In silico prediction of Tumor Associated Antigens' immunogenicity

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NEW TRENDS IN BIOMATHEMATICS Applications in Oncology and Immunology Over Over Yourdshop And a Magna - Engegneria "Tablo Potomenia" Universitä dagi Statik Meditarcana Vi & Devende 1: Regis Edates

modeling biological processes with ABMs



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



1

## 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



# Choice of the HLAs

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% of individuals carrying the allele





## **TCR-peptides affinity prediction**



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<sup>[4]</sup>

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<sup>[\*]</sup>

<sup>&</sup>lt;sup>[1]</sup> 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 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**



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



• (3.5) (b)

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

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

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% of individuals that have the allele

### HLA-I peptides (CTL peptides)

AOZOI Number of strong binders Number of weak binders 6	MVP_001039.1 prostate-specific antigen isotorm i preproprotein inomo sapiens) MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGQVLVHPQWVLTAAHCIRNK SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP
A0301 Number of strong binders : Number of weak binders 10	>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens] MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK 3SVILLGRHSLFHPEDTGQVFQVSHSFPHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD LPTQEPALGTTCYASGWGSIEPEEFLTPKKLOCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP
B3501 Number of strong binders 8	>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens] MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHS0PW0VLVASRGRAVCGGVLVHP0WVLTAAHCIRNK SVILLGRHSLFHPEDTG0VF0VSHSFFHPLYDMSLLKNRFLRPGDDSSHDLMLLRLSEPAELTDAVKVMD LPT0EPALGTTCYASGWGSIEPEELTPKKL0CVDLHVISNDVCAQVHP0KVTKFMLCAGRWTGGKSTCS GDSGGPLVCNGVL0GITSWGSEPCALPERFSLYTKVVHYRKWIKDTIVANP
B5101 Number of strong binders Number of weak binders 13	>NP_001639.1 prostate-specific antigen isoform 1 preproprotein [Homo sapiens] MWVPVVFLTLSVTWIGAPLILSRIVGGWECEKHSQPWQVLVASRGRAVCGGVLVHPQWVLTAAHCIRNK SVILLGRHSLFHPEDTGQVFQVSHSFFHPLYDMSLLKNRELRPGDDSSHDLMLLRLSEPAELTDAVKVMD LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS GDSGGPLVCNGVLQGITSWGSEPCALPERPSLYTKVVHYRKWIKDTIVANP

### HLA-II peptides (HTL peptides)

DRB1_0301	>NP_001639.1 prost <u>ate-specif</u> ic antigen isoform 1 preproprot <u>ein [Homo sapie</u> ns]
Number of strong binders: 3	MWVPVVFLTLSVTWIGAAPLILSRIVGGWECEKHSOPWOVI VASRGRAVCGGVLVHPOWVLTAAHCIRNK
Number of weak binders. 21	
	GDSGGPLVCNGVLQGITSWGSEPCALPERPS <mark>LYTKVVHYKKWIKDTIVAN</mark> P

DRB1\_1601

Number of strong binders: 8 Number of weak binders: 18 >NP\_001639.1 prostate-specific antigen\_isoform\_1 preproprotein [Homo sapiens] MWVP/VFLTL\$VTWIGAAPL1LSRIVGGWECEKHSOPWOV\_VASRGRAVCGGVLVHPOWVLTAAHCIRNK SVILLGRH\$LFHPEDTGQVFQVSHSFPHPLYPMSLLKWFFLRPGDD\$\$HDLMLLRL\$EPAELTDAVKVMD LPTQEPALGTTCYASGWGSIEPEEFLTPKKLQCVDLHVISNDVCAQVHPQKVTKFMLCAGRWTGGKSTCS GDSGGPLVCNGVLQGITSWGSEPCALPERP\$LYTKVVHYFKWIKDTIVANP

## 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
- tosis: B cells phagocyte, internalise, process and present viral peptides on class II HLA

- 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 I is a parameter representing the efficiency of interferon in activating 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)
- sytosis: 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 becomes anergic upon interaction of its TCR with the HLA-pepide complex
  - tion by APCs: activated Th interacting with antigen presenting cells (M, DC) Th sti 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
  - ation 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

tion: 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 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/dayg 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 18. 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<sup>+</sup> 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.





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#### **Tumor Associated Antigen**

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

#### 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
- 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

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#### 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  $\alpha$  chain; Green areas=contact points with the TCR  $\beta$  chain.



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Strong binding peptides Weak binding peptides Threshold for HLA-A\*02:01 Aff(nM) %Rank\_aff Com HLA Identity Pred Thaif(h) %Rank\_S 1-log50k peptide CLPP ILDEVLVHL HLA-A\*02:01 ILDKVLVHL CLPP 0.899 6.54 0.40 0.703 24.98 1.00 0.742 0.77 W8 8 CIPPY TLDCVLVHI. 0.80 HLA-A\*02:01 ILDCVLVH CLPPN 0.879 5.36 0.60 0.757 13.90 0.781 0,75 WB CEA INIGVLVGV CEA 0.977 29.43 0.851 5.02 0.15 0.876 0,05 SB HLA-A\*02:01 IMIGVLVGV 0.01 9 CEAV IIIGALVGV HLA-A\*02-01 IIIGALVGV CEAV 0.927 9.10 0.20 0.755 14.23 0.80 0.789 0.5 WA gp100 CRUMEN 0.812 7.67 0.829 0,32 58 02:01 MLGTHTME 0.30 gp1 10 MLGTHAMLY 80100N HLA-A\*02:01 MLGTHAMLV gp100V 0.794 3.00 1.20 0.747 15.38 0.80 0.756 0,86 WB tyr MLLAVLYCL HLA-A\*02:01 MLLAVLYOL 0.945 12.30 0.12 0.840 5 62 0.16 Tumor cell inoculation No treatment (notreatment) control Random peptide (wrongpep) control Vaccine (variant) Wild type vaccination (wt) control D7 D10 D13 D16 D19 01 SLAEDDVV KIF20A' 0.686 1.84 2.50 0.672 0.675 HLA-A\*02:01 KMDAEHPEL 0.617 0.643 47.73 1.50 1,73 Secerni: 0.512 1.03 4.50 SECV1 18 MDAEHPGL HLA-A\*02:01 RMDAEHPGL SECV1 0.492 0.98 4.50 0.532 158.63 3.00 0.524 SECV2 KMDEEHPGL HLA-A\*02:01 KMDEEHPGL 1,18 SECV2 0.555 1.18 4.00 0.701 25.45 1.00 0.672 HLA-A\*03:01 LASFKSFLK HLA-A\*03:01 LASFKSFLK RG55 RG55 0.177 0.40 3.50 0.542 142.57 0.80 0.469 0.95 19 HLA-A\*03:01 LAAFKSFLK RGS5V RGS5V 0.220 0.46 3.00 0.563 113.17



Prediction of cross-reactive antitumor T cell response





### **CD8 (virtual) ablation experiment**

## 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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