In silico prediction of Tumor Associated Antigens' immunogenicity

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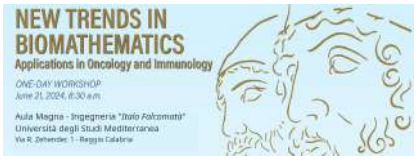
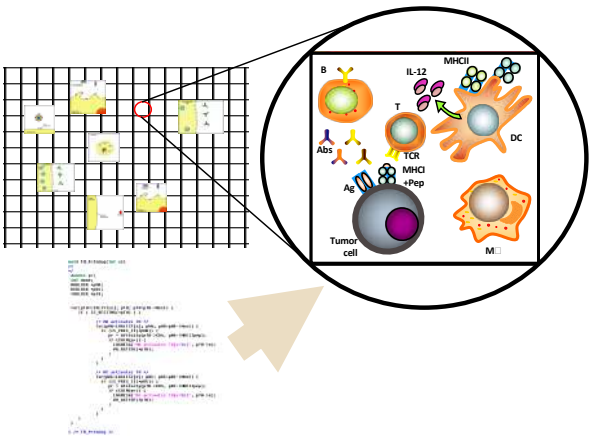
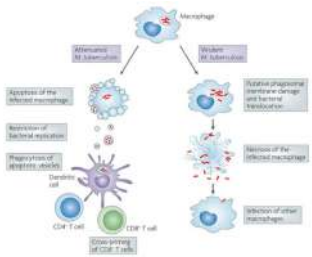


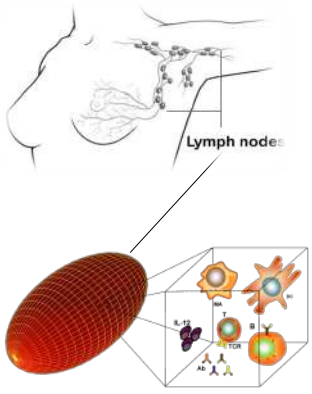
modeling biological processes with ABMs





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

stochastic ABM of the immune response



Cells

- B-cell
  - B-1
    - B-2
    - PLB
- CD4 T-cell
  - Th1
  - Th2
  - Th17
  - Treg
- CD8 T-cell
- NK
- Mφ
  - M1
  - M2

DC

EP (virus target)

Immunoglobulins

- IgM
- IgG
  - IgG1
  - IgG2
- IC

Interleukins/cytokines

- IL-2
- IL-4
- IL-12
- IL-10
- IL-18
- IL-23
- IL-1β
- IFN-β
- IFN-γ
- TNF-α
- TGF-β
- Danger

Antigens

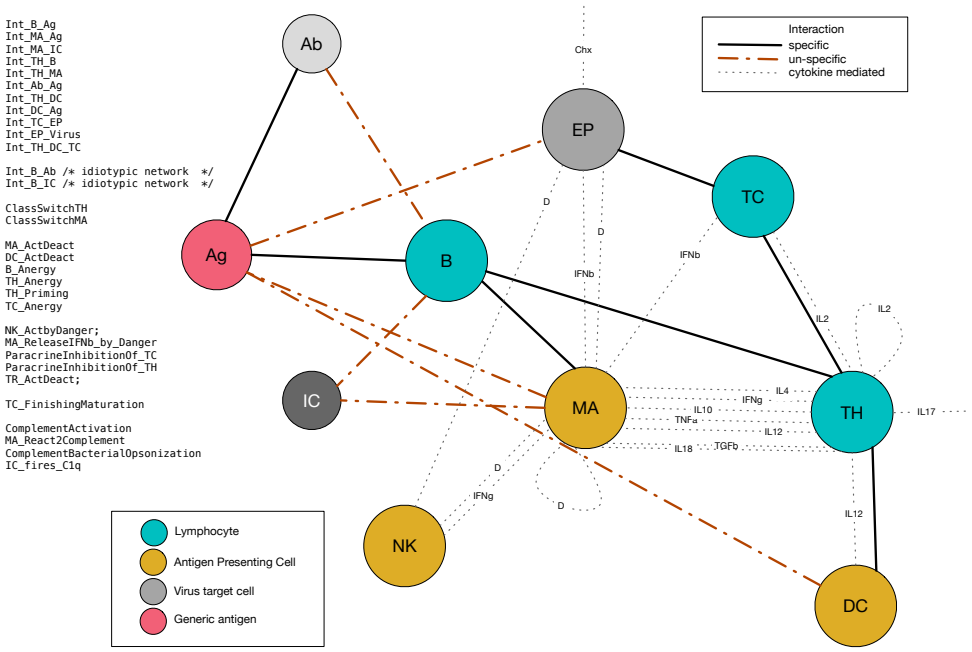
- vaccines
- virus
- bacteria
- cancer cells
- ...

Immunological features

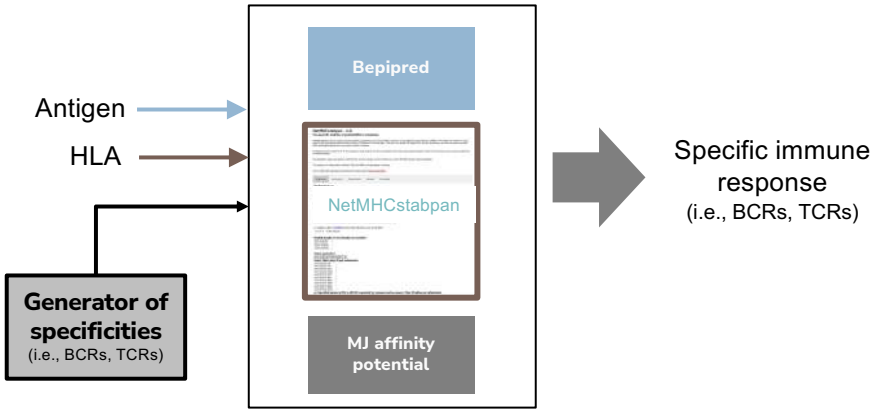
- Clonal selection theory (random generation of TCRs and BCRs)
- Clonal deletion theory (thymic pos/neg selection of T-cells)
- Innate + adaptive immunity
- Humoral + cellular immunity
- Immunological memory
- Homeostasis
- Affinity maturation and hypermutation
- Peptide digestion and presentation (class-1, class-2 MHC)
- Cytokine activation/inhibition of immune cells' functions
- Matzinger's danger signal theory
- T-cells replicative senescence (the Hayflick limit)
- T-cell anergy (lack of second signal)
- B-cell anergy (overstimulation)
- Peripheral tolerance by Tregs
- IFN-Induced Attrition of CD8 T cells
- Idiotypic network

