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Oral Presentation

Immunoinformatics analysis of Wilms' Tumor Protein to Design Multiepitope Peptide-Based Vaccine Against Breast Cancer for Indonesian Population

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Breast cancer is one of the leading cancer-related deaths among females in Indonesia. The use of cancer vaccine as immunotherapy to elicit T-cell responses such as CD8+ cytotoxic T-cells (CTL) and CD4+ helper T-cells (HTL) is on the rise. CTL will kill cancer cells and HTL will orchestrate the immune responses to cancer. T-cell recognises peptide antigen as a complex with HLA molecule on the surface of the cancer cells. Effective induction of protective T-cell immunity with the absence of autoimmunity required specific target antigens that are overexpressed in tumor cells but not in healthy cells. In this study, Wilms' tumor protein 1 (WT1) was proposed as the target for the cancer vaccine since it is overexpressed in breast cancer cells. The immunoinformatics tools netCTLpan and netMHCIIpan were employed to identify WT1 CTL and HTL epitopes, respectively. The epitopes were specifically predicted as peptides that bind to HLA alleles predominant in the Indonesian population to design a vaccine for this populat ion. The peptides generated were then evaluated for the immunogenicity and the ability to induce IFNγ production. Vaccine constructs containing the selected promiscuous CTL and HTL epitopes were assembled using appropriate peptide linkers and maltose-binding protein (MBP) was used as the adjuvant. Further in-silico evaluation showed that the vaccine construct is antigenic, non-allergenic, able to elicit CTL and HTL responses, and covers 99.95% of the Indonesian population. The current insilico analysis results can be used as the basis for developing a multiepitope peptide-based vaccine for breast cancer in Indonesia.

Keywords: WT1 (Wilms' Tumor Protein 1), breast cancer, immunoinformatics, vaccine design, cytotoxic T cells, helper T cells, epitopes

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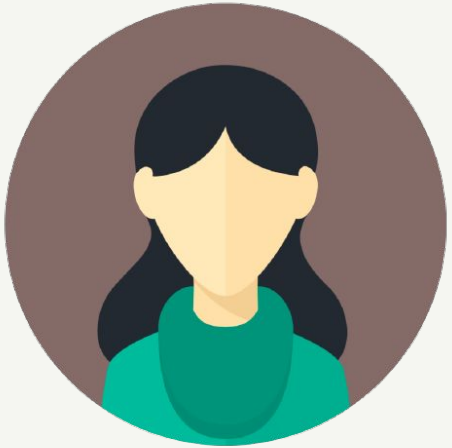
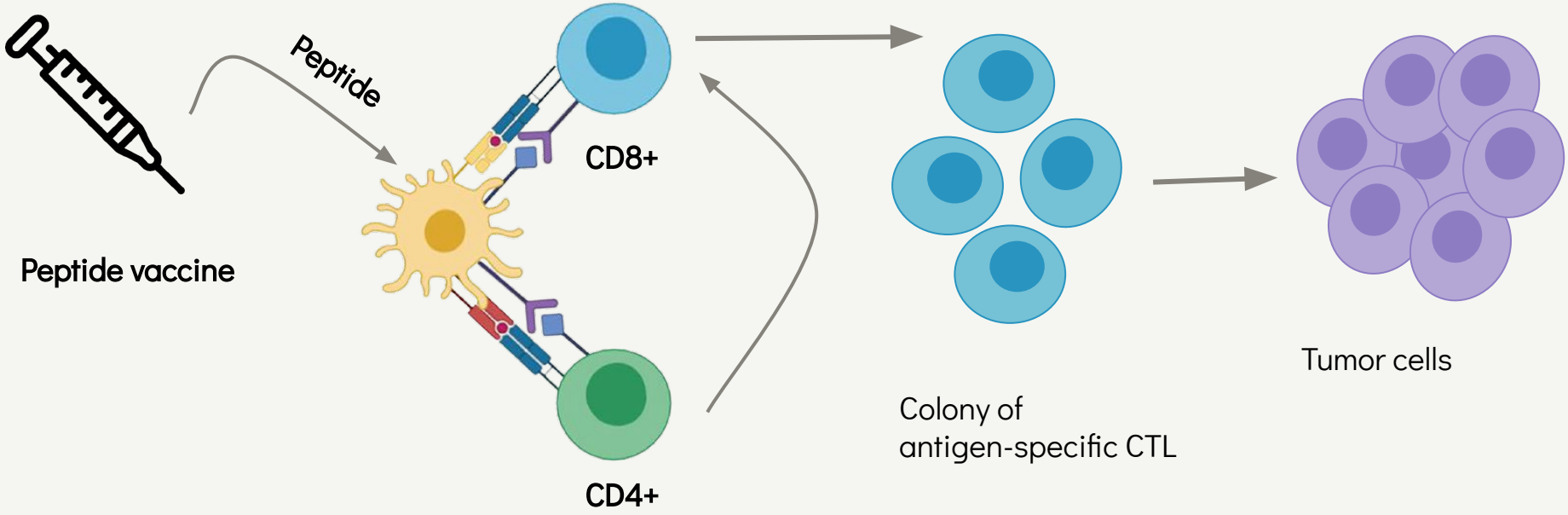


