



## Interactive Report

The myNEO ImmunoEngine performs an extensive analysis to detect, identify, and rank neoantigens that can serve as immunogenic targets for immunotherapy. Therefore, the ImmunoEngine renders a tremendous amount of data. To summarise this data and to allow visual exploration of the identified mutations and neoantigens, an interactive report is generated.

These guidelines provide an overview of the functionalities of the interactive report and a use case example. The report was generated using publicly available data from Peng *et al.*<sup>1</sup> and can be accessed online:

<http://myneo-demo.herokuapp.com/>

Login: myneo

Password: myneo\_demo

## General Outline

The interactive report is composed of multiple tabs:

- General Information
- Genomic Variants
- Neoantigens
- Window
- Differential Expression
- TME
- ImmunoEngine

The **General Information tab** contains available information of the patient, the patient's HLA-type, the numbers of identified variants and neoantigens, and a circular plot visualising the genomic variants.

The **Genomic Variant tab** provides more detailed information for every variant type: SNVs, indels, gene fusion, neoisoforms, transposable elements and structural variants. Per variant type, a table summarising all relevant metrics is available. Using the range slider above the table, the variants can be filtered on the desired variant score range, if available. Furthermore, if a variant is selected, the table below provides the resulting neoepitopes, both MHCI and MHCII predicted neoepitopes, ranked on Neoantigen Score. Additionally, in the SNV&indel subtab, a violin plot visualises the (sub)clonal populations of the identified mutations which allows to select a mutation that appears in a large fraction of the cancer cells.

The most important tab, however, is the **Neoantigen tab** which provides elaborate information on the most promising 100 neoantigens. On top, a table summarises all relevant metrics for the top 100 neoantigens. The selection box allows to examine both MHCI and MHCII predicted neoepitopes. Furthermore, a dropdown menu allows to specify the HLA-type for which the most promising 100

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<sup>1</sup> S. Peng *et al.*, "Sensitive Detection and Analysis of Neoantigen-Specific T Cell Populations from Tumors and Blood," *Cell Rep.*, vol. 28, no. 10, pp. 2728-2738.e7, 2019.



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neoantigen candidates should be displayed. For example, if one prefers neoantigens directed against HLA-A24:02, one can simply select this HLA-type in the dropdown menu.



**Note:** the option "Consensus alleles" only takes into account consensus HLA-types, as shown in the HLA-type table on the **General Information tab**.

Below the top 100 table, 3 text boxes provide all information on gene level, neoantigen level and variant level for the selected neoantigen. For example, using this information, one is able to retrieve the variant which caused the neoantigen and the corresponding information, such as allelic frequency etc. Using this information, an oncologist will be able to select a set of highly potential neoantigens that can be used for a personalised vaccine in an efficient and convenient manner.

For the construction of the actual vaccine, it is of high interest to not only look at the best neoantigens, but also at their neighbourhood. A peptide might be prioritised if the variant results in multiple neighbouring neoantigens causing an immune response directed against the tumour. Therefore, the **Window tab** provides peptides of 21, 25, 27 and 31 amino acids containing multiple predicted neoantigens. The selection box allows to specify the size of the window, the neoepitope type (MHCI and/or MHCII), the HLA-type, the score calculation and the variant type. Furthermore, the box below provides information on the affected genes.

The **Differential Expression tab** allows more careful investigation of the expression level of known cancer genes, MHC related genes, CTA genes and affected genes in the top 100 neoantigens. The percentile expression plot on top visualises if genes are highly (red) or lowly (blue) expressed compared to normal tissue. The interactive table lists all genes with corresponding expression values (TPM and CPM) and percentile ranks. Upon clicking on a gene in the table, additional gene information is displayed. This includes a plot of the CPM sample distribution in the reference tissue with a line indicating where the patient ranks. Another plot shows the median expression CPM of all available tissues with a line indicating the patient's CPM.

The **TME tab** contains information on the tumour microenvironment. A table is provided containing estimated fractions of immune cells. Moreover, the selected immune cell fraction can be compared to other cancer samples using the distribution plot. Using the checkbox one, or multiple, cancer types can be selected for comparison. The black, dotted line represents the cell fraction of the patient's sample. Furthermore, a bar plot is provided visualising the cell fractions of the patient's sample. This bar plot can be compared to bar plots of other cancer types. In addition, a heatmap summarises all cell fractions for different cancer types.

