InCob2019
Special Session
Bioinformatics in Indonesia

Oral Presentation

T cell epitope prediction of cancer antigens restricted by HLA A*24:07

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Abstract

T cells are the primary effector cells of tumor immunity. T cells recognized peptides derived from tumor antigens wich is presented as a complex with HLA (histocompatibility leukocyte antigen - HLA) on the surface of target cells. HLA-A*24:07 is the major HLA alleles in Indonesia. HLA A*24:07 is considered unique and not a member of the HLA A*24 supertype, which is represented by HLA A*24:02. The two molecules, A*24:02 and A*24:07, share high homology except for one amino acid residue number 70. Residue number 70 in A*24:02 is H and in A*24:07 is Q. This residue happens to be in the peptide binding groove of the HLA allele, and affecting at least 3 out of 6 peptide binding pockets. HLA-A*24:07 is a less characterized allele, and at the moment, there is only one T-cell epitopes reported to be restricted by this HLA allele (IEDB website). The paper will present the peptide binding motif of HLA A*24:07, the benchmark of online T cell epitope prediction server, and the use of prediction server to predict peptide derived from cancer antigens that will bind to HLA A*24:07.

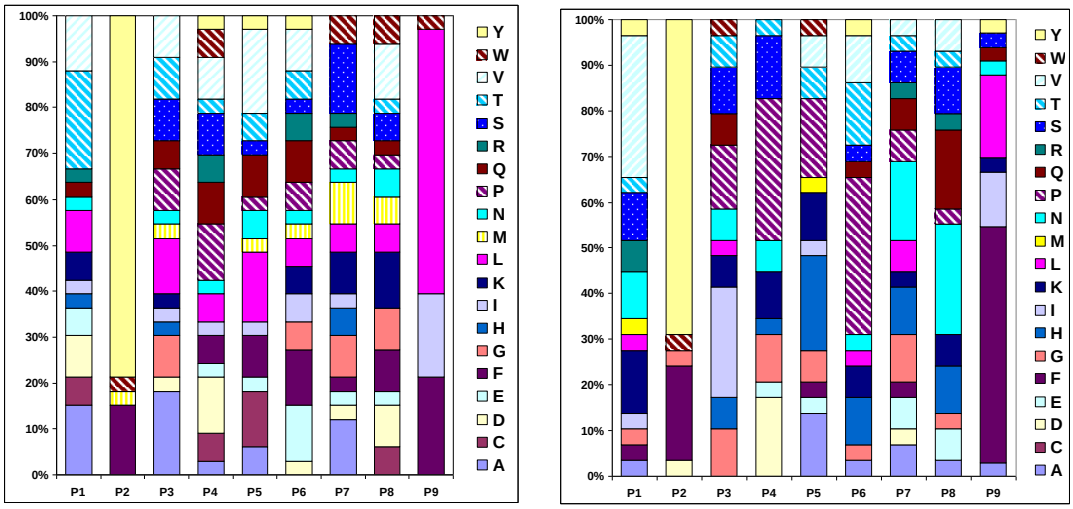

Fig 1. Peptide binding motif of HLA-A*24:02 (left panel) and HLA-A*24:07 (right panel).

