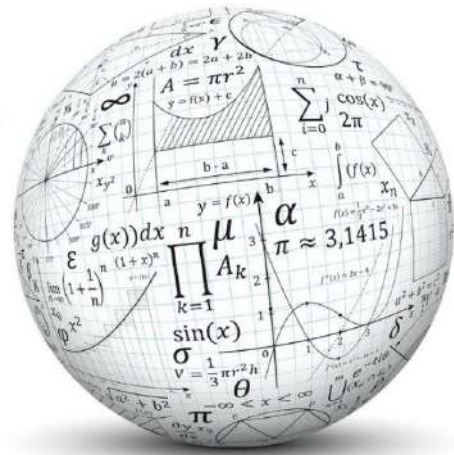


In silico prediction of Tumor Associated Antigens' immunogenicity

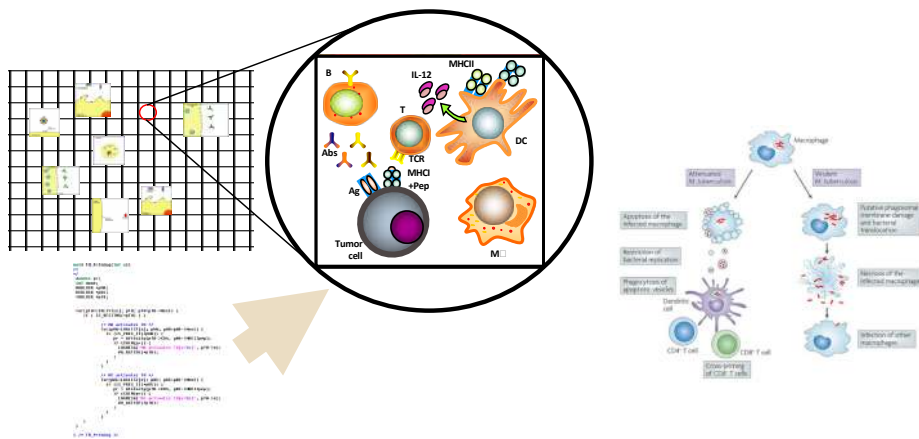
Filippo Castiglione



National Research Council of Italy, Italy

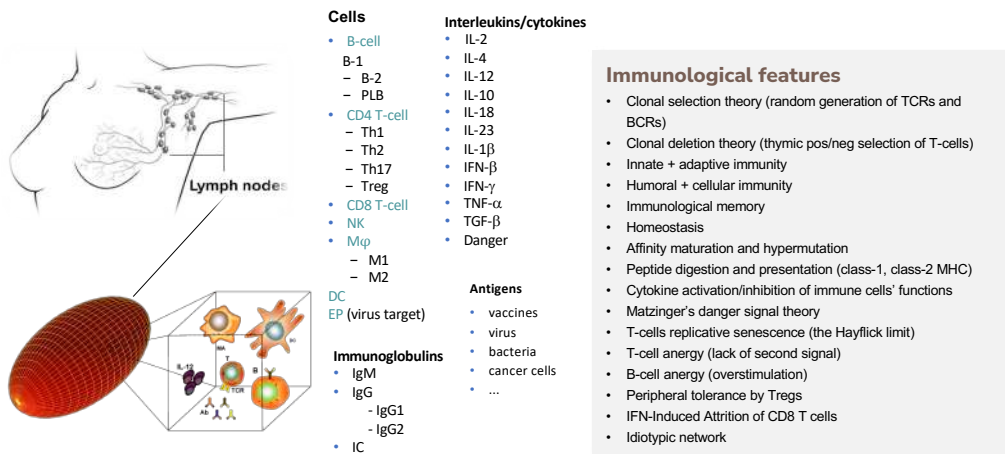


modeling biological processes with ABMs



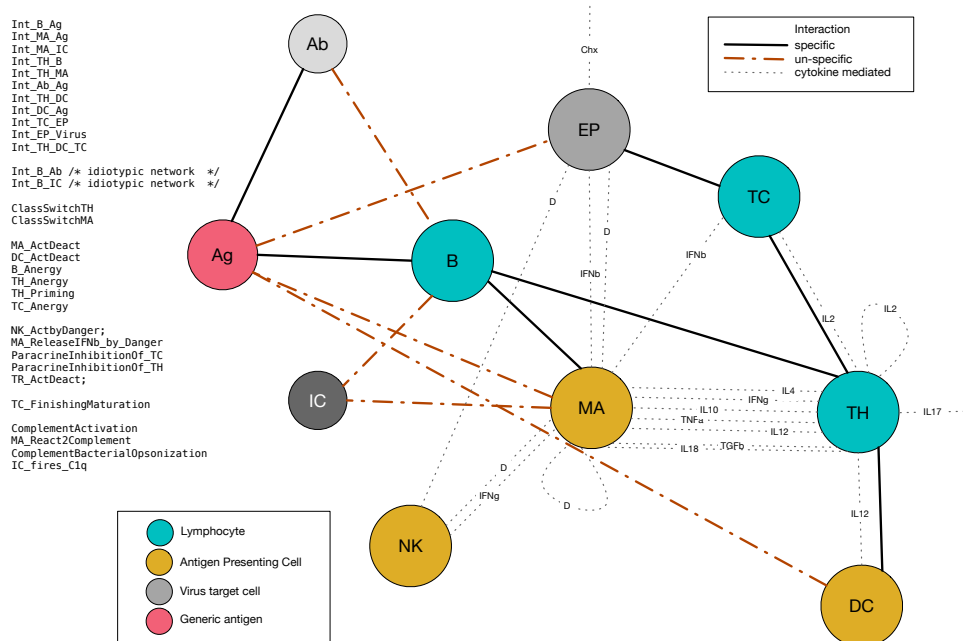
Agent-based Models (ABM) are mainly mechanistic models but may contain subprocesses described phenomenologically

stochastic ABM of the immune response

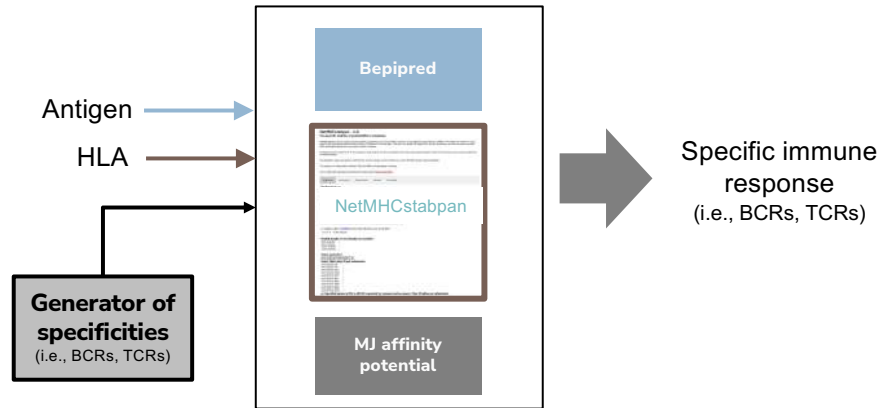


Celada F, Seiden PE. A computer model of cellular interactions in the immune system. *Immunology Today* 1992; 13:56-62

Castiglione F, Celada F. *Immune System Modeling and Simulation*. CRC Press, Boca Raton, London, New York, 2015



exploiting ML predictions



5

NIH National Library of Medicine
National Center for Biotechnology Information

Protein

prostate-specific antigen isoform 1 preproprotein [Homo sapiens]

NCBI Reference Sequence: NP_001639.1

FASTA

GenPept

Log in

Search

Change region shown

Analyze this sequence

Run BLAST

Identify Conserved Domains

Show in Genome Data Viewer

Protein 3D Structure

Crystal Structure Of Human Prostate Specific Antigen (Psa) In Fab Sandwich With PDB: 3QJM

Source: Homo sapiens, Mus musculus

Method: X-Ray Diffraction

Resolution: 3.2 Å

See all 4 structures.

