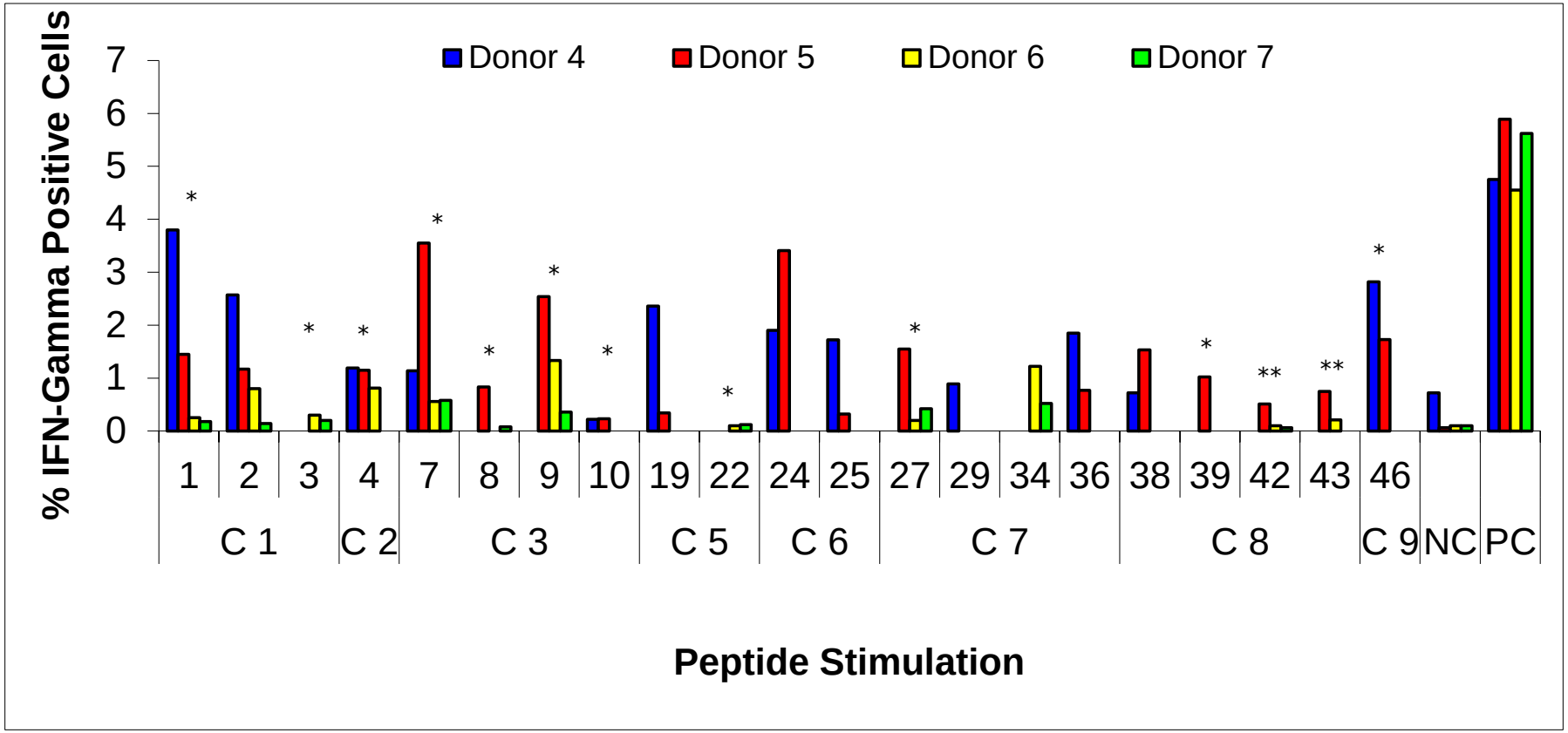
- Fast on-rates for peptides 4, 10 and 24 (*)
- Slow second phase off-rates for peptides 22, 39 and especially 43 (#)

Summary of REVEAL™ Assays

Quick Scores for single phase peptides, Affinity Scores for peptides with 2-phase off-rates