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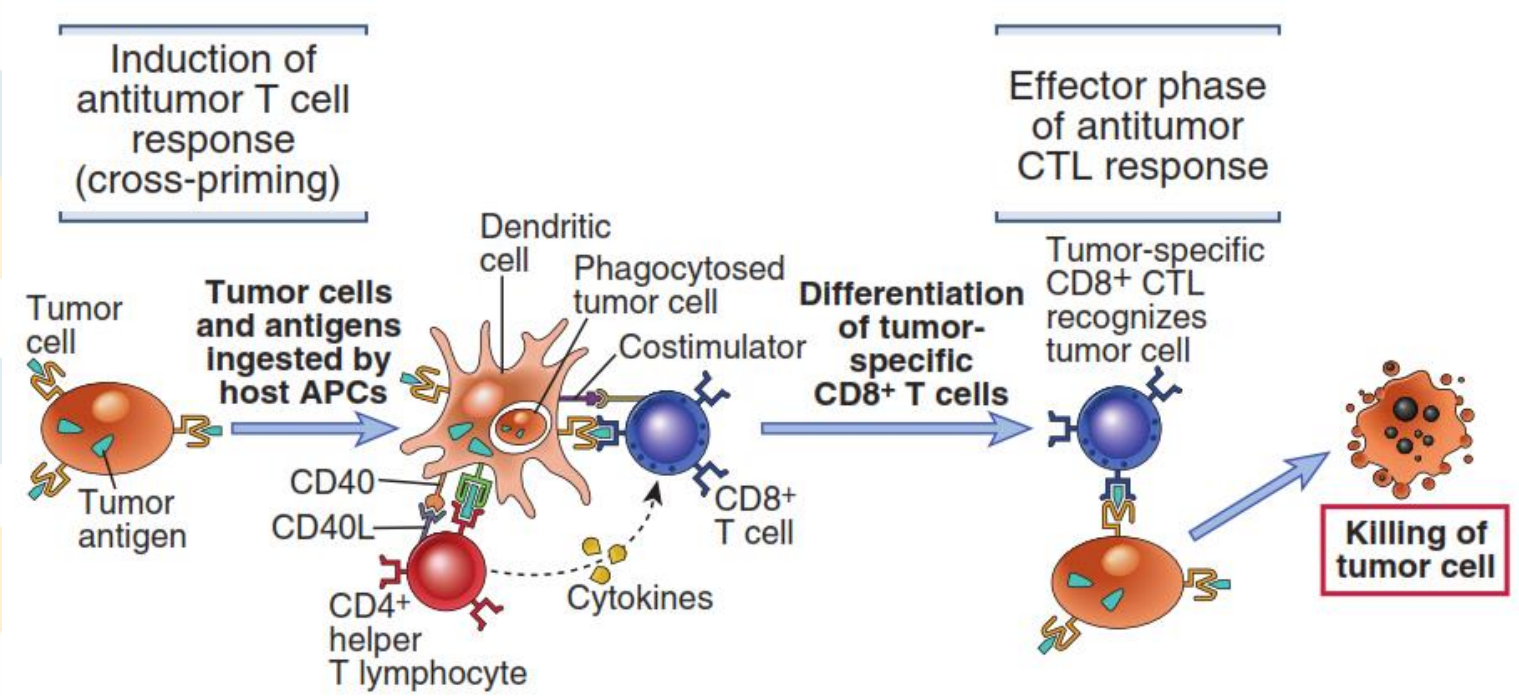
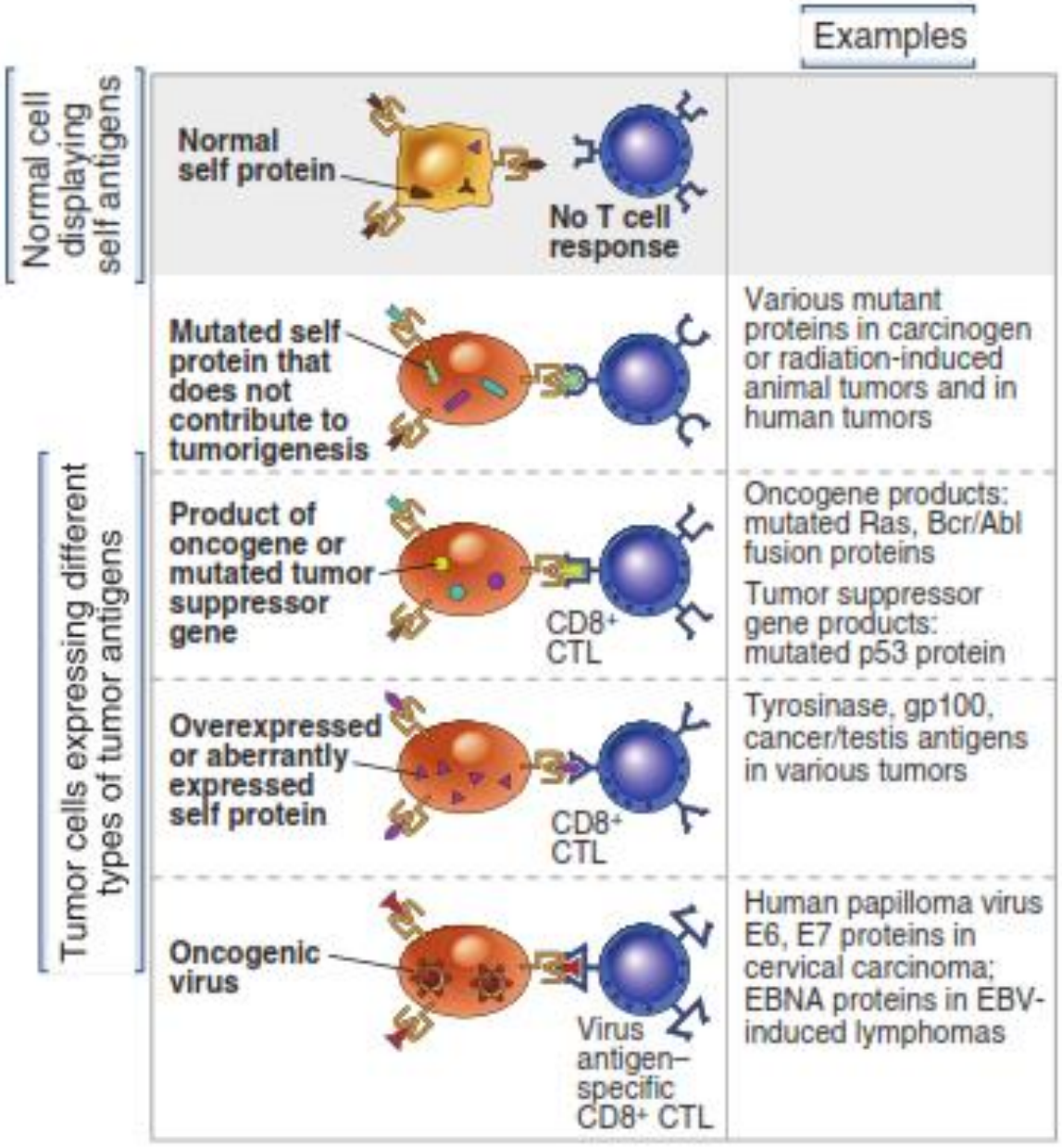
Cell mediated immunity toward cancer



CTL recognizes complex of peptide and MHC molecule.
The peptide is derived from cancer antigen.