Finally, the last tab is the **ImmunoEngine tab** which provides an overview of the ImmunoEngine pipeline.



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## Use Case Example

In this use case example, we will more thoroughly evaluate the properties of the most promising neoantigen candidate (FFYLGSAHYHA). In addition, we will discuss the tumour's microenvironment and expression profile.

### ImmunoEngine Report



- General Information
- Genomic Variants
- Neoantigens**
- Window
- Differential Expression
- TME
- ImmunoEngine

#### Neoantigen Top 100

MHCI MHCII

Consensus alleles

Net peptide	Norm peptide	HLA allele	Neoantigen Score	Peptide Score	SARA Event Score	Gene Symbol	Mut Rank	Variant Type
FFYLGSAHYHA	FLYLGSAHYHA	HLA-C03:03	0.951	1	0.902	DUSP4	0.07	SNV_inde1
VEILPFFYL	VEILPFLYL	HLA-B44:02	0.9467	0.9913	0.902	DUSP4	0.09	SNV_inde1
GEVLLHAF	GEVLLHAF	HLA-B44:02	0.9375	0.9913	0.8836	KIF1C	0.09	SNV_inde1
GEVLLHAF	GEVLLHAF	HLA-B44:02	0.9375	0.9913	0.8836	KIF1C	0.09	SNV_inde1
FYLGSAHYHA	LYLGSAHYHA	HLA-C03:03	0.9344	0.9668	0.902	DUSP4	0.14	SNV_inde1
FFYLGSAHYHA	PFLYLGSAHYHA	HLA-C03:03	0.8841	0.8661	0.902	DUSP4	0.36	SNV_inde1
YLWRVDFI	DLWRVDFI	HLA-B15:07	0.881	0.9814	0.7805	USP7	0.11	SNV_inde1
EIIPIQCIAR	KIIPQCIAR	HLA-A68:01	0.8699	0.8881	0.8517	RPF1	0.31	SNV_inde1
AAADSPFPH	AAADSPVPH	HLA-C03:03	0.8639	0.9766	0.7512	SSBP4	0.12	SNV_inde1
GEVLLHAF	GEVLLHAF	HLA-B44:02	0.86	0.8363	0.8836	KIF1C	0.43	SNV_inde1
GEVLLHAF	GEVLLHAF	HLA-B44:02	0.86	0.8363	0.8836	KIF1C	0.43	SNV_inde1
YLWRVDFI	DLWRVDFI	HLA-A02:01	0.8594	0.9383	0.7805	USP7	0.2	SNV_inde1
FAVLEALALR	YAVLEALALR	HLA-A68:01	0.8589	0.8705	0.8474	COP57A	0.35	SNV_inde1



**Note:** it should be noted that FFYLGSAHYHA is considered the best neoantigen targeting a consensus HLA-type, more specifically HLA-C03:03. If we want to select a neoantigen directed against another HLA-type, the dropdown menu on top can be used.

**Gene Information:**

Gene: DUSP4

The protein encoded by this gene is a member of the dual specificity protein phosphatase subfamily. These phosphatases inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues. They negatively regulate members of the mitogen-activated protein (MAP) kinase superfamily (MAP3K/ERK/SAPK/JNK, p38), which are associated with cellular proliferation and differentiation. Different members of the family of dual specificity phosphatases show distinct substrate specificities for various MAP kinase, different tissue distribution and subcellular localizations, and different modes of inducibility of their expression by extracellular stimuli. This gene product inactivates ERK1, ERK2 and JNK, is expressed in a variety of tissues, and is localized in the nucleus. Two alternatively spliced transcript variants encoding distinct isoforms, have been observed for this gene. In addition, multiple polyadenylation sites have been reported. [provided by RefSeq, Jul 2008]

**Gene Description:**

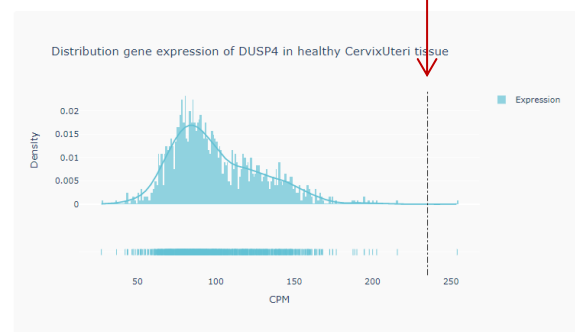
**Second Gene:** Only for gene fusions.

