Interrogating the antigen specific T cell recognition of cancer

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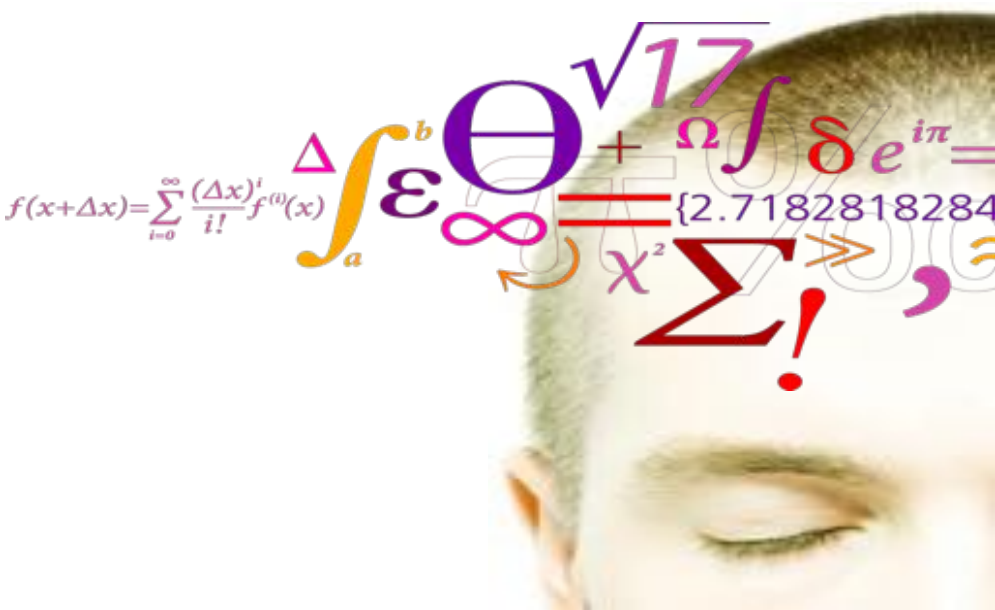
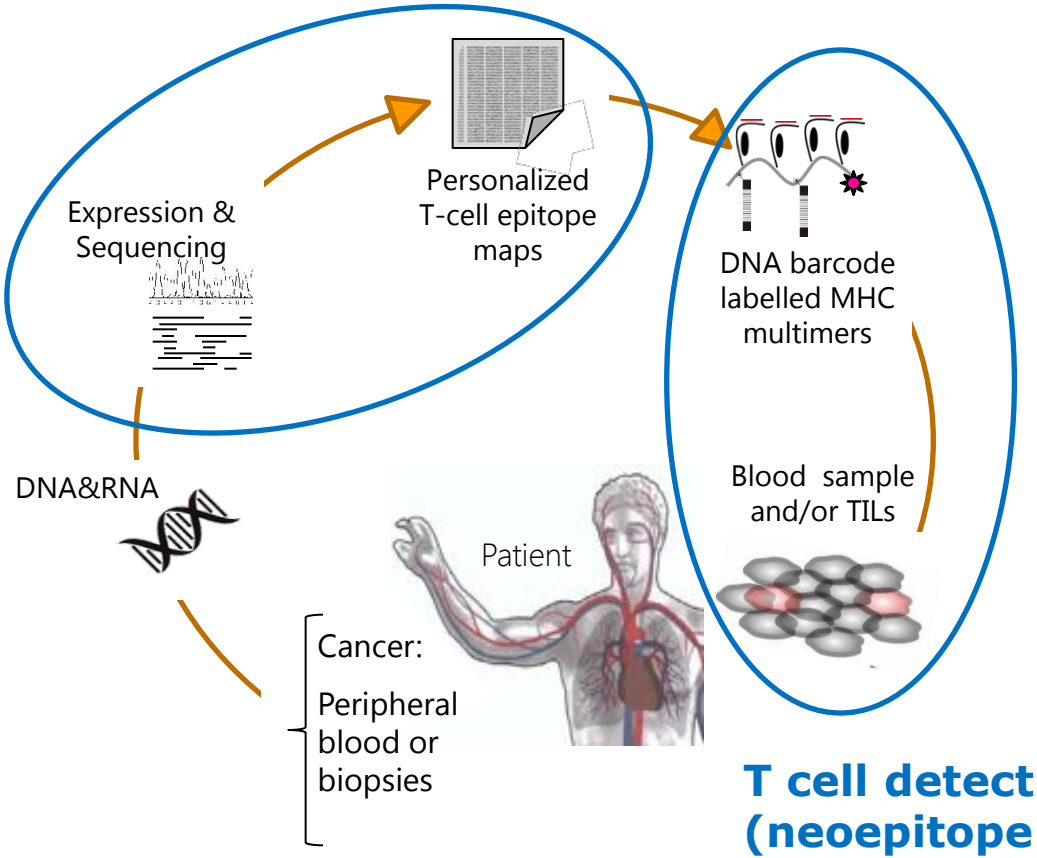


Neoepitope prediction



T cell detection (neoepitope immunogenicity)

- Technologies for T cell detection
- Neoepitope immunogenicity
- TCR fingerprinting

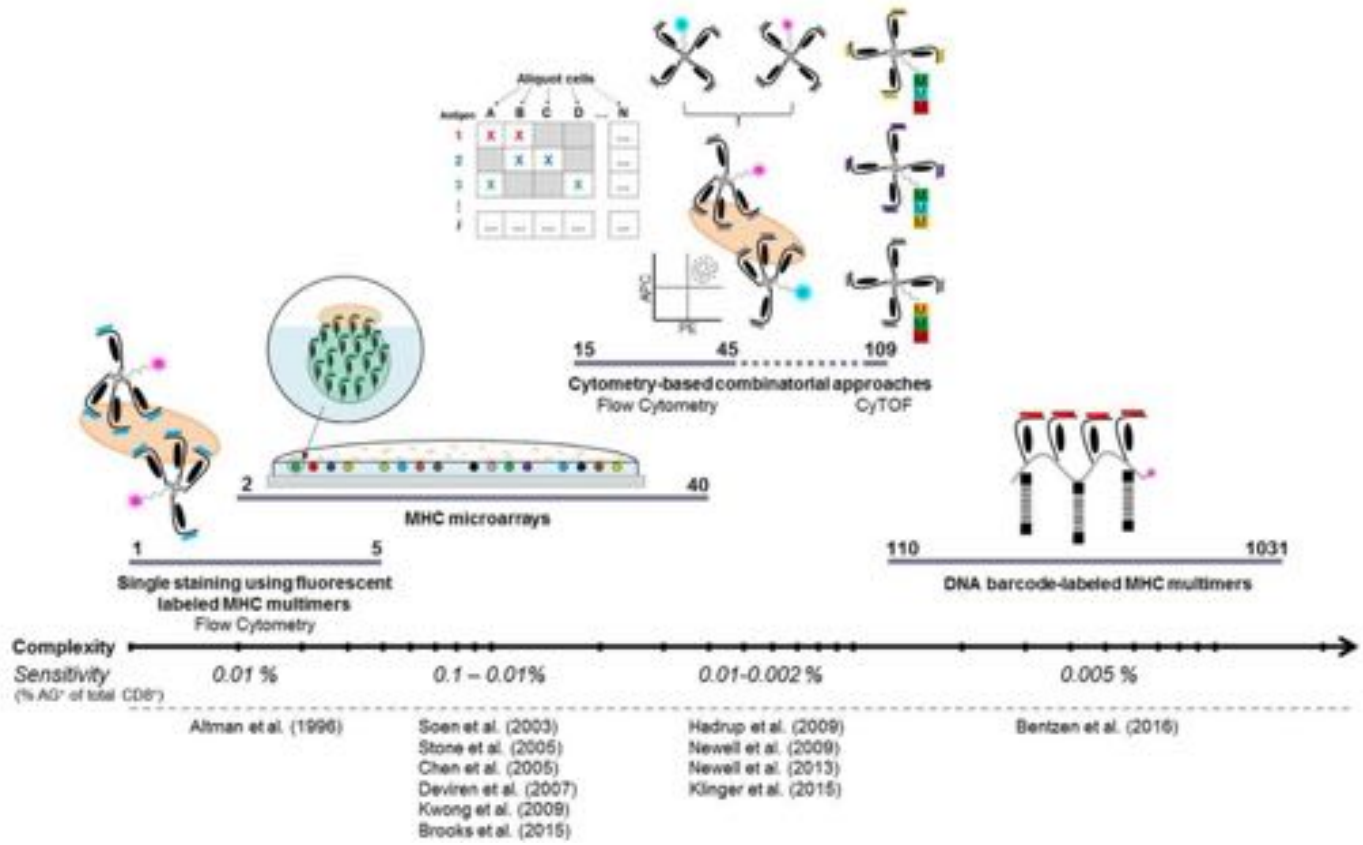
Mind the gap in neoepitope prediction

First and last author	Journal	Tumor type	Patients	Peptides tested	T cell responses	Test method	Peptide lengths
Robbins et al. Rosenberg	Nat Med	SKCM	3	227	10	ELISPOT	9–10
van Roij et al. Schumacher	J Clin Oncol	SKCM	1	-	1	FLT	9
Wick et al. Nelson	Clin Cancer Res	HGSC	3	109	1	ELISPOT	8–11
Rajasagi et al. Wu	Blood	CLL	2	48	3	ELISPOT	9–10
Lu et al. Robbins	Clin Cancer Res	SKCM	2	10	2	ELISA	8–11
Snyder et al. Chan	N Engl J Med	SKCM	1	-	1	ICS	9
Rizvi et al. Chan	Science	NSCLC	1	-	1	FLT	9
Cohen et al. Robbins	J Clin Invest	SKCM	8	427	9	FLT	9–10
Kalaora et al. Samuels	Oncotarget	SKCM	1	2	1	ICS	9, 11
McGranahan et al. Swanton	Science	NSCLC	2	642	3/8	FLT / BLM	9–11
Strønen et al. Schumacher	Science	SKCM	4	56	11	FLT	9–11
Bassani-Sternberg et al. Krackhardt	Nature Commun	SKCM	1	8	2	MS-FLT	8–10,12
Bentzen et al. Hadrup	Nat Biotechnol	NSCLC	2	703	9	BLM	9–11
TOTAL			**24**	**1874**	**53**		

