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Oral Presentation

Immunoinformatics design of a multi-epitope peptide-based ovarian cancer vaccine targeting Mucin1

Putri Ashiila, Herdiana Ronsumbre, Vanessa Reba, Marsia Gustiananda

Department of Biomedicine, School of Life Sciences,
Indonesia International Institute for Life Sciences,
Jl Pulomas Barat Kav 88, Jakarta Timur, 13210, Indonesia

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Presenting Author: putri.ashiila@student.i3l.ac.id;
*Corresponding Author: marsia.gustiananda@i3l.ac.id

Ovarian cancer is the most fatal gynecological malignancy and the fifth main cause of cancer deaths in women in the developed world. In Indonesia alone, new cases of ovarian cancer reach the number of 14,896 cases each year according to the Global Cancer Observatory. Immunotherapy using cancer vaccines is a promising treatment. Cancer vaccine works by activating cytotoxic T-cells that are responsible for cancer cell elimination and helper T-cells that are important for cytokine production. T-cells recognize peptides epitopes derived from the cancer antigen which is presented by HLA molecules on the surface of the cancer cells. We combined immunoinformatics with an *in-silico* vaccine design to construct a Mucin1-based cancer vaccine for the Indonesian population. The sequence of Mucin1 protein was obtained from NCBI databases and evaluated for CTL epitopes using netCTLpan and HTL epitopes using netMHCIIpan. The epitopes were predicted to bind to HLA alleles of the Indonesian population. B cell epitopes were predicted by the Bepipred server in IEDB. A total of ten T-cell epitopes were chosen for vaccine construct based on the highest immunogenicity scores (0.09837 - 0.19463), IFN gamma epitope score (0.3072045 - 1.0216963), and population coverage score (99.62%). Five immunogenic B cell epitopes were also included. Two vaccine constructs were made by incorporating maltose-binding protein from *Bacillus sp.* and *E. coli* as an adjuvant. The epitopes were linked together using appropriate linkers. Vaccine constructs evaluation by Vaxijen, AntigenPro, AllerTop, and Protparam showed that both constructs are antigenic, non-allergenic, and have good stability.

Keywords: B-cell epitopes, HTL epitopes, CTL epitopes, Mucin1, vaccine design, immunoinformatics, ovarian cancer

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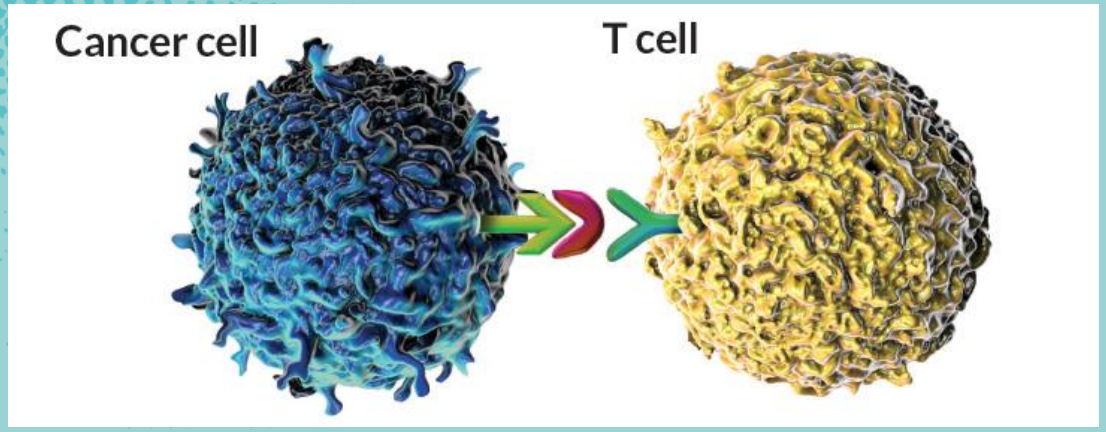
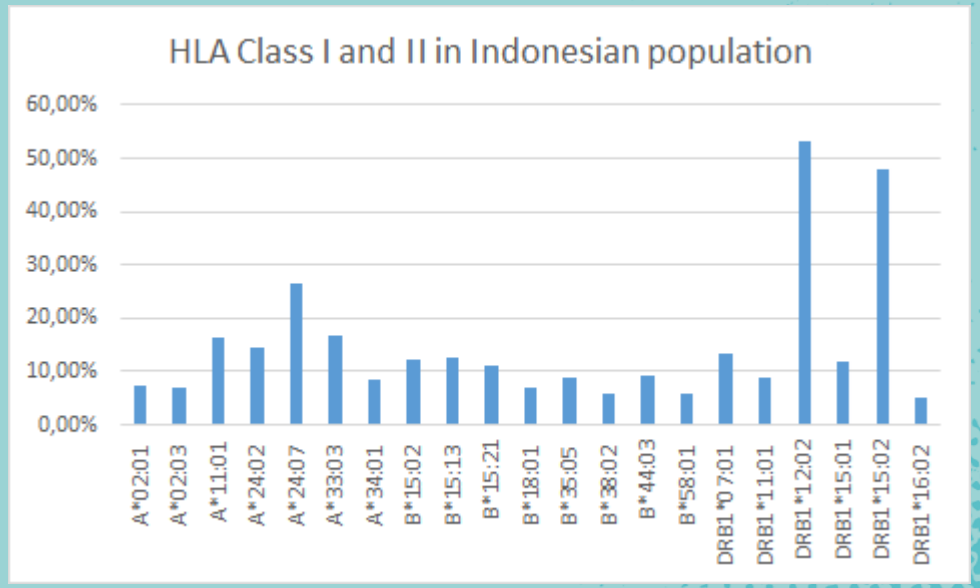
Ovarian Cancer