**Second Gene Description:** Only for gene fusions.

The selected neoantigen results from a missense variant (SNV) on chromosome 8, genomic position 29338474. The affected gene is the dual specificity protein phosphatase 4 (DUSP4). Additional information on this gene is provided in the gene information box.

Gene	Percentile	TPM	CPM
ADCY1	0.5	9.24	6.71
AK3	0	15.40	31.64
ANKRA2	0.02	6.56	7.55
BRAF	0	5.68	20.69
CHD8	0.23	17.28	83.4
CHKA	1	38.08	33.93
CDG4	0.86	39.85	73.86
COP57A	0.71	68.75	74.77
CSS1L	0.89	46.76	111.03
DLG1	0.99	50.64	97.64
DST	0	32.78	165.67
<b>DUSP4</b>	<b>1</b>	<b>61.26</b>	<b>235.16</b>
DYN	0.91	33.58	43.12
FAM84B	0.28	2.5	8.22
FASTKD1	0.78	11.6	20.34

To examine the expression level of the affected DUSP4 gene, we can switch to the **Differential Expression** tab and take a look at the Top 100 genes. We can see here that this gene has very high expression values and a percentile rank of 1. This indicates that the DUSP4 gene is highly expressed compared to normal tissue, which can also be observed in the distribution plot.





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If we switch back to the **Neoantigen tab**, we can see that the selected neoantigen has a very high ARA Event Score indicating that the variant is a high-confidence variant with a high expression. Indeed, if we examine the variant information box, we can see a high allelic frequency (AF), both on DNA and RNA level, and a high expression level. Moreover, a high Peptide Score is observed indicating that the peptide has a high probability of being presented on the tumour's cell surface. Hence, also a high Neoantigen Score can be observed. Again, we can confirm this examining the neoantigen information box.

Norm_MHCrnk	1.45
Mut_peptide	FFYLGSAVHA
Mut_MHCAffinity	1.5
Mut_MHCrnk	0.07
Gene_ID	ENSG00000120875
Transcript_ID	ENST00000240100,ENST00000240101
Amino_Acid_Change	L/F
Allele_Frequency	0.644

cellular_prevalence_sequenza	0.981
DNA_AF_tumor	0.644
DNA_RD_tumor	75
DNA_AF_normal	0
DNA_RD_normal	98
RNA_FILTER	PASS
RNA_AF_tumor	0.677
RNA_RD_tumor	966

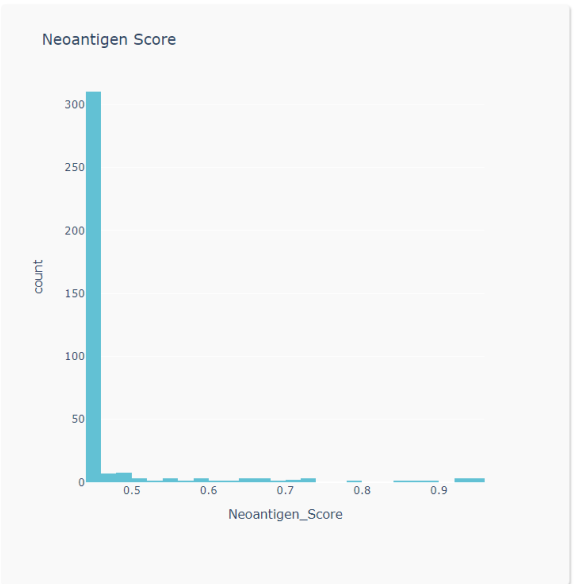
If we want more information on the point mutation causing this neoantigen, we can switch to the **Genomic Variants tab** and look for the mutation in the SNV&indel list. If we browse to the right of the table, we can find more information on the affected genes and transcripts, clinical significance and known variants. Furthermore, when we select the variant, we can see all additional predicted peptides and their corresponding Peptide and Neoantigen Score, and MHC rank. Of course, our selected neoantigen ranks on top.