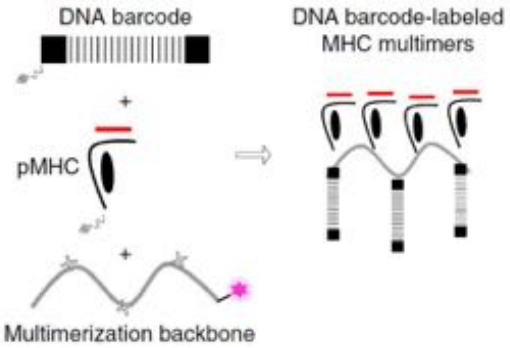
~3% of all predicted neoepitopes give rise to a T cell response

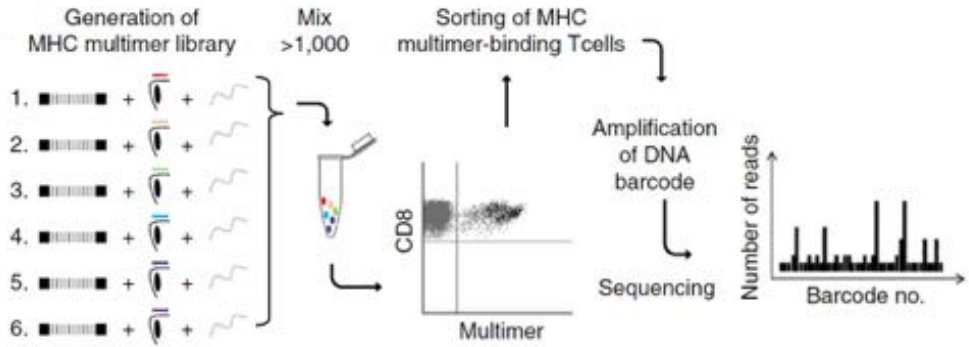
Bjerregaard AM, submitted

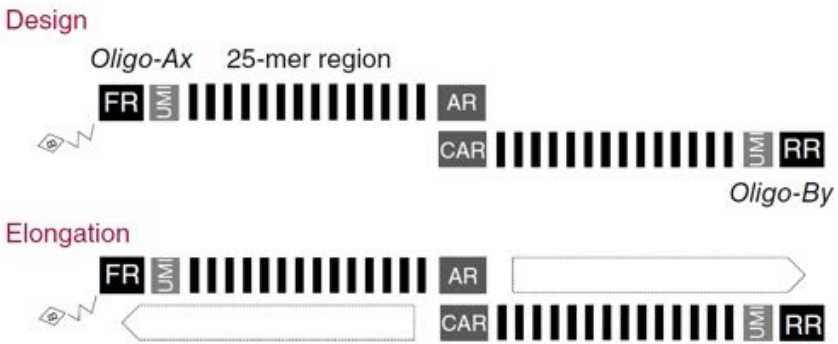
Evolution of T cell detection technologies

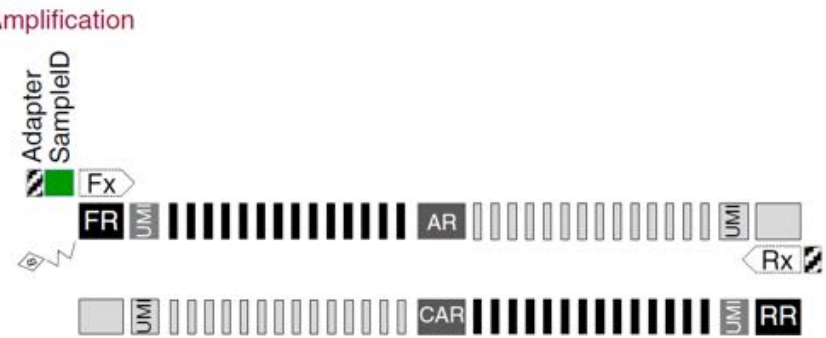


Bentzen & Hadrup, CII, 2017



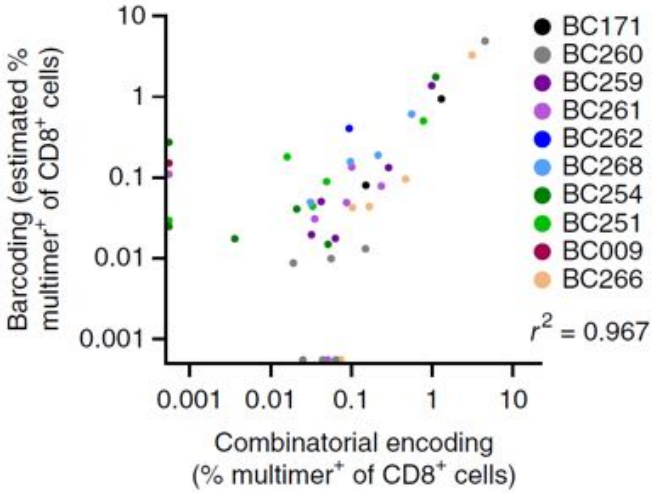




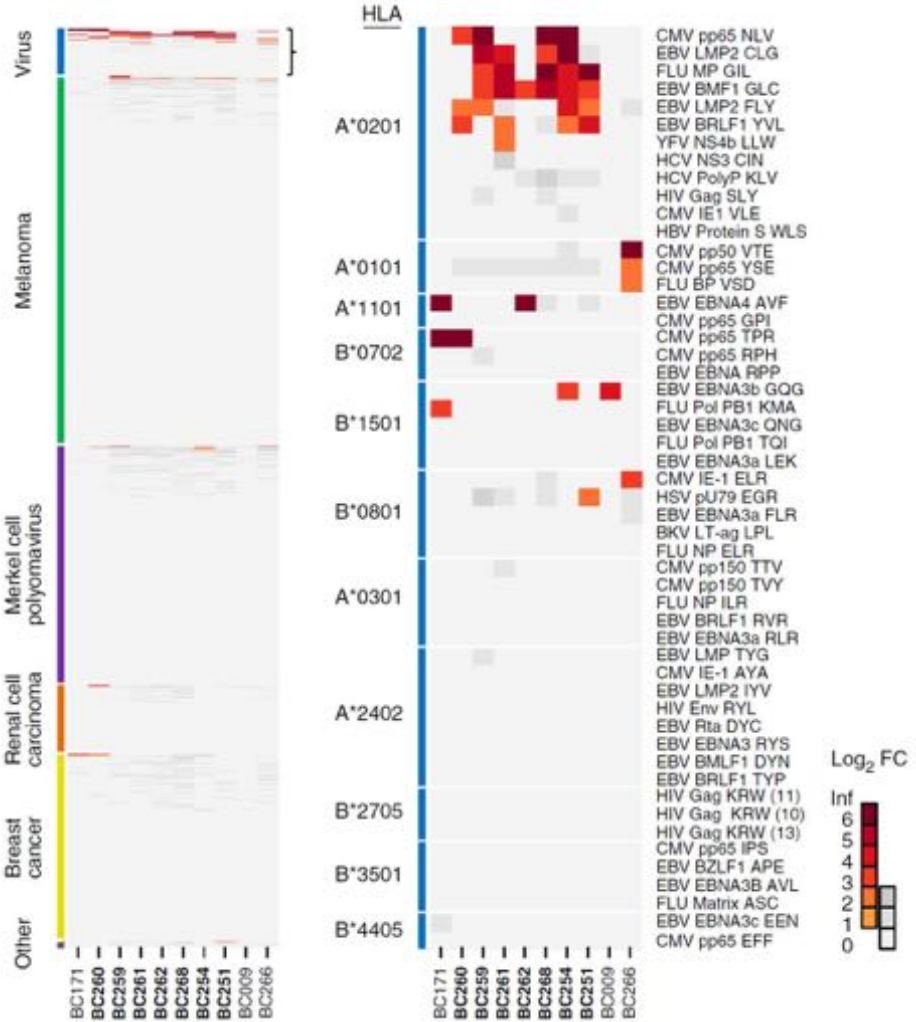


Bentzen et al. Nat. Biotechnology 2016

Correlation to state-of-the-art method:

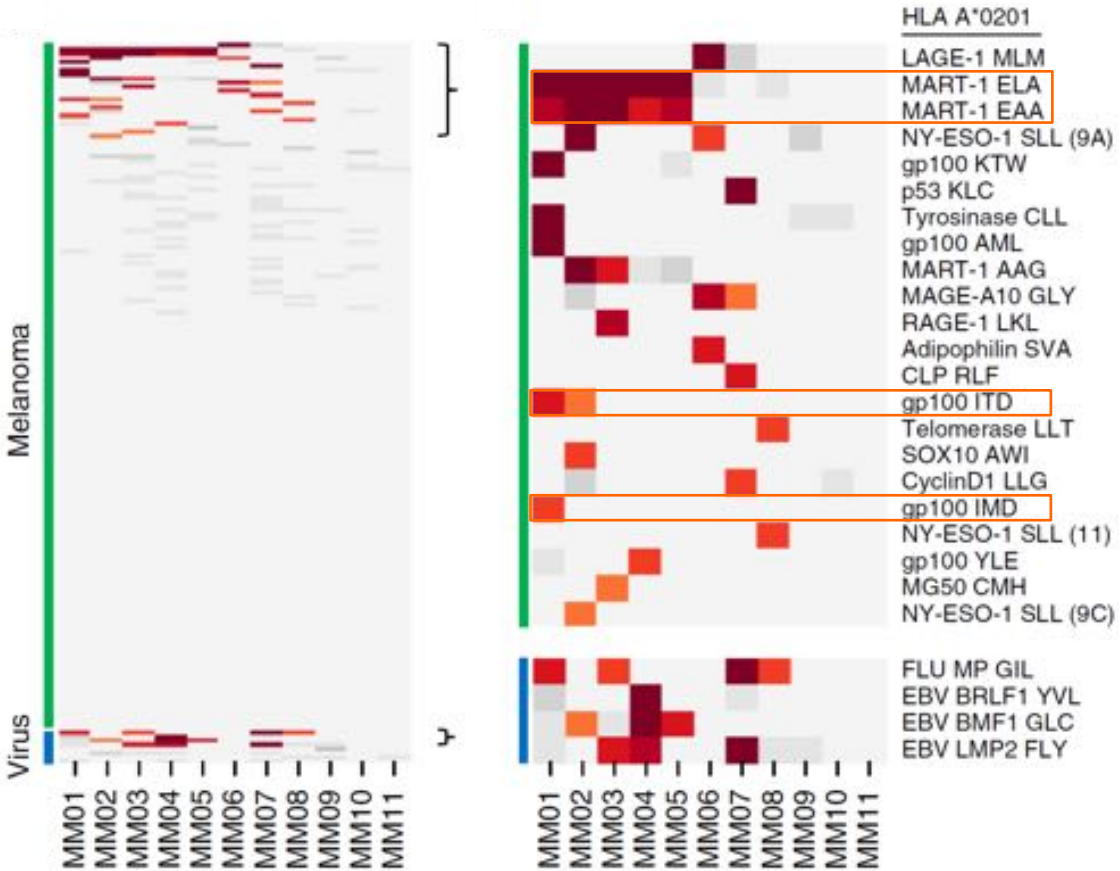


10 healthy donors screened with a library of 1031 pMHC complexes

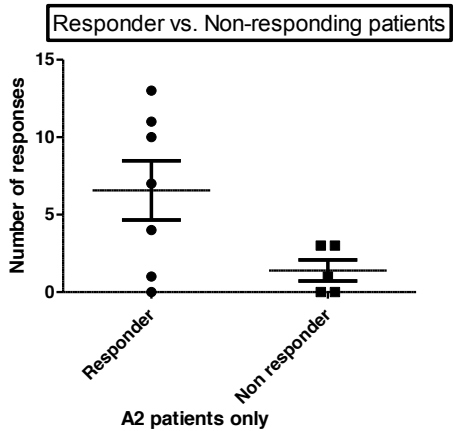


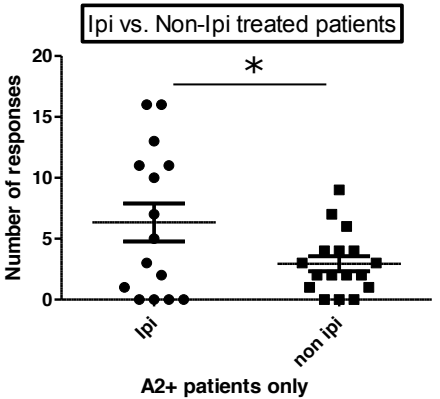
Bentzen et al. Nat. Biotechnology 2016

Analysis of Melanoma TIL against 175 HLA-A2 shared antigen epitopes



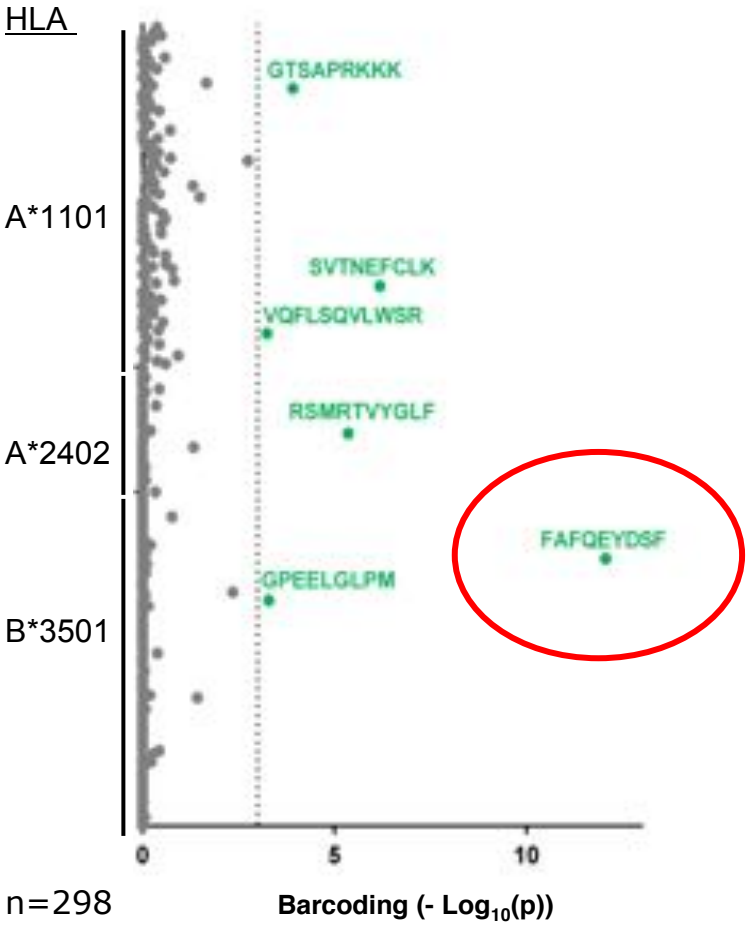
Bentzen et al. Nat. Biotechnology 2016

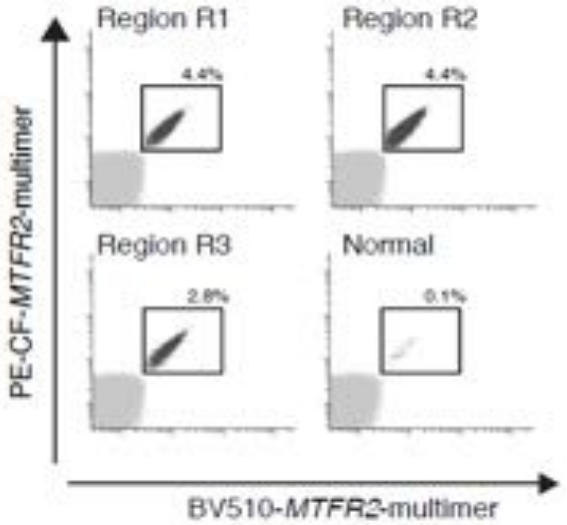




Bjørn et al. Oncotarget 2017

Neoepitope recognition in NSCLC

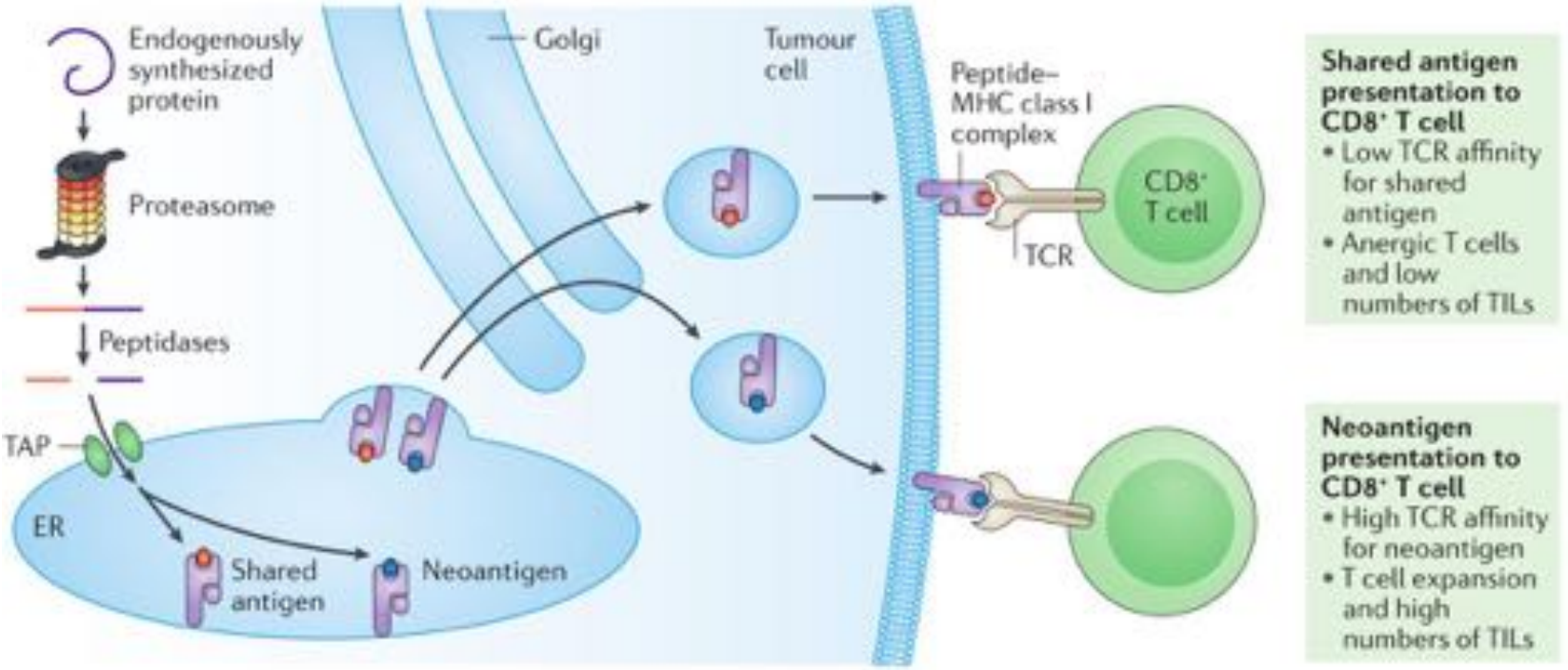




MTFR2 (FAFQE**Y**DSF)