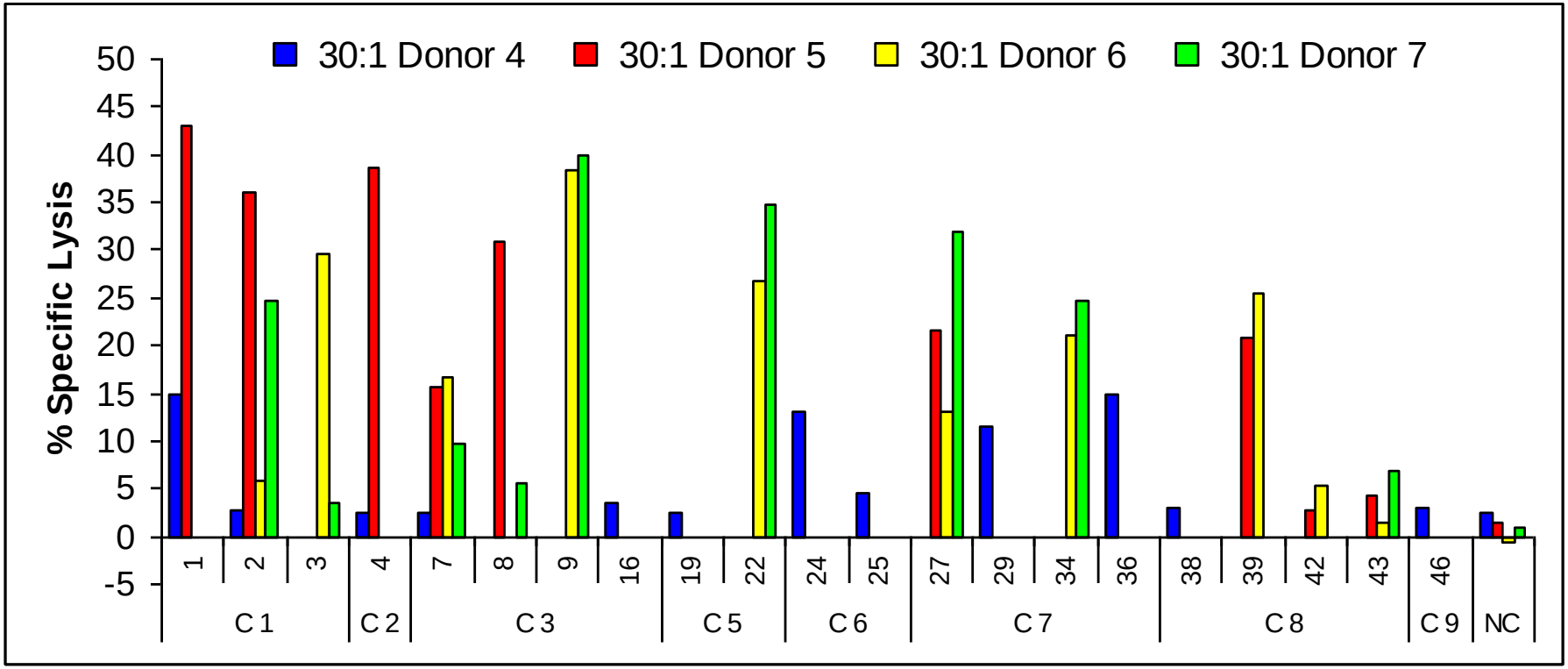
Peptides with Intermediate Affinity Can Stimulate T Cells from Normal Donors



* High-scoring peptides
** Highest affinity peptides

3 rounds of *in vitro* stimulation on dendritic cells pulsed with peptides

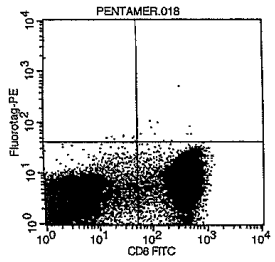
Peptides with Intermediate Affinity Can Induce CTL in Normal Donors



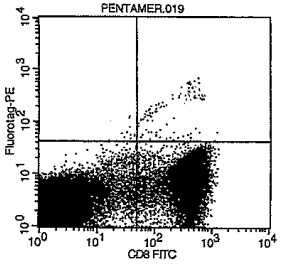
Four rounds of *in vitro* stimulation on dendritic cells pulsed with peptide
NIH:OVCAR-3 ovarian cancer cells as targets