- The most fatal gynaecological malignancy in women in the developed world
- In Indonesia, the cases reach 14,896 each year
- There are more than 30 types of ovarian cancer
- The most common is Epithelial Ovarian Cancer (EOCs)


Cancer cell
T cell


HLA Class I and II in Indonesian population
60,00%
50,00%
40,00%
30,00%
20,00%
10,00%
0,00%
A*02:01
A*02:03
A*11:01
A*24:02
A*24:07
A*33:03
A*34:01
B*15:02
B*15:13
B*15:21
B*18:01
B*35:05
B*38:02
B*44:03
B*58:01
DRB1*07:01
DRB1*11:01
DRB1*12:02
DRB1*15:01
DRB1*15:02
DRB1*16:02

Aim

Fill the gap in research

Study

MUC1 has been studied before, but not on ovarian cancer

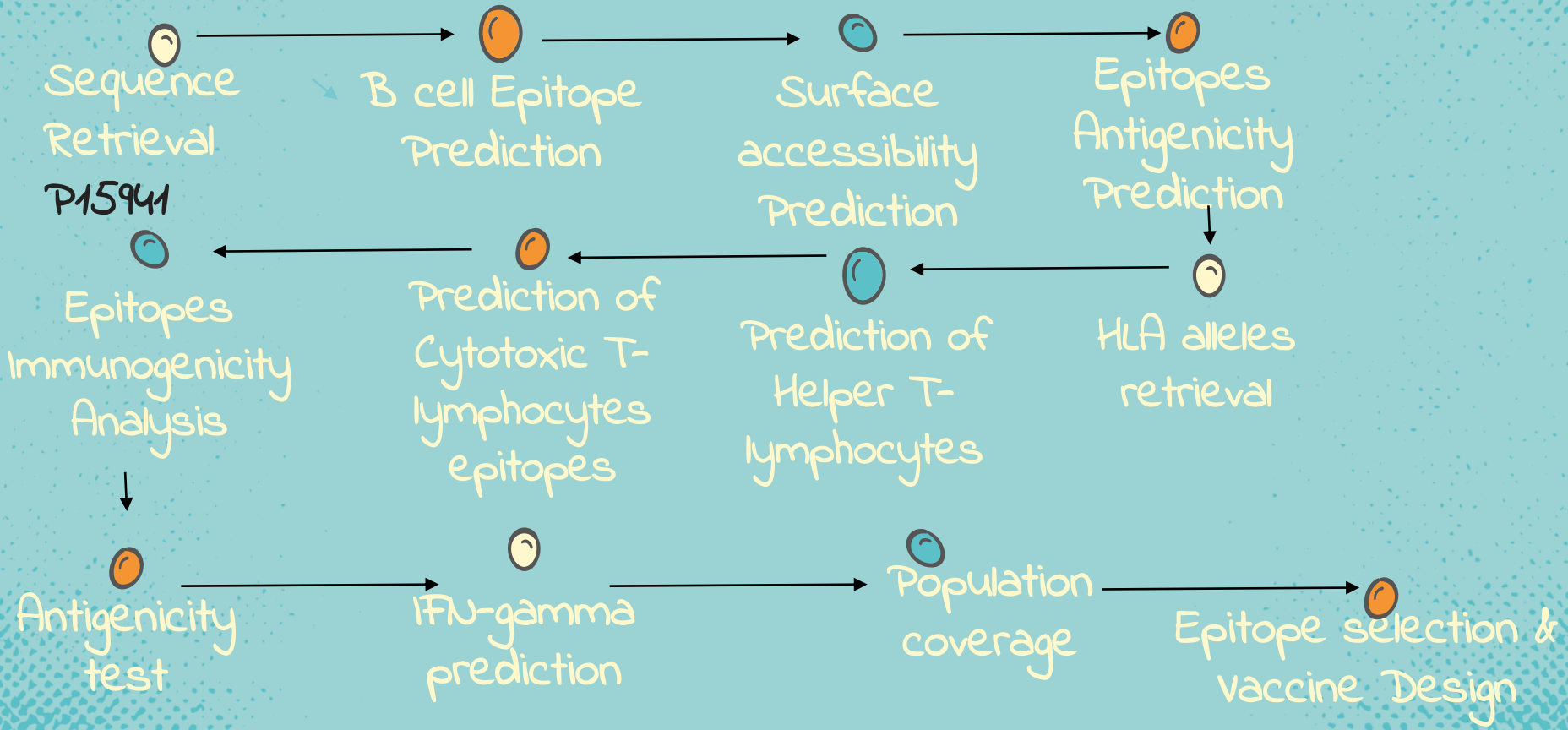
Targets

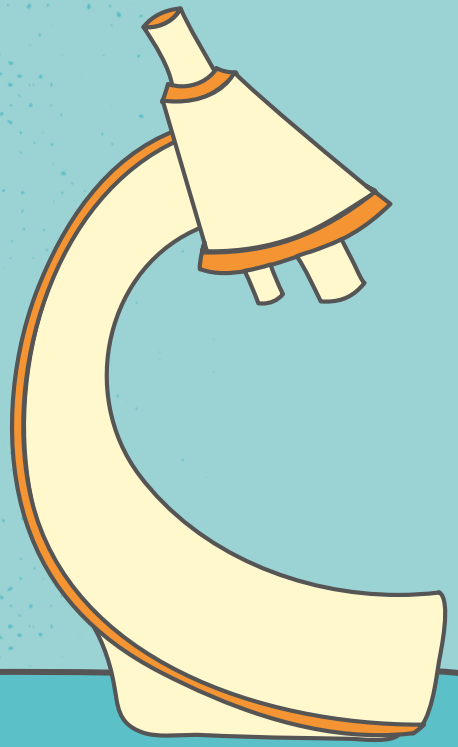
The specific targets that the MUC1 TAA will bind to is yet to be specified.

Population

The studies previously done were also not specified to a particular population, and this study is focused on Indonesian population