Articles about the KLK3 gene

Reference sequence information

RefSeq genomic sequence

See the genomic reference sequence for the KLK3 gene (NG_011903.1).

Choice of the HLAs

HLA database
www.allelefrequencies.net

class I
 HLA-A*02:01 44%
 HLA-A*03:01 17.4%
 HLA-B*35:01 20.9%
 HLA-B*51:01 28.7%

class II
 DRB1*03:01 33.1%
 DRB1*16:01 33.1%

% of individuals carrying the allele

The screenshot shows the Allele*Frequencies website interface. At the top, there are search filters for Population (United Arab Emirates pop 2), Country (All countries), Score of alleles (All scores), Region (All regions), Ethnic Origin (All ethnic), Type of Study (All studies), Sample Size (All), Sample Year (All years), Level of resolution (All), and Population standard (Gold only, Gold and Silver, All). Below the filters is a table with columns: Line, Allele, Population, % of individuals that have the allele, Allele Frequency (in decimals), Sample Size, IMG7/HLA1 Database, Distribution, Haplotype Association, and Notes. The table lists 13 alleles, with HLA-A*02:01 having the highest frequency at 44.0%.

Line	Allele	Population	% of individuals that have the allele	Allele Frequency (in decimals)	Sample Size	IMG7/HLA1 Database	Distribution	Haplotype Association	Notes
1	A*02	United Arab Emirates pop 2	44.0	0.2920	373	See	See	See	
2	A*11	United Arab Emirates pop 2	19.2	0.1020	373	See	See	See	
3	A*03	United Arab Emirates pop 2	17.4	0.0910	373	See	See	See	
4	A*08	United Arab Emirates pop 2	16.1	0.0860	373	See	See	See	
5	A*26	United Arab Emirates pop 2	14.2	0.0740	373	See	See	See	
6	A*01	United Arab Emirates pop 2	12.1	0.0620	373	See	See	See	
7	A*31	United Arab Emirates pop 2	11.8	0.0610	373	See	See	See	
8	A*24	United Arab Emirates pop 2	10.2	0.0520	373	See	See	See	
9	A*30	United Arab Emirates pop 2	9.7	0.0500	373	See	See	See	
10	A*32	United Arab Emirates pop 2	7.5	0.0390	373	See	See	See	
11	A*23	United Arab Emirates pop 2	4.2	0.0210	373	See	See	See	
12	A*21	United Arab Emirates pop 2	3.6	0.0190	373	See	See	See	
13	A*29	United Arab Emirates pop 2	3.2	0.0180	373	See	See	See	

Prediction of potential linear B-cell epitopes

The screenshot shows the BepiPred-2.0 web interface. An arrow labeled 'Antigen' points to the 'Submit data' button. Another arrow labeled 'epitopes list and scores' points to the output area. The interface includes a header for DTU Health Tech and navigation tabs for News, Education, Research, Collaboration, Services and Products, and About Us.

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVFLTLSVTWIG**AAPLILSRI**VGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
 SVILLGRHSL**FHPEDT**GQVFQVSH**SFPHPLYDMSLLKNRFLRPGDSSH**DLMLRLSEPAELTDAVKVMD
 LPTQEPALGTTTCYASGWGSI**EPEEFLTPKKL**QCVDLHVIS**NDVCAQVHPQKVKTF**MLCAGRWT**GGKSTCS**
GDSGGPLVNCNGLVQGITSWG**SEPCALPER**PSLYTKV**VHYRKWIKDT**IVANP

Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 2017 (Web Server issue). doi: 10.1093/nar/gkx352

TCR-peptides affinity prediction

<https://services.healthtech.dtu.dk/service.php?NetMHCstabpan-1.0>
<https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-4.0>

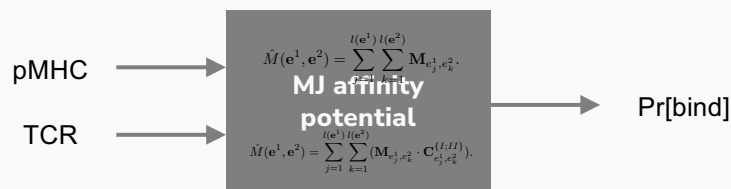


M. Rasmussen, E. Fenoy, M. Harndahl, A. Bregnballe Kristensen, I. Kallehaug Nielsen, M. Nielsen, S. Buus. Pan-specific prediction of peptide-MHC-I complex stability; a correlate of T cell immunogenicity. *J Immunol* August 15, 2016, 197 (4) 1517-1524; doi:10.4049/jimmunol.1600582

Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M. Improved prediction of MHC II antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data. *J Proteome Res* 2020 Apr 30. doi: 10.1021/acs.jproteome.9b00874.

pMHC-TCR affinity calculation (and BCR-epitope)

Miyazawa-Jernigan TCR-MHCpep contact potential [4]



The work of Miyazawa and Jernigan on protein energy potentials provides a method for assessing the chance of direct interactions among proteins. The protein-protein potential concept was derived from the analysis of 3-dimensional structures in which the relative positions of amino acids were determined.

The contact potential matrix estimated by Miyazawa and Jernigan reflects the entropy between two residues. Low entropy means that the two residues have low energy and, therefore, that interaction between them is possible.

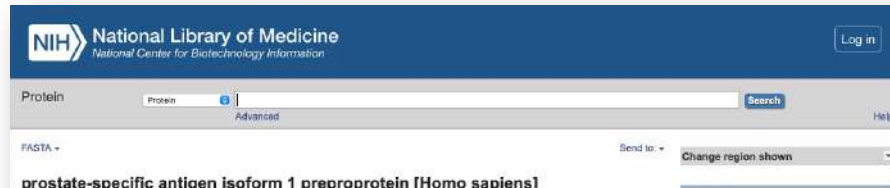
Application 1

Computational modeling of prostate cancer (active) immunotherapy

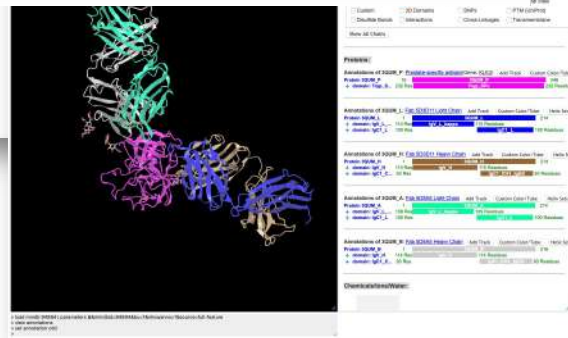
KLK3

- The introduction of testing for prostate-specific antigen (PSA), a member of the fifteen-gene family of kallikrein-related peptidases and also known as kallikrein-related peptidase 3 (KLK3), in blood has revolutionized both the detection and management of prostate cancer.
- Initially identified in 1966, PSA (*KLK3*), a 33-kDa glycoprotein secreted by prostatic epithelial cells, was first characterized in 1971 by Hara et al. in forensic studies as a marker for human semen^[1]