Breast Cancer

	New cases				Deaths			
Cancer	Number	Rank	(%)	Cum.risk	Number	Rank	(%)	Cum.risk
Breast	65 858	1	16.6	4.83	22 430	2	9.6	1.78
Cervix uteri	36 633	2	9.2	2.69	21 003	3	9.0	1.73
Lung	34 783	3	8.8	1.54	30 843	1	13.2	1.39
Liver	21 392	4	5.4	0.92	20 920	4	8.9	0.91
Nasopharynx	19 943	5	5.0	0.75	13 399	5	5.7	0.56
Colon	17 368	6	4.4	0.74	9 444	8	4.0	0.38
Non-Hodgkin lymphoma	16 125	7	4.1	0.66	9 024	9	3.8	0.38
Rectum	16 059	8	4.0	0.68	8 342	10	3.6	0.35
Leukaemia	14 979	9	3.8	0.52	11 530	6	4.9	0.42
Ovary	14 896	10	3.8	1.09	9 581	7	4.1	0.77
Prostate	13 563	11	3.4	1.47	4 863	13	2.1	0.38
Thyroid	13 114	12	3.3	0.49	2 224	19	0.95	0.07

(Globocan, 2020)

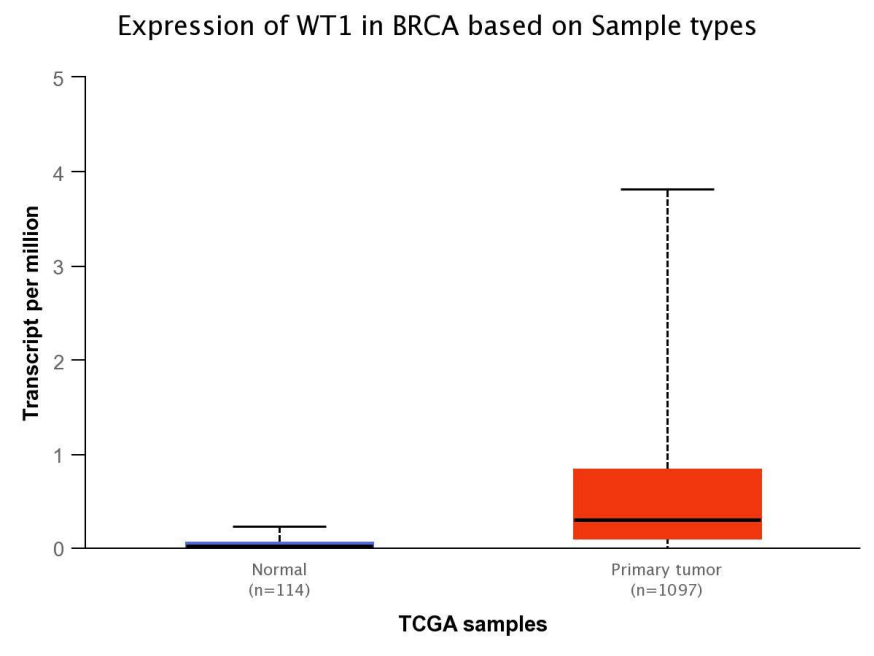
Cancer Vaccine



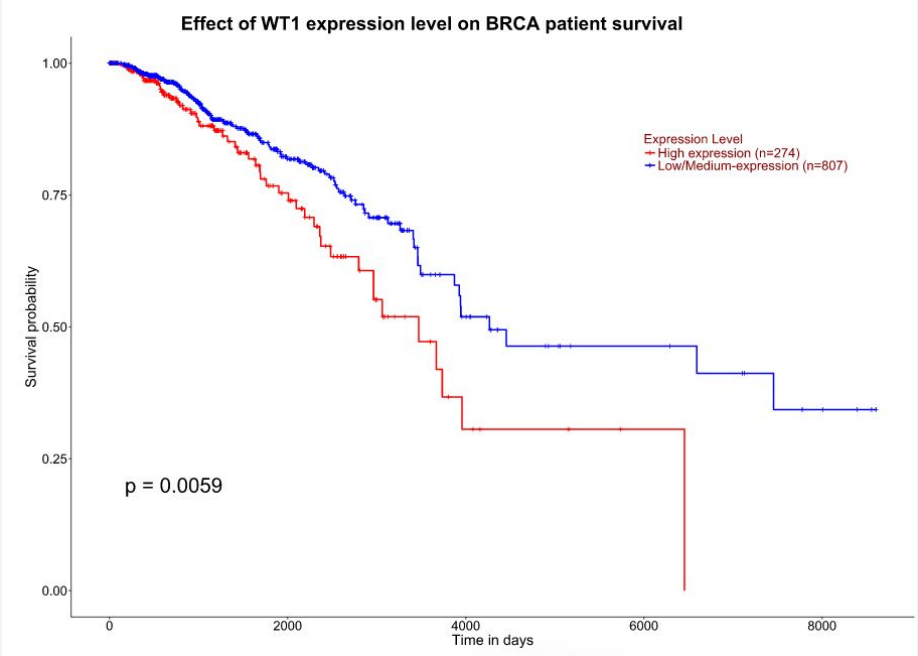
(Busselaar et al.,2020; Bartnik et al., 2012)

Wilm's tumor gene 1 (WT-1) as tumor associated antigen (TAA)

Overexpressed in breast cancers

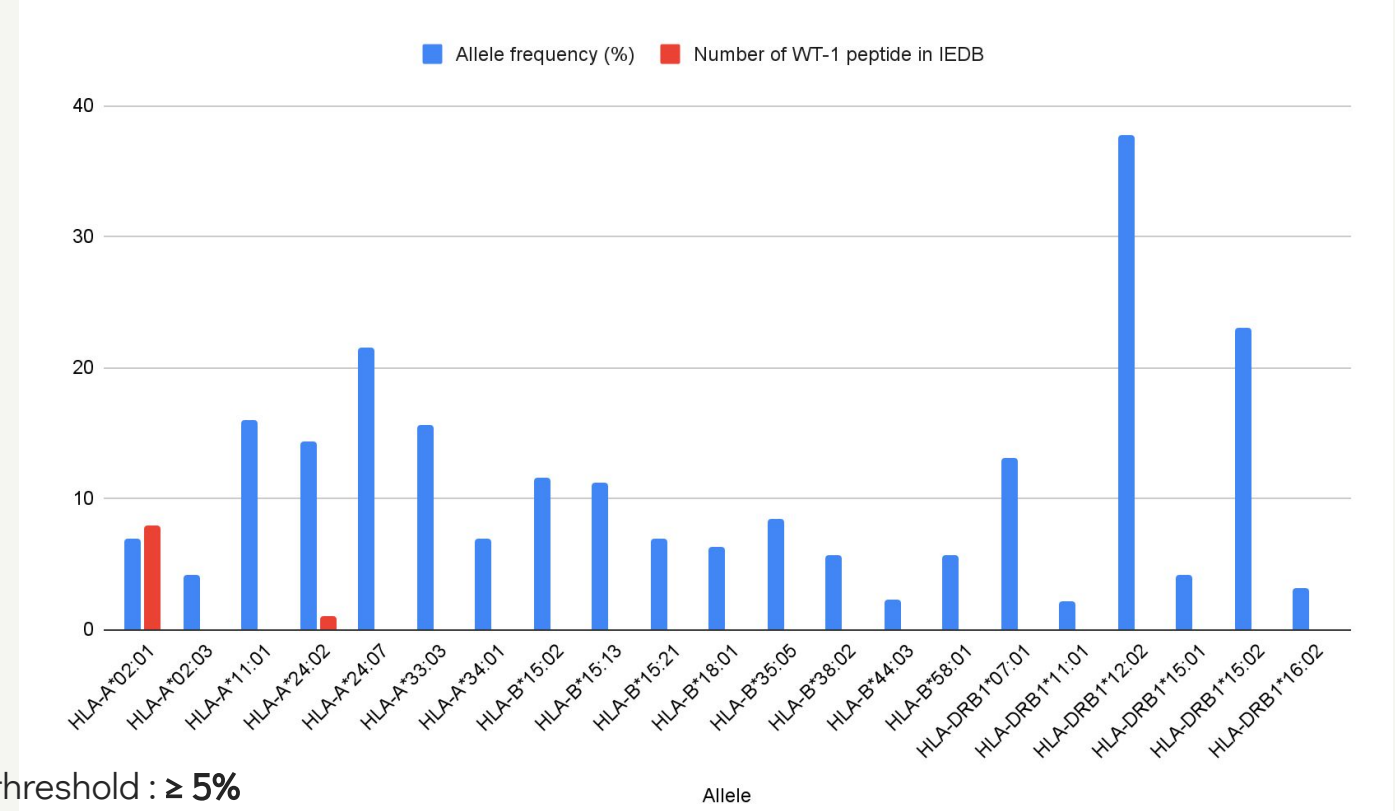


Expression of WT1 related to poor outcome



(Chandrashekar et al., 2017)

