Therapeutic Targets Prediction of Tumor Specific Antigen for Vaccine Design

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Submitted by:

Sunil kumar
2K16/BIO/07

Under the Supervision of

Dr. ASMITA DAS

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

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DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, Sunil Kumar , Roll No. 2K16/BIO/07 student of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled "**Therapeutic Targets Prediction of Tumor Specific Antigen for Vaccine Design**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi **SUNIL KUMAR**

Date:

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the project dissertation titled "**Therapeutic Targets Prediction of Tumor Specific Antigen for Vaccine Design**" which is submitted by Sunil kumar, Roll No. 2K16/BIO/07, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master in Technology (Bioinformatics), is a record of a project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for ant Degree or Diploma to this University or elsewhere.

Place: Delhi **(Dr. ASMITA DAS)**

Date: **SUPERVISOR**

Assistant Professor

Department of Biotechnology

Delhi Technological University

ABSTRACT

The difficulty of treating cancer lies in their similarity with normal cells in terms of antigen expression. Cancer cells are transformed cells that gains immortality by mutagenic transformation of normal cells. The cross reactivity of surface antigens on tumor cells and normal cells makes them difficult to be identified by immune system. However, T cytotoxic cells, NK cells and dendritic cells play important role in immune response to tumor cells. T cell epitopes of tumor specific antigens were predicted as vaccine candidates so as to increase immunity against tumor cell. Tumor cells express tumor specific antigen peptide on their surfaces which are recognised by T-cytotoxic cells and hence give immune response against tumor. Dendritic cell phagocytose the tumor cell and present their peptides to the T-helper cell and hence result in tumor rejection. MHC class I binding prediction tool and $CD8^+$ immunogenicity prediction tool were used to predict class I epitopes. MHC class II binding prediction tool and $CD4^+$ immunogenicity prediction tool were used to predict class II epitopes. Population coverage of the epitopes of both MHC I and MHC II binding peptide was calculated in Indian population. Top ten population covered epitopes were selected for each antigen. Epitopes were predicted for ACTN4, BRAF, CAMEL, CASP5, CASP8, CDC27, CDK4, CDKN2A, CTNNB1, EEF2, FLT3, GPNMB, HSPA1B, KRAS, MUM1, PAPOLG, TPI1, UBXD5. A combinatorial epitope prediction would be effective in designing multifactorial immune stimulation response against tumor specific antigen.

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SUNIL KUMAR

(2K16/BIO/07)

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LIST OF ACRONYMS

Abbreviation	Full Form
MHC I	Major Histocompatibility Complex I
MHC II	Major Histocompatibility Complex II
CD4	Cluster of Differentiation 4
CD8	Cluster of Differentiation 8
CASP5	Caspase-5 precursor
APC	Antigen-presenting cell
ORF	open reading frame
HLA	human leukocyte antigen
DNA	Deoxyribonucleic Acid
mRNA	Messenger RNA

1 Introduction

Tumor is the abnormal mass of tissue when uncontrolled cell division takes place. Tumour classified in two types benign and malignant. Benign is that tumor which cannot form cancer. Malignant is that tumor which can form cancer. Malignant is also known as neoplasm[1][2][3].

Tumor antigen is the protein product of the tumor cell, which is identified by immune cell. By identification of tumor antigen immune cell respond to the tumor cells and try to eliminate these cells. Tumor antgen is classified into three different type, these are unique antigens, shared antigen, unclassified[4]. Here we studied unique antigen which is further classified into substitutional mutation, alternative ORF, intron encoding, chromosomal translocation, internal tandem repeat[5]. There are no. of antigen in these like in substitution mutation ACTN4, BRAF, CASP8, CDC27 etc., in alternative ORF CASP5, CDKN2A,OGT etc., in intron encoding MUM1, in chromosomal translocation ABL-BCR, BCR-ABL, DEK-CAN etc., internal tandem repeat FLT3[6][7].

The tumor unique antigens are the main target of the immunogenic response against the tumor cell. These are the unique antigens (not present on the normal cell) so if immunotherapy using these as targets for immune cells, then there is no harmful effect of it on the normal cell[8].

MHC class l present the short peptide on the surface of tumor cell. T cytotoxic cell recognise this peptide and MHC I complex and give immune response against it[9]. MHC class II present the short peptide on the surface of tumor cell. T helper cell recognise this peptide and MHC II complex[10]. Helper T cell never give direct immune response instead of that they help in the activation of cytotoxic T cell and macrophages to give immune response to target cell. Or the T helper cell stimulate B cells to secrete antibodies. APCs ingest microbes, degrade them, and export small part of it(antigen) to the cell surface with the help of MHC Il. This MHC Il and peptide complex then recognise by the receptors present on the helper cell surface known as $CD4^+$ receptors[10]. But this is not the only signal which is required for the activation of T cell. It required one more signal for its activation. It must be given by one of these two signal either by stimulation by a cytokine or by a stimulatory signal that is between B7 and CD28. B7 found on the surface of APCs. Receptor protein CD28 found on the surface

of helper T cell. So, when the first signal and one of second signal are received then the helper T cell becomes activated[11][12].

So, Here we have to predict the epitopes, which can bind to receptors of both T helper cell as well as T cytotoxic cell. There are lots of tool which can use to predict MHC I and MHC Il binding peptides[13][14][15].

They give an accuracy of about 90-95%. Then we have to select those alleles which cover most of the population frequency. So the vaccine we want to form can be bind to the most HLA alleles. We can collect these data from different papers in which population frequency of different HLA alleles given[16].

Once the epitope selection takes place, then we can select a set of those epitope which cover atleast 80% of the population. So the vaccine we formed from these epitopes can be effective on the large population size.

2 Review of literature

2.1 Cancer

Cancer is a disease in which the abnormal cell growth takes place which destroy the body tissue in which their growth starts. these abnormal cells migrate from the tissue in which their growth starts to other parts of the body.this is not in benign tumors,which do not spread to other parts of the body. The difficulty of curing a cancer is similar to the difficulty of getting rid of weeds. Symptoms of cancer may include abnormal bleeding, a lumb, unexplained weight loss, prolonged cough, and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes[17]. Over 100 types of cancers affect humans. Few of them listed below.

Brain tumors-Some brain tumors are benign other are malignant. They are common tumor of childhood. There are many kinds of brain tumors, their classification depends on the histology and location within the brain[1].

Germ cell tumor- Germ cell tumor are those tumor which developes from the germ cells. In the developing embryo germ cells migrate to the ovaries or testicles[18] .

Breast cancer- Cancer which is begin when the breast cells grow uncontrolled, they formed a mass or sheet of cells known as tumor. All through breast cancer most commonly spreads to near by lymph nodes. It can also spread to other body parts. This is known as fourth stage breast cancer[19].

Prostate cancer- The prostate is a walnut-sized gland which is present behind the base of a man's penis, in front of the rectum, and below the bladder. Cancer starts when cells in the prostate grow uncontrolled, and formed a tumor[20].

Bladder cancer- bladder cancer starts when healthy cells in the bladder lining, most commonly urothelial cells, change and grow out of control, forming a mass called a tumor[21].

Different type of bleader cancers are Urothelial carcinoma, Squamous cell carcinoma, Adenocarcinoma.

Kidney cancer- cancer in which cells of one or both the kidney grow uncontrolled. And the formed a cluster of these abnormal cells known as a renal cortical tumor. Different types of kidney cancer are Renal cell carcinoma, Urothelial carcinoma, Lymphoma, Sarcoma**.**

Thyroid cancer- it begins when cells in thyroids grow out of control and form a mass known as tumor. Thyroid gland contains follicular and C cells.different types of thyroid cancer are Papillary thyroid cancer, Follicular thyroid cancer, Hurthle cell cancer.

2.2 Different cancer treatment therapy and their problems in cancer treatment

As we all know that prevention is better than cure, so it's better to prevent the chance of occurrence of cancer by avoiding use of carcinogenic products in daily life, for example tobacco smoking. It is our industrialised society which provokes such kind of unhealthy lifestyle and make humans more prone to cancers.

2.2.1 Surgery

Early detection of primary tumors and their removal before they reach metastatic state can be a life saver as in many cases of cervical cancer. Betterment in the area of cancer diagnosis can prove to be a milestone in preventing deaths caused by cancer every year. There is a need of highly efficient molecular assays for early detection of cancer so that tumor can't reach malignant state[22][19].

2.2.2 Chemotherapy

Tumours that became malignant are very difficult to eradicate. In many cases, it is generally seen that some of the cancerous cells were always remain and can't be completely removed even after surgery or chemotherapeutic destruction and eventually got resistant to chemotherapy. These cells proliferate again to become full grown tumors. Certain procedures like chemotherapy and use of anti-cancerous drugs proves to be very effective for the patients but in some patients, these shows the reverse effect and patients can't even withstand the toxicity generated by them in the body[23].

2.2.3 Radiation

Cancer targeting therapies utilise the properties like genetic instability of cancerous cells which help them to distinguish between normal cells and cancerous cells. Conventional treatment of cancer involves use of drugs and ionising radiation which targets DNA and the chromosomal machinery of cancerous cells and the cell ultimately dies of heavy DNA damage. Normal cells, when subjected to radiations, arrest their cell cycle till they repair DNA damage. So, defects in cell-cycle checkpoints in cancerous cells can be utilised to make effective drugs for better treatment of cancer. As these properties are very helpful for treating cancer but some of them makes cancerous cell resistant to any kind of treatment. For instance, defect in cell death program of cancerous cells makes them to escape apotosis and hence became a major concern in cancer treatment. This all shows that cancerous cells are different from each other, have different types of defects and response to anti-cancer therapy, so they can't be treated with single therapy and thus a patient need combination anti-cancer treatment[24].

Moreover, in many cases it is seen that a patient treated with single anticancer drug, develops resistance for that drug as well as to other drugs also, making the case very severe and complicated. Multidrug resistance associated with cancer is due to a gene called, *Mdr1,* this gene is related with pumping out of the lipophilic drugs from cell and hence, preventing them to gather into them. Scientific communities are working hard to find better treatment and diagnosis for cancer., moreover they also working hard to expand survival of patients. Certain methods are developed to treat cancer and to improve patient's life, for instance, to treat breast cancer, researchers used Tamoxifen which is an estrogen antagonist and is capable of blocking synthesis of estrogen which help in delaying or preventing recurrence of breast cancer[25][26].

2.3 Progression of Cancer Therapies

Various approaches have been discovered for targeted drug delivery to cure cancer. One of these studies include exploitation of a protein called Her2, which are present on cancerous cell's surface. Antibodies against various surface proteins like Her2, armed with anti-cancer drugs or enzymes which help in cleaving the existing prodrug in the system are raised which binds to the protein on the surface of cells via diffusion and release drugs at that site, which specifically kills the cancer cells[27]. But this technique is not viable for all the cancerous cells so, another technique used to target cancerous cells have been developed, for instance,

utilisation of cancer cell property i.e., loss of p53. Many viruses produce proteins that bind and help inactivating the p53 of host's cell[20]. This property enables viruses to inactivate the p53-mediated host cell's defense and replication of their own genome in host cell. They replicate continuously and burst out of the cell when their number increases so much, resulting in death of cell. Using this mechanism, adenoviruses are produced lacking gene for p53 blocking protein, which infects only those cells lacking activity of p53 gene, in this way cancer cell can be targeted and killed. These type of treatments for tumor destruction are under clinical trials and their efficacy is yet to be tested in humans, but still they raise hopes for patients having multi-drug resistance. Accurate diagnosis of cancer at an early stage is crucial for cancer treatment and treatment efficacy in patients. mRNA of cancerous tissue can be analysed by using DNA microarray for early detection of cancer, in this technique expression of genes are checked in single go and then the expression profiles of genes are compared with expression profiles of normal control tissue.

All these advancements are creating hopes for the cancer patients for better treatment and high life expectancy for those which were already cured from the diasese. The craving to comprehend that drives essential research will unquestionably uncover better approaches to utilize our insight into the cell for compassionate objectives, in connection to tumor, as well as with respect to infectious disease, psychological instability, horticulture, and different regions that we can barely predict.

2.4 Tumor antigens

Tumor antigens are the protein product of the tumor cells. These antigens may or may not be present on the normal cells. Some of these antigens are present on the normal cell also. But their expression is more in tumor cell as compare to the normal cell[5]. These are known as tumor shared antigen. But there are some unique antigen which are present only on the tumor cells not on the normal cell are known as tumor unique antigens. These antigens are the main source for the immunogenic response against the tumor cell. These are the unique antigens (not present on the normal cell) so if we immunotherapy using these as targets for immune cells, then there is no harmful effect of it on the normal cell.

Type of Unique Tumor Antigen	Name of Unique Tumor Antigen
Substitution Mutation	ACTN4,BRAF,CASP8,CDC27,CDK27,CDK4,EEF2,EFTUD2,FN1, GPNMB,MUM3,MYO1B,PAPOLG,UBXD5,SNRPD1
Alternative ORF	CASP5,CDKN2A,OGT,CAMEL
Intron Encoding	MUM1
Chromosomal Translocation	ABL-BCR,BCR-ABL,DEK-CAN,ETV6-AML1,LDLR-FUT,NPM1-ALK1,PAX3-FKHR,PML-RARA

2.5 Importance of tumor specific antigen in cancer treatment

MHC class l presents the short peptide on the surface of tumor cell. T cytotoxic cell recognise this peptide and MHC I complex and give immune response against it. MHC class II present the short peptide on the surface of tumor cell. T helper cell recognises this peptide and MHC II complex. Helper T cell never give direct immune response instead of that they help in the activation of cytotoxic T cell and macrophages to give immune response to target cell. Or the T helper cell stimulates B cells to secrete antibodies. APCs ingest microbes, degrade them, and export small part of it(antigen) to the cell surface with the help of MHC II. This MHC II and peptide complex then recognise by the receptors present on the helper cell surface known as $CD4^{+}$ receptors. But only this signal is not sufficient for activation of T cell but they required one more signal this provided by either by stimulation by a cytokine or by a costimulatory signal that is between B7 found on the surface APCs and the receptor protein CD28 found on the surface of helper T cell. So, when the first signal and one of second signal were received then the helper T cell becomes activated[28].

3 METHODOLOGY

3.1 DATA COLLECTION

Accession id. of tumor specific antigen and their isomers were retrieve from TANTIGEN database. There were 45 tumor specific antigens and their name listed below.

Table 1 list of all tumor specific Antigens

ACTN4	FN1	NFYC	SNRPD1	CAMAL	PAX3-FKHR
BRAF	GPNMB	NRAS	TPI1	MUM1	PML-RARA
CASP8	HHAT	OS9	TRAPPC1	ABL-BCR	SYT-SSX1
CDC27	HSPA1B	PAPOLG	UBXD5	BCR-ABL	SYT-SSX2
CDK4	KRAS	LPGAT1	ZUBR1	DEK-CAN	FLT3
CTNNB1	ME1	PRDX5	CASP5	ETVC-AMLY	-
EEF2	MUM3	PTPRK	CDKN2A	LDLR-FUT	-
EFTUD2	MYO1B	SIRT2	OGT	HPM1-ALK1	-

A python code had to be run for the collection of sequences of these tumor specific antigen.

3.1.1 Python code for antigen sequence collection

```
import urllib.request

from bs4 import BeautifulSoup
```

```
def read_file(f):
    file = open(f)
    file_contain = file.read()
    return file_contain.split()
def deleteContent(pfile):
    pfile.seek(0)
    pfile.truncate()
main_url = 'http://cvc.dfci.harvard.edu/cvccgi/tadb/displayAntigen.pl?ACC='
ac_id_list = read_file('ac_id.txt')
f = open('result.txt', 'w')
deleteContent(f)
count=0
for ac_id in ac_id_list:
    page = urllib.request.urlopen(main_url+ac_id)
    soup = BeautifulSoup(page, 'html.parser')
    table = soup.find("table")
    last_row = table("tr")[-2]
    seq = last_row('td')[-1]
    seq_a = seq.get_text()
    f.write(ac_id+'\n')# python will convert \n to os.linesep
    f.write(seq_a+'\n'+'\n')
    count=count+1
    print('ac_id '+str(count)+ '   done')
```

```
    #seq_a = ''.join(str(e) for e in seq_a)

    #seq_f=''.join(list(seq_a))

f.close()

print('done')
```

Consenses sequences were find by multiple sequence allingment of all the isomers of that tumor specific antigens.

We selected the complete sequence of one isomer because that was approximatly common in all the isomers of that antigen.

3.2 Epitope prediction of T-cytotoxic cell

The antigen sequences are first devided into small peptide which binds to MHC1 then these MHC1-peptide complexs bind to the $CD8^+$ receptor of T-cytotoxic cell. So we first predicted MHC1 binding peptide then $CD8^+$ receptors binding.

3.2.1 MHC I binding peptide prediction

We use IEDB prediction tool Tepitool for the prediction of MHC I binding peptide. because this is a user friendly tool. And we can manually select the different attributes here.

3.2.1.1 Alleles selection

A set of alleles selected on the bases of their population frequencies. We use 38 most aboundant alleles set of HLA class l which covers almost 99% population of the world.

3.2.1.2 IC^{50} value selection

The ic^{50} value of all these alleles are more than 100 nm , so we select a threshold of less than or equal to 100 nm for best prediction results.

3.2.1.3 Prediction method selection

We used netMHC1pan method for the prediction of mhc1 binding peptides. This is the only method which predict peptide on the bases of IC^{50} value.

3.2.2 $CD8^+$ immunogenicity prediction

We used IEDB CD8+ immunogenicity prediction tool with the default setting. In this tool automatically masking of 1,2 and C-terminal of amino acid take place. Prediction score is calculated for all MHC I binding peptide and select only those peptide whose prediction score is more than zero.

3.2.3 Population coverage of T-cytotoxic epitope

We used POPULATION ANALYSIS tool for the calculation of population coverage of selected peptide and select those peptide which cover most of the population.

3.3 T-helper cell epitope prediction

Tumor antigen are the internal peptides so presented by mhc1 but these tumor cell phegocytosis by dendritic cells which are antigen presenting cells . they present these antigens peptides to the T-helper cell.

We have to predict mhcll binding peptides followed by their $CD4^+$ immunogenicity prediction.

3.3.1 MHC Il binding peptide prediction

We use IEDB prediction tool Tepitool for the prediction of MHC Il binding ppeptide. And we can select different attributes here.

3.3.1.1 Alleles selection

We use 26 most aboundant alleles of MHC Il. These alleles IC^{50} value threshold cutoff selected here is 1000.

3.3.1.2 Prediction method selection

We use here a set of methods for prediction of most promisiouse binding of HLA ll alleles. In this only those peptide are selected which bind to atleast 50% of alleles from these 26 alleles.

3.3.2 $CD4^+$ immunogenicity prediction

We use IEDB prediction tool for the prediction of immunogenicity of the MHC Il binding peptides. In this immunogenicity score of all the peptide are given and we have to select only those peptide which have immunogenicity score more than zero.

3.3.3 Population coverage

We used IEDB population coverage prediction tool for the analysis of population coverage in india. We selected those peptide which cover most population.

3.4 Combined population coverage of MHC1 and MHC11

We calculated combined population coverage of selected peptide of both mhc1 and mhc11 by population analysis tool.

4 Results

4.1 DATA COLLECTION

Accession id. of tumor specific antigen and their isomers were retrieve from TANTIGEN database. There were 45 tumor specific antigens.

A python code had to be run for the collection of sequences of these tumor specific antigen.

For these 45 sequences we have to select the consense sequence which we find with the multiple sequence allingment of all the isomers of that protein. This data is also available on the TANTIGEN database.

We get 17 antigens consense sequence which can be selected for the further prediction. For all other protein we either not able to get full length sequence or they have too short sequence so we cant predict targets from them.

In some cases we select the complete sequence of one isomer that is known as reference sequence. In other cases we select the peptide part of the sequences which is common in most of the isomers.

From these sequences we have to predict the mhcl binding peptide and mhcII binding peptide.

4.2 T-cytotoxic cell epitope prediction

The antigen sequences are first devided into small peptide which binds to MHC1 then these MHC1-peptide complexs bind to the $CD8^+$ receptor of T-cytotoxic cell. So we first predicted MHC1 binding peptide then $CD8^+$ receptors binding.

4.2.1 MHC I binding predictions

Now from these consensus sequences we have to predict the mhc1 binding peptide. We predicted them on the bases of their IC^{50} value. We selected only those peptides whose predicted IC^{50} value <100. Because we have to select best binders. For this we used tepitool as a prediction tool. It include 6 steps in it to predict mhc1 binding peptides.

4.2.1.1 Input antigen sequence

In first step we have to upload the file containing sequence of the tumor antigen in fasta format.