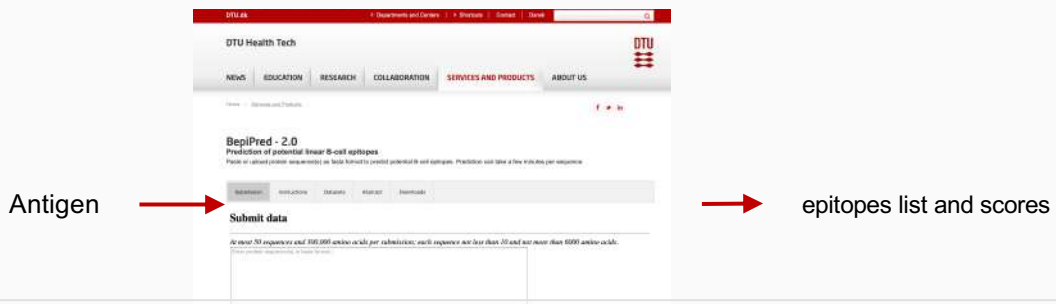
^[1] M. Hara, Y. Koyanagi, T. Inoue, and T. Fukuyama, "Some physico-chemical characteristics of "gamma-seminoprotein", an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII," *Japanese Journal of Legal Medicine*, vol. 25, no. 4, pp. 322– 324, 1971 (Japanese).



```
>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVFLTSLVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGVFQVSHSFPHPLYDMSLLKNRFLRPGDSSHDMLLLRLEPAELTDAVKVMD
LPTQEPALGTTTCYASGWGSIPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP
```



Prediction of potential linear B-cell epitopes



```
>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVFLTSLVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGVFQVSHSFPHPLYDMSLLKNRFLRPGDSSHDLMLLLRLEPAELTDAVKVMD
LPTQEPALGTTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP
```

Strong binder peptides were selected with a predicted affinity value <100nM and stability >1hr

Prediction of T-cell peptides

T-cell peptides in the study were predicted with the servers

- NetMHCpan-4.1
 - ▣ <https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1>
- NetMHCIIpan-3.2
 - ▣ <https://services.healthtech.dtu.dk/services/NetMHCIIpan-3.2>

T-cell peptides affinity prediction

<https://services.healthtech.dtu.dk/service.php?NetMHCstabpan-1.0>

<https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-4.0>



M. Rasmussen, E. Fenoy, M. Harndahl, A. Bregnballe Kristensen, I. Kallehauge Nielsen, M. Nielsen, S. Buus. Pan-specific prediction of peptide-MHC-I complex stability; a correlate of T cell immunogenicity. *J Immunol* August 15, 2016, 197 (4) 1517-1524; doi:10.4049/jimmunol.1600582

Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M. Improved prediction of MHC II antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data. *J Proteome Res* 2020 Apr 30. doi: 10.1021/acs.jproteome.9b00874.

Most prevalent HLAs in the UAE



Source: HLA database
(www.allele-frequencies.net)

class I

HLA-A*02:01	44%
HLA-A*03:01	17.4%
HLA-B*35:01	20.9%
HLA-B*51:01	28.7%

class II

DRB1*03:01	33.1%
DRB1*16:01	33.1%

Line	Allele	Population	% of individuals that have the allele	Allele Frequency (in_decimals)	Sample Size	IMGT/HLA ¹ Database	Distribution ²	Haplotype ³ Association	Notes ⁴
1	A*02	United Arab Emirates pop 2	44.0	0.2520	373	See	See	See	
2	A*11	United Arab Emirates pop 2	16.3	0.1030	373	See	See	See	
3	A*03	United Arab Emirates pop 2	17.4	0.0910	373	See	See	See	
4	A*01	United Arab Emirates pop 2	16.1	0.0840	373	See	See	See	
5	A*28	United Arab Emirates pop 2	14.3	0.0740	373	See	See	See	
6	A*01	United Arab Emirates pop 2	12.1	0.0620	373	See	See	See	
7	A*23	United Arab Emirates pop 2	11.8	0.0610	373	See	See	See	
8	A*24	United Arab Emirates pop 2	10.2	0.0520	373	See	See	See	
9	A*30	United Arab Emirates pop 2	9.7	0.0500	373	See	See	See	
10	A*32	United Arab Emirates pop 2	7.5	0.0380	373	See	See	See	
11	A*23	United Arab Emirates pop 2	6.2	0.0310	373	See	See	See	
12	A*31	United Arab Emirates pop 2	5.6	0.0280	373	See	See	See	
13	A*29	United Arab Emirates pop 2	3.2	0.0180	373	See	See	See	

% of individuals that have the allele

HLA-I peptides (CTL peptides)

A0201

Number of strong binders 1
Number of weak binders 6

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVVFL**FL**TL**S**VTW**I**GAAPLILSRIVGGWECEK**HSQPWQVLV**ASRGRAVCG**VLVHPQWV**TAAH**C**IRNK
 SVILLGRHSLF**HP**EDT**G**QV**F**QV**S**HS**F**PH**PL**YD**M**SL**L**KNR**FL**RP**GD**SS**H**DL**ML**LR**L**SEPA**EL**TD**AV**K**V**MD
 LPTQEPALGTT**C**YAS**G**W**S**IE**P**EE**FL**TP**KK**L**Q**CV**DL**H**V**IS**ND**V**CA**Q**V**HP**Q**K**V**TK**F**ML**C**AGR**WT**GG**K**ST**C**S
 GDSGGPLVCNGVLQGITSWGSEPCALPER**PS**LY**TKV**V**H**Y**R**K**W**IK**D**TIVANP

A0301

Number of strong binders 3
Number of weak binders 10

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVVFL**FL**TL**S**VTW**I**GAAPLILSRIVGGWECEK**HSQPWQVLV**ASRGRAVCG**GV**LV**HP**QW**V**LTA**A**H**C**IRNK
 SVI**LL**GRHSLF**HP**EDT**G**QV**F**QV**S**HS**F**PH**PL**YD**M**SL**L**KNR**FL**RP**GD**SS**H**DL**ML**LR**L**SEPA**EL**TD**AV**K**V**MD
 LPTQEPALGTT**C**YAS**G**W**S**IE**P**EE**FL**TP**KK**L**Q**CV**DL**H**V**IS**ND**V**CA**Q**V**HP**Q**K**V**TK**F**ML**C**AGR**WT**GG**K**ST**C**S
 GDSGGPLVCNGVLQGITSWGSEPCALPER**PS**LY**TKV**V**H**Y**R**K**W**IK**D**TIVANP

B3501

Number of strong binders 6
Number of weak binders 8

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVVFL**FL**TL**S**VTW**I**GAAPLILSRIVGGWECEK**HSQPWQVLV**ASRGRAVCG**GV**LV**HP**QW**V**LTA**A**H**C**IRNK
 SVILLGRHSLF**HP**EDT**G**QV**F**QV**S**HS**F**PH**PL**YD**M**SL**L**KNR**FL**RP**GD**SS**H**DL**ML**LR**L**SEPA**EL**TD**AV**K**V**MD
 LPTQEPALGTT**C**YAS**G**W**S**IE**P**EE**FL**TP**KK**L**Q**CV**DL**H**V**IS**ND**V**CA**Q**V**HP**Q**K**V**TK**F**ML**C**AGR**WT**GG**K**ST**C**S
 GDSGGPLVCNGVLQGITSWGSEPCALPER**PS**LY**TKV**V**H**Y**R**K**W**IK**D**TIVANP

