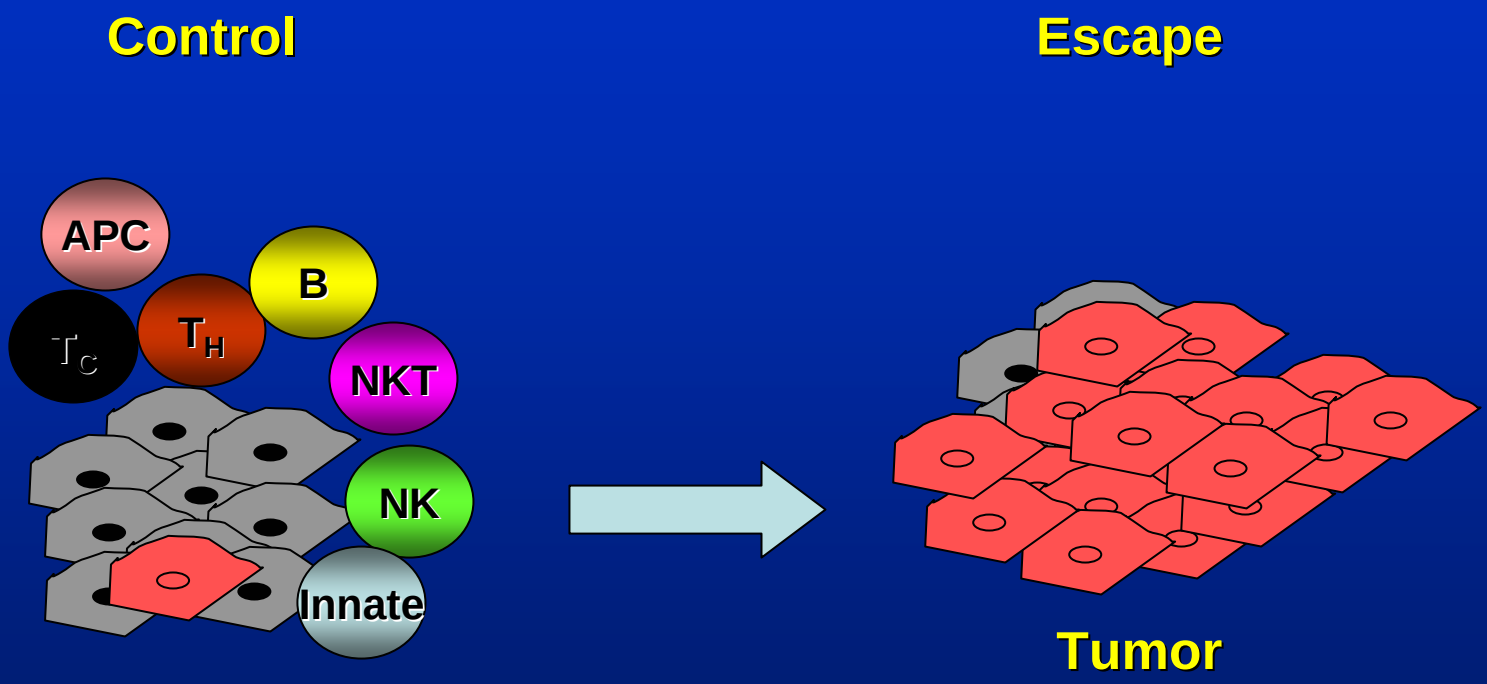
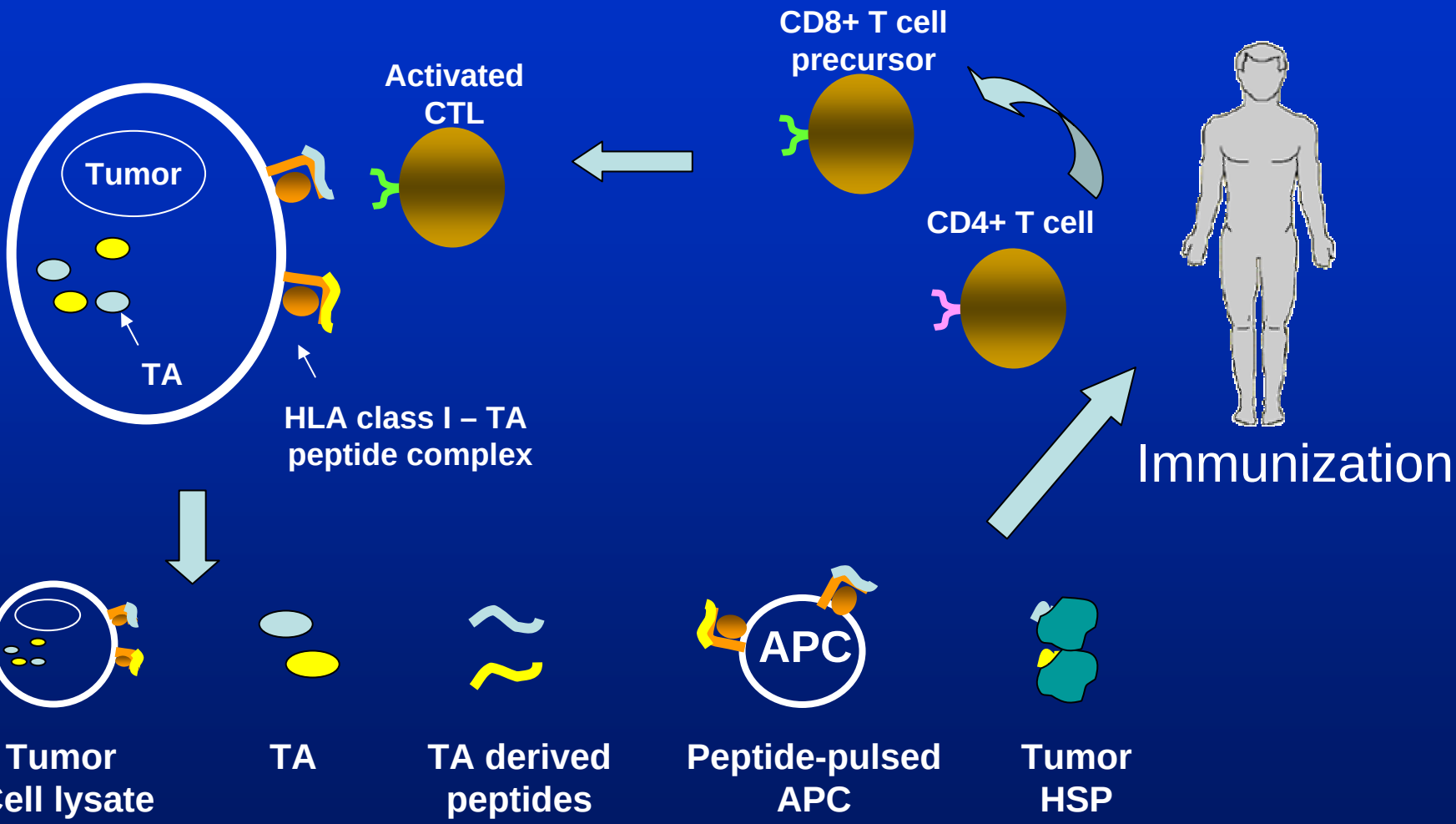
Immune surveillance: The immune system can recognize and destroy nascent malignant cells



T cells are believed to play a major role in controlling tumor growth.

T cell-based Immunotherapy



T cell-based immunotherapy clinical trials: lessons learned

- Immunization strategies have been successful in eliciting tumor antigen-specific CTL in at least a proportion of the immunized patients

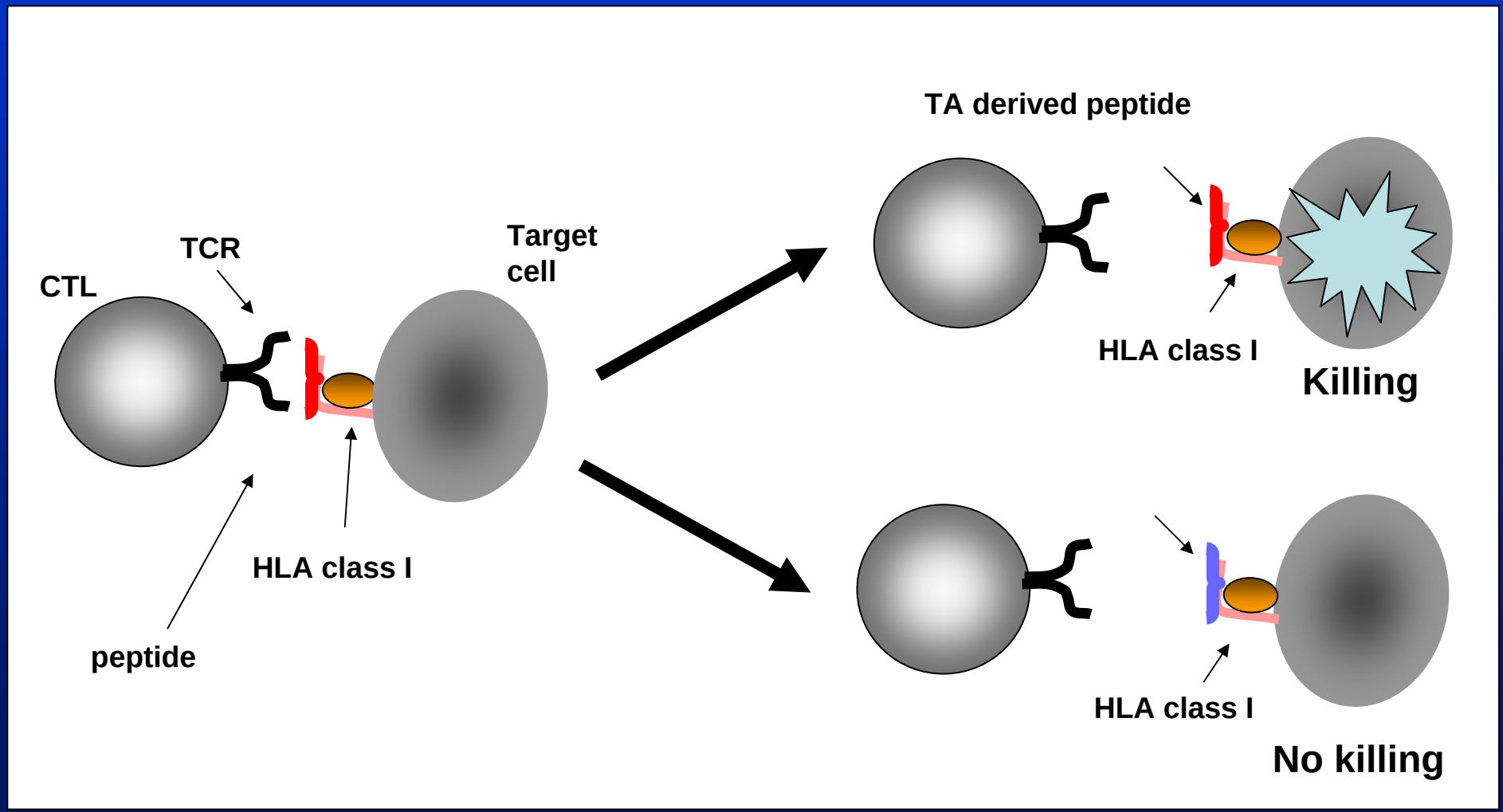
- Disappointing clinical response rates have been obtained

- A tumor antigen-specific CTL immune response is often not accompanied by a clinical response