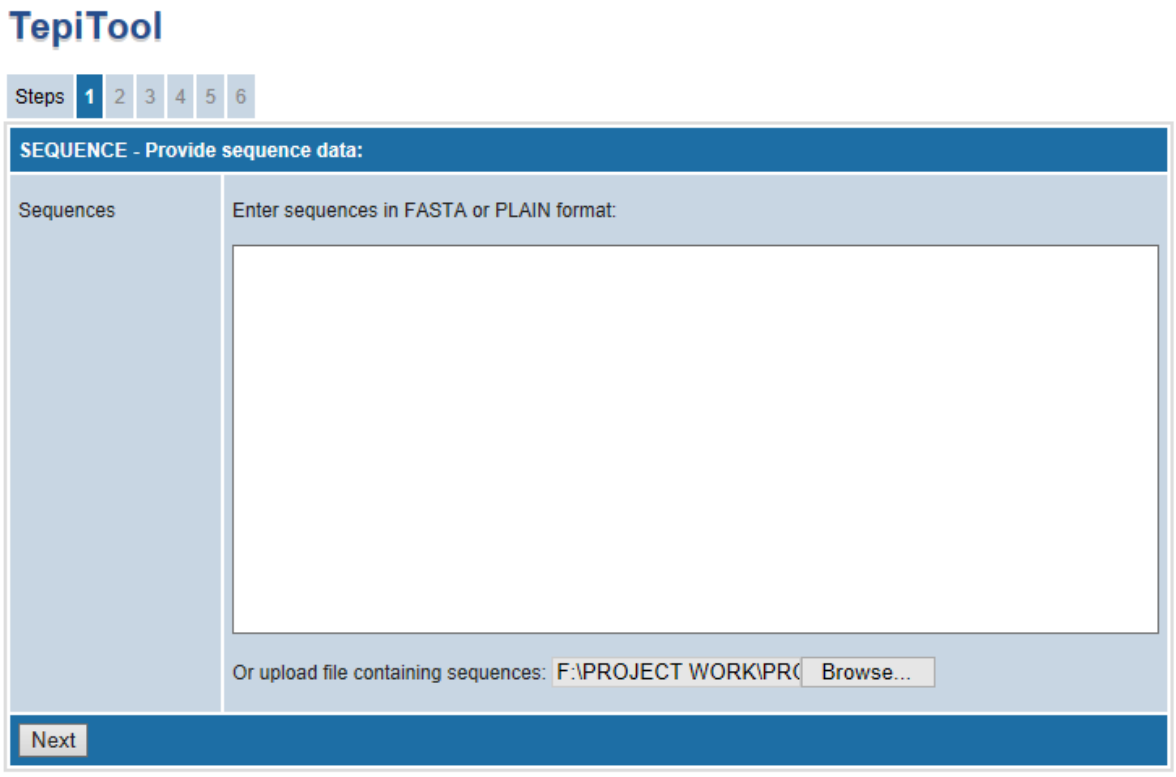


Figure 1 Tepitool prediction – input sequence

4.2.1.2 Selection of host species and MHC allele class

In second step we have to select the host species in our case that is human.

And we have to select mhc allele class in this case that is mhc1 allele.

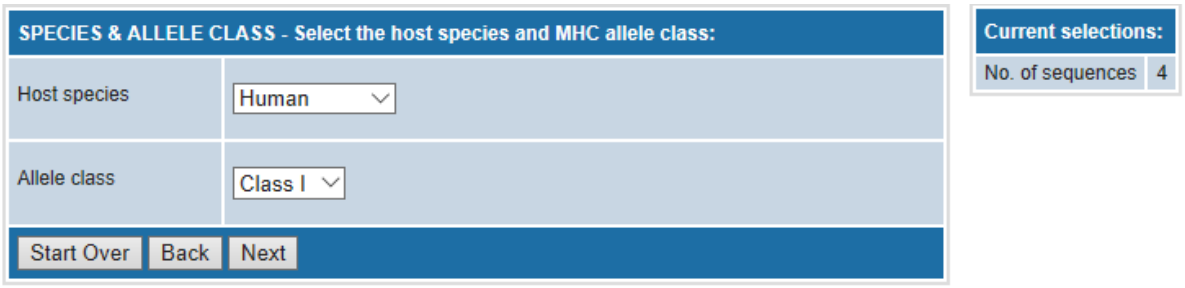


Figure 2 Tepitool prediction- species selection & alleles selection

4.2.1.3 Input list of selected alleles

In third step we have to put the list of mhc1 allele which have to bind to the peptide so we select the 38 most frequently occurring alleles in human population.

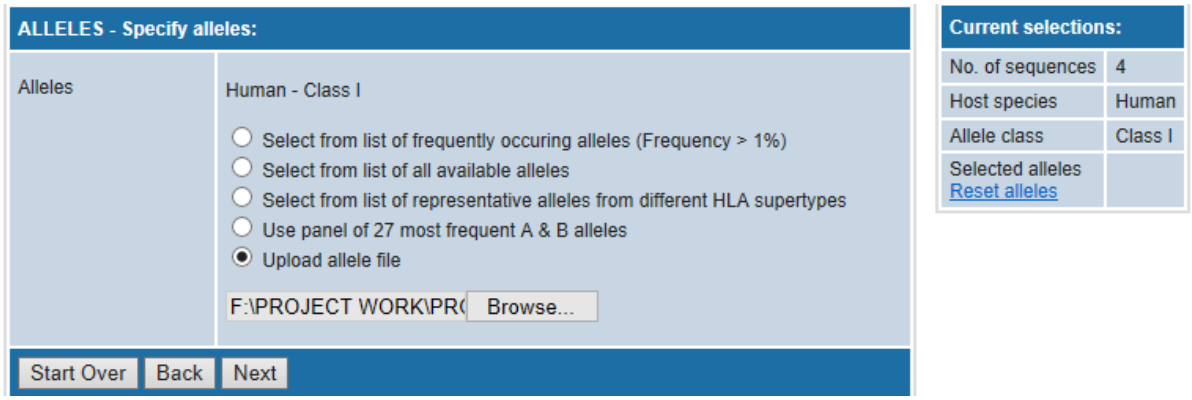


Figure 3 Tepitool prediction- input alleles list

4.2.1.4 Selection of peptides to be included in prediction

In forth step we have to select peptide which have to be included in prediction. We have to remove duplicate to reduce the no. of peptide. Have we have only predict 9 mer peptides only.

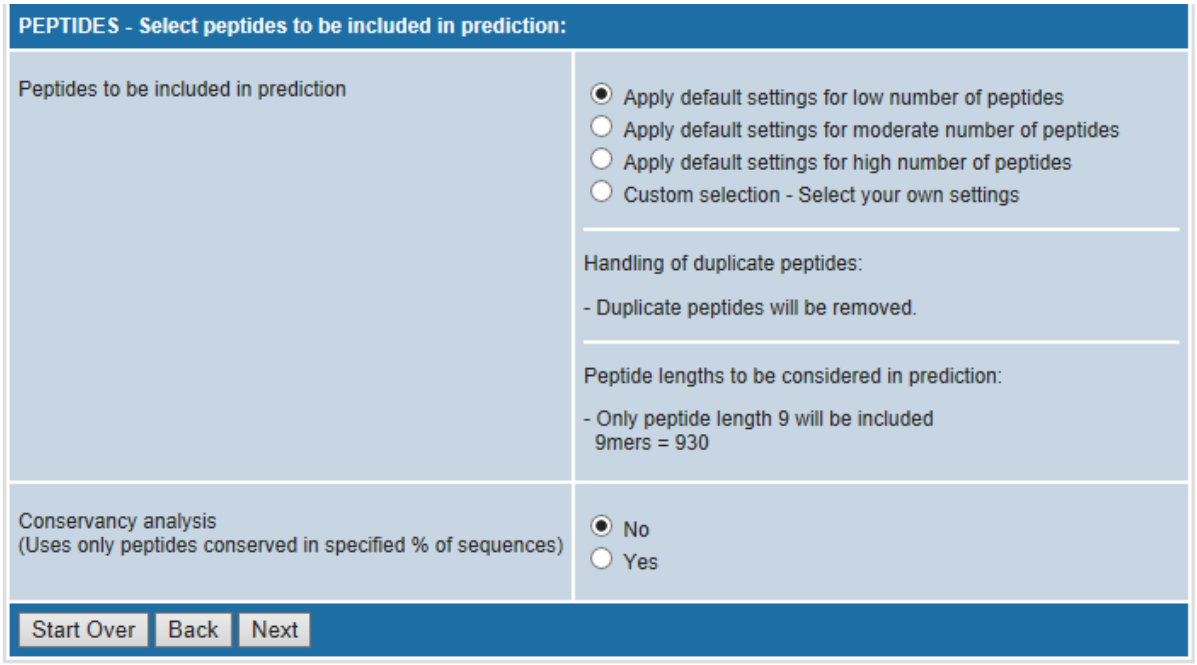


Figure 4 Tepitool prediction- selection of peptide

4.2.1.5 Select prediction & peptide selection and cutoff values

In this step we have to prediction and peptide selection methods and cutoff values. We select peptide on the base of predicted IC_{50} value and select the cutoff value 100.

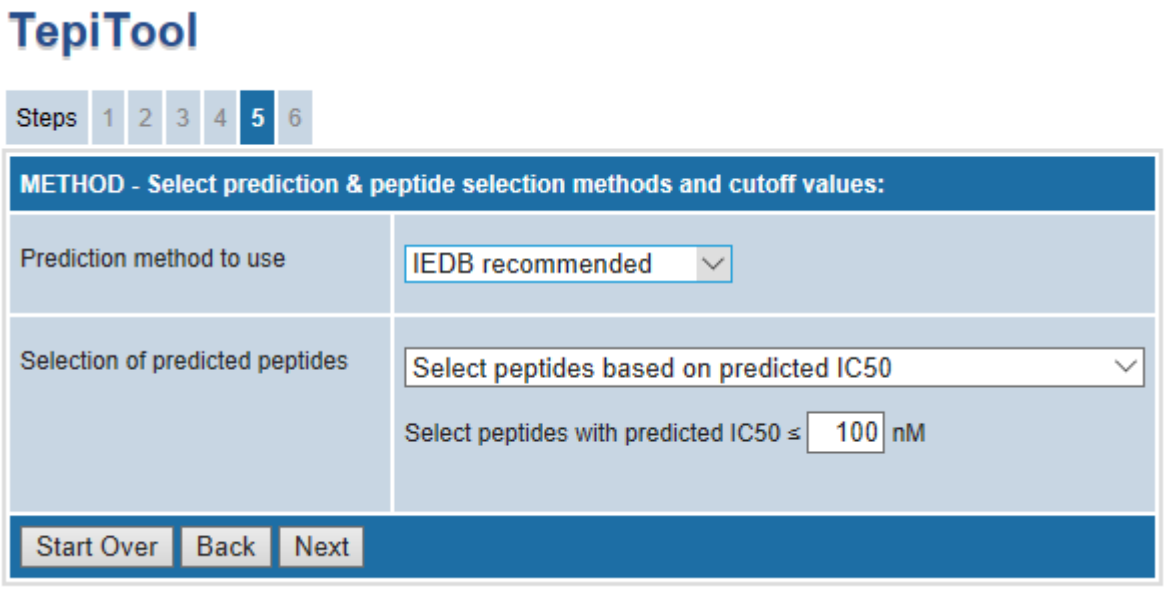


Figure 5 Select prediction method & selection of predicted peptide

4.2.1.6 Summary of all the selections & Antigen name

In this step we have to review all the sections. Than enter Antigen details and submit data.

Summary:	
No. of sequences	17
Host species	Human
Allele class	Class I
Alleles	1.A*01:01 2.A*02:01 3.A*02:03 4.A*02:06 5.A*03:01 6.A*11:01 7.A*23:01 8.A*24:02 9.A*25:01 10.A*26:01 11.A*29:02 12.A*30:01 13.A*30:02 14.A*31:01 15.A*32:01 16.A*33:01 17.A*68:01 18.A*68:02 19.B*07:02 20.B*08:01 21.B*14:02 22.B*15:01 23.B*18:01 24.B*27:05 25.B*35:01 26.B*35:03 27.B*38:01 28.B*39:01 29.B*40:01 30.B*40:02 31.B*44:02 32.B*44:03 33.B*46:01 34.B*48:01 35.B*51:01 36.B*53:01 37.B*57:01 38.B*58:01
Duplicate peptides	Removed
Peptide lengths selected	9mers
Approx no. of peptides included	823
Peptide overlap	N/A (all possible nmers are included in class I)
Conservancy analysis	No
Prediction method	IEDB recommended
Peptide selection criterion	Based on predicted IC50 (Cutoff selected = 500nM)
Job details:	
Job name (optional)	CASP8

Figure 6 tepitool- summary of all selection

After that we have to download results. You can get them on your mail id too.

4.2.1.7 Results of MHC I Binding peptide

No. of MHC I binding peptide for each tumor antigen listed in the table below.

Table 2 No. of predicted peptide and alleles combination for each antigen

ANTIGEN NAME	NO. OF PREDICTED PEPTIDE & ALLELES COMBINATION		ANTIGEN NAME	NO. OF PREDICTED PEPTIDE & ALLELES COMBINATION
ACTN4	156		CTNNB1	158
BRAF	124		EEF2	202
CAMEL	26		FLT3	67
CASP5	60		GPNMB	164
CASP8	84		HSPA1B	86
CDC27	179		KRAS	37
CDK4	76		MUM1	110
CDKN2A	13		PAPOLG	142
TPI1	47		UBXD5	93

For example, we show here results of **ACTN4** MHCI Binding results

Table 3 Result of MHC I binding peptide and alleles combination of ACTN4

Peptide	IC50	Allele
MTLGMIWTI	3.9	HLA-A*02:06
MTLGMIWTI	5.7	HLA-A*32:01
MTLGMIWTI	7.9	HLA-A*68:02

KQLEAIDQL	8	HLA-A*02:06
FTAWCNSHL	8.3	HLA-A*68:02
TAWCNSHLR	8.4	HLA-A*68:01
YVSSFYHAF	8.6	HLA-B*35:01
HAANQSYQY	9.5	HLA-B*35:01
LAFNALIHR	9.9	HLA-A*68:01
MTLGMIWTI	10.1	HLA-A*02:01
KLRLSNRPA	10.1	HLA-A*30:01
FIVHTIEEI	10.2	HLA-A*02:06
ETATLSDIK	10.3	HLA-A*68:01
MTYVSSFYH	10.8	HLA-A*68:01
CQRKTAPYK	11.1	HLA-A*30:01
AIMTYVSSF	11.4	HLA-B*15:01
HSRREALEK	11.7	HLA-A*30:01
GEAEFNRIM	12	HLA-B*40:01
IMTYVSSFY	12.1	HLA-A*30:02
FIVHTIEEI	12.2	HLA-A*02:03
ETAANRICK	12.9	HLA-A*68:01
MVSDINNGW	13.1	HLA-B*58:01
GLVTFQAFI	13.4	HLA-A*02:03
KALDFIASK	13.5	HLA-A*11:01
TIARTINEV	13.6	HLA-A*02:03
NAFEVAEKY	13.7	HLA-B*35:01
AEFNRIMSL	14.4	HLA-B*40:01
TLSDIKALI	14.7	HLA-A*02:03
YEKLASDLL	14.8	HLA-B*40:01
KALDFIASK	16.7	HLA-A*30:01
RTIPWLEDR	17.1	HLA-A*31:01
HTNYTMEHI	17.2	HLA-A*68:02
TIARTINEV	17.8	HLA-A*68:02
KLEDFRDYR	18.1	HLA-A*31:01
DAVPGALDY	18.1	HLA-B*35:01
YVSSFYHAF	20.1	HLA-B*15:01
AIDQLHLEY	20.2	HLA-A*01:01
QAFIDFMSR	20.2	HLA-A*68:01
MTLGMIWTI	21.1	HLA-B*58:01
LSHLKQYER	21.6	HLA-A*31:01
MVSDINNGW	22	HLA-B*53:01
REREAILAI	22.9	HLA-B*40:01
LAFNALIHR	23.1	HLA-A*31:01
LPKPERGKM	23.5	HLA-B*07:02
HTIEEIEGL	23.5	HLA-A*68:02
SAKEGLLLW	24.1	HLA-B*58:01
IMTYVSSFY	24.2	HLA-B*15:01

KTFTAWCNS	24.4	HLA-A*30:01
KLASDLLEW	24.5	HLA-B*58:01
HLMEDYEKL	25.3	HLA-A*02:06
HLMEDYEKL	25.5	HLA-A*02:01
YEEWLLNEI	26.6	HLA-B*40:01
EIVDGNAKM	26.7	HLA-A*26:01
KLMLLLEVI	27.1	HLA-A*02:03
GLVTFQAFI	27.2	HLA-A*02:01
FIVHTIEEI	28.3	HLA-A*02:01
KMRVHKINN	29.6	HLA-A*30:01
KLMLLLEVI	29.7	HLA-A*02:01
MEEIGRISI	30.1	HLA-B*40:01
AIMTYVSSF	30.7	HLA-A*32:01
KVQQLVPKR	31.5	HLA-A*31:01
TAWCNSHLR	32	HLA-A*31:01
MTYVSSFYH	32	HLA-A*11:01
AVPGALDYK	32	HLA-A*11:01
QTKLRLSNR	32.5	HLA-A*31:01
QMQEFRASF	32.5	HLA-B*15:01
VPKRDHALL	32.6	HLA-B*07:02
RQKASIHEA	32.9	HLA-A*30:01
KMLDAEDIV	34.4	HLA-A*02:01
WTIILRFAI	34.7	HLA-A*68:02
KMLDAEDIV	35.6	HLA-A*02:06
FMPSEGKMV	35.8	HLA-A*02:03
QIINSKWEK	35.8	HLA-A*68:01
HLMEDYEKL	36.8	HLA-A*02:03
YCIARMAPY	37.5	HLA-B*35:01
KTIQEMQQK	38.1	HLA-A*11:01
QIINSKWEK	38.8	HLA-A*11:01
KLMLLLEVI	39.3	HLA-A*02:06
ISWKDGLAF	39.6	HLA-B*58:01
RQFASQANV	42.8	HLA-A*02:06
TAWCNSHLR	44	HLA-A*33:01
LLLDPAWEK	46.1	HLA-A*11:01
ELIEYDKLR	46.8	HLA-A*68:01
TADQVIASF	46.8	HLA-B*35:01
LEVISGERL	47.6	HLA-B*40:01
NFITAEELR	47.7	HLA-A*33:01
YETATLSDI	48.2	HLA-B*40:01
GMIWTIILR	48.6	HLA-A*31:01
MTYVSSFYH	48.6	HLA-A*31:01
RIAESNHIK	48.7	HLA-A*11:01
RASFNHFDK	48.7	HLA-A*30:01

KLMLLLEVI	49.6	HLA-A*32:01
VISGERLPK	50.4	HLA-A*11:01
HTIEEIEGL	51.6	HLA-A*02:06
FTAWCNSHL	52.7	HLA-A*02:06
SAKEGLLLW	52.9	HLA-B*57:01
WLLNEIRRL	53.1	HLA-A*02:03
MTLGMIWTI	53.7	HLA-A*02:03
VQNFHISWK	54.4	HLA-A*03:01
MTLGMIWTI	55.1	HLA-A*23:01
LAIHKEAQR	55.2	HLA-A*68:01
VQNFHISWK	55.3	HLA-A*11:01
FTAWCNSHL	55.8	HLA-A*02:03
KQQSNEHLR	56.2	HLA-A*31:01
RQFASQANV	56.2	HLA-A*02:03
SAMEDLQDM	58.3	HLA-B*35:01
MVSDINNGW	60.1	HLA-B*57:01
SNRPAFMPS	60.2	HLA-A*30:01
ILRFAIQDI	61	HLA-A*02:03
NVNKALDFI	62.6	HLA-A*68:02
QMQEFRASF	63.1	HLA-A*32:01
RASFNHFDK	64.3	HLA-A*11:01
RAAPFNNWM	65.2	HLA-B*58:01
TPQIINSKW	66.3	HLA-B*53:01
KALDFIASK	67.4	HLA-A*31:01
LIHRHRPEL	67.5	HLA-B*08:01
WLLNEIRRL	68	HLA-A*02:01
YVSSFYHAF	68.1	HLA-A*23:01
IMTYVSSFY	68.2	HLA-A*03:01
QLIQEALIF	69.7	HLA-B*15:01
VLAGDKNFI	70.3	HLA-A*02:03
ALIRKHEAF	71.4	HLA-B*15:01
TIARTINEV	71.6	HLA-A*02:06
ISWKDGLAF	73.1	HLA-B*35:01
VPQKTIQEM	73.8	HLA-B*07:02
TLSDIKALI	75.3	HLA-A*02:01
ISWKDGLAF	75.9	HLA-B*15:01
KALDFIASK	77.9	HLA-A*03:01
LAFNALIHR	78.3	HLA-A*33:01
KSFSTALYG	78.4	HLA-A*30:01
APYQGPDAV	78.6	HLA-B*07:02
CLISLGYDV	80.2	HLA-A*02:01
HAANQSYQY	81.2	HLA-A*30:02
LSHLKQYER	83.7	HLA-A*68:01
RIAESNHIK	84.4	HLA-A*30:01

QTKMEEIGR	85	HLA-A*68:01
ALGSLTHSR	85.5	HLA-A*31:01
KQLEAIDQL	86.1	HLA-A*02:01
CLISLGYDV	86.2	HLA-A*02:03
KACLISLGY	87.7	HLA-A*30:02
AEFNRIMSL	88.2	HLA-B*44:03
VVGPWIQTK	88.4	HLA-A*11:01
YCIARMAPY	88.4	HLA-A*26:01
TLEDQLSHL	91.9	HLA-A*02:03
LTHSRREAL	92.4	HLA-B*08:01
FIASKGVKL	92.6	HLA-A*02:03
RLSNRPAFM	93.1	HLA-A*02:03
QTKLRLSNR	93.9	HLA-A*33:01
LISAHDQFK	93.9	HLA-A*68:01
GPDAVPGAL	94.6	HLA-B*07:02
WLLNEIRRL	95.7	HLA-A*02:06
FITAEELRR	96.8	HLA-A*68:01
DQAEYCIAR	96.8	HLA-A*33:01
LEAIDQLHL	99.3	HLA-B*40:01
FIVHTIEEI	99.6	HLA-A*68:02
MQQKLEDFR	99.7	HLA-A*31:01

4.2.2 Class 1 immunogenicity prediction

These MHC I binding ligands have to bind to the $CD8^+$ receptors of the cytotoxic T cell . All these peptid- mhc complex does not have the ability to bind with the $CD8^+$ receptor so they can produce immune response. So for that we have to find immunogenicity.