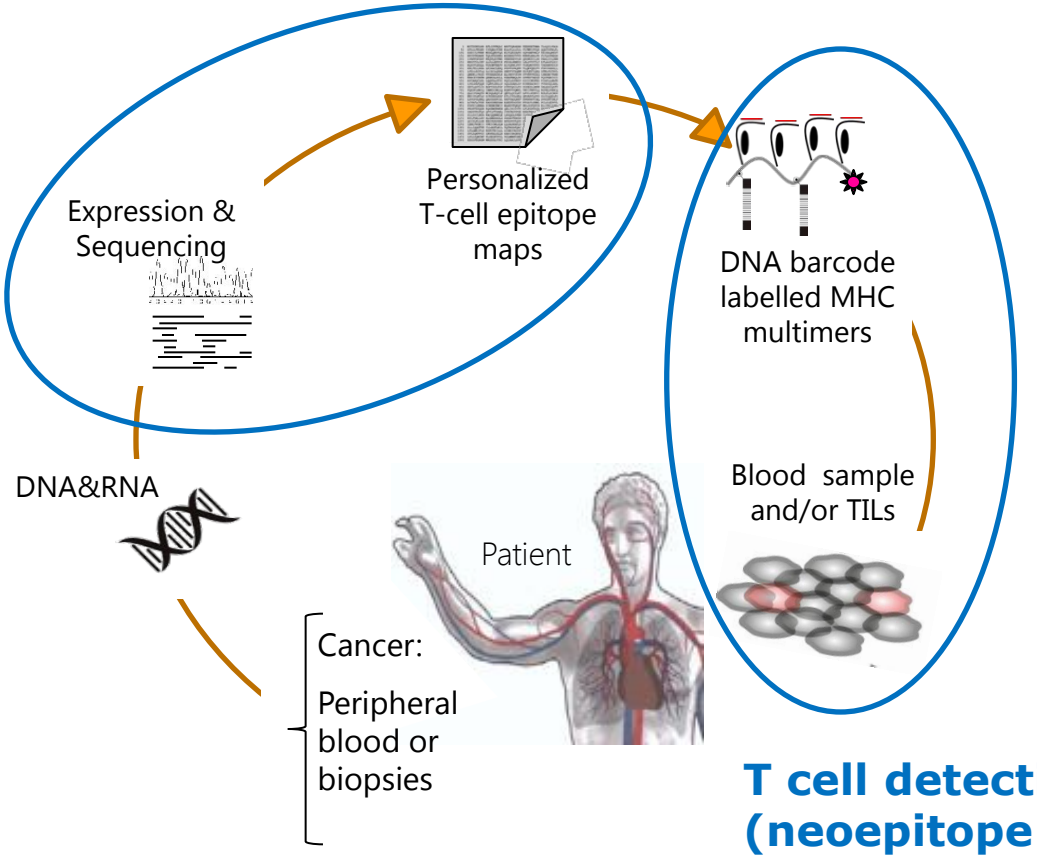
McGrananham, Science 2016 and Bentzen et al. Nat. Biotechnology 2016

What determines neoepitope immunogenicity?



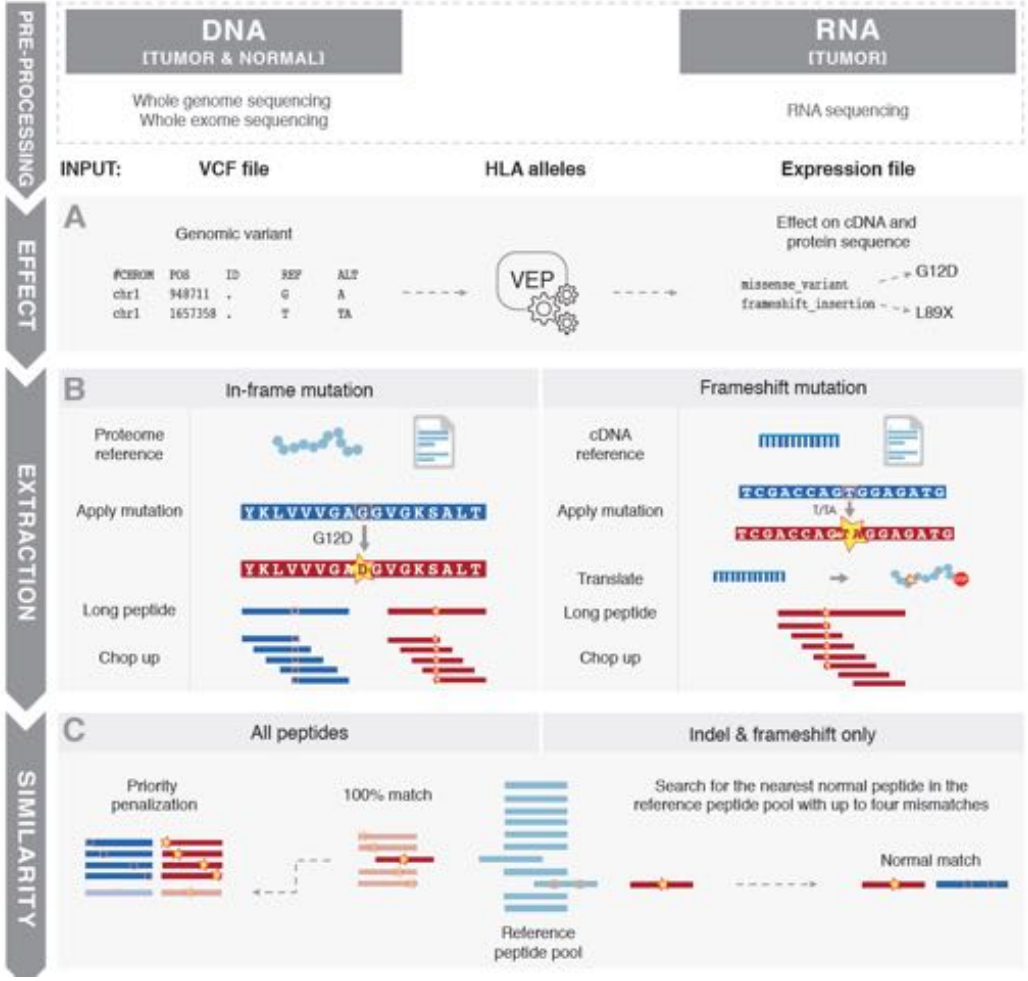
Yarchoan M & Jaffee EM. *Nat Rev Cancer* (2017) **17**:209–222.

Neoepitope prediction



T cell detection
(neoepitope immunogenicity)

1. Identification of cancer-specific genetic alterations



NGS pipeline:

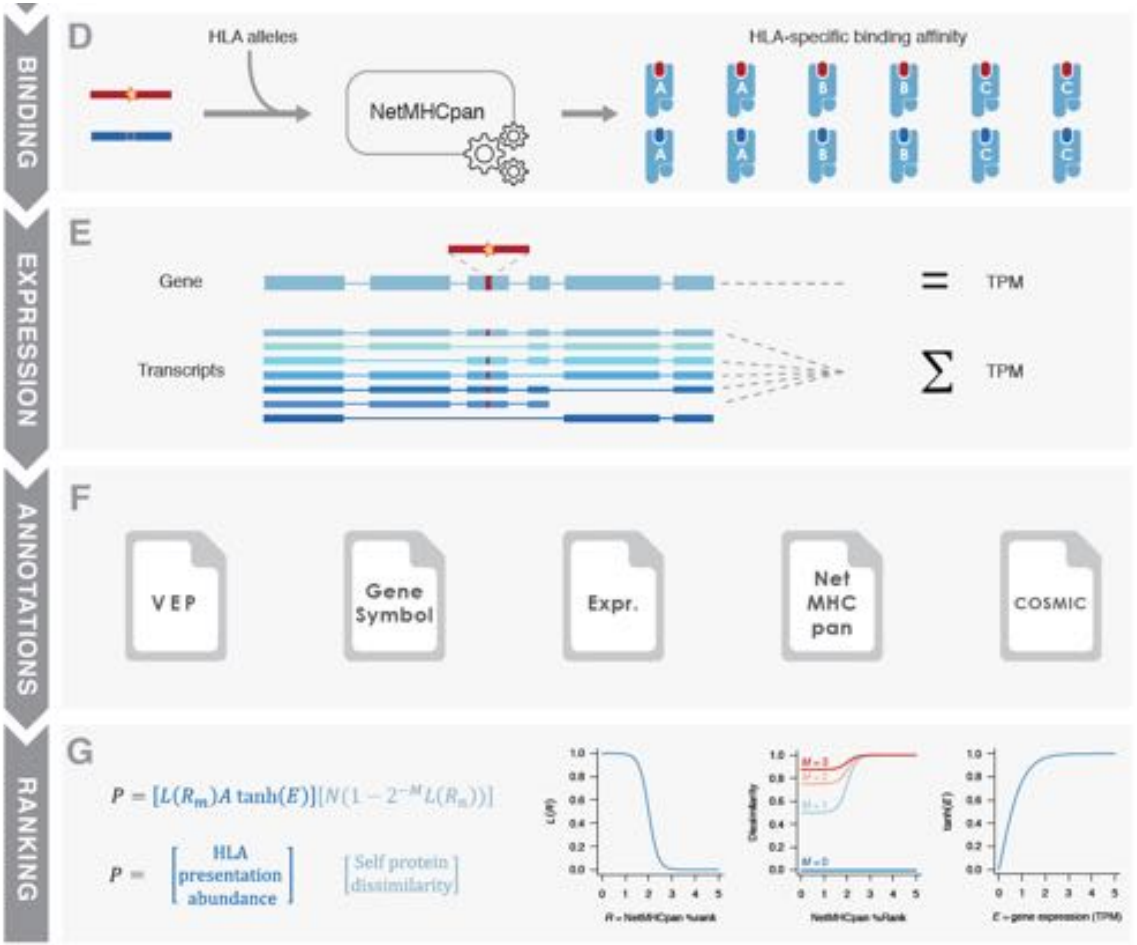
MuPeXI

(Mutant Peptide Extractor and Informer)