B5101

Number of strong binders 1
Number of weak binders 13

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVVFL**FL**TL**S**VTW**I**GAAPLILSRIVGGWECEK**HSQPWQVLV**ASRGRAVCG**GV**LV**HP**QW**V**LTA**A**H**C**IRNK
 SVILLGRHSLF**HP**EDT**G**QV**F**QV**S**HS**F**PH**PL**YD**M**SL**L**KNR**FL**RP**GD**SS**H**DL**ML**LR**L**SEPA**EL**TD**AV**K**V**MD
 LPTQEPALGTT**C**YAS**G**W**S**IE**P**EE**FL**TP**KK**L**Q**CV**DL**H**V**IS**ND**V**CA**Q**V**HP**Q**K**V**TK**F**ML**C**AGR**WT**GG**K**ST**C**S
 GDSGGPLVCNGVLQGITSWGSEPCALPER**PS**LY**TKV**V**H**Y**R**K**W**IK**D**TIVANP

HLA-II peptides (HTL peptides)

DRB1_0301

Number of strong binders: 3
Number of weak binders: 21

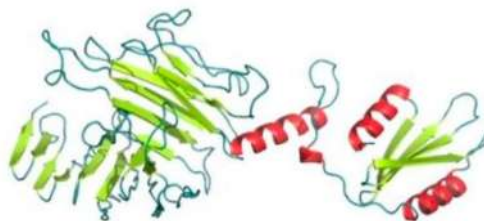
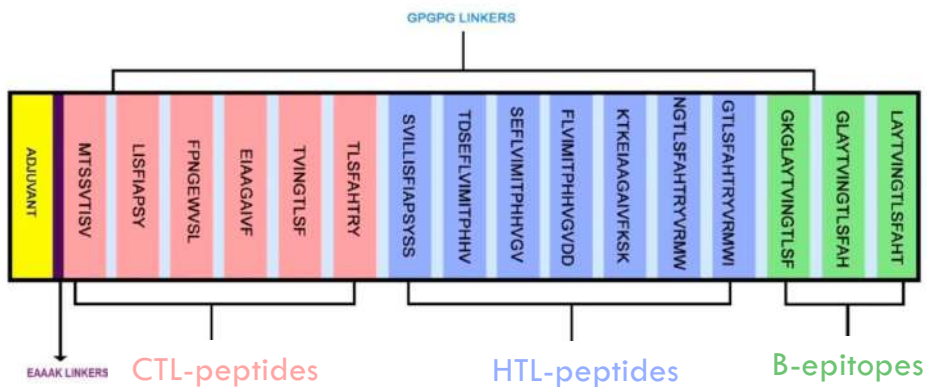
>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVFLTSLVTWIGAAPLILSRIVGGWECEKHSOPWVIVASRGRAVCGGVLVHPQWVLTAAHCIRNK
 SVTLLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLRLSEPAELTDAVKVMD
 LPTQEPALGTTTCYASGWSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
 GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

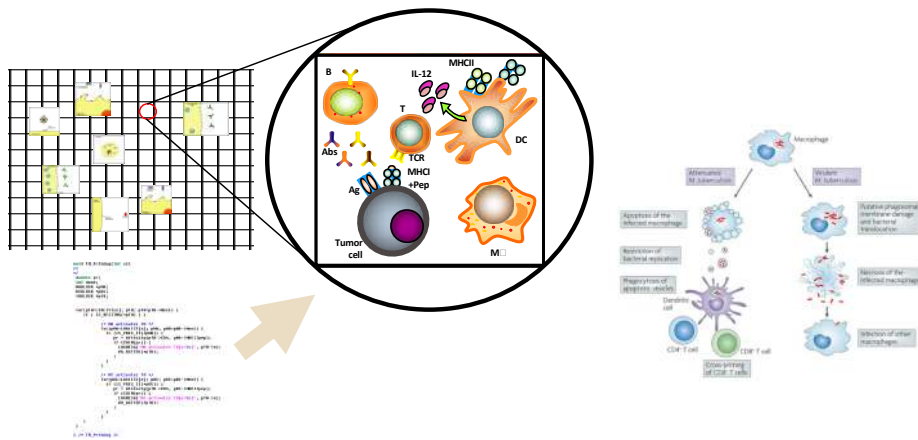
DRB1_1601

Number of strong binders: 8
Number of weak binders: 18

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
 MWVPVFLTSLVTWIGAAPLILSRIVGGWECEKHSOPWVIVASRGRAVCGGVLVHPQWVLTAAHCIRNK
 SVTLLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLRLSEPAELTDAVKVMD
 LPTQEPALGTTTCYASGWSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
 GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

Multi-epitope subunit vaccine

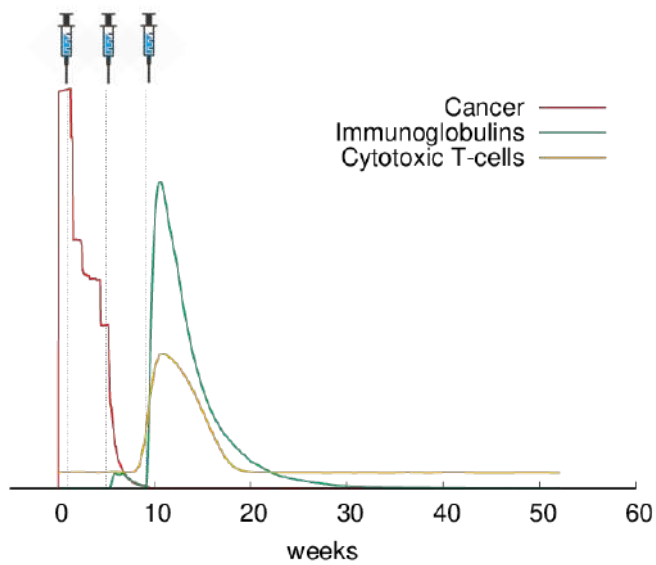
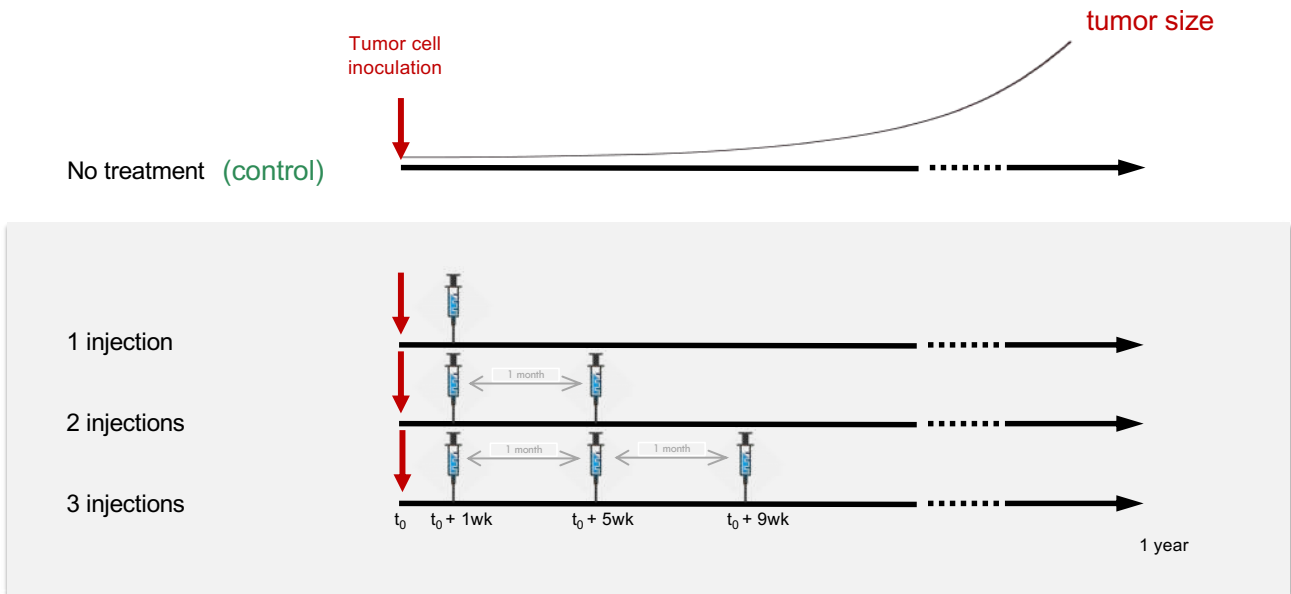