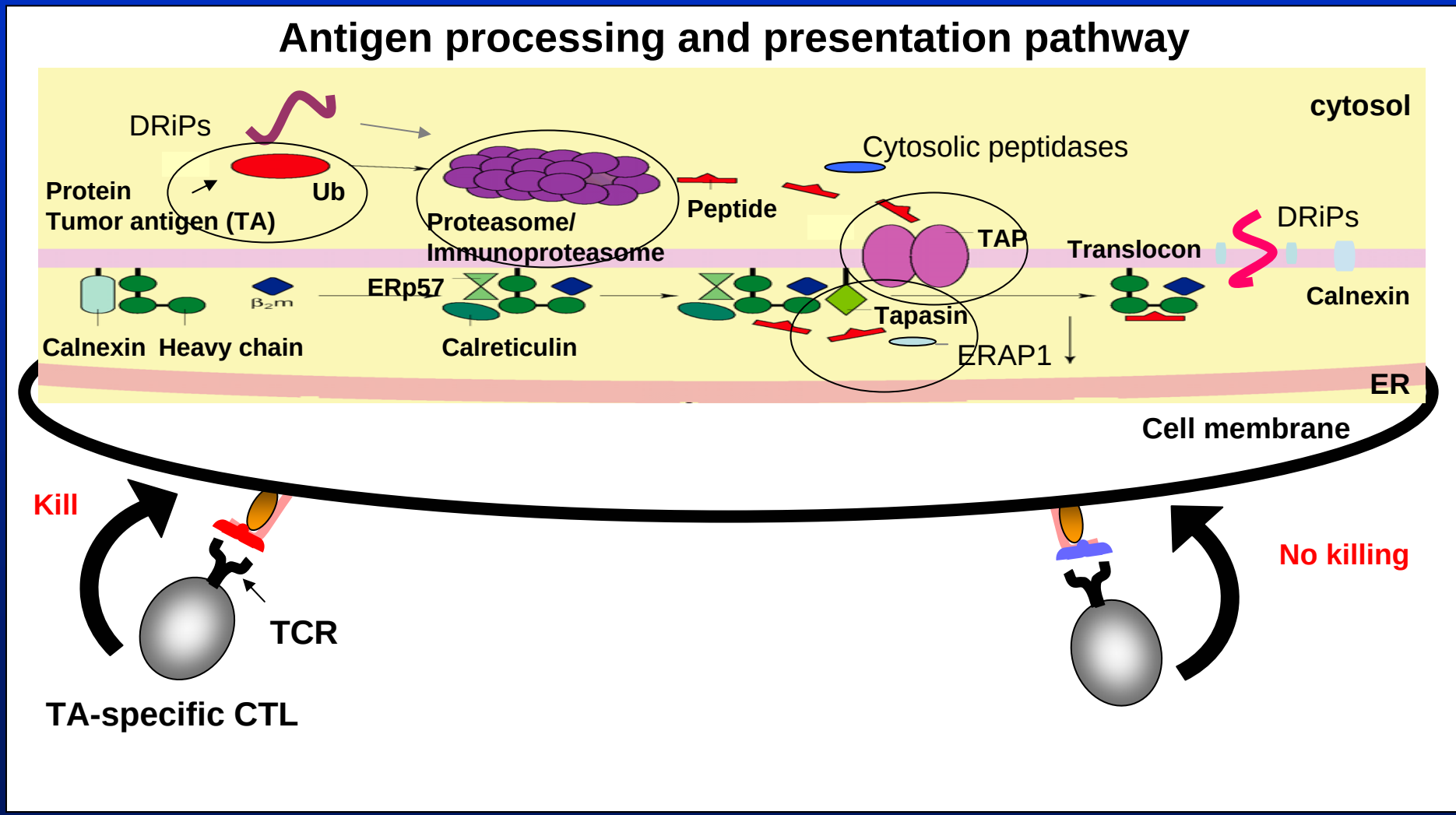
Why does a TA-specific CTL immune response not correlate with clinical response in patients with malignant disease treated with immunotherapy?

- defects in CTL
- resistance of tumor cells to CTL recognition

HLA class I antigen-peptide complex expression is necessary for tumor antigen derived recognition by CTL



How are HLA class I antigen-tumor antigen peptide complexes generated?

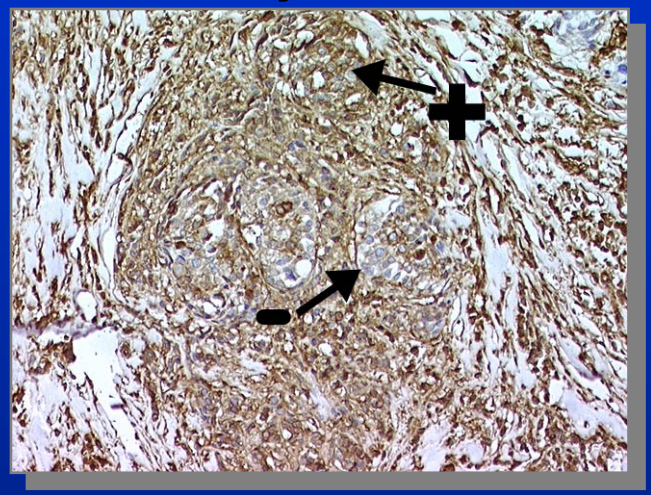


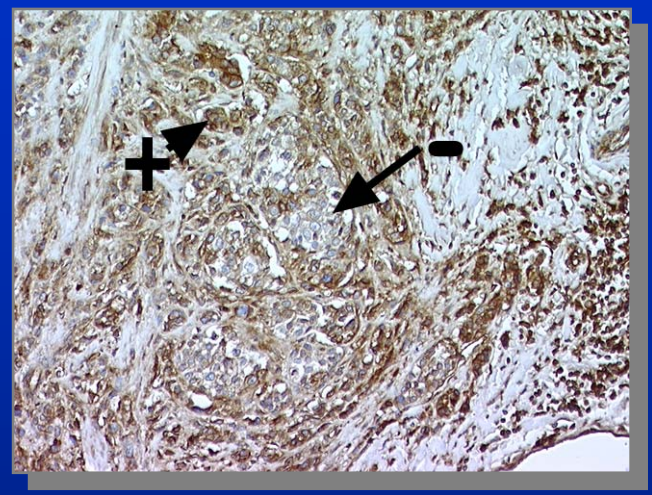
Immunohistochemical staining of formalin fixed, paraffin embedded malignant lesions by HLA class I specific mAb

Heavy chain

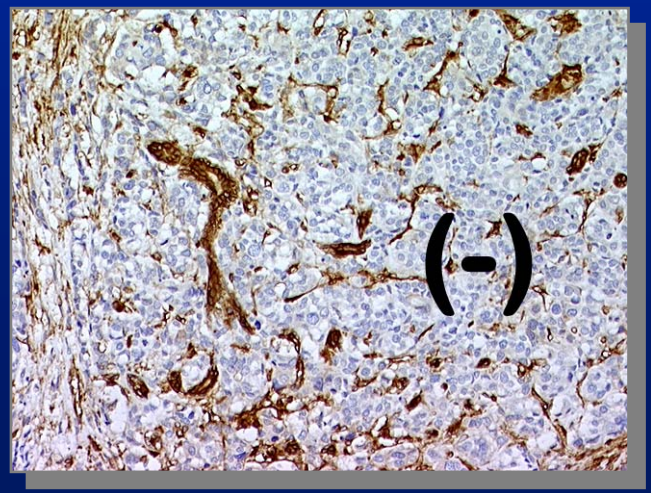
β_2-microglobulin

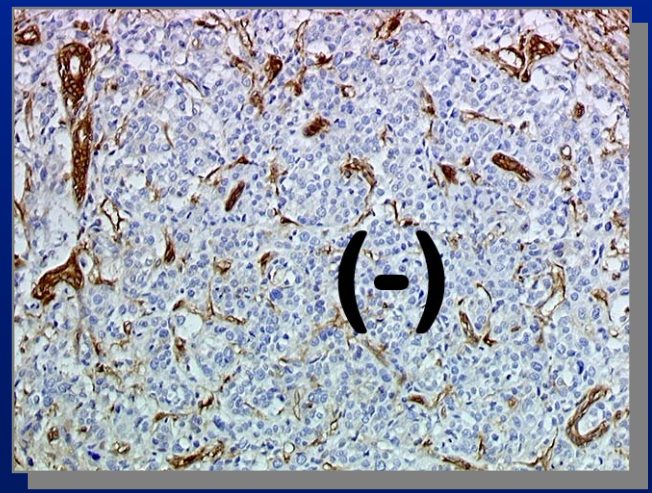
Heterogeneous expression





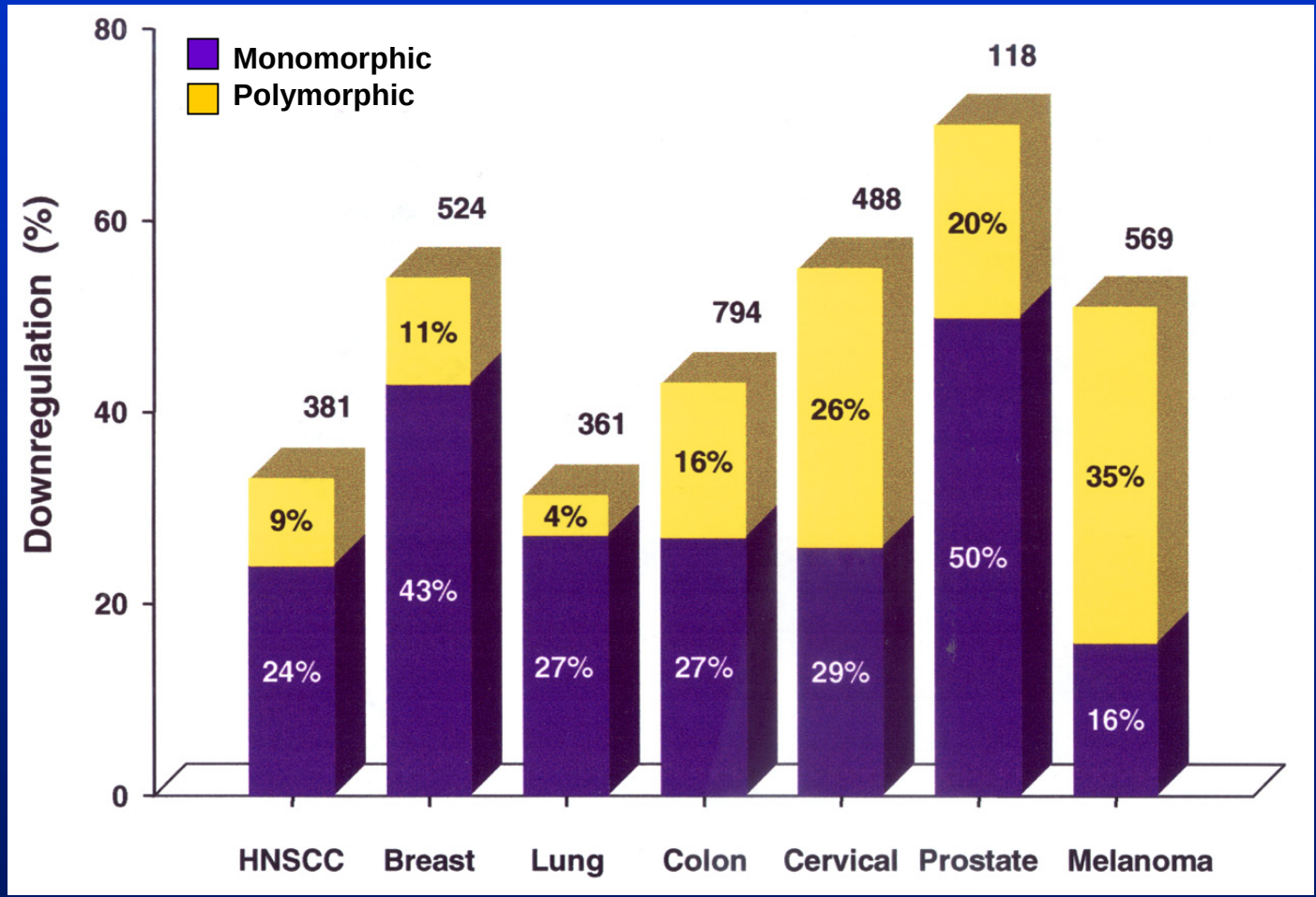
Loss of expression in undifferentiated cells