http://www.cbs.dtu.dk/services/MuPeXI

Bjerregaard et al. CII, 2017

2. Neo-epitope immunogenicity



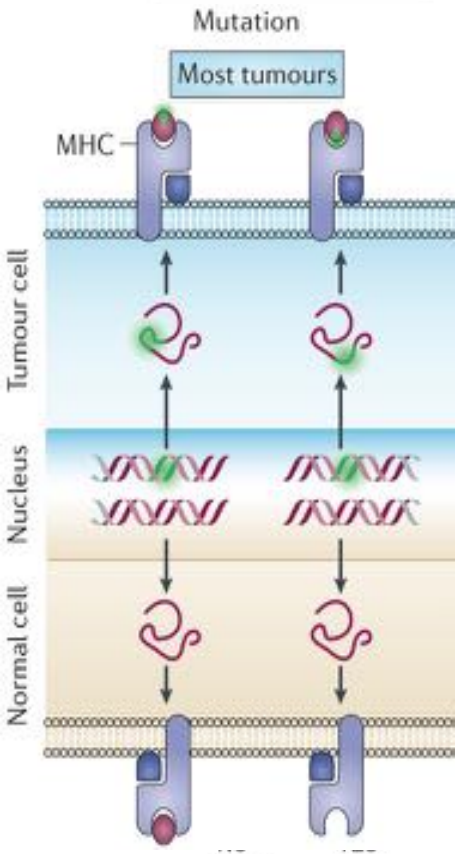
MHC binding

Gene expression level

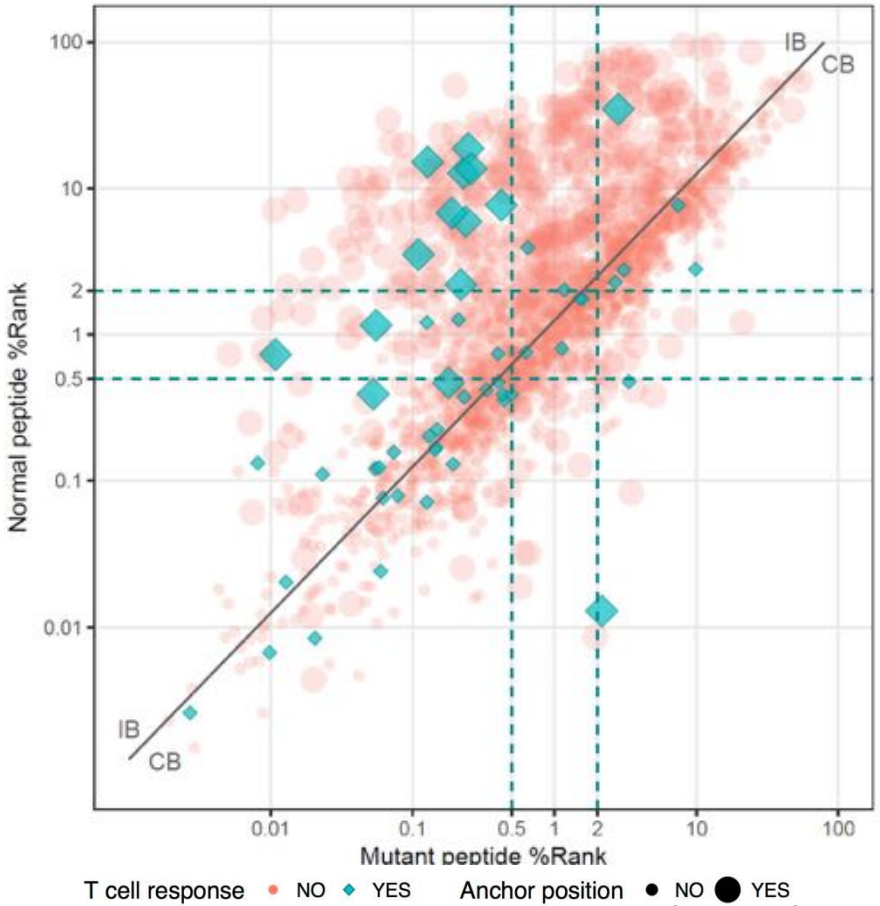
Mutation characteristics (position/self-similarity)

Selective advantage & genetic heterogeneity

Neoepitopes are stronger HLA binders than neopeptides

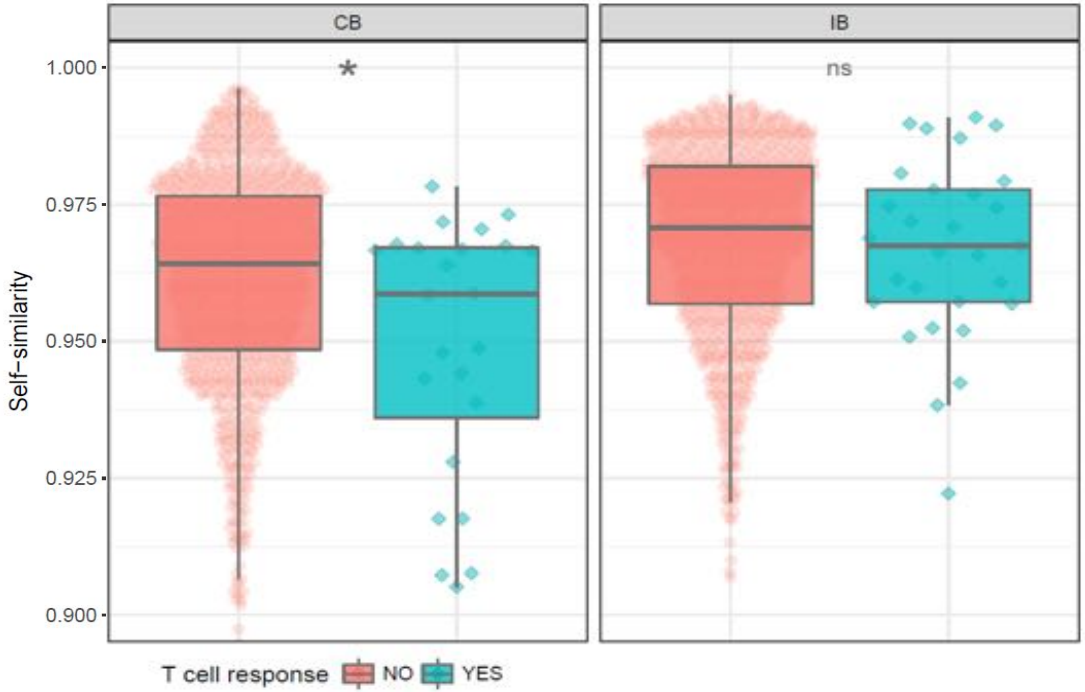


Coulie et al. Nature Reviews. Cancer. 2014

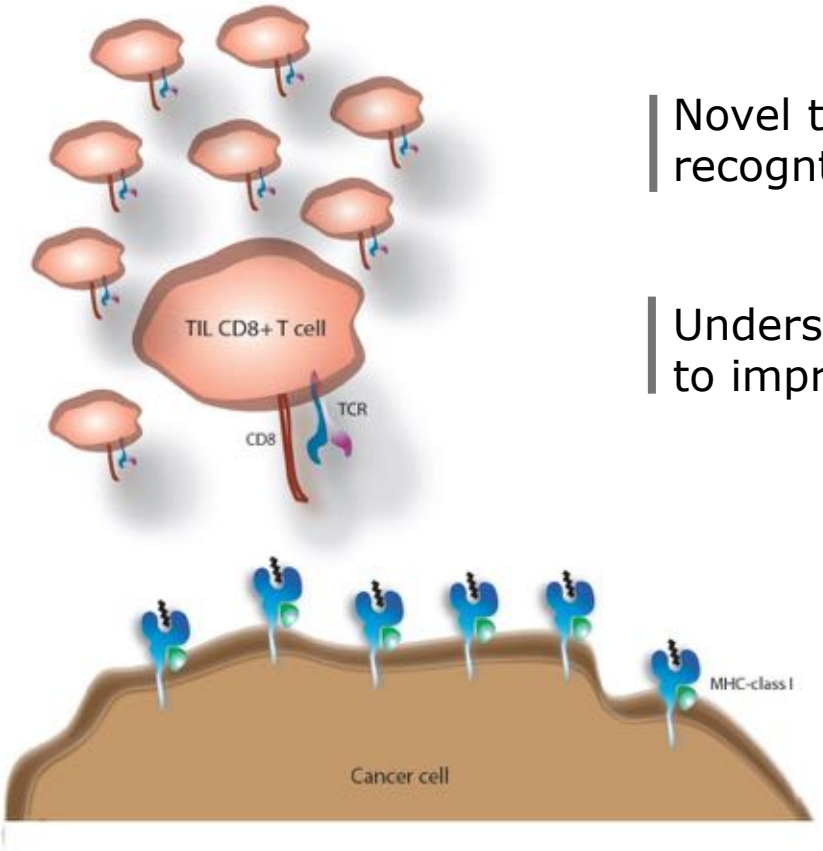


Bjerregaard AM et al. Frontiers Imm. 2017

Loss of self-similarity contributes to immunogenicity specifically for conserved binders