For immunogenicity prediction we select all the unique peptide sequence from the MHC I binding results. Then use them as the input sequence for immunogenicity prediction.

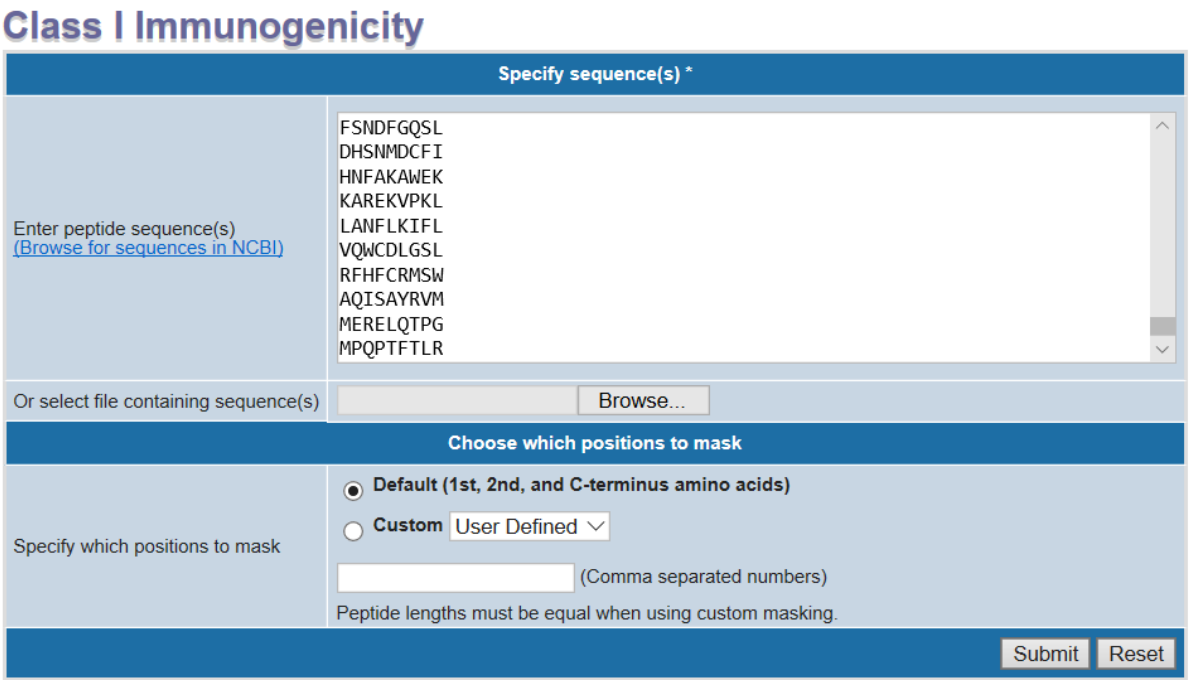


Figure 7 CD8+ Immunogenicity prediction

4.2.2.1 CD8⁺ Immunogenicity Prediction for tumor antigens binding to MHC I

Immunogenicity of these peptide is predicted on the bases of immunogenicity score. We selected only those peptide which have immunogenicity score more than zero.

Table 4 Immunogenic peptide no. for each antigen

ANTIGEN NAME	NO. OF IMMUNOGENIC PEPTIDE		ANTIGEN NAME	NO. OF IMMUNOGENIC PEPTIDE
ACTN4	39		CTNNB1	34
BRAF	56		EEF2	16
CAMEL	32		FLT3	25
CASP5	28		GPNMB	19
CASP8	19		HSPA1B	25
CDC27	30		KRAS	20

CDK4	15		MUM1	18
CDKN2A	13		PAPOLG	142
TPI1	47		UBXD5	93

Results of ACTN4 Immunogenicity Prediction are listed below.

Table 5 Results of ACTN4 immunogenicity prediction

Peptide	Score
GMIWTIILR	0.53501
HTIEEIEGL	0.47103
FIVHTIEEI	0.35203
GEAEFNRIM	0.3428
WTIILRFAI	0.3367
RTIPWLEDR	0.33476
REREAILAI	0.29443
NFITAEELR	0.29263
LLLDPAWEK	0.29019
YEEWLLNEI	0.28719
WLLNEIRRL	0.28659
TIARTINEV	0.2809
KTFTAWCNS	0.27439
VVGPWIQTK	0.26574
FITAEELRR	0.2646
MEEIGRISI	0.2638
VQNFHISWK	0.26228
HSRREALEK	0.25235
KMLDAEDIV	0.24755
KLEDFRDYR	0.2341
RAAPFNNWM	0.23341
ILRFAIQDI	0.21318
KQLEAIDQL	0.21157
RASFNHFDK	0.20001
MTLGMIWTI	0.1944
KALDFIASK	0.19436
DQAEYCIAR	0.19428
LIHRHRPEL	0.19194
LAFNALIHR	0.19037
ETAANRICK	0.17531
NAFEVAEKY	0.17428
QMQEFRASF	0.16223

GLVTFQAFI	0.15884
LEVISGERL	0.13286
QLIQEALIF	0.12937
GPDAVPGAL	0.12779
VISGERLPK	0.11078
MVSDINNGW	0.10647
RLSNRPAFM	0.08117
QTKMEEIGR	0.07717
DAVPGALDY	0.07567
AVPGALDYK	0.07472
LEAIDQLHL	0.06872
VPKRDHALL	0.06067
QAFIDFMSR	0.05886
FTAWCNSHL	0.05628
RIAESNHIK	0.05132
LTHSRREAL	0.05051
SAKEGLLLW	0.03747
ALIRKHEAF	0.02309
HLMEDYEKL	0.02037
RQKASIHEA	0.01935
KLMLLLEVI	0.01922
LISAHDQFK	0.00869

4.2.3 Population coverage results of class1 epitopes

T cells recognize a complex between a specific major histocompatibility complex (MHC) molecule and a particular pathogen-derived epitope. A given epitope will elicit a response only in individuals that express an MHC molecule capable of binding that particular epitope. MHC molecules are extremely polymorphic and over a thousand different human MHC (HLA) alleles are known. A disproportionate amount of MHC polymorphism occurs in positions constituting the peptide-binding region, and as a result, MHC molecules exhibit a widely varying binding specificity. In the design of peptide-based vaccines and diagnostics, the issue of population coverage in relation to MHC polymorphism is further complicated by the fact that different HLA types are expressed at dramatically different frequencies in different ethnicities. Thus, without careful consideration, a vaccine or diagnostic with ethnically biased population coverage could result[29].

Table 6 ACTN4 Class 1 population coverage

Epitope list	Population coverage
Epitope #1: KALDFIASK	42.27%
Epitope #2: MTYVSSFYH	35.68%
Epitope #3: VQNFHISWK	29.40%
Epitope #4: MTLGMIWTI	27.76%
Epitope #5: LAFNALIHR	20.14%
Epitope set	**67.53%**

Table 7 BRAF class 1 population coverage

Epitope list	Population coverage
Epitope #1: RSNCPKAMK	42.27%
Epitope #2: MIKLIDIAR	20.14%
Epitope #3: SLYHHLHII	19.18%
Epitope #4: TLAFCDFCR	20.14%
Epitope #5: SFQSDVYAF	18.18%
Epitope set	**74.03%**

Table 8 CAMEL class 1 population coverage

Epitope list	Population coverage
Epitope #1: FLMAQGAML	18.89%
Epitope #2: RMAVPLLRR	37.98%
Epitope #3: RPWKRSWSA	6.24%
Epitope #4: LMAQEALAF	10.14%
Epitope #5: CTSRCLSRR	19.31%
Epitope set	**63.92%**

Table 9 CASP5 class 1 population coverage

Epitope list	Population coverage
Epitope #1: SVLRAFAAR	36.41%
Epitope #2: KSFEVPQAK	33.99%
Epitope #3: LLYDTIFQI	19.18%
Epitope #4: SSTPHNVSW	13.77%
Epitope #5: NVSWRDRTR	10.44%
Epitope set	**68.43%**

Table 10 CASP8 class 1 population coverage

Epitope list	Population coverage
Epitope #1: IINNHNFAK	42.27%
Epitope #2: TVNNCVSYR	36.41%
Epitope #3: ILTEVNYEV	15.90%
Epitope #4: LSFLKELLF	13.77%
Epitope #5: MLEESNLSF	10.14%
Epitope set	**70.16%**

Table 11 CDC27 class 1 population coverage

Epitope list	Population coverage
Epitope #1: HSGHFVALK	41.71%
Epitope #2: CIFAEMFRR	36.41%

Epitope #3: RIYSYQMAL	24.10%
Epitope #4: NPHKRISAF	12.03%
Epitope #5: LTPVVVTLW	13.77%
Epitope set	**72.51%**

Table 12 CDK4 class 1 population prediction

Epitope list	Population coverage
Epitope #1: HSGHFVALK	41.71%
Epitope #2: CIFAEMFRR	36.41%
Epitope #3: RIYSYQMAL	24.10%
Epitope #4: NPHKRISAF	12.03%
Epitope #5: LTPVVVTLW	13.77%
Epitope set	**72.51%**

Table 13 CDKN2A lass 1 population coverage

Epitope list	Population coverage
Epitope #1: LPVDLAEEL	6.84%
Epitope #3: DWLATAAAR	0.92%
Epitope #4: LTRPVHDAA	5.47%
Epitope set	**12.77%**

Table 14 CTNNB1 class 1 population coverage

Epitope list	Population coverage
Epitope #1: IMRTYTYEK	42.27%
Epitope #2: TTAPSLSGK	37.40%
Epitope #3: RLHYGLPVV	20.92%
Epitope #4: YAAAVLFRM	20.22%
Epitope #5: ATYAAAVLF	16.30%
Epitope set	**69.79%**

Table 15 EEF2 class 1 population coverage

Epitope list	Population coverage
Epitope #1: IMRTYTYEK	42.27%
Epitope #2: TTAPSLSGK	37.40%
Epitope #3: RLHYGLPVV	20.92%
Epitope #4: YAAAVLFRM	20.22%
Epitope #5: ATYAAAVLF	16.30%
Epitope set	**69.79%**

Table 16 FLT3 class 1 population coverage

Epitope list	Population coverage
Epitope #1: RMPEAAPPV	15.90%
Epitope #2: KTYQGSYGF	20.70%
Epitope #3: CTYSPALNK	41.71%
Epitope #4: RVRAMAIYK	42.27%
Epitope #5: TAKSVTCTY	10.14%
Epitope set	**71.44%**

Table 17 GPNMB class 1 population coverage

Epitope list	Population coverage
Epitope #1: TIVEGILEV	15.90%
Epitope #2: ITFAVNLIF	20.70%
Epitope #3: YVLNGTFSL	24.01%
Epitope #4: RTFNGSGTY	45.21%
Epitope #5: YVFHTLGQY	13.29%
Epitope set	**63.02%**

Table 18 HSPA1B class 1 population coverage

Epitope list	Population coverage
Epitope #1: LLLLDVAPL	15.90%
Epitope #2: AEAYLGYPV	7.03%
Epitope #3: RTTPSYVAF	20.70%
Epitope #4: VIAGLNVLR	19.31%
Epitope #5: GVMTALIKR	35.68%
Epitope set	**62.71%**

Table 19 KRAS class 1 population coverage

Epitope list	Population coverage
Epitope #1: KSFEDIHHY	13.77%
Epitope #2: CVFAINNTK	37.40%
Epitope #3: LVREIRQYR	20.14%
Epitope #4: CLLDILDTA	15.90%
Epitope #5: QYMRTGEGF	18.18%

Epitope set	**73.39%**

Table 20 MUM1 class 1 population coverage

Epitope list	Population coverage
Epitope #1: YAADISYPV	20.93%
Epitope #2: KMKGFTVSL	21.86%
Epitope #3: RSFEVGMLV	22.76%
Epitope #4: GMLVWHKHK	12.30%
Epitope #5: LSSSFTCEK	31.61%
Epitope set	**64.35%**

Table 21 PAPOLG class 1 population coverage

Epitope list	Population coverage
Epitope #1: HLMPIITPA	15.90%
Epitope #2: CTIPTVVGR	36.41%
Epitope #3: KIFTFGSYR	50.13%
Epitope #4: RSDFFQSFF	20.59%
Epitope #5: YPNAAASTL	9.49%
Epitope set	**74.52%**

Table 22 TPI1 class 1 population coverage

Epitope list	Population prediction
Epitope #1: HLMPIITPA	15.90%
Epitope #2: CTIPTVVGR	36.41%
Epitope #3: KIFTFGSYR	50.13%
Epitope #4: RSDFFQSFF	20.59%
Epitope #5: YPNAAASTL	9.49%
Epitope set	**74.52%**

Table 23 UBXD5 class 1 population coverage

Epitope list	Population coverage
Epitope #1: RTLEPIPLK	42.27%
Epitope #2: SAFEIFSTF	22.85%
Epitope #3: RLYPNGVPF	9.13%
Epitope #4: MTAEKFLNR	36.41%
Epitope #5: MAFMTRKLW	14.60%
Epitope set	**64.77%**

4.3 T-helper cell epitope prediction

Tumor antigen are the internal peptides so presented by mhc1 but these tumor cell phegocytosis by dendritic cells which are antigen presenting cells . they present these antigens peptides to the T-helper cell.

We have to predict mhcll binding peptides followed by their $CD4^+$ immunogenicity prediction.

4.3.1 MHC II binding prediction

Now from these consensus sequences we have to predict the mhc11 binding peptide. We predicted them on the bases of their IC^{50} value. We selected only those peptides whose predicted IC^{50} value <200. Because we have to select best binders. For this we used tepitool as a prediction tool. It include 6 steps in it to predict mhc11 binding peptides.

4.3.1.1 Input antigen sequence

We have to select the sequence file of the tumor antigen in the fasta format.

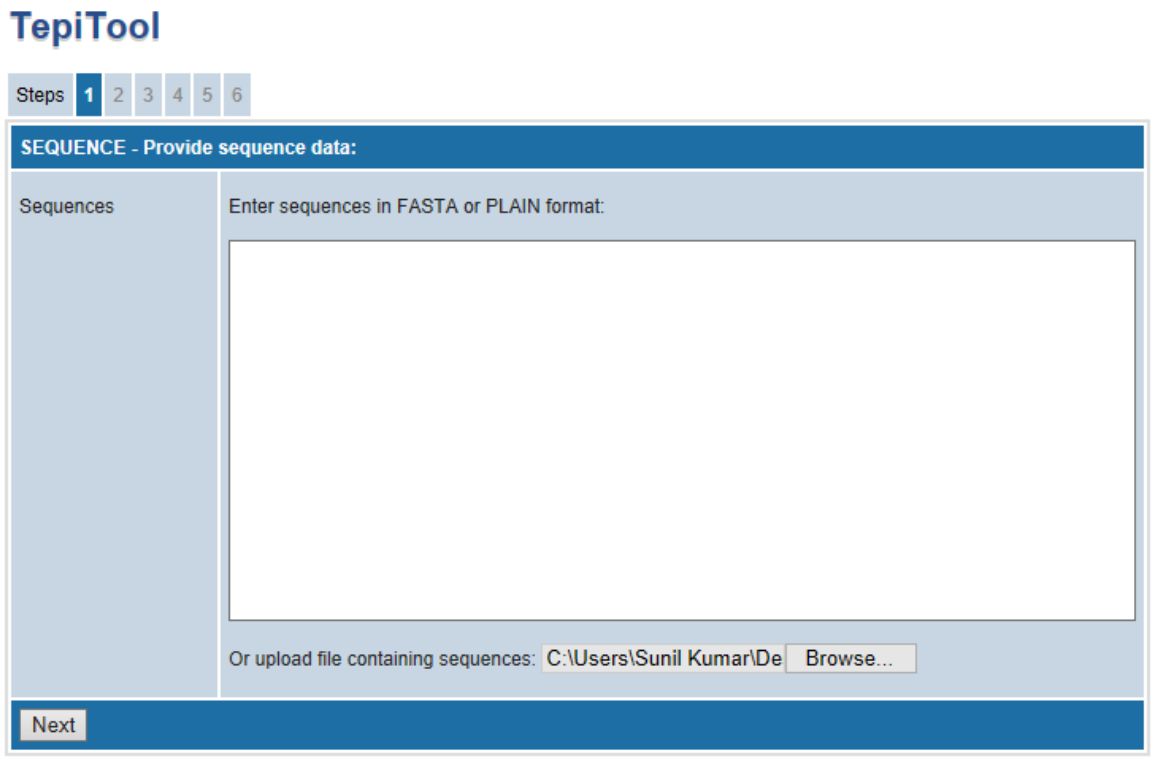


Figure 8 tepitool mhc11 binding prediction - input sequence

4.3.1.2 Selection of host species and MHC allele class

Here we have to select the host species in our case that is human.

And we have to select mhc allele class in this case that is MHC II class.

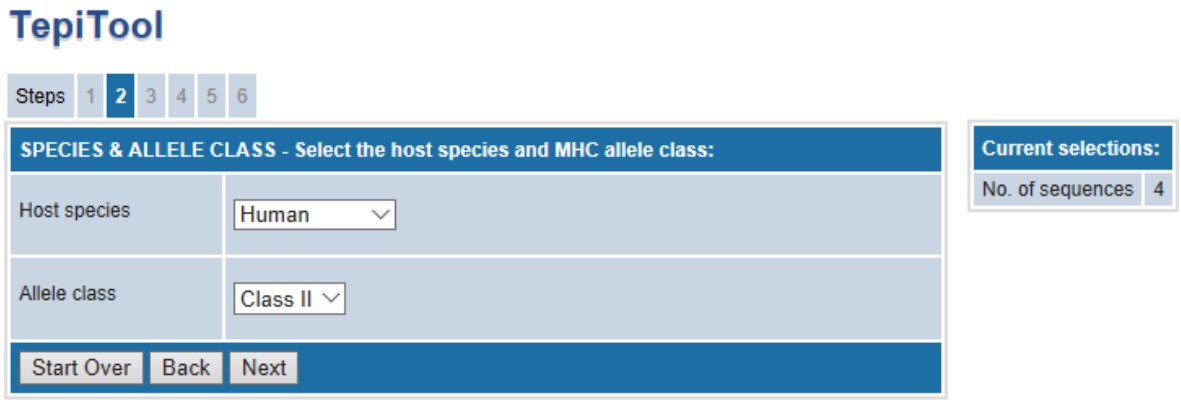


Figure 9 tepitool mhc11 binding prediction-species & alleles selection

4.3.1.3 Selection of MHC II alleles

Here we have to select MHC II alleles which have to bind to the peptide in this case we select 26 most frequent occurring allele.