#ID	ARA Event Score	CHROM	POS	REF	ALT	Gene Symbol	cluster_id	sequenza	cellular prevalence	DN
8:29338474:G:A	0.982	8	29338474	G	A	DUSP4	1		0.981	
17:5081279:A:G	0.884	17	5081279	A	G	KIF1C	1		0.98	
2:96287122:G:A	0.862	2	96287122	G	A	SNRNP200	1		0.98	
1:84490400:A:G	0.852	1	84490400	A	G	RPF1	1		0.98	
12:6729257:A:T	0.847	12	6729257	A	T	COP57A	1		0.981	
1:155057077:C:T	0.845	1	155057077	C	T	ADAM15	1		0.98	
2:208477924:C:T	0.84	2	208477924	C	T	SPATS2L	1		0.98	
1:151176898:C:T	0.821	1	151176898	C	T	VPS72	1		0.979	
8:38827247:C:T	0.784	8	38827247	C	T	TACC1	1		0.981	

Neoantigen resulting from selected genomic variant

MHC I  MHC II

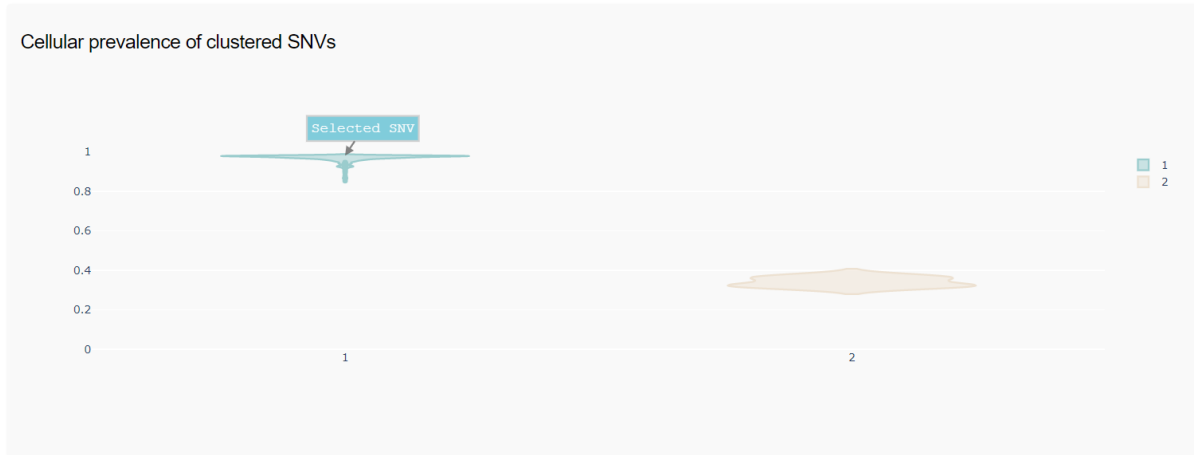
#Mut_peptide	HLA_allele	Neoantigen_Score	Peptide_Score	MHC Affini
FFYLGSAVHA	HLA-C03:03	0.951	1	1.5
VEILPFFYL	HLA-B44:02	0.947	0.991	911.39
VEILPFFYL	HLA-B44:09	0.947	0.991	911.39
FYLGSAVHA	HLA-C03:03	0.934	0.967	1.66
FYLGSAVHA	HLA-C03:292	0.932	0.962	19.46
ILPFFYLGSA	HLA-C03:292	0.92	0.938	22.23
PFFYLGSAVHA	HLA-C03:03	0.884	0.866	2
FFYLGSAVHA	HLA-C03:292	0.876	0.849	33.47
PVEILPFFYL	HLA-C03:292	0.849	0.796	39.94
GPVEILPFFYL	HLA-C03:292	0.788	0.675	56.7
PVEILPFFYL	HLA-C03:03	0.737	0.572	3.5
FYLGSAVHAA	HLA-C03:03	0.723	0.544	3.71
GPVEILPFFYL	HLA-C03:03	0.72	0.539	3.74
FYLGSAVHAA	HLA-C03:292	0.718	0.533	82.18
ILPFFYLGSAV	HLA-B15:07	0.702	0.502	98.89





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Furthermore, we can observe that the selected mutation is present in clonal population 1, which has a high cellular prevalence around 0.98. This means, that this mutation is present in almost all cancer cells, which makes it a very interesting candidate mutation to target for vaccination.