Pentamer Staining of Early T Cell Lines

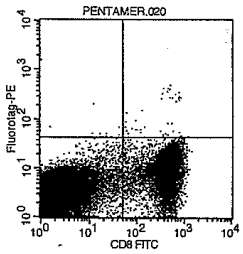
Fluorotag/no pentamer



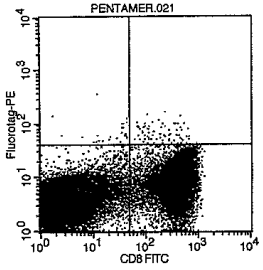
Pentamer #2



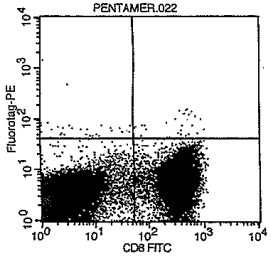
Pentamer #3



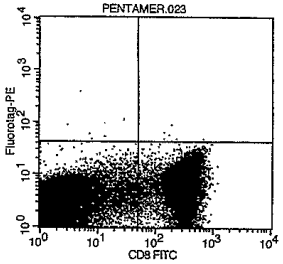
Pentamer #7



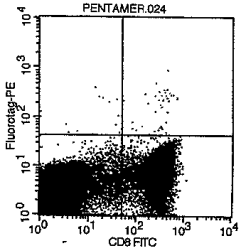
Pentamer #9



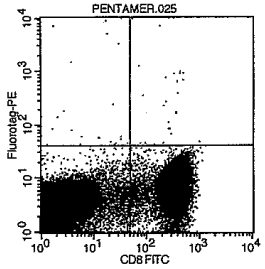
Pentamer #22



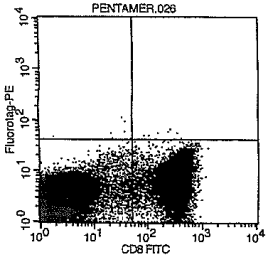
Pentamer #27



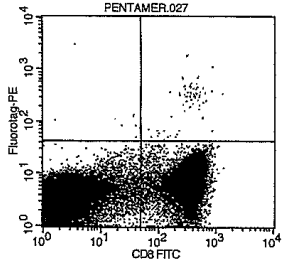
Pentamer #34



Pentamer #42