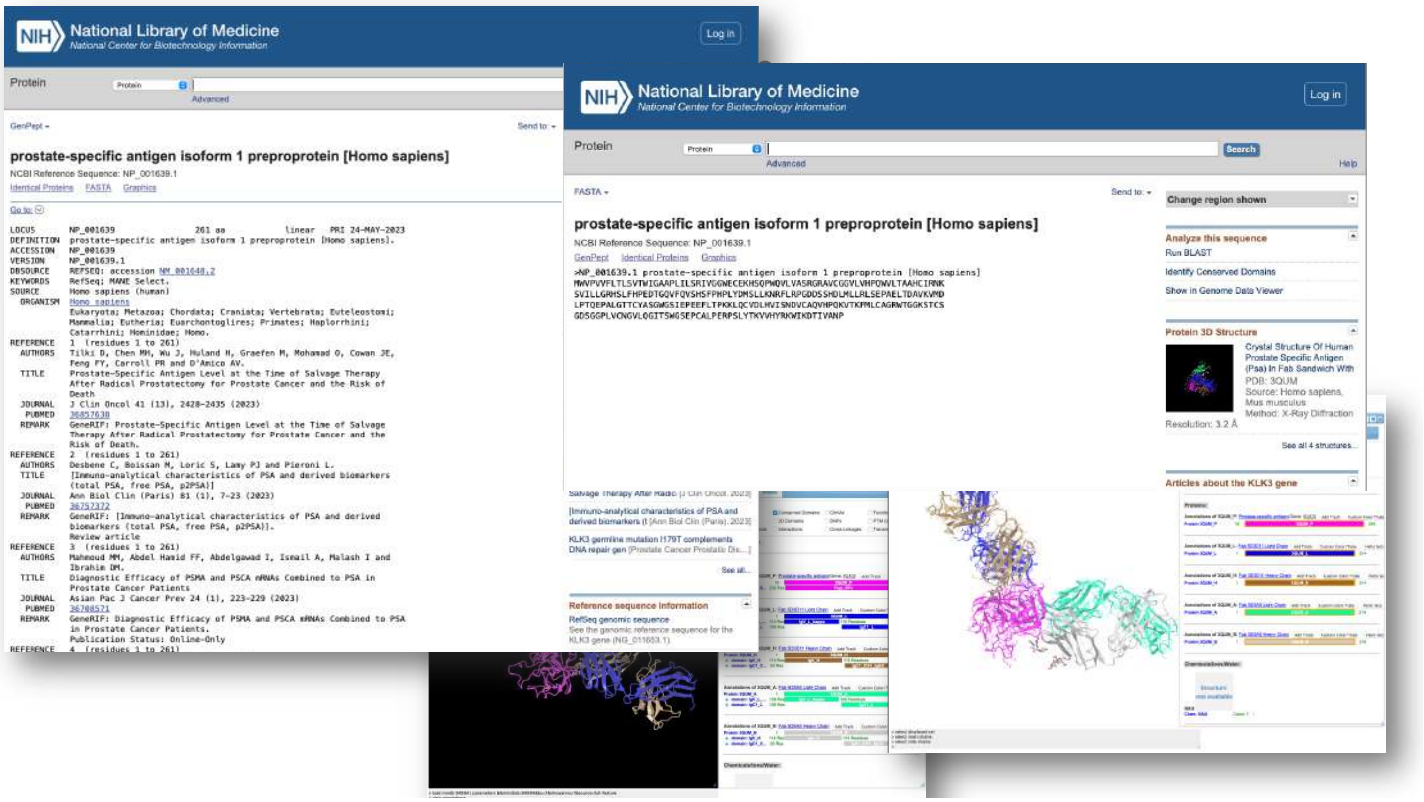
Celada F, Seiden PE. A computer model of cellular interactions in the immune system. Immunology Today 1992; 13:56-62

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



exploiting ML predictions





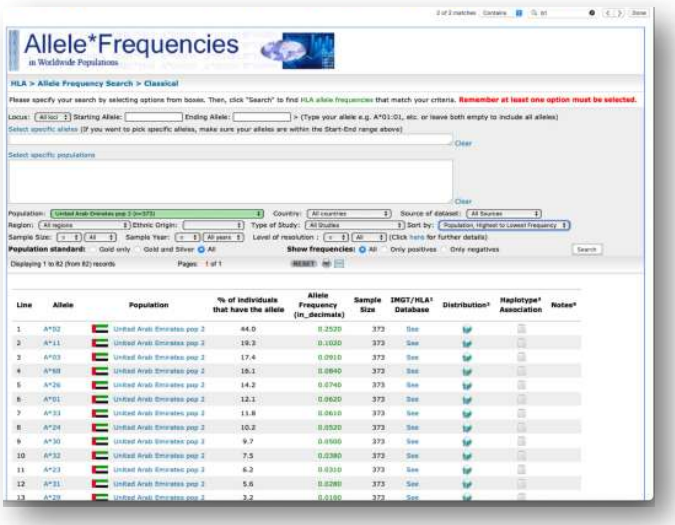
Choice of the HLAs

HLA database
www.allelefrequencies.net

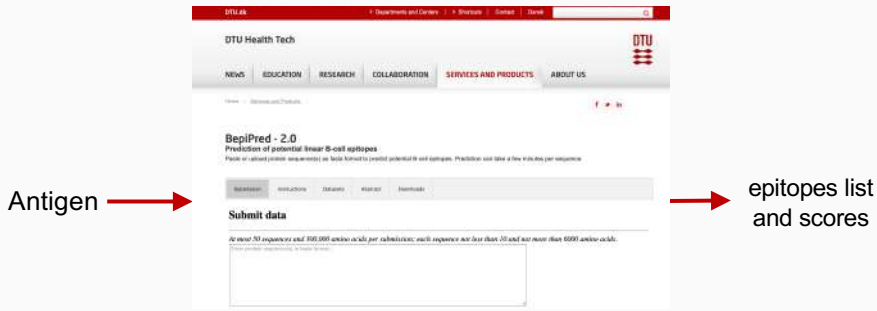
class I
  HLA-A02:01 44%
  HLA-A03:01 17.4%
  HLA-B35:01 20.9%
  HLA-B51:01 28.7%

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

% of individuals carrying the allele



Prediction of potential linear B-cell epitopes



>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

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

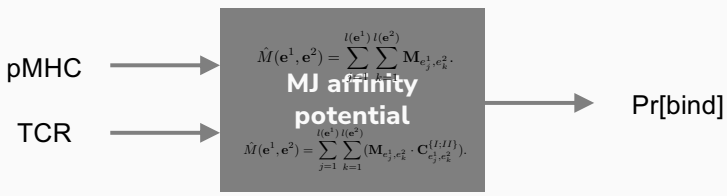
https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-4.0

M. Rasmussen, E. Fenoy, M. Harndahl, A. Bregnballe Kristensen, I. 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.

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.