Bjerregaard AM et al. Frontiers Imm. 2017

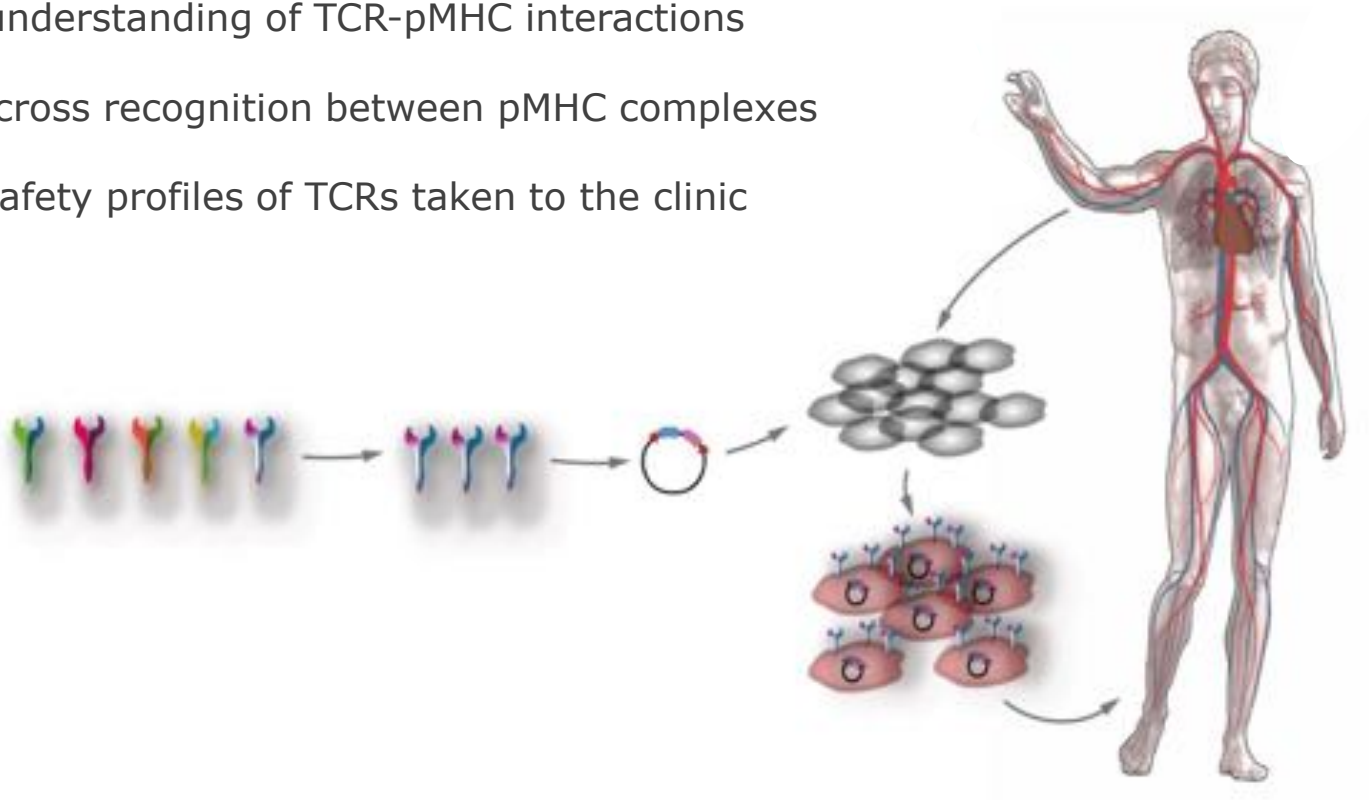


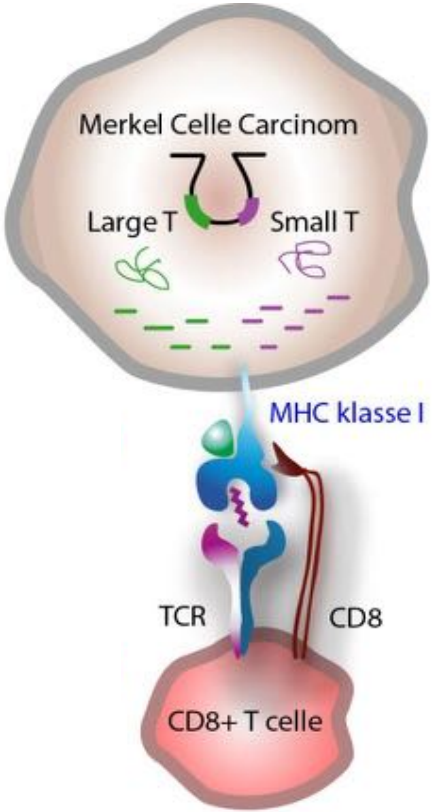
- Novel tools to analyze for T cell recogntion using large libraries of pMHC

- Understanding neoepitope immunogenicity, to improve neoepitope prediction

TCR fingerprinting

- Increased understanding of TCR-pMHC interactions
- `Rules´for cross recognition between pMHC complexes
- Improved safety profiles of TCRs taken to the clinic





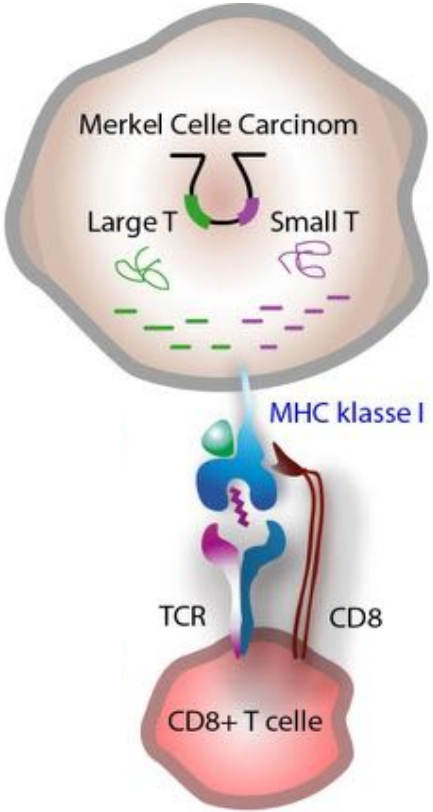
2 TCRs:

HLA-B0702 LTA (APNCYGNIPL)

HLA-B2401 LTA (EWWRSGGFSF)

12 T cell clones:

HLA-A0201 LTA (KLLEIAPNC)



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HLA-B0702 LTA (APNCYGNIPL)

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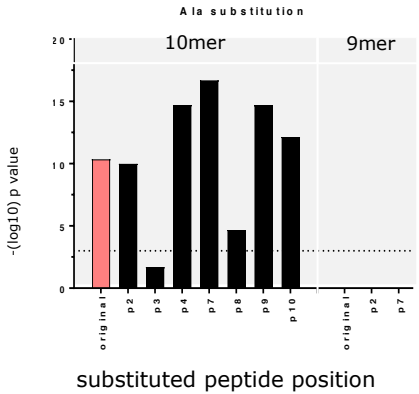
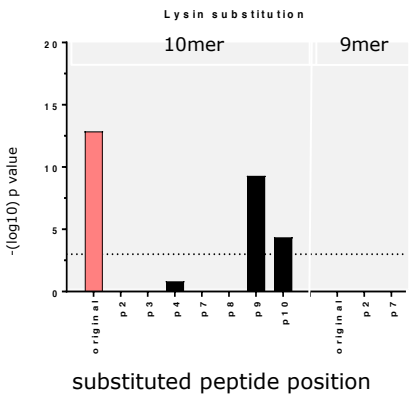
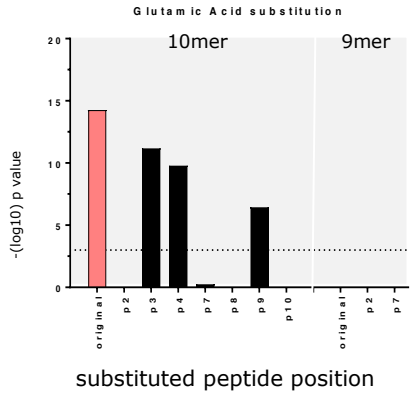
12 T cell clones:

HLA-A0201 LTA (KLLEIAPNC)

Alanine substitution describes only partially the TCR interaction requirements



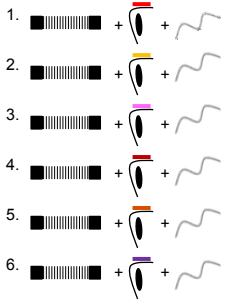


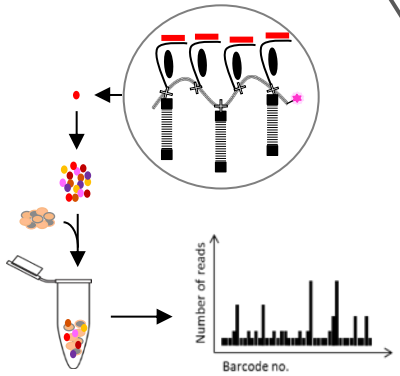


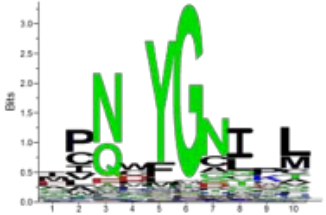
Such L et al. unpublished

A

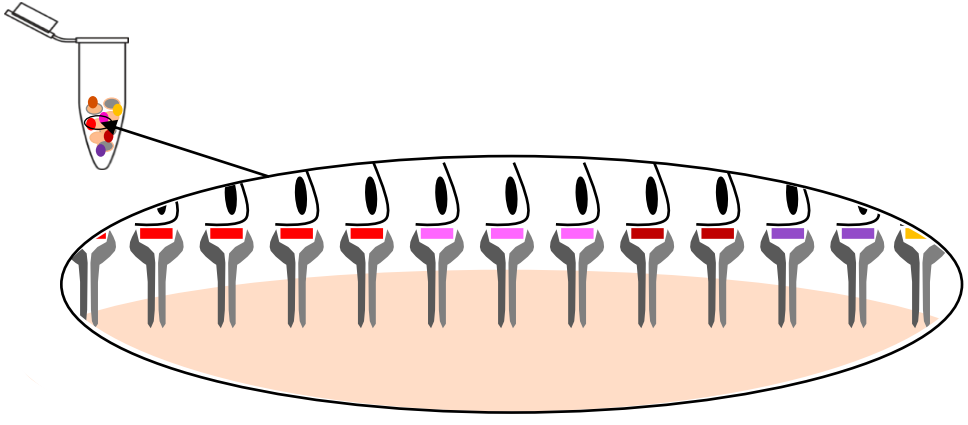
Peptide sequence								
p1	p2	p3	p4	p5	p6	p7	p8	p9
p1*	p2	p3	p4	p5	p6	p7	p8	p9
p1	p2*	p3	p4	p5	p6	p7	p8	p9
p1	p2	p3*	p4	p5	p6	p7	p8	p9
p1	p2	p3	p4*	p5	p6	p7	p8	p9
p1	p2	p3	p4	p5*	p6	p7	p8	p9
p1	p2	p3	p4	p5	p6*	p7	p8	p9
p1	p2	p3	p4	p5	p6	p7*	p8	p9
p1	p2	p3	p4	p5	p6	p7	p8*	p9
p1	p2	p3	p4	p5	p6	p7	p8	p9*