Methods Overview





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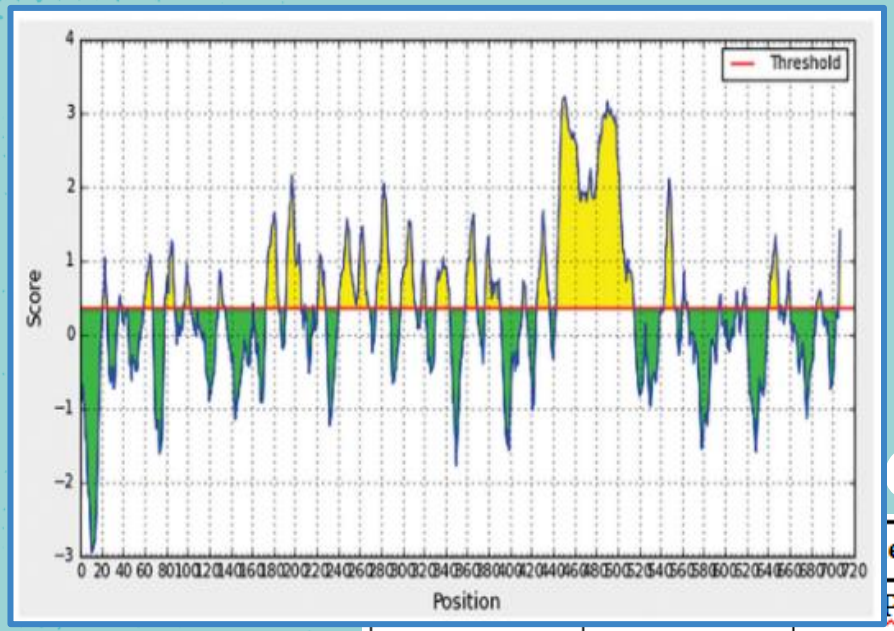
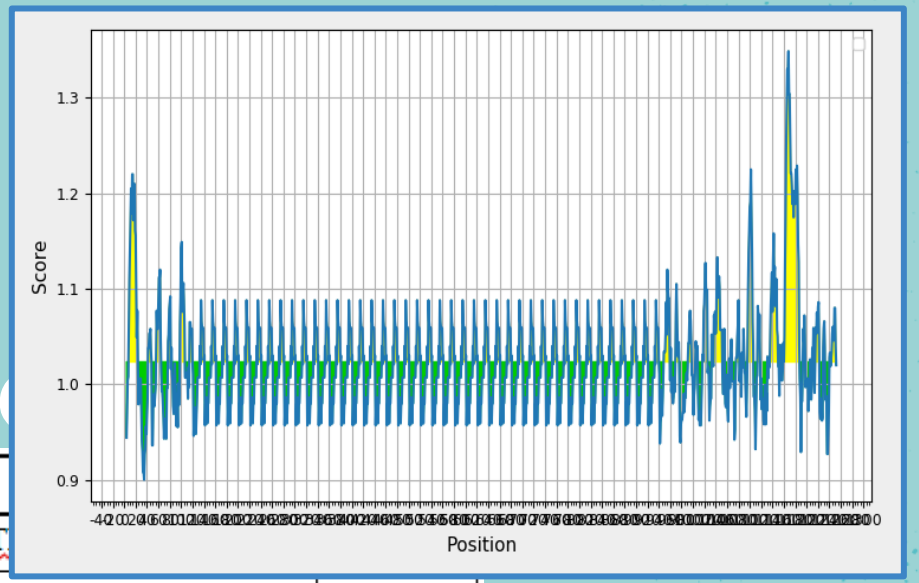
Result & Discussion

Prediction of B-cell Epitopes

Prediction of linear B-cell epitopes



Prediction of epitopes antigenicity



135	141	AHGVTSA	7
950	958	STAPPVHNV	9
1017	1025	THHSSVPPL	9
1141	1149	VSDVPFPFS	9

Prediction of CTL Epitopes and Immunogenicity

35 peptides were identified as epitopes of cytotoxic T-lymphocytes

Five peptides were chosen based on the immunogenicity score and its ability to interact with major alleles in Indonesia population.

Start	End	Epitopes	Allleles	Immunogenicity score
10	18	FLLLLLTVL	HLA-A*02:01	0.1221
1036	1044	LSTGVSFFF	HLA-A*24:02,HLA-A*24:07,HLA-B*15:13,HLA-B*58:01	0.09837
1041	1049	SFFFLSFHI	HLA-A*24:02,HLA-A*24:07	0.10697