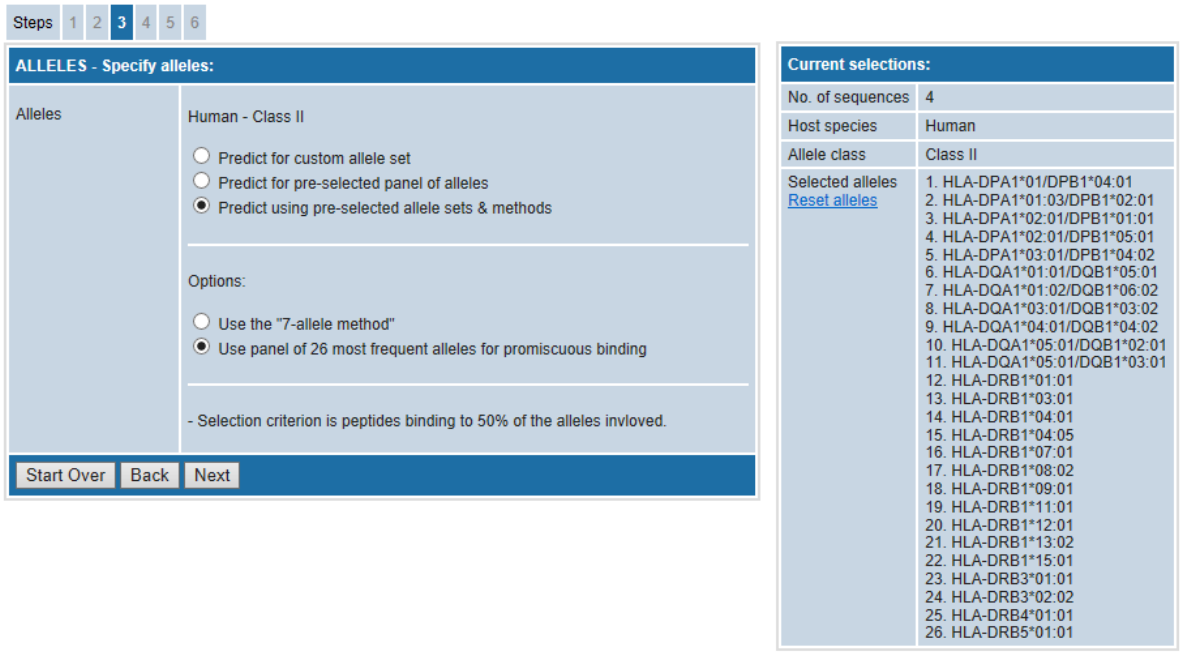


Figure 10 alleles selection

4.3.1.4 Selection of peptide

In this step we have to select peptide which have to be binding to the mhc alleles. We have to remove duplicate peptides. No. of overlapping peptides for 15 mer peptide to be generated (peptide length is fixed at 15 for class 2).

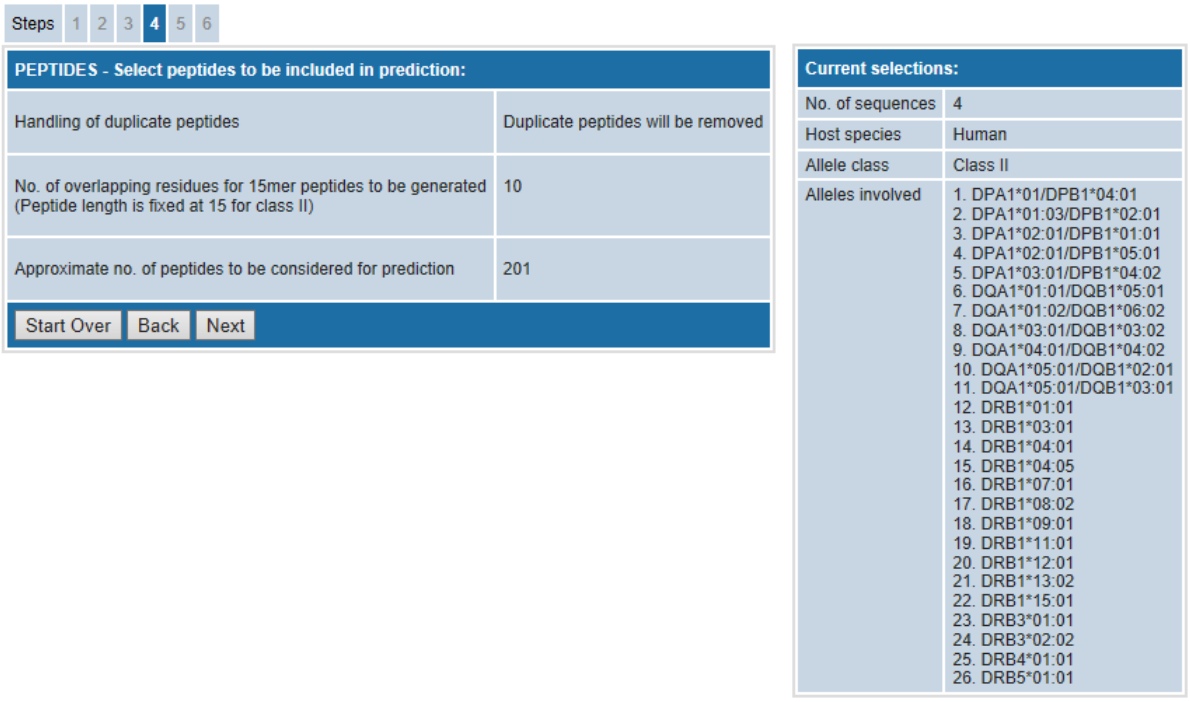


Figure 11 selection of peptide to be included in prediction

4.3.1.5 Selection of prediction method and cutoff values

Prediction method in this case is the IEDB Recomanded and selection of predicted peptide is based on promiscuity based on No. of alleles binding. Peptide considered as binders if it binds to 50% of 26 most frequently occurring alleles.

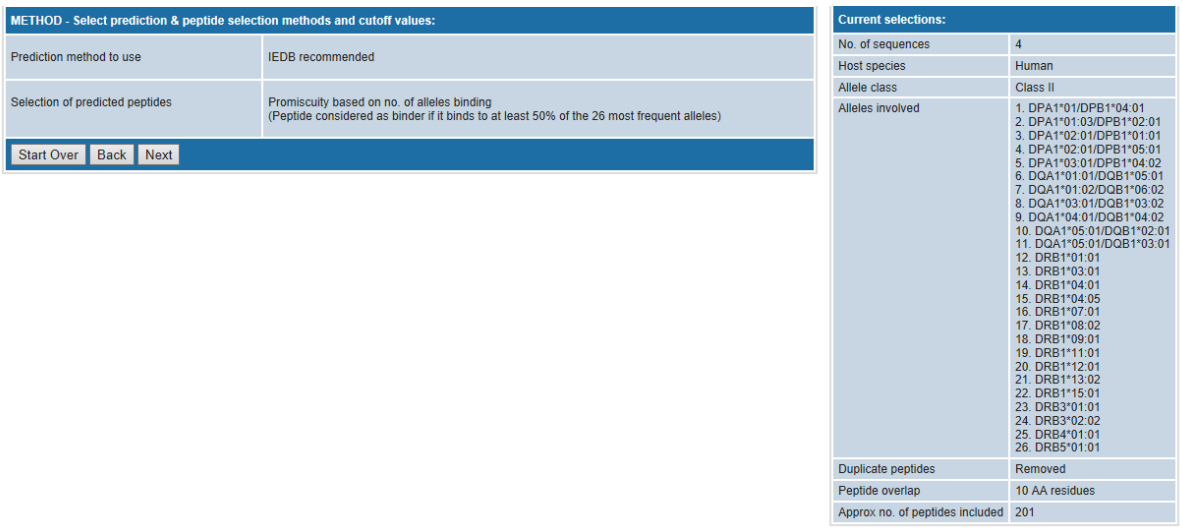
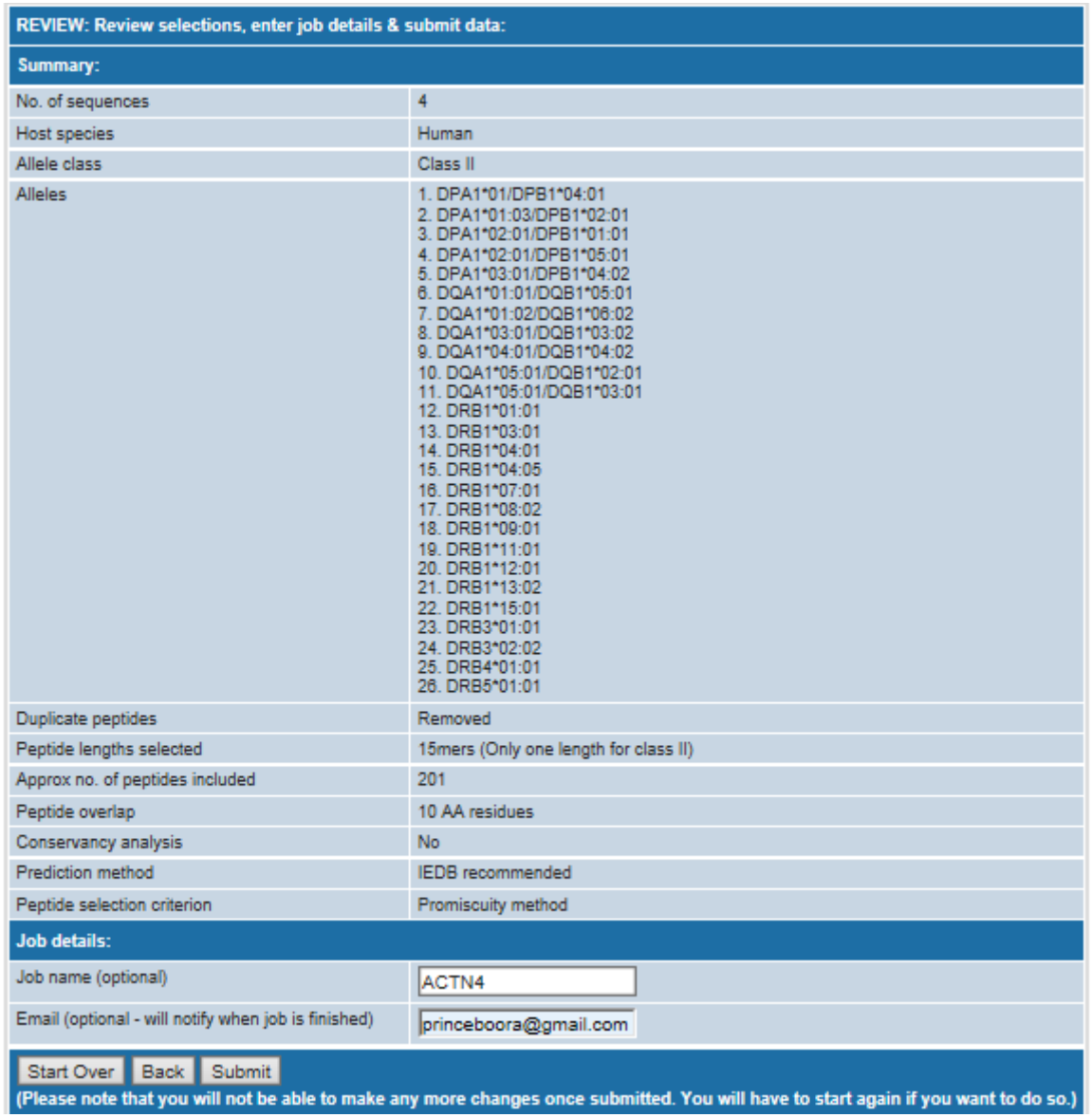


Figure 12 prediction method and cutoff value

4.3.1.6 Review of all the selection and Enter Antigen name

In this step we have to review selection, enter Antigen name in job detail& submit data.



REVIEW: Review selections, enter job details & submit data:	
Summary:	
No. of sequences	4
Host species	Human
Allele class	Class II
Alleles	1. DPA1*01/DPB1*04:01 2. DPA1*01:03/DPB1*02:01 3. DPA1*02:01/DPB1*01:01 4. DPA1*02:01/DPB1*05:01 5. DPA1*03:01/DPB1*04:02 6. DQA1*01:01/DQB1*05:01 7. DQA1*01:02/DQB1*06:02 8. DQA1*03:01/DQB1*03:02 9. DQA1*04:01/DQB1*04:02 10. DQA1*05:01/DQB1*02:01 11. DQA1*05:01/DQB1*03:01 12. DRB1*01:01 13. DRB1*03:01 14. DRB1*04:01 15. DRB1*04:05 16. DRB1*07:01 17. DRB1*08:02 18. DRB1*09:01 19. DRB1*11:01 20. DRB1*12:01 21. DRB1*13:02 22. DRB1*15:01 23. DRB3*01:01 24. DRB3*02:02 25. DRB4*01:01 26. DRB5*01:01
Duplicate peptides	Removed
Peptide lengths selected	15mers (Only one length for class II)
Approx no. of peptides included	201
Peptide overlap	10 AA residues
Conservancy analysis	No
Prediction method	IEDB recommended
Peptide selection criterion	Promiscuity method
Job details:	
Job name (optional)	ACTN4
Email (optional - will notify when job is finished)	princeboora@gmail.com

Start Over | Back | Submit

(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)

Figure 13 Review all selection and job details

4.3.1.7 Results of MHC II binding prediction peptide

These are the no. of peptide and allele combination for each of selected antigen.

Table 24 No. of MHC11 Binding peptide for each antigen

ANTIGEN NAME	NO. OF PREDICTED PEPTIDE & ALLELES COMBINATION		ANTIGEN NAME	NO. OF PREDICTED PEPTIDE & ALLELES COMBINATION
ACTN4	267		CTNNB1	296
BRAF	168		EEF2	152
CAMEL	134		FLT3	103
CASP5	287		GPNMB	159
CASP8	178		HSPA1B	172
CDC27	115		KRAS	126
CDK4	137		MUM1	250
CDKN2A	154		PAPOLG	208
TPI1	270		UBXD5	125

4.3.2 Class ll immunogenicity prediction

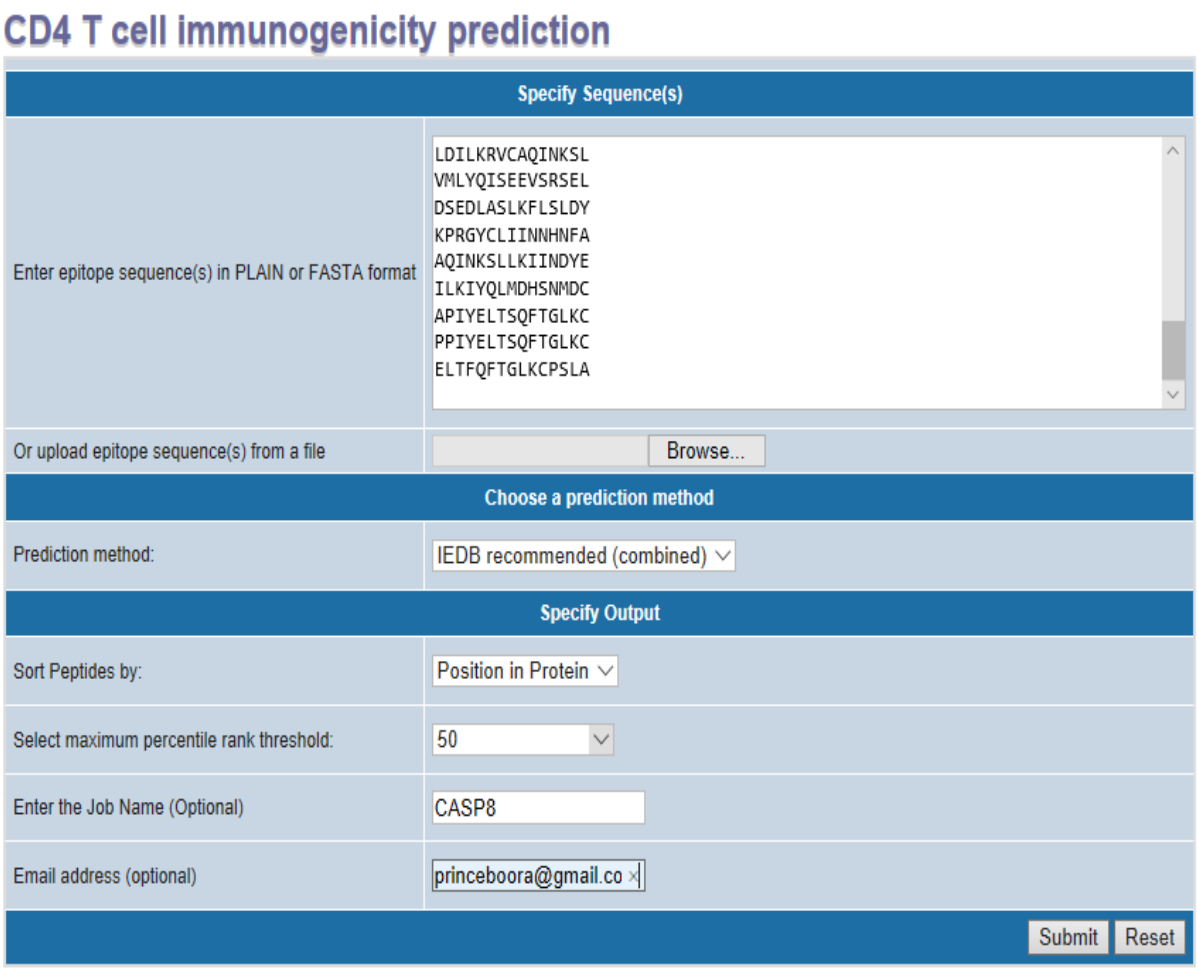


Figure 14 CD4+ Immunogenicity prediction

Table 25 MHC11 binding peptide and allele combination

Peptide	Allele	ic50
ALDFIASKGVKLVSI	HLA-DRB1*01:01	3.51
KAIMTYVSSFYHAFS	HLA-DRB1*12:01	5.35
LAFNALIHRHRPELI	HLA-DRB1*12:01	5.75
ALDFIASKGVKLVSI	HLA-DRB1*07:01	5.87
ALDFIASKGVKLVSI	HLA-DRB5*01:01	5.88
LEINFNTLQTKLRLS	HLA-DRB5*01:01	6.75
SDLLEWIRRTIPWLE	HLA-DRB1*12:01	7
LAFNALIHRHRPELI	HLA-DRB5*01:01	7.31
TLGMIWTIILRFAIQ	HLA-DRB1*12:01	7.47
LQTKLRLSNRPAFMP	HLA-DRB1*12:01	7.65
ALDFIASKGVKLVSI	HLA-DRB1*12:01	7.85
WLLNEIRRLERLDHL	HLA-DRB1*12:01	8
TLSDIKALIRKHEAF	HLA-DRB1*12:01	8.06

SSFYHAFSGAQKAET	HLA-DRB5*01:01	8.54
LEINFNTLQTKLRLS	HLA-DRB1*12:01	8.54
EHLRRQFASQANVVG	HLA-DRB1*01:01	10.56
ALDFIASKGVKLVSI	HLA-DRB1*09:01	13.15
ADQVIASFKVLAGDK	HLA-DRB1*12:01	13.41
KEGLLLWCQRKTAPY	HLA-DRB1*12:01	13.58
LAFNALIHRHRPELI	HLA-DRB1*11:01	13.81
RVGWEQLLTTIARTI	HLA-DRB1*01:01	14.72
SSFYHAFSGAQKAET	HLA-DRB1*01:01	14.79
DFRDGLKLMLLLEVI	HLA-DRB1*12:01	16.58
LEINFNTLQTKLRLS	HLA-DRB1*01:01	17.38
REAILAIHKEAQRIA	HLA-DRB1*12:01	19.38
LMLLLEVISGERLPK	HLA-DRB1*12:01	20.08
QLSHLKQYERSIVDY	HLA-DRB1*12:01	20.83
KNFITAEELRRELPP	HLA-DRB5*01:01	21.14
MEDYEKLASDLLEWI	HLA-DRB1*01:01	21.66
SSFYHAFSGAQKAET	HLA-DQA1*05:01/DQB1*03:01	22.71
KAIMTYVSSFYHAFS	HLA-DPA1*01:03/DPB1*02:01	22.89
LEINFNTLQTKLRLS	HLA-DRB1*11:01	23.38
AGNGAGGGGSMGDYM	HLA-DQA1*05:01/DQB1*03:01	24.63
ALDFIASKGVKLVSI	HLA-DRB3*02:02	26.89
ALDFIASKGVKLVSI	HLA-DRB1*11:01	28.11
ALDFIASKGVKLVSI	HLA-DRB1*15:01	28.3
SNPYTTVTPQIINSK	HLA-DRB1*01:01	28.41
RPAFMPSEGKMVSDI	HLA-DRB1*01:01	28.71
IILRFAIQDISVEET	HLA-DRB1*12:01	29.94
ALGSLTHSRREALEK	HLA-DRB1*12:01	30
IDQLHLEYAKRAAPF	HLA-DRB1*12:01	30.81
SGLVTFQAFIDFMSR	HLA-DPA1*01:03/DPB1*02:01	31.39
LMLLLEVISGERLPK	HLA-DRB1*01:01	31.61
IDQLHLEYAKRAAPF	HLA-DRB1*01:01	32.54
SYQYGPSSAGNGAGG	HLA-DQA1*05:01/DQB1*03:01	32.81
KAIMTYVSSFYHAFS	HLA-DRB1*15:01	32.9
IDQLHLEYAKRAAPF	HLA-DRB5*01:01	33.65
AEFNRIMSLVDPNHS	HLA-DRB1*12:01	34.37
QAEYCIARMAPYQGP	HLA-DRB1*12:01	34.49
RVGWEQLLTTIARTI	HLA-DRB1*12:01	34.95
GKMRVHKINNVNKAL	HLA-DRB1*12:01	35.98
ALDYKSFSTALYGES	HLA-DRB1*01:01	36.51
DLQDMFIVHTIEEIE	HLA-DPA1*02:01/DPB1*01:01	36.64
LAFNALIHRHRPELI	HLA-DRB1*01:01	37.31
TLSDIKALIRKHEAF	HLA-DRB1*11:01	37.63
TAANRICKVLAVNQE	HLA-DRB1*12:01	37.72
LQTKLRLSNRPAFMP	HLA-DRB1*03:01	37.8