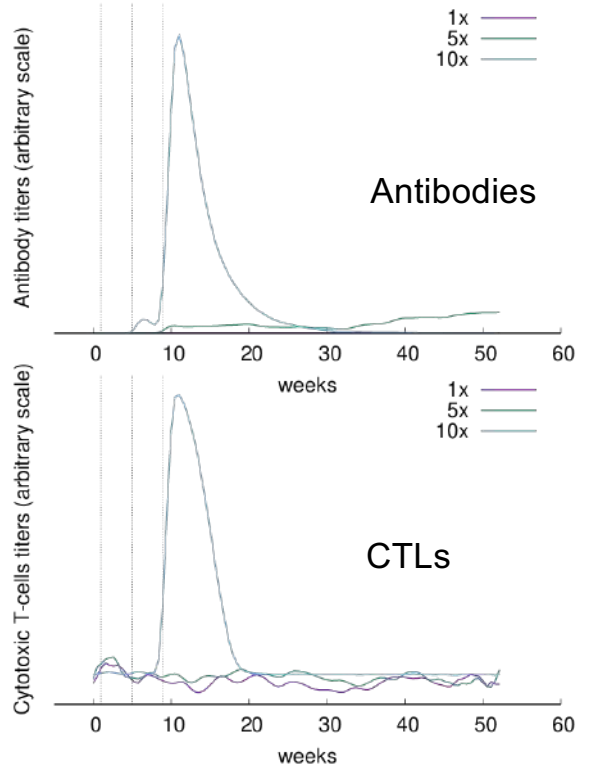
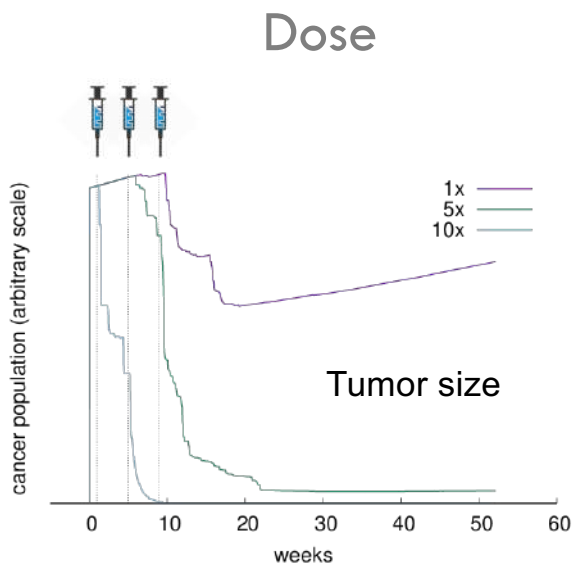
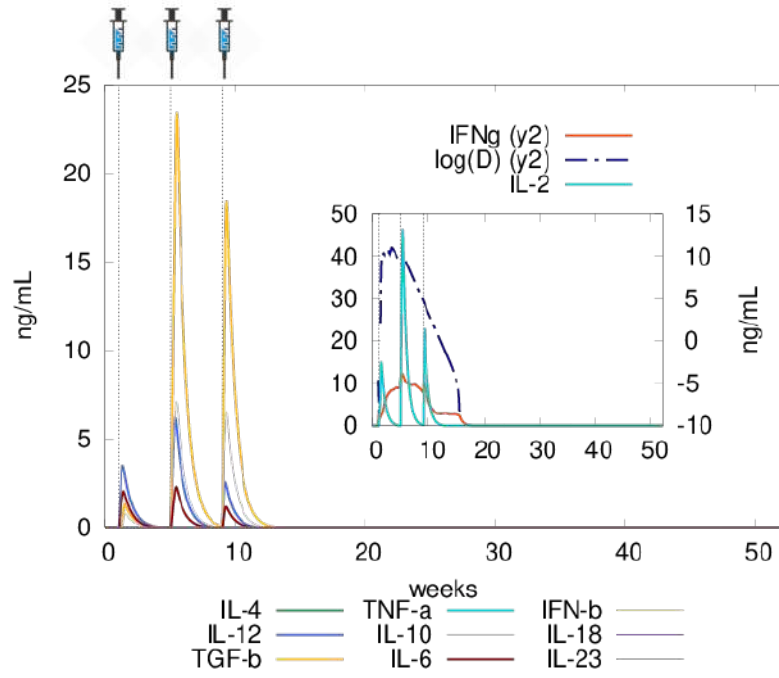