1140	1148	SVSDVPFPF	HLA-A*34:01,HLA-B*15:02,HLA-B*15:13,HLA-B*15:21,HLA-B*35:05,HLA-B*58:01	0.0907
1171	1179	VALAIVYLI	HLA-A*24:07	0.19463

Prediction of HTL Epitopes & IFN Gamma Score

30 peptides that have strong binding were identified

Five peptides were chosen for vaccine construct based on the Interferon gamma score and its ability to interact with major alleles in Indonesia population.

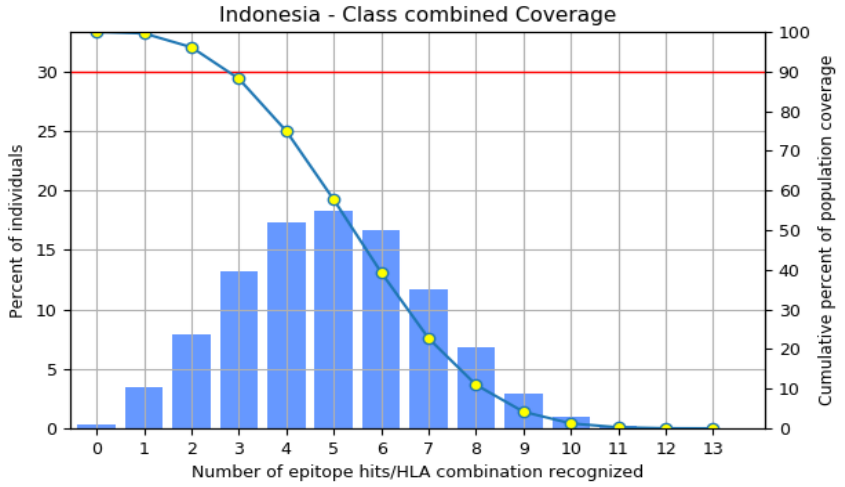
Start	End	Epitopes	Core	Alleles	IFN gamma score
1062	1076	STDYYQELQRDISEM	YQELQRDIS	HLA-DRB1*11:01	0.59555444
1083	1097	QGGFLGLSNIKFRPG	FLGLSNIKF	HLA-DRB1*12:02,HLA-DRB1*15:02,HLA-DRB1*16:02	0.43650965
1129	1143	ASRYNLTISDVSVSD	YNLTISDVS	HLA-DRB1*07:01	0.3072045
1145	1159	PFPFSAQSGAGVPGW	FSAQSGAGV	HLA-DRB1*16:02	1.0216963
1203	1217	YHPMSEYPTYHTHGR	MSEYPTYHT	HLA-DRB1*15:01,HLA-DRB1*15:02	0.50297389