Miyazawa S, Jernigan RL (2000) Identifying sequence-structure pairs undetected by sequence alignments. Protein Eng 13: 459–475

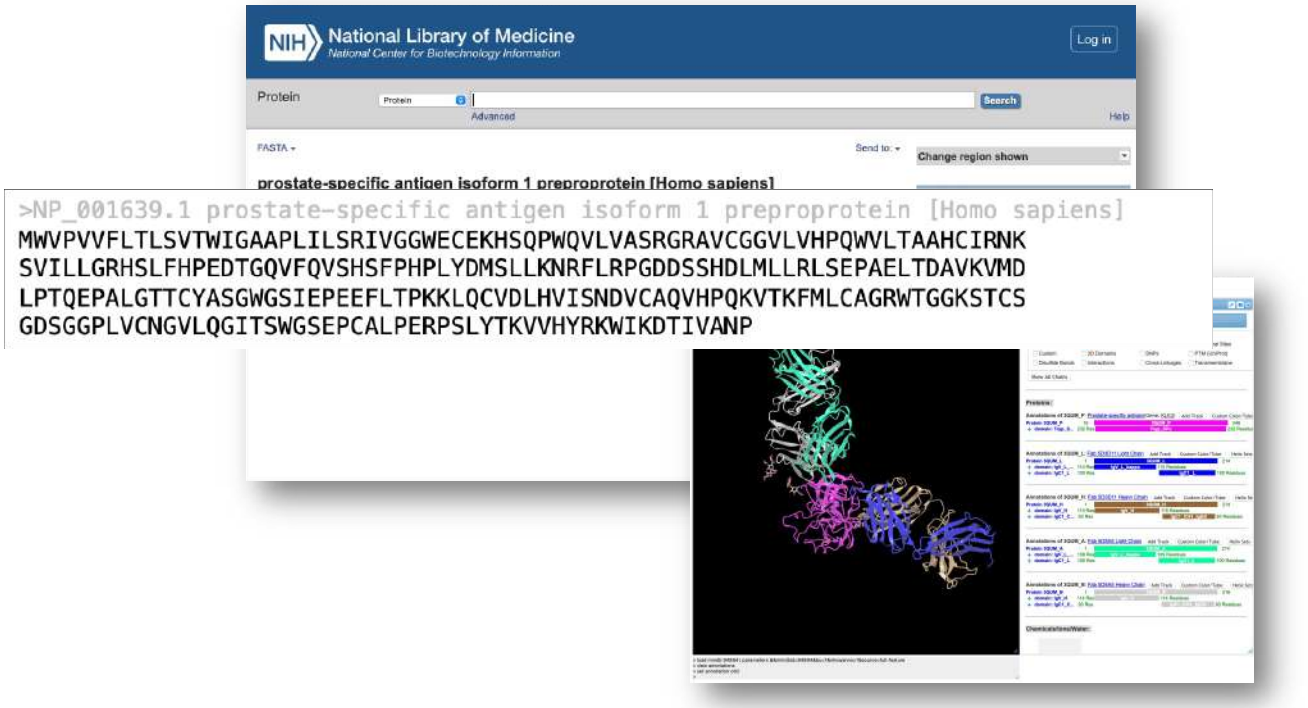
Application 1

Computational modeling of prostate cancer (active) immunotherapy

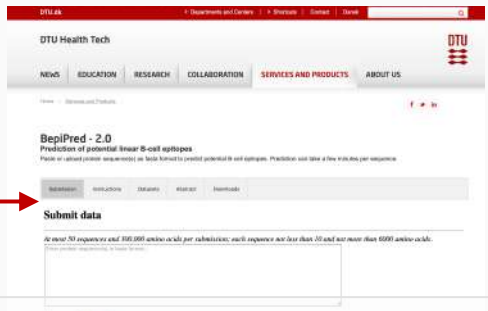
KLK3

- 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[*]

[*] 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).



Prediction of potential linear B-cell epitopes



Antigen → epitopes list and scores

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MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

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

Prediction of T-cell peptides

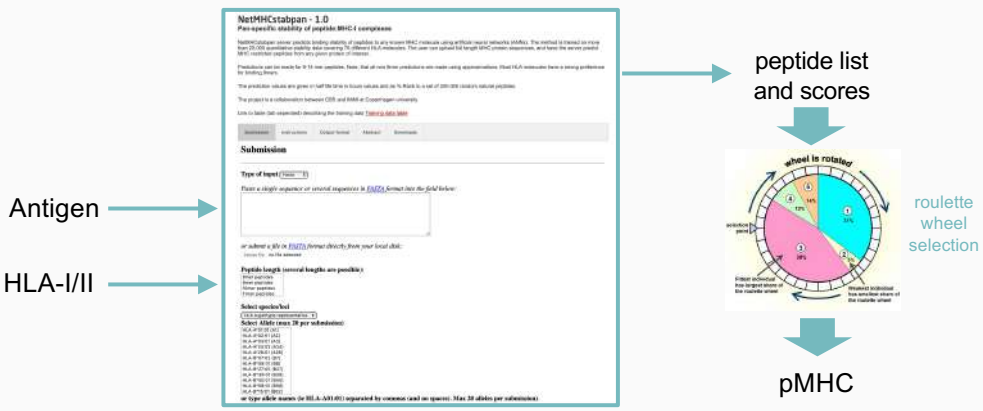
T-cell peptides in the study were predicted with the servers