LQTKLRLSNRPAFMP	HLA-DRB1*01:01	39.31
SNHIKLSGSNPYTTV	HLA-DRB1*13:02	39.57
SGLVTFQAFIDFMSR	HLA-DPA1*02:01/DPB1*01:01	40.26
QAEYCIARMAPYQGP	HLA-DRB1*01:01	40.56
RKTFTAWCNSHLRKA	HLA-DRB1*12:01	41.37
RKTFTAWCNSHLRKA	HLA-DRB1*01:01	41.4
SNHIKLSGSNPYTTV	HLA-DRB1*01:01	41.56
SGLVTFQAFIDFMSR	HLA-DRB1*12:01	41.7
LQTKLRLSNRPAFMP	HLA-DRB1*11:01	42.49
DFRDYRRVHKPPKVQ	HLA-DRB5*01:01	43.18
DFRDGLKLMLLLEVI	HLA-DPA1*03:01/DPB1*04:02	43.66
DFRDGLKLMLLLEVI	HLA-DPA1*02:01/DPB1*01:01	43.73
LAEKFRQKASIHEAW	HLA-DRB5*01:01	46.22
QDRVEQIAAIAQELN	HLA-DQA1*05:01/DQB1*03:01	46.24
SSFYHAFSGAQKAET	HLA-DRB1*09:01	46.55
LEINFNTLQTKLRLS	HLA-DRB1*07:01	46.66
KAIMTYVSSFYHAFS	HLA-DPA1*02:01/DPB1*01:01	46.76
DLQDMFIVHTIEEIE	HLA-DPA1*01:03/DPB1*02:01	47.9
EKVQQLVPKRDHALL	HLA-DRB1*12:01	49.35
DLQDMFIVHTIEEIE	HLA-DPA1*03:01/DPB1*04:02	50.7
KNFITAEELRRELPP	HLA-DPA1*02:01/DPB1*01:01	50.72
KNFITAEELRRELPP	HLA-DRB1*12:01	50.74
KGVKLVSIGAEEIVD	HLA-DRB1*01:01	51.66
PEEFKACLISLGYDV	HLA-DRB1*01:01	51.96
TLSDIKALIRKHEAF	HLA-DRB5*01:01	52.86
KAIMTYVSSFYHAFS	HLA-DRB5*01:01	54
SNHIKLSGSNPYTTV	HLA-DRB1*07:01	54.97
KAIMTYVSSFYHAFS	HLA-DRB1*01:01	55.51
RKTFTAWCNSHLRKA	HLA-DRB5*01:01	55.52
EHLRRQFASQANVVG	HLA-DRB1*04:01	56.04
KAIMTYVSSFYHAFS	HLA-DRB1*07:01	56.64
PYKNVNVQNFHISWK	HLA-DRB1*12:01	57.11
LAEKFRQKASIHEAW	HLA-DRB1*12:01	57.34
ADQVIASFKVLAGDK	HLA-DRB1*01:01	58.53
TLGMIWTIILRFAIQ	HLA-DPA1*03:01/DPB1*04:02	58.72
AEFNRIMSLVDPNHS	HLA-DRB1*01:01	58.89
LAFNALIHRHRPELI	HLA-DRB1*07:01	59.59
QDRVEQIAAIAQELN	HLA-DQA1*04:01/DQB1*04:02	59.85
SNPYTTVTPQIINSK	HLA-DRB1*07:01	60.71
SGLVTFQAFIDFMSR	HLA-DPA1*03:01/DPB1*04:02	60.93
LAEKFRQKASIHEAW	HLA-DRB1*01:01	61.62
SGAQKAETAANRICK	HLA-DQA1*05:01/DQB1*03:01	62.32
QDRVEQIAAIAQELN	HLA-DQA1*03:01/DQB1*03:02	62.58
LEINFNTLQTKLRLS	HLA-DPA1*02:01/DPB1*01:01	64.17

QDRVEQIAAIAQELN	HLA-DQA1*01:02/DQB1*06:02	64.4
ALDFIASKGVKLVSI	HLA-DRB1*04:01	65.35
RKTFTAWCNSHLRKA	HLA-DRB1*07:01	65.66
LEINFNTLQTKLRLS	HLA-DRB1*04:05	68.19
LEINFNTLQTKLRLS	HLA-DRB3*02:02	68.78
EHLRRQFASQANVVG	HLA-DQA1*05:01/DQB1*03:01	69.42
KPNLDLLEQQHQLIQ	HLA-DRB1*12:01	69.78
LAFNALIHRHRPELI	HLA-DRB1*15:01	69.92
ALGSLTHSRREALEK	HLA-DRB5*01:01	70.27
KAIMTYVSSFYHAFS	HLA-DRB1*11:01	70.67
LMLLLEVISGERLPK	HLA-DPA1*02:01/DPB1*01:01	71.91
KAIMTYVSSFYHAFS	HLA-DPA1*03:01/DPB1*04:02	73.36
RKTFTAWCNSHLRKA	HLA-DRB1*04:05	73.85
NYTMEHIRVGWEQLL	HLA-DRB1*12:01	74.26
EHLRRQFASQANVVG	HLA-DRB1*12:01	75.13
TLGMIWTIILRFAIQ	HLA-DPA1*02:01/DPB1*01:01	75.23
RLERLDHLAEKFRQK	HLA-DRB1*12:01	75.7
ALDFIASKGVKLVSI	HLA-DRB1*13:02	77.08
DLQDMFIVHTIEEIE	HLA-DQA1*05:01/DQB1*02:01	78.27
SSFYHAFSGAQKAET	HLA-DRB1*11:01	80.09
AEFNRIMSLVDPNHS	HLA-DRB1*04:05	81.03
KNFITAEELRRELPP	HLA-DRB1*03:01	81.21
RDLLLDPAWEKQQRK	HLA-DRB1*03:01	81.68
IILRFAIQDISVEET	HLA-DPA1*02:01/DPB1*01:01	82.43
QDRVEQIAAIAQELN	HLA-DRB1*01:01	82.7
ALDYKSFSTALYGES	HLA-DRB1*04:05	82.92
ALDYKSFSTALYGES	HLA-DRB1*07:01	83.18
EHLRRQFASQANVVG	HLA-DRB1*09:01	83.47
ALDFIASKGVKLVSI	HLA-DQA1*05:01/DQB1*03:01	84.21
SNHIKLSGSNPYTTV	HLA-DRB3*02:02	85.09
HISWKDGLAFNALIH	HLA-DRB1*01:01	87.65
APYQGPDAVPGALDY	HLA-DQA1*05:01/DQB1*03:01	87.69
DFRDYRRVHKPPKVQ	HLA-DRB1*11:01	89.74
ALDFIASKGVKLVSI	HLA-DRB1*04:05	89.89
SSFYHAFSGAQKAET	HLA-DRB1*07:01	89.97
MEDYEKLASDLLEWI	HLA-DPA1*02:01/DPB1*01:01	91.17
TPQIINSKWEKVQQL	HLA-DRB1*12:01	91.65
KAIMTYVSSFYHAFS	HLA-DRB1*04:05	93.15
KNFITAEELRRELPP	HLA-DPA1*03:01/DPB1*04:02	93.49
GKMRVHKINNVNKAL	HLA-DRB5*01:01	93.55
IILRFAIQDISVEET	HLA-DRB4*01:01	93.6
NNWMESAMEDLQDMF	HLA-DQA1*05:01/DQB1*02:01	94.2
SNHIKLSGSNPYTTV	HLA-DRB1*12:01	94.46
HISWKDGLAFNALIH	HLA-DQA1*05:01/DQB1*03:01	94.51

LEINFNTLQTKLRLS	HLA-DPA1*03:01/DPB1*04:02	95.94
KEGLLLWCQRKTAPY	HLA-DRB1*11:01	95.97
LQTKLRLSNRPAFMP	HLA-DRB3*02:02	96.01
LEINFNTLQTKLRLS	HLA-DRB1*04:01	96.52
KNFITAEELRRELPP	HLA-DPA1*01:03/DPB1*02:01	97.06
LEINFNTLQTKLRLS	HLA-DRB1*15:01	97.27
LMLLLEVISGERLPK	HLA-DPA1*03:01/DPB1*04:02	98.14
ALDYKSFSTALYGES	HLA-DRB1*09:01	98.27
ALDYKSFSTALYGES	HLA-DPA1*01:03/DPB1*02:01	98.75
PEEFKACLISLGYDV	HLA-DRB1*12:01	99.28
QAEYCIARMAPYQGP	HLA-DQA1*05:01/DQB1*03:01	99.91
IDQLHLEYAKRAAPF	HLA-DRB1*11:01	101.12
LQTKLRLSNRPAFMP	HLA-DRB5*01:01	101.71
TEKQLEAIDQLHLEY	HLA-DRB1*12:01	101.95
DFRDGLKLMLLLEVI	HLA-DPA1*01:03/DPB1*02:01	102.22
TLGMIWTIILRFAIQ	HLA-DPA1*01:03/DPB1*02:01	102.45
QQHQLIQEALIFDNK	HLA-DPA1*02:01/DPB1*01:01	102.48
ALDYKSFSTALYGES	HLA-DRB1*04:01	103.39
DFRDGLKLMLLLEVI	HLA-DRB1*01:01	104.35
EHLRRQFASQANVVG	HLA-DRB5*01:01	105.77
HEAFESDLAAHQDRV	HLA-DRB1*01:01	106.83
RKTFTAWCNSHLRKA	HLA-DRB1*04:01	107.43
LAEKFRQKASIHEAW	HLA-DRB1*11:01	107.68
WLLNEIRRLERLDHL	HLA-DPA1*02:01/DPB1*01:01	108.08
SNHIKLSGSNPYTTV	HLA-DRB1*15:01	108.67
RKTFTAWCNSHLRKA	HLA-DRB1*15:01	108.74
EHLRRQFASQANVVG	HLA-DRB1*04:05	109.06
QAEYCIARMAPYQGP	HLA-DRB5*01:01	110.18
QDRVEQIAAIAQELN	HLA-DQA1*05:01/DQB1*02:01	110.41
RKTFTAWCNSHLRKA	HLA-DRB1*11:01	110.49
HEAFESDLAAHQDRV	HLA-DRB1*04:01	110.69
IILRFAIQDISVEET	HLA-DRB1*01:01	111.04
EHLRRQFASQANVVG	HLA-DRB1*07:01	111.15
TAANRICKVLAVNQE	HLA-DRB1*01:01	111.61
IILRFAIQDISVEET	HLA-DPA1*03:01/DPB1*04:02	112.4
SNHIKLSGSNPYTTV	HLA-DRB1*04:01	113.03
TEKQLEAIDQLHLEY	HLA-DPA1*02:01/DPB1*01:01	113.81
LMLLLEVISGERLPK	HLA-DRB1*07:01	114.02
SDLLEWIRRTIPWLE	HLA-DRB1*11:01	115.14
WLLNEIRRLERLDHL	HLA-DRB1*11:01	116.71
RPAFMPSEGKMVSDI	HLA-DRB1*07:01	116.93
SSFYHAFSGAQKAET	HLA-DRB1*04:01	117.14
RHRPELIEYDKLRKD	HLA-DRB1*12:01	117.49
WLLNEIRRLERLDHL	HLA-DRB4*01:01	118.65

LEINFNTLQTKLRLS	HLA-DRB1*09:01	119.39
ADQVIASFKVLAGDK	HLA-DRB1*07:01	120.01
SNPYTTVTPQIINSK	HLA-DRB1*09:01	120.6
ALGSLTHSRREALEK	HLA-DRB1*11:01	121.56
DLQDMFIVHTIEEIE	HLA-DRB1*12:01	122.55
ALDYKSFSTALYGES	HLA-DPA1*02:01/DPB1*01:01	124.7
WLLNEIRRLERLDHL	HLA-DRB1*03:01	124.83
IEEIEGLISAHDQFK	HLA-DRB1*01:01	125.71
WLLNEIRRLERLDHL	HLA-DRB1*01:01	126.04
IILRFAIQDISVEET	HLA-DQA1*05:01/DQB1*02:01	128.23
ALDFIASKGVKLVSI	HLA-DPA1*02:01/DPB1*01:01	131.17
KPNLDLLEQQHQLIQ	HLA-DRB4*01:01	132.36
RPAFMPSEGKMVSDI	HLA-DRB1*09:01	132.49
ALDYKSFSTALYGES	HLA-DRB1*12:01	132.64
RVGWEQLLTTIARTI	HLA-DRB1*07:01	132.73
FKVLAGDKNFITAEE	HLA-DRB1*01:01	133.1
KGVKLVSIGAEEIVD	HLA-DQA1*05:01/DQB1*02:01	136.13
AEFNRIMSLVDPNHS	HLA-DRB1*04:01	136.16
QQHQLIQEALIFDNK	HLA-DPA1*03:01/DPB1*04:02	136.5
QAEYCIARMAPYQGP	HLA-DRB1*11:01	137.45
SGLVTFQAFIDFMSR	HLA-DRB1*01:01	137.67
GKMRVHKINNVNKAL	HLA-DRB1*13:02	138.05
NAFEVAEKYLDIPKM	HLA-DPA1*01:03/DPB1*02:01	138.6
LEINFNTLQTKLRLS	HLA-DRB4*01:01	138.94
LAFNALIHRHRPELI	HLA-DRB1*08:02	139.12
DFRDYRRVHKPPKVQ	HLA-DRB1*12:01	139.4
ALDYKSFSTALYGES	HLA-DRB5*01:01	140.35
KNFITAEELRRELPP	HLA-DRB1*01:01	141.71
ADQVIASFKVLAGDK	HLA-DRB1*15:01	143.32
SGLVTFQAFIDFMSR	HLA-DQA1*01:01/DQB1*05:01	144.36
ALDFIASKGVKLVSI	HLA-DPA1*03:01/DPB1*04:02	144.69
SGLVTFQAFIDFMSR	HLA-DQA1*05:01/DQB1*02:01	144.85
SDLLEWIRRTIPWLE	HLA-DPA1*02:01/DPB1*01:01	146.91
SNHIKLSGSNPYTTV	HLA-DRB1*03:01	147.3
LQTKLRLSNRPAFMP	HLA-DRB1*15:01	148.03
RLERLDHLAEKFRQK	HLA-DRB5*01:01	148.45
LMLLLEVISGERLPK	HLA-DRB5*01:01	148.93
SSFYHAFSGAQKAET	HLA-DRB1*04:05	149.06
WLLNEIRRLERLDHL	HLA-DPA1*03:01/DPB1*04:02	149.96
EQMQEFRASFNHFDK	HLA-DRB1*01:01	150.8
LAFNALIHRHRPELI	HLA-DRB3*02:02	150.9
SNHIKLSGSNPYTTV	HLA-DQA1*05:01/DQB1*03:01	151.09
RPAFMPSEGKMVSDI	HLA-DRB5*01:01	151.59
GKMRVHKINNVNKAL	HLA-DRB1*01:01	151.97

RVGWEQLLTTIARTI	HLA-DRB1*04:05	152.61
NYTMEHIRVGWEQLL	HLA-DRB1*01:01	152.8
SNHIKLSGSNPYTTV	HLA-DRB1*09:01	153.61
LAFNALIHRHRPELI	HLA-DRB1*09:01	155.12
QQHQLIQEALIFDNK	HLA-DRB1*12:01	155.38
QQHQLIQEALIFDNK	HLA-DRB1*01:01	155.58
TAANRICKVLAVNQE	HLA-DQA1*01:02/DQB1*06:02	155.63
AEKGYEEWLLNEIRR	HLA-DPA1*02:01/DPB1*01:01	156.64
ALDFIASKGVKLVSI	HLA-DRB1*03:01	157.13
VENQILTRDAKGISQ	HLA-DRB1*12:01	158.17
LQTKLRLSNRPAFMP	HLA-DRB1*13:02	159.54
MEDYEKLASDLLEWI	HLA-DPA1*01:03/DPB1*02:01	161.16
SNHIKLSGSNPYTTV	HLA-DRB5*01:01	161.34
QAEYCIARMAPYQGP	HLA-DRB1*07:01	161.36
KGVKLVSIGAEEIVD	HLA-DRB1*12:01	161.4
RKTFTAWCNSHLRKA	HLA-DRB1*09:01	164.42
FDKDHGGALGPEEFK	HLA-DQA1*05:01/DQB1*03:01	164.46
IDQLHLEYAKRAAPF	HLA-DRB3*02:02	165.06
SSFYHAFSGAQKAET	HLA-DRB1*12:01	165.65
LEINFNTLQTKLRLS	HLA-DRB1*03:01	166.49
LQTKLRLSNRPAFMP	HLA-DRB1*07:01	167.61
LMLLLEVISGERLPK	HLA-DPA1*01:03/DPB1*02:01	168.76
SGLVTFQAFIDFMSR	HLA-DRB1*04:05	168.79
MEDYEKLASDLLEWI	HLA-DPA1*03:01/DPB1*04:02	171.85
KAIMTYVSSFYHAFS	HLA-DRB1*09:01	172.18
NAFEVAEKYLDIPKM	HLA-DPA1*02:01/DPB1*01:01	172.54
RVGWEQLLTTIARTI	HLA-DRB1*04:01	173.57
ADQVIASFKVLAGDK	HLA-DRB1*11:01	173.74
EQMQEFRASFNHFDK	HLA-DRB1*04:05	173.96
TLGMIWTIILRFAIQ	HLA-DRB1*15:01	173.99
EQMQEFRASFNHFDK	HLA-DRB1*07:01	176.97
LEINFNTLQTKLRLS	HLA-DPA1*02:01/DPB1*05:01	177.33
TLGMIWTIILRFAIQ	HLA-DRB1*01:01	177.81
SSFYHAFSGAQKAET	HLA-DRB1*15:01	177.99
AEFNRIMSLVDPNHS	HLA-DRB4*01:01	178.25
EEIVDGNAKMTLGMI	HLA-DQA1*05:01/DQB1*03:01	179.22
IILRFAIQDISVEET	HLA-DRB1*04:05	180.36
CNSHLRKAGTQIENI	HLA-DRB1*01:01	180.85
IILRFAIQDISVEET	HLA-DPA1*01:03/DPB1*02:01	181.32
ADQVIASFKVLAGDK	HLA-DQA1*01:02/DQB1*06:02	181.67
REAILAIHKEAQRIA	HLA-DRB5*01:01	182
KEAQRIAESNHIKLS	HLA-DRB1*12:01	182.08
EQMQEFRASFNHFDK	HLA-DRB1*12:01	183.66
EHLRRQFASQANVVG	HLA-DRB1*11:01	184.02

RVGWEQLLTTIARTI	HLA-DRB1*09:01	185.54
LAEKFRQKASIHEAW	HLA-DRB1*07:01	185.58
QAEYCIARMAPYQGP	HLA-DRB1*09:01	186.77
QKTIQEMQQKLEDFR	HLA-DRB1*12:01	187.13
NYTMEHIRVGWEQLL	HLA-DPA1*02:01/DPB1*01:01	187.47
EQMQEFRASFNHFDK	HLA-DRB1*04:01	187.55
TLGMIWTIILRFAIQ	HLA-DRB1*11:01	190.74
AEFNRIMSLVDPNHS	HLA-DRB1*07:01	191
EKVQQLVPKRDHALL	HLA-DRB1*01:01	192.01
AEKGYEEWLLNEIRR	HLA-DPA1*01:03/DPB1*02:01	192.17
KGVKLVSIGAEEIVD	HLA-DQA1*05:01/DQB1*03:01	192.62
REAILAIHKEAQRIA	HLA-DRB1*11:01	192.97
ALGSLTHSRREALEK	HLA-DRB1*03:01	194.73
EHLRRQFASQANVVG	HLA-DRB3*02:02	194.75
REAILAIHKEAQRIA	HLA-DRB4*01:01	196.3
LMLLLEVISGERLPK	HLA-DRB4*01:01	196.82
LAFNALIHRHRPELI	HLA-DRB4*01:01	198.71
PEEFKACLISLGYDV	HLA-DRB1*07:01	199.56
TEKQLEAIDQLHLEY	HLA-DPA1*03:01/DPB1*04:02	199.88

4.3.2.1 Results of CD4+ Immunogenicity Prediction

$CD4^+$ Immunogenicity prediction peptides were selected based on their prediction score which should be more than zero. No. of peptide for each antigen are listed below.