events / rules

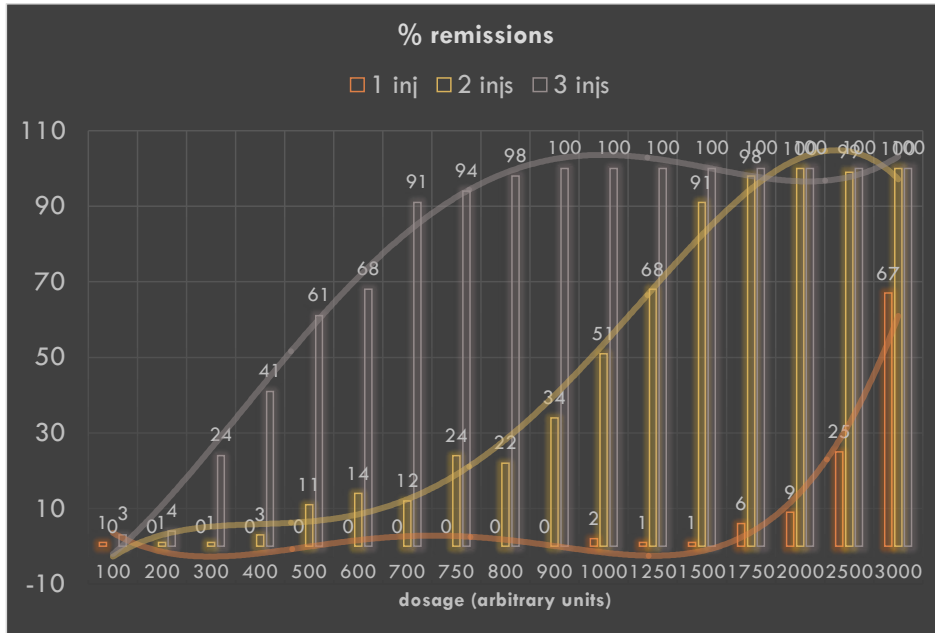
- Injection:** A dose $V(0) = V$ is injected into the simulated volume representing 10 microliters discretised in $L \times L \times L = 10 \times 5 \times 5 = 250$ lattice points.
- B phagocytosis:** B cells phagocyte, internalise, process and present viral peptides on class II HLA
- Response to Danger:**
 - NK response: Natural killer cells (NKs) release IFNg upon bystander stimulation by danger
 - M response: Macrophages (M) respond to danger (e.g., DAMPs) via TLR4 releasing TNFa and IL-6
- M activation:** macrophages become activated by IFNg (activated M have a greater phagocytic activity). This is modeled as a Bernoulli event with parameter $p = c \times e^{I/E}$, where $c = 0.9$, I is local concentration of IFNg (i.e., in lattice site x) and E is a parameter representing the efficiency of interferon in activating M
- Active M**
 - M phagocytosis: M internalise, process and present viral peptides on class II HLA; in presence of IFNg they release IL-12; they also release TNFa
 - DC activation: M release TNFa which activate dendritic cells (DC)
- DC phagocytosis & endocytosis:** DC phagocyte, internalise, process and present viral peptides on class II HLA (exocytic pathway) but also on class I HLA (endocytic pathway)
- Th activation:** in presence of danger signal, resting T helper lymphocytes are activated by interaction with peptide-bound HLAs on professional antigen presenting cells (M and DC, mainly DC) surface by means of specific interaction with their T-cell receptors (TCR); if no danger is present, the Th cells become anergic upon interaction of its TCR with the HLA-peptide complex
- Th stimulation by APCs:** activated Th interacting with antigen presenting cells (M, DC)
 - Th duplication: start clone expansion; 50% of the daughter cells become memory cells
 - Th cells release IL-2
 - M release IL-6
 - Th1 release IFNg
 - Th2 release IL-4
 - release IL-12 in presence of high local concentration of IFNg
 - Treg release TGFb and IL-10
- Th stimulation by APCs:** activated Th interacting with antigen presenting cells (M, DC)
 - Th duplication: start clone expansion; 50% of the daughter cells become memory
 - Th cells release IL-2
 - M release IL-6
 - Th1 release IFNg
 - Th2 release IL-4
 - Th release IL-12 in presence of high local concentration of IFNg
 - Treg release TGFb and IL-10
- Th differentiation:** depending on the local concentration of IFNg, IL-10, IL-4, IL-6, IFNb, IL-12, IL-18, IL-2, TGFb and IL23, active T helper cells undergo class switch into Th1 and Th2
- B differentiation:** B cells differentiate to antibody-secreting plasma B cells (PLB), 50% of duplicating B cells become PLBs. If the B lymphocyte is a memory cells then it generates 80% of PLBs
- Isotype switch:** B cells perform immunoglobulin class switching, that is, change production of immunoglobulin from the isotype IgM to the isotype IgG. This is modeled as a Bernoulli event with parameter p depending on the local concentration of IL-2
- Antibodies production:** Plasma cells secrete antibodies at a rate of about 2 ng/day_B
- Humoral response:** antibodies inhibit viral particles by opsonization; the result are the immuno-complexes that are eventually cleared by macrophages
- Tc activation:** in presence of IL-2, resting cytotoxic T cells (Tc) are activated by the interaction of their TCR with DC presenting on class I HLA the viral peptides but only in presence of IL-2
- Tc duplication:** activated Tc interact with cancer cells presenting viral peptides on class I HLA molecule
 - Tc start duplication. 50% of the daughter cells become memory cells
- Cytotoxic response:** activated Tc kill infected CC (this will further release danger signal)

Tested protocols





Dose escalation experiment



Application 2

identification of viral antigens sharing sequence and structural homology with tumor-associated antigens

in-silico prediction of TAA immunogenicity (active immunotherapy)

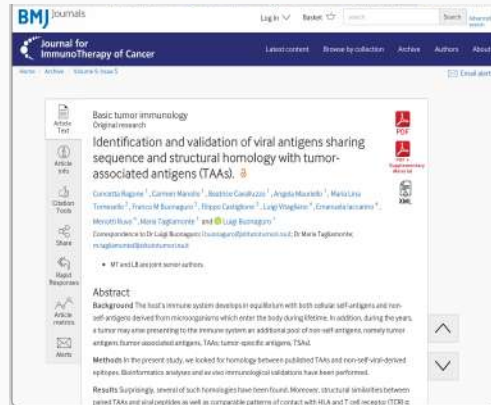
Identification and validation of viral antigens sharing sequence and structural homology with tumor-associated antigens (TAAs)

Methods We looked for homology between published TAAs and non-self-viral-derived epitopes.

Results Several homologies (structural similarities) have been found between paired TAAs and viral peptides. These show eliciting cross-reacting CD8⁺ T cell responses which possibly drive the fate of cancer development and progression.

Conclusions An established antiviral T cell memory may turn out to be an anticancer T cell memory, able to control the growth of a cancer developed during the lifetime if the expressed TAA is similar to the viral epitope.

C. Ragone, et al. Identification and validation of viral antigens sharing sequence and structural homology with tumor associated antigens (TAAs). *J Immunotherapy of Cancer*. 9:e002694 (2021)



Cancer antigenic peptide database

<https://caped.icp.ucl.ac.be/Peptide/list>

Mutation	Tumor-specific	Differentiation	Oversampled	Potential	Gene/Protein	HLA ID	HLAN ID	Peptide Sequence	Position	Lymphocyte Stimulator	Reference	Key ID
AP01	colorectal cancer				AP01	A24	20	ATLEDAKRF	79-87	peptide	Shibata, 2011	
AP02	melanoma				AP02	D81	18	YSDYMLPAGTYVH		antigenic tumor cells	Wang, 2009	
B-RAF	melanoma				B-RAF	054	24	EDLVYKSDGLATDSEKSGEHRDFGLD	595-614	peptide	Shayakh, 2024	
ICE-1/IRF1 nuclear protein (IRF1)	chronic myeloid leukemia				ICE-1/IRF1 nuclear protein (IRF1)	88	14	GRKGGKAL	922-935	peptide	Yoshida, 1988	
						064	24	ATGKGGKALQHPKLS	800-816	peptide	Bosch, 1986	
						42	48	SSAKLGRPL	800-814	peptide	Yoshida, 1988	
						099	3	ATGKGGKALQHPKLS	800-816	peptide	Watanabe, 2012	
HER2/neu	melanoma				HER2/neu	428	30	EYLDSEK	29-37	antigenic tumor cells	Hudson, 1998	
CD47	ovoid, gastric, and endometrial carcinoma				CD47	42	43	FLRGGYV	67-74	peptide	Schultze, 2005	
CD47	head and neck squamous cell carcinoma				CD47	805	30	FFRDSYK	476-484	antigenic tumor cells	Mandrycky, 1981	
CD47	melanoma				CD47	094	24	FRGGKALQHPKLS	795-771	antigenic tumor cells	Wang, 1993	
CDK12	melanoma				CDK12	411	19	EALDLTK	800-813	antigenic tumor cells	Hudson, 2010	
CDK4	melanoma				CDK4	43	44	ATGKGGKALQHPKLS	33-32	antigenic tumor cells	Wu, 1986	
CDKN2A	melanoma				CDKN2A	411	18	YVDPATVAL	155-153	antigenic tumor cells	Wang, 2006	