HLA allele frequency in Indonesian population and WT-1 Peptides that are reported to bind to the allele



Allele frequency threshold : **≥ 5%**

(http://www.allelefrequencies.net)

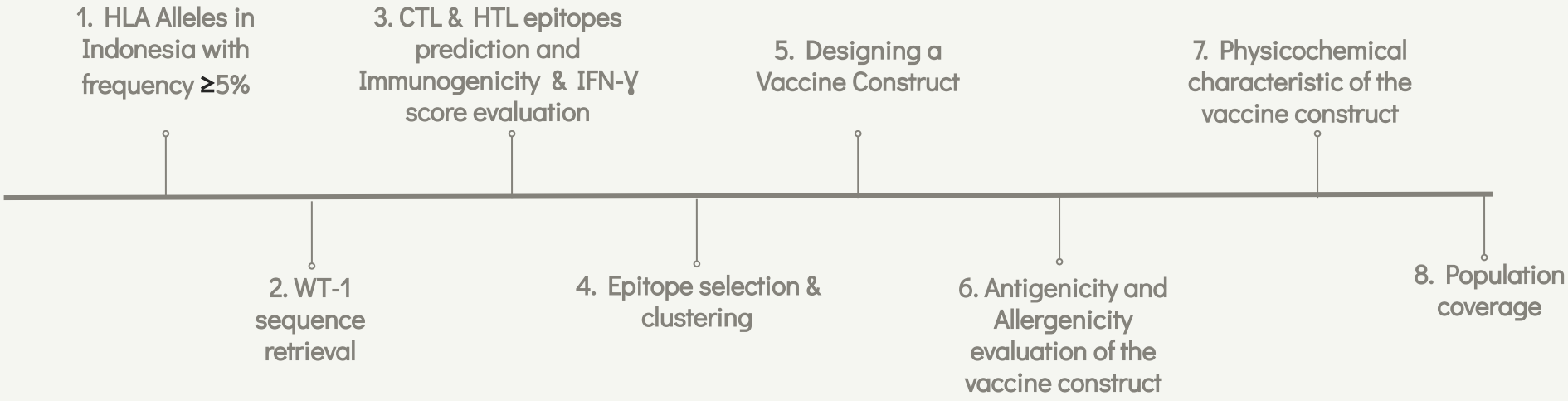
(http://www.iedb.org/)

Objectives

To find peptides from WT-1 that are presented by HLA alleles predominant in Indonesian population using immunoinformatics tools.

To construct multi-epitope peptide based vaccine targeting WT-1 for breast cancer immunotherapy.

Research Scope



T cell epitopes prediction

Complete amino acid sequences of Wilms' tumour protein (UniProt ID : P19544)
Consist of 449 amino acids

83 CTL epitopes (9-mer) generated from WT-1 using the NetCTLpan webserver

26 epitopes are immunogenic towards the CTL

immunogenic CTL epitopes with IC50 less than 500nM were chosen

31 HTL (15-mer) epitopes predicted using the NetMHCIIpan webserver

Tested positive for their IFN-gamma induction capability

HTL epitopes that had the ability to induce IFN-gamma release

Chosen to be clustered

(https://services.healthtech.dtu.dk/service.php?NetCTLpan-1.1)
(http://tools.iedb.org/immunogenicity/)
(http://www.cbs.dtu.dk/services/NetMHCpan-4.0/)
https://services.healthtech.dtu.dk/service.php?NetMHCIIpan-4.0)
(http://crdd.osdd.net/raghava/ifnepitope/predict.php)