Population coverage

The population coverage analysis was found to be 92.06% for MHC Class 1 and 95.26% for MHC Class 2 which indicated a high percentage.

Epitopes	Allleles
FLLLLLTVL	13.07%
LSTGVSFFF	71.53%
SFFFLSFHI	58.77%
SVSDVPFPF	72.96%
VALAIVYLI	38.75%
STDYYQELQRDISEM	4.83%
QGGFLGLSNIKFRPG	84.51%
ASRYNLTISDVSVSD	20.49%
PFPFSAQSGAGVPGW	5.41%
YHPMSEYPTYHTHGR	47.35%

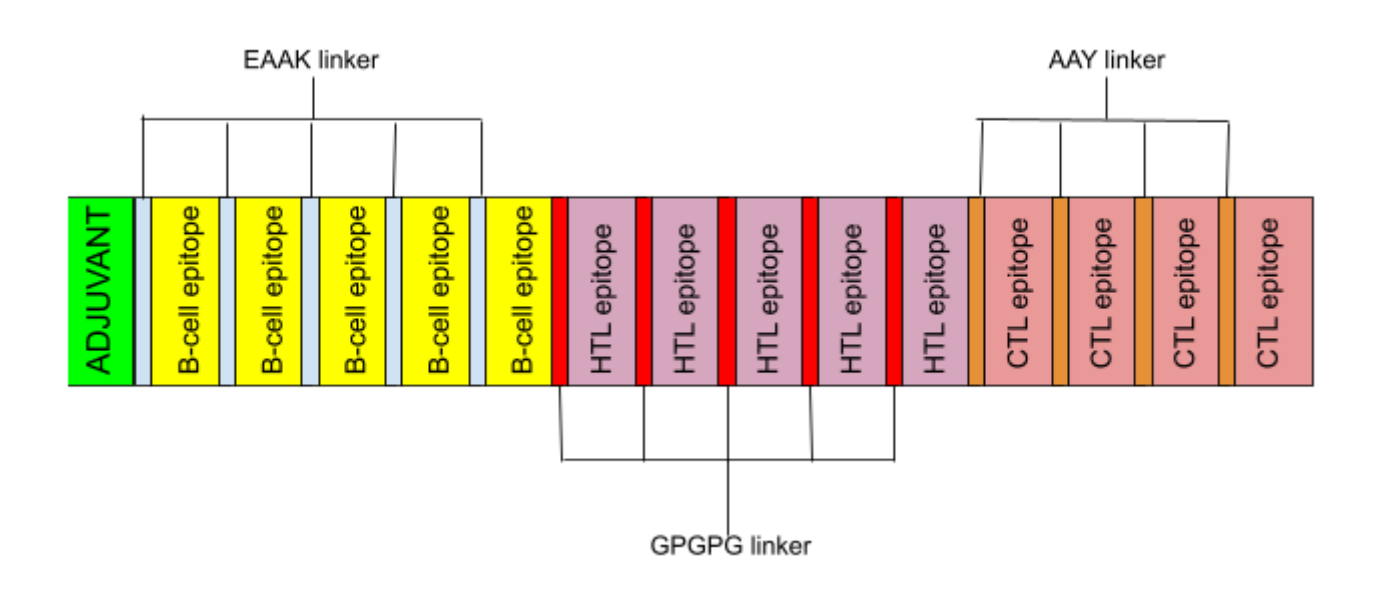
MHC class	Coverage	Average hit	PC90
combined	99.62%	4.95	2.77