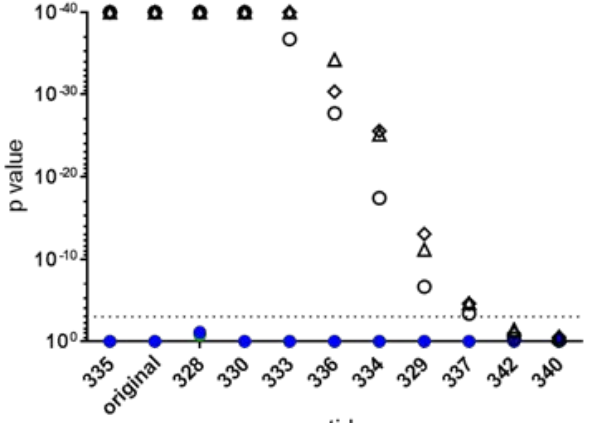




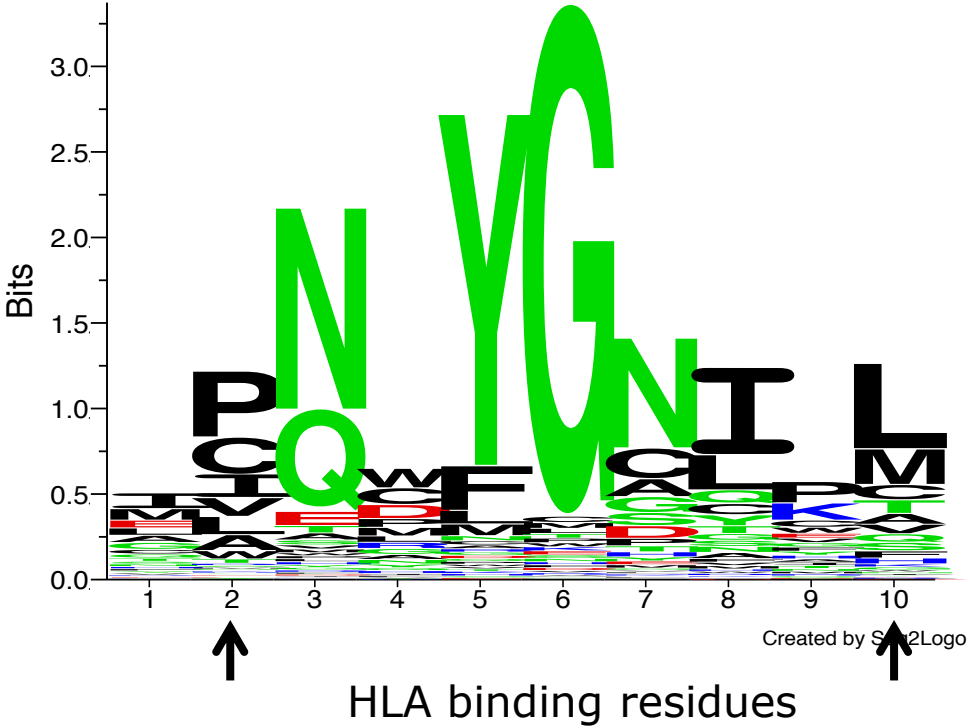
B

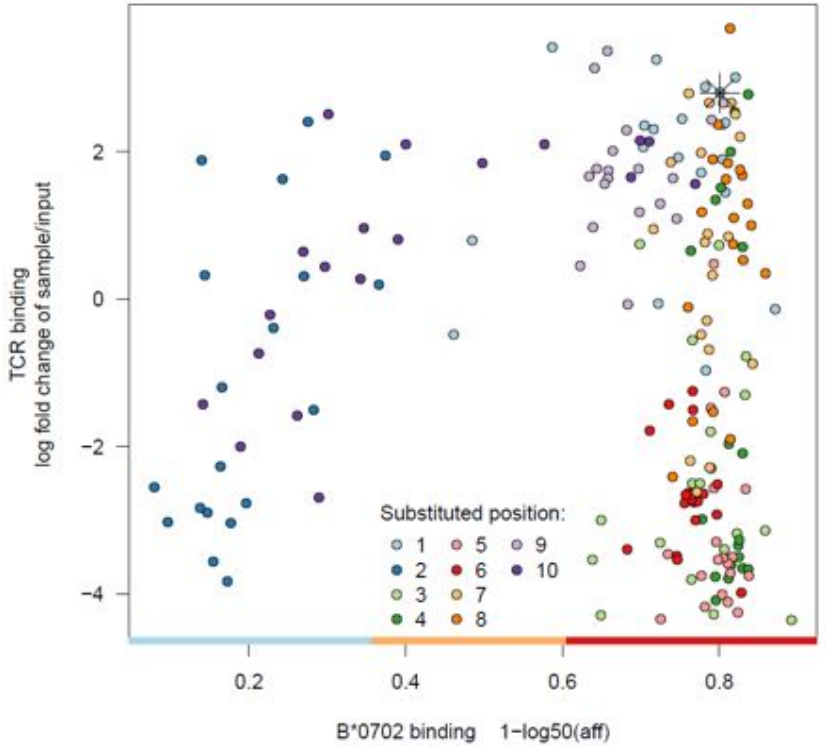


C

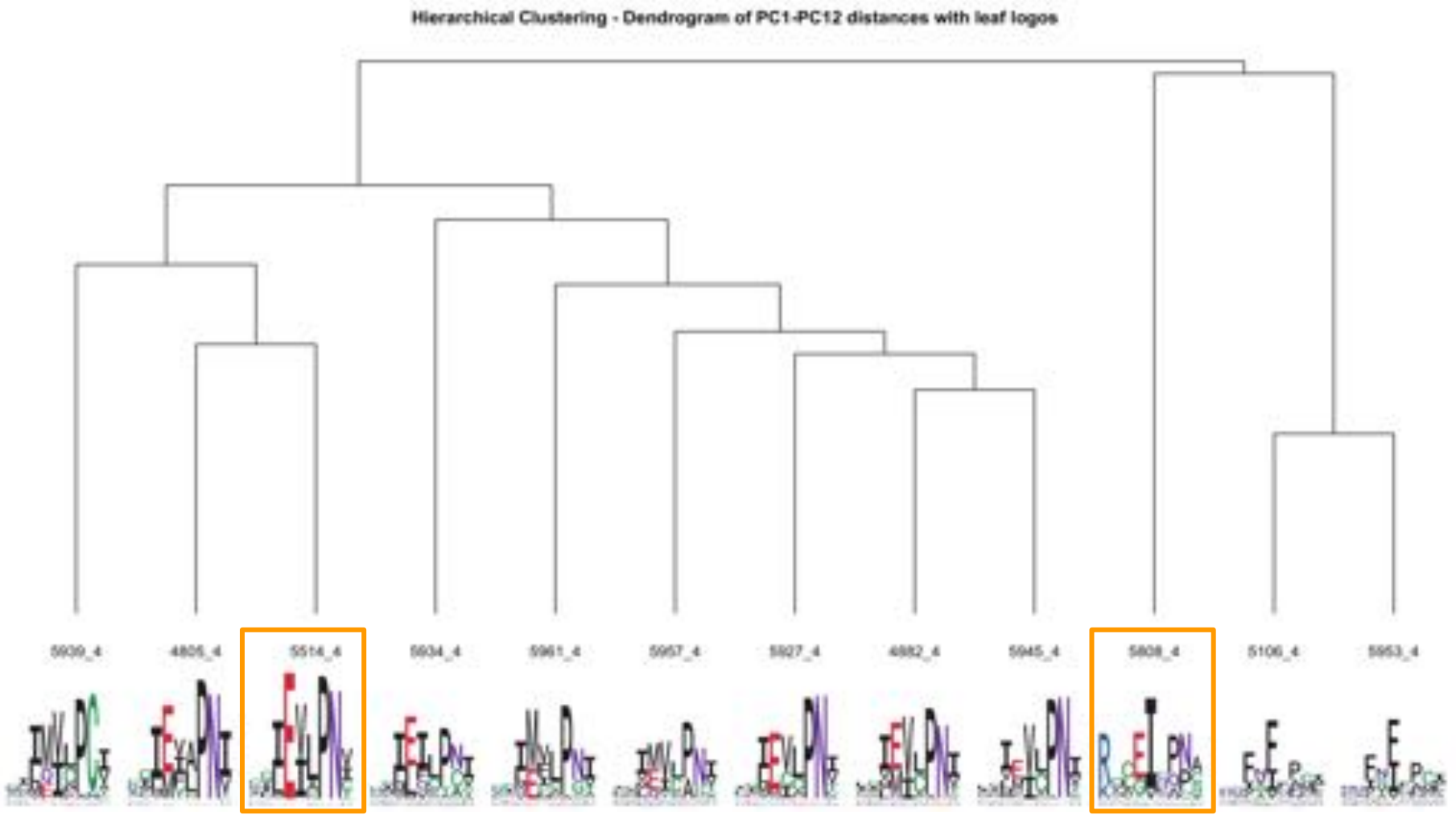


TCR fingerprinting HLA-B0702 LTA (APNCYGNIPL)