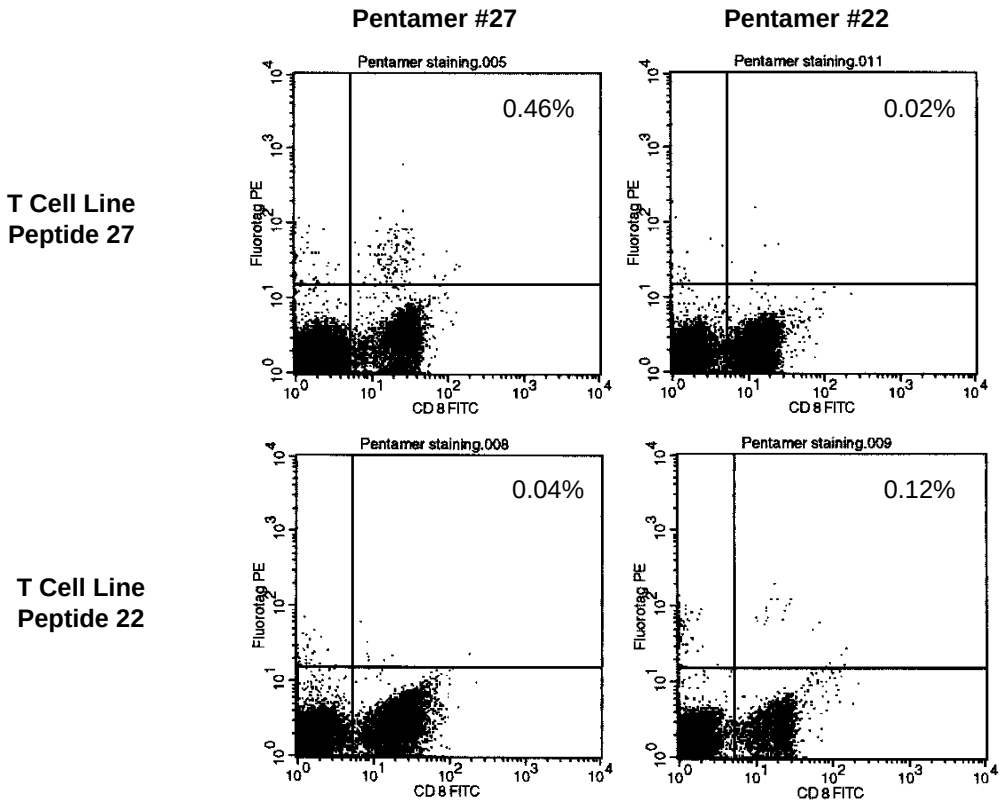
Control Pentamer Flu



<u>Donor 6</u>:
positive in <u>ICC assay</u> for peptides 2, 3, 4, 7, 9, 27 and 34
Positive in <u>CTL assay</u> for peptides 2, 3, 7, 9, 22, 27, 34, 39

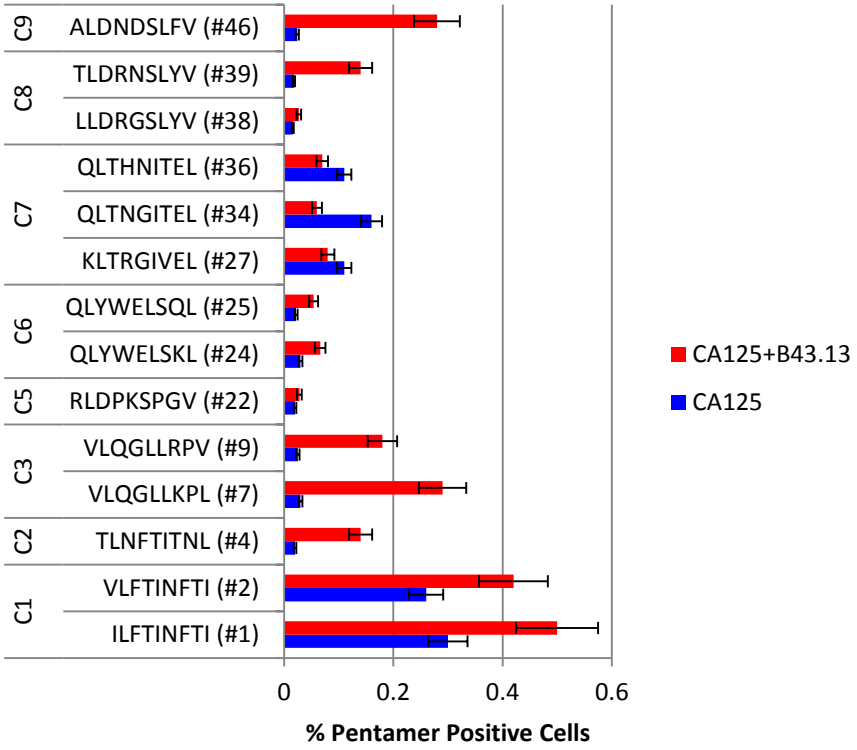
Pentamer Staining
T cell lines specific for peptides 1, 2, 3, 7, 9, 22, 27, 34, and 42 as well as control T cells were stained with custom-made unlabeled pentamers (ProVE) specific for that peptide, followed by washing and incubation with anti-human CD8-FITC and Fluorotag-PE