- 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

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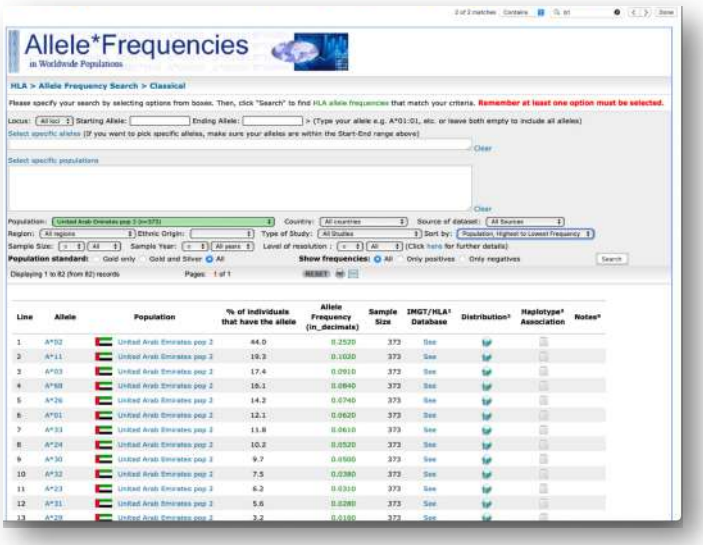
Most prevalent HLAs in the UAE



Source: HLA database
(www.allelefrequencies.net)

class I

HLA-A02:01	44%
HLA-A03:01	17.4%
HLA-B35:01	20.9%
HLA-B51:01	28.7%

class II

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

% 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]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

A0301

Number of strong binders 3
Number of weak binders 10

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

B3501

Number of strong binders 6
Number of weak binders 8

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

B5101

Number of strong binders 1
Number of weak binders 13

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

HLA-II peptides (HTL peptides)

DRB1_0301

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

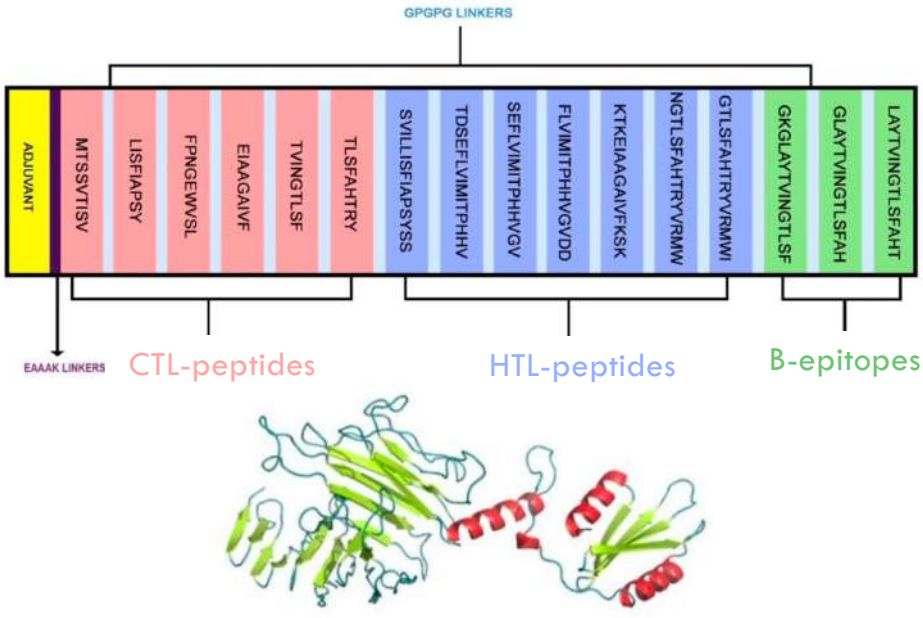
>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

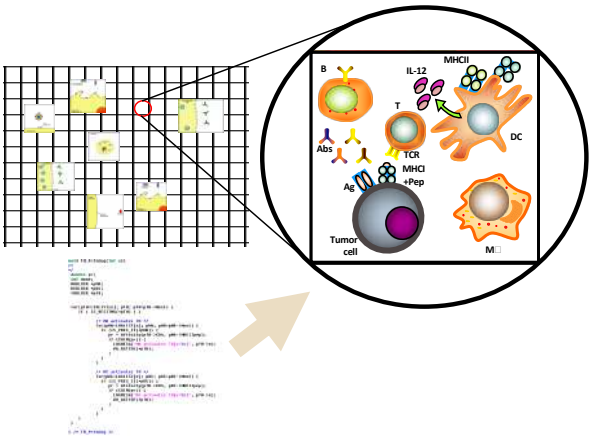
DRB1_1601

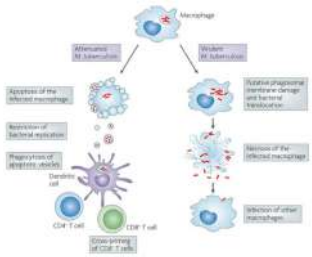
Number of strong binders: 8
Number of weak binders: 18

>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens]
MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK
SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD
LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS
GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

Multi-epitope subunit vaccine







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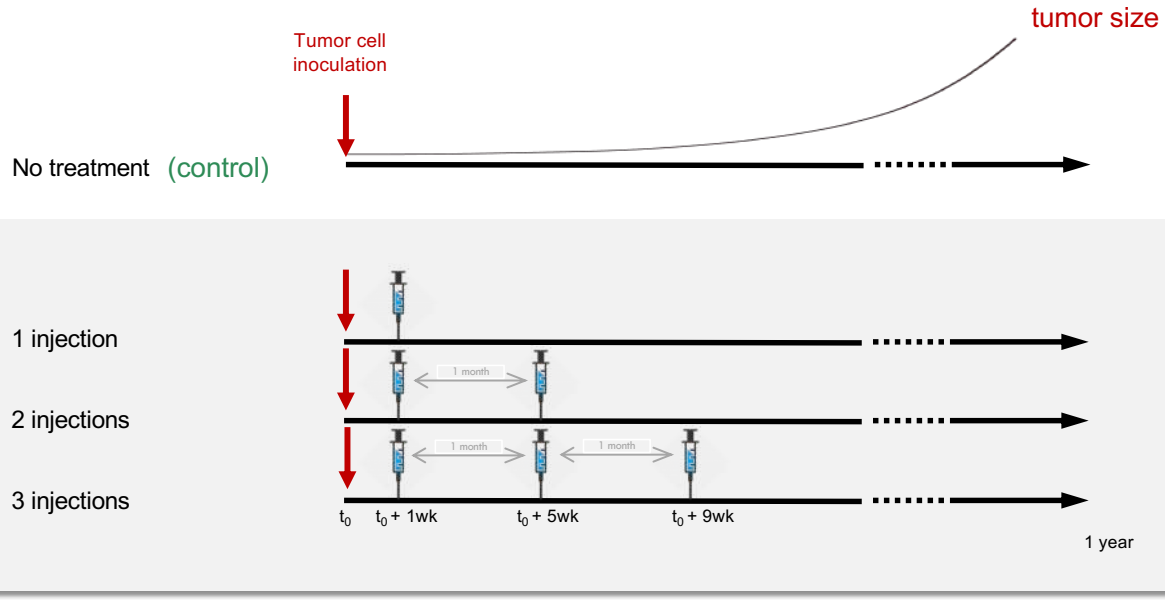
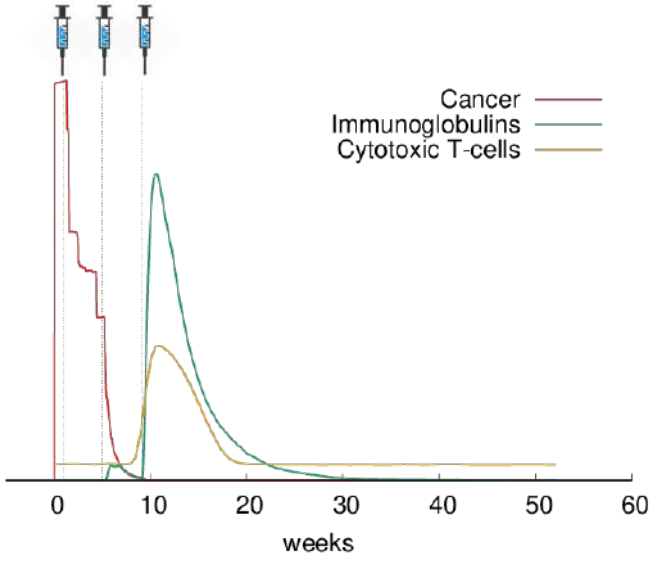
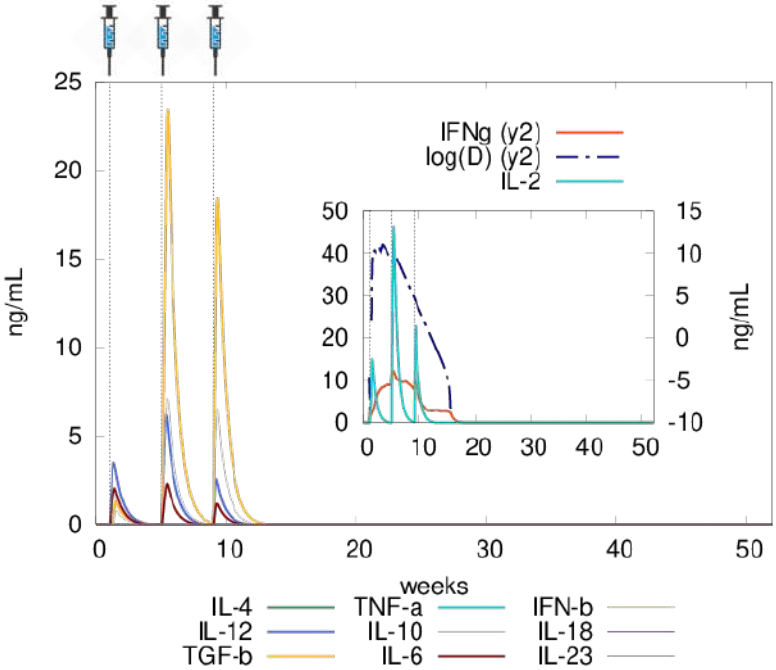
events / rules

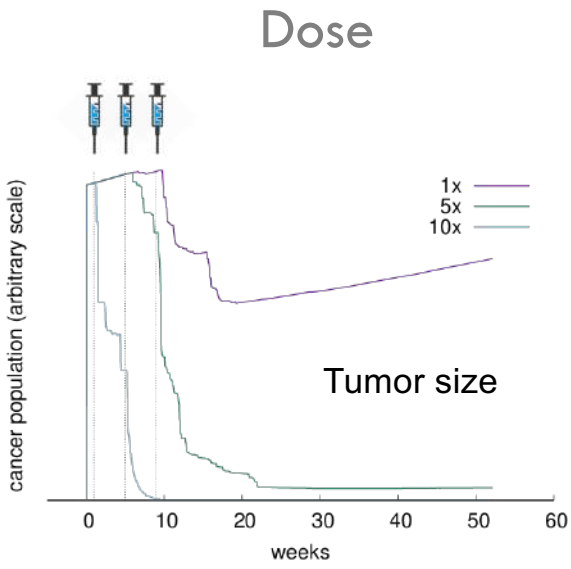
1. Injection: A dose $V(0) = V$ is injected into the simulated volume representing 10 microliters discretised in $L \times L \times L = 10 \times 5 \times 5 = 250$ lattice points.
2. B phagocytosis: B cells phagocyte, internalise, process and present viral peptides on class II HLA
3. Response to Danger:
   - 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
4. 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 I. is a parameter representing the efficiency of interferon in activating M
5. 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)
6. 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)
7. 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 becomes anergic upon interaction of its TCR with the HLA-pepide complex
8. 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
9. 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
10. Th stimulation by B: activated Th interacting with B cells
    - B duplication: stimulate B cells to clone expansion; 50% of the daughter cells become memory
    - Th duplication: start clone expansion; 50% of the daughter cells become memory
    - Th release IL-2, IL-12
    - Th1 release IFNg
    - Th2 release IL-4
    - Treg release TGFb and IL-10
11. 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
12. 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
13. 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
14. Antibodies production: Plasma cells secrete antibodies at a rate of about 2 ng/day$_g$
15. Humoral response: antibodies inhibit viral particles by opsonization; the result are the immuno-complexes that are eventually cleared by macrophages
16. 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
17. 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
18. Cytotoxic response: activated Tc kill infected CC (this will further release danger signal)

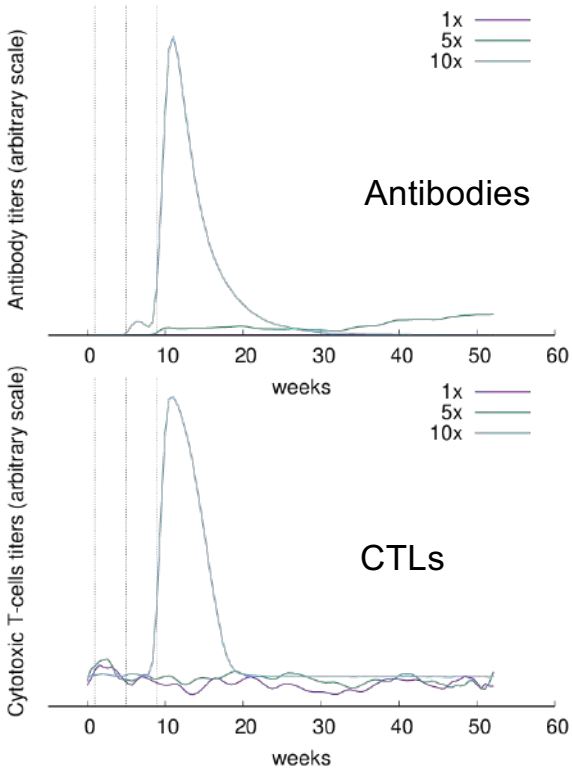
Tested protocols



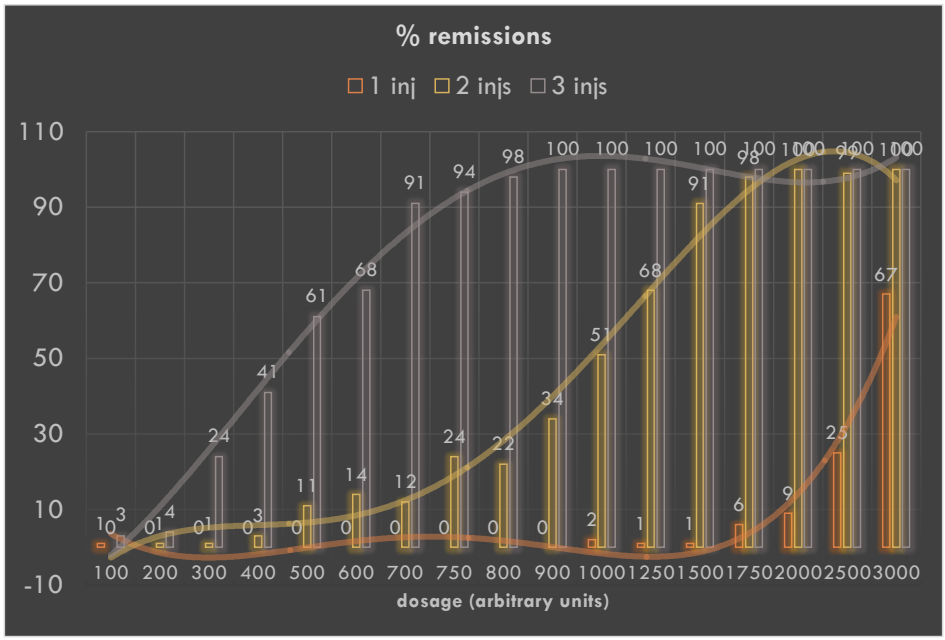



IFNg (y2)
log(D) (y2)
IL-2
ng/mL
weeks
IL-4
IL-12
TGF-b
TNF-a
IL-10
IL-6
IFN-b
IL-18
IL-23


Dose
1x
5x
10x
cancer population (arbitrary scale)
Tumor size
weeks


1x
5x
10x
Antibody titers (arbitrary scale)
Antibodies
weeks
Cytotoxic T-cells titers (arbitrary scale)
CTLs
weeks

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)

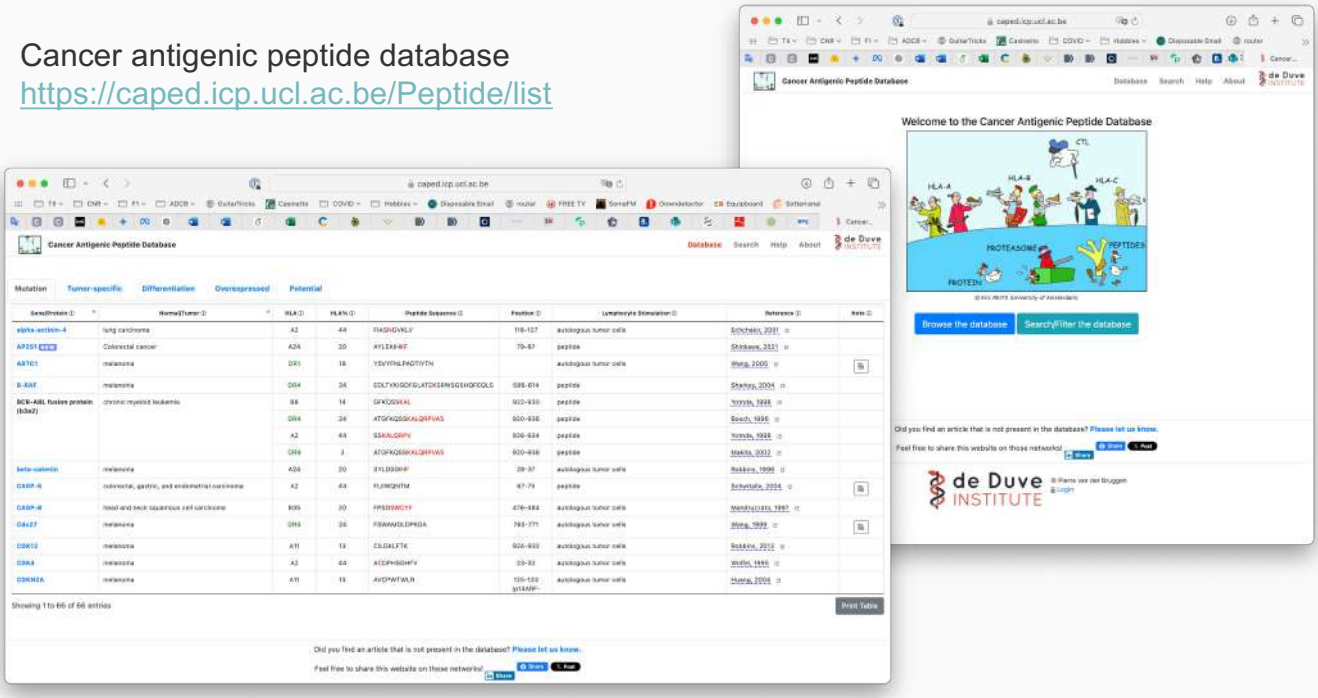
Methods We looked for homology between published TAAs and non-self-*viral-derived* epitopes.

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



Tumor Associated Antigen		*Viral protein*
• ***CLPP***		• ***E1 HPV***
• ***Gp100***		• ***UL20 HCMV***
• ***HSPH1***		• ***large tegument protein HSV-2***
• ***HEPACAM***		• ***polyprotein encephalomyelitis virus***
• ***CD274***	versus	• ***ENV HIV***
• ***MUC1***		• ***ORF46 HHV8***
• ***KIF20A***		• ***Env HIV***
• ***Tyrosinase***		• ***Gag HIV and Env HERV***
• ***CEA***		• ***Env HIV***
• ***Telomerase***		• ***Env HIV***
• ***Secernin1***		• ***PolB1 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

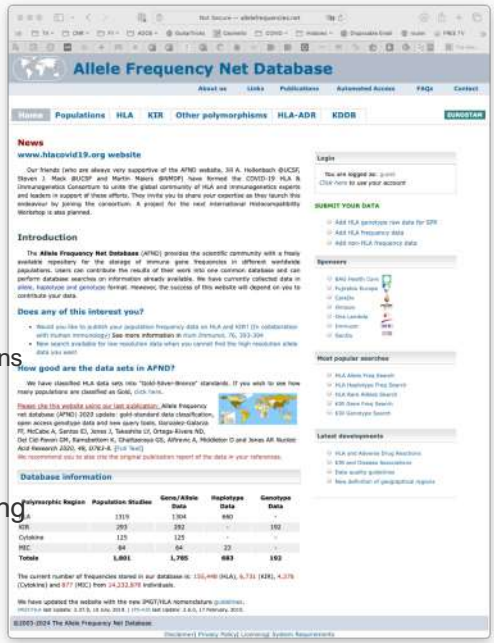
Class I alleles (http://www.allelefrequencies.net)

- 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

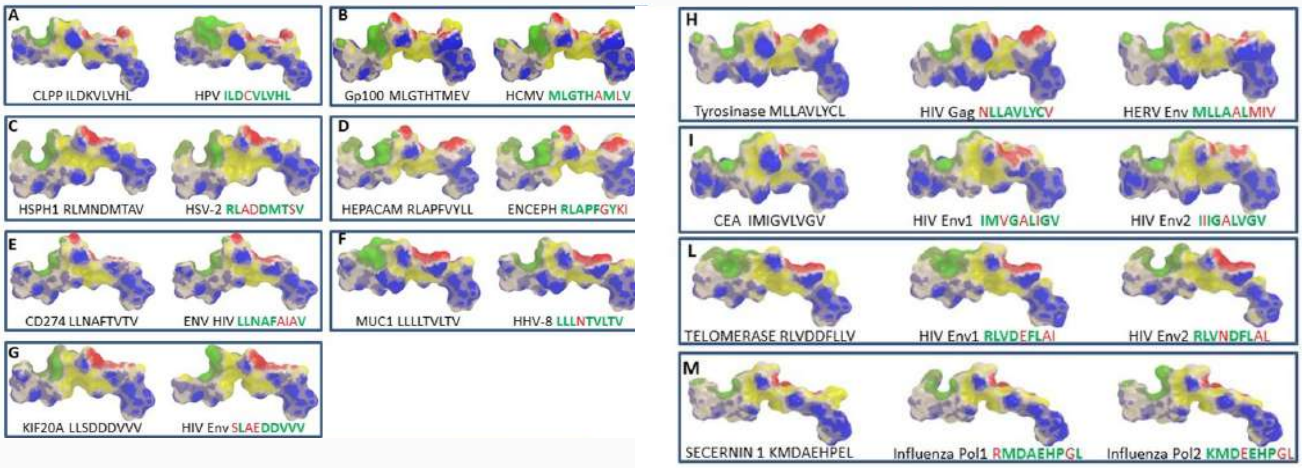
have been selected with a predicted affinity value <100 nM (strong binders, SBs: netMHCpan)



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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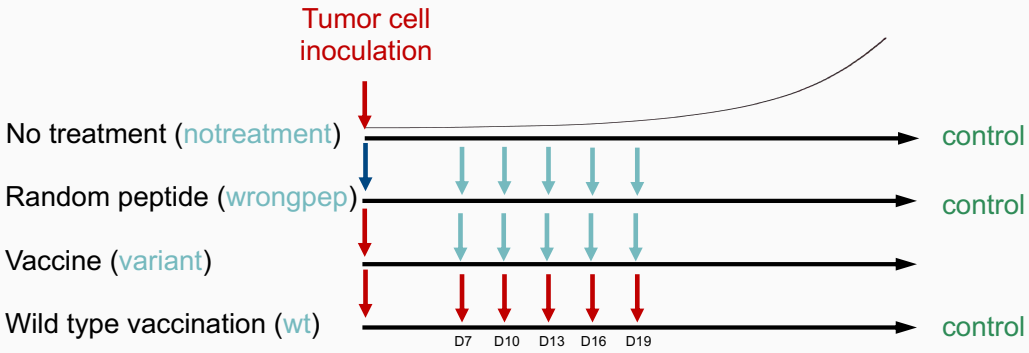
Threshold for Strong binding peptides 0.500
Threshold for Weak binding peptides 2.000

HLA-A*02:01

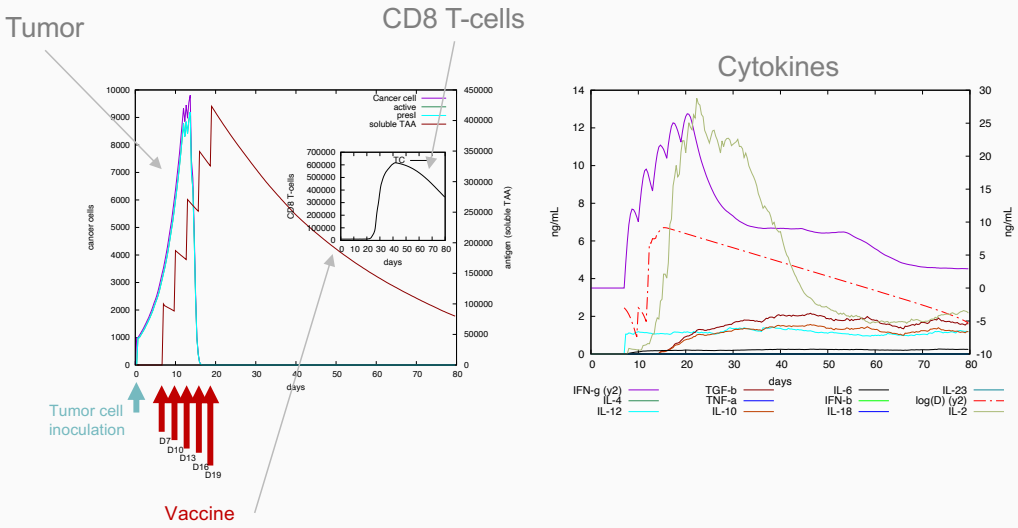
PROTOCOL		peptide	HLA	peptide	Identity	Pred	Thalf(h)	%Rank_S	1-log50k	Aff(nM)	%Rank_aff	Combined	ombined_%ran	Bin	dLevel
8	CLPP	ILDKVLVHL	HLA-A*02:01	ILDKVLVHL	CLPP	0.899	6.54	0.40	0.703	24.98	1.00	0.742	0,77	<=	WB
	CLPPV	ILDCVLVHL	HLA-A*02:01	ILDCVLVHL	CLPPV	0.879	5.36	0.60	0.757	13.90	0.80	0.781	0,75	<=	WB
9	CEA	IMIGVLVGV	HLA-A*02:01	IMIGVLVGV	CEA	0.977	29.43	0.01	0.851	5.02	0.15	0.876	0,05	<=	SB
	CEAV	IIIGALVGV	HLA-A*02:01	IIIGALVGV	CEAV	0.927	9.10	0.20	0.755	14.23	0.80	0.789	0,5	<=	WB
10	gp100	MLGTHTMEV	HLA-A*02:01	MLGTHTMEV	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	tyrosine	0.945	12.30	0.12	0.840	5.62	0.17	0.861	0,16	<=	SB



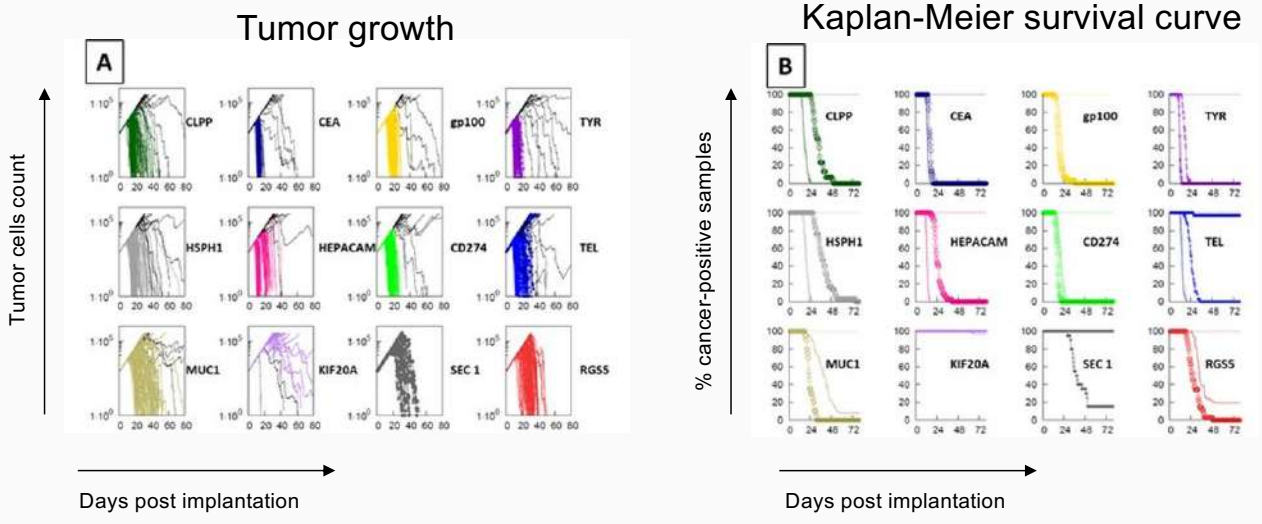
			HLA-A*02:01	SLAEDDVVV	KIF20A	0.686	1.84	2.50	0.672	34.69	1.50	0.675	1,63	<=	WB
18	Secernin 1	KMDAEHPEL	HLA-A*02:01	KMDAEHPEL	Secerni	0.512	1.03	4.50	0.643	47.73	1.50	0.617	1,73	<=	WB
	SECV1	RMDAEHPGL	HLA-A*02:01	RMDAEHPGL	SECV1	0.492	0.98	4.50	0.532	158.63	3.00	0.524	3,21		
	SECV2	KMDEEHPGL	HLA-A*02:01	KMDEEHPGL	SECV2	0.555	1.18	4.00	0.701	25.45	1.00	0.672	1,18	<=	WB

HLA-A*03:01

PROTOCOL															
19	RGS5	LASFKSFLK	HLA-A*03:01	LASFKSFLK	RGS5	0.177	0.40	3.50	0.542	142.57	0.80	0.469	0,95	<=	WB
	RGS5V	LAAFKSFLK	HLA-A*03:01	LAAFKSFLK	RGS5V	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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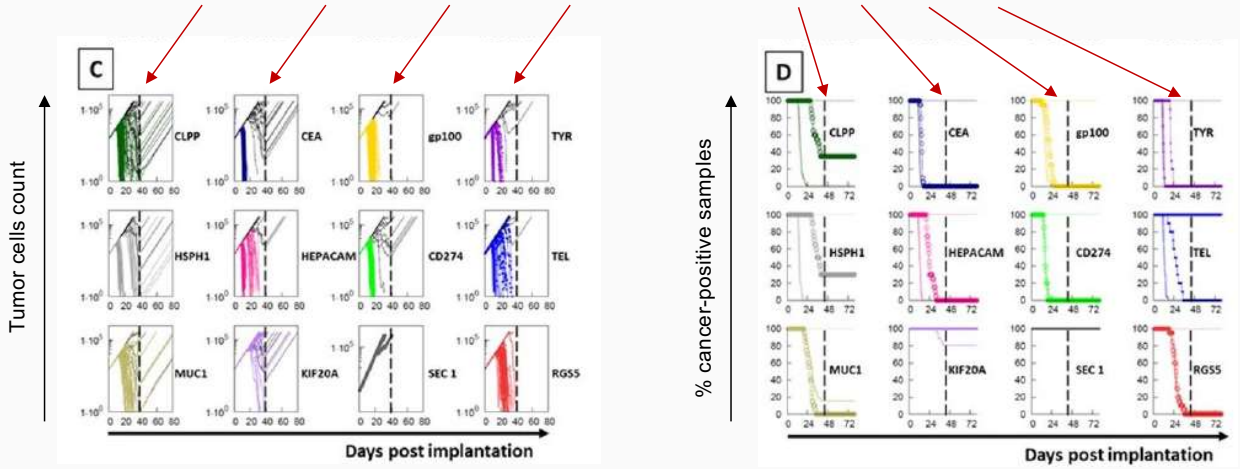
CD8 (virtual) ablation experiment



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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