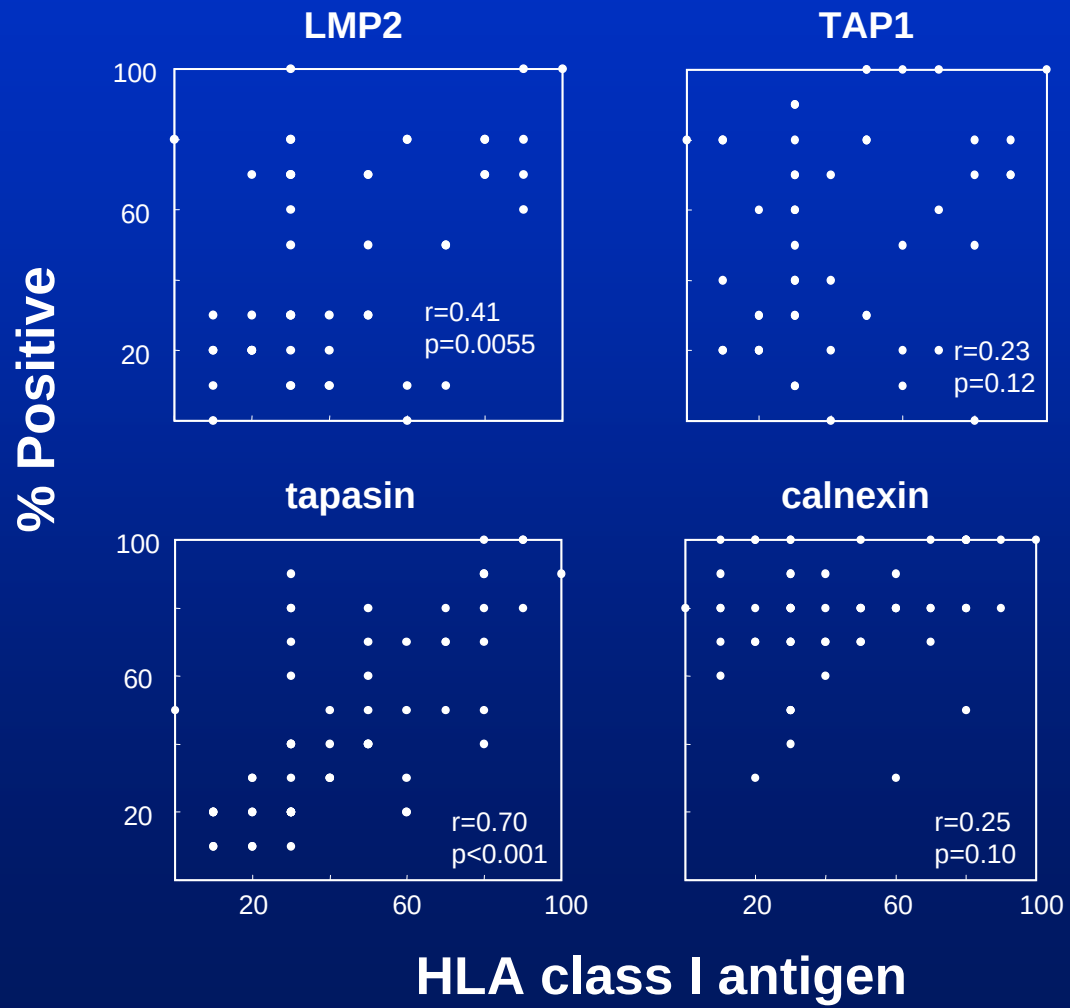


Serial Sections of a Breast Carcinoma Lesion

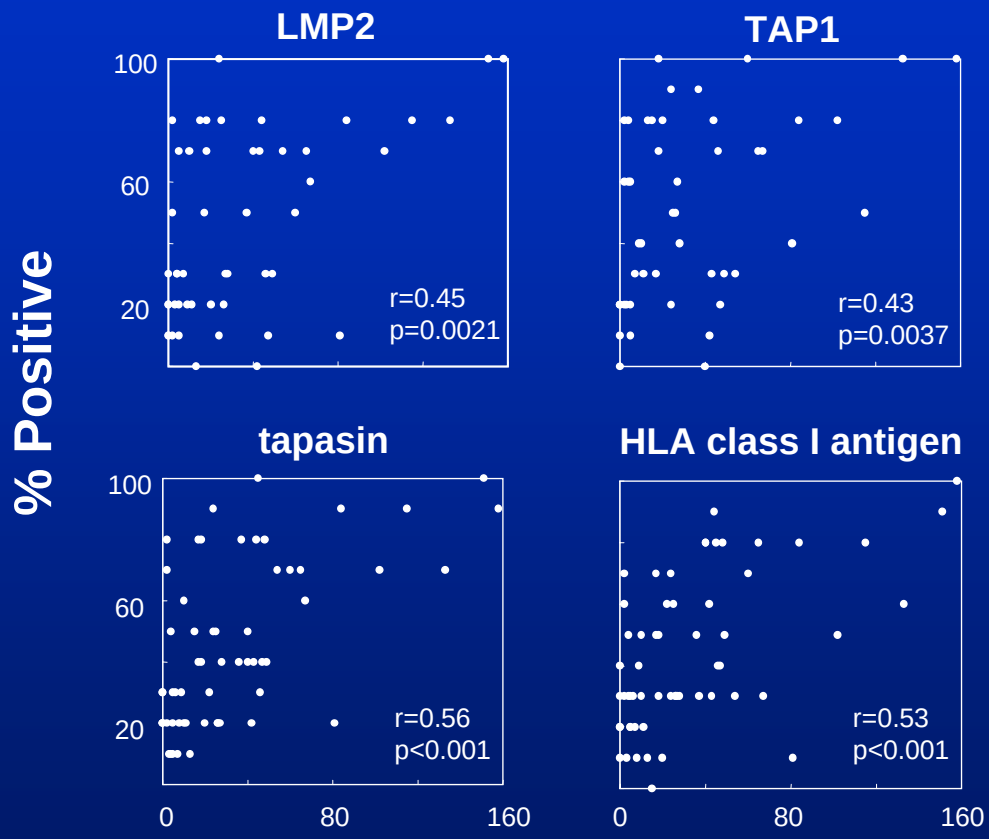
Different frequency of HLA class I antigen downregulation in different tumor types



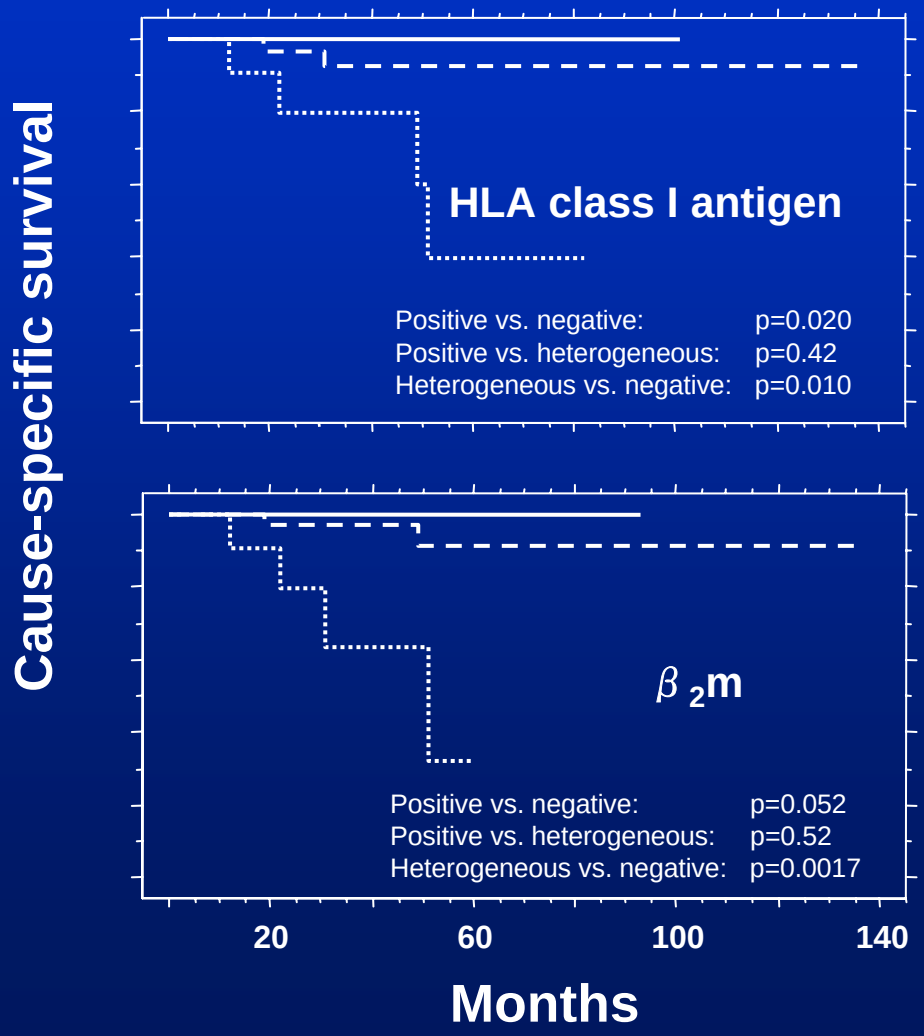
Correlation of LMP2 and tapasin expression with HLA class I antigen expression in primary laryngeal squamous cell carcinoma lesions



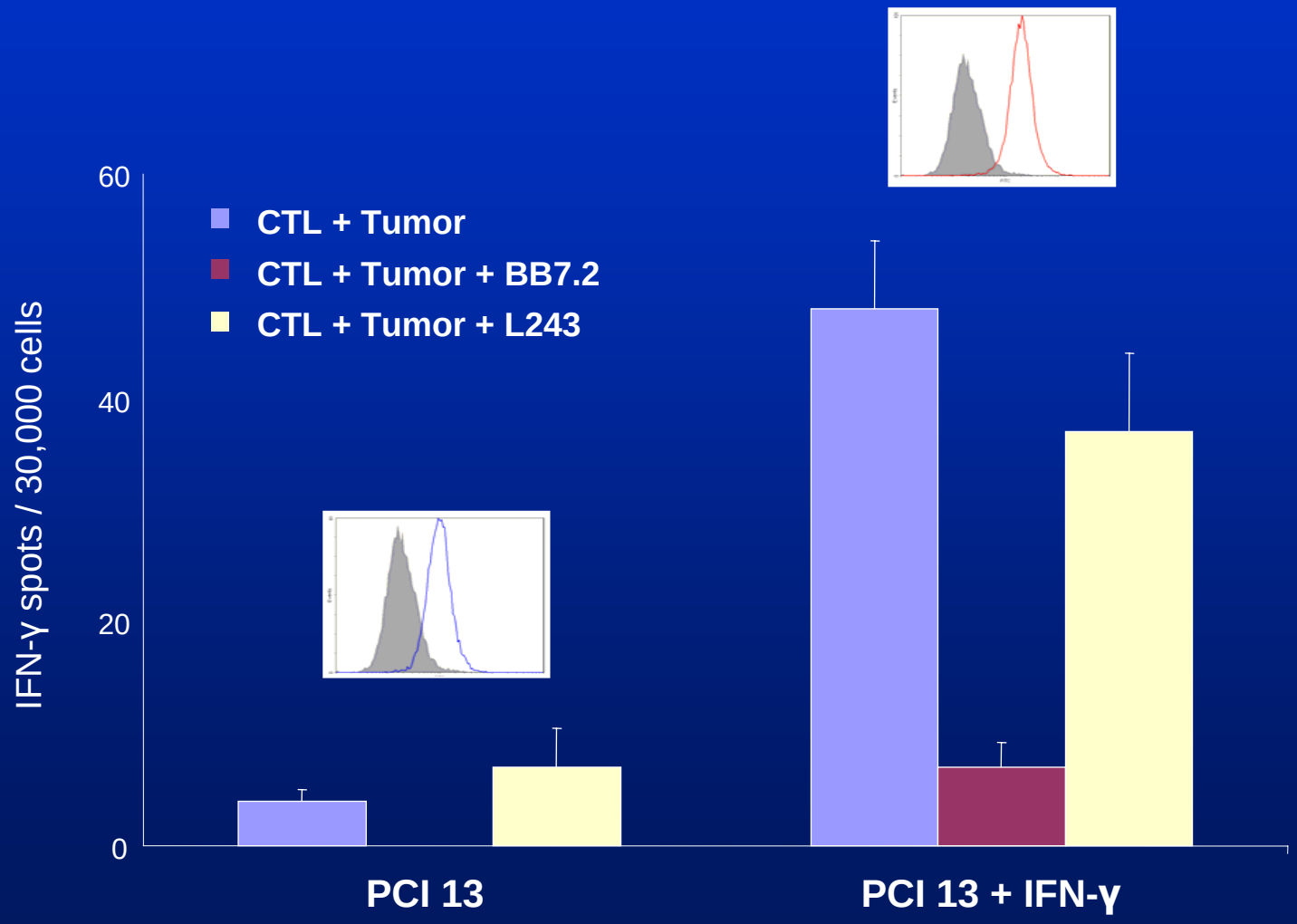
Association of APM component and HLA class I antigen expression with CD8+ T cell infiltration in primary laryngeal squamous cell carcinoma lesions



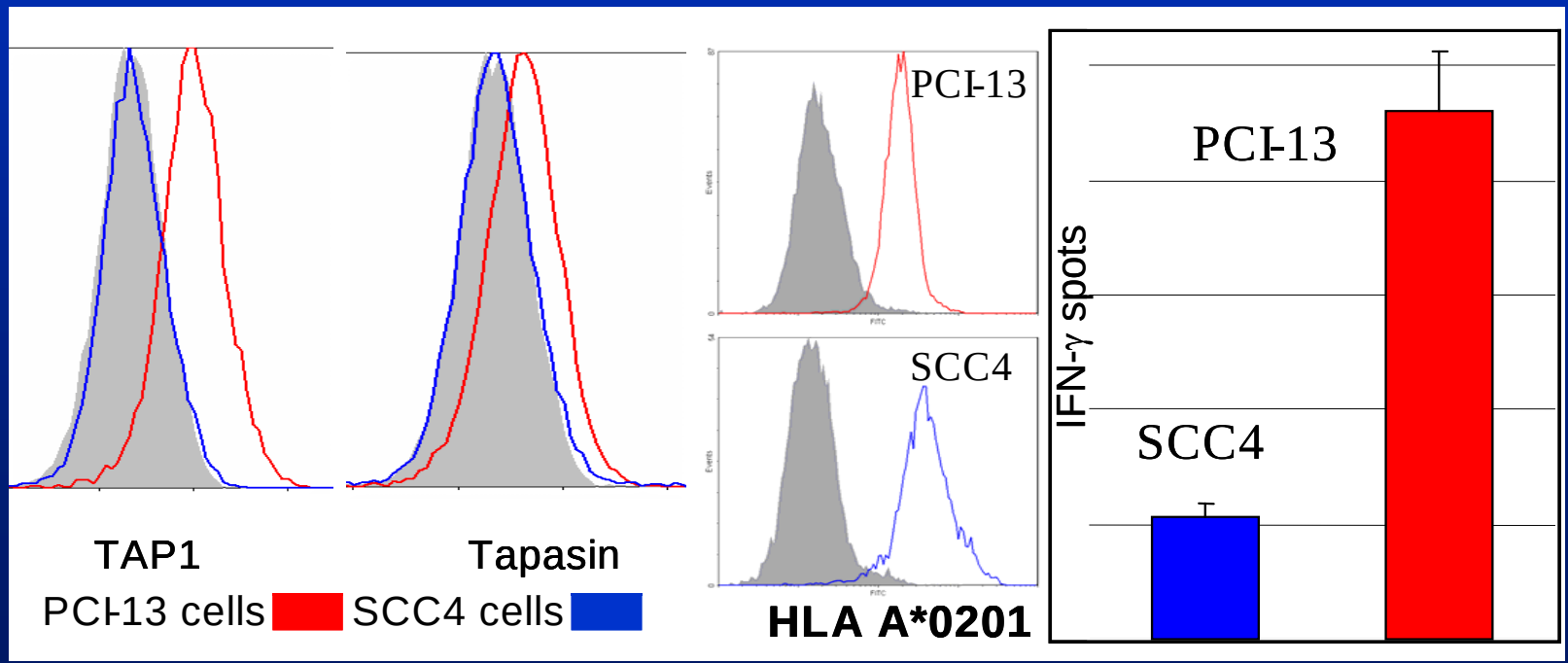
Association of HLA class I antigen expression and CD8+ T cell infiltration in primary laryngeal squamous carcinoma lesions with poor survival