Cancer antigens - proteins



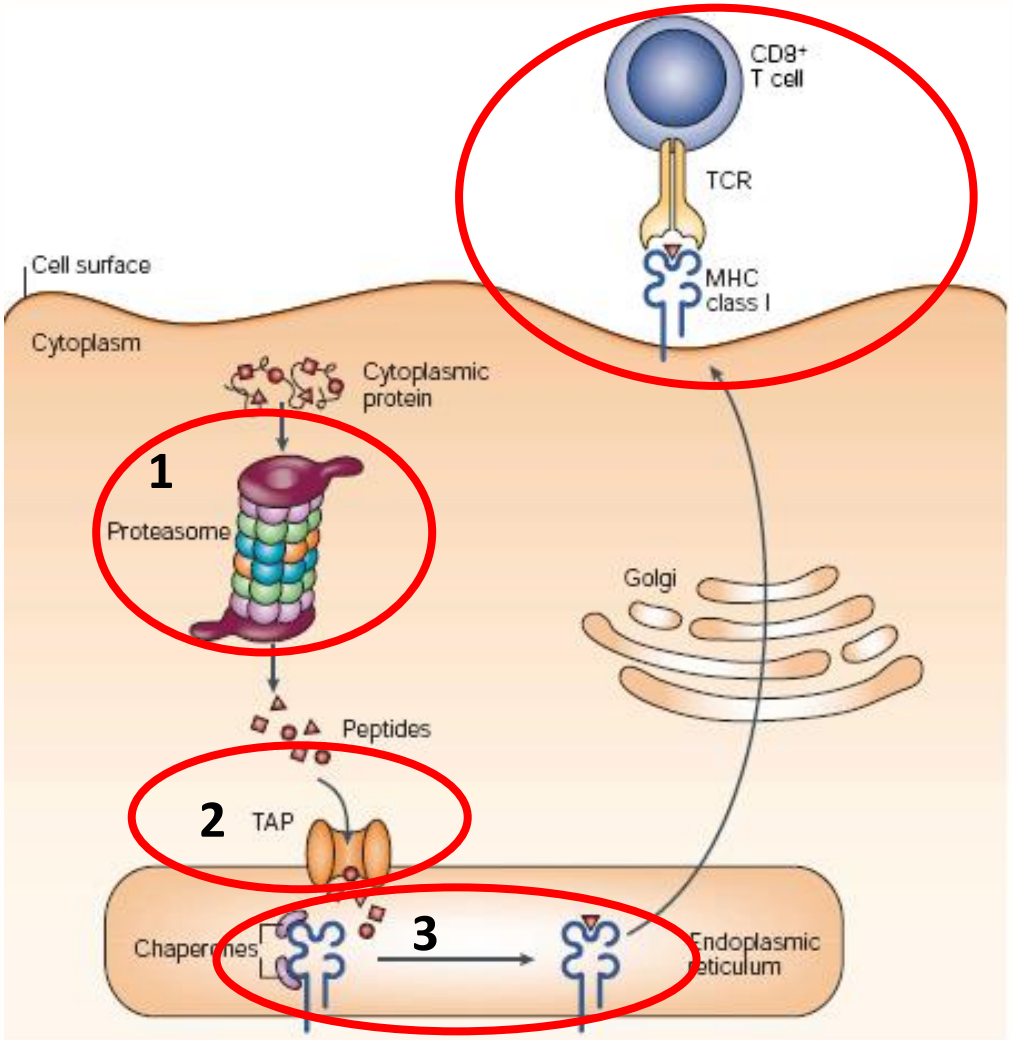
Over express by cancer cells but not by normal somatic cells → good candidate for antigens → “off the shelf”

Which tumor peptides will become T-cell epitopes and recognized by T-cells?

Inside the tumor cells, proteins will undergo…….

1. Proteasomal cleavage
2. Transport into the ER
3. Binding by MHC molecules

- 1,2,3 is not random, it can be modelled, so we can predict using computer algorithms which peptides will be presented to T-cells → **Immunoinformatics**
- Step 3, binding to MHC, is the most important



Nature Reviews | Immunology

Major Histocompatibility Complex (MHC), known as HLA (Human Leukocyte Antigen)

- A set of molecules displayed on cell surfaces
- Present peptide for recognition by T-cells
- Two classes of MHC are important for antigen presentation: MHC Class I and MHC Class II
- **The most polymorphic molecules, important for recognition of the pathogen**
- The naming of HLA