Table 26 Results of CD4+ Immunogenicity prediction

ANTIGEN NAME	NO. OF PREDICTED IMMUNOGENIC PEPTIDE		ANTIGEN NAME	NO. OF PREDICTED IMMUNOGENIC PEPTIDE
ACTN4	39		CTNNB1	27
BRAF	42		EEF2	20
CAMEL	48		FLT3	39
CASP5	43		GPNMB	65
CASP8	41		HSPA1B	28
CDC27	22		KRAS	37
CDK4	25		MUM1	44
CDKN2A	40		PAPOLG	42
TPI1	31		UBXD5	35

4.3.3 Class 11 population coverage results

Table 27 ACTN4 Class 11 population coverage results

Epitope list	Population coverage
Epitope #1: SSFYHAFSGAQKAET	62.92%
Epitope #2: ALDFIASKGVKLVSI	74.63%
Epitope #3: LEINFNTLQTKLRLS	72.48%

Epitope #4: LQTKLRLSNRPAFMP	71.56%
Epitope #5: SNHIKLSGSNPYTTV	67.53%
Epitope set	**74.63%**

Table 28 CAMEL class 11 population coverage results

Epitope list	Population coverage
Epitope #1: AVPLLRRMEGAPAGP	42.07%
Epitope #2: FLMAQGAMLAAQERR	52.55%
Epitope #3: MLMAQEALAFLMAQG	38.46%
Epitope set	**62.92%**

Table 29 CASP5 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: GAHYDIVGMKRLLQG	59.23%
Epitope #3: STFLVLMSHGILEGI	51.04%
Epitope #4: RDMESVLRAFAARPE	60.61%
Epitope #5: DFIAFCSSTPHNVSW	65.43%
Epitope #6: LWVRDSPASLAVISS	54.94%
Epitope set	**74.63%**

Table 30 CASP8 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: SFLKELLFRINRLDL	59.87%
Epitope #2: DALMLFQRLQEKRML	37.47%
Epitope #3: RAQISAYRVMLYQIS	59.87%
Epitope #6: RGYCLIINNHNFAKA	74.63%

Epitope #8: DLASLKFLSLDYIPQ	51.74%
Epitope set	**74.63%**

Table 31 CDC27 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: KEAINILSHLPSHHY	65.84%
Epitope #8: TTLWHLQKDVALSVL	74.63%
Epitope #11: QTFKFTSLQNFSNCL	62.92%
Epitope #14: AAIWQALNHYAYRDA	62.92%
Epitope #17: YAYAYTLLGHEFVLT	61.24%
Epitope set	**74.99%**

Table 32 CDK4 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: PHSGHFVALKSVRVP	65.84%
Epitope #2: RISAFRALQHSYLHK	63.35%
Epitope #4: SYQMALTPVVVTLWY	62.92%
Epitope #6: DFGLARIYSYQMALT	61.24%
Epitope #7: RQFLRGLDFLHANCI	62.92%
Epitope set	**65.84%**

Table 33 CDKN2A class 11 population coverage results

Epitope list	Population coverage
Epitope #1: EEVRALLEAGALPNA	9.12%
Epitope #2: LDTLVVLHRAGARLD	59.23%
Epitope set	**59.23%**

Table 34 CTNNB1 class 11 population coverage results

Epitope list	Population coverage
Epitope #2: GQYAMTRAQRVRAAM	74.99%
Epitope #3: EKLLWTTSRVLKVLS	74.63%
Epitope #4: QALVNIMRTYTYEKL	59.87%
Epitope #5: WPLIKATVGLIRNLA	72.71%
Epitope #19: GLIRNLALCPANHAP	65.43%
Epitope set	**74.99%**

Table 35 EEF2 class 11 population coverage results

Epitope list	Population coverage
Epitope #3: PLMMYISKMVPTSDK	59.87%
Epitope #4: GRVFSGLVSTGLKVR	55.10%
Epitope #5: GGIYGVLNRKRGHVF	59.68%
Epitope #13: CITIKSTAISLFYEL	65.43%
Epitope #16: NRLYMKARPFPDGLA	62.92%
Epitope set	**74.99%**

Table 36 FLT3 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: APPQHLIRVEGNLRV	71.56%
Epitope #2: RAMAIYKQSQHMTEV	12.26%
Epitope #3: TYQGSYGFRLGFLHS	60.61%
Epitope #4: VTCTYSPALNKMFCQ	40.16%
Epitope set	**72.71%**

Table 37 GPNMB class 11 population coverage results

Epitope list	Population coverage
Epitope #1: TVISLLVYKKHKEYN	37.47%
Epitope #2: DPASPLRMANSALIS	74.63%
Epitope #3: LSVFLNRAKAVFFPG	74.13%
Epitope #5: DVDEMCLLTVRRTFN	37.47%
Epitope #6: GNVVRSKGLSVFLNR	63.19%
Epitope set	**74.63%**

Table 38 HSPA1B class 11 population coverage results

Epitope list	Population coverage
Epitope #1: GLNVLRIINEPTAAA	64.13%
Epitope #2: QNKRAVRRLRTACER	27.20%
Epitope #3: TKDAGVIAGLNVLRI	51.04%

Epitope #4: GGVMTALIKRNSTIP	14.96%
Epitope #7: VGVFQHGKVEIIAND	40.16%
Epitope set	**65.84%**

Table 39 KRAS class 11 population coverage results

Epitope list	Population coverage
Epitope #1: TLVREIRQYRLKKIS	69.29%
Epitope #2: ALTIQLIQNHFVDEY	64.13%
Epitope #3: GFLCVFAINNTKSFE	64.13%
Epitope set	**73.52%**

Table 40 MUM1 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: PLEELAYRRSLRVAL	73.60%
Epitope #2: IGWCVSLITDYRVRL	69.84%
Epitope #3: GFTVSLKSLKHFDCK	59.23%
Epitope #5: KGAESHLRAILKSRK	59.68%
Epitope #9: LEYYAADISYPVRKS	43.67%
Epitope set	**74.99%**

Table 41 PAPOLG class 11 population coverage results

Epitope list	Population coverage
Epitope #1: FRLTLRAVKLWAKRR	71.40%
Epitope #3: KYRHYIVLTASASTE	65.84%
Epitope #6: VESKIRVLVGNLERN	61.58%
Epitope #16: LRSLDIRCIRSLNGC	47.68%
Epitope #17: LGIIFRRVENAESVN	51.88%
Epitope set	**74.99%**

Table 42 TPI1 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: GELIGTLNAAKVPAD	51.19%
Epitope #2: ESDELIGQKVAHALA	54.42%
Epitope #5: FHFAALYISGQWPRL	62.92%
Epitope #7: SKVVLAYEPVWAIGT	27.42%
Epitope #11: LRGWLKSNVSDAVAQ	15.61%
Epitope set	**65.43%**

Table 43 UBXD5 class 11 population coverage results

Epitope list	Population coverage
Epitope #1: IPLKLYRNGIMMFDG	61.86%
Epitope #3: FPSELQRLYPNGVPF	40.16%
Epitope #7: KAALLLRARRAPKSS	69.67%
Epitope #8: EQAFLLMMQPDNTIG	15.73%
Epitope #12: DASAFEIFSTFPPTL	39.24%
Epitope set	**74.99%**

4.4 Combined population coverage results of MHC class l and class ll epitopes

The cumulative Population protection coverage of class I epitopes and class II epitopes was calculating using the IEDB PC tool. The epitopes with the combination of HLA alleles is selected so that the population coverage at least >80% achieved. So different combination of epitopes are test for the achievement of highest population coverage.

4.4.1 ACTN4's selected epitopes

Table 44 ACTN4 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: KALDFIASK	42.27%
Epitope #2: MTYVSSFYH	35.68%
Epitope #3: VQNFHISWK	29.40%
Epitope #4: MTLGMIWTI	27.76%
Epitope #5: LAFNALIHR	20.14%
Epitope #6: SSFYHAFSGAQKAET	62.92%
Epitope #7: ALDFIASKGVKLVSI	74.63%
Epitope #8: LEINFNTLQTKLRLS	72.48%
Epitope #9: LQTKLRLSNRPAFMP	71.56%
Epitope #10: SNHIKLSGSNPYTTV	67.53%
Epitope set	**93.51%**

4.4.2 CAMEL's selected epitope

Table 45 CAMEL MHC class I and class II epitope's population coverage

Epitope list	Population coverage
Epitope #1: FLMAQGAML	18.89%
Epitope #2: RMAVPLLRR	37.98%
Epitope #3: RPWKRSWSA	6.24%
Epitope #4: LMAQEALAF	10.14%
Epitope #5: CTSRCLSRR	19.31%
Epitope #6: AVPLLRRMEGAPAGP	42.07%
Epitope #7: FLMAQGAMLAAQERR	52.55%

Epitope #8: MLMAQEALAFLMAQG	38.46%
Epitope set	**87.32%**

4.4.3 CASP5's selected epitopes

Table 46 CASP5 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: SVLRAFAAR	36.41%
Epitope #2: KSFEVPQAK	33.99%
Epitope #3: LLYDTIFQI	19.18%
Epitope #4: SSTPHNVSW	13.77%
Epitope #5: NVSWRDRTR	10.44%
Epitope #6: GAHYDIVGMKRLLQG	59.23%
Epitope #7: STFLVLMSHGILEGI	51.04%
Epitope #8: RDMESVLRAFAARPE	60.61%
Epitope #9: DFIAFCSSTPHNVSW	65.43%
Epitope #10: LWVRDSPASLAVISS	54.94%
Epitope set	**88.64%**

4.4.4 CASP8's selected epitopes

Table 45 CASP8 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: IINNHNFAK	42.27%
Epitope #2: TVNNCVSYR	36.41%
Epitope #3: ILTEVNYEV	15.90%
Epitope #4: LSFLKELLF	13.77%
Epitope #5: MLEESNLSF	10.14%

Epitope #6: SFLKELLFRINRLDL	59.87%
Epitope #7: DALMLFQRLQEKRML	37.47%
Epitope #8: RAQISAYRVMLYQIS	59.87%
Epitope #9: RGYCLIINNHNFAKA	74.63%
Epitope #10: DLASLKFLSLDYIPQ	51.74%
Epitope set	**87.36%**

4.4.5 CDC27's selected epitopes

Table 47 CDC27 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: HSGHFVALK	41.71%
Epitope #2: CIFAEMFRR	36.41%
Epitope #3: RIYSYQMAL	24.10%
Epitope #4: NPHKRISAF	12.03%
Epitope #5: LTPVVVTLW	13.77%
Epitope #6: KEAINILSHLPSHHY	65.84%
Epitope #7: TTLWHLQKDVALSVL	74.63%
Epitope #8: QTFKFTSLQNFSNCL	62.92%
Epitope #9: AAIWQALNHYAYRDA	62.92%
Epitope #10: YAYAYTLLGHEFVLT	61.24%
Epitope set	91.5%

4.4.6 CDK4's selected epitopes

Table 48 CDK4 MHC class 1 and class 11 epitope's population prediction

Epitope list	Population coverage
Epitope #1: HSGHFVALK	41.71%
Epitope #2: CIFAEMFRR	36.41%
Epitope #3: RIYSYQMAL	24.10%
Epitope #4: NPHKRISAF	12.03%
Epitope #5: LTPVVVTLW	13.77%

Epitope #6: PHSGHFVALKSVRVP	65.84%
Epitope #7: RISAFRALQHSYLHK	63.35%
Epitope #8: SYQMALTPVVVTLWY	62.92%
Epitope #9: DFGLARIYSYQMALT	61.24%
Epitope #10: RQFLRGLDFLHANCI	62.92%
Epitope set	94.41%

4.4.7 CDKN2A's selected epitopes

Table 49 CDKN2A MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: LPVDLAEEL	6.84%
Epitope #2: DWLATAAAR	0.92%
Epitope #3: LTRPVHDAA	5.47%
Epitope #4: EEVRALLEAGALPNA	9.12%
Epitope #5: LDTLVVLHRAGARLD	59.23%
Epitope set	67.81%

4.4.8 CTNNB1's selected epitopes

Table 50 CTNNB1 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: IMRTYTYEK	42.27%
Epitope #2: TTAPSLSGK	37.40%
Epitope #3: RLHYGLPVV	20.92%
Epitope #4: YAAAVLFRM	20.22%
Epitope #5: ATYAAAVLF	16.30%
Epitope #6: GQYAMTRAQRVRAAM	74.99%
Epitope #7: EKLLWTTSRVLKVLS	74.63%
Epitope #8: QALVNIMRTYTYEKL	59.87%
Epitope #9: WPLIKATVGLIRNLA	72.71%
Epitope #10: GLIRNLALCPANHAP	65.43%
Epitope set	91.62%

4.4.9 EEF2's selected epitopes

Table 51 EEF2 class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: IMRTYTYEK	42.27%
Epitope #2: TTAPSLSGK	37.40%
Epitope #3: RLHYGLPVV	20.92%
Epitope #4: YAAAVLFRM	20.22%
Epitope #5: ATYAAAVLF	16.30%
Epitope #6: PLMMYISKMVPTSDK	59.87%
Epitope #7: GRVFSGLVSTGLKVR	55.10%
Epitope #8: GGIYGVLNRKRGHVF	59.68%
Epitope #9: CITIKSTAISLFYEL	65.43%
Epitope #10: NRLYMKARPFPDGLA	62.92%
Epitope set	85.41%

4.4.10 FLT3's selected epitopes

Table 52 FLT3 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: RMPEAAPPV	15.90%
Epitope #2: KTYQGSYGF	20.70%
Epitope #3: CTYSPALNK	41.71%
Epitope #4: RVRAMAIYK	42.27%
Epitope #5: TAKSVTCTY	10.14%
Epitope #6: APPQHLIRVEGNLRV	71.56%
Epitope #7: RAMAIYKQSQHMTEV	12.26%
Epitope #8: TYQGSYGFRLGFLHS	60.61%
Epitope #9: VTCTYSPALNKMFCQ	40.16%
Epitope set	86.13%

4.4.11 GPNMB's selected epitopes

Table 53 GPNMB MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: TIVEGILEV	15.90%
Epitope #2: ITFAVNLIF	20.70%
Epitope #3: YVLNGTFSL	24.01%
Epitope #4: RTFNGSGTY	45.21%
Epitope #5: YVFHTLGQY	13.29%
Epitope #6: TVISLLVYKKHKEYN	37.47%
Epitope #7: DPASPLRMANSALIS	74.63%
Epitope #8: LSVFLNRAKAVFFPG	74.13%
Epitope #9: DVDEMCLLTVRRTFN	37.47%
Epitope #10: GNVVRSKGLSVFLNR	63.19%
Epitope set	89.68%

4.4.12 HSPA1B's selected epitopes

Table 54 HSPA1B MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: LLLLDVAPL	15.90%
Epitope #2: AEAYLGYPV	7.03%
Epitope #3: RTTPSYVAF	20.70%
Epitope #4: VIAGLNVLR	19.31%
Epitope #5: GVMTALIKR	35.68%
Epitope #6: GLNVLRIINEPTAAA	64.13%
Epitope #7: QNKRAVRRLRTACER	27.20%
Epitope #8: TKDAGVIAGLNVLRI	51.04%
Epitope #9: GGVMTALIKRNSTIP	14.96%
Epitope #10: VGVFQHGKVEIIAND	40.16%
Epitope set	86.34%

4.4.13 KRAS's selected epitopes

Table 55 KRAS MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: KSFEDIHHY	13.77%
Epitope #2: CVFAINNTK	37.40%
Epitope #3: LVREIRQYR	20.14%
Epitope #4: CLLDILDTA	15.90%
Epitope #5: QYMRTGEGF	18.18%
Epitope #6: TLVREIRQYRLKKIS	69.29%
Epitope #7: ALTIQLIQNHFVDEY	64.13%
Epitope #8: GFLCVFAINNTKSFE	64.13%
Epitope set	87.68%

4.4.14 MUM1's selected peptide

Table 56 MUM1 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: YAADISYPV	20.93%
Epitope #2: KMKGFTVSL	21.86%
Epitope #3: RSFEVGMLV	22.76%
Epitope #4: GMLVWHKHK	12.30%
Epitope #5: LSSSFTCEK	31.61%
Epitope #6: PLEELAYRRSLRVAL	73.60%
Epitope #7: IGWCVSLITDYRVRL	69.84%
Epitope #8: GFTVSLKSLKHFDCK	59.23%
Epitope #9: KGAESHLRAILKSRK	59.68%
Epitope #10: LEYYAADISYPVRKS	43.67%
Epitope set	91.56%

4.4.15 PAPOLG's selected epitopes

Table 57 PAPOLG MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: HLMPIITPA	15.90%
Epitope #2: CTIPTVVGR	36.41%
Epitope #3: KIFTFGSYR	50.13%
Epitope #4: RSDFFQSFF	20.59%
Epitope #5: YPNAAASTL	9.49%
Epitope #6: FRLTLRAVKLWAKRR	71.40%
Epitope #7: KYRHYIVLTASASTE	65.84%
Epitope #8: VESKIRVLVGNLERN	61.58%
Epitope #9: LRSLDIRCIRSLNGC	47.68%
Epitope #10: LGIIFRRVENAESVN	51.88%
Epitope set	87.11%

4.4.16 TPI1's selected epitopes

Table 58 TPI1 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population prediction
Epitope #1: HLMPIITPA	15.90%
Epitope #2: CTIPTVVGR	36.41%
Epitope #3: KIFTFGSYR	50.13%
Epitope #4: RSDFFQSFF	20.59%
Epitope #5: YPNAAASTL	9.49%
Epitope #6: GELIGTLNAAKVPAD	51.19%
Epitope #7: ESDELIGQKVAHALA	54.42%
Epitope #8: FHFAALYISGQWPRL	62.92%
Epitope #9: SKVVLAYEPVWAIGT	27.42%
Epitope #10: LRGWLKSNVSDAVAQ	15.61%

Epitope set	86.73%

4.4.17 UBXD5's selected epitopes

Table 59 UBXD5 MHC class 1 and class 11 epitope's population coverage

Epitope list	Population coverage
Epitope #1: RTLEPIPLK	42.27%
Epitope #2: SAFEIFSTF	22.85%
Epitope #3: RLYPNGVPF	9.13%
Epitope #4: MTAEKFLNR	36.41%
Epitope #5: MAFMTRKLW	14.60%
Epitope #6: IPLKLYRNGIMMFDG	61.86%
Epitope #7: FPSELQRLYPNGVPF	40.16%
Epitope #8: KAALLLRARRAPKSS	69.67%
Epitope #9: EQAFLLMMQPDNTIG	15.73%
Epitope #10: DASAFEIFSTFPPTL	39.24%
Epitope set	88.45%

5 Conclusion

The tumor specific antigens are the main target of the immunogenic response against the tumor cell. These are the unique antigens (not present on the normal cell) so if immunotherapy using these as targets for immune cells, then there is no harmful effect of it on the normal cell. T cell epitopes were predicted for 17 tumor specific antigens. Ten epitopes for each antigen were selected which cover maximum population. These are the epitopes for each antigen.

ANTIGEN NAME	SELECTED EPITOPE SEQUENCE
ACTN4	KALDFIASK,MTYVSSFYH,VQNFHISWK,MTLGMIWTI,LAFNALIHR,SSFYHAFSGAQKAET,ALDFIASKG VKLVSI,LEINFNTLQTKLRLS,LQTKLRLSNRPAFMP,SNHIKLSGSNPYTTV
BRAF	FLMAQGAML,RMAVPLLRR,RPWKRSWSA,LMAQEALAF,CTSRCLSRR,AVPLLRRMEGAPAGP,FLMAQG AMLAAQERR,MLMAQEALAFLMAQG
CAMEL	SVLRAFAAR,KSFEVPQAK,LLYDTIFQI,SSTPHNVSW,NVSWRDRTR,GAHYDIVGMKRLLQG,STFLVLMS HGILEGI,RDMESVLRAFAARPE,DFIAFCSSTPHNVSW,LWVRDSPASLAVISS
CASP5	IINNHNFAK,TVNNCVSYR,ILTEVNYEV,LSFLKELLF,MLEESNLSF,SFLKELLFRINRLDL,DALMLFQRLQE KRML,RAQISAYRVMLYQIS,RGYCLIINNHNFAKA, DLASLKFLSLDYIPQ
CASP8	HSGHFVALK,CIFAEMFRR,RIYSYQMAL,NPHKRISAF,LTPVVVTLW,KEAINILSHLPSHHY,TTLWHLQKD VALSVL,QTFKFTSLQNFSNCL,AAIWQALNHYAYRDA,YAYAYTLLGHEFVLT
CDC27	HSGHFVALK,CIFAEMFRR,RIYSYQMAL,NPHKRISAF,LTPVVVTLW,KEAINILSHLPSH,TTLWHLQKDVA LSVL,QTFKFTSLQNFSNCL,AAIWQALNHYAYRDA,YAYAYTLLGHEFVLT
CDK4	HSGHFVALK,CIFAEMFRR,RIYSYQMAL,NPHKRISAF,LTPVVVTLW,PHSGHFVALKSVRVP,RISAFRALQ HSYLHK,SYQMALTPVVVTLWY,DFGLARIYSYQMALT,RQFLRGLDFLHANCI
CDKN2A	LPVDLAEEL,DWLATAAAR,LTRPVHDAA,EEVRALLEAGALPNA,LDTLVVLHRAGARLD
TPI1	HLMPIITPA,CTIPTVVGR,KIFTFGSYR,RSDFFQSFF,YPNAAASTL,GELIGTLNAAKVPAD,ESDELIGQKVA HALA,FHFAALYISGQWPRL,SKVVLAYEPVWAIGT,LRGWLKSNVSDAVAQ
CTNNB1	IMRTYTYEK,TTAPSLSGK,RLHYGLPVV,YAAAVLFRM,ATYAAAVLF,GQYAMTRAQRVRAAM,EKLLWT TSRVLKVLS,QALVNIMRTYTYEKL,WPLIKATVGLIRNLA,GLIRNLALCPANHAP
EEF2	IMRTYTYEK,TTAPSLSGK,RLHYGLPVV,YAAAVLFRM,ATYAAAVLF,PLMMYISKMVPTSDK,GRVFSGL VSTGLKVR,GGIYGVLNRKRGHVF,CITIKSTAISLFYEL,NRLYMKARPFPDGLA
FLT3	RMPEAAPPV,KTYQGSYGF,CTYSPALNK,RVRAMAIYK,TAKSVTCTY,APPQHLIRVEGNLRV,RAMAIYKQ SQHMTEV,TYQGSYGFRLGFLHS,VTCTYSPALNKMFCQ
GPNMB	TIVEGILEV,ITFAVNLIF,YVLNGTFSL,RTFNGSGTY,YVFHTLGQY,TVISLLVYKKHKEYN,DPASPLRMAN SALIS,LSVFLNRAKAVFFPG,DVDEMCLLTVRRTFN,GNVVRSKGLSVFLNR
HSPA1B	LLLLDVAPL,AEAYLGYPV,RTTPSYVAF,VIAGLNVLR,GVMTALIKR,GLNVLRIINEPTAAA,QNKRAVRRL RTACER,TKDAGVIAGLNVLRI,GGVMTALIKRNSTIP,VGVFQHGKVEIIAND
KRAS	KSFEDIHHY,CVFAINNTK,LVREIRQYR,CLLDILDTA,QYMRTGEGF,TLVREIRQYRLKKIS,ALTIQLIQNHF VDEY,GFLCVFAINNTKSFE
MUM1	YAADISYPV,KMKGFTVSL,RSFEVGMLV,GMLVWHKHK,LSSSFTCEK,PLEELAYRRSLRVAL,IGWCVSLIT DYRVRL,GFTVSLKSLKHFDCK,KGAESHLRAILKSRK,LEYYAADISYPVRKS
PAPOLG	HLMPIITPA,CTIPTVVGR,KIFTFGSYR,RSDFFQSFF,YPNAAASTL,FRLTLRAVKLWAKRR,KYRHYIVLTAS ASTE,VESKIRVLVGNLERN,LRSLDIRCIRSLNGC,LGIIFRRVENAESVN
UBXD5	RTLEPIPLK,SAFEIFSTF,RLYPNGVPF,MTAEKFLNR,MAFMTRKLW,IPLKLYRNGIMMFDG,FPSELQRLYP NGVPF,KAALLLRARRAPKSS,EQAFLLMMQPDNTIG,DASAFEIFSTFPPTL

We can further analyse these T cells epitopes for B cell epitope prediction which would give epitopes predicted to stimulate both cell mediated and humoral immune response.