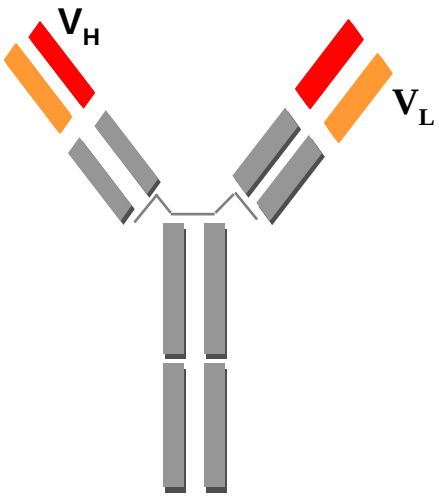
Restoration by IFN-γ of recognition of SCCHN PCI 13 cells by HLA class I antigen restricted, TA-specific CTL.

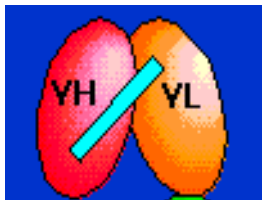


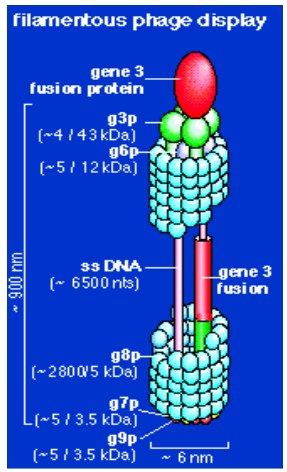
Relationship between upregulation of TAP1 and tapasin level and recognition of IFN-γ treated SCCHN cells PCI-13 and SCC4 by HLA class I antigen restricted, TA-specific CTL



Phage display antibody libraries





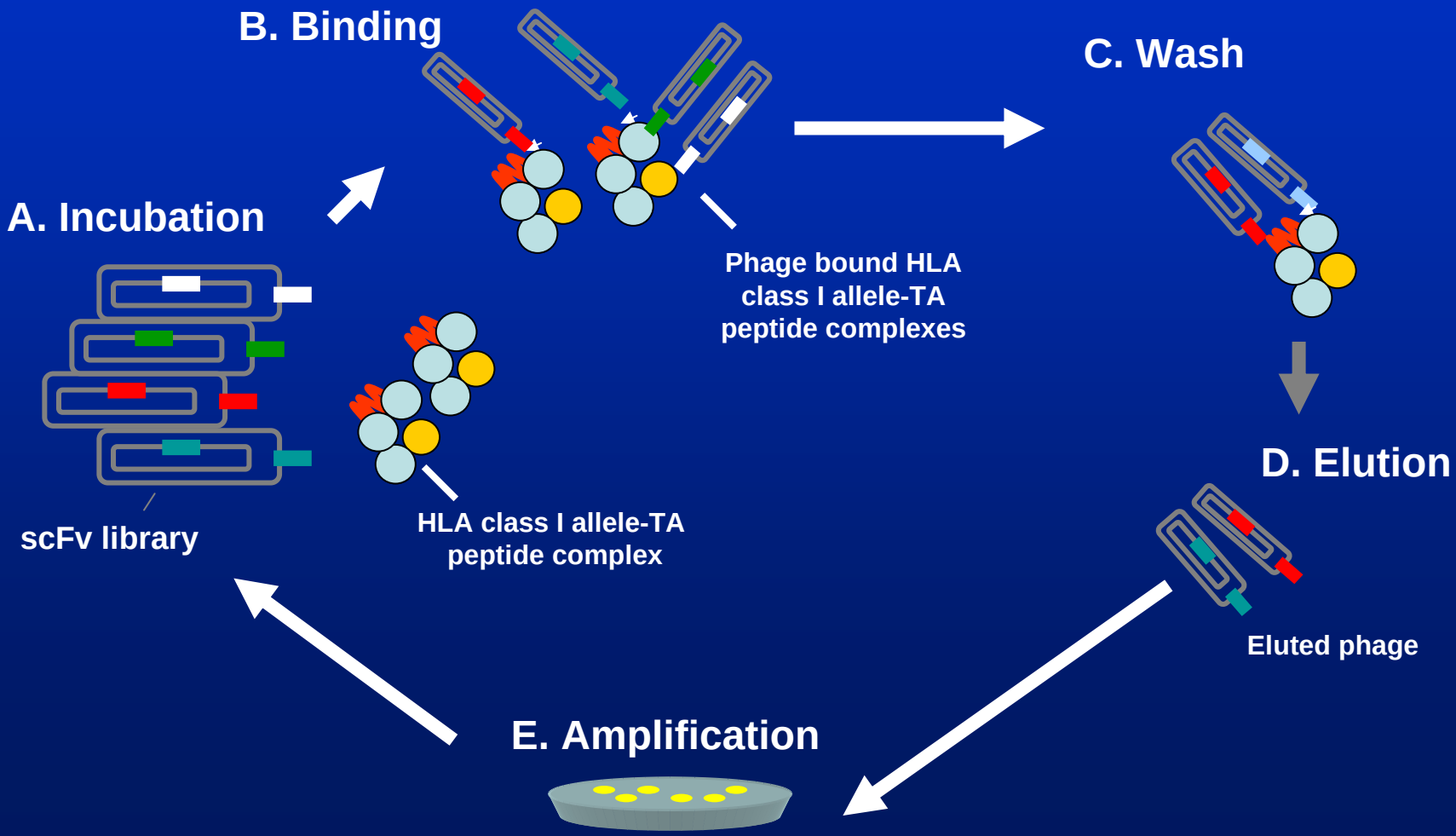


Immunoglobulin

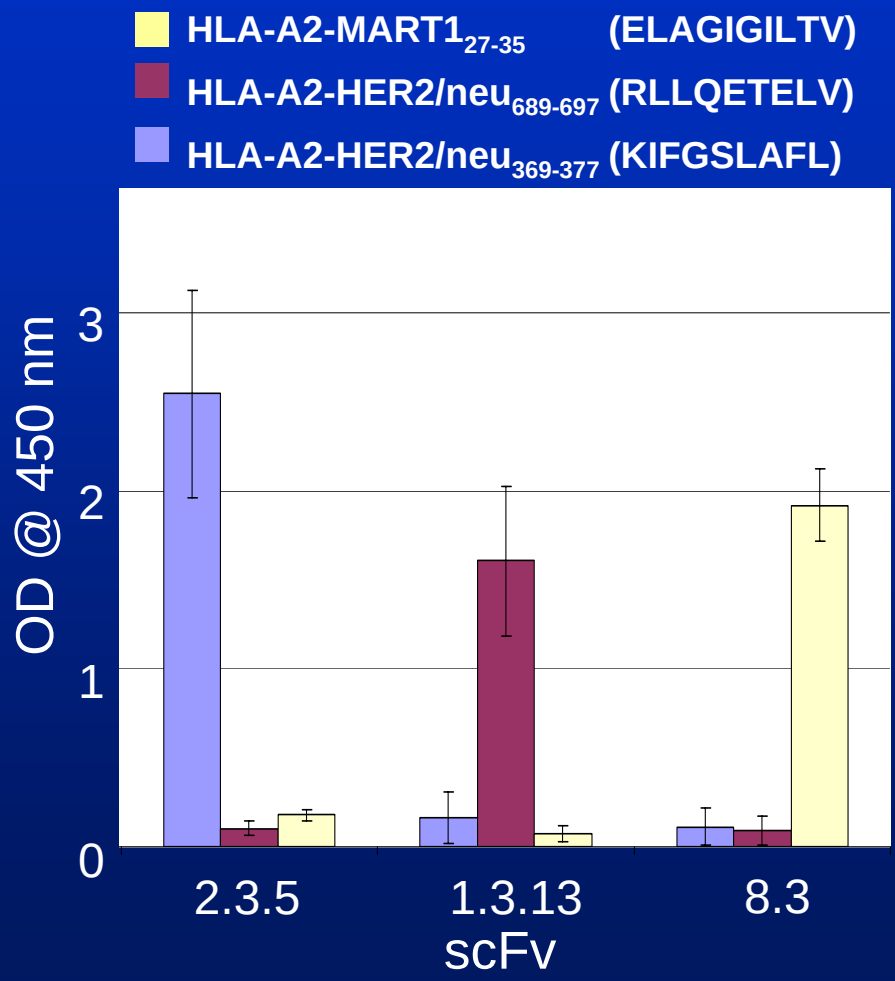
Semi-synthetic single chain fragment of antibody variable region (scFv)

phage displayed scFv

Panning phage display antibody libraries with HLA class I allele-TA peptide complexes



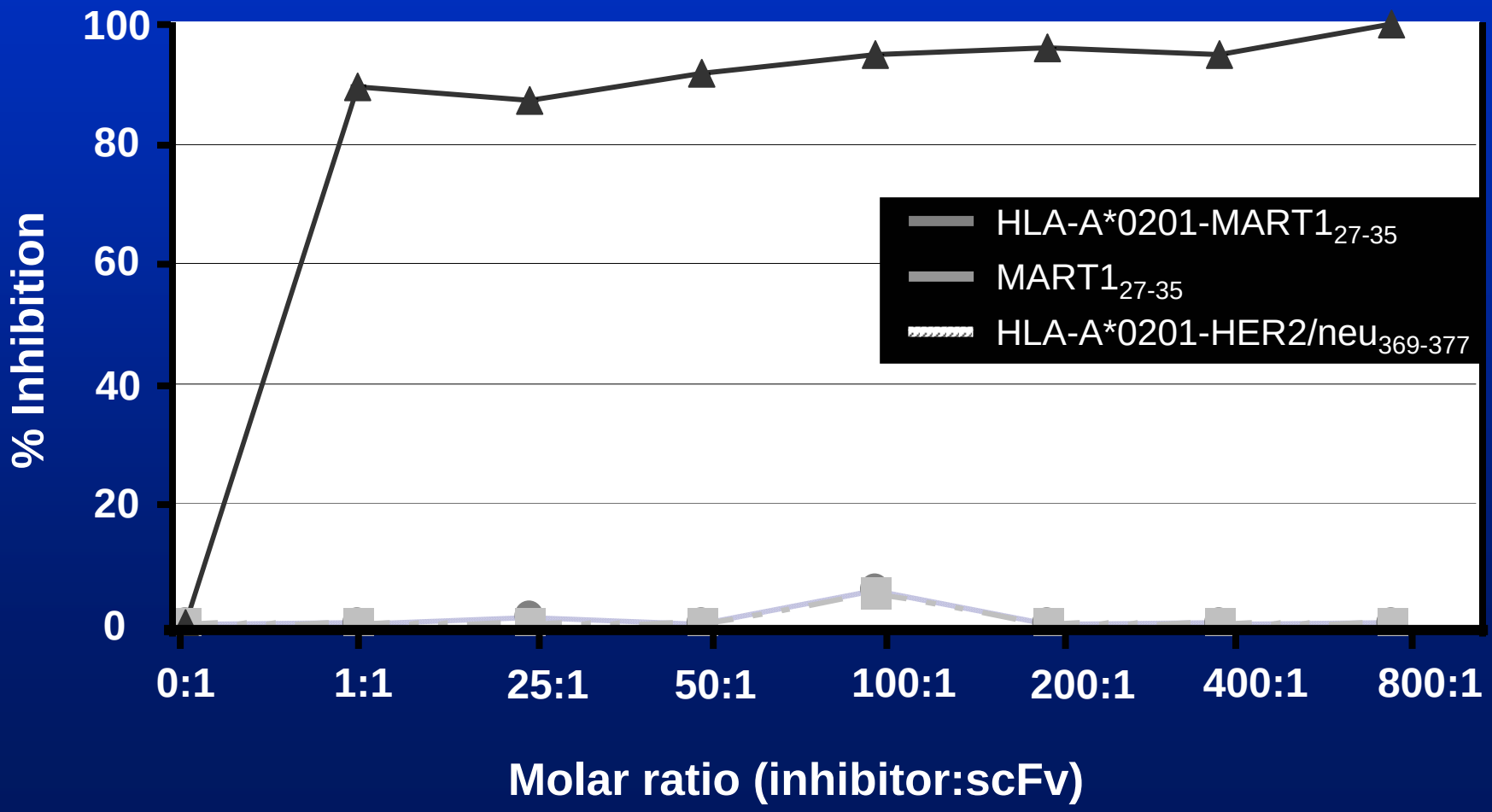
Enriched phage displayed scFv recognize purified HLA-A*0201-peptide complexes