If we now would like to construct a vector for a vaccine containing a peptide sequence of 25 amino acids, we can look for our neoantigen in the **Window tab**. Here, we can see that the window containing the best neoantigen also ranks as the best window. Furthermore, we can see that 36 additional neoantigens (MHCI) are included in this window which will also serve as targets for vaccination.

General Information
Genomic Variants
Neoantigens
Window
Differential Expression
TME
ImmunoEngine

Select a window size:

21AA  25AA  27AA  31AA

Select a MHC type:

MHCI  MHCII  ALL

Specify HLA-type:

Select...

Window score calculation:

Mean of Neoantigen Scores  
 Max of Neoantigen Scores  
 Supermax of Neoantigen Scores

Select a variant type:

Missense Variants  
 Gene Fusions  
 Neoisoforms  
 Transposable Element  
 Frameshift Variants

Filter Score:

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

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### Selection Table

#Window	%Average_score_MHCI	#Number_MHCI	#ID	#Variant_Type
HDQGGPVEILPFFYLGSAHYAARRD	0.477	30	8:29338474:G:A	missense
SQQQVYRDIGEEVLLHAFEGYVNCI	0.46	30	17:5001279:A:G	missense
ELIRMPKNGKTIYKYVHLFPKLELS	0.442	30	2:96287122:G:A	missense
VVTLAAKVKCIPFVAVLLEALLRNV	0.441	30	12:6729257:A:T	missense
SHVYVRRGLALKEIIPQCIARDFTD	0.43	30	1:84490400:A:G	missense
NSPCPGPAPAKICIMEASTDFLPG	0.424	30	1:155057077:C:T	missense
QHAADTSEARPFMSSVGRVSCNLC	0.424	30	2:200477924:C:T	missense
SDDATFEENFPQRRPKVPVREVCV	0.42	30	1:151176898:C:T	missense
TEELNLRSLVPPKAAPKSPCS	0.416	27	1:151176898:C:T	missense
NSELPTAKEYFRYLVRVDVIFCDK	0.403	30	16:8899702:C:A	missense
ITVNIYPPQRSISDITLMSFSVI	0.402	30	16:8899702:C:A	missense
RDGHATDEEKLAFTSCGQKSAGAEV	0.394	30	8:38827247:C:T	missense
ITTHFELKHLSSRDLLRDWILRGTE	0.39	30	9:4740973:C:T	missense
ITTHFELKHLSSRDLLRDWILRGTE	0.39	30	9:4740973:C:T	missense
KNIRRRVVDALNLLHAWIISKEKK	0.389	30	13:113633938:G:C	missense
FNVVAALAAADCFMCMADGDTMAA	0.300	30	10:10420005:C:T	missense

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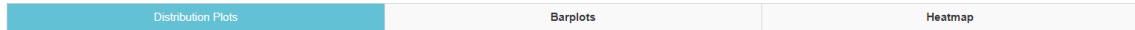


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Finally, we want to assess the tumour microenvironment to check for presence of immunostimulatory immune cells. In the **TME tab**, we observe low estimated cell fractions of CD8+ and CD4+ T cells, suggesting that this tumour might be a cold tumour.

## Tumor Microenvironment Screening



### Cell Fractions

cell_type	cell_fraction
T cell CD8+	0.0208
T cell CD4+	0.0325
T cell regulatory (Tregs)	0.0119
B cell	0.0015
Myeloid dendritic cell	0.0125
Macrophage	0.0023
Neutrophil	0.0598
Cancer associated fibroblast	0.0081
Endothelial cell	0.0144
Uncharacterized cells	0.8369

### Cell Type

Please select a cell type.

T cell CD8+

Please select a cancer type.

Skin Cutaneous Melanoma

Distribution of TCGA samples