Pentamer Staining of T Cell Lines Is Specific

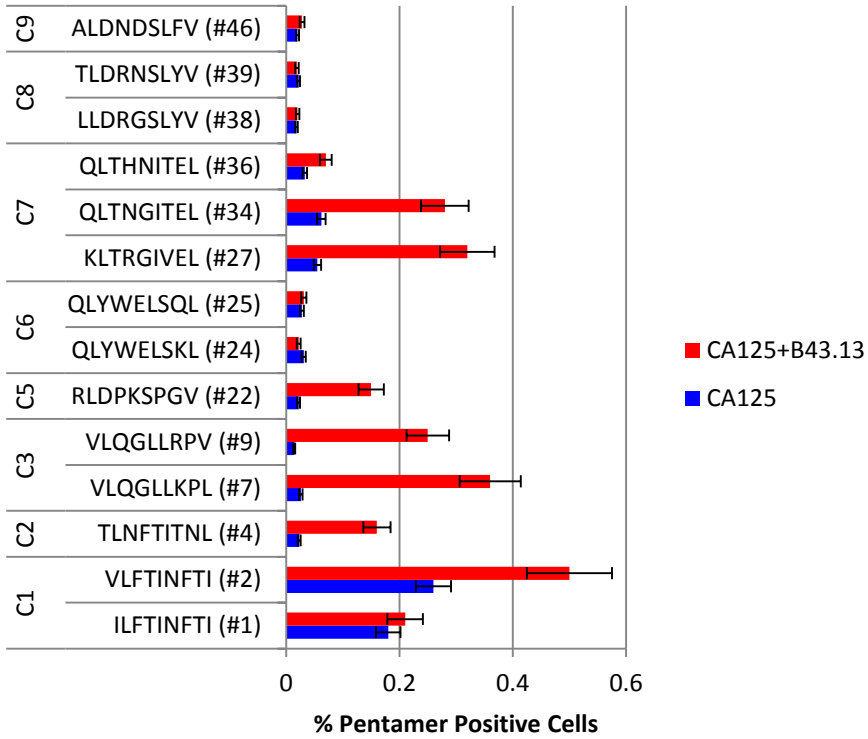


Pentamer Staining
T cell lines specific for peptides 22 & 27 were stained with custom-made unlabeled pentamers (ProVE), followed by washing and incubation with anti-human CD8-FITC and Fluorotag-PE

Peptide Presentation by Dendritic Cells Processing CA125 or CA125-MAb-B43.13 Immune Complexes