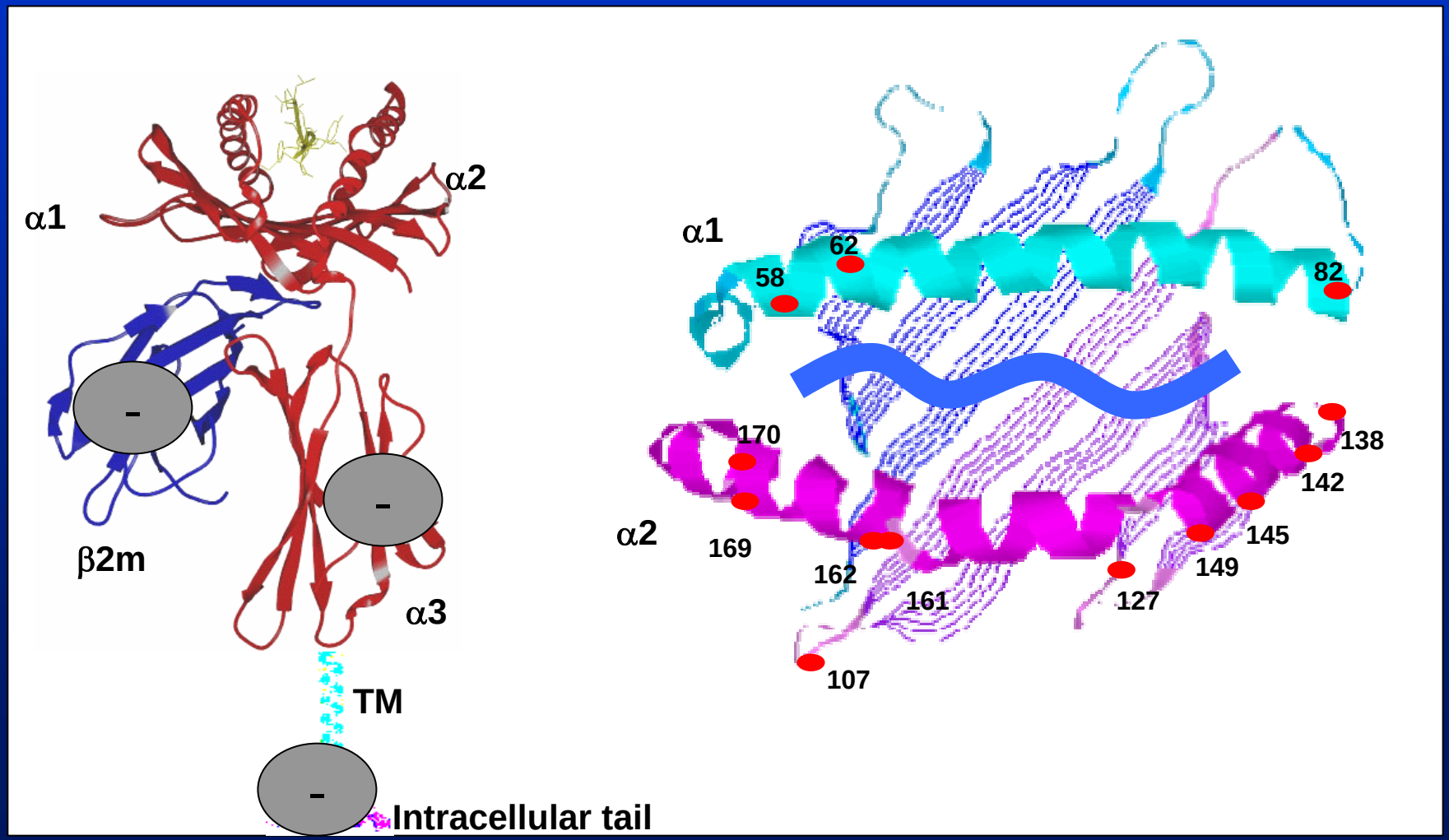
Isolation of unique HLA class I allele-TA peptide complex specific scFv

Clone	Heavy chains[a]			Light chains		
	Family	Segment	CDR3	Family	Segment	CDR3
HLA-A*0201-MART1$_{27-35}$-specific scFv						
8.3	VH3	DP-45	**ARSSSLCTWGQ**	Vκ2	DPK-15	MQALQTQC
24.3	VH3	DP-45	**ARSSSLCTWGQ**	Vλ3	DPL-16	NSRDSSGF
25.3	VH3	DP-45	**ARSSSLCTWGQ**	Vκ3	DPK-1D	QQYDNLPS
HLA-A*0201-HER2/neu$_{369-377}$-specific scFv						
2.3.5	VH3	DP-13*01	**AGPAGAGPWGQ**	Vκ2	DPK-29*01	MQSIQLHT
2.4.38	VH3	DP-13*01	**AGPAGAGPWGQ**		DPL-19*01	NSRDSSGNHPDV
HLA-A*0201-HER2/neu$_{689-697}$-specific scFv						
1.3.13	VH3	DP-23*01	ARGEFRTYFPT	Vκ1	DPK-39*01	QQANSFLSST

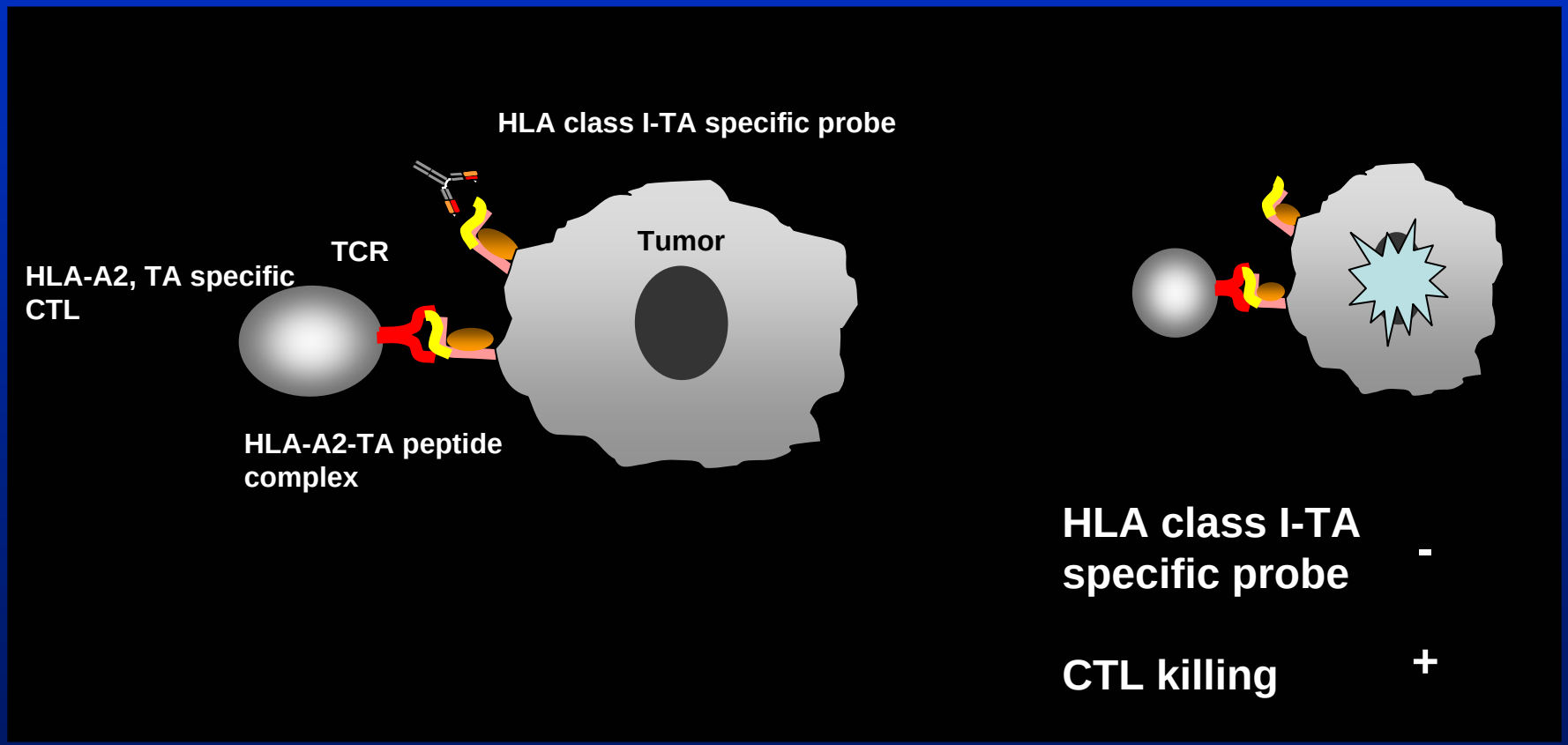
scFv 8.3 does not bind to MART1$_{27-35}$ peptide alone



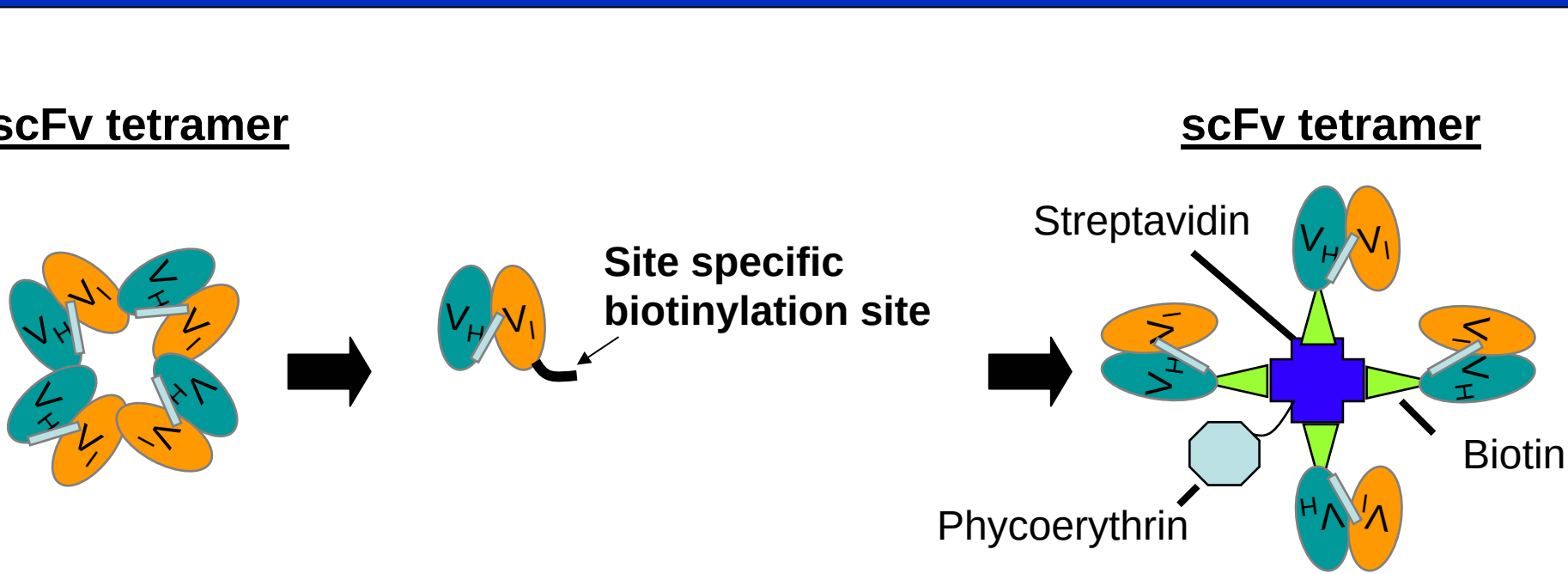
scFv 8.3 binds to a determinant located on the α1/α2 domains of HLA-A*0201 and MART1$_{27-35}$ peptide