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7 Appendix

Table 60 CD8+ binding Immunogenic peptide of ACTN4

Peptide	Score
GMIWTIILR	0.53501
HTIEEIEGL	0.47103
FIVHTIEEI	0.35203
GEAEFNRIM	0.3428
WTIILRFAI	0.3367
RTIPWLEDR	0.33476
REREAILAI	0.29443
NFITAEELR	0.29263
LLLDPAWEK	0.29019
YEEWLLNEI	0.28719
WLLNEIRRL	0.28659
TIARTINEV	0.2809
KTFTAWCNS	0.27439
VVGPWIQTK	0.26574
FITAEELRR	0.2646
MEEIGRISI	0.2638
VQNFHISWK	0.26228
HSRREALEK	0.25235
KMLDAEDIV	0.24755
KLEDFRDYR	0.2341
RAAPFNNWM	0.23341
ILRFAIQDI	0.21318
KQLEAIDQL	0.21157
RASFNHFDK	0.20001
MTLGMIWTI	0.1944
KALDFIASK	0.19436
DQAEYCIAR	0.19428
LIHRHRPEL	0.19194
LAFNALIHR	0.19037
ETAANRICK	0.17531
NAFEVAEKY	0.17428
QMQEFRASF	0.16223
GLVTFQAFI	0.15884
LEVISGERL	0.13286
QLIQEALIF	0.12937
GPDAVPGAL	0.12779
VISGERLPK	0.11078
MVSDINNGW	0.10647
RLSNRPAFM	0.08117

QTKMEEIGR	0.07717
DAVPGALDY	0.07567
AVPGALDYK	0.07472
LEAIDQLHL	0.06872
VPKRDHALL	0.06067
QAFIDFMSR	0.05886
FTAWCNSHL	0.05628
RIAESNHIK	0.05132
LTHSRREAL	0.05051
SAKEGLLLW	0.03747
ALIRKHEAF	0.02309
HLMEDYEKL	0.02037
RQKASIHEA	0.01935
KLMLLLEVI	0.01922
LISAHDQFK	0.00869

Table 61 CD8+ binding Immunogenic peptide of BRAF

peptide	score
AIPEEVWNI	0.41667
WMAPEVIRM	0.28046
KSIIHRDLK	0.26958
WLTGEELHV	0.24799
LTTHNFVRK	0.21413
APNVHINTI	0.21344
MIKLIDIAR	0.2045
GEELHVEVL	0.20032
KTRHVNILL	0.1893
DQIIFMVGR	0.18046
IGWDTDISW	0.16856
SGSFGTVYK	0.16632
TLAFCDFCR	0.16618
SLYHHLHII	0.15747
IVRVFLPNK	0.14876
KQRTVVPAR	0.14842
SILWMAPEV	0.13426
EAYEEYTSK	0.12967
IQAGGYGAF	0.12778
VEVLENVPL	0.12201
FMVGRGYLS	0.1202
DLIRDQGFR	0.10484
KTPIQAGGY	0.10275
VPARCGVTV	0.1017
LAFCDFCRK	0.10029

FQTEDFSLY	0.09905
KMLNVTAPT	0.09317
EIPDGQITV	0.07768
SIPIPQPFR	0.06952
FVRKTFFTL	0.06928
SSLYHHLHI	0.06417
TVVPARCGV	0.06336
GLIPECCAV	0.05615
ILASIELLA	0.05424
GSGSFGTVY	0.04731
KSIPIPQPF	0.04696
ILWMAPEVI	0.03148
QGMDYLHAK	0.00144

Table 62 CD8+ Immunogenic peptide of CAMEL

peptide	score
MAQEALAFL	0.19223
AAQERRVPR	0.19063
MLAAQERRV	0.10744
CLSRRPWKR	0.09928
AMLAAQERR	0.07957
RMAVPLLRR	0.05388
RCLSRRPWK	0.04911
LMAQEALAF	0.04397
APRGVRMAV	0.01448

Table 63 CD8+ binding immunogenic peptide of CASP5

peptide	score
MPTIERATL	0.34844
CPREEFLRL	0.34613
NVSWRDRTR	0.30683
MSHGILEGI	0.26806
LYDTIFQIF	0.26606
SVLRAFAAR	0.25266
FITELITCF	0.22909
LLYDTIFQI	0.21532
ESVLRAFAA	0.21113
AQMPTIERA	0.20966
RLALIICNT	0.20714
HLMEIFRKV	0.20123
RTRGSIFIT	0.19164
TLTRDFYLF	0.18688

RATLTRDFY	0.17508
TIFQIFNNR	0.152
LPARNGAHY	0.1423
KVIIVQACR	0.1098
KSFEVPQAK	0.09361
SVCKIHEEK	0.06855
ESAESTNIL	0.06119
STPHNVSWR	0.05131
LLQGLGYTV	0.03716

Table 64 CD8+ binding Immunogenic peptide of CDC27

peptide	score
NLLDIFIEM	0.42934
NPAEGTWYI	0.36777
IPDEADFLL	0.25925
DILTILTEV	0.24588
YIPDEADFL	0.24017
ILTEVNYEV	0.20284
NLYDIGEQL	0.19944
RYIPDEADF	0.19387
CTVEQIYEI	0.18201
QSLRERCPR	0.14272
RLDLLITYL	0.14112
FLKELLFRI	0.13855
IINNHNFAK	0.13846
DAGALTTTF	0.13155
NMDCFICCI	0.11523
KPRGYCLII	0.06495
DIFIEMEKR	0.06262
LITYLNTRK	0.05499
GMATVNNCV	0.04891
RINRLDLLI	0.04422
LTSQFTGLK	0.0024

Table 65 CD8+ binding immunogenic peptide of CDK4

peptide	score
SEVRRIENY	0.32188
VQAAIWQAL	0.31528
EVRRIENYR	0.28511
TLLGHEFVL	0.27917
LACFRNAIR	0.25539
HEFVLTEEL	0.24828

SLVYFLIGK	0.24536
RNAIRVNPR	0.22394
KLAEGEQIL	0.2207
IYSTTLWHL	0.21856
YLPDDEEPI	0.21259
YAYRDAVFL	0.21255
AYRDAVFLA	0.2084
TPSFGILPL	0.20654
ASVLFANEK	0.20611
YTLLGHEFV	0.20159
AVFLAERLY	0.19639
REHDIAIKF	0.18557
FLAERLYAE	0.17315
STTLWHLQK	0.17055
DSSIISEGK	0.15839
VLLCHIGVV	0.15165
NTGWVLCQI	0.15047
ILSGGVFNK	0.14728
ALACFRNAI	0.13857
HYAYRDAVF	0.1374
LVYFLIGKV	0.13368
KALACFRNA	0.13337
RAYFELSEY	0.12254
SLLGGPAAL	0.10894
AYTLLGHEF	0.10834
ALMNFSWAM	0.10456
SVLLCHIGV	0.09531
VLCQIGRAY	0.09398
QIKEAIDKR	0.08685
AEVHSEEAL	0.08646
EVTPILAQT	0.08594
RIFSEVRRI	0.08181
DIAIKFFQR	0.07794
KISTITPQI	0.07446
NYAYAYTLL	0.06988
SLLGHVYCK	0.06624
KESLVYFLI	0.06418
RSLLGGPAA	0.06364
RVNPRHYNA	0.06069
QALNHYAYR	0.04877
WQALNHYAY	0.04543
IQVDPNYAY	0.03857
VPLGTGTSI	0.0363
EAINILSHL	0.03513

QIQAFNLQK	0.03264
SSRLFTSDS	0.02952
FLLATCYYR	0.01754
VLQEPVQAA	0.01631
SLSLNPFLW	0.01072
TLSLLGHVY	0.00766
SLAEMHFQK	0.00402
DSACFTLSL	0.00297
MSLLREMGK	0.00149

Table 66 CD8+ Binding immunogenic peptide of CDKN2A

peptide	score
VAEIGVGAY	0.28974
TSRTDREIK	0.28844
PVVVTLWYR	0.26708
KVTLVFEHV	0.25524
TPVVVTLWY	0.25174
HSGHFVALK	0.22295
TLWYRAPEV	0.20455
ALLRRLEAF	0.1958
KLADFGLAR	0.19442
LWYRAPEVL	0.18716
TVREVALLR	0.17874
EAFEHPNVV	0.17847
LTPVVVTLW	0.14328
CIFAEMFRR	0.13861
ALTPVVVTL	0.13802
RQFLRGLDF	0.11274
FGLARIYSY	0.11167
VGAYGTVYK	0.1112
REVALLRRL	0.10545
FEHPNVVRL	0.09698
QMALTPVVV	0.08786
GAFPPRGPR	0.08688
LMRQFLRGL	0.06728
LQSTYATPV	0.04487
YQMALTPVV	0.02287
MLTFNPHKR	0.01496
RLMDVCATS	0.01047

Table 67 CD8+ biding immunogenic peptide of CTNNB1

peptide	score

LPVDLAEEL	0.20475
ATAAARGRV	0.19773
DWLATAAAR	0.16628
LTRPVHDAA	0.11787
DVARYLRAA	0.11728
VARYLRAAA	0.10688

Table 68 CD8+ binding immunogenic peptide of EEF2

peptide	score
VLYEWEQGF	0.33154
CQVGGIEAL	0.31314
QVGGIEALV	0.29549
DVHNRIVIR	0.29227
REDITEPAI	0.28667
VLFYAITTL	0.2531
MAWNETADL	0.24541
MVSAIVRTM	0.22049
GLNTIPLFV	0.21516
YAAAVLFRM	0.21087
NTNDVETAR	0.21029
FLAITTDCL	0.20818
TYAAAVLFR	0.18807
FPETLDEGM	0.18594
KLLWTTSRV	0.18425
REQGAIPRL	0.18076
VQNCLWTLR	0.16764
ALVRTVLRA	0.16302
RAQRVRAAM	0.15928
TQFDAAHPT	0.15607
EALVRTVLR	0.15324
IEALVRTVL	0.14734
HAVVNLINY	0.14674
QVADIDGQY	0.14642
NIQRVAAGV	0.14433
IMRTYTYEK	0.14418
ATYAAAVLF	0.14146
GVATYAAAV	0.14087
AQNAVRLHY	0.13573
IVIRGLNTI	0.13506
YAITTLHNL	0.13314
RTVLRAGDR	0.13103
TQQQFVEGV	0.103
LVQNCLWTL	0.10257

GPQALVNIM	0.10213
NAVRLHYGL	0.10181
ETARCTAGT	0.10164
NTIPLFVQL	0.0986
CTAGTLHNL	0.09768
ASRHAIMRS	0.09477
NVVTCAAGI	0.08961
APLREQGAI	0.0884
EASRHAIMR	0.07643
AQRVRAAMF	0.07599
MLKHAVVNL	0.07057
LLNDEDQVV	0.06496
YPVDGLPDL	0.06188
ETADLGLDI	0.05972
MTRAQRVRA	0.05717
RAAMFPETL	0.04674
MQALGLHLT	0.04492
RLHYGLPVV	0.0441
KEASRHAIM	0.03786
ALVNIMRTY	0.03755
IPLFVQLLY	0.02952
LLYSPIENI	0.02753
GLLGTLVQL	0.02502
CAAGILSNL	0.02256
RVRAAMFPE	0.02129
CALRHLTSR	0.00564
YAMTRAQRV	0.00177

Table 69 CD8+ binding immunogenic peptide of FLT3

Peptide	score
GLHGWAFTL	0.41861
KGRFYAFGR	0.28643
ESFGFTADL	0.26862
ETRFTDTRK	0.25628
RFYAFGRVF	0.25187
VVRVAVEAK	0.24266
TLHADAIHR	0.23952
AVMRRWLPA	0.23815
TTFEHAHNM	0.2306
SVVAGFQWA	0.22763
QLILDPIFK	0.22392
QVVGGIYGV	0.22246
GLKEGIPAL	0.20253

KAGIIASAR	0.19459
GELHLEICL	0.19322
FSSEVTAAL	0.17967
NVNVIISTY	0.17738
MMGRYVEPI	0.17636
RIVENVNVI	0.16537
CVQTETVLR	0.16386
DSVVAGFQW	0.15606
VLRQAIAER	0.15514
VIAHVDHGK	0.15343
RLMEPIYLV	0.14863
MVNFTVDQI	0.1434
KMDRALLEL	0.13608
AEARKIWCF	0.1355
EEDHACIPI	0.13294
VTDGALVVV	0.12792
DPVLGTVGF	0.12642
KPIQRTILM	0.11942
KQRARYLAE	0.11659
WLPAGDALL	0.11619
FTVDQIRAI	0.11474
LILDPIFKV	0.106
QVVAETRKR	0.10449
IMIDPVLGT	0.10402
RYFDPANGK	0.10069
AIAERIKPV	0.10065
IPTARRCLY	0.09911
FPQCVFDHW	0.09617
GIYGVLNRK	0.08744
KSTAISLFY	0.08488
KLPRTFCQL	0.0833
LLQMITIHL	0.08306
GLVGVDQFL	0.07922
MIDPVLGTV	0.07708
KVFDAIMNF	0.07172
LPVNESFGF	0.06726
WQILPGDPF	0.06538
FQWATKEGA	0.05037
ILTDITKGV	0.03886
TAISLFYEL	0.03151
KQFAEMYVA	0.03057
RSNTGGQAF	0.02696
TFEHAHNMR	0.02554
SQVAGTPMF	0.01035

MYVAKFAAK	0.00885
AYLPVNESF	0.00719
YLKPIQRTI	0.00576
EVSARQELK	0.00505

Table 70 CD8+ binding immunogenic peptide of GPNMB

Peptide	score
RPILTIITL	0.33012
LQIRGRERF	0.29174
NSFEVRVCA	0.23101
EMFRELNEA	0.23018
GSYGFRLGF	0.20606
ELNEALELK	0.20433
LSPDDIEQW	0.18242
RELNEALEL	0.17336
GSDCTTIHY	0.15851
RMPEAAPPV	0.15624
MPEAAPPVA	0.11429
APAAPTPAA	0.09131
YFTLQIRGR	0.0774
LLGRNSFEV	0.05835
EAAPPVAPA	0.05614
RVRAMAIYK	0.03216
NTFRHSVVV	0.02481
GTRVRAMAI	0.02023
YQGSYGFRL	0.00187

Table 71 CD8+ binding immunogenic peptide of GPNMB

Peptide	score
ATITIVEGI	0.35502
RWNFIYVFH	0.34506
RRWNFIYVF	0.32567
TIVEGILEV	0.32157
SLIDFVVTC	0.2759
NFIYVFHTL	0.23986
LYYFLGFLL	0.23002
FAVNLIFPR	0.21369
RAKAVFFPG	0.21209
ESSLIDFVV	0.20854
NITFAVNLI	0.19542
NSSDETFLK	0.19498
LSVFLNRAK	0.18085

ITFAVNLIF	0.17988
LMEVTVYRR	0.17782
SVGCLAIFV	0.1635
HGRAYVPIA	0.15983
VTVYRRHGR	0.1559
YYFLGFLLL	0.1542
QLMEVTVYR	0.15317
KEDANGNIV	0.14447
CQINRYGHF	0.13111
FLGFLLLAA	0.12106
YPVWKRGDM	0.11657
GLSVFLNRA	0.11618
LLLAARLPL	0.10675
RYGHFQATI	0.10421
HTVNHTYVL	0.09593
ISVGCLAIF	0.09534
NLIFPRCQK	0.08574
HTYVLNGTF	0.07473
YVVTDQIPV	0.07086
DVYVVTDQI	0.06812
EMCLLTVRR	0.06216
YVLNGTFSL	0.06157
LGFLLLAAR	0.06148
YVFHTLGQY	0.05883
RVSVNTANV	0.04732
LPIMFDVLI	0.02974
SADPYVYNW	0.0244
ALVGSNITF	0.01531
FVTVISLLV	0.01217
IISDPTCEI	0.00736

Table 72 CD8+ binding immunogenic peptide of HSPA1B

Peptide	score
SLFEGIDFY	0.38415
ITRARFEEL	0.35977
TIDDGIFEV	0.3451
KMKEIAEAY	0.30454
YVAFTDTER	0.28044
EPTAAAIAY	0.26208
NAVITVPAY	0.23748
TAAAIAYGL	0.23518
LLQDFFNGR	0.22326
YPVTNAVIT	0.19559

KQTQIFTTY	0.19128
IINEPTAAA	0.18027
MAKAAAIGI	0.17642
RAVRRLRTA	0.1718
EIAEAYLGY	0.15851
RLRTACERA	0.15795
LSGIPPAPR	0.15022
AVITVPAYF	0.14288
EAVAYGAAV	0.13695
ATKDAGVIA	0.13492
GVIAGLNVL	0.12379
AQIHDLVLV	0.11527
EGIDFYTSI	0.11214
NQPGVLIQV	0.1049
CVGVFQHGK	0.1046
ASLEIDSLF	0.10153
QPGVLIQVY	0.09338
VQRERVSAK	0.09005
FYTSITRAR	0.07881
LLLLDVAPL	0.07224
YLGYPVTNA	0.06432
VIAGLNVLR	0.05827
AEAYLGYPV	0.02048
LEQVCNPII	0.01375
RTACERAKR	0.01169
MVQEAEKYK	0.01134

Table 73 CD8+ binding immunogenic peptide of KRAS

Peptide	score
KSFEDIHHY	0.33183
YMRTGEGFL	0.28011
RQRVEDAFY	0.27814
IQNHFVDEY	0.26053
FYTLVREIR	0.25262
SFEDIHHYR	0.24001
CVFAINNTK	0.2181
LVREIRQYR	0.19595
CLLDILDTA	0.17928
QYMRTGEGF	0.16908
FVDEYDPTI	0.13855
IQLIQNHFV	0.10713
KTRQRVEDA	0.08696
KSALTIQLI	0.06038

GETCLLDIL	0.03359

Table 74 CD8+ binding immunogenic peptide of MUM1

Peptide	score
KYPFWPAVV	0.3766
LLPEAIICA	0.34135
LPEAIICAI	0.30411
RIRFILDVL	0.2966
RTNGDRIRF	0.24488
ESHLRAILK	0.19241
RSRAARDRA	0.19195
SAVDEVDYK	0.18864
VPAAPLEEL	0.17383
KLVEYIVKA	0.14467
KPSRWLQTF	0.12856
AESHLRAIL	0.12755
YVTCVETYL	0.1234
GMLVWHKHK	0.12099
RPHRRRPCV	0.12084
CVSLITDYR	0.11784
FLEYYAADI	0.10799
RVLLYHEPR	0.09011
KTAEEKYIK	0.08259
ILDVLLPEA	0.07664
KEIFDNQLL	0.07227
GVYQEVGAK	0.07006
EVGMLVWHK	0.0682
LLYHEPRSF	0.06543
RSFEVGMLV	0.05617
AARDRANQK	0.05321
KMKGFTVSL	0.05282
SFAGSFLEY	0.04504
RIHHKNWTL	0.03658
AIICAISAV	0.03557
LEDEGQLDL	0.03551
CAISAVDEV	0.03091
FAGSFLEYY	0.03043