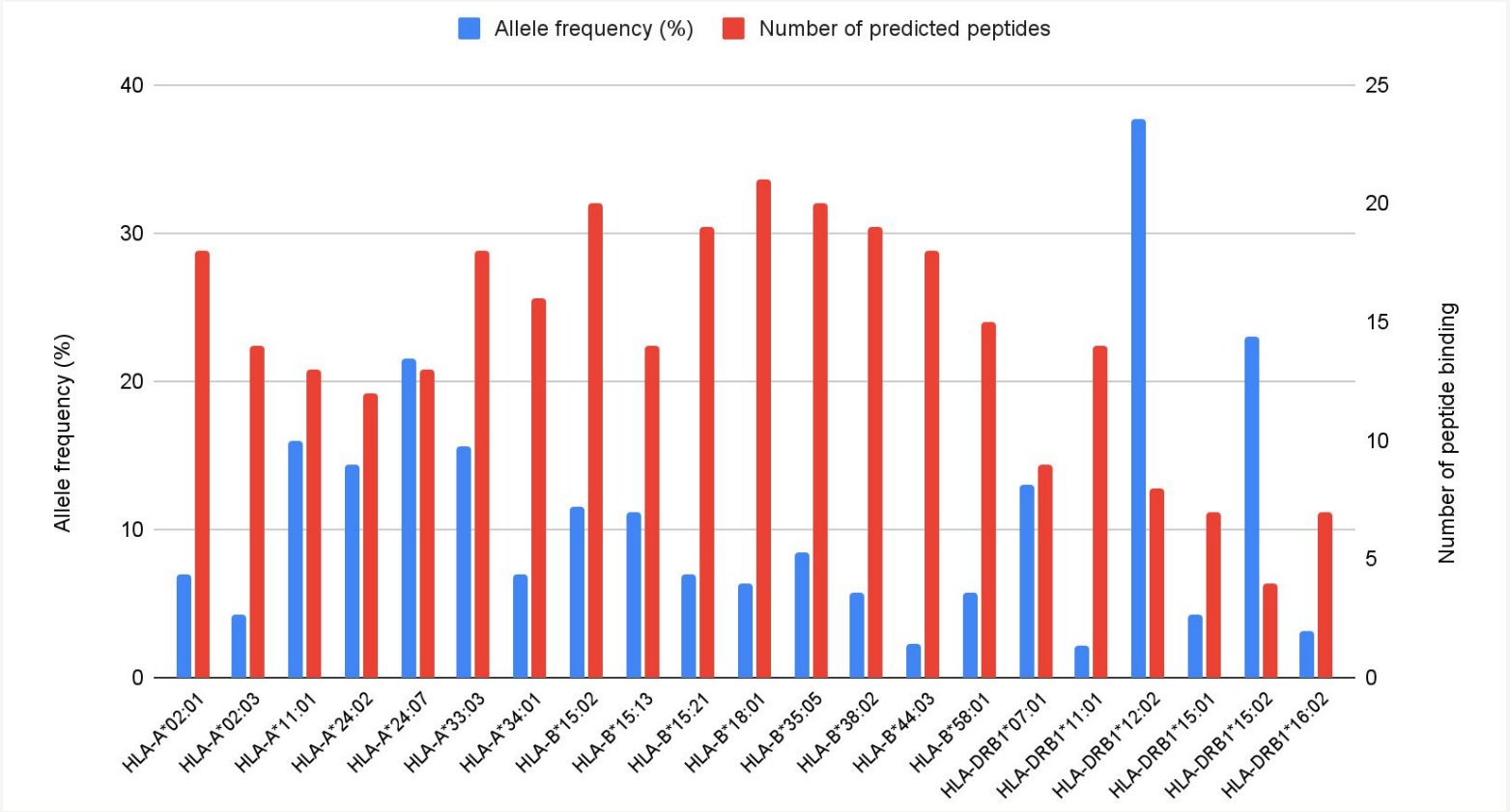
Selection criteria CTL epitopes :

- Nonamer
- Percent Rank (5%)
- IC50 < 500nM

Selection criteria HTL epitopes :

- 15-mer
- SB (<2%), WB (<5%)
- IFN-gamma (+)