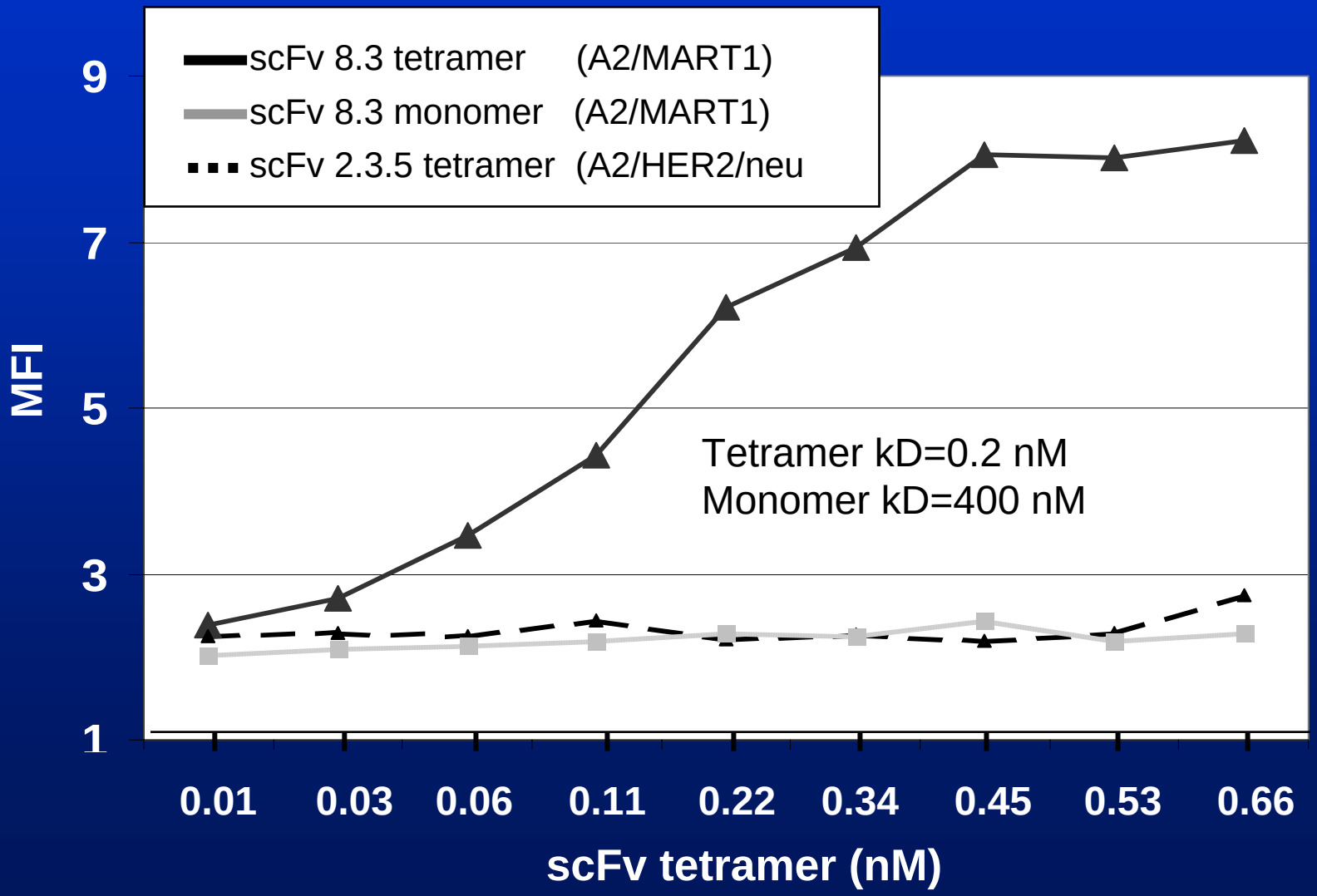
Can we enhance the sensitivity of HLA class I allele-TA peptide complex specific probes?



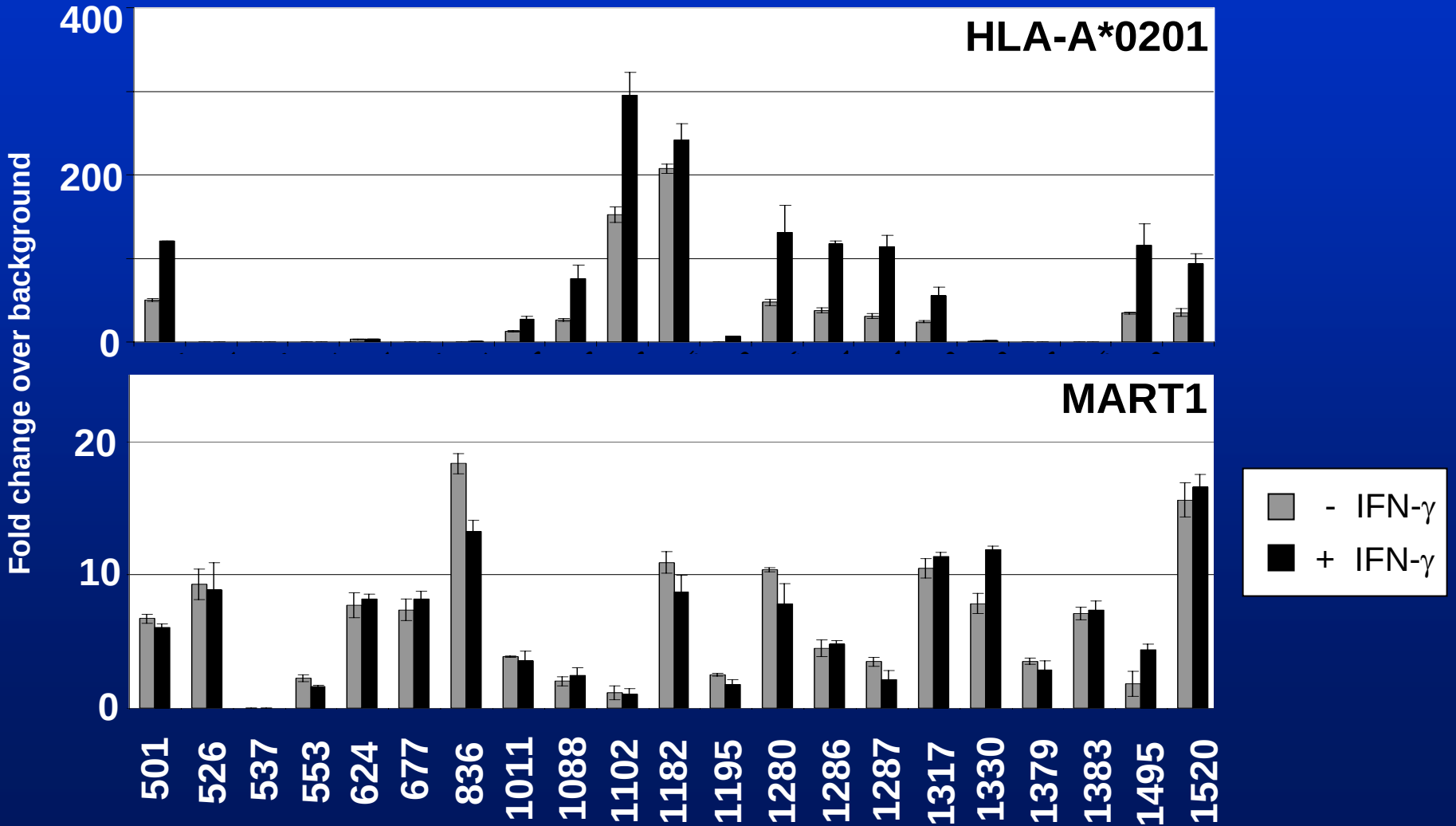
Generation of HLA class I allele-TA peptide complex specific scFv tetramers



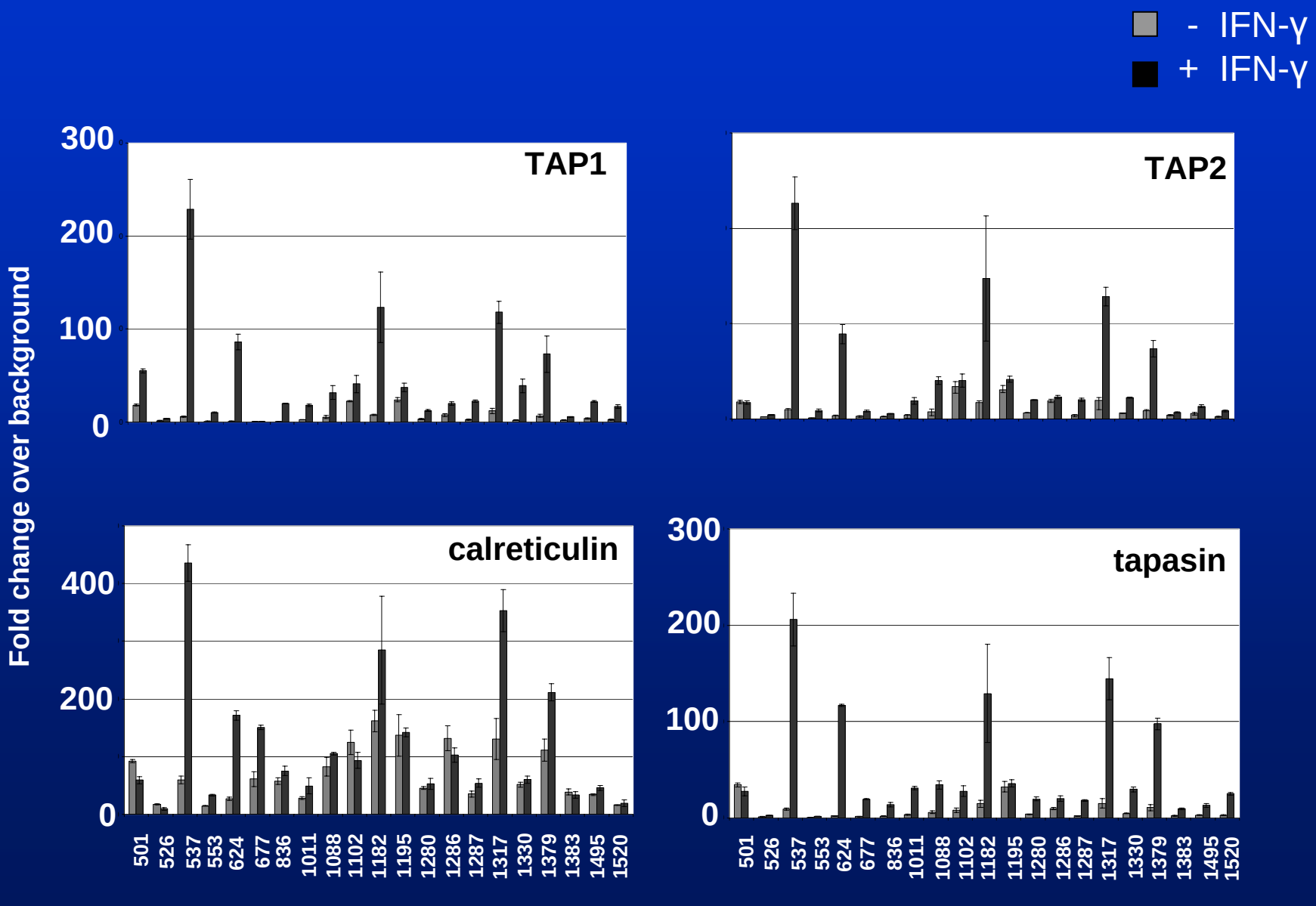
scFv tetramerization enhances their ability to detect HLA class I allele-TA peptide complexes on target cells