Tumor Associated Antigen

- **CLPP**
- **Gp100**
- **HSPH1**
- **HEPACAM**
- **CD274**
- **MUC1**
- **KIF20A**
- **Tyrosinase**
- **CEA**
- **Telomerase**
- **Secernin1**

versus

Viral protein

- **E1 HPV**
- **UL20 HCMV**
- **large tegument protein HSV-2**
- **polyprotein encephalomyelitis virus**
- **ENV HIV**
- **ORF46 HHV8**
- **Env HIV**
- **Gag HIV and Env HERV**
- **Env HIV**
- **Env HIV**
- **PoIB1 influenza**

Viral nanomer peptides for the four most prevalent MHC class I alleles where chosen by homology search (BLAST) and selected by NetMHCstapan v.1.0

31

Class I alleles (<http://www.allelefrequencies.net>)

- HLA-A*01:01
- HLA-A* 02:01
- HLA-A* 03:01
- HLA-A* 24:02

altogether cover about 50% of the world population

- ~ 60% of the European as well as the north American Caucasian populations
- ~ 50% of the Japanese population
- ~ 30% of the Chinese population
- ~ 20% of the Indian population

have been selected with a predicted affinity value <100 nM (strong binders, SBs: netMHCpan)

The screenshot shows the Allele Frequency Net Database (AFND) website. The main content area displays a table titled "Database information" with the following data:

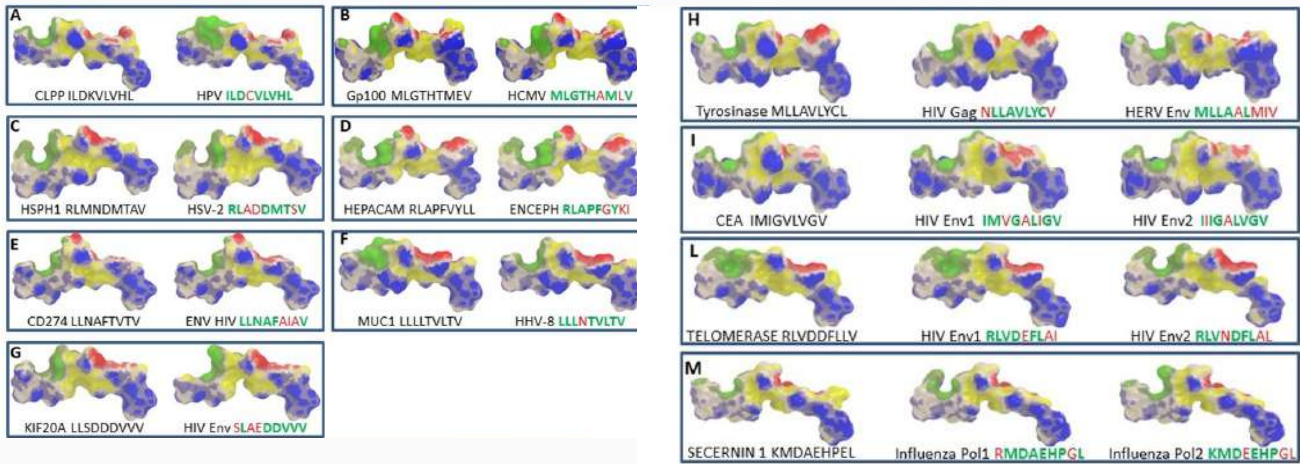
Geographic Region	Population Size	Gene/Allele Data	Haplotype Data	Genotype Data
AF	1,118	1,564	660	-
EUR	293	292	192	-
COL	125	125	-	-
CHC	94	64	33	-
Total	1,630	1,785	683	193

Below the table, it states: "The current number of frequencies stored in our database is: 135,490 (HLA), 6,731 (KIR), 4,276 (Cytokine) and 977 (MHC) from 24,232,878 individuals." It also mentions an update to the nomenclature and a copyright notice for 2015.

Homologies between TAAs and viral peptides

Structural predicted conformation of the paired viral and tumor-associated antigen peptides bound to the HLA-A*02:01.

Blue areas = contact points with HLA molecule;
 Red areas = contact points with the TCR α chain;
 Green areas = contact points with the TCR β chain.