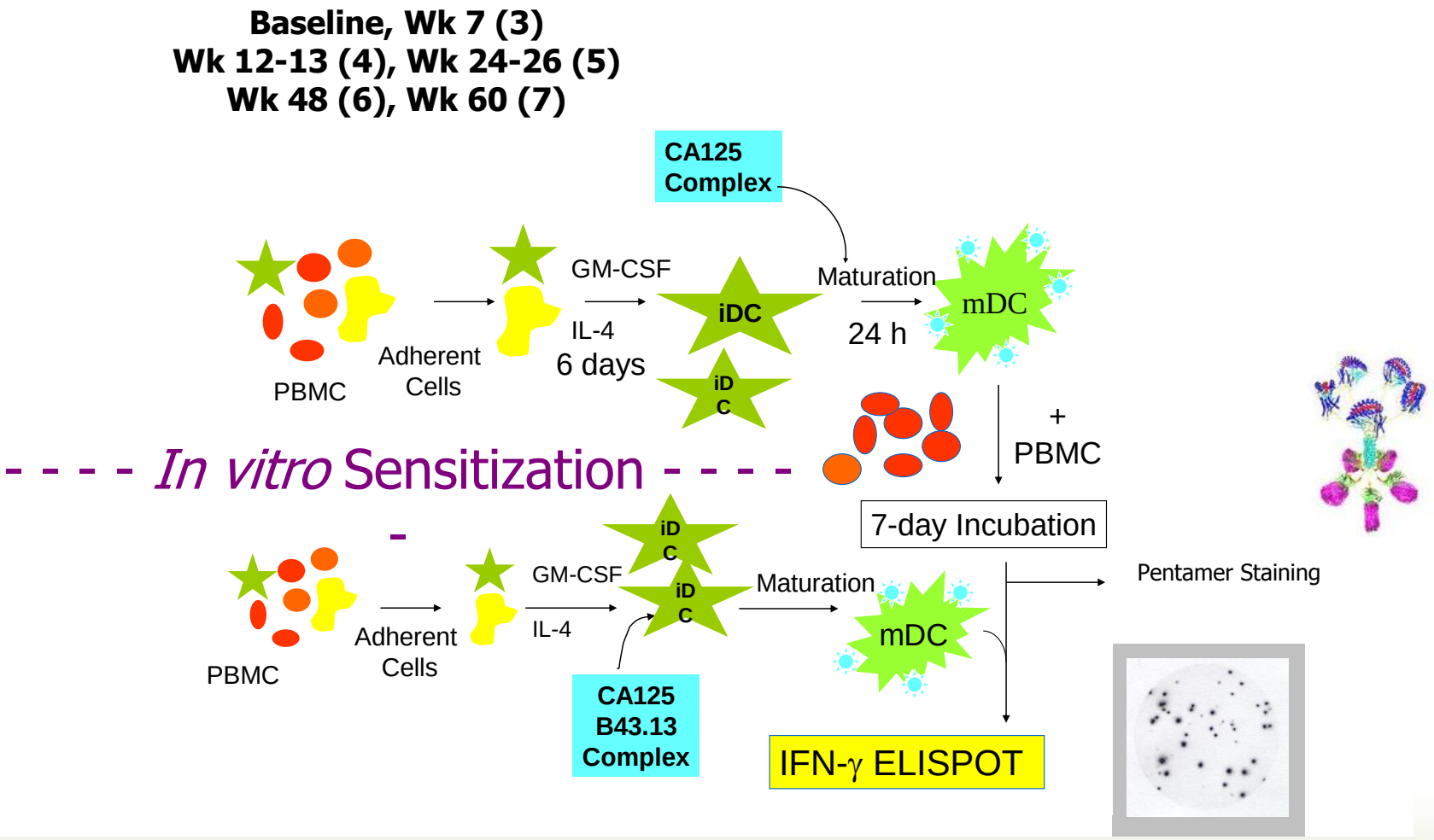


Donor 4

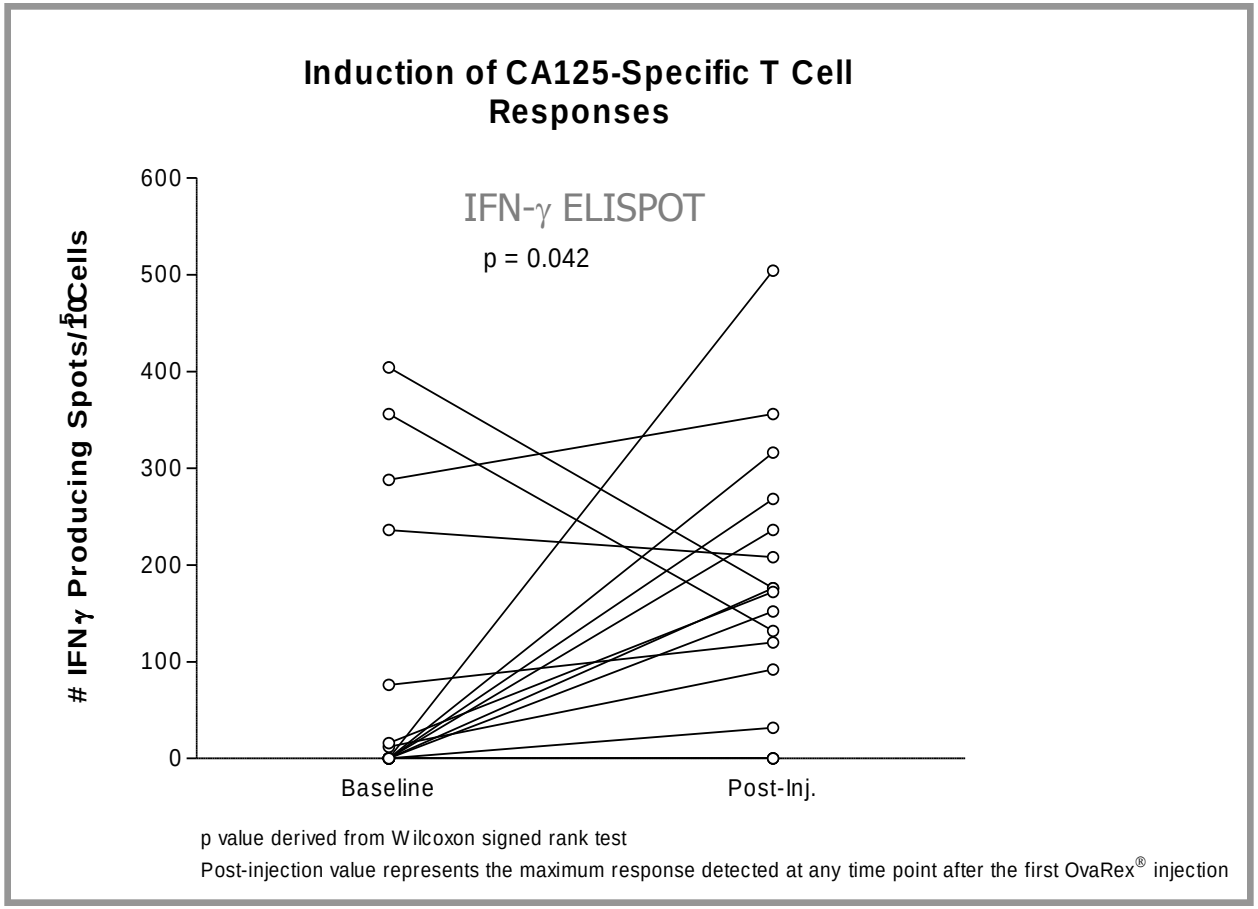


Donor 6

Optimized IFN-γ ELISPOT Assay Used for Patient Sample Analysis



CA125-Specific T Cell Induction in a Phase II OvaRex® Study



- Typed patients for HLA-A*0201 allele: 6 of 14 patients tested positive
- Tested cryopreserved PBMC for pentamer-positive cells after one round of *in vitro* sensitization

Peptide Recognition by Patient T Cells after Processing of CA125 or CA125-MAb-B43.13 Immune Complexes

No. of responding patients (>2x background) of 6 HLA-A*0201 patients

	Pentamer	CA125 processed Pentamer positive	CA125+B43.13 IC processed Pentamer positive
1	ILFTINFTI (#1)	3	4
	VLFTINFTI (#2)	4	5
2	TLNFTITNL (#4)	0	1
3	VLQGLLKPL (#7)	0	3
	VLQGLLRPV (#9)	0	2
5	RLDPKSPGV (#22)	0	1
6	QLYWELSKL (#24)	0	1
7	KLTRGIVEL (#27)	1	3
	QLTNGITEL (#34)	2	2
	QLTHNITEL (#36)	1	1
8	TLDRNSLYV (#39)	0	3
9	ALDNDSLFV (#46)	0	2

Conclusions

- The REVEAL and ProVE™ epitope discovery system reduced the amount of peptides and aided their prioritization in T cell induction assays, although many of the algorism-predicted peptides were confirmed.
- Pentamers could be synthesized for peptides with at least intermediate affinity.
- We were not able to stimulate T cells with the very high affinity peptides for this tumor antigen, likely due to clonal deletion of the precursors.
- Many of the intermediate affinity peptides were able to stimulate and expand T cells, including some T cell lines with CTL activity.
- Natural antigen-processing was distinct for antigen alone vs. immune complexes, indicating that the antibody alters presentation of CA125.
- Responses to the various peptides were donor- and patient-dependent; however, several peptides stimulated T cells from a broader patient pool. Those peptides might be most useful for immune monitoring.
- Several pentamers showed positive staining with HLA-A*0201 PBMC samples from patients post OvaRex® treatment.

Acknowledgements

Peptide synthesis, pentamers, REVEAL and ProVE epitope discovery

ProImmune

Antigen-Presentation Assays (United Therapeutics)

Selena Kansal

Fuxiang Hou

Graham Daley

Patient Immune Monitoring

Theresa Whiteside – Immune Monitoring Lab, University of Pittsburgh

Clinical Trial

Christopher Nicodemus (United Therapeutics)

Alan Gordon (U.S. Oncology, Texas Oncology PA, Dallas)

THANK YOU!