Vaccine Design

Two vaccine constructs were made with different adjuvant:

1. Maltose/maltodextrin-binding protein from *Bacillus sp.*
2. Maltose/maltodextrin-binding periplasmic protein from *Escherichia coli*



Vaccine construct with MBP from *Bacillus* sp. as adjuvant

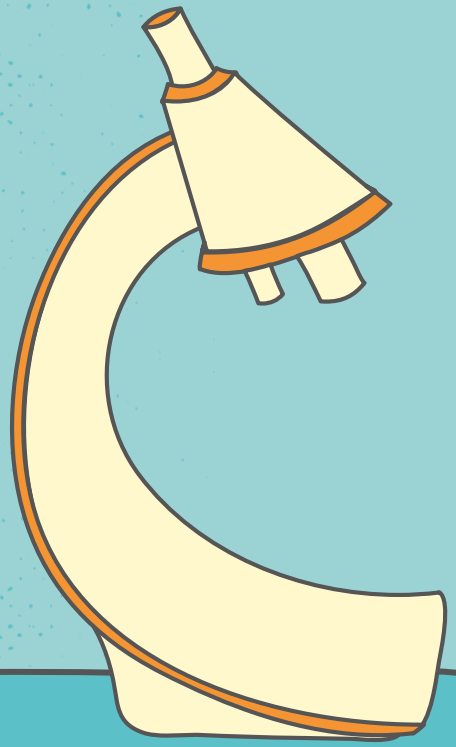
MKKGFSLLSLITMFLMIILLAACAPEREEEAVTTDTNDGEADQPEELTIWANDREEQLEAIEKIANDYTEQTGINVKVETKPMMDQLQ
ELSLAGPEGNGPDLFFQPHDQIGNIVAQGLADPLTLSDDELSNYASSSIDAVTYEFEGETDIYGIPAVIETYGIFYNKEIVPEAPETIRIRS
LEAAAKVTSVPVTRPEAAAKGAHGVTSAEAAAKSTAPPVHNVEAAAKTHHSSVPPLEAAAKVSDVPFPFSEAAAKSTDYYQELQRD
ISEMGPGPGQGGFLGLSNIKFRPGGPGPGASRYNLTISDVSVSDGPGPGPFPFSAQSGAGVPGWGPGPGYHPMSEYPTYHTHGRGPGP
GFLLLLLTVLAAYLSTGVSFFFAAYSFFFLSFHIAAYSVSDVPFPFAAYVALAIVYLI

Number of amino acids: 412
Vaxijen score = 0.4318 (Probable NON-ANTIGEN)
AntigenPro score = 0.797712
Allergenicity = non-allergen
Physicochemical characteristic :
Molecular weight =43846.21
Theoretical pI = 4.50
Instability index = 39.32 (stable)

Vaccine construct MBP from *Escherichia coli* as adjuvant

MKIKTGARILALSALTTMMFSASALAKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIF
WAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALM
FNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWS
NIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAAT
MENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITKEAAAKVTSVPVTREAAAKAHGVTSAEAAAKSTAPP
VHNVEAAAKTHHSSVPPLEAAAKVSDVPFPFSEAAAKSTDYYQELQRDISEMGPGPGQGGFLGLSNIKFRPGGPGPGASRYNLTISDV
SVSDGPGPGPFPFSAQSGAGVPGWGPGPGYHPMSEYPTYHTHGRGPGPGFLLLLLTVLAAYLSTGVSFFFAAYSFFFLSFHIAAYSVSD
VPFPFAAYVALAIVYLI

Number of amino acids: 625
Vaxijen score = 0.4321 (Probable NON-ANTIGEN)
AntigenPro score = 0.912902
Allergenicity = non-allergen
Physicochemical characteristic :
Molecular weight = 67015.27
Theoretical pI = **5.86**
Instability index = 25.53 (Stable)



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Conclusion

Mucin-1 protein that is overexpressed in epithelial ovarian cancer is a potential target for developing T-cell epitope based vaccines.

We found peptides from Mucin-1 presented by HLA allele that are immunogenic, able to induce IFN gamma production,

The multi-epitope based vaccine constructs targeting Mucin-1 is antigenic, non-allergenic, and stable.

This immunoinformatic result can be the basis for *in vitro* and *in vivo* experiments in order to develop vaccine and immunotherapy for cancer

Thank You

Do you have any questions?

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