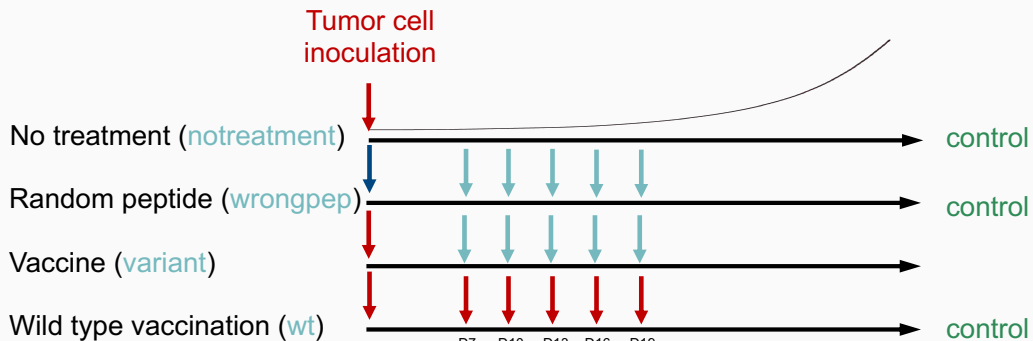


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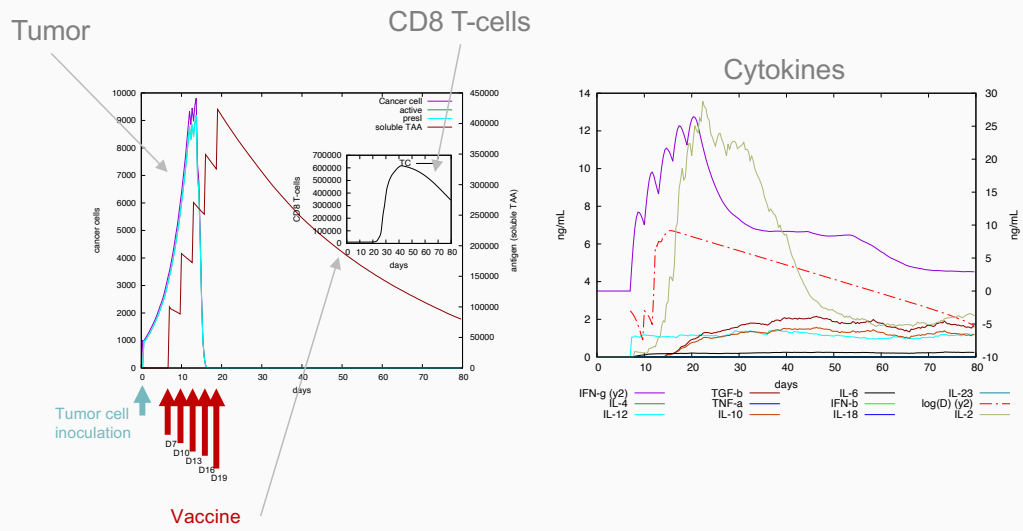
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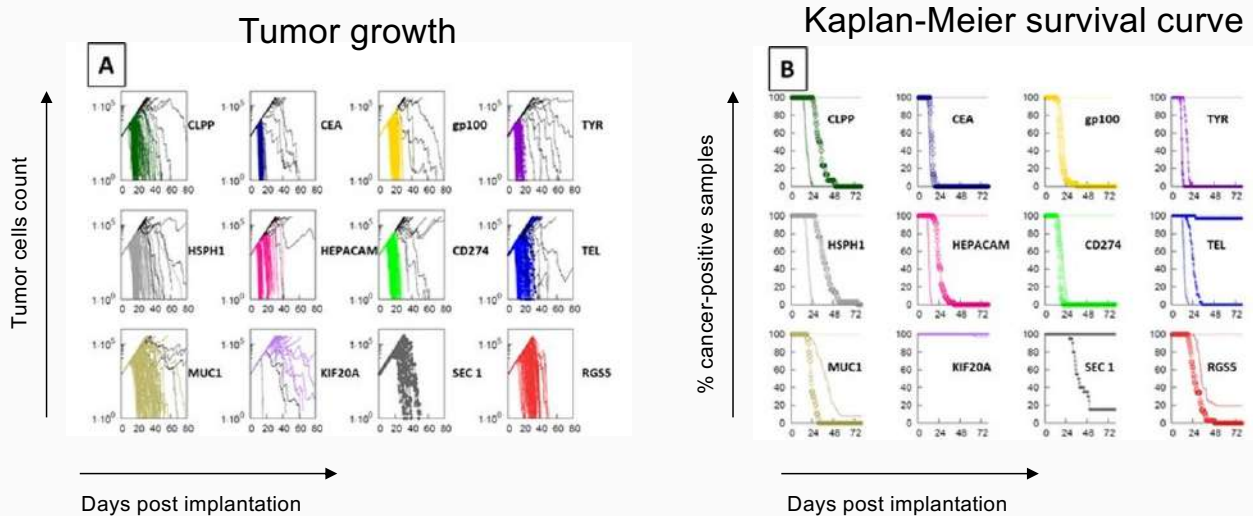
		Threshold for		Strong binding peptides		Weak binding peptides											
		HLA-A*02:01		0.500		2.000											
PROTOCOL		HLA	peptide	Identity	Pred	Thalf(h)	%Rank_5	1-log50k	Aff(nM)	%Rank_eff	Combined	combined_%rank	Bin	dLevel			
8	CLPP	ILDKVLVHL	HLA-A*02:01 ILDKVLVHL	CLPP	0.899	6.54	0.40	0.703	24.98	1.00	0.742	0.77	<=	WB			
	CLPPV	ILDCVLVHL	HLA-A*02:01 ILDCVLVHL	CLPPV	0.879	5.36	0.60	0.757	13.90	0.80	0.781	0.75	<=	WB			
9	CEA	IMIGVLGVV	HLA-A*02:01 IMIGVLGVV	CEA	0.977	29.43	0.01	0.851	5.02	0.15	0.876	0.05	<=	SB			
	CEAV	IIGALGVV	HLA-A*02:01 IIGALGVV	CEAV	0.927	9.10	0.20	0.755	14.23	0.80	0.789	0.5	<=	WB			
10	gp100	MLGTHMEV	HLA-A*02:01 MLGTHMEV	gp100	0.897	6.40	0.40	0.812	7.67	0.30	0.829	0.32	<=	SB			
	gp100V	MLGTHAMLV	HLA-A*02:01 MLGTHAMLV	gp100V	0.794	3.00	1.20	0.747	15.38	0.80	0.756	0.86	<=	WB			
	tyrosinase	MLLAVLYCL	HLA-A*02:01 MLLAVLYCL	tyrosinase	0.945	12.30	0.12	0.840	5.62	0.17	0.851	0.16	<=	SB			



		Threshold for		Strong binding peptides		Weak binding peptides											
		HLA-A*03:01		0.500		2.000											
PROTOCOL		HLA	peptide	Identity	Pred	Thalf(h)	%Rank_5	1-log50k	Aff(nM)	%Rank_eff	Combined	combined_%rank	Bin	dLevel			
18	Secernin 1	RMDAHPPEL	HLA-A*02:01 KMDAHPPEL	Secernin 1	0.512	1.03	4.50	0.643	47.73	1.50	0.617	1.73	<=	WB			
	SECV1	RMDAHPGL	HLA-A*02:01 RMDAHPGL	SECV1	0.492	0.98	4.50	0.532	158.63	3.00	0.524	3.21	<=	WB			
	SECV2	KMDAHPGL	HLA-A*02:01 KMDAHPGL	SECV2	0.555	1.18	4.00	0.701	25.45	1.00	0.672	1.18	<=	WB			
			HLA-A*02:01 SLAEDDVVV	KIF20A	0.686	1.84	2.50	0.672	34.69	1.50	0.675	1.63	<=	WB			
19	RG55	LASFKSLK	HLA-A*03:01 LASFKSLK	RG55	0.177	0.40	3.50	0.542	142.57	0.80	0.469	0.95	<=	WB			
	RG55V	LAAPKSLK	HLA-A*03:01 LAAPKSLK	RG55V	0.220	0.46	3.00	0.563	113.17	0.80	0.494	0.94	<=	WB			



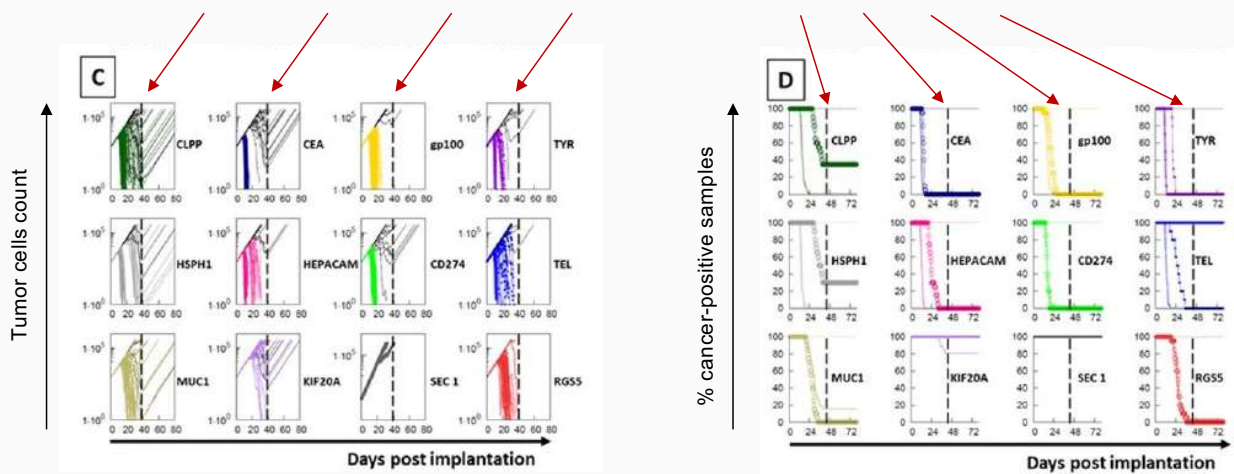
Prediction of cross-reactive antitumor T cell response



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CD8 (virtual) ablation experiment



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Conclusions

- Computer simulations and immuno-informatics prediction tools enable in-silico trials
- They provide comprehensive control over the configuration of virtual experiments and in-depth analysis of the outcomes. For example, this includes
 - ▣ selecting the haplotype
 - ▣ determining the vaccine's subunits
 - ▣ assessing the impacts of modifying the injection schedule
 - ▣ conducting dose-escalation studies
- While they represent an approximation of reality, they can serve as a valuable tool in the clinical optimization of immunotherapies

Thank you for listening

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