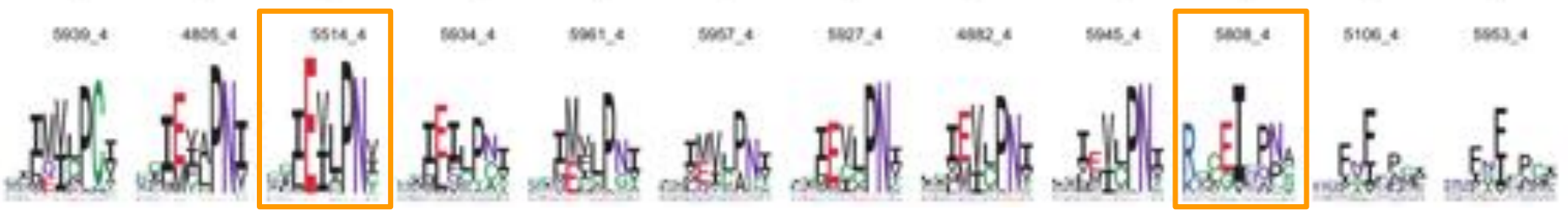




Such L et al. unpublished

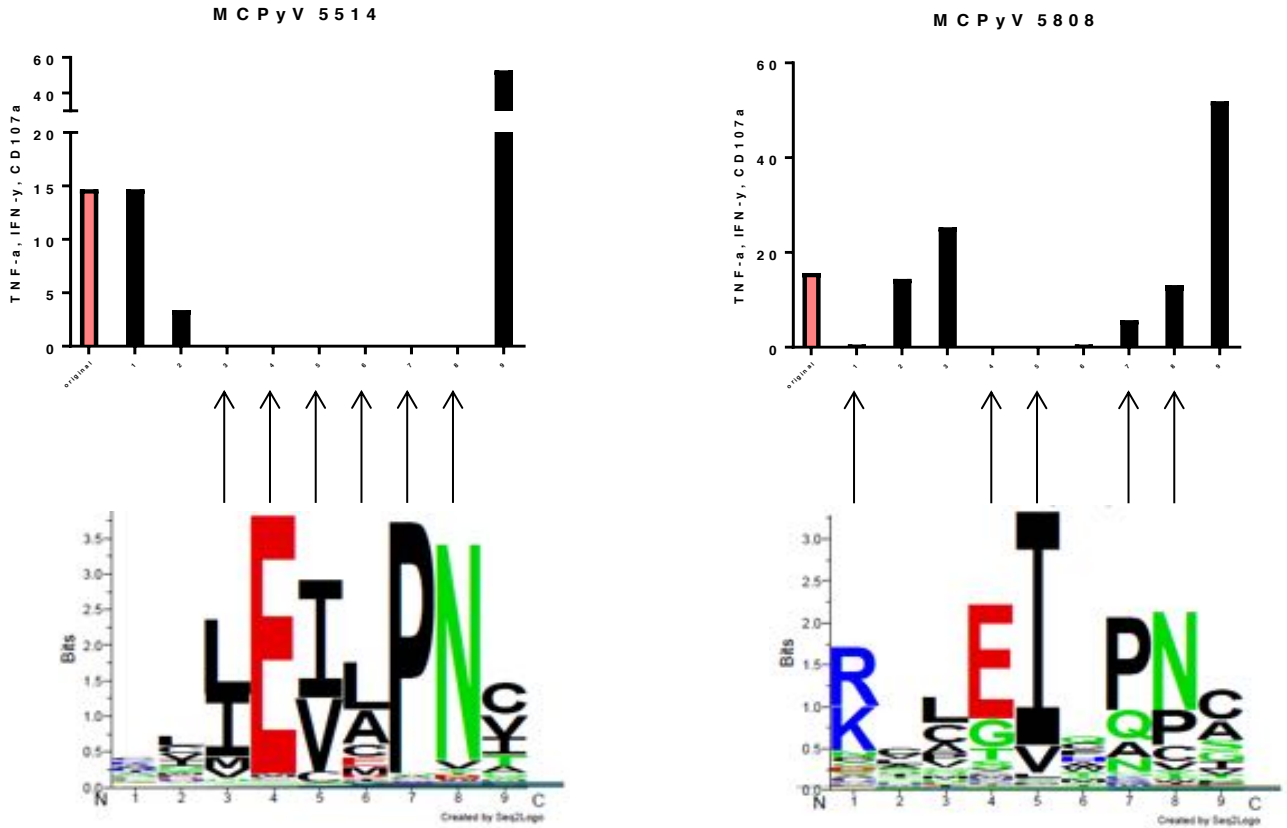

Hierarchical Clustering - Dendrogram of PC1-PC12 distances with leaf logos
5939_4
4805_4
5514_4
5934_4
5961_4
5957_4
5927_4
4882_4
5945_4
5808_4
5106_4
5953_4

Patient	Clone	TRA sequence	TRB sequence	EC50
w678	1	CVLNNNDMRF	CAIRQFDANTGELFF	0,47
	1.5	unresolved by NGS	CAIRQFDANTGELFF	0,84
	2	CAFRVSHDMRF	CASSIIAGSSYNEQFF	0,01
	3	CVVATYSGGGADGLTF	CASSIIAGSSYNEQFF	0,02
	4	CVVATYSGGGADGLTF	CASSSGNPSTDTQYF	0,02
w876	2	CVVTEYSGGGADGLTF	CASRGQNTGELFF	1,20
	5	CAYNQGGKLIF	CASSVLNTGELFF	0,11
	5.5	unknown alpha	CASSVLNTGELFF	0,14
	6	CVVPLYSSASKIIF	CASSDTPDLNTEAFF	0,03
	9	CVGNNNDMRF	CAIRRQDQNTGELFF	0,13
w830	4	CVLNNNDMRF	CASSILGASNQPQHF	1,10
w683	1	CVVALYSGGGADGLTF	CASRSQNYYGYTF	0,83



Validation based on functional recognition

ICS following Alanine substitution, (Gly sub for pos 6)



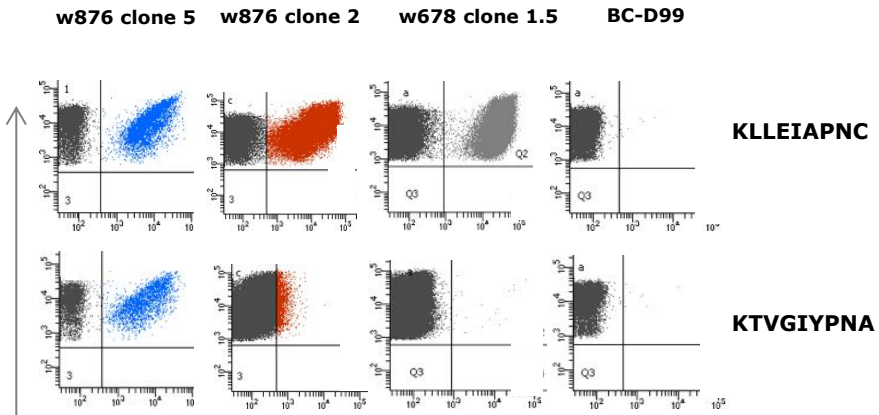
Such L et al. unpublished

Potential cross-recognition in the human proteasome

KLL-clone	sequence	p-value	gene	protein name
5808	RTCEIQGWC	1.51e-07	P2RX3	Purinergic receptor P2X 3
	KWQEIIQNC	2.48e-07	SLF2	SMC5-SMC6 complex localization factor 2
	KTVGIYPNA	2.87e-07	ST6GALNAC3	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 3
	RLVGIHQNG	3.07e-07	WLS	Wntless Wnt ligand secretion mediator
	RNCTITANA	3.64e-07	CD27	CD27 molecule
	RTCEIWSWC	4.2e-07	P2RX6	Purinergic receptor P2X 6
	FICEICNNG	4.92e-07	PLEKHM3	Pleckstrin homology domain containing M3
	KTCEIFGWC	5.47e-07	P2RX1	Purinergic receptor P2X 1
	CVVEICPPA	5.47e-07	PXDNL	Peroxidasin like
	RQLEIANNS	5.52e-07	IFT122	Intraflagellar transport 122
5514	NLMEVMPNI	4.14e-08	RAD9B	RAD9 checkpoint clamp component B
	TVMEVMVNV	2.39e-07	RYR1	Ryanodine receptor 1
	LVLEVDPNI	2.57e-07	KRT8	Keratin 8
	CIQEVEVNC	3.06e-07	PRAMEF25	PRAME family member 25
	LNLEVDPNI	3.63e-07	TTBK2	tau tubulin kinase 2
	KVKEVCPNV	3.77e-07	FAR2	Fatty acyl-CoA reductase 2
	IPMEILPNV	7.57e-07	GK	Glycerol kinase
	RIIEVAPQV	1.04e-06	SEPHS1	Selenophosphate synthetase 1
	RSLEVLWNV	1.11e-06	PCSK7	Proprotein convertase subtilisin/kexin type 7
	KILEICDNV	1.14e-06	TNMD	Tenomodulin

Blasted on FIMO (Find Individual Motif Occurrences)
Grant et al, Bioinformatics 2011

Evaluation of cross-recognition



Gene	Protein name	Organism	Function	Pathway	Tissue expression	Expressing cell types	Staining intensity
ST6GALNAC3	ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	Human	Sialyltransferase	Protein glycosylation	Brain	Neuronal cells	Low
					Endocrine tissue	Glandular cells	Low
					Gastrointenstinal tract	Glandular cells	Low
					Breast and female reproductive system	Squamous epithelial cells	Low
					Muscle tissue	Myocytes	Medium-high

In summary

Large library screening for pMHC recognition of T cells
- **Shared antigens**
- **Neoantigens**

Identify novel antigens & understand immunogenicity

TCR fingerprinting
- **Implications for clinical safety**