  example

HLA-A*24:07

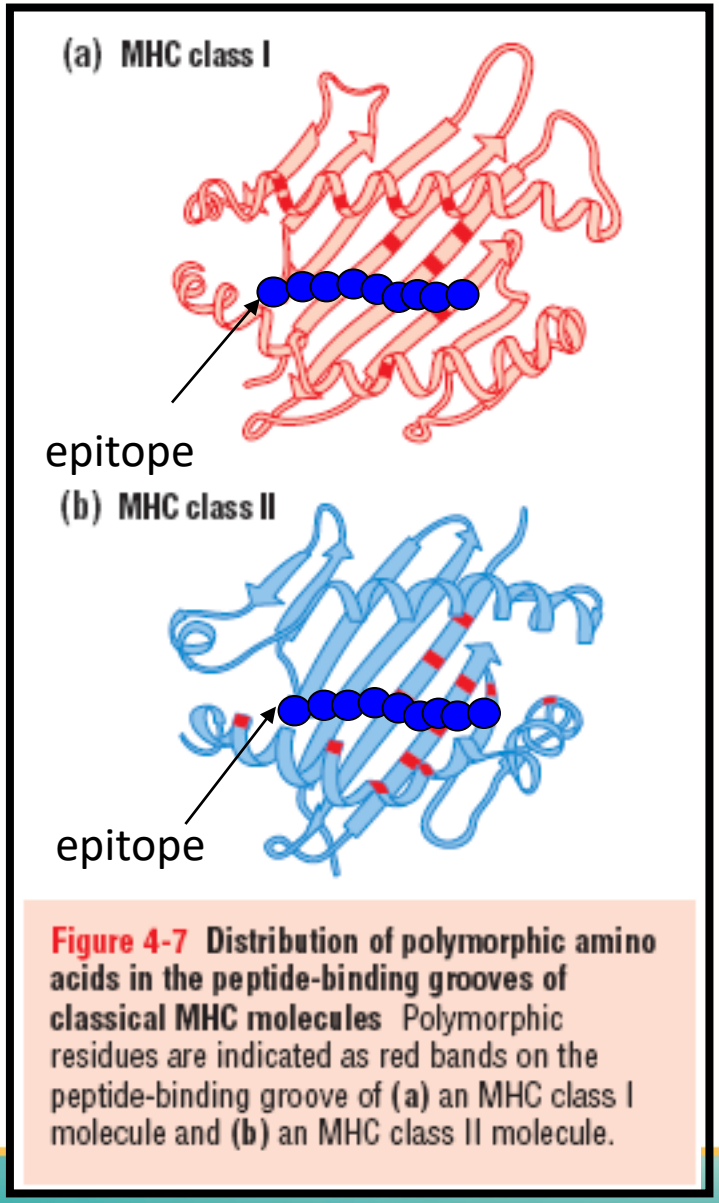
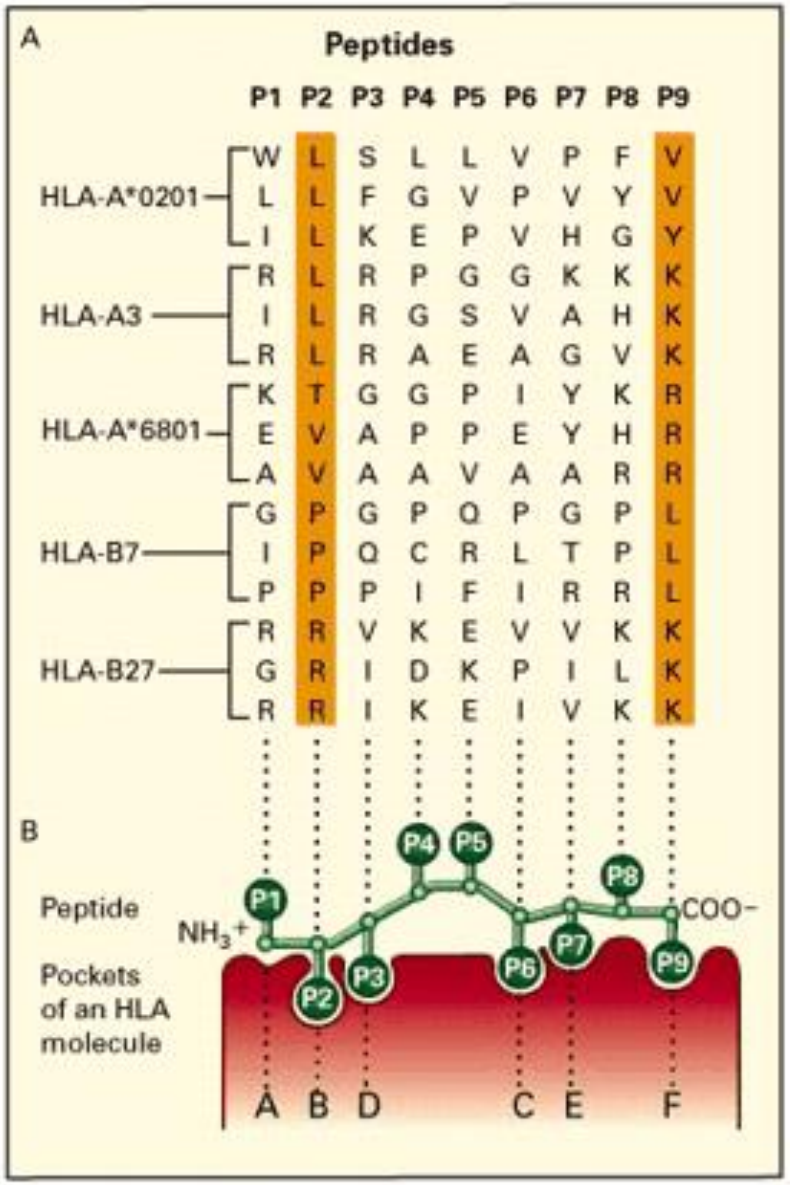


Figure 4-7 Distribution of polymorphic amino acids in the peptide-binding grooves of classical MHC molecules Polymorphic residues are indicated as red bands on the peptide-binding groove of **(a)** an MHC class I molecule and **(b)** an MHC class II molecule.

Specificity of peptide binding to HLA molecules



- Typically, an HLA class I molecule has six pockets (A-F).
- The side chains that fit into them serve as the peptide's anchors.
- Each HLA type has its own preference peptide motif.
- Computational methods were developed to predict whether peptide will bind to HLA molecules using quantitative matrices.
- Matrices are generated from experimental binding data of large ensemble of sequence variants (Sette et al ., 1989; Ruppert et al., 1993; Parker et al., 1994; Gulukota et al., 1997; Bhasin and Raghava 2003) .