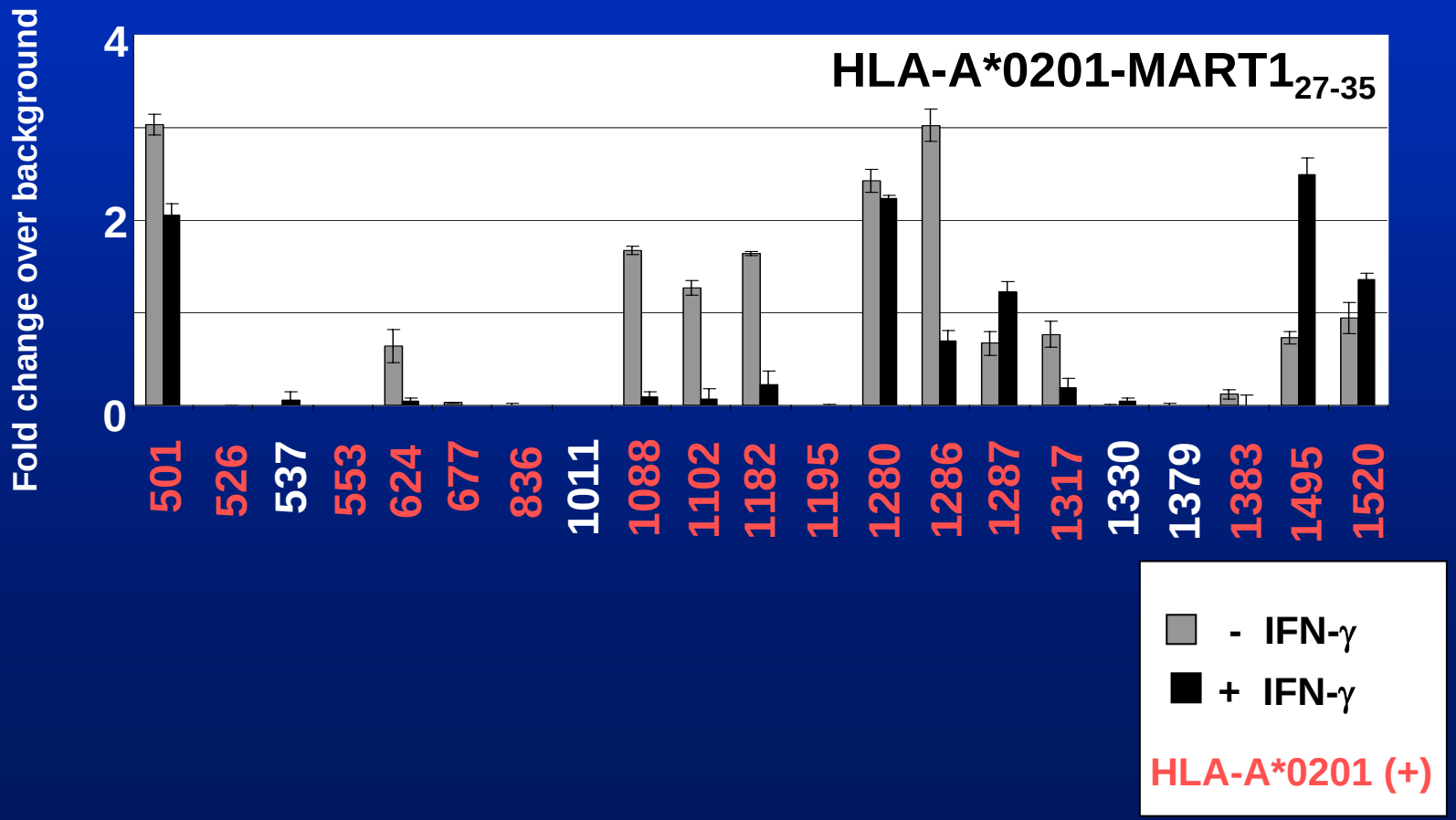
Heterogeneous HLA-A*0201 surface expression and intracellular MART1 protein expression in human melanoma cells



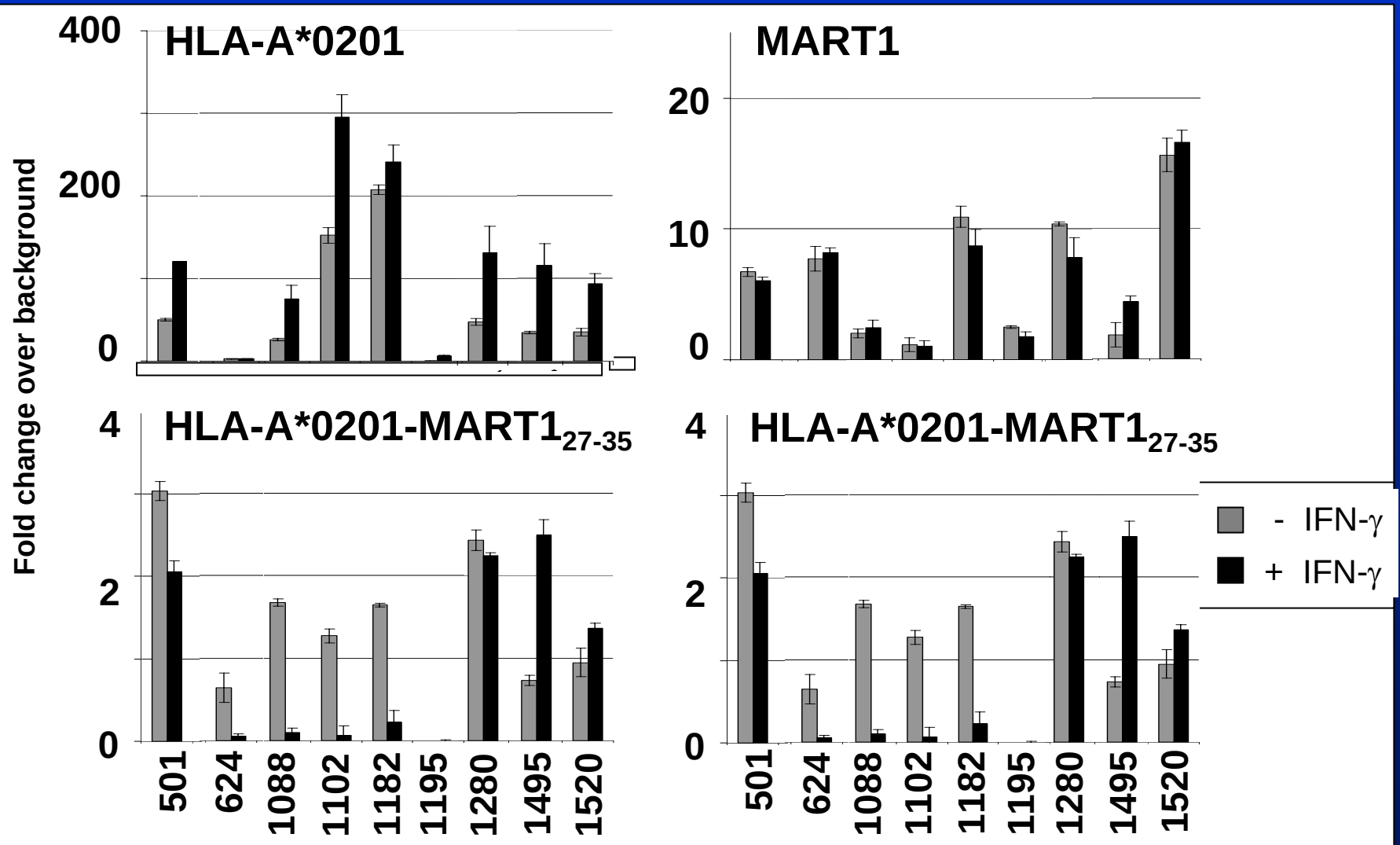
Heterogeneous APM component expression in human melanoma cells



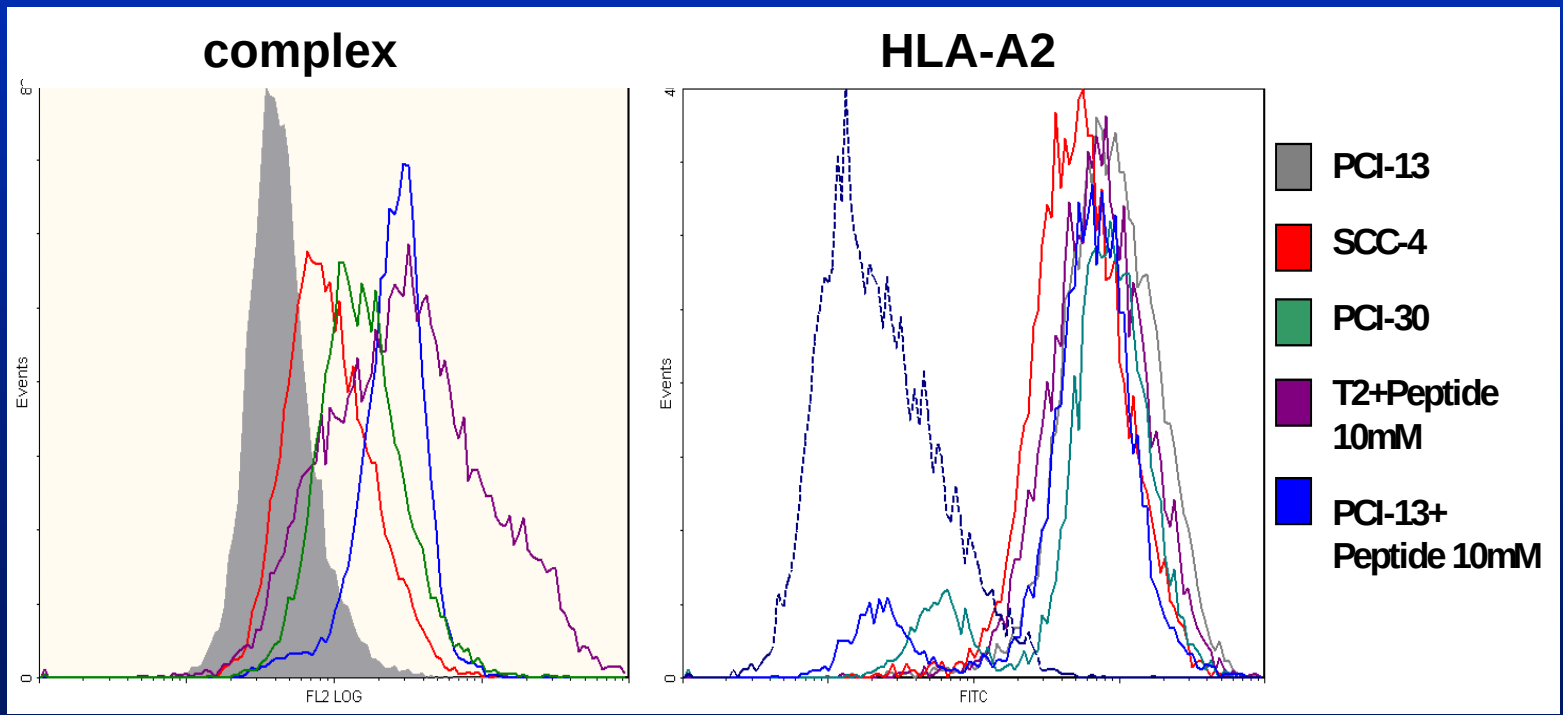
Heterogeneous HLA-A*0201-MART1$_{27-35}$ peptide complex expression on human melanoma cells



Lack of correlation between HLA-A*0201, MART1 and HLA-A*0201-MART1$_{27-35}$ peptide complex expression



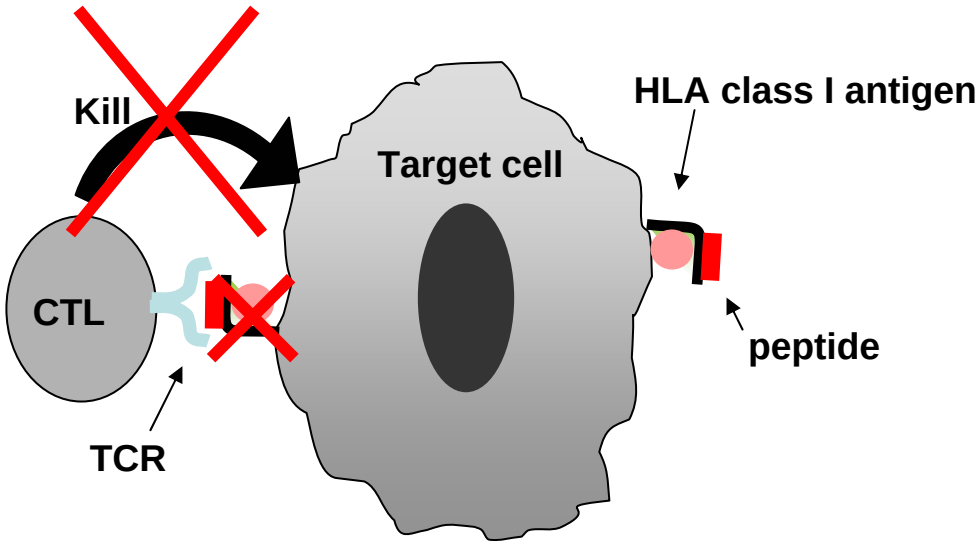
Lack of relationship between HLA-A2 antigen and HLA-A2 antigen-HER2/neu $_{369-377}$ peptide complex expression by SCCHN cell lines