Table 75 CD8+ binding immunogenic peptide of PAPOLG

Peptide	score
FLGIIFRRV	0.45864
WFLGIIFRR	0.41442

SMWFLGIIF	0.40088
MWFLGIIFR	0.36584
LERNEFITL	0.35299
LMPIITPAY	0.30996
SPKEIDHIY	0.28629
IIFRRVENA	0.26006
GMKIEATHV	0.24991
LSIPVIGAK	0.24898
VTDEILHLV	0.24793
LVKEWISDV	0.24507
MPIPTIDTS	0.23652
AVEDAFVPV	0.23148
RSLDIRCIR	0.2293
VEDAFVPVI	0.21419
HLMPIITPA	0.213
STEENHLEW	0.20654
KYRHYIVLT	0.19939
NSAEPAAVI	0.19662
VPANNIRVI	0.19297
NMLGFLGGV	0.18246
IQSFTDTVY	0.17966
SVVATVGGK	0.17783
CVAPRHVER	0.17573
ESVNIDLTY	0.17069
QSFTDTVYR	0.16788
CTIPTVVGR	0.16334
FLGGVSWAM	0.13937
KLLEPPNFF	0.13885
ETFRLTLRA	0.1367
EIDHIYTQK	0.13095
SYRLGVHTK	0.12748
RTVMVEEFK	0.12405
RVLVGNLER	0.11399
KIRVLVGNL	0.11122
NSIRLTLNR	0.10788
YVSMWFLGI	0.10594
FQKYRHYIV	0.08177
KIFTFGSYR	0.08118
WEWPNPVLL	0.07236
KFIRLESTF	0.06179
RSDFFQSFF	0.05874
KIEATHVKK	0.04896
FIRLESTFK	0.04857
KQLHHYLPA	0.04113

TLVHKFFLV	0.03847
IFTFGSYRL	0.03479
LVHKFFLVF	0.03246
LGFLGGVSW	0.02992
DFFQSFFEK	0.02784
MLVARTCQL	0.02653
AVKLWAKRR	0.01961
RQANNINML	0.01711
FLVFSKWEW	0.01254
STSTRTVMV	0.00454

Table 76 CD8+ binding immunogenic peptide of TPI1

Peptide	score
LAYEPVWAI	0.33741
STRIIYGGS	0.32524
EFVDIINAK	0.308
PTAYIDFAR	0.28112
SLGELIGTL	0.27751
KFFVGGNWK	0.2684
VLAYEPVWA	0.2603
AEFHFAALY	0.24792
GVIACIGEK	0.24245
ALAEGLGVI	0.18873
SLKPEFVDI	0.17434
VVLAYEPVW	0.14118
GSVTGATCK	0.12355
ELIGTLNAA	0.12206
KVVLAYEPV	0.11488
FTGEISPGM	0.09606
VAQSTRIIY	0.07253

Table 77 CD8+ binding immunogenic peptide of UBXD5

Peptide	score
EVIDIRGPI	0.26596
SAFEIFSTF	0.26161
NTIGDVRAL	0.2043
RTLEPIPLK	0.19579
RPHPAEATL	0.18739
LLRARRAPK	0.18183
ETPTLAAER	0.15301
FSTFPPTLY	0.13544
LVVEGDTQV	0.13311

TPVPGGARL	0.1304
SENGEQAFL	0.12188
TLRPHPAEA	0.11822
MMFDGPFQP	0.114
LQAAGLVPK	0.10299
YLEDGLDPF	0.08962
AALLLRARR	0.08642
KLYRNGIMM	0.0862
VMDASAFEI	0.0796
KTVSEHGER	0.06198
SPNTPAPPL	0.04715
KAALLLRAR	0.04684
FMTRKLWDL	0.04414
RLYPNGVPF	0.0416
STFPPTLYQ	0.04106
MFDGPFQPF	0.03646
YPNGVPFKV	0.03456
KLWDLEQQV	0.01223
ALEDLVQTL	0.0078
MTAEKFLNR	0.00051

Table 78 CD4+ binding Immunogenic peptides of ACTN4

Peptide	Percentile Rank
GKMRVHKINNVNKAL	23.587
SNHIKLSGSNPYTTV	32.7632
TLGMIWTIILRFAIQ	33.05048
FKVLAGDKNFITAEE	33.17528
LQTKLRLSNRPAFMP	34.26484
LAFNALIHRHRPELI	35.76636
ALDFIASKGVKLVSI	37.33184
LEINFNTLQTKLRLS	39.95052
LMLLLEVISGERLPK	40.37924
TLSDIKALIRKHEAF	40.52832
ADQVIASFKVLAGDK	40.924
SDLLEWIRRTIPWLE	40.99596
QAEYCIARMAPYQGP	41.17148
KAIMTYVSSFYHAFS	41.81516
SGLVTFQAFIDFMSR	42.00952
WLLNEIRRLERLDHL	42.05976
RPAFMPSEGKMVSDI	42.91768
DFRDGLKLMLLLEVI	43.1136
IDQLHLEYAKRAAPF	43.37248
AEFNRIMSLVDPNHS	43.38452

EHLRRQFASQANVVG	43.7606
IILRFAIQDISVEET	43.81512
RVGWEQLLTTIARTI	44.41228
DFRDYRRVHKPPKVQ	45.56864
SNPYTTVTPQIINSK	46.52952
RKTFTAWCNSHLRKA	46.96408
KEGLLLWCQRKTAPY	47.78912
MEDYEKLASDLLEWI	48.1924
IEEIEGLISAHDQFK	48.2928
REAILAIHKEAQRIA	48.32588
KNFITAEELRRELPP	48.37136
KEAQRIAESNHIKLS	48.3766
ALDYKSFSTALYGES	48.47536
EQMQEFRASFNHFDK	48.65716
KGVKLVSIGAEEIVD	48.9882
SSFYHAFSGAQKAET	49.5446
HEAFESDLAAHQDRV	49.81208
TAANRICKVLAVNQE	49.9598

Table 79 CD4+ binding immunogenic peptides of BRAF

Peptide	Percentile Rank
RDQIIFMVGRGYLSP	36.9836
NRDQIIFMVGRGYLS	37.674
WNIKQMIKLTQEHIE	44.63252
KPIVRVFLPNKQRTV	30.37304
ASIELLARSLPKIHR	42.02156
SIELLARSLPKIHRS	41.66092
QIIFMVGRGYLSPDL	39.02032
VWNIKQMIKLTQEHI	43.92124
QKPIVRVFLPNKQRT	33.26168
SHQFEQLSGSILWMA	47.56444
NIKQMIKLTQEHIEA	45.02556
DQIIFMVGRGYLSPD	37.43728
IKQMIKLTQEHIEAL	41.005
PQKPIVRVFLPNKQR	35.23376
GSHQFEQLSGSILWM	48.51236
KFEMIKLIDIARQTA	37.79772
SSSLSVLPSSLSVFQ	46.73436
NNRDQIIFMVGRGYL	38.53112
VGVLRKTRHVNILLF	38.41032
IELLARSLPKIHRSA	42.9814
HQFEQLSGSILWMAP	48.501
PIVRVFLPNKQRTVV	30.49688

SSSSLSVLPSSLSVF	46.52252
LASIELLARSLPKIH	41.72768
VRDSLKKALMMRGLI	41.4066
RDSLKKALMMRGLIP	43.28604
GVLRKTRHVNILLFM	41.41812
TKFEMIKLIDIARQT	38.80244
SSLSVLPSSLSVFQN	46.82576
FEMIKLIDIARQTAQ	38.60368
EVGVLRKTRHVNILL	39.76348
ILASIELLARSLPKI	43.80436
DSLKKALMMRGLIPE	45.95828
SGSHQFEQLSGSILW	48.61552
IIFMVGRGYLSPDLS	39.85416
ETKFEMIKLIDIARQ	42.70924
VLRKTRHVNILLFMG	46.26388
KQMIKLTQEHIEALL	45.0758
AQGMDYLHAKSIIHR	43.32312
ELLARSLPKIHRSAS	45.8688
EMIKLIDIARQTAQG	41.6676
GMDYLHAKSIIHRDL	42.33268
IVRVFLPNKQRTVVP	34.472
EVWNIKQMIKLTQEH	48.14424
SPQKPIVRVFLPNKQ	42.78356
QILASIELLARSLPK	46.45164
SSSSSLSVLPSSLSV	48.59576
QGMDYLHAKSIIHRD	43.65448
QFEQLSGSILWMAPE	49.38016
EEVWNIKQMIKLTQE	46.59164
PQILASIELLARSLP	45.6398
FPQILASIELLARSL	46.21196
NEVGVLRKTRHVNIL	39.45476
MDYLHAKSIIHRDLK	40.41556
MIKLIDIARQTAQGM	41.70944
SLSVLPSSLSVFQNP	46.26732
PKAMKRLMAECLKKK	44.53624
TTHNFVRKTFFTLAF	42.23844
THNFVRKTFFTLAFC	45.74812
LLARSLPKIHRSASE	46.76928
KAMKRLMAECLKKKR	44.81476
PEEVWNIKQMIKLTQ	46.9402
CPKAMKRLMAECLKK	44.50316
LTTHNFVRKTFFTLA	43.05432
TVRDSLKKALMMRGL	44.46024
IVLYELMTGQLPYSN	47.67368

SGSILWMAPEVIRMQ	35.79272
QMIKLTQEHIEALLD	49.05464
DYLHAKSIIHRDLKS	41.2646
LRKTRHVNILLFMGY	48.3388
GIVLYELMTGQLPYS	45.63144
TAQGMDYLHAKSIIH	45.20456
LSGSILWMAPEVIRM	35.42044
VLYELMTGQLPYSNI	47.54948
NCPKAMKRLMAECLK	47.6476
LDLLFVSKFFEHHPI	43.6122
GSILWMAPEVIRMQD	37.47388
DQLDLLFVSKFFEHH	47.79856
PLTTHNFVRKTFFTL	45.43448
QLDLLFVSKFFEHHP	47.8576
IETKFEMIKLIDIAR	43.436
LFPQILASIELLARS	44.52912
KNEVGVLRKTRHVNI	41.62772
FGIVLYELMTGQLPY	44.98324
FCDFCRKLLFQGFRC	46.73888
AFCDFCRKLLFQGFR	45.65976
YLHAKSIIHRDLKSN	41.9502
HLHIIETKFEMIKLI	41.48112
VNILLFMGYSTKPQL	39.7764
NILLFMGYSTKPQLA	37.35764
HHLHIIETKFEMIKL	39.30028
LYHHLHIIETKFEMI	41.16256
AMKRLMAECLKKKRD	47.86016
FCRKLLFQGFRCQTC	48.52724
PLFPQILASIELLAR	44.16048
TRHVNILLFMGYSTK	45.3142
SILWMAPEVIRMQDK	39.83852
ILLFMGYSTKPQLAI	37.03884
LYELMTGQLPYSNIN	47.68424
LHIIETKFEMIKLID	47.02776
VTVRDSLKKALMMRG	48.03852
IFMVGRGYLSPDLSK	45.59872
GSSLYHHLHIIETKF	47.07456
KIGDFGLATVKSRWS	44.035
SSLYHHLHIIETKFE	45.72412
YHHLHIIETKFEMIK	39.66908
SNCPKAMKRLMAECL	46.99632
VRVFLPNKQRTVVPA	40.39868
HVNILLFMGYSTKPQ	42.013
RHVNILLFMGYSTKP	43.1812

SLYHHLHIIETKFEM	43.3374
QLSGSILWMAPEVIR	35.73552
LLFVSKFFEHHPIPQ	48.18904
INNRDQIIFMVGRGY	44.16976
IGDFGLATVKSRWSG	43.84608
HIIETKFEMIKLIDI	42.49952
KTRHVNILLFMGYST	45.9494
LQAFKNEVGVLRKTR	40.23312
LLFMGYSTKPQLAIV	42.56748
GDFGLATVKSRWSGS	45.6822
FKNEVGVLRKTRHVN	45.62276
IIETKFEMIKLIDIA	45.30072
VPLTTHNFVRKTFFT	43.74404
NYDQLDLLFVSKFFE	48.78876
AFGIVLYELMTGQLP	46.5064
MKRLMAECLKKKRDE	49.57188
IKLIDIARQTAQGMD	42.29688
RPLFPQILASIELLA	47.4554
QLQAFKNEVGVLRKT	36.99056
FSLYACASPKTPIQA	48.41092
IPEEVWNIKQMIKLT	48.444
AFKNEVGVLRKTRHV	46.65164
DFGLATVKSRWSGSH	46.81608
QQLQAFKNEVGVLRK	38.75704
NVPLTTHNFVRKTFF	43.20796
DFSLYACASPKTPIQ	48.808
ERPLFPQILASIELL	47.52852
GTDFSVSSSASMDTV	48.33136
VNYDQLDLLFVSKFF	49.20612
CCAVYRIQDGEKKPI	48.50972
TDFSVSSSASMDTVT	48.58376
EDFSLYACASPKTPI	48.0136
QSDVYAFGIVLYELM	46.8246
ILWMAPEVIRMQDKN	39.28452
CVNYDQLDLLFVSKF	49.01728
LSVLPSSLSVFQNPT	47.26236
VAVKMLNVTAPTPQQ	43.195
CAVYRIQDGEKKPIG	48.86096
NGTDFSVSSSASMDT	48.44264
GNGTDFSVSSSASMD	48.38848
DVYAFGIVLYELMTG	48.91616
AVKMLNVTAPTPQQL	44.5182
DVAVKMLNVTAPTPQ	41.60884
KVRSNCPKAMKRLMA	49.49316

VYAFGIVLYELMTGQ	47.34308
FGLATVKSRWSGSHQ	47.303
EQLSGSILWMAPEVI	37.11848
LFMGYSTKPQLAIVT	44.18852
YAFGIVLYELMTGQL	46.97444
LGNGTDFSVSSSASM	49.25592
GDVAVKMLNVTAPTP	46.94268
GSLTNVKALQKSPGP	45.0166
PGSLTNVKALQKSPG	44.53312
ENVPLTTHNFVRKTF	45.76688
PQQLQAFKNEVGVLR	40.06216
HGDVAVKMLNVTAPT	45.54636
KLIDIARQTAQGMDY	44.62732
KLLFQGFRCQTCGYK	46.52444
AIPEEVWNIKQMIKL	49.89508
VKMLNVTAPTPQQLQ	45.394
YSFQSDVYAFGIVLY	48.11972
HAKSIIHRDLKSNNI	48.5898
AKSIIHRDLKSNNIF	46.86572
LTNVKALQKSPGPQR	49.4486
RVFLPNKQRTVVPAR	46.13428
SIIHRDLKSNNIFLH	36.0666
LENVPLTTHNFVRKT	48.00136
IIHRDLKSNNIFLHE	36.66296
WHGDVAVKMLNVTAP	45.11144
KGKWHGDVAVKMLNV	44.68456
EELHVEVLENVPLTT	48.90488
PEVIRMQDKNPYSFQ	37.77152
KSNNIFLHEDLTVKI	42.10308
KSIIHRDLKSNNIFL	46.13996
IGPQILTSPSPSKSI	48.35396
APEVIRMQDKNPYSF	37.78192
KWHGDVAVKMLNVTA	44.84764
EVIRMQDKNPYSFQS	42.68824
FMGYSTKPQLAIVTQ	44.85972
LKSNNIFLHEDLTVK	40.6282
RDLKSNNIFLHEDLT	45.21548
YKGKWHGDVAVKMLN	44.5316
HRDLKSNNIFLHEDL	39.7684
VYKGKWHGDVAVKML	45.09028
GKWHGDVAVKMLNVT	43.7508
KMLNVTAPTPQQLQA	47.69552
SNNIFLHEDLTVKIG	41.7342
VLENVPLTTHNFVRK	48.86428

APNVHINTIEPVNID	44.33776
DLKSNNIFLHEDLTV	48.51896
LSKVRSNCPKAMKRL	44.31572
SAPNVHINTIEPVNI	43.8624
SKVRSNCPKAMKRLM	47.52568
NNIFLHEDLTVKIGD	42.995
PNVHINTIEPVNIDD	42.89024
NVHINTIEPVNIDDL	41.68616
MAPEVIRMQDKNPYS	39.69608
GEELHVEVLENVPLT	47.274
IHRDLKSNNIFLHED	38.95944
DLSKVRSNCPKAMKR	46.47604
NIFLHEDLTVKIGDF	42.96868
VNIDDLIRDQGFRGD	48.20376
GRGYLSPDLSKVRSN	49.3584
PVNIDDLIRDQGFRG	47.92876

Table 80 CD4+ binding immunogenic peptides of CAMEL

Peptide	Percentile Rank
AVPLLRRMEGAPAGP	45.60228
FLMAQGAMLAAQERR	39.8082
MLMAQEALAFLMAQG	48.6942

Table 81 CD4+ binding immunogenic peptides of CASP5

Peptide	Percentile Rank
GAHYDIVGMKRLLQG	43.71768
CCCHLMEIFRKVQKS	40.97168
STFLVLMSHGILEGI	38.24128
RDMESVLRAFAARPE	42.96952
DFIAFCSSTPHNVSW	42.82164
LWVRDSPASLAVISS	45.05436
EFLRLCKKNHDEIYP	49.5854
LLYDTIFQIFNNRNC	19.82444
SIFITELITCFQKYS	47.86508

Table 82 CD4+ Binding immunogenic peptides of CASP8

Peptide	Percentile Rank
PHSGHFVALKSVRVP	36.34056
RISAFRALQHSYLHK	37.18724

TVREVALLRRLEAFE	41.49208
SYQMALTPVVVTLWY	45.92228
ETIKDLMRQFLRGLD	41.43556
DFGLARIYSYQMALT	38.32648
RQFLRGLDFLHANCI	41.19992
AQLLLEMLTFNPHKR	37.65044
LTFNPHKRISAFRAL	35.27328
WYRAPEVLLQSTYAT	45.60188
KPENILVTSGGTVKL	39.88088
LRRLEAFEHPNVVRL	45.81536

Table 83 CD4+ binding immunogenic peptides of CDC27

Peptide	Percentile Rank
IPRLVQLLVRAHQDT	44.34424
GQYAMTRAQRVRAAM	43.53636
EKLLWTTSRVLKVLS	31.01528
QALVNIMRTYTYEKL	40.17936
WPLIKATVGLIRNLA	39.58072
YGLPVVVKLLHPPSH	45.89528
VDSVLFYAITTLHNL	38.72348
LVKMLGSPVDSVLFY	43.94408
AITTLHNLLLHQEGA	48.07272
AGGLQKMVALLNKTN	45.0484
VALLNKTNVKFLAIT	33.49848
SKLIILASGGPQALV	41.92404
AKMAVRLAGGLQKMV	47.46816
VHNRIVIRGLNTIPL	38.63044
LLLHQEGAKMAVRLA	49.28308
NEGVATYAAAVLFRM	47.48088
QNAVRLHYGLPVVVK	42.1772
LHILARDVHNRIVIR	32.8494
GLIRNLALCPANHAP	41.85064
ALRHLTSRHQEAEMA	43.5466
RLVQNCLWTLRNLSD	43.58684
EGLLAIFKSGGIPAL	35.87312
VVVNKAAVMVHQLSK	44.85432
MRSPQMVSAIVRTMQ	46.29784
ENIQRVAAGVLCELA	49.01372
ILAYGNQESKLIILA	47.58904
LKHAVVNLINYQDDA	39.07404
VQLLYSPIENIQRVA	44.48368
GLLGTLVQLLGSDDI	47.2946
PLREQGAIPRLVQLL	48.32768

TSSLFRTEPMAWNET	47.43544
SAIVRTMQNTNDVET	49.4026
AITTDCLQILAYGNQ	45.52

Table 84 CD4+ Binding immunogenic peptides of UBXD5

Peptide	Percentile Rank
IPLKLYRNGIMMFDG	34.74268
RLRTLEPIPLKLYRN	43.98716
FPSELQRLYPNGVPF	46.7522
EMERFLSDYGLQWVG	48.02436
RDILDGFFPSELQRL	47.472
NGIMMFDGPFQPFYD	48.85808
KAALLLRARRAPKSS	39.0608
EQAFLLMMQPDNTIG	41.67856
DDTLTLQAAGLVPKA	48.41524
DNTIGDVRALLAQAR	46.03724
QEIVVETPTLAAERE	48.60964
DASAFEIFSTFPPTL	41.32472
PKFVIRQGEVIDIRG	42.99528
LAQARVMDASAFEIF	46.4134
GEVIDIRGPIRDTLQ	42.37004