Klein and Sato, 2000
http://kdcp.hallym.or.kr/immunogenetics/NEJM-HLA.htm

HLA allele types of Indonesian population

57 HLA Class I alleles and 20 HLA Class II alleles were identified

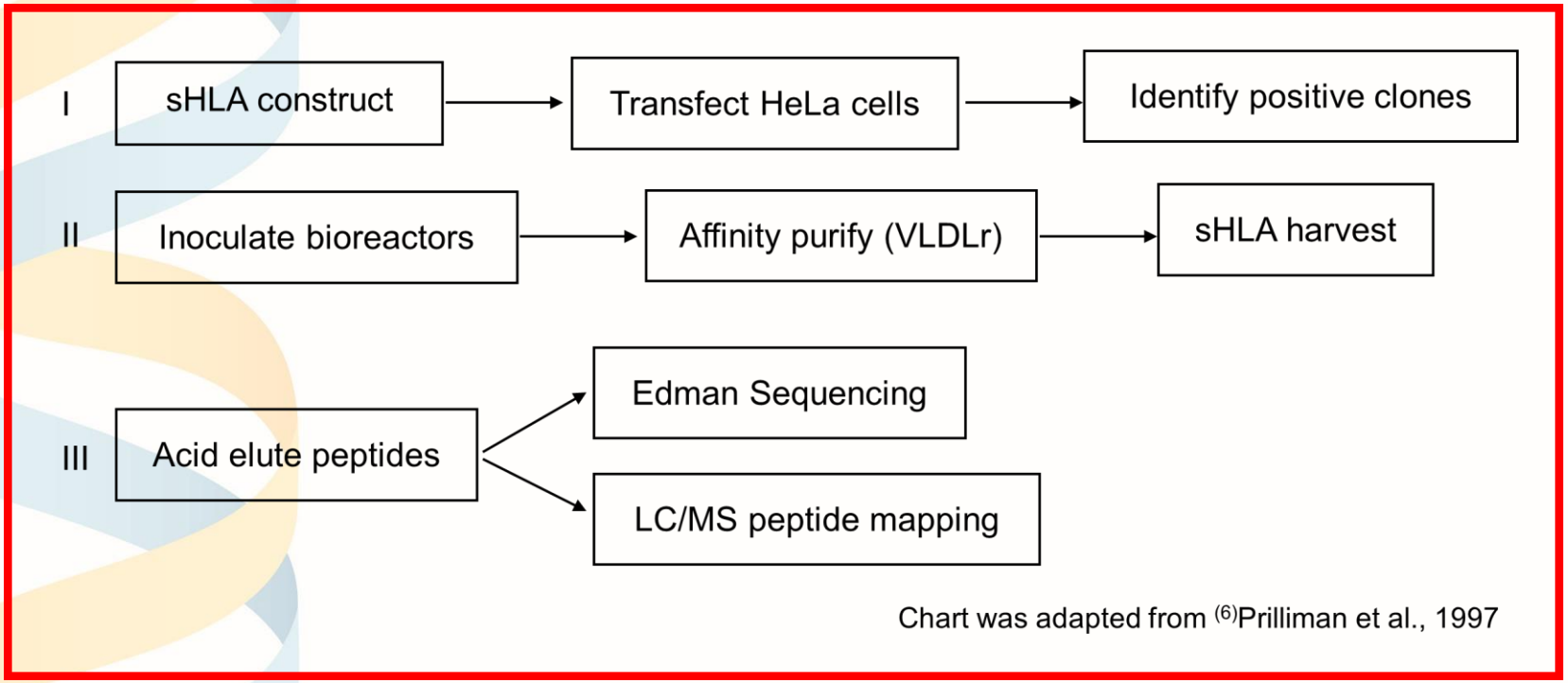
Major HLA Allele	Allele Frequency (%)
A*24:07	**21.52**
A*11:01	16.03
A*33:03	**15.61**
A*24:02	14.35
B*15:02	**11.6**
B*15:13	**11.2**
DRB1*12:02	**37.8**
DRB1*15:02	23
DRB1*07:01	13.1

Yuliwulandari et al., 2008

Aims

- Testing the accuracy of the immunoinformatics prediction server for peptide binding to HLA-A*24:07 → comparing experimentally identified peptides and the predicted peptides.
- Identify and characterize the epitopes derived from cancer antigens that are relevant to the HLA alleles found in Indonesian population → HLA- A*24:07

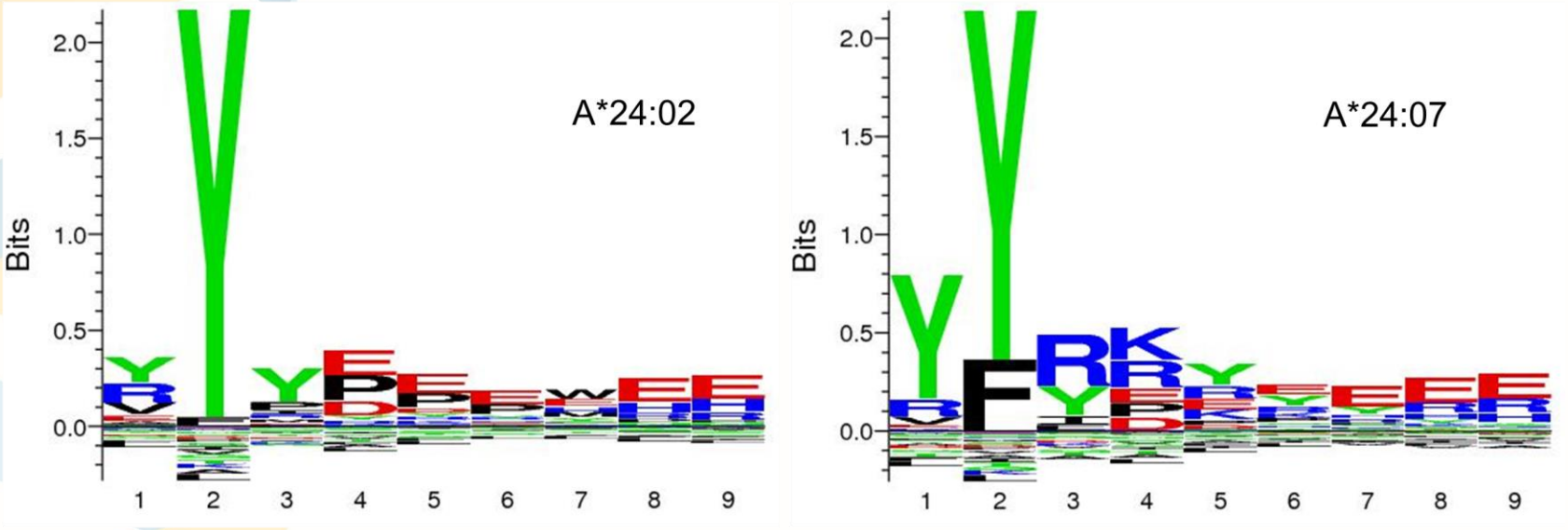
Direct discovery of peptide presented by HLA-A*24:07