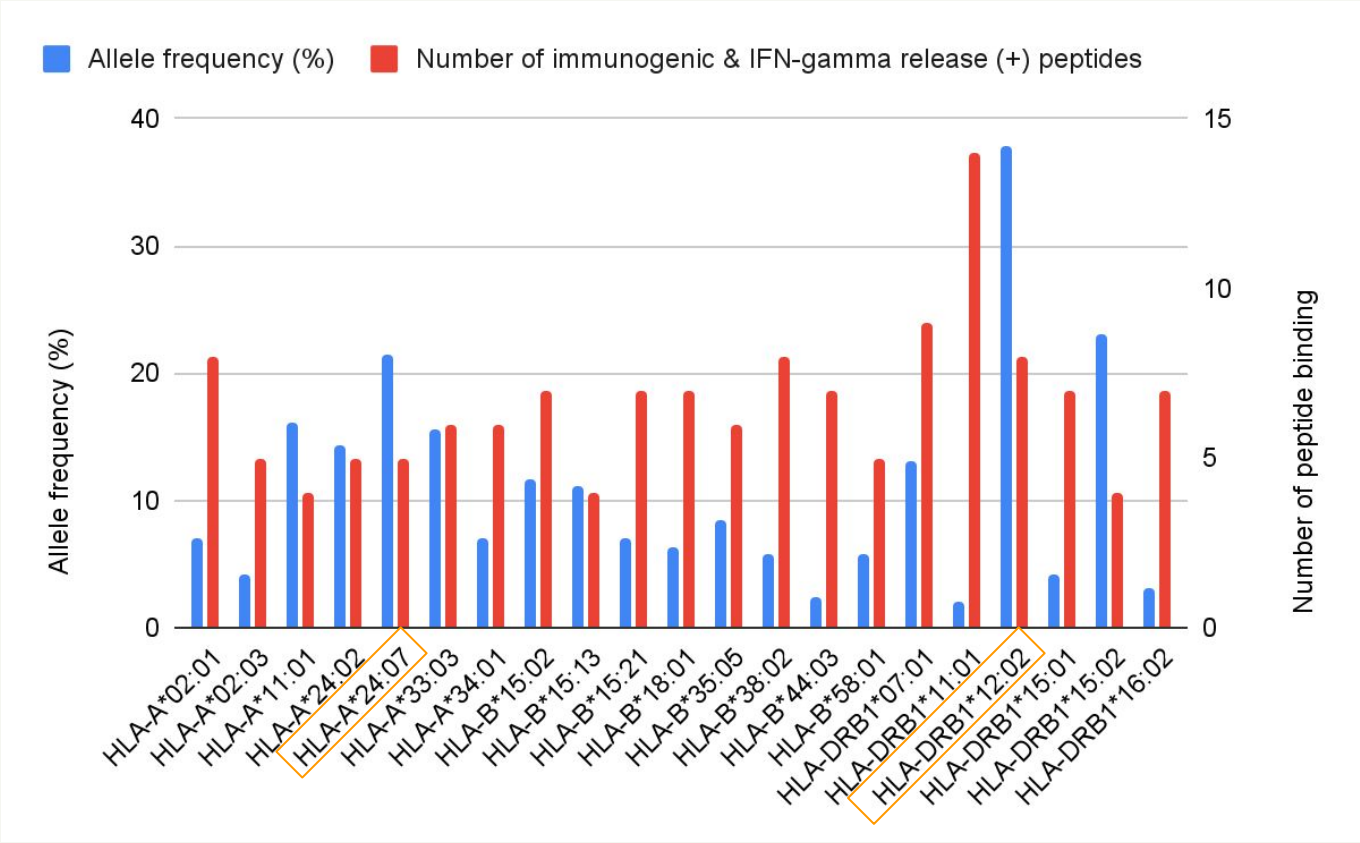
HLA Allele Frequency in Indonesian Population



Allele frequency threshold : **≥ 5%**

(Yuliwulandari et al., 2009)

HLA-Allele Frequency in Indonesian Population and WT-1 peptides that are predicted to bind to the alleles



Chosen and clustered epitopes for vaccine construct

Table 1. The epitopes clusterization of CTL and HTL epitopes for vaccine construct. The bold epitopes indicate consensus sequences.

Sequence	HLA allele
HGVFRGIQDVRRVPGVAP	HLA-A*02:01, HLA-A*02:03, HLA-A*11:01, HLA-A*34:01, HLA-B*15:02, HLA-B*15:13, HLA-B*15:21, HLA-B*35:05, HLA-B*58:01, HLA-DRB1*11:01, HLA-DRB1*12:02, HLA-DRB1*16:02
RMFPNAPYLPS	HLA-A*02:01, HLA-A*02:03, HLA-A*24:07, HLA-B*35:05
GQFTGTAGACR	HLA-A*02:03, HLA-A*33:03
NQMNLGATLK	HLA-A*11:01, HLA-B*38:02, HLA-B*15:02
TPSYGHTPSHHAAQF	HLA-DRB1*07:01, HLA-DRB1*15:02, HLA-DRB1*16:02
DLNALLPAV	HLA-A*02:01, HLA-A*02:03
VTFDGTPSY	HLA-A*11:01, HLA-A*33:03, HLA-A*34:01, HLA-B*15:02, HLA-B*15:13, HLA-B*15:21, HLA-B*18:01, HLA-B*35:05, HLA-B*44:03, HLA-B*58:01
RIHTHGVFR	HLA-A*11:01, HLA-A*33:03
HSFIKQEPSWGGAEP	HLA-DRB1*15:01, HLA-DRB1*15:02, HLA-DRB1*16:02

(http://tools.iedb.org/cluster/)