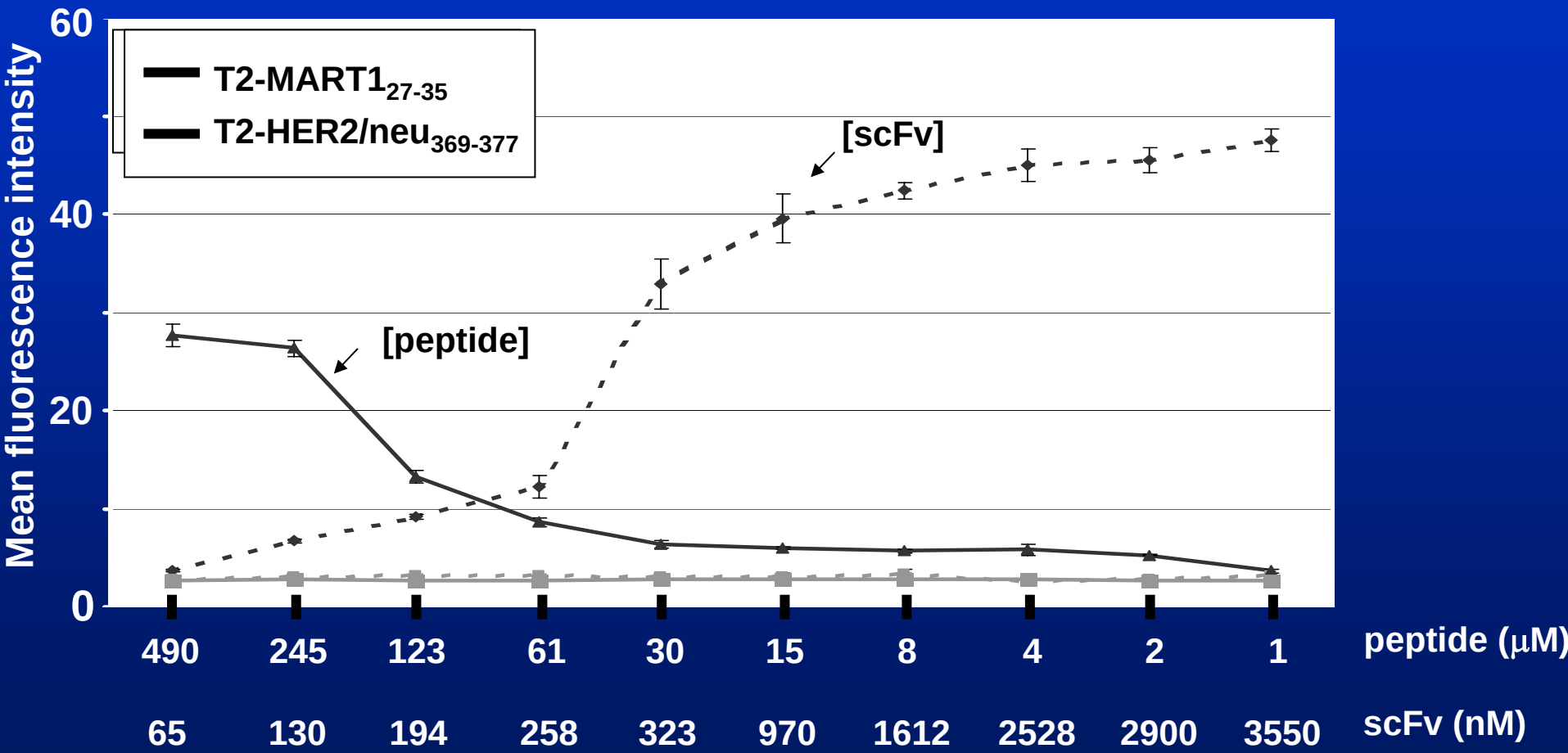
Conclusions

- The level of APM components and HLA class I antigens markedly vary in malignant cells
- Measure of the level of APM component and HLA antigen expression provides only limited information about their functional properties
- The level of HLA class I antigen-tumor antigen peptide complexes on tumor cells does not correlate with the level of APM components, HLA class I antigens and tumor antigens
- These results stress the need to measure the level of HLA class I antigen-tumor antigen peptide complexes on tumor cells to characterize their interactions with CTL

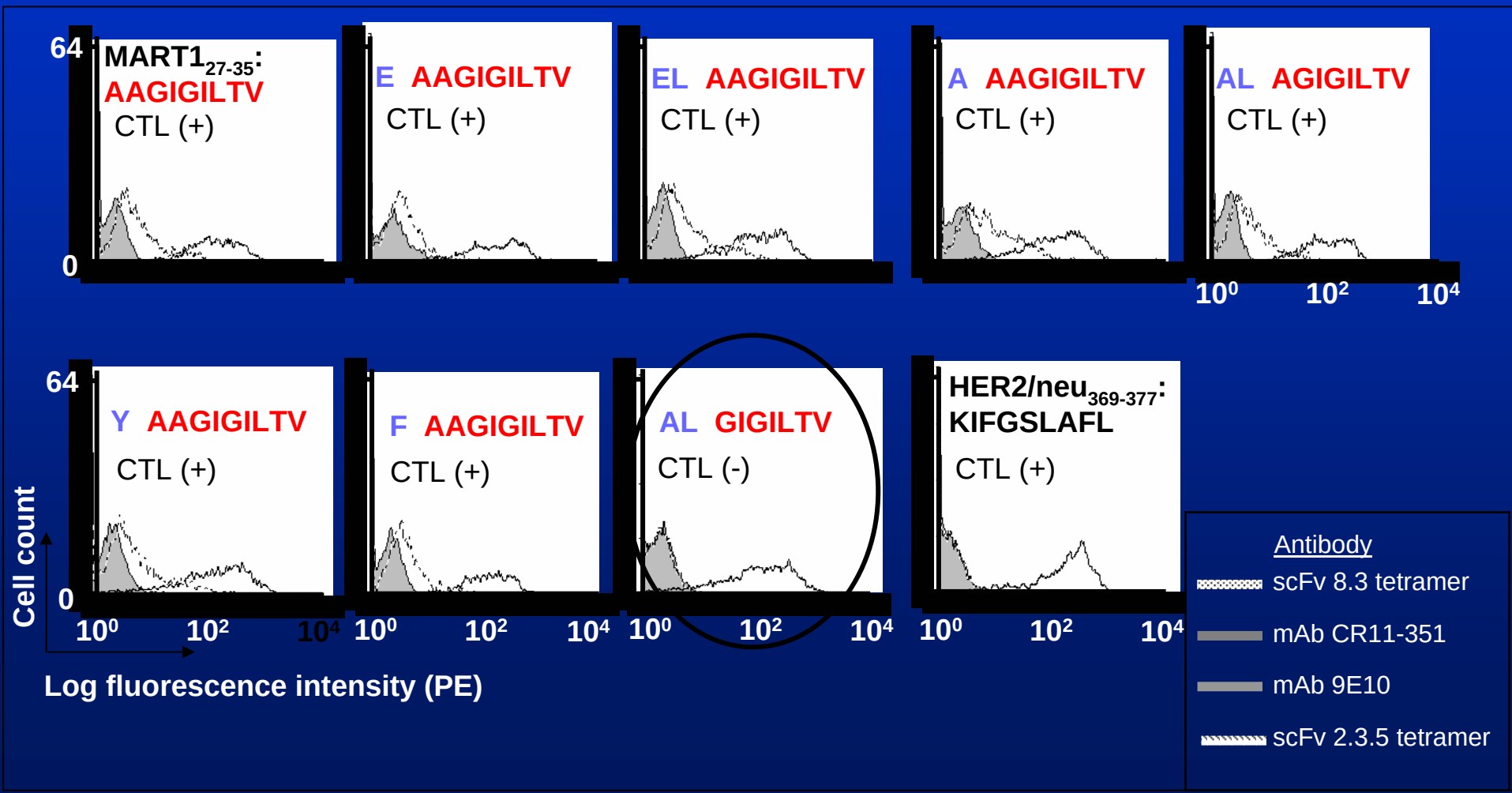
HLA class I antigen-peptide complexes mediate recognition of target cells by cytotoxic T lymphocytes (CTL)



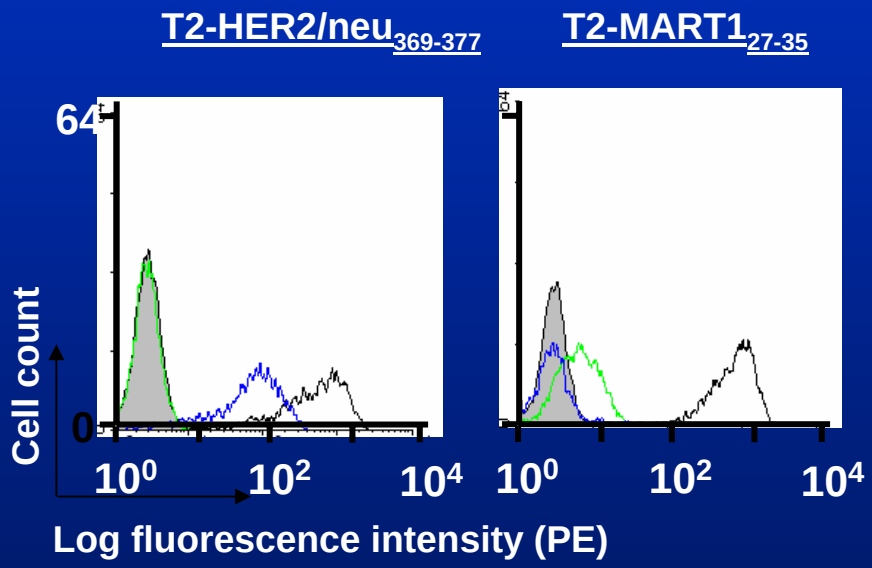
Reactivity of scFv 8.3 with peptide pulsed T2 cells is dependant on scFv & MART1$_{27-35}$ concentration



scFv 8.3 mimics the reactivity of HLA-A*0201-MART1$_{27-35}$-specific TCR

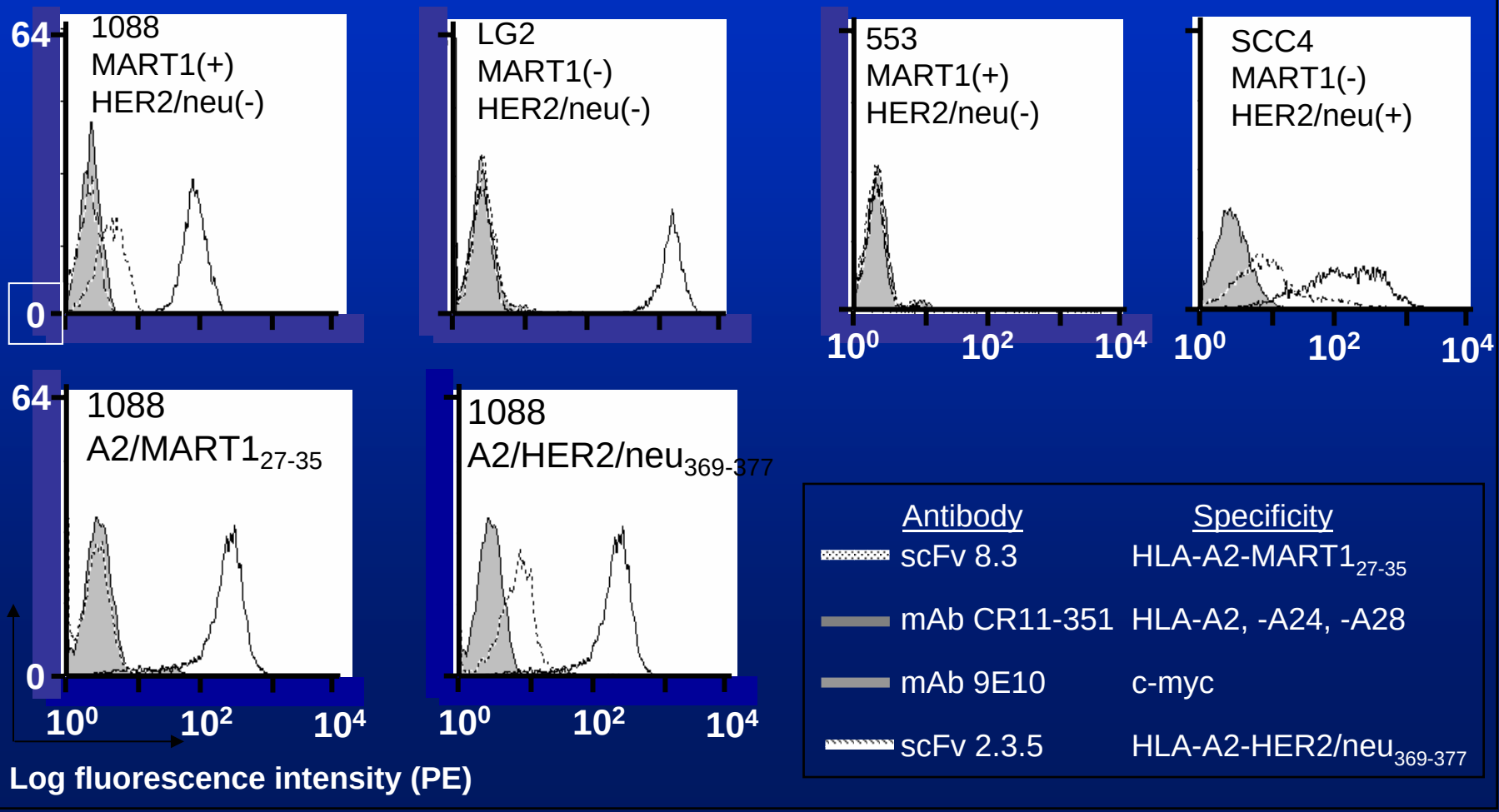


Enriched phage displayed scFv recognize peptide pulsed T2 cells



Antibody	Specificity
scFv 8.3	HLA-A2-MART1$_{27-35}$
scFv 2.3.5	HLA-A2-HER2/neu$_{369-377}$
mAb CR11-351	HLA-A2, -A24, -A28
mAb 9E10 JH1	c-myc
scFv JH1	Human VEGF

HLA class I allele-TA peptide complex specific scFv tetramers retain their specificity



Lack of correlation between APM component and HLA-A*0201-MART1$_{27-35}$ peptide complex expression