- HeLa cells produce soluble HLA-A*24:07 molecules.
- Identify peptides that are associated with the HLA-A*24:07 using Edman Sequencing and LC/MS peptide mapping

Gustiananda, Marsia & Cox, Daryl & Mcmurtrey, Curtis & W, Kenneth & Jackson, Ken & Mojsilovic, Danijela & Bardet, Wilfried & Schafer, Fredda & Cate, Steven & Yaciuk, Jane & Kaabinejadian, Saghar & Hildebrand, William. (2013). Motifs of the naturally processed peptides presented by HLA-A*24:07. Frontiers in Immunology. 4. 10.3389/conf.fimmu.2013.02.01033.

Edman Sequencing of eluted peptides



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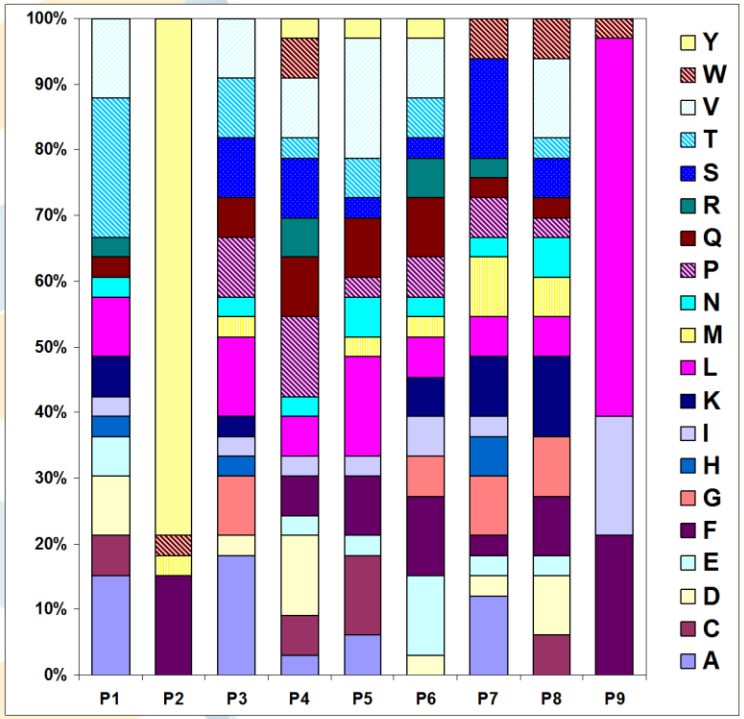
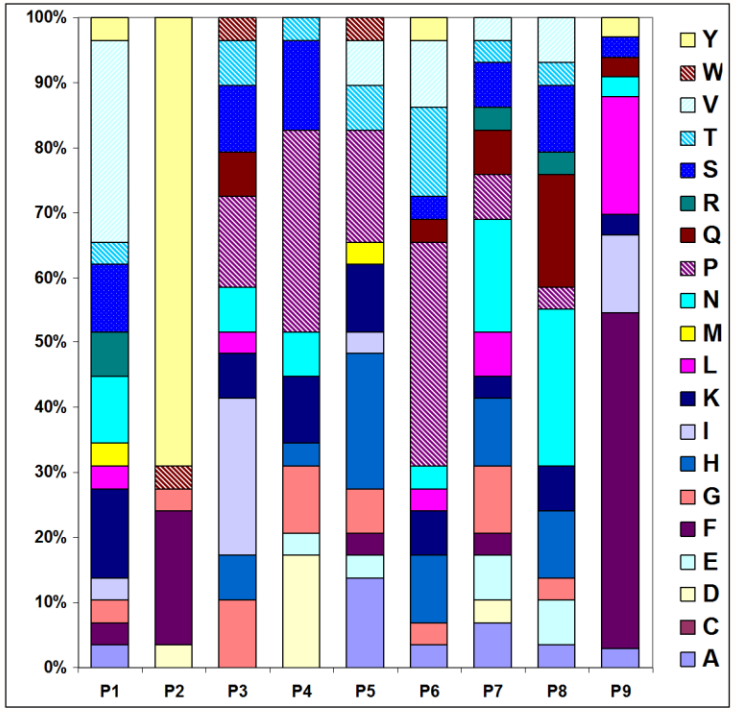
Edman pool sequencing analysis of peptides eluted from both A*24:02 and A*24:07 demonstrated amino acid preferences at position P2 are tyrosine and phenylalanine (Y and F). This result is consistent with the previous report of A*24 peptide motif.

Gustiananda, Marsia & Cox, Daryl & Mcmurtrey, Curtis & W, Kenneth & Jackson, Ken & Mojsilovic, Danijela & Bardet, Wilfried & Schafer, Fredda & Cate, Steven & Yaciuk, Jane & Kaabinejadian, Saghar & Hildebrand, William. (2013). Motifs of the naturally processed peptides presented by HLA-A*24:07. Frontiers in Immunology. 4. 10.3389/conf.fimmu.2013.02.01033.

Peptide binding motif of A*24:02



Peptide binding motif of A*24:07



Peptide binding motif of A*24:07 generated from the MS sequencing were compared to the motif of A*24:02 (derived from the Syfpeithy database). The prefered amino acid at P2 (Y and F) are the same between A*24:02 and A*2407, with Y dominating over F. In both A*24:02 and A*24:07, the prefer amino acid at P9 are L, I, and F. In A*24:02 L is dominant over F and I, but in A*24:07 F is dominant over L and I. The number of possible amino acid at P2 and P9 in A*24:07 is higher than in A*24:02. However, the auxilliary anchor P3 and P6 in A*24:02 is more variable than in A*24:07.