Vaccine Design Construct

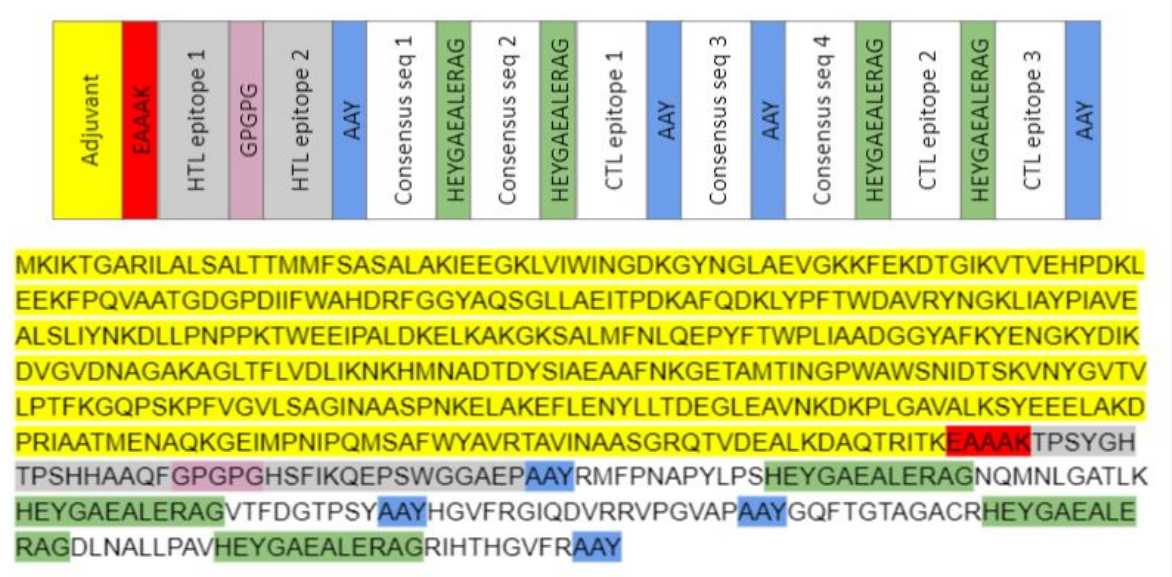


Figure 3. The schematic image of vaccine construct based on Wilms' Tumor Protein (WT1). The vaccine construct consists of maltose-binding protein (MBP) (yellow) as adjuvant and EAAAK (red), GPGPG (purple), HEYGAEALERAG (green), and AAY (blue) linkers.

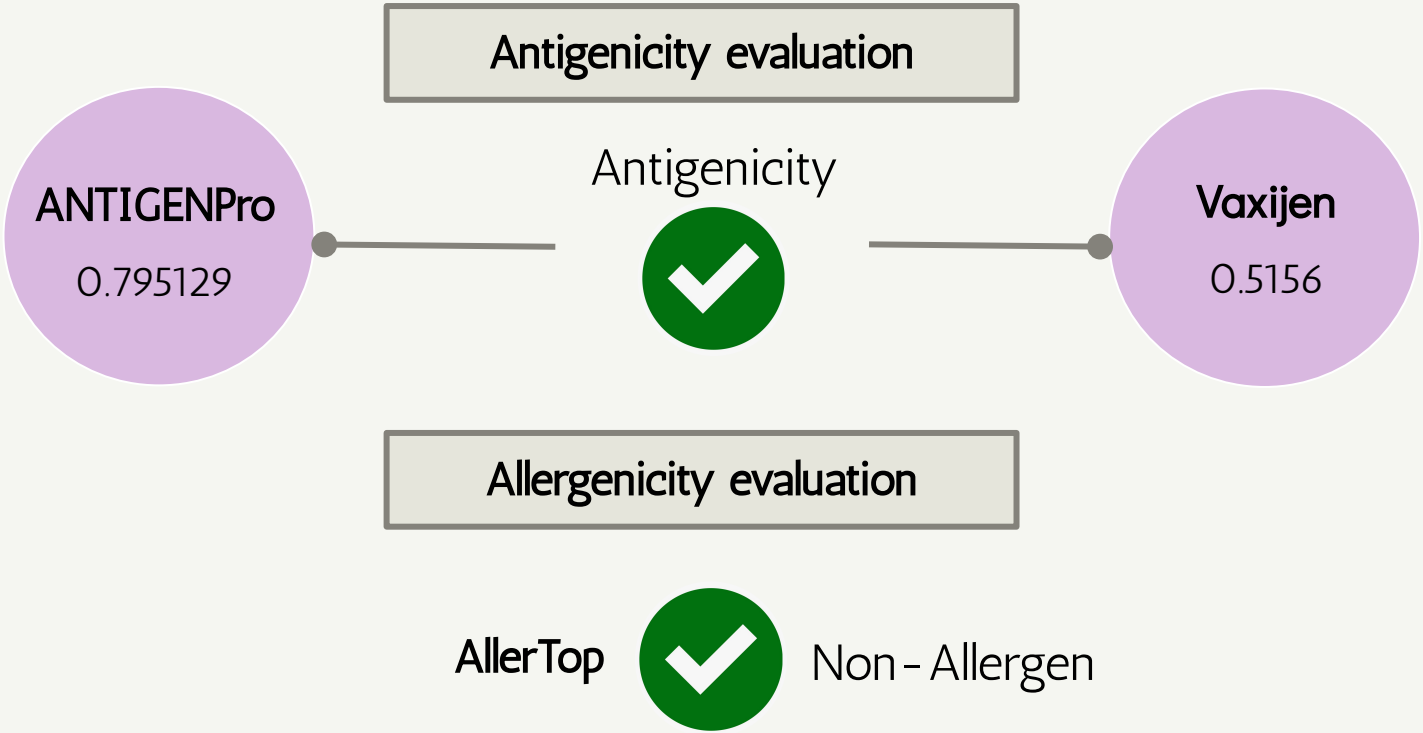
Adjuvant : Maltose-binding protein (MBP) (Uniprot: P0AEX9) → TLR2/TLR4 agonist

Linkers :

- The adjuvant and the N-terminus of the vaccine construct → EAAAK linker
- The CTL epitopes and consensus sequences → AAY and HEYGAEALERAG linkers
- The HTL epitopes → GPGPG linkers

(Jiang et al., 2017; Parvizpour et al., 2019)

Antigenicity & Allergenicity Evaluation of vaccine construct



(http://scratch.proteomics.ics.uci.edu/)

(http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html)

(https://www.ddg-pharmfac.net/AllerTOP/)

Physicochemical Properties of Vaccine Construct

Table 2. The physicochemical properties of the vaccine construct predicted by Protparam server.

Amino acid	573
Molecular weight	62.17 kDa
Predicted pI	5.93
Evaluated half-life	30 hours in mammalian reticulocytes (in vitro) >20 hours in yeast (in vivo) >10 hours in E.coli (in vivo)
Evaluated aliphatic index	77.16 → thermostable
GRAVY	– 0.314 → hydrophilic
Instability index score	20.99 → stable

https://web.expasy.org/protparam/)

Population coverage

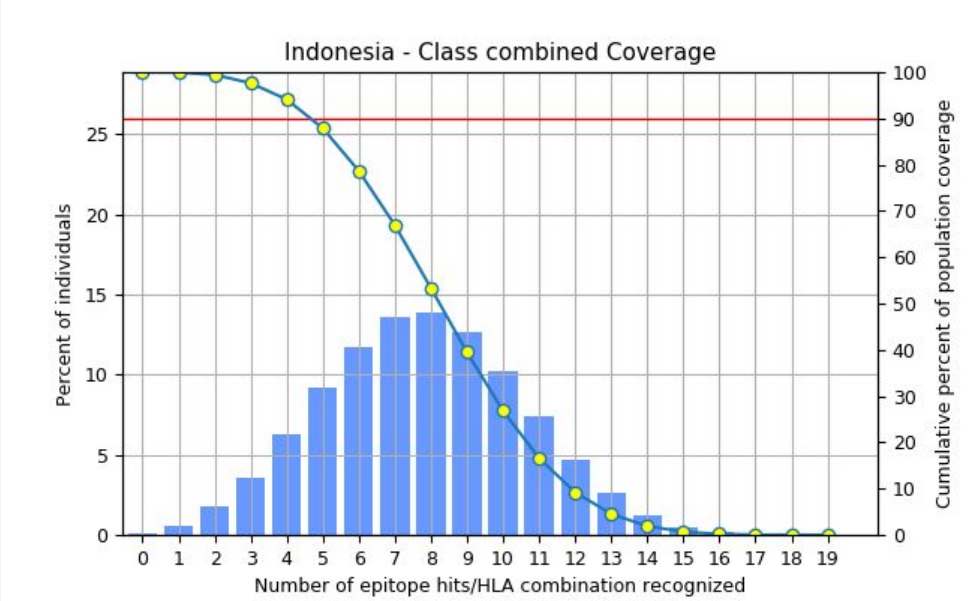


Table 3. Epitope population coverage for Indonesia population from IEDB.

MHC Class	Coverage	Average Hit	PC90
Class combined	99.95%	7.77	4.66

(http://tools.iedb.org/population/)

Conclusion

WT-1 peptides that bind to the predominant HLA alleles of Indonesian population have been identified using immunoinformatics method. The peptides are immunogenic and can induce IFN-ɣ production (induce TH1 responses).

The multi-epitope peptide-based vaccine construct based on WT-1 with maltose-binding protein (MBP) as adjuvant were designed through immunoinformatics approach. The vaccine construct were shown to be immunogenic and non-allergenic. This construct may have a promising potential for breast cancer vaccine for Indonesia population. However, further research, including *in vitro* and *in vivo* evaluation, on its efficacy and safety still have to be done.

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Thank You

Do you have any question?