Gustiananda, Marsia & Cox, Daryl & Mcmurtrey, Curtis & W, Kenneth & Jackson, Ken & Mojsilovic, Danijela & Bardet, Wilfried & Schafer, Fredda & Cate, Steven & Yaciuk, Jane & Kaabinejadian, Saghar & Hildebrand, William. (2013). Motifs of the naturally processed peptides presented by HLA-A*24:07. Frontiers in Immunology. 4. 10.3389/conf.fimmu.2013.02.01033.

658 self Peptides presented by HLA-A*24:07

Sequence	Protein Accession	Length
QYDSTHGKF	gi|31645	9
RVIISAPSA	gi|31645	9
VINGNPITI	gi|31645	9
GIALNDHFV	gi|31645	9
AGIALNDHF	gi|31645	9
ALNDHFVKL	gi|31645	9
GFYPAEITL	gi|88192431	9
FYPAEITLT	gi|88192431	9
YPAEITLTW	gi|88192431	9
FLSELTQQL	gi|5542151	9
NVGWNNSTF	gi|5542151	9
VVPDQLMAF	gi|5542151	9
PMFIVNTNV	gi|5542151	9
IVNTNVPRA	gi|5542151	9
SFYPAEITL	gi|453648	9
ILPVGAANF	gi|203282367	9
YTDKVVIGM	gi|203282367	9

Comparison of predicted and experimentally identified peptides

	Experimental	Predicted			
Protein Accession	Peptide	Peptide	MHC	%Rank	Epitope
gi|8922756	SYSNAPQHI	SYSNAPQHI	0,50600	0,30	E
		QYINIYKTV	0,44100	0,80	E
		CTSLNFHLI	0,47700	0,80	E
		AYYVAFLPL	0,47900	0,20	E
		VWINAFVML	0,53000	0,15	E
		IYLLVALFI	0,63100	0,30	E
		SHARFYFLF	0,54500	0,10	E
		VFYQLYSLL	0,43000	0,40	E
		LFCNYYVLF	0,47300	0,20	E
		NYYVLFKLL	0,41800	0,40	E
		SYPLNSYEL	0,48300	0,20	E
gi|51471973	VYPPHSHSI	VYPPHSHSI	0,61300	0,05	E
		YFIAVLLLL	0,43700	0,30	E
		PYFIAVLLL	0,38800	0,80	E
gi|52632385	GYGPPPPHY	GYGPPPPHY	0,10500	4,00	
		IYSITTDVL	0,43800	0,40	E
		ITTDVLYTI	0,41900	0,40	E
		THLNNNFMF	0,34200	0,80	E
		IYIAGHPAF	0,58500	0,05	E
gi|62089178	KYKPAVNQI	KYKPAVNQI	0,57300	0,10	E
gi|662994	KYQEVTNNL	KYQEVTNNL	0,56500	0,10	E
gi|48146455	YYQDTPKQI	YYQDTPKQI	0,51900	0,30	E
		FYPAEWQDF	0,43500	0,80	E
gi|119613125	VYLHHNFP	VYLHHNFP	0,11100	50,00	
		PFPVYIAF	0,29700	0,80	E
		DYLVHSSF	0,32500	0,80	E
		MYSVLAVL	0,44700	0,30	E
		MWRDTVPF	0,32400	0,80	E
gi|163931089	VYHKPPNAF	VYHKPPNAF	0,39400	0,30	E
		KYESIIATL	0,52800	0,10	E
		KYPNKYESI	0,59300	0,10	E
gi|1000094	KYISGPHEL	KYISGPHEL	0,66600	0,05	E
		RQQRSFQTL	0,34300	0,80	E
		AFAKEHQEF	0,36500	0,80	E
		SYNAQLVQL	0,33700	0,80	E
		KYTALEQKL	0,34700	0,80	E
		VYRQSLEKL	0,35500	0,80	E
		QYLSENEQW	0,37400	0,80	E

- **Out of 658 experimental identified peptides, 449 of them is predicted as T cell epitopes by prediction server netCTLpan 3.1 → 68,24%**
- **netCTLpan use structural similarity with HLA-A*24:02 to predict peptide binding to HLA-A*24:07.**
- **The experimental peptides are among the highest rank of the predicted epitopes**

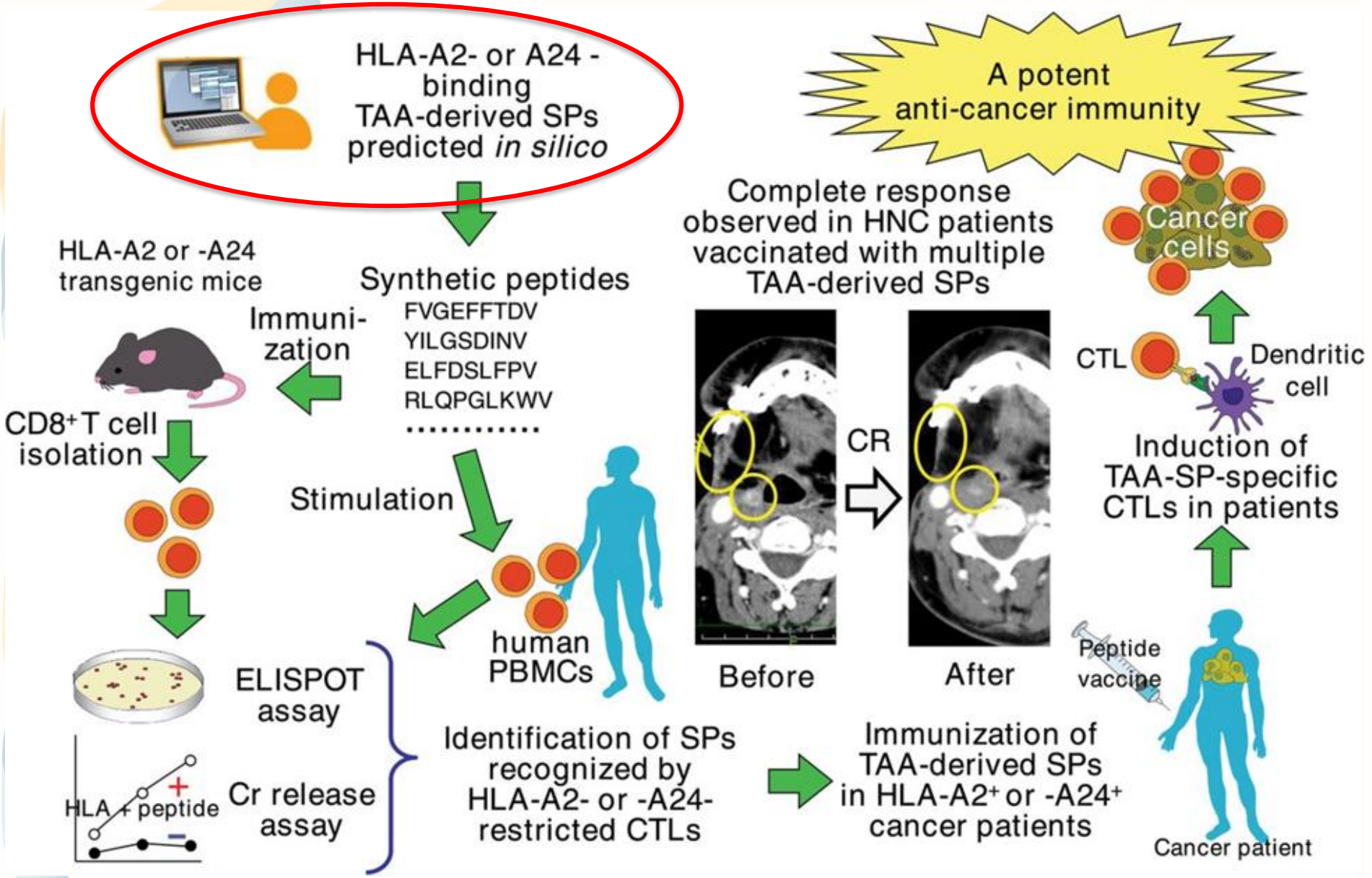
netCTLpan 3.1 was used to predict T cell epitopes from cancer antigens

Antigen	Pos	HLA	Peptide	Score	Aff(nM)	%Rank	BindLevel
ACTL8	28	HLA-A*24:07	VFPNIVNYL	0,447	396,7	0,4	<= SB
CD45	240	HLA-A*24:07	LYNKETKLF	0,47716	286,3	0,3	<= SB
CD45	341	HLA-A*24:07	RFQCGNMIF	0,62374	58,6	0,05	<= SB
CD45	451	HLA-A*24:07	PYTKYVLSL	0,44813	391,9	0,4	<= SB
CD45	544	HLA-A*24:07	LQYSTDYTF	0,42932	480,4	0,5	<= SB
CD45	652	HLA-A*24:07	LFLAEFQSI	0,48052	276,1	0,3	<= SB
CD45	659	HLA-A*24:07	SIPRVFSKF	0,41446	564,2	0,5	<= SB
CD45	862	HLA-A*24:07	TYIGIDAML	0,44467	406,9	0,4	<= SB
CD45	901	HLA-A*24:07	QYILIHQAL	0,43987	428,6	0,4	<= SB
CD45	1016	HLA-A*24:07	KYINASFIM	0,58951	84,9	0,09	<= SB
CD45	1025	HLA-A*24:07	SYWKPEVMI	0,48763	255,6	0,25	<= SB
CD45	1098	HLA-A*24:07	TYTLRVFEL	0,53205	158,1	0,17	<= SB
CD45	1117	HLA-A*24:07	VYQYQYTNW	0,55753	120	0,12	<= SB
CD45	1119	HLA-A*24:07	QYQYTNWSV	0,56638	109	0,12	<= SB
CD45	1217	HLA-A*24:07	QYQFLYDVI	0,6346	52,1	0,05	<= SB
CTA83	3	HLA-A*24:07	FYLLLASSI	0,55441	124,1	0,12	<= SB
MAGEB4	142	HLA-A*24:07	KYKEHFPEI	0,59536	79,7	0,08	<= SB
MAGEB4	229	HLA-A*24:07	IYDGKRHLI	0,4191	536,5	0,5	<= SB
PRDM13	120	HLA-A*24:07	WYSNSLAQW	0,50007	223,4	0,25	<= SB
PRDM13	297	HLA-A*24:07	FYPGVRSAF	0,41201	579,3	0,5	<= SB
PRDM13	488	HLA-A*24:07	AYYPLKLHF	0,62588	57,3	0,05	<= SB
PRDM13	501	HLA-A*24:07	KYPESISYF	0,70328	24,8	0,01	<= SB
TLX3	55	HLA-A*24:07	TYPSLPASF	0,6091	68,7	0,07	<= SB
TLX3	62	HLA-A*24:07	SFAGLGAPF	0,45424	366,9	0,4	<= SB
TNFAIP8	107	HLA-A*24:07	SFHQVDYTF	0,68297	30,9	0,02	<= SB

Proteins that are overexpressed in cancer cells, but not in adult somatic normal cells.

- ACTL8
- CD45
- MAGEB4
- PRDM13
- TLX3
- TNFAIP8

Personalized cancer vaccine?